Non-Invasive Hemoglobin Measurement Technique

A Project Report

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THESIS CERTIFICATE

This is to certify that the thesis titled **Non-Invasive Hemoglobin Measurement Technique**, submitted by **Shashi Kumar**, to the Indian Institute of Technology, Madras, for the award of the degree of **MASTER OF TECHNOLOGY**, is a bona fide record of the research work done by him under our supervision. The contents of this thesis, in full or in parts, have not been submitted to any other Institute or University for the award of any degree or diploma.

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ABSTRACT

KEYWORDS: Blood Test ; Hemoglobin; PPG;Non Invasive. Non

Invasive medical diagnosis is rapidly gaining popularity as its not painful and dangerous. This project mainly presented the principle and prototyping the non invasive hemoglobin measurement technique. For this PPG corresponding to two wavelengths (650nm and 850nm) are obtained and analyzed for hemoglobin measurement. The prototype included a finger lobe fitted with transmission type photodetector and two SMD leds to illuminate finger tip. A PCB is designed to acquire photocurrent and filter the raw signal to finally get desired PPG on LabView.

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ABBREVIATIONS

PPG	Photoplethysmogram
PCB	Printed Circuit Board
RBC	Red Blood Cells
WBC	White Blood Cells
IR	Infra Red
LED	Light Emitting Diode
SMD	Surface Mount Devices

CHAPTER 1

Introduction

1.1 Blood and its content

Blood is essential fluid which is responsible for transportation of nutrients and oxygen to the various body cells and helps us get rid of wastes like carbon dioxide, ammonia and other wastes from cells. Blood is vital in maintaining body temperature by acting as heat sink. Blood accounts for $7 - 8\%$ of a healthy body weight of human. Blood consists of **plasma** which is blood fluid and accounts for 55% of blood. Rest 45% of blood is composed of different kinds of blood cells and platelets. The three kinds of blood cells are RBC($> 44\%$) and WBC and Platelets (nearly 1%).

Plasma are yellow coloured fluid which is made up of mostly water (92% of plasma). Plasma contains waste and dissipated proteins, carbon dioxide, harmones, minerals ions, glucose etc. Plasma is main medium through which these wastes are excreted to excretory organs.All the cells of the blood i.e RBC, WBC and platelets are suspended in plasma. Plasma offers no attentuation to wavelenghts above visible lights. This characteristic of plasma is of great significance to us and we will discuss this more in further chapters.

Blood consists nearly 45% of RBC , Red Blood Cells or erythrocytes. Stem cells present in bone marrow produces red blood cells. RBC carries oxygenated blood from lungs to cells and carries CO_2 to lungs to excrete out of body. RBC consists of haemoglobin which is protein molecule that makes up 95% of a RBC. Every RBC has about 270 millions of iron rich hemoglobin molecules.Oxygenated RBCs provide red colour to the blood.Haemoglobin content in RBC defines the oxygen carrying capacity of blood. WBCs and platelets are also very important constituents of blood. WBC have primary responsiblity to defend our immune system. They help in removal of any bacteria,viruses and fungi by binding to the proteins present in these harmful foreign microorganisms. WBCs helps in getting rid of foreign matter such as dust, asbestos and dead

cells in body. Platelets have primary function to clot blood around wounds by coagulating chemicals which cause clots to form in the blood and thereby stops blood loss.

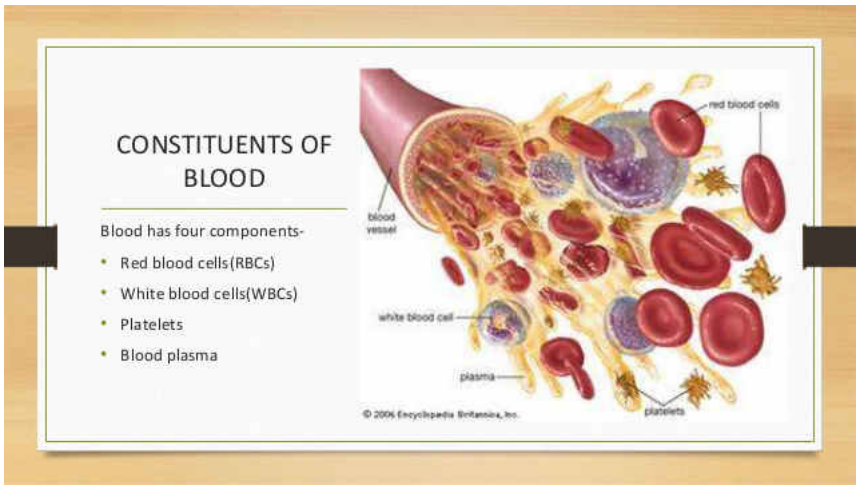

Figure 1.1: Consituents of Blood; source:Encyclopedia Britannica Inc.

1.1.1 Importance of Haemoglobin

Importance of blood in medical condition and disease diagonosis is quite known to everyone. Scientists and engineers around the world are using cutting edge technology to make blood tests simpler , quicker and more accurate. Blood test is a complex procedure which requires specialised equipments and technicians. Even before blood is tested , it is spun very fast to separate the blood cells from the plasma(fluid part) of blood,creating a serum or plasma sample. Shape and size of blood cells are then identified by using sophisticated devices and chemical reactions are done to know the concentration of certain molecules like haemoglobin which is extemely important in function of blood and health of individual and therefor it is of great importance in medical and disease diagonosis. So blood tests are a vital part of the diagnostic process, helping physicians and doctors make the correct diagnosis and determine the appropriate course of treatment.

Whenever a physician or doctor sugests a blood tests , measurement of haemoglobin level in blood is of utmost importance. A haemoglobin diagonistic test measures the amount of haemoglobin in the blood. Haemoglobin is protein present in the blood which carries oxygen from the respiratory organs to all the parts of the body tissues and cells. Haemoglobin protein releases the oxygen in cells and tissues and allow aer-

obic respiration(respiration in presence of oxygen) to provide energy and power up the functions of cells and tissues for various metabolic activities. Haemoglobin also carries back CO_2 back from organs and tissues back to lungs. Iron is one of the key component for the manufacture of protein haemoglobin. But only small amount of iron is required by bone marrow for producing RBCs and haemoglobin.

Low blood cell count in blood test or if hemoglobin measuement test reveals that hemoglobin level in blood is lower than normal, then the medical condition is called anemia. That means oxygen carrying capacity of blood is lower than desired. If bone marrow which produces red blood cells is not able to produce RBCs fastenough or if there is increase in reduction of RBCs due to bleeding or other medical conditions, then the overall number of RBCs and hemoglobin will drop, resulting in anemia. Higher level of haemoglobin in test reveals and indicates towards the blood disorder polycythemia vera. Smoking, dehydration and living in high altitude can also influence to higher haemoglobin content in blood. In higher altitute blood dissolves more oxygen molecules. Oxygen is the most essential element required for living humans beings. Brain damage, organ failure and death can result if an adequate supply of oxygen is not circulated throughout the body to vital organs and tissues. Some conditions affect RBC production in the bone marrow and may cause an increase or decrease in the number of mature RBCs released into the blood circulation. Other conditions may affect the lifespan of RBCs in the circulation.

Normal hemoglobin levels differs between males and females, ranges from:
12-16 g/dL for women and
13-18 g/dL for men

1.2 Techniques of Hb measuremt

Haemoglobin is conventionally measured using invasive method where blood is required to taken from body as sample for blood test. Needles are used for extraction of blood from patients which is usually painful and unwelcomed . Moreover, use of needle increases the chances of infection. There are many biochemical methods and techniques which require sample of blood and therefore are invasive methods of haemoblobin mea-

surement. Some of the invasive techniques are discussed in next section.

1.2.1 Invasive Techniques

1. Haematocrit method for measuring hemoglobin
2. Hemoglobin measurement through spectrophtometer

Haematocrit method

The hematocrit is measurement of volume of red blood cells compared to the total volume of blood. This method is called as packed cell volume method or spun crit. In this invasive method a small amount of blood is taken from patient (about .005 to 0.1ml) and is placed in a thin capilllary tube , the tube is sealed with wax or clay and then is placed in a centrifuge at 10,000 rpm for about 5 minutes so as to separate the blood into its major components, namely, plasma, red blood cells and the rest. The red cells collected at the bottom form a red column and this layer is separated by a straw coloured serum coloumn in a small area composed of WBCs. Measurement of height of the total blood in the capillary tube which includes red cells, white cells, and serum makes it 100%. The height of the red cell column divided by the height of the total fluid in the capillary tube equals the hematocrit (percentage of RBC's in the total blood volume).

Limitations of this method

Both the hemoglobin and the hematocrit are based on volume of blood and consequently dependent on volume of plasma. If a patient is severely dehydrated, the hemoglobin and hematocrit will appear higher than if the patient were normovolemic; if the patient is fluid overloaded, they will be lower than their actual level. Therefore this is not very efficient method of measurement of haemoglobin and is also invasive.

Spectrophotometer based measurements

Spectrophotometry is method of measurement of absorption of light by measuring the attenuation or change in its intensity, as beam of light passes though sample solution. The basic principle is that each compound absorbs or transmits light over a certain range

of wavelength. This technique can be used to find the absorption of a solute in a solvent. In this method a solute whose absorption is to be measured is dissolved in solvent and then a monochromatic light is passed through solution kept in a cuvette as shown in the figure 1.2. Light of certain wavelength and intensity is passed through cuvette and then its attenuation is measured by applying the Beer Lambert's Law. The output light gets attenuated and its intensity is given by :

$$I_{o\lambda} = I_{i\lambda} e^{\varepsilon_\lambda cl} \tag{1.1}$$

$I_{o\lambda}$ is intensity of output light after absorption by solute
$I_{i\lambda}$ is intensity of incident light
λ is wavelength of light incident
ε_λ is the extinction coefficient of the solute at the wavelength λ
c is concentration of solute
l is path length of light

Limitaton of this method is that we have to assume that light is not attenuated by solvent. Also we need to know the extinction coefficient of the solute at the wavelength λ . While measuring hemoglobin content a modified method of spectrophotometry is used . First we should have a standard sample whose concentration c_s is known and is tested with spectrohotometer and its absorption is determined as :

$$A_s = ln\frac{I_{o\lambda}}{I_{i\lambda}}|_{standardsolution} = \varepsilon_\lambda c_s l \tag{1.2}$$

Then the absorbance of the unknown specimen possessing a concentration c_x is determined using an identical cuvette.

$$A_x = ln\frac{I_{o\lambda}}{I_{i\lambda}}|_{unknownsolution} = -\varepsilon_\lambda c_x l \tag{1.3}$$

Dividing equation 1.2 by equation 1.3, we get:

$$\frac{A_s}{A_x} = \frac{\varepsilon_\lambda c_s l}{\varepsilon_\lambda c_x l} = \frac{c_s}{c_x} \tag{1.4}$$

$$c_x = \frac{A_s}{A_x} c_s l \tag{1.5}$$

Thus using the comparison method and a standard specimen, the concentration of unknown specimen can be easily determined using equation 1.5 without the knowledge on either extinction coefficient or path length.

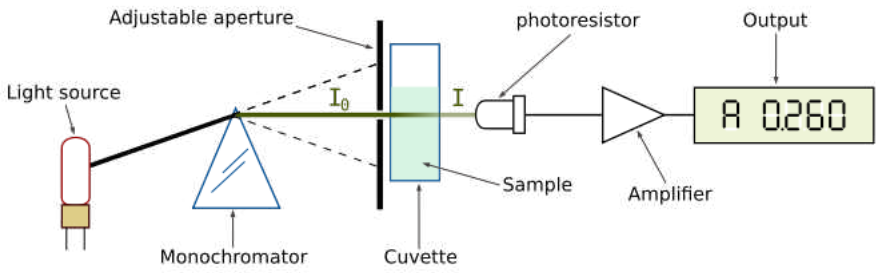

Figure 1.2: Figure depicting Spectrophotometer Principle ;image source :wikipedia

Hemiglobin cyanide (HiCN) method

The principle of the cyanmethemoglobin method is conversion of hemoglobin to cyanmethemoglobin by the addition of potassium cyanide and ferricyanide whose absorbance is measured at 540 nm in a photoelectric calorimeter against a standard solution.

