Universidade do Minho
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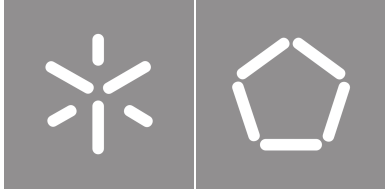
Leonardo Lopes Ramalho

Smart Acquisition System Development for Multiple Readout from Quantum Dots' Fluorescence

October, 2022



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Smart Acquisition System Development for Multiple Readout from Quantum Dots' Fluorescence

Master Thesis
Master in Physics Engineering
Specialization in Devices, Microsystems and Nanotechnologies

Work developed under the supervision of:
Paulo Mateus Mendes

October, 2022

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Abstract

Smart Acquisition System Development for Multiple Readout from Quantum Dots' Fluorescence

The increasing demand for rapid testing stimulates the development of new techniques in clinical diagnosis. When compared to other conventional methods, lateral flow immunoassay has many advantages such as low cost, easy operation, friendly use and quick response. However, it can only give a qualitative or semi quantitative result.

Improving the sensibility of the test result and make it quantifiable is a very challenging field of research. Recently, luminescent nanoparticles have been used, and low detection limits have been obtained. One example is quantum dots, which are ideal fluorescent labels and can improve widely the detection sensitivity thanks to their narrow emission spectra, broad excitation range and excellent brightness.

This work was focused on making a reader that could read strips targeting biomarkers of COPD (Chronic Obstructive Pulmonary Disease). The new reader was created without a motor dependency, which generally takes a great part of the battery power and the reader's size. It consists of an acquisition board, a system board and an optical filter. Several simulations and practical tests were made to complete its instrumentation circuit. In order to get dynamic results, a Python Graphic User Interface was made, making it easier for the user to analyse.

At last, the reader could perform a 563 ms read, sending the information wirelessly to the computer using a BLE protocol. The device has a changeable sensitivity through programming and a directly proportional response to light intensity.

Keywords: acquisition system, BLE, LFIA, microcontroller, COPD, quantum dots, quantum dot's fluorescence

Resumo

Desenvolvimento de um Sistema de Aquisição Inteligente para Leitura Múltipla da Fluorescência de Pontos Quânticos

A crescente necessidade para testes rápidos estimulou o desenvolvimento de novas técnicas na área clínica. Quando comparado com outros métodos convencionais, o imunoensaio de fluxo lateral possui muitas vantagens tais como baixo custo, fácil operação, uso amigável e resposta rápida. No entanto, só pode dar um resultado qualitativo ou semi quantitativo.

Melhorar a sensibilidade do resultado do teste e torná-lo quantificável é uma área de investigação desafiante. Recentemente, nanopartículas luminescentes têm sido utilizadas, e foram alcançados limites mínimos de deteção. Um exemplo são pontos quânticos, que são marcadores fluorescentes ideais e podem melhorar amplamente a sensibilidade de deteção graças aos seus espectros de emissão estreitos, ampla faixa de excitação e excelente brilho.

Este trabalho teve como foco fazer um leitor que pudesse ler tiras direcionadas aos biomarcadores de DPOC (Doença Pulmonar Obstrutiva Crónica). O novo leitor foi criado sem dependência motora, o que geralmente consome grande parte da energia da bateria e do tamanho do leitor. É constituído por uma placa de aquisição, uma placa de sistema e um filtro ótico. Várias simulações e testes práticos foram realizados para completar o seu circuito de instrumentação. Para obter resultados dinâmicos, foi feita uma Interface Gráfica de Utilizador em Python, facilitando a análise do utilizador.

Por fim, o leitor conseguia realizar uma leitura em 563 ms, enviando as informações por ligação sem fio BLE para o computador. O dispositivo tem uma sensibilidade variável por meio de programação e uma resposta diretamente proporcional à intensidade da luz.

Palavras-chave: sistema de aquisição, BLE, LFIA, microcontrolador, PDOC, quantum dots, quantum dots' fluorescence

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1 Introduction

It is now generally acknowledged that up to 70% of clinical decisions are variably influenced by the results of diagnostic testing, so that this branch of science and medicine has an undisputed value in patient care. In fact, an improved diagnostics could lead to earlier detection of pathologies and appropriate intervention, thus improving health outcomes and minimizing the costs of disease and its complications [2].

There are numerous factors that contributed to this impressive trend of growing scientific and clinical interest, including a major understating of disease mechanisms and individual patient responses to therapy, along with remarkable technological advances in laboratory techniques, which have progressed much faster than other diagnostic disciplines, such as diagnostic imaging, and simplified the measurement of a vast array of biomarkers.

1.1 Chronic Obstructive Pulmonary Disease

Nowadays, there is an increasing number of people diagnosed with COPD (Chronic Obstructive Pulmonary Disease). This acronym is a general name for multiple chronic inflammatory lung diseases that leads to irreversible airflow obstruction and even respiratory failure. It is recorded as the third leading cause of death worldwide [3]. Due to its importance, it is relevant to track the condition of the patients regularly. To achieve this, it is necessary to implement a system that can give a quick, reliable and user-friendly result of the test.

Among analytical tests available for point-of-care diagnostics, the lateral flow immunoassay (LFIA) stands out for its low-cost, speed, portability, and ease of use. By its nature, this paper-based instrument is qualitative, intended to provide a positive/negative reading. LFIA, coupled to a quantitative readout device that did not compromise its advantages, would be a powerful tool for many clinical and biological applications, enabling a disease progression understanding.

1.2 Motivation and Objectives

The main motivation of this dissertation was to create a portable fluorescence reader that can read COPD biomarkers, such as, C-Reactive protein and procalcitonin from a saliva sample, using a lateral flow immunoassay with quantum dots as readout.

There is already a developed reader which intellectual property is not owned 100% by Sensovann. It is the company's interest to create a new reader technology, with no dependency on third parties.

The existing reader shows important disadvantages, as it is bulky, heavy, and hard to handle. A smaller device will be much better to use and to offer to the community. The existing reader uses a motor to move the strip in order to analyse it. This uses a lot of power from the battery, and contributes the most to the reader's size. Eliminating this motor was a major improvement on the reader's battery life and dimensions.

The final goal for this project was to create a new smaller reader with quick, reliable feedback.

In order to accomplish this, it was necessary to:

- Develop a smaller and easier to handle reader;
- Propose an alternative readout method, able to eliminate the motor's dependency;
- Develop a new optical acquisition instrumentation system to read the strips;
- Program a microcontroller to control the new proposed readout methodology, convert its data from analog to digital format and send it to other devices by Bluetooth.

1.3 Contributions

During the work of this dissertation, several contributions were made and will be listed below:

- A new instrumentation setup was created for a fluorescence reader.
- A filter, inspired in parallel hole collimator, was created to filter the non-orthogonal light.
- A new design for a reader was created, making it smaller, lighter and easier to handle than the previous version.
- A communication protocol between the reader and the computer using BLE technology was created, sending in words of 3 bytes data information and location information.
- A reader for LFIAs with quantum dots' fluorescence was created, with changeable sensibility, working on the visible spectrum and a quick response ($\approx 563\ ms$).

1.4 Thesis Organization

This document follows the following structure:

- In the first chapter, a contextualization of diagnose tests and the importance of their accuracy is introduced, alongside with a short explanation of COPD and its diagnosing significance. This chapter ends with the motivation and contributions of this thesis.
- The second chapter contains a review of different types of diagnostic tests, more specifically LFIA, and their possible labels for targeting.
- Chapter 3 explains the work done to accomplish the acquisition system. A photodetector was selected, an instrumentation setup was created and simulated, and an electronic circuit was made.
- The next chapter, chapter 4, contains the reader's creation process, from the microcontroller's selection to the reader's casing and components placement. The device's characterization is also performed to provide a complete analysis of the reader.
- Lastly, the fifth chapter is constituted by the conclusion and the future work of this project.

2

Disease Diagnostic Tests

The biggest problem of chronic diseases, such as COPD, is that they usually are not diagnosed at early stages, when it is easily treatable, but better detected when the disease has reached a more complicated stage. For this reason, low cost and quick tests are more required and there is a strong motivation to improve them, giving a quicker and more reliable result.

2.1 Lateral Flow Immunoassay

Lateral flow technology has been available for a long time and these days it got more popular with SARS-CoV-2 rapid tests [4]. They have attracted interest due to their friendly user formats, short assay times, little interferences, low costs, and being easily operated by non-specialized personnel.

In simple words, a lateral flow immunoassay is an easy to handle diagnostic device used to confirm the presence or absence of a target analyte, such as pathogens or biomarkers in humans or animals, or contaminants in water supplies [5].

These tests usually have a control line to confirm if the test is working properly, along with one or more test lines. They can be qualitative and read easily (with an answer yes or no), or they can provide quantitative data when combined with a reader technology.

Commonly, the LFIA technology comprises cellulose and nitrocellulose membranes, coloured nanoparticles, and antibodies as recognition elements. When a sample is added, it will flow laterally along the test device, passing through the conjugate pad into the nitrocellulose membrane and then onto the absorbent pad. (Figure 1)

The sample pad acts as the first stage of the absorption process, and in some cases contains a filter, to ensure the accurate and controlled flow of the sample.

The conjugate pad, which stores the conjugated labels and antibodies, will receive the sample. If the target is present, the immobilized conjugated antibodies and labels will bind to the target by immune-affinity and continue to migrate along the test.

As the sample moves along the device, the antibody-target complexes moving through the nitrocellulose membrane will bind to the second antibody anchored at the test line. A colored line will form, and

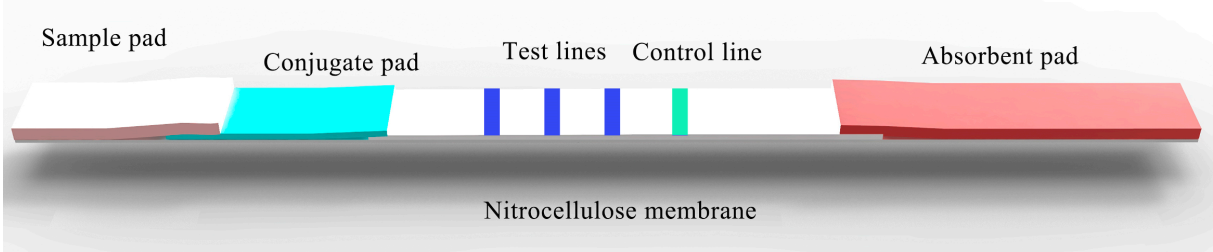


Figure 1: LFIA Strip Schematic

the intensity of the line color will vary depending on the quantity of the present target.

Some targets may require quantification to determine target concentration. This is where a rapid test can be combined with a reader to provide quantitative results.

The sample will pass through the nitrocellulose membrane into the absorbent pad. The absorbent pad will absorb the excess sample. The specification of the absorbent pad will have an impact on the volume of sample that the test can incorporate [6].

2.2 Labels used in LFIA's development

Many LFIA tests are qualitative and semi-quantitative. A number of researchers developed quantitative LFIAs by coupling a signal transducer, such as optical, electrical or magnetic reader. Also, these transducers convert the coloured zones into digital signals.

In optical detection, an array scanner is used as a transducer, and is a high-cost device, restricting the applications. The electrochemical detection is suitable for quantitative analysis, but it requires an additional dissolution step, since the usage of strong acids and toxic mercury limits the applications [7]. In colorimetric detection, the quantitative test results are based on the reading of coloured bands with a portable strip reader. The peak areas are correlated with the intensities of the band and indicate the concentration of analyte in the sample. In multiplex detection, a strip reader records multiple bands simultaneously and shows the individual peak for each band.

Nanomaterials have attracted great interests because of its signal amplification property to achieve high sensitivity and selectivity for target analysis in LFIA. There are many examples of lateral flow assays that combine nanomaterials with immunochromatographic tests, and which can be used for clinical, environmental, and food analysis. Nanoparticles (gold nanoparticles, carbon nanoparticles), luminescent nanoparticles (quantum dots, fluorescent quenching material, up-converting phosphor), superparamagnetic nanoparticles, liposome and enzymes are used as a label in the LFIA development. Giving all the information, such as, production cost, sensibility, testing time, among other, the quantum dots seem to be the most promising label for a LFIA strip.

2.2.1 Gold Nanoparticles

Colloidal gold or gold nanoparticles are commonly used as detector reagent in the LFIA strip for visualization of signals. Vivid and strength red colour enhances visual detection. Other unique properties are the high chemical stability, large specific area, easy synthesis, low cost and easy preparation steps. These properties make the analysis time short and provide reliable analysis on-site [8–11].

The shape, size and stability of AuNPs (Gold Nanoparticles) are key parameters that affect the success of the LFIA. The size and colour of AuNPs depend on the amount of sodium citrate that is used in the reduction of gold [12]. AuNPs with the diameter smaller than 15 nm were small for generating a strong colour; however, nano-colloidal gold with a diameter of 20 nm was used as detector reagent. AuNPs with the diameter larger than 60–70 nm were easily self-aggregated [13] and unstable.

Gold nanoparticle-labelled lateral flow immunoassay (AuNP-LFIA) is an effective method that is widely used in many fields. But the traditional AuNP-LFIA is limited due to its low sensitivity. New methods have been developed for improving sensitivity, such as silver deposition, and signal amplification of large AuNPs. Silver deposition requires additional pretreatment methods and signal amplification of large AuNPs method is simply amplifying the signals.

2.2.2 Quantum Dots

Recently, in a number of studies, fluorescent nanoparticles are used rather than colorimetric markers and low detection limits are obtained [14].

Quantum dots are ideal fluorescent labels and have been widely used to improve the detection sensitivity of LFIA owing to their narrow emission spectra, broad excitation range and high fluorescent quantum yields. Quantum dots have excellent brightness, size-tunable fluorescence emissions, large absorption coefficients, good stability, and high signal-to-noise ratio. The excellence of brightness and exceptional stability of quantum dots enable them to be detected and be quantified sensitively.

For simultaneous detection of several compounds in a sample, two different formats of LFIA have been developed. In the first format, LFIA includes different lines for each analyte, this is, the different antibodies of analytes are immobilized in different lines. In the second format, LFIA includes one line for both analytes, this is, all the antibodies are in the same line.

