DEVELOPMENT OF 3D PRINTED SPECTROSCOPY INSTRUMENTATION FOR MEDICAL APPLICATIONS

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İZMİR

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ABSTRACT

DEVELOPMENT OF 3D PRINTED SPECTROSCOPY INSTRUMENTATION FOR MEDICAL APPLICATIONS

The aim of this thesis is technically to produce a national production basement on analytical device manufacturing. In addition, recent advances in the evaluation of absorbance, reflection and fluorescence spectrum measurements in the UV-VIS range (vascular volume, oxygenation, extra cellular matrix, metabolic redox states, cellular proliferation) give us valuable measurements about the diagnosis, prognosis and treatment of some cancer types (*1*). Thus for every doctor and for every medical laboratory a cheap, portable, handy, and enough accurate UV-VIS spectrometer has become an up to date demand. Therefore production of an analytical VIS-spectrometer is planned as a first step on the way of our aim, by using our laboratory facilities and by using very common optical, electronical, mechanical components in the market around.

Optical box design was get from an internet open source and printed in our laboratory by 3D-printer (Stratasys-Objet 30). A general purpose plastic mounting box (15x9x5 cm) is used for electronic part of the spectrometer. These two box is adjusted to each other to become as a single cabinet for spectrometer. Optical components are mounted, aligned and coupled to electronic circuit. PC and Microcontroller programs are written and loaded in our laboratory. During the calibration of our device, its performance is compared with the technical specifications of commercial VIS-spectrometers. As a last step; the produced device is used and tested in our laboratory.

ÖZET

MEDİKAL UYGULAMALAR İÇİN YERLİ SPEKTROSKOPİ CİHAZI GELİŞTİRİLMESİ

Bu tezin amacı, teknik olarak analitik cihaz imalatı üzerine ulusal bir üretim temeli oluşturmaktır. Ek olarak UV-VIS aralığında absorbans, yansıma ve floresan spektrum ölçümlerinin değerlendirilmesiyle ilgili son gelişmeler (vasküler hacim, oksijenasyon, hücre dışı matris kapsamı, metabolik redoks durumları, hücresel proliferasyon) bize bazı kanser türlerinin tanısı, seyri ve tedavisi hakkında değerli ölçümler vermektedir. Bu nedenle, her doktor ve her tıbbi laboratuar için ekonomik, taşınabilir, kullanışlı ve yeterince hassas bir UV-VIS görünür spektrometresi güncel bir talep haline gelmiştir. Bu sebepler değerlendirildiğinde, laboratuar imkanlarımızdan yararlanılarak ve piyasada çok yaygın olan optik, elektronik, mekanik bileşenler kullanılarak bir VIS spektrometre üretimi amacımıza giden yolda ilk adım olarak planlanmıştır.

Optik kutu tasarımı internette açık bir kaynaktan alındı ve laboratuvarımızda 3D yazıcı (Stratasys-Object 30) ile basıldı. Spektrometrenin elektronik kısmı için genel amaçlı bir plastik montaj kutusu (15x9x5 cm) kullanıldı. Bu iki kutu, spektrometre için bir bütün oluşturacak şekilde birleştirildi. Optik bileşenler optik kutuya yerleştirildi, hizalandı ve elektronik devreye bağlandı. PC ve mikrodenetleyici programları laboratuarımızda yazıldı ve yüklendi. Kalibrasyon yapılırken cihazımızın performansı ticari VIS spektrometrelerin teknik özellikleri ile karşılaştırıldı.

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CHAPTER 1

INTRODUCTION

1.1.History of Spectrometer.

UV-VIS Spectrometer is one of the most important instruments for development of bioscience. The first sold spectrometer in 1941 was developed by Beckman and colleagues. Between 1950-1970 mass production of devices reduced the UV-VIS spectrometer costs and new technologies were introduced into this area just like photo diode arrays. These improvements were dropped down the scanning time of whole wave length range from minutes to seconds. Using PCs improved the data acquisition and data processing in 1980s. In 1990s and 2000s external softwares, PC controls and other improvements in technology have enabled to measure micro volume liquid samples in biotechnology.

1.2.Theory of Spectrometer and Spectroscopy.

1.2.1.Electromagnetic (EM) radiation.

Light is a form of electromagnetic(EM) energy. It has both wave and particle properties. The properties such as reflection, refraction, and diffraction are wave properties. Other properties such as emission, absorbtion, collision are particle properties. This dual model of light or electromagnetic(EM) radiation is very useful for us to describe behaviours of EM radiation or light. (Figure.1)

1.2.2.Wave properties of electromagnetic (EM) radiation.

EM radiation has two oscillating components; electric and magnetic fields. These vector fields are perpendicular to each other and they are also perpendicular to wave propagation direction. EM wave or light propagates with a constant velocity of

$3.00X10^8$ m/s. An EM wave or light is characterised by some fundamental properties. They are velocity, amplitude, frequency, phase angle, polarisation, propagation direction, wave length. EM radiation can be characterised by an equation:

$$L_I = L_o \sin(2\pi ft + \Theta) \qquad \text{eqn 1.1}$$

L_I ……Magnitude of incident electric field of wave at time (t).

L_o ……Maximum value of electric field.

f …….Frequency of wave.

t …….Time.

Θ…….Phase angle of wave.

Wavelength of EM wave or light symbolised by λ. λ is the distance between successive peaks of the wave. It is measured in nanometers for UV andVIS radiation and measured in microns for IR radiation. The relationship between velocity of EM radiation and its wave length is given by the equation:

$$c = f\lambda \qquad \text{eqn 1.2}$$

c …….Velocity of light wave (EM radiation).

f …….Frequency of light wave.

λ ……Wavelength of light wave.

Infrared radiation characterised by wave number ($\bar{\upsilon}$).

$$\bar{\upsilon} = 1/\lambda \qquad \text{eqn 1.3}$$

1.2.3.Particle properties of electromagnetic (EM) radiation.

The easiest way to understand the interaction between EM radiation and matter is assuming the radiation consists of energetic particles are called photon. When a

collision occurs between photon and matter, the energy of photon absorbed by sample. The photon energy is related to its wavelength and frequency.

$$E_P = hf = h\frac{c}{\lambda} = hc\bar{\upsilon} \qquad \text{eqn 1.4}$$

E_P ……..Photon energy.

h ……...Planck constant (6.626×10^{-34} J.s)

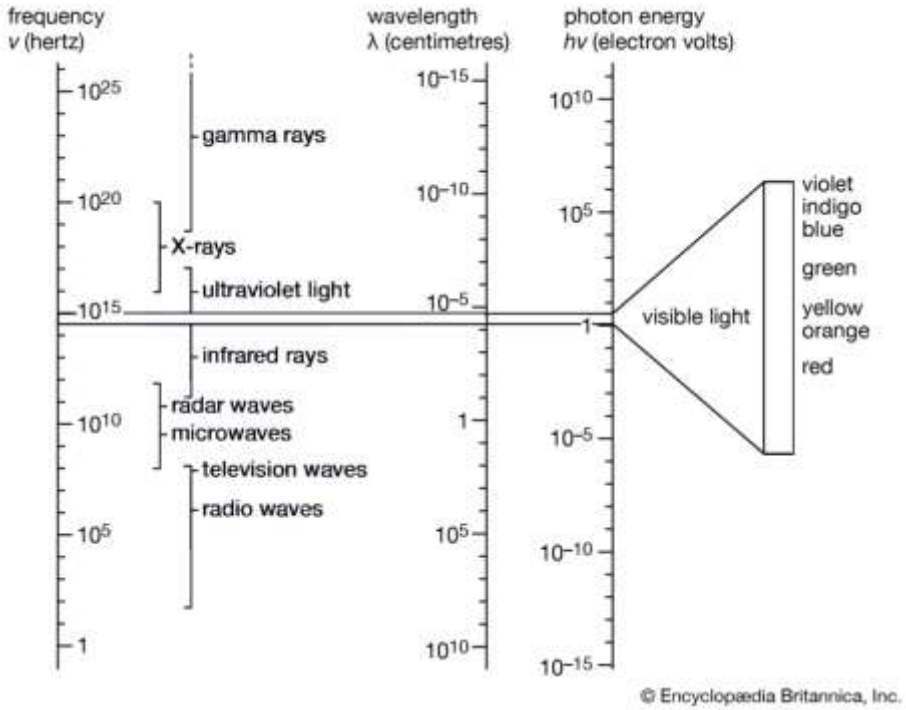

Figure 1.1. Electromagnetic Radiation

1.2.4.Spectroscopy, Spectrometry, Spectra, Absorbance.

Electromagnetic radiation interacts with matter. Analysis of this interaction is called spectroscopy. Today it includes the study of the emission and absorption of light and other radiation by matter.

Spectroscopy and spectrometry are used in our daily life interchangeably, but they do not have the same meaning exactly. Spectrometry is the measurament of

interaction of electromagnetic radiation with matter or system and calculation to obtain information. Shortly; it is a method of studying spectra.

Spectra is the plot of some measured values of absorbed or emitted electromagnetic radiation by a sample versus wavelengths or energy levels of electromagnetic radiation.

The most commonly used spectroscopies are ultraviolet, visible, and infrared. The approximate wavelength values of these regions are shown in Table.1.1.

Range	Wavelength
Ultraviolet	100nm-400nm
Visible	400nm-700nm
Infrared	700 nm-1mm

Table 1.1. Range wavelengths

Photon-sample interaction is necessary for a spectroscopic measurement. Because of this interaction some changes ocur in values of characteristic properties of light or EM radiation. Absorbing of visible light photons excites molecule valance electrons to higher energy levels. Absorbing infrared radiation cause to increase vibrational energy of chemical bounds of a molecule. After these kind of interaction the number of photons decreases or vanishes passing through sample. The measurement of this change is called absorbance. A plot of absorbance as a function of wavelength or wave energy is called absorbance spectrum.

1.2.5.Qualitative analysis

Energy transitions ocur between electronic energy levels of molecules and atoms in UV-VIS spectroscopy. Specially in organic molecules UV-VIS range photons excites the electrons in energy levels.

There are some defined peaks in UV-VIS spectra because of definite electronic transitions dominates this region. Instead of sharp peaks, wide hump curves are observed in UV-VIS region. There are some explanations for this observation:

Vibrational and rotational energy levels overlap with electronic transition energy levels. Another explanation for wide peaks is being in very close positions of sample molecules to each other just like in a liquid or in a solution. Gas phase gives sharp peaks than liquid phase or solution for a given molecule.(Figure.2)

1.2.6.Quantitative analysis

Absorbance measurements of a sample gives us right knowledges about sample. Absorbance values are predicted by measurements of intensity of light at given wavelegths. If we measure the intensity at a given wavelength with and without sample, the ratio of these values gives us transmittance,T.(Figure.3)

$$T = I_T / I_0 \qquad \text{eqn 1.5}$$

I_T ……Intensity with sample

I_0 ……..Intensity without sample

Absorbance defined as

$$A = - \log T \qquad \text{eqn 1.6}$$

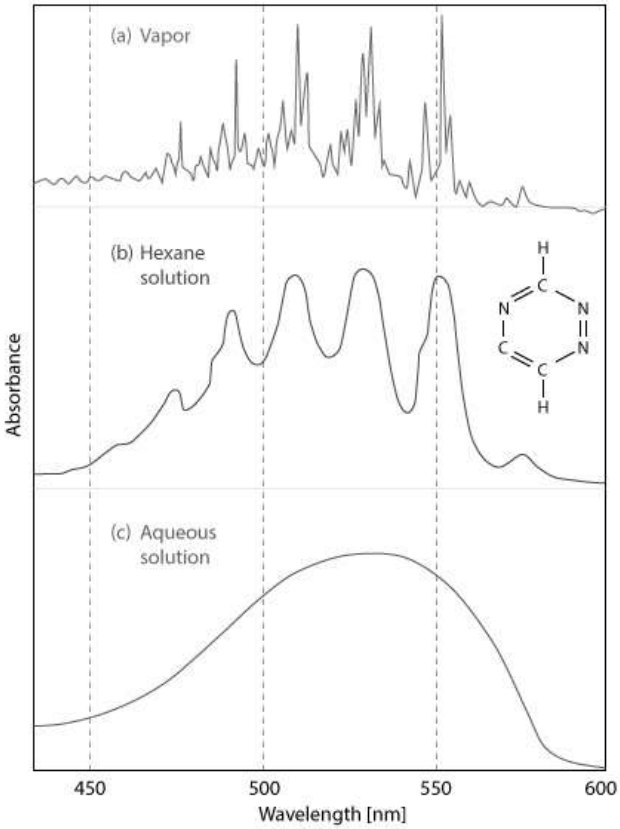

Figure 1.2. Solution effect on absorbance spectra[2]

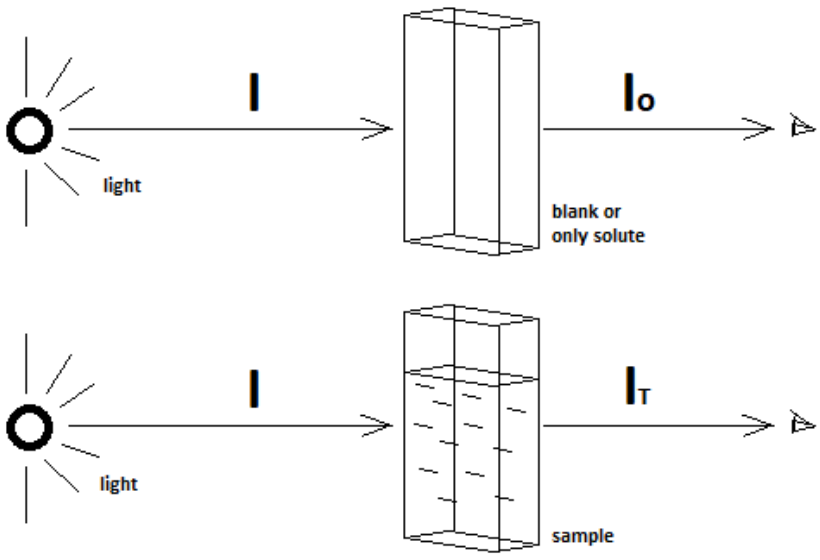

Figure 1.3. Transmittance of light

The Beer-Lambert law relates eqn.6 to concentration of sample as

$$A = \varepsilon dC \qquad \text{eqn 1.7}$$

ε ……..Molar absorbtivity

d ……..Optical path

C……..Concentration

1.2.7.Applications

UV-VIS absorbtion analysis is very common measurement because many organic and inorganic compounds have absorbtion bands in this region. Besides this if a sample does not have absorption band in UV-VIS range it can be react with another species which have absorbing band in UV-VİS region. Analysis for narcotics, for drag testing, blood alcahol test, water, waste water,agriculture and medical application tests are some practical applications of UV-VIS absorbtion analysis.

1.2.8.Instrumentation

The basic solution as a device for UV-VIS spectrometry is a light spectrometer that detects the wavelength components of light and their amplitude changes.

During last ten years a considerable increase is observed in the numbers of biotechnology and bioengineering laboratories and also departments using spectroscopy. UV-VIS spectroscopy is a well known analytical technic. It has widespread usage in industry sectors such as food and beverage production, pharmaceutical and environmental testing. Spectrometer is the device enables researchers to get great knowledge about samples. Spectrometers are the basic devices for laboratories specially when they are combined with electronic and personnel computers. There are a few spectrometer configurations; single beam, split beam, dual beam.

Single beam spectrometers are cheap and simple devices. They are efficient devices as work done per unit time because spectra measurements are obtained very quickly. CCD cameras are more suitable for single beam spectrometers. Referance and sample spectras are measured seperately in these spectrometers. If the time difference between measurements is prolonged, erroneous measurements may be made due to changes in the operation of the light source.

Dual beam spectrometers have been developed in order not to be affected by changes in the light source due to time-dependent differences. In dual beam spectrometers, the incoming light beam is directed alternately on the referance and the sample by using a chopper mechanism.

In split beam spectrometers, the referance and sample spectra are measured simultaneously by identical beams created by splitting the light coming from the soutce into two beams. More optical and electronic components are used in dual beam spectrometers and in split beam spectrometers.(*10-11*)

1.3.Spectrometer components.

There are three type components of a spectrometers: Optical components, Mechanical components, and Electronical components.

1.3.1.Single slit.

The light enters into spectrometer through an aligned slit (Figure.4). The angle of entering light is controlled by slit. Slit widths are changed from 5 µm to 800 µm and heights are changed 1mm to 2 mm. It must be choosed appropriately for the application. The width of entrance slit must be proportional to detector pixel size because of it effects the resolution of spectrometer.

The light enters into spectrometer from slit. If slit width is larger than wavelength of light, Light beam or wave continues to propagate in the same direction (Figure1.4.). Light is diffracted, if slit width equals or less than wavelength of it (Figure 1.5)

