Bachelor in Biomedical Engineering

Design and Development of a LEDs' Roulette for its Application in Microscopy

Bachelor Thesis

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ABSTRACT

The main goal of this experiment was the creation of a LED roulette that will be used in microscopy in such a way that samples can be analyze not only with white light, but also with LEDs with different wavelengths. Moreover, this experiment was focused in the creation of a calibration protocol for the LEDs, based on a sensor (photodetector), in such a way that it is possible to quantify as a function of the distance between the light source and the sample, as well as taking into account the aperture angle of the light source, the energy that will arrive to the sample for its posterior analysis. Therefore, this protocol would be used as a baseline for other experiment so that the correct components, the ones that better fits the needs of the new test, can be selected.

Chapter 1. Introduction

1. Motivation

When analyzing the condition of a tumor, usually it is needed some structural information (where it is located), as well as some functional information (referred to its level of activity). Therefore, if traditional medical imaging systems were applied, since they are focused just in a single imaging technique, the sample needs to be analyzed by at least two different imaging systems, each one of them providing a set of images containing just a single type of information (one system focused in structural images and the other focused in functional images). However, during the last few decades the medical imaging field has been evolving basically due to the great advances in technology, and now the main tool in medical imaging is based in systems which associate different imaging techniques in a unique system, in such a way that it provides a single set of images representing a combination of images containing different type of information (structural plus functional information).

Moreover, two main goals in which medical imaging systems are focused are: the creation of new devices that can be considered 'a la carte', meaning that they are modular systems in which the user is able to select the specific components that better fits to their corresponding needs, in such a way that the results of the experiments are much more accurate; and the creation of a new medical system (eHealth), new devices connected to the DICOM system, which will allow the exchange of information between different labs and/or doctors, as well as the shearing of medical images and diagnosis (information in general) between patients and hospitals.

The project that is going to be presented in this document is going to be oriented to the medical applications of imaging systems, basically in the creation of new and enhanced medical imaging systems that will be consider modular.

1.1. Objectives

In this document it is going to be presented an experiment that is focused in the creation of a LEDs roulette for its application in microscopy, in such a way that samples are no longer analyzed just by white light, but also they are studied by a wide variety LEDs with different wavelengths. Moreover, such roulette will be associated to a motor, which will make possible the selection of the specific LED that will be used at each moment. Therefore, the results that are obtained by this type of systems are much more complete, meaning that more information

can be provided. However, in order to achieve the main goal of creating the whole imaging system, there are different small objectives that must be overcome.

The first objective is the design and development of some mechanical components. At the beginning of the experiment, just the LEDs and the motor existed, so it was essential the design of some mechanical pieces, such as the mount of each individual LED, as well as the platform of the whole group of LEDs that will be also inserted in the motor.

The second objective is the creation of a calibration protocol. Since the developed system is going to be based in modular systems, it is important to be able of changing the different components. For that reason the calibration protocol is required based on a sensor; it will provide information related to the amount of energy that is scattered as a function of the distance between the sample and the source. Therefore, comparing the energy curve of a light source with the energy curve obtained during the calibration protocol, the user is able to select the proper source that better fits within its experiment.

And the last objective is focused in the creation of two different programs: one of these programs is focused in the rotation of the motor, thus in the selection of the proper LED at each moment; and the other program is oriented in the control of the intensity of each individual LED, in such a way that it makes possible the utilization of this systems for the analysis of all types of samples, even those that are really fragile or unstable.

1.2. Planning

As it has previously mentioned, the aim of this experiment is the creation of a LEDs roulette, and in order to achieve this main goal, there exist different steps that must be complete: the design of some new structural components that will allow the attachment of the whole group of LEDs in a unique element, which, in turn, will be connected to the motor; the design and development of a calibration protocol based on a photodetector, in such a way that it is possible to quantify the amount of light that is scattered as a function of the distance between the light source and the sample; and finally, the development of programs so that the intensity of each LED can be controlled individually, as well as the rotation of the motor.

Taking into account the different objectives that are found in this project, it is important to create a schedule, in such a way that all or at least most of the objectives are achieved. The following planning will specify how the time was programmed for each specific task.

| *Design and development of the different mechanical elements* |
| *System mounting* |
| *Design and development production of the amplification circuit for the photodetector (including code)* |
| *Measurements related with the sensor/LEDs characterization, as well as calibration curve* |
| *Intensity control program* |
| *Motor control program* |

February

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2. Background

As main definition, medical imaging systems refers to devices that are focused in processes and techniques used for creating images about the human body, or at least some of its parts, with clinical purposes. However, there exist some other imaging devices that are not focused in the human body directly, but in samples that are obtained from it; therefore, it is useful to make a small classification regarding the object that is going to be observed and analyzed.

2.1. Imaging Systems: Samples Analysis

There are many different imaging techniques that are focused in the analysis and observation of samples, from fluoroscopy to magnetic resonance, including echography; the technique used will depend on the sample type. However, when it is required the analysis or observation of samples, it is done basically by means of microscopy[1]. Convectional microscopy is a really common technique for nearly any type of sample due to the fact that it does not pose at risk is integrity, and is because such advantage why microscopy has always been a really useful imaging procedure. For that reason, and although technology have been evolving, the essence of this technique has always be the same[2]; therefore, microscopy systems can be considered standard equipment.

Conventional microscopy refers interchangeably to the use of basically two different types of microscopes, optical microscopes and electronic microscopes, which work in a similar way, but with some differences (Image 1). On one hand, optical microscopes are based on a system of lenses (basically two lenses: the objective and the eyepiece) and a source of visible light, in such a way that this source illuminates the sample using visible light photons and thus forming an image on the objective that is magnified and projected towards the eyepiece[3]. And on the other hand, electronic microscopes are similar to the optical ones, but with a difference in the light source; instead of using visible light, they are based on electron beams[4]. The electron beams have advantages and disadvantages with respect visible light: one advantage is the wavelength, which, since it has a lower value, it allows or represents higher levels of magnification; and the disadvantage is basically the aperture angle, that, since it is smaller, it will limit the resolution degree.
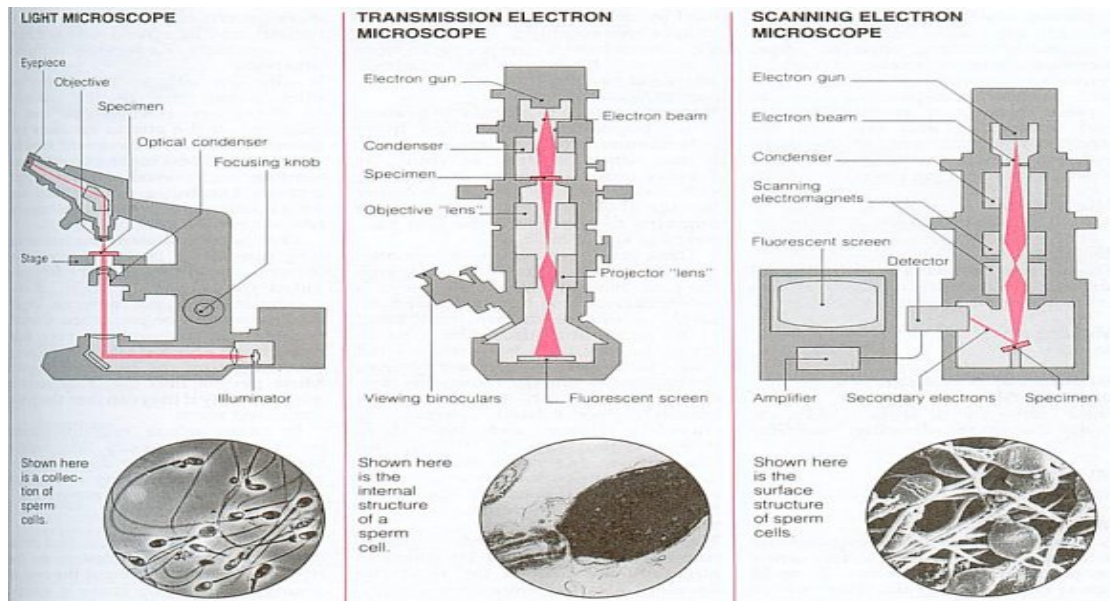
Image 1. Comparison between the different types of conventional microscopes

However, there exist some specific situations and/or experiments performed at both hospitals and investigation labs, in which convectional microscopy is not sufficient in order to obtain useful images that can be employed for the acquisition of precise and coherent results. This lack can be a consequence not only of the way in which the information is processed, but also it may be due to the mounting set itself. This refers to the fact that in some specific scenarios for special experiments it is impossible to use convectional microscopes due to the impossibility to include the microscope device in the mounting set directly; because of the dimensions of the microscope, it cannot be inserted in the set, hence it cannot be used.

For that reason, trying to solve this type of problems, nowadays there exist companies and labs which are focus in the customization of medical systems, that is, they designs and develops imaging devices, as well as their corresponding software tools, that adjust to the necessities of different labs, making possible the achievement of special mounting sets that are much more useful because of their specificity.

2.2. Future Incorporations

Thanks to the ability of digitalization, images can be acquired with a higher resolution degree, in such a way that the clinical diagnosis is more accurate. Also, this characteristic makes possible a simpler access to information from many different locations, as well as it represent a significant space saving, which can be also be associated with an increase in the productivity of labs and hospitals. This digitalization step represents the major advantage since it is possible the shearing of the medical history of patients between different specialists (Image 2).

Nowadays there exists the DICOM[5] (digital and communication in medicine) technology which allows the interchange of different medical tests and results, and that is thought to be focus to the processing, visualization and storage of different medical information that is possible thanks to the information and communication technologies. However, in this case it still requires a direct contact between the doctors and the patients.

Therefore, the future seems to be eHealth (Image 3), which can be considered any type of medical activity that does not implies a physical contact between the patient and the expert. This new tool focused in providing medical assistance without taking into account the distance is possible thanks to information and communication technologies, which will allow the communication between different specialist in such a way that they are able to contrast information or to put in common their different points of view about the condition of a single patient, as well as it makes possible the realization of medical consultations and diagnosis (even surgeries) no matter the distance and in real time.
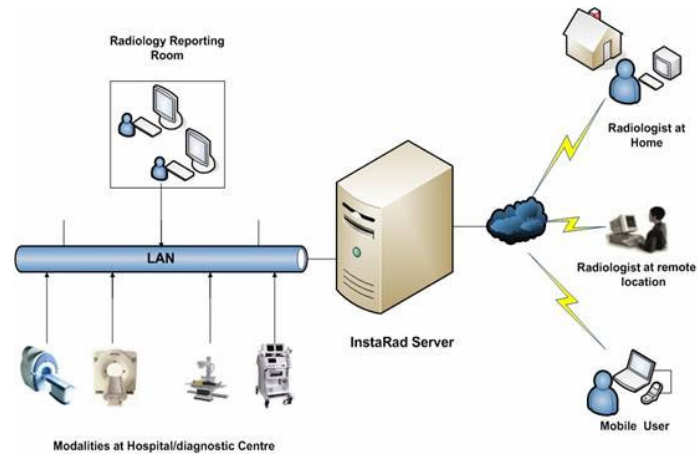
Image 3. eHealth Scheme

The information and communication technologies allow basically the easy access of information in nearly any format in a fast way. Also, they have some characteristics that play an essential role for this easy and fast access of information:

- <u>No material</u>: data is not a material thing so it is possible to store large amount of information in small devices, as well as to access to any type of information from many different locations far away.
- <u>Instantaneous</u>: it is possible to ensure an instantaneous communication and information shearing no matter the distance
- <u>Interactivity</u>: it allows a bidirectional communication between different individuals or groups by means of emails, webs, foros, conferences…
- <u>Automatic functions</u>: it is possible to program some processes that will be performed in an automatic way by a computer, ensuring safety and effectiveness.

With this eHealth tool there are three main activities that can be done without giving any importance to the distance between the patient and the doctor: the first is tele-medicine, which makes possible the delivery of a second opinion from a specialist (complementary and instantaneous services); the second one is tele-diagnosis, which allows the immediate diagnosis of the condition of a patient by an expert; and the third one is the electronic recipe, which represents basically the pharmaceutical prescription of medicines in such a way that the patient does not need to go to the hospital.

Chapter 2. Materials & Methods

1. Description

The experiment that is going to be presented in this document is going to be focused in microscopy, specifically in the implementation of advanced medical imaging systems. Indeed, the idea is based on the customization of medical systems, that is, the designs and developments of imaging devices, as well as their corresponding software tools, in such a way that they adjust to the necessities of different labs, making possible the achievement of special mounting sets that are much more useful because of their specificity. Furthermore, it is important to highlight that the developed systems need to have basically two important advantages: the first one is that they should be user-friendly, that is, they should be easy to use even for people that do not have a deep knowledge in the medical imaging field; and the other one is that this type of systems could be considered modular equipment, in such a way that the user (the hospital or the investigation team) is able to select the components that better fits to it particular experiment and/or needs.