In the haemiglobincyanide method, first the blood sample is processed so that the cell membrane of all the red blood cells ruptures and the haemoglobin molecules are released. The sample with the freed haemoglobin molecules then goes through a lysing process in which a lysing agent is mixed with the sample. The lysing agent extracts the haemoglobin molecules from the ruptured RBCs. After the extraction of haemoglobin molecules, a reagent known as the Drabkin's reagent is used. Iron, sodium bicarbonate, potassium cyanide constitute the Drabkin's reagent. This reagent chemically converts the haemoglobin molecule to haemiglobin cyanide (HiCN) molecules. The absorbance of the processed sample containing the HiCN is then determined using comparison

method of spectrophotometry. In India approximately 70% of laboratories still use direct cyanmethemoglobin method (HiCN)for hemoglobin estimation especially in rural areas.

Limitations There is a need to have non cyanide methods to avoid environmental pollution by cyanide reagents. A study was conducted in a tertiary care hospital in Mumbai which compared cyanide method (HiCN) with non-cyanide methods (alkaline hematin method and alkaline borate method). The results showed excellent correlation among the three methods. The cyanide free methods also have advantages from safety standpoint as well as cost when compared to the standard HiCN method . Another disadvantage of HiCN method is presence of turbidity which results in an inaccurate estimate when absorbance is measured at single wavelength of 540nm. Hemocue hemoglobin photometer now widely used compensates for this. The system consists of disposable microcuvettes containing dry reagents and a photometer. Blood is placed in the microcuvette which reacts with sodium deoxycholate, releasing hemoglobin by hemolysing erythrocytes. Then sodium nitrite converts hemoglobin to methemoglobin which, together with sodium azide, gives azidemethemoglobin. The absorbance is measured at two wavelengths (565nm and 880nm) in order to compensate for turbidity.

1.2.2 Non-Invasive Techniques

Non invasive techniques to measure hemoglobin concentration was first presented by Massimo in 2000's and technique and accuracy has been constantly improved in recent years. The principle involved in the non invasive hemoglobin measurement is presented in this thesis.

1.3 Objective

The objective of the work presented in this thesis is to detail the basics of technique involved in non invasive hemoglobin measurement. For this we obtain (Photoplethysmography)PPG, which is the key process in determining the hemoglobin concentration in blood.PPG is non-invasive measurement of signal proportional to change in volume of blood in one heart beat cycle. PPGs are obtained by illuminating light on a part of the

body and detecting the amount of absorbtion of light by blood through photodetector. The photodetector can be reflective type or transmissive type. The PPG obtained using reflective type photodiode is called reflectance PPG and one which detects the transmitted light through body is called transmission PPG. In the work presented in the thesis we have used transmission type PPG because it is difficult to use reflective type photodetector due to low depth penetration of light inside body. To obtain PPG we are illuminating light on the finger and obtained the PPG on LabView. We used DAQ 6008 to acquire signal from photodetector after filtering out low DC and slow varying frequency components on LabView. We have used red and infrared led fixed on appropriate lobe for illuminating finger tip and used photodetector on other side for photodetector to detect transmitted light passed through finger. Most of the photons (nearly 90%) detected by detector would have passed through epidermis, dermis, soft bones and these photons accounts for the DC component obtained in the PPG. Approximately 9.9% of the light passes blood and accounts for the slow varying frequecy component in PPG. This slow varying component in PPG is not useful for non-invasive hemoglobin measurement. Only the 0.1% of the photons detected by photodetector contribute to the pulsatile part of the PPG and this component of PPG would have passed through arterial blood and they possed characteristics of arterial blood flow and frequecy components dictated by the heart rate . PPGs are very important for many biological medical diagnosis as informations like heart rate and its variations can be observed from PPG. Respiratory rate, blood pressure ,and oxygen saturation are some other vital informations that can be extracted from PPG signal. As it would be shown in this thesis information on concentration of hemoglobin can also be derived from the PPG signal.

1.4 Organisation of thesis

The first chapter gives an introduction to the blood and its constituents and discusses the importance of hemoglobin measurement. The second chapter describes mathematical model which can be used to measure concentration of hemoglobin after extraction of data from PPG. Third chapter describes various units for construction of system to obtain PPG ,starting from sensor head to DAQ for acquiring signal from circuit. Fourth and last chapter details the results obatined during project and conclusion of the project.

CHAPTER 2

PPG and haemoglobin measurement

2.1 Various layers inside finger tip

As illustrated in figure 2.1 ,we have mostly six major layers inside finger which causes attenuation to monochromatic light passed through finger tip. These layers are epidermis, dermis, various tissues, softbones, veins and arteries. A PPG is variation of high frquecy part of output signal obtained when a monochromatic light of particular wavelength is illuminated on the body. A photo-detector is used to detect the output current signal which is equivalently converted to voltage by either transconductance amplifier or LOG amplifier as used in our case. This signal is then passed through appropriate filter which gives the high pulsatile portion of signal called PPG. The intensity of the light that has passed through arterial blood would posses pulsatile nature due to blood volume changes caused becuase of the pumping of the heart. During systole there is an increase in the blood volume which reduces the amount of photons falling on phtoetector. During diastole there is reduction in the volume of the arterial blood and this increases the amount of light reaching the phtodetector. This is the basic biological process involved when light is illuminated on finger tip to obtain the PPG.

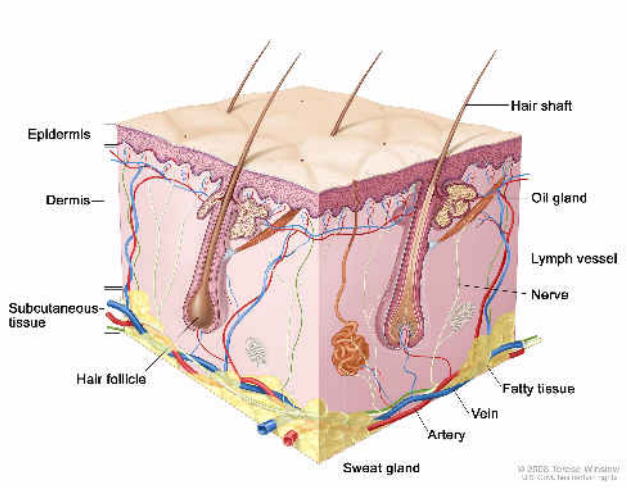

Figure 2.1: Various biological layers inside finger tip

2.2 Components of PPG

A photoplethysmogram (PPG) is an optically obtained plethysmogram, which is a volumetric measurement of an organ. A PPG is often obtained by using a pulse oximeter which illuminates the skin and measures changes in light absorption. Skin is normally so richly perfused that it is relatively easy to detect the pulsatile component of the cardiac cycle. The DC component of the signal can be accounted on the bulk absorption of the skin tissue, while the AC component is due to variation in blood volume in the skin caused by the pressure pulse of the cardiac cycle.

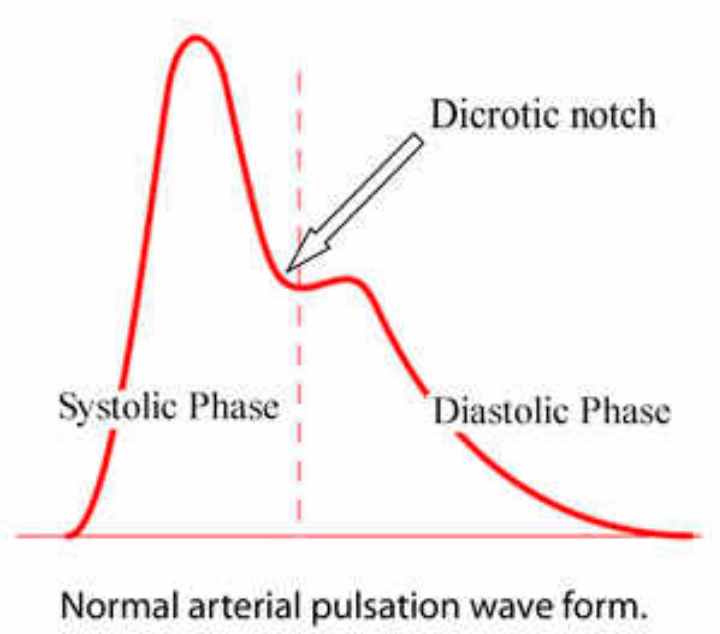

Figure 2.2: A typical PPG

The height of AC component of the photoplethysmogram is proportional to the pulse pressure, the difference between the systolic and diastolic pressure in the arteries. A typical PPG waveform is shown in figure2.2. Different wavelength of light show different attenuation characteristics when light is passed through finger. Also when light passes through the finger from one side, light is absorbed by epidermis, dermis, soft bones, veins and artery and each of these show different attenuation characteristic

Attenuation by various layers of finger can be summarized in following equation

$$\nu_{o\lambda} = K_{S\lambda} I_{\lambda} e^{[\varepsilon_{E\lambda} C_E + \varepsilon_{D\lambda} C_D + \varepsilon_{T\lambda} C_T + \varepsilon_{B_o\lambda} C_{B_o} + \varepsilon_{V_b\lambda} C_{V_b} + \varepsilon_{A_b\lambda} C_{A_b}] T_F} \tag{2.1}$$

Large portion of signal obatined has DC component caused due to attenuation by epidermis, dermis and soft bones $\nu_{o\lambda}|_{DC}$. A small portion of PPG varies very slowly due to blood flowing in veins $\nu_{o\lambda}|_{slowvarying}$. Most important part of PPG is highy pulsatile part which is consitute of higher frequecy component of PPG $\nu_{o\lambda}|_{pulsatile}$.

$$\nu_{o\lambda}|_{DC} = K_{S\lambda} I_\lambda e^{[\varepsilon_{E\lambda} C_E + \varepsilon_{D\lambda} C_D + \varepsilon_{T\lambda} C_T] T_F} \tag{2.2}$$

$$\nu_{o\lambda}|_{slowvarying} = K_{S\lambda} I_\lambda e^{[\varepsilon_{V_b\lambda} C_{V_b}] T_F} \tag{2.3}$$

$$\nu_{o\lambda}|_{highlypulsatile} = K_{S\lambda} I_\lambda e^{[\varepsilon_{A_b\lambda} C_{A_b}] T_F} \tag{2.4}$$

2.3 Attenuattion characteristics of various wavelengths

Blood being very essential for life, it has both very special fluidic and optical properties. Apart from plasma, blood contains various scattering bodies, mainly red blood cells (RBCs) significantly influence the behavior of light scattering. Blood have different components which have different characteristic with different wavelength of light. As we know blood has mainly plasma and RBC in the following section we will discuss the attenuation characteristic of different wavelength of light when passed through these two major components of blood.

On this basis, new procedures and sensors are being developed for optical and noninvasive determination of several blood parameters. Our area of interest is determination of hemoglobin content in blood through noninvasive technique. For this we are going to exploit the optical properties of blood to different wavelength of light. Plasma which is a clear liquid and is main constituent of blood shows attenuation characteristic (absorbtion by plasma) as shown in 2.3. As shown in figure plasma has high absorbtion coefficient for lights of low wavelength (lower than 600nm) but has very small absorption for lights of higher wavelength (above 600nm). To study about hemoglobin and

blood cells, it is essential to exploit this absorption characteristic that plamsa shows no absorption espcially in the visible and near infrared regions.