An example of the first format was developed in [15], which is a "traffic light" format lateral flow immunoassay and in this case, the LFIA detected three different antibiotics. Antibodies against antibiotics were conjugated with water-soluble quantum dots with emission maximum at either 525, 585 or 625 nm, making the strip with the colours green, yellow and red respectively, like a traffic light. The quantitative detection was based on the colour intensities of the corresponding test lines. In Figure 2, it is possible to see this format of multiple detection. In A, the strip is presented before performing any test. B shows the results for the sample containing the first antibiotic, this is, the green target. And in C, it is a strip used with a sample containing the second and third antibiotic, this is, the yellow and the red targets.

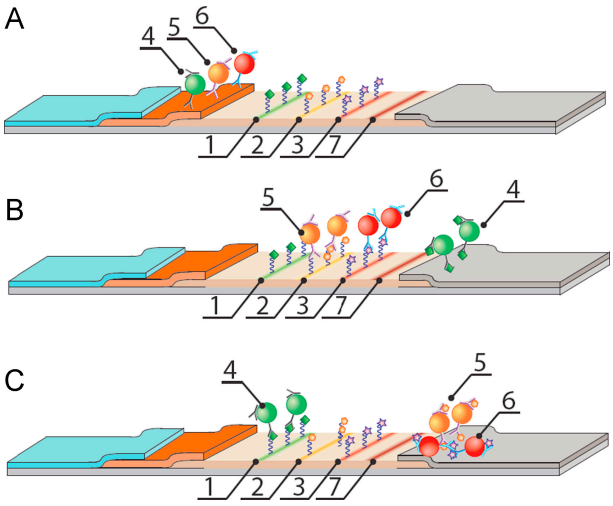


Figure 2: Principle of detection of three antibiotics using "traffic light" [15]

In Figure 3 is represented a schematic illustration for the test strip for the second case. For the simultaneous detection, one test and one control line were constructed on the nitrocellulose membrane. The fluorescence intensities of tumour markers captured on the test and control lines were measured simultaneously by using a strip reader.

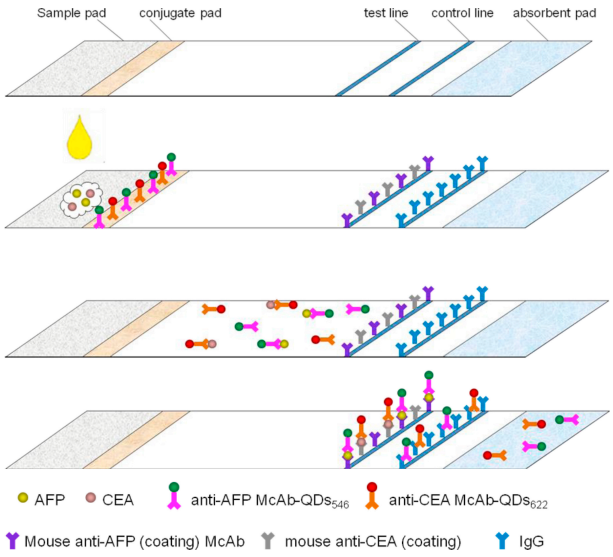


Figure 3: Schematic illustration of simultaneous detection with only one test line [16]

2.2.3 Superparamagnetic nanoparticles

Superparamagnetic nanoparticles (MNPs) are new labelling materials to develop a LFIA. They increase the sensitivity almost 10 to 100 times. Additionally, magnetic signals are produced by MNPs, and they are stable over long periods of time. They have low background noise, because the magnetic material does not exist usually in the environment or in the tested samples [17].

Most studies have shown that super-paramagnetic particles are new attractive material to construct a LFIA, which will replace traditional labels due to its stable magnetic signal which can be entirely captured by devices, thus improving the sensitivity of LFIA. The reasons for using these particles as labels are their unique properties, such as magnetism in strong magnetic fields, and this specific property prevents aggregation and precipitation [17]. The small size of magnetic nanoparticles (MNPs) enables short detection time. The increased magnetite content of MNPs provides a stronger signal; also, the magnetite content has positive linear relationship with the signal intensity [18].

In [19], a LFIA using magnetic particles is presented exploiting spontaneous magnetic switching, a superparamagnetic characteristic, to produce a quantifiable electromagnetic induction in an alternating current carrier. This approach is practical as it does not require the application of external magnetic fields.

2.2.4 Enzymes

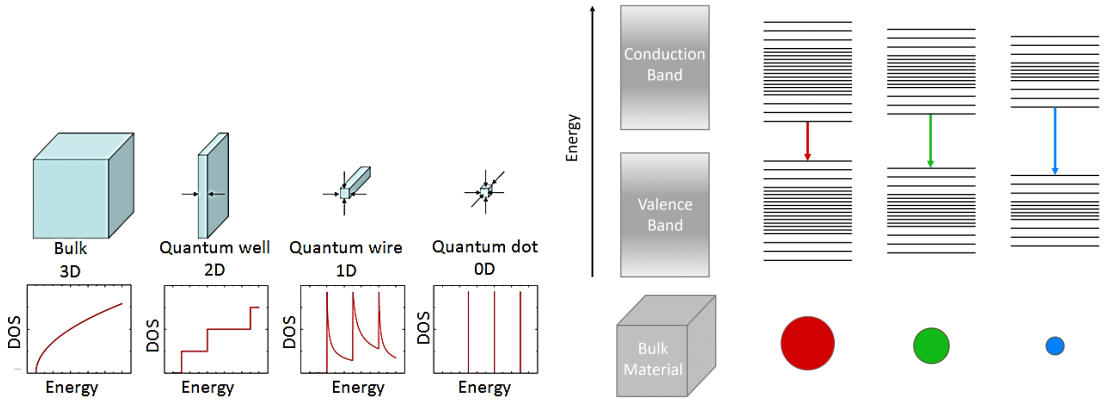
In the literature, three types of test strips have been used. The most common strip is based on capturing an analyte by an antibody labelled with colloidal gold, latex bead or fluorescent dyes to form antibody-antigen complex. This forming complex binds to other immobilized antibody on TL and CL lines. The second type of strip is based on chemical reactions which gives a colour change. The third type is based on the enzyme-substrate reactions for colour development. The formed colour can be visualized by naked eye and standard colour chart. This leads to imprecision caused by large variation in judgment due to human errors. Quantifications of signal by readers have been also developed, for example the refractometer for determining the optical density, the electrochemical signal for measuring the conductivity of metal labels and stripping voltammetric detection of metal ion labels. However, the requirements of expensive reading device make the assay costly less beneficial.

2.2.5 Liposomes

Liposomes are sphere-shaped artificial vesicles consisting of one or more phospholipid bilayers. Owing to their size and hydrophobic and hydrophilic character (besides biocompatibility), they have been particularly used in drug delivery. Liposomes' properties differ considerably with lipid composition, surface charge, size, and the preparation method. They are very stable, and their large internal volume provides interaction with several biological elements, such as peptides, hormones, antibodies, sugars, and nucleic acids. These properties make them useful in field-portable or point-of-care sensor systems. But, these come with some major disadvantages such as the structural weakness toward detergents, pH changes which is an adverse effect on the ionic charges in the phospholipid bilayer, and osmotic pressure related to swelling or crenation [20].

2.3 Quantum Dots

Quantum dots are semiconductor particles with a size of a few nanometres. The selection of quantum dot materials has primarily been driven by the ability to prepare particles with the desired optical properties [21]. These luminescent nanoparticles are really special because they are particles confined to three dimensions, which makes its density of states unique. The energy levels are very discrete and precise (Figure 4(a)) and the energy gap changes according to the quantum dot's dimension. (Figure 4(b))



(a) Evolution of the density of states (DOS) with dimensionality of the system [22]

(b) Relation between the size of QD and its band gap and color of fluorescence [23]

Figure 4: Quantum Dots Properties

Quantum dots also have an optical property, which is the main interest in this project. Quantum dots' fluorescence is, in simple words, when a quantum dot is illuminated, absorbing the energy from the photons, it emits the light back in a different wavelength. This wavelength is related to its energy gap and, as mentioned above, is directly associated with its size. (Figure 4(b))

One of the most known applications are quantum dots LEDs. These can reach from 1250 to 67840 cd/m^2 of luminance [24]. Looking from the photodetector's perspective, a group of quantum dots can be seen as a light source with isotropic properties and a coherent intensity of light, therefore, a light source such as a torch or a smartphone can be used as a replacement.

2.4 Reading Quantum Dots' Fluorescence

In order to read the fluorescence of quantum dots, a preliminary light emission is necessary to give energy to the quantum dots. In this case, the reader has an emitter to excite the quantum dot and a receiver to collect the light. Usually, the wavelengths of the excitation light and emission light are well-defined and really distinct [25]. This is a good advantage feature on behalf of the specific energy gap, which defines the wavelength of emission light and minimizes any interference between the LED and the photodetector [26].

The emission light, on a macroscopic scale, is isotropic, this is, the radiation propagates in the same intensity when measured in different directions. Although there are many advantages to acquire the light,

the disadvantages can't be ignored. It is possible to implement the whole optical system (emitter and receiver) in the same plane, however, if the strip has several test lines, crosstalk may lead to interference in the light collected by photodetector, due to the present simultaneous multiple lines.

For this project, the LFIA strips were already manufactured, and they use quantum dots as label for the targets. They are core/shell quantum dots made from CdSe/ZnS. The absorption wavelength is around 365 nm, this is, at UV light, and the emission peak wavelength is at 610 nm, which is the visible spectrum, the colour red. Figure 5 is a spectrum from typical quantum dots that were manufactured.

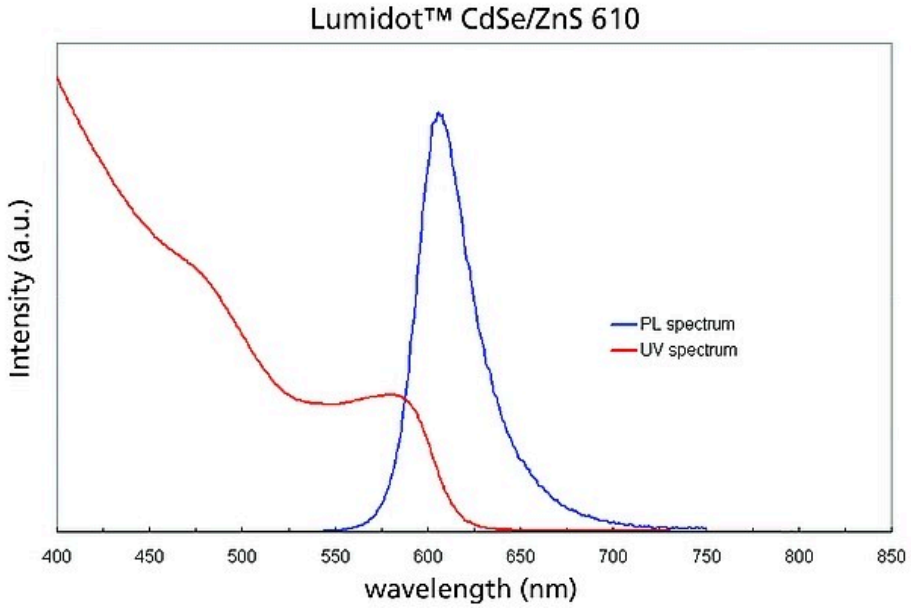


Figure 5: Absorption and Emission spectrum of CdSe/ZnS quantum dots

3

Acquisition System

An existing prototype that makes a reliable and quick result has been accomplished. However, the reader is bulky, heavy, and not user-friendly. In order to read all the strip's T-Lines (test lines), this prototype uses a black box with the optical system, which has the emitter and the detector, and a motor to make the strip move, in other words, it is the opposite of a scanner, the sensor is fixed in the same place and the strip moves to be scanned.

3.1 Reader Architecture

Below, Figure 6 represents a schematic of this working reader.

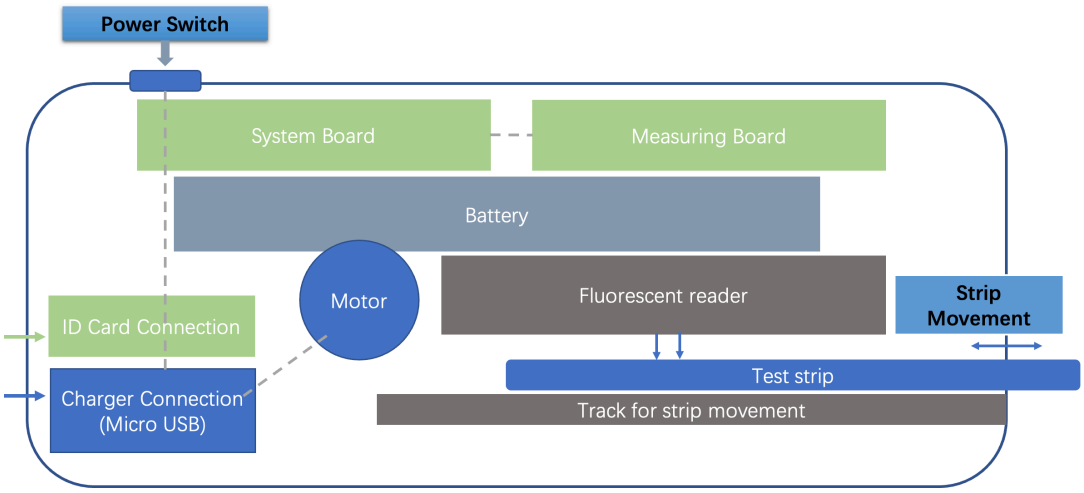


Figure 6: Reader Block Diagram, showing a general overview of the device's architecture

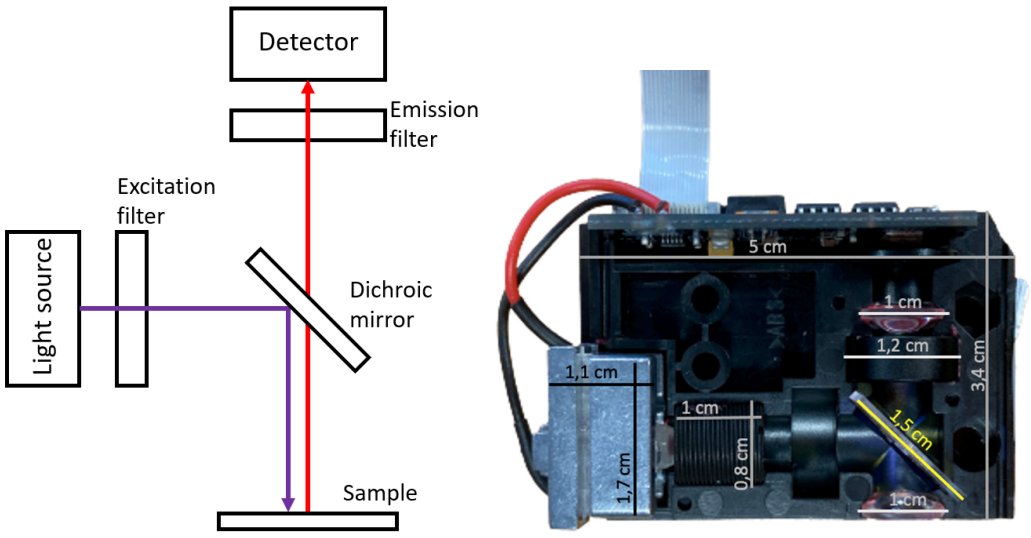
The reader is composed by a battery and its charging port, a system board, a measuring board, the optical system (black box), a motor and a platform to place and move the strip.