So at the end, the main goal is the design and development of systems 'a la carte' meaning that they give the chance to the different laboratories to obtain imaging systems that adjust to their specific necessities. For that reason, this imaging company is searching innovations and tries to improve its pre-existing systems and this project is focused in such idea of innovation.

The experiment that is going to be presented is based on the design and development of a LEDs roulette that will be used in microscopy, in such a way that the samples can be analyzed, not only with white light, but also with a variety of LEDs with different wavelengths (Image 4). Moreover, following the concepts previously mentioned, the new LEDs system has the characteristic of modularity, which means that it has different components that can be swapped in order to better fit the requirements of the user.
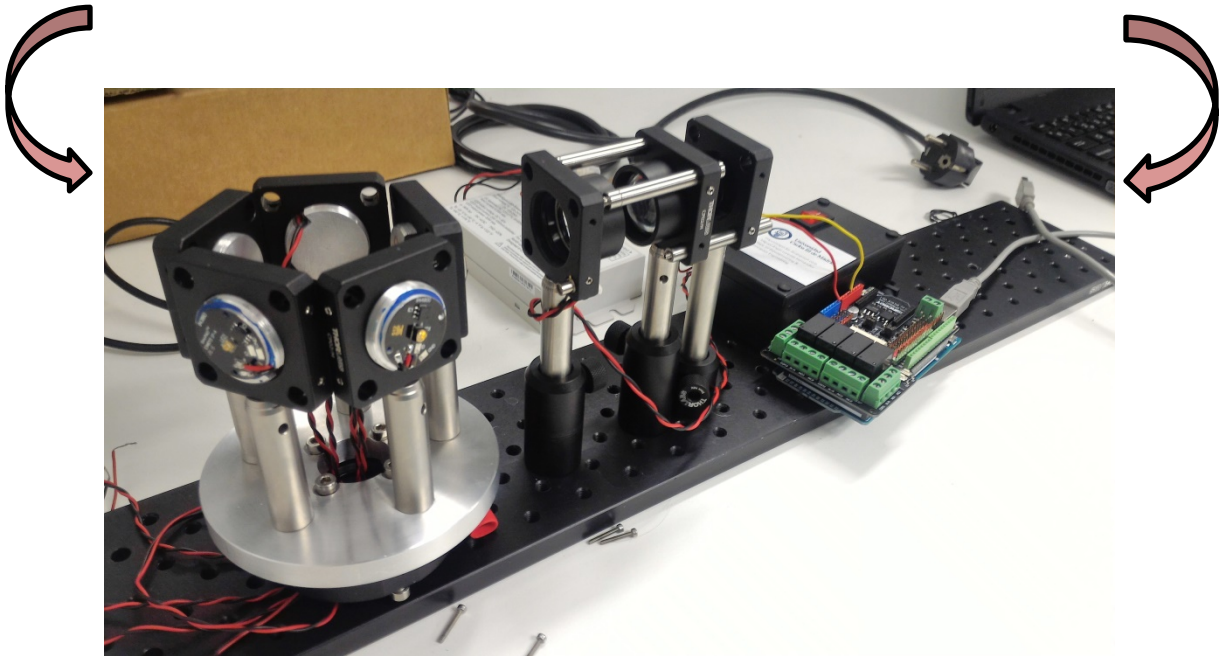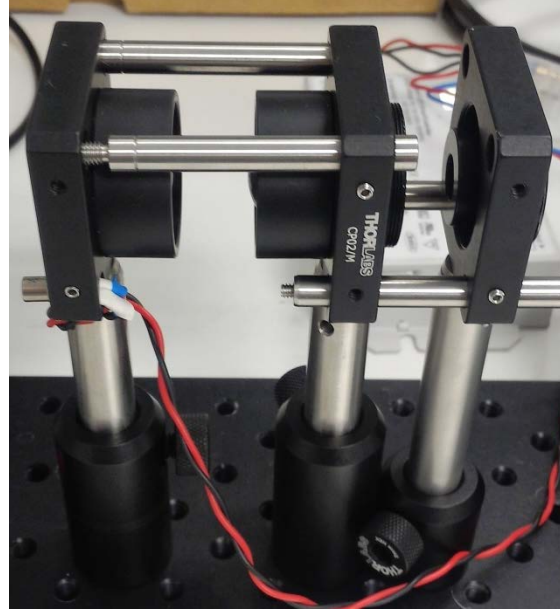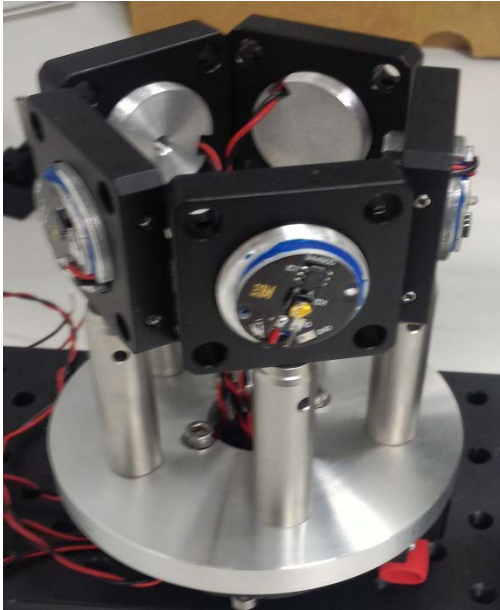
Image 4. Complete LEDs roulette with each individual component

2. Design and Development of Components

This first step focused in the design and development of components is crucial for performing the experiment since such new pieces are mechanicals part that will allow the integration of the different elements for the correct functioning of the system; indeed, these mechanical parts are two: a platform for the group of LEDs and the individual LEDs mount. This new

components were designed by AutoCAD[6], an advanced software tool focused in computer assisted drawing that is used for 2D and 3D modelling of planes.

2.1. LEDs Platform

Initially, this LEDs platform was thought to contain both, the LEDs themselves and the lenses (Image 5). Therefore, it was thought as the combination of two different elements; one internal, fixed plate (red) in which the lenses would be screw, and another external, mobile plate (green) where the different LEDs would be place. Moreover, these two elements needed to be associated in such a way that they could be attached to the motor that would be used for the movement of the system. Furthermore, trying to save some money, the idea of printing the plates by a 3D printer was mooted.
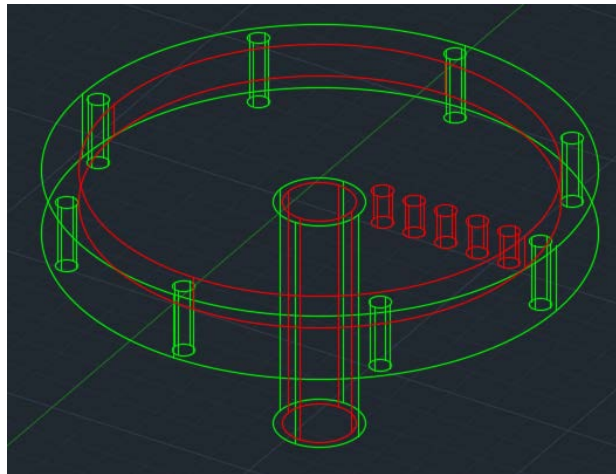
Image 5. First approach for the LEDs platform.

However, this whole idea for the LEDs platform was discarded because different reasons: the first one arises due to the impossibility to associate both elements and the motor so that the internal plate remains fixed; another reason is because the 3D printing, a process which provides a plastic piece which has not the enough resistance to resist the weight of the whole set of LEDs and lenses (poor stability); and the third reason was the huge size of the whole set-ups, which makes the system really unstable.

Once this first approach was discarded, it was presented the idea of changing the orientation of the system, that is: the LEDs point outwards, instead of inwards; and the lenses are placed outside rather than in the interior of the roulette. For that reason, it was thought to create a unique platform for the LEDs that will be associated to the motor, while the lenses and the optical fiber were placed on a separate element (Image 6). At the end, the base for the different LEDs is basically a plate formed by a metal, particularly aluminum, which will be joined to the motor that will be used for making possible the rotation. This plate will contain

two types of holes: one group of holes will be located at the external region and they will be used for screwing the different LEDs; and a second group located in the internal region that will be used for joining the plate to the motor.
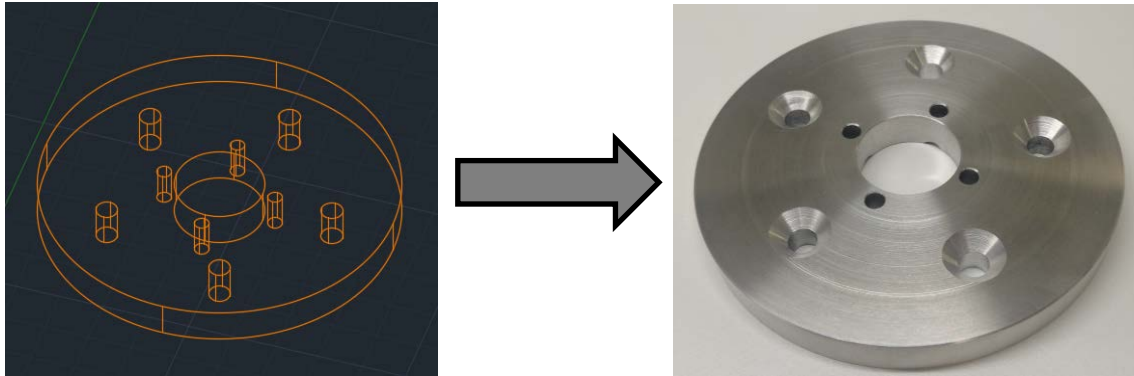

Image 6. Design and development of the final LEDs platform; from the computer design to the final component

The system of lenses is basically formed by two aspheric lenses, and the reason why these aspheric lenses are specifically used is because the large aperture angle of the LEDs; the light emitted by each LED is scattered a lot, so although it is needed to capture as much light as possible through the optical fiber for creating high quality images, large amounts of light are missed (Image 7). However, by means of the aspheric lenses what is done is to focus the light beam coming from the LEDs, and although this collimation is limited, it is really effective, which means that the energy that scatters when the light arrives the optical fiber is minimum in contrast with the situation in which the lenses are not placed.
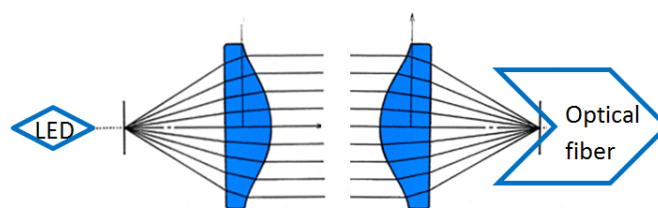
Image 7. This is how the mounted system looks like; light exits the LED and then it is collimated towards the optical fiber

2.2. LEDs Mount

On the other hand, the individual mount for each LED was designed in a more straight-forward manner. It is basically a cylinder that must fit in a cage CP02, so it is important that the cylinder contain an external screw MS1. Also, this component needs to have a small notch that allows the passage of the electrical cables that power the LED (Image 8).
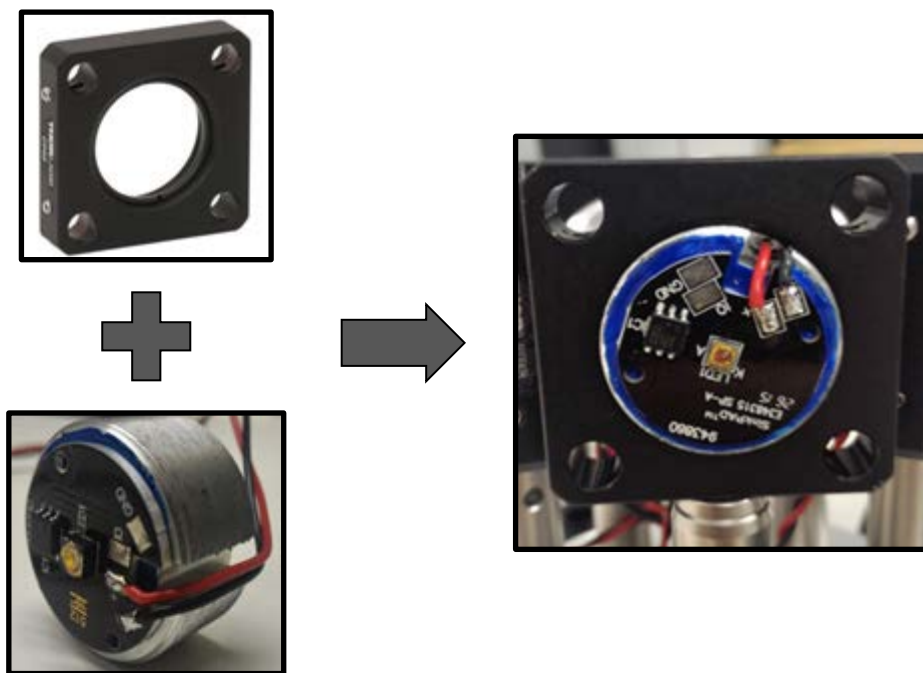
Image 8. Representation of how the different LEDs are mounted

However, there was a consideration that must be taken into account while performing this design. It was important to be conscious about the kind of LEDs that would be used during the experiment, which had the peculiarity that they are high power LEDs, meaning that they warm-up a lot. Because this specific characteristic it was necessary the incorporation of a refrigeration element that allows the reduction of the temperature of the LEDs by dissipating the heat generated. However, in order to avoid the incorporation of an external element, what has been done is to use as refrigerator the LED mount itself due to the fact that it is formed by a material such as aluminum, which enhance heat dissipation and thus helps the LEDs refrigeration.