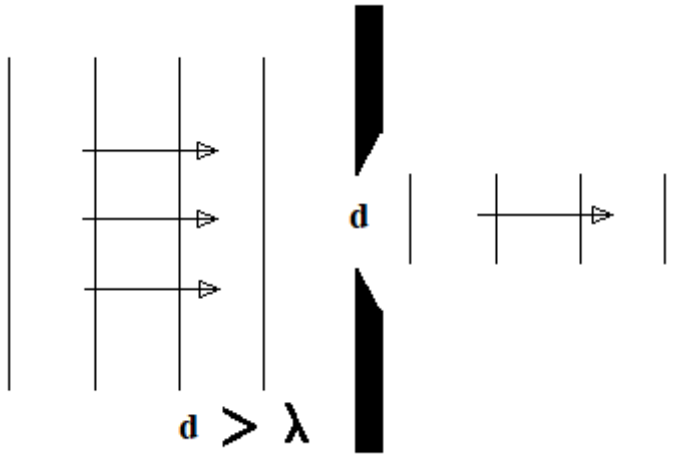

Figure 1.4. Light propagates without diffraction

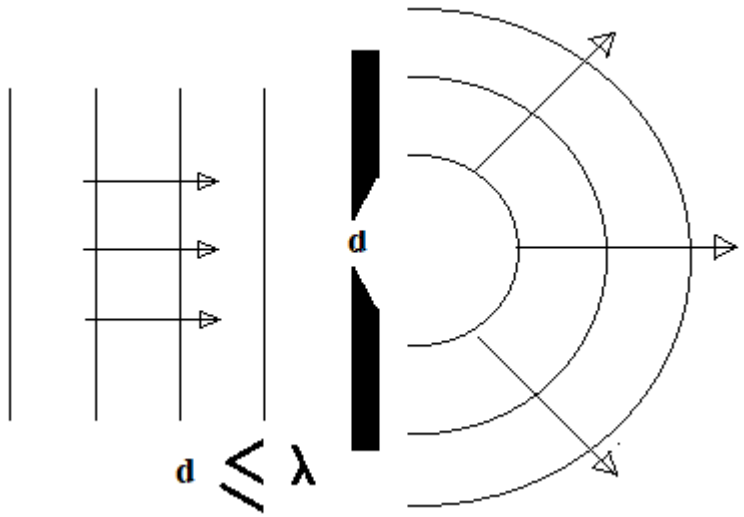

Figure 1.5. light diffracted

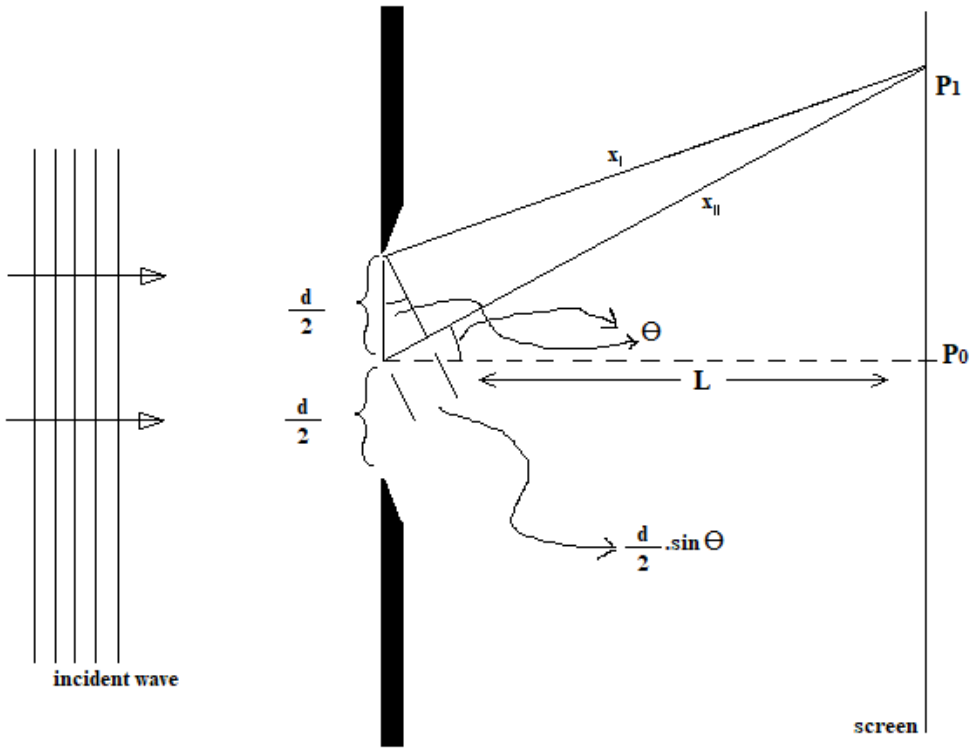

Figure 1.6. First minima position

The condition for the first minima is

$$\frac{d}{2}\sin\Theta = \frac{\lambda}{2}$$

$$d.\sin\Theta = \lambda \qquad \text{eqn 1.8}$$

A general Formula can be written as

$$d.\sin\Theta = m\lambda \qquad m = 1,2,3,4\ldots\ldots \qquad \text{eqn 1.9}$$

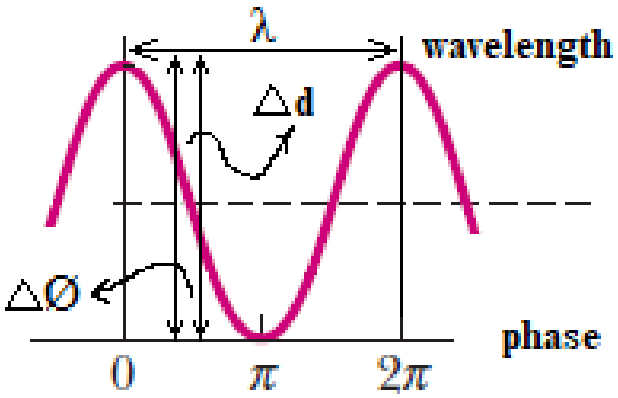

Figure 1.7. Wavelength and phase relation

From Figure.1.6. and Figure 1.7. an equation can be written

$$\frac{Ø}{2\pi} = \frac{d.\sin\theta}{\lambda} \qquad \text{eqn 1.10}$$

$$\frac{Ø}{2} = \frac{\pi d}{\lambda} \sin\Theta \qquad \text{eqn 1.11}$$

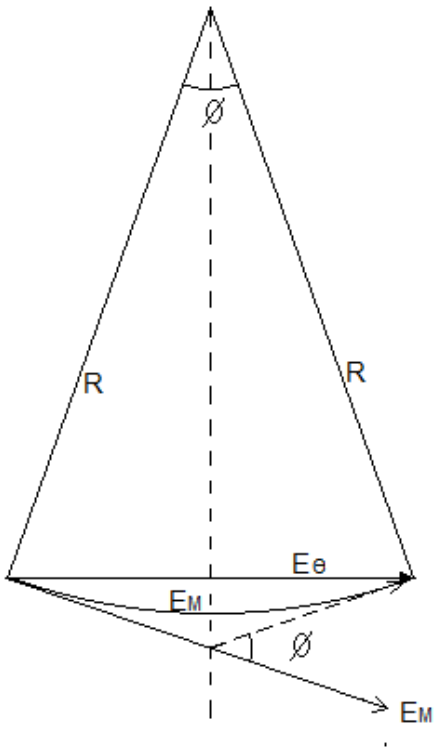

Figure 1.8 Light intensity phasor diagram

We assume that the slit width consist of point light sources arranged side by side. In order to calculate the light intensity at a specified point on the diffraction pattern, the sum of the phasors corresponding to these sources gives us the light intensity at that point. In this case, we need to add the electric field phasors of each point source at the specified point. The sum of these phasors forms E_M in the center of the diffraction pattern. If we select a different point from the center of the diffraction pattern to calculate, the sum of these phasors will be E_θ. Θ is the angle of the point we have chosen to the center of the diffraction pattern(Figure 1.6)(Figure 1.8). Ø is the phase angle of the light at the point we have chosen(Figure 1.8). Now we can write down following formulas:

$$\text{Sin}\,\frac{1}{2}\,Ø = \frac{E_\theta}{2R}$$

$$Ø = \frac{E_M}{R}$$

By solving these equations for R we can obtain fallowig Formula;

$$\frac{E_\theta}{E_M} = \frac{sin\frac{Ø}{2}}{\frac{Ø}{2}}$$

It is known that intensity of an EM wave is proportional to the square of E field of EM wave.(*13*)

$$\frac{I_\theta}{I_M} = \frac{\left(sin\frac{Ø}{2}\right)^2}{\left(\frac{Ø}{2}\right)^2} \qquad \text{eqn 1.12}$$

1.3.2.Mirrors.

Collimating mirror (Figure 1.9) collimates light passing through slit into spectrometer. Divergent light beam becomes a paralel light beam after collimation and is directed towards grating(Figure 1.10).

Figure 1.9 Concave mirrors (*3*)

Diffracted light produces diffraction patern as iluminated and not illuminated lines prallel to slit(Figure 1.10) on collimating mirror. We can evaluate formulas for configuration.

Figure 1.10 The image on collimating mirror

Fallowing rules are Fundamentals for concave mirror imaging.

1 –A light beam hits the mirror by passing through center point of mirror is reflected back in the same way(Figure 1.11 beam 1).

2 –A light beam hits the mirror by approaching parallel to center line of mirror is reflected to focal point(Figure 1.11 beam 2).

3 –A light beam hits the mirror at the vertex point by an angle is reflected by an equal angle to center line(Figure 1.11 beam 3)

These rules are valid for reverse directions of light beam. By using Figure 1.11. a general Formula for concave mirrors can be written(eqn.13):

$$\frac{1}{f} = \frac{1}{o} + \frac{1}{i}$$ eqn 1.13

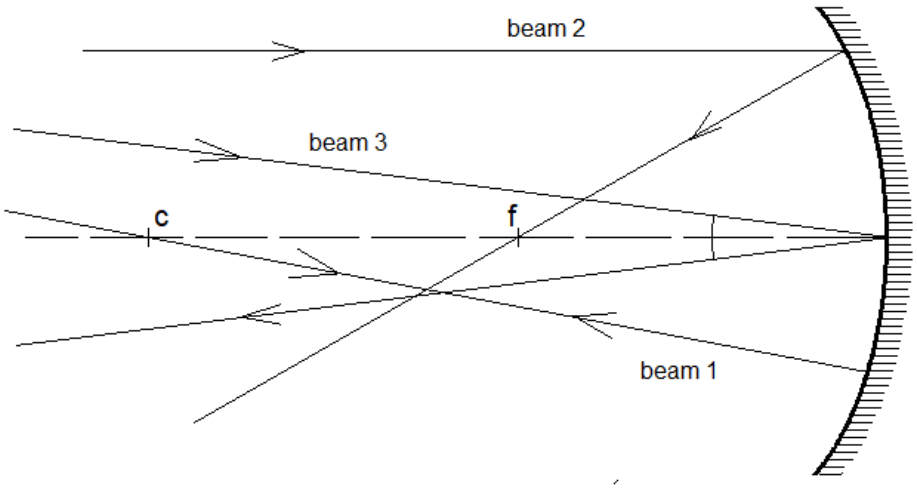

Figure 1.11 Special light beams for concave mirror

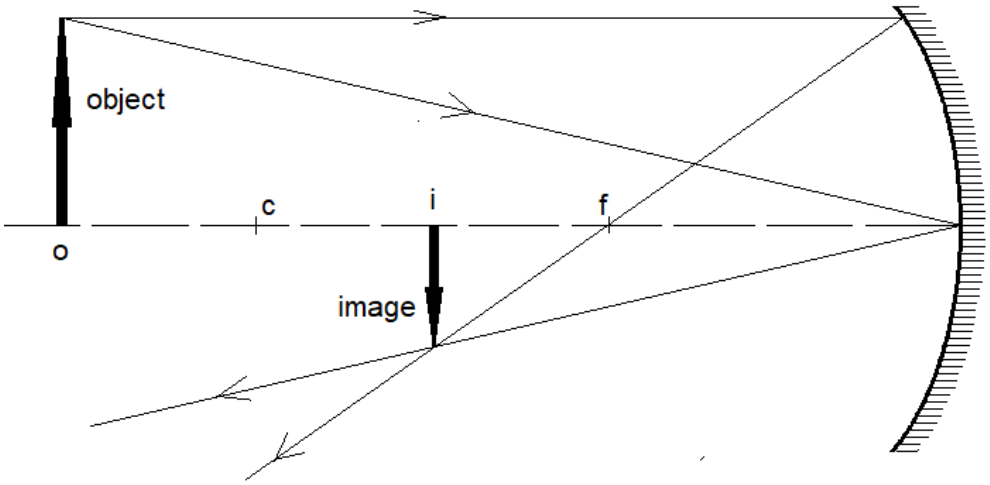

Figure 1.12 Concave mirror image formation

1.3.3.Gratings.

Grating is a dispersive component of spectrometer(Figure 1.13). Prisms are used to dispers light, but dispersion is nonlinear in prisms and light is partially absorbed in them. Gratings eliminate these problems. The dispersion of light by grating may visually resemble its dispersion in a prism, but its formation is very different. Light is dispersed linearly and absorption losses are minimized by reflection. A diffraction grating is made of thin reflective surfaces with very narrow width comparable to wavelengths. We can define three type of gratings according to their manufacturings; ruled gratings, interferance gratings, replicated gratings and two types according to their shapes; plain gratings, concave gratings. Some other clasifications may also be possible.

Figure 1.13 Gratings (*3*)

Reflection grating is a collection of reflecting surfaces with a proportional width to wavelength(λ). Incoming and diffracting wave fronts can be seen in (Figure 1.14). Path difference between waves diffracted from successive grating surfaces is equal to $d(\sin\alpha + \sin\beta)$. If this difference is equal to λ or its multiples they constitute contructive interference.

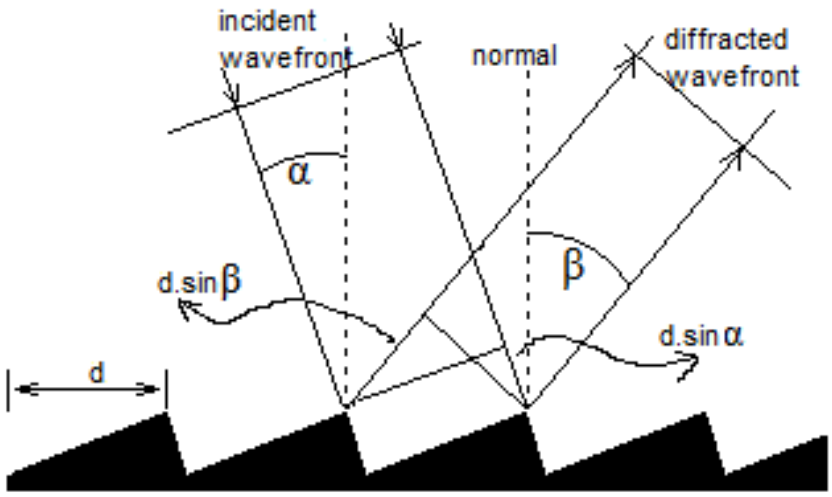

Figure 1.14 Diffraction geometry of grating

The general grating equation can be written by using the sketch Figure 1.14

$$m\lambda = d\,(\sin\alpha + \sin\beta) \qquad \text{eqn 1.14}$$

m……..diffraction order

d……...groove width

α……...incoming light angle

β……...diffracted light angle

Angular dispersion.

Angular dispersion of a grating is the value of angular seperation between different wavelengths. If we differantiate both sides of eqn.11, we can find the dispersion. It is the change in diffraction angle wih respect to change in wavelength. Angular dispersion increases if the Groove width is reduced. This means that for a given order m the angular seperation between wavelengths increases.

$$D = \frac{d\beta}{d\lambda} = \frac{m}{d.\cos\beta} \qquad \text{eqn 1.15}$$

D……..angular dispersion

Linear dispersion.

It is the product of angular diffraction and focal length of grating or system.

$$r.D = \frac{r.m}{d.\cos\beta} \qquad \text{eqn 1.16}$$

r……..focal length of system.

Resolving power

It is the grating seperation power to distinguish very adjecant wavelengths that they have average wavelength λ. It is a dimensionless quantity.

$$R = \frac{\lambda}{\Delta\lambda} \qquad \text{eqn 1.17}$$

Δλ……resolution limit

Another equation can be derived as

$$R = Nm \qquad \text{eqn 1.18}$$

m……..diffraction order

N……..total illuminated grooves

Equation 15 shows us that to increase resolving power, the groove density must be increased.(*12*)

Focusing mirror (Figure 1.9) focuses dispersed spectrum of light coming from grating onto detector and a linear rainbow image (Figure 1.15) occurs on detector CCD camera (Figure 1.16).

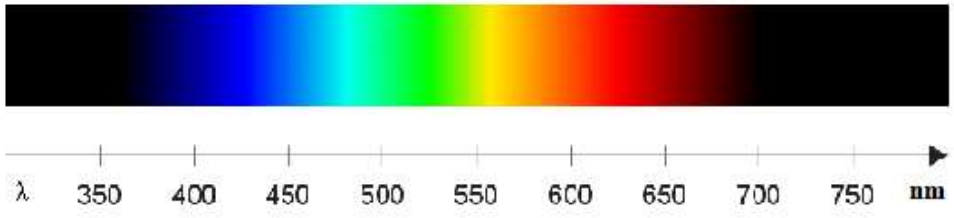

Figure 1.15 Visible spectrum (Rainbow)

1.3.4.CCD cameras.

Today CCD cameras(Fig1.15.) are used mostly as visible detector. They are used to convert the photon energy into digital datas by taking advantage of photoelectric effect. CCD cameras are integrated circuits printed on a slicon substrate. They are made of slicon pixels organised as matrix. Each pixel acts as an electronic well. Illumination causes photoelectric effect in slicon matrix and creates free charges. Those free charges proportional to incoming light energy, fill into wells. When pixels are read one by one in order; the picture or graph can be drawn easily.

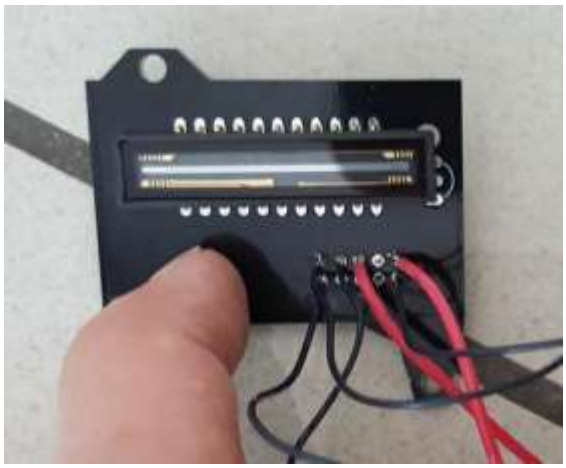

Figure 1.16 Linear CCD camera ToshibaTCD1304DG
(forum.pjrc.com)

"The Rain Drop Bucket" analogy helps to visualize the working principle of a CCD. Rain drops with different intensity correspond to striking light as photon pockets to CCD, and buckets correspond to photosensitive pixels on CCD. Buckets with different amounth of rain move on conveyor belts in paralel lines. They are poured into another serial conveyor bucket line, and finally they are poured into a calibrated bucket and registered one by one. On CCD; light energy is transduced into electrical charges in CCD semicinductor and, they are accumulated under CCD pixels. These accumulated charges are shifted in CCD semiconductor. These analog charge amounts are converted into digital signals by ADC (Analog-Digital Converters) and registered (Figure 1.17, Figure 1.18,Figure 1.19).

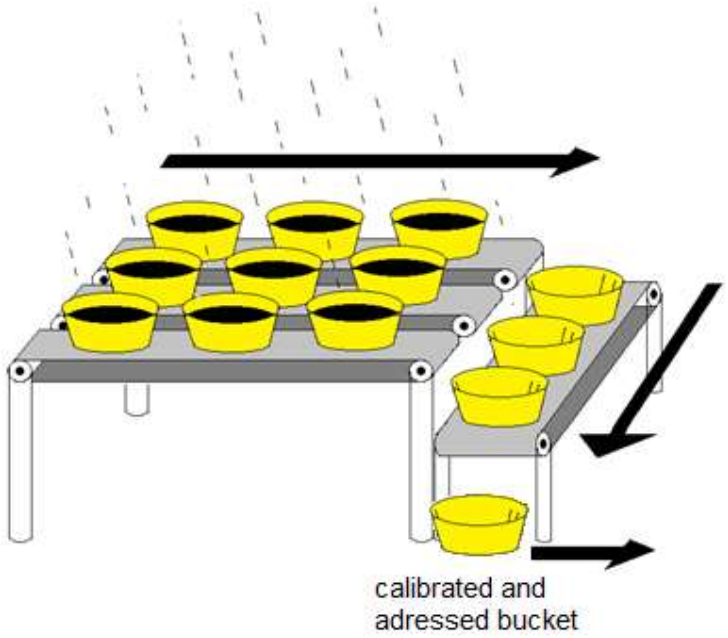

Figure 1.17 Rain drop bucket model

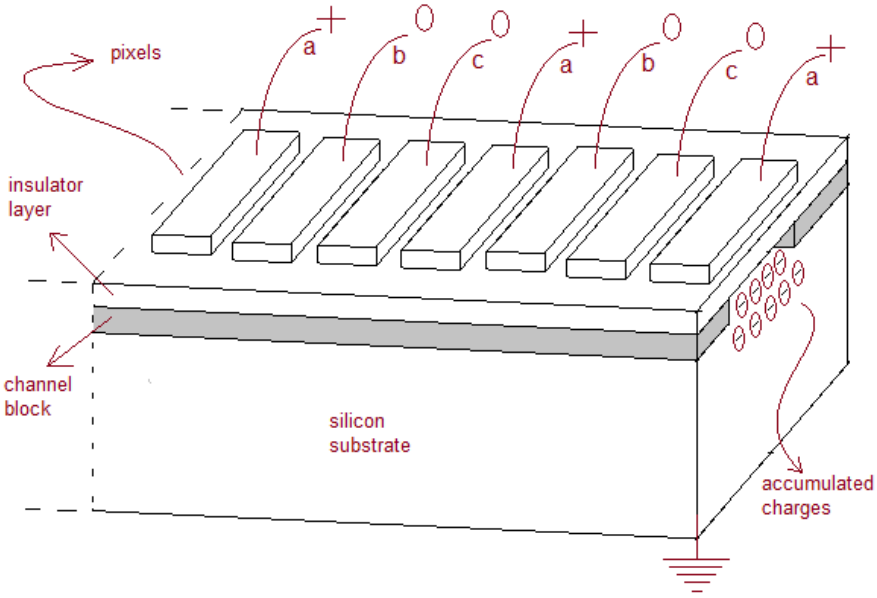

Figure 1.18 Linear CCD structure

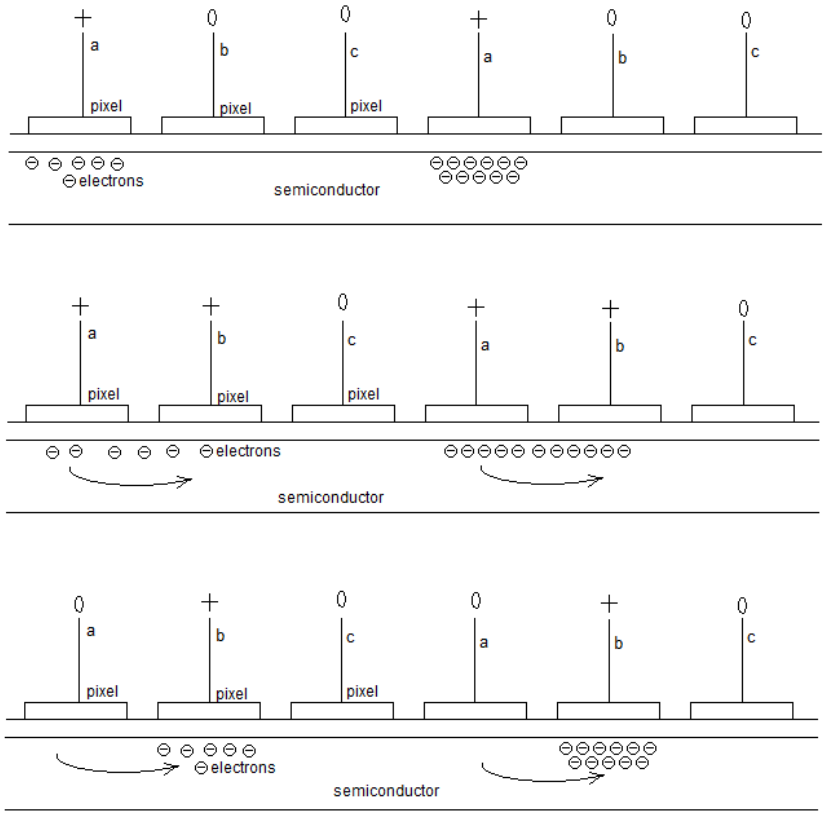

Figure 1.19 Working principle of CCD cameras

1.3.5.Microprocessors Microcontrollers and electronics.

