CHRISTOPHER JOHANNES GULL

A NOVEL LOW-COST CHLOROPHYLL FLUORESCENCE SENSOR FOR EARLY DETECTION OF ENVIRONMENTAL POLLUTION

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Ciência da Computação, para obtenção do título de *Magister Scientiae*.

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APROVADA: 03 de julho de 2017.

Davies William de Lima Monteiro

Marcos Augusto Menezes Vieira

Eduardo Gusmão Pereira
(Coorientador)

José Augusto Miranda Nacif
(Orientador)

Dedicated to all those who encouraged me to seek my dreams and who helped me realise them. Also dedicated to those who helped me through the struggles of finding my way in a new country, new culture and new language, and to those who believed in me when I wanted to give up and go home. This dissertation reflects the hardship, the joy and the passion of everything I have learned and experienced in my host country – Brazil.

Do not go where the path may lead.
Go instead where there is no path,
and leave a trail.

(Ralph W. Emerson)

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Biography

Christopher J. Gull, born 郭俊文 on June 30, 1987 in colonial Hong Kong, and adopted by Finnish parents, Tor G. Gull and Inger E. Gull, on November 23, 1991. Grown up in Helsinki, Finland, and Amersfoort, The Netherlands, where he graduated B.Eng. (Hons) in Advanced Sensor Applications at Hanze University of Applied Sciences, Groningen, on July 20, 2012. He holds a patent for an invention while working at Royal Philips Electronics N.V., and quit his job to pursue his Master's degree in Computer Science at *Universidade Federal de Viçosa* in Viçosa, Minas Gerais, Brazil. As a polyglot, he started learning Portuguese, his seventh spoken language, upon commencing his Master's studies in Brazil.

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Abstract

GULL, Christopher Johannes, M.Sc., Universidade Federal de Viçosa, July, 2017. **A Novel Low-Cost Chlorophyll Fluorescence Sensor for Early Detection of Environmental Pollution.** Advisor: José Augusto Miranda Nacif. Co-advisor: Eduardo Gusmão Pereira.

Pollution seriously affects all living organisms as well as economies directly or indirectly relying on natural growth resources. Monitoring the environment for stresses in plants, caused by pollutants, is necessary in order to anticipate and counteract the adverse effects before they manifest as visible damage. Failure to constantly monitor plants results in decreased crop growth, damage to ecosystems, health-related issues, and, ultimately, economic losses. Especially around affected areas, such as waste deposits, mining activities and factories, but also in and around urban areas, it is important to acknowledge the potential environmental issues that may arise from human activities. Among the consequences we find acid rain, heavy metal contamination, surface ozone, changes in temperature, and drought, contributing to alterations in plant physiology, specifically chlorophyll content and photosynthetic efficiency. Measuring plant efficiency, thus health, using commercial fluorometers, such as PAM (pulse-amplitude modulation) devices, presents a challenge, since cost, complexity and the measurement methods make real-time monitoring a difficult proposition. Although such devices are high-precision instruments, they are merely able to provide ‘snapshots’ of small areas. This makes it difficult to understand the health of plants over large areas and over extended periods of time, frequently resulting in actions taken only after significant changes to plants and productivity. One solution would be for a farmer in an area impacted by pollution to acquire multiple of these devices and to employ a workforce dedicated solely to monitoring plant health, but this is costly and inefficient. Another solution would be to simplify the devices with which to measure, and use a multitude of these. Indeed, in this work, we focus on solving this problem, by reducing costs and complexity, and eliminating the need for human input in the measurement process. We propose a system of low-cost chlorophyll fluorescence sensors able to monitor a large number of individual plants at the same time and wirelessly. These sensors have been designed, prototyped and built from the ground up to provide reasonable accuracy, and capacity for discriminating between plants subjected to stress from non-stressed plants. Where our sensor system, the CFY (chlorophyll fluorescence yield) Sensor, lacks in accuracy, it compensates with a multitude of potential simultaneous measurements from an array of sensors within a network. For this reason, the sensor prototype is inherently designed for wireless sensor networks (WSN). Using two species of plants, *Clusia hilariana* and *Paspalum densum*, we have built, tested and verified our methodologies and prototype sensors through a series of experiments. Through these, we observed significant results when employed in an emulated sensor network using one sensor on a large number of plants over extended periods of time. Differentiating the stressed group from the control group

was possible, in addition to rapid and well before any visible damage had manifested on leaves. We conclude that it is indeed possible to not only detect plant stress using low-cost methods, but also to do so automatically and in real-time, allowing for early-detection of pollution and providing e.g. a farmer enough time to resolve problems before they become irreversible and costly.

Resumo

GULL, Christopher Johannes, M.Sc., Universidade Federal de Viçosa, julho de 2017. **Um Sensor Novo de Baixo Custo para Fluorescência da Clorofila para a Detecção Precoce de Poluição Ambiental.** Orientador: José Augusto Miranda Nacif. Coorientador: Eduardo Gusmão Pereira.

A poluição afeta seriamente todos os organismos vivos, assim como economias que dependem, diretamente ou indiretamente, de recursos naturais. O monitoramento ambiental de estresses das plantas, causadas pelos poluentes, é necessário para antecipar e evitar os efeitos negativos antes que se manifestem como danos visíveis. A ausência de monitoramento constante das plantas resulta em diminuição do crescimento das culturas, danos aos ecossistemas, problemas de saúde, e, no fim das contas, perdas econômicas. Especialmente ao redor das áreas afetadas, como, por exemplo, depósitos de resíduos, mineradoras e indústrias, mas também dentro e ao redor das áreas urbanas, é importante reconhecer os problemas potenciais do meio ambiente que possam surgir por causa das atividades humanas. Entre as consequências estão chuva ácida, contaminação de metais pesados, ozônio superficial, modificações de temperatura, e seca, causando alterações na fisiologia vegetal, especificamente na eficiência fotossintética e conteúdo de clorofila. A medição da eficiência fotossintética das plantas, i.e. vitalidade, usando fluorômetros comerciais tais como aparelhos PAM (modulação de amplitude de pulso), torna-se um desafio, uma vez que custo, complexidade e os métodos de medição tornam difíceis o monitoramento em tempo real. Apesar dos aparelhos possuírem alta precisão, podem meramente fornecer uma medição “instantânea” de áreas pequenas. Assim, torna-se difícil o entendimento da vitalidade e eficiência das plantas em grandes áreas e períodos longos, resultando, muitas vezes, que ações sejam tomadas apenas após mudanças significativas nas plantas e na produtividade. Uma solução para o produtor em áreas impactadas por poluentes poderia ser a obtenção de vários desses aparelhos e empregar trabalhadores dedicados exclusivamente para o monitoramento de saúde das plantas, mas isto é caro e ineficiente. Outra solução seria simplificar os equipamentos de medição, e usar vários deles. De fato, neste trabalho nós nos concentramos na resolução deste problema, reduzindo o custo e a complexidade, e eliminando a necessidade de intervenção humana no processo de medição. Propõe-se um sistema de sensores de fluorescência de clorofila de baixo custo que pode monitorar, simultaneamente e sem fios, várias plantas individuais. Estes sensores foram desenvolvidos, prototipados e construídos do zero para dar precisão razoável, com a capacidade de diferenciar entre plantas submetidas à estresse e sem estresse. Nos casos em que o sistema, o Sensor CFY (rendimento fluorescência da clorofila), não possua alta precisão, o sistema compensa em várias medições simultâneas de uma rede de sensores. Isto é, o protótipo do sensor é, inerentemente, desenvolvido para ser usado em redes sem fio (WSN). Usando duas espécies de plantas, *Clusia hilariana* e *Paspalum densum*, foi construído, testado e verificado as nossas metodologias e o nosso protótipo através de uma série de experimentos.

Baseado nisso, foram observado resultados significativos quando utilizamos o sensor em uma rede de sensores emulado, usando um sensor único em várias plantas durante um longo período de tempo. Foi possível a discriminação entre plantas nos grupos de estresse e as do controle, assim como a descoberta rápida bem antes de danos se manifestarem nas folhas. Concluímos que é, de fato, possível a detecção da estresse nas plantas utilizando métodos de baixo custo, assim como fazê-lo automaticamente e em tempo real, permitindo a detecção precoce de poluição e fornecendo, por exemplo, tempo suficiente para um produtor resolver os problemas antes de eles se tornarem irreversíveis e dispendiosos.

1. General Introduction

Monitoring the environment on a large scale is challenging using traditional fluorometers and methods. Due to the inability of properly accounting for environmental pollution, it is a factor contributing to substantial economic losses and health-related problems. Apart from the detrimental effects on human health (Rabl & Spadaro, 2000), research on the effect of pollutants, e.g. surface Ozone, in Europe, Asia and the United States have demonstrated that damage to plants causes increased susceptibility to diseases, decreased growth, and reduced crop and forest yields (Mauzerall & Wang, 2001), in additional to long-term effects on ecosystems (EPA, 2006). Consequently, environmental changes lead to annual losses of tens of billions of dollar in the United States alone (Field *et al.*, 2007; Statista, 2012).

Cost, small-scale and non-real-time measurements are factors that contribute to the difficulty for a user to understand changes and stress factors over time. Especially in areas known to be susceptible to contamination from industries or mining, it is vital to monitor and understand regional and temporal variations. Using commercial fluorometers makes such a task daunting, because they cannot provide real-time monitoring. Other solutions exist, but are less common, experimental and/or cutting edge, making them costlier. Hence, there is an opportunity for innovation using simpler technology and novel techniques to aid e.g. farmers in tasks such as crop area selection.

This dissertation is structured as follows: the introduction summarises our proposed solution, highlighting the contributions of this work. Chapters 2 and 3 constitute two papers that have been submitted, and present details about the evolution of our solution focussed on issues related to complexity, cost, and automation. However, while the chapters have a common goal, the approaches are quite different, with the focus of Chapter 2 merely proving the ability to read two different chlorophyll fluorescence (CF) signals of different intensities, whereas Chapter 3 is based around real-world experiments with statistical significance in mind. Chapter 4 concludes this work and presents future directions.

1.1 General Biology Concepts

All plants use light for energy production through photosynthesis. Light energy is captured in the chlorophyll pigments in leaves, where photochemical reactions use electrons previously elevated to an excited state by photons. While leaves need sunlight for production, an excess in light may cause damage to the organism, which is avoided through dissipating excess energy through a few measurable processes. Among these we find chlorophyll fluorescence (CF), which is the re-emission of energy in the upper red-light spectrum, around 690-735 nm (Lichtenthaler, 1988). CF represents a small percentage of the overall energy used or dissipated, but can be very useful in gauging the photosynthetic activity of a plant.

Kautsky & Hirsch (1931) was the first team to discover the phenomenon of increased levels of CF upon stress in plants. The team used light stress to observe that a plant's activity can be quantified *in vivo* when it is rapidly brought from the dark into the light. Prior to this, plants had been measured *in vitro*, using destructive means, e.g. chemical, to detect problems, such as pollution. The 1931 discovery led to what is termed the Kautsky Effect, which effectively describes a basic relationship between the measurable fluorescence quantum yield (i.e. efficiency in converting light to energy) and electron transport rate. Using modern equipment, it is possible to measure this quantum yield by exciting a leaf with artificial light and measuring the levels of CF. Such equipment normally measures (using red or blue light) the top (adaxial) side of leaves where chlorophyll pigments are in high concentration.

Further details can be found in Sections 2.3 and 3.3.

1.2 Proposed Solution

In this dissertation, we have solved the problems in question using several novel approaches. Not only have we developed hardware capable of measuring chlorophyll fluorescence (CF) at a lower cost, but because of the limitations when faced with non-state-of-the-art technology, we needed to resort to unconventional methods that, while they may initially seem counter-intuitive, solved several problems and even improved on a number of potential issues with traditional methods. Of particular interest is the sensor's ability to not only measure CF, but to also distinguish between the CF emitted from normally functioning plants and from plants suffering from effects of pollution (acid rain). Therefore, the goal of the project has been the low-cost monitoring of environmental pollution that could be useful in an array of situations.