2.3. Calibration Protocol

The second step required in order to achieve the correct functioning of the LEDs roulette is a calibration protocol that is based in a sensor, particularly in a photo-detector (Image 9), which will provide information related with the amount of energy that will be dissipated as a function of the distance between the light source and the sensor/sample, of the aperture angle of the light source, and the angle in which the light beam hits the sensor/sample. Then, this information can be used by future user in such a way that they can have an idea of the different elements and light sources that should be used in their corresponding experiments that better fits their specific needs, as well as the proper location in which the sample should be placed in order to obtain the best images as possible. It means, 'a grosso modo' that if we

place the sample to close to the light source, a saturated image will be obtained (white image) and even if the light beam is really intense or has a large energy value, it may damage the sample; on the other hand, if we place the sample too far away from the source of light, few energy will reach the sample hence the image will not be created (black image) (Image 10). Therefore, in order to reach the starting point for performing the experiment related with the quantification of the energy that is dissipated, there are needed several components or elements such as an amplification circuit, some software tools (Arduino, MatLab) and a laser control system.

Image 9. Photo-diode that will be used in the experiment
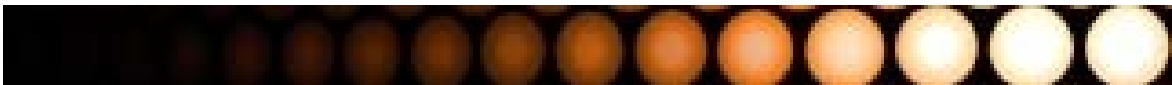
Image 10. It shows how an image would look like as a function of the energy that hits the sample[7]

2.2.1. Laser System

Starting with the laser, it is a system controlled by means of a designed program in LabView, a software tool that allows the development of different systems, including simulations, and that can be used in several applications by using a visual programming language. The laser itself is a high power, high stability laser, which means that the energy that it emits is nearly constant in time (Image 11). Also it has the particular characteristic that the light beam does not scatter too much due to it small aperture angle, what means that is possible to focus the laser towards the sensor in a more precise way (few light scatters, which represent a non-meaningful amount of energy); hence much more accurate and useful data can be obtain.
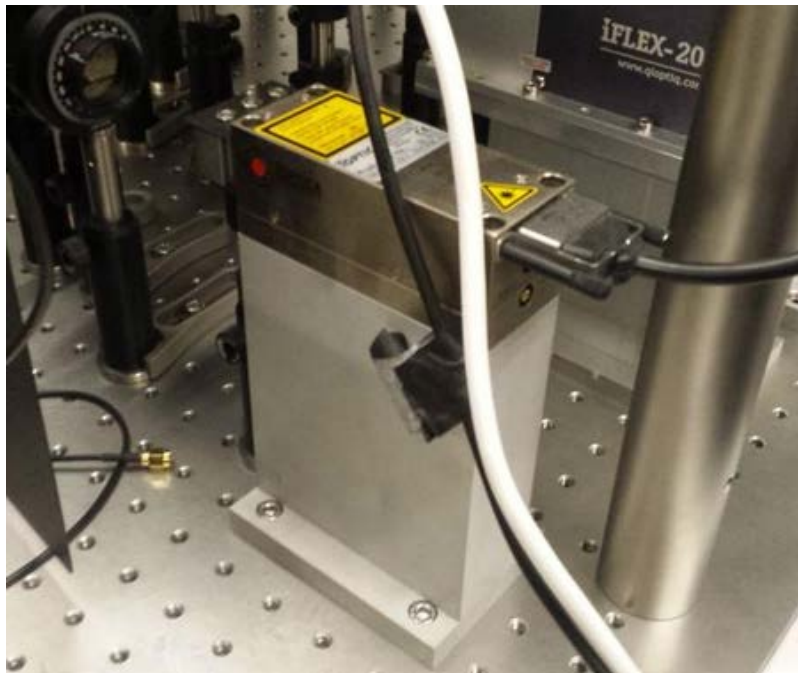
Image 11. Laser System used to perform the experiment

2.2.2. Amplification System

The next component that can be seen is the amplification circuit, which is vital for this experiment since it makes possible the collection of significant results. During this experiment it was observed that the amount of energy obtained by means of the photodetector was really low, in the millivolts range. So in order to collect meaningful data that can be used in further analysis what is done is to incorporate an amplification circuit, that is, the information acquired by the sensor leave the millivolts range and move towards the volt scale (Image 12). Moreover, this amplification circuit was compacted and introduced in a small cage containing the corresponding external connections, in such a way that the circuit can be used in many different situations and experiments, and employing diverse components (this circuit allows the utilization of different sensors and elements).

Image 12. It is the scheme of the circuit described, with the references of the corresponding elements and components that have been used
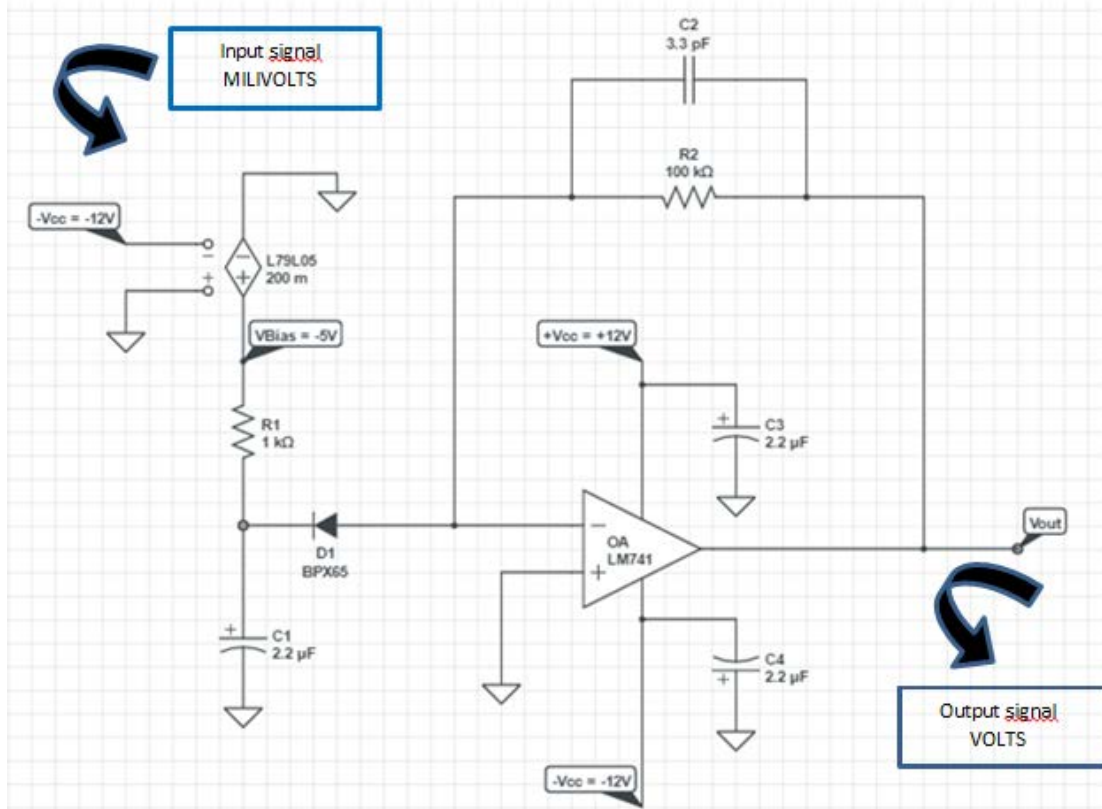
It is possible to observe that some filters have been included in the circuit, in such a way that they help to the reduction of noise that arise due to unwanted light signals and due to the 50 Hz signal from the electrical grid. Furthermore, it is necessary to make some clarifications about the components that have been included in it design and development (Image 13).

- There is a component (RAC06) that allows the utilization of the local power of 220V for power the circuit; therefore, it avoids the use of external power supplies. This RAC is basically a converter, from AC-220V to DC-12V.

- In order to perform the measurements by means of Arduino, it is needed a positive output signal. However, since in this circuit it is used an operational amplifier, which has a negative gain, it is crucial to power the photodetector with a negative voltage (-Vbias). Moreover, since it is required a high gain in order to obtain large output values, what is done is to select the proper resistors, so that the gain is 100.

$$Vout = -\frac{R2}{R1} Vin \quad \rightarrow \quad Vout = -\frac{R2}{R1}(-Vbias) > 0$$

$$Gain = \frac{Vout}{Vin} = \frac{R2}{R1} = 100 \quad \rightarrow \quad R2 = 100 * R1$$

- Avoiding the utilization of several power supplies (one to power the operational amplifier and another one as input signal for the sensor) what has been done is to include a ramification using a 7905, a component that permits the division of the 12V coming from the RAC06, converting them into 5V. At the end two paths are obtained; one path in which the 12V coming from the RAC power directly the operational amplifier, and another path in which the 12V enter the 7905, where they are transformed into 5V, that represent the Vbias, which is used to power the sensor. Actually, as it has been previously mentioned, the input signal that powers the sensor must be negative due to the fact that the gain of the amplifier is negative. Therefore, since we need a negative Vbias, the voltage that enters the 7905 is not the 12V from the RAC, but the -12V.
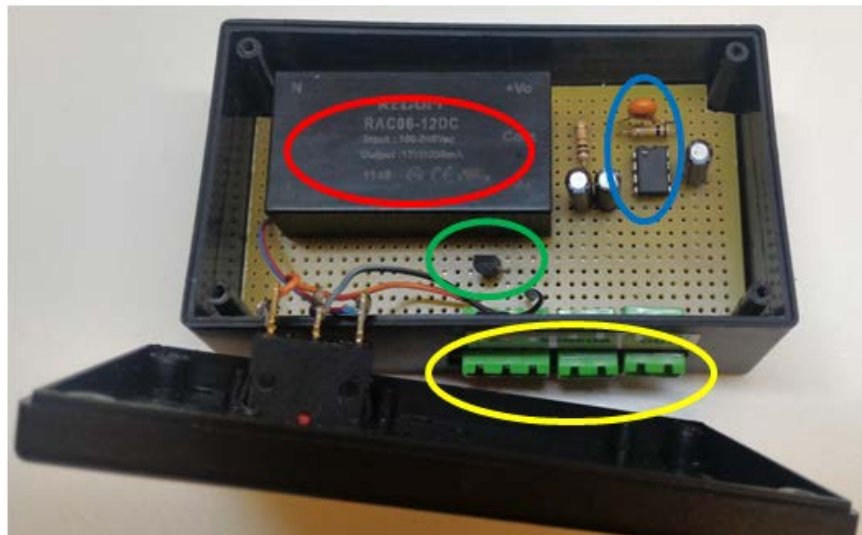
Image 13. Red = RAC06 (AC/DC converter); Blue =Operational Amplifier; Green = 7905 (Voltage ramification); and Yellow = External Connections

2.2.3. Software Tools

Finally, the last component that is found are the software tools that are going to be used to perform the experiment. At this point it is necessary to make a small distinction; two different computer programs are going to be used: Arduino[8], which is focused just to the visualization of the obtained information, and MatLab[9], which is focused in the compilation, processing and storage of data. Moreover, it is important the order in which these two programs are used in order to obtain, manipulate and store the information in a correct form; it is require the utilization of Arduino before MatLab.