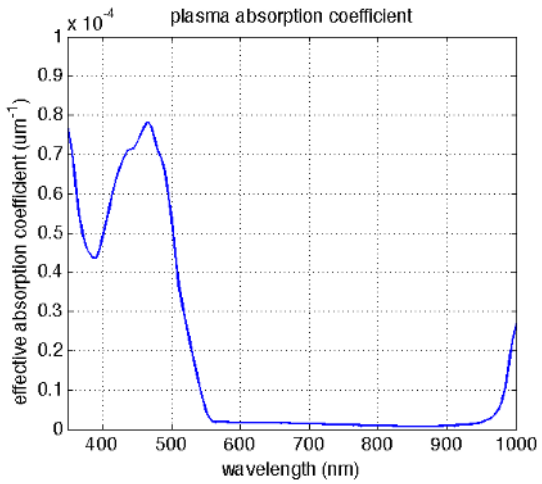

Figure 2.3: Absorption by plasma present in blood v/s wavelength of light

Oxygenated blood shows different absorption characteristic than plasma, shown in the figure 2.4 and de-oxygenated blood shows absorption characteristics as shown in figure 2.5. So we can clearly analyze that we should use lights in the range of wavelength (600-950) for studying the attenuation of light passed through blood for calculation of hemoglobin content in blood. Also at this wavelength there is clear contrast in the attenuation between oxygenated and deoxygenated hemoglobin. At 660nm attenuation by Hb is large compared to HbO and at 850nm attenuation by HbO is larger compared to Hb.

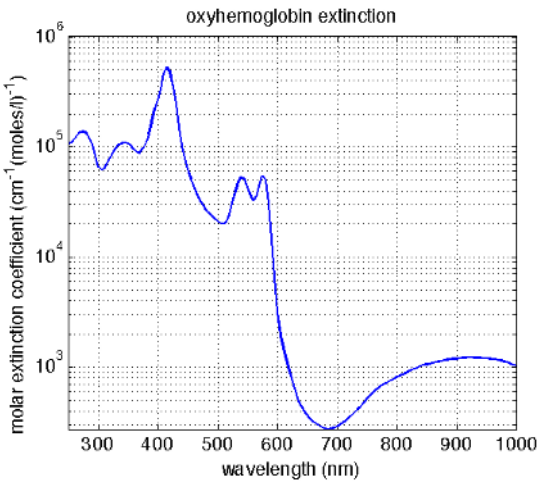

Figure 2.4: Absorption by Oxygenated hemoglobin v/s wavelength of light

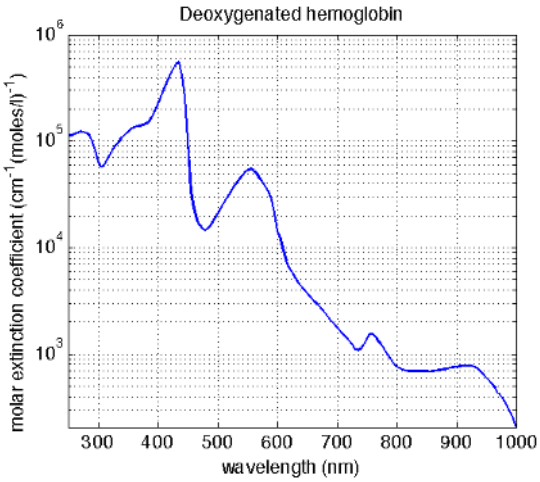

Figure 2.5: Absorption by de-oxygenated hemoglobin v/s wavelength of light

2.3.1 Mathematical model used for hemoglobin measurement

The photo detector current is converted to voltage which is mathematically modelled as :

$$\nu_{o\lambda} = K_{S\lambda} I_\lambda e^{-[\varepsilon_{E\lambda} C_E + \varepsilon_{D\lambda} C_D + \varepsilon_{T\lambda} C_T + \varepsilon_{B_o\lambda} C_{B_o} + \varepsilon_{V_b\lambda} C_{V_b} + \varepsilon_{A_b\lambda} C_{A_b}] T_F} \tag{2.5}$$

$\nu_{o\lambda}$ is voltage output of log amplifier

$K_{S\lambda}$ is sensitivity of photodetector

I_λ is the photocurrent generated by photodiode

C_E, C_D, C_T, C_{B_o},C_{V_b}, C_{A_b} are the cell concentrations of epidermis, dermis, tissues, softbones, venous bloodand arterial bloodrespectively

T_F is path length of light inside the finger

$\varepsilon_{E\lambda}$, $\varepsilon_{D\lambda}$, $\varepsilon_{T\lambda}$, $\varepsilon_{B_o\lambda}$,$\varepsilon_{V_b\lambda}$, $\varepsilon_{A_b\lambda}$ are the extinction coefficients

Taking natural logarithm to the above equation which models unfiltered voltage signal from detector

$$ln(\nu_{o\lambda}) = ln(K_{S\lambda} I_\lambda) - (\varepsilon_{E\lambda} C_E + \varepsilon_{D\lambda} C_D + \varepsilon_{T\lambda} C_T + \varepsilon_{B_o\lambda} C_{B_o}) T_F - (\varepsilon_{V_b\lambda} C_{V_b}) T_F - (\varepsilon_{A_b\lambda} C_{A_b}) T_F (2.6$$

As it is already discussed $(\varepsilon_{E\lambda} C_E + \varepsilon_{D\lambda} C_D + \varepsilon_{T\lambda} C_T + \varepsilon_{B_o\lambda} C_{B_o}) T_F$ this component is DC and is undesired and is to be removed. $(\varepsilon_{V_b\lambda} C_{V_b}) T_F$ is also undesired part

and is also supressed through appropriate filter. The desired component which gives PPG is $(\varepsilon_{A_b\lambda}C_{A_b})T_F$. We are using two wavelenths RED and IR to claculate the concentration of hemoglobin. So we will extract only the pulsatile part of the detector after applying natural logarithm to $ln(\nu_{o\lambda})$

$$ln(\nu_{o\lambda}) = (\varepsilon_{A_b\lambda}C_{A_b})T_F \tag{2.7}$$

The RED and IR PPGs obtained are as follows

$$ln(\nu_R) = (\varepsilon_{HbR}C_{Hb} + \varepsilon_{HbOR}C_{HbO})T_F \tag{2.8}$$

$$ln(\nu_{IR}) = (\varepsilon_{HbIR}C_{Hb} + \varepsilon_{HbOIR}C_{HbO})T_F \tag{2.9}$$

Here $ln(\nu_R)$ and $ln(\nu_{IR})$ are the logarithmic ouput of only pulsatile part of photodetector voltage which is called PPG. The ε_{HbOR} and ε_{HbR} are the extinction coefficients of the oxygenated and deoxygenated hemoglobin respectively at red wavelength. Similarly ε_{HbOIR} and ε_{HbIR} are the extinction coefficient of the oxygenated and deoxygenated hemoglobin respectively at infrared wavelength . C_{HbO} and C_{Hb} are the molar concentration of the oxygenated and deoxygenated hemoglobin respectively in arterial blood. Using these equations we have three unknowns, namely C_{Hb}, C_{HbO} and path length T_F but we have only two equations.

Let us assume a quantity $Q = C_{HbO}/C_{Hb}$ which is the ratio of the concentration of the oxy hemoglobin to deoxy hemoglobin. To obtain Q, we divide equation 2.8 by equation 2.9 to get :

$$\frac{\nu_R}{\nu_{IR}} = \frac{(\varepsilon_{HbR}C_{Hb} + \varepsilon_{HbOR}C_{HbO})}{(\varepsilon_{HbIR}C_{Hb} + \varepsilon_{HbOIR}C_{HbO})} \tag{2.10}$$

Dividing the numerator and denominator of the equation 2.10 by C_{Hb} we get :

$$\frac{\nu_R}{\nu_{IR}} = \frac{(\varepsilon_{HbR} + \varepsilon_{HbOR}C_{HbO}/C_{Hb})}{(\varepsilon_{HbIR} + \varepsilon_{HbOIR}C_{HbO}/C_{Hb})} \tag{2.11}$$

and substituting $Q = C_{HbO}/C_{Hb}$ we get:

$$\frac{\nu_R}{\nu_{IR}} = \frac{(\varepsilon_{HbR} + \varepsilon_{HbOR}Q)}{(\varepsilon_{HbIR} + \varepsilon_{HbOIR}Q)} = R \tag{2.12}$$

So from the above equation 'Q' can be calculated as given below by equation 2.13

$$Q = \frac{(\varepsilon_{HbR} - R\varepsilon_{HbIR})}{(R\varepsilon_{HbOIR} - \varepsilon_{HbOR})} \tag{2.13}$$

Substituting the value of Q in eqauations 2.8 and 2.9 we get :

$$\nu_R = (\varepsilon_{HbR} + \varepsilon_{HbOR}Q)C_{Hb}T_F \tag{2.14}$$

and

$$\nu_{IR} = (\varepsilon_{HbIR} + \varepsilon_{HbOIR}Q)C_{Hb}T_F \tag{2.15}$$

In eqaution 2.14 and 2.15 the only unknown variables are C_{Hb} and T_F.Though we have two equations and two unknowns , a unique solution cannot be obtained as the variables are multiplicative. Thus in order to estimate concentration of hemoglobin we only need to solve for the unknown variable T_F.

CHAPTER 3

CONSTRUCTION OF PROTOTYPE

3.1 The PPG Sensor Head

PPG sensor head is an arrangement made up of plastic clip, on whose one side two SMD light emitting diodes are fixed and on other side, a photodetector is placed in such an arrangement that we can place finger tip on the LEDs and get transmitted photons detected by the photodetector. The pictorial representation of the sensor head is shown in figure 3.1. The two SMD LEDs chosen are Red(660nm) and Infrared(850nm). These wavelength are chosen because they don't show attenuation by plasma. Hence any absorption due to the arterial blood would be dictated by RBC. Photodetectors are chosen with appropriate sensitivity as it determines the photocurrent and also photodetector which has lowest dark current is chosen for best result to get PPG. Initially a probe was procured from local company which was designed for four channels or say four wavelengths. But the probe didn't work well and eventually we procured sensor lobe with department but having only two LEDs, red and infrared.

Figure 3.1: Finger lobe

3.2 Signal Conditioning Circuit

The signal conditioning circuit has many sub units, each of them serves important role in conditioning of signals emanating from LEDs, which is first detected by photo detector and the output is sent to LOG112 and then, LOG112 sends the output to filter circuit and finally the signal is viewed on LabView acquired by DAQ 6008. The various subunits of signal conditioning circuit are as following

1. I to V convertor(LOG112) The output of photodetector is very small current of order micro amps which is converted to voltage and amplified by LOG amplifier as shown in equations below. The voltage can be mathematically modelled as :

$$\nu_{o\lambda} = K_{D\lambda} I_\lambda e^{[\varepsilon_{D\lambda} C_D + \varepsilon_{T\lambda} C_T + \varepsilon_{B_o\lambda} C_{B_o} + \varepsilon_{V_b\lambda} C_{V_b} + \varepsilon_{A_b\lambda} C_{A_b}] T_F} \tag{3.1}$$

The photodetector current can be modelled as :

$$I_{o\lambda} = I_\lambda e^{[\varepsilon_{D\lambda} C_D + \varepsilon_{T\lambda} C_T + \varepsilon_{B_o\lambda} C_{B_o} + \varepsilon_{V_b\lambda} C_{V_b} + \varepsilon_{A_b\lambda} C_{A_b}] T_F} \tag{3.2}$$

The output of photodetector is current ,so we need an I to V converter . LOG112 serves as the best converter incorporating logarithmic compression. LOG112 functions as transimpedence amplifier.This ensures proper and efficient conversion of optical signal impinging on photodetector into an equivalent output voltage. LOG112 is a versatile integrated circuit that compute the logarithimic or log ratio of an input current relative to reference current.It features an internal 2.5V voltage reference that may be used to generated a precision current reference using an external resistor.

As shown in the figure we have used a potential divider to control the reference current. The R2 and R3 resistor as shown in the figure 3.2 are in the ratio of 3:1 so that voltage developed accross common junction of R1, R2 and R3 is 0.66V and we chose a reference resistor of 1 to 2 mega ohms so that I_{ref} is smaller than photocurrent from detector.