The system board controls the entire reader and makes the bridge between the user and the board. It has a power switch to preserve battery, two LEDs to indicate the power state and the Bluetooth connection,

a microcontroller, several surface mounted devices for the instrumentation and connectors for the other board (measuring board) and a motor.

The measuring board is made only to excite and collect the fluorescence from the quantum dots. It has a UV LED with its own LED drive, so the intensity of the excitation light is always constant and the photodetector where its peak of sensibility is around the quantum dots' emission light wavelength.

3.2 Optical system



(a) Block Diagram (b) Photo

Figure 7: Optical System

In Figure 7(b), it is possible to see the existing prototype reader's optical system. It is composed by a UV LED (on the left) with its own lens to focus the excitation beam. In the middle there is a dichroic mirror which reflects light below approximately $425\ nm$ and lets pass through the emission light. Figure 8 shows its transmission rate versus the wavelength. On top, there is a red filter to eliminate any light noise and increase the photodetector sensibility

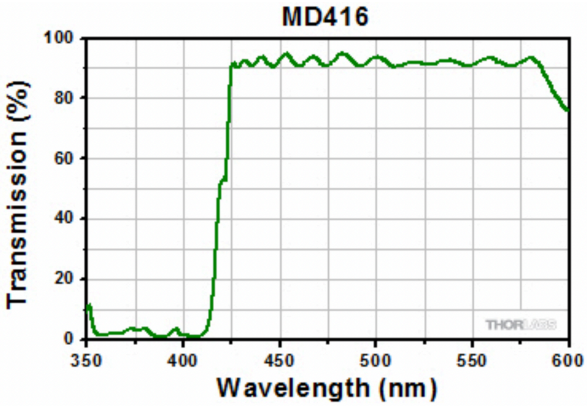


Figure 8: Spectrum of Dichroic Mirror [27]

and a small lens to focus the light to the photodetector. At the end, there is a photodiode to convert the light into current that later will be transformed to voltage.

As can be seen in Figure 7(a), the excitation light starts in an orthogonal direction. Only then is reflected in the dichroic mirror to reach the sample and initiate the fluorescence process. Then the emission light passes through the same mirror and reaches the photodetector.

This optical system is made in this way to maximize the distance between the emitter and the receiver. Thus, there is no interference in the photodetector from the UV LED.

3.3 Proposed Approach

After the reader was disassembled, studied and analysed, this project motivation came from the need to develop a new, quick, reliable and user-friendly reader, with quick feedback. Hereupon, the main objective was to propose and implement a new reader that is able to read multiple test lines in one strip, allowing to remove the motor dependency, leading to a significant system miniaturization.

Figure 9 is a schematic of the system that was intended to be implemented.

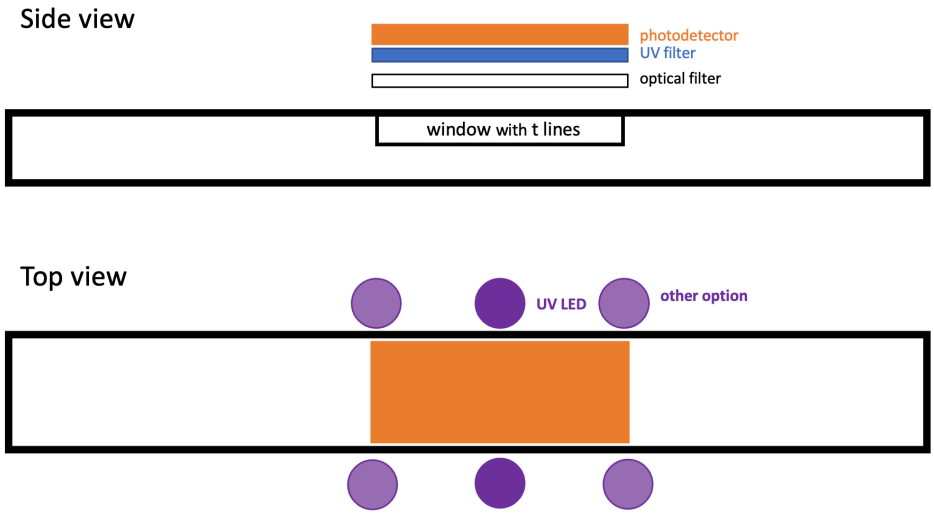


Figure 9: New Reader Building Blocks

The UV LEDs were placed in the same plane as the photodetector, however the number of LEDs was determined based on its emission angle to create a coherent and well-distributed light.

The main goal of this project was to create a new and better reader without the motor dependency and capable of connecting wirelessly to a smartphone app by Bluetooth. To fulfil all these requirements, a system was built with a photodetector, a microcontroller and a Bluetooth module. Since the smartphone app was still underdevelopment, a python graphic interface was created to test the communication, replacing the smartphone with a computer. In Figure 10 it is possible to see a block diagram representing the reader's operation.

The computer sends a wireless signal to start the reading to the Bluetooth module, the microcontroller receives this information and sends the start signal to the photodetector. The photodetector gives the

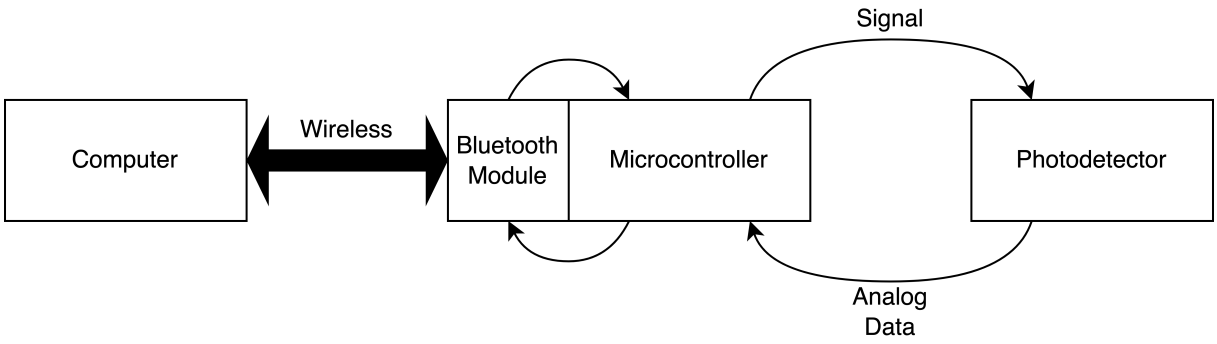


Figure 10: Reader's Block Diagram

voltage, related to the light intensity, to the microcontroller that sends it, with the Bluetooth module's help, back to the computer where the user can analyse the data. This process will be explained with more detail in chapter 4.

3.4 Photodetector

The first component to find for the reader was the photodetector. Given that the emission wavelength of the Quantum Dots was $610\ nm$, to get the best possible result, the main goal was to find a photodetector with its sensitivity peak close to the same value. Several different types of photodetectors were found. Table 1, shows their main features, allowing a side by side comparison.

Table 1: Found photodetectors and their main features

Name	Range (nm)	Sensitivity	Sensitivity peak	610 nm sensitivity	Angle of detection
TEPT4400	440-800	$2\ \mu A/lx$	570 nm	95%	$60°$
TEPT5600	440-800	$3,5\ \mu A/lx$	570 nm	95%	$40°$
SFH3711	470-670	–	570 nm	75%	$120°$
S9219	380-780	$0,24\ \mu A/W$	550 nm	40%	$60°$
SFH2240	400-690	$7,3\ nA/lx$	620 nm	95%	$120°$
SFH2440	400-690	$9,4\ nA/lx$	620 nm	95%	$120°$
SFH2716	350-1000	–	620 nm	98%	$120°$

As shown in Figure 11, the idea was to acquire three photodetectors and place them exactly above the test lines to get their light. For this configuration, the best photodetector would be the one with the highest sensitivity, however, with the lowest angle of detection, in order to prevent cross talking, this is, the first photodetector collecting light from the second test line. Given these requisites, the best photodetector from Table 1 is the TEPT5600.

During the time of execution of this project, the requirements were changed, and the new reader is supposed to be capable to read strips with two, three or four lines, this is, one, two or three test lines for the different biomarkers and one control line.

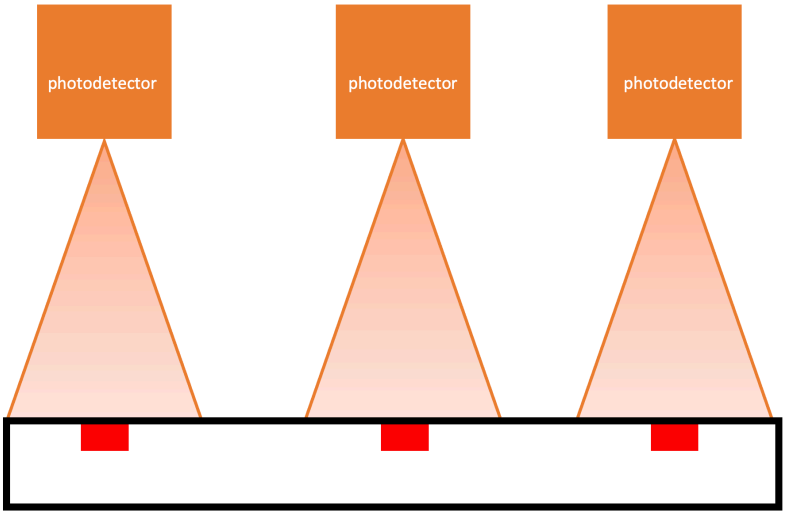


Figure 11: Initial photodetector placement

At that moment, the configuration in Figure 11 only allows to read strips with three lines, which are the ones that exist at that moment. However, new strips had been made to cover all the three biomarkers, so these new strips will have 4 lines to read. Furthermore, it was required that the reader could be versatile and could read any type of strips.

As it is possible to see in Figure 12, the strips have the same "readable window" and only the space between the lines change. Keeping the idea to remove the motor dependency and make a static solution, the chosen photodetector is no longer a valid option.

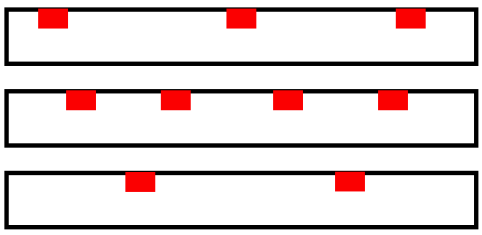


Figure 12: Possible places for the lines in the readable window

To get around this problem, a different type of photodetector was found. A NMOS Image Sensor. It is an array of photodiodes, with a built-in Current Voltage Converter. With this new configuration, no matter what version of strip, the reader can detect all the lines and get the information about the biomarker's concentration, without the motor dependency.

Looking at the dimensions, the photodetector found (S3901-512Q) has $25,6\ mm$ of length where the readable window has $18\ mm$. Each line has approximately $1\ mm$ of width, and the photodetector has $45\ \mu m$ wide photodiodes spaced by $5\ \mu m$.

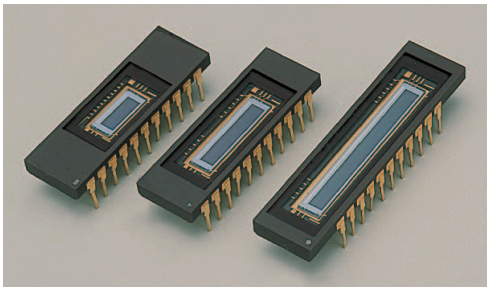


Figure 13: NMOS Image Sensors

This photodetector was a good solution for the new requirements, however, due to the semiconductor shortage, the delivery time for S3901-512Q was not suitable to meet the work plan. So, as an alternative, a smaller photodetector was used to test if the proposed solution worked. Summing up, the S3904-512Q photodetector was used, which is shorter with only $12,8\ mm$ of length, but with more pixels with $20\ \mu m$ wide photodiodes.

To test the photodetector, all the pins were connected, as the datasheet says. As an output, the photodetector gave $0\ V$ when it got to light's saturation and roughly $1,64\ V$ when no light was detected.

Since, the photodetector's goal is to give a directly proportional relation, this is, more light gives more voltage, an inverter configuration is needed. The final goal of the photodetector's output is to get to a microcontroller's ADC, so, it is the best interest to get the output range between $0\ V$ and $5\ V$. To accomplish this, an inverter amplifier with a voltage offset was used, using resistors of $10\ k\Omega$ and $27\ k\Omega$, making a gain of 2,7 and a voltage offset of $1,25\ V$.

To get these values, some simulations were made in software Tina-TI.