- Arduino represents an open code platform that has a particular characteristic; anyone can have a free access to both the hardware and software (Image 14). Moreover, this

company designs and commercialized some plates containing integrated circuits and including a microprocessor, that permits processing many different type of information for performing further processes. In this case, the process that is going to be performed is the conversion of an analog information or signal (volts) provided by the photodetector to a digital information or signal (bits), so that the final results are going to be printed and visualized on the screen.
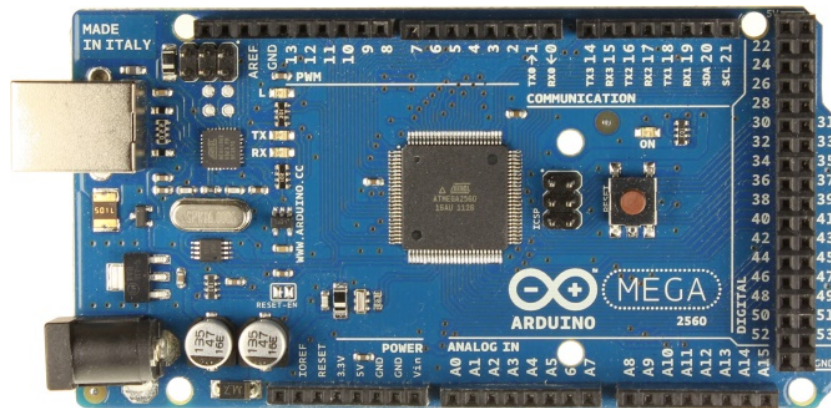

Image 14. Example of an Arduino plate

Therefore, in order to perform the process, the conversion, previously described, it is needed the direct connection of the sensor to the Arduino plate, in such a way that using the following Arduino script (Image 15) it is possible to change the analog signal acquired by the sensor into a digital signal.

Moreover, it is important to talk about the delay time, which represents basically the period of time that the Arduino program needs in order to reset the script. Usually, this delay time is settled by the user, by changing it value directly in the script. However, there are some cases in which the this time lapse is set to zero, meaning continuous exposure, which can also be translated in a constant acquisition of information by the sensor. Nevertheless, there is a non-written rule that does not allow this constant exposure of the sensor by means of Arduino; instead, when the delay is settle in zero, there is a minimum time required by the Arduino plate itself for re-starting the program; an unknown period of time that will be calculated by MatLab.

```
Fotodetector §

int FD;                 // Variable that is going to be used for the representation of the digital values

void setup() {
  Serial.begin(9600);
}

void loop() {           // Process that is going to be performed:
  FD = analogRead(A0);  //   the analog signal is going to be measured and transformed into digital
  Serial.print(FD);     //   the digital value is going to be displayed in the screen
  Serial.print("\n");
  delay(0);             //   the delay represent the period of time for reset of the program
}
```

Image 15. Arduino Script

- MatLab is a mathematical software tool that allows not only the visualization of information, but also admits the manipulation and the representation of data by means of diverse mathematical operations and graphs. This software has some limitations, basically due to the fact that it requires licensing; in contrast than Arduino, that is a free program, in the case of MatLab it is needed a permission from the company itself or from the seller in order to employ this program. However, if licensing is not possible, there exist some other options that are free, such as Genius and SageMath, that although they are not as powerful as MatLab, they are going to report really similar results to those obtained by MatLab.

So returning to the experiment itself, it is important to perform some clarifications regarding the MatLab program that is going to be used (Image 16). Therefore, analyzing the script:

```
close all;
clear all;
clc;

%Inicializar puerto serial
delete(instrfind({'Port'},{'COM3'}));
puerto_serial = serial('COM3');
puerto_serial.BaudRate = 9600;
warning('off','MATLAB:serial:fscanf:unsuccessfulRead');

%Abrir puerto serial
fopen(puerto_serial);
i = 1;
t = 0;
Nmeas = 100;
exposure = 1; % in seconds

%Fichero de texto
```

```matlab
fichero_texto = fopen([num2str(now) '.txt'],'w');

% Bucle (cronometrado)
for i=1:Nmeas
    [voltage(i),sigma(i),Nvals(i)]=read_fotodetector(puerto_serial,exposure);
    figure(1)
    errorbar(1:i,voltage,sigma)
    axis([0 Nmeas 0 12.5])
    xlabel('Tiempo (s)')
    ylabel('Voltaje (V)')
    title(i)
    pause(0.1)
    fprintf(fichero_texto,'%12.8f %12.8f %12.8f %12.0f\n',[now; voltage(i); sigma(i);
Nvals(i)]);
    if ~rem(i,10), disp('press any key'),pause, end;
end

%Cerrar fichero de texto
fclose(fichero_texto);

%Cerrar puerto serial
fclose(puerto_serial);
delete(puerto_serial);
```

Image 16-a. MatLab main script

```matlab
function [volts,sigma,iv] = read_fotodetector(serial_port,exposure)

iv = 0;

tic
while toc < exposure
    iv = iv+1;
    fotodetector = fscanf(serial_port,'%d')';
    if fotodetector
        voltage = fotodetector(1)*12/1024;
        v(iv) = voltage;
    end
end

volts = mean(v);
sigma = std(v);

return
```

Image 16-b. MatLab function

The first thing that is found is a command that allows the connection between the Arduino plate and the MatLab software, in such a way that the information is no longer visualized in the Arduino screen, but it is manipulated by MatLab (Image 17). Once

these two elements are connected, the required measurements can be performed in order to create the corresponding calibration protocol. Moreover, in order to obtain such data in the most accurate way as possible, in the MatLab code it is important to introduce some different parameters which represent different characteristics and/or specifications of both the plate and software of the Arduino platform.

```
%Inicializar puerto serial
delete(instrfind({'Port'},{'COM3'}));
puerto_serial = serial('COM3');
puerto_serial.BaudRate = 9600;
warning('off','MATLAB:serial:fscanf:unsuccessfulRead');
```

Image 17. Commands connecting the Arduino plate and MatLab.

On one hand, one of these parameters refers to the exposure time of the photodetector. This period represents the time during which the detector is opened and exposed to light, or said in a different manner, it shows the time during which the sensor is acquiring information/measuring. As said before, in this experiment it is wished to perform a continuous acquisition; therefore, this time lag is determined by the Arduino plate due to the fact that the user settles the delay as zero. However, since this time is unknown, it is required its calculation to stablish the time of exposure for the program. Such calculation is performed just by introducing a clock inside the MatLab script, indeed inside the function that is located inside the loop, while the program is being running (Image 18). At the end, after performing this step, the value obtained for the delay required by the Arduino plate was 0.01 seconds.

```
tic
while toc < exposure
    iv = iv+1;
    fotodetector = fscanf(serial_port,'%d')';
    if fotodetector
        voltage = fotodetector(1)*12/1024;
        v(iv) = voltage;
    end
end
```

Image 18. Clock the will provide the time value required to reset the program

On the other hand, the second parameter that can be observed in the script is referred to the number of measurements that need to be performed each second in order to obtain coherent and accurate results, and it appears as a consequence of the exposure time. As it has been shown, it is necessary a setup in which is possible to perform

measurements in a continuous manner. However, since Arduino is employed, the acquisition time cannot be infinite, but there is a time lag that is determined by the Arduino plate which is 0.01 seconds to reset the program. Therefore, making some calculations it is possible to obtain that it is required to perform a hundred measurements per second in order to obtain exact results, in such a way that in each second 100 measurements are obtained, each one of those represent data collected by the sensor in an exposure time of 0.01 seconds.

```matlab
%Abrir puerto serial
fopen(puerto_serial);
i = 1;
t = 0;
Nmeas = 100;
exposure = 1; % in seconds
```

Clock value = 0.01 s → time lag between two different measurements

Exposure = 1 s
Nmeas = 100 → number of measurements acquired each second;

Image 19. Number of measurements performed each second

Once these parameters are known, it is possible to perform accurate measurements, as well as to represent and store such information. In order to perform this two processes, inside the MatLab script it is introduced a command, in fact a loop, which allows the real-time representation and storage of the voltage obtained through the detector as a function of time.

Graphically, what this loop does it to create a diagram where a single dot, whose value is determined by averaging the complete set of a hundred measurements, is represented each second. Moreover, there exist a command inside this loop that is used to freeze the script every 1000 measurements or 10 seconds, which represent an advantage due to the fact that with this pause it is possible to change the intensity or the energy of the laser, thus it is possible to include the measurements of many different intensities in a single graph, in other words, it is possible to be conscious of how the acquired data is varying in a graphical way, just observing the graph that is being created.

On the other hand, numerically speaking, what this loop does it to create a text file where all the measurements are going to be collected. The selection of a text file is not random, but is due basically to the fact that it provide some advantages while storing; with MatLab is relatively easy to write inside a text file in an structured ordered way, and such structure will allow that, once the file is completely created, all the information is much more accessible (easy to select just a range of values that are interesting) for posterior analysis.

```matlab
% Bucle (cronometrado)
for i=1:Nmeas
    [voltage(i),sigma(i),Nvals(i)]=read_fotodetector(puerto_serial,exposure);
    figure(1)
    errorbar(1:i,voltage,sigma)
    axis([0 Nmeas 0 12.5])
    xlabel('Tiempo (s)')
    ylabel('Voltaje (V)')
    title(i)
    pause(0.1)
    fprintf(fichero_texto,'%12.8f  %12.8f  %12.8f  %12.0f\n',[now; voltage(i); sigma(i); Nvals(i)]);
    if ~rem(i,10), disp('press any key'),pause, end;
end
```

Image 20. Measurements loop

Chapter 3. Results

As it has been previously mentioned, the experiment has as main goal the creation of a LEDs roulette that will be used in microscopy (Image 21). Moreover, it is wished to create a calibration protocol by means of a sensor, in such a way that the system developed can be apply in many different experiments due to the fact that it gives the possibility of changing the light sources so that they fit the specific needs of the new tests. Therefore, since the calibration protocol is based on a photodetector, it is necessary a previous characterization of the sensor, in such a way that knowing the conditions in which the sensor is able to compile more information, it can be situated in the proper place with the correct orientation, so that the amount of energy that is scattered along the path can be measured in a more efficient and accurate manner; between the place where the light beam is generated by the source until it hits the sample.
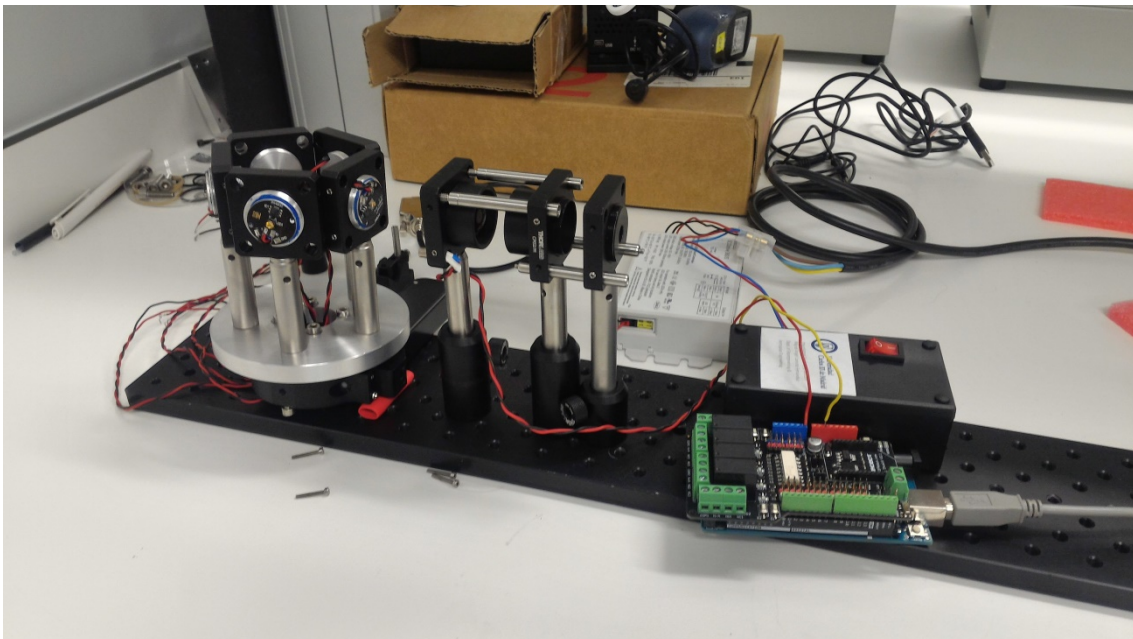

Image 21. Complete system that is developed; final design of the LEDs roulette

1. Sensor Characterization

This sensor characterization process will provide two different types of results since it allows the laser characterization, as well as the sensor characterization. To do so, it is going to be used a high power and high stability laser, a specific type of laser for which it is possible to have a theoretical approximation of how the sensor will behave and also an estimation of the results that should be obtained. Therefore, comparing these ideal results with those obtained during the experiments, it is possible to demonstrate and be conscious of the correct functioning of the sensor.

Starting with the step of the experiment that is focused in the laser characterization, since it is used a high stability and high power laser the theoretical results should be more or less constant when the intensity of the light and the distance between the laser and the sensor are maintained constant[10].

For that reason, while performing the experiment there were three key points that must be ensure in order to guarantee the correct laser characterization. The first one is achieved thanks to the characteristics of the laser, the small aperture angle, which permits the focalization of the light source towards the sensor. The second is based on the intensity of the light that should also be constant; although the intensity of the laser can be changed, what would be useful for further experiments, in this case it is needed a permanent value. And finally, the last one is related with position of the sensor; results are the same no matter the distance between the sensor and the light beam because of the high stability and high power characteristics of the laser, but they vary as a function of the angle by which the light hits the sample, that is, as the light beam moves away from the perpendicular of the sensor, the amount of energy that is obtained is reduced, hence the results will differ from the theoretical estimations.