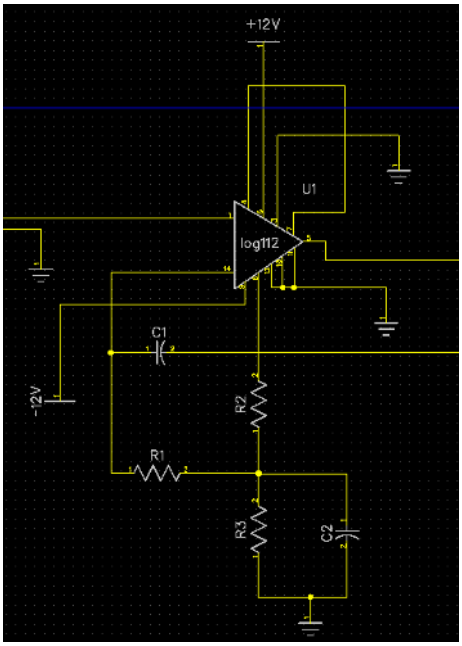

Figure 3.2: Potential divider for LOG112 amplifier

Equation describing the output of log amplifier is given below. The output is taken at PIN-5 of log amplifier. Also PIN 4 and PIN 7 are shorted so that no PIN is left floating. Interestingly after shorting PIN4 and PIN7 $R_2 = 0$, V_{o3} gives same output asV_{LOGOUT}

$$V_{LOGOUT} = (0.5)LOG(I_{photodetectorcurrent}/I_{referencecurrent}) \tag{3.3}$$

$$V_{o3} = K(0.5)LOG(I_{pc}/I_{rc}) \tag{3.4}$$

where $K = 1 + R_2/R_1$

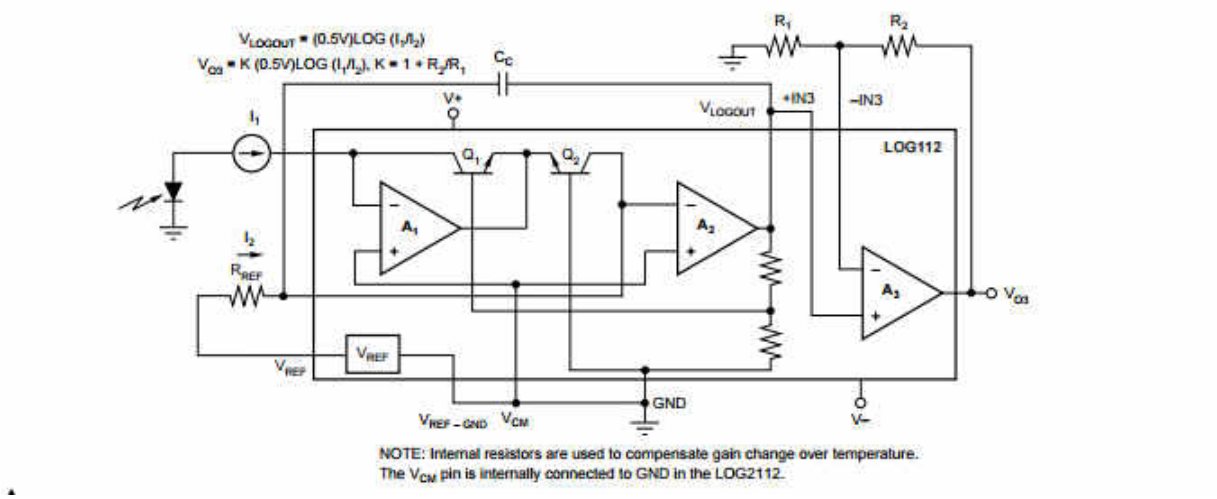

Figure 3.3: LOG112 internal circuit arrangement

There are several advantages of using LOG112, as it allows accurate measurement of low level signals due to characteristics of low DC offset voltage. Frequency compensation for LOG112 is obtained by connecting a capacitor between output PIN 5 and pin where reference current is fed i.e PIN 14. The size of capacitor is a function of the input currents as shown in the typical characteristic curve as shown in the figure 3.4 . Larger value of C_c make LOG112 more stable but reduces the frequecy response. Compensation capacitor C_c determines the frequency response and the settling time. For our case we used optimum value of the compensation capacitor of value 12pf.

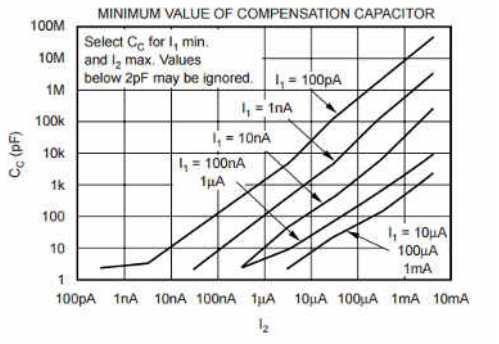

Figure 3.4: Compensation capacitor based on reference current and detecor current

In the present case we are supposed to get highly pulsatile portion of PPG. But if we use normal I to V converter which doesn't have logarithimic compression, the ratio of desired pulsatile portion to undesired DC or slow varying AC portion is too high

making the further processing a challenge. Once we apple logarithmic compression using LOG112, I to V converter we get rid of majority of the unwanted DC portion of PPG. The is due to fact that the output of LOG112 is $log(I_{pc})$ - $log(I_{rc})$, where I_{pc} is the photodiode current and I_{rc} is the reference current DC current as shown in the equation above. The value of $I_{reference}$ is chosen such that the logarithmic amplifier suppresses most of the undesired DC and slow varying AC part from the PPG.

2. LEDs driver(OPA551) In our signal conditioning circuit we are using low cost operational amplifier OPA551 which has high voltage and high current capability. This IC drives the LEDs. The current to the LEDs are time sliced with help of control signals to AO and A1 of ADG409. These control signals are provided by microcontroller PIC16F628A,about which we will discuss in detail in next sections.

3. The demultiplexing unit The photodetector senses all the four optical signals emanating from LEDs and produces a photocurrent which is then sent to LOG112(I to V converter)and then to filtering unit about which we will discuss in next section. Since there are 4 LEDs and we require 4 different PPGs as output, therefore at any time the output of the photodiode will correspond to only one of the wavelengths(red, infrared, blue and green). Thus the output of the LOG112 will also possess the time sliced (multiplexed) red, infrared, blue and green PPG signals and these have to be demultiplexed. For this purpose we are using sample and hold IC SMP04 which gets its control signal from PIC microcontroller. Whenever the red LED is switched ON, the sample and hold circuit S/H_1 is enabled (kept in sampling mode) and S/H_2, S/H_3 and S/H_4 is disabled (kept in hold mode) using the control signals C1, C2, C3 and C4. For this condition, the output of the LOG112 (corresponding to the photons due to the red LED is passed on to the red PPG channel and other 3 PPG channel holds the value attained prior to the switching ON of the red LED.

Similarly whenever the infrared LED is switched ON, the sample and hold circuit S/H_2 is enabled (kept in sampling mode) and S/H_1,S/H_3 and S/H_4 is disabled (kept in hold mode) using the control signals C1, C2, C3 and C4. For this condition, the output of the LOG112 (corresponding to the photons due to the infrared LED is passed on to the infrared PPG channel and other 3 PPG channel holds the value attained prior to the switching ON of the yellow LED.

The process is repeated for green and blue LEDs as well and we get PPGs corresponding to green and blue LEDs at the ouput of sample and hold circuit ,ready to be fed into the filter unit. Thus the output of S/H_1 contains the red PPG, with major portion of DC removed but possessing a residual DC and slow varying AC and the pulsatile signal at the heart rate. Similarly the output of S/H_2 contains the red PPG, with major portion of dc removed but possessing a residual dc and slow varying ac and the important pulsatile signal at the heart rate and same goes for S/H_3 and S/H_4 which gives green and blue PPG respectively.

4. Filter Unit The output volatge of LOG112 due to the illumination by the red LED is collected by the output of the sample and hold S/H_1 and is fed to a multiple feedback low pass filter possessing a cut-off frequency of 0.3 Hz. Thus LPF extracts the residual dc and slow varying components from the red PPG. The subtractor circuit as show in the figure 3.5 subtracts the residual dc and slow varying components of the red PPG extracted by LPF and provides at its output only the pulsatile portion of the logarithm applied red PPG. The output of subtractor is further band limited and amplified by second lowpass filter to obtain red PPG ν_R. The cut-off frequency of second low pass filter is chosen to be 20 Hz so as to ensure that all the harmonics of the red PPG (pulsatile portion) is retained and at the same time unwanted noise and interference (especially the power supply interference) are filtered out from ν_R. Similarly the output volatge of LOG112 due to the illumination by the red LED is collected by the output of the sample and hold S/H_2 and is fed to a multiple feedback low pass filter possessing a cut-off frequency of 0.3 Hz. Thus LPF extracts the residual dc and slow varying components from the red PPG. The subtractor circuit as show in the figure 3.5 subtracts the residual dc and slow varying components of the red PPG extracted by LPF and provides at its output only the pulsatile portion of the logarithm applied infrared PPG. The output of subtractor is further band limited and amplified by second lowpass filter to obtain infrared PPG ν_Y. The cut-off frequency of second low pass filter is chosen to be 20 Hz so as to ensure that all the harmonics of the yellow PPG (pulsatile portion) is retained and at the same time unwanted noise and interference (especially the power supply interference) are filtered out from ν_Y.Similarly same filtering processing is done for the other channels namely green and blue which is acquired from LOG112 output by sample and holds S/H_3 and S/H_4respectively.

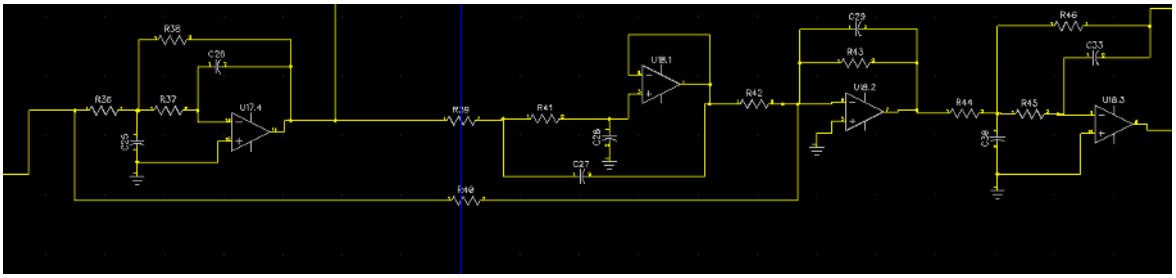

Figure 3.5: PIC16F628A IC Pin configurations

4. Timing and contol unit The overall sequencing for time slicing the LEDs and the de-multiplexing the output of LOG112 is implemented using a timing and control logic. The logic of the timing and control logic unit is shown in figure**??**. At power ON condition, all three signals C1, C2, C3 and C4 are set to zero. For this condition the red LED is turned on and other LED are off and both the sample and hold circuitry are kept on hold mode. After a delay of 0.5ms, C1 is set to one putting the sample and hold S/H_1 in the sampling mode and the output of the LOG112 that contains transient free sample of the logarithm applied red PPG is connected to the red PPG channel. This condition is held for a period of 1ms and at the end of 1ms, C1 is set to zero bringing S/H_1 to hold mode. The above process is repeated endlessly to obtain the red,infrared,blue and green PPG signals. The intentional time gap between the transition of the LED switching and the sampling process ensures that the output is unaffected by the transients that can occur when switching from one LED to the other, especially in the response of the LOG112 IC. Each LED is ON for 2ms and again is programmed ON after 6ms. Samples are taken for each LED after every 7ms of hold time. So, samples are read from a single LED for 1ms and then is kept on hold for 7ms and then again is sampled and its photocurrent data is taken.

3.3 Data Aquisition System

The red,infrared,green and blue PPG signals ν_R, ν_I, ν_G and ν_B obtained are sampled using a data acquisition system NIDAQ6008 manufactured by National Instruments. The DAQ system is interfaced to windows PC and configured and controlled through a suitable virtual instrument software developed using the NI LabVIEW platform. Samples of ν_R, ν_I, ν_G and ν_B are were acquired at the rate of 200 Samples/s and further pro-

cessing to extract an empirical equation for determining T_F as well as the haemoglobin concentration C_{Hb} were also achieved through suitable virtual instruments (software) created in the NI LabVIEW platform. It has 8 analog inputs (12-bit, 10 kS/s) and 2 static analog outputs (12-bit); 12 digital I/O and 32-bit counter.It is bus-powered for high mobility and has built in signal connectivity.

