

**AM5023- PHYSIOLOGICAL MEASUREMENTS AND  
INSTRUMENTATION LABORATORY**

**AM ELECTRONICS - LABORATORY REPORT**

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**DEPARTMENT OF APPLIED MECHANICS &  
BIOMEDICAL ENGINEERING**

**INDIAN INSTITUTE OF TECHNOLOGY, MADRAS**

# MEASUREMENTS USING OSCILLOSCOPE

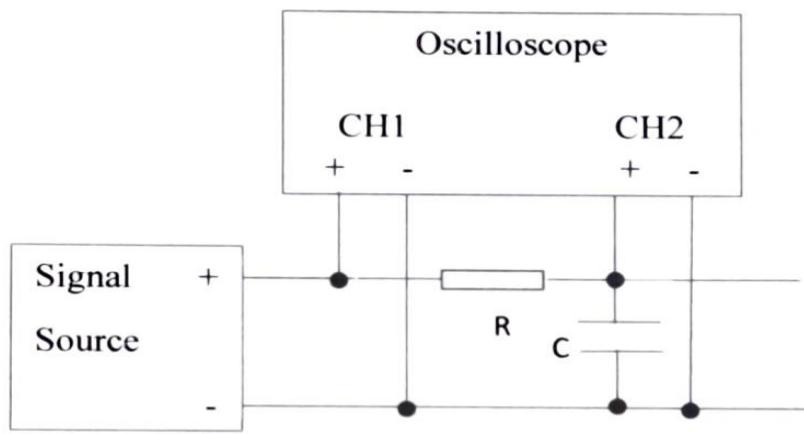
## Aim :

To become familiar with basic front panel controls of Oscilloscopes and use for measurements.

## Materials Required :

Signal Source, Oscilloscope with probes, Bread board, Resistor and Capacitor.

## Circuit Diagram and Measurement Setup :



## Useful Calculations :

For Voltage measurement : (No.of divisions on vertical axis) x (Volts / division)

For Frequency measurement :

Time = (No.of divisions on horizontal axis) x (Time / division)

Frequency = (1/ Time) Hz

Cut-off frequency of Low pass filter =  $(1/2\pi RC)$  Hz

**Table for Measurement :**

Sl.No	Signal Source		Oscilloscope			
	Amplitude (p-p)	Frequency	Horizontal axis measurement		Vertical axis measurement	
			Time	Frequency	CH1	CH2
1	5V	50 Hz	20 ms	50 Hz	5V	4.7 V
2	5V	100 Hz	10 ms	100 Hz	5V	4.2 V
3	5V	500 Hz	2 ms	500 Hz	5V	1.7 V
4	5V	1 KHz	1 ms	1 KHz	5V	0.9 V
5	5Vpp+ 1VDC	1K Hz	20 ms	50 Hz	5V	4.7 V

**Procedure :**

Make connections as per circuit diagram on bread board. Connect signal source and oscilloscopes to the circuit as per the measurement set-up. Using oscilloscope (with out Auto scale option), Set signal source amplitude and frequency as per the table values. Use the trigger option(s) for the stable display of signal on screen of Oscilloscope. Tabulate the horizontal and vertical axis measurements from oscilloscope. While following fifth row of Table use AC/ DC coupling options of oscilloscope to see only AC or AC+DC signal.

**Results / Observations :**

- Become familiar with voltage, time and frequency measurements using basic front panel controls of oscilloscope without using auto-scale option.
- Additionally, frequency response of first-order, R-C low pass filter is studied.
- In place of R-C filter, any circuit like Instrumentation amplifier, filter or any Op-amp based amplifier may be placed to study its characteristics.

# INSTRUMENTATION AMPLIFIER

## Aim:

To build and test the Instrumentation amplifier using op-amps.

## Materials Required:

Multi-output Power Supply (DCPS), Signal Source, Oscilloscope with probes, Bread Board, Resistor (10 kohm – 7Nos) and IC LM358-2 Nos or LM324-1 No.

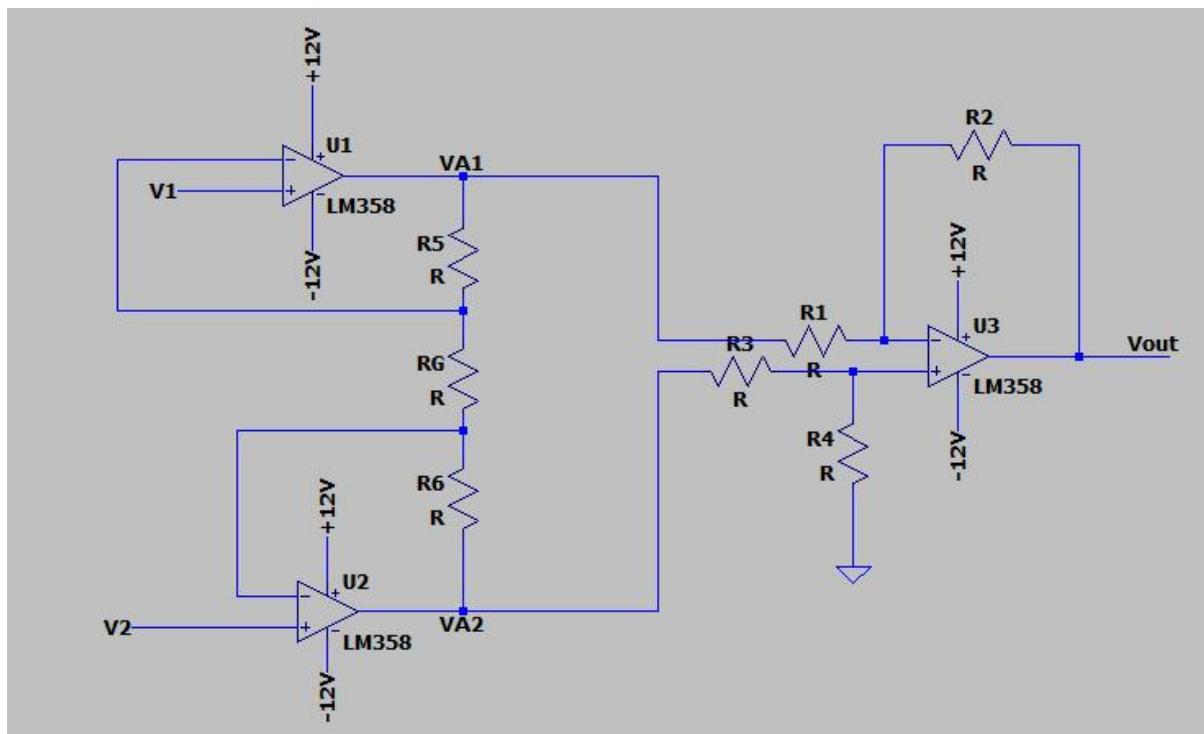
## Theory of Operation:

In practical applications like industrial controllers and home appliances, to control and/ or display the physical quantities like light level, temperature, pressure, weight, humidity, flow of gas or water...etc, it's measurement is very important. Transducers are widely used to measure these quantities. Transducers are the sensors that convert physical quantity into electrical quantity (directly or indirectly). As the output of the transducers are very low like in the order of  $\mu\text{V}$  to  $\text{mV}$ , it must be amplified before fed to display or next processing circuit. The amplifier should also provide the following features for accurate and reliable measurement for better control and display. Those are

- Programmable gain
- High CMRR-Common Mode Rejection Ratio (ability to reject the common noise)
- Stable gain with low temperature sensitivity
- Low DC offset against temperature and time
- Low output impedance and high input impedance

One such a amplifier is Instrumentation Amplifier (INA) using three op-amps. The typical circuit diagram of INA is given in Figure-1. In this circuit, the first stage consists of amplifiers A1 and A2, are in Non-inverting amplifier configuration and sharing common gain resistor  $R_G$ . Second stage is typical differential amplifier. Now a days, INA is available in single chip with all required components/ circuits are integrated and require only minimal external components.

**Circuit diagram:**



**Table for measurement:**

Sl. No.	Inputs		Output
	V1	V2	
1	5V DC	4V DC	-2.77 V
2	5V DC	6V DC	3.11 V
3	4V DC	5V DC	3.08 V
4	0V	1Vpp-1kHz sine	$V_{OD} = 2.6 \text{ V}$
5	1Vpp-1kHz sine	1Vpp-1kHz sine	$V_{oc} = 0.4 \text{ mV}$

## **Useful design Equations:**

### **A3 amplifier stage:**

For  $R_1 = R_3$  and  $R_2 = R_4$ ,  $V_{out} = (R_2 / R_1) * (V_{A2} - V_{A1})$

### **First stage amplifier:**

For,  $R_6 = R_5$

$$V_{A1} = \{(R_5 + RG) * V_1 - (R_5 * V_2)\} / RG$$

$$V_{A2} = \{(R_5 + RG) * V_2 - (R_5 * V_1)\} / RG$$

### **Combined circuit:**

$$V_{out} = (R_2 / R_1) * (1 + 2R_5/RG) * (V_2 - V_1)$$

### **Procedure:**

Calculate the resistance values for overall gain of 3. Using these resistance values, make connections as per the circuit diagram on bread board. Give the input voltages  $V_1$  and  $V_2$  as per the Table. Record the amplifier response ( $V_{out}$ ). Calculate the following values from the observations.

$$CMRR = 20\log|A_d / A_c| \text{ dB}$$

$$A_d = V_{OD} / (V_1 - V_2) \text{ and } A_c = V_{OC} / (V_1 \text{ or } V_2)$$

### **Results / Observations:**

- Wired and tested the Instrumentation Amplifier (INA).
- The theoretical gain of INA = **3** and observed gain = **2.9**.
- Observed CMRR = **76.25 dB**.

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**NANOMOLECULAR- LABORATORY REPORT**

Submitted by: DINESH KUMAR M

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## **BIOCOMPATIBILITY ASSAY – MTT ASSAY**

### **Aim:**

To measure cellular metabolic activity as an indicator of cell viability, proliferation and cytotoxicity.

### **Materials Required:**

Cell lines and cell culture reagents

Cells in appropriate medium

Drug/Test Compound

Adjustable pipettes and a repeat pipettor

MTT Reagent

Flat bottom 96-well microtiter plate for culturing the cells

IX PBS (Phosphate Buffered saline)

DMSO (Dimethyl Sulfoxide)

96-well plate reader capable of measuring the absorbance

### **Theory Of Operation:**

This colorimetric assay is based on the reduction of a yellow tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide or MTT) to purple formazan crystals by metabolically active cells. The viable cells contain NAD(P)H-dependent oxidoreductase enzymes which reduce the MTT to formazan. The insoluble formazan crystals are dissolved using a solubilization solution and the resulting coloured solution is quantified by measuring absorbance at 500-600 nanometres using a multi-well spectrophotometer. The darker the solution, the greater the number of viable, metabolically active cells.

### **Procedure:**

For the determination of the cytotoxic effect of Test compound on target cells

## Preparation of cells:

Always use freshly harvested cells for assay. Seed the cells in a cell culture flask or dish in an amount appropriate for the assay and incubate at 37°C in a 5% CO<sub>2</sub> environment. Allow the cells to grow up to 24 hours or till confluence is reached.

Assay controls Include appropriate assay controls i.e.

1. Medium control (medium without cells)
2. Cell control (medium with cells but without the experimental drug/compound)
3. Vehicle control (medium with the cells and solvent in which the experimental drug or compound is dissolved)

Harvest the cells and use for the assay. (Note: Quantity of the cell suspension to be seeded in the medium depends upon doubling time of individual cell lines and seeding density to be used in assay).

Seed 200µl cell suspension in a flat bottom 96-well plate at the optimized cell density.

Incubate the plate at 37°C in a 5% CO<sub>2</sub> atmosphere for 24 hrs.

After the incubation period, remove the plates from incubator and treat with test compound at respective concentration and time intervals.

After the incubation period, remove the existing medium from the plate and wash the cells gently with 1X PBS and remove the PBS, add 100 µl of the MTT labelling reagent (final concentration 5mg/ml) to each well.

Return the plates to the incubator and incubate for 2 to 4 hours.

(Note: Incubation time varies for different cell lines. Incubation time should be kept constant while making comparisons. Some cell lines may require incubation for up to 24 hours.)

Remove the plate from incubator after incubation and gently remove the MTT reagent in the plate and add 100 µl of DMSO to each well, provide a gentle shake on the gyratory shaker to enhance uniform dispersion of reagent.

Read the absorbance on spectrophotometer or an ELISA reader by using 570 nm as primary filter.

Subtract the average absorbance values of the blank wells from control wells and corresponding experimental wells.

% Viability = Mean OD (sample)/ Mean OD(Control) x 100

Note: Assay should be done in triplicates.

## **Results / Observations:**

Absorbance values that are lower than the control cells indicate a reduction in the rate of cell proliferation. Conversely a higher absorbance rate indicates an increase in cell proliferation. Rarely, an increase in proliferation may be offset by cell death; evidence of cell death may be inferred from morphological changes.

Applications:

- Cell proliferation: Quantification of changes in proliferative activity of cells caused by trophic factors, cytokines, and growth promoters.
- Cell cytotoxicity: Evaluation of effects of inhibitors or inducers of apoptosis, cytotoxic reagents, carcinogens and toxins.
- Drug discovery: High-throughput screening of various anti-cancer drugs

## Troubleshooting Points

**Problem:** Blanks (medium only) give high absorbance readings

Cause	Remedy
The medium is contaminated with cells/bacteria/yeast (visible under microscope).	Discard. Check medium before plating Use sterile technique for cell plating in biological hood. Use sterile 96-well plate.
The medium contains ascorbic acid.	Incubate plate in the dark. Find alternative medium if possible.

**Problem:** Absorbance readings too high.

Cause	Remedy
Cell number per well too high.	Decrease cell density at plating.
Contamination of culture with bacteria or yeast.	Discard. View wells prior to addition of MTT Reagent to check for contamination.

**Problem:** Absorbance readings are too low.

Cause	Remedy
Cell number per well is too low.	Increase cell density at plating.
Incubation time for reduction of MTT is too short. No purple colour visible in cells when viewed under microscope.	Increase incubation time with MTT Re-agent until purple color is evident inside cells when viewed under microscope. Longer incubation of up to 24 hours may be required for some cell types.
Incubation time for solubilization of formazan dye too short (intact cells with intracellular dye visible when viewed under the microscope).	Increase incubation time with Detergent Reagent or incubate at 37°C. View under microscope to ensure no crystals remain out of solution.
Cells not proliferating due to improper culture conditions or inadequate time of recovery after plating.	Check that culture conditions (medium, temperature, humidity, CO <sub>2</sub> , etc.) are appropriate. View cells periodically to check condition.

	Increase time in culture after plating for cell recovery.
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**Problem:** Replicates have different values.

Cause	Remedy
Inaccurate plating or pipetting.	Increase accuracy of cell plating, check accuracy of pipette.

# **DILUTIONS AND MOLARITY CALCULATIONS/ SAFE LAB PRACTICES**

## **DILUTION**

To prepare a fixed amount of dilute solution, we have a formula

$$C_1 \cdot V_1 = C_2 \cdot V_2$$

Where,

$V_1$  denotes the volume of stock solution needed to make the new solution

$V_2$  is the final volume of the solution

$C_1$ = concentration of the stock solution

$C_2$ = Final concentration of stock solution

## **MOLARITY**

Molarity (M)= no. of moles of solute/ volume of solution

As we know,

Moles= Mass/molar mass

Molarity(M)= (mass of the solute(W) /molar mass of the solute(M)) \*volume of solution (V)

$$= \text{mass of solute} \cdot 1000 / (\text{molar mass of the solute} \cdot V \text{ (ml)})$$

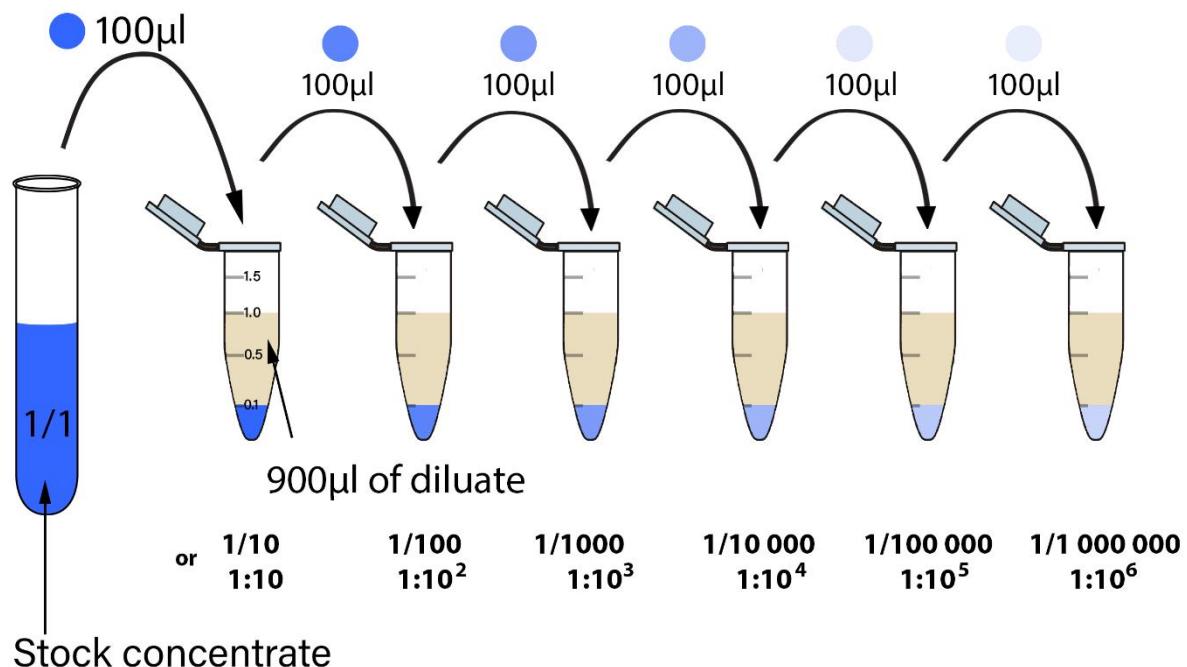
The units of molarity are mol/ L or mol/dm<sup>3</sup>

## **MOLALITY**

Molality (m) = No. of moles of solute/ mass of solvent in Kg

$$m = \text{mass of solute} \cdot 1000 / (\text{Molar mass of solute} \cdot \text{Mass of solvent in g})$$

## SERIAL DILUTION



## DILUTION FACTOR

After dilution, the dilution factor (or dilution ratio) represents how much of the original stock solution remains in the entire solution. It's usually expressed as a ratio, although it can also be expressed as an exponent.

The part of the stock solution to the part of the dilutant added (S: D) or the part of the stock solution to the part of the total solution (S: T) is described by the solution factor, which can be expressed as a ratio or an exponent.

Below mentioned are the steps to calculate the dilution factor by hand:

- Find any two of the following three values: stock solution volume (stock), dilutant solution volume (dilutant), and total solution volume (total). This can be done either theoretically (before conducting the experiment) or experimentally (after the experiment)
- With this equation, we can find the third volume using the two volumes: stock + dilutant = total. This step may not be necessary if we know which notation we want to use (S:D or S: T), but it is included for completeness
- Convert the numbers to the same units as each other.
- We decide which notation we require:
  - S:D = set the stock and dilutant amount values as ratio stock: dilutant.

- S: T = set the stock and total amount values as a ratio- stock: total.
- If necessary, we find the Greatest Common Factor to cancel down the fractions.

## **EXAMPLES**

Note: We divide the final volume by the initial volume.

$$DF = V_f/V_i$$

Example 1:

When a 0.1 ml aliquot of a specimen is added to 9.9 ml of diluent, what is the dilution factor?

Solution:

$$V_f = \text{aliquot volume} + \text{diluent volume} = (0.1 + 9.9) \text{ ml} = 10.0 \text{ ml}$$

$$Df = 10/0.1 = 100$$

Thus, we have diluted the sample by a factor of 100.

The denominator of a fraction is frequently the dilution factor. A DF of 100, for example, indicates a dilution of 1:100

## **SAFE PIPETTING PRACTICES:**

### **USAGE**

1. Pre-wet tip. Aspirate liquid into the tip and dispense two or three times before taking up the desired volume.
2. Immerse to appropriate depth during aspiration. Immerse the tip below the meniscus. Large volume pipettes should be immersed to 5- 6 mm, while smaller volume pipettes should be immersed by to 2- 3 mm.
3. Pause consistently after aspiration and before removing the tip from the liquid. The liquid will continue moving into the tip after the plunger stops, so this consistent pause before removing the tip from the liquid will ensure the amount of liquid drawn into the pipette is accurate.
4. Use consistent plunger pressure and speed.
5. Aspirate sample at a 90-degree angle and dispense at a 45-degree angle. These angles are optimal for ensuring that all material is drawn up into, and expelled from, the tip.

6. Reduce heat transfer by holding the pipette loosely and setting down when not in use.

## **STORAGE**

1. Store pipettes vertically to avoid contamination.
2. Store pipetted dialed to highest volume setting.
3. Store away from heat and moisture.
4. Pipetted tips should be removed prior to storage.
5. Keep pipettes clean and free of contaminants.
6. When cleaning pipettes with bleach solution, do not saturate as fluid can make its way to the piston and cause corrosion.

## **CLEANING LABORATORY GLASSWARE**

Stir bars, spatulas, funnels, flasks, beakers, and other reusable equipment

1. To remove organic residues, rinse glassware briefly with an organic solvent (acetone or ethanol). The used rinse will then be discarded into the organic waste.
2. Use warm tap water and a brush with soapy water to scrub the inside of curved glassware. This waste water can go down the sink.
3. Remove soapsuds with deionized water to avoid harsh water stains. To expedite the glass-drying process, rinse again with acetone to remove water. The residual acetone will go into the organic waste container.

## **Practice Questions**

1. Determine the number of grams of NaHCO<sub>3</sub> that are in one liter of a 2.1 M solution

Molecular wt of NaHCO<sub>3</sub> - 84.01g

M = no. of moles / vol in L

2.1M = 2.1 moles / 1L

Moles = mass ÷ molar mass

2.1moles = mass / 84.01

mass = 2.1 × 84.01 mass = 176.421g

2. You have to make 500mL of a 0.5M BaCl<sub>2</sub>. You have 2.0M barium chloride solution available. Determine how to make the needed dilution.

$$C_1V_1 = C_2V_2$$

$$0.5 \times 500 = 2.0 * x$$

$$x = (0.5 * 500) / (2.0 * 1000)$$

$$= 5/40$$

$$= 125\text{ml}$$

# POLYMERASE CHAIN REACTION (PCR)

## AIM

Amplify the nucleotide sequence of a desired gene.

## MATERIALS REQUIRED

- Sterile distilled water
- 10X Taq buffer
- dNTPs
- Primers (both Forward and Reverse)
- Taq DNA polymerase
- Template DNA

## INTRODUCTION

PCR is an in vitro method of enzymatic synthesis of specific DNA fragments developed by Kary Mullis in 1983. It is very simple technique for characterizing, analysing and synthesizing DNA from virtually any living organism (plant, animal, virus and bacteria). The purpose of a PCR is to make a huge number of copies of a gene. This is necessary to have enough starting template for sequencing or for gene cloning. Any sequence of DNA can be amplified if the flanking sequences are known. Based on the flanking sequence, the primers 1 and 2 must be designed and used for PCR amplification of the desired gene. PCR amplification is done by the following steps:-

**The cycling reactions:** There are three major steps in a PCR, which are repeated for 25 or 30 cycles. This is done on an automated cycler, which can heat and cool the tubes with the reaction mixture in a very short time.

### Denaturation at 95°C

During the denaturation, the double strand melts open to single stranded DNA, all enzymatic reactions stop (for example: the extension from a previous cycle).

### Annealing at 50-65°C

Ionic bonds are constantly formed and broken between the single stranded primer and the single stranded template. The more stable bonds last a little bit longer (primers that fit exactly) and on that little piece of double stranded DNA (template and primer), the polymerase can attach and starts copying the template. Once there are a few bases built in, the ionic bond is so strong between the template and the primer, that it does not break anymore.

### Extension at 72°C

This is the ideal working temperature for the polymerase. The primers, where there are a few bases built in, already have a stronger ionic attraction to the template than the forces breaking these attractions. Primers that are on positions with no exact match, get loose again (because of the higher temperature) and don't give an extension of the fragment. The bases (complementary to the template) are coupled to

the primer on the 3' side the polymerase adds dNTP's from 5' to 3', reading the template from 3' to 5' side, bases are added complementary to the template).

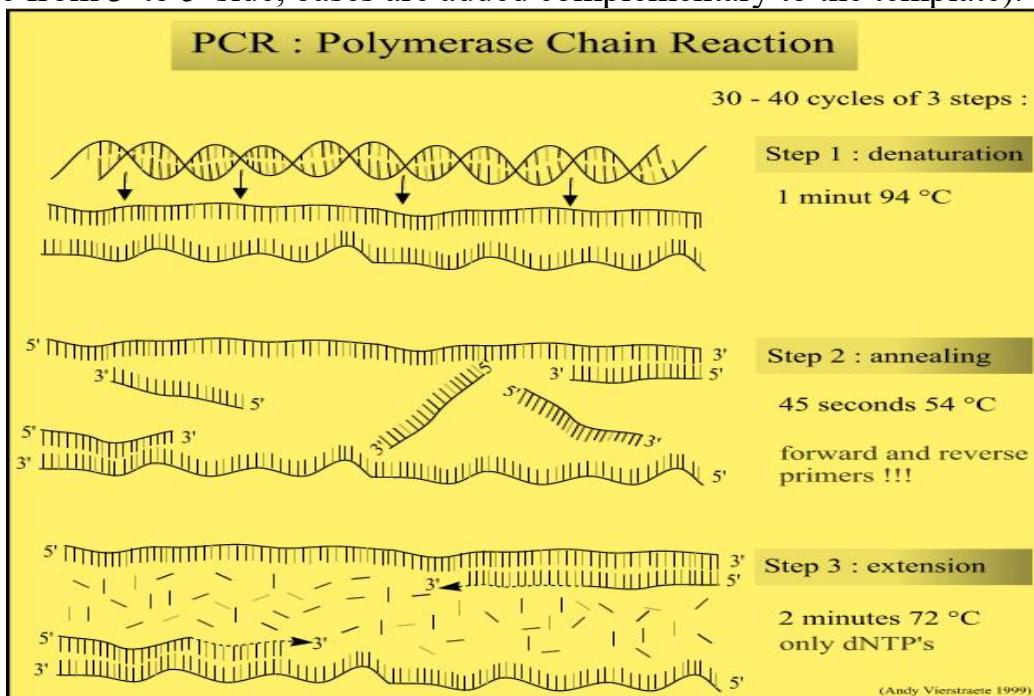


Fig.01-Different steps in PCR

## EXPONENTIAL AMPLIFICATION

Both strands are copied during PCR, so there is an exponential increase of the number of copies of the gene. Suppose there is only one copy of the wanted gene before the cycling starts, after one cycle, there will be 2 copies, after two cycles, there will be 4 copies, and three cycles will result in 8 copies and so on.

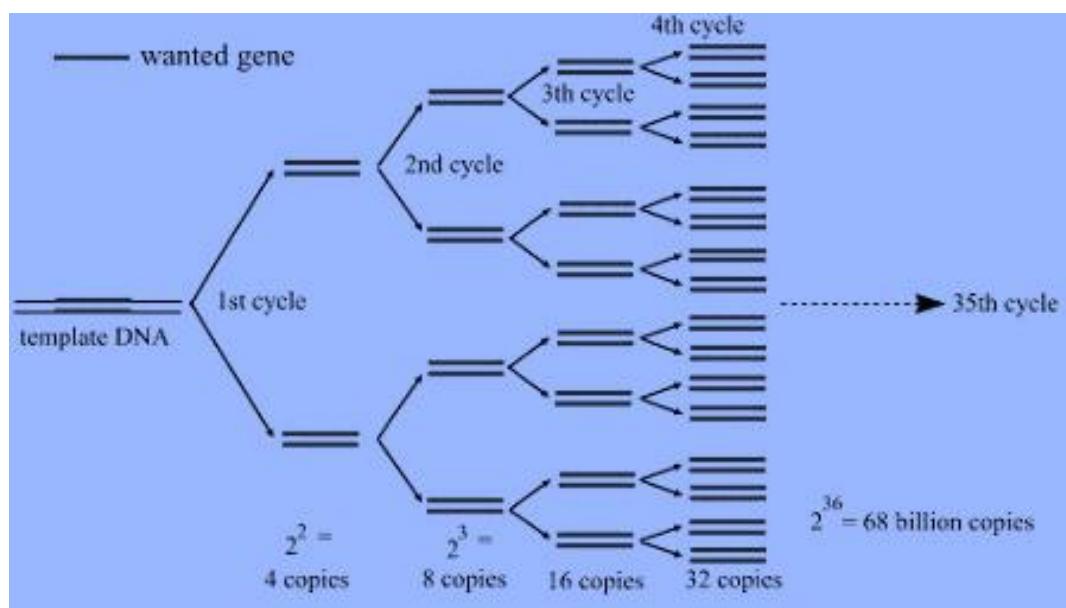


Fig.02-Exponential amplification of the gene in PCR

## **PROCEDURE**

- Gently vortex and briefly centrifuge all solutions after thawing.
- To a 0.2 ml PCR microfuge tube add the following contents

Content	Volume
Sterile distilled water	12.8 µl
Taq buffer (10X)	2.0 µl
2 mM dNTPs mix	2.0 µl
Forward Primer (10 to 15 pM)	1.0 µl
Reverse Primer (10 to 15 pM)	1.0 µl
Taq polymerase (1.25 U/50 µl)	0.2 µl
Template DNA	1.0 µl
Total volume	20.0 µl

- Gently vortex the sample and briefly centrifuge to collect all drops from walls of tube.
- Overlay the sample with half volume of mineral oil or add an appropriate amount of wax. This step may be omitted if the thermal cycler is equipped with a heated lid.
- Place the tubes into the slots of thermocycler and run for 35 to 40 cycles with the following PCR condition:-

**Step 1:** 95° C for 10 mins

**Step 2:** 95° C for 1 min

**Step 3:** Annealing temp (55-60) for 1 min 3 0)

**Step 4:** 72°C for 1 min

**Step 5:** 35 time's step 2

**Step 6:** 72 °C for 10 mins

**Step 7:** 4 °C forever

**Step 8:** End

## **RESULT**

Familiarised various steps involved in PCR reaction and the related equipment.