Microprocessors are very small digital circuits with a CPU (Central Processing Unit), memory and input / output units that processes the comands sent to it.

Microcontroller is a computer that takes an external data (program) into its memory, compiles it, and then outputs it. They communicate with the real world using sensors and actuators. Microcontroller based embedded systems can be defined as special pupose computer systems used to perform a specific task. Microcontrollers work more successfuly in real time applications. Microcontrollers can do this in very small sizes and with less energy consumption. They allow us to control electronic systems.

Microprocessor and Microcontroller shuld not be confused. Microcontrollers also include microprocessors in their structures. There are also serial and paralel ports, counters and converters in microcontrollers.

There are the following units in the structure of microcontrollers;

- CPU
- RAM
- ROM
- I / O Ports
- Serial and Parallel Ports
- Counters
- A / D (Analog to Digital) and D / A (Digital to Analog) converters

Microcontrollers are used to communicate with and control the working systems. Texas Instruments Tiva C Series TM4C123G microcontroller (Figure 1.20) is used in this project. Other type microcontrollers also can be used.

Texas Instruments Launch Pad TM4C123G is an evalution board which is built with two ARM Cortex- M4 microprocessors(TM4C123GH6PM) one for debugging.The Launch Pad block structure and properties are given in folloving figure (Figure 1.21). TI TM4C123G Launch Pad is a low cost development board produced for TM4C123GH6PMI microcontrollers. The important features in this design are: microcontroller USB 2.0 connection, hibernation module, PWM module for motion control, programable user buttons, red-green-blue LED applications and connection sockets to other peripheral units.

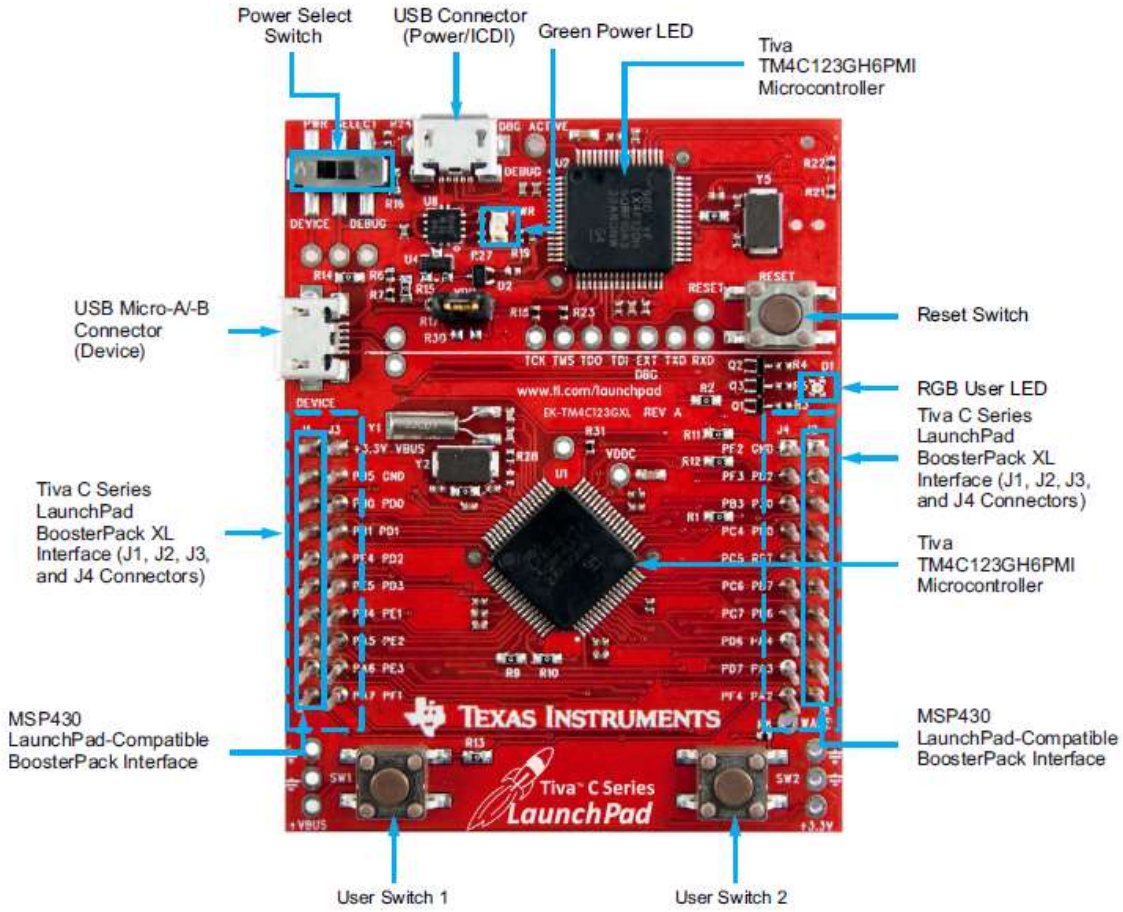

Figure 1.20 Tiva C Series TM4C123G LaunchPad Evaluation Board

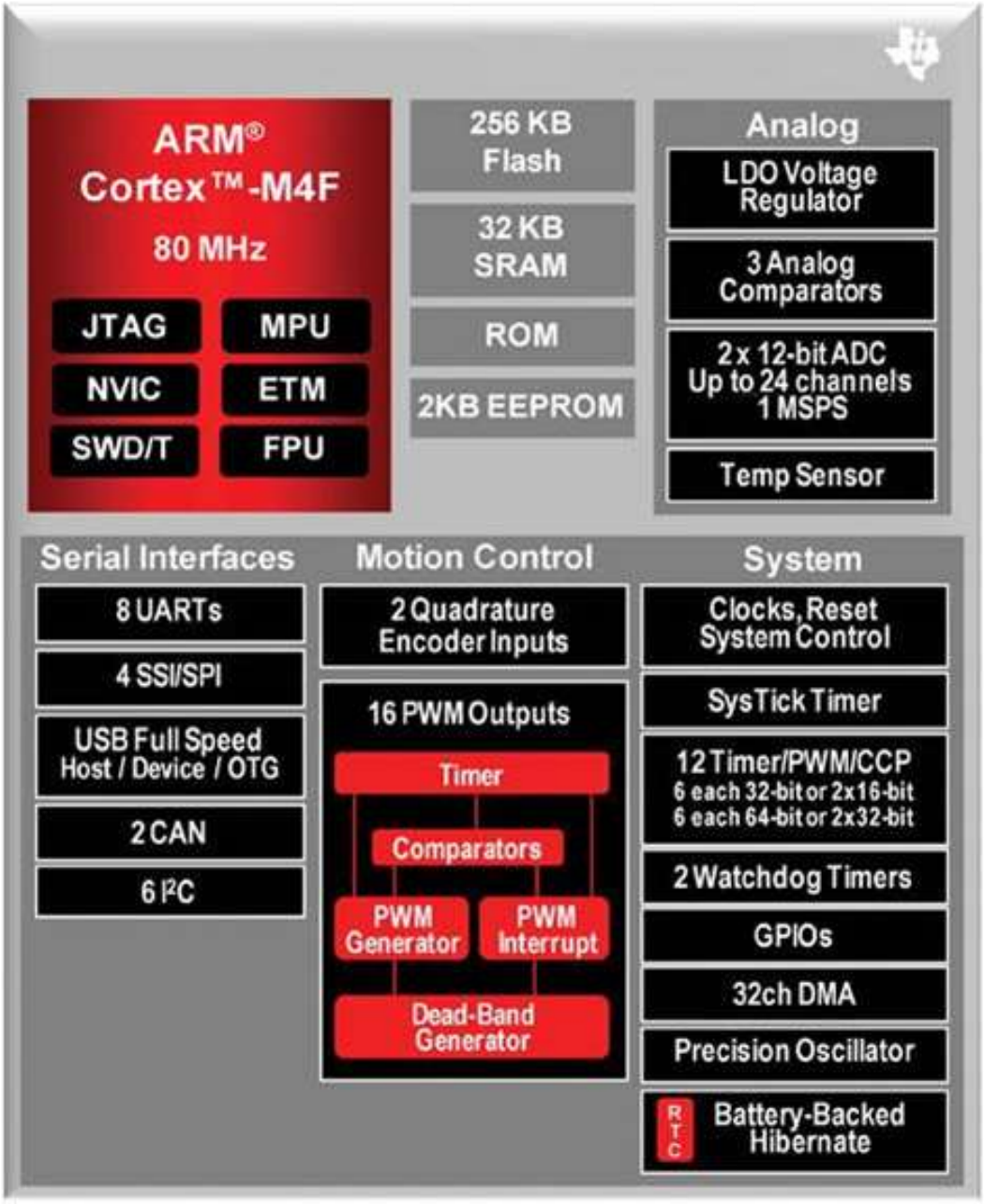

Figure 1.21 Structure and properties of Launch Pad TM4C123G.

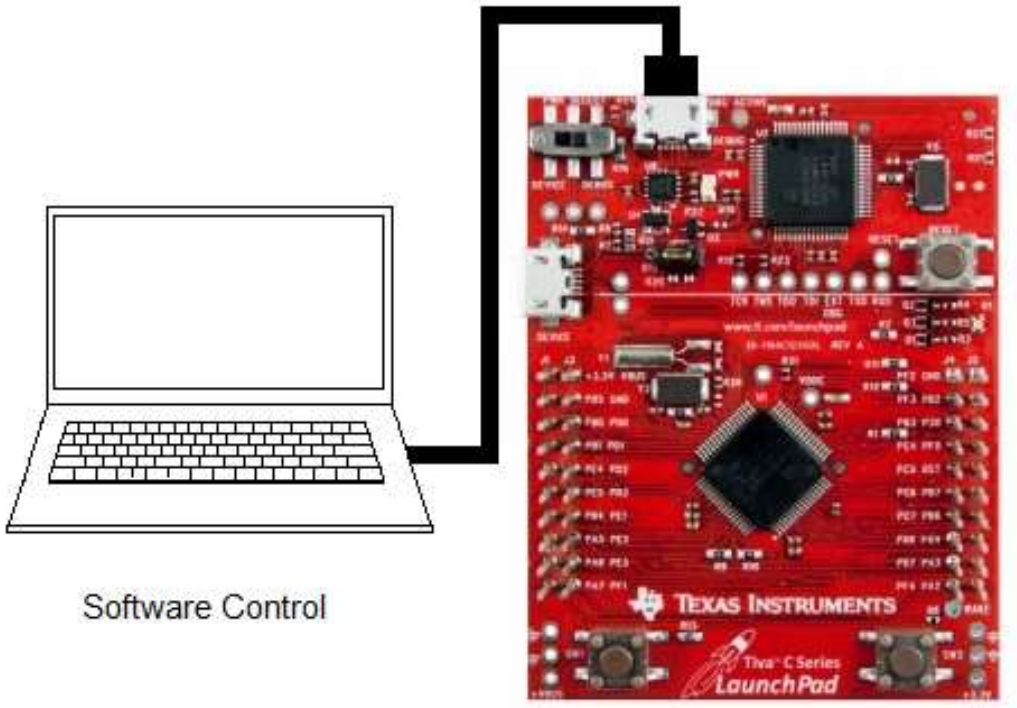

Figure 1.22 Development environment

There are some Integrated Development Environments(IDE) as mentor embedded, IAR systems, ARM-KEIL, and code COMPOSER studio. We used code COMPOSER studio to develop a software for microcontroller in this thesis. CCD working signals are produced and datas coming from CCD are sent to PC by microcontroller(Figure 1.22).

CHAPTER 2

CURRENT PROBLEM AND OUR SOLUTION

2.1. Handheld Spectrometers

The history of portable spectroscopy is started with paint analyzers in 1990s and covered the analyzing alloys by using miniaturized X-ray tube as a source and a silicon PIN diode as detector (XRF analyzers). It is reported that Thermo Fisher Scientific had produced 35,000 handheld XRF analyzers since 1995. After the distruction of the World Trade Center in New York city on September 11,2001, the portable and handheld spectrometers started to have a place on market. The demand was to detect and identify explosives, chemical menaces, etc. Producer companies applied a policy to develope technology and transfered it industrial and civil markets such as agricultural, chemical, pharmaceutical, and clinical. Practical portable NIR devices produced in 2000-2005.

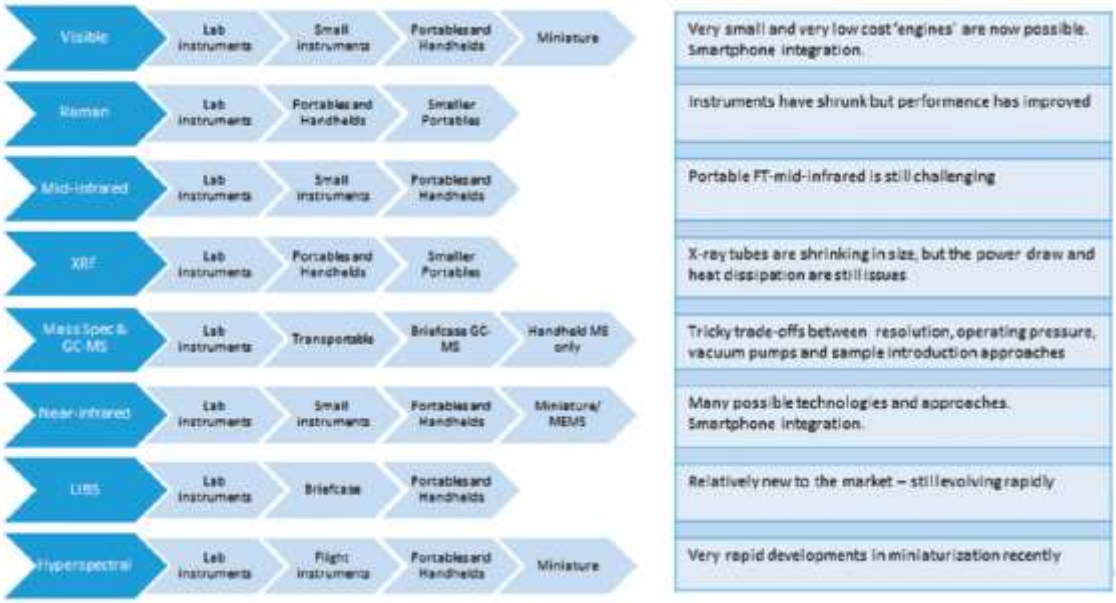

Figure 2.1. Development of portable spectrometers by scientific instrument companies (*4*)

Besides all those developments for miniaturization some types of spectrometers still is not smaller than book dimensions and some types of them stil is not smaller than briefcase such as GC-MS devices

2.2. Usage and Applications

The operators of these devices are usually non-specialists, people of other disciplines or non-scientists. As a result critical questions of end users of portable handheld spectrometers in field:

Is there something?

What is this?

If there is something; is it what we are looking for?

What is the amounth?

Those needs directed the producers and some types of spectrometers are developed as miniature spectrometers, smart phone spectrometer prototypes and low cost spectrometers, limited to special purposes. Smart phone spectrometer developments focused on medical diagnosis and clinical usage to produce solutions without applying to a well equipped laboratory. Another developmental branch is attempting of consumers to predict food quality, freshness, ripeness and contamination includes allergens, pathogens, pesticides.

2.2.1. UV-VIS (250 nm – 700 nm) Spectrometers

These spectrometers are most improved in miniaturization and widely used. This kind of portable instruments are present in market with prices 1000 $ US as lowest cost and some more complicated ones are 3000 – 5000 $ US. There are clinical analyzers working by the same principle. They are used for quantitative analysis of proteins and nucleic acids.

2.2.2. VIS-NIR (400 nm – 1500 nm) Spectrometers

They are used to measure alcahol amounth of beverages and to measure clorophyl, antocyanins, lycopen, caretenoids and moisture. They have applications in health care as to measure hemoglobin, oxyhemoglobin, tissue hydration.

2.3. Market Research

Trading volume on portable spectrometer was USD 1.24 billion in 2017 and it is estimated that it will be USD 2.15 billion with a CAGR of 10.06 % (Compound Annual Growth Rate) at the end of 2023 (Figure 2.2.). Market preference for low cost and quick result created the demand for portable spectrometers. The demand comes off pharmaceutical, food and agricultural industries. Miniaturization trend will increase the growing up of demend. Recent years, increasing governmental regulations on pharmaceutical productions around the world affected the demand positively. The demand of portable spectrometers increased also for molecular spectroscopy and medical screening, specially in North America and Europe(*5*).