3.4 Microcontroller

We need microcontroller which can send control signals to four differential channels of ADG409(multiplexer) and based on selection of differential channel can control four sample and hold channels of SMP04(used as multichannel Data Acquisition System). Six control signals C1, C2, C3, C4, C5 and C6 are generated by PIC16F628A microcontroller which controls various timing and control functions of our signal conditioning circuit. C1, C2, C3 and C4 are generated and goes to control four different sample and holds of SMP04 . C5, C6 are fed to the ADG409 at A0 and A1 respectively.Therefore C5 and C6 control-signals, control the four different channels of ADG409 namely S1A, S2A, S3A, S4A. Each differential channels have two sets namely A and B. So ADG409 have (S1A, S1B); (S2A, S2B); (S1A, S1B) and (S1A, S1B) as four differential channel which thereby function as high performance multiplexer.This differential channels and S/H's of SMP04 together allow only one of four LEDs to produce its PPG after passing through appropriate filter circuit to filter out low frequecy components.

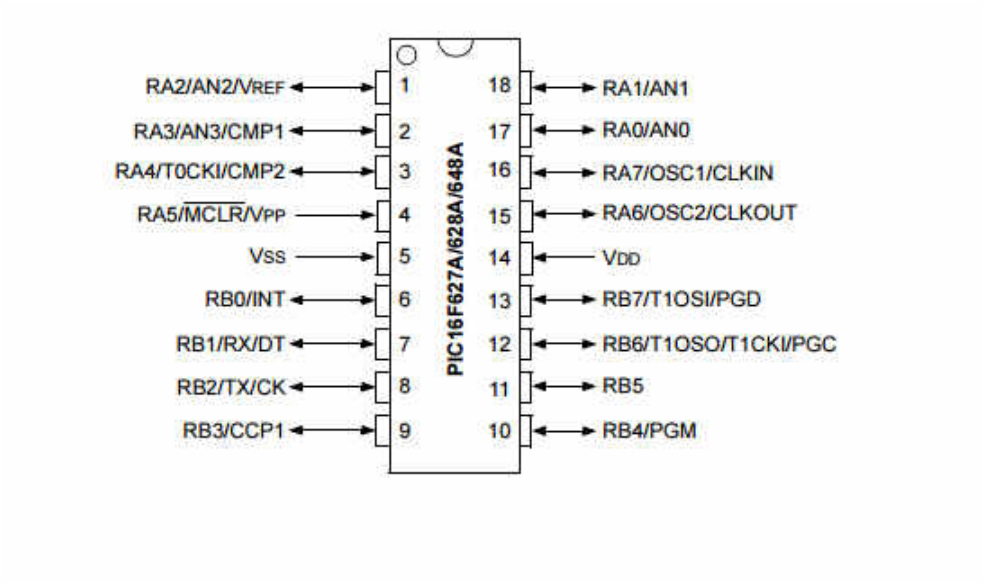

Figure 3.6: PIC16F628A IC Pin configurations

3.4.1 Control Process

Channel-Red If the A0=0 and A1=0, which means when control signal C5=0 and C6=0 then (S1A,S1B) differential channel is ON and other channels are OFF. Also based on this selection one of the sample and hold (S/H_1) are switched on via help of control signal C1=1 and rest all other sample and hold control signals are C2=C3=C4=0. This differential channel and S/H_1 together allow only red LED to produce its PPG after passing through appropriate filter circuit to filter out low frequecy components.

Channel-infrared Similarly If the A0=0 and A1=1, which means when control signal C5=0 and C6=1 then (S2A,S2B)differential channel is ON and other channels are OFF and based on this selection one of the sample and hold(S/H_2) are switched ON via help of control signal C2=1 and rest all other sample and hold control signals are OFF C1=C3=C4=0. This differential channel and S/H_2 together allow only infrared LED to produce its PPG after passing through appropriate filter circuit to filter out low frequecy components.

Channel-Blue If the A0=1 and A1=0, which means when control signal C5=1 and C6=0 then (S3A,S3B)differential channel is ON and other channels are OFF. Also based on this selection one of the sample and hold (S/H_3) are switched on via help of

control signal C3=1 and rest all other sample and hold control signals are C1=C2=C4=0. This differential channel and S/H_3 together allow only blue LED to produce its PPG after passing through appropriate filter circuit to filter out low frequecy components

Channel-Green If the A0=1 and A1=1, which means when control signal C5=1 and C6=1 then (S4A,S4B) differential channel is ON and other channels are OFF. Also based on this selection one of the sample and hold (S/H_4)are switched on via help of control signal C4=1 and rest all other sample and hold control signals are C1=C2=C3=0. This differential channel and S/H_3 together allow only green LED to produce its PPG after passing through appropriate filter circuit to filter out low frequecy components.

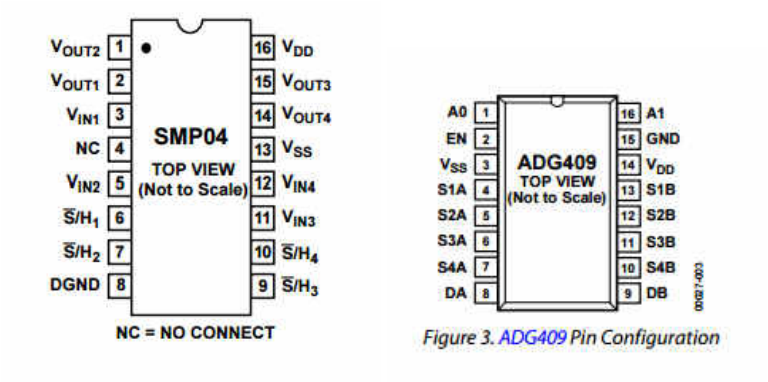

Figure 3.7: Pin configuration of SMP04 and ADG409

Above process is schematically represented in the figure 3.8 . The PIC microcontroller controls the SMP04 S/H_s and multiplex ADG409 channels. The LED driver shown in schematic drives the LEDs . The photo sensor feeds the ouput to LOG112 (I to V convertor) which then goes to filter circuit through sample and hold channels. This process continues for entire timing and control logic till PPG corresponding to all four wavelength is obtained.Table 3.1 depicting the confiuration and logic of all devicess used for signal and control purpose.

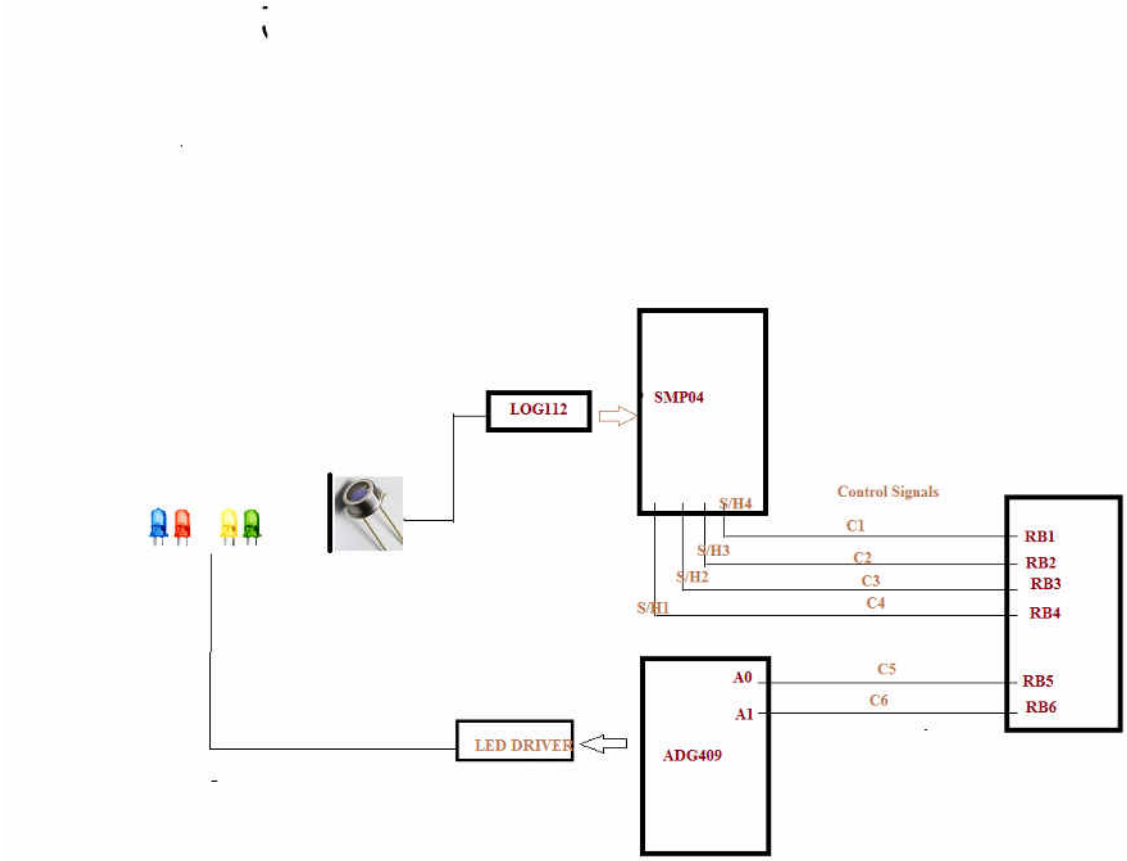

Figure 3.8: Microcontroller functioning overview

3.4.2 SMP04

The SMP04 is CMOS quad sample and hold amplifier. It is a 16 pin IC having 4 signal input $V_{IN1}, V_{IN2}, V_{IN3}, V_{IN4}$ and 4 output pins $V_{out1}, V_{out2}, V_{out3}, V_{out4}$. There are 4 sample and hold pins$(S/H_1, S/H_2, S/H_3, S/H_4)$. The sample and hold pins are connected to programmable pins of PIC16F628 as shown in figure 3.8. And the signal input pins are connected to output of LOG112(I to V) convertor. Depending upon the value of control singnals C1, C2, C3 and C4 one of the sample and hold is ON and rest is OFF. The ouput of SMPO4 is conected to the filter circuit to filter out low frequency components from PPG signals and we get only high frequency pulsating part of PPG.

3.4.3 ADG409

ADG409 is high performance analog multiplexer.It has four differential channels. Each channel controls, when are different LEDs are to be powered through driver, after receiving cotrol bits A0, A1. DA and DB are the output pins of the ADG409 . Output of DA is input to the LED Driver OPA551 and output of OPA551 drives 4 different LEDs. An EN pin in the IC is used to enable or disable the device. When EN is low, the device is disabled and all switches are off. When high, Ax logic inputs(A0,A1) determine on switches.

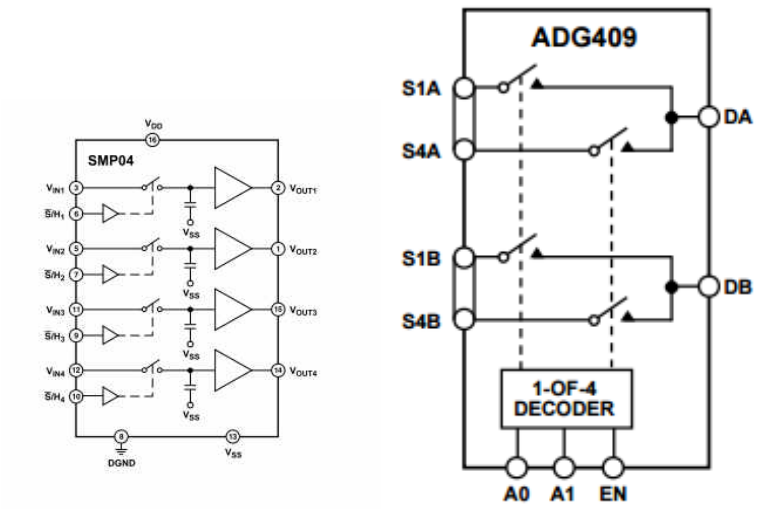

Figure 3.9: Internal circuit arrangement for SMP04 and ADG409

3.4.4 PIC-Peripheral Interface Controller IC

The PIC16F628A are 18-pin flash-based members of the versatile PIC16F series of family of low-cost, high-performance, CMOS, fullystatic, 8-bit microcontrollers. Flash devices can be erased and reprogrammed electrically. This allows the same device to be used for prototype development,pilot programs and production. A further advantage of the electrically erasable flash is that it can be erased and reprogrammed in circuit, or by device programmers. PIC16F628A has two ports, PORT-A and PORT-B. In our case we are using only PORT-B which has 6 bidirectional I/O port. These port can be software programmed for interupt.RA5 is master clear port and it should be kept high for normal operation of the IC. When configured as low it reset the device. But voltage on $MCLR/V_{pp}$ must not exceed V_{DD} during normal device operation. It has internal

oscillator of 4MHz apart from ports for use of external crystal oscillator which we don't require.