 Once these requirements were achieved, the experiment could be performed. This test consist basically in maintaining the laser on around an hour in such a way that it is focused towards the photo-detector; therefore, the sensor is able to measure by means of the previously described MatLab program all the energy values that are emitted by the laser during such hour. Remembering the MatLab script, there is a command for pause the program that in this case is not used. Moreover, the information related with this energy quantification was also represented in a graph, so it was possible to compare the ideal estimation (Image 22) with the experimental results (Image 23).

This experiment was performed two times: a first time in which the laser is cold, meaning that the laser was turned on and the measurements started to be acquired immediately; and another time in which the laser is warm, which represents the concept of turning on the laser, and after while the measurements were taken. These two processes were performed basically because, although a high power and high stability laser was used, when light sources are turned on they usually required a small period of time so that they achieve the complete, high stable energy value. However, comparing the results of both experiments did not show any evidence of such behavior.
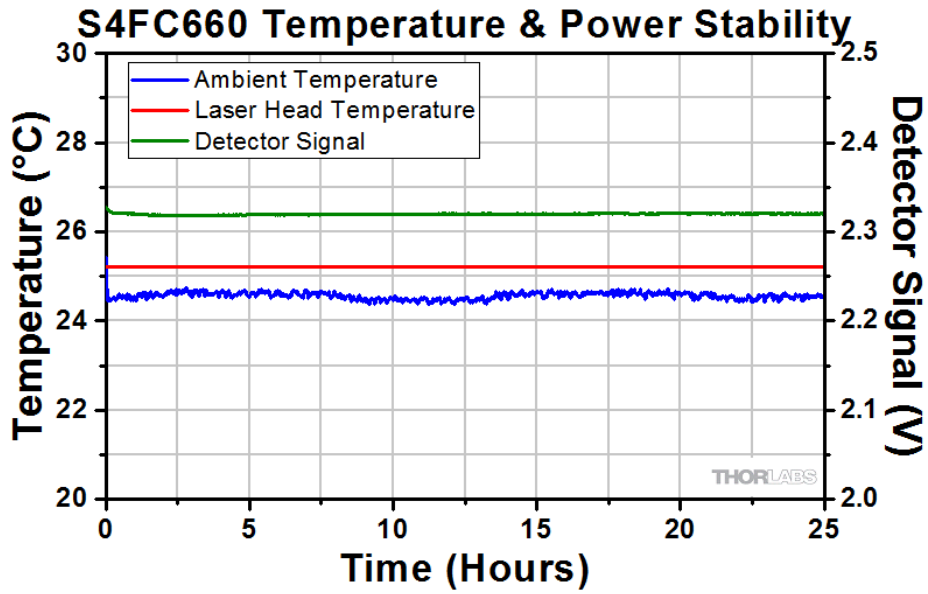
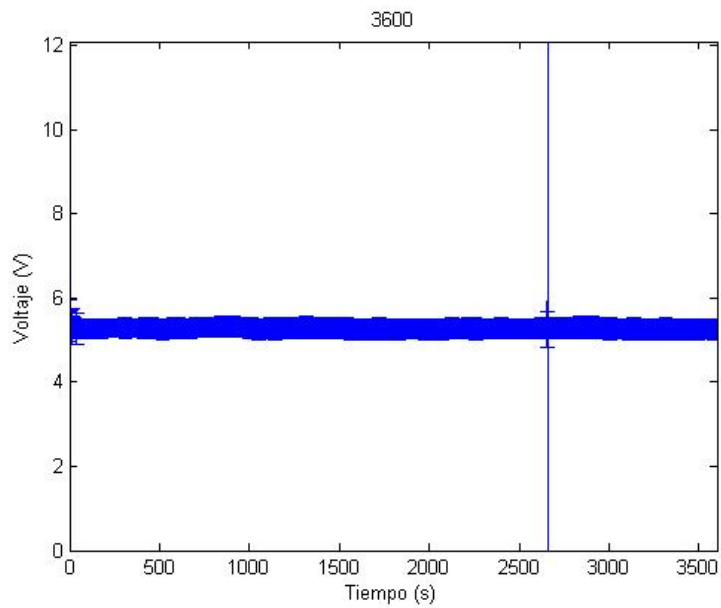**Image 22. Theoretical behavior of the laser[12]**

Image 23-a. Results obtained for the laser behavior when it was warm
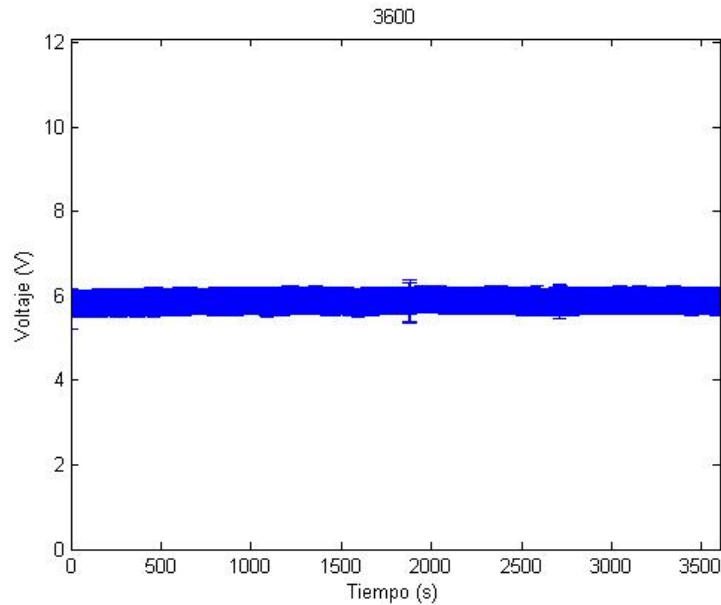
Image 23-b. Results obtained for the laser behavior when it was cold

Once the laser has been characterized, the next step for the calibration protocol is the sensor characterization itself. This task will provide as result something like a calibration curve, representing the way in which the acquired energy varies as a function of the intensity of the light source. Then, this curve will be used as a standard for comparing different light sources hence the users are able to select the components, the proper lights, that better fits their corresponding experiment needs.

In this step, it was also necessary some key parameter; the distance and the position of the sensor needs to be also constant. Therefore, while performing the experiment different measurements were obtained, as much as a hundred measurements each second. Then, the MatLab program is responsible for the representation of the average value of the complete set of the hundred measurements, in such a way that each second a single dot is displayed. Moreover, every ten seconds measuring the same value, the same energy level produced by the laser, the MatLab code is paused in such a way that it gives the chance to the user to change the intensity of the laser and then continues measuring another ten seconds in such new energy level. With this pause what is achieved is the fact that it is possible to represent different energy levels in the same graph, so it is possible to observe graphically how the sensor is acquiring energy levels as a function of time while varying the intensity of the laser.

Moreover, this experiment was also performed two times, but in this case what changes is the environment, meaning that one of the tests will be performed with the ambient light on (Image 24-a) while the other test will be produce without the ambient light (Image 24-b). Comparing both calibration curves, they seem to be the same with a slightly difference in the saturation point, which is reached in a more accurate way in the case in where the light is not presented.
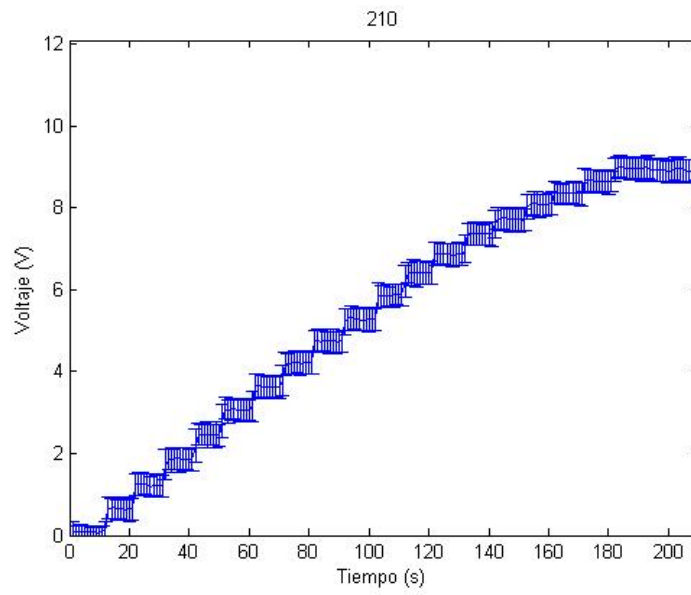
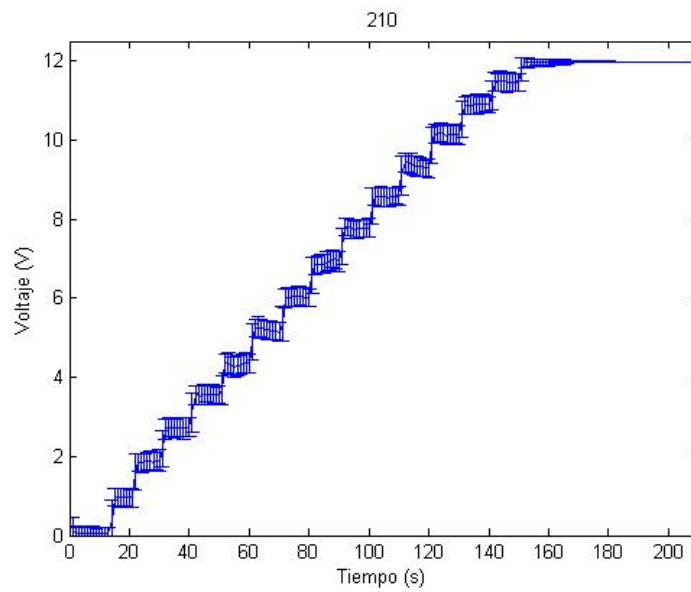**Image 24-a. Calibration curve with light**

Image 24-b. Calibration curve without light

2. LEDs Characterization

Once the sensor has been characterized so that is known how the acquired information regarding the energy varies as a function of the intensity of the light source, it is time to move

towards the LED characterization step. At this point, the experiment will be focused in the LEDs roulette; therefore, it is required the incorporation of the sensor inside the system, in fact, it was incorporated between the LED and the first aspheric lens.

During this experiment what was going to be measured is the amount of light that is scattered between the light source and the sample, quantification that will depend on three main parameters:

- Aperture angle of the LEDs (light source): as the aperture angle is increased, more energy/light will be scattered. This concept can be properly described by the Lambert Law
- Intensity of the light source: it is the same as in the previous section in which the calibration curve of the laser was obtained; as the intensity is decreased, more energy is scattered.
- Distance between the sensor and the light source: in the case of the laser this distance was non-significant because of the high stability characteristic of the laser, which can be translated into a more or less collimated light beam so that the light is focused directly and perpendicularly towards the sensor. However in this case, the distance between the LEDs and the sensor is a really important parameter due to the fact that light is not collimated; as the distance is increased, more light is scattered.

After taking into account all this parameters and conditions, two different processes were performed.