# SYNTHESIS OF HEMATITE NANOPARTICLES AND CHARACTERIZATION

## INTRODUCTION

Hematite ( $\alpha\text{-Fe}_2\text{O}_3$ ) is a stable iron oxide nanoparticle with low cost, high corrosion resistance, thermal stability, conductivity, n-type semiconducting and magnetic properties. Owing to varying particle size, shape, magnetic and physical properties,  $\alpha\text{-Fe}_2\text{O}_3$  nanoparticles have been considered as a polymer reinforcement.

## AIM

To synthesize spherical hematite nanoparticles and undergo characterization of nanoparticles

## MATERIALS REQUIRED

Iron Nitrate (  $\text{Fe}(\text{NO}_3)_3$  )

Barium Chloride (  $\text{BaCl}_2$  )

Polyvinyl Pyrolydine ( PVP )

## THEORY OF OPERATIONS

Formation of the uniform  $\alpha\text{-Fe}_2\text{O}_3$  ellipsoidal particles are obtained by forced hydrolysis, which is obtained by precipitation of iron (III) perchlorate in the presence of urea. Urea ( $\text{CO}(\text{NH}_2)_2$ ) liberates hydroxide ions which is used to promote the precipitation of metal oxides when heated in aqueous solutions. The addition of phosphate resulted in ellipsoidal  $\alpha\text{-Fe}_2\text{O}_3$  particles whose axial ration increased with the relative phosphate to iron amount. The particle shape from the tiny spheres evolved to ellipsoids could be explained through the aggregation of the initially precipitated particles. This led to a reduction in the observed particle count as aging time increased. The growth of particles occurs through the aggregation of much smaller primary particles or through the diffusion of solutes from the solution to the particle's surface.

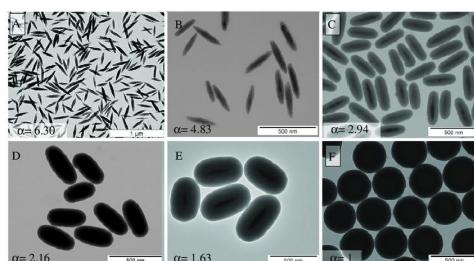


Fig. 1 : Ellipsoidal hematite nanoparticles

## **PROCEDURE**

**Synthesis of hematite nanoparticles** (Here hematite acts as a targeted drug delivery carrier; a cargo)

1. Ellipsoidal hematite nanoparticles are produced by combining 0.5388 mmoles of  $\text{Fe}(\text{ClO}_4)_3 \cdot 6\text{H}_2\text{O}$ , 2.497 mmoles of  $\text{NH}_2\text{CONH}_2$ , and 0.13 mmoles of  $\text{NaH}_2\text{PO}_4$  in 25 mL of Milli-Q water.
2. The solution is placed in a hot air oven and incubated at  $100 \pm 2^\circ\text{C}$  for 24 hours.
3. After incubation, the solution should change color to reddish-brown, confirming hematite nanoparticle synthesis.
4. Next, the solution is centrifuged at 8,000 rpm for 30 minutes.
5. The resulting pellets are washed repeatedly with Milli-Q water until the supernatant becomes clear.
6. Finally, the pellets are redissolved in Milli-Q water to create a nanoparticle suspension.

## **CHARACTERIZATION OF HEMATITE NANOPARTICLES**

### **UV-V is spectroscopy**

The UV-Visible absorption spectra of the hematite nanoparticles were acquired by subjecting the HNP suspension to a scan within the wavelength range of 200 to 1100 nm using a UV spectrophotometer.

### **Scanning electron microscopy**

The process of sample preparation involved depositing a droplet of the HNP solution onto a  $10 \times 10 \text{ mm}^2$  glass slide. To enhance conductivity, a 120s gold sputter coating was applied. High-resolution scanning electron microscopy (SEM) was then utilized for imaging.

## **PARTICLE SIZE HISTOGRAM AND CALCULATION**

### **Calculation**

To determine the mass of each compound when combined in a 25 ml solution, we'll use the given quantities in moles and their respective molar masses.

### **Given**

- $\text{Fe}(\text{ClO}_4)_3 \cdot 6\text{H}_2\text{O}$ : 0.5385 mmoles

- NH<sub>2</sub>CONH<sub>2</sub>: 2.497 mmoles
- NaH<sub>2</sub>PO<sub>4</sub>: 0.13 mmoles

## Solution

### Molar masses:

- Fe (ClO<sub>4</sub>)<sub>3</sub>.6HO

Molar mass of Fe = 55.845 g/mol

Molar mass of Cl = 35.453 g/mol Molar

mass of O = 15.999 g/mol Molar mass of

H<sub>2</sub>O = 18.015 g/mol

Fe (ClO<sub>4</sub>)<sub>3</sub>.6HO = Molar mass of Fe + 3 \* Molar mass of Cl + 6 \* Molar  
mass of O + 6 \* Molar mass of H<sub>2</sub>O

Molar mass of Fe (ClO<sub>4</sub>)<sub>3</sub>.6HO = 55.845 + 3 \* 35.453 + 6 \*  
15.999 + 6 \* 18.015 = **419.32 g/mol.**

- NH<sub>2</sub>CONH<sub>2</sub>

Molar mass of N (Nitrogen) = 14.007 g/mol Molar

mass of H (Hydrogen) = 1.008 g/mol Molar mass

of C (Carbon) = 12.011 g/mol Molar mass of O

(Oxygen) = 15.999 g/mol

Molar mass of NH<sub>2</sub>CONH<sub>2</sub> = Molar mass of N + 2 \* Molar mass of H +  
Molar mass of C + 1 \* Molar mass of O

Molar mass of NH<sub>2</sub>CONH<sub>2</sub> = 14.007 + 2 \* 1.008 + 12.011 + 15.999

Molar mass of NH<sub>2</sub>CONH<sub>2</sub> = **60.056 g/mol**

- NaH<sub>2</sub>PO<sub>4</sub>.

Molar mass of Na (Sodium) = 22.99 g/mol Molar

mass of H (Hydrogen) = 1.008 g/mol

Molar mass of PO<sub>4</sub> (Phosphate group) = **94.971 g/mol**

Molar mass of NaH<sub>2</sub>PO<sub>4</sub> = Molar mass of Na + Molar mass of H<sub>2</sub> +  
Molar mass of PO<sub>4</sub> Molar mass of NaH<sub>2</sub>PO<sub>4</sub> = 22.99 + 2.016 + 94.971

Molar mass of NaH<sub>2</sub>PO<sub>4</sub> = **119.976 g/mol**

1. Mass of Fe (ClO<sub>4</sub>)<sub>3</sub>.6HO:

$$\text{Mass} = \text{Number of moles} \times \text{Molar mass}$$

$$\text{Mass} = 0.5385 \text{ mmoles} \times 419.32 \text{ g/mol} = \mathbf{225.919 \text{ mg}}$$

2. Mass of NH<sub>2</sub>CONH<sub>2</sub>:

$$\text{Mass} = \text{Number of moles} \times \text{Molar mass}$$

$$\text{Mass} = 2.497 \text{ mmoles} \times 60.056 \text{ g/mol} = \mathbf{149.899 \text{ mg}}$$

3. Mass of NaH<sub>2</sub>PO<sub>4</sub>:

$$\text{Mass} = \text{Number of moles} \times \text{Molar mass}$$

$$\text{Mass} = 0.13 \text{ mmoles} \times 119.976 \text{ g/mol} = \mathbf{15.597 \text{ mg}}$$

## PARTICLE SIZE CALCULATION FROM IMAGE J

The image is loaded in Image J and the scale is set according to the scale bar in the image. Then the image is filtered using band pass filtered and then thresholded to get the edges. Then The particle analysis is performed.

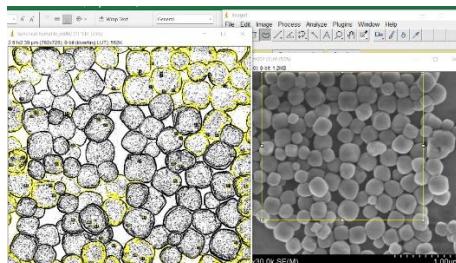


Fig. 2. Getting particle area from Image J

The area obtained from the Image J was used to calculate the particle diameter and then the particle size distribution was plotted in Origin as shown in the figure below.

	Area	Mean	Min	Max	Angle	Length
1	9.734E-4	156.619	131.209	202	18.838	0.295
2	5.883E-4	164.095	114.000	178.409	36.027	0.178
3	8.023E-4	181.130	125.333	223.018	14.036	0.243
4	0.002	157.629	117.000	212.000	7.289	0.281
5	0.002	157.629	117.000	212.000	7.289	0.281
6	0.001	148.608	120.208	212.333	16.750	0.350
7	8.986E-4	150.558	130.307	187.514	7.549	0.273
	Area	Mean	Min	Max	Angle	Length
1	0.001	127.485	102	155.149	16.587	0.321
2	0.001	131.789	109.154	162.000	14.859	0.331
3	7.689E-4	162.233	123.000	192.795	-8.130	0.231
4	8.117E-4	157.349	123.500	193.154	-27.937	0.243
5	0.001	146.565	115.353	203.667	-2.291	0.329
6	8.010E-4	154.523	117.500	198.770	19.654	0.242

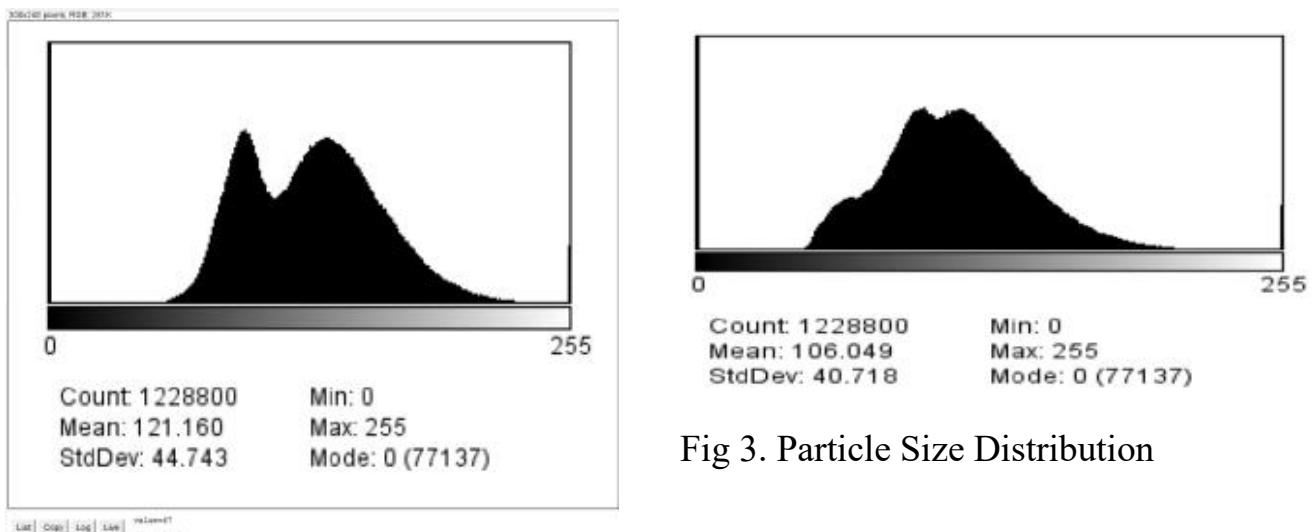


Fig 3. Particle Size Distribution

## PARTICLE MORPHOLOGY

The precise calculation and control of  $\text{Fe}(\text{ClO}_4)_3 \cdot 6\text{H}_2\text{O}$ ,  $\text{NH}_2\text{CONH}_2$ , and  $\text{NaH}_2\text{PO}_4$  masses are pivotal in tailoring particle morphology. These meticulous measurements directly influence nanoparticle size, shape, and surface characteristics, crucial in crafting effective drug delivery systems. Such control ensures reproducibility and consistency, essential for optimizing nanoparticles' properties for targeted therapeutic applications.

## RESULTS/ OBSERVATION

In summary, ellipsoidal hematite nanoparticles, serving as targeted drug carriers, are successfully synthesized through a meticulous process involving precise quantities of  $\text{Fe}(\text{ClO}_4)_3 \cdot 6\text{H}_2\text{O}$ ,  $\text{NH}_2\text{CONH}_2$ , and  $\text{NaH}_2\text{PO}_4$  in a Milli-Q water solution. The controlled incubation at  $100 \pm 2^\circ\text{C}$  for 24 hours yields a distinct reddish-brown color, confirming nanoparticle formation. Centrifugation and thorough washing result in purified pellets, later redissolved in Milli-Q water, ultimately producing a nanoparticle suspension ready for potential applications in targeted drug delivery systems.

**AM5023- PHYSIOLOGICAL MEASUREMENTS AND  
INSTRUMENTATION LABORATORY**

**BIOMEDICAL SIGNAL PROCESSING - LABORATORY  
REPORT**

Submitted by: DINESH KUMAR M

Registration no: AM23M022



**DEPARTMENT OF APPLIED MECHANICS &  
BIOMEDICAL ENGINEERING**

**INDIAN INSTITUTE OF TECHNOLOGY, MADRAS**

# **ELECTROCARDIOGRAM MEASUREMENT**

## **AIM:**

To understand Electrocardiography (ECG) measurement techniques, principles, and the interpretation of ECG waveforms using limb leads.

## **OBJECTIVES:**

- To understand the anatomy and physiology of the heart and the generation of electrical signals in the cardiac cycle.
- To record and interpret normal and abnormal ECG waveforms using limb leads.
- To understand the clinical significance of ECG measurements.

## **APPARATUS REQUIRED:**

- ECG Machine
- ECG Electrodes (limb electrodes)
- Electrode gel or paste
- ECG paper

## **THEORY:**

### **Heart:**

The human heart is an electrical organ that generates electrical impulses, causing it to contract rhythmically. These electrical impulses can be recorded using an electrocardiogram (ECG). In Einthoven's triangle, the focus is on recording ECG signals using limb leads. Limb leads I, II, and III create a triangle on the body's surface, capturing electrical activity from different angles.

### **Principles:**

- Electrical activity originates in the sinoatrial (SA) node and travels through the heart.
- Limb electrodes placed on the wrists and ankles capture electrical signals, creating Einthoven's triangle.
- Different limb leads provide a comprehensive view of the heart's electrical activity.

- The ECG waveform consists of P, QRS, and T waves, corresponding to atrial depolarization, ventricular depolarization, and ventricular repolarization.

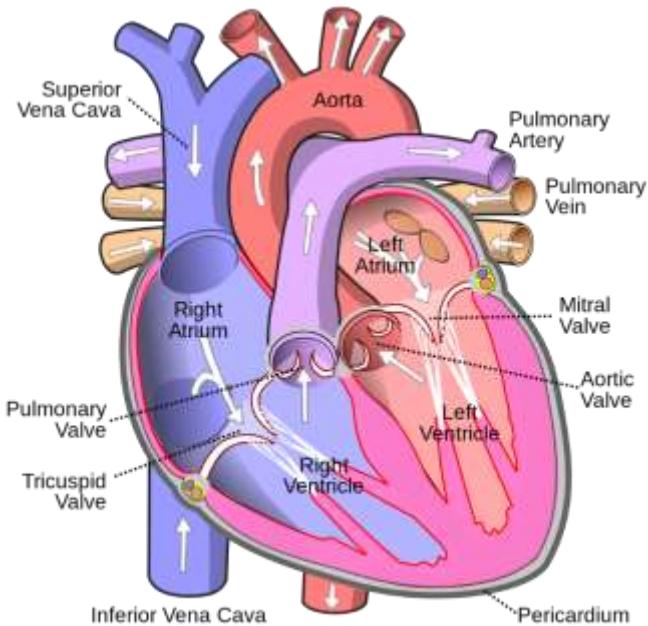


Fig 1. Anatomy of the Heart

## METHODS:

### 1. Preparation:

- Ensure the ECG machine is functioning correctly and connected to a power source.
- Check and calibrate the ECG machine if necessary.
- Clean the skin at the electrode placement sites on the wrists and ankles with alcohol swabs to remove oils and debris.
- Apply electrode gel or paste to the electrode sites to ensure good electrical contact.
- Attach limb electrodes to the wrists and ankles as per standard placement.

### 2. Creating Einthoven's Triangle:

**Limb Lead I:** Attach one electrode to the right wrist and another to the left wrist.

**Limb Lead II:** Attach one electrode to the right wrist and another to the left ankle.

**Limb Lead III:** Attach one electrode to the left wrist and another to the left ankle.

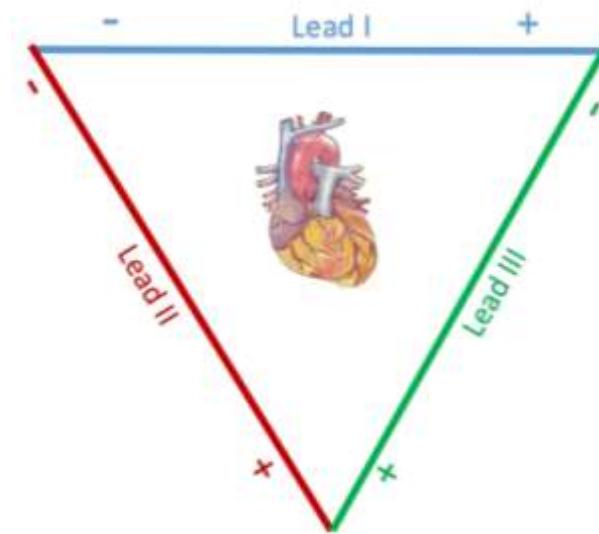


Fig 2. Einthoven's Triangle

### 3. ECG Recording:

- Set the ECG machine to standard parameters (paper speed, voltage, lead configuration) if it's not set default.
- Start the recording and allow the ECG to run for a sufficient duration to capture a representative cardiac cycle.

### 4. Analysis and Interpretation:

- Examine the ECG waveform for P waves, QRS complexes, and T waves.
- Measure the duration and amplitude of these waves.
- Identify any abnormalities, such as arrhythmias or bundle branch blocks.
- Interpret the findings based on clinical knowledge and reference materials.

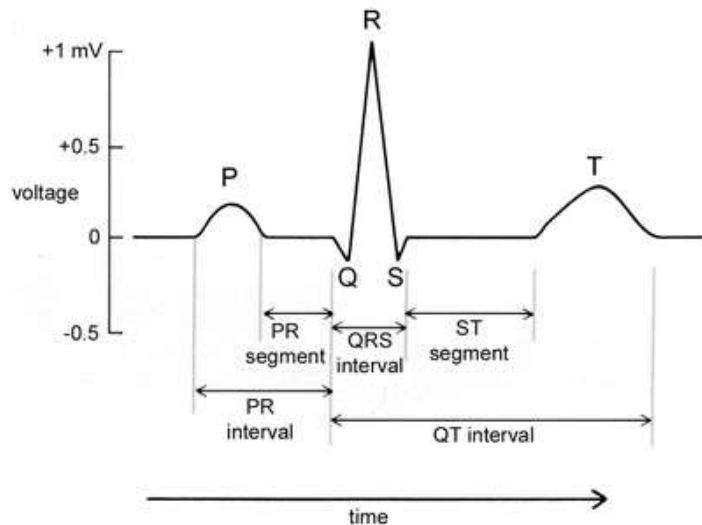


Fig 3. ECG Waveform

Remove electrodes and clean the subject's skin.

Turn off the ECG machine and disconnect all electrodes and leads.

### OBSERVATION:

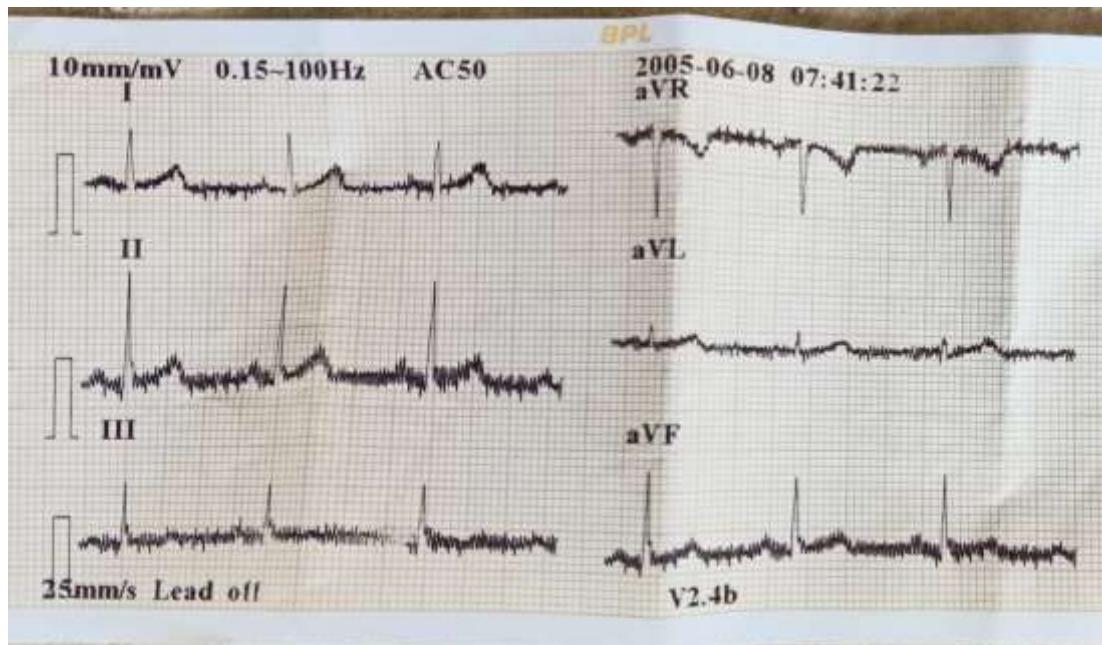


Fig 4. ECG Recording of the subject

Sex	:	Male
BP	:	mmHg
Height	:	cm
Weight	:	kg
HR	:	82 bpm
P Dur	:	80 ms
PR int	:	137 ms
QRS Dur	:	80 ms
QT/QTC int	:	344/404 ms
P/QRST axis	:	62/49/27 °
R/S amp	:	0.000/0.021 mV
S+R amp	:	0.021 mV
R/S amp	:	0.000/0.029 mV

Fig 5. Results Obtained

### **Calculations:**

5 major boxes of electrocardiograph = 1 s

1 major box of electrocardiograph = 0.2 s

R-R interval = number of small boxes between the two R peaks x 0.2 s

$$= 4 \times 0.2 = 0.8 \text{ s}$$

Heart Rate = 60/ R-R interval

$$60/0.8 = 75 \text{ bpm}$$

### **CONCLUSION:**

Through ECG measurements with limb leads and the application of Einthoven's triangle, we obtained valuable data. Our analysis of the ECG waveforms enabled us to calculate the R-R interval, thus allowing for the accurate determination of the heart rate.

# **PURE TONE AUDIOMETRY MEASUREMENT**

## **AIM**

In this study, we assess an individual's auditory capacity using an audiometer, specifically evaluating their hearing through two methods:

- Bone conduction
- Air conduction.

## **OBJECTIVE**

To gain insight into the proper utilization of an audiometer for evaluating air and bone conduction in an individual with typical hearing.

## **APPARATUS REQUIRED**

- An audiometer (Sau Maico-MA 42) equipped with a pure tone generator
- A bone conduction oscillator designed for cochlear function assessment
- An attenuator for adjusting sound intensity
- A microphone for conducting speech tests
- Headphones for evaluating air conduction

## **THEORY**

The ear, a sophisticated organ responsible for hearing and balance, comprises three main sections: the outer, middle, and inner ear. Its essential components include:

**Outer Ear:** This section encompasses the visible pinna and the ear canal, which collects sound waves and guides them into the ear canal. Scientifically known as the external acoustic meatus, the ear canal extends from the pinna to the eardrum and features unique skin and tiny hairs that act as filters for dust and dirt.

**Middle Ear:** Housing the eardrum, ossicles (small bones), and the Eustachian tube connecting to the throat, the middle ear plays a vital role in equalizing air pressure. The eardrum is a thin, cone-shaped membrane that separates the middle ear from the outer ear. When sound waves reach it, it starts to vibrate. Within the middle ear, three tiny bones, namely the hammer (Malleus), anvil (Incus), and stirrup (Stapes), work together to amplify and transmit these vibrations. The hammer is connected to the eardrum, the anvil connects the

hammer to the stirrup, and the stapes, the smallest bone in the human body, connects to the oval window.

**Inner Ear:** This is a delicate sensory organ containing the cochlea (responsible for hearing), semicircular canals (critical for balance), and the vestibule (responsible for orientation and sensing gravity). The cochlea, resembling a spiral seashell, is filled with fluid and lined with specialized cells that detect sound waves and convert them into electrical signals. This intricate process not only translates sound into neural impulses but also contributes to our overall sense of balance.

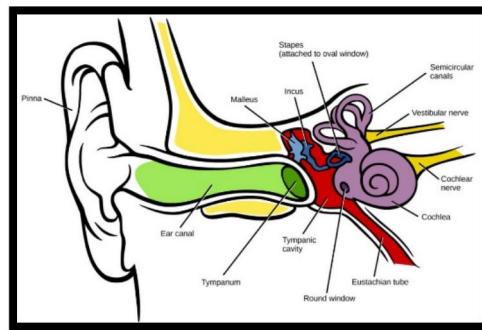


Figure 1. The anatomy of the ear.

## TRANSMISSION OF SOUND IN THE COCHLEA

The transmission of sound in the cochlea involves these key steps:

1. **Sound Entry:** Sound waves enter through the oval window in the cochlea, initially collected by the middle ear's ossicles (hammer, anvil, and stirrup).
2. **Cochlear Fluid Movement:** Sound waves cause the fluids (perilymph and endolymph) inside the cochlea to move, generating pressure waves along the cochlear duct.
3. **Basilar Membrane Vibration:** The cochlea's coiled structure includes the basilar membrane, which vibrates in response to fluid waves, with specific regions vibrating to different sound frequencies.
4. **Hair Cell Activation:** Above the basilar membrane lies the organ of Corti, housing thousands of sensory hair cells. These cells bend when the basilar membrane vibrates, leading to electrical signals.
5. **Neural Transmission:** Bending hair cells open ion channels, allowing ions to enter and create electrical signals. These signals activate auditory nerve fibers connected to the hair cells.

6. Auditory Pathways: Auditory nerve fibers form the auditory nerve, transmitting impulses to the brainstem and onward to auditory processing centers, such as the auditory cortex, where sound is perceived.

## STRUCTURE OF BASILAR MEMBRANE (DIFFERENT FREQUENCIES)

The basilar membrane isn't consistent but varies in stiffness and width, enabling the detection of different frequencies. The basal section near the oval window is sensitive to high-pitched sounds due to its rigidity, while the apical end, farther away, is responsive to low-pitched sounds because it's more flexible. This organization along the basilar membrane influences how we perceive pitch. Auditory nerve fibers transmit these distinctions to the brain, allowing us to recognize and differentiate various pitches and frequencies.