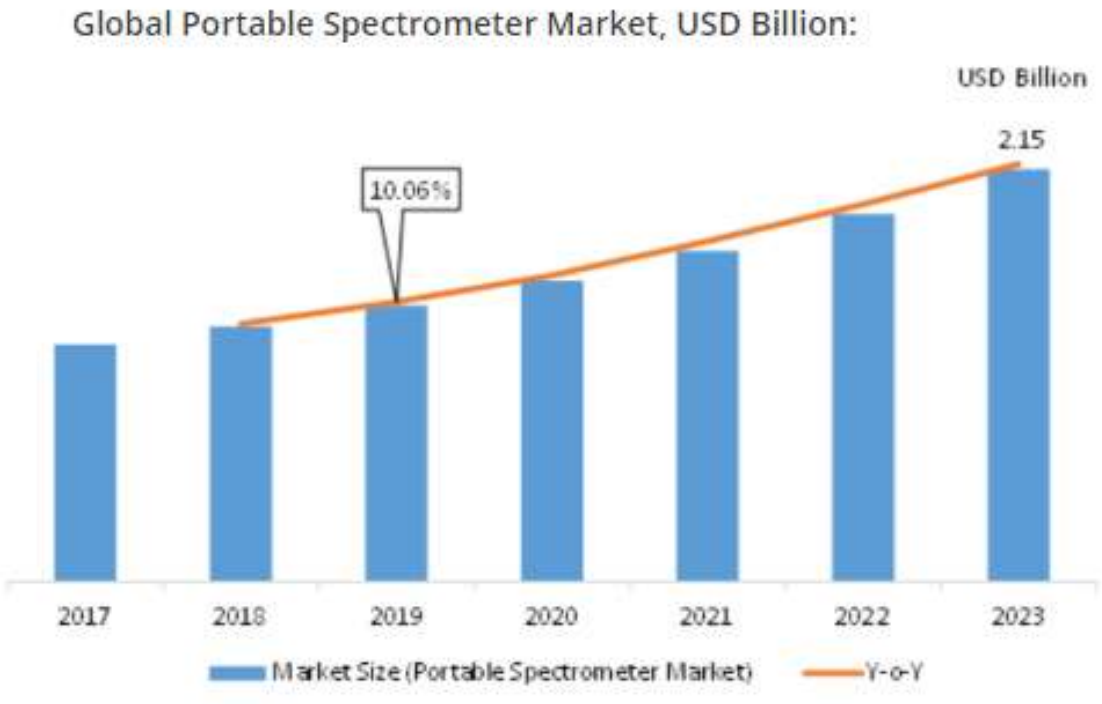

Figure 2.2. Trading volume on portable spectrometer(*4*)

2.4. Conclusion and Solution

Handheld and portable spectrometers are developed and produced by improvements in photonics, optics, mechanics and electronics. Commercial portable instruments are produced working on spectrum from X-ray, UV-VIS, NIR, MIR, FIR, Raman and LIBS to Terahertz region. Because of the operators of these devices are usually non-specialists, operators from different disciplines or non-scientists: These operators need accurate resuls but not spectra to determine the actions to be performed after measurement. As a result, data banks, libraries, and periodic calibration operations are needed for devices internally or externally to maintain quality control and tracebility. However these operations and services increase costs of devices.

In this thesis we aimed bioengineering(biotechnological, medical, clinical) applications of spectrometers and to produce a national production basement on analytical device manufacturing. Additionally recent developments on evaluation of absorbance, reflectance and fluorescent spectra measurements in UV-VIS range such as vascular volume, oxigenation, extracellular matrix extent, metabolic redox states, and cellular proliferation give us valuable measures about tissue properties and diagnosis, prognosis, and treatment of some cancer types(*1*). Thus for every doctor and for every medical laboratory a cheap, portable, handy, and enough accurate UV-VIS spectrometer has become an up to date demand. On the other hand research on this market showed us to take part in this new market is a chance for our national profitability.Therefore production of an analytical VIS-spectrometer is planned as a first step on the way of our aim, by using our laboratory facilities and by using very common optical, electronical, mechanical components in the market.

To reach our goal we decided to produce a VIS-spectrometer which has technical specifications comparable with a commercial spectrometer just like “Ocean Optics USB-2000”

CHAPTER 3

CONSTRUCTION

3.1. Optic box and components.

We produced optical mounting box in our laboratory by using 3D printer(Stratasys-Objet 30). We bought slit, concave mirrors, grating and electronic components from the market around us. Mirrors and grating have been aligned after the optical installation.Visible spectra is focused on CCD camera.In reality, this alignment and focusing process takes a lot of time and needs patience. We have four variables for our alignment and focusing process; two mirrors, grating and CCD camera. These must be aligned for each trial until a clear image of spectra is obtained. We used a micro controller Texas Instrument's "Tiva C Series TM4C123G Launch Pad Evaluation Board" to drive CCD camera and to collect datas from CCD camera. We used a PC and LabView program for processing collected datas to obtain spectras.In this study we observed that all mechanical and optical components can be produced in our laboratories by proper conditions also. (*6, 7, 8, 9*).

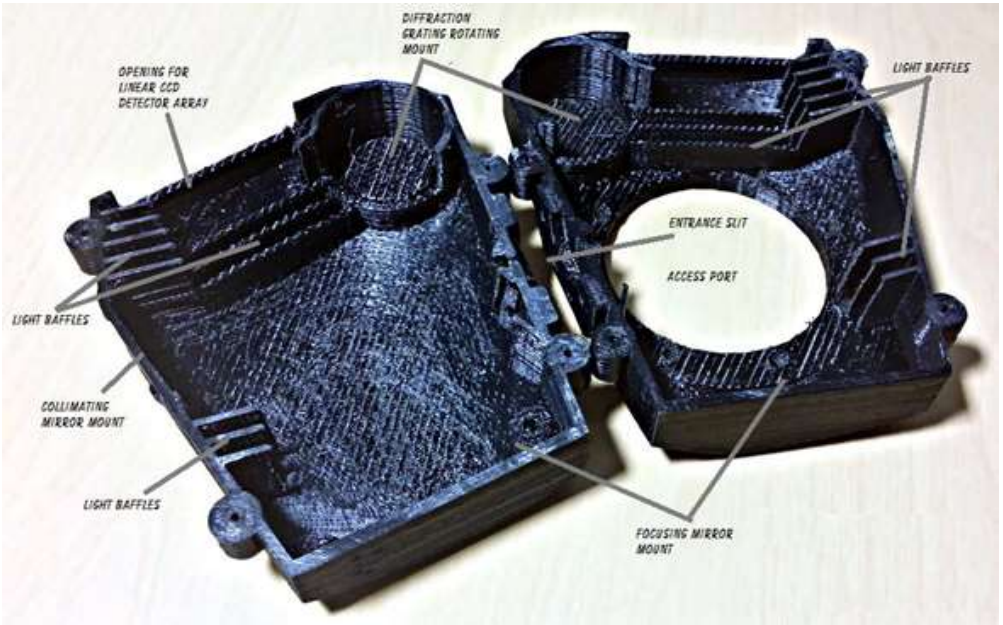

Figure 3.1. Bottom part is on left, top part is on right.

Figure 3.2. Collimating mirror and holder.
Holder is printed by 3D printer.

Figure 3.3. Grating and focusing mirror and their holders.
Holders are printed by 3D printer.

Scientific mirrors are designed precisely enough to be almost perfect. The material used in making the mirror affects the quality of the mirror. In mirror materials, smooth and opaque surface feature is preferred rather than transparency. The change in the thickness of the material used in mirror production and the coating thickness along the mirror surface causes image defects. Therefore, front surface reflective coatings have begun to be preferred in mirrors and coating techniques have been developed.

The most basic material known as the mirror base is glass and when polished, a smooth surface is obtained. When metal coating is applied, a very smooth mirror surface is formed. Since glass can be molded into different shapes, it is very suitable for

making special shaped mirrors. Very common mirror bases are borosilicate (pyrex) glasses or fused silica (quartz) glasses. In order to make mirrors these bases prepared in different forms must be coated. The most common coating is metallic coating. Metals such as silver, gold, chrome and aluminum are used.

Figure 3.4. Slits

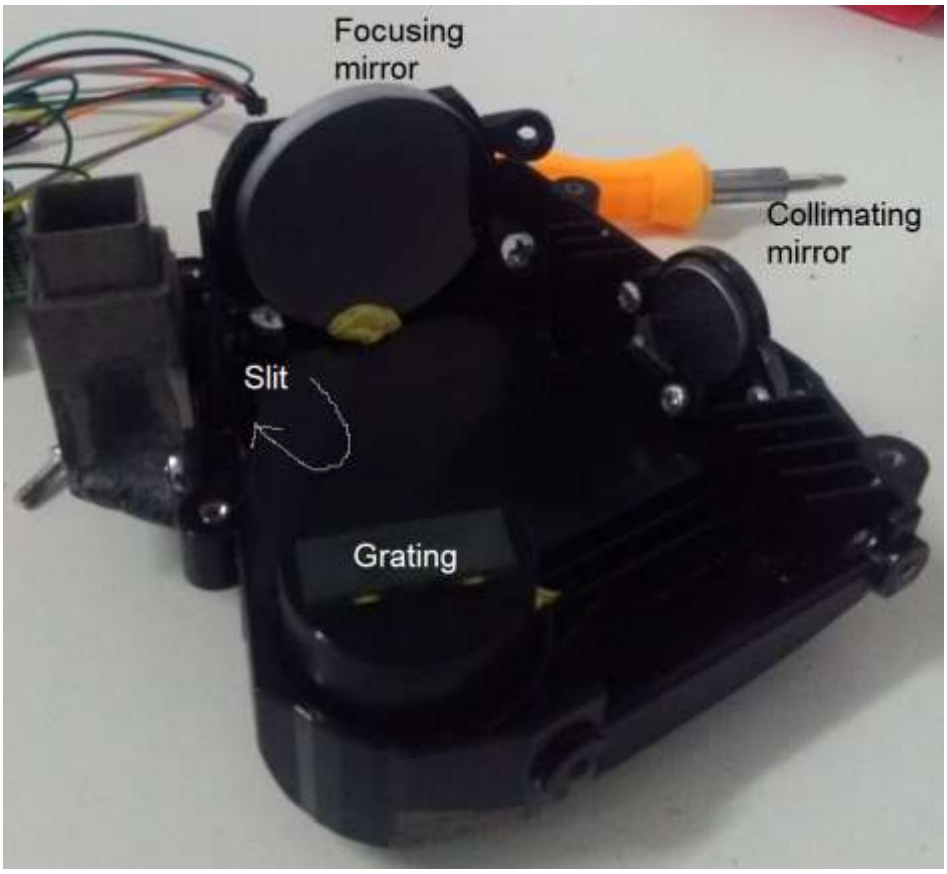

Figure 3.5. Placements of optical components in optical box

Figure 3.6. Optical box

3.2. Electronic box and components.

In order for the CCD camera not to be affected by the led light on Launch Pad TM4C123G, we arranged the electronic box in two sections. We mounted the CCD camera in the first compartment, and the Launch Pad TM4C123G in the second compartment.

Figure 3.7. Electronic box

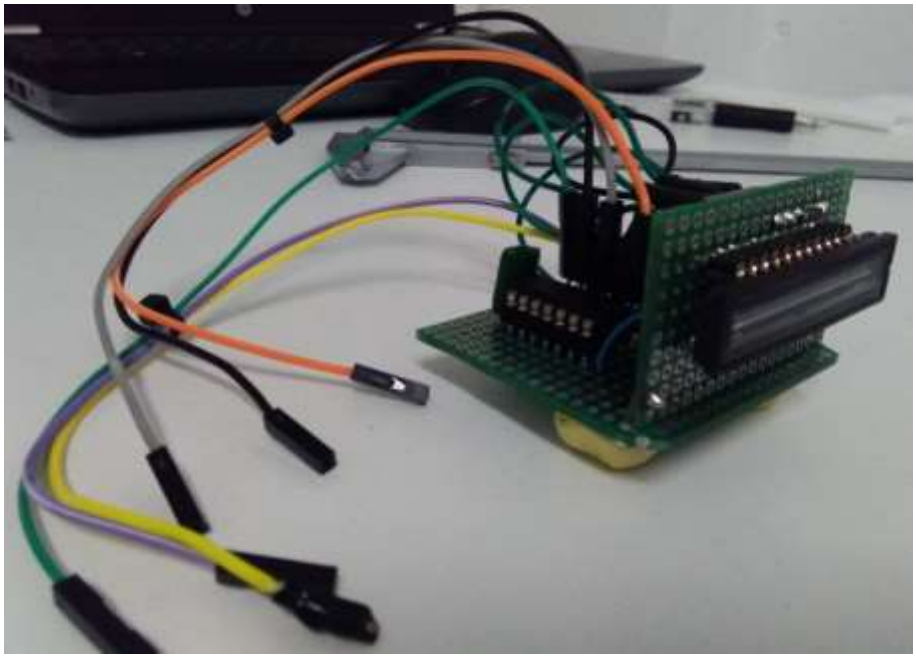

Figure 3.8. CCD camera and driver circuit

Figure 3.9. CCD camera slider bed

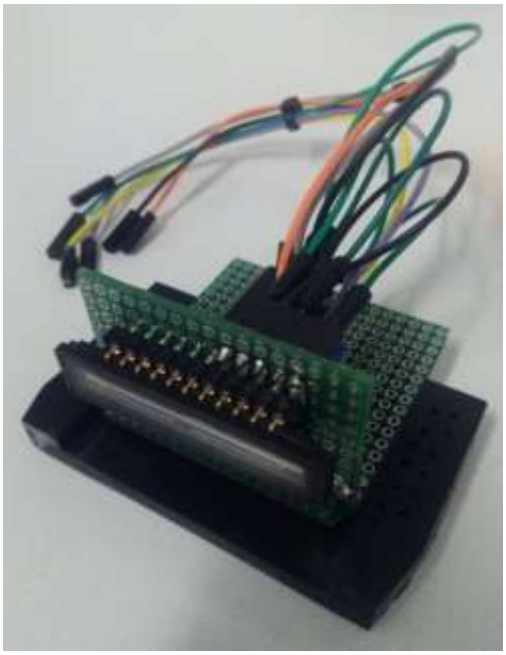

Figure 3.10 CCD camera and driver circuit on slider bed.

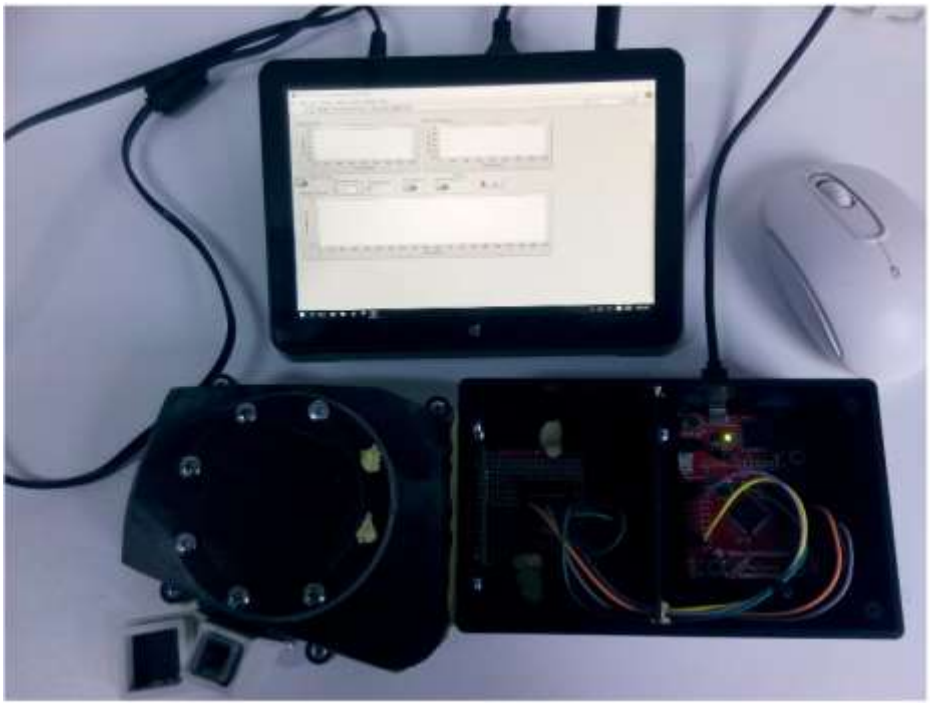

Figure 3.11 Completed set-up of our Prototype Spectrometer.

3.3. Simulation.

Spectromeers are analytical devices that perform chemical and biological analysis by using optical techniques. TracePro is an opto-mechanical software to perform illumination and optical analysis.

TracePro supports the transition process from basic design to production. Its 3D CAD interface is compatible with other mechanical design programs. TracePro provides a design environment that minimizes the time to finalize the design. It creates its models by impoorting lens designs or CAD files and by making solid geometry models. Created models can be modified by scaling, rotating and shifting operations in the interface. Basic parts such as cylinders, spheres, cones and optical elements such as reflectors, frensel lenses can be added.

TracePro imports interactive drawings quickly and uses them to create sophisticated geometries. Visualization options covers wireframe, hidden lines, silhouette, solid and realistic rendering. Rotation, zoom, pan and other manipulation techniques can be used. Several models can be open at the same time and several views of the same model can be open at the same time. To copy and paste objects is posible from one model to another by keystrokes or menu pics.

3.3.1. Optical Properties

Many different material and surface properties can be used for models. Optical properties can be categorised as:

- Material properties as index of refraction, absorbtion, briefringence.
- Aperture diffraction
- Surface properties as reflectance coefficient, surface absorption, surface scattering.
- Bulk scatter
- Mueller matrix
- Surface source
- Temperature distribution

- Thin film stacks for modeling multilayer optical coatings, including anti-reflection coatings, bandpass filters and cut off filters.

Random and periodic surface arrays can be created by using Reptile feature. TracePro analysis mode provides both visual and quantitative analysis of surfaces and objects to measure the feasibility of a design. TracePro simulates light intensity, brightness, illumination and luminous flux on a model or selected surface by Monte Carlo method.

3.3.2. Light Sources

Light sources are modelled by emitting light beams. Light beams are defined by using combinations of three methods.

Grid – Light beams profile are defined by wave lengths, distance, beam orientation, polarization state and degree of polarization.

Surface – Light beams profile are defined by angular distribution and emission spectrum from surfaces of objects using luminous flux or irradiance. Surfaces can be categorized as blackbody or graybody radiators and combination of them as light sources.

Ray file – Light beams profile are defined by XYZ coordinates starting points, direction vectors, polarization states, wavelength datas and initial flux values.

3.3.3. Result analysing tools of TracePro

• Irradiance/Illuminance Maps show irradiance or illuminance, CIE, and true color maps of light incident on, absorbed by, or exiting a selected surface.

• Luminance/Radiance Maps

• 3D Irradiance Plots

• Candela Plots

• Polarization Maps chart

• Incident Ray Tables

• Ray History Tables

TracePro has symmetric 2D and non-symmetric 3D optimizers. These optimizers offers rapid determination of starting design segment points and control. The

optimizers enables ray tracing to analyse the operability of starting design. They supply continious monitoring and readjustments of design.

TracePro simulates energy propagation from any optical or illuminating system directly or via reflection, refraction from surfaces and materials. 3D visualization graphics with linear and log scale supply easy understanding of optical energy fluxes on any surface.

3.3.4. Reporting

TracePro produces several reports as flux reports of surface areas, number of incident ligt rays, incident and absorbed light energy for given sources of wave lengths.

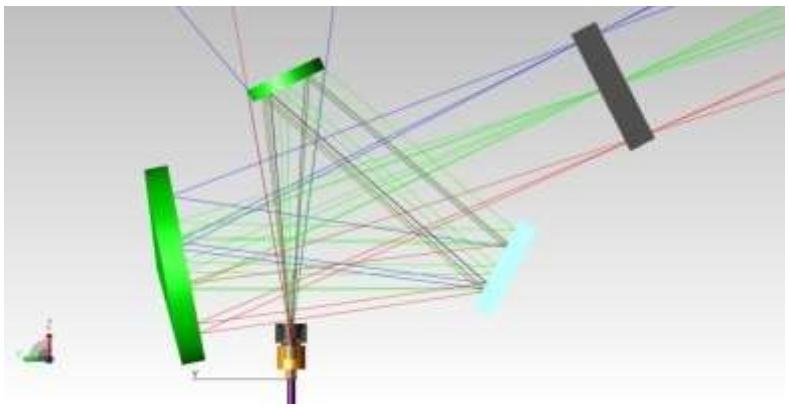

Figure 3.12. Simulation image created by TracePro for our device.