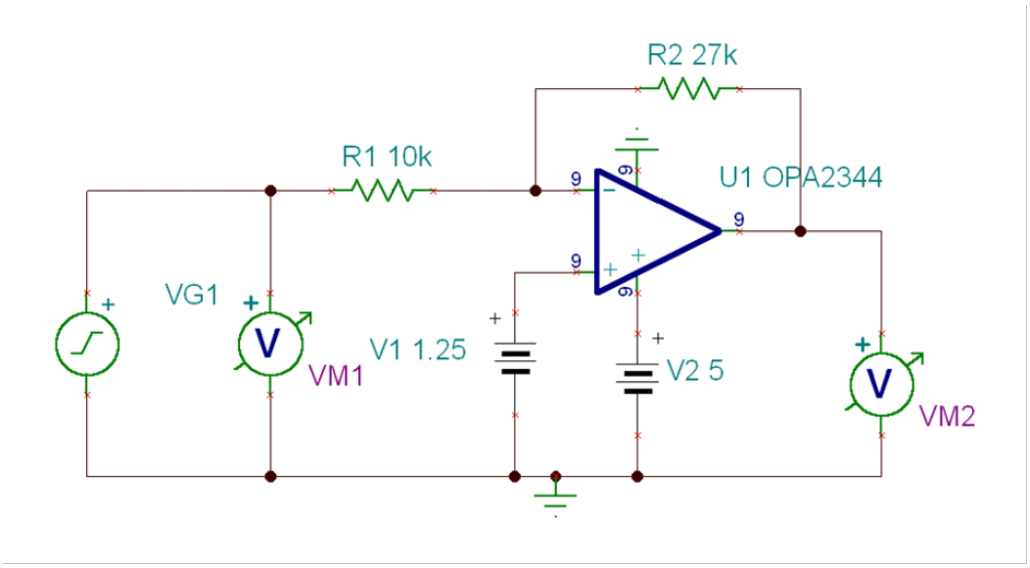


Figure 14: Circuit Diagram for simulation on Tina-TI

In Figure 14 it is possible to see the circuit that was used to make the simulation and in Figure 15 the oscilloscope signal of the input and output signal.

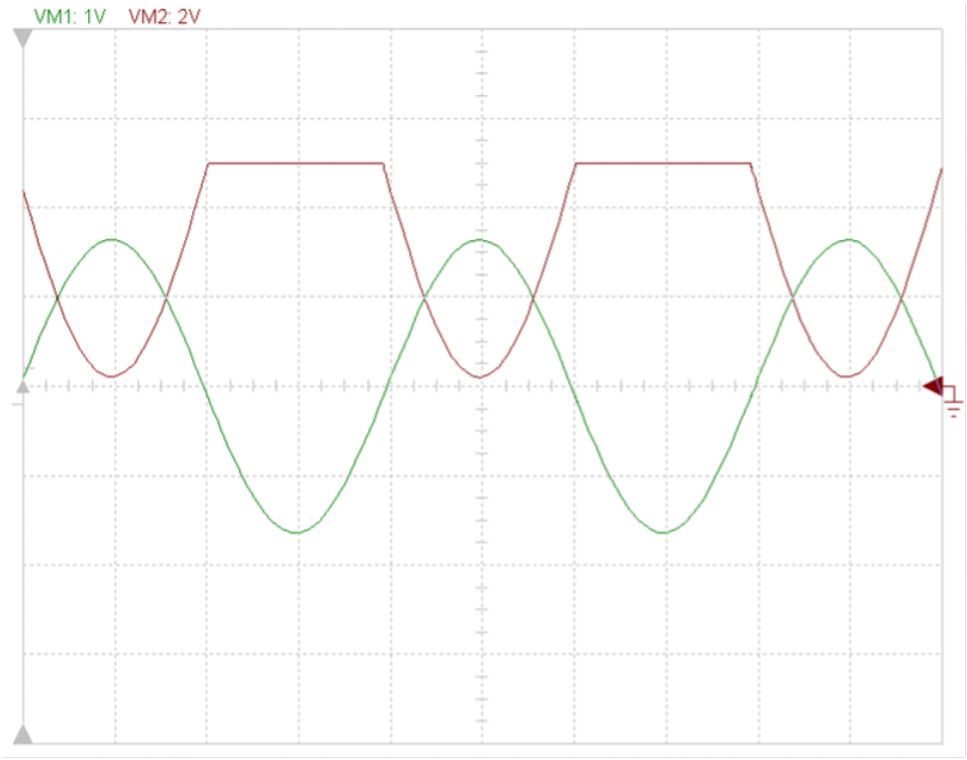


Figure 15: Oscilloscope signal of the input (green) and output (red) signal

To simulate the signal that comes from the photodetector, a sinusoidal signal of $1,64\ V$ of amplitude was created. After some time of trial and error, a perfect offset voltage was achieved, $1,25\ V$. As it is possible to see in Figure 15, the output signal is no longer reaching negative voltage and has a range between $0,210\ V$ and $4,63\ V$. It is noteworthy that the output signal reaches positive saturation level, however it is irrelevant since the photodetector cannot give negative voltage.

3.5 Conclusion

Summing all up, an already developed reader was disassembled, studied and used as inspiration to create a new one.

To make this new reader, a different type of photodetector was needed. That photodetector was found and tested, and with some amplification, it can give a directly proportional response between the light and the voltage, making this last one reaching from $0,210\ V$ to $4,63\ V$.

4

Acquisition Module

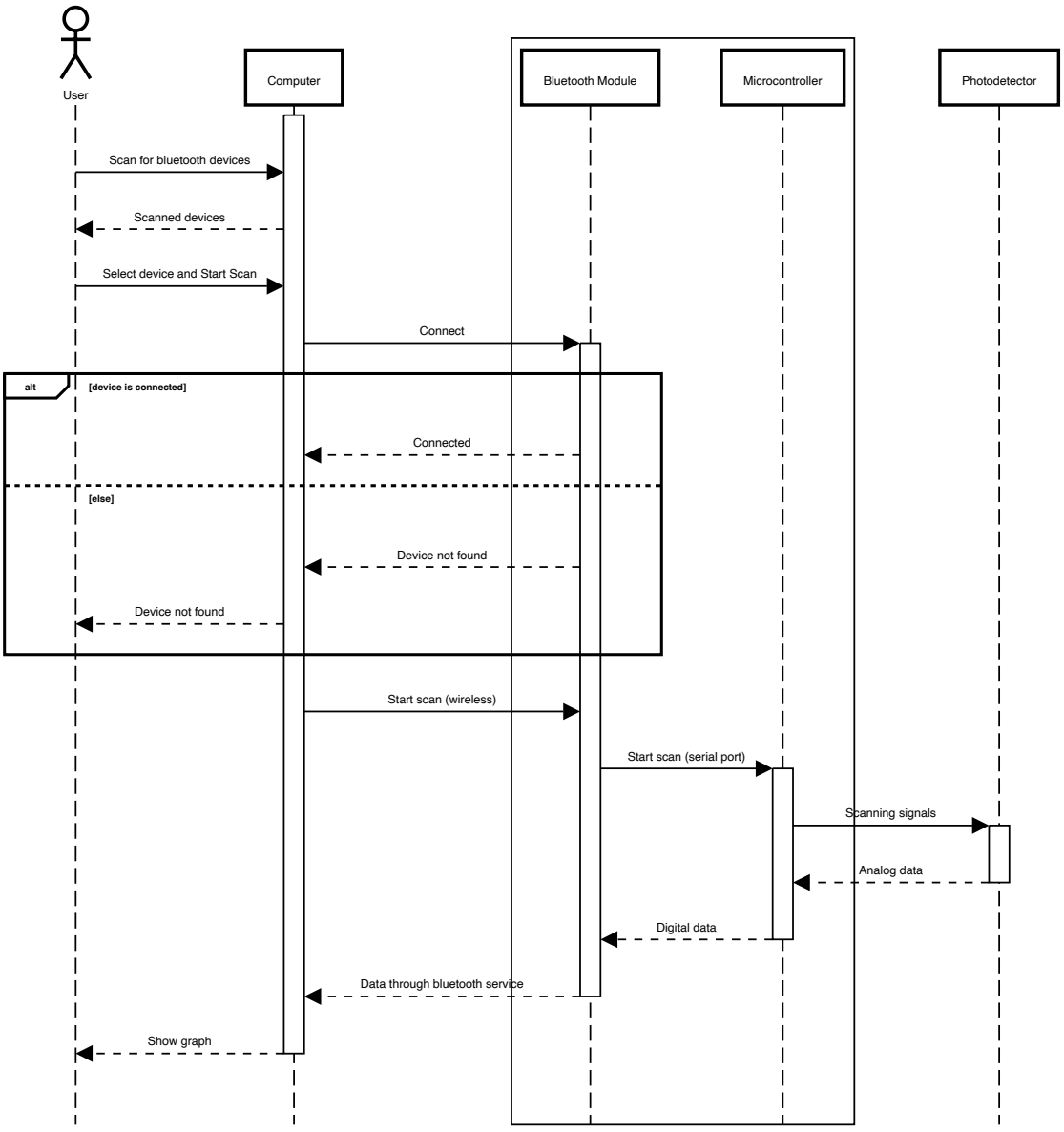


Figure 16: Sequence Diagram of the whole project

For the reader to work, a user and a computer are needed.

Figure 16 shows a sequence diagram of the reader's operation. Starting with the user, he scans for Bluetooth devices using the computer. After getting the list of devices, selects the desired device, which in this case is called "KOLS reader". Then, after connecting successfully with the device, it sends the signal to start the reading. The Bluetooth module transmits this information to the microcontroller that initiates the reading by sending the signal to the photodetector. In the meantime, the photodetector is giving back the information needed, which is collected by the microcontroller's ADC. This value is then sent to the Bluetooth module that sends wirelessly to the computer. Finally, the computer crunches the data and shows a graph, so the user can see and analyse.

All the code can be consulted in Appendix A.

4.1 Microcontroller

In order to control the photodetector and to make the user experience the easiest possible, a *CURIOSITY HIGH PIN COUNT (HPC) DEVELOPMENT BOARD* from Microchip was acquired.

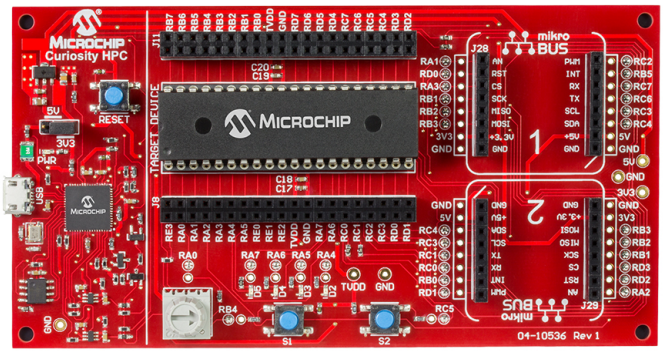


Figure 17: Curiosity High Pin Count Development Board

This board came with a $PIC18F47Q10$ microcontroller, which is a chip with several features, from which we can highlight:

- Selectable clock up to $64\ MHz$
- Three 8-bit Timers
- $128K$ Bytes Program Flash Memory
- Operating Voltage between $1,8\ V$ and $5,5\ V$
- Two Enhanced USART
- 35 I/O Pins
- 10-Bit Analog-to-Digital Converter with Computation (ADCC)

It is also important to mention that the HPC Development Board has two buttons, four LEDs and a potentiometer to debug and check if the program is working. It also has an Integrated Programmer/Debugger with USB Interface which makes the integration seamless with MPLAB X IDE and Code Configurator and Mikrobus™ support that was used for the Bluetooth Module, which is going to be discussed in section 4.2.

The microcontroller programming was based on the photodetector's datasheet [28].

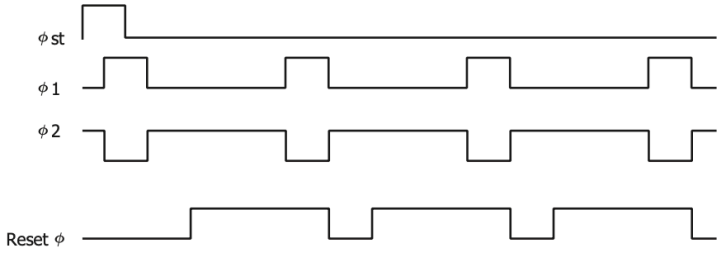


Figure 18: Signal required by the photodetector

In Figure 18, it is possible to see the four signals that are needed to get the read from the photodetector and how they need to be relatively to each other. To accomplish this, four different ports were used to send the signal (as can be seen in Figure 25).

To create a similar signal as in Figure 18, three functions were created.

A function named *tick()* was created to control the output of each port.

```
void tick(int times){
    int i;
    for(i=0;i<times;i++){
        __delay_us(100);
    }
}
```

tick() uses the assembly function *__delay_us()* that makes the microcontroller wait the given amount of time in microseconds.

A simple function of printing was created using the predefined function *EUSART1_Write()*, called *print()*.

```
void print(char* str){
    int i;
    for(i=0;str[i]!='\0';i++){
        EUSART1_Write(str[i]);
    }
}
```

Another function was created, called *read*, which can be seen in Appendix A. Its purpose is to do the whole reading process, since the beginning until the testing if the reading is complete. It uses a variable called *stop* to get the value from *End of scan* pin. This pin is always at $5\ V$ except when the scan is over, which goes to $0\ V$. Since the ADC's voltage reference is $5\ V$, that means that *stop* will be always 255 until the scan has ended.

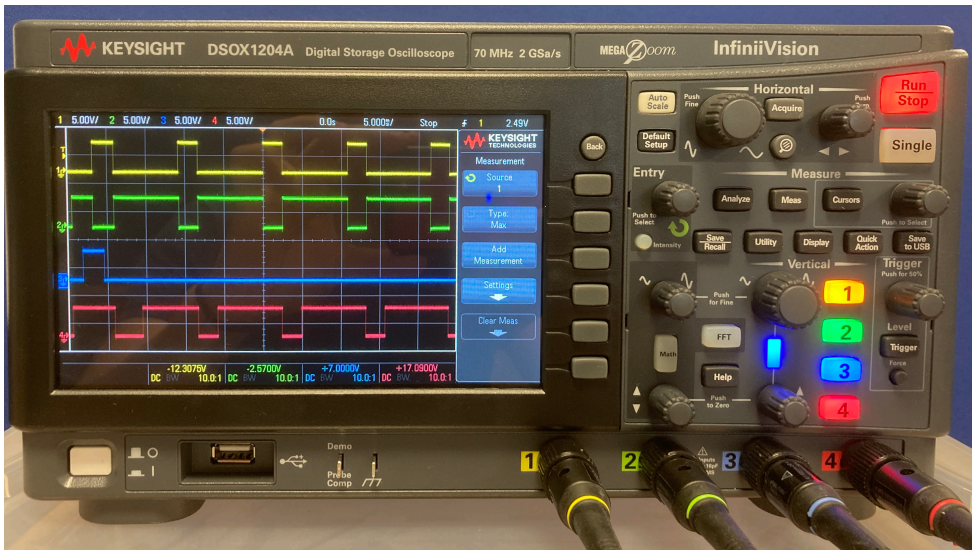


Figure 19: Photo of the oscilloscope

Before connecting to the photodetector, an oscilloscope was used to test if all four signals were working properly. Figure 19 is a photo of the oscilloscope with the reading signals. Where channel 1 is signal $\phi 1$, channel 2 is signal $\phi 2$, channel 3 is signal ϕst and channel 4 is signal $Reset\phi$. Concluding, Figure 19 is similar to Figure 18.