Throughout the project, we have worked on building prototypes, testing them, iterating on them with improvements, and re-testing. In total, we iterated through six prototypes, with a number of sub-iterations. All of them were based around Texas Instruments MSP430-series microcontrollers, primarily the Launchpad versions. The prototyping began with a simple breadboard setup using only a pair of LEDs and some transistors coupled with the sensor. Subsequent prototypes were implemented on prototyping boards. Each sub-iteration presented minor changes to major prototypes to correct or improve on the original idea. The fifth iteration underwent a number of major corrections and improvements before becoming the idea that we finally implemented in the sixth iteration. This prototype was entirely designed in a CAD application, and also underwent a number of sub-iterations. The PCB that we designed was sent off for printing and then manually mounted into a working sensor, complete with a custom 3D-printed casing. The casing was designed and printed externally based on the requirements from our sensor. While still a prototype, this version is ready to be deployed into the field for large-scale testing, bearing in mind the inherent limitations.

We believe that our sensor system compares favourably to other systems and to works by other authors, since our solution achieves similar functionality without the complexity briefly discussed in the following methods. A method by Lichtenthaler & Miehé (1997) used cameras and laser-induced fluorescence to detect CF, while measuring CF in the Fraunhofer lines using hyperspectral imaging was done by Meroni *et al.* (2008). More traditional solutions also exist, such as the well-proven PAM (pulse-amplitude modulation) fluorometers. These are examples of technologies that are used to filter out the background radiation from the CF signal of leaves. Our contribution represents a practical solution to this to avoid the need to compete with the background radiation, avoiding the need for precision instruments and instead focussing on the application, i.e. large-scale and real-time.

Additionally, emerging technologies, similar to our solution, also exist. Wireless sensor networks (WSNs) for 'Smart cultivation management practices' have been in use by Kameoka *et al.* (2017) for several years, using various types of sensors and measuring techniques ranging from X-ray to thermal imaging. The work by the team proves the importance of WSNs in modern monitoring systems.

1.3 A Low-Cost Chlorophyll Fluorescence Sensor System

This paper was submitted to the VI Brazilian Symposium on Computing Systems Engineering (SBESC 2016) as a work-in-progress, where it was accepted and presented in João Pessoa, Paraíba, Brazil. We treated the topic through the view of 'acceptable trade-offs' and 'bare essentials'. We were seeking the best ways to measure CF using much less costly methods compared to common fluorometers, while maintaining the capability of distinguishing two plants with different photosynthetic efficiencies. We also emphasised that the system had to be built using off-the-shelf components, as these are cheap, easy to find and already standardised. Essentially, it was the inherent problems of using non-customised parts that presented the challenges we needed to solve and verify, using means that were not in line with recommended or traditional methodologies. One of the fundamental challenges of measuring CF will always be interference from other light sources. As the CF emitted from a leaf is vastly inferior in intensity than that of e.g. sunlight, methods of filtering out the ambient interference is important.

The solution that we devised to the interference problems was first tested in the prototype covered in the paper, using abaxial-reading measurements, although not explicitly part of the contribution. Prior to this, we had been using a number of other methods, such as keeping the excitation light and sensor on different sides of the leaf. In this version, we had also constructed the first rudimentary leaf holder, using material from a carton packaging. Using this, we designed an experiment to test the hand-built sensor. The experiment revolved around the locally-grown *Clusia hilariana*, which we exposed to acid rain stress to compare CF, hence photosynthetic activity, to that of control plants. These tests were one-off tests, thus with no repetitions or statistical significance. The latter was, however, not the aim, as we only sought to find out the raw capabilities of the system in order to learn about the weak points.

As expected, we had a number of issues with the results, primarily stemming from the rudimentary prototype. However, we were able to produce some conclusions based on the data we obtained. The sensor was capable of recognising the two different states of a plant, termed the dark adapted and light adapted state, in which a plant displays varying degrees of CF depending on the light condition. We observed that light stressed plants were clearly exhibiting a higher fluorescence in its maximum dark-adapted (DA) fluorescence (Fm'), due to lowered photochemical activity resulting in higher proportion of energy being emitted back. Since we used the Mini-PAM for comparison, we were able to conclude that the influence of our chosen stressor, acid (as acid rain is a common problem in nature), is detectable, even with our sensor system.

As a work-in-progress, the main contribution of the first article was, therefore, providing a rudimentary sensor system with which we could study the capabilities of the most important components and hardware. Using this knowledge, we were able to continue researching and improving our solutions and methods.

1.4 Early Detection of Pollution Using a Low-cost Chlorophyll Fluorescence Sensor System

This paper will be submitted to the international journal 'Computers and Electronics in Agriculture'. It is based on experiments with two different revisions of the same prototype concept, where revision A was hand-built and revision B was manufactured. This paper serves to prove the feasibility of the sensor system as a complete solution to autonomous environmental monitoring. We achieved this by using one sensor on multiple plants to simulate a network of sensors.

The sensor systems concept described in this paper is aimed at testing the coherency and the overall capability of providing simple and reliable measurements. This system provides complete wireless autonomy using very low-powered components able to function for weeks on a Li-Po battery. Indeed, the major contribution of this paper, as well as dissertation, was building and verifying a complete sensor node suited for simple deployment in the field, complete with user-end data management software.

Due to the complexity, our sensor system is, in fact, divided into two parts: the sensor itself, and the data handling and wireless IoT node. It was decided that the best approach was to use a very light and small sensor, while keeping all other communication and power modules away from the leaf being measured. As such, we have the CFY Sensor tethered to an IoT module through serial communication, for a stable connection. This also means that a user could be free to choose their own communication platform for use with our CFY Sensor.

Another major contribution of this paper is the official introduction of abaxial-surface measurements. Abaxial leaf surfaces are the inferior part of leaves, i.e. the side of the leaf facing away from sunlight (as opposed to adaxial leaf surfaces facing towards the sun), and is naturally shielded from direct sunlight. In addition to attenuating this radiation, abaxial changes in CF, particularly in thicker leaves, will respond quicker to photosynthetic efficiency changes than in adaxial surfaces. Since our sensor works best with thicker kind of leaves, being able to measure near the stomata makes sure that we can more closely follow a plant's ability to assimilate carbon (Gaastra, 1959), which is a direct response to inefficient photosynthesis and stress. Since our focus lies heavily on being able to detect stress before visual symptoms, it is certainly of added value to be able to measure the immediate responses of plants rather than waiting for the effect of CO_2 variations to reach all parts of a leaf. Although the CFY Sensor receives a lower CF signal with the inherently lower chlorophyll content in abaxial surfaces, we believe that the trade-off is justified. Through the obtained results in the second paper, we discovered that the amount of CF is sufficient for finding discrepancies in stressed and non-stressed plants.

Indeed, the results we obtained from the 9-day long laboratory experiment with our CFY Sensor and with the Mini-PAM revealed that even with our relatively mild stress treatment, a clear difference could be observed with both sensors. In addition to this, we were able to observe a recovery in all stressed plants, measured using both sensors. This is also a proof of the accuracy of the sensor, since we could see both a decline in photosynthetic production as well as a subsequent recovery in the stressed plants.

Statistical analysis also became fundamental at this stage. For a good analysis, the experiment needed statistically relevant data, meaning we carried out experiment on several plants with repetitions. Five plants from the species *Paspalum densum* were chosen for both the stress and control group, totalling ten plants. This species is commonly used in the revegetation of iron mining areas. During the one-and-a-half-week experiment, the plants had already responded to the stress within the first few days. For the obtained data, we carried out Cochran's Q and Bartlett's test for homogeneity of variance, Lilliefors normality test, the Tukey test, and a regression and correlation analysis. We also studied the data graphs, in which clearly significant data points based on the standard error was observed.

The Tukey tests revealed a number of interesting conclusions. Since we had to make two different Tukey tests, due to different amounts of data points between the CFY Sensor and Mini-PAM, the most interesting results stem from the data analysis of the CFY Sensor results. Here, we were able to see significant data within a matter of days after beginning the application of acid rain, and well before any visual symptoms began manifesting. The comparison of statistical significance between the two sensors also confirmed that both measured similar CF variations and with similar significance when comparing treatment and control. While not explicitly a correlation, this does provide a type of a correlation.

The regression and correlation analyses revealed the relationship between the data collected by the Mini-PAM and the CFY Sensor. Since we did not measure adaxial surfaces with our sensor system, we have approximately twice the amount of data points for the Mini-PAM. Therefore, we also obtained two different correlations. Plotting the raw data from the CFY Sensor (abaxial) against that of the adaxial data of the Mini-PAM yielded the highest correlation.

We also did a few preliminary real-life tests with the sensor in the field in order to test the sensor system's capability for continuous measurements in outside a laboratory. Plotting and analysing the data from two plants with different photosynthetic efficiency was conclusive, in that in most cases, the stressed plant exhibited a lower quantum yield (efficiency).

1.5 Contributions

The main contributions of this work can be summarised as follows:

1. Low-cost sensor node prototype for measuring chlorophyll fluorescence in plants, using off-the-shelf materials;
2. Alternative measurement techniques using abaxial leaf surfaces;
3. Sensor nodes enable real-time, remote and autonomous monitoring for large areas and long periods of time, and the use of wireless sensor networks (WSN) for large data, statistics and data mining, aiding in prediction;
4. Early-detection of stress factors caused by environmental pollution, for preventing damage to plants and crops, providing recommendations for action;

2. A Low-Cost Chlorophyll Fluorescence Sensor System

2.1 Abstract

Chlorophyll fluorescence (CF) yield is a well-known method of measuring plant health. Currently, many solutions exist for measuring CF, but the application methods are complex and costs are high. We propose a solution in which we utilise off-the-shelf components in an affordable package, which is able to measure CF. The prototype that was built demonstrates the ability of discriminating between stressed and non-stressed plants, and shows potential as a low-cost fluorometer. As the focus is less on accuracy and more on relative results and cost, this opens up the possibility of using multiple sensors in a wireless sensor network (WSN). In this paper, we explain the underlying methodology and its relation to known methods, and verify the system in its usefulness as a low-cost CF sensor.

2.2 Introduction

Plant health is an important aspect in a broad sense. Whether we consider agriculture or environmental issues, monitoring the conditions are not only useful, but indeed necessary. In the US alone, each year environmental factors cause the loss of tens of billions of dollars in the agricultural sector (Field *et al.*, 2007; Statista, 2012), and much could be improved in this regard. In 1931, Kautsky and Hirsch were the first to outline a non-invasive technique for measuring plant health using chlorophyll fluorescence (CF) (Nickelsen, 2015). Not only can CF be measured without harmful effects, but it is a well-known and well-tested method providing robust monitoring. As such, countless studies have been done on how to best measure the chlorophyll activity (or lack thereof) in a wide range of species and conditions, using many methods of exciting the chlorophyll.

The methods have been utilised in the development of products that quickly and accurately allow a user to gauge the environment through plants, enabling near-immediate actions that can optimise e.g. the efficiency for a farmer. However, existing solutions for CF measurements generally have a few common problems, such as complexity, user-friendliness and cost. As most fluorometers available on the market are based on the deep understanding of plant physiology and its processes, we have products that are able to tell us a wide range of parameters very accurately and precisely. However, this leads to users needing not only a deep understanding of the equipment, but also the physiology of plants, before understanding whether or not there is a problem.

In this paper, we propose an alternative solution. Our objective is to filter out all the “nice to haves” and provide a system in which we are able to quickly, and using low-cost methods, monitor and understand plant health without a deep know-how. This means finding acceptable trade-offs that aim to emulate somewhat the professional systems, but using alternative resources and techniques. As this brings with it its compromises, we need to justify the losses; however, being of a low-cost nature, it also opens up the possibilities for automated monitoring and wireless sensor networks

(WSNs). The working method exploits induced CF, i.e. when an artificial light source is used as a cause of excitation. The amount of reflected light at a certain wavelength is measured, and further analysis reveals the physiological state of the plant. The simplicity and low-cost of the proposed system strive to achieve accuracy in terms of relative presence of stress in plants.