On one hand it was done a LEDs characterization, which is basically the quantification of the energy captured by the sensor, thus scattered. This experiment was performed following the same instructions as in the case of the laser characterization; the distance between the light source, the LEDs, and the sensor will be maintain constant, as well as its intensity, which will be settled to its maximum value. This process was repeated for each different LED (Image 25):
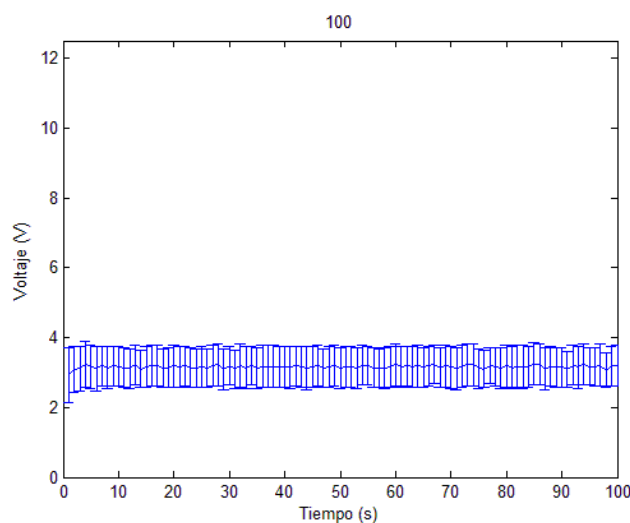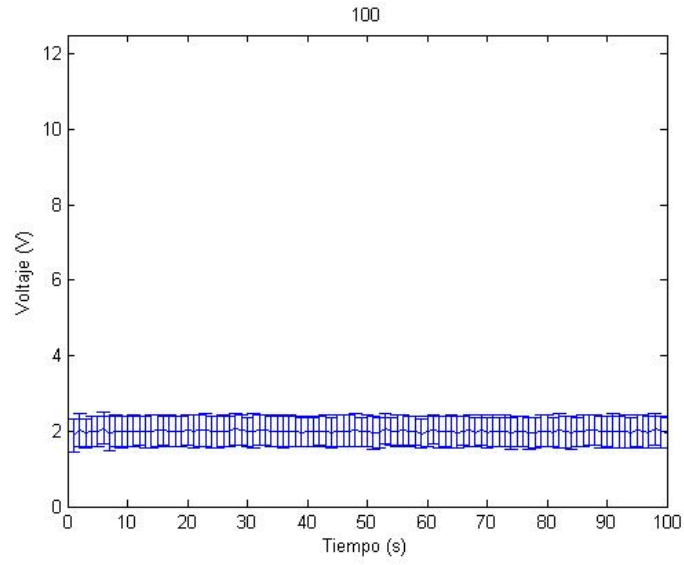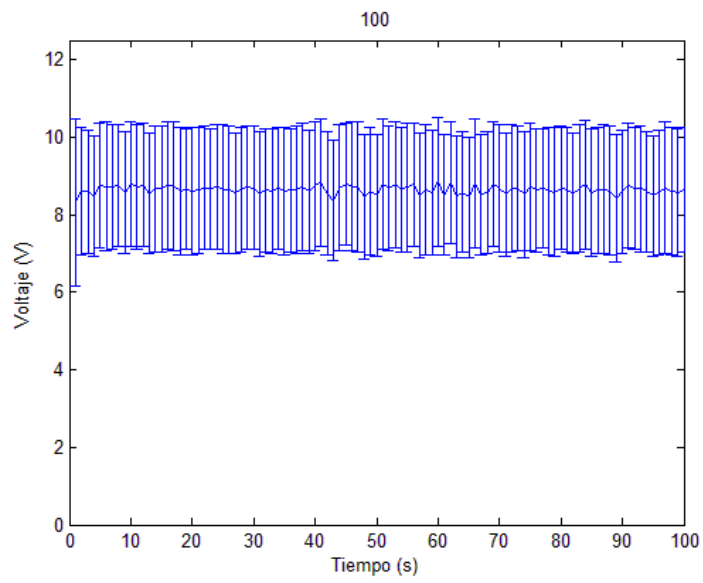

Image 25-a. Characterization of the 470nm LED (BLUE)

Image 25-b. Characterization of the 530nm LED (GREEN)

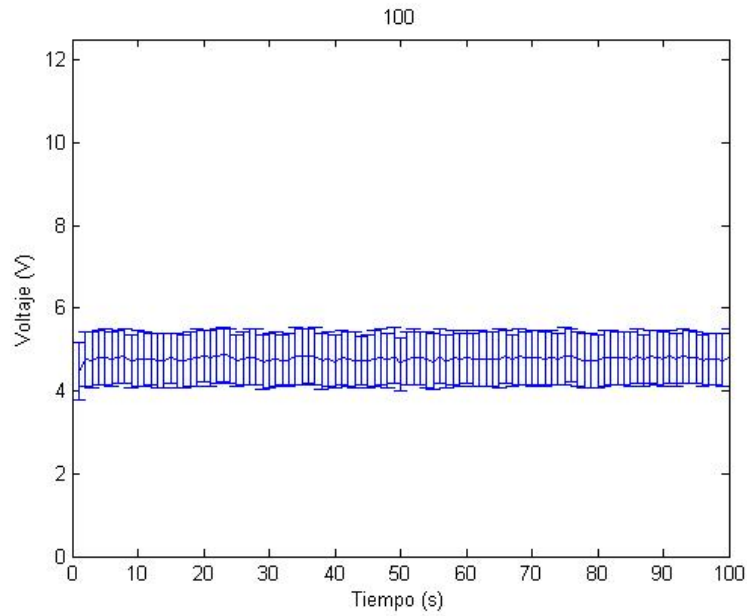**Image 25-c. Characterization of the 595nm LED (RED)**

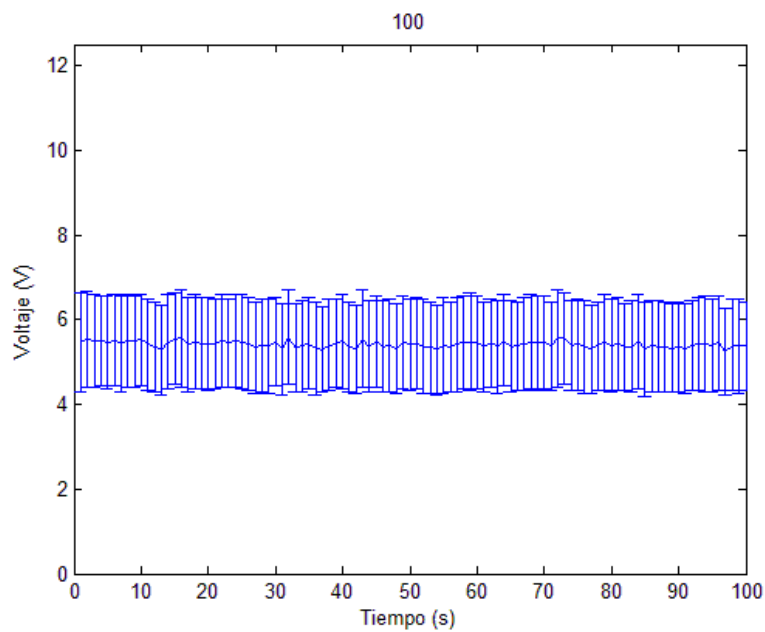**Image 25-d. Characterization of the 625nm LED (YELLOW)**

Image 25-e. Characterization of the 810nm LED (UV)

And on the other hand it was performed a measurements map, which can be considered a correspondence of the calibration curved obtained by the use of the laser. In the case of such curve, it was obtained by measuring the energy as a function of the intensity of the laser, but without changing the position of the sensor (distance). However, in the case of the LEDs, what is done is to obtain a measurement map, which will provide a similar result, but with the

difference that in this case the intensity is going to be constant while the distance between the light source and the sensor vary.

Moreover, these changes in the distance were performed in two different ways; therefore in each case, when the results were obtained, it was possible to compare them with the theoretical or ideal results, in such a way that it was likely to observe and study how the acquired energy varies as a function of the distance.

- 1D Measurements Map: in this first process the light beam is always hitting the sample in a perpendicular way, but the sensor is moving closer and further (Image 26). Therefore, it is possible to contrast such curve with the experimental results (Image 27); there would be an exponential decay of the energy reaching the sensor, as the distance is increased.
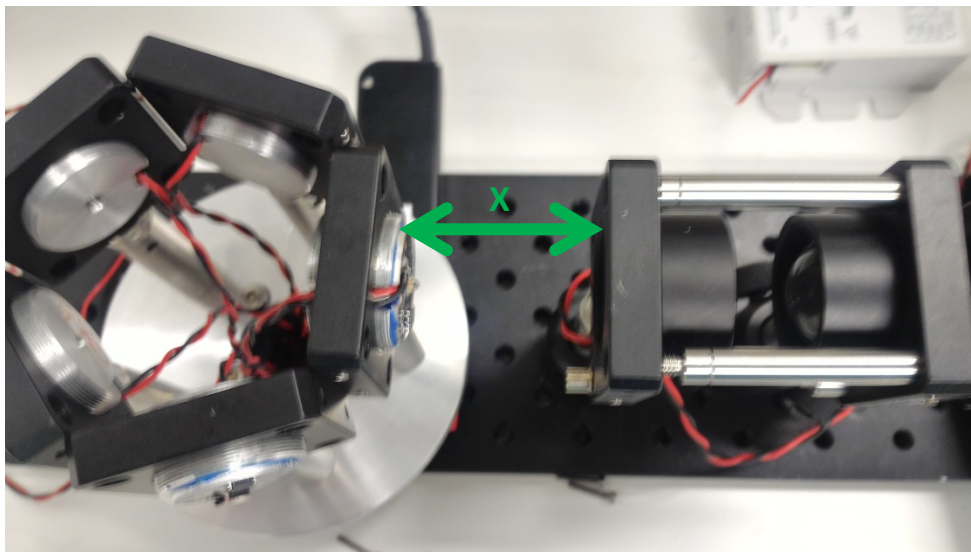

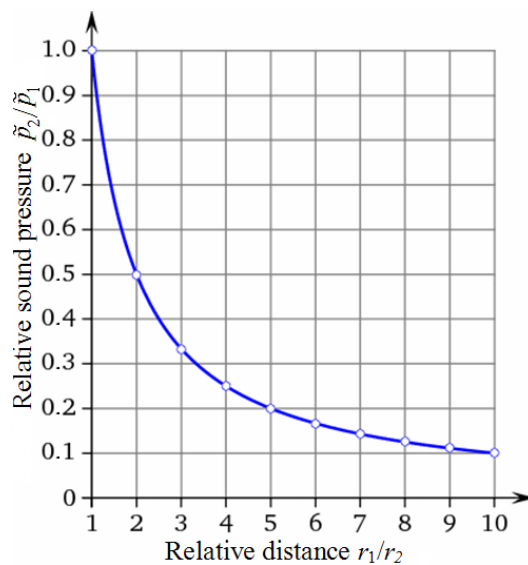**Image 26-a. Representation of the movement performed in order to carry out the experiment**

Image 26-b. Theoretical results of the way in which light is scattered as a function of the distance[11]

Distance (cm)	Energy (V)
1	Saturated
2.5	12
4	6.5
6.5	3.5
8	2
9.5	1.5
11	1
12.5	0.75
14	0.5
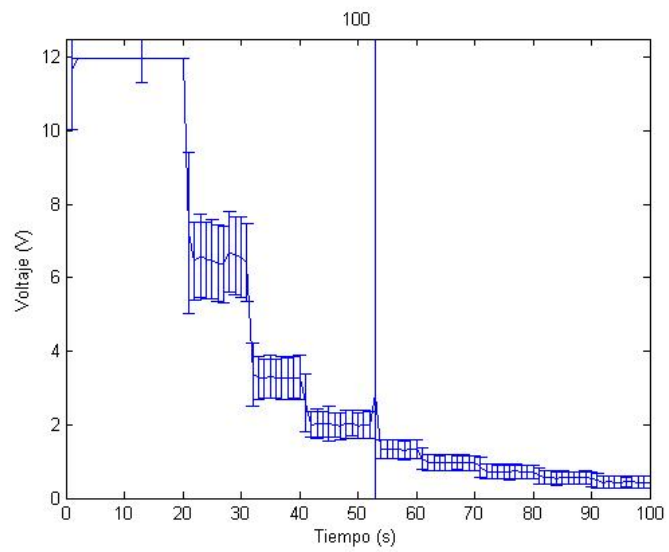
Image 27-a. Scattered light curve corresponding to the 470nm LED (BLUE)

Distance (cm)	Energy (V)
1	Saturated
2.5	11
4	4.5
6.5	2.25
8	1.75
9.5	1.5
11	1
12.5	0.75
14	0.5
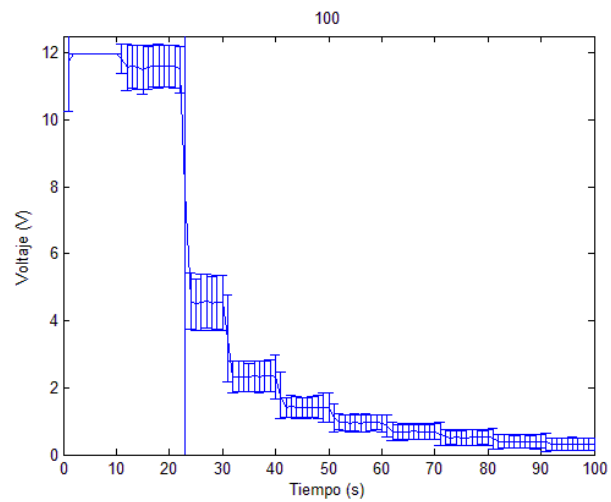
Image 27-b. Scattered light curve corresponding to the 530nm LED (GREEN)

Distance (cm)	Energy (V)
1	Saturated
2.5	12
4	9
6.5	5.5
8	3.5
9.5	2.25
11	2
12.5	1.75
14	1
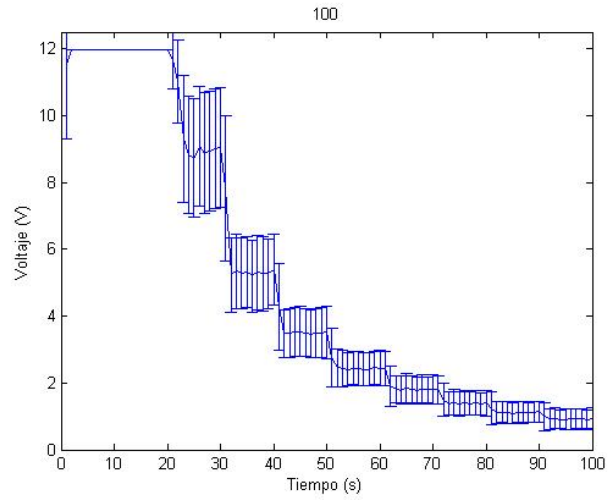
Image 27-c. Scattered light curve corresponding to the 595nm LED (RED)

