POLLUTION SENSORS: MEASURING CHLOROPHYLL FLUORESCENCE IN PLANTS

BY

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ABSTRACT

POLLUTION SENSORS: MEASURING CHLOROPHYLL FLUORESCENCE IN PLANTS

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This paper describes the research on a small, cheap and autonomous fluorometer, to detect present stress in plants. The implementation of such a system would indirectly utilize plants as biosensors. The project work concentrates on different design aspects of the conceptual model of the required fluorometer as well as on validation of the chosen hardware and method of measurement. Results from conducted research and validation reveal that chlorophyll fluorescence yield can be correctly identified with absence/level of presence of stress but efficiency of a plant cannot be determined because of low digital resolution of the proposed setup. This paper outlines electrical, mechanical and software design of a fluorometer with the aforementioned specifications.

DECLARATION

I hereby certify that this report constitutes my own product, that where the language of others is set forth, quotation marks so indicate, and that appropriate credit is given where I have used the language, ideas, expressions or writings of another.

I declare that the report describes original work that has not previously been presented for the award of any other degree of any institution.

Signed,

Minko Todorov Minkov:

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CHAPTER 1 – RATIONALE

Project Background

Nowadays the technique of chlorophyll fluorometry has become an eminent tool for characterization of plant ecophysiology[1]. Presence of fluorometers eases the quantification of the process as it gives identification of the stress the plan is exposed to[2]. Thus, improvements in this field could lead to better portrayal of the plant photosynthetic and ecophysiological states. With this project, we strive to produce a small, portable and cheap system, to detect and quantify chlorophyll fluorescence (from now on abbreviated as CF). The system needs to collect information on the fluorescence capabilities of a plant, thus indirectly yielding results about the stress the plant experiences, as present stresses in the micro environmental vicinities of a plant are directly related to CF outputs.[1] Our design strives to incorporate factors with diverse background restrictions. Nevertheless, a resulted sensor node would operate easily with a wide range of plants. This way the implementation of such a system could make the utilization of plants as biosensors possible, leading to numerous advantages both from scientific and social points of view.

Reason for Research

Not many improvements have been achieved in the area of CF measurements, since the discovery of the phenomenon in the early thirties[16]. Even though improvements in technology have indeed affected factors such as precision, accuracy and ease of deployment, methods of data collection themselves have not been affected in a great matter.[2]

The implementation of this project would lead to an innovative, environmental and socially relevant solution to a problem with exponentially increasing importance – pollution and the detection of different types of pollutions. Further research and improvements in this field could lead to small, cheap and easily deployed sensor network. This way plants could indicate about different types of present pollutions and their levels in a non-destructive way.

The idea of this project is to create a sensor network to transmit and analyze data about CF, by deployment of multiple sensor nodes on a field. The current fluorometers are too big to represent a single sensor node (incorporation of a screen and buttons causes the size of a fluorometer to increase).[2] Thus, the main idea is to create a small sensor node, which could be clipped on a plant's leave. The collected data would be wirelessly transferred, thus a decrease of the node's size.

Fluorometers on contemporary market could have a price between 450 and 4900 BRL [4][5]. With this project we strive to produce a much cheaper sensor node, which would be able to produce the same result. Moreover, current fluorometers require presence and interaction of a user in order for the data to be collected. This project strives to create an automated system, which would constantly collect data by itself.

The main reason for research is the need for more flexible equipment, to be deployed on the field and thus analyze and present data about the ecophysiological state of plants. This research is initialized by the presence of a causal problem – that is, the technical research and implementation of new, custom specified fluorometer is the solution to a practically defined problem. The very final outcome of the whole project would be a sensor network of nodes, to measure chlorophyll fluorescence. The sensor network would consist of multiple stationary sensor nodes to continuously monitor the plants. In terms of the phenomenon's type, the theory behind the main working method is induced CF – artificial source of light strikes upon the leaves of plants, thus they emit waves at specific wavelengths. This wavelength emission could be presented on a graph (time vs. fluorescence (in mV)). The difference between the global maximum and minimum in this graph is related to the photosynthetic state of the plant. Thus, one can make conclusions about the stresses the plant is exposed to.

In conclusion, three main input characteristics are presented in the beginning of the system's development – low price (not specifically identified), small size – the system needs to be small enough in order to be clapped directly onto a plant's leaf, and autonomous design, which would allow the system to independently take measurements at specified time.

Desired Output and Requirements

The research question, as established prior to the initialization of my graduation project, is:

How to design the hardware and the software of a sensor system to measure chlorophyll fluorescence? What factors must be taken under consideration for the system to be reliable and produce validated outcomes?

The first direct output filed of this research question should answer the question "How" – how is the design shaped; how do the research and implementation of this project influence the final output system; how is chlorophyll fluorescence detected; how should I define the tools and processes required for proper project implementation.

The second direct output field of this research question should answer the question "What" – what are the factors behind CF, which I need to take into consideration; what is important, when designing the system; what biological principles outline the creation process; what should be done for proper validation.

This research question subsequently outlines the two very basic project procedures/phases to follow – research phase and construction phase. The research phase of this project would require acquiring of theoretical background in the field of CF itself, proper equipment, tools and components definition and relation between background factors and system design. The construction phase would incorporate physical implementation of the design, resulted after completion of the research phase. The final steps of the construction phase consist of validation of the system on several different levels and recommendation for continuation of system improvements.

Deliverables

This graduation project would also serve as the initial project phase of Christopher Gull's Masters Project at UFV Viçosa . All project matters and requirements have been discussed both with him and Mr. José Nacif, my company project supervisor. Figure 1 outlines the incorporation of my graduation project in the whole project of creation of a small and cheap fluorometer.

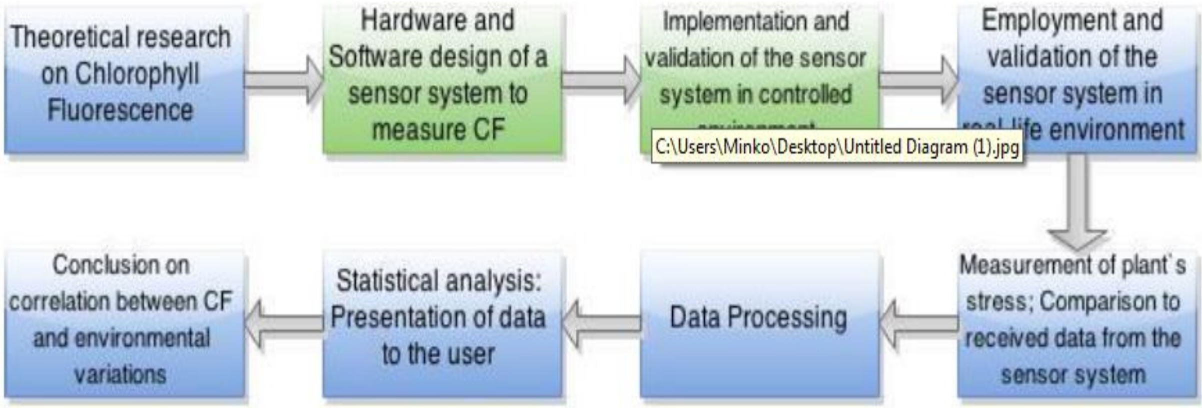

Figure 1 – Flowchart of the development of a sensor system to measure CF in plants. Green fields depict my scope of work and its incorporation in theoverall project, depicted by blue fields.

This construction of work and cooperation requires preliminary classification of my activities and responsibilities in terms of scope of project work. Table 1 represents the main inside and outside scopes of work, required for the implementation of my graduation project. This list has been adjusted throughout the project itself, as provision of information caused clarification on numerous project-related topics.

Inside of scope	Outside of scope
Conduct research on appropriate excitation source	**Production of deep, theoretical research on the topic of chlorophyll fluorescence itself**
Find a small and cheap sensor to measure chlorophyll fluorescence and implement light filters to measure light in the far visible spectrum	**Implementation of sensor network nor its deployment on the field. I will be working on the design, implementation and lab verification of a single sensor node.**
Build a sensor system, possibly with custom PCB, which would act as a non-modulated fluorometer	**Design or produce of a new sensor to measure light reemission**

Program the sensor system	**Validation of the sensor system in real-life environment**
Incorporate wireless transmitter in the PCB design	**Conclusions on statistical data from the taken measurements**
Validate the sensor system in controlled environment	**Presentation of data to the user**
Document progress and work outcomes	**I will not be differentiating between different types of stresses, nor will I be working on characterization of a new type of stress**
Research on methods of CF measuring	
Research on the topic of powering and incorporate batteries into the PCB design	

Table 1 – Inside and outside scopes of work throughout the research and construction initial phases of the project

Document Overview

The remaining of this report is organized as follows:

Chapter 2: Situational and Theoretical Analysis. The chapter provides background information on the phenomenon of CF and defines the most important characteristics in the field of CFY measurements.

Chapter 3: Conceptual Model. This chapter establishes factors and characteristics of the system precisely, as resulted by theoretical research, and portrays methods of approach towards design construction and physical implementation.

Chapter 4: Research Design: This chapter reveals how the results from the proposed definitions are implemented.

Chapter 5: Research Results and Discussion. This chapter presents the final results of this project, including mechanical design, design of electronic schematics, programming configurations and final definition of all important factors.

Chapter 6: Recommendations and Conclusions. This chapter interprets the results from the previous one, outlining the most important fields of progress and summarizing the period of project work. The chapter also states the answer to the already defined research question.

CHAPTER 2 – SITUATIONAL AND THEORETICAL ANALYSIS

The following chapter presents theoretical background behind the discussed phenomenon of CF which serves as base for establishment and realization of the most important factors to be taken into consideration throughout the implementation of the proposed system.

Introduction

Chlorophyll fuorescence is simply the light re-emitted from the surface of a plant's leaves, when light strikes. Unlike reflection, which is caused by chlorophyll molecules and thus the re-mitted light peaks in the green spectrum of visible light, the CF occurs at wavelengths of around 700 nanometers[14]. This output wavelength is driven by input light with wavelength of approximately 400 nanometers. Three possible scenarios are present when light strikes upon a leaf: it either drives photosynthesis, stimulates the dissipation of excess energy in the form of heat or the CF itself. And since those three process “contest” each other, quantification of one of them could lead to information yield for the other two. Thus, measurements of CF could also identify the photosynthetic and chemical efficiency and state of heat dissipation of a plant [1][12][13] A fluorometer is a device, which detects and measures the chlorophyll fluorometry at a certain plant. The principal way of work could be described as follows: light source emits wavelengths in the whole spectrum; the emitted light is then filtered (by a filter or a monochromator), so that only specific portion of the spectrum hits the plant leaf; then the fluorescence occurs: the plant re-emits light at higher frequency; the re-emitted light is again filtered and measured by a detector. Of course, differerent structures of fluorometers are present, but this is the basic form of construction.

Researches show that there is a direct correlation between measured CF and presence of stress. [9] Moreover, the type of stress could be timely identified, thus preventions can occur at early state. In other words, CF not only reveals the efficiency of photosynthesis in a plant, but it also directly describes the plant's health. Pure light reectance is also a source of ecophysiological state, but CF might be a more precise signal for the plant's state and thus it could explain presence of stress at early phases. In simpler words, by the method of chlorophyll fluorometry, we can deduct on stress presence prior to any visible changes. In a nutshell, quantification of CF could lead to the direct implementation of diffeerent plants as biosensors to characterize their own microenvironments and thus detect presence of different kinds of stresses at early stages.

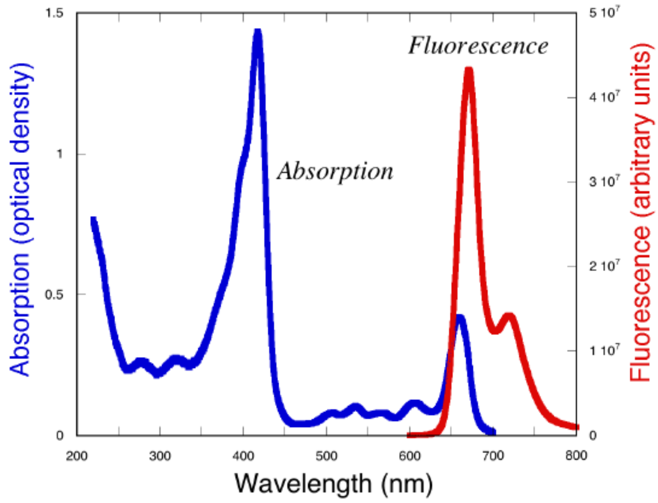

Figure 2 - Absorption and Fluorescence global maxima

In recent years, measurements in the area of CFY have become an eminent part of the investigation of plants' photosynthetic performance and capabilities. [7][8]Moreover, incorporation of different measurement techniques have made it possible for further analysis of several different characteristics of plants.

Theory Behind the Phenomenon

This sub-chapter provides a short summary of the main biological and mathematical theoretical principles, behind the phenomenon of CF.

From biological point of view, measurements of CF are directly related to Photosystems I and II, more specifically to Chlorophyll a, as it mostly absorbs energy from wavelengths in the violet-blue range of the visible light spectrum [22][23] Moreover, Chlorophyll a reflects light in the green visible spectrum, thus contributing to the observable green colour of plants.