The contribution of this paper is an innovative, environmental, and socially relevant solution in terms of alternative, low-cost methods of stress detection in plants. The result of this is a prototype using off-the-shelf components, demonstrating sufficient discrimination between stressed and non-stressed plants.

This paper is structured as follows: Section 2.3 covers the basics of chlorophyll and its systems that provide the ability to measure CF; Section 2.4 studies related works upon which this paper is based; Section 2.5 describes how we designed and built our prototype sensor; Section 2.6 analyses the results obtained by the prototype; and finally, Section 2.7 concludes this paper.

2.3 Background

In this section, we will cover the important considerations when building a fluorometer. It is vital to understand the mechanics of fluorescence and how these relate to plant stress, so that the designing of the sensor system and interpretation of data are appropriate.

2.3.1 Photosynthesis and Fluorescence

Photosynthesis occurs in various parts of a leaf. Of interest to us are the photosystems and the chlorophyll types. Typically, fluorometers focus on photosystem II (PSII) and chlorophyll *a* (Chl.a), since PSII is the first stage in photosynthesis. Chl.a is present in PSII of all organisms that produce oxygen, and is the dominating pigment (Cambell & Farrell, 2009; Zude, 2009). The pigment is an electron donor, and as such, plays a vital importance in photosynthesis and in the gauging of fluorescence.

Plant leaves absorb light in the visible light spectrum (Harbinson & Rosenqvist, 2003), but of particular importance are the blue and red wavelengths due to the high absorption peaks in those spectra (Lambers *et al.*, 2008). Fluorometers generally exist in two variants: blue and red wavelength excitation. The blue spectrum generally incites a higher fluorescence (Zude, 2009), and as such, it can be beneficial to use the former. Indeed, this is what we focus on in this paper, as we shall discuss in Section 2.5.

Upon light excitation, a leaf will re-emit absorbed light in the red region, generally in the 660-800 nm spectrum, peaking at around 690 nm and 735 nm (Jones & Vaughan, 2010). This relationship between CF and efficiency is approximately inversely proportional (Lichtenthaler, 1992), and is therefore a powerful technique for monitoring plant health (Shultz & Hoffman, 2016).

2.3.2 The Kautsky Effect

In 1960, Kautsky *et al.* (1960) described a mechanism – the Kautsky Effect – where the reaction centre of PSII has a low efficiency due to the reduction of electron acceptors between PSII and PSI. The result of this is the closing of reaction centres whereby the currently excited electrons fall back to ground state and emit energy, among others, as fluorescence, peaking around a second (the other competing factors being heat and the process or photosynthesis). CF will continue until the first electron carrier manages to pass the electron onto the next carrier. During this time, CF gradually falls as PSII becomes increasingly more efficient in two ways. Firstly, the electron carriers become more efficient, causing an increase in electrons that are able to move on to PSI, termed photochemical quenching (PQ). The second factor is the increase in efficiency of heat conversion, termed non-photochemical Quenching (NPQ) (Heinz Walz GmbH, 1999). These factors cause the gradual decrease in CF as the photosystem acclimates (Maxwell & Johnson, 2000). Figure 1 shows a graphical representation of the two adaptation states as well as the four basic parameters that can be measured to calculate efficiency (yield).

2.3.3 Quantum Yield

In the striving for understanding plant health, it is important to understand what quantum yield (QY) means for a plant. Since QY is a direct indicator of a plant's ability to photosynthesise, it means that as long as a plant photosynthesises effectively, the loss as heat or CF should be low. In other words, the re-emission of absorbed light is low when the electron transport is optimal in the electron transport chain (Kautsky *et al.*, 1960).

In the dark-adapted state (DAS), the plastoquinone pool in the electron transport chain re-oxidises, providing a reference point for absolute minimum and maximum CF (Maxwell & Johnson, 2000). This is achieved after tens of minutes devoid of light in the leaf area to be measured (Baker & Ort, 1992). This means that we are able to obtain F_0 (absolute minimum fluorescence) and F_m (absolute maximum fluorescence), from which we can calculate the maximum quantum yield, F_v/F_m, of photosystem II (1).

$$\frac{F_v}{F_m} = \frac{F_m - F_0}{F_m} \tag{1}$$

where $F_v = F_m - F_0$.

Likewise, there is a light-adapted state (LAS), where the maximum CF in the presence of light, F_m', will be lower due to a higher photosynthetic efficiency, and where the steady-state fluorescence, F_s, provides a ground-level for CF in the presence of light. Using above parameters, we can calculate the light-adapted quantum yield, Φ_{PSII} (2).

$$\Phi_{PSII} = \frac{F'_m - F_t}{F'_m} \tag{2}$$

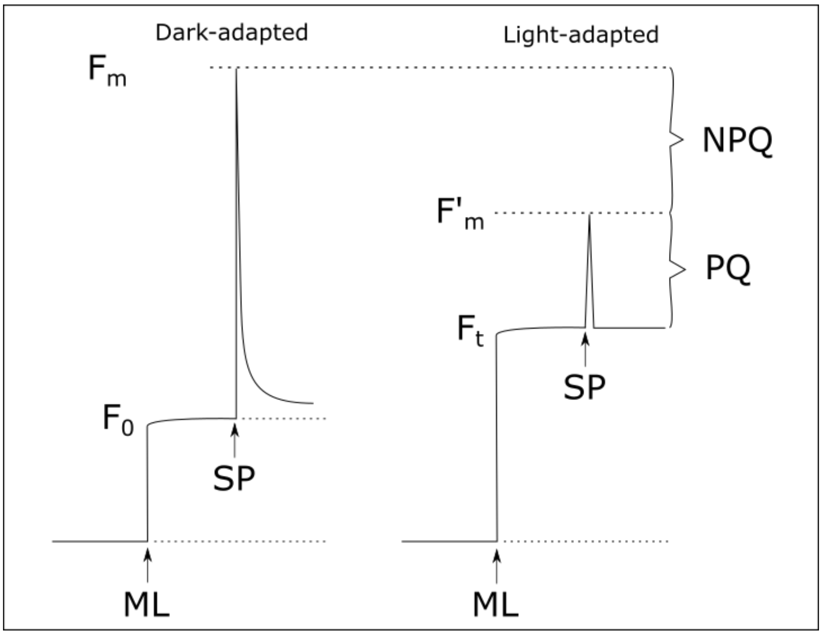

Figure 1: Fluorescence emission in dark and light-adapted leaves. The Kautsky Effect describes a phenomenon whereby the maximum fluorescence in dark (Fm) and maximum fluorescence in light adapted (Fm') leaves will differ vastly upon applying a saturation pulse (SP). The measuring light (ML) is applied to gauge the minimum CF (F0) and steady state CF (Ft). Non-photochemical quenching (NPQ) and photochemical quenching (PQ) are a result of increased PSII efficiency. This diagram was adapted from works by Kautsky et al. (1960), Krause & Weis (1991) and Lichtenthaler & Miehé (1997).

2.4 Related work

The general use for commercial fluorometers is gauging stress in plants. Kancheva *et al.* (2008) describe some typical factors such as heat and heavy metal contamination in the soil, which influence a plant's ability to maintain productivity. The authors used varying concentrations of heavy metals, subsequently measuring the plants' responses through UV light excitation and measurements in the far-red light spectrum. The results manifested as an increase in chlorophyll fluorescence, which is an appropriate response to a decrease in photosynthetic efficiency. Since responses can be measured through CF before external signs manifest, Kancheva *et al.* (2008) used this as early stress detection.

Various methods for exciting the chlorophyll exist. Ultraviolet light is commonly used, as well as infrared and white light. Modern equipment frequently use LED light sources, but some applications also apply ultra-bright Xenon lights (Buschmann & Lichtenthaler, 1998; Papageorgiou & Govindjee, 2004). These methodologies go deep into aspects of plant responses in order to precisely and accurately understand the reactions. Some go so far as to apply varying types of light sources and even varying types of light pulses in order to minimise approximations and over- or underestimations. Loriaux *et al.*, (2013) used such a technique, known as multi-phase flash in order to correct for underestimation of maximum light-adapted quantum yield.

Some studies have also succeeded in correlating photosynthesis and carbon fixation (Edwards & Baker, 1993; Genty *et al.*, 1989), but with the conclusion that this may not always be correct, depending on the situation, making it difficult to universally correlate various parameters with carbon fixation (Fryer *et al.*, 1998).

The differentiation between causes of stress is, however, difficult in some cases. High salinity in water and absence of water, for instance, lead to similar physiological response in a plant, whereas the stresses caused by shading and limitation or excess amounts of water cause considerable differences (Bote & Struik, 2011; Stockle & Dugas, 1992). Thus, the nature of the stress itself can be ambiguous. Furthermore, literature in the field of CF mainly discusses the topic when a single type of stress is present. The effect of multiple types of stress and their influences upon CF is yet to be researched.

2.5 Methodology

In this section, we discuss how we determined the proposed sensor setup, taking into account the basic concepts that need to be considered in the quantification of CF, i.e. light source type, wavelength, duration, and light source intensity (Berg *et al.*, 2002). Here, we also discuss the test setup used in the experiments.

2.5.1 Sensor Setup

Modern industrial fluorometers are able to distinguish characteristics and calculate parameters related to CF beyond simply the determination of quantum yield; however, our focus on low-cost and simplicity strive to narrow down the design of the proposed system exclusively to quantum yield monitoring. In this regard, all decisions taken during the design and construction have meant maximising the potential of the available hardware.

In order to reduce cost, many of the components used are complete packages or embedded that only require a small amount of external components. Also taking into consideration power requirements, we have done research into the best choices for light excitation sources. The following component choices have been made based on certain requirements.

2.5.1.1 LEDs

As the light intensities should be sufficient to saturate the photochemistry, the choice of light source is of particular interest to us. According to Strasser *et al.*, (1988), blue light has a higher excitability on chlorophyll than red or white light, yielding the ability of saturating the system using less power. This tends to shift the emission spectrum somewhat (Lambers *et al.*, 2008), but for our purposes, it is advantageous, as the fluorescence emission spectra becomes broader, making detection of CF easier, particularly with low-cost methods.

Another reason to use blue LEDs is the fact that excitation and emission can be easily separated using low-cost filters. In the event of IR excitation light, the filtering ability is more crucial, requiring precision filters that we cannot afford. In our prototype, we use narrow-wavelength 470 nm Luxeon Rebel blue LEDs (Lumileds, San Jose/CA, USA).

2.5.1.2 Sensor

The sensor – Texas Instruments OPT101 – that we use is a monolithic photodiode containing a transimpedance amplifier. As the package is complete, no external conditioning is essentially necessary in order to function. The spectral response of the sensor ranges from 300 nm to 1100 nm, with its highest response between 700 and 950 nm, and is therefore an optimal sensor for this project, as CF is measured in the red/infrared spectrum.

In addition, photodiodes possess high linearity in its input-output transfer function, which, together with its typically fast response, makes it an appropriate choice for the design of the proposed system (Texas Instruments, 2015). The disadvantage of using a complete package is that it is less flexible.

2.5.1.3 Filters

Filters are necessary, despite the sensor being less sensitive in the blue region. In order to minimise measurement of reflected UV light or light from other undesired light sources, light is filtered through two cut-off filters. These filters are primary red and primary green filters (Paton Hawksley, Bristol, UK), and are used in tandem in order to shape the response curve appropriately.

2.5.1.4 Microcontroller

The UV LEDs and the sensor are both connected to the microcontroller that we use. This device is a low-cost Launchpad MSP430G2553 (Texas Instruments, Dallas/TX, USA), featuring two 16-bit timers and a 10-bit analogue-to-digital converter (ADC). We have programmed one of the timers to pulse-width modulate (PWM) the LEDs in order to control the intensity (necessary for obtaining the various parameters mentioned in Section 2.3). The microcontroller is also responsible for acquiring the signal from the sensor and converting it into values that we can send via serial to a computer for processing and analysis.

The firmware is programmed in C using IAR Embedded Workbench. The timers are programmed to turn the light source on and off, i.e. modulate the light appropriately for the different types of measurements, over the course of sampling. The device is in a low-power state apart from when we take readings, as initiated by a serial connection interrupt. The ADC passes on its data directly to the serial handler, thus we store no values on the device. We send all the raw data to a PC for analysis, and no analysis is done in the microcontroller itself.