To test if the microcontroller was getting the voltage from the photodetector, a test was made.

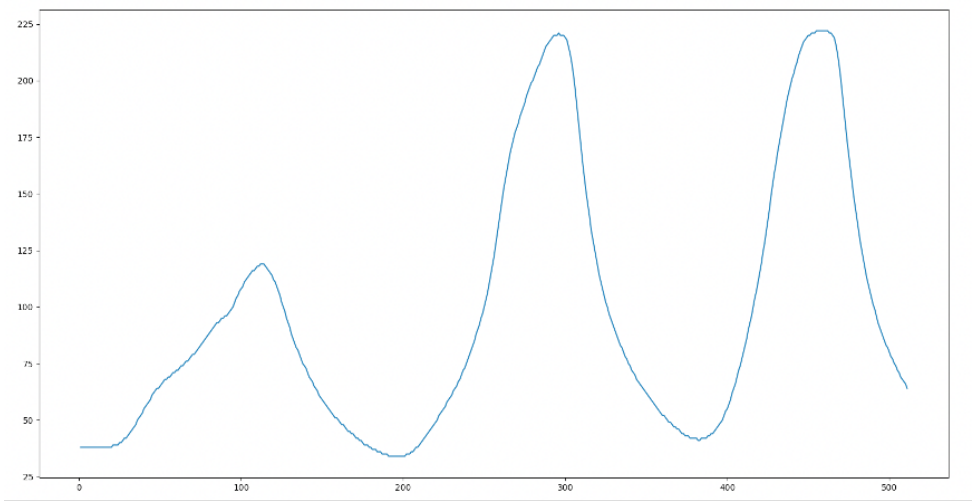


Figure 20: Graph from 1st test

Figure 20 is a graph obtained by making a reading from the photodetector when the smartphone's

flashlight is moving closer and then further away three times. And sending the data by serial port to the computer.

With this test, it is possible to conclude that the photodetector can detect light and its response is directly proportional and that the microcontroller is receiving the data correctly.

4.2 Bluetooth Module

To make the communication between the cellphone and the reader wireless, a bluetooth module was selected with a mikroBUS™ interface and a RN4020 module.

Figure 21: BLE2 Click

The main feature to select this module was the Microchip Low-energy Data Profile (MLDP) that makes the reader working as a wireless with serial communication protocol.

This module can be programmed using the UART serial communication port. To make this process the easiest possible, the USB Interface from the HPC Development Board and a terminal called CoolTerm were used.

Figure 22 is a photo of the layout used to program the Bluetooth module. The orange and white cables are for receiving and transmitting pins, respectively. All the red wire are connected to $3,3\ V$ and all the blue wire are connected to ground.

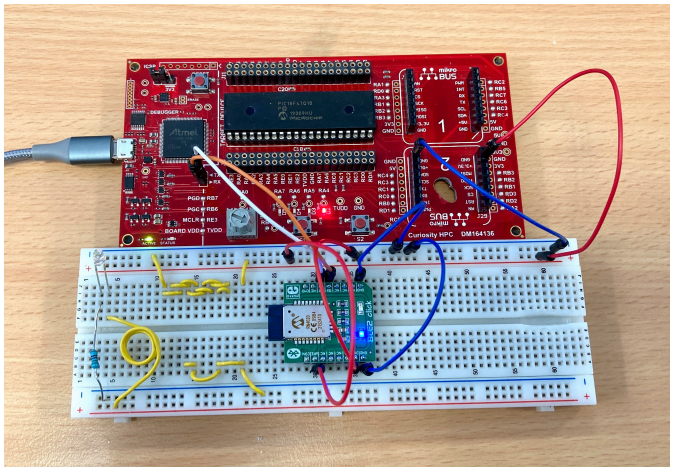


Figure 22: Photo of setup to program the Bluetooth module

For the purpose of this project, the Bluetooth module's programming was very simple.

First, it was set the baud rate. In this case, the baud rate suggested in the datasheet was used, this is 115200.

Second, the needed services were selected. Since only the information from the photodetector is needed on the smartphone or computer, only device information and a private service were selected.

Then, a private service is created with two characteristics, one for reading and other for writing.

At last, the features of the module are selected, in this case, only *Auto Advertisement* was selected.

In Figure 23, it is possible to see a print screen of CoolTerm and all the commands made to program the Bluetooth module. At the end, an LS command is executed to check if everything was created as expected.

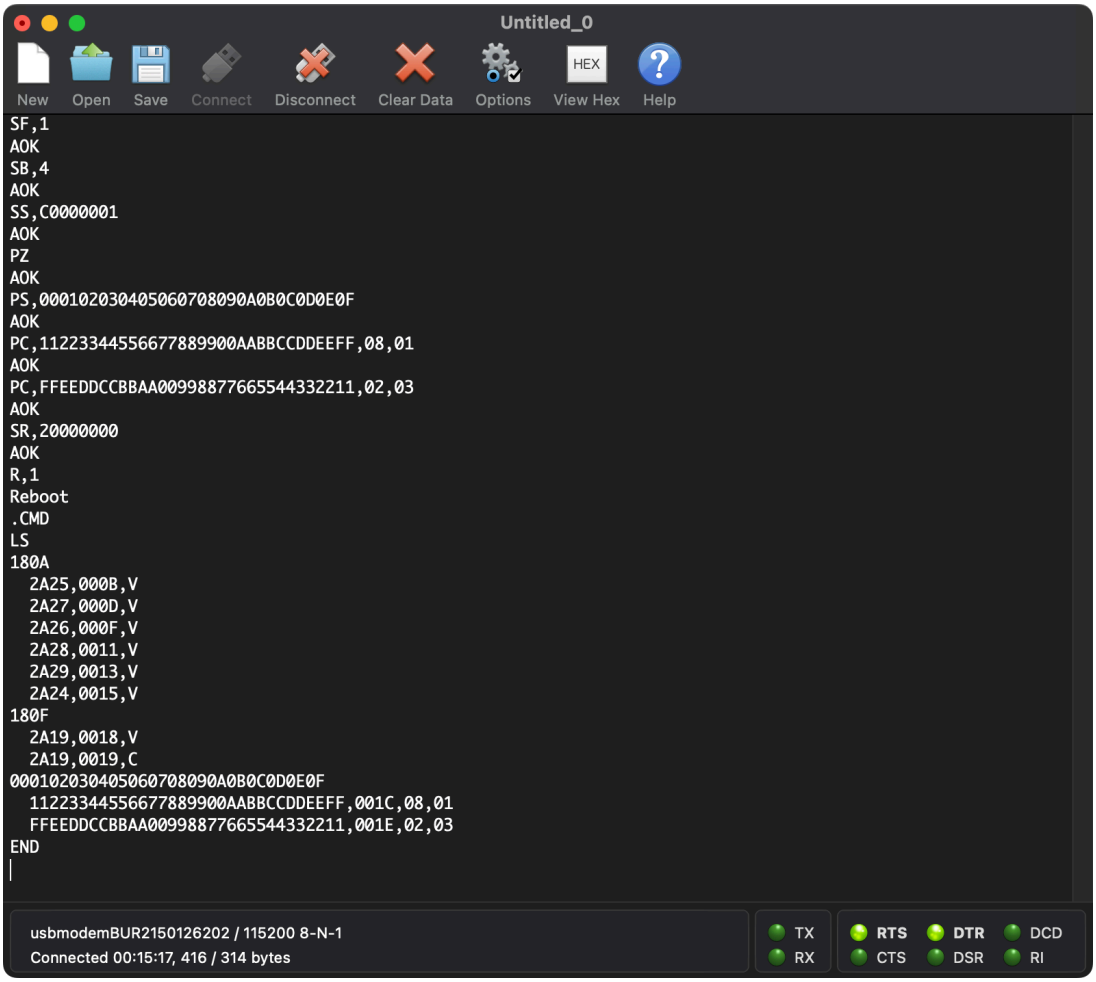


Figure 23: Programming of the Bluetooth module

4.3 UV LED

The UV LED was selected based on two main variables: emission wavelength and angle of emission.

Figure 24: MT3650W3-UV

Knowing that the Quantum Dots' excite at $365\ nm$ and emit at $610\ nm$, a LED was chosen with the following features.

Table 2: Main features of selected UV LED

Peak Emission Wavelength	Power Output	Reverse Current	Half Intensity Beam Angle
$365\ nm$	$1,5\ mW$	$80\ mA$	$\pm 45^{\circ}$

4.4 Circuit

Figure 25 shows a schematic of the main components of the project.

Looking at the microcontroller, it has 4 pins without connection (RA4 to 7). This is represented in this way because these pins were used only for debugging with the LEDs inlaid in the development board. Then, there are 3 connections to the *BLE2 Click* using ports RA1, RD0 and RC2. These connections are for waking and change states of the Bluetooth module that was discussed in section 4.2, and are also made through the PCB of the development board. The Bluetooth module has 2 more connections for the serial port communication with the microcontroller. On the right side of the microcontroller, it is possible to see 4 ports dedicated to send the signal to the photodetector (RB0 to 3). And a last pin (RB5) to control the UV LEDs.

The photodetector and the microcontroller are connected by two wires. The first is from the *End of scan* pin to RA3. The purpose of this connection is to tell the microcontroller when the scan is over. The second connects the *Active Video* pin to RA2, passing through some amplification.

An inverting amplifier configuration was chosen to make the photodetector's output voltage directly proportional to the intensity of light. For this, a rail to rail OpAmp was used, more precisely, an OPA2344.

To make the voltage offset, a potentiometer connected to $5\ V$ and ground was used. To know what position should the potentiometer be, a simulation in Tina-TI was made.

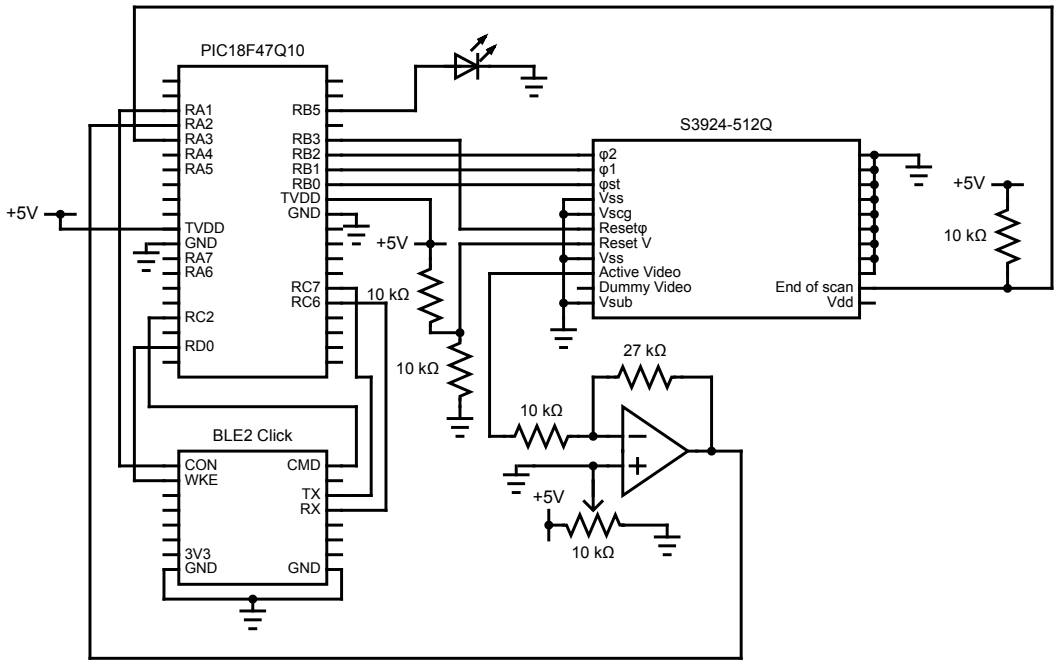


Figure 25: Circuit Diagram of major reader's components

Summing up, the Bluetooth module is attached to the development board, where the microcontroller is. Then, the microcontroller sends the signal by four channels to the photodetector. Hereafter, the photodetector sends the signal to the microcontroller, passing through an amplification system with a gain of 2,7 and a voltage offset of $1,25\ V$.

In Figure 26, it is possible to see a photo of the setup working. Here, the UV LED got replaced by a blue LED to test if the system was working.

4.5 Reader's casing

To assemble every component into one and create a first prototype, a team of USN bachelor's students from Mechanical Engineering was recruited to help creating the case of the reader.

Figure 27 shows the final product.

In order to create the case, several goals had to be achieved:

- The case had to be lighter, easier to handle and more stable;
- All the electronic components inside needed to be securely attached;
- The reader required a power button and a charging port;
- The photodetector needed to be perfectly aligned with the strip's window.