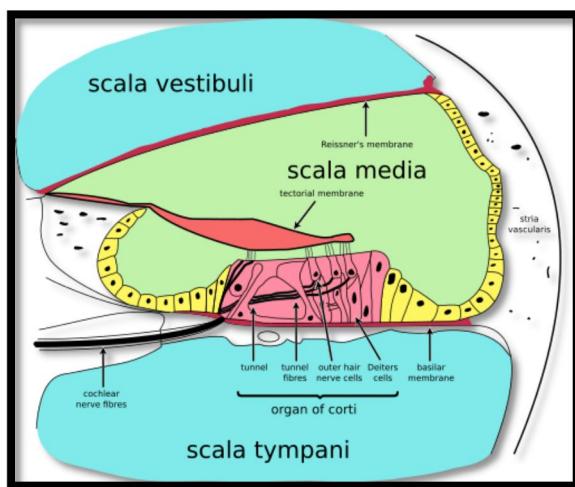


Figure 2. Basilar membrane

## AIR CONDUCTION AND BONE CONDUCTION

Air conduction and bone conduction are two different ways that sound travels to the inner ear. With air conduction, sound goes through the ear canal and makes the eardrum vibrate. The middle ear's ossicles then amplify and send these vibrations to the cochlea, where hair cells convert them into signals for the brain. On the other hand, bone conduction doesn't use the outer or middle ear. Instead, it sends vibrations directly to the skull bones, which then stimulate the cochlea. This method is helpful in cases of conductive hearing loss.

loss or issues with the outer or middle ear, letting people hear sounds without the usual air-conducted route.

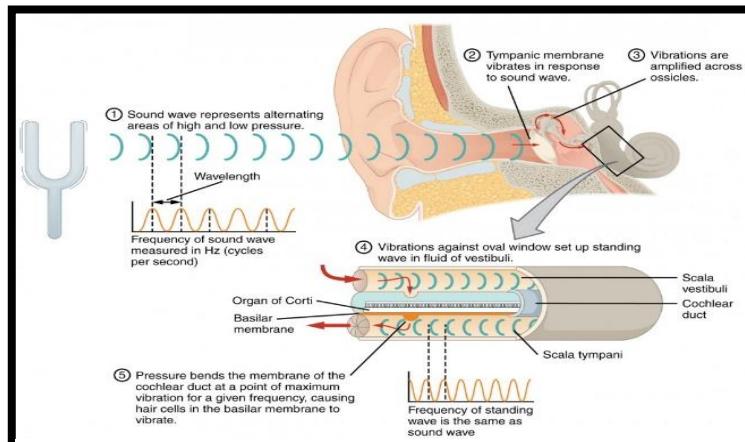


Figure 3. Air and bone conduction

## AUDIOMETRY

The electric audiometer, a 1930s innovation, is vital for precise hearing assessment, measuring thresholds across a frequency range of 125 to 8,000 Hz. It includes an oscillator, amplifier, and attenuator. The audiogram, with "zero dB" marking normal hearing, is the international standard since 1964. Pure-tone audiometry involves separate ear tests in a quiet room, with subjects signaling when they hear a tone 50% of the time. Thresholds, like a 40-dB level for 4,000 Hz, help shape an audiogram, crucial for hearing issue diagnosis. The audiogram highlights hearing levels by frequency, vital for diagnosis in cases of hearing difficulties. It also incorporates bone conduction for assessing middle ear transmission issues and explores recruitment.



Figure 4. Audiometer

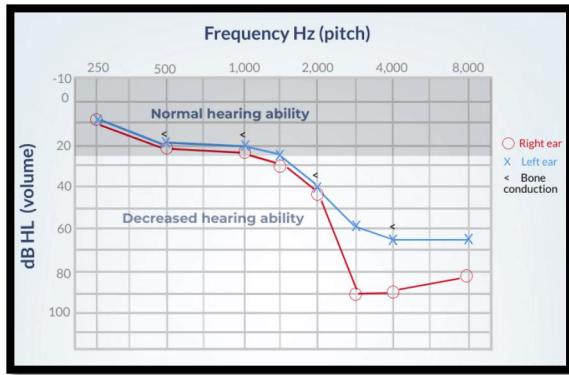


Figure 5. Frequency heard by right and left ear respectively.

## METHODOLOGY

1. **Seating Position:** The individual should be comfortably seated at a minimum distance of 1 meter (about 3.3 feet) from the audiometer device. This distance is maintained to prevent any proximity-related influence on the individual's responses during the hearing test.
2. **Ear Clarity:** Prior to the test, it is essential to ensure that there are no obstructions interfering with the proper placement of the earphones over the patient's ears. Any items like hats or eyeglasses that might impede the correct fit of the headphones should be removed.
3. **Proper Headphone Placement:** Verify that the headphones are correctly positioned over the person's ears to guarantee an accurate test. For the bone conduction, it should be on the mastoid bone.
4. **Earphone Color Coding:** Employ a color-coded system to differentiate the right ear from the left ear. Typically, the red earphone is placed over the right ear, and the blue earphone is placed over the left ear, ensuring consistency and accuracy in the testing process.
5. **Headband Adjustment:** Adjust the headband of the headphones to ensure that the receivers (the speakers within the earphones) are positioned at the correct height, ensuring a secure and comfortable fit for the patient.

## **Reading:**

**Patient Response Switch:** Advise the individual to activate the patient response switch or button when they detect a tone, even if it's faint. This is necessary to ensure accurate recording of the patient's responses by the audiologist.

## **RESULTS**



## **CONCLUSION**

### **Air Conduction**

A typical hearing sensitivity pattern is evident, with lower frequencies requiring higher sound intensities (in dB) for audibility, while higher frequencies are heard at lower intensities.

Remarkably good hearing sensitivity at 8000 Hz is observed, with an incredibly low minimal audible level of 0 dB.

## **BONE CONDUCTION**

Sensitivity to bone conduction varies across frequencies, with some frequencies showing better sensitivity (lower minimal audible levels) and others indicating poorer sensitivity (higher minimal audible levels).

An unusual occurrence at 4000 Hz in the right ear shows negative minimal audible levels, potentially suggesting a testing artifact or issue.

These findings provide valuable insights into the individual's hearing thresholds at different frequencies. However, the variations in bone conduction results may warrant further examination or reevaluation to ensure data accuracy. A comprehensive interpretation and clinical assessment are necessary to determine the implications of these results for the individual's overall hearing health.

# **DESIGN OF INSTRUMENTATION AMPLIFIER**

## **AIM**

To design an instrumentation amplifier and assess its amplification ability for biomedical signals.

## **OBJECTIVE**

- Design the instrumentation amplifier meeting the specifications of voltage gain, input impedance, output impedance, CMRR and bandwidth suitable for amplifying the biomedical signals.

## **APPARATUS REQUIRED**

- LM 324
- Resistors
- Function Generator and Oscilloscope
- Breadboard and connecting wires

## **THEORY**

An instrumentation amplifier is a kind of differential amplifier that can be built with three operational amplifiers, as shown in Fig1. It is used to amplify very low-level signals, rejecting noise and interference signals. The essential characteristics of a good instrumentation amplifier are as follows:

1. Inputs to the instrumentation amplifiers will have low signal energy. Therefore,
2. it should have high and accurate gain
3. The gains should be easily adjustable using a single control.
4. It must have high input impedance and low output impedance to prevent loading.
5. It should have high CMRR since the transducer output will usually contain
6. common mode signals, such as noise when transmitted over long wires.
7. It must also have a high slew rate to handle sharp rise times of events and
8. provide maximum undistorted output voltage swing

## PROCEDURE

- Design instrumentation amplifier for suitable gain:

$$\text{Gain} = R_3/R_2[1 + (2R_1/R_{\text{gain}})]$$

- Rig up the circuit as per Fig.1, using IC741's and the designed  $R_1$ ,  $R_2$ ,  $R_3$  and  $R_{\text{gain}}$  resistors.
- Use a Function generator to feed input voltages and an oscilloscope to measure the output voltage of the instrumentation amplifier.
- Find the differential mode voltage gain  $A_d = V_{\text{out}}/V_1 - V_2$  by using the output voltage  $V_{\text{out}}$  obtained due to the unequal voltages  $V_1$  and  $V_2$  at the input of the instrumentation amplifier.

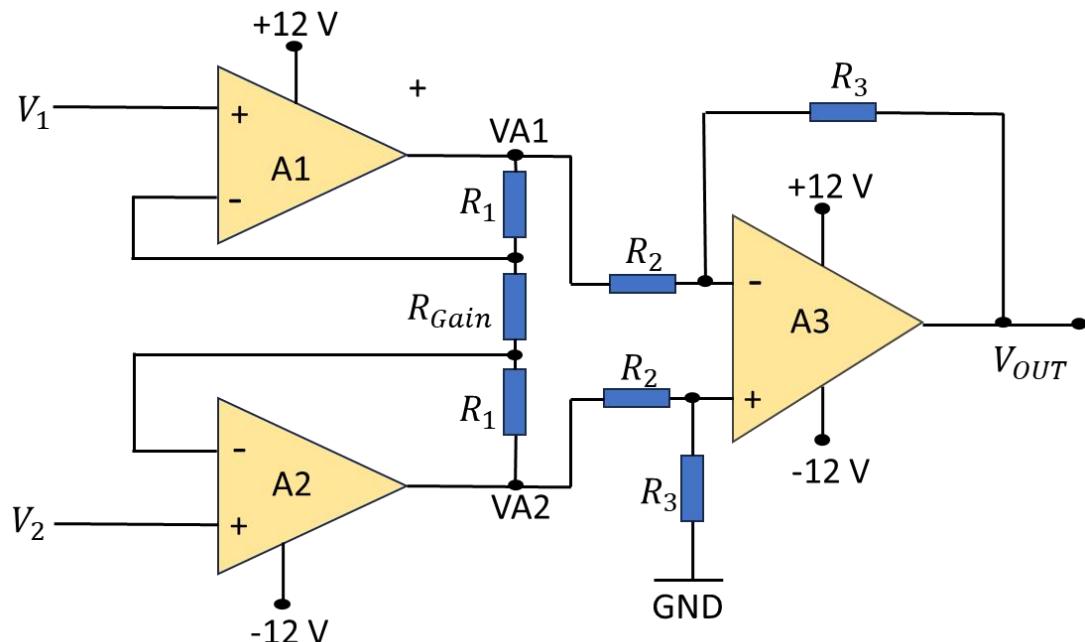


Figure 1. Instrumentation Amplifier

- Find the common mode gain voltage  $A_c = V_{\text{out}}/V$ , by feeding a common voltage to the  $V_1$  and  $V_2$  terminals of Fig.1.
- Compute the common mode rejection ratio  $\text{CMRR} = A_d/A_c$

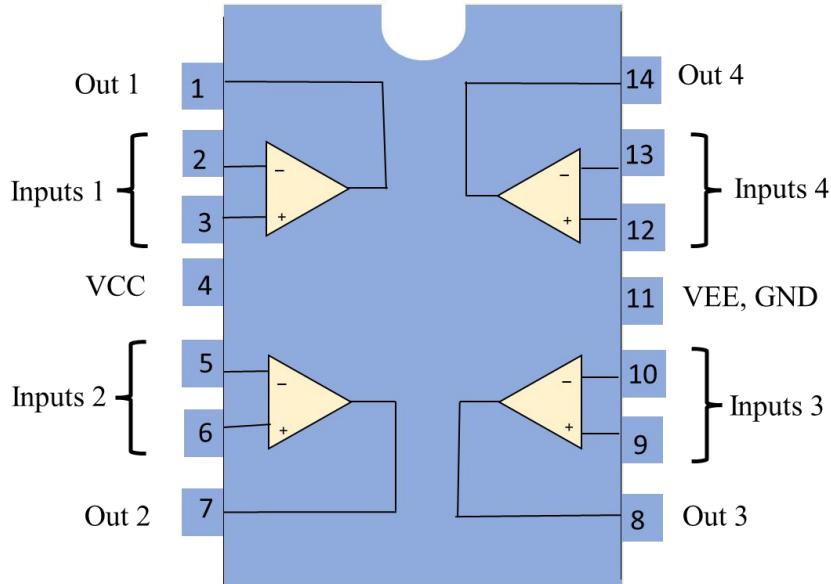


Figure 2. Pin Diagram of LM324

- Measure input impedance of the instrumentation amplifier by connecting the additional resistor,  $R_{test}$  at the non-inverting terminal of one of the input op-amp by giving the non-inverting terminal of other input op-amp as shown in Fig.3.

$$\text{Input Impedance} = R_{test} (V_2/(V_1-V_2))$$

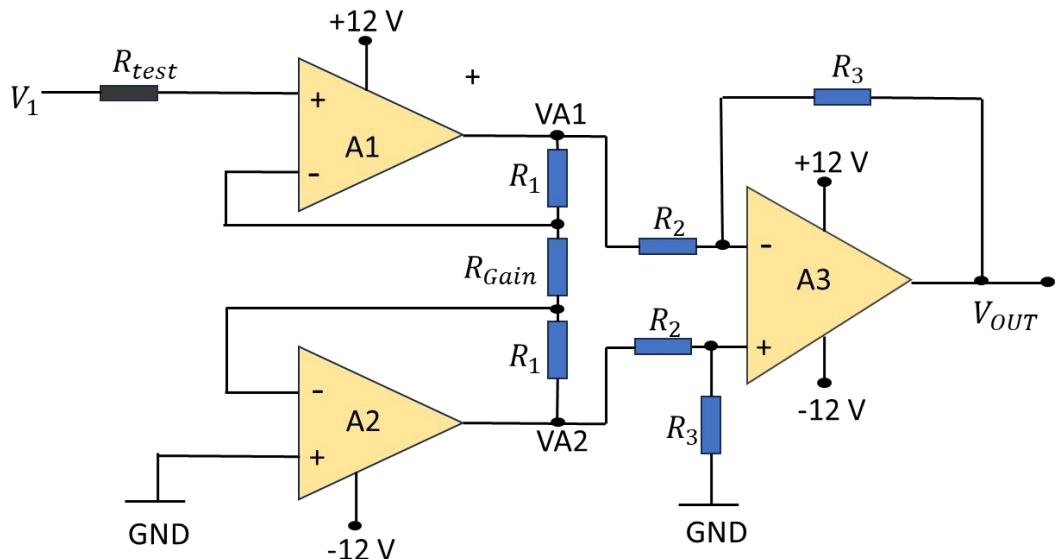


Figure 3. Measuring the input impedance of Instrumentation Amplifier

- Measure the output impedance of instrumentation amplifier by connecting a load resistor  $R_{load}$  at the output terminal Out, as shown in Fig.4.

$$\text{Output Impedance} = R_{load} ((V_{open}/V_{load})-1)$$

Where  $V_{open}$  is the open circuit output voltage measured at terminal Out when  $R_{load}$  is not connected, and  $V_{load}$  is the voltage measured at terminal Out when  $R_{load}$  is connected.

- Plot the frequency response characteristics by measuring the gain of the amplifier at frequencies range from 100Hz to 10KHz and calculate the bandwidth value.

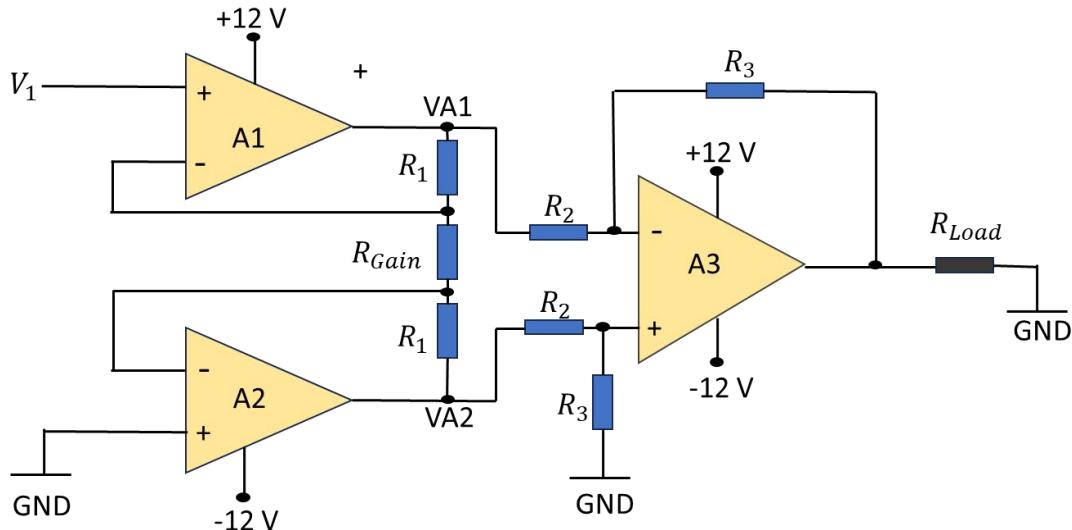


Figure 4. Measuring the output impedance of Instrumentation Amplifier

## RESULTS & OBSERVATION

- For gain =100, the resistors  $R_1= 100\text{k}\Omega$ ,  $R_2 = 100\text{k}\Omega$ ,  $R_3 = 100\text{k}\Omega$  and  $R_{gain}= 2\text{k}\Omega$
- Differential mode voltage gain  $A_d = 93.3$
- Common mode voltage gain  $A_c = 0.06$ .
- $\text{CMRR} = A_d/A_c = 93.3/0.06 = 1555$
- Input Impedance =  $13.65 \text{ k}\Omega$  & Output Impedance =  $500\Omega$ .
- Frequency Response for  $V_{in} = 30 \text{ mV}$

Table 1: Frequency Response

Frequency	Output voltage
200Hz	2.8 V
500 Hz	2.8 V
1 kHz	2.8 V
2 kHz	2.8 V
5 kHz	2.6 V
10 kHz	2.2 V

20 kHz	1.6 V
50 kHz	0.8 V
100 kHz	0.5 V
200 kHz	0.4 V
300 kHz	0.4 V
500 kHz	0.3 V
1 MHz	0.3 V

Upper cut-off frequency  $f_H = 13.6$  kHz, Lower cut-off frequency is  $f_L$  is 0 Hz and -3dB bandwidth =  $f_H - f_L = 0$  to 13.6 kHz.

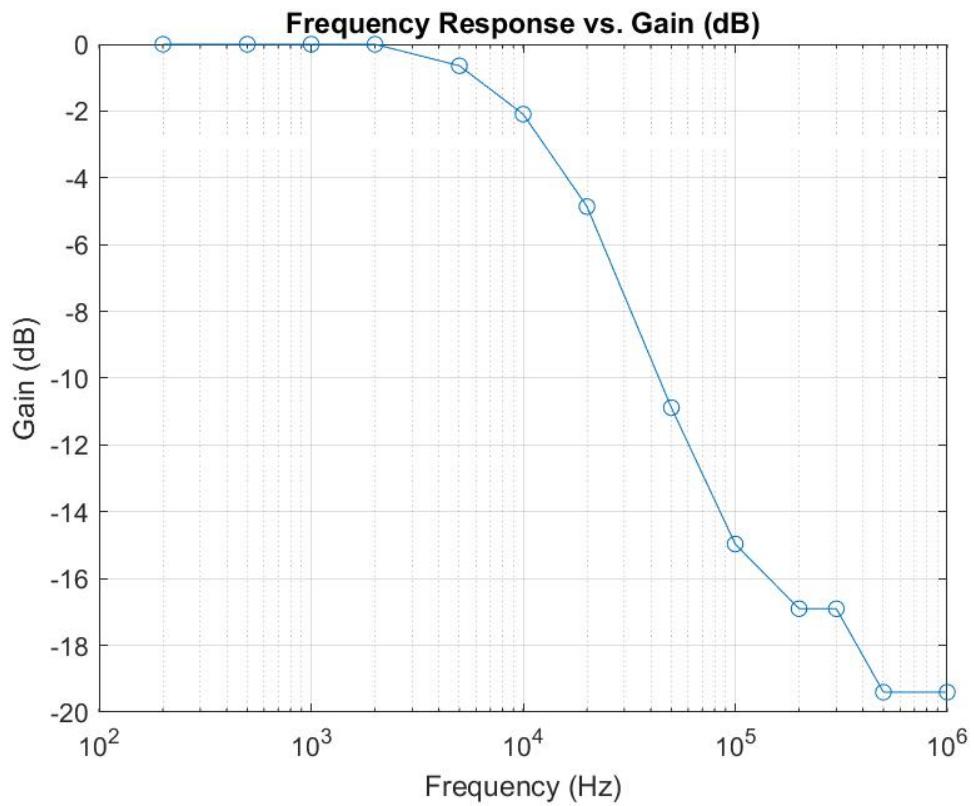


Figure 5. Frequency response of the instrumentation amplifier.

## MATLAB CODE

```
frequency = [200, 500, 1000, 2000, 5000, 10000, 20000, 50000, 100000,  
200000, 300000, 500000, 1000000];  
voltage = [2.8, 2.8, 2.8, 2.8, 2.6, 2.2, 1.6, 0.8, 0.5, 0.4, 0.4, 0.3, 0.3];  
  
reference_voltage = 2.8;  
gain_dB = 20 * log10(voltage ./ reference_voltage);  
semilogx(frequency, gain_dB, 'o-');  
xlabel('Frequency (Hz)');  
ylabel('Gain (dB)');  
title('Frequency Response vs. Gain (dB)');  
grid on;
```

## CONCLUSION

The preamplifier meets the requirements necessary for acquiring bio signals is designed.

## **SSVEP ANALYSIS OF EEG**

### **AIM**

To understand the steady state visually evoked potentials (SSVEPs) from EEG during visual stimulation

### **OBJECTIVE**

To obtain the frequencies in the EEG signal using MATLAB

### **APPARATUS REQUIRED**

- EEG
- System with visual stimulation
- Electrode gel
- Laptop
- MATLAB software

### **THEORY**

SSVEPs, or steady-state visually evoked potentials, refer to brain signals that align with the frequency of a visual stimulus, typically one that flickers consistently. This rhythmic stimulus has the ability to synchronize neural activity in the occipital lobe, which is closely linked to the visual cortex. Properly executed, the correlation between the frequency of the neural activity and the flickering stimulus allows for real-time determination of which stimulus a participant is observing. This is achievable by presenting a selection of stimuli flickering at distinct rates, with each SSVEP uniquely corresponding to the observed stimulus. In neuroscience, clinical neurology, and psychology, SSVEPs serve as an objective biomarker, particularly in studies related to the visual system. Additionally, SSVEPs are widely utilized in the realm of Brain-Computer Interface (BCI) research due to their reliability, accuracy, high information transfer rate, straightforward setup, and minimal training requirements.

### **PRINCIPLE OF EEG**

EEG, or electroencephalogram, is a non-invasive neuroimaging technique that records electrical activity in the brain. The principle behind EEG is based on the detection and measurement of electrical potentials generated by the synchronized activity of large groups of neurons. Electrodes are placed on the scalp to capture the collective electrical signals produced by neural firing.

As neurons communicate with each other, they generate electrical impulses. These electrical signals produce voltage fluctuations that can be detected on the scalp. EEG measures these voltage changes over time, providing a dynamic representation of brain activity.

## **TYPES OF EEG WAVES**

EEG recordings reveal different types of brain waves, each associated with specific mental states, activities, or conditions. These waves are categorized based on their frequency and amplitude. The main types of EEG waves include:

### **1. Delta Waves (0.5-4 Hz):**

- Delta waves are slow, high-amplitude waves.
- They are typically associated with deep sleep and certain pathological states.

### **2. Theta Waves (4-8 Hz):**

- Theta waves are slower than alpha waves and are associated with drowsiness, relaxation, and the early stages of sleep.

### **3. Alpha Waves (8-13 Hz):**

- Alpha waves are present during relaxed wakefulness and are dominant in the posterior regions of the brain.
- They are often associated with a state of calmness and alert relaxation.

### **4. Beta Waves (13-30 Hz):**

- Beta waves are associated with active, alert, and focused states of consciousness.
- They are prominent during tasks requiring attention and mental effort.

### **5. Gamma Waves (30-40 Hz and above):**

- Gamma waves are associated with higher cognitive functions, including perception, learning, and problem-solving.
- They are involved in integrating information across different brain regions.

Understanding the patterns and variations of these EEG waves provides valuable insights into brain function, cognitive states, and neurological disorders.

## 10-20 ELECTRODE PLACEMENT

The 10-20 system is a standardized method for electrode placement in electroencephalography (EEG). This system is widely used to ensure consistency in electrode positioning across different individuals. The name "10-20" refers to the fact that electrodes are placed at intervals of either 10% or 20% of the total front-back or right-left distance on the scalp. The distances are measured based on the individual's head circumference.

Electrodes are labeled with letters to denote their specific locations, such as F (frontal), C (central), P (parietal), O (occipital), and T (temporal). Common electrode sites in the 10-20 system include Fp1, Fp2, F7, F8, F3, F4, C3, C4, P3, P4, O1, O2, T3, T4, T5, and T6, among others. This standardized system facilitates consistency in EEG data collection and analysis across different research studies and clinical settings. It ensures that electrodes are placed in similar locations on the scalp for different individuals, allowing for meaningful comparisons and interpretations of EEG recordings.

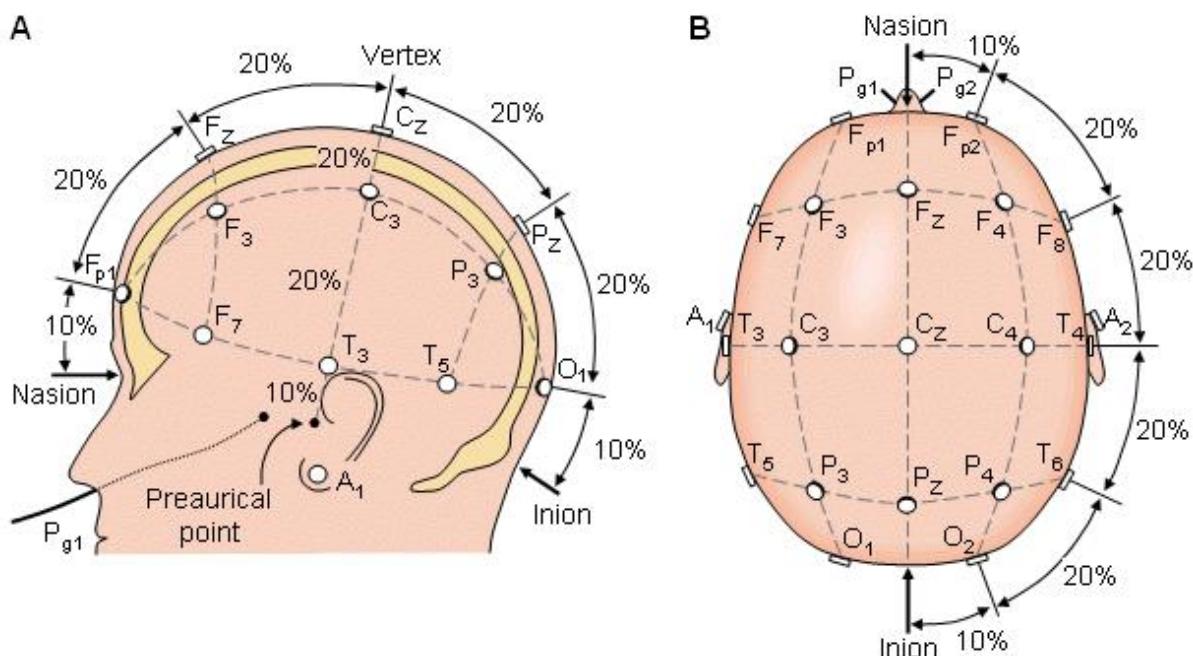


Figure 1: 10-20 electrode placement

## METHOD

- The EEG electrode is placed at the occipital lobe, using the 10-20 electrode placement principle.
- The subject is allowed to rest and sit still with no disturbances
- The subject is asked to sit in front of a screen which shows flickering cubes at different rates.

- The subject is asked to focus at one of the cubes and then is asked to change his focus to another square without informing.
- The EEG signal is recorded at a sampling rate of 250 Hz.