2.5.1.5 Software

In order to receive and process the data, we have designed a simple program with a user interface in the C# language. This program receives serial data and converts it into information that we can analyse graphically. The software also commands the microcontroller to execute various types of measurements with predefined sampling times and PWM.

2.5.2 Prototype

The prototype, CFY Sensor, in Figure 2 and Figure 3, is built atop the microcontroller, using prototyping boards and through-hole components (except the LEDs). At the bottom is the microcontroller, on top of which the prototyping shield is mounted, containing the sensor, sensor housing and the transistors. On top of this board are the filters, and at the top, there is another board with the LEDs. This board has a cutout hole to let fluorescence emission reach the sensor, passing through the filters immediately beneath. On this board, a leaf holder has been mounted, which was constructed from cardboard.

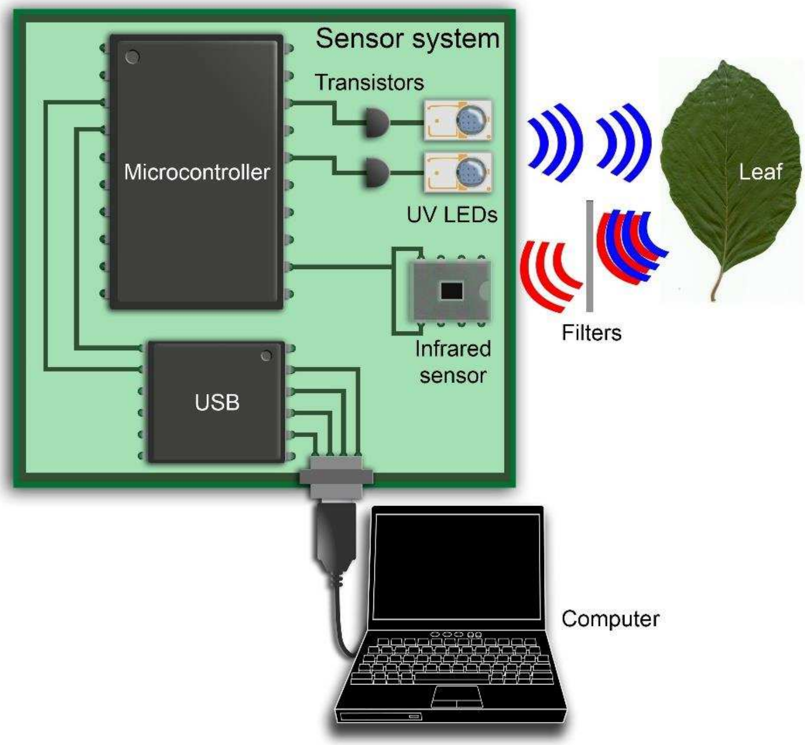

Figure 2: A simplified overview of the chlorophyll fluorescence sensor prototype. In the actual prototype, the microcontroller is located on its own circuit board.

The prototype was built to test leaves for Chlorophyll Fluorescence in a controlled environment, placing leaves directly on top of the sensor setup. The LEDs are PWM driven through two transistors controlled by a timer in the microcontroller, and this timer also synchronises the ADC, such that conversions are done on positive edges of the signal. This means that the light pulses from the LEDs should, in theory, be synchronised with the readings. We do not employ filters on the LEDs themselves, as they are narrow-spectrum light sources, as defined in Section 2.5. However, the filters in use are placed atop the sensor (and below the LEDs) enabling UV light to reach leaves, and emitted

IR light to pass through the filters. In order to shield the sensor from stray-light, there is a small housing built around the sensor, with a small opening right above the sensor.

The microcontroller is powered directly off of the USB bus, and the operational voltage of the entire prototype is 3.3V, as the this is the microcontroller's reference voltage. As such the LEDs are also operated at 3.3V.

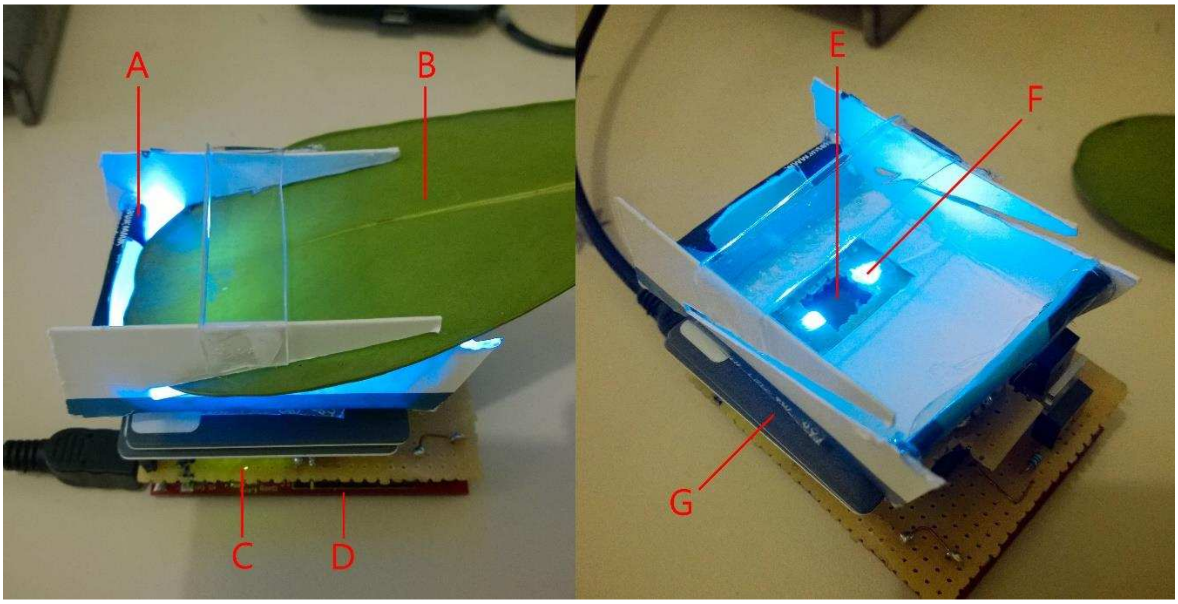

Figure 3: Prototype with and without leaf. (A) Leaf holder. (B) Leaf. (C) Shield with sensor and transistors. (D) Microcontroller board. (E) Opening above sensor for fluorescence from leaf. (F) Blue LED. (G) Optical filters.

2.5.3 Test Setup

We have done two different types of tests using the prototype. These tests both aim to gauge the sensor's viability under laboratory conditions. We have carried out tests on *Clusia hilariana*, a Crassulacean Acid Metabolism (CAM) plant. We have chosen this species due to its sensitivity to environmental changes, which can serve as early stress warning for other plants (Pereira *et al.*, 2008).

An important consideration is the fact that fluorescence manifests similarly in different types of plants, thus individual and physiological characteristics of a plant (such as size, colour, shape, etc.) used for the CFY sensor validation do not significantly influence the outputs (Bolhar *et al.*, 2008).

The saturation pulse (SP) for our system is around 2250 µmol m^{-2} s^{-1}, while the measuring light (ML) for light and dark adapted are about 1200 and 35 µmol m^{-2} s^{-1}, respectively. The saturation pulse is sufficient, as literature has shown intensity as low as 507 µmol m^{-2} s^{-1} to be ample (Kancheva et al., 2008). Many fluorometers have SP intensities of many thousand µmol m^{-2} s^{-1}, but the low-power requirements of the proposed system dictate that we not use such high intensities.

Since the yields we obtain may not be very accurate, the direct comparison to professional equipment is rather limited; however, the aim is not accuracy, but relative comparisons. If, between leaves with different stresses, it is possible to observe a difference in CF, then the sensor works sufficiently well to monitor leaf stress. Hence, the data we obtain does not necessarily reflect the true values we would obtain with professional fluorometers. Indeed, the yield calculations will be very different due to our sensor system's insensitivity to low-level fluorescence measurements, as we shall discuss in the Section 2.6. Nonetheless, we shall first discuss the experimental setup.

2.5.3.1 Sensor verification

In order to test the sensor's viability as a fluorometer, we have carried out tests on leaves in both DAS and LAS. This means subjecting leaves to various lighting conditions in order to obtain the various parameters used for calculating yield. For DAS and LAS, we adapted leaves to the appropriate light conditions for 20 and 10 minutes, respectively, before taking measurements.

The experiments were done in a laboratory under controlled lighting. Both measurements were done using the three different excitation light intensities specified previously. In DAS, we use a measuring light (ML) of very low intensity to obtain a fluorescence that is weakly influenced by the ML (as low as possible, within our sensor's range), whereas the saturation pulse (SP) aims to saturate the photosystem and induce a high level of CF. From this, we can calculate maximum yield using Equation (1). LAS uses the same maximum intensity, but a brighter steady-state light to obtain F_s. From this, we can calculate the DAS yield using Equation (2). A graphical representation of the results can be seen in Figure 4.

2.5.3.2 Stress measurements

The *Clusia hilariana* leaves were subjected to at least 6 hours of exposure to acidic solutions of pH 2.11 and pH 3.45. For both stresses, we used five leaves each, totalling ten leaves. The experiments were carried out in a laboratory in the presence of artificial (fluorescent tube) light. The leaves were tested in succession by inserting the leaves into the fixed leaf holder seen in Figure 3 (A). The excitation time was 1 second, including ML and SP.

2.6 Results

This section analyses the data obtained by the two experimental setups as defined in Section 2.5.

2.6.1 Sensor Verification

When verifying the sensor, we measured CF on the same leaf and without any chemical stress. The results can be seen in Figure 4, and highlight the differences in fluorescence in DAS and LAS. The aim was to test if a significant difference could be observed between the two states using the current setup, and indeed, we can observe a relatively large difference.

It is possible to observe a strong difference between F_m (maximum fluorescence) and F_m' (maximum fluorescence in the presence of light). The quantum yields we observe here relate solely to light-stress. The maximum QY is noticeably higher for the same leaf during the DAS than in LAS, but the latter is not a good true measure for photosynthetic activity. The F_m is expectedly high, and higher than F_m', since the leaf is fully *oxidised* in its dark-adapted state (Baker & Ort, 1992), causing low efficiency (see Section 2.3). The F_0 is very low, as it is only weakly excited by the ML (measuring light).

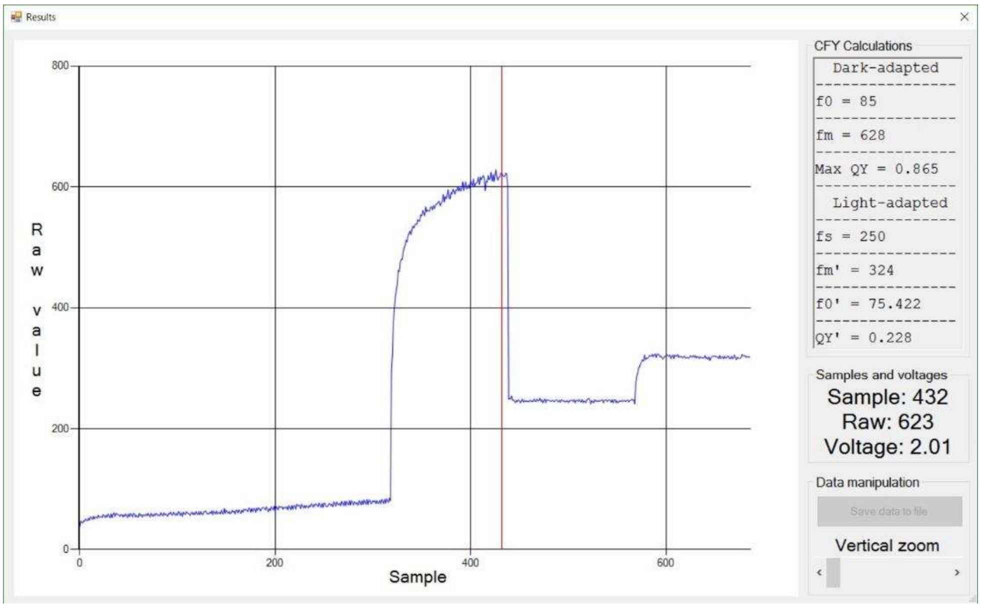

Figure 4: CF plot response from sensor system software on sensor verification on a leaf in both dark (until first peak, around sample 435) and light adapted state. For both measurements, the same saturation pulse is used to obtain Fm and Fm', but to obtain F0 (sample 0~320), a very low-intensity, ideally non-photosynthetically inducing light is used, while Ft (sample 435~570) uses a more intense excitation light. This graph is a typical response of the Kautsky Effect, demonstrating varying degrees of CF at two different light adaptation states of the same plant undergoing brief light stress.