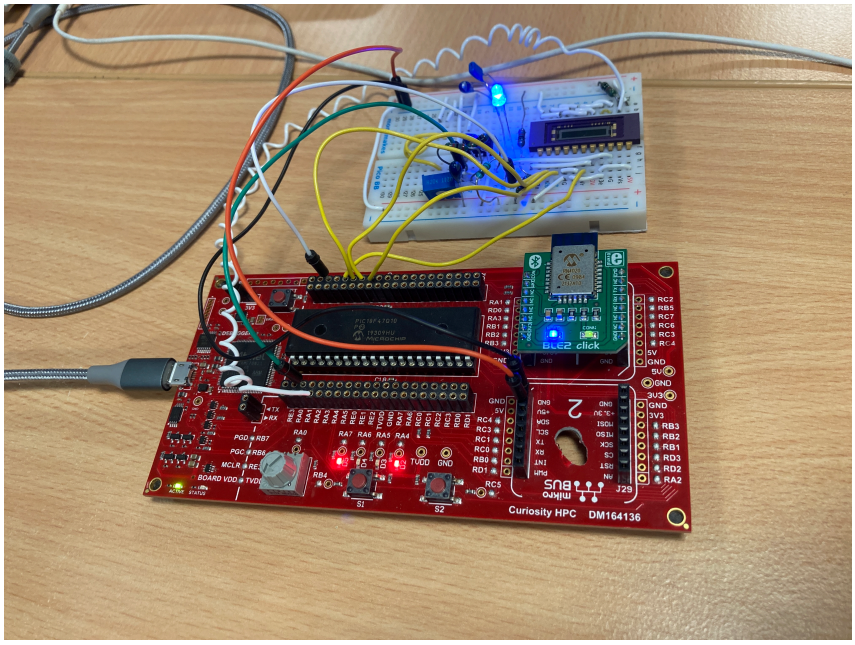


Figure 26: Photo of the whole setup

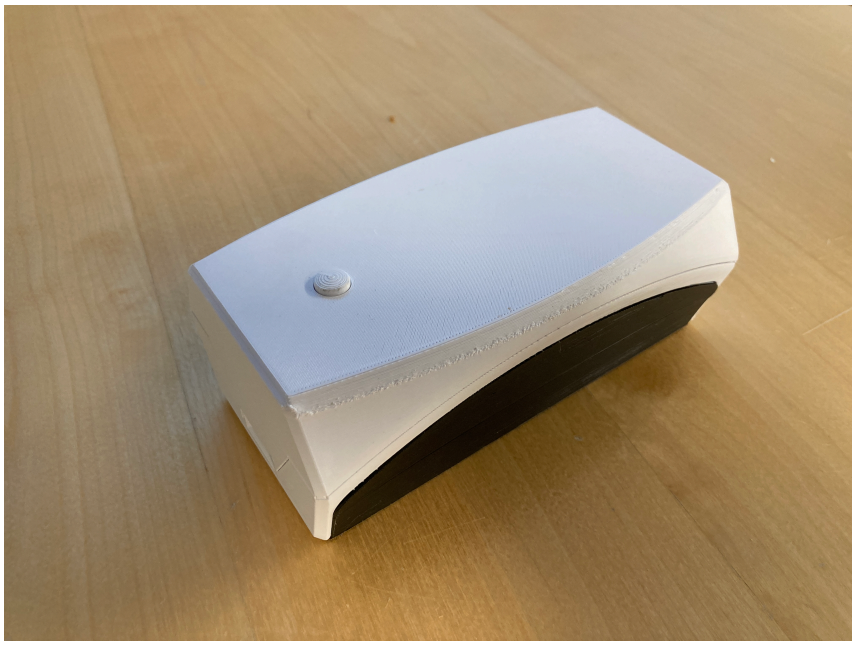
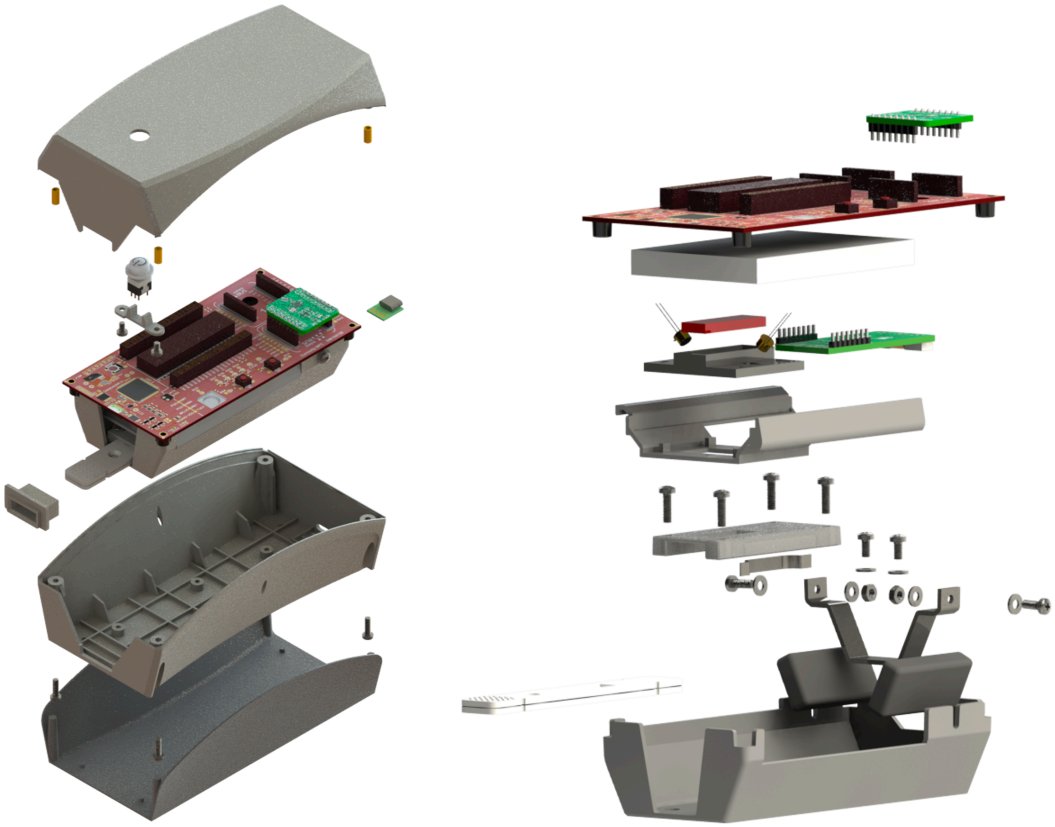


Figure 27: Photo of the whole setup

The assembling instructions are shown in Figure 28, where Figure 28(b) shows how to assemble the internal structure and place perfectly all the electronic components and Figure 28(a) indicates how to cover the internal structure with an external case.

More detailed assembling instructions can be found in Appendix B.



(a) External Case (b) Internal Case

Figure 28: Assembling instructions

Another obstacle needed to overcome was the prevention of cross talk between the lines. For this, a filter, inspired in a parallel hole collimator, was created to let only the perpendicular light pass through.

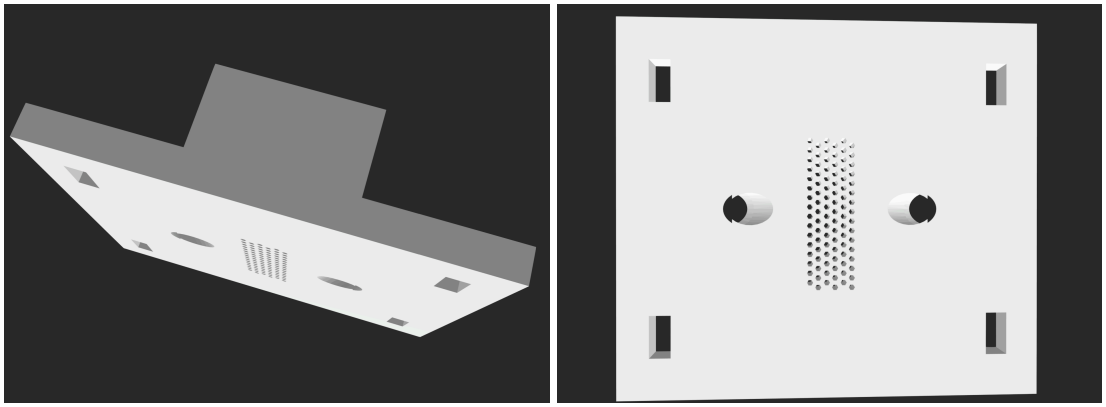


Figure 29: Filter's 3D model

Figure 29 shows, from two different points of view, the created filter to prevent cross talking. The four outside holes are for placement to the structure in the reader. The two round holes are for the two UV LEDs placement. And in the middle there is an array of tiny holes, where the photodetector would be and receive the light that it is directly below it.

Figure 30 represents a schematic of how the filter works and prevents the cross talk.

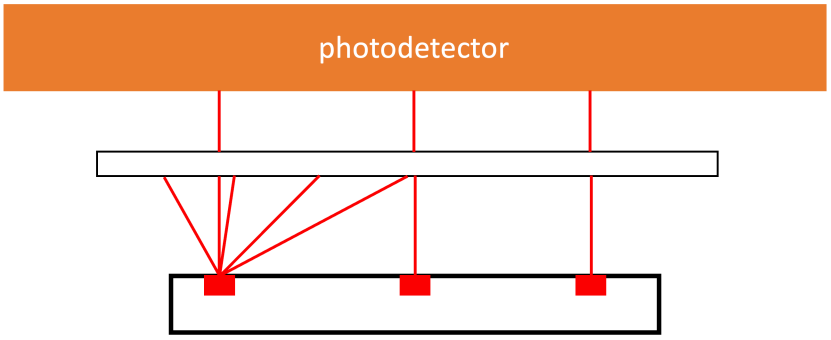


Figure 30: Schematic of cross talk prevention

A test was made to verify if the light could pass with a small tilt of the light source. Figure 31 is two pictures from that test. Where the left one is when the light source is perpendicular to the filter and the light can be seen on the table. And the right one is when the light source has a small tilt of approximately 2 degrees and the light cannot be seen below the filter.

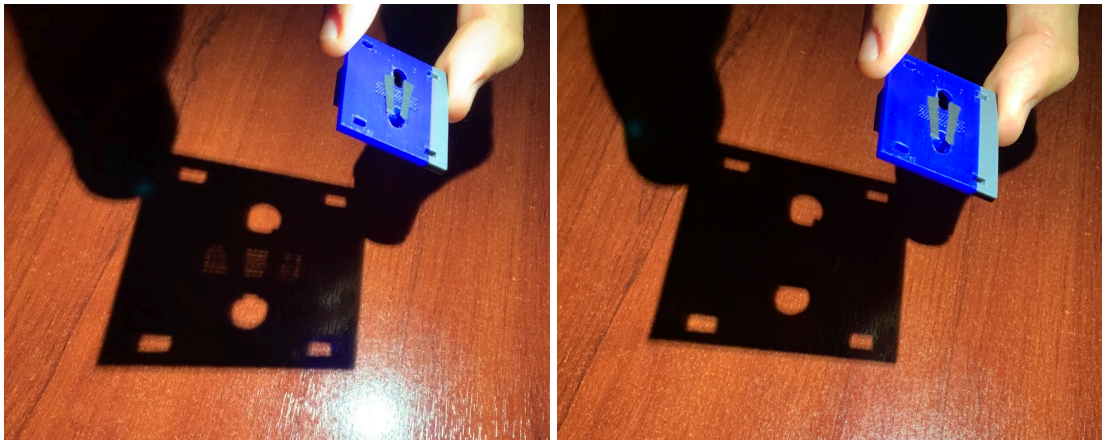


Figure 31: Filter testing

To conclude, the filter works for a normal light source, therefore, it will work with a strip where the light source are quantum dots.

4.6 Python GUI

A python program with a graphic user interface (GUI) was created to collect the data and show it to the user in the form of a graph. At a first step, the serial port to receive the data, using the package *serial*.

Figure 32 shows the GUI that uses the serial port to collect the data.

At the bottom there are 5 buttons. The first one, *Begin*, is to start the reading and collecting the data. The *Clear* button is to clean the graph for a second scan. The third button closes correctly the program.

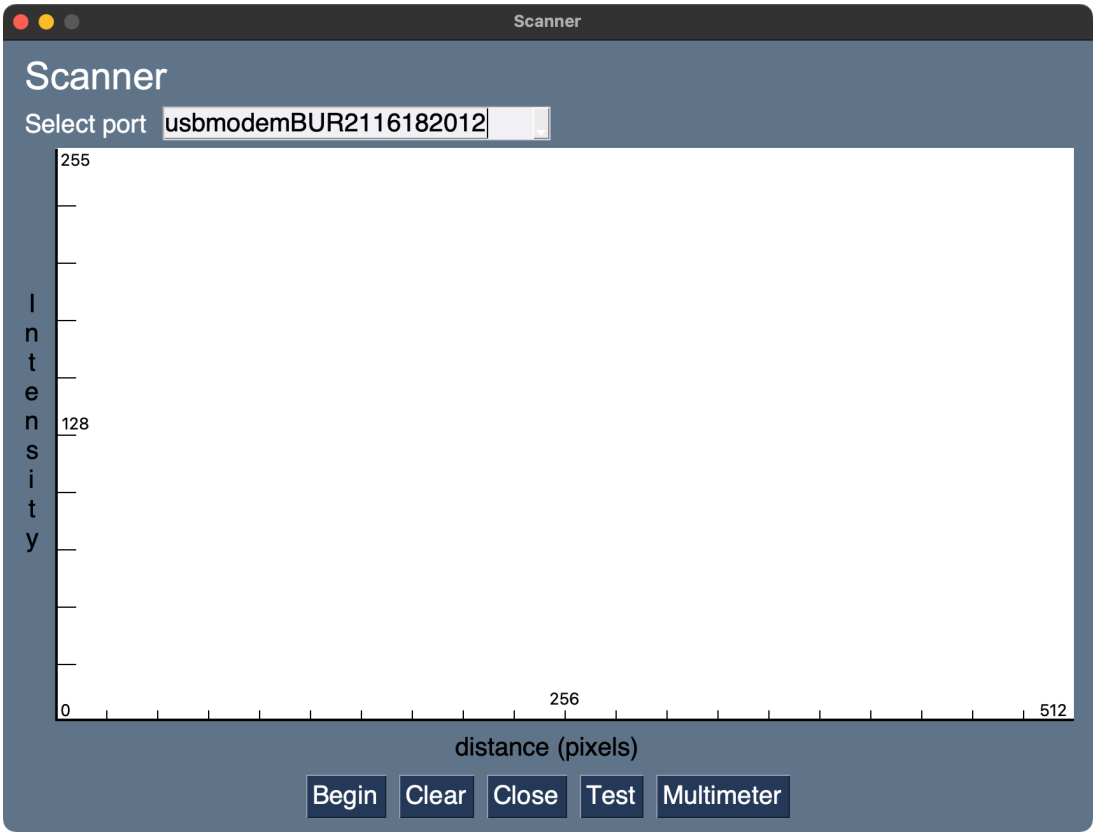


Figure 32: Serial port GUI

The last two buttons were created to debug. The first one was to test if the connection with the serial port was correctly made, sending the number 10 and the board turning on or off one of its LEDs. The purpose of the last button is to know how much voltage is in one pin, printing the value to the console.