Distance (cm)	Energy (V)
1	Saturated
2.5	12
4	8
6.5	4
8	2.25
9.5	1.75
11	1
12.5	0.75
14	0.5
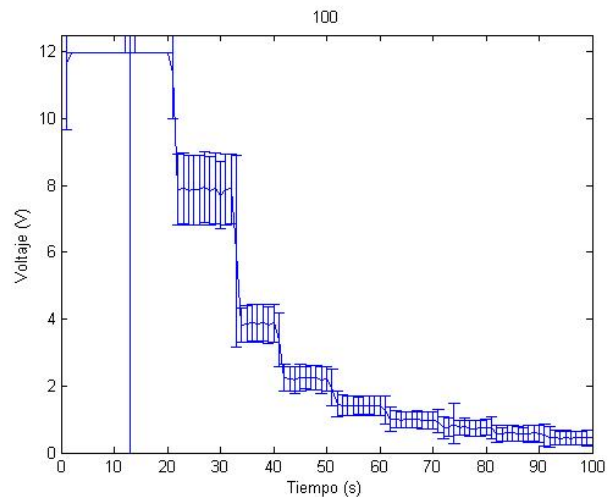
Image 27-d. Scattered light curve corresponding to the 625nm LED (YELLOW)

Distance (cm)	Energy (V)
1	Saturated
14	11
16.5	9.5
18	7.75
19.5	6.25
21	5.75
22.5	4.25
24	4
26.5	3.5
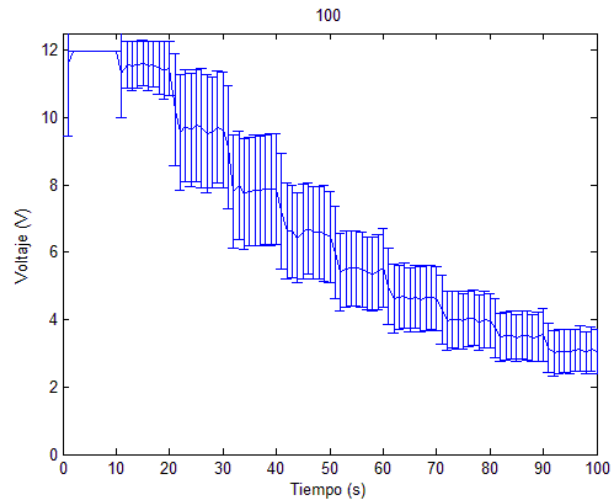
*it was needed to change the range of distance in this particular case due to the fact that with the initial values the light did not scatter enough so that the results were meaningful

Image 27-e. Scattered light curve corresponding to the 810nm LED (UV)

- 2D Measurements Map: in this second process the light beam is always pointing the same place, but the sensor is moving on a perpendicular plane (Image 28). In this case, it is necessary to highlight the fact that the sensor is always located at the same distance I the principal axis; indeed, it is stablish at a distance of 4.5 cm. Then, the obtained results can be compared with such theoretical results (Image 29); as the sensor moves out from the position in which it is perpendicular to the light beam, the less energy it is able to acquire.
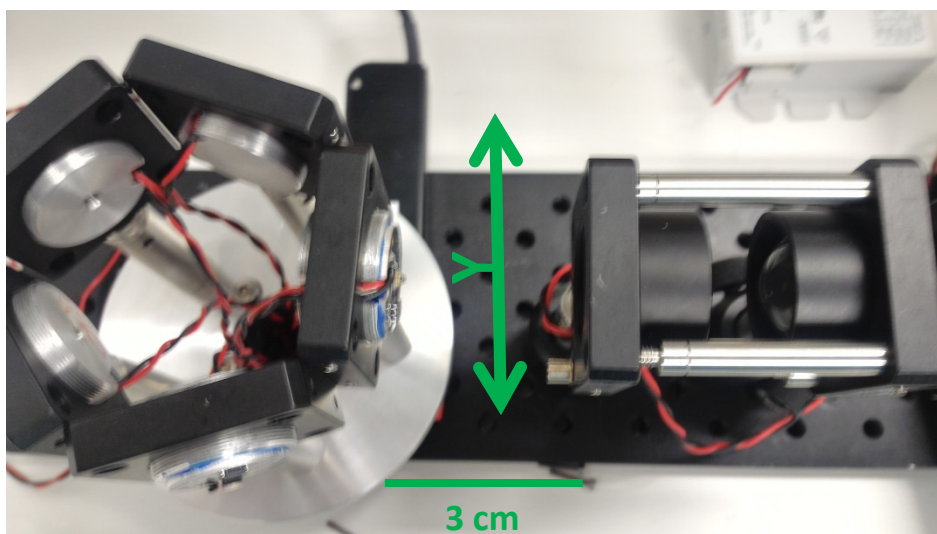

Image 28-a. Representation of the movement performed in order to carry out the experiment

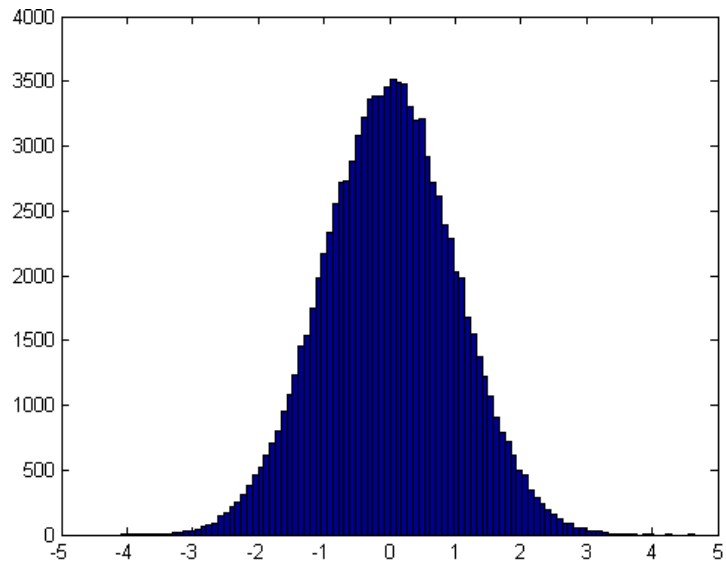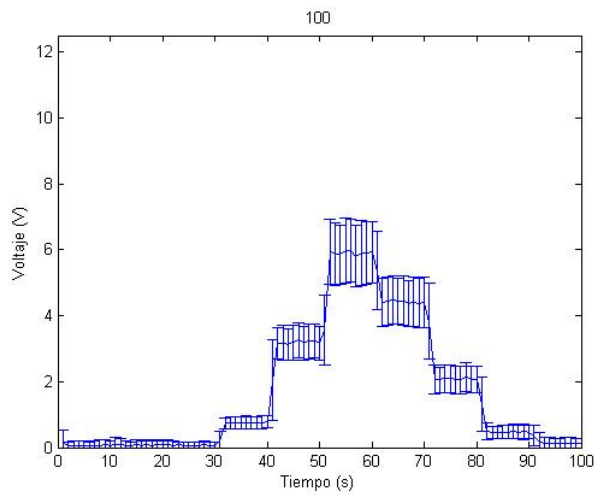**Image 28-b. Theoretical results of the way in which light is scattered as a function of the distance (perpendicular)[11]**

Distance (cm)	Energy (V)
-6	0
-4.5	0
-3	1
-1.5	3.25
0	5.75
1.5	4.25
3	2.25
4.5	0.25
6	0

Image 29-a. Scattered light curve corresponding to the 470nm LED (BLUE)
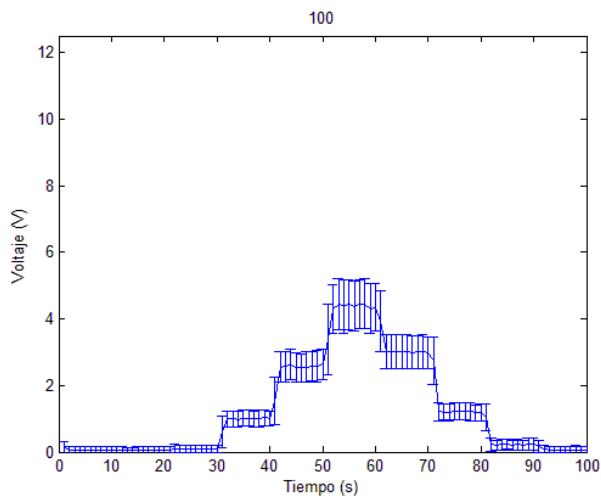
Distance (cm)	Energy (V)
-6	0
-4.5	0.25
-3	1
-1.5	2.5
0	4.25
1.5	3
3	1.5
4.5	0.5
6	0

Image 29-b. Scattered light curve corresponding to the 530nm LED (GREEN)
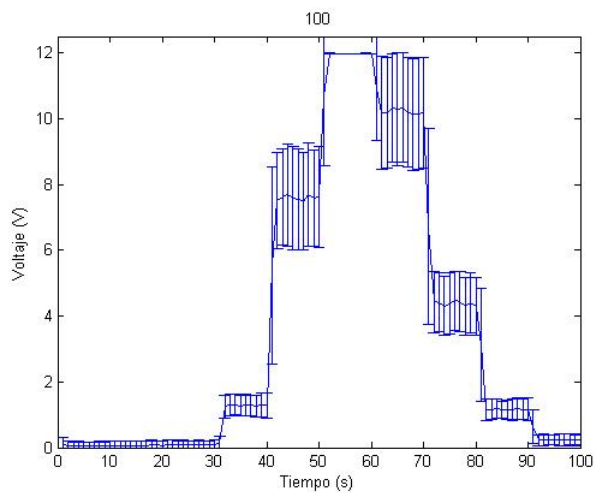
Distance (cm)	Energy (V)
-6	0
-4.5	0
-3	1
-1.5	7.75
0	12
1.5	10
3	4.25
4.5	1
6	0

Image 29-c. Scattered light curve corresponding to the 595nm LED (RED)
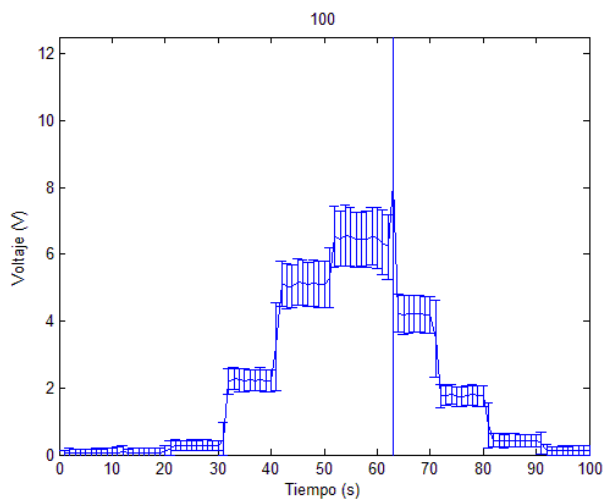
Distance (cm)	Energy (V)
-6	0
-4.5	10.252
-3	2.25
-1.5	5
0	6.25
1.5	4.25
3	1.75
4.5	0.5
6	0

Image 29-d. Scattered light curve corresponding to the 625nm LED (YELLOW)
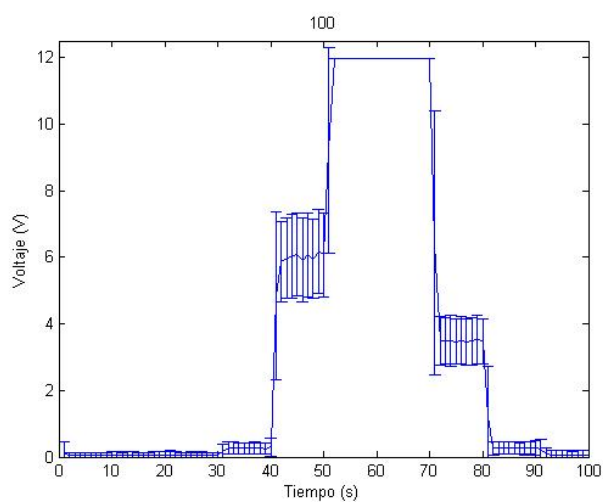
Distance (cm)	Energy (V)
-6	0
-4.5	0
-3	0.25
-1.5	6
0	12
1.5	12
3	3.75
4.5	0.5
6	0

Image 29-e. Scattered light curve corresponding to the 810nm LED (UV)

*note: the error bars that are represented in the graphs increase as the measured intensity becomes higher due to the fact that the values measured by the Arduino spans a wider range of values; since the values vary a lot, the calculated standard deviation will be higher.