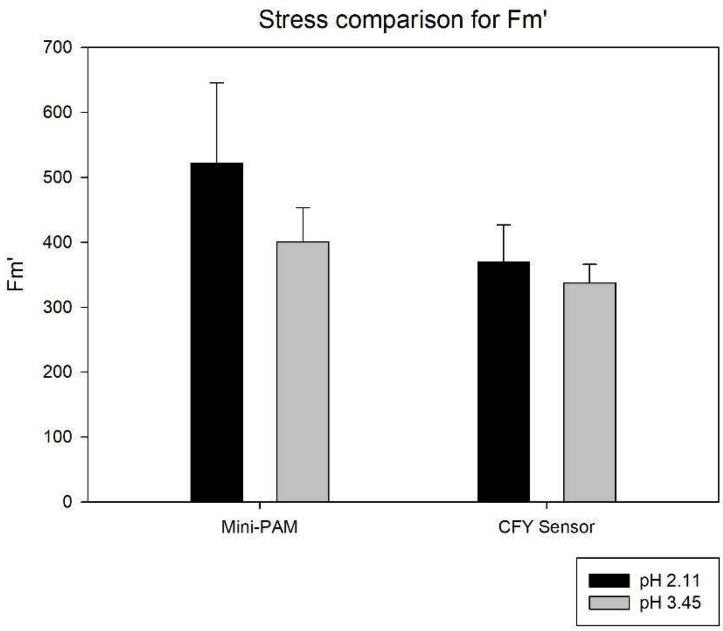

Figure 5: Experimental results from Mini-PAM and CFY Sensor, comparing Fm', the maximum fluorescence in light-adapted leaves, with margin of error. It is possible to observe a higher CF with both devices in the leaves that had been exposed to a more acidic solution (pH 2.11).

2.6.2 Stress Measurements

In Figure 5, we have charted the maximum fluorescence measured with both our benchmark fluorometer, the Mini-PAM (Heinz Walz, Effeltrich, Germany), as well as with our prototype, the CFY Sensor. The reason that we have chosen to analyse F_m' is because it shows the most characteristic changes in relation to stress, as discussed in Section 2.3.3. As can be noted in the comparison graph, in all but one case, our sensor indicates a higher CF (thus, lower efficiency) in the leaves that had been exposed to a more acidic solution. Due to constraints with our sensor, the differences in maximum CF between the stressed and non-stressed leaves is less distinguished than with the Mini-PAM; however, it is still possible to discriminate between them.

2.7 Conclusion

In this paper, we proposed an alternative, low-cost method of measuring chlorophyll fluorescence. As common fluorometers have their strengths and weaknesses, so does our fluorescence sensor. The prototype we built and tested presents a potential leap forward in reducing costs and focussing on the essentials of stress measurements, in such a way that the compromises introduced can be overcome by understanding what aspects of the system is reliable enough in use. Naturally, in this paper, we have worked solely in a laboratory and in controlled environments, but the results we have obtained show promising signs that could be exploited on a large scale in e.g. wireless sensor networks.

The prototype uses off-the-shelf components, featuring near-complete packages that need basic interfacing. The choice of LEDs and sensor was important to this project, as simplicity and power requirements had precedence, as long as it was within functional parameters. Additionally, the choice of filters and methods of inciting fluorescence were also based on previous works and well-known methods used by professional fluorometers.

The results obtained showed positive outcomes in a number of aspects. Firstly, we were able to clearly distinguish a leaf in its dark-adapted state from its light-adapted equivalent. Secondly, the stress tests showed a noticeable decrease in efficiency in the leaves that had been subjected to a more acidic solution. This manifested as an increase in fluorescence, indirectly providing information about the electron transport efficiency, and, therefore, rate of photosynthesis.

Future work involves better signal conditioning and improved methods of inciting fluorescence, in such a way that we get closer to the real values that would normally be obtained by a professional fluorometer. Further studies are also necessary with larger samples. The necessity of additional sensors might also be considered, such as ambient temperature and light sensors, as these factors are known to cause differences in fluorescence depending on, among other, the time of day, temperature and location (shade, direct sunlight) (Bote & Struik, 2011; Heinz Walz GmbH, 1999; Willits & Peet, 2001), and may need to be accounted for using multiple sensor data.

We envision a use case for this type of sensor system, where we employ a network of these over large areas, and monitor both local clusters and a network as a whole constantly and over long periods of time. The data could be statistically analysed and long-term changes observed. Warnings about potential problems could thus be provided, without user intervention.

2.8 Acknowledgments

We thank the research agencies FAPEMIG, CAPES, CNPq and GAPSO for their financial support.

3. Early Detection of Pollution Using a Low-Cost Chlorophyll Fluorescence Sensor System

3.1 Abstract

Environmental pollution is a serious issue that affects all organisms directly or indirectly. Our actions in nature affect everybody, leading to health problems and even the loss of life. Additionally, pollution causes losses in businesses directly relying on natural resources, such as agriculture, with decreased production and increased losses from e.g. acid rain, global warming and heavy metals. The contributions of our work include constructing and verifying a low-cost chlorophyll fluorescence (CF) sensor node capable of early detection of environmental pollution through photosynthetic efficiency using non-destructive and novel measures. Our results indicate that the sensor prototype is capable of autonomously measuring CF, at a fraction of the price and complexity compared to that of commercial equipment. These solutions are typically both expensive and do not operate in real-time, making it difficult to monitor large areas and predict or detect environmental problems. Indeed, stress detection before visual symptoms is a challenge using traditional methods, and we believe that it is possible to automate the processes using low-cost and emerging technologies, as well as large-scale implementation.

Keywords: pollution, sensor, chlorophyll fluorescence, low-cost, real-time

3.2 Introduction

The effects of environmental pollution range from inconvenient to detrimental. The monitoring of the environment is therefore vital to all organisms and ecosystems. As the effects of pollution are detectable before visual symptoms and damage, the possibility of using these as biosensors is an interesting option that has already been used for decades in environmental and agricultural monitoring using various methods and technologies. This allows us to survey a potential or currently used area in order to select or determine its suitability for crops.

Monitoring plant health can be done through non-destructive chlorophyll fluorescence measurements. Kautsky & Hirsch (1931) was the first team to realise that plants show increased fluorescence upon external light stimuli on dark-adapted plants, and that this fluorescence varies with chlorophyll content and photosynthetic activity of a plant. Contrast this with more traditional methods, whereby chlorophyll content measurements done *in vitro* cause irreversible changes, meaning that plants using this destructive method are no longer in their natural state and habitat. Therefore, the team's discovery has been crucial, as it has paved way for all modern equipment that employs non-destructive measuring techniques. Indeed, the technological advancements have resulted in measurement equipment that can monitor plant health and activity rapidly, and *in vivo*.

Today, we have a large assortment of commercial fluorometers at our disposal; however, as with all professional equipment, there can be a steep learning curve, not to mention high costs and complexity (Flexas *et al.*, 2000; Lichtenthaler & Miehé, 1997; Meroni *et al.*, 2008). This can be beneficial in a traditional setup, but as technology advances, our methods for gathering data progresses concomitantly. With the increasingly more capable low-powered hardware, the need for advanced computing systems is becoming less necessary, and with the rapid growth of Internet of Things (IoT), the need for manually monitoring plant health diminishes. Among the challenges that could benefit from using such technology and infrastructure, we find environmental monitoring in urban regions (Lambrechts and Sinha 2016; McLean *et al.* 2016), pollution sensing in industrial areas (Çağrı & Hancke, 2013; Kumar, 2004; Saha *et al.*, 2017), air pollution impacts (Clarke, 1997; Izuta, 2017), automation and precision in agriculture (Barcelo-Ordinas *et al*. 2013; Gutiérrez *et al*. 2014; Lichtenberg *et al.* 2015; Park *et al*. 2011), and other abiotic stress factors (Cohen, Alchanatis, Meron, Saranga, & Tsipris, 2005; Tuteja & Gill, 2016). In all scenarios, the monitoring of environment is generally labour-intensive and expensive, and this presents a challenge that we can face using smarter technology that has become available and economically feasible in the past decade (Hwang & Chen, 2017; Perera, 2017).

The contributions of this paper are providing a low-cost sensor prototype and proving its feasibility. The prototype provides a complete platform, including hardware and software, for doing *in vivo* measurements on plants. Our contributions extend to proving the feasibility of non-conventional methods of measuring chlorophyll fluorescence using abaxial leaf surfaces (underside), with adaxial surface measurements being the traditional method. Hence, this work aims to simplify and automate chlorophyll fluorescence measurements using a low-cost fluorescence sensor system.

The objective of this work is to develop and verify a low-powered sensor node able to monitor environmental pollution through photosynthetic efficiency using chlorophyll fluorescence detection. Bearing in mind the low-cost factor, our solution does, by design, forego the cutting-edge technology that can be found in commercial equipment. However, our goal is to test and verify that the sensor node can sufficiently differentiate between plant affected and not affected by stress, such as pollution, and to do so early on before this begins to manifest in the physiology of plants. To that end, we performed experiments comparing and verifying the capabilities of our prototype wireless fluorescence sensor, the ‘CFY Sensor’ (chlorophyll fluorescence yield sensor), against a commercial fluorometer, the Mini-PAM (Heinz Walz, Effeltrich, Germany). Our experiments on live *Paspalum densum* plants, using acid rain simulation as the stressor, revealed, through statistical analysis, that our sensor has potential in stress detection.

This paper is structured as follows: Section 3.2 introduces the paper; Section 3.3 outlines the background necessary for understanding chlorophyll fluorescence and its role in measuring plant health; Section 3.4 covers the design and functionality of our prototype sensor; Section 3.5 explains the experimental setup; In Section 3.6, we analyse the results obtained by both sensors; In Section 3.7, we outline a general algorithm for detection stress based on provided data; In Section 3.8, we discuss the significance of our results; and finally, Section 3.9 concludes this paper.

3.3 Background

In this section, we present the underlying mechanics of plant physiology. As chlorophyll fluorescence is based on a non-destructive probing, we expect externally observable responses. Indeed, chlorophyll fluorescence (CF) has long been a preferred and accurate method for monitoring plant health.

3.3.1 Photosynthesis and Fluorescence

Plants absorb light throughout the photosynthetically active radiation (PAR) spectrum (400-700 nm) (Jones & Vaughan, 2010). However, the blue and red spectra are mainly used in photosynthesis, while green is reflected, as plants exhibit absorption maxima in the 430 and 660 nm region (Walker, 1992). Of particular importance is the lower-end of the light spectrum, where wavelengths are short and carry a high amount of energy that can cause energy excess.

Fluorescence occurs mainly in the chlorophyll *a* (chl.a.) pigments of photosystem II (PSII). As it is the dominant pigment, important to oxygen-producing organisms in general, its responses are a useful measure of plant activity and health. At any given point, plants will always emit a small amount of fluorescence peaking at around 690 nm and 735 nm (Lichtenthaler, 1988) and at around 0.6-3% of the absorbed light (Krause & Weis, 1991) due to a sub-100% light quanta utilisation (Björkman & Demmig, 1987). This can become affected during stress, as we shall outline in the following subsections. The approximately inversely proportional relationship between CF and photosynthetic efficiency (CO_2 assimilation) can then be exploited for non-invasive measurements of physiological condition (Lichtenthaler, 1992; Papageorgiou & Govindjee, 2004).