Photosystems I and II is the place where chlorophyll collects light and as result releases electrons into the electron transport chain. As electrons are brought from ground to excited state, this occurs in the reaction centers of the aforementioned photosystems. A transition to the mathematical approach of understanding CFY, is the further elaboration on the topic of dark adaptation. As it serves as a reference points for further measurements, its determination is crucial when one describes the underlying phenomenon. During dark adaptation, the CFY of a plant is at its minimum (a proper descriptive relation could be represented by the dark current in photodetectors). Determination of the efficiency of the plant system, which is related to presence of ambient stress, greek letter PSII incorporates usage of pulses of high energy levels in order to stimulate CFY. This allows for quantification of Fm – the maximum CFY and Ft – the normal CFY under actinic light.

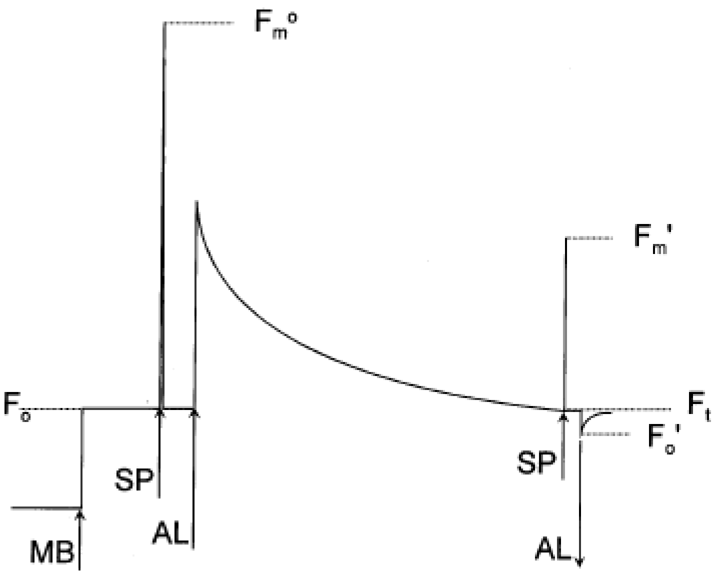

Figure 3 - Graphical representation of typical CFY. When measurement light (MB) is turned on, the process of CFY commences as F0, the minimum CFY level is measured. Presence of highly saturated light (SP) allows for measurement of the maximum levels of yield Fm0. A second saturation pulse allows for determination of Fm' – the maximum level of CFY in the light to be measured. Right before the source of light is switched off, quantification of the normal CFY level under actinic light is determined

Once all aforementioned parameters have been obtained, the determination of the system's efficiency could be calculated as:

$$\varphi psII = \frac{F'm - Ft}{F'm}$$

, with Fm' being the maximum CFY in light adapted plant, as opposing to Fm0, being the maximum CFY in dark adapted plant. Further elaboration on this relation would lead to calculation of the proportion of light, absorbed by Chlorophyll a in PSII. Low values of plant efficiency reveal that the plant itself is most likely under stress.

Contemporary Situation in the Field of CF Measurements

Technically, there are two types of fluorometers when it comes to underlying method of operation – a non-modulated fluorimeter gives information only about the difference between the global maximum and minimum in the graph; a modulated fluorimeter also gives other parameters and this way the type of stress could be characterized.

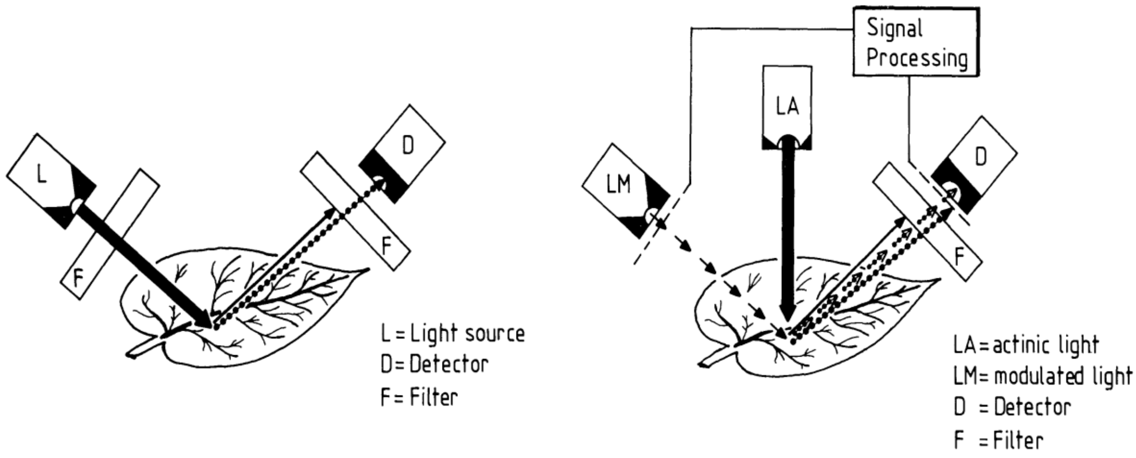

Figure 4 - Left: A non-modulated fluorometer. Constant light beam is emitted and read by the photodetector. Right: A modulated fluorometer. Pulses of light are applied and synchronized with the working mode of the photodetector. Source: Bolhar. H. R. et al. (2008). Chlorophyll Fluorescence as a Probe of the Photosynthetic Competence of Leaves in the Field: A Review of Current Instrumentation. Functional Ecology. 3 (3), 497-514.

The topic of CF was not that much improved for the last 40-50 years, since it was discovered – the application is still new, so is the equipment used. This present difficulties in characterizing the different types of stresses - for instance, stress caused by too high salinity in plenty of water and the absence or the little amount of water produce the same physiological response in the plant – a common signal is recorded therefore. Thus, even a modulated fluorimeter cannot differentiate between those types of stresses. On the other hand, stress caused by shading and strass caused by too much water are easily differentiated. A completely other example is the stress, caused by sulfur dioxide and nitric oxide – pollutants emitted in the air by factories – there is not enough information on this type of pollution and thus, it cannot be characterized at the very moment.

Another issue in understanding the relation between CF and the type of introduced stress is that in articles, discussing the topic of CF, present only on kind of stress to the plant. No articles discuss the relation between CF and the presence of two different, distinguishable types of stresses. Moreover, in most of the articles on the topic of CF, the authors use CFY indirectly in order to conduct their research. Therefore, the articles do not strive for precise clarification on the topic of CF itself. One example is the output CFY presented in arbitrary units instead of mV. Two articles are close to the research in this project – "Chlorophyll fluorescence as a plant stress indicator" and "Chlorophyll fluorescence as a quantitative measure of plant stress" by Kancheva et. al. (2008). The authors quantify their setup, input units and output CFY. Nevertheless, the system they used for measurements taking remains unclear.

Underlying factors of CF Measurements

The following sub-chapter presents the most important factors to be taken into consideration during the implementation of the project's system. Via theoretical research, background of the phenomenon and consultation with a professional in the field of plant ecophysiology, the following sub-chapter unifies and presents a final hypothesis to be further developed in Chapter 3.

The required sensor node characteristics were discussed with a professional in the field of plant ecophysiology on the base of already conducted researches and published articles. In terms of technical characteristics, five major categories were defined, where restrictions outlay the initial states of the desired outcomes.

- *Light Source Wavelength*

Fluorescence, as a physical phenomenon, is most basically explain by emission of light at certain wavelength and its reflectance at wavelength, higher than the emitted one.[21] In the case of CF, light emission wavelength has been shown to be in the 400-420nm region [2]

- *Duration of Excitation*

Direct exposure to light drives CF. A short period of exposure of 0.6 seconds is enough for the phenomenon to be initialized [9]. Higher values of exposure times would only lead to higher power requirements, not influencing the required output.

- *Frequency of Measurements*

The field that is mostly influenced by the characterization of this variable is the software development of the system itself. Frequency of measurements also determine the amount of data to be analyzed per unit time as well as power requirements for the system. Both fields are strictly subjective for the requirements of this project.

- *Light Source Intensity*

The intensity of the light, directly incident on a plant's leaf and thus driving CF, (in other words, the amount of photons striking a leaf) needs to meet a minimum value in order to be able to initialize the whole process . Once this minimum value has been reached, all electrons would be brought to excited state, all action centers would be closed and CF would occur. [22][23]

- *Measurements Duration*

Like frequency of measurements, this field is directly related to the software development of the system. Furthermore, data quantity and power dissipation are also directly linked to duration of measurements per unit time. It has been established that for the needs of this project measurements duration of one to three hours in the middle of the day are sufficient for proper data processing and analysis.

A really important notice for further analysis of technical characteristics and requirement of the resulted system is the fact that CF output is the same for different types of plants. Thus, we do not take personal/ecophysiological/environmental characteristics of different plants into consideration during the portrayal of the needs of this system. The resulted system shall apply to different types of plants the very same way, with the same approach, data processing and analysis. [6][10][11]

Hardware Requirements

Three main components are required for the implementation of the sensor system – a light source (laser or LED for instance), a filter and a detector of quantity of electrons emitted. The operation of the system is as follows: the source of light emits light, which stimulates CF, which is afterwards filtered by the filter. Then the detector reads the quantity of emitted light and sends it wirelessly to a station. It is a good idea to research if light source and filter can come into one component.

Another vital remark is required – a modulated fluorimeter is more complex into its design and operation. If presence of external light (sunlight) is presented, it would influence the outcomes. On the other hand, the simpler non-modulated fluorometer does not require complete darkness. Contemporary solutions in the field of CF measurements propose three main types of light excitation: LED light induced CF, laser induced CF and halogen light induced CF.

When it comes to input characteristics two main types of fluorometers are present on contemporary market – spectrofluorometers and filter fluorometers. [2] The first one is identified as spectrofluorometer and it includes an excitation monochromator and an emission monochromator. Thus, a spectrofluorometer provides a range of adjustable wavelengths, exciting CF over the very same area. Main disadvantages of spectrofluorometers, compared to filter fluorometers, are higher prices and lower sensitivity and specificity.

A filter fluorometer, on the other hand, inputs light of single wavelength. It measures the ability of a sample (in this case – a leaf) to emit the input light and reflect light at lower wavelength. It is a good choice, when one would quantify CF in plants. With this project we are interested into the implementation of a filter fluorometer, in order to quantitatively define and understand the fluorescence-pollution relation.

The underlying working principle of a filter fluorometer presents a one-way flow of light, changing its physical characteristics when it hits the leaf. A light source emits light in a portion of different wavelengths. An excitation filter is present in order to block the not required wavelengths, as incoming from the light source (the aforementioned two components could be easily unified as a single one, by the introduction of an embedded filter to the input light source, or, as in the case of single-wavelength LEDs, the light source itself inputs light of single wavelength). The light then reaches the surface of the sample cell (leaf), where the chemical process of fluorescence occurs. This results in emission of light, at lower wavelength, when compared to the input source light. Since the input-output wavelength light relation is known, the presence of an emission filter is required when one would like to quantify only the incoming light, produced by the process of CF itself. An important remark is the angular displacement between the light source and light detector. [10] The most common solution is a 90-degree angular displacement, as the resulted CF could be thought of as the continuous reflection of the input incident light.

Once the emission light is filtered, a detector reads it quantitatively. Depending on the type of light detectors, further filtering and/or amplification by means of hardware applications could be required. Throughout the implementation of this project, we follow the described basic working method of a filter fluorometer.

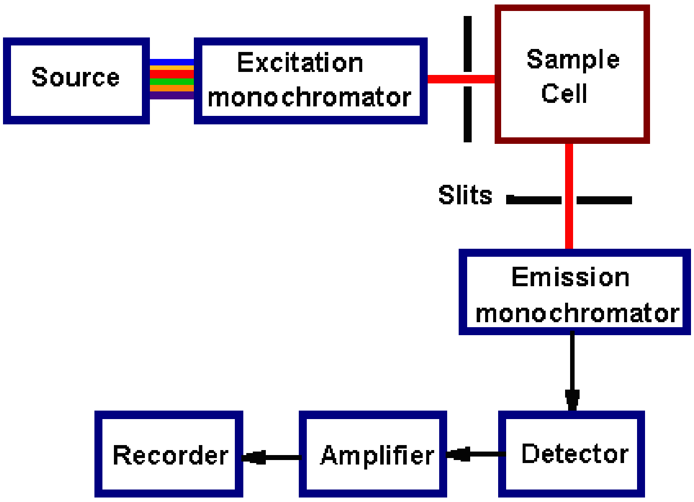

Figure 5 - Basic underlying operation of a filter fluorometer

Mechanical Orientation and Design

Bolhar. H. R. et al. (2008) propose physical light source-light detector orientation of 90 degrees (as depicted on Figure 4). The proposed solution is suitable for the needs of this project, as it does not introduce great deviations in any of the three dimensions. Striving for a system with minimalistic size, the proposed physical orientation would contribute to the specification of the final system's size. As one of the most important requirements for the design of the final sensor node is small size, mechanical characterization plays a vital role in this project. Contemporary fluorometers mostly rely on human interaction for the results to be produced. Moreover, they have significant dimensioning in the both cases, when being presented as a stationary system and as a hand-held device.

Originating from the input concept of small size, the sensor node needs to be directly clapped onto a plant's leave. Thus, once a node is attached to a plant, it would continuously and autonomously record levels of CFY. A few direct conclusions could be drawn from the desired clip-like mechanical design: firstly, the node must not present any damage to the leaf itself because of the clipping mechanism and secondly, the system must be light enough so that it stays on the leaf without causing it to bend. This leads to a requirement of a light, yet, durable material to be used for the physical construction of the sensor node.