## MATLAB CODE

```

clear all;
close all;
clc;
% Load EEG data from .mat file
data=load("F:\IITM\Physiological measurements lab\EEGRAW.mat");
my_data=data.AM23M025;
time=(1:length(my_data))/250;
% Example: Bandpass filter between 1-30 Hz
figure();
plot(time,my_data);
title('EEG Signal');
ylabel('Electrode Potential in mV');
xlabel('time');

%sampling rate is 250 Hz
low_freq = 1; % Set your desired low cutoff frequency in Hz
high_freq = 40; % Set your desired high cutoff frequency in Hz
sampling_rate=250;
% Design a bandpass filter
[b, a] = butter(4, [low_freq, high_freq]/(sampling_rate/2), 'bandpass');

% Apply the bandpass filter
eeg_data_filtered = filtfilt(b, a, my_data);
% Example: Calculate FFT for each epoch
fft_data = fft(eeg_data_filtered);
% Example: Find peaks in the FFT
fft_result = fft_data(1:length(fft_data)/2); % Keep only the positive frequencies
% Calculate the frequency axis
% Calculate corresponding frequencies
frequencies = (0:length(fft_result)-1)*(sampling_rate/length(fft_result));

% Plot the spectrum
figure;
plot(frequencies, abs(fft_result));
xlabel('Frequency (Hz)');
ylabel('Magnitude');

```

```

title('Frequency Spectrum of EEG Signal');
% Identify SSVEP frequencies
% You may need to set a threshold or use peak detection to identify SSVEP
% frequencies

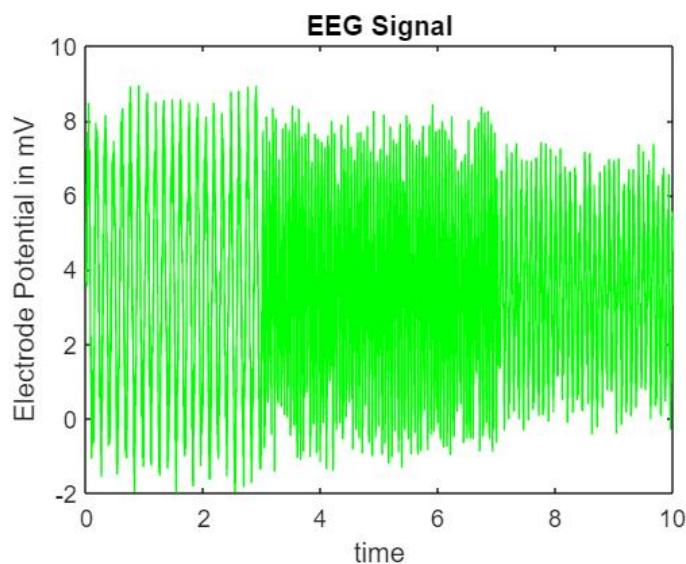
% Example: Assume SSVEPs occur between 5 and 20 Hz
ssvep_min_freq = 5;
ssvep_max_freq = 20;
ssvep_indices = find(frequencies >= ssvep_min_freq & frequencies <=
ssvep_max_freq);
ssvep_magnitudes = abs(fft_result(ssvep_indices));

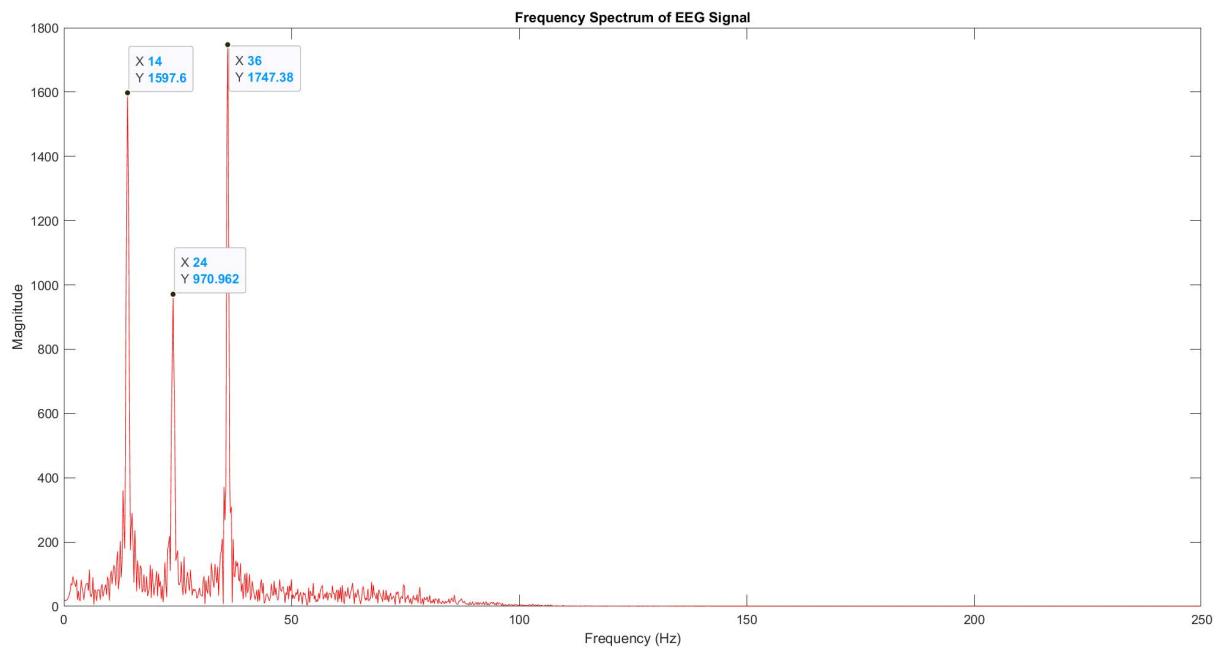
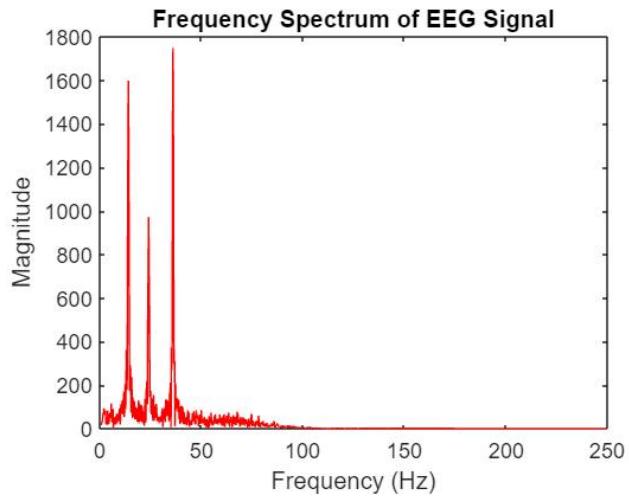
% Identify the dominant SSVEP frequency
[max_magnitude, max_index] = max(ssvep_magnitudes);
dominant_ssvep_frequency = frequencies(ssvep_indices(max_index));

fprintf('Dominant SSVEP Frequency: %.2f Hz\n', dominant_ssvep_frequency);

```

## RESULTS





## RESULT

Dominant SSVEP Frequency: 14.00 Hz and 36.00 Hz.

From the frequency spectrum, we can find three major peaks at **14Hz, 24 Hz and 36 Hz**.

## **CONCLUSION**

In this experiment, we used EEG to obtain the steady-state visually evoked potentials and then used MATLAB to obtain the dominant frequency in the spectrum of the EEG signal. This can be used in brain computer systems to execute tasks.

**AM5023- PHYSIOLOGICAL MEASUREMENTS AND  
INSTRUMENTATION LABORATORY**

**BIOSENSORS LABORATORY REPORT**

Submitted by: DINESH KUMAR M

Registration no: AM23M022



**DEPARTMENT OF APPLIED MECHANICS &  
BIOMEDICAL ENGINEERING**

**INDIAN INSTITUTE OF TECHNOLOGY, MADRAS**

# PHYSIOLOGICAL pH MEASUREMENT

## Aim:

To understand the concept of blood pH and its importance.

Background: Blood pH is very tightly maintained between 7.36 and 7.44. An increase or decrease in blood pH results in physiological conditions known as alkalosis and acidosis respectively. Various acids are produced in our body during metabolism and it is important to remove/convert them efficiently to maintain a homeostatic balance of pH.



CA - Carbonic anhydrase

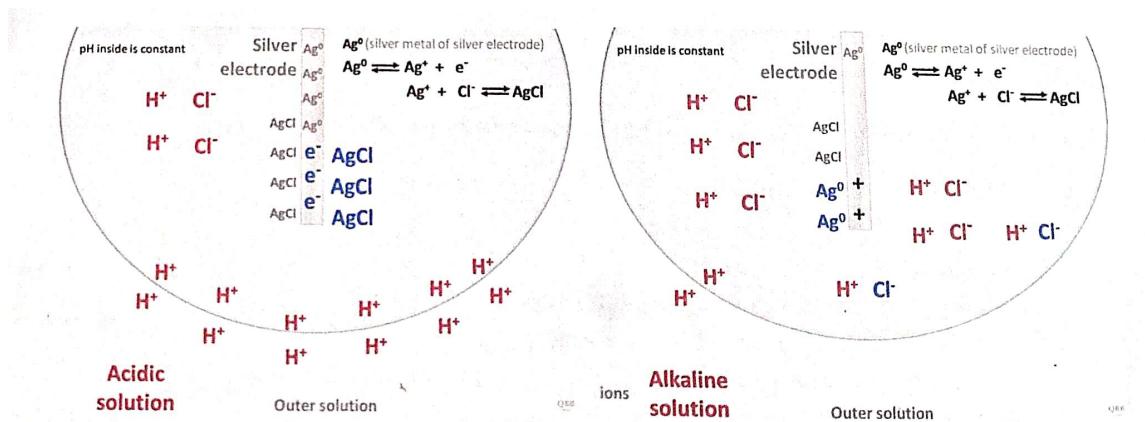
The CO<sub>2</sub> levels are maintained in the lungs whereas the HCO<sub>3</sub><sup>-</sup> levels are maintained in the kidneys. The lungs can help regulate blood pH rapidly through the process of exhaling CO<sub>2</sub> whereas the kidneys regulate the pH of the blood by excreting acids in urine. They also produce and regulate bicarbonate, which helps maintain a healthy blood pH.

## Objective:

To measure the pH of an unknown sample using a pH meter

## Working Principle:

The pH probe consists of 2 electrodes (reference and glass electrode). Both electrodes have silver wire coated with silver chloride. The glass electrode is filled with 0.1 N HCl whereas the reference electrode is filled with saturated KCl. The bottom part of the glass electrode has a thin circular glass bulb (which comes in contact with the sample) that specifically binds H<sup>+</sup> ions from both sides (without making them cross). The voltage across these 2 electrodes is measured via a voltmeter (Note: The voltage across the reference electrode is always constant).



The concentration of H' ions in the outer solution affects the binding of H<sup>+</sup> ions in the inner hydration layer. This binding or release of H ions in turn affects the oxidation/reduction of silver ions. The oxidation/reduction events cause electrons either to be gained or lost by the electrode which changes the voltage of the electrode.

### Apparatus required:

Kim wipes, Glass containers, deionized water, pH Meter, water waste beaker

### Procedure:

1. Switch on the main power supply to the instrument.
- 2 Press the ON/OFF switch to display "MEAS" pH and temp mode on the screen.
3. Allow the instrument to stabilize for at least 15 min before performing daily calibration.
4. Calibrate the instrument with the standard buffer solution, pH 4,7 and 10.
5. Press the CAL/MEAS button. "CAL" mode and pH 7, 10, and 4 will be displayed on the screen.
6. Always start with neutral pH standard buffer pH 7, remove the pH electrode from the storage solution, and rinse it with purified DI water 2-3 times. Clean the excess water on the pH electrode by wipe with tissue paper and inserting the pH electrode into the solution.
7. Stir the standard pH 7 solution with a pH electrode and keep the pH electrode in the solution.
8. Allow the instrument to stabilize the pH and press the "Enter" button to calibrate the pH 7 buffer.

9. Remove the pH electrode and rinse with DI water. Clean the excess water on the pH electrode by wipe with tissue paper
10. In the instrument, pH 4 or pH 10 will be displayed. Insert the pH electrode in either pH 4 or 10 standard buffer to calibrate the instrument for the same pH 7 buffer. Follow the steps steps 5 to 7.
11. Once the instrument is calibrated with pH 4,7 and 10 standard buffer the instrument is automatically set to measurement "MEAS" mode. Now the instrument is ready to measure the sample buffer solution for pH adjustment.
12. Insert the pH electrode into the buffer solution to check the pH. Stir the buffer solution with the pH electrode and keep the pH electrode and allow it to stabilize the pH, the instrument will display ready on the screen, record the pH.
13. Remove the pH electrode and rinse with DI water. Clean the excess water on the electrode by wipe with tissue paper between measurements.
14. After completion of the pH measurement insert the pH electrode in the storage solution.
15. Switch OFF the instrument and clean the workspace.

### **Results and Observation:**

1. Successfully performed calibration using standard solutions of pH- YES/NO
2. The pH of the unknown sample is 7.21

### **Precautions:**

1. The glass electrode is extremely fragile so it to be handled with care.
2. Use of gloves is mandatory while operating the pH meter.
3. Handle buffer solutions with care. The solutions may cause eye and skin irritation. And maybe harmful if swallowed or inhaled.
4. Buffers should always be read at accurate pH
5. Do not immerse electrodes in the buffer solutions before rinsing the electrodes thoroughly with deionized water.
6. Do not mix buffers with other solutions or contaminate with samples.

**AM5023- PHYSIOLOGICAL MEASUREMENTS AND  
INSTRUMENTATION LABORATORY**

**BIOMEDICAL ULTRASOUND - LABORATORY REPORT**

Submitted by: DINESH KUMAR M

Registration no: AM23M022



**DEPARTMENT OF APPLIED MECHANICS &  
BIOMEDICAL ENGINEERING**

**INDIAN INSTITUTE OF TECHNOLOGY, MADRAS**

# **DETERMINATION OF THE CHARACTERISTICS OF A TISSUE LIKE MEDIUM USING ULTRASOUND**

## **AIM**

To determine the speed of sound, acoustic impedance, amplitude attenuation factor and the amplitude attenuation coefficient.

## **OBJECTIVE**

To understand how we view the tissues from ultrasound and how the image is constructed.

## **APPARATUS REQUIRED**

- Tissue mimicking Phantom
- L11-5v Ultrasound Transducer (Verasonics Inc., WA.USA)
- Vantage 64 scanner (Verasonics Inc., WA.USA)
- Vernier Caliper
- Weighing balance

## **BACKGROUND**

The sounds which have a frequency greater than 20kHz, i.e., above the human hearing limit comprise the ultrasound. Thus, the characteristics of propagation of ultrasound are the same as that of any other sound except for the frequency dependent characteristics. Hence ultrasound, just like any other sound, is the longitudinal propagation of alternating compressions and rarefactions in the medium of propagation. The speed at which sound propagates is a property of the medium and so is the amount of attenuation.

Speed of Sound – The speed of sound in a material depends on its compressibility ( $\kappa$ ) and density ( $\rho$ ).

$$c = \sqrt{\kappa \rho}$$

However, a simple distance-time-based calculation is possible where a short US pulse is passed through the medium of known distance and the time of echo from the bottom of the medium is noted. Then, the speed of sound is measured as:

$$c = 2dt$$

Attenuation - When the sound wave travels through a medium, it loses energy exponentially with respect to the distance of the travel. This phenomenon is known as attenuation and includes the losses due to scattering, absorption and mode conversion (refraction).

If  $A_0$  is the initial amplitude and  $A_z$  the amplitude at distance  $z$  in the medium, then

$$A_z = A_0 e^{-\mu_a z}$$

where  $\mu_a$  is the amplitude attenuation factor given by rearranging as shown;  
 $a = \frac{1}{z} \ln \left( \frac{A_0}{A_z} \right)$

The attenuation coefficient can be calculated by

$$\alpha = 20 (\log_{10} e) \mu_a \approx 8.7 \mu_a$$

## PROCEDURE

- The transducer is connected to the ultrasound scanner and ensure that it is placed intact in the slot.
- The phantom is placed on a level, regular surface such as the bottom of a flat container.
- The transducer is placed on the top of the phantom, with care taken that the transducer face is parallel to the surface of the phantom.
- Water is poured such that the transducer-phantom interface is submerged to avoid impedance mismatch due to air being present in between the transducer and the phantom.
- Center frequency is set by the user which will give rise to a sampling frequency 4 times that of center frequency.
- The MATLAB environment is run.
- Echo (reflected waves) known as raw data is detected by the transducer and is beamformed to get the B-mode image
- Data is saved into a .mat file and MATLAB is used to perform the calculations.
- The A line is plotted and the peak intensity values are found.
- The calculations have been for characterization

## MATLAB CODE

```
%OPEN THE DATA
load("phantom_raw_data.mat");
data = rf_bbf(:,64,64);
plot(data);
title("A-line");
xlabel('Samples'); ylabel('Amplitude');
%calculation
fs = 20*(10^6); %Hz
```

```

n = size(rf_bbf);
%transmit time
[pks,locs] = findpeaks(data);
[sorted_pkssorted_idx] = sort(pks,'descend');
sorted_locs = locs(sorted_idx);
[pks1,locs1] = findpeaks(data(1:100));
[sorted_pkssorted_idx] = sort(pks1,'descend');
sorted_locs1 = locs1(sorted_idx);
n1 = sorted_locs(1);
n2 = sorted_locs1(1);
A1 = sorted_pkssorted_idx(1);
A2 = sorted_pkssorted_idx(1);
transmit_time = (1/fs)*(n1-n2);
%phantom paramters
dim = [0.08,0.05,0.05];
mass = 0.209;
%speed
speed = 2*dim(3)/transmit_time;
%time of flight
t1 = n1/fs;
t2 = n2/fs;
%depth
d1 = speed*t1/2;
d2 = speed*t2/2;
%attenuation factor
ua = -(1/(d1-d2))*log(A2/A1);
%attenuation coefficient
a = 8.7 * ua;
%density
den = mass/(dim(1)*dim(2)*dim(3));
%acoustic impedance
Z = den * speed;
fprintf('Sampling frequency = %.0f Hz\n', fs);
fprintf('Sampling time = %.8f s\n', 1/fs);

fprintf('No. of Samples = %d\n', n(1));
fprintf('Transmit time = %.8f s\n', transmit_time);
fprintf('Speed = %.4f m/s\n', speed);
fprintf('Attenuation factor = %.4f\n', ua);
fprintf('Attenuation coefficient = %.4f\n', a);
fprintf('Density of Phantom = %d kg/m3\n', den);
fprintf('Acoustic Impedance = %.0f Pa-s/m3\n', Z);

```

## OBSERVATION

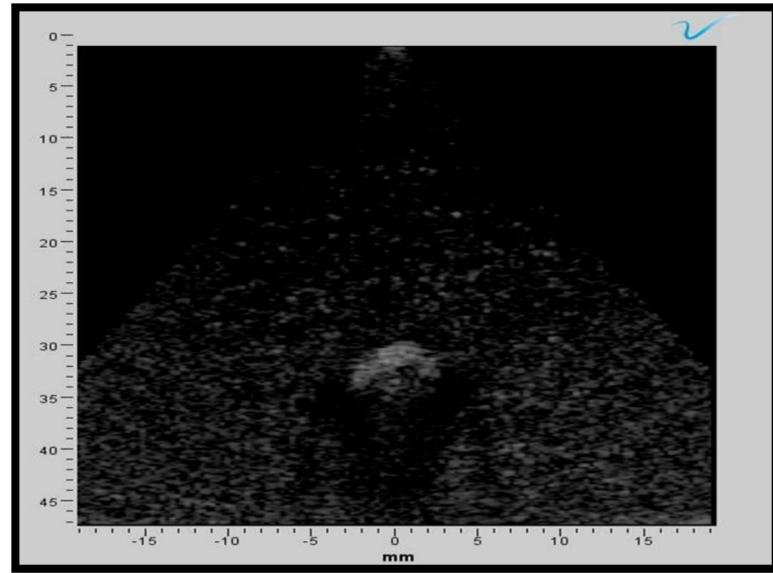


Figure 1 Verasonics B-mode image

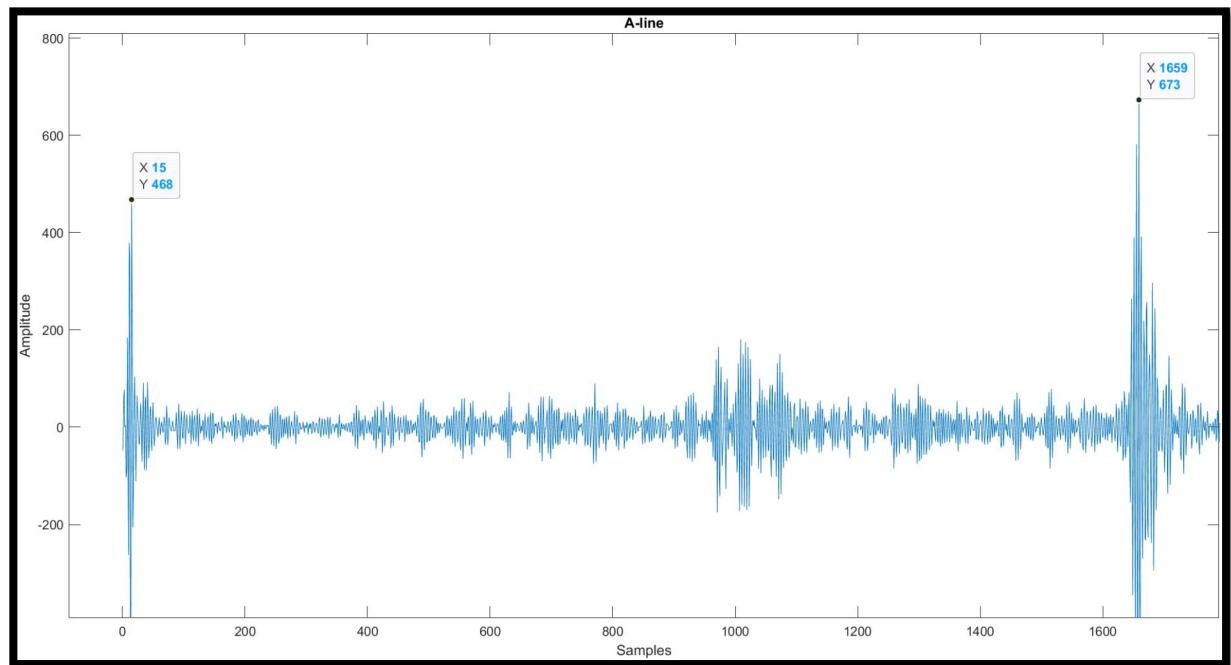


Figure 2: plot of A-line

## CALCULATION

The dimension of the phantom is 8 cm x 5 cm x 5cm and the mass is 209 grams. In this case d is 5 cm.

## RESULT

```
Sampling frequency = 20000000 Hz
Sampling time = 0.00000005 s

No. of Samples = 2048
Transmit time = 0.00008220 s
Speed = 1216.5450 m/s
Attenuation factor = 7.2655
Attenuation coefficient = 63.2102
Density of Phantom = 1045 kg/m3
Acoustic Impedance = 1271290 Pa-s/m3
```

## CONCLUSION

In this experiment we learned how to calculate the characteristics of a tissue such as the attenuation factor, acoustic impedance, speed of sound through the phantom tissue. We therefore get a proper understanding of how ultrasound travels in the body, gets absorbed and reflected and then generates signals which are processed to form images.

**AM5023- PHYSIOLOGICAL MEASUREMENTS AND  
INSTRUMENTATION LABORATORY**

**BIOPHOTONICS - LABORATORY REPORT**

Submitted by: DINESH KUMAR M

Registration no: AM23M022



**DEPARTMENT OF APPLIED MECHANICS &  
BIOMEDICAL ENGINEERING**

**INDIAN INSTITUTE OF TECHNOLOGY, MADRAS**

# ASSESSMENT OF VARIATION IN SKIN CONDUCTANCE UNDER VARIOUS PHYSIOLOGICAL CONDITIONS

## OBJECTIVE

To measure the changes in skin conductance stemming from variations in sweat gland activity induced by diverse external stimuli factors like emotions, stress, temperature, etc.

## THEORY

Skin conductance variation, or Galvanic skin response (GSR) or Electrodermal activity (EDA), quantifies the electrical conductance between two points on the skin. Skin conductance changes due to the activity of sweat glands and the resulting moisture content on the skin. Here are some reasons: emotional arousal, stress, physiological arousal, temperature, pain or discomfort, cognitive and mental processes, etc.

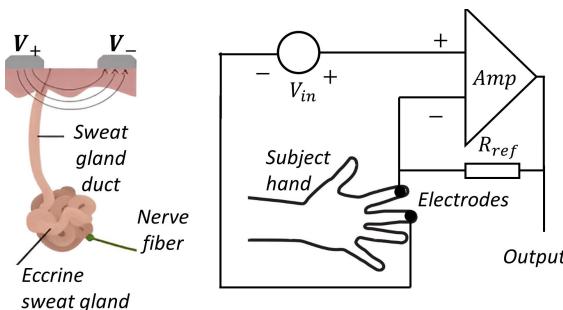


Figure 1: Principle of skin conductance measurement.

## EXPERIMENTAL SETUP

The block diagram of the experimental setup is depicted in Figure 2. It contains two electrodes, a GSR amplifier, a Data acquisition system, and a computer with Lab Chart data analysis software. In GSR measurements, electrodes are affixed to the subject's fingers. A consistent, minimal voltage is applied across the electrodes. The resulting current, influenced by skin resistance fluctuations, allows computation of the skin's conductance alterations. These real-time data are then graphically represented using Lab Chart software, enable analysis of skin conductance changes in response to emotional or stressful stimuli.

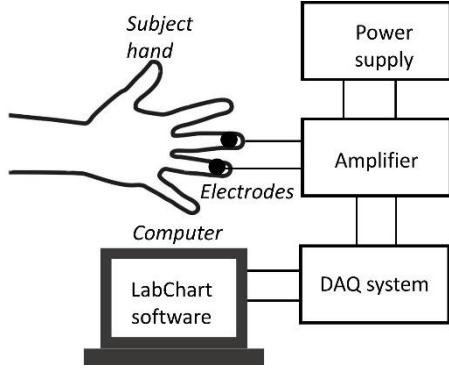


Figure 2: Block diagram for GSR measurement.

### GSR signal pattern

The formulas for computing conductance ( $G(t)$ ) and conductance level ( $CL$ ) are:

$$G(t) = \frac{1}{V_{in}/i(t)}; CL = \int_{t_1}^{t_2} G(t) \quad (1)$$

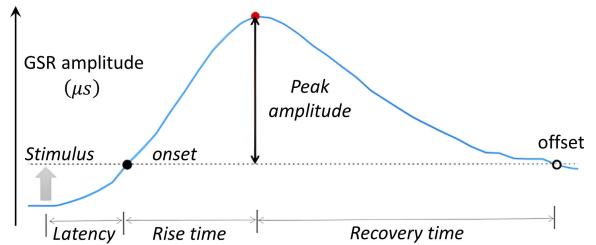


Figure 3: GSR response pattern and metrics.

The typical GSR pattern and its metrics is depicted in Figure 3. Latency Time: Duration between stimulus onset and skin conductance response initiation, indicating the speed of physiological reaction to stimuli. Rise Time: Speed of skin conductance changes from baseline to peak during a response; faster rise times signal rapid physiological reactions. Recovery Time: Speed of skin conductance returning to baseline after a response; shorter times imply efficient emotional arousal regulation.