3.4. Programming

To controll whole system a PC is used with a software LabVIEW program. Besides this a C program is used to program the microcontroller “Texas Instruments Tiva C Series TM4C123G LaunchPad Evaluation Board”.

3.4.1. C Programming

C programming language is a general programming language. Different programs can be written in different domains as operating systems, numeric computing,

graphic applications by C programming. It is showed that C is agreeable, ingenious, and versatile programming language. C compilers and operating systems commonly used for several machines. Written programs for a machine in C can be run easily on other machines without changing or only by small changings. It is easy to learn and supplies a wide operation range from high level to low level approaching to assembly.

3.4.2. Embedded System and Embedded C

An embedded system is composed of a hardware and its related software. Both components are restiricted or specialised for a certain mission. Embedded systems have a vide application range in our daily life. They can be used in industria machines, otomobiles, medical devices and many other machines and systems.

C programming is a general programming language, on the contrary embedded C is an extention set of language makes C programming more convenient for embedded systems. A report was released fort his C language extention in 2004, and revised in 2016. Embedded C gives more skills to developers but it is necessary to know the hardware structure of used system.

3.4.3. LabVIEW

LabView is an authoratative graphical programming language. It is compatible with data acquisition, processing and storage. LabView supplies results to user by an interface “Front Panel”.

CHAPTER 4

CALIBRATION

4.1. What is Calibration?

Calibration is the act of comparison between measurement values of Device Under Test (DUT) with the measurement values of Standard device (10 times accurate than DUT) or well known values of some standarts (like atomic emission values).

4.2. Is Calibration Important?

Calibration is crucial if measurament is important, wherever it is. Periodic calibrations and validations are vital to get dependable measurement values and to be confident. We can quantify and control measurement errors and uncertainties by calibration. The accuracy of all measuring devices is degraded over time by some factors as electric and mechanical shocks and chemical impacts (etc). Calibration improves the accuracy of measurements and determines the tracebility to national or international standards. Calibration offers us international reliability.

4.3. How Often to Calibrate?

The differences or shifts of measurement values are observed and compared over time. The calibration period can be predicted according to those measurement shifts.

4.4. Wavelength Calibration of Spectrometer.

Grating spectrometers disperses light into wavelengths and they are recorded by CCD cameras. Resolution of spectrometers depends on entrance slit, Groove density of grating, imaging mirror, andpixel width of CCD camera. Every pixel corresponds to a wavelength. This correspondance is defined and restricted by resolution. The

relationship between pixels and wavelengths is defined by calibration procedure. There are some standards to calibrate spectrometers like mercury lamp emission, deuterium lamp emission, holmium oxide transmission light with didmium filters. The well known Standard is Hg-argon light source spectra.

The polynomial method with proper constants is used as a conventional calibration method. Spectral peak values of Standard sources used as referance points of polynomial and by using this polynomial the other wavelength positions are predicted. This polynomial applies interpolation and extrapolation to predict different matching points other than referances, but this operation lowers the accuracy because the used polynomial is just an approximation of the relationship between pixels and wavelengths. Therefore some deviations occur for distant points other than referances. It is possible to improve this method by using interferometry by producing equidistant calibration lines. Besides these bandwidth also effects calibration accuracy.

Another calibration method is to find a formula or formulas by using system parameters to establish a relationship between pixel numbers and wavelengths. By using geometric optics an analytical calibration model can be established between pixels and wavelengths and optical system parameters.

4.4.1. First Wavelength Calibration Study

The pixel numbers of our device must be matched with wavelength values. Three type of LEDs (Light Emitting Diode) as red, yellow, blue, are bought from market(Electronic Component Supliers). Spectras of LED's are get from Ocean Optics USB2000 spectrometer and our prototype spectrometer. The wavelength peak values and FWHM (Full Width Half Maximum) values from USB2000 are matched with reciprocal pixel numbers from prototype spectrometer(Table.2). Calibration line and its equation are obtained.

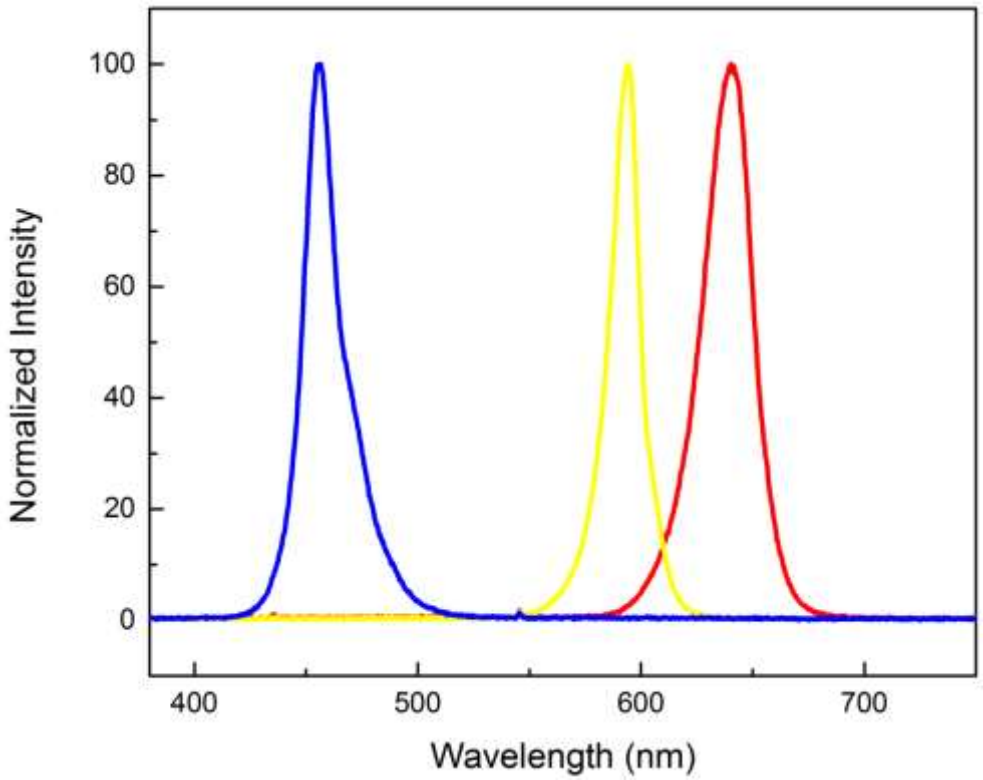

Figure 4.1. Led spectras by Ocean Optics USB2000 Spectrometer

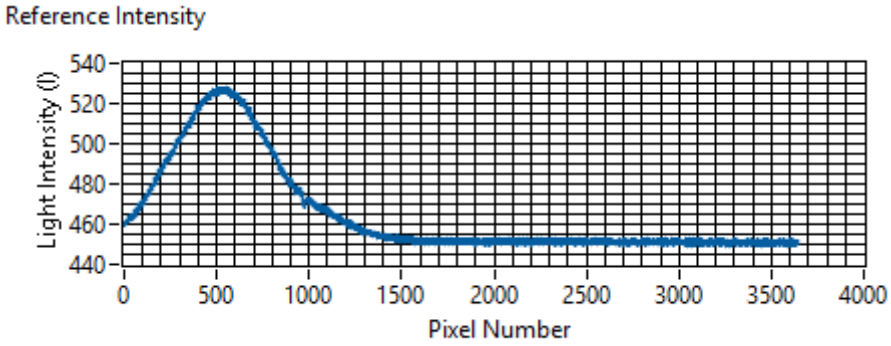

Figure 4.2. Red led spectra by Prototype Spectrometer

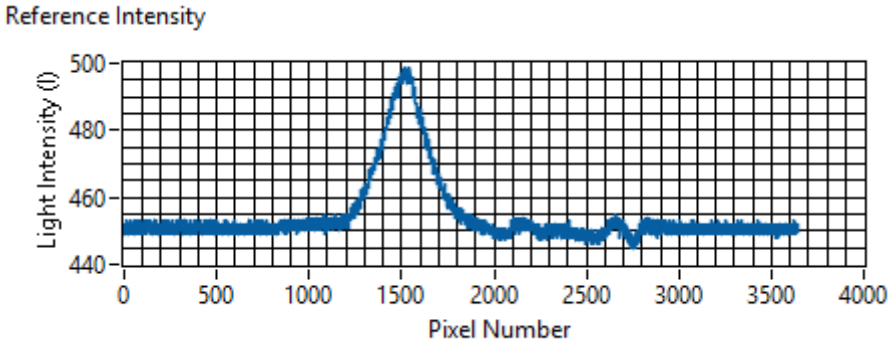

Figure 4.3. Green led spectra by Prototype Spectra

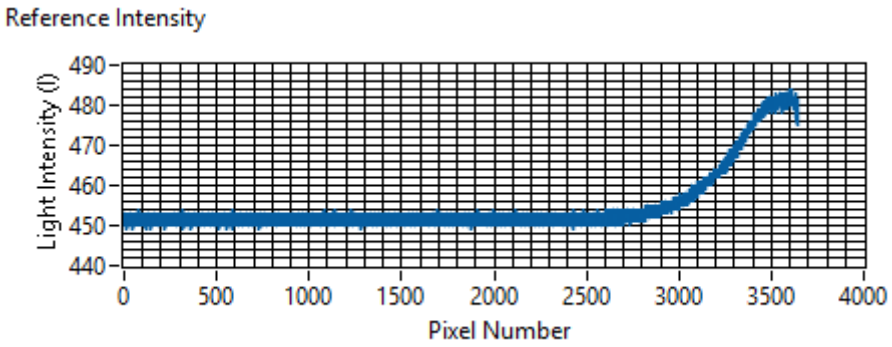

Figure 4.4. Blue led spectra by Prototype spectrometer

Wavelength (nm)	Pixel
455,99	3570
466,1	3267
586,06	1671
594,46	1536
600,37	1382
625,57	850
640,27	540
651,47	210

Table 4.1. First calibration datas

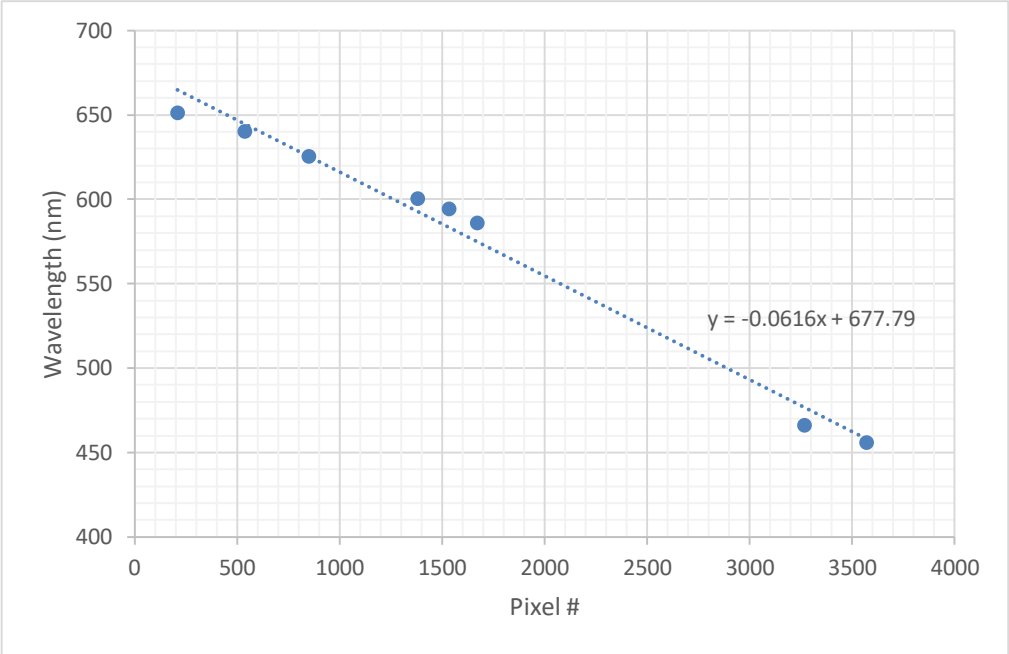

Figure 4.5. Calibration line and equation

Line equation gives us reciprocal wavelengths of CCD pixel numbers.

$$y = - 0.061\ x + 677.7 \qquad \text{eqn 4.1}$$

y……Wavelength

x……Pixel numbe

4.4.2. Second Wavelength Calibration Study

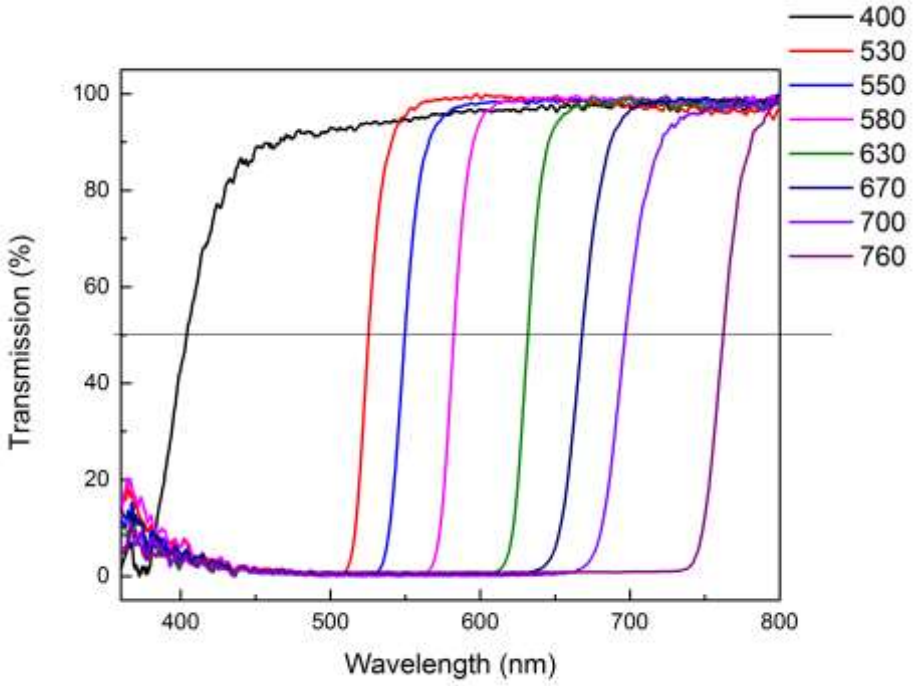

Figure 4.6. Transmission spectras of high pass optical filters taken by Ocean Optics USB2000 spectrometer.

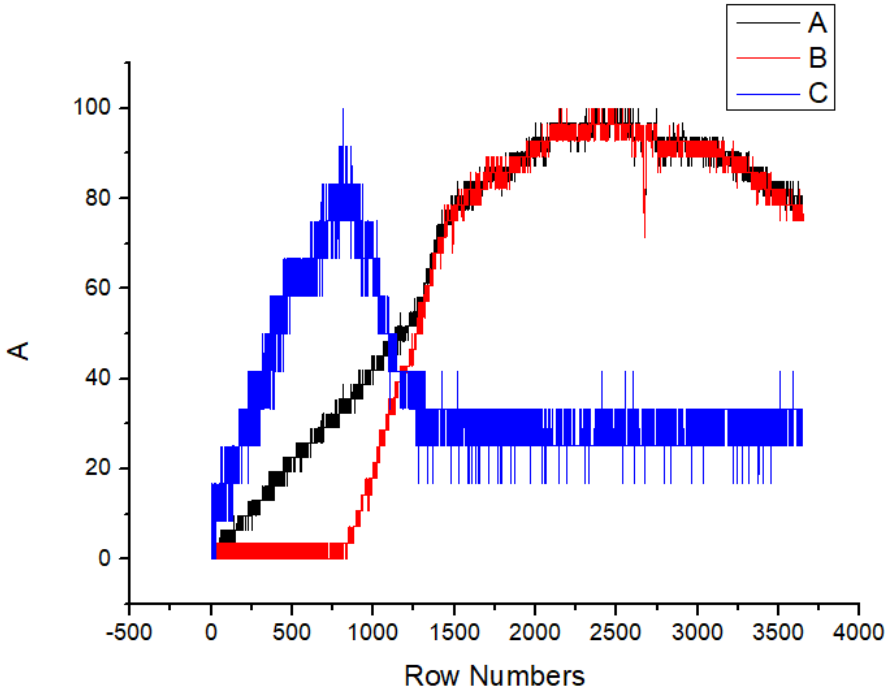

Figure 4.7. 530 nm high pass fitler graphs taken by prototype after 2nd alignment.
[referance (black), transmission (red), absorbtion (blue)]

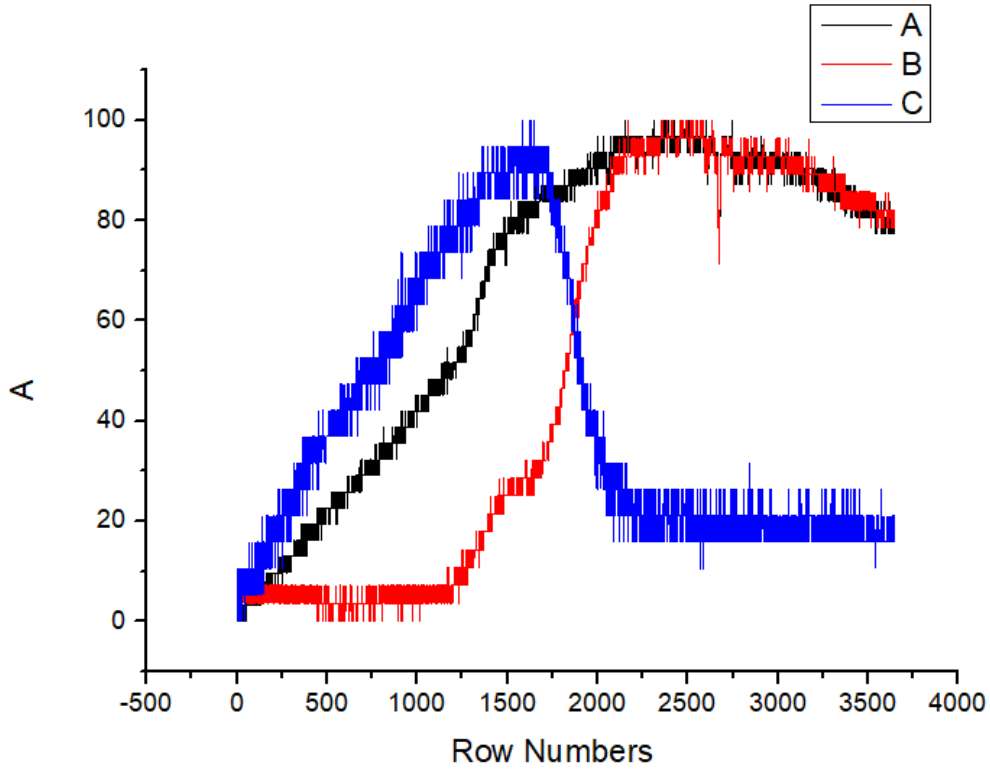

Figure 4.8. 580 nm high pass filter graphs taken by prototypeafter 2nd alignment.
[referance (black), transmission (red), absorbtion (blue)]

After an alignment another calibration line graph is obtained by using transmission spectras of high pass optical filters. Transmission and absorption spectras are get from both USB2000 and prototype spectrometer. The HM (Half Maximum) wavelengths and pixel numbers are matched(Table 4.2.).