Design Determining Factors

Resulted output of the theoretical analysis lead to determination of the most vital and important factors, to be taken under consideration throughout the development of the design process in regards to the sensor node. Appendix A elaborates on the further choices made.

Factor	Influence
Light Source Wavelength	Direct correlation with CFY

Light Source Intensity	Direct correlation with CFY/Powering of the system
Duration of Excitation	Direct correlation with CFY/Powering of the system
Type of light source	Direct correlation with CFY
Frequency of measurements	Desired amount of data to be analyzed
Duration of measurements	Desired amount of data to be analyzed
Type of data transmission	Size of system/Price of components
Storage of data	Size of system/Price of components
Powering of system	Size of system/Price of components
Method of excitement	Direct correlation with CFY/Powering of the system

Table 2 – Most important factors outlining CFY measurements

As already stated a few characteristics in the mechanical design of the sensor node need to be taken into consideration:

- Presentation of a 90 degree light-source-light detector orientation, for ease of measuring and minimization of the system's size
- The design of the system, and more specifically the part to interact as a clip with the leaf itself, must not in any way disturb nor harm the structure of the plant
- Once attached, the sensor node must not present any additional stress to the plant, caused by its weight

Thus, the produced data allows for construction of a hypothesis for the implementation of this system. With the implementation of the project itself, we strive to produce a small, cheap device, which would be able to serve as a non-modulated field fluorometer. The sensor node needs to be small enough, so that it could easily be clipped directly onto a plant's leaf, without introducing any additional stress to the leaf itself. As this would only be the first prototype of the system, it would not excel in complexity, but it would rather prove the very basic point of CFY detection and would provide base for further development.

CHAPTER 3 – CONCEPTUAL MODEL

The following chapter presents the resulted conceptual model of the sensor node, specifying and quantifying the described required characteristics from the previous chapter.

Calculating Efficiency

This first prototype of a CF sensor node would be able to calculate only the photosynthetic efficiency of a plant. Thus, it needs to be able to output two values – F0 – the minimum level of fluorescence and Fm' – the maximum level of yield. Moreover, in order for those two values to be produced, two light input pulses would need to be applied. The first one (depicted as MB on Figure 4) is the measurement light required to initialize the process of CFY. The intensity of this light input is kept low and it is required only for the establishment of the ground levels of yield. The second light pulse would be one of high intensity (SP) and it would result into a short, high value of maximum CFY. Post processing of information would yield to determination of the plant's efficiency.

Methods of CF Measurements

Different methods of CF measurements have been applied since its discovery, which build on the fluorescence induction technique. The different methods strive to give true estimates of different CF variables.

The first one is a Rectangular Single Flash (RSF) method, which applies a signal of the same intensity for a duration of 400 to 1200 ms. The second possible method is the Flash Train (FT) method. [19]It relies on impulses of varying intensities with a two minute interval between each pulse, applied at random order. The third proposed methods is a Multiphase Single Flash (MSF) method. With this method, light of high intensity is applied for 250ms; afterwards input amplitude declines linearly for 500 ms and at the end there is a return to the initial amplitude for 250 more ms. MSF has been proven to output the best true estimates of different CF variables, while RSF leads to large under estimates of those variables. Moreover, the FT is a long, time-consuming method, and it is not desired for the needs of this project.

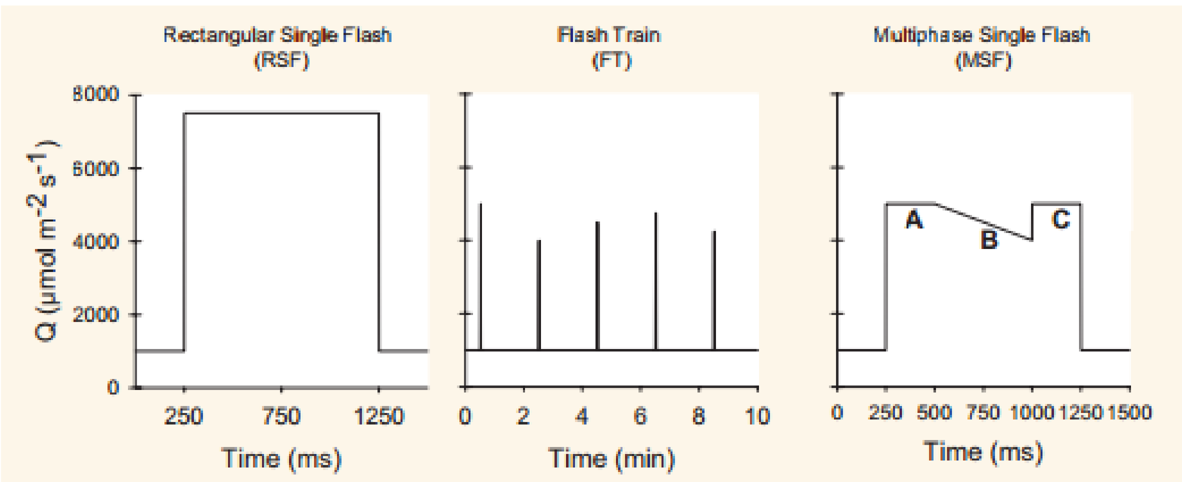

Figure 6 – Three main types of light input to drive CF

Another method, proven to output reliable results is the Fast Repetition Rate method (FRR). It relies on generation of single-turnover (ST) and multiple-turnover (MT) flashes. FRR method proves to yield maximum fluorescence. [20] The collective name for all aforementioned methods for CFY is Multiphase Flash Methodology (MPF). The underlying idea is that high values of CFY could be achieved by presence of artificial source of light, at wavelengths lower than those of the fluorescence itself.

Taking into consideration the simplicity of the first prototype of the resulted sensor node, RSF has been established as underlying method of CF excitement, mainly because it would be straightforward to be produced from software point of view and because of the short period of excitement, it would not require great amounts of input power.

Light Source Wavelength

As CF excitation peaks in the 400 nm region [2] light source with this intensity would best fit our requirements, as it would drive CF at its maximum. This could be achieved either by usage of ultraviolet single-color source or by usage of multi-color (RGB) one. Those two possibilities would eliminate the need for an excitation filter. The latter would require precise determination of the transition between input light wavelength and corresponding lead currents.

Usage of purely white light as source would also drive photosynthesis, as it would contain portions of the required excitation wavelengths. In this case an excitation filter would need to be applied.

Single-color ultraviolet light input has been established as main light source in our sensor node, mainly because of the resulted ease of operation, fixed wavelength and redundancy of an excitation filter.

Light Source intensity

Light source intensity plays a vital role in CFY measurements. The firstly applied measurement pulse requires really low levels of intensity. Values of 2-3 $\mu mol.m^{-2}.s^{-1}$ have been established for the testing phase of the prototype. The light pulse to drive maximum levels of CFY, on the other hand has significantly higher values of intensity. R.H. Kancheva et. al. (2007) used values of 507 $\mu mol.m^{-2}.s^{-1}$. Bolhar et. al. (2008) use a set of three different fluorometers, with values of excitation light intensities of 600, 660 and 10 000 $\mu mol.m^{-2}.s^{-1}$ respectively. Tennessen et. al. use values of 1000 $\mu mol.m^{-2}.s^{-1}$ Further elaboration on this parameter yields that commercial fluorometers have typical values of light intensity of about 12 000 $\mu mol.m^{-2}.s^{-1}$

The conducted research outputs that values of the second light pulse to be applied could be as low as 507 $\mu mol.m^{-2}.s^{-1}$. Such intensity has been proven to be high enough in order to drive the process of CFY. In our sensor node we strive to use as less power as possible and since higher levels of light intensity would require higher levels of power input, we strive to keep the light source intensity at minimum. Value of 3000 $\mu mol.m^{-2}.s^{-1}$ has been established to be used in this first fluorometer’s prototype. [6][10][11]

Duration of Excitation

Rosema and Zahn (1997) use consecutive excitation pulses of 10 ns each. A vital remark is that in their paper they discuss laser induced fluorescence. Kancheva et. al. (2008) on the other hand input light pulses of 1.5

seconds in order to drive the CFY. Consultations with a professional in the field of plant ecophysiology resulted in establishment of 0.6 seconds as excitation length for both CFY excitation pulses.

Type of Light Source

In terms of light source, there exist several different possibilities. [2] The first one is the usage of xenon lamps, as they propose wide range of input wavelengths and have the ability to mimic natural light. A mercury vapor lamp is usually more intense that a xanon one. It furthermore has longer lifecycle. Nevertheless, the aforementioned two types of light input usually come in packages, with too great dimensions, compared to the requirements of this project.

Two more appropriate choices for light input, driving CFY, are lasers and LEDs. Lasers have already been proven to yield desirable results, establishing the bases of laser-induced chlorophyll fluorescence. [7] They propose high light intensity over small CF excitation area. Nevertheless, due to the size requirements for this project, laser-induced CF is not suitable for further implementation. LEDs have the best required characteristics, combining high light intensity, low cost and small size. Going further into analysis, there exist three main excitation methods, in terms of LED excitation. The most straightforward method, as described by the basic working operation of a filter fluorometer, is the usage of a white LED, with incorporation of a filter, blocking all wavelengths but the 400 nm one. The second, more appropriate method, excludes the incorporation of a filter and presents the usage of a RGB LED, as it could be programmed to emit light at desired wavelength. Nevertheless, direct translation between RGB 8-bit values and energy of light in terms of wavelength (frequency) is not straightforward and would require further tests. The third and most suitable choice of LED is a single-color ultraviolet LED, as it emits light of the required input wavelength and excludes the incorporation of an excitation filter. This both reduces size and programming complexity of the system.

Excitation Profile

With the provided information so far we can deduct on the characteristics of the light input to drive CFY in our sensor node. The input light would have intensity of 3000 $\mu mol.m^{-2}.s^{-1}$ (lower values would also be acceptable throughout validation and tests, as long as they prove to drive CFY). Ultraviolet LED would be used as light source, at input wavelengths of 400-420 nm and method of excitation of RSF. The working operation of the LED would comprise of light exposure of a measuring (low intensity) light for 0.6 seconds, followed by a pause of 0.5 seconds. Then the high intensity light pulse would be applied for a period of 0.6 seconds. Due to the two very different levels of light intensities there arises the question about precise mode of operation of the LED light source. Two possibilities exist – the first one would be the incorporation of a digital potentiometer, to control the resistance accompanying the LED, thus controlling the current flowing through it. This would result into different levels of light intensities applied at desired times, which could be achieved by software means in cooperation with a proper microcontroller. The second possibility for application of light at two different intensities is the introduction of two different LED sources, each of them at fixed light intensity. Then one of them would always act as a measuring light, while the other one would serve as excitation for the yield of maximum CFY. As the presence of a digital potentiometer (and the resulted influence on programming complexity of the chosen microcontroller) would make the implementation of the first prototype of the system more difficult, usage of two LEDs at fixed values of light intensities is the preferred method of work.

Frequency of Measurements

The minimum frequency of measurements is every 15 minutes. Any higher frequency, which would not disturb the requirements of the functionality of the whole system would be considered as expanded way of approach.

Duration of Measurements

As this is directly related to power consumption, we might not be able to take measurements throughout the whole day. Therefore, we have defined a period of one to three hours in the middle of the day, when measurements will be taken, as CFY produces best outputs in the middle of the day [1](article from Fenna). Measurements more than three hours per day would be considered as expanded feature of the system. In the ideal way, we would strive for constant output of data, throughout the whole day.

Data Storage Transmission and Design

Figure 7 presents the working method of the output system.

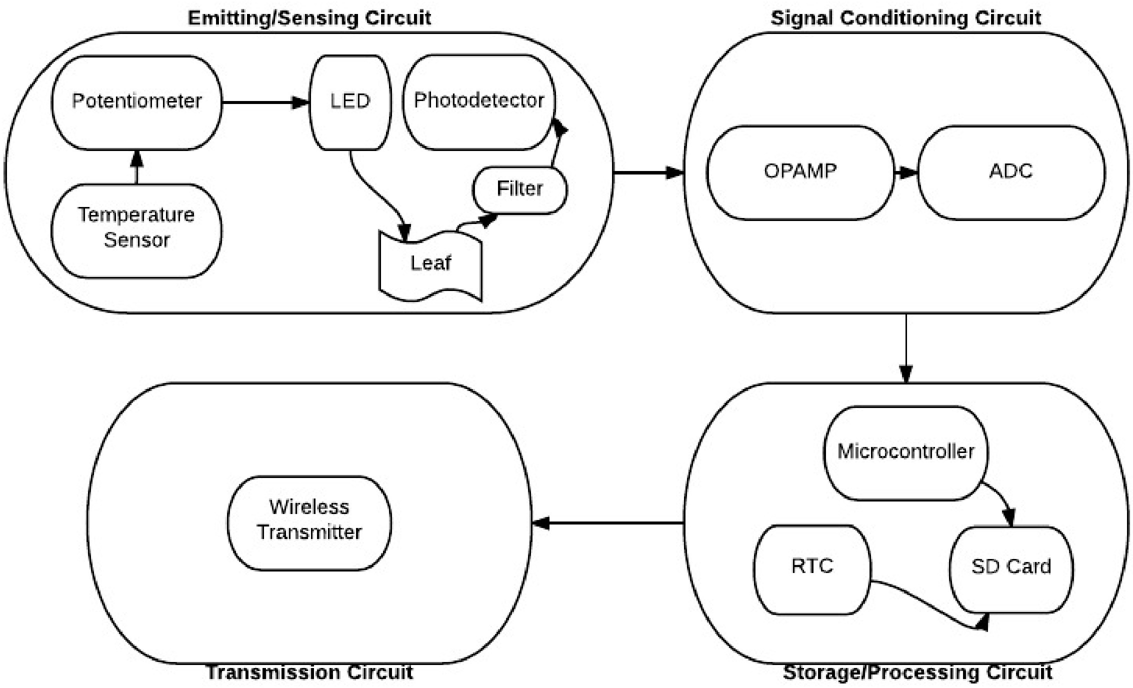

Figure 7 – The four main parts of the final system

Four main components comprise the working method of the system. The first one is the Emitting/Sensing circuit. It is responsible for data acquisition. Apart from the three very basic components for measurements of CFY – light source, filter and light detector – it also contains a potentiometer (not implemented for the first prototype, but rather used as a recommendation for further developments). This circuit also contains a temperature sensor, as CFY behaves differently under different temperatures (again, not implemented in the prototype and used as recommendation).