Table 3.1: Various PIN arrangement in tabulated form

$ControlBits$	$ADG409ChannelSelected$	$SMP04Channel$	$Colour$
00	S1A PIN-4	S/H$_1$ $PIN-6$	RED
01	S2A PIN-5	S/H$_2$ $PIN-7$	IR
10	S3A PIN-6	S/H$_3$ $PIN-9$	GREEN
11	S4A PIN-7	S/H$_4$ $PIN-10$	BLUE

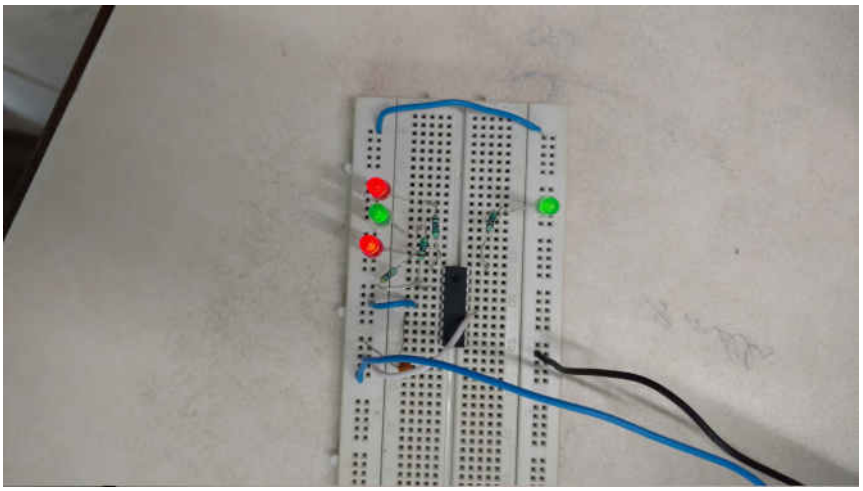

Figure 3.10: Testing of microcontroller

CHAPTER 4

Results and Conclusions

4.1 Results

For obtaining results stage by stage execution of cicuit was done. First a circuit was build on breadboard with simple transimpedance amplifier and simple low pass filter and a subtractor and results are recorded as shown in figure 4.1. Then a circuit a build with simple logarithmic amplifier for converting the photocurrent and sending of voltage signal to filter unit of circuit. There are various advantages of using a log amplifier as discussed in previous chapters. The results are shown in figure 4.5. In final experiement two obtain two PPGs red and infrared is obtained. The circuit is designed and build on PCB with help of dip trace software. The plan was to implement four channels and four PPG but due to some technical error in the finger lobe acquired we had to use department's finger lobe with red and IR LED channel. The results for the two channels is done and PPG are acquired on LabView as shown in the figure. Figure 4.6 shows the PPG when infrared LED is ON and figure 4.7 shows PPG when red LED is ON.