## MATLAB CODE:

```
csvFile = 'C:\Users\DineshKumar\OneDrive\Desktop\E1.csv';
data = csvread(csvFile);
% E1 DATA PLOT
x = data(:, 1);
y = data(:, 2);
scatter(x,y);
xlabel('TIME');
ylabel('GSR AMPLITUDE ');
title('GSR Response pattern E1');
grid on;
```

## %E2 DATA PLOT

```
a = data(:, 6);
b = data(:, 7);
scatter(a, b);
xlabel('TIME');
ylabel('GSR AMPLITUDE ');
title('GSR Response pattern E2');
grid on;
```

```
c = data(:, 11);
d = data(:, 12);
scatter(c, d);
xlabel('TIME');
ylabel('GSR AMPLITUDE ');
title('GSR Response pattern E3');
grid on;
```

```
e = data(:, 16);
f = data(:, 17);
```

```

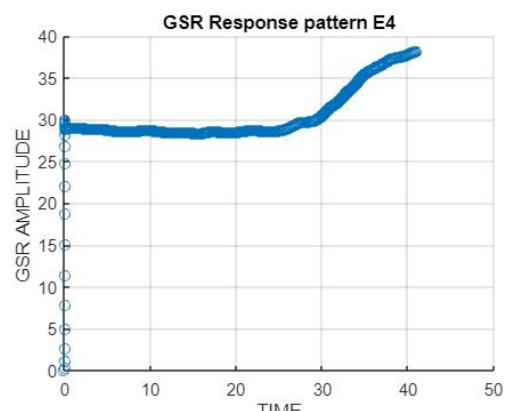
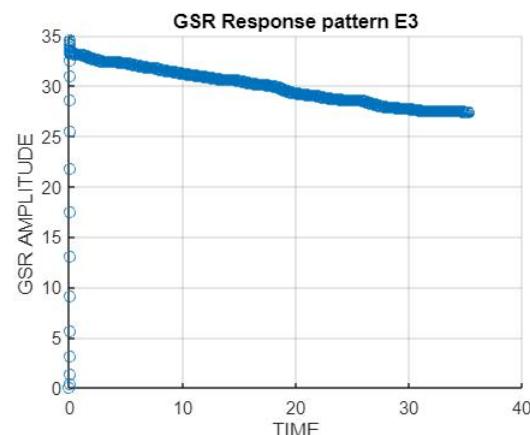
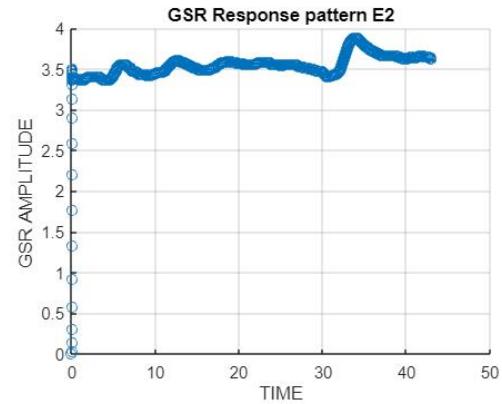
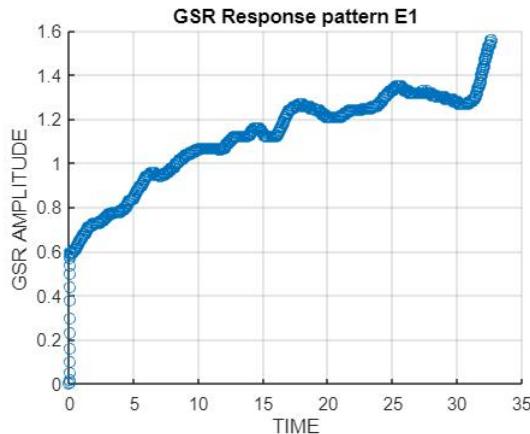
scatter(e, f);
xlabel('TIME');
ylabel('GSR AMPLITUDE ');
title('GSR Response pattern E4');
grid on;

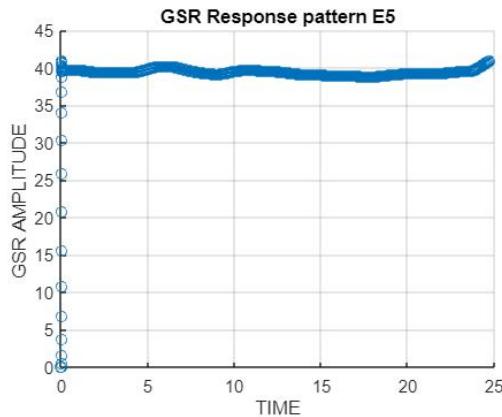
```

```

g = data(:, 20);
h = data(:, 21);
scatter(g, h);
xlabel('TIME');
ylabel('GSR AMPLITUDE ');
title('GSR Response pattern E5');
grid on;

```





## INFERENCE

The study aimed to understand how Galvanic Skin Response (GSR) relates to emotions, cognitive load, habituation, and biofeedback.

## OUTCOME

GSR increased with fear and stress, indicating emotional arousal.

GSR correlated with cognitive load, showing its sensitivity to mental effort.

Habituation led to decreased GSR after repeated exposure to the **same** stimulus.

GSR biofeedback was effective for stress management.

## CONCLUSION

GSR is a valuable tool for measuring emotional arousal and cognitive load.

Habituation should be considered in GSR experiments.

GSR can assist in stress management through biofeedback.

# **BLOOD PRESSURE MEASUREMENT USING A PIEZOELECTRIC SENSOR AND ITS APPLICATION**

## **OBJECTIVE**

To simulate the blood pressure variations with changes in the vessel diameter and blood flow rate.

## **THEORY**

Blood pressure measurement using a piezoelectric sensor involves a sensor that generates an electric signal in response to pressure. Piezoelectric sensors operate on the principle of the piezoelectric effect, which describes the generation of an electric signal in certain materials (quartz crystals and various ceramics, possess a non-centrosymmetric crystal structure, where the positive and negative charges within the crystal are not symmetrically distributed) when subjected to pressure. The magnitude of the generated electric signal (millivolts to volts) is directly proportional to the applied pressure.

## **EXPERIMENTAL SETUP**

The block diagrams of the experimental setup are depicted in Figure 1 and Figure 2 (Calibration and Testing). It contains a piezoelectric sensor, a bridge amplifier, a data acquisition system, a converging-diverging flow nozzle, a syringe pump, a micro-tubule, a micro-needle, a large diameter tubule, two syringes, and a computer with Lab Chart data analysis software. The piezoelectric pressure sensor undergoes calibration through integration with a sphygmomanometer setup.

The flow nozzle interfaces one end with the sphygmomanometer and the other with a syringe, while the piezoelectric sensor is affixed to the flow nozzle. This sensor output is channeled into a bridge amplifier for signal amplification. The amplified signal is then routed to Power Lab for data acquisition. Power Lab, linked to a computer via USB, interfaces with Lab Chart for signal visualization.

The syringe is manipulated during calibration to raise manometric pressure to 100 mmHg, and the corresponding voltage alteration is recorded. Simultaneously, the voltage corresponding to 0 mmHg is recorded. These values facilitate unit conversion, enabling data representation in mmHg units on the graph.

Post-calibration, the syringe, now filled with water, is mounted onto a syringe pump. This syringe interfaces with the inlet of a converging-diverging flow-type nozzle. Subsequently, signal acquisition is performed under six distinct scenarios.

The flow rates are maintained at 50ml/hr. and 100 ml/hr. Pressure measurements are taken for three distinct cases over approximately one minute: open-ended, connected to a large diameter tubule, and connected to a micro-tubule.

Steps:

- Lab chart software- new file- channel settings
- Calibrate the sensor at 0 mmHg.
- Make the output voltage to zero if it has some error by applying negative pressure.
- Convert the unit to mmHg.
- **With calibration 0 mV was equal to 0 mmHg and 4.04 mV was equal to 100 mmHg.**

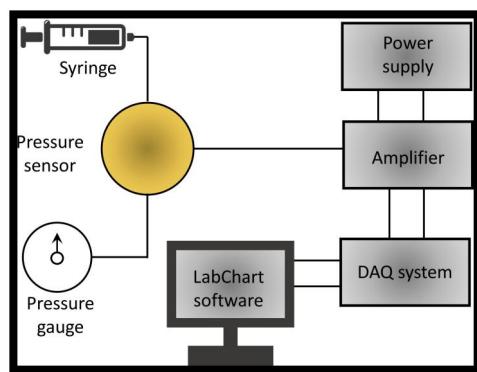


Figure 1: Block diagram for calibration.

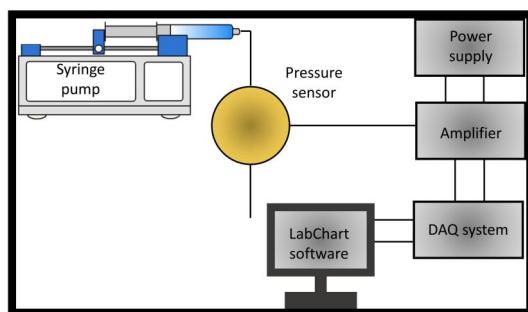


Figure 2: Block diagram for testing .

## MATLAB CODE

```
csvFile = "F:\IITM\Physiological measurements lab\piezotrans.xlsx.csv";
data = readmatrix(csvFile);
x = data(:, 2);
y = data(:, 3);
plot(x,y);
xlabel('TIME');
ylabel('PRESSURE (mmHg)');
title('CASE1');
grid on;
```

```
a = data(:, 6);
b = data(:, 7);
plot(a, b);
xlabel('TIME');
ylabel('PRESSURE (mmHg)');
title('CASE2');
grid on;
```

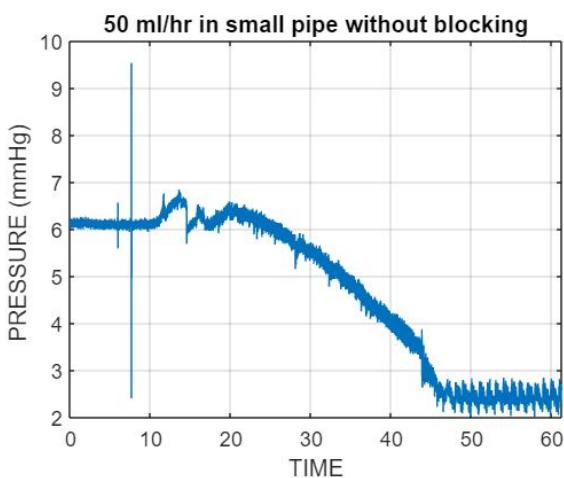
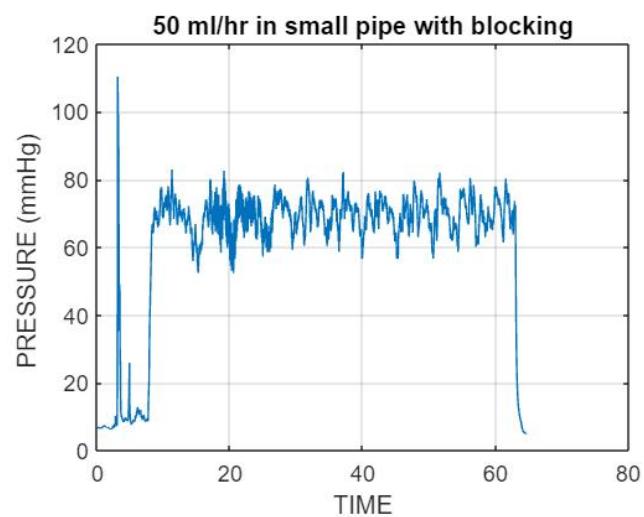
```
c = data(:, 10);
d = data(:, 11);
plot(c, d);
xlabel('TIME');
ylabel('PRESSURE (mmHg)');
title('CASE3');
grid on;
```

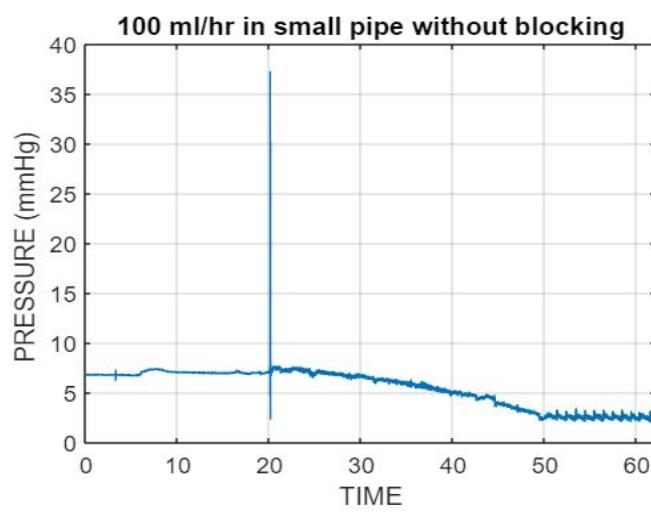
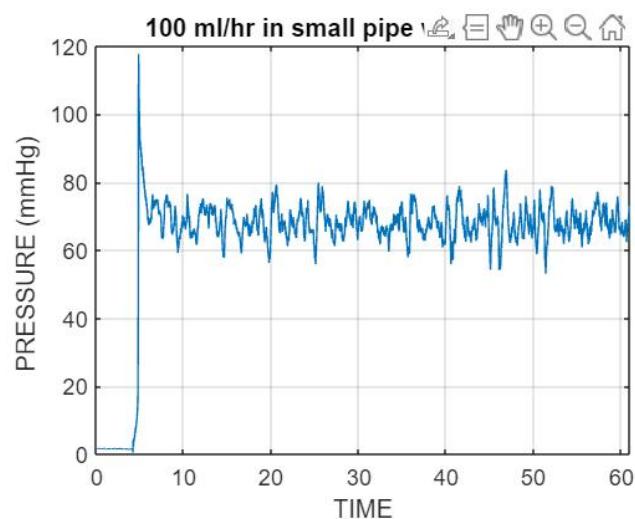
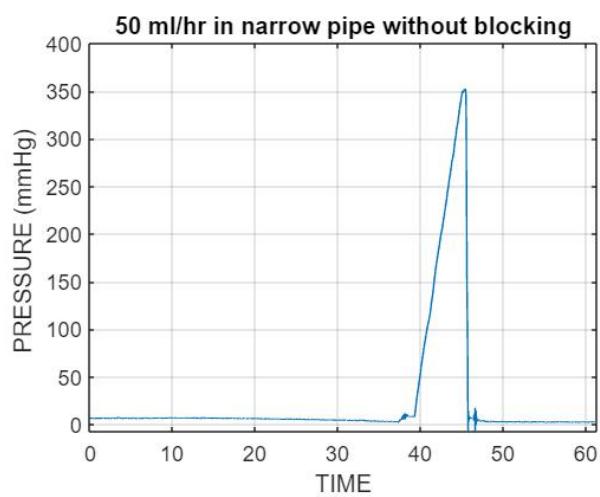
```
e = data(:, 14);
f = data(:, 15);
plot(e, f);
xlabel('TIME');
ylabel('PRESSURE (mmHg)');
title('CASE4');
grid on;
```

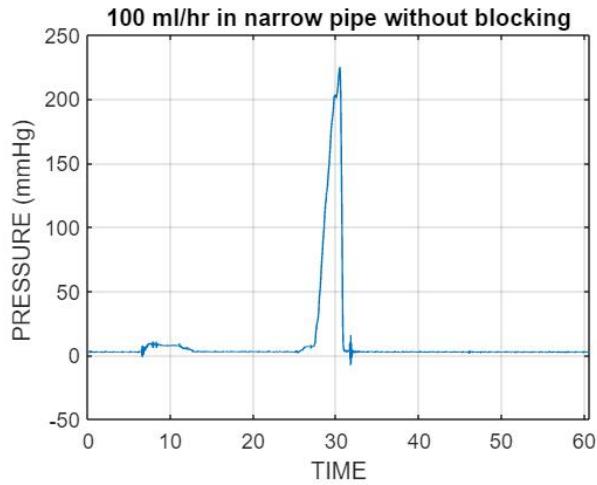
```
g = data(:, 18);
h = data(:, 19);
plot(g, h);
xlabel('TIME');
ylabel('PRESSURE (mmHg)');
```

```
title('CASE5');  
grid on;
```

```
o = data(:, 22);  
l = data(:, 23);  
plot(o, l);  
xlabel('TIME');  
ylabel('PRESSURE (mmHg) ');  
title('CASE6');  
grid on;
```







## INFERENCE

When the flow rate increases the pressure increases. When the tube is blocked the pressure tends to increase higher and higher.

## OUTCOME

The change in the tubule diameter will cause a pressure change in the waterflowing through the sensor. The pressure changes for the two diameters and the flow rate need to be noted.

**AM5023- PHYSIOLOGICAL MEASUREMENTS AND  
INSTRUMENTATION LABORATORY**

**NIID- LABORATORY REPORT**

Submitted by: DINESH KUMAR M

Registration no: AM23M022



**DEPARTMENT OF APPLIED MECHANICS &  
BIOMEDICAL ENGINEERING**

**INDIAN INSTITUTE OF TECHNOLOGY, MADRAS**

# BLOOD PRESSURE MEASUREMENT AND ANALYSIS OF BP VARIATIONS UNDER DIFFERENT PROTOCOLS

## Aim

The aim of this experiment is to record BP measurement with sitting, supine and standing position.

## Objective

To use digital sphygmomanometer to analysis data under different condition.

## Apparatus required

- BP cuff (Medium size)
- Digital sphygmomanometer (Company: Circa, Model: Eris Microlife)
- Clock

## Theory

Most automated NIBP devices are based on oscillometry, measures mean arterial pressure and use an algorithm to estimate the systolic and diastolic blood pressure. The cuff is inflated above systolic pressure and then deflates either continuously or in a stepwise manner. As the cuff pressure decreases, at a rate about 4 mm Hg per second, below occlusive pressure, blood starts flowing through the artery and causes detectable oscillation. The pulse pressure wave and the gauge pressure in the occluding cuff are detected and converted into an electronic signal by a transducer. The pressure at which the peak amplitude of arterial pulsations occurs corresponds closely to directly measured mean arterial pressure (MAP), and values for systolic and diastolic pressure are derived.

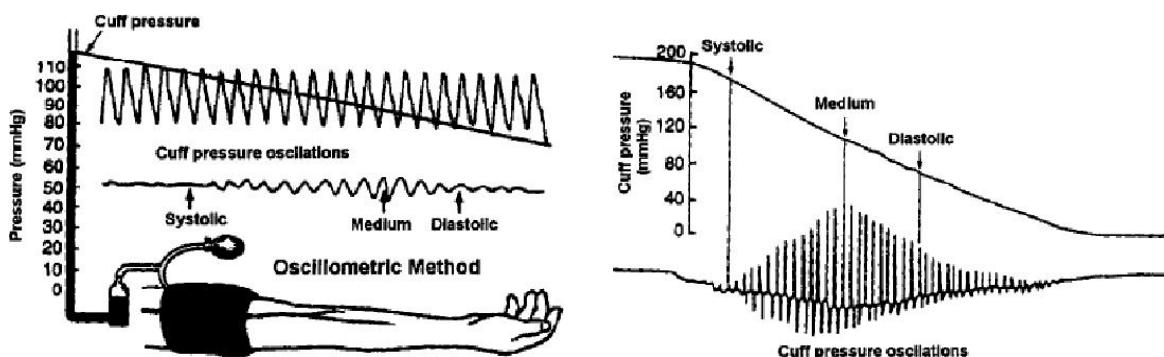


Fig 1. Blood pressure determination using oscillometric method

## Method

Follow the instruction written in manual. BP should not be taken on empty stomach. Take rest 5 min before taking BP measurement. BP cuff should be placed over the Brachial artery. Cuff should not be too loose or too tight. Digital sphygmomanometer does not require a stethoscope and it's reading doesn't depend on the hearing ability of observer. It automatically inflates the cuff till artery gets occluded after that cuff is deflated automatically.

Reading is taken for 3 time to get better accuracy.

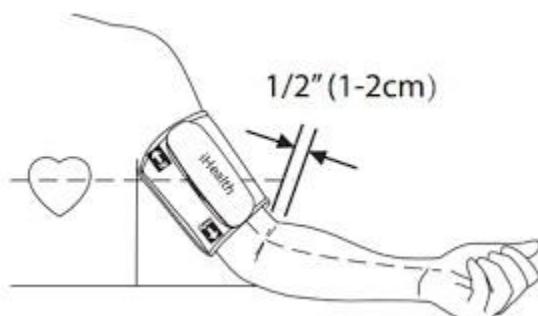


Fig 2. Cuff position

BP is taken two times a day in standing, sitting and supine position. Data is recorded for 10 days to observe the variation in BP under different condition.

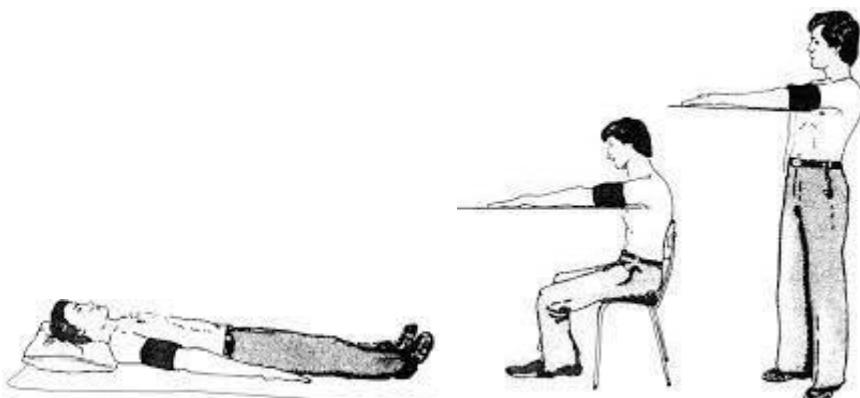


Fig 3. Different Position for BP measurement

Sitting blood pressure is taken from the left arm, flexed at the elbow and supported at the heart level on the chair. After at least one minute of standing, the blood pressure is then taken standing, with the arm supported at the elbow.

The cuff should be at the heart level. After one minute of rest, the blood pressure was subsequently taken supine position.

## Results

Systolic Pressure							
		Day time (9:00 AM to 10:00AM)			Evening Time (4:00 PM to 5:00PM)		
Date	Reading	Sitting	Supine	Standing	Sitting	Supine	Standing
25/10/23	1	99	98	107	103	101	104
	2	100	95	107	101	102	104
	3	96	99	106	100	98	106
	Average	98.33	97.33	106.67	101.33	100.33	104.67
27/10/23	1	105	102	108	105	101	108
	2	104	101	100	103	108	107
	3	105	103	102	101	101	111
	Average	104.67	102	103.33	103	103.33	108.67
30/10/23	1	104	100	102	101	99	102
	2	105	98	99	100	96	110
	3	102	101	106	100	92	100
	Average	103.67	99.67	102.33	100.33	95.67	104
31/10/23	1	97	100	101	93	94	96
	2	95	101	99	91	94	96
	3	95	97	99	92	95	98
	Average	95.67	99.33	99.67	92	94.33	96.67
01/11/23	1	111	105	115	100	101	100
	2	107	104	106	98	102	97
	3	107	103	103	95	99	100
	Average	108.33	104	108	97.67	100.67	99
03/11/23	1	99	96	97	89	90	97
	2	98	96	102	91	93	94
	3	96	98	105	91	92	93
	Average	97.67	96.67	101.33	90.33	91.67	94.67
06/11/23	1	105	98	108	100	98	103
	2	102	96	102	104	100	105
	3	97	97	105	101	99	101
	Average	101.33	97	105	101.67	99	103
07/11/23	1	94	97	97	99	96	96
	2	95	96	104	98	95	107

	3	90	95	98	98	96	98
	Average	93	96	99.67	98.33	95.67	100.33
08/11/23	1	99	112	104	98	98	98
	2	102	112	106	102	104	99
	3	96	106	102	97	99	101
	Average	99	110	104	99	100.33	99.33

Diastolic Pressure							
		Day time (9:00 AM to 10:00AM)			Evening Time (4:00 PM to 5:00PM)		
Date	Reading	Sitting	Supine	Standing	Sitting	Supine	Standing
25/10/23	1	69	65	71	68	68	76
	2	72	69	76	68	68	74
	3	71	72	74	64	65	73
	Average	70.67	68.67	73.67	66.67	67	74.33
27/10/23	1	69	62	71	70	67	74
	2	65	64	71	71	68	76
	3	64	65	65	71	63	80
	Average	66	63.67	69	70.67	66	76.67
30/10/23	1	66	65	65	67	61	71
	2	64	66	70	70	63	72
	3	66	67	73	68	62	61
	Average	65.33	66	69.33	68.33	62	68
31/10/23	1	62	62	67	62	61	69
	2	62	63	66	74	61	73
	3	64	62	71	65	64	71
	Average	62.67	62.33	68	67	62	71
01/11/23	1	73	65	71	64	69	71
	2	72	65	68	68	67	70
	3	67	63	69	67	67	71
	Average	70.67	64.33	69.33	66.33	67.67	70.67
03/11/23	1	64	62	73	64	62	66
	2	63	67	68	61	64	67
	3	64	66	70	61	67	68
	Average	63.67	65	70.33	62	64.33	67
06/11/23	1	64	62	66	71	67	75
	2	62	63	72	65	67	75

	3	64	62	72	68	69	72
	Average	63.33	62.33	70	68	67.67	74
07/11/23	1	62	62	70	69	64	68
	2	62	64	69	66	64	74
	3	70	60	70	63	61	75
	Average	64.67	62	69.67	66	63	72.33
08/11/23	1	68	72	72	72	70	70
	2	65	71	73	70	69	70
	3	70	69	72	70	71	69
	Average	67.67	70.67	72.33	70.67	70	69.67

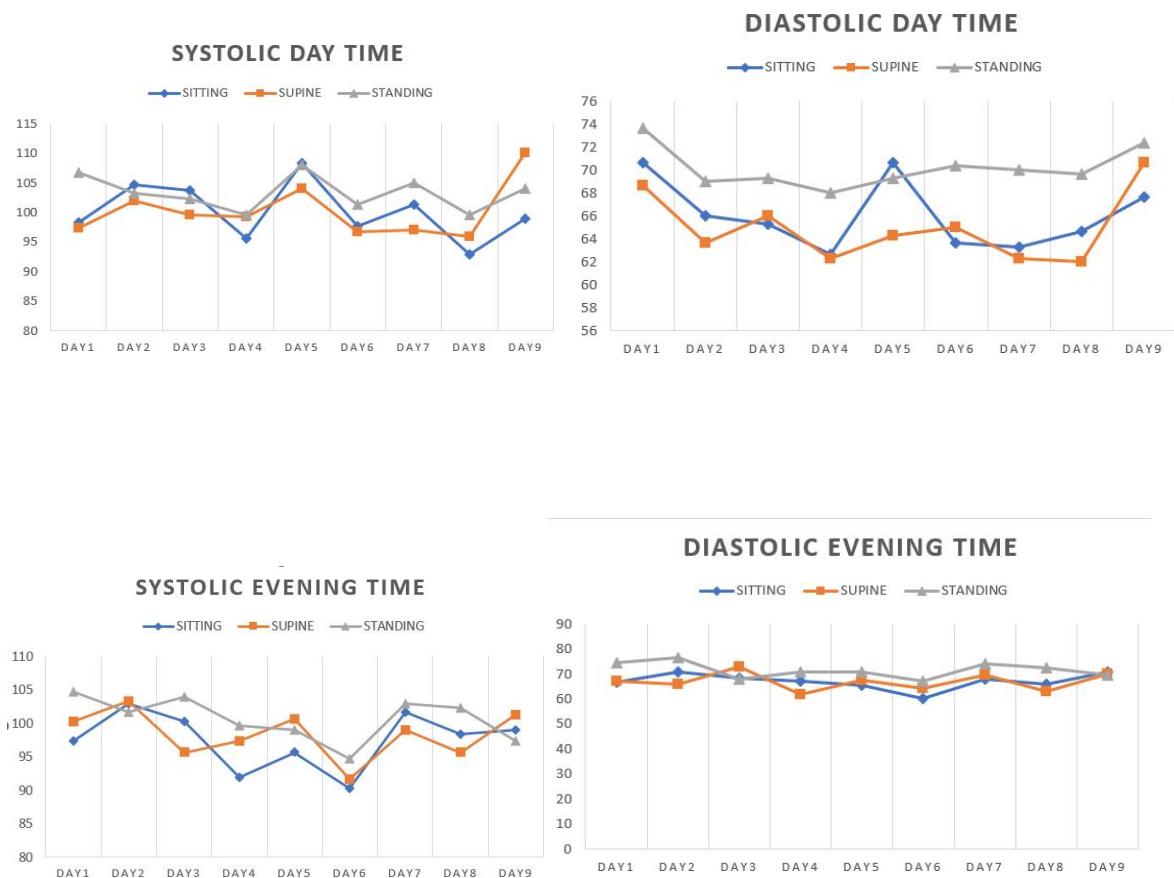


Fig 4. Plot for different position for BP Measurement during (a) Day Time and (b) Evening Time

## **Observation**

- It is observed that the systolic and diastolic blood pressure is the highest in supine position compared the other positions.
- From the result it is also visible that blood pressure tends to drop in the standing position compared with the sitting and supine.
- Effect of daytime and evening time is also seen in experiment. In evening blood pressure seems to drop.

## **Conclusion**

Blood pressure is critical indicator for cardiovascular, renal and other diseases. It is essential to consider the patient position and time while assessing the blood pressure. Patient position and time of measurement affects the BP measurements.

# ELECTRICAL SAFETY TEST

## AIM

To test electrical safety of patient monitor according to IEC 60601-1 electrical safety standard.

## OBJECTIVE

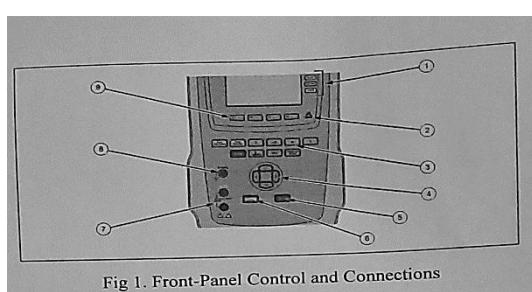
To perform electrical safety test for electrical medical devices such as patient monitor

To develop an understanding of globally recognized electrical safety standards such as IEC 60601.

## APPARATUS REQUIRED

The Fluke Biomedical ESA615 Electrical Safety Analyzer (the Product) is a full-featured compact, portable analyser, designed to verify the electrical safety of medical devices. The Product does these tests:

- Line (Mains) voltage
- Ground Wire (Protective Earth) resistance
- Equipment current
- Insulation resistance
- Ground (Earth) leakage
- Chassis (Enclosure) leakage
- Lead to Ground (Patient) and Lead to Lead (Patient Auxiliary) leakage
- Lead isolation (Mains on applied parts leakage)
- Differential leakage
- Direct equipment leakage
- Direct applied part leakage
- Alternative equipment leakage
- Alternative applied part patient leakage
- Point to point leakage, voltage, and resistance
- ECG simulation and performance waveforms



<b>Item</b>	<b>Name</b>	<b>Description</b>
1	Equipment Outlet Configuration Buttons	Controls the configuration of the equipment outlet, Open, closes the neutral and ground connection, and reverses the polarity of the neutral and hot connection.
2	High Voltage Indicator	Illuminates when high voltage is applied to the ECG/Applied Parts posts or L1 and L2 of the Test Receptacle,
3	Test Function Buttons	Selects the Product test functions
4	Navigation Buttons	Cursor control buttons for navigating menus and list.
5	Test Button	Starts selected tests.
6	Enter Button	Sets the highlighted function.
7	Input Jacks	Test lead connectors.
8	Nulling Jack	Connection to zero test lead resistance.
9	Function Softkeys	Keys F1 through F4 are used to select from a number of selections that show in the LCD display above each function softkey.