The second main circuitry is the Signal Conditioning circuit, which is responsible for data amplification and digitalization. Exact amplification would depend on the chose light detector. Digitalization is performed via an analogue-to-digital converter, which could be either present as a separate electronic component, or embedded in the microcontroller to be used.

These two circuits together represent the small sensor node to be clapped onto a leaf. The minimalistic approach in terms of node's size mean that only measurements will be taken on place. The data is further passed to the Storage/Processing circuit, where a Real Time Clock presents each measurement with a time stamp. The data is stored on a SD card and is then passed to the Transmission circuit, which transfers the data to a remote system, where it would be further analyzed and processed.

The original concept of the sensor node suggests post analysis of data. All measurements taken would need to be transmitted to a remote station, where processing, powering, analysis and visualization of data would take place. This arises the question of how exactly data would be stored and transmitted.

Figure 8 presents the operating model of several sensor nodes. Since a sensor node needs to be as small and as light as possible, it needs to contain as few electronic components as possible.

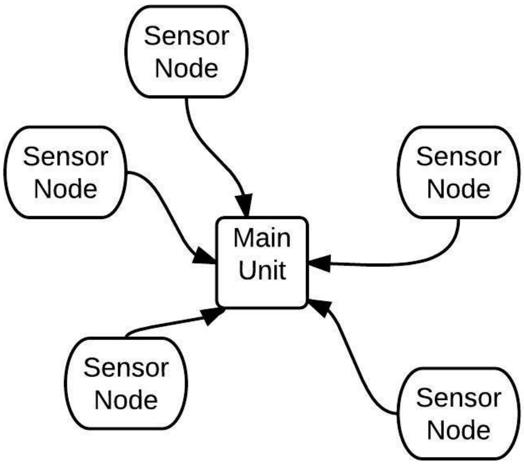

Figure 8 – Basic cooperation between sensor nodes and a main unit

The depicted star configuration allows for multiple sensor nodes to share a single data memory, which is required due to the high prices of SD cards (availability of a single card per a sensor node would immensely raise the price per node). Thus, several sensor nodes would collect and send data to the main unit, each of them controlled via a microcontroller. The main unit itself comprises power input, a second microcontroller to handle the incoming data, an RTC for time stamping, an SD card to store the incoming data and a wireless transmitter to later transmit the data to a remote station.

An important remark must be done in terms of method of data transmission. Constant flow of data would require great amounts of power, as every time a wireless transmitter is turned into active mode, it requires great amounts of current for it operation. Instead, the collected data would be stored on the SD card and transmitted at once at certain periods of time. This would increase data traffic, nevertheless, it would lower power dissipation.

The first possibility for a wireless transmission medium is Wi-Fi. With its 2.4 GHz of frequency it would be an appropriate medium for CFY measurements transfer. Nevertheless, network coverage is not guaranteed at the place where the sensor nodes would be incorporated. And since the output must be applicable to places of different kind, Wi-Fi transmission would not be appropriate implementation.

The second option of data transmission medium is radio wavelengths via short-range devices at lower frequency than Wi-Fi. And since such short range devices can transmit to a few hundred meters, their successful operation directly depends on the distance between the sensor nodes and the station for post-processing. Therefore, the distance between the nodes and the post-processing station must not exceed the transmission range of the device. Such a device could be found at low price, small and compact package, low input voltage, and different transmission wavelengths.[29]

System Powering

Throughout design of electronic schematics it is vital to implement a single input voltage reference. Input voltage of 5 V is a typical value – light sources and light detectors operate with values of 5V. Nevertheless, microcontrollers typically require input voltage of 3.3V, therefore, this would require the implementation of a voltage regulator in the design.

Batteries could come in different sizes and packages. Since power to a sensor node would be supplied from the main unit, the limitations of size do not apply here so greatly. Therefore, price is the only limiting factor, when choosing batteries. Cheap button batteries could be used in series in order to produce the required 5V.[30]

Light Detector

There are four general choices to consider when choosing a type of light detector to be implemented in this sensor node – photodiodes, photoresistors, phototransistors and specialized active electronic components.

- Photodiodes

Photodiodes are semiconductor devices, which respond to light energy. The incoming photos from the incident light are absorbed by a photodiode and generate flow of current in an external circuit. Photodiodes are highly linear, thus the generated current is proportional to the incident light energy. This allows calibration for measurements with really high precision. [24]

Photodiodes have very fast response – in order of nanoseconds and can be used as light sensor is myriad of applications. Nevertheless, photodiodes are not as sensitive as phototransistors and thus, implementation of an operational amplifier must be incorporated in the circuit design.

- Photoresistors

Photoresistors are also known as light dependent resistors. They could be used to detect the presence or absence of light, or quantify it. In a nutshell, a photoresistor's resistance decreases as the incident light increases. Used together with a voltage source, a photoresisor could be indirectly used for light intensity measurements.

Photoresistors' response depends on light wavelength. Moreover, their response is not linear. While photodiodes and phototransistors are purely semiconductor devices, which directly rely on light in order to produce electron flow, photoresistors are passive components, which lack a PN-junction. The resulting resistance might vary significantly, even if light source intensity is kept at constant levels. Thus, photoresistors are best used for detection/absence of light, not for precise light level measurements and are not appropriate implementation for the needs of our project. [26]

- Phototransistors

A phototransistor is a semiconductor device, which acts as a light sensor. It is formed by a basic transistor with a transparent cover, which provides much greater sensitivity than photodiodes and photoresistors.

Phototransistors are still cheap, compared to photoresistors and photodiodes. Two main advantages bring phototransistors forward – they do not need additional circuitry in order to produce measurable levels of electron flow (the device itself has high gain) and they are more precise compared to the previous two choices. Nevertheless, the main flaw of phototransistors is their non-linear relation between incident light and output voltage. This would require for precise calibration of one.

- Other types of photodetectors

Specialized active electronic components to measure light intensity are available. They come in different forms, sizes, sensitivity, et cetera. Furthermore, whilst the aforementioned electronic components could be strictly defined in distinctive categories, other types of photodetectors may have their method of light measurement/indication completely different from one type of photodetector to another.

According to my research, two main reasons indicate that we should not stick to this option – firstly, most of the photodiodes come in too large dimensions for the needs of this project; secondly, their price range tends to be significantly higher than the ones of the aforementioned components. On the positive hand, some photodetectors can provide extremely high sensitivity, nevertheless in this case they would disrupt the whole basic idea behind this project, by being too big and way too expensive.

- Conclusion

A photodiode would be implemented as the sensing part for this prototype, because of its linearity in input/output ratio. This would ease implementation, validation and data analysis. The chosen photodiode would need to operate with an operational amplifier.

CHAPTER 4 – RESEARCH DESIGN

The following chapter describes how the conceptual model would be tested and how the research would strive to answer the defined research question.

Method of CF Measurement and Duration of Excitation

The excitation method of 0.6 seconds of low intensity light – 0.5 seconds pause – 0.6 seconds of high intensity light would be done with the usage of a MSP430G2553 microcontroller, directly embedded on a Texas Instruments LaunchPad.

Figure 9 - TI LaunchPad with MSP430G2553 microprocessor with 16 MHz frequency, 16KB flash and 8channel 10-bit ADC. The microcontroller development board has analogue pins to be connected to the photodetector and to supply input power to the light source. The board has a button available, which could trigger an event. Thus, LED light pulses could be manually initialized.

- Light Source Wavelength

As already discussed, a single-color LED would be used, therefore there would be no need for calibration and measurements of a RGB one. Input wavelength could be verified with proper equipment, if would be available.

- Light Source Intensity

Light source intensity would be measured with HD 2102.2 photo-radiometer. It could successfully measure light intensities in the 400-1000 nm spectrum. A measurement in a dark room would provide the required data for light intensities of the different LED sources tested. Furthermore, HD 2102.2 can directly quantify light intensity in µmol.m^{-2}.s^{-1} so post-calculations would not be necessary.

Figure 10 - HD 2102.2 photo-radiometer

- Mechanical Design and Electronics Schematics

Mechanical design and electronics schematics will be externally validated by engineers. The mechanical design will need to meet the established criteria for size and structure. Electronics schematics would provide the base for further development of a PCB board, to be implemented on the mechanical design. The combination of two would result in a final sensor node. Thus, the design of the electronic schematics would provide simplicity and would incorporate as few elements as possible, as it needs to meet the criteria of small size, and therefore weight.

- Validation

The aforementioned equipment would be used for system validation. The validation would strive to test different light source inputs as well as different types of filters, in order to yield the best results. The very final validation would incorporate measurements of CFY on leaves, experienced different levels of stress. Thus, comparing the data with literature and/or a commercial fluorometer (double tailed t-test would serve for data comparison in the presence of a fluorometer to compare with) validation of the used photodetector would be achieved. Factors to be taken into consideration in the performance of the photodetectors would be sensitivity and noise.

CHAPTER 5 – RESEARCH RESULTS AND DISCUSSION

Electronic Components and Schematics

Electronics schematics were produced via Altium Designer. Figure *** depicts the resulted schematics.

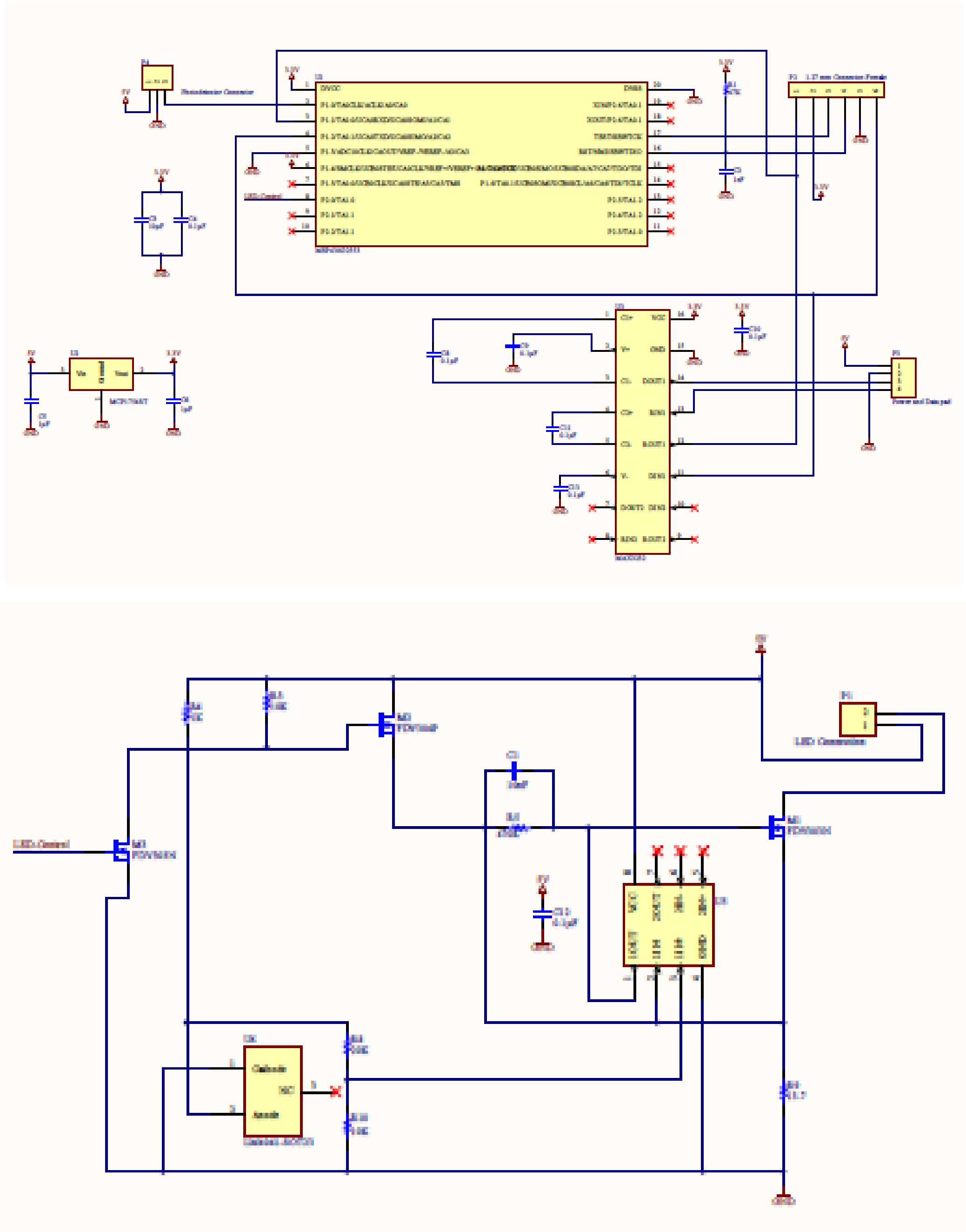

Figure 11 – Top: Connections of microcontroller, transceiver, voltage regulator and connectors

Bottom: LED circuit to produce stable LED flash light

Digikey was chosen as main electronics components supplier, because of the quick delivery of required components and professional services. Prior to the order of materials, a Bill of Materials was composed, which could be found in Appendix D. As already stated, MSP430G2553 was the chosen microcontroller to be implemented both in the sensor node and during validation of the first prototype of the system.