Chapter 4. Conclusion

1. Conclusion

As a conclusion it is important to talk about the validity of the results obtained. As it is possible to observe in previous sections, the results obtained from the experiments performed for both the characterization of the laser end the sensor can be compared with their corresponding theoretical results, and they fit. Moreover, regarding the maps of measurements that were performed, they seem to fit with the curves that correspond to ideal situations. Therefore, it is possible to conclude that the used photodetector, as well as the developed amplification circuit act in a similar way to pre-existing sensors, thus making possible the acquisition of information in an accurate manner.

Furthermore, it is important to highlight the utility of the complete system developed, which will provide advantages for studying different samples. On one hand, the LEDs roulette will allow the analysis of samples not only with white light, but also with some other light sources with different wavelengths due to the fact that the motor in which the roulette is inserted will makes possible the selection of the LED that will be used at each moment. And on the other hand, by changing the position of the sample, further or closer to the light source, it is possible to control the amount of energy that each LED will provide, as well as the energy that reaches the sample, thus allowing the utilization of the whole set of light sources for the analysis of any type of samples, including those that are unstable or are easily degraded.

2. Future Work

During this experiment, like in many other cases, some problems regarding the time employed in each task arise, basically due to the design and improvement of the different mechanical components, as well as due to the development of some components required for creating the calibration protocol, such as the amplification circuit. Therefore, looking for the scheduling that was performed at the beginning of the project, it is possible to observe that one of the objectives that were described have not being done: the creation of the scripts for controlling the intensity of each individual LED, as well as for controlling the rotation of the motor. For that reason, as future work, it would be interesting the idea of creating these two programs.

2.1. Intensity

On one hand, the step related with the intensity control is important due to the fact that controlling the intensity of each individual LED will avoid the step of changing the position of the sample itself; therefore this new system could be employ for the analysis of a wide range of samples, even for those that can be consider fragile, unstable, or that may degrade easily without varying its location. At the end, it is possible to select the amount of energy that will reach the sample, in such a way that it provides the best images as possible while it does not pose in risk the integrity of the sample.

This program could be done by means of Arduino software and a shielded controller, in such a way that it is possible to re-use the Arduino plate that is been employed. Moreover, if this approach is followed, here it is attached an estimation of how the circuit should look like (Image 30), meaning that there exists a scheme of how the LEDs should be connected to the Arduino plate in order to ensure the functioning of the system.
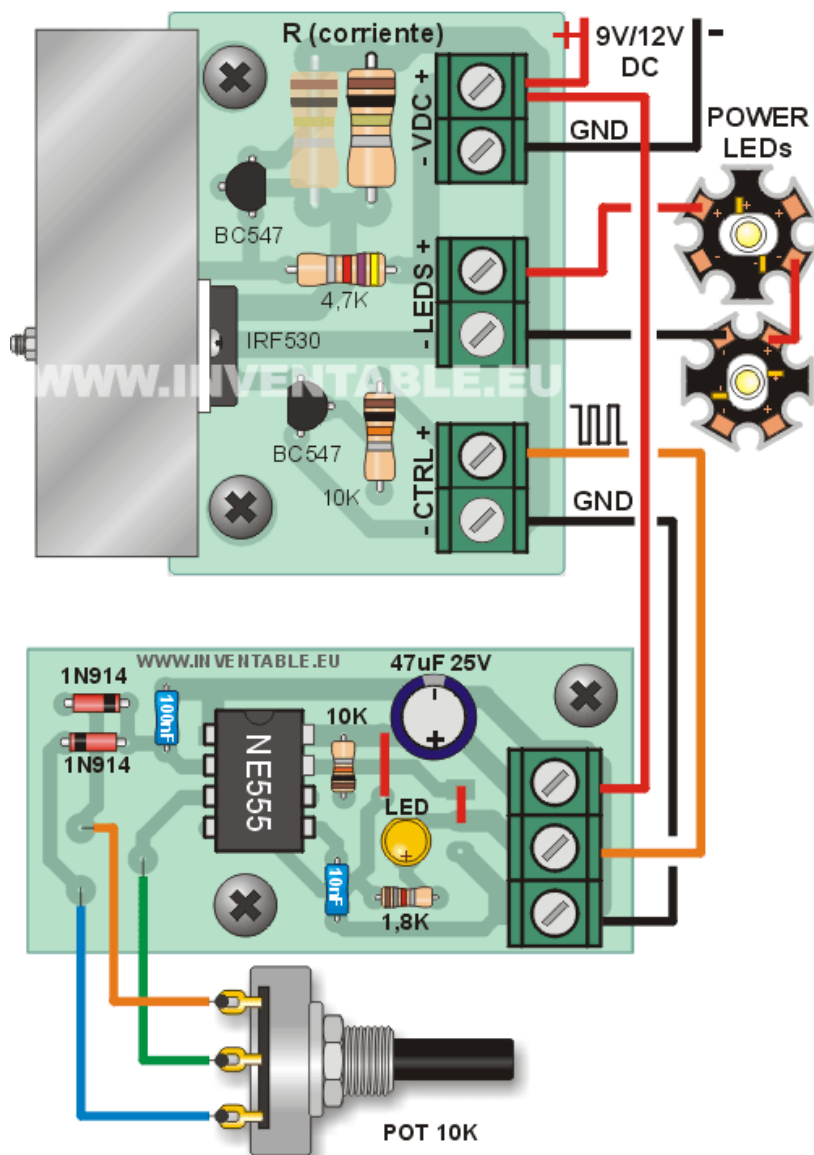

Image 30. Scheme of how the connections should be done in order to control de intensity of the LEDs

2.2. Motor

On the other hand, the program related with the rotation of the motor is also a key point (Image 31), in such a way that it is possible to move the LEDs roulette for selecting the corresponding LED that would be use at each moment. The selection of the LED that is going to be employed at each instant also represents a great advantage of this system regarding the samples that can be analyzed due to the fact that not all the samples have the same luminous characteristics, so they will be excited by different lights/wavelengths. Therefore, high quality images are going to be obtained no matter the characteristics of the samples, thanks to the different applied lights.

Image 31. Motor whose rotation is responsible for the selection of the LED that is going to be used at each moment

This program could be developed by means of LabView software; since there is no an initial version of the program to be tested, in this case it should be developed from the very beginning.

2.3. Measurements Maps

A final process that may be done in the future is a measurements map, which can be described as the representation of the different values obtained by the sensor as a function of the distance between the light source and the sensor, as well as a function of the distance between perpendicular rays of light and the perpendicular of the sensor. This type of maps can be also considered a calibration diagram since it is possible to have a 3D representation of how the intensity of light is scattered by combining the results obtained previously, the 1D measurement map and the 2D measurement map. In such way it is possible to reach even more accurate results than in the situation in which just one axes is taken into account (Image 32).
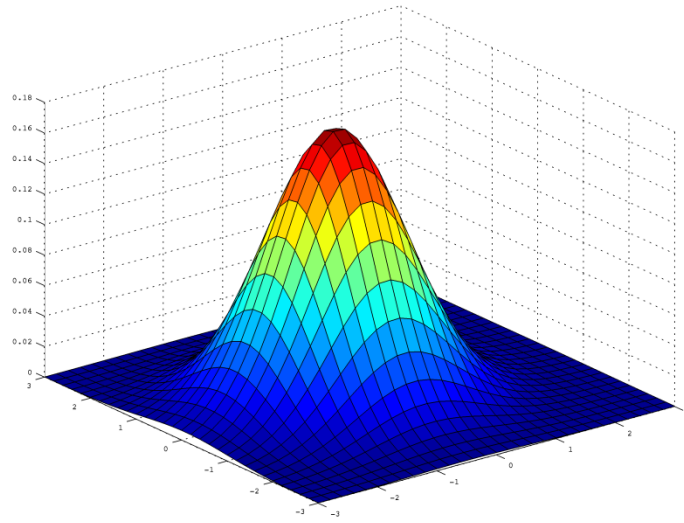
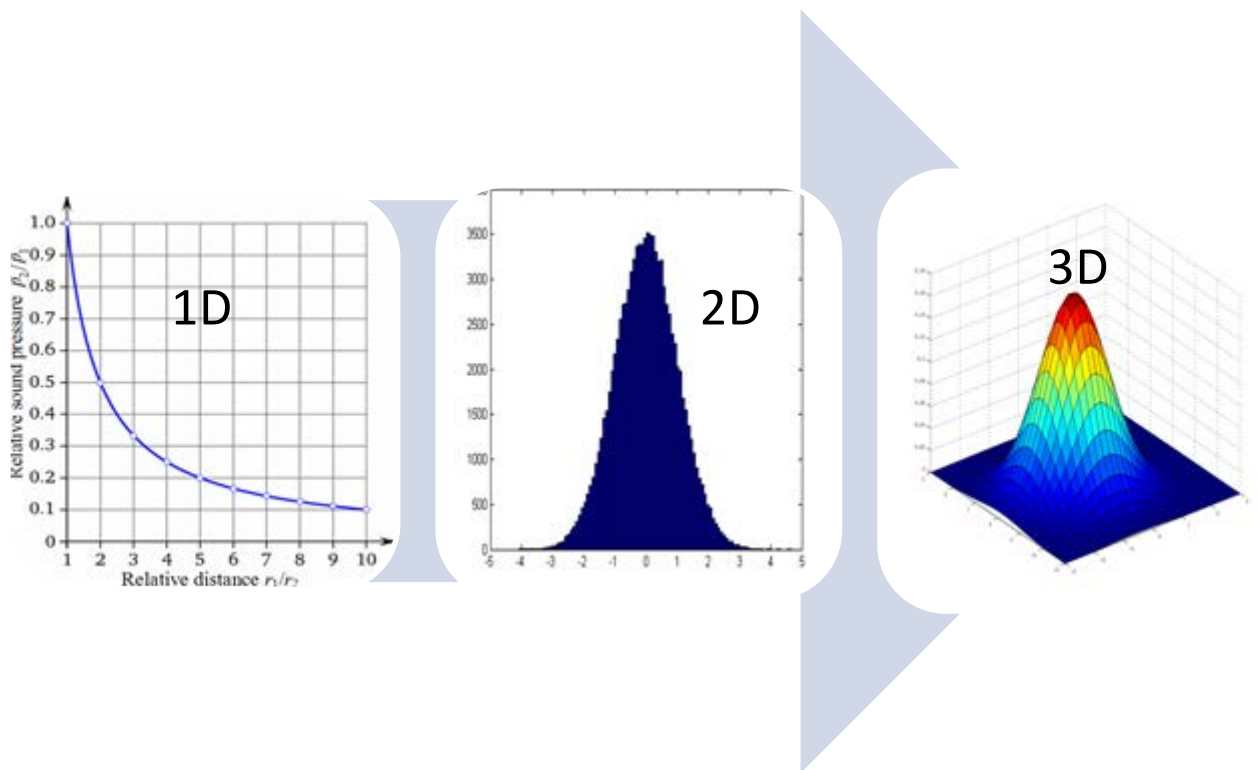**Image 32-a. Final representation of how a measurement map should be.**

Image 32-b. Evolution scheme of measurement maps

Chapter 5. Socioeconomic Impact

Although there are some aspects that need some changes, this system is almost complete, meaning that at this point it works properly and providing coherent and useful results. Therefore, this new system can be considered a revolution for microscopy due to the fact that samples are no longer analyze just by withe light, but also they can be studied by a wide variety of light sources with different wavelengths, hence several light can be used at the same time. Moreover, the intensity control will avoid the degradation of fragile samples that may be damage due to the application of high energy source.

Another thing that must be emphasized is the calibration protocol; with such information, the users of the roulette can change the light sources in such a way that they are able to select the proper components so that they fulfill the needs of the experiment. Therefore, the results obtained will be more accurate.

The last that can be highlight is based in the components of the system rather than in its results; the photodetector and its corresponding amplification circuit provide real time results that can be measured and visualized in such a way that they can be compare with the results that may be obtained by means of pre-exiting sensor. Therefore, the new developed sensor will compete with pre-existing photo-detectors basically due to its low production cost; its commercialization will have a huge impact in the market.

Chapter 6. Budget

1. Human Costs

Status	Number	Cost per Hour (€)	Time Investment (Hours)	Cost (€)
Supervisor	1	35	150	5250
Student	1	15	600	9000
			TOTAL	***14250***

2. Material Costs

Status	Number	Individual Cost (€)	Cost (€)
Computer	1	500	500
Arduino Plate	1	50	50
Sensor	3	8	25
Circuit Components	3	8	25
LEDs	5	100	500
Laser System	1	1000	1000
Mechanical Components	2	50	100
		TOTAL	***2200***

3. Other Costs

Basically it is represented by the 21% of IVA (taxes) that must be paid corresponding to both the human costs and the material costs. At the end, the final values are: 3454.5€ and 462€.

4. Total costs

	Total Cost per Section (€)
Human Costs	*14250*
Material Costs	*2200*
Other Costs	*3916.5*
TOTAL	***20366.5***

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