<b>Item</b>	<b>Name</b>	<b>Description</b>
1	Equipment Outlet	Equipment outlet, specified to the version of the product, which supplies a DUT connection.
2	USB A Controller Port	For external keyboard or barcode reader.
3	USB Device Port (Mini B-style connector)	Digital connection to control the Product from a PC or instrument controller.
4	Fuse Access Door	Equipment outlet fuse access.
5	Tilt Stand	Holds the Product in a tilted position.
6	SD Card Slot	SD Memory Card access.
7	AC Power Switch	Turns ac power on and off.

8	Power Input Connector	A grounded male three-prong (IEC 60320 C19) connector that accepts the line-power cord.
9	ECG/Applied Parts Jacks	Connection posts for Device under Test (DUT) applied parts, such as ECG leads. Used to test for leakage current through leads and to supply ECG signals and performance waveforms to a DUT.
10	Banana Jack to ECG Adaptor	Adapter to connect ECG snap lead to the product.

Fig 4. Side and Top-Panel Connections.

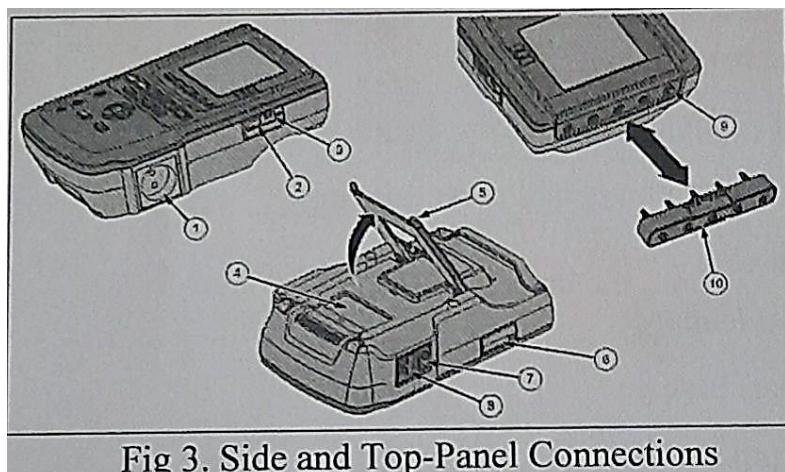


Fig 3. Side and Top-Panel Connections

## PATIENT MONITOR

Patient monitors are devices used to measure, record, and display various patient parameters. Much as heart rate and rhythm, SpO<sub>2</sub>, blood pressure, temperature, respiratory rate, blood pressure, blood oxygen saturation, etc., to keep a track of the patient's health and provide them with high-quality health care. Patient monitors are most often used in hospitals but are also frequently found in the homes of patients who suffer from a chronic illness, diabetes, etc., in order to keep an eye on their vitals and to detect further complications.

Nihon Kohden Vismo PVM-2703 is a 10-inch bedside patient monitor with high accuracy and simple operation. It measures seven parameters: ECG (3 or 6 electrodes), SpO<sub>2</sub>, NIBP, Impedance respiration, and temperature, IBP or CO<sub>2</sub>.

## METHOD

- How to Connect a DUT to the Product-
- Figure 5 shows a Device Under Test (DUT) connected to the test receptacle, applied parts posts, and a connection to the enclosure or protective earth ground of the DUT.
- How to Turn On the Product-
- Push the power switch found on the left-side panel so the "I" side of the ac power switch is down. The Product does a series of self-tests and then shows a message when the self-tests and then shows a message when the self-test has completed successfully.
- How to Access the Product Functions.
- For each test and setup function, the Product uses a series of menus to access different Product tests and setup variables. The More softkey lets you access more menus related to the test. When you push a softkey (F1 through F4) below a test name, the Product sets up for or does the selected test.
- How to Set Up the Product.
- There are a number of Product parameters that are adjusted through the setup function. To access the first Setup menu, push SETUP. Set the Operator Name, Date, Time, and Test Standard (IEC 60601-1),
- Ground wire resistance test- Follow instructions on the screen and zero the test lead..
- Connect the test lead back to the DUT and begin the test.
- Once the test has ended, results are stored in a SD Card and can be viewed in a computer later.

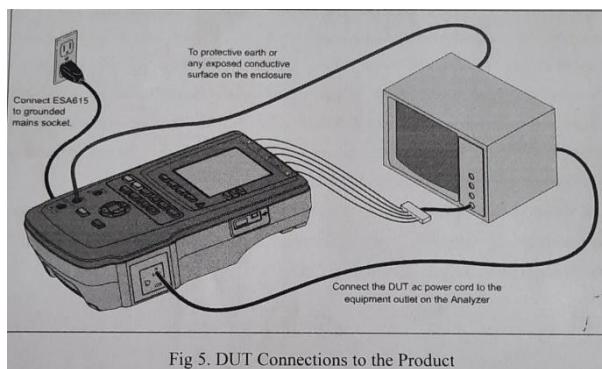
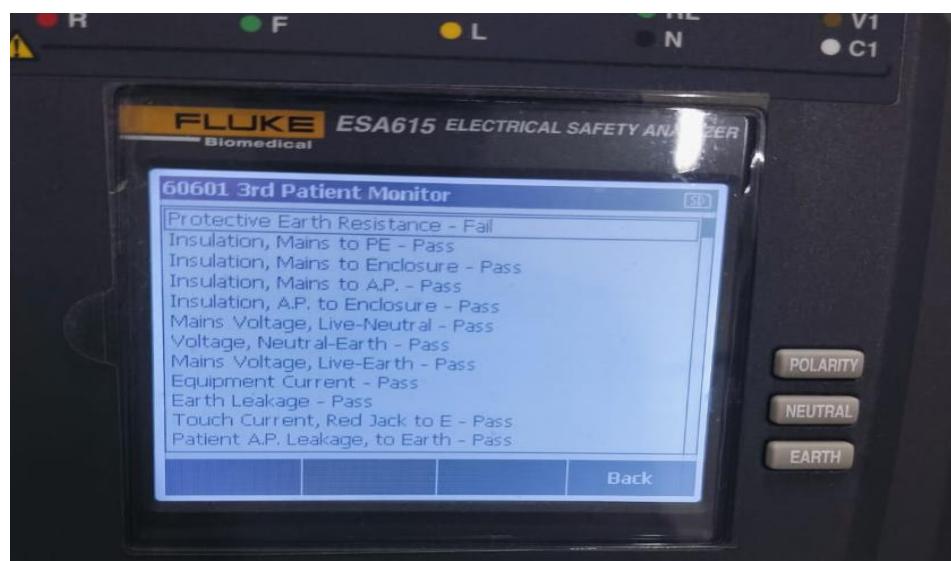


Fig 5. DUT Connections to the Product

## OTHER STANDARDS FOR EQUIPMENTS

Standard	Description	Most Current Version
ISO 13485	Medical Device Quality Management Systems	ISO 13485:2016
ISO 14971	Medical Device Risk Management	ISO 14971:2019
ISO 9001	Business Quality Management Systems	ISO 9001:2015
ISO 62304	Software for Medical Devices	ISO 62304:2006
ISO 10993	Biological Evaluation of Medical Devices (23 Parts)	Various Parts
ISO 15223	Symbols for Medical Device Labels and Information	Two Parts
ISO 11135	Ethylene Oxide Sterilization of Medical Devices	ISO 11135:2014
ISO 11137	Sterilization of Medical Devices Using Radiation	Various Parts
ISO 11607	Sterilized Product Packaging for Medical Devices	Various Parts
IEC 60601	Safety and Performance of Medical Electrical Equipment	Country-specific versions

## OBSERVATION



## **CONCLUSION**

- The device under test either passed or failed the electrical safety test.
- The device can be said to comply to the recognized electrical safety standards for medical devices, such as IEC 60601-1.

# **MEASUREMENT OF THE SPATIAL VARIATION OF PLANTAR TISSUE VISCOELASTICITY IN NORMAL SUBJECTS**

## **Aim**

To measure plantar tissue viscoelasticity from anatomical locations of interest using myotonometry and shore durometer.

## **Objective**

- To identify and mark the anatomical locations on the plantar aspect
- To record myotonometric parameters from the plantar anatomic sites
- To measure hardness of the plantar anatomic sites using shoremeter
- To compare the shore hardness and myotonometric parameters
- To analyse the spatial variation of plantar viscoelastic measurements

## **Apparatus Required**

- Mytonometer
- Shore Durometer
- MATLAB

## **Theory**

Plantar soft tissues have an inhomogeneous collagen-rich architecture with inclusions of fat globules. The compliance of the plantar soft tissue allows the dissipation of the ground impact loads on the foot during ambulation. Thus, it provides a cushioning effect against the plantar pressure loads during daily movements which protects the deep tissue structures from stress- induced damage.

The cushioning effect of plantar is dependent on the viscoelastic properties of the plantar tissues. Measurement of plantar tissue viscoelasticity has been predominantly done using manual palpation in the clinical practice hitherto. However, quantification of mechanical properties of plantar tissue for reliable assessment of the state of the tissues. Static indentation technique is a direct way of measuring target tissue property, where the deformation of tissue against imposed load is taken as the measure of tissue stiffness. Shore durometer works in the principle of measuring the spring deformation in response to imposed load. Type A durometer is customary device for measuring rubber elastomer hardness, and it has been predominantly used in the field of the biomedical

domain. The measurements are made in terms of the hardness scale of 1 to 100, with increasing score indicating higher stiffness. The hardness score is directly proportional to Young's modulus of the material.

Myotonometry is, on the other hand, a dynamic indentation technique that measures the inertial oscillation of the tissue under input impulse load. In this technique, a 0.4 N impulse load is applied on the tissue using an automatic electromagnetic linear actuator for 15 ms. The resulting free oscillation is recorded using an accelerometer for the consequent 400 ms. The acceleration signal is further analysed to compute the myotonometric parameters such as Oscillation Frequency, Logarithmic Decrement, Dynamic Stiffness and Relaxation Time. Oscillation Frequency (OF) is the peak frequency of the oscillation determined from the power spectrum of the myotonometry signal. Logarithmic Decrement (LoD) determines the order of decrement from the first to second positive peak and can be mathematically expressed as  $\text{LoD}=\ln a/\sqrt{a_3}$ . Dynamic Stiffness (DS) is the characteristic resistance of the tissue against any imposed deformation and can be estimated as  $\text{DS}=m.a_1\Delta l$  where m is the mass of the probe. Relaxation Time (RT) is the measure of the time scale required for the deformed tissue to attain the original shape and is defined as

$$\text{RT} = t_R - t$$

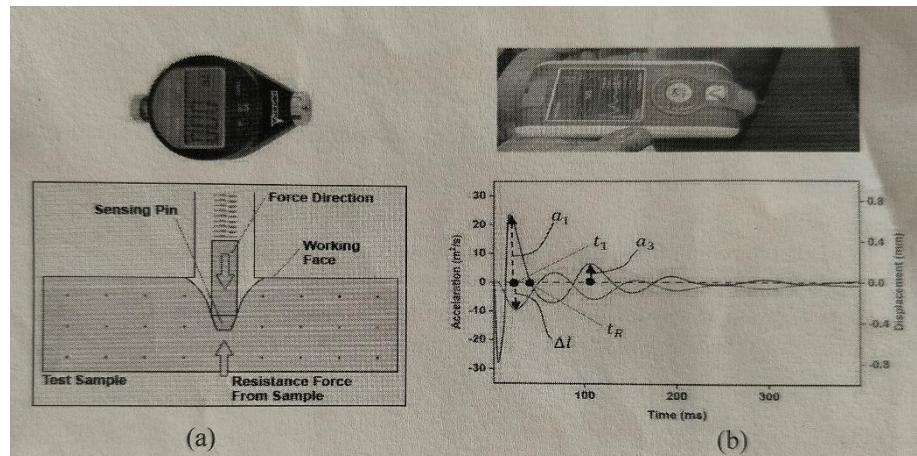


Figure 1. Working principles of the (a) Shore Durometer and (b) Myotonometer

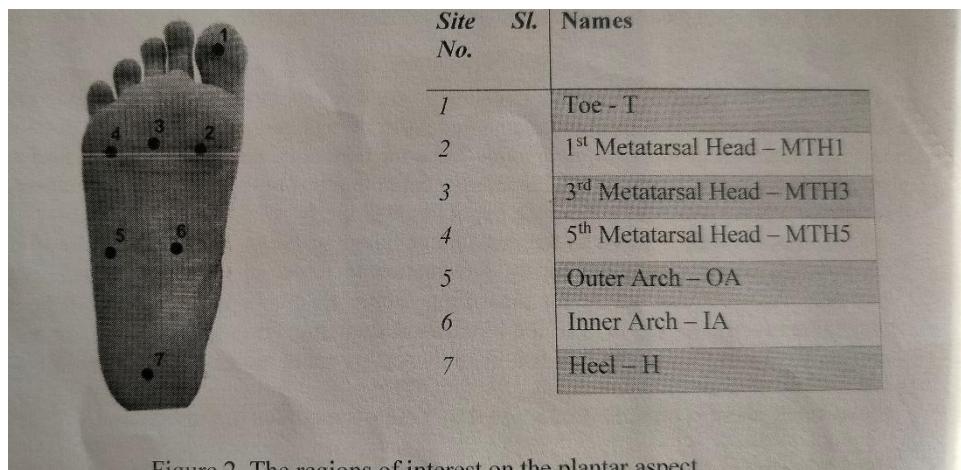


Figure 2. The regions of interest on the plantar aspect

The methodology of the experiment is as follows

1. 15 minutes of rest is provided for the subjects before the experiment commences
2. The plantar aspect is cleaned with a wet cloth and the seven anatomical sites are marked for the reference of measurements.
3. The hardness of the anatomical sites is recorded using Shore Durometer
4. The myotonometric parameters are also consequently recorded from all the seven anatomical sites
5. The choice of order for the foot is randomized, but nevertheless, measurements is to be carried out from both right and left feet.

**Table 1. Spatial distribution of the mechanical properties of the plantar soft tissue measured by the mytonometer and the durometer.**

Foot	Site	Mytonometer				Duromter
		OF (Hz)	LoD	DS (N/m)	RT (ms)	
Right	T	24	2.94	486	9.8	14.5
	MTH1	23.3	1.59	458	11.2	18.5
	MTH3	27.4	1.41	551	9.6	15.5
	MTH5	28.1	1.52	524	9.4	18

	OA	31.5	1.96	701	6.9	27
	IA	27.7	1.55	638	8.6	21.5
	H	30.3	1.71	658	7.6	23.5
Left	T	29.5	2.9	492	9.3	13.5
	MTH1	28.9	1.83	561	9.7	17
	MTH3	31	1.52	630	8.4	15
	MTH5	26.1	2.22	517	9.5	15
	OA	30.4	1.31	663	7.3	24
	IA	26.2	1.72	607	9.3	19
	H	39.1	1.87	932	4.7	24.5

## PYTHON CODE FOR PLOTTING THE GRAPH

```

import numpy as np
import matplotlib.pyplot as plt

# Defining the parameters for the right foot
hardness_right = [14.5, 18.5, 15.5, 18, 27, 21.5, 23.5];
oscillation_freq_right = [24, 23.3, 27.4, 28.1, 31.5, 27.7, 30.3];
Dynamic_stiffness_right = [486, 458, 551, 524, 701, 638, 658];
Log_decrement_right = [2.94, 1.59, 1.41, 1.52, 1.96, 1.55, 1.71];
Relapse_time_right = [9.8, 11.2, 9.6, 9.4, 6.9, 8.6, 7.6];
#defining the parameters for right foot
hardness_left = [13.5, 17, 15, 15, 24, 19, 24.5];
oscillation_freq_left = [29.5, 28.9, 31, 26.1, 30.4, 26.2, 39.1];
Dynamic_stiffness_left = [492, 561, 630, 517, 663, 607, 932];
Log_decrement_left = [2.96, 1.83, 1.52, 2.22, 1.91, 1.72, 1.87];

```

```

Relapse_time_left = [9.3,9.7,8.4,9.5,7.3,9.3,4.7];

# Combine data for both feet
hardness = np.concatenate((hardness_right, hardness_left))
oscillation_freq = np.concatenate((oscillation_freq_right, oscillation_freq_left))
Log_decrement = np.concatenate((Log_decrement_right, Log_decrement_left))
Relapse_time = np.concatenate((Relapse_time_right, Relapse_time_left))
Dynamic_stiffness=np.concatenate((Dynamic_stiffness_right,Dynamic_stiffness_left))

# Scatter plot with different markers
plt.figure(1)
plt.scatter(hardness[:7], oscillation_freq[:7], marker='o', label='Right Foot')
plt.scatter(hardness[7:], oscillation_freq[7:], marker='s', label='Left Foot')
plt.title('Hardness vs. Oscillation Frequency')
plt.xlabel('Hardness (HA)')
plt.ylabel('Oscillation Frequency (Hz)')
plt.legend()

# Calculate and plot regression lines
coeffs = np.polyfit(hardness, oscillation_freq, 1)
x_fit = np.linspace(min(hardness), max(hardness), 100)
y_fit = np.polyval(coeffs, x_fit)
plt.plot(x_fit, y_fit, 'r--', linewidth=2)

# Calculate Pearson correlation coefficient
R_value_HoF = np.corrcoef(hardness, oscillation_freq)[0, 1]

```

```

# After plotting your data points, add the R value to the graph:

# Add the R value to the top of the graph

plt.text(20, max(oscillation_freq) - 1, fR = {R_value_HoF:.2f}', fontsize=12,
ha='right', va='top')

plt.show()

# Scatter plot for Hardness vs. Logarithmic Decrement

plt.figure(2)

plt.scatter(hardness[:7], Log_decrement[:7], marker='o', label='Right Foot')
plt.scatter(hardness[7:], Log_decrement[7:], marker='s', label='Left Foot')
plt.title('Hardness vs. Logarithmic Decrement')
plt.xlabel('Hardness (HA)')
plt.ylabel('Logarithmic Decrement')
plt.legend()

# Calculate and plot regression lines

coeffs = np.polyfit(hardness, Log_decrement, 1)
x_fit = np.linspace(min(hardness), max(hardness), 100)
y_fit = np.polyval(coeffs, x_fit)
plt.plot(x_fit, y_fit, 'r--', linewidth=2)

# Calculate Pearson correlation coefficient

R_value_HLD = np.corrcoef(hardness, Log_decrement)[0, 1]

# Add the R value to the graph (right side)

plt.text(25, 2.5, fR = {R_value_HLD:.2f}', fontsize=12, ha='right', va='top')

plt.show()

# Scatter plot for Hardness vs. Dynamic Stiffness

```

```
plt.figure(3)

plt.scatter(hardness[:7], Dynamic_stiffness[:7], marker='o', label='Right Foot')
plt.scatter(hardness[7:], Dynamic_stiffness[7:], marker='s', label='Left Foot')
plt.title('Hardness vs. Dynamic Stiffness')
plt.xlabel('Hardness (HA)')
plt.ylabel('Dynamic Stiffness (N/m)')
plt.legend()
```

```
# Calculate and plot regression lines
coeffs = np.polyfit(hardness, Dynamic_stiffness, 1)
x_fit = np.linspace(min(hardness), max(hardness), 100)
y_fit = np.polyval(coeffs, x_fit)
plt.plot(x_fit, y_fit, 'r--', linewidth=2)
```

```
# Calculate Pearson correlation coefficient
R_value_HDS = np.corrcoef(hardness, Dynamic_stiffness)[0, 1]
```

```
# Add the R value to the graph (right side)
plt.text(25, 950, f'R = {R_value_HDS:.2f}', fontsize=12, ha='right', va='top')
plt.show()
```

```
# Scatter plot for Hardness vs. Relapse Time
plt.figure(4)

plt.scatter(hardness[:7], Relapse_time[:7], marker='o', label='Right Foot')
plt.scatter(hardness[7:], Relapse_time[7:], marker='s', label='Left Foot')
plt.title('Hardness vs. Relaxation Time')
plt.xlabel('Hardness (HA)')
```

```

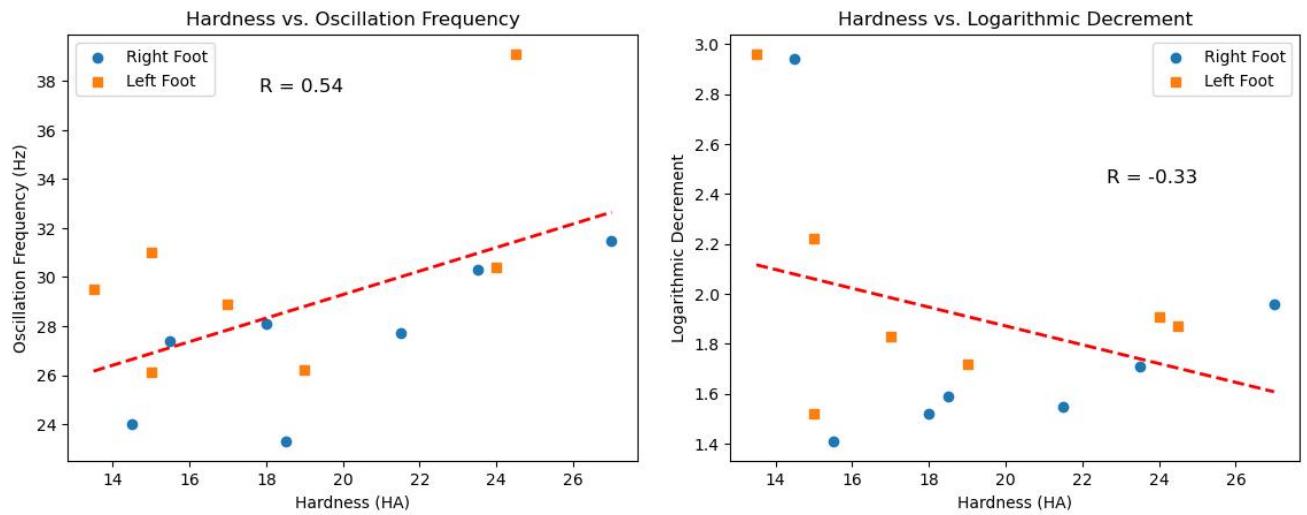
plt.ylabel('Relaxation Time (ms)')
plt.legend()

# Calculate and plot regression lines
coeffs = np.polyfit(hardness, Relapse_time, 1)
x_fit = np.linspace(min(hardness), max(hardness), 100)
y_fit = np.polyval(coeffs, x_fit)
plt.plot(x_fit, y_fit, 'r--', linewidth=2)

# Calculate Pearson correlation coefficient
R_value_HRT = np.corrcoef(hardness, Relapse_time)[0, 1]

# Add the R value to the graph (right side)
plt.text(25, 10, f'R = {R_value_HRT:.2f}', fontsize=12, ha='right', va='top')
plt.show()

```



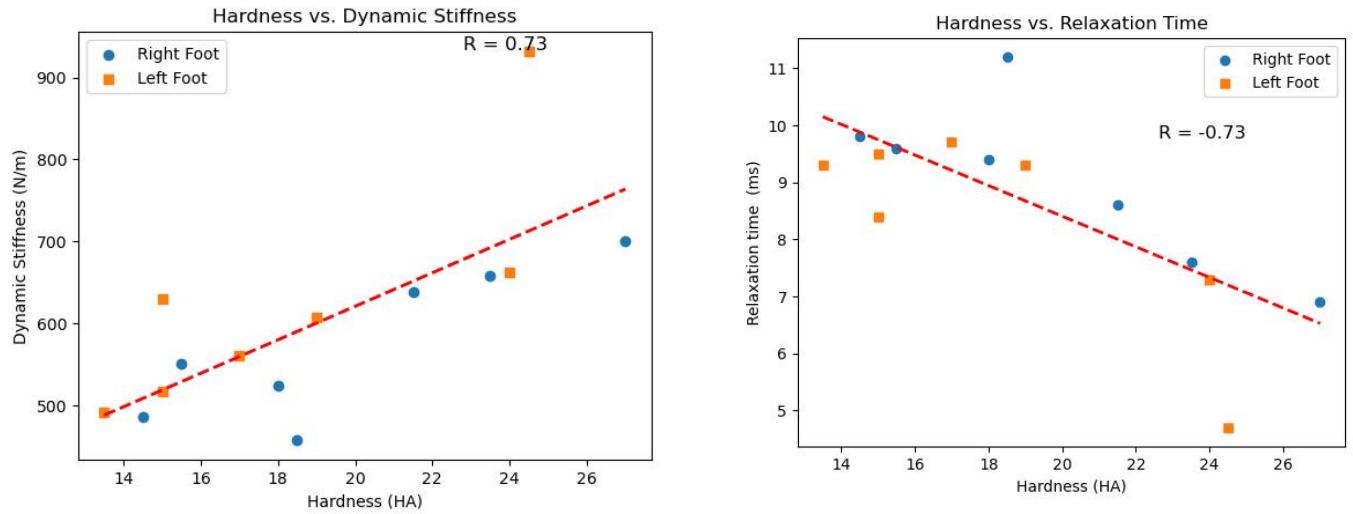


Figure 3. Variation of the myotonometric parameters with the hardness measurements using Shore Durometer (a) Oscillation Frequency, (b) Logarithmic Decrement, (c) Dynamic Stiffness, and (d) Relaxation Time.

## RESULT

The highest correlation is for the Hardness vs dynamic stiffness = 0.73

## CONCLUSION

Plantar tissue mechanical properties vary spatially, with different tissue properties in the forefoot, midfoot, and hindfoot region. The measurements from different techniques to characterize tissue properties are comparable to each other.

# **RECORDING AND ANALYSIS OF SURFACE EMG SIGNALS WITH VARRYING GRIP STRENGTH**

## **AIM**

To record and analyse EMG signals from flexor muscle with varying grip strength to identify variations in dominant and non-dominant hand.

## **OBJECTIVES**

- To record EMG signals using signal acquisition system with minimum artifacts
- To identify correlation in EMG signals with varying grip strength for dominant and non-dominant hands.

## **APPARATUS REQUIRED**

- Ag-AgCl foam disc-type surface electrodes
- Gauze for skin preparation
- Computer
- Hand dynamometer (kg).
- Biopac Science Lab system (MP40 and software)
- MATLAB SOFTWARE

## **THEORY**

Skeletal muscles are responsible for numerous activities that are performed in our daily life such as locomotion and posture maintenance. They play a vital role in force control for making precise or powerful movements. It consists of fibres that are innervated by a-motor neurons for producing these movements. Surface electromyography (SEMG) is a non-invasive technique that records muscle activity with the help of surface electrodes. Measurement of maximal grip strength (MGS) is an essential element to follow people during growth, ageing, injury, rehabilitation, training or therapeutic trials. Its measurement is performed using dynamometers, which estimate the muscle strength primarily generated by the flexor muscles of the hand and the forearm.

## **METHOD**

### **EMG electrode placement**

The signals are attained from the participants' dominant hand. The criteria for the selection of subjects include no experience in weight training and no neuromuscular disease history. An informed consent of the subject is taken, since pain may be induced during the exercise which might last for a few days. Before recording the signal, the skin is abraded and cleaned in order to provide better electrode skin interface. The alcohol is widely used for cleaning the skin

in order to eliminate the wetness or sweat and reduce skin impedance. Instructions about the task are provided to subjects prior to start of the experimentation. The signals are acquired in bipolar electrode configuration with two surface electrodes kept on flexor muscle belly at an interelectrode distance of 2 cm based on Surface Electromyography for Non Invasive Assessment of Muscles (SENIAM) standards.