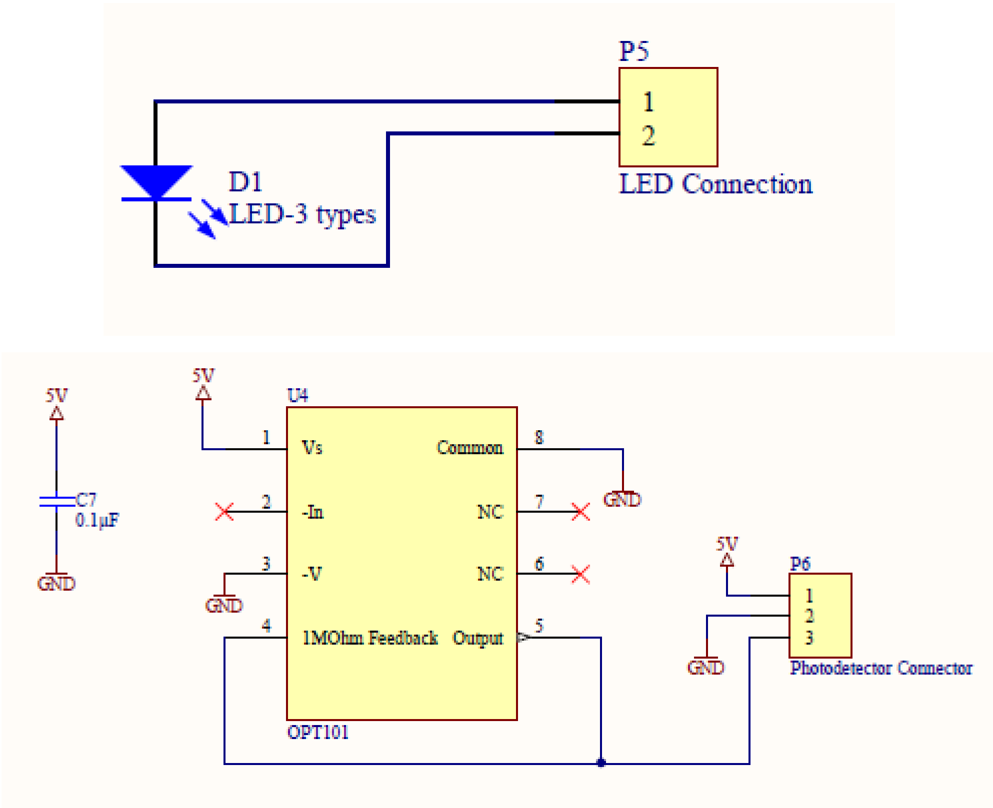

Figure 12 – Top: LED connection

Bottom: Photodetector connection

The node would receive input voltage of 5V, which is required for the operation of the photodiode and the LED. The presence of a voltage regulator makes sure to supply constant voltage of 3.3V to the microcontroller and transceiver. The presence of MAX3232 transceiver would transfer CFY measurements from the sensor node to the main unit. Part of the circuit makes sure that the LED is supplied with the correct amount of current and that it operates at correct frequency of light impulses (lower image). For that purpose a simulation was performed on LTSpice, in order to show how the circuit would operate and what the resulted LED light sequence would look like.

The simulation on LTSpice acts as a validation technique for LED circuit performance. The final version of the schematics was verified and validated by José Nacif and Adriano Cardoso.

The chosen photodiode was OPT101 [31]. It has highly linear light intensity-output voltage relation, which makes it suitable for the current setup. Its benefits include responsivity of 83% at the 700 nm spectrum, small size and built-it amplifier. OPT101 also comes in 8-DIP package, which makes it suitable for implementation onto a breadboard.

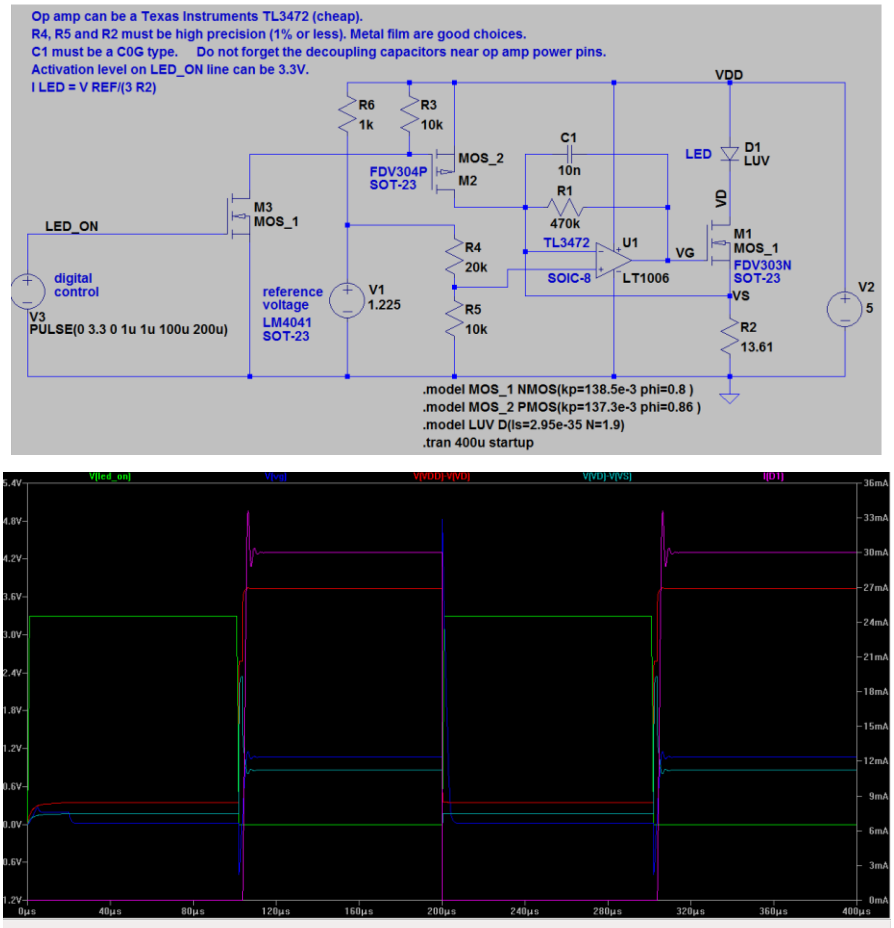

Figure 13 – Top: LED circuit on LTSpice

Bottom: Resulted simulation. Green line represents current flowing through the LED

Setup

Validations in controlled environment were performed with different LEDs as light sources and different filters. LaunchPad was programmed to send the required 0.6-second signals to the two LEDs. Appendix E contains additional pictures of the constructed setup.

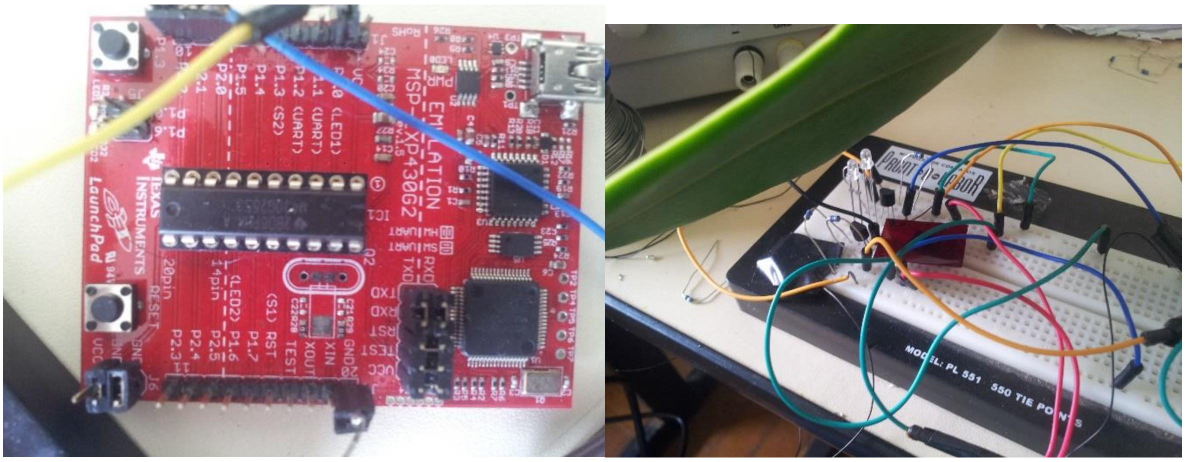

Figure 14 - Left: LaunchPad by Texas Instruments. The microcontroller used is MSP430G2553. The pad uses digital ports for communication with the photodiode. LEDs are turned on at the proper sequence every time a button on the pad is pressed (top left). Data communication of measurements flows from the microcontroller to a PC via Serial port, every 50 msec. The embedded Analogue-to-Digital converter on the microcontroller enables measurement resolution of 10 bits.

Right: Basic setup used throughout validation and tests. The two LEDs are supplied with current by the LaunchPad. Combination of transistors and resistors make sure to supply 5V input to the LEDs, triggered by the 3.3V input from the LaunchPad. A filter is applied directly onto the photodetector.

Mechanical Design

The mechanical design of the system was developed via AutoCAD software. It presents different views on how the node would be implemented on a plant. The developed soft link at the part where the node is attached to the plant and its relatively large area make sure that the plan remains unharmed. The presence of a moving bridge (via a spring in order to cause pressure onto the plant and thus clip the whole node) makes sure that the current light source-light detector mechanical orientation meets the requirements of 90 degrees orientation.

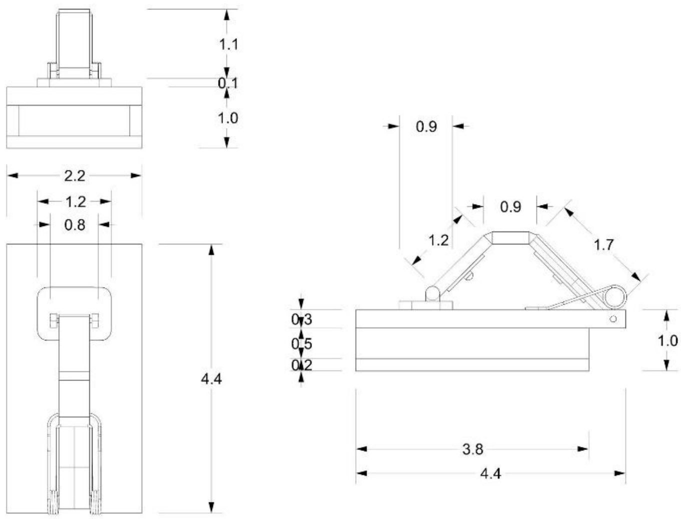

Figure 15 – Three point of view of the mechanical design with dimensions

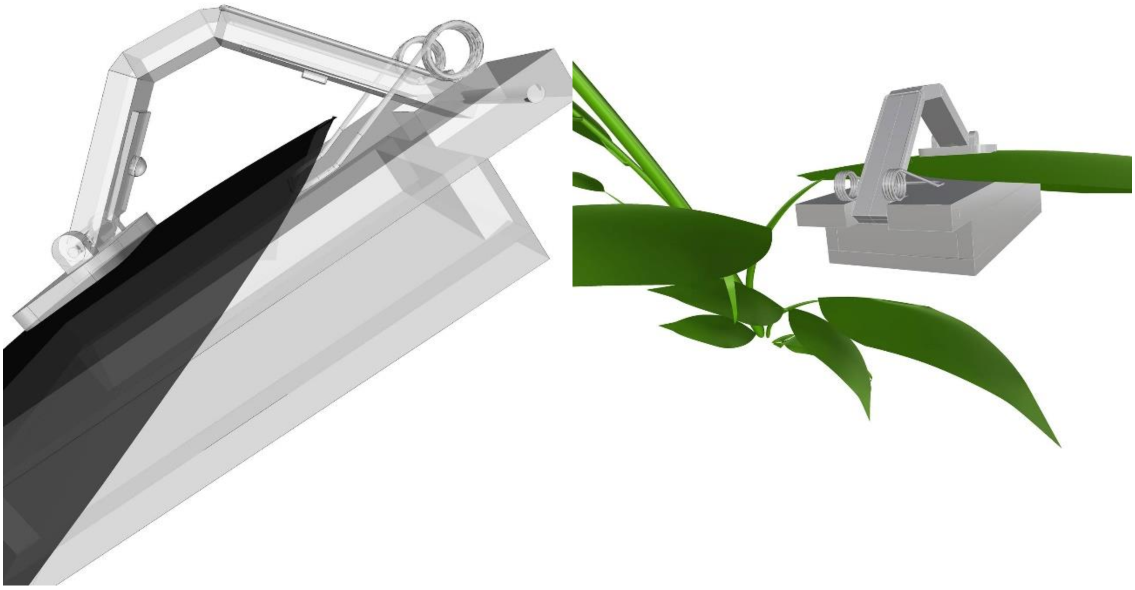

Figure 16 – Demonstration of how the sensor node would be attached to a plant.