4.1.1 Experiement with simple transimpedance amplifier

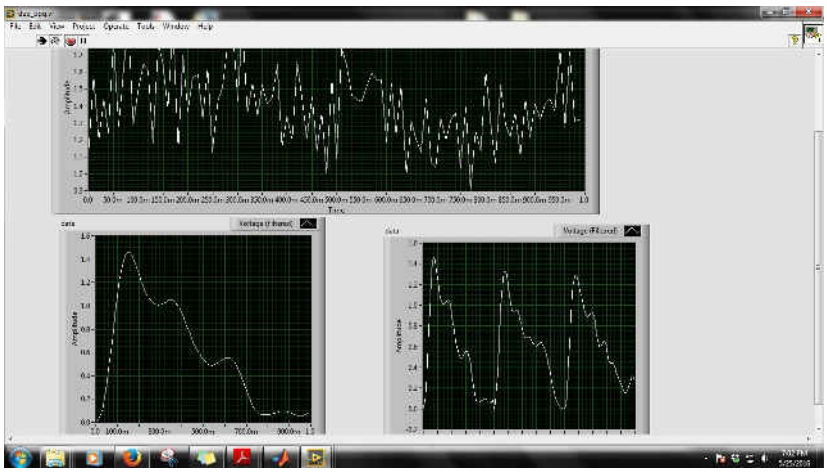

Figure 4.1: white light ppg with transimpedance amplifier

4.1.2 Experiement with simple one cahnnel LOG amplifier

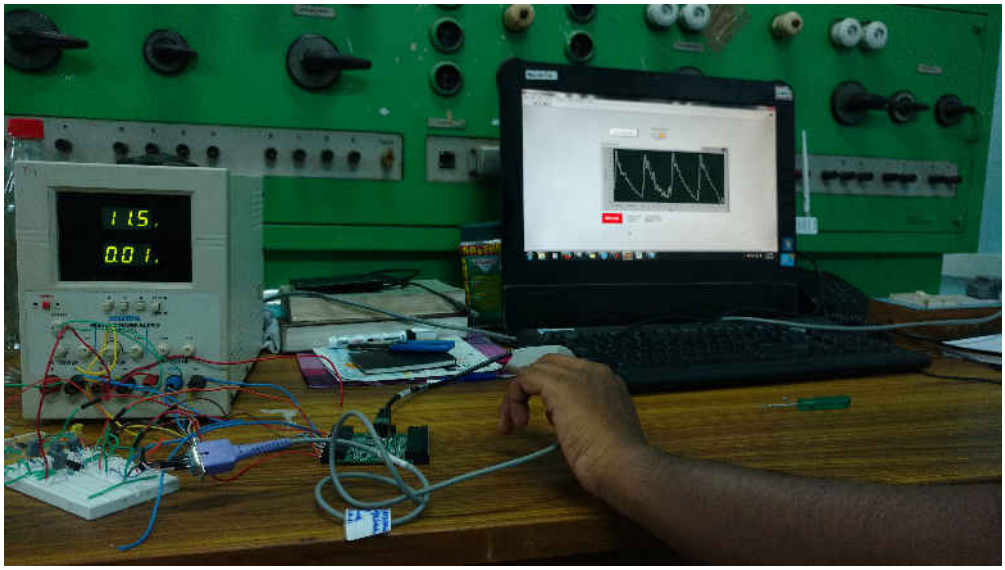

Figure 4.2: Experiemental setup

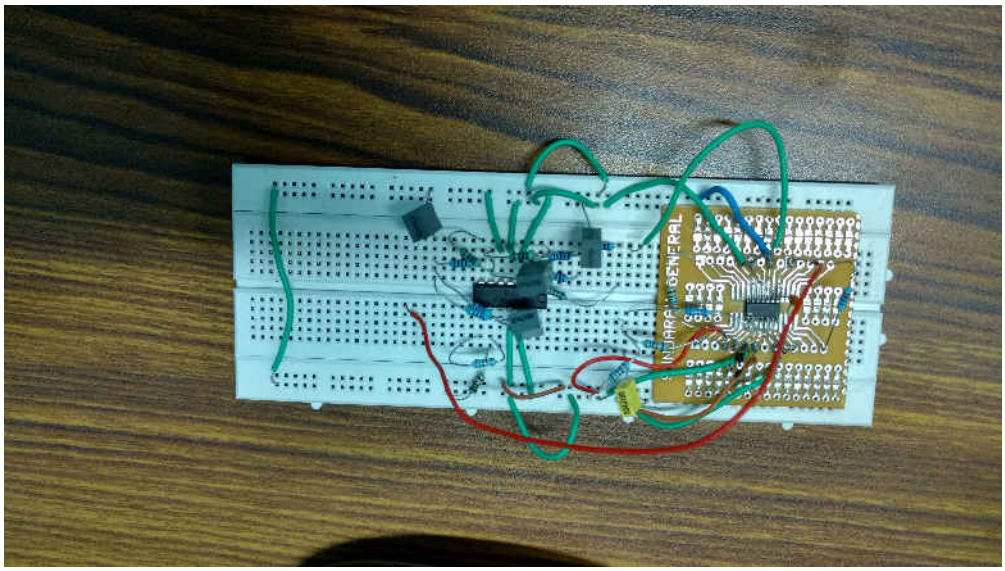

Figure 4.3: Circuit used with LOG112 amplifier

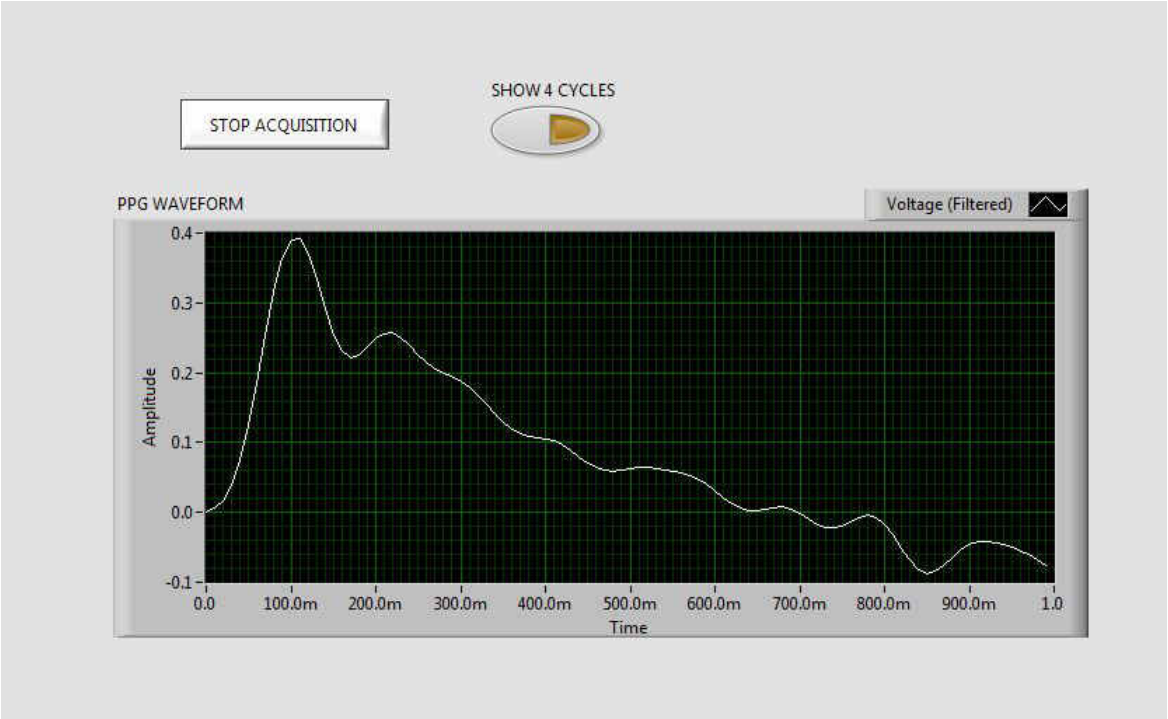

Figure 4.4: PPG obatined with log amplifier

4.1.3 Two channels implemented on PCB with LOG amplifier

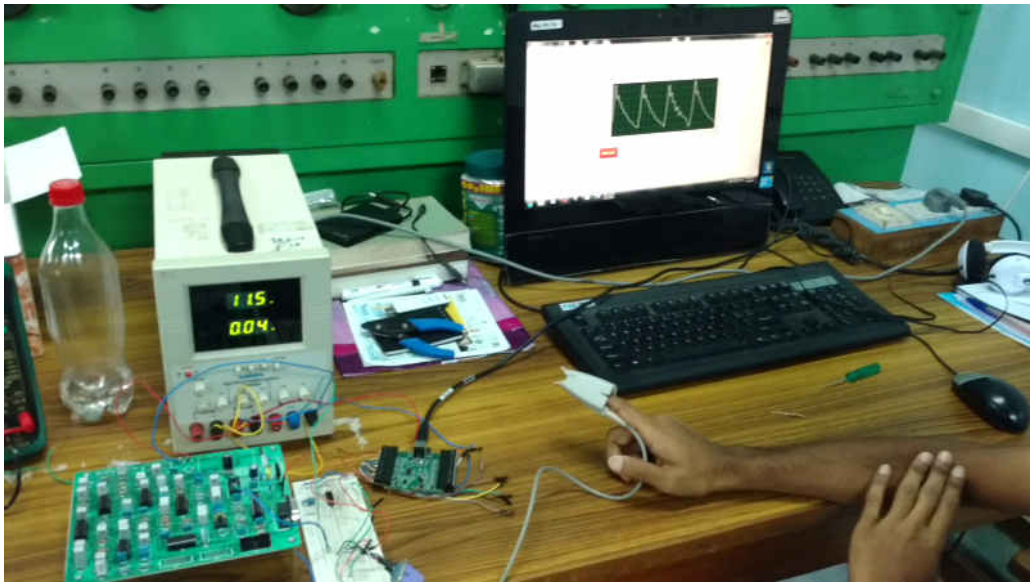

Figure 4.5: Experiemental setup on PCB

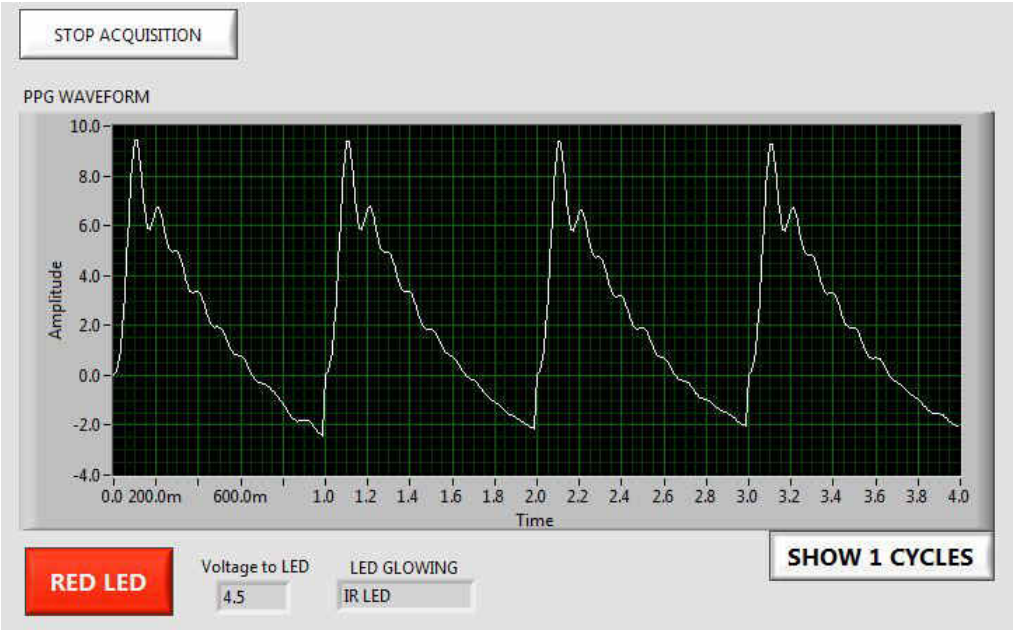

Figure 4.6: IR PPG obtained

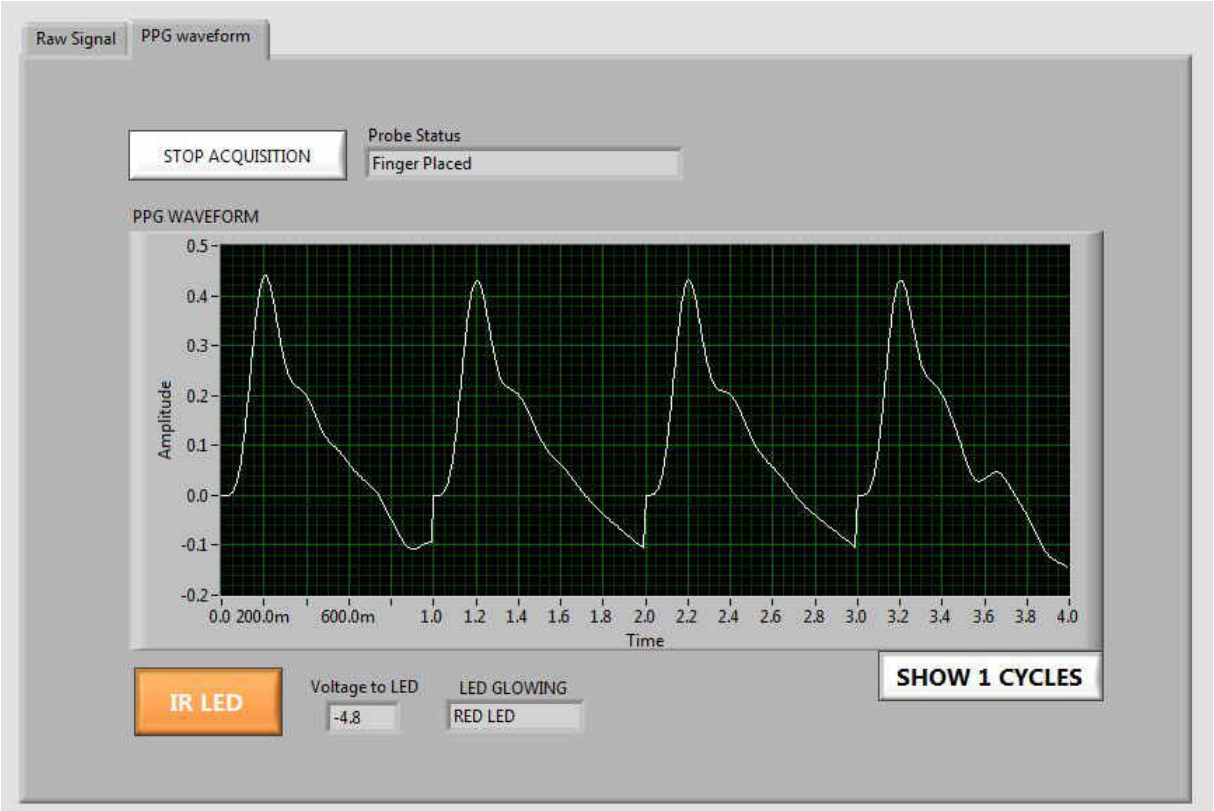

Figure 4.7: RED PPG obtained

4.2 Conclusion

The red and infra-red are used because of their difference in absorbance at those two frequencies between oxygenated and unoxygenated hemoglobin. The IR has higher penetration depth inside skin and therefore have higher amplitude in PPG graph as compared to PPG obtained when red LED is ON. With the help of mathematical model discussed in chapter 2, we can now calculate the concentration of hemoglobin in blood as we can now calculate R as shown in equation 4.1. It is important to note that R value varies from person to person based on skin colour to various other factors.

$$R = \frac{\nu_R}{\nu_{IR}} \tag{4.1}$$

Thus the value Q can be now be calculated as shown in equation :

$$Q = \frac{(\varepsilon_{HbR} - R\varepsilon_{HbIR})}{(R\varepsilon_{HbOIR} - \varepsilon_{HbOR})} \tag{4.2}$$

$$ln(\nu_R) = (\varepsilon_{HbR} + \varepsilon_{HbOR}Q)C_{Hb}T_F \tag{4.3}$$

and

$$ln(\nu_{IR}) = (\varepsilon_{HbIR} + \varepsilon_{HbOIR}Q)C_{Hb}T_F \tag{4.4}$$

The ε_{HbOR} and ε_{HbR} are the extinction coefficients of the oxygenated and deoxygenated hemoglobin respectively at red wavelength.Similarly ε_{HbOIR} and ε_{HbIR} are the extinction coefficient of the oxygenated and deoxygenated hemoglobin respectively at infrared wavelength . C_{HbO} and C_{Hb} are the molar concentration of the oxygenated and deoxygenated hemoglobin respectively in arterial blood. These parameters are constant at particular wavelengths and thus only unknown quantity left is path length which can't be calculated by analytical means but experiements validate theory that non-invasive determination of heamoglobin content in blood is possible using PPG signals.

APPENDIX A

Programming PIC IC

Programming a PIC microcontroller involves two major steps

1) Generatinng hex bit file

2)Buring hex bit file to IC and programming it.

A.0.1 Generating Hex File

MPLAB X IDE is official software program that can be installed on a PC for development of applications for Microchip microcontrollers . This provides a single integrated environment for development and debug platform for embedded microcontroller. But here the main source code is written in Assembly language which is not very user friendly and difficult to understand. However MPLAB X has official compiler which would allows to write code in C and generate hex file to program PIC IC. There are mainly two official MPLAB C compiler ,first being provided by HI-TECH PICC compiler and other one being the XC8(For 8 bit MCU) as shown in fiureA.1. Both the compilers are instaled seperately and later MPLAB links and detect with compiler automatically.

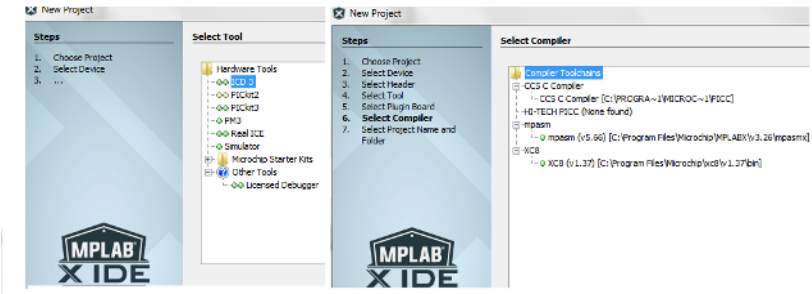

Figure A.1: MPLAB front interface

CCS C Compiler But since our PIC Microcontroller is very basic micrcontroller and it is easiest to use CCS C Compiler.It is very subtle for beginners . CCS stands

for Custom Computer Services. They have lot of build in libraries which enable us to program a PIC Microcontroller easily with compilcated internal archietecture. We don't need to configure bits to program the internal archietecture. The software allow us to configure bits. Here we are using internal clock. If you want to use external clock, there is a option for that also. But for our need we are going to use 4MHz internal clock. We write the code and get the hex file in our project folder after succesfully compile the correct code and running building option for hex file generation as shown is figure A.3.