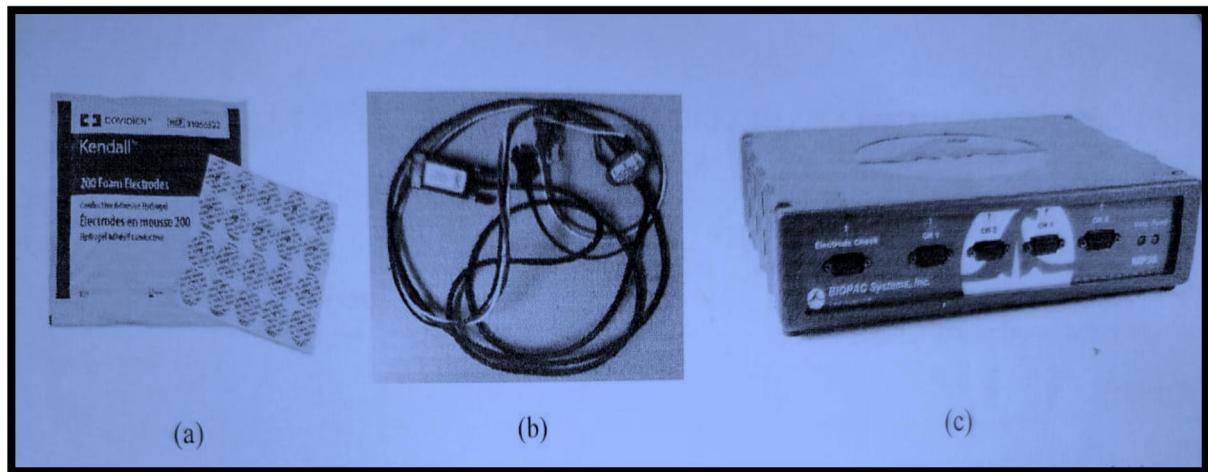


Fig. 1. Signal acquisition (a) Ag-AgCl Surface electrodes (b) Shielded cables and (c) BIOPAC bioamplifier system.

### sEMG SIGNAL ACQUISITION

The participants are advised to stand straight on the insulated platform to isolate from the ground. The sEMG signals are acquired with BIOPAC MP36 data acquisition system which is approved by Food and Drug Administration. The bioamplifier has signal to noise ratio, differential mode input impedance and common mode rejection ratio of 89 dB, 2 MS2 and 110 dB respectively. The 24 bits resolution analog to digital converter is present in the acquisition system and the gain is set to 1000. The signals are acquired with the sampling rate of 10 KHz. In the offline analysis, the signal is down sampled to 1000 Hz, in order to reduce the computational head.

### HAND GRIP STRENGTH MEASUREMENT

The height and weight of the subjects were recorded as well as anthropometric hand data were measured by the experimenter using a standard 1000-mm tape measure. The circumference of the forearm was defined as the perimeter of the largest part of the forearm, located over the bulk of the brachioradialis muscle, at the proximal quarter of the whole forearm length (Fig. 2a). The circumference of the hand was measured as the perimeter of the middle part of hand, located at the two major transverse palmar creases ("heart line" and "head line") (Fig. 2b). Hand length was defined as the distance from the tip of the middle finger to the midline of the distal wrist crease (Fig. 2c). All

anthropometric data were measured to the nearest millimetre with the forearm and hand in an outstretched and supinated position. Dominant side was defined as the hand with which the subject writes.

## EXPERIMENTAL SETUP

- Before applying electrodes to the subject, it is first important to properly prepare and clean the electrode sites
- Let the areas dry before attaching the electrodes
- Two surface electrodes kept on flexor muscle belly at an interelectrode distance of 2 cm
- A reference electrode is positioned in the elbow
- Setup the Biopac signal acquisition system. Insert the connectors on the red and black electrode lead wires into the matching sockets on the EMG cable
- The subjects are instructed to maintain an upright posture with the minimal torso and the upper arm distance. They are advised not to rest the elbow on the hip
- The upper arm is maintained in vertical position and the forearm is held in supine position.
- Subjects were verbally encouraged to produce their maximal grip strength (MGS). Two trials were first recorded, consisting of a 2-4-second maximal contraction, with a 30-second rest period between each trial. If the relative difference between these two MGS was within 10%, no additional trial was required.
- This is repeated for 50% and 10% MGS in stages, noting down the values of dynamometer.
- Raw signals are pre-processed and analysed using MATLAB.

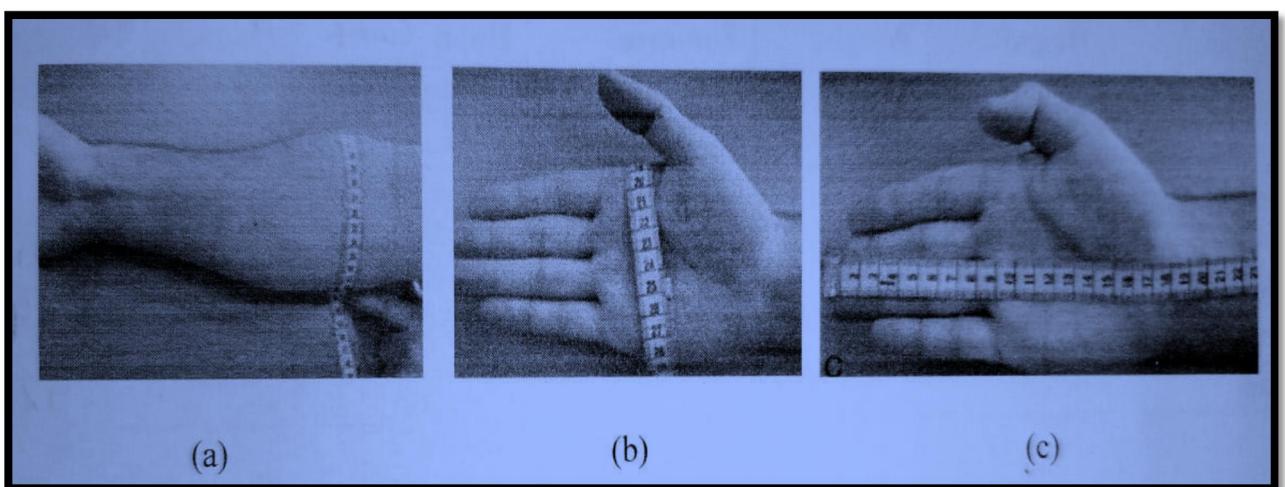


Fig. 2. Measurements of anthropometric characteristics of hand and forearm including (a) forearm circumference. (b) hand circumference and (c) hand length.

## PLACEMENT OF ELECTRODES FOR RECORDING EMG

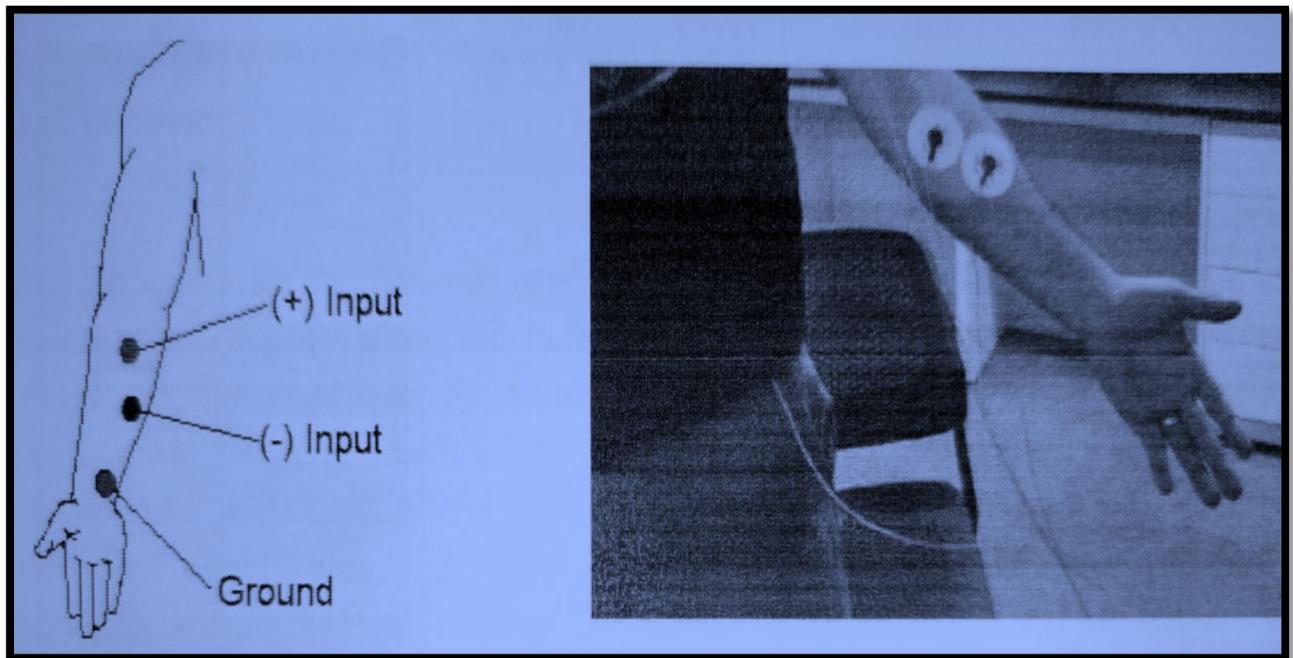


TABLE 1: ANTHROPOMETRIC MEASUREMENTS

Subject	Height(c)	Weight(l)	Upper Forearm Circumference (cm)	Lower forearm Circumference (cm)	Hand Length (cm)
<b>Dominant Hand</b>	-	-	26.1	17.6	31
<b>Non-Dominant Hand</b>	-	-	25	16.1	31

### MATLAB CODE:

```
% Load the EMG signal from the .mat file
emg_signal=load("F:\IITM\Physiological measurements lab\EMG
data\KaviDom.mat"); % Replace 'your_emg_data.mat' with the actual filename
my_data=emg_signal.data;
% Plot the original EMG signal
figure;
plot(my_data);
title('Original EMG Signal');
xlabel('Sample Number');
ylabel('Amplitude');
```

```

grid on;
%separating the peaks based on activity for grip strength
force_max=my_data(28356:38695);%maximum grip strength
force_50=my_data(39000:48000);%50% of maximum grip strength
force_10=my_data(48000:57077);%10% of maximum grip strength
%for maximum grip strength
%RMS Value
rms_value_max = rms(force_max);
disp(['RMS Value for maximum grip strength for dominant hand: ', num2str(rms_value_max)]);
%Mean absolute value
mav_value_max = mean(abs(force_max));
disp(['Mean Absolute Value (MAV) for maximum grip strength for dominant hand: ', num2str(mav_value_max)]);
%Max amplitude
max_amplitude_max = max(abs(force_max));
disp(['Maximum Amplitude for maximum grip strength for dominant hand: ', num2str(max_amplitude_max)]);
%Zero crossing rate
zero_crossings_max = sum(abs(diff(sign(force_max))) > 0);
zero_crossing_rate_max = zero_crossings_max / (length(force_max) - 1);
disp(['Zero-Crossing Rate for maximum grip strength for dominant hand: ', num2str(zero_crossing_rate_max)]);

```

```

%for 50 % of Maximum grip strength

%RMS Value
rms_value_50= rms(force_50);
disp(['RMS Value for 50 % of maximum grip strength for dominant hand: ', num2str(rms_value_50)]);
%Mean absolute value
mav_value_50= mean(abs(force_50));
disp(['Mean Absolute Value (MAV) for 50 % of maximum grip strength for dominant hand: ', num2str(mav_value_50)]);
%Max amplitude
max_amplitude_50 = max(abs(force_50));
disp(['Maximum Amplitude for 50 % of maximum grip strength for dominant hand: ', num2str(max_amplitude_50)]);
%Zero crossing rate
zero_crossings_50 = sum(abs(diff(sign(force_50))) > 0);
zero_crossing_rate_50 = zero_crossings_50 / (length(force_50) - 1);

```

```
disp(['Zero-Crossing Rate for 50 % of maximum grip strength for dominant hand:: ', num2str(zero_crossing_rate_50)]);
```

```
%for 10 % of Maximum grip strength
%RMS Value
rms_value_10= rms(force_10);
disp(['RMS Value for 10 % of maximum grip strength for dominant hand: ', num2str(rms_value_10)]);
%Mean absolute value
mav_value_10= mean(abs(force_10));
disp(['Mean Absolute Value (MAV) for 10 % of maximum grip strength for dominant hand: ', num2str(mav_value_10)]);
%Max amplitude
max_amplitude_10 = max(abs(force_10));
disp(['Maximum Amplitude for 10 % of maximum grip strength for dominant hand: ', num2str(max_amplitude_10)]);
%Zero crossing rate
zero_crossings_10 = sum(abs(diff(sign(force_10))) > 0);
zero_crossing_rate_10 = zero_crossings_10 / (length(force_10) - 1);
disp(['Zero-Crossing Rate for 10 % of maximum grip strength for dominant hand:: ', num2str(zero_crossing_rate_10)]);
% Sample EMG signal and time vector (replace with your actual data)
fs = 1000; % Sampling frequency in Hz
% Calculate the RMS of the EMG signal in a sliding manner
windowSize = 100; % Choose an appropriate window size
rmsValues_max = zeros(1, length(force_max) - windowSize + 1);

for i = 1:length(rmsValues_max)
    rmsValues_max(i) = rms(force_max(i:i+windowSize-1));
end
% Create a time vector for RMS values
timeRMS_max = (0:length(rmsValues_max)-1) / fs;

%for 50% force
rmsValues_50 = zeros(1, length(force_50) - windowSize + 1);

for i = 1:length(rmsValues_50)
    rmsValues_50(i) = rms(force_50(i:i+windowSize-1));
end

% Create a time vector for RMS values
timeRMS_50 = (0:length(rmsValues_50)-1) / fs;
```

```

%for 10% force
rmsValues_10 = zeros(1, length(force_10) - windowSize + 1);

for i = 1:length(rmsValues_10)
    rmsValues_10(i) = rms(force_10(i:i+windowSize-1));
end

% Create a time vector for RMS values
timeRMS_10 = (0:length(rmsValues_10)-1) / fs;

% Plot the RMS values with respect to time
figure;
subplot(3,2,1);
plot(force_max);
xlabel('Time (ms)');
ylabel('EMG Signal(mV)');
title('EMG Signal Over Time for maximum grip strength');
subplot(3,2,2);
plot(timeRMS_max, rmsValues_max);
xlabel('Time (s)');
ylabel('RMS of EMG Signal (mV)');
title('RMS of EMG Signal Over Time for maximum grip strength');
subplot(3,2,3);
plot(force_50);
xlabel('Time (ms)');
ylabel('EMG Signal(mV)');
title('EMG Signal Over Time for 50 % of maximum grip strength');
subplot(3,2,4);
plot(timeRMS_50, rmsValues_50);
xlabel('Time (s)');
ylabel('RMS of EMG Signal (mV)');
title('RMS of EMG Signal Over Time for 50 % of maximum grip strength');
subplot(3,2,5);
plot(force_10);
xlabel('Time (ms)');
ylabel('EMG Signal(mV)');
title('EMG Signal Over Time for 10 % of maximum grip strength');
subplot(3,2,6);
plot(timeRMS_10, rmsValues_10);
xlabel('Time (s)');
ylabel('RMS of EMG Signal (mV)');
title('RMS of EMG Signal Over Time for 10 % of maximum grip strength');

```

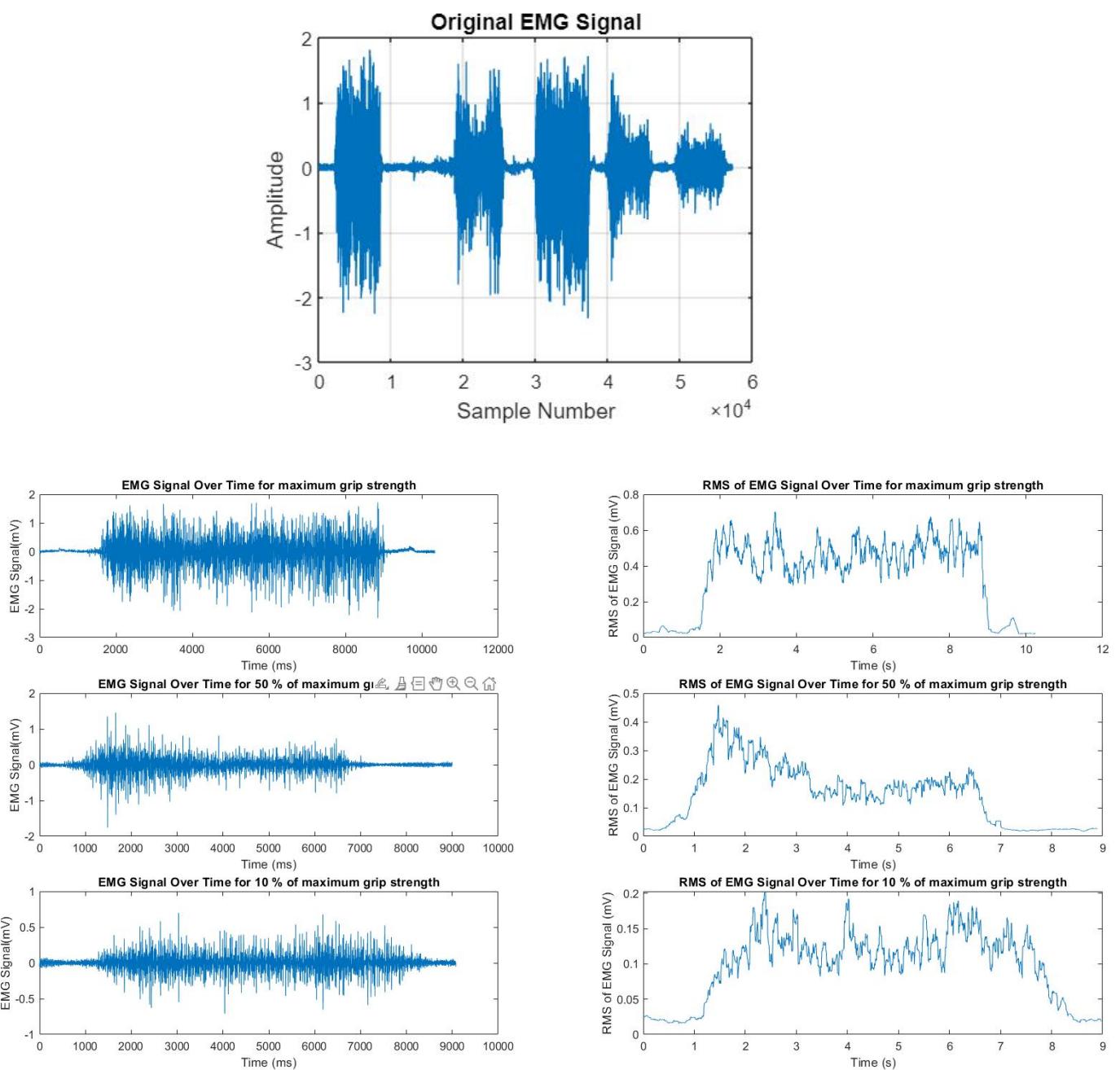


Figure 4: Representative of raw sEMG signals recorded and RMS of EMG Signal recorded for **Dominant Hand**

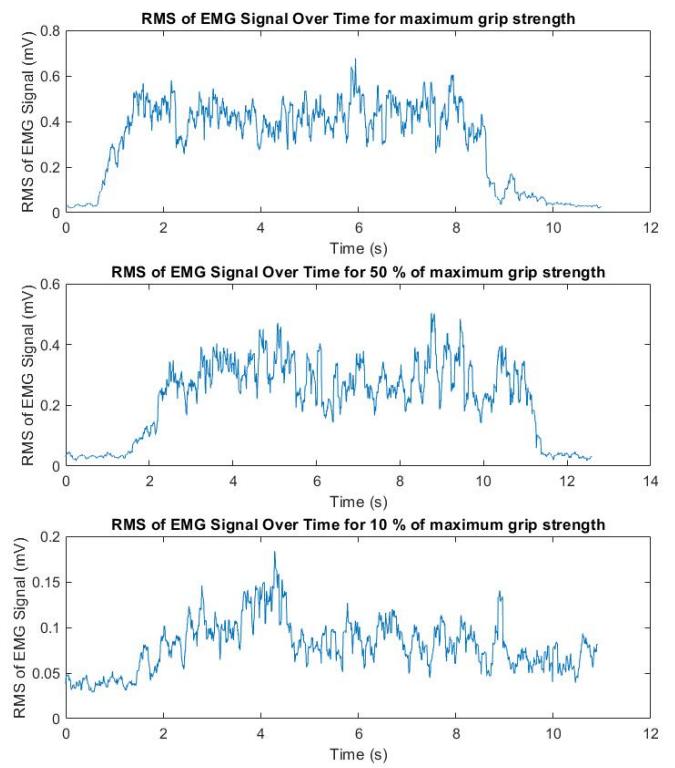
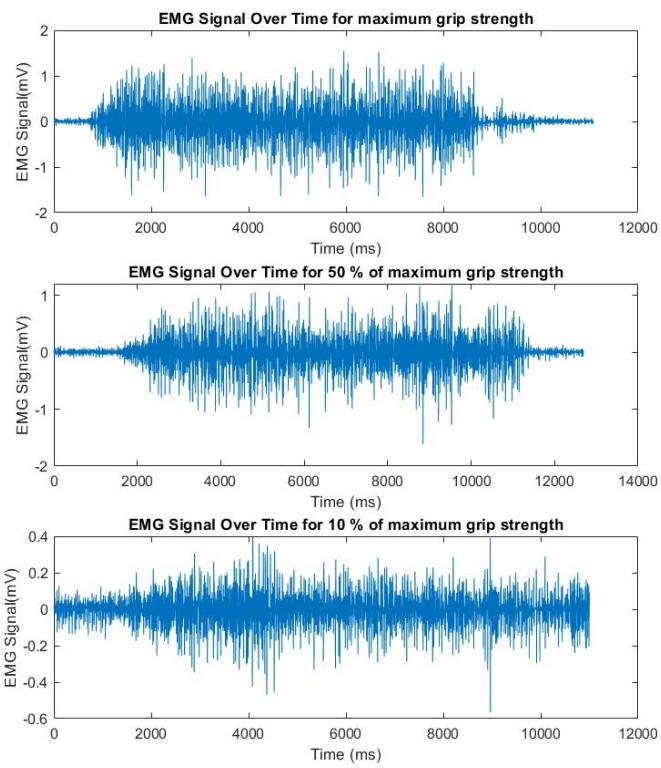
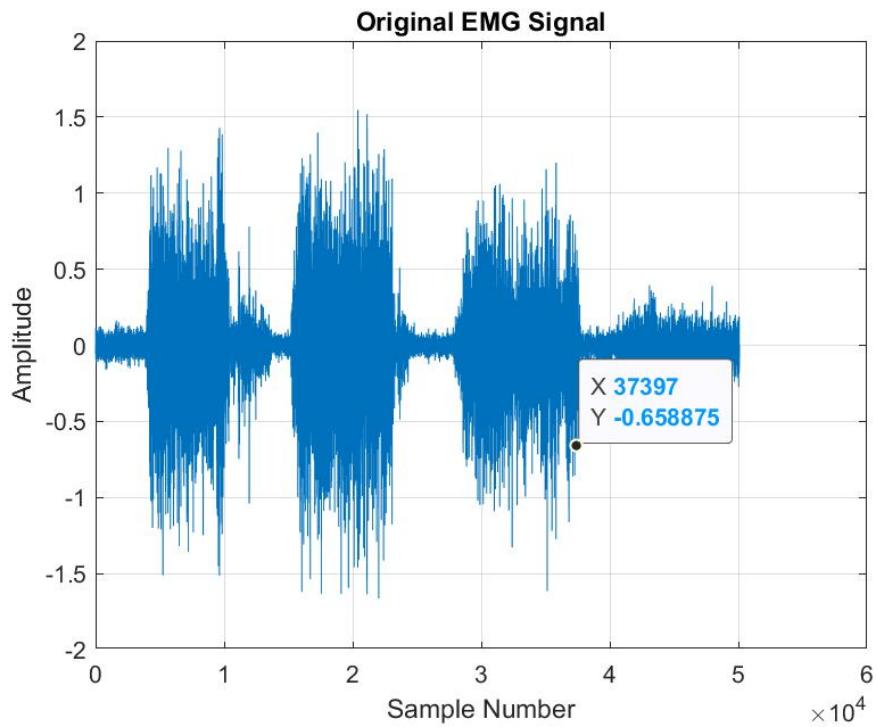


Figure 5: Representative of raw sEMG signals recorded and RMS of EMG Signal recorded for **Non-dominant Hand**

## Result:

Subject	Grip Strength	Grip Strength (Kg)	RMS Value (mV)	Mean Absolute Value	Maximum Amplitude (mV)	Zero Crossing Rate
Dominant Hand	Maximum (100%)	63	0.40464	0.25495	2.3224	0.1889
Non-dominant Hand	Maximum (100%)	63	0.36058	0.24292	1.6641	0.14091
Dominant Hand	50%	31.5	0.17683	0.10626	1.756	0.21556
Non-dominant Hand	50%	31.5	0.25489	0.16944	1.6159	0.13507
Dominant Hand	10%	6.3	0.10941	0.070974	0.70984	0.21747
Non-dominant Hand	10%	6.3	0.084501	0.061312	0.56732	0.19389

**Table 2: Observations Recorded**

- From the table it is obvious that the RMS value and the mean absolute value decreases as the grip strength decreases.

## CONCLUSION

Time domain features are extracted from the pre-processed EMG signals to identify variations in signals recorded from non-dominant hand of a subject with varying grip strength.

# **SEGMENTATION OF RETINAL BLOOD VESSELS FROM FUNDUS IMAGES USING MATLAB**

## **AIM**

To segment and validate the segmentation of retinal blood vessels from fundus images using

Image Processing and Computer Vision toolbox in MATLAB.

## **OBJECTIVE**

To segment retinal blood vessels from fundus images using MATLAB

## **APPARATUS REQUIRED**

- Laptop
- MATLAB software

## **THEORY**

The separation of blood vessels in the retina is a major aspect of detecting ailment and is carried out by segregating the retinal blood vessels from the fundus images. The retina is a type of photosensitive tissue that lines the interior layer of the eye. Retinal blood vessels are a part of the central retinal artery, vein, and their branch. Any changes in these retinal vessels in terms of their morphology or topography are employed to identify some pathology such as diabetic retinopathy. Fundus cameras are most commonly used to obtain images of the retina. The images obtained by the fundus camera are known as fundus images. It contains not only the image of the retina but also some diagnostic information about the retina. To measure the alterations that occur in blood vessels, a blood vessel segmentation image has to be developed from the obtained fundus image. This is usually performed by expert medical practitioners manually. These limitations in retinal manual retinal segmentation such as inter-rater variability and error rates have led to development of automation in retinal segmentation. Analyzing the retinal image can hold a crucial role in identifying and classifying retinal diseases such as age-based macular degeneration, diabetic retinopathy (DR), retinoblastoma, macular bunker.

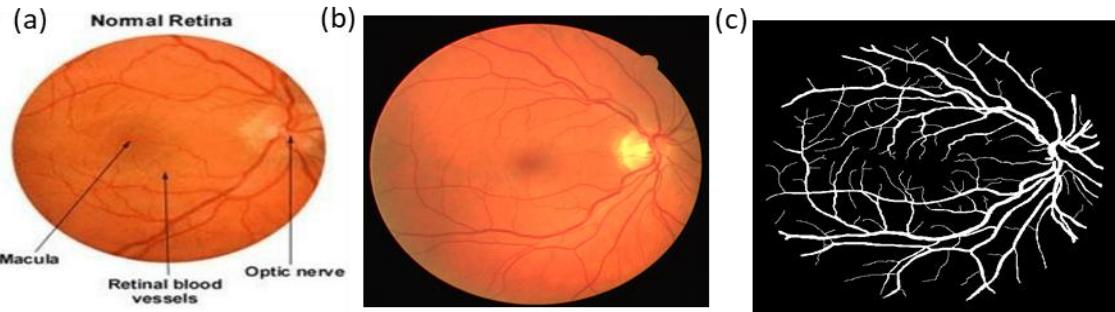


Fig 1. The images correspond to (a) Normal retina, (b) Fundus image. (c) Retinal vessels

## METHOD

Download and install Image Processing and Computer Vision' toolbox, and launch MATLAB. Load the RGB fundus image and the ground truth image provided. Ground truth image refers to the image which contains the retinal vessels extracted by manual process. Convert the fundus image to grayscale image. The contrast enhancement is performed to enhance the image resulting in better segmentation followed by background noise removal and thresholding. The morphological process in MATLAB further cleans the obtained image. Generate the binary mask of the segmented region by the sequences provided. The performance of retinal segmentation methods is measured regarding segment pixels. So segmented pixels are differentiated for vessels and non-vessels or backgrounds. Validate the segmentation result using the Dice coefficient overlap measure and Jaccard index as follows:

$$\text{Dice coefficient} = 2 * |Y \cap X| / |X| + |Y|$$

$$\text{Jaccard index} = |X \cap Y| / |Y \cup X|$$

where Y and X denote the segmented region and corresponding ground truth, respectively.

Repeat the same experiment for other images to extract retinal blood vessels.