More pictures are available in Appendix F

Validation Results

The first phase of the validation consisted of determination of the most appropriate filter to be implemented for further purposes. Four different types of red filters/materials were acquired and tested. Two of them showed no filtering abilities and were discarded for further implementation. The two other filters showed good performance in filtering out light wavelengths not in the red/far red spectrum.

The second phase of the validation tested different LED sources for their output light intensity. This was achieved with HD 2102.2 photo-radiometer. The experiment was performed in a dark room. Light intensity was measured at distance of 5 mm from the LED sources. Circuit resistance used was 4.7 Ohm.

The last phase of the validation strived to measure CFY in differently stressed plants and compare the results with already known from literature. For this purpose three leaves of a plant were obtained. Stress was introduced to two of the leaves. Then repeated measurements were taken with the constructed setup, CFY on each of the leaves was recorded and visualized. CFY was detected from the upper surface of the plants after 2 minutes of predarkening (Kancheva et. al. (2008) suggest values of 3 minutes). Input power was delivered via a fixed power source of 5V.

Figure 17 - The tree leaves used for tests. The leaf on the left is completely healthy and stress was introduced to the other two by presence of excessive amounts of energy in a microwave. The change of color clearly indicates the presented stress. The leaf on the middle was exposed to high levels of energy for 6 seconds. The leaf on the right was exposed to high levels of energy for 10 seconds.

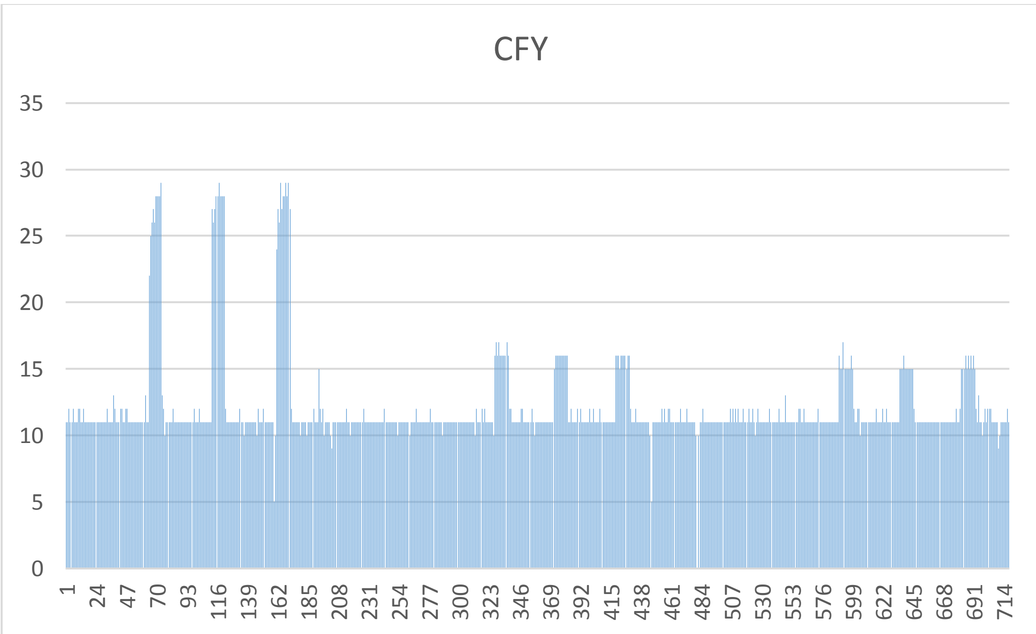

Figure 18 - Graph of CFY, produced after application of RSF light input to three differently stressed leaves (results in 10-bit digital values). CFY was initialized three consecutive times on each leaf. Results on left are produced from the healthy leaf; results in the middle – leaf with 6-second exposure to high levels of energy; results on the right come from the leaf with 10-second exposure to high levels of energy. The graph clearly indicates fluctuations in photodetector measurements. The offset with value of 10 is clearly visible and confirms datasheet input [31]

Discussion

The graph clearly indicates that the healthy leaf leads to higher levels of CFY, while the ones where stress was introduced output lower levels of CFY. It could be also deducted that the three measurements performed in the middle have, on average, higher values of CFY, which correctly indicates that this is the leaf, less exposed to stress.

Nevertheless, the current graph yields results far from expected. Firstly, the presented inconsistency of photodetector measurements is clearly visible, both during CFY peaks and non-excitation. This constant fluctuation has a digital value of 1, which could be explained with the working operation of the photodetector itself (this is typical fluctuation in the operation of a sensor, measuring environmental characteristic). This test was performed several times, yielding the very same results. The current graph shows different values of CFY for the three different measurements taken on the healthy leaf. The visible difference between those values is greater than 1 bit. This leads to the conclusion that measurements need to be taken with higher frequency than the used 20 Hz.

Furthermore, there is no visible effect from the low-intensity light pulse generated by the first LED. Looking at the expected results on Figure 4, we are supposed to see increased output (F0) when the first LED shines upon the leaf. There is no such result visible here. This indicates of low digital resolution – the used 10-bit ADC resolution, embedded on MSP430G2553 cannot sustain the presented requirements, of measurements of F0. Therefore, the current setup can indicate levels of Fm’, but cannot indicate levels of Fm’. Thus, with the usage of OPT101 and digital resolution of 10 bits, determination of a plant’s efficiency is not possible. The current setup does introduce the possibility for differentiation between stressed and non-stressed plants (and corresponding level of stresses), nevertheless, it did not reach the required performance of successfully determining a CF variable of a plant.

CHAPTER 6 – RECOMMENDATIONS AND CONCLUSIONS

The results of this project's research and implementation answered the introduced research question:

How to design the hardware and the software of a sensor system to measure chlorophyll fluorescence? What factors must be taken under consideration for the system to be reliable and produce validated outcomes?

The implementation of this project served as base input for the further continuation and research on the topic of construction of a small, cheap and autonomous fluorometer. The most important factors to be taken into consideration throughout the design and implementation of a sensor node were established, quantified and compared with results from scientific articles. Light source intensity, light source wavelength, duration of excitation and method of excitation were shown to be crucial in the design of a fluorometer. Theoretical research led to understanding the phenomenon of CFY, its role in society and the link between its characteristics and the produced design requirements for the output sensor node. Electronics schematics, software and mechanical design were produced in order to outline and demonstrate the results from theoretical research. The hardware and software of a system to measure chlorophyll fluorescence, according to the given input characteristics of size, price and autonomy were designed and validated accordingly. Both electronics schematics and mechanical design were constructed following the minimalistic requirement of size.

The requirement for a cheap sensor node highly restricted choice of components during construction of bill of materials. A final cost of almost thirty US dollars (cost for all components required for a single sensor node plus a wireless transmitter and batteries) was achieved, where the used photodetector comprises for around 28% of the total cost. The resulted graph clearly indicates for the need of highly better resolution of analogue to digital conversion. MSP430G2553 does sustain 12-bit resolution, nevertheless, the low intensity of the first light impulse would require resolution even higher than this. A recommendation for future development is research either in a new microcontroller to be implemented or in additional high-precision ADC, to be used together with a microcontroller.

Implementation of a temperature sensor is a vital recommendation for the continuation of this project. As LED output wavelength, photodetector output characteristics and behavior of CFY itself differ with changes in temperature, implementation of a temperature sensor in a control loop, together with a microprocessor would lead to correction in the taken CFY measurements. Such a sensor would no need to be of great accuracy (therefore, price) as changes in any of the aforementioned characteristics occur only with great shifts in temperature.

A recommendation for power implementation is usage of multiple batteries inside the very same main unit. The star structured physical relation of several sensor nodes sharing the processing capabilities of a single main unit introduce ease in terms of power maintenance (once the system is out of power, only batteries in the main unit would need to be changed; if batteries were present in every sensor node, that would result in greater human interaction). On the other hand the power requirements of multiple sensor nodes would result in low operation time, if only a few batteries are present. Therefore, as size/weight of a main unit is not vital in its design, the usage of multiple batteries at the same time is a recommendation.

The research and implementation of this sensor system showed that high sensitivity in sensing is crucial in the design of a sensor node, in order for outcomes to be reliable. Future validation with a commercial fluorometer would need to prove the validity of the output data itself. As the current system has shown to distinguish between absence and presence of different level of stress, future work will need to be oriented towards improvements in sensitivity and relation with data, acquired from another system. The requirement for higher sensitivity would affect the total cost of the system, thus revealing that measurements of CFY cannot be achieved with little resources. The results from this research serve as a good base for continuation and development of the current progress.

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APPENDIX A – DESIRED AND MINIMALISTIC MODELS OF THE RESULTED FLUOROMETER

Desired Minimalistic Model

As the name suggests, this situation would define the project as successful but it would not be the best resulted system itself. With the desired minimalistic system we would be able to measure CF correctly and reach the

desired outcomes. After discussions and taking under consideration the time available for project work we decided that I would be working on the desired minimalistic system and any other improvement would lead us closer to the desired expanded system.

Light Source Wavelength

Three possibilities are present in total: white light source: this is good for CFY as it contains the wavelengths, required for excitation; red light source: also appropriate as CF excitation occurs in that part of the light spectrum;

combination of 90% blue and 10% red light. The minimalistic implementation of light source wavelength is white light, as it is most common and easiest to incorporate.

Light Source Intensity

This factor is a constant for both the minimalistic and expanded systems with a value of 3000 µmol.m-2.s-1.

Duration of Excitation

This factor is a constant for both the minimalistic and expanded systems: 0.6 seconds of excitation is enough for CFY to occur.

Type of Light Source

Three possibilities are present in total: white light, transmitted via fiberoptics; LED uorescence; Laser induced uorescence. LED uorescence is our minimalistic choice of type of light source, as it is the easiest to implement

in terms of size and costs

Frequency of Measurements

The minimum frequency of measurements is every 15 minutes

Duration of Measurements

As this is directly related to power consumption, we might not be able to take measurements throughout the whole day. Therefore, we have defined a period of one to three hours in the middle of the day, when measurements

will be taken.

Type of Data Transmission

Transmission via Wi-Fi, Radio frequency or Bluetooth are our options. The final choice would rely mainly of size of the transmitter, as it has to be directly incorporated onto the PCB

Storage of Data

This factor is a constant for both the minimalistic and expanded systems. As transmission of big data once per day would require less energy than multiple transmission of small data (since bursts of energy are required every time at the beginning of transmission from the module) we are going to implement an SD card onto the system

Powering of System

The minimalistic way of implementation is pure incorporation of batteries onto the system's PCB This type of system would rely on RSF method, as rectangular input wave is easier to implement. Furthermore, a system with such a design would be considered as a non-modulated fluorometer.

Desired Expanded System

The expanded system would generally show better functionality and results. Nevertheless, we might not be able to construct it before the end of the project but its characteristics have been established.

Light Source Wavelength

The expanded way of approach consists of 90% of blue and 10% of red light mixture. This combination has been proven to work best in terms of CFY[6][10].

Light Source Intenisty

This factor is a constant for both the minimalistic and expanded systems with a value of 3000 µmol.m-2.s-1.

Duration of Excitation

This factor is a constant for both the minimalistic and expanded systems: 0.6 seconds of excitation

Type of Light Source

Fiber optics are not suitable for the needs of this project, as this implementation would be costly and time inefficient. Nevertheless, I would research on laser induced CF and this may be our expanded way of approach.

Frequency of Measurements

The minimum frequency of measurements is every 15 minutes. Any higher frequency, which would not disturb the requirements of the functionality of the whole system would be considered as expanded way of approach.

Duration of Measurements

As this is directly related to power consumption, we might not be able to take measurements throughout the whole day. Therefore, we have defined a period of one to three hours in the middle of the day, when measurements will

be taken. Measurements more than three hours per day would be considered as expanded feature of the system. In the ideal way, we would strive for constant output of data, throughout the whole day.

Type of Data Transmission

Transmission via Wi-Fi, Radio frequency or Bluetooth are our options. The final choice would rely mainly of size of the transmitter, as it has to be directly incorporated onto the PCB.

Storage of Data

This factor is a constant for both the minimalistic and expanded systems. As transmission of big data once per day would require less energy than multiple transmission of small data (since bursts of energy are required every time at the beginning of transmission from the module) we are going to implement an SD card onto the system.

Powering of System

Desired Expanded Model would refer to different way of powering: a centralizedpower source to be connected with each node of the network. This type of system would rely on MSF method. As it is more difficult to implement than the RSF methods (because of the linear decrease of input signal) it would also require more electronics onto the PCB. Moreover, such a system would be considered as a modulated uorometer.

APPENDIX B – CALCULATIONS REGARDING MINUMUM LIGHT SOURCE INTENSITY (LOGBOOK)

What we start with is the intensity of the light, striking the leaf at its surface, which is 3000 micromoles per square meter per second (at least this value; we could of course use higher values – current fluorometers use values up to 12 000 micromoles per square meter per second, but we also strive for lowest power consumption and the proposed value of 3000 micromoles per square meter per second is good enough to drive CFY).