3.3.2 The Kautsky Effect

Kautsky & Hirsch (1931) were the first to describe that plants displayed an increased fluorescence level upon being dark-adapted followed by intense light irradiation. Later, Kautsky *et al.* (1960) was also the first team to outline the basic relationship between electron transport rate and fluorescence yield.

Termed the Kautsky Effect, the phenomenon stems from the chlorophyll *a* (Chl.a) pigments in photosystem II. This response was proven to be a defence mechanism in response to light-induced oxidative damage. CF is thus a result of the reduction of electron acceptors between photosystems II and I due to closed PSII reaction centres. This causes Chl.a pigments, previously elevated to their singlet excited state, Chl.a*, to fall back to ground state, since there is an excess in absorbed energy that cannot be used in photosynthesis or sufficiently dissipated by heat, and may therefore cause damage to leaves. Quenching, which occurs in competing photochemical (qP) and non-photochemical (NPQ) forms, is responsible for reducing the amount of fluorescence as a leaf gradually becomes more photosynthetically efficient through increased electron transport rate (ETR). Therefore, in a healthy leaf, the amount of qP and NPQ gradually causes a decrease in CF as a plant adapts to light, but in stressed or damaged plants, this behaviour will deviate significantly. This difference can be quantified using light pulses to briefly saturate the photochemistry (Müller *et al.* 2001), as further elaborated upon in the next subsection. Figure 6 demonstrates the Kautsky Effect, i.e. the varying intensities of CF in dark-adapted (DA) and light-adapted (LA) leaves, upon applying a measuring light (ML) and a light-stress saturating pulse (SP), as well as shows the theoretical estimations of quenching.

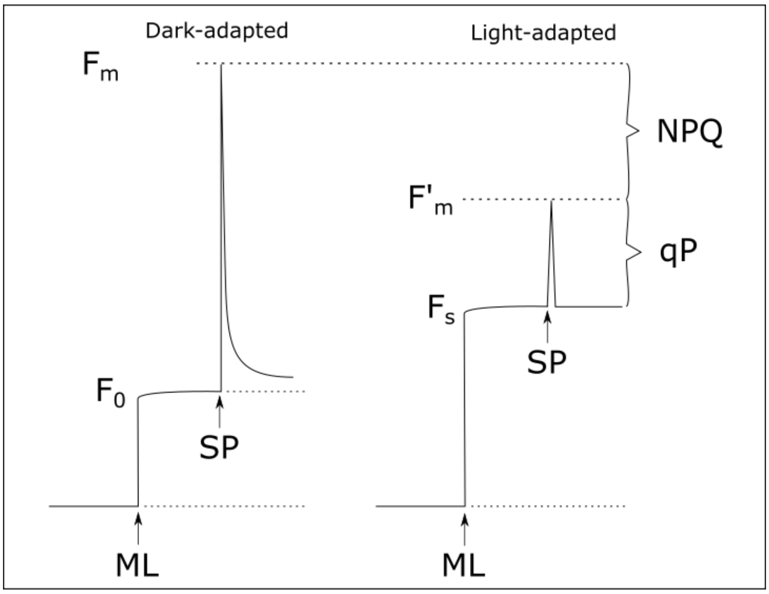

Figure 6: The Kautsky Effect demonstrated through dark and light-adapted leaf fluorescence emission. Leaves adapted to the light conditions will exhibit different responses to light pulses than that of dark-adapted leaves. The saturation pulse (SP) is applied briefly to both types of measurements in order to obtain Fm and Fm', hence separating NPQ (non-photochemical quenching) and qP (photochemical quenching). The measuring light (ML) measures F0 and Fs. This diagram was adapted from works by Kautsky et al. (1960), Krause & Weis (1991) and Lichtenthaler & Miehé (1997).

3.3.3 Gauging Efficiency

The plant responses will vary depending on the photosynthetic activity. The various adaptation states and light-induced reactions can be described through the quantum yield (QY). This is calculated separately for dark and light-adapted leaves. As a plant's ability to photosynthesise effectively is reflected in the yield, we can quickly and with simple measurements understand a plant's current condition. This means that with efficient photosynthesis, a plant emits low amounts of fluorescence, and QY reflects the proportion of light absorbed by the chlorophyll in PSII, from which the electron transport rate can be calculated (Maxwell & Johnson, 2000).

As there are two competing factors in the process of quenching, we need to eliminate one of these factors while minimally influencing the other. Bradbury and Baker (1981) and Quick and

Horton (1984) devised a method for transiently eliminating the effect of photochemical quenching on CF, so that fluorescence due to only one factor can be measured. This method relies on eliminating one factor (qP), and is important for *in vivo* measurements, as photochemically reducing chemicals (e.g. herbicides) are unfeasible in non-destructive measurements. From this, we can obtain various fluorescence parameters.

In the DA state, we are able to measure the absolute minimum and maximum CF. The minimum CF, F_0, is the base-level fluorescence of the plant in complete absence of actinic light. The maximum CF, F_m, is the fluorescence in the complete absence of photochemical quenching, i.e. no photosynthesis. This re-oxidation of the plastoquinone pool in the electron transport chain occurs after 10-20 minutes in the absence of light. The result is that when a saturating light occurs, we obtain the potential maximum quantum efficiency, F_v/F_m, i.e. resulting from only changes in NQP efficiency (Kitajima & Butler, 1975). This value decreases upon exposure to stress. Based on obtained fluorescence, we can calculate the maximum quantum yield of a leaf or plant using Equation (1).

$$\frac{F_v}{F_m} = \frac{F_m - F_0}{F_m} \tag{1}$$

where $F_v = F_m - F_0$.

In the LA state, we obtain F_s, or the steady-state CF, while F_m' represents the maximum fluorescence, both in the presence of light. F_m' will be lower than F_m due to increased levels of NPQ and qP through increased PSII efficiency (see Figure 6), while F_s will be higher than F_0 due to a higher number of closed PSII reaction centres. From the measurements, we can calculate the effective PSII quantum yield in LA state ($\Phi_{PSII} = \Delta F/F_m$') (Genty *et al.* 1989) using Equation (2).

$$\Phi_{PSII} = \frac{F'_m - F_t}{F'_m} \tag{2}$$

Furthermore, it is possible to calculate the qP, or the fraction of open reaction centres upon strong light excitation, using the DA parameters and the Oxborough-Baker approximation. However, this is out of the scope of our research; for further information, refer to the works of Baker and Oxborough (1997) and Baker and Oxborough (2004).

3.4 Prototype Development & Design

The entire sensor system is based around microprocessors from Texas Instruments. We chose these due to their relative ease-of-use while maintaining access to low-level features, as well as low-cost. We use two different platforms: one for the sensor, and one for the wireless node. We do this because the sensor itself will be attached to leaves and needs to be very small and light. The other microprocessor, found in the IoT module, is more complex, and is housed in a separate unit at the base of the plant. This unit also contains the Li-Po battery to power both platforms, and could be extended to include energy-harvesting devices. We utilise serial communication between the two platforms.

3.4.1 CFY Sensor

Our CFY Sensor, which is responsible for the measurements, is based around a Texas Instruments microcontroller, with a sensor, a pair of high-intensity LEDs, an OPAMP, voltage regulators, LED driver and optical filters; listed below. It should be noted that two revisions exist, Rev. A and Rev. B, of which the former was hand-built on a prototyping board with a wooden casing, while the latter is a prototype designed and printed on a PCB with a 3D-printed plastic casing. Both revisions use the same principle and equivalent components, with the exception of the LED driver, which drives six LEDs in Rev. A, while only two LEDs in Rev. B (using a higher current); see Figure 7 for a comparison.

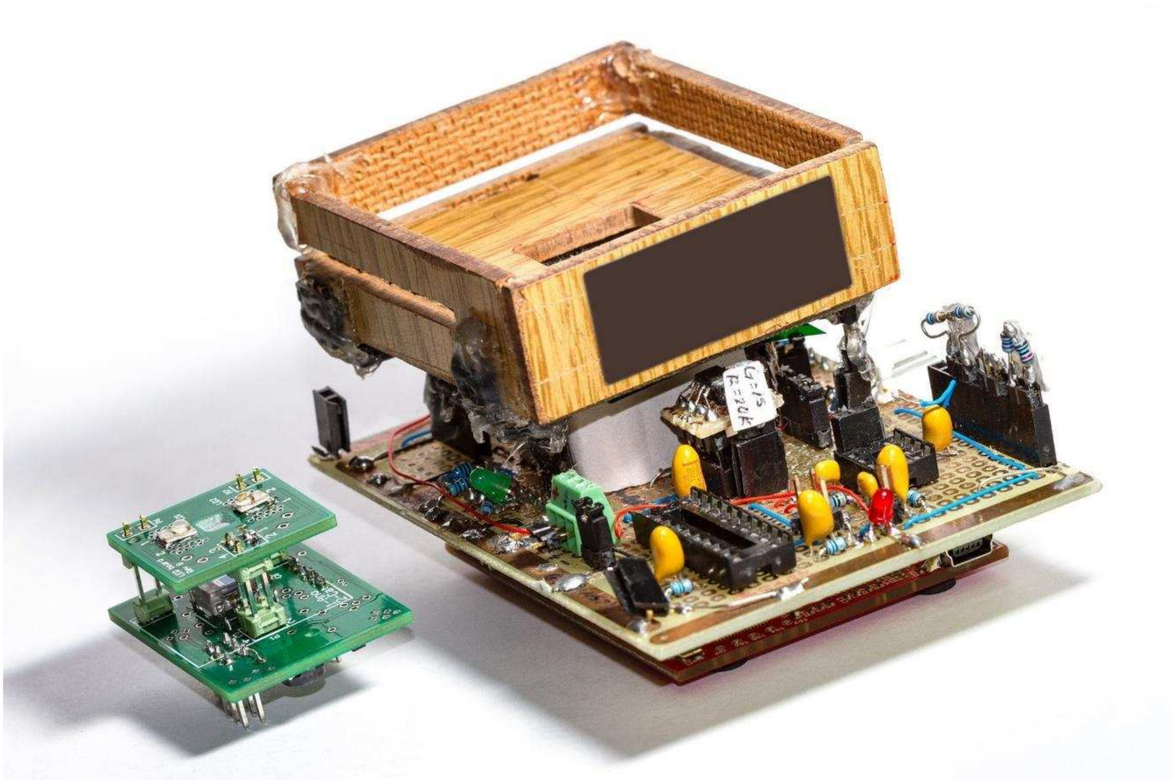

Figure 7: CFY Sensor Rev. A (right) and Rev. B. (without casing and filters).

3.4.1.1 Microcontroller

The Texas Instruments 16-bit, 16MHz MSP430G2553 is a microcontroller with two user-configurable timers, interrupts, 10-bit ADC and PWM capability, among other. PWM allows us to vary the average light intensity of the LEDs, and to pulse-amplitude modulate the light in an attempt at differentiating background radiation from fluorescence signals.

3.4.1.2 LEDs

We use LEDs of type Luxeon Rebel Blue (1A max current), since the blue spectrum is more energetic than the traditionally used red spectrum, allowing for lower-powered LEDs using a higher-energy wavelength (Zude, 2009) (as further explained in Section 3.5).

In Rev. A, we use six LEDs limited to 20mA each.

In Rev. B, we use a pair of LEDs limited to 75mA each.

3.4.1.3 Sensor

The sensor is a Texas Instruments OPT101 monolithic photodiode package containing a transimpedance amplifier. The sensor's spectral response ranges from 300 nm to 1100 nm, with peak response between 700 and 950 nm, which is within the wavelength that we focus on for the CFY Sensor (see below).

3.4.1.4 Optical filters

Three different filters are used in the sensor system: primary red, primary green (both by Paton Hawksley) and a heat absorbing filter (Schott KG1 in Rev. A, and KG3 in Rev. B due to improved heat absorption). In Figure 8 is a graph with all three filter responses, forming a type of bandpass filter in the upper red spectrum. Naturally, overall transmission will be lower with the number stacked of filters.