## MATLAB CODE:

```
Test_image=imread("F:\IITM\Physiological measurements
lab\images_retinal_seg\retina_images\1.tif");
GT= imread("F:\IITM\Physiological measurements
lab\images_retinal_seg\ground truth images\1.tif");
```

```
Resized_Image = imresize(Test_image,[584 565]);
```

```

Converted_Image=im2double(Resized_Image);

Lab_Image=rgb2lab(Converted_Image);
fill=cat(3,1,0,0);
Filled_Image=bsxfun(@times,fill,Lab_Image);
Reshaped_Lab_Image=reshape(Filled_Image,[],3);

[C, S]=pca(Reshaped_Lab_Image);
S=reshape(S,size(Lab_Image));
S=S(:,:,1);
Gray_Image=(S-min(S(:)))./(max(S(:))-min(S(:)));

Enhanced_Image=adapthisteq(Gray_Image,'NumTiles',[8 8],'nbins',128);
Avg_Filter=fspecial('average',[9 9]);
Filtered_Image=imfilter(Enhanced_Image,Avg_Filter);

substracted_Image=imsubtract(Filtered_Image,Enhanced_Image);
level=Threshold_Level(substracted_Image);

figure,subplot(221),imshow(Test_image)
title('Test image 1')
subplot(222), imshow(Filtered_Image)
title('Filtered Image')
Binary_Image=imbinarize(substracted_Image,level-0.008);
subplot(223),imshow(Binary_Image)
title('Binary Image')
Clean_Image=bwareaopen(Binary_Image,50);
subplot(223),imshow(Clean_Image)
title('clean image')
Complemented_Image=imcomplement(Clean_Image);
subplot(224),imshow(Complemented_Image)
title('Complemented image')
%%%
similarity = dice(im2double(Clean_Image),im2double(GT))
Jrrad=jaccard(im2double(Clean_Image),im2double(GT))

%%%
function level=Threshold_Level(Image)

Image=im2uint8(Image(:));
[Histogram_count,Bin_Number]=imhist(Image);
i=1;

```

```

Cumulative_Sum = cumsum(Histogram_count);
T(i)=(sum(Bin_Number.*Histogram_count))/Cumulative_Sum(end);
T(i)=round(T(i));
Cumulative_Sum_2= cumsum(Histogram_count(1:T(i)));
MBT=sum(Bin_Number(1:T(i)).*Histogram_count(1:T(i)))/Cumulative_Sum_2(end);
Cumulative_Sum_3= cumsum(Histogram_count(T(i):end));
MAT=sum(Bin_Number(T(i):end).*Histogram_count(T(i):end))/Cumulative_Sum_3(end);
i=i+1;
T(i)=round((MAT+MBT)/2);
while abs(T(i)-T(i-1))>=1

```

```

Cumulative_Sum_2= cumsum(Histogram_count(1:T(i)));
MBT=sum(Bin_Number(1:T(i)).*Histogram_count(1:T(i)))/Cumulative_Sum_2(end);

```

```

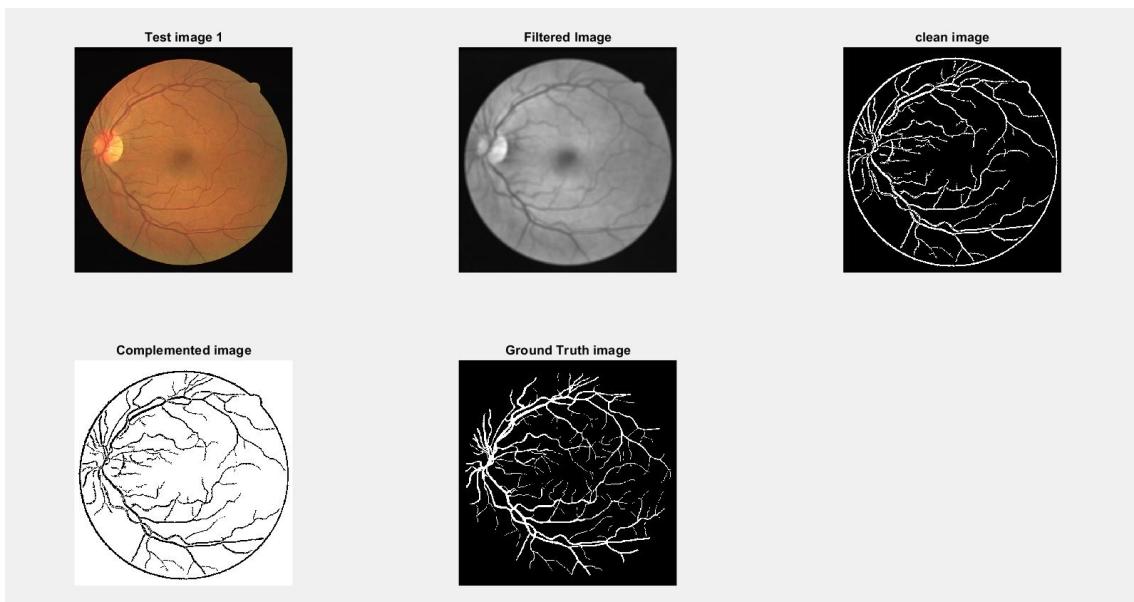
Cumulative_Sum_3= cumsum(Histogram_count(T(i):end));
MAT=sum(Bin_Number(T(i):end).*Histogram_count(T(i):end))/Cumulative_Sum_3(end);

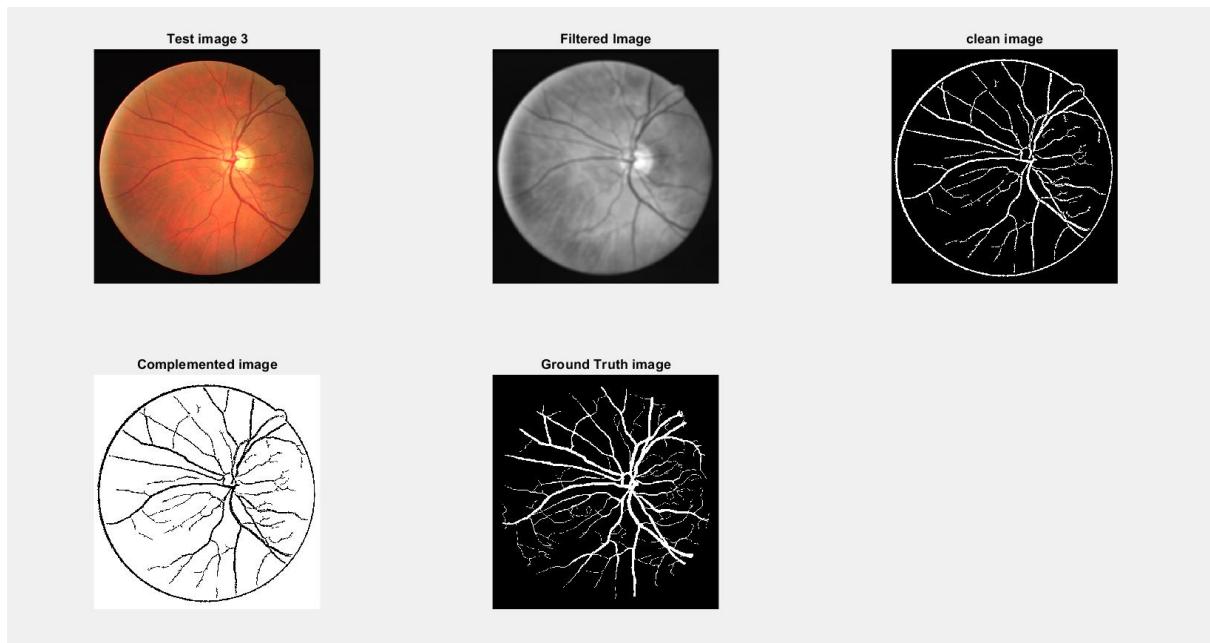
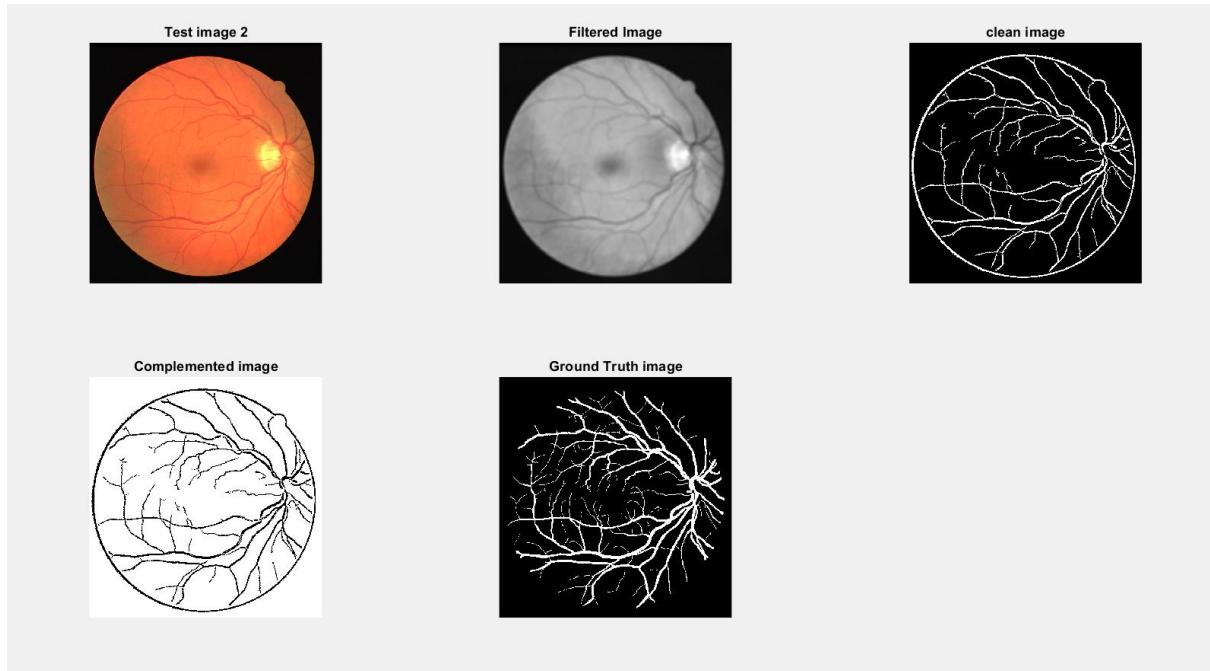
```

```

i=i+1;
T(i)=round((MAT+MBT)/2);
Threshold = T(i);
end
level=(Threshold-1)/(Bin_Number(end)-1);
end

```





## **RESULTS AND SCORES**

<b>Images</b>	<b>Dice coefficient</b>	<b>Jaccard index</b>
Test Image 1	0.6555	0.4876
Test Image 2	0.6392	0.4698
Test Image 3	0.6191	0.4484

## **CONCLUSION**

Retinal vessel segmentation is performed on fundus images by setting appropriate parameters and sequences of filtering and morphological process using MATLAB code. It is observed that these vessels can be accurately segmented from fundus images and is validated by measures such as Dice and Jaccard scores by comparing it with the ground truth mask.

**AM5023- PHYSIOLOGICAL MEASUREMENTS AND  
INSTRUMENTATION LABORATORY**

**HUMAN IN LOOP CYBER PHYSICAL SYSTEMS -  
LABORATORY REPORT**

Submitted by: DINESH KUMAR M

Registration no: AM23M022



**DEPARTMENT OF APPLIED MECHANICS &  
BIOMEDICAL ENGINEERING**

**INDIAN INSTITUTE OF TECHNOLOGY, MADRAS**

# **USE OF IMU SENSORS TO CLASSIFY BOXING PUNCH**

## **AIM**

Automatic classification of boxing punches to help athletes improving their punch performance.

## **OBJECTIVE**

- To record the IMU signal and use machine learning model to classify boxing punch

## **APPARATUS REQUIRED**

- MMRL – METAMOTIONRL IMU Sensor
- Metabase mobile application (Data acquisition)
- Computer system with programming language
- Velcro wrist strap
- Sampling Frequency=200Hz

## **THEORY**

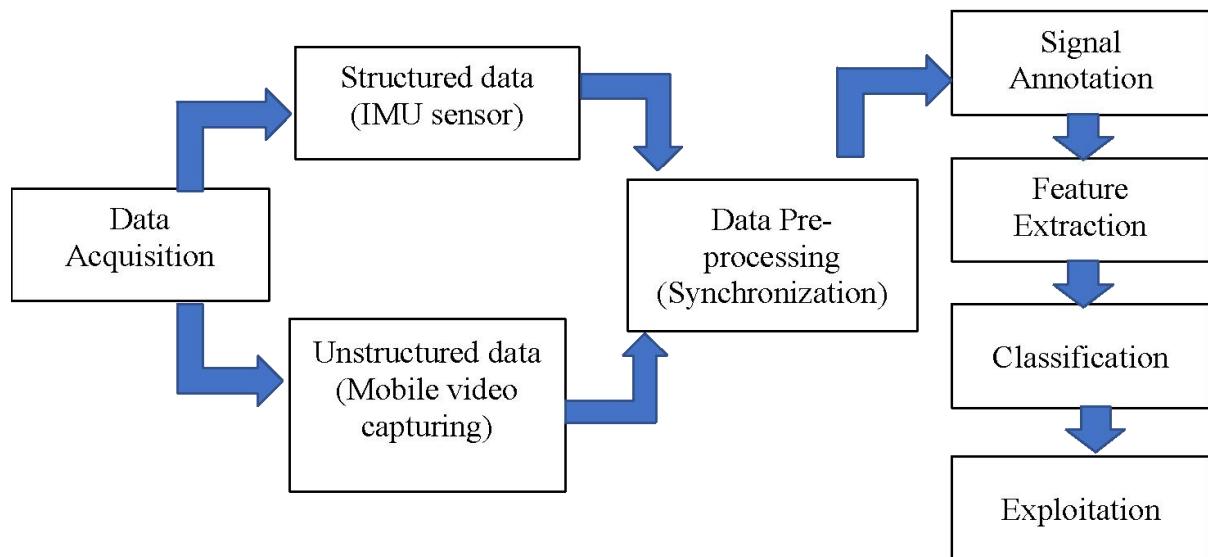
Boxing is a physically demanding combat sport. Boxers rely on a combination of strength, coordination, speed, and stamina to succeed in impacting the opponent while evading an adversary's punches. A successful performance requires the ability to deliver precise punches above the belt, to the head or the torso, without being punched back. There are four main types attacking techniques using punches: Jab, cross, hook and uppercut. From punch classification, we get a lot of useful information about their effectiveness, repeatability, strength and individual profile.

Wearable inertial sensors are fast becoming a validated technology to provide data for athlete performance analysis in a range of sports. The advancement in technology resulted in relatively low-cost wearable, unobtrusive inertial sensors and global positioning system (GPS) units and are more readily available for coaching teams. Machine learning and artificial intelligence (AI) techniques are then used to process output data from these sensors. Such techniques consist of classification models to predict boxer's performance. An abundance of scientific literature and commercialized technology have used signals obtained by wearable inertial sensors to identify activity type and intensity in sport and general living.

The monitoring of acceleration or magnitude of sporting motions is often realized by wearing inertial measurement units (IMUs) on the wrist/hand and shank/foot. As a faster, more reliable and cost-efficient strategy for activity and motion analysis, wearable IMUs benefit a lot from the significant reduction in sensor volume and price and are playing a more and more important role in the

field of sports analytics. Commonly, an IMU consists of an accelerometer for measuring linear acceleration, a gyroscope for angular acceleration and sometimes a magnetometer for a magnetic field. Moreover, three-dimensional sensors are favorable due to their ability to capture parameters along the three axes and provide detailed and useful component data for orientation and kinematics studies. The combination of multifarious sensors ensures the system robustness and accuracy of captured data and then improves the validity and reliability of activity detection and analysis.

## FLOW CHART



## METHOD

- Wrap the IMU sensor on the wrist to acquire data.
- Integrating both IMU sensor and Metabase mobile application through Bluetooth connectivity.
- Configure the IMU sensor (200 Hz sampling frequency,  $\pm 16$  g accelerometer,  $\pm 2000$  deg/s gyroscope)
- Record the signals like accelerometer (x,y,z axis) and gyroscope(x,y,z axis). Simultaneously record the video through mobile camera.
- Wireless transmission of csv file from IMU sensor to PC for processing.
- Synchronized both acquired signal and captured video.
- Extracting temporal or spectral or spectrotemporal features.
- Classify the punches using machine learning model.

## RESULTS

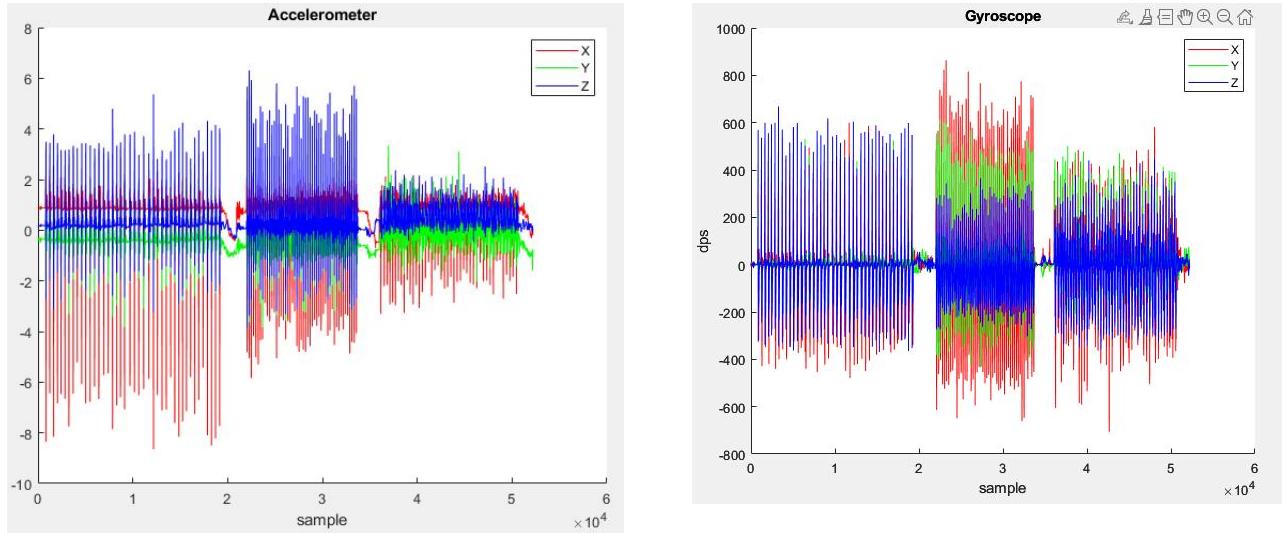


Fig 1: Jab punch signal

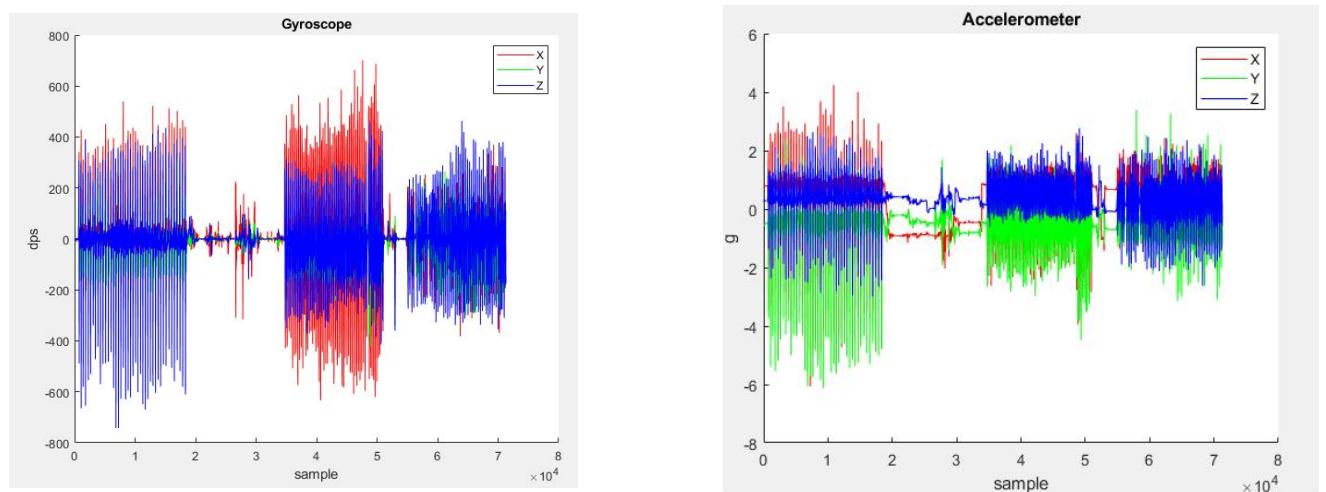


Fig 2: Cross punch signal

## MACHINE LEARNING MODEL: SVM KERNEL

Class 0 = No punch

Class 1 = Jab

Class 2 = Hook

### Learning Model:

```
In [1]: import pandas as pd
import numpy as np
from sklearn.svm import SVC
from sklearn.metrics import classification_report, confusion_matrix
import matplotlib.pyplot as plt
get_ipython().run_line_magic('matplotlib', 'inline')

In [2]: irisdata = pd.read_csv('F:\\IITM\\Physiological measurements lab\\boxing imu\\feattimepyth.csv')

In [3]: from sklearn.model_selection import train_test_split
x = irisdata.drop('class', axis=1)
y = irisdata['class']
x_train, x_test, y_train, y_test = train_test_split(x, y, test_size = 0.20)

In [4]: kernels = ['Polynomial', 'RBF', 'Sigmoid','Linear']#A function which returns the corresponding SVC model
def getclassifier(ktype):
    if ktype == 0:
        # Polynomial kernel
        return SVC(kernel='poly', degree=8, gamma="auto")
    elif ktype == 1:
        # Radial Basis Function kernel
        return SVC(kernel='rbf', gamma="auto")
    elif ktype == 2:
        # Sigmoid kernel
        return SVC(kernel='sigmoid', gamma="auto")
    elif ktype == 3:
        # Linear kernel
        return SVC(kernel='linear', gamma="auto")

for i in range(4):
    # Separate data into test and training sets
    x_train, X_test, y_train, y_test = train_test_split(x, y, test_size = 0.20)# Train a SVC model using different kernel
    svclassifier = getclassifier(i)
    svclassifier.fit(x_train, y_train)# Make prediction
    y_pred = svclassifier.predict(X_test)# Evaluate our model
    print("Evaluation:", kernels[i], "kernel")
    print(classification_report(y_test,y_pred))
```

Evaluation: Polynomial kernel				
	precision	recall	f1-score	support
0	1.00	0.97	0.98	30
1	1.00	1.00	1.00	23
2	0.97	1.00	0.98	32
accuracy			0.99	85
macro avg	0.99	0.99	0.99	85
weighted avg	0.99	0.99	0.99	85

Evaluation: RBF kernel				
	precision	recall	f1-score	support
0	0.00	0.00	0.00	22
1	0.00	0.00	0.00	32
2	0.36	1.00	0.53	31
accuracy			0.36	85
macro avg	0.12	0.33	0.18	85
weighted avg	0.13	0.36	0.19	85

Evaluation: Sigmoid kernel				
	precision	recall	f1-score	support
0	0.00	0.00	0.00	24
1	0.00	0.00	0.00	28
2	0.39	1.00	0.56	33
accuracy			0.39	85
macro avg	0.13	0.33	0.19	85
weighted avg	0.15	0.39	0.22	85

Evaluation: Linear kernel				
	precision	recall	f1-score	support
0	0.94	0.97	0.95	31
1	1.00	1.00	1.00	18
2	0.97	0.94	0.96	36
accuracy			0.96	85
macro avg	0.97	0.97	0.97	85
weighted avg	0.97	0.96	0.96	85

```
In [5]: from sklearn.model_selection import GridSearchCV

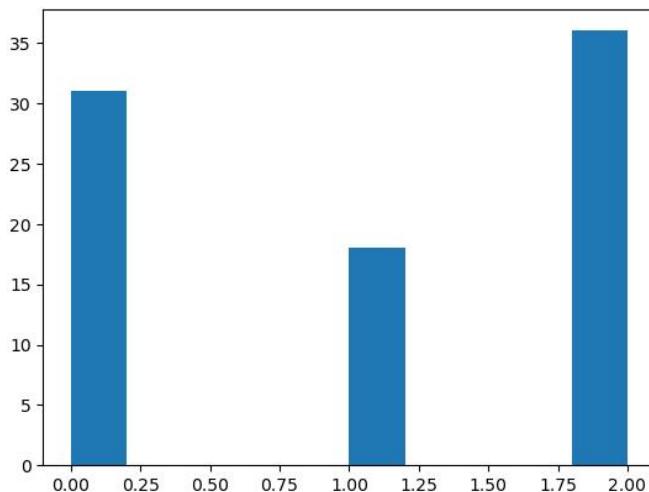
param_grid = {'C': [0.1, 1, 10, 100], 'gamma': [1, 0.1, 0.01, 0.001], 'kernel': ['rbf', 'poly', 'sigmoid']}
grid = GridSearchCV(SVC(), param_grid, refit=True, verbose=2)
grid.fit(X_train, y_train)

print(grid.best_estimator_)

grid_predictions = grid.predict(X_test)
print(confusion_matrix(y_test, grid_predictions))
print(classification_report(y_test, grid_predictions))

plt.hist(grid_predictions)
plt.show()

SVC(C=0.1, gamma=1, kernel='poly')
[[30  0  1]
 [ 0 18  0]
 [ 1  0 35]]
      precision    recall   f1-score   support
          0       0.97     0.97     0.97      31
          1       1.00     1.00     1.00      18
          2       0.97     0.97     0.97      36
      accuracy                           0.98      85
     macro avg       0.98     0.98     0.98      85
 weighted avg       0.98     0.98     0.98      85
```



**Figure: Histogram**

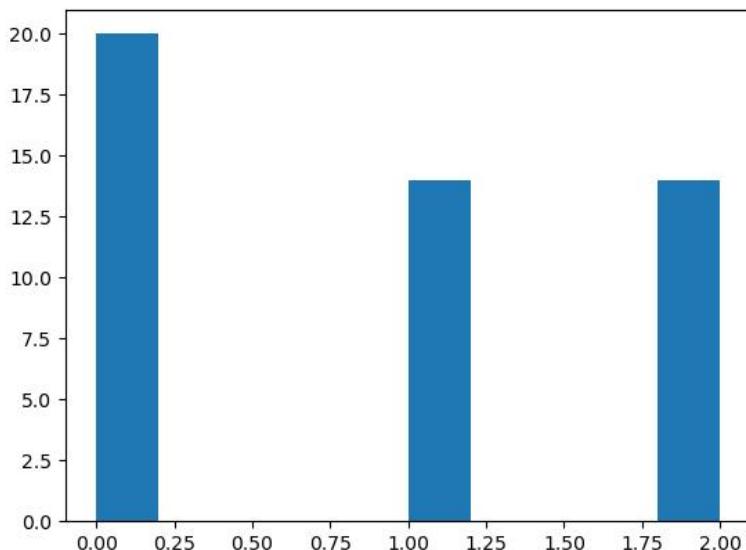
```
In [6]: xx = pd.read_csv("F:\\IITM\\Physiological measurements lab\\boxing imu\\Testingtimepyth.csv")
yy = pd.read_csv("F:\\IITM\\Physiological measurements lab\\boxing imu\\Testing outputpyth.csv")
grid_predictions1 = grid.predict(xx)
print(confusion_matrix(yy,grid_predictions1))
print(classification_report(yy,grid_predictions1))
print(grid_predictions1)
print(yy)

plt.hist(grid_predictions1)
plt.show()

xx1 = pd.read_csv("F:\\IITM\\Physiological measurements lab\\boxing imu\\Testingtimepyth.csv")
yy1 = pd.read_csv("F:\\IITM\\Physiological measurements lab\\boxing imu\\Testing outputpyth.csv")
grid_predictions2 = grid.predict(xx1)
print(confusion_matrix(yy1,grid_predictions2))
print(classification_report(yy1,grid_predictions2))
print(grid_predictions2)

[[18  2  4]
 [ 0 12  0]
 [ 2  0 10]]
      precision    recall   f1-score   support
          0       0.90      0.75      0.82      24
          1       0.86      1.00      0.92      12
          2       0.71      0.83      0.77      12

   accuracy                           0.83      48
  macro avg       0.82      0.86      0.84      48
weighted avg       0.84      0.83      0.83      48
```



**Figure: Histogram**

## **HYPERPARAMETERS**

Kernel = ‘poly’

C = 0.1

Gamma = 1

## **CONCLUSION**

The results indicate that the machine learning models can classify boxer punches with a high degree of accuracy. Therefore, automatic punch classification can be used to evaluate and automatically label complex pad-work combinations executed in training, bypassing cumbersome notional analysis and video labelling. There is potential for the punch types to be paired with other biofeedback metrics such as punch impact acceleration, velocity, approach angle, torso angular velocity, and rotation angle, calories burnt and fatigue level.