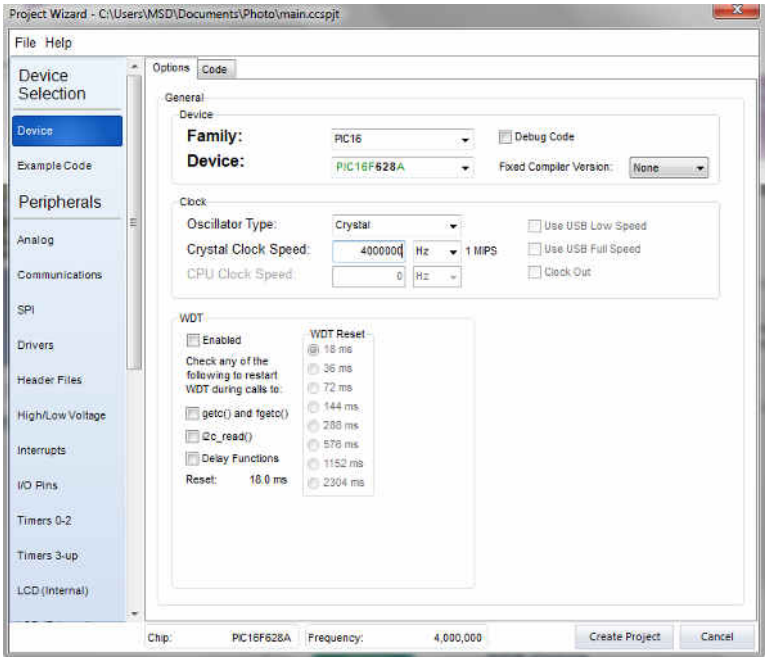

Figure A.2: Setting up internal clock frequency

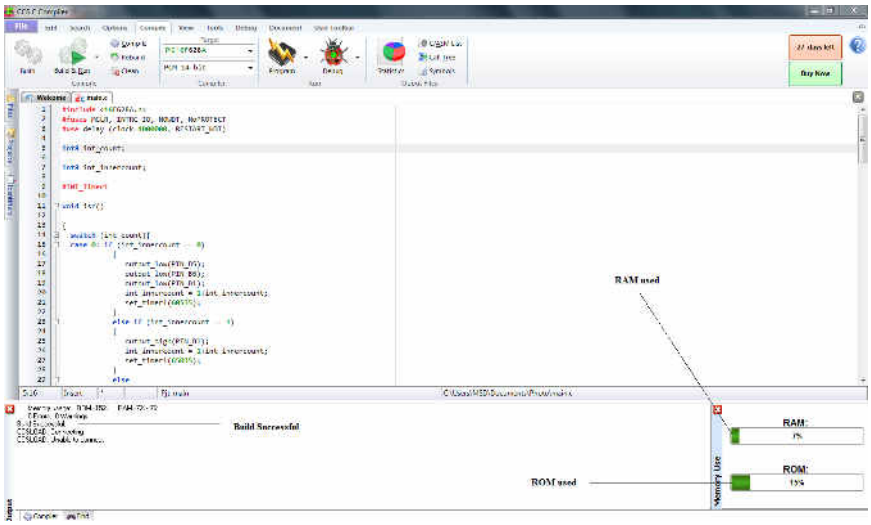

Figure A.3: Compile and Generation of Hex file

A.0.2 Burning the Hex file to IC

To burn the hex fie to PIC IC we need external hardwares. Microchip provides user with development board or programmer kits like Real ICE , PICkit2, PICkit3 With software to burn the hex file after placing the PIC Microcontroller on Development board or programmer kit.

SUPERPRO 280U SuperPro 280 U is universal Microcontroller Programmer which is provided by Xeltec. For our purpose we used this Programmer kit. It also came with Software to burn hex file and programm the PIC IC. However Super Pro 280 is very old programmer and its software supports only windows XP and lower windows version.

APPENDIX B

Schematic developed for PCB

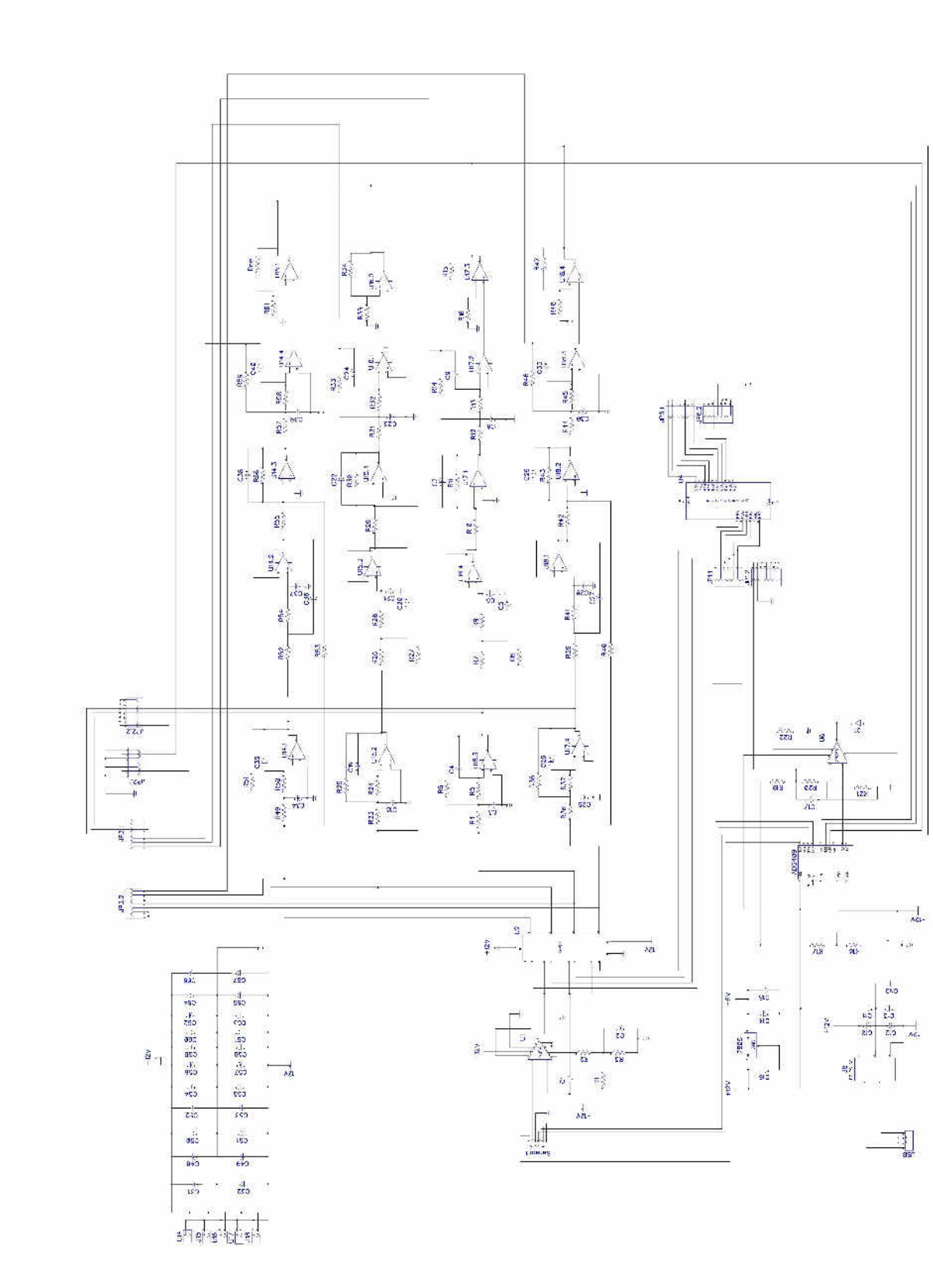

Figure B.1: Schematic for experiement

APPENDIX C

PCB used for project

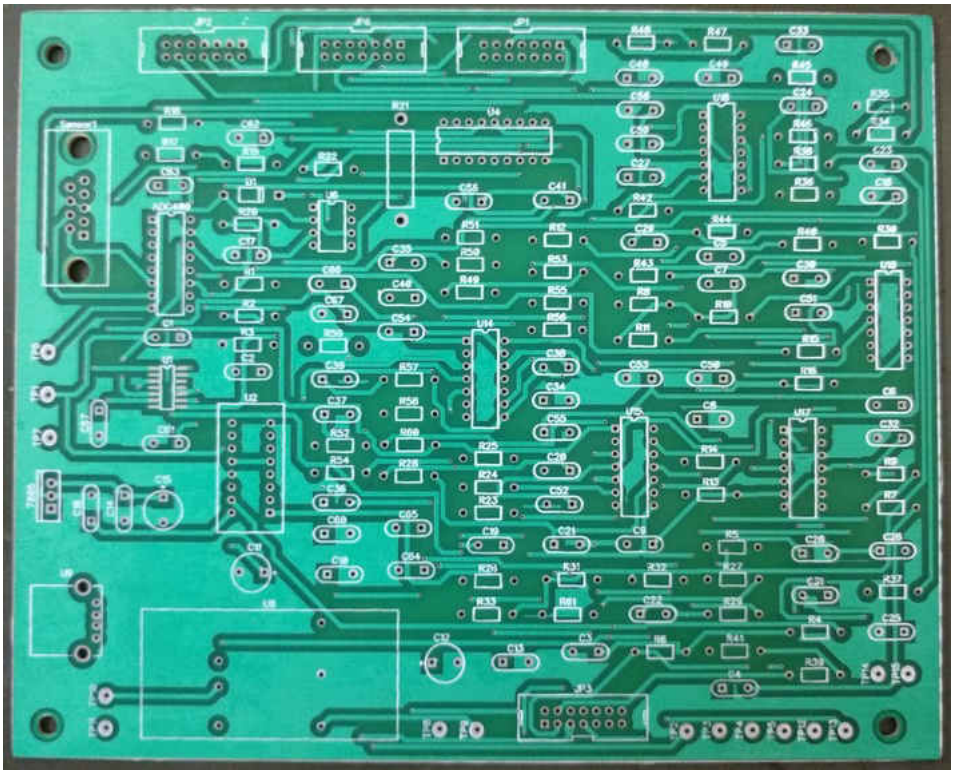

Figure C.1: Printed Circuit Board

APPENDIX D

VI developed on LabView

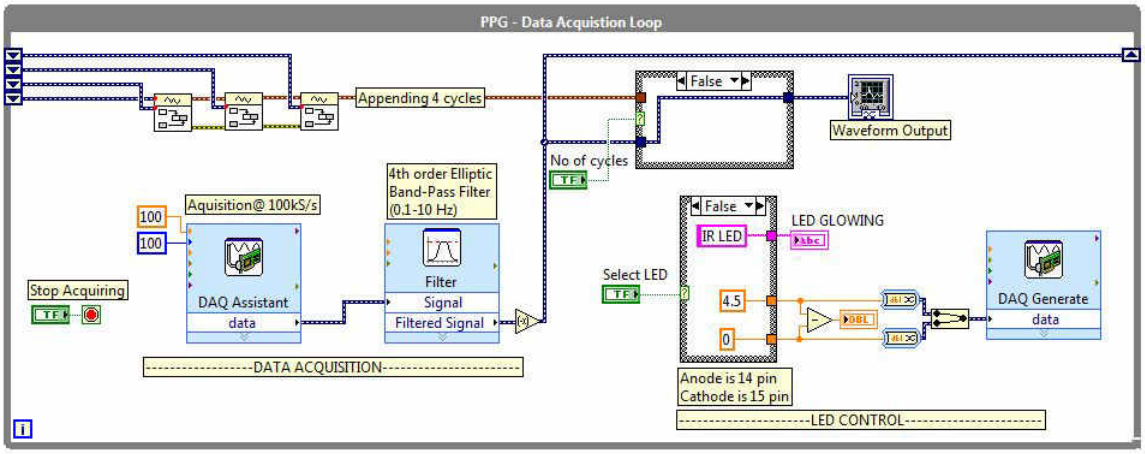

Figure D.1: VI developed for experiements

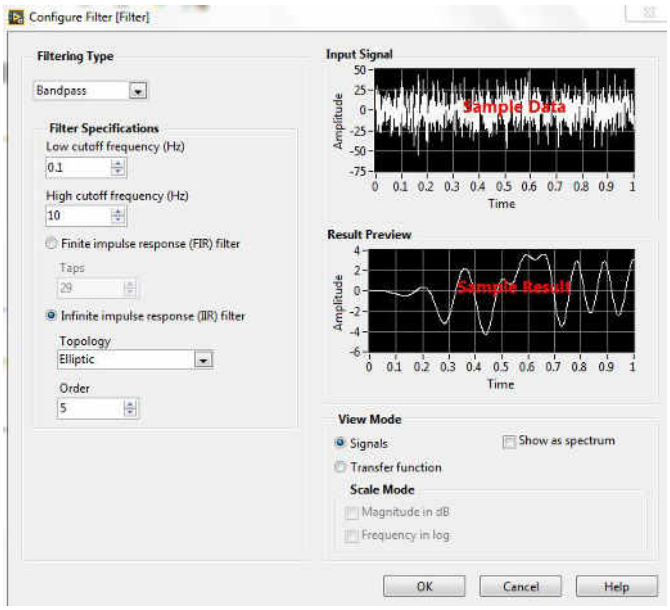

Figure D.2: Filter Details

APPENDIX E

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