After getting the Bluetooth module, an improvement to the program was made to make it wireless. For this step, the packages *bleak* and *asyncio* were used to connect to Bluetooth devices.

Comparing with Figure 32, the GUI shown in Figure 33 has some major improvements. At the top, it has a button to scan for Bluetooth devices next to a drop list to select the desired device.

At the bottom, some buttons were removed. The debugging buttons are no longer needed, and the *Clear* button was merged with the *Begin* one, this is, every time a reading is started, the program cleans the previous graph first.

4.7 Communication protocol

The communication between the computer and the reader is made differently in each way.

When the reader is waiting for instructions, it sets the pin RC2 to 1. This means that the MLDP mode from the Bluetooth module is activated. Everything written in the private service is sent by serial port to the microcontroller. Receiving this information, the microcontroller can start the reading.

However, the MLDP mode only works in one way, from the computer to the reader. In order to get

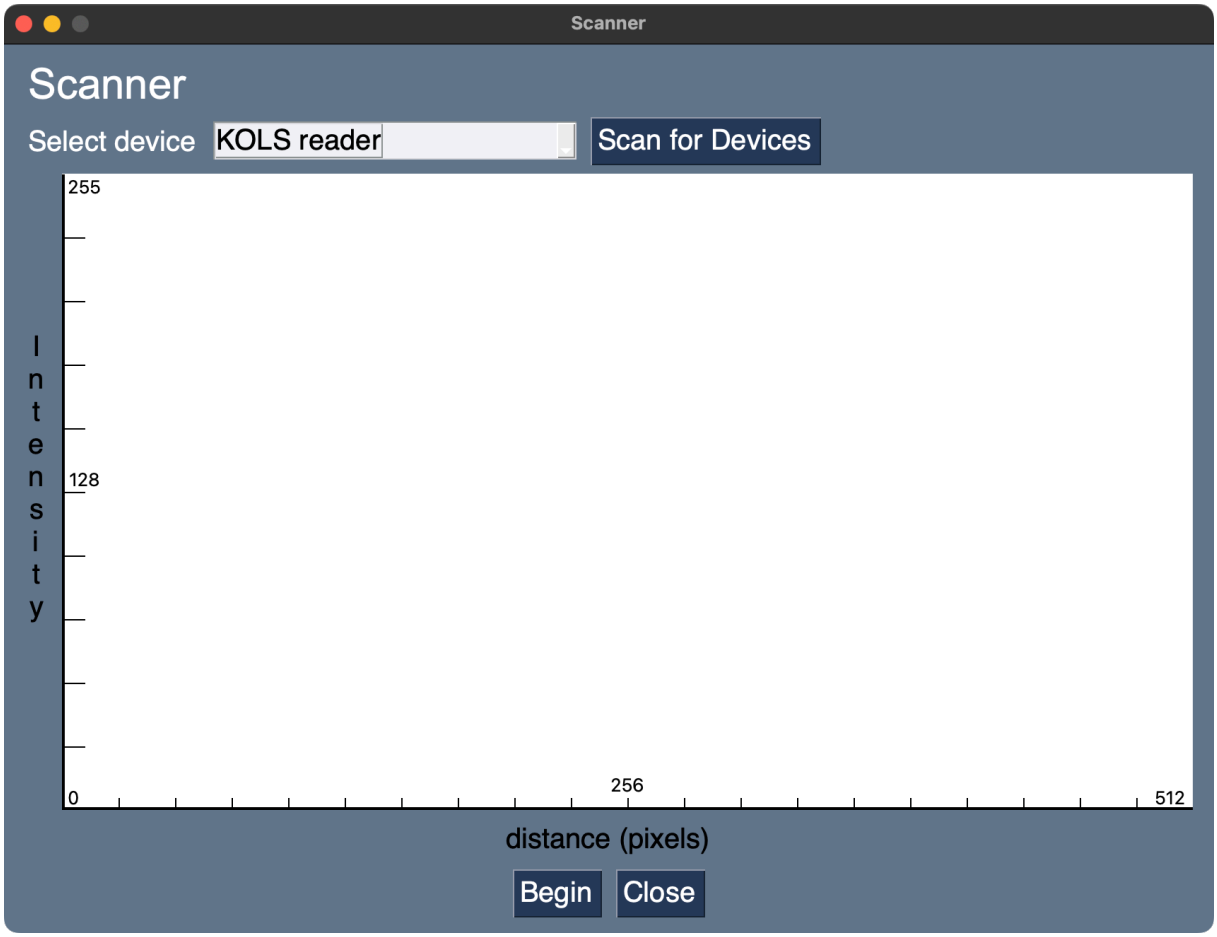


Figure 33: Bluetooth GUI

the information from the reader, a private service was created with 3 bytes of memory. In this way, it is possible to transmit the data, which is only 1 byte, and tell which position of the array is with the other two bytes.

In Figure 34, it is possible to see how the data was stored in the private service, where which rectangle represents one bit. Since we have 512 values of information, 2 bytes are needed to know the location.

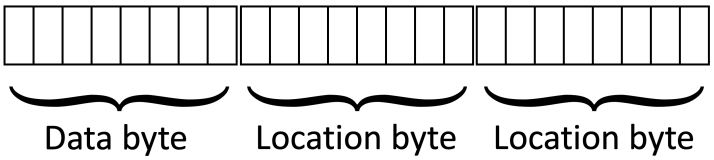


Figure 34: Data schematic

To make this number, in the microcontroller, this is, in C, a type int variable was created called *counter* that increments each time a value is read from the photodetector. Getting the data and the location bytes together can be done by simply writing first the data byte, and then the location bytes.

```
str[20];
print("SWH,0018,");
sprintf(str, "%d", data);
print(str);
sprintf(str, "%d", counter);
print(str);
print("\r\n");
```

To collect the data on the computer, this is, in Python, after connecting to the Bluetooth device some calculations are needed. First, the information is received in string type, which is necessary to convert to int. In order to separate the data byte from the location type, it is only needed to perform a division by $2^{16} = 65536$. The quotient is the data, and the remainder is the order where data is going to be placed in the array.

```
number = name.encode("utf-8").hex()
data = number // 65536
order = number % 65536
array[order] = data
```

To summarize, Figure 35 shows a flowchart representing the algorithm used.

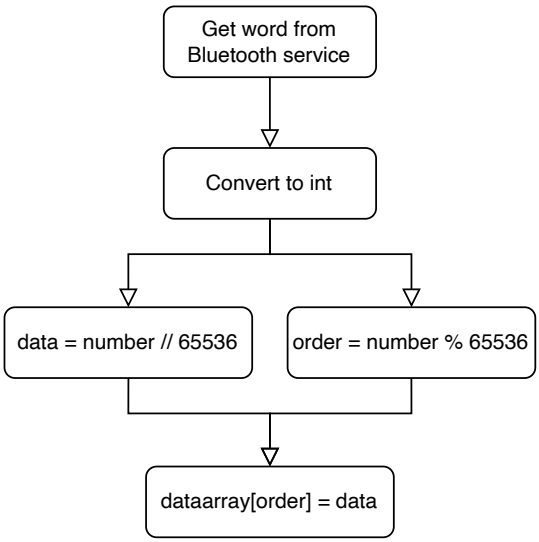


Figure 35: Flowchart of algorithm used to collect the data

4.8 Material and software used

The practical part of this project was possible to execute thanks to professor Raymond from USN lending most electronic components (resistors, capacitors, OpAmps and wire) and an oscilloscope to verify the signals. The main components were acquired by Sensovann, such as the microcontroller, the Bluetooth module and the photodetector. When it comes to software, for this project MPLAB X IDE and Microchip® Code Configurator were used to program the microcontroller, and Visual Studio Code was used to program the GUI (Graphic User Interface). This last one was programmed in the Python language, using the packages: serial, numpy, PySimpleGUI, glob, asyncio and bleak.

4.9 Performing a reading

Summarizing everything, a reader was created to read the fluorescent light from quantum dots placed on a strip.

To get a result, firstly, a saliva sample is collected and placed on the strip. Then, after approximately 15 minutes, all the sample was spread through the strip, where the antibodies capture the targets in several orthogonal and parallel lines. At this moment, the strip is ready to be read, there is 2, 3 or 4 lines of targets bound with quantum dots.

The strip is inserted inside the reader, and using the smartphone or the computer, the user indicates to start the reading. A word is sent to the device to start the process. The microcontroller first lights up the UV LEDs to induce the fluorescence, and then sends four signals to the photodetector. (Figure 19) These signals give the clock time that the photodetector collects light from a photodiode and moves to the next one. This process can start immediately since the fluorescence is instantaneous. After that, each voltage obtained from a photodiode is sent to a Bluetooth service, placing the value following the location of the photodiode.

In the smartphone or computer, after sending the starting signal, it waits until the first value is written at the Bluetooth service and then starts collecting the value and the position and organize them in an array. This process usually takes roughly 563 ms. Finally, the smartphone or computer shows a graph with the values obtained from the reading.

After this, the strip is obsolete and should be discharged in its proper place.

The voltage that the photodetector gives as output for each photodiode is in DC, so the system's frequency response is determined by the shift from each photodiode. In this case, the photodetector shifts to the next photodiode with a period of $1,1\ ms$, therefore the frequency of shifting is $f = \frac{1}{1,1} = 909\ Hz$. The OpAmp used in this project's circuit has a GBP (Gain Bandwidth Product) of 1 MHz. So, the system is capable of giving a linear response to the circuit frequency. Since the time each photodiode is collecting light is controlled by the clock signals that the microcontroller sends, the sensibility can be changed for whatever it is needed, compromising of course the time of a full read of the strip.

5

Conclusions and Future Work

5.1 Conclusion

Diagnosing diseases has been proven to improve the accuracy of clinical decisions. Due to its importance, a great investment on research and manufacturing diagnosing tests is being made.

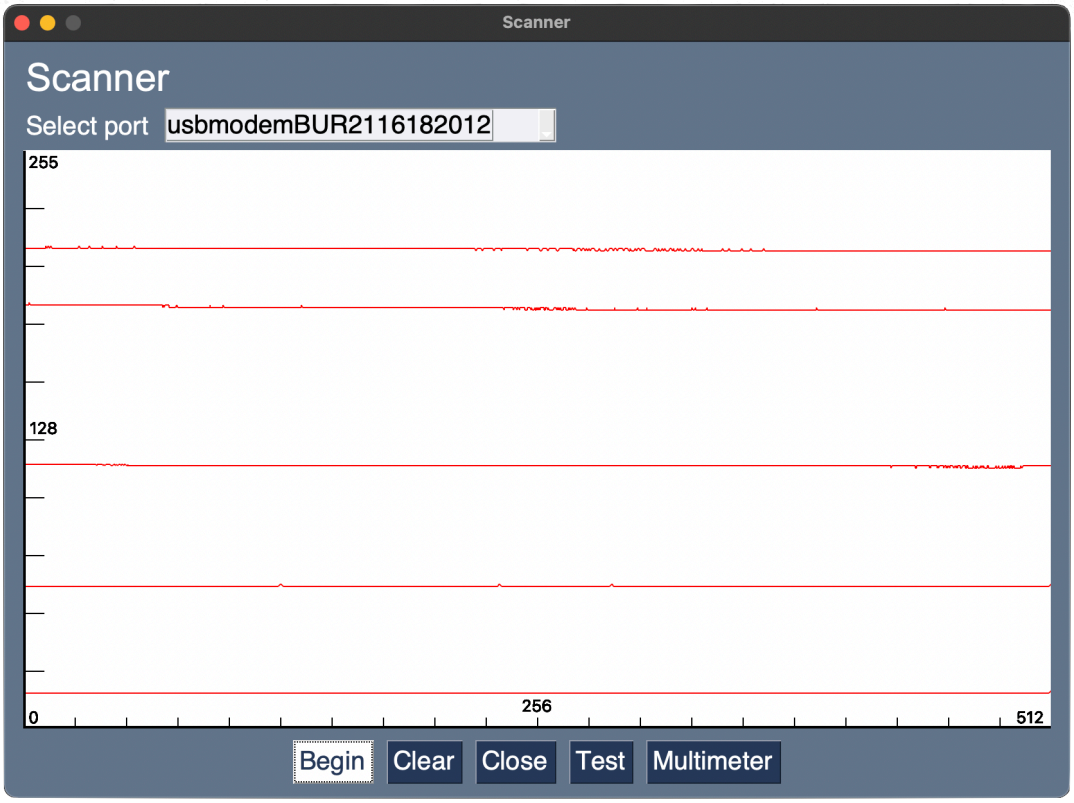


Figure 36: Light intensity test

In this work, firstly, literature review was performed on diagnosing tests, more specifically, LFIA tests, to better understand the possibilities and difficulties on this subject. Secondly, a reader's prototype was disassembled and studied to inspire and to learn how to improve for a new prototype. After that, a prototype for a reader that can read light intensity from quantum dots' fluorescence was made using a different paradigm, controlled with a microcontroller and sending information by Bluetooth.

Due to work plan incompatibility, strips with quantum dots lines were not available, so tests were made with a light source that, from the photodetector's perspective, have a similar behaviour. With a light source fixed in the same place, changing its intensity 5 times, it was possible to get a graph with all the horizontal lines at different levels, proving that the more light inflicted on the photodetector, the higher the value it gets. (Figure 36)

Since the photodetector is controlled by the reading clock signals sent by the microcontroller. This last one can manipulate the time between readings from each photodiode in the photodetector. Therefore, the photodetector's sensibility can be controlled and change through microcontroller programming.

Summing all up, a device was created targeted to read LFIA labelled with quantum dots. It has two UV LEDs (with a wavelength of 365 nm) for quantum dots' absorption, a photodetector that cover all the strip's testing window and a microcontroller paired with a Bluetooth module to control the device and communicate with an external one, a computer or a smartphone. This device has two major advantages, its sensibility can be regulated through microcontrollers programming, and the photodetector can detect any wavelength in the visible spectrum, so any strips labelled with quantum dots that have an absorption spectrum in the UV range can be used with this device. It doesn't need a motor to function, therefore its size is significantly smaller than the competition.