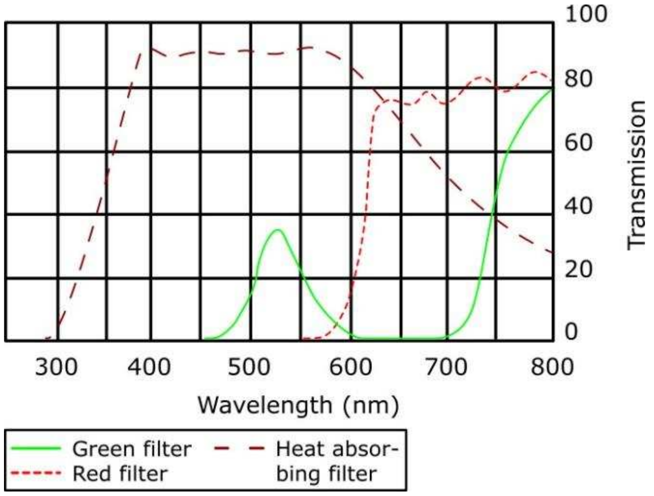

Figure 8: Three filters superimposed to form a bandpass filter at around 700-750. The heat-absorbing filter curve represents the transmission characteristics for Schott KG3.

3.4.1.5 LED driver

Rev. A uses a CAT4238 high efficiency 10 LED boost converter for driving six LEDs in series at a maximum of 30mA per LED, directly controlled through PWM.

Rev. B uses a Texas Instruments LM3405 constant current buck regulator for LEDs, with a total maximum output of 1A. The current can be directly controlled by PWM. The LED driver is used with two LEDs in parallel.

3.4.1.6 Voltage regulators

The sensor system uses two 150mA Texas Instruments LP3985 ultra-low dropout voltage regulators. One is used exclusively for the LED driver, while the other feeds the rest of the system. In the prototypes, the LED current is limited by the voltage regulators.

In Figure 9 is a high-level overview of the complete sensor system concept, and in Figure 10 is a photograph of Rev. B sensor system prototype. See Appendix A for a detailed overview of the electronic circuit.

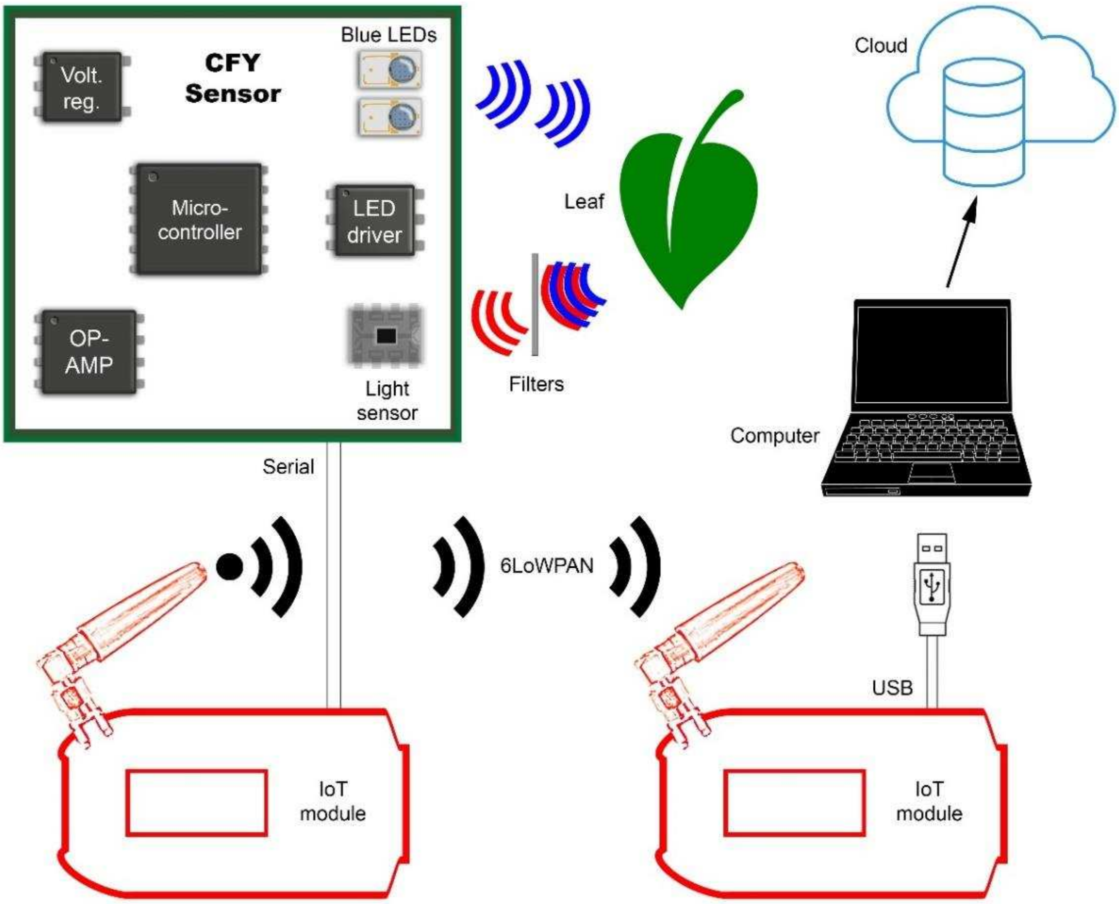

Figure 9: A simplified overview of the setup concept. The sensor system is completely self-contained and only requires power and serial communication with an IoT (internet of things) module. The module is responsible for triggering periodic measurements, as well as transmitting this data to an off-site location, where another IoT module transfers data to a PC over USB. The data can then be processed and stored in the cloud for access by other devices.

The sensor system is programmed to carry out various types of measurements with varying frequencies, PWM duty cycles and duration. Since we need to determine the quantum yield in both dark-adapted and light-adapted leaves, the sensor needs to be programmed accordingly. Table 1 outlines the various parameters.

Table 1: Frequency and timing parameters for sensor. These parameters were configured in the microcontroller to achieve the desired combination of excitation light intensities and pulse durations. The duration is based on standard measurement timings of common fluorometers. The rest of the parameters were obtained through testing using a professional photo/radiometer (Delta Ohm HD2102.2, Caselle di Selvazzano, Italy).

Measurement	Frequency	Duty cycle	Pulse duration (µs)	Duration (ms)
F_0	100 Hz	1%	100	750
F_m, F_m'	4 KHz	80%	200	750
F_s	400 Hz	8%	200	40,000 + 750*

**Steady-state CF is first pre-adapted with a constant 40-second light pulse, and then measured over 750 ms.*

All of the parameters have been adjusted to maintain a 100 or 200 µs pulse for all types of measurements. This is 1-2 orders of magnitude higher than what a commercial PAM fluorometer uses (typically less than 10 µs), but as we use low-cost components, we are limited by what both the microcontroller and IC components are capable of. While commercial PAM fluorometers use very short pulses and high frequencies to achieve modulation and saturation light, we use lower frequencies and longer pulses to reach a similar result. Since this results in the loss of modulation efficacy, we have devised an alternative method of measuring the fluorescence that minimises the influence of background radiation, as described in Section 3.4.4.

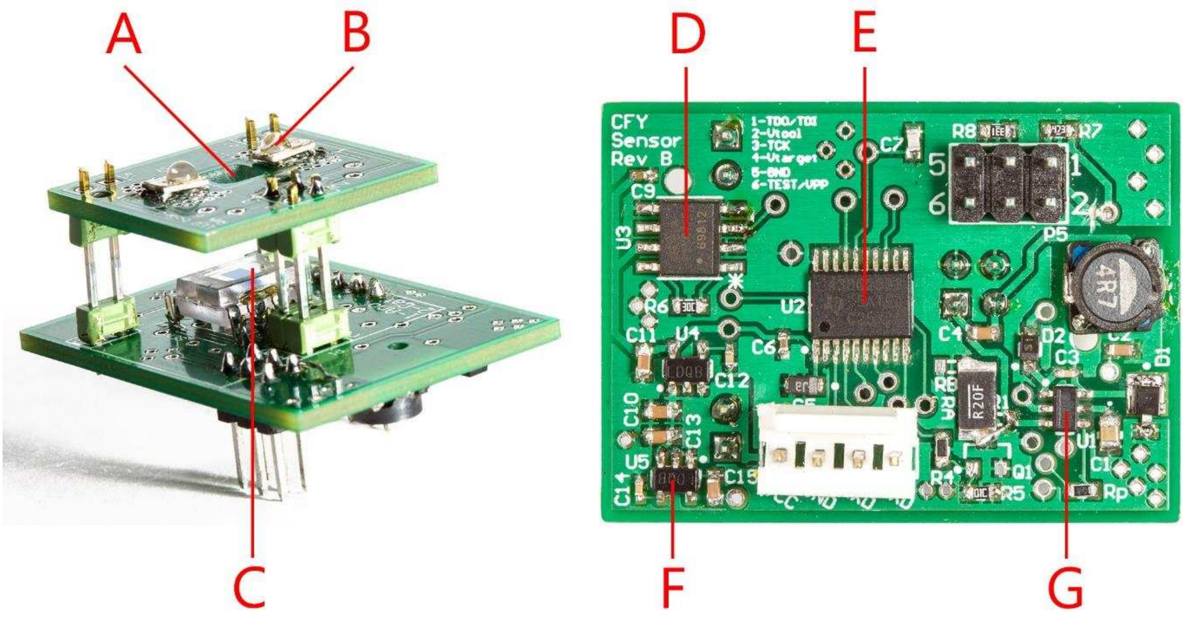

Figure 10: CFY Sensor prototype Rev. B without casing and optical filters. The design is stacked to allow sensor and LEDs to be separated vertically by optical filters. Note that Rev. A is essentially identical, but unoptimised. (A) PCB cutout opening above sensor for received CF from leaf. (B) Blue LED. (C) Sensor. (D) Amplifier. (E) MSP430G2553 microcontroller. (F) voltage regulator. (G) LED driver.

3.4.2 IoT Platform

The data collected by the sensor is communicated via serial to the IoT (internet of things) module. This platform contains an ARM Cortex-M3 based TI CC2538 system-on-chip (SoC), with a 12-bit ADC, 2.4GHz and sub-GHz IPv6-compatible radio, real time clock, among other. This platform uses the open-source Contiki OS for IoT, utilising the C language. As this is a complete platform, all communication and protocols are managed by the device as a black-box, and we are able to take advantage of APIs (application protocol interfaces) to communicate over 6LoWPAN (IPv6 over low power wireless personal area network), as well as create mesh networks for use in wireless sensor networks (WSN). This is of particular importance, as WSNs typically cover large areas where many sensors are unable to directly communicate with a sink node or host. However, for our prototype, we have not implemented this, as we have been testing on a limited scale.

The data received from the sensor is sent to the sink node over UDP (user datagram protocol) and broadcasting protocols. As such, for testing, we have not implemented mesh networking nor advanced features such as TCP or cryptosecurity. Such features are relatively easy to add or update in future improvements. However, we do employ data management, real-time clock, interrupts, voltage and temperature sensors, timeout timers, and management of the radios. In the next subsection, we outline the finite state machine that has been employed in order to take a measurement, process and transmit the data.

3.4.3 Sensor System Behaviour

In this section, we describe the finite state machine (FSM) that is used to control the system behaviour of a sensor node. Figure 11 shows an FSM diagram.

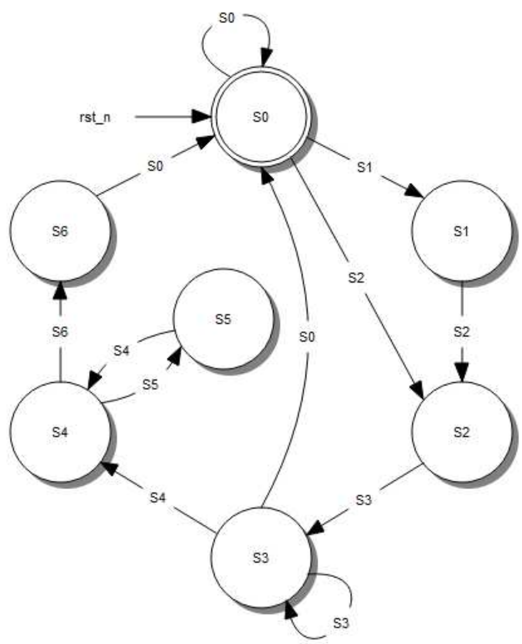

Figure 11: Finite state machine diagram of the sensor system.