Pixel No	Wavelength
2650	524.770
2275	549.235
1775	582.262
1050	631.192
550	668.000
125	696.020

Table 4.2. Second calibration datas

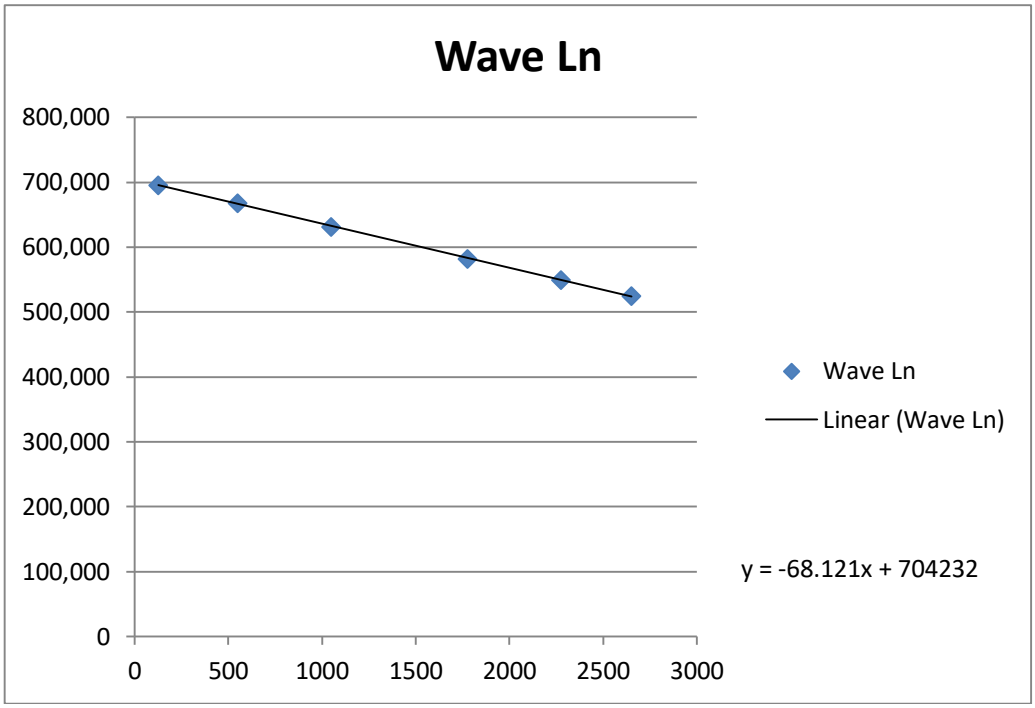

Figure 4.9. Calibration line and equation

4.4.3. Third Wavelength Calibration Study

Optical components and CCD camera realigned. Laser sources are used to predict wavelength range adjustments of our prototype spectrometer. We have seen in the first and second calibration studies that in order for it to be a valid calibration and to be compared with other spectrometers we had to find internationally valid standads. Seeing the characteristic wave lengths of mercury emission on NIST's (NIST: National Institute of Standards and Technology) website, the problem was solved. We used domestic fluorescence lamp to observe the characteristic mercury emissions in our third calibration study. We verified our work by comparing the spectra image obtained from the Ocean optics USB 2000 spectrometer with our constructed spectrometer spectra image. Later on we matched the pixel numbers with the wavelengths using the mercury emission peak values we got from NIST's website and performed the wavelength calibration. Having sharp peaks and stable, constant wavelengths, these light sources are prefered for calibration. After the 3rd calibration and alingment study, we can see the improvement of the spectral line quality in Figure 4.18 and Figure 4.19.

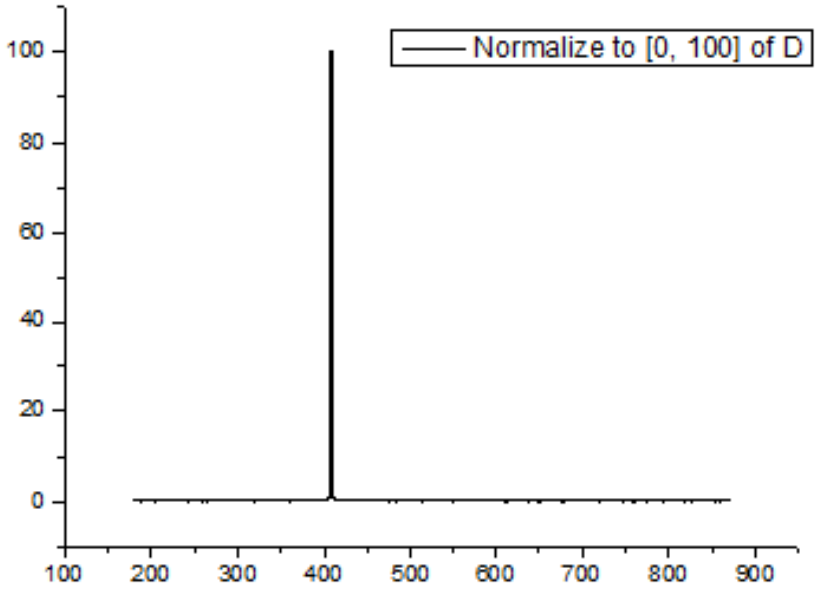

Figure 4.10. Blue Laser Transmission graph taken by Ocean Optics USB2000 spectrometer.

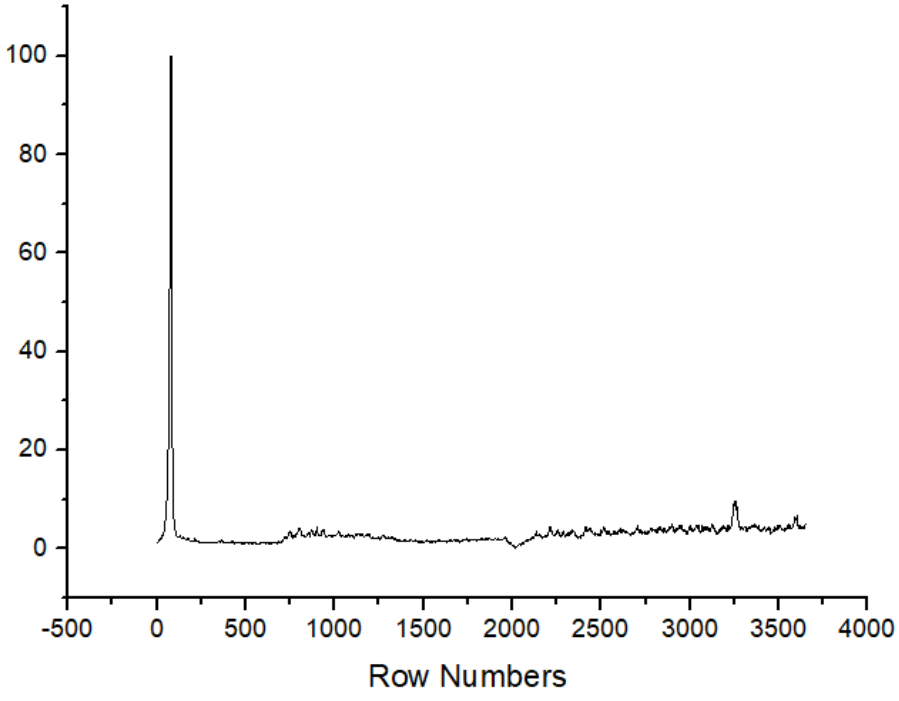

Figure 4.11. Blue Laser Transmission graph taken by prototype

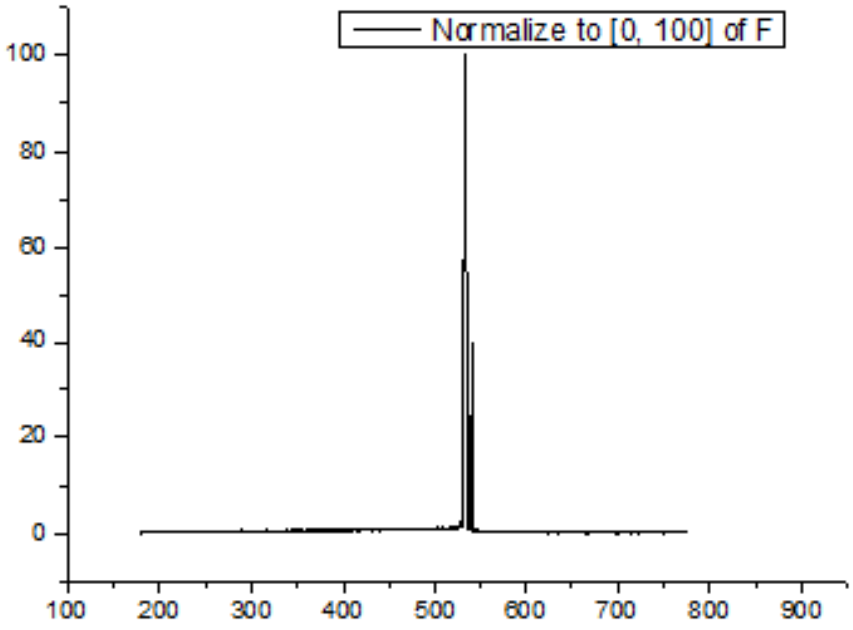

Figure 4.12. Green Laser Transmission graph taken by Ocean Optics USB2000 spectrometer.

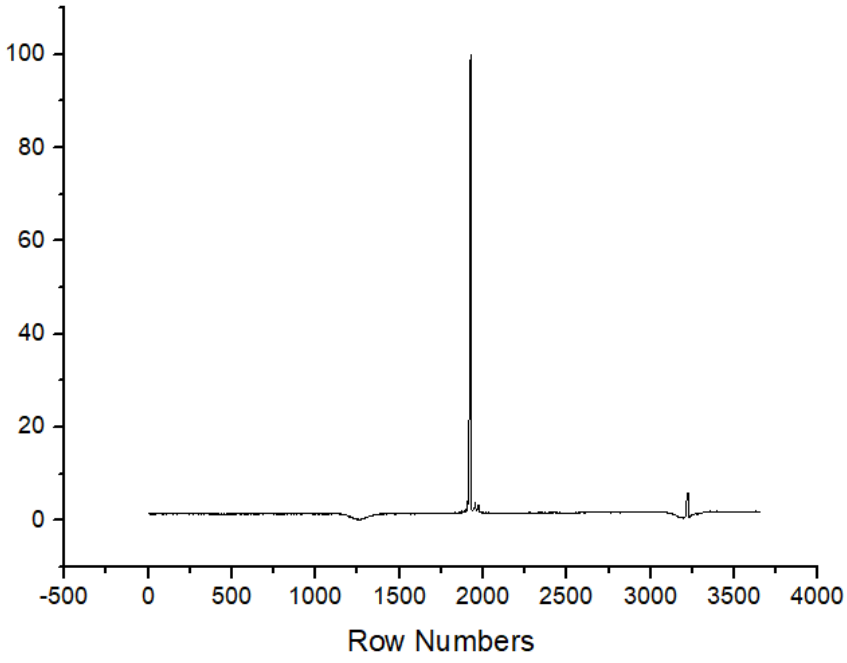

Figure 4.13. Green Laser Transmission graph taken by prototype.

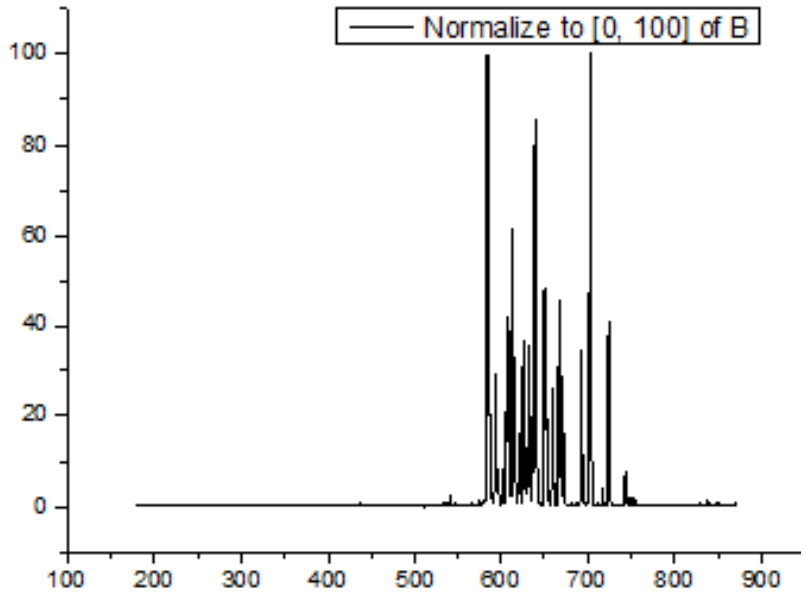

Figure 4.14. Neon bulb emission spectra.
(Measurement by Ocean Optics USB2000 spectrometer)

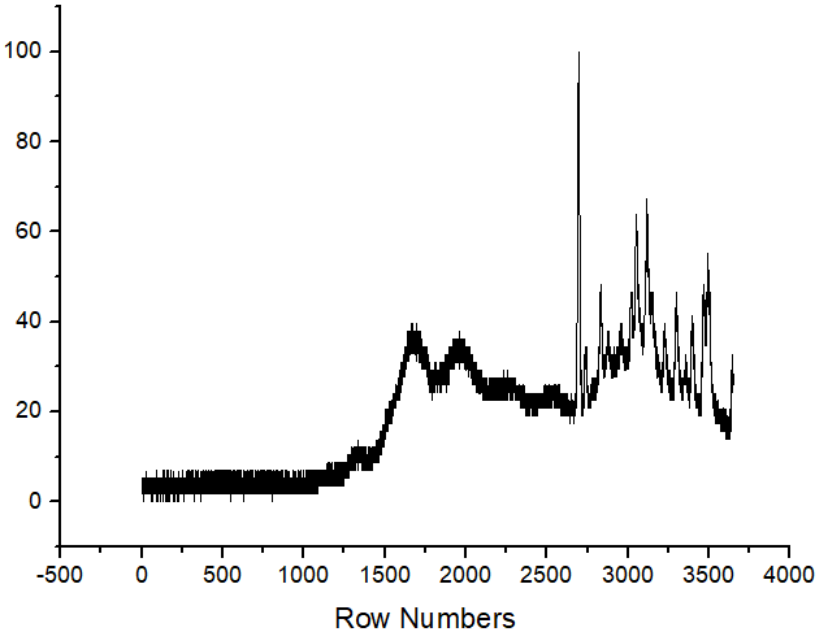

Figure 4.15. Neon bulb emission spectra.
(Measurement by Prototype spectrometer)

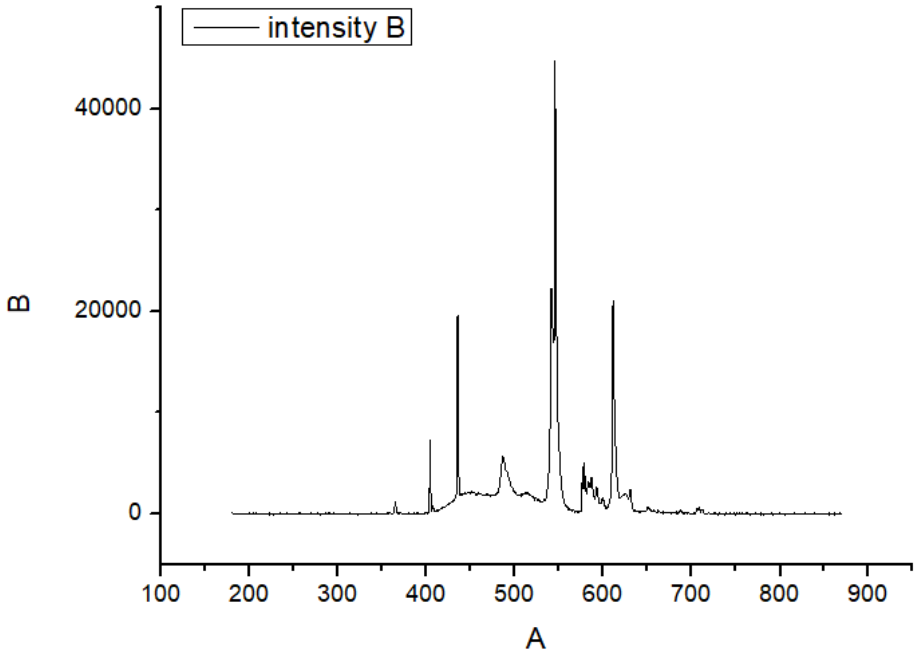

Figure 4.16. Domestic white fluorescent bulb emission spectra.
(Measurement by Ocean Optics USB2000 spectrometer)

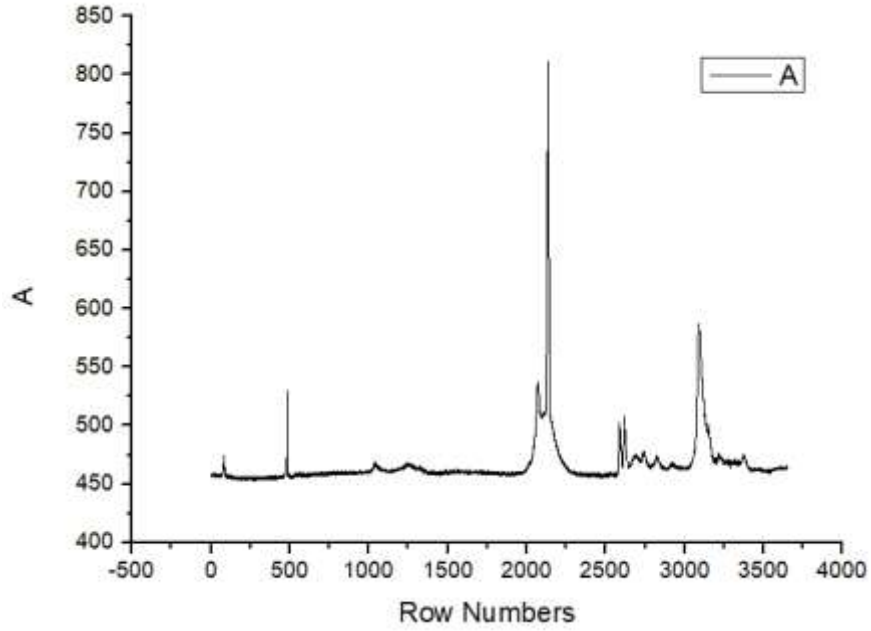

Figure 4.17. Domestic white fluorescent bulb emission spectra.
(Measurement by prototype spectrometer.)