5.2 Future Work

A working prototype was made, giving a value of voltage in response to the light intensity. However, more work could be done to improve the device.

The most important, the reader should be tested with strips that have quantum dots lines. Giving the value of minimum and maximum light intensity that the strips can give. Furthermore, a relation between biomarker's concentration and light intensity, so the final result can be in biomarker's concentration instead of the value of voltage the ADC reads. Figure 37 shows a possible output from the reader, using a strip with quantum dots as input.

Figure 37: Hypothetical test with strips

The reader's final goal is to be portable. In the current state, the reader can receive and send data wirelessly, but it still needs to be connected by cable to get power. In a future work, batteries implementation is something that needs to be performed to make the reader 100% portable.

Lastly, to make the reader even smaller and lighter, a shift from development board and breadboard to PCB (Printed Circuit Board) is necessary. This will reduce drastically the reader's height, and this will make the job of creating a case easier and simpler.

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A

Programming Code

A.1 Microcontroller Code

```
void print(char* str)
{
    int i;

    for(i=0;str[i]!='\0';i++)
    {
        EUSART1_Write(str[i]);
    }
}

void tick(int times){
    int i;
    for(i=0;i<times;i++)
    {
        __delay_us(100);
    }
}

void read()
{
    char str[10];
    static uint8_t adcResult;

    // start
```

```
LATB5 = 0;
LATB0 = 0;
LATB1 = 0;
LATB2 = 1;
LATB3 = 1;

tick(1);

//phi start
LATB5 = 1;
LATB0 = 1;

tick(1);

//first impulse
LATB2 = 0;
LATB1 = 1;

tick(1);

//end of start
LATB0 = 0;

tick(1);

LATB1 = 0;
LATB3 = 0;
LATB2 = 1;

tick(3);

LATB3 = 1;

tick(3);

int stop = 255;
int counter = 0;
while(stop>200)
```

```
    {
        adcResult = ADCC_GetSingleConversion(PHOTO_CHANNEL) >> 2;
        stop = ADCC_GetSingleConversion(EOS_CHANNEL) >> 2;
        print("SHW,0018,");
        sprintf(str, "%d", adcResult);
        print(str);
        sprintf(str, "%d", counter);
        print(str);
        print("\r\n");
        counter++;

        LATB1 = 1;
        LATB2 = 0;
        LATA6 = 1;

        tick(2);

        LATB3 = 0;
        LATB1 = 0;
        LATB2 = 1;
        LATA6 = 0;

        tick(2);

        LATB3 = 1;
        tick(4);
    }

    LATB = 0;
    print("SHW,0018,000000001111111111111111\r\n");
}

void main(void)
{
    SYSTEM_Initialize();

    uint8_t n;
    char str[20];
```

```
    RD0 = 1;
    RC2 = 1;

    while (1)
    {
        if(PIR3bits.RC1IF)
        {
            n = EUSART1_Read();
            if(n==11)
            {
                RC2 = 0;
                read();
                RC1 = 1;
            }
        }
    }
}
```

A.2 Computer Code

```
from ast import If
import serial
import numpy as np
import PySimpleGUI as sg
import glob
import asyncio
import bleak

def make_vertical(title):
        new_title = ""
        for letter in title:
            new_title += f"{letter}\n"
        return new_title

buttons = [
```

```
    [sg.Button(button_text='Begin'), sg.Button(button_text='Close')]
]

newdevices = []
deviceslist = []

layout = [
    [sg.Text('Scanner', font = ("Arial", 30))],
    [sg.Text('Select device'), sg.Drop(values = newdevices,
    ↪  key='devices', size=20), sg.Button(button_text='Scan for
    ↪  Devices')],
    [sg.Text(make_vertical('Intensity'), justification='center',
    ↪  text_color='black'), sg.Graph(canvas_size=(800, 450),
                    graph_bottom_left=(0, 0),
                    graph_top_right=(100, 100),
                    background_color='white',
                    key='graph')],
    [sg.Push(), sg.Text('distance (pixels)', justification='center',
    ↪  text_color='black'), sg.Push()],
    [sg.Push(), sg.Column(buttons, element_justification='center'),
    ↪  sg.Push()]
]

errorLayout =  [
    [sg.Text('Please scan for devices first')],
    [sg.Button(button_text='Dismiss', key='Dismiss')]
]

scanLayout = [
    [sg.Text('Please choose a device from the list')],
    [sg.Button(button_text='Dismiss', key='Dismiss')]
]

window = sg.Window('Scanner', layout, default_element_size=(20,1),
↪  font=("Helvetica", 20))
graph = window['graph']
```

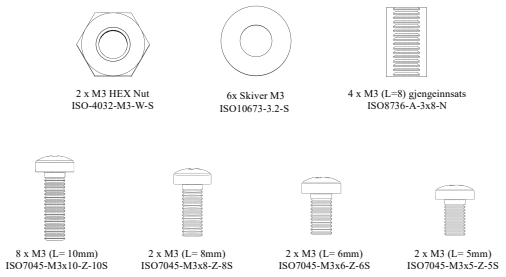
```
dataRangeMin = 0
dataRangeMax = 100
dataNum = 10000

def drawPlot(y):
    y = np.array(y)
    x = np.linspace(0, 100, len(y))
    y = y/255*100
    x_ant = x[0]
    y_ant = y[0]
    for i in range(1, len(x)):
        graph.DrawLine((x_ant, y_ant), (x[i],y[i]), color='red')
        x_ant = x[i]
        y_ant = y[i]
    drawAxis()

def drawAxis():
    graph.DrawText("255", (2, 98))
    graph.DrawText("128", (2,52))
    graph.DrawText("0", (1,2))
    graph.DrawText("512", (98,2))
    graph.DrawText("256", (50,4))
    graph.DrawLine((0,0), (0,100))
    graph.DrawLine((0.1,0), (0.1,100))
    graph.DrawLine((0,0.3), (100, 0.3))
    graph.DrawLine((0,0.4), (100, 0.4))

    for i in range(0,100, 10):
        graph.DrawLine((0,i), (2,i))

    for i in range(0,100,5):
        graph.DrawLine((i,0), (i,2))

stop = False
while not stop:
    window.Finalize()
    graph.erase()
```

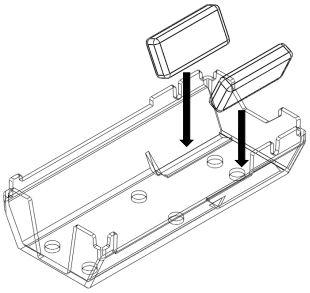
```
    drawAxis()
    event, values = window.Read()

    if(event == "Begin"):
        devicename = values['devices']
        if (newdevices == []):
            error1 = sg.Window('ERROR', default_element_size=(40,10),
            ↪  font=("Helvetica", 20)).Layout(errorLayout)
            stop1 = False
            while not stop1:
                event1, values1 = error1.Read()
                if event1 == sg.WIN_CLOSED or event1 == 'Dismiss':
                    stop1 = True
            error1.Close()
        else:
            if(devicename==''):
                error2 = sg.Window('Scanned Devices',
                ↪  default_element_size=(40,10), font=("Helvetica",
                ↪  20)).Layout(scanLayout)
                stop2 = False
                while not stop2:
                    event2, values2 = error2.Read()
                    if event2 == sg.WIN_CLOSED or event2 == 'Dismiss':
                        stop2 = True
                error2.Close()
            else:
                for d in deviceslist:
                    if(devicename == d.name):
                        ble_address = d.address
                async def main():
                    device = await
                    ↪  bleak.BleakScanner.find_device_by_address(ble_address,
                    ↪  timeout=20.0)
                    async with bleak.BleakClient(device) as client:
                        dataarray = np.zeros(512)
                        stop = 0
                        first = await client.read_gatt_char(23)
```

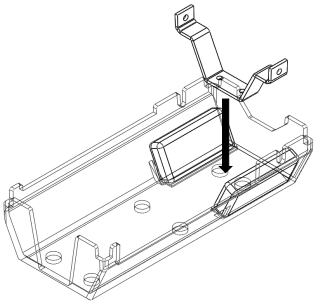
```
                    await client.write_gatt_char(23, bytearray("11",
                    ↪  encoding='utf-8'))

                    while(first == await client.read_gatt_char(23)):
                        pass

                    while(stop == 0):
                        name_bytes = await client.read_gatt_char(23)
                        name = bytearray.decode(name_bytes)
                        number = name.encode("utf-8").hex()
                        if(number!=65535):
                            data = number//65536
                            order = number%65536

                            dataarray[order] = data
                        else:
                            stop = 1

                    drawPlot(dataarray)
                asyncio.run(main())

    if(event == "Scan for Devices"):
        newdevices = []
        async def main():
            devices = await bleak.BleakScanner.discover(1)
            for d in devices:
                if(d.name != 'Unknown'):
                    newdevices.append(d.name)
                    deviceslist.append(d)
        asyncio.run(main())
        window.find_element('devices').Update(values=newdevices)

    if event == sg.WIN_CLOSED or event == 'Close':
        stop = True

window.Close()
```

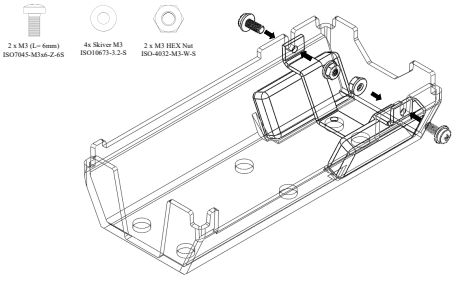
B

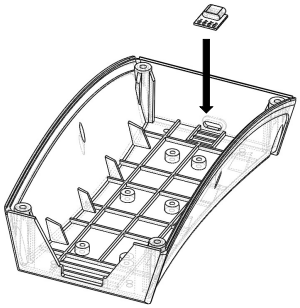
Assembling instructions

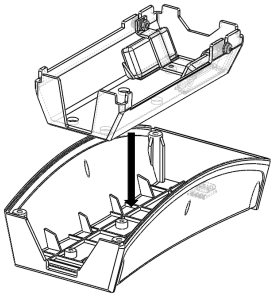


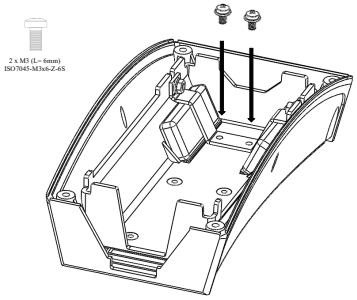


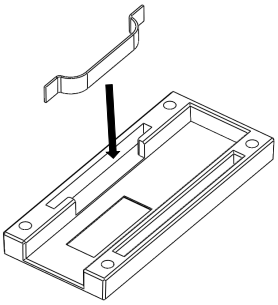


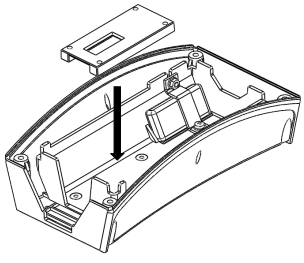


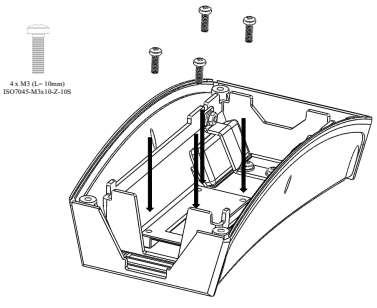


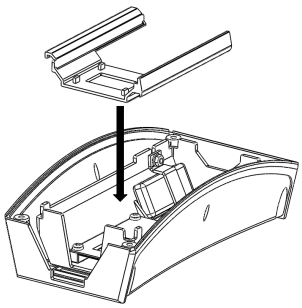


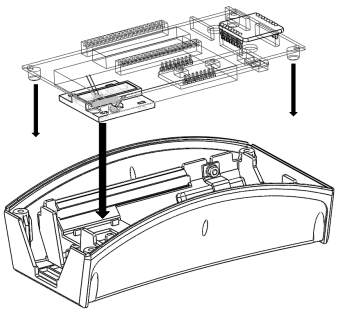

2 x M3 (L= 6mm)
ISO7045-M3x6-Z-6S

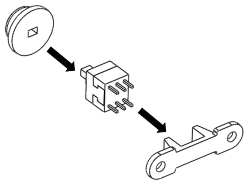


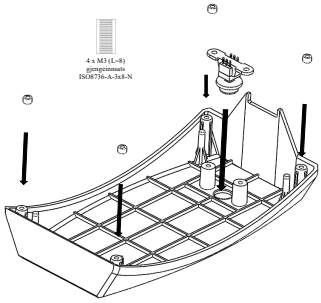


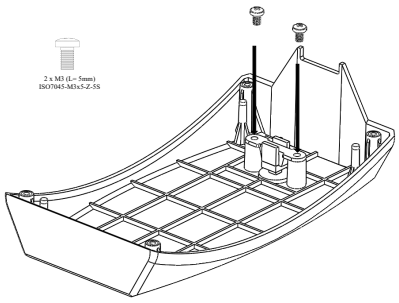

4 x M3 (L= 10mm)
ISO7045-M3x10-Z-10S

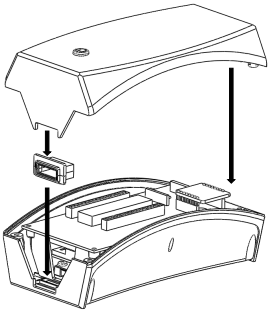


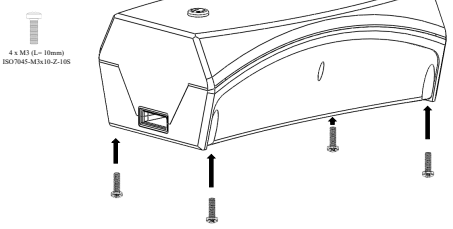





4 x M3 (L=8)
ISO8736-A-3x8-N


2 x M3 (L= 5mm)
ISO7045-M3x5-Z-5S




4 x M3 (L= 10mm)
ISO7045-M3x10-Z-10S