Since light intensity of LEDs (our light source) is given in mcd (micro candelas) we need to transform in the appropriate units. The problem is that transformation from ppf (micromoles per square meter per second) to lux/mcd (light intensity of LEDs in datasheets is given in mcd) requires knowledge on the source of light, as different light sources have possess different energies. This is indeed a problem, as electroluminescence (the type/method of light emitted from LEDs) is not on the list. This raises the question if fluorescent light source would be a better option, as it would possess the required light energy and direct conversion would be much easier. The answer to this question is 'no, fluorescent light source would not be a better option', mainly because of the requirement for a really cheap sensor node. Thus we are left with the problem of transformation between ppf and lux, when the type of light source is electroluminescence. The given light sources vary between 99 and 260 thousand lx for different input light types.

Nevertheless, I can indirectly correlate electroluminescent to incandescent types of light. According to a source a 12W LED uses around 25% of the energy of a 60W incandescent light bulb. Therefore, a 20W LED would use around 25% of the energy of a 100W incandescent light bulb, which would produce 150 000 lx (conversion from 3000 ppf). Therefore a 20W LED would produce 150 000lx/4 = 37 500 lx. Therefore, if we multiply x/20 by 37 500 we would receive the required intensity in lx, where x is the power usage of the LED in W. An RGB LED with power consumption of 0.3 W would produce luminance of about 562.5 lx, which would result in light intensity of the source of 56.2 mcd, for a distance between light source and leaf of 1 cm.

A general formula would be: x/20*37,500*l2 mcd for the intensity of the LED, where x is the power consumption of the LED in W, and l is the distance between the LED and the leaf in meters.

Nevertheless, this calculation takes into consideration device power consumption, not production and because of the fact that the power consumption/power emission ration of LEDs differs for the different types/brands of LEDs, at this point I cannot directly relate/calculate required light intensity of light input source. What I can do is calculate light input intensity for incandescence – thus an electroluminescent light source with the same power consumption would produce greater amounts of energy – therefore the required threshold of 3000 micromoles per square meter per second would be reached.

Therefore, our calculation leads to a light source intensity of 3750 mcd. This leads us to high power single color LEDs.

Apart from LED calibration via the sensor that we are going to use, we have two ways to vary the intensity of the light striking a leaf – distance from the leaf and applied forward current. Therefore, if forward current vs light intensity graph is not presented in the datasheets of the LED we are going to use, I will need to calibrate this as well (produce the graph in order to establish a mathematical relation between light intensity and forward current).

My research in Farnell showed that for Low-Power Single Color LEDs (forward current of about 20-30 mA), the lowest available dominant wavelength is 465 nm – more than we require, but might work.

Since Eduardo proposed the usage of white LED, a difference of +/- 40-50 nm would not be a great issue, in my opinion.

RGB LEDs have different peak wavelengths at different anodes. Therefore, I do not think I can apply the relation between wavelength and RGB values I found. Moreover, Farnell proposes only one RGB LED with a value for the blue anode of 430 nm (the minimum one present), but the total light intensity of this one is around 73 mcd, so this is not enough (this is about Low Current RGB LEDs). High Current RGB LEDS propose a minimum wavelength possible of about 460 nm, so it would not make any sense to take RGB LED and adjust it to 460 nm, instead of simply taking a 460 single color LED. Conclusion: there is not an appropriate RGB LED we can use (I included one in the components list to exemplify what I mean to state).

Sometimes light intensity of LEDs is given in mW/sr instead of mcd.

If we take two points on the graph (30mA for 25 C and 20 ma for 50C), we result into a current/temperature graph equation: $C= -0.4xT + 40$, where T is the surrounding temperature and C is the forward current in the LED. (This formula applies for forward current of 30mA, even though the other tests were made @ 20mA, which is a bit strange for me). There is no evidence that the same relation would apply at different initial forward currents. So, I will finish the calculations with a forward current of 3.5 V @ 30mA. It is important to notice, that in this case the luminous intensity rises up to 1.5 times the typical value, so 1.5x3.1 = 4.65 cd, which is good for us (we will adjust intensity with the distance). I do not take into consideration the ambient temperature/luminous intensity relation, as the output value of luminous intensity does not vary much in the portion of degrees we are interested in (20 – 50 degrees).

APPENDIX C – HISTORY OF CF

If we take a dark-adapted plant (dark adaptation in plants is a technique, which provides a non-stress reference for further analysis – it could be thought as ground in voltage measurements [15]) and induce constant source of light upon its leaves, the phenomenon of CF occurs. The energy of light directly excites an electron from ground state to an excited state in chlorophyll molecules. As already discussed, CF is one of the three paths occurring during de-excitation of the electrons.

Professor Hans Willem Kautsky was the first one to describe the process of CF in 1931, together with A. Hirsh. The method used by Professor Kautsky is also called "fluorescence induction technique". [16] Nevertheless, this observation was performed on a dark-adapted plant, exposed to a constant source of light.

The characteristics changes, caused by the dark-adaptation of a plant have been named 'fluorescence transient' . As the needs of this project do not allow us to previously dark-adapt the plants to be measured (as they are in a field under the direct presence of sunlight) this method of measurement is not suitable. We are going to rely on another type of measurement , which does not require dark adaptation [17].

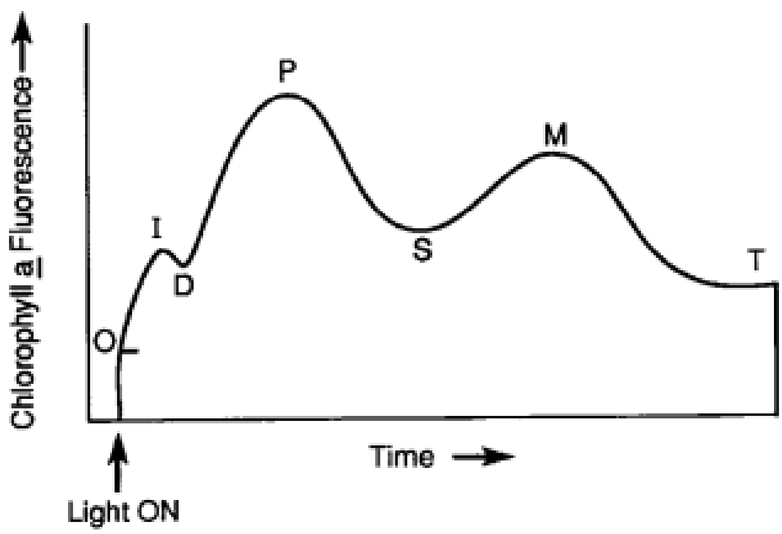


Figure 5 - The fluorescence transient, as described by Kautsky and Hirsh in 1931. The CFY increases from point O to point I, afterwards it decreases to point D, increases to the maximum value of P and starts decreasing again (first wave). The presence of a local maximum in point M is to show the transient response of the system until point T (second wave). If additional waves are transient waves are observed, they are labeled as S1, M1, S2, M2, etc.

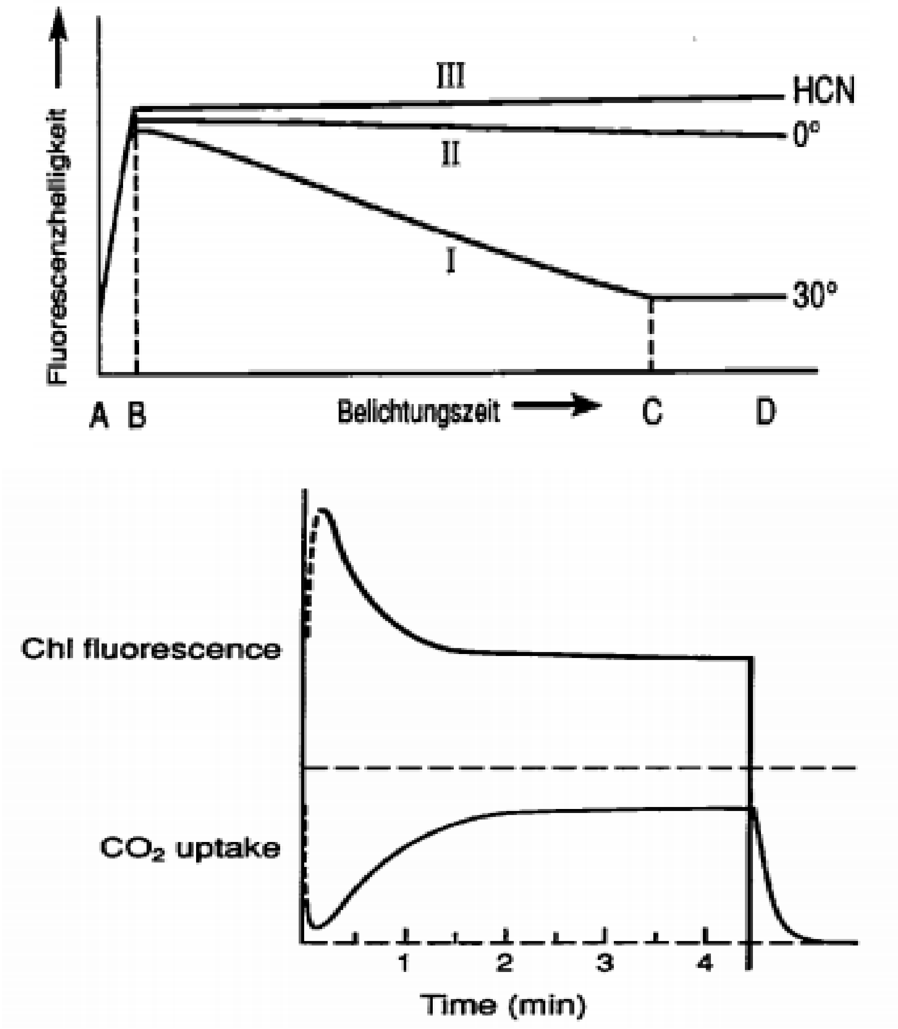


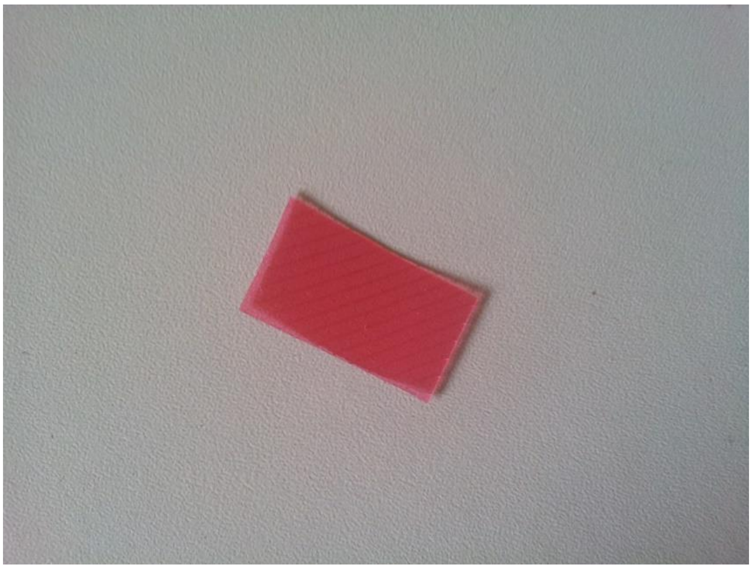
Figure 6 - Top: With their single-page paper [16], Kautsky and Hirsh managed to determine a few basic relations, caused by the phenomenon. Firstly, it was established that CFY increases to a maximum, after which it declines until the steady-state of the system has been achieved. The process occurs in a matter of minutes. Secondly, the repetition of the experiment, with changes in the input parameters showed that no change is observed in the initial rising period of CFY (the corresponding image shows graph of CFY with change in temperature and presence of hydrogen cyanide as poison). Thus, it was deducted that this portion of the graph was considered to be related to the primary reaction of photosynthesis.