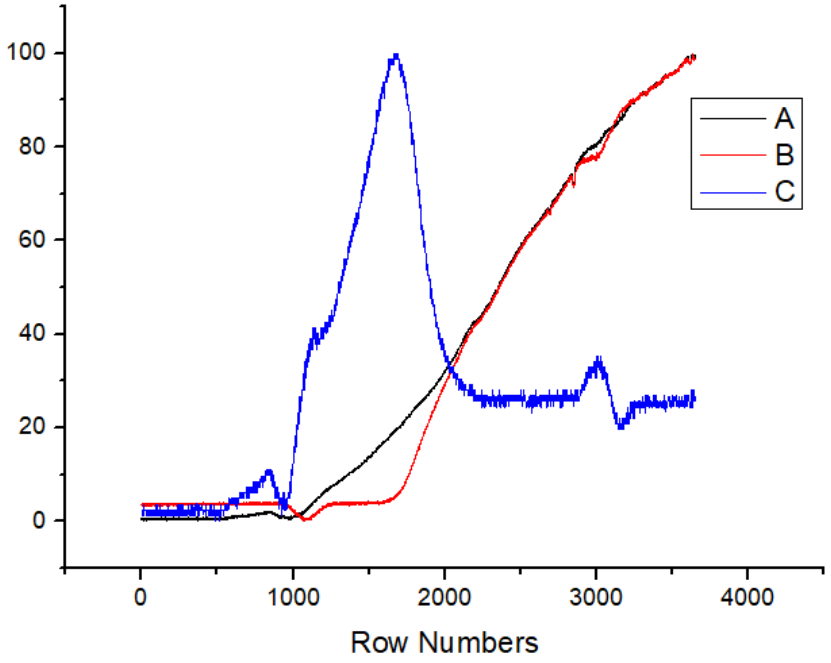

Figure 4.18. 530 nm High pass Filter graph taken by prototype after 3rd alignment.
(Black-Referance, Blue-Absorbtion, Red-Transmission)

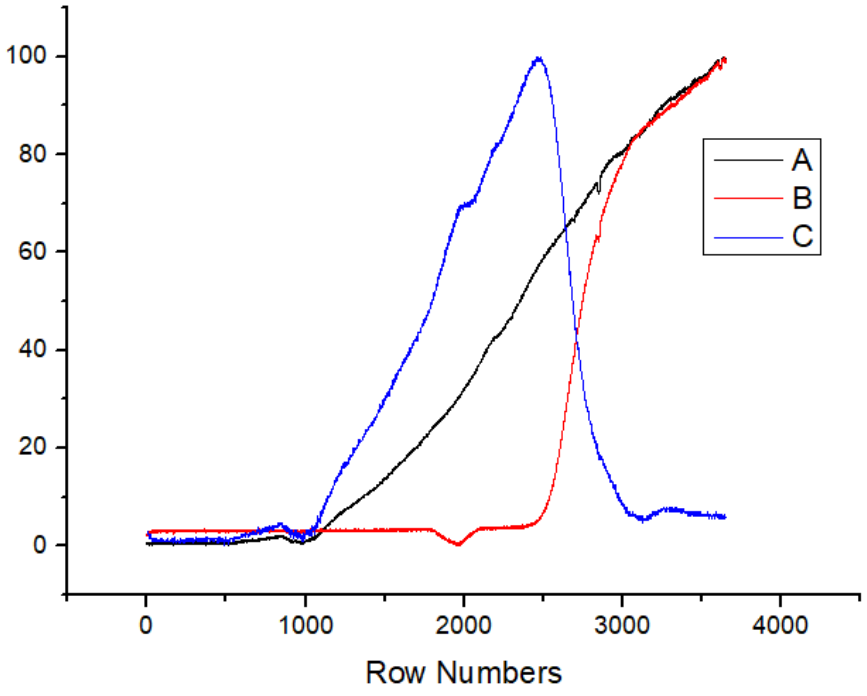

Figure 4.19. 580 nm High pass Filter graph taken by prototype after 3rd alignment.
(Black is referance, Blue is absorbtion, Red is transmission)

Row numbers	Wavelengths (nm)
3566	404.656
3166	435.833
2400	487.700
1513	546.074
1062	576.960
1032	579.066
910	587.000
560	611.000
276	630.000

Table 4.3. Matching points datas for 3rd calibration.

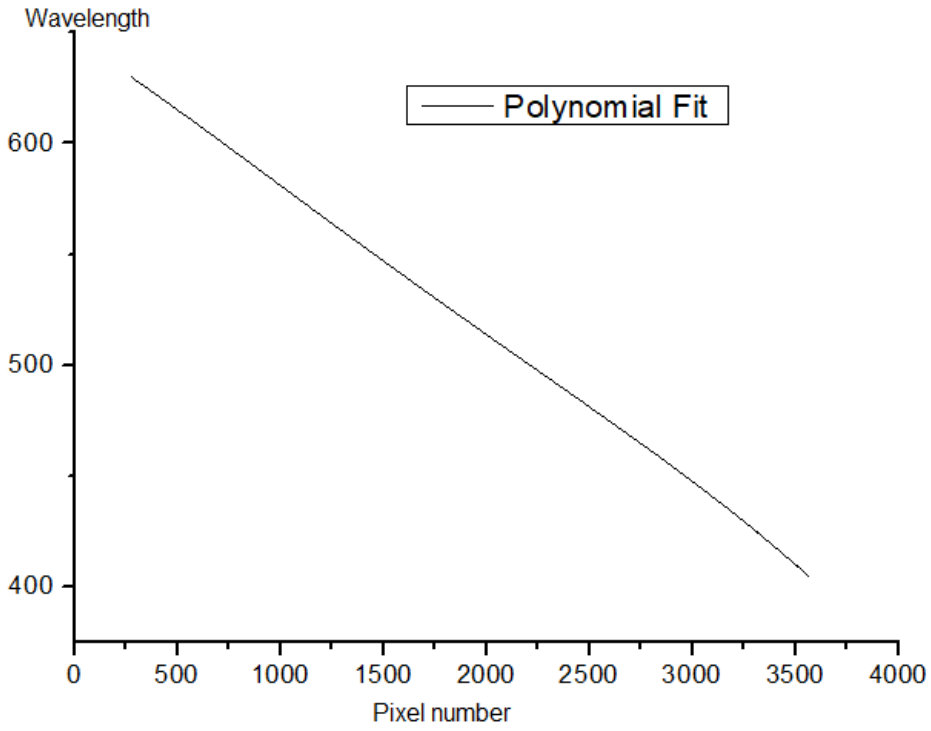

Figure 4.20. Pixel/Wavelength matching graph for 3rd calibration.

Matching polynomial of wavelengths:

$\lambda = -8.23229\,E^{-13}\,x^4 + 5.29101\,E^{-9}\,x^3 - 1.06664\,E^{-5}\,x^2 - 0.05992\,x + 647.17236$

Reciprocal wavelength values are calculated for pixel numbers after finding calibration polynomial. From now on, these values will be used as X axis instead of pixel numbers. As a first application; in Figure 4.21 amplitude values of domestic fluorescent bulb with respect to wavelength values are seen.

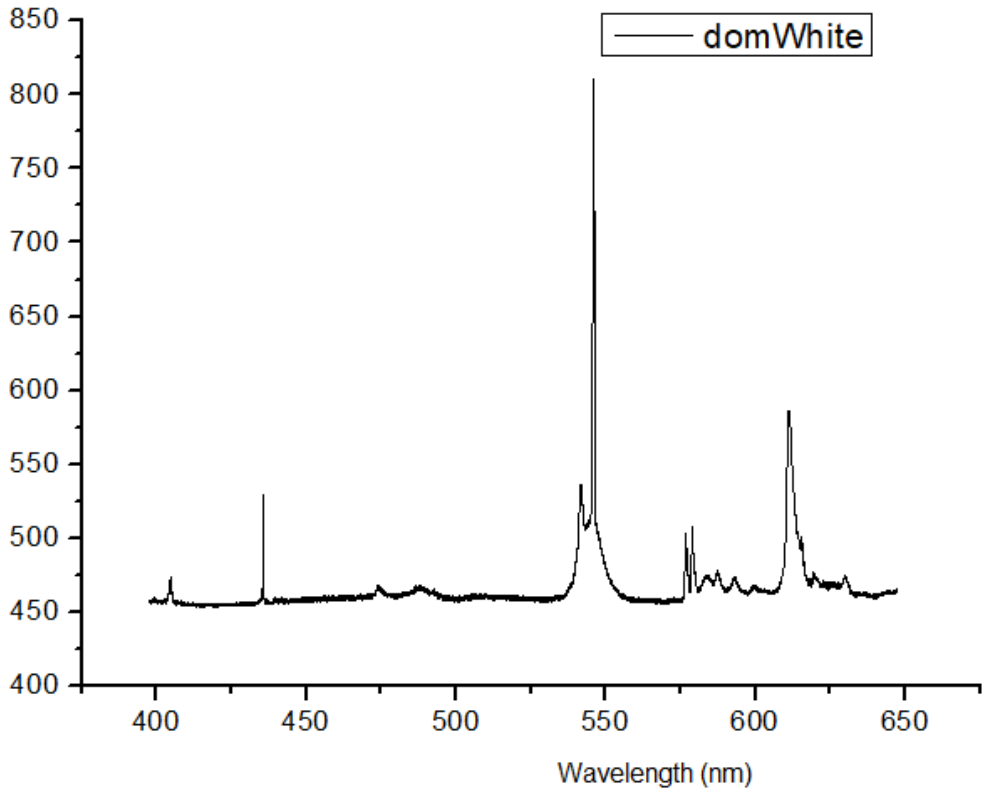

Figure 4.21. Domestic white fluorescent graph after wave length calibration.

4.5. Amplitude calibration

UV-VIS spectras are excited by incident radiation and substance outher shell or bonding electrons are forced to higher energy states. UV-VIS spectral bands are generally wide bands and they do not give us exact values for identification but they are very convenient for quantitative analysis.

Most UV-VIS analysis are relative measurements. System is calibrated by known values of substance at the beginning and a calibration curve obtained. By using this calibration curve unknown quantity of substance is measured. The Beer-Lambert law is the main principle of absorbance spectroscopy.

$$A_\lambda = \log_{10}\left(\frac{1}{T_\lambda}\right) \qquad \text{eqn 4.2}$$

A_λ ……Absorbance
T_λ ……Transmittance

$$T_\lambda = \frac{I_\lambda}{I_{\lambda 0}} \qquad \text{eqn 4.3}$$

I_λ ……..measured light energy (transmitted) at wave length λ.
$I_{\lambda 0}$ …….incoming light energy at wave length λ.

Absorbation olso proportional to path length of light in sample and concentration.

$$A_\lambda = \varepsilon_\lambda . c . b \qquad \text{eqn 4.4}$$

ε_λ …….molar absorptivity
c ……...concentration of solution(mol/L)
b ……...path length in solution(cm)

If we write concentration in gr/L, ε_λ becomes a_λ (absorptivity).

To perform amplitude calibration; we prepared three different solutions. Because of Most UV-VIS analysis are relative measurements.

4.5.1. Apparatus

Volumetric flasks, Pipettes, 1 cm quartz cuvettes.

Used solutions as Standard

Fallowing solutions are prepared.

23.26 gr $CuNO_3$ / 100 ml water (1M)
29.10 gr $CoNO_3$ / 100 ml water (1M)
26.29 gr $NiSO_4$ / 100 ml water (1M)

50 ml $CuNO_3$ (1M) + 50 ml water = 100 ml $CuNO_3$ (0.5M)
50 ml $CoNO_3$ (1M) + 50 ml water = 100 ml $CoNO_3$ (0.5M)
50 ml $NiSO_4$ (1M) + 50 ml water = 100 ml $NiSO_4$ (0.5 M)

10 ml $CuNO_3$ (1M) + 90 ml water = 100 ml $CuNO_3$ (0.1M)
10 ml $CoNO_3$ (1M) + 90 ml water = 100 ml $CoNO_3$ (0.1M)
10 ml $NiSO_4$ (1M) + 90 ml water = 100 ml $NiSO_4$ (0.1M)

5 ml $CuNO_3$ (1M) + 95 ml water = 100 ml $CuNO_3$ (0.05M)
5 ml $CoNO_3$ (1M) + 95 ml water = 100 ml $CoNO_3$ (0.05M)
5 ml $NiSO_4$ (1M) + 95 ml water = 100 ml $NiSO_4$ (0.05M)

1 ml $CuNO_3$ (1M) + 99 ml water = 100 ml $CuNO_3$ (0.01M)
1 ml $CoNO_3$ (1M) + 99 ml water = 100 ml $CoNO_3$ (0.01M)
1 ml $NiSO_4$ (1M) + 99 ml water = 100 ml $NiSO_4$ (0.01M)

4.5.2. Procedures

23.26 g $CuNO_3$ is dissolved in 100 mL water and prepared 1M $CuNO_3$ solution. 29.1 g $CoNO_3$ is dissolved in 100 mL water and prepared 1M $CoNO_3$ solution. 26.29 g $NiSO_4$ is dissolved in 100 mL water and prepared 1M $NiSO_4$ solution.

Secondly 50 mL, 1M solutions are diluted with 50 mL water and prepared 0.5M solutions for each compound.

As a third step 10 mL, 1M solutions are diluted with 90 mL water and prepared 0.1M solutions for each compound. By applying same procedure we prepared 0.05M and 0.01M solutions of each compound also.

These solutions are tested by prototype spectrometer. The same solutions are tested by shimadzu spectrometer and resultant graphs are used as conrol group. Figure 4.22, Figure 4.24, Figure 4.26.