Bottom: Kautsky and Hirsh's experiments also proved the anti-parallel relation between Chlorophyll a fluorescence and carbon dioxide uptake. This inverse correlation between CF and photosynthesis was later quantitatively proven by MacAlister and Myers in 1940. [17]

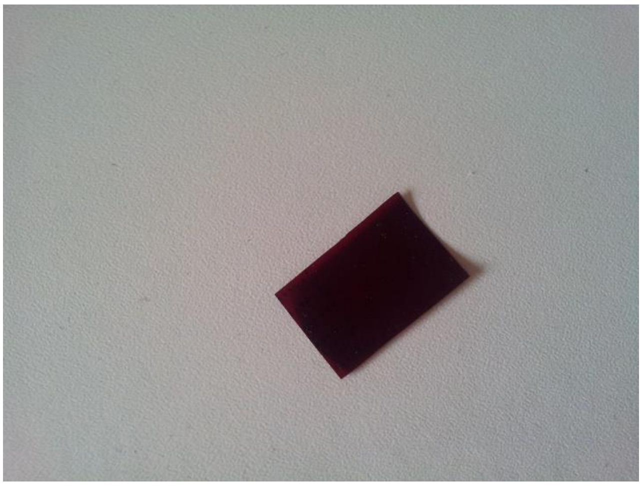
APPENDIX D – BILL OF MATERIALS

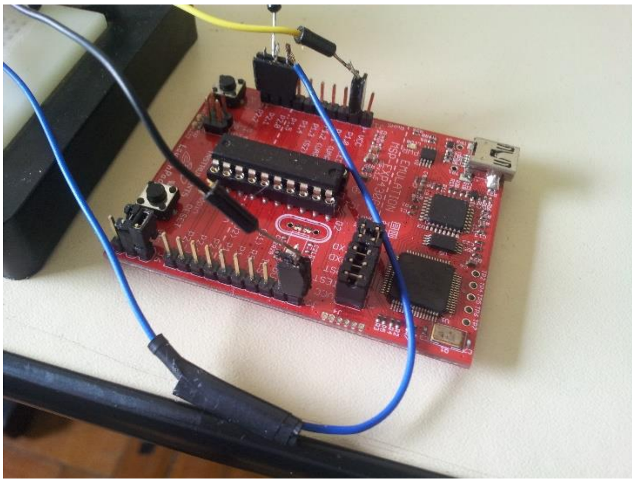
Part	Comment	Designator	Value	P...	Price	Q	Part Num...	M...	Matc...	Part ...	Part N...	Footprint
Capacitor	0.1µF	C4, C7, C8, C9, C10, C11, C12, C13	0.1µF		0.10	8	http://www.digi					CAP-Ceramic-0805
Capacitor	1µF	C5, C6	1µF		0.10	2	http://www.digi					CAP-Ceramic-0805
Connector	LED Connection	P1, P5	2-pin Female AWG22		0.40	2	http://www.digi	http://w	http://www			
Connector	Photodetector Connector	P4, P6			0.54	2	http://www.digi	http://w	http://www			
NMOS Trans	FDV303N	M1, M3		SOT23	0.38	2	http://www.digi					FDV303N-SOT23
Resistor	10K	R3, R10	10K		0.61	2	http://www.digi					RES-PCF-0805-10
Capacitor	10nF	C1	10nF		0.32	1	http://www.digi					CAP-Ceramic-0805
Capacitor	10µF	C3	10µF		0.19	1	http://www.digi					CAP-Ceramic-0805
Capacitor	1nF	C2	1nF		0.29	1	http://www.digi					
Connector	1.27 mm Connector-Female	P2	6-pin-Female		3.02	1	http://www.digi	http://w				
Connector	Power and Data pad	P3	4-pin Female 22AWG		0.39	1	http://www.digi	http://w				
LED	LED-3 types	D1	SMD;THD		1.88	1	http://www.digi			http://www	http://www.	
Microcontroll	MSP430G2553	U1		20-TS	2.59	1	http://www.digi					MSP430G2553
OPAmp	TL3472	U5		SOIC	0.68	1	http://www.digi					TL3472-SOIC
Photodetect	OPT101	U4	Active	8-DIP	7.02	1	http://www.digi					DIP-8
PMOS Trans	FDV304P	M2		SOT23	0.38	1	http://www.digi					FDV304P-SOT23
Reference V	LM4041-SOT23	U6	1.225V-Fixed	SOT23	0.73	1	http://www.digi					LM4041-SOT23
Resistor	13.7	R9	13.7		0.10	1	http://www.digi					
Resistor	1K	R4	1K		0.61	1	http://www.digi					RES-PCF-0805-1K
Resistor	20K	R8	20K		0.61	1	http://www.digi					RES-PCF-0805-20
Resistor	470K	R5	470K		0.10	1	http://www.digi					
Resistor	47K	R1	47K		0.10	1	http://www.digi					
Transceiver	MAX3232	U3		TSSOP	1.87	1	http://www.digi					MAX3232-TSSOP
Voltage Reg	MCP1754ST	U2	5Vin-3.3Vout	SOT-2	0.48	1	http://www.digi					MCP1754ST

APPENDIX E – SETUP

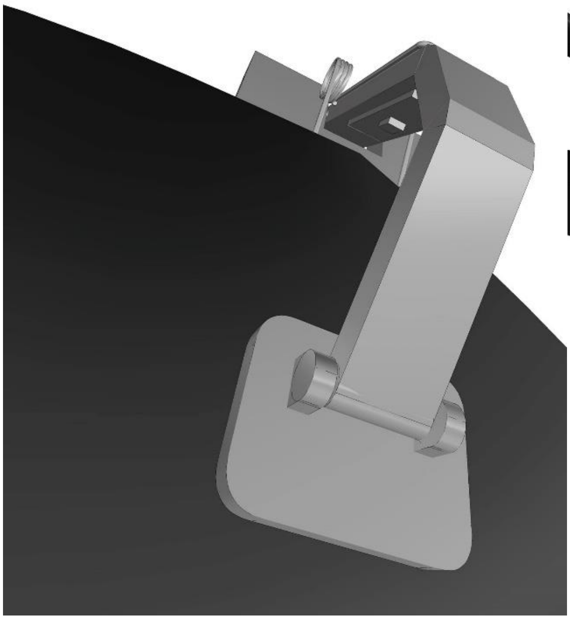


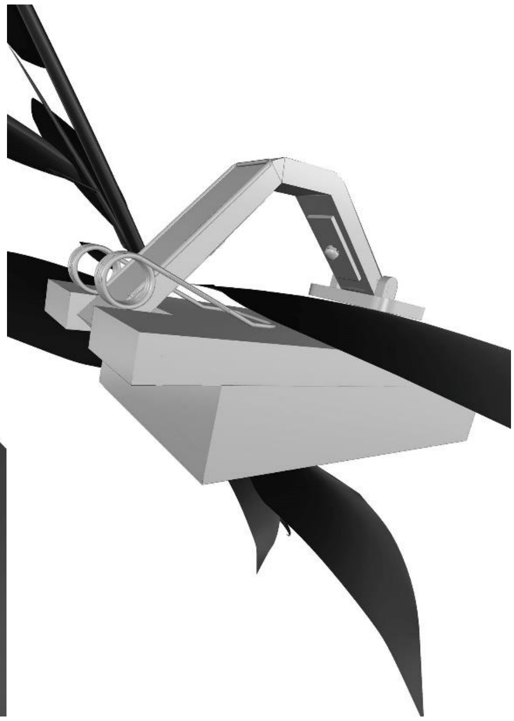

FILTRE DE COULEUR
PRIMAIRE

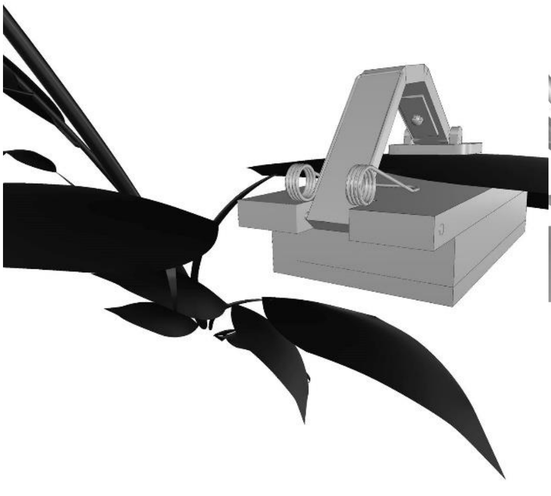


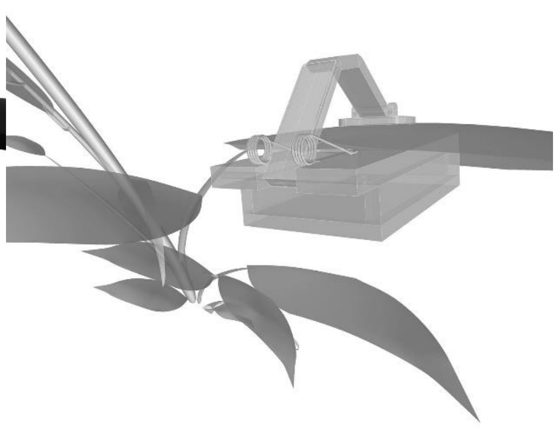


APPENDIX F – MECHANICAL DESIGN









APPENDIX G – SOURCE CODE

Appendix G.1 – Configuration of MSP430G2553

The following code was burned on the MSP430G2553 on LunchPad during validation and tests. The code provides the required sequence of LED light inputs and it makes sure that light intensities are transmitted via Serial port.

```
#include "msp430g2553.h"
#include "msp430g2553_isr.c"

#define LED1 0x01 //PIN 0 ON THE BOARD ON PORT 2
#define LED2 0x02  //PIN 1 ON THE BOARD ON PORT 2
#define PIN_ANALOGIC_READ 0x01 // PIN 0 ON THE BOARD ON PORT 1
#define BUTTON 0x08
void delay(unsigned long ms);
void ledConf();
void led1On();
void led1Off();
void led2On();
void led2Off();
void Timer0_A3_init();
void ADC10_init();
void BCSplus_init();
void USCI_A0_init();
void startConversion();
void stopConversion();
void GPIO_init();

int main( void )
{
  // Stop watchdog timer to prevent time out reset
  WDTCTL = WDTPW + WDTHOLD;
  GPIO_init();//set pins
  BCSplus_init();//set clock
  USCI_A0_init();//set rs232
  ADC10_init();//set adc
  Timer0_A3_init();//set timer
```

```
  ledConf();//set leds
  startConversion();
  while(1){
    if(!(P1IN & BUTTON)){
      led2On();
      delay(600);
      led2Off();
      delay(500);
      led1On();
      delay(600);
      led1Off();
      delay(500);
    }
  }

}
void delay(unsigned long ms)
{
  while(ms--)
  {
    __delay_cycles(1000);
  }
}
void ledConf()
{
  P2DIR |= LED1+LED2;
  P2OUT &= ~(LED1+LED2);
}
void led1On(){
  P2OUT |= LED1;
}
void led1Off()
{
  P2OUT &= ~LED1;
}
void led2On(){
  P2OUT |= LED2;
```

```
}
void led2Off()
{
  P2OUT &= ~LED2;
}
void Timer0_A3_init(void)
{
  TA0CCTL0 = CM_0 + CCIS_0 + OUTMOD_0 + CCIE;
  TA0CCTL1 = CM_0 + CCIS_0 + OUTMOD_7;
  TA0CCR0 = 599;
  TA0CCR1 = 299;
  TA0CTL = TASSEL_1 + ID_0 + MC_1;
}
void ADC10_init(void)
{
  ADC10CTL0 &= ~ENC;
  ADC10CTL0 =  SREF_0 + ADC10SHT_0 + ADC10ON;
  ADC10CTL1 = CONSEQ_0 + ADC10SSEL_0 + ADC10DIV_0 + SHS_0 + INCH_0;
  ADC10AE0 = PIN_ANALOGIC_READ;
  ADC10CTL0 |= ENC;
}
void BCSplus_init(void)
{
  BCSCTL2 = SELM_0 + DIVM_0 + DIVS_0;

  if (CALBC1_1MHZ != 0xFF) {
    DCOCTL = 0x00;
    BCSCTL1 = CALBC1_1MHZ;      /* Set DCO to 1MHz */
    DCOCTL = CALDCO_1MHZ;
  }
  BCSCTL1 |= XT2OFF + DIVA_0;
  BCSCTL3 = XT2S_0 + LFXT1S_2 + XCAP_1;
}
void USCI_A0_init(void)
{

  UCA0CTL1 |= UCSWRST;
```

```
  UCA0CTL1 = UCSSEL_2 + UCSWRST;
  UCA0MCTL = UCBRF_0 + UCBRS_3;
  UCA0BR0 = 208;
  UCA0TXBUF =0;
  UCA0CTL1 &= ~UCSWRST;

}
void startConversion()
{
  TACTL |= MC_1;

  // Enter LPM3 with global interrupts enabled
  __bis_SR_register(GIE);
}
void stopConversion()
{
  __bic_SR_register(GIE);
}
void GPIO_init(void)
{
  P1OUT = 0;
  P1SEL = BIT1 + BIT2 + BIT6;
  P1SEL2 = BIT1 + BIT2;
  P1DIR = BIT6;
  P1IES = 0;
  P1IFG = 0;
  P2IES = 0;
  P2IFG = 0;
  P1REN |= BIT3;
  P1OUT |= BIT3;
}

#include <stdio.h>

#pragma vector=TIMER0_A0_VECTOR
__interrupt void TIMER0_A0_ISR_HOOK(void)
{
```

```
  ADC10CTL0 |= ENC + ADC10SC;
  while ((ADC10CTL0 & ADC10IFG) == 0);
  while (!(IFG2 & UCA0TXIFG)); // Poll TXIFG to until set
    UCA0TXBUF = (ADC10MEM>>8);
  while (!(IFG2 & UCA0TXIFG)); // Poll TXIFG to until set
    UCA0TXBUF =  ADC10MEM;
}
```

Appencix G.2 – Visualization of Results

The following code was used (Processing) in order to successfully translate the two separate bits of data to a valid 10-bit value and write the results on an Excel sheet for visualization purposes.

```
String values=
"";
int final_v;
String[] split_values=values.split(" ");
String[] first_b=new String[split_values.length/2];
String[] second_b=new String[split_values.length/2];
String[] final_values=new String[split_values.length/2];
int first_byte, second_byte;

for (int i=0; i<split_values.length; i+=2) {
  first_byte=int(split_values[i]);
  first_b[i/2]=str(first_byte);
}
for (int i=1; i<split_values.length; i+=2) {
  second_byte=int(split_values[i]);
  second_b[(i-1)/2]=str(second_byte);
}
for (int i=0; i<split_values.length/2; i++) {
  final_v=256*int(first_b[i])+int(second_b[i]);
  println(final_v);
  final_values[i]=str(final_v);
}
saveStrings("Values.csv", final_values);
```