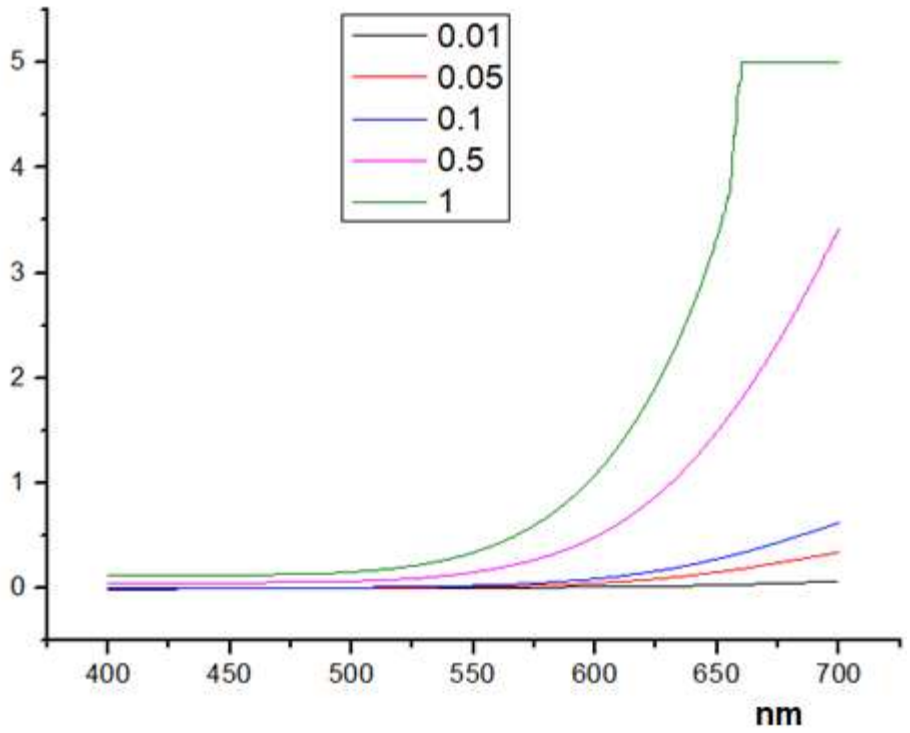

Figure 4.22. $CuNO_3$ referance spectra by shimadzu

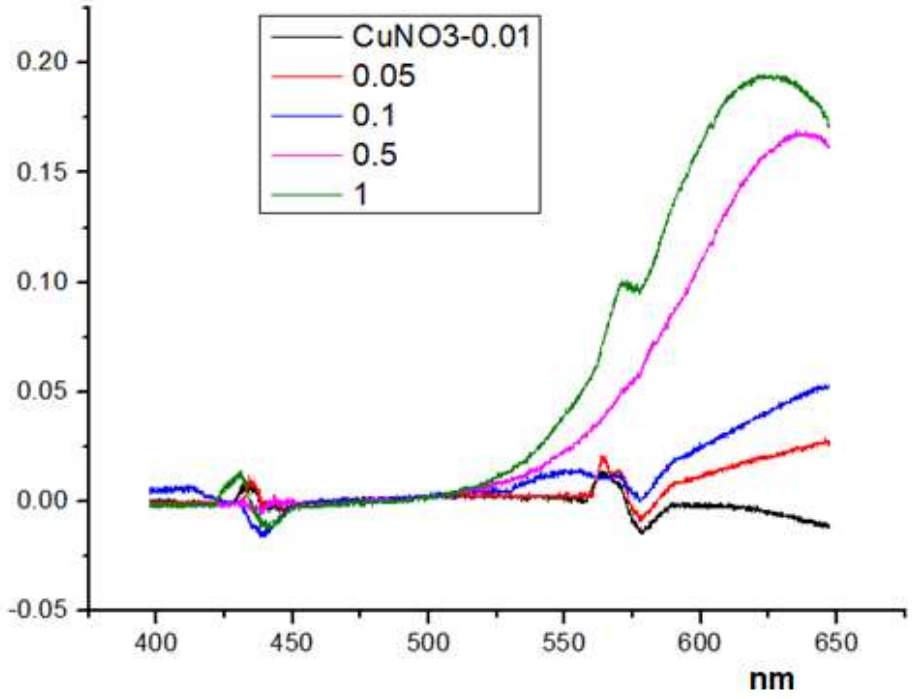

Figure 4.23. $CuNO_3$ spectra by protype

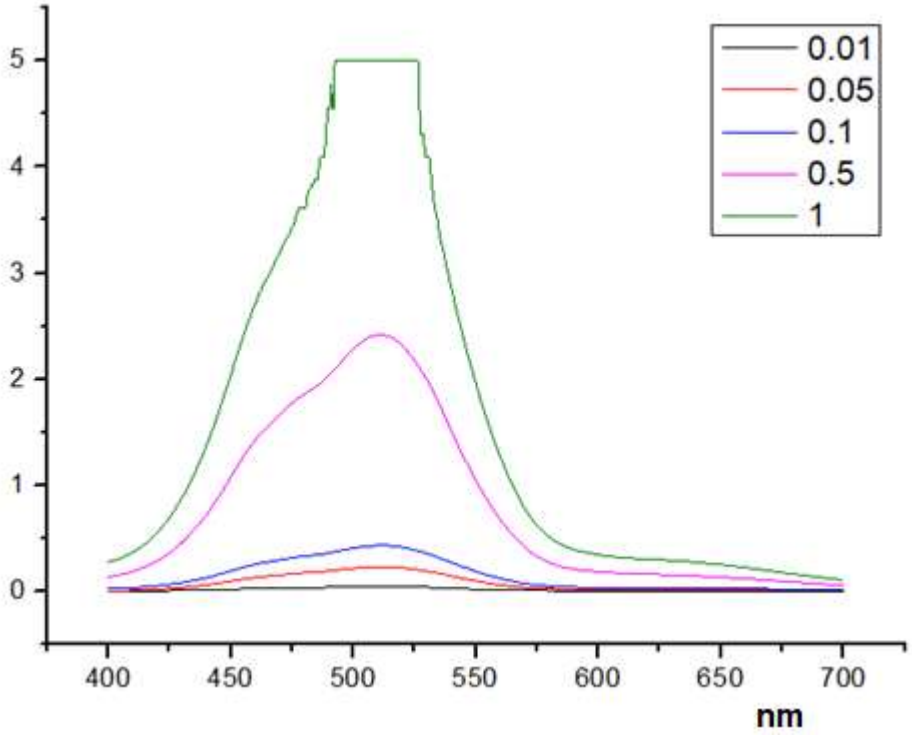

Figure 4.24. $CoNO_3$ referance spectra by shimadzu

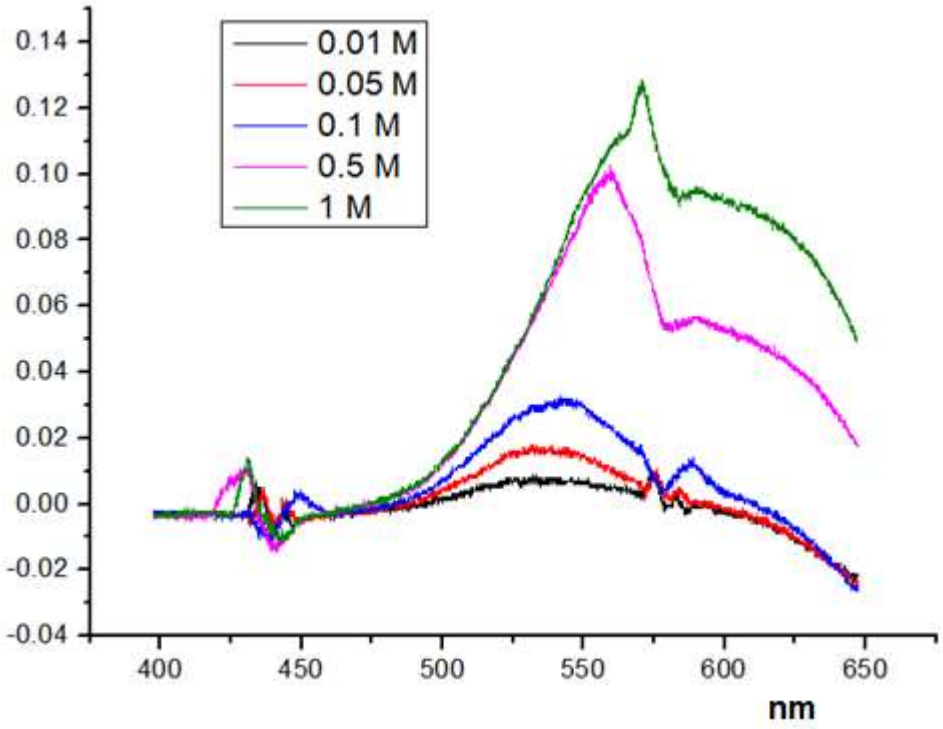

Figure 4.25. $CoNO_3$ spectra by prototype

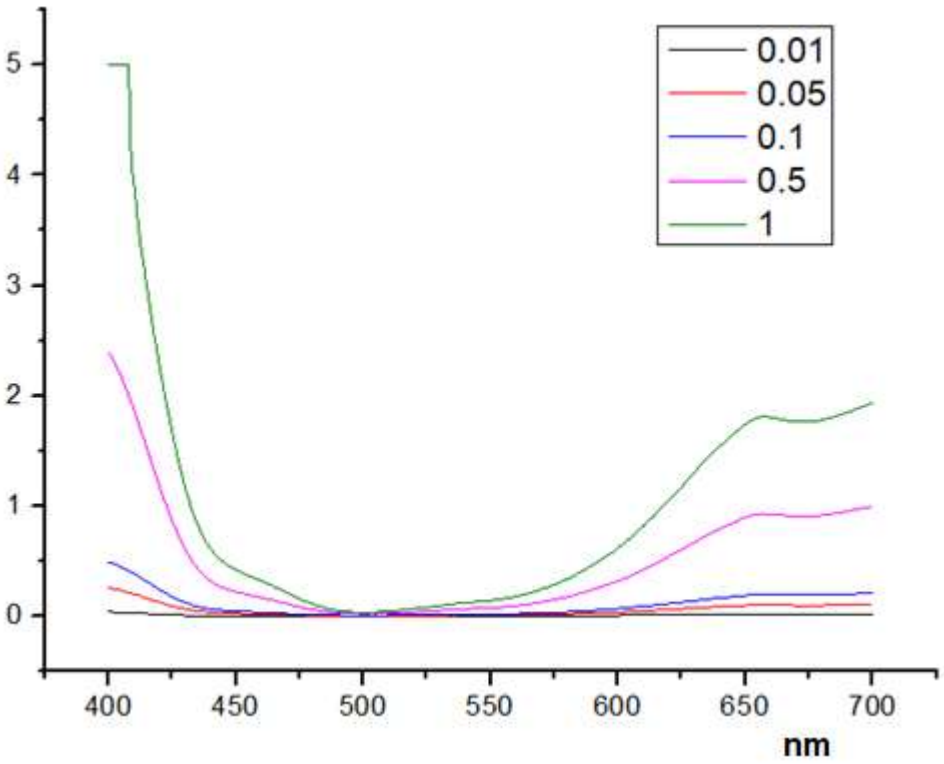

Figure 4.26. $NiSO_4$ Referance spectra by shimadzu

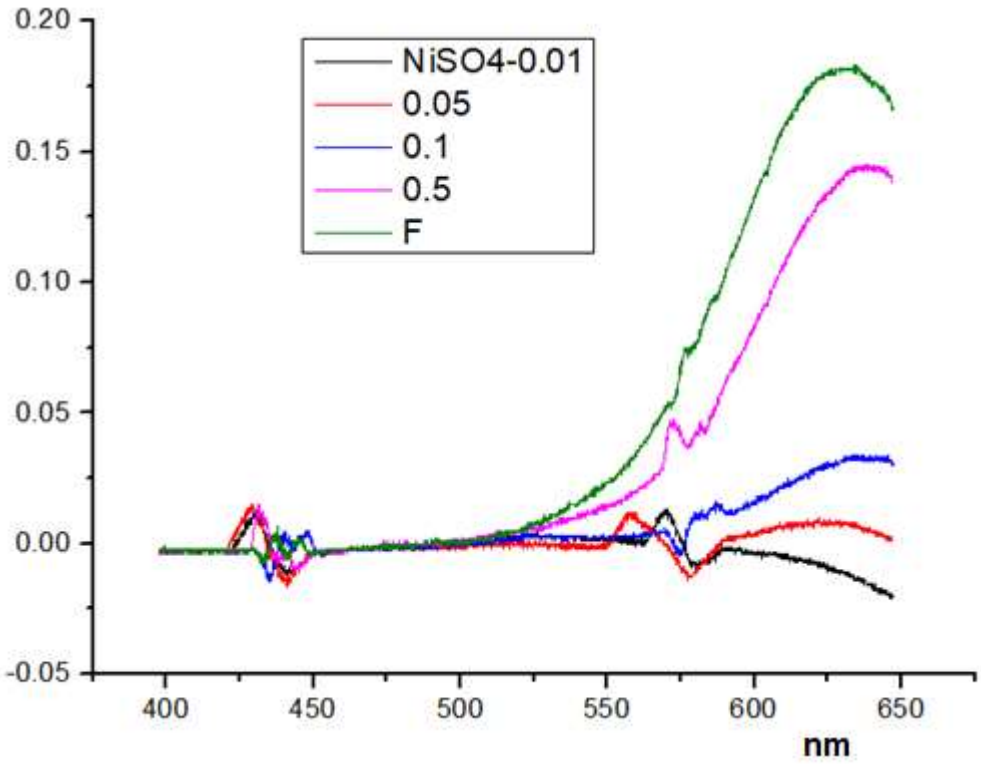

Figure 4.27. $NiSO_4$ spectra by prototype

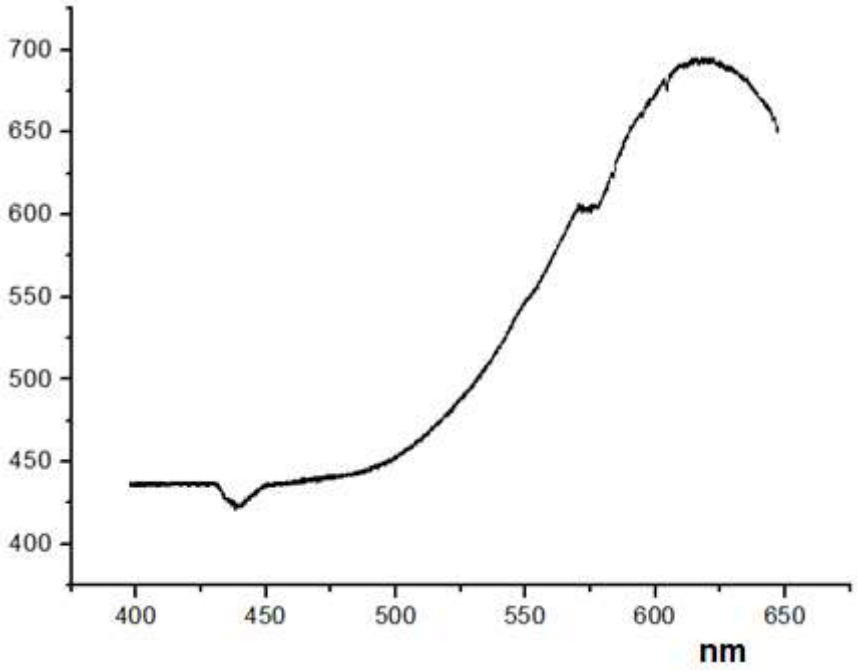

Figure 4.28. Referance light source spectra

Wave length peak (nm)	624.91	637.08	645.12	645.9
Absorbance arbitrary unit (au)	0.194	0.168	0.052	0.027
Molarity (M)	1	0.5	0.1	0.05

Table 4.4. CuNO3 Solutions absorbation values.

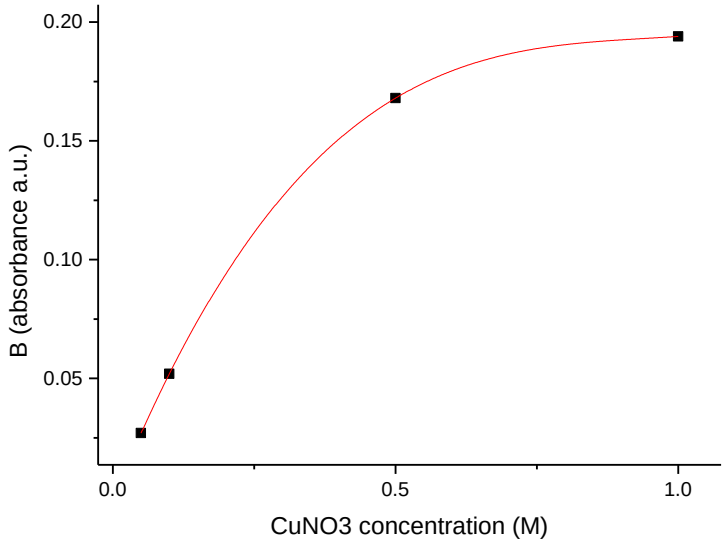

Figure 4.29. CuNO3 Absorbance / Concentration graph

Wave length peak (nm)	563.99	559.43	544.09	532.47	532.4
Absorbance arbitrary unit (au)	0.112	0.099	0.031	0.017	0.008
Molarity (M)	1	0.5	0.1	0.05	0.01

Table 4.5. CoNO3 Solutions absorbation values.

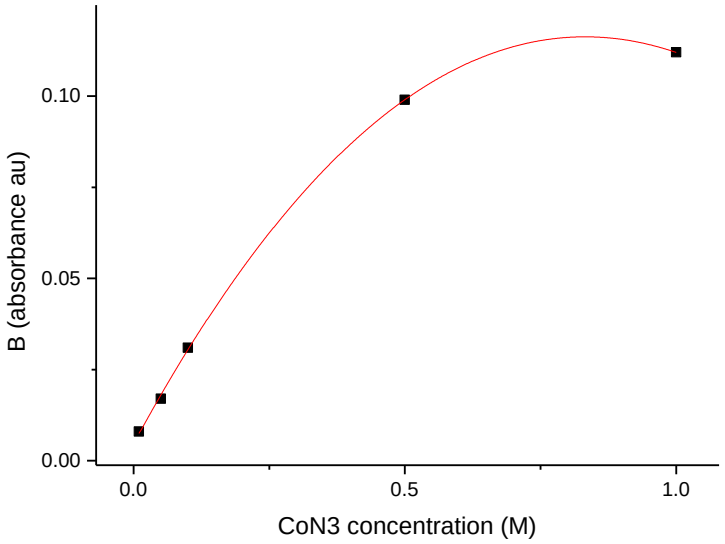

Figure 4.30. CoNO3 Absorbation / Concentration graph

Wave length peak (nm)	631.79	638.4	634.99	625.5
Absorbance arbitrary unit (au)	0.182	0.144	0.033	0.008
Molarity (M)	1	0.5	0.1	0.05

Table 4.6. NiSO4 Solutions absorbation values.

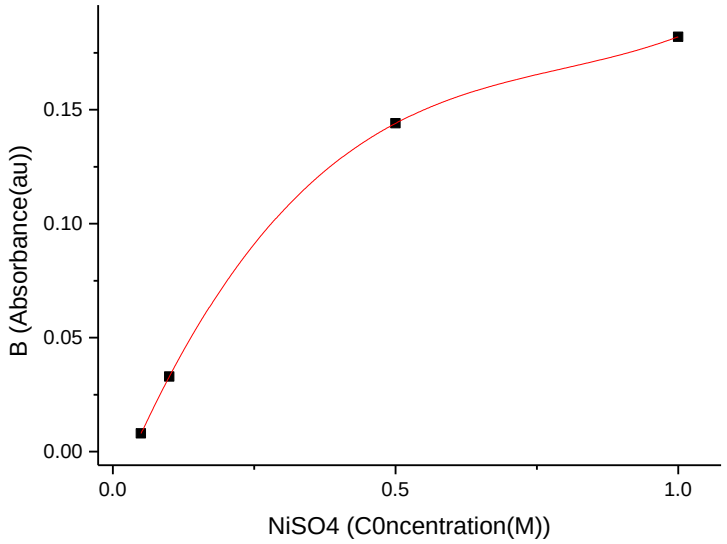

Figure 4.31. NiSO4 Absorbation / Concentration graph

4.6. Comparison

4.6.1 Spectral Resolution

We can measure spectral resolution at FWHM of the peak by using a single mode laser source or narrow peaks of a characteristic emission wave lengths. We can calculate spectral resolution of a VIS spectrometer also by following Formula related to system parameters:

$$\Delta\lambda = \frac{RF * \Delta\lambda * w_s}{n * w_p} \qquad \text{eqn 4.5}$$

δλ…….Spectral Resolution
RF……Resolution factor
Δλ…….Spectral Range of Spectrometer
w_s…….Slit Width
w_p…….Pixel width
n……Pixel number of detector

If slit width approximately equal to pixel width we can accept RF = 3. We can calculate the spectral resolution of our prototype spectrometer theoretically.

$$\Delta\lambda = \frac{3 * 250 * 10}{3648 * 8} = 0.25699 \text{ nm}$$

Optical resolution is also measured on Domestic White Flourescent Bulb spectras of Ocean Optics USB 2000 spectrometer(we use in our laboratory) and our Prototype spectrometer.

USB2000

λ = 545.68485 nm FWHM = 3.34152 nm

PROTOTYPE

λ = 546.74753 nm FWHM = 0.85864 nm

4.6.2 Signal to Noise Ratio(S/N)

The S/N ratio is evaluated as sensitivity measure of a spectrometer. S/N ratio determines whether the spectrometer meets our needs or not at given range. Basically S/N ratio is calculated as dividing peak signal value by near by noise value in same spectra.

Definition of signal: Mean value of maximum signal in spectra at a given time interval.

Definition of noise: Peak to peak (P-P) or root mean square (RMS) value changings of max signal. In other words, standard deviation of max signal value at the same time interval

In our measurement set up, a stable light source was needed. Ocean Optics halogen light source (HL- 2000) was used and assumed it was stable. According to this assumption, it was accepted that the signal deviations were created by our device as noise.

Sixty(60) consecutive spectra values was recorded with random time intervals. The mean value of maximum spectra signals and their Standard deviation was calculated.

S_{mean}= 747.8833

δ = 7.41527

$$SNR = \frac{S_{mean}}{\delta} \qquad \text{eqn 4.6}$$

SNR = 100.8572

PHYSICAL	**USB2000**	**HR4000**	**PROTOTYPE**
Dimensions:	89.1 x 63.3 mm x 34.4 mm	148.6 x 104.8 mm 45.1 mm	
Weight:	190 g	570 gr	
DETECTOR			
Detector:	SonyILX511B Linear silicon CCD array	Toshiba TCD1304AP Linear CCD array	Toshiba TCD1304AP Linear CCD array
Detector range:	200-1100 nm	200-1100 nm	400-630 nm
Pixels:	2048 pixels	3648 pixels,	3648 pixels,
Pixel size	14 µm x 200 µm	8 µm x 200 µm	8 µm x 200 µm
OPTICAL BENCH			
Design:		f/4,Symmetrical crossed Czerny-Turner	f/4, crossed Czerny-Turner
Focal Length:		101.6 mm input 101.6 mm output	100mm
Entrance Aperture:		5, 10, 25, 50, 100, 200 µm wide slits	10 µm
Grating options:		14 gratings, UV-VIS	1200 gr
Order-sorting filters:		filters	none
Fiber optic connector		SMA 905	none
SPECTROSCOPIC			
Optical resolution:	~0.1-10.0 nm FWHM (configuration dependent)	~0.02-8.4 nm FWHM (configuration dependent)	~0.86 nm FWHM at 546.748 nm
Signal-to-noise ratio:	250:1 (full signal)	300:1 (at full signal)	100:1
A/D resolution:	16 bit		
Dark noise:	50 RMS counts	12 RMS counts	
Dynamic range:	8.5 x10^7 system 1300:1 for a single acquisition	2 x 10^8 system 1300:1 for a single acquisition	
Integration time:	1 ms – 65 seconds	3.8 ms to 10 seconds	
Stray light:	<0.05% at600nm; <0.10% at435 nm		
Corrected linearity:	>99%		

Table 4.7. Comparison with Ocean Optics spectrometer models USB2000 and HR4000

CHAPTER 5

CONCLUSION

In this thesis, we identified a problem in our branch and proposed a solution to this problem. As a solution to the problem, we produced a prototype VIS spectrometer with our laboratory facilities.

During production, we have seen that if suitable conditions are met, the device can be produced entirely by our laboratory facilities, except for electronic components used in production.

During the assembly of optical components, we found that the optical box, whose design was obtained from an internet open source, limits the alignment and adjustment of the optical components. In future studies, we decided to design the optical box ourselves in accordance with our optical components.

We compared our prototype spectrometer specifications with the USB2000 and HR4000 models of Ocean Optics. In our calculations, the signal-to-noise ratio of the prototype spectrometer was low. This low result is due to our electronic and optical deficiencies. Since the width of the spectral image formed on the CCD camera is much larger than the pixel dimentions of the CCD camera, most of the light energy received by the spectra is wasted. This problem was solved by mounting special lenses on the CCD camera in commercial spectrometers. This special type lens is not used in our design.

Some differences can be observed between the spectras of the referance spectrometers and the spectras of the prototype spectrometer. On the screen of commercial spectrometer (observation on USB2000), the amplitude of the liğht coming from the light source appears to be the same for all wavelengths. Absorbance spectra images of them are also shaped accordingly. Actually the situation is a little different; the energies of the wavelengths from the light source differ across the range (Figure 4.28). However, spectras prepared by assuming that all wavelengths are of equal energy provides more comfortable and meaningful images to the operator. As the second differance, another absorbance is observed in the 400-450 nm range in the NiSO4 spectra of the shimadzu spectrometer. Since there is no emission at these wavelengths in the light source we use, this part is not observed in prototype spectra. As the third difference, two phantom figures appear in the prototype spectra. These figures move

together to the right or left over the spectra with a constant distance between them. For example; if they were observed on the right side of the spectra in the first sampling, they appear in the middle of the spectra in the second sampling and on the left side of spectra in the third sampling.

This observations makes us think that these phantom figures are caused by electronic signals that enable the CCD camera to be read.

As a solution:

- First, the electronic circuit must be checked and made necessary modifications.
- Second, used software must be developed
- Optical design must be modified.

CHAPTER 6

FUTURE PERSPECTIVES

As long as human being exist, the need for biotechnology will continue. In addition, the healthcare sector and technological developments in this sector will continue with the existence of human beings. Considering these issues, the need for analytical devices in biotechnology and biomedical fields will continue.

First of all, I think that this work will form a good basis for our future work. I hope that analytical device production will start and develop with the continuation of our work. We are not considered late to start working in this field.

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