3.4.3.1 Standby

State S0 is the low-powered mode where no measurements and no data transmissions take place. No processes are running except for periodical radio activity checking (at 8 Hz). The system waits for an interrupt from radio, timer or button. Consequently, this is also the final state after a measurement.

3.4.3.2 Measurements

States S1-S3 are responsible taking a measurement, depending on what command was received either over radio (from master) or from its own timer interrupt. If timer interrupt was fired, we need to check the time (S1); if $18 < t < 6$, then notify S2 to take F_v/F_m measurements; else, notify S2 to take Φ_{PSII} measurements. If we received a command over radio, then relay this directly to CFY Sensor (skip S1), given that it is a valid command. During the measurement (S3) by the CFY Sensor, the IoT module will stay awake and wait for data from CFY Sensor, or timeout and go back to sleep if sensor is disconnected or faulty.

3.4.3.3 Save and transmit data

The final states (S4-S6) are responsible for storing and transmitting data. The data from CFY Sensor transmits, over serial, to the IoT module, which, subsequently, stores the data in flash memory, if applicable, and transmits the data wirelessly over 6LoWPAN (IPv6 over IEEE 802.15.4) to master or sink node.

3.4.4 Abaxial CF Measurements

Since background radiation is much larger than the fluorescence signal we can obtain from leaves, one must resort to PAM, other advanced methods, or find new solutions. We propose using abaxial surface CF measurements, i.e. the underside of leaves, facing away from sunlight, instead of adaxial surfaces directly exposed to sunlight. This method has not been applied before, as leaves generally contain a lower concentration of chlorophyll in abaxial surfaces, resulting in lower CF during measurements compared to that of adaxial surfaces. However, this is an optimal solution for our purposes, as it allows us to mount the sensor upside-down, i.e. on the underside of the leaf and away from excess light that interferes with measurements. This can be achieved using a small chamber that is open at the top. The chamber attaches to leaves, leaving the top part of leaves exposed to natural light, while the bottom part of the leaf (partially) shields the sensor. The chamber houses all the necessary electronics for measuring abaxial CF; see Figure 12 for a CAD model of the sensor and its housing.

Abaxial measurements also provide a number of benefits, especially in thicker leaves. As the stomata are located in these surfaces, CO_2 is initially absorbed by the neighbouring chlorophyll before reaching chlorophyll closer to adaxial surfaces (Gaastra, 1959). This means that abaxial chlorophyll responds faster to stresses induced by e.g. pollutants, as we are more closely coupling biochemical and light capturing efficiency. As a result of our solution, stress symptoms become detectable at an earlier stage while the background radiation is attenuated. We therefore believe that abaxial measurements show real potential within our scope for autonomous monitoring.

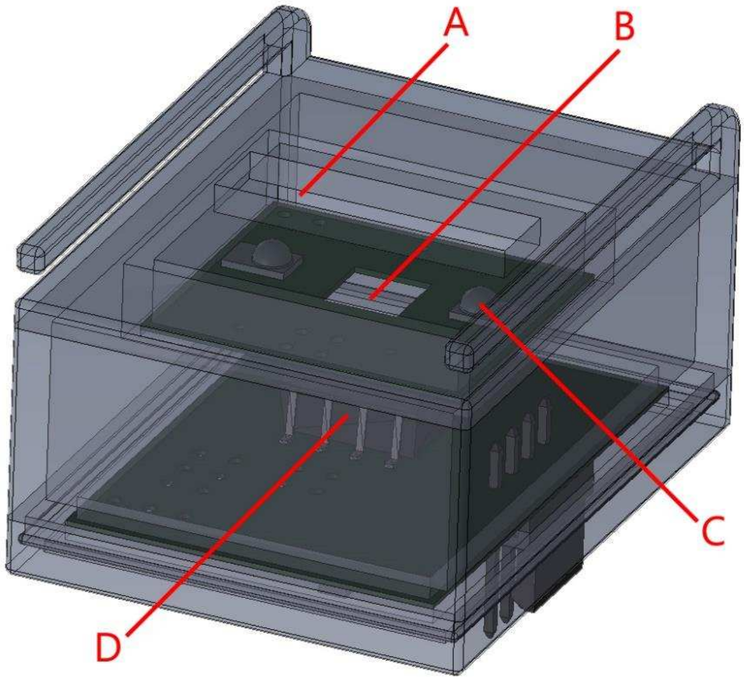

Figure 12: A CAD model of Rev. B prototype with its casing that is attached on the abaxial surfaces of leaves. (A) Case opening for LED excitation light to leaf, and fluorescence from leaf. (B) PCB cutout opening above sensor for received fluorescence signal. (C) Blue LED. (D) Sensor.

3.5 Experimental setup

3.5.1 CFY Sensor

CFY Sensor Rev. A measures steady state fluorescence (F_s) and light-adapted maximum fluorescence (F_m') at 145 µmol $m^{-2}s^{-1}$ and 730 µmol $m^{-2}s^{-1}$ intensities, respectively (we omit light intensities for dark-adapted measurements f_0 and F_m, as these were not carried out). Rev. B measures F_0 at 9 µmol $m^{-2}s^{-1}$, F_s at 115 µmol $m^{-2}s^{-1}$, and both F_m and F_m' using 1260 µmol m^{-2} s^{-1} of light. The system is configured to automatically measure plant activity every hour when set to auto-sampling.

The light-adapted parameter F_s was obtained during 0.75 seconds after a 40-second steady-state adaptation time, after which the sensor measured the F_m' during another 0.75 seconds. The same procedure is applied to dark-adapted measurements, with the omission of a 40-second steady state light. From the measured parameters, we can calculate the effective quantum yields previously elaborated on in Section 3.3.

The blue wavelength was chosen for the sensor system as the blue colour is one of two regions in the absorption spectrum of chlorophyll *a*. The light absorption in leaves is also higher at this wavelength than in the red spectrum. In addition, the blue spectrum is more energetic, aiding in maintaining low-power consumption while enabling a high level of excitation. There are some limitations of using blue light instead of red light, most notably in the types of parameters that are possible to measure. However, the basic parameters needed for yield calculations can be done more efficiently in the blue spectrum, and, as such, is advantageous for our application.

Note that all experiments except tests in the field (greenhouse) were carried out using CFY Sensor Rev. A prototype.

3.5.2 Mini-PAM

The Mini-PAM (Heinz Walz, Effeltrich, Germany) fluorometer was configured to measure, in dark-adapted state, the minimum (F_0) and maximum fluorescence (F_m) using a red measuring light (650nm) of 0.15 µmol $m^{-2}s^{-1}$ and a saturating light of >5000 µmol $m^{-2}s^{-1}$ for 0.8 seconds. For LA measurements, the plants were exposed to red light with a calibrated intensity of 1000 µmol $m^{-2}s^{-1}$ for 40 seconds to achieve F_s, followed by saturating light to obtain F_m'. All plant measurements, except in DA state, were carried out for both adaxial and abaxial surfaces. In DA leaves, we only made adaxial measurements.

From the measured parameters, we can calculate the effective quantum yields previously elaborated on in Section 3.3. Due to the use of red excitation light in the Mini-PAM, the device is capable of measuring and calculating additional parameters not possible when measuring in the blue spectrum, for example, the minimum fluorescence level in the presence of light (f_0'). In our tests, we have disregarded all non-comparable parameters and calculations.

3.5.3 Plants

In our experiment, we used the *Paspalum densum* Poir (Poaceae) Panicoideae. This family of monocotyledonous flower plant (*gramineae*) is tolerant to soil with elevated iron content, playing an important role in the revegetation of areas impacted by iron mining (Souza, 2016).

3.5.3.1 Experimental conditions

The experiment was set up in small greenhouses at Universidade Federal de Viçosa – Campus Florestal (19°53'08.9"S e 44°25'02.2"W). The *Paspalum densum* seedlings were obtained from original matrices at said campus. The experiment took place in March 2017, with conditions approaching winter with lower temperatures and less overall sunlight, which lowers plants' photosynthetic activity and potentially results in additional stress caused by large temperature differences. This may influence the results to a certain degree, although also uniformly across all plants, but because all measurements were taken under similar conditions for both Mini-PAM and CFY Sensor, this factor can be disregarded.

For the seedlings, tillers collected from the matrices were separated and transplanted directly into five-litre vases, containing a 1:2 proportion of soil and manure. The plants were cultivated in a greenhouse for three months, watered daily and acclimatised before the start of the experiments.

3.5.3.2 Experimental design

Two experiments were set up to test the CFY Sensor: a laboratory test using CFY Sensor Rev. A, and a field test using CFY Sensor Rev. B. The laboratory test was designed to provide data for comprehensive analysis, while the field test was designed to provide an idea of the sensor's capabilities in the real world and in the field. The latter was time-restricted, since one sensor had to be used on multiple plants over long periods of time.

A total of ten *Paspalum densum* plants were used during the 9-day laboratory experiment with CFY Sensor Rev. A. The experiment was set up in a randomised block design with two treatments and five repetitions for each species. The two treatments consisted of exposing the plants to simulated acid rain of pH 2.3, with a pH 6.5 exposure as control.

For the control solution, deionised water was used, while diluted sulphuric acid (H_2SO_4) constituted the acid rain solution. The choice for this composition is due to the fact that it is the naturally most abundant constituent of acid rain. For the solution application, a 20-litre manual sprayer (Jacto Clean XP20) was used together with a Teejet (XR11002) tip, with a calibrated average application of 700 ml (0.2 gallons) min^{-1}. The plants were subjected to daily treatments (between 7am and 8am) for one minute, and measured daily on fully expanded leaves throughout the experiment using the Mini-PAM and the CFY Sensor.

For the field test, two plants were used, with measurements each spanning 40 hours (in succession) using CFY Sensor Rev. B. Prior to the test, 20 plants had been previously subjected to heat and light stress for a month, having initially been acclimated to indirect sunlight for more than two months. Two plants were selected based on their measured plant activity, using the Mini-PAM, of which one indicated normal activity (i.e. good light and heat acclimatisation) and another indicated lower activity (i.e. poor acclimatisation, thus high stress). The test was intended to be a real-world test on two plants of different activity, using the CFY Sensor Rev. B, but the experiment was limited by the availability of multiple sensor nodes, which means statistical analysis is not possible.

3.5.3.3 Statistical analysis

We recorded a total of nine days using our CFY Sensor and eight days using the Mini-PAM. Each measurement session (day) contains ten measurements in two treatment groups, and each parameter test contains a maximum of 90 data points.

Every experiment followed a randomised block design with two pH rain simulation solutions (pH 6.5 and 2.3), with five repetitions per treatment. The data was grouped into sub-divided parcels, with each parcel representing the time of sampling. All data was subjected to an analysis of variance, and the means were compared using Tukey's test at 5% probability using the statistical analysis software, SAEG 9.1-UFV (Fundação Arthur Bernardes, UFV, Viçosa, 2007). Prior to the Tukey test, all data sets were checked for their homogeneity of variance (Cochran's Q and Bartlett's tests) and normality (Lilliefors test).

3.6 Results

The focus of the research and results of the present work is on validating the sensor system, mainly using Rev. A prototype. As such, the experiments carried out have been focussed on comparing the CFY Sensor system to that of our reference equipment (Mini-PAM). Of particular interest were statistical analyses (correlation, Tukey test) and early stress detection observation by comparing stress-treated plants with control plants, of which the latter was done both in the laboratory and in the field.

For practical reasons when using the CFY Sensor, we measured only the abaxial leaf surfaces. Since we have used the Mini-PAM to gauge CF in both adaxial and abaxial surfaces, we assume any correlation and statistical significance within and between our prototype sensor's abaxial readings and readings from the Mini-PAM to be indicative of a functioning abaxial-measuring sensor. See Appendix B for detailed statistical analysis of the CFY Sensor data.

3.6.1 Tukey test

For the analyses, we carried out Tukey tests after the initial tests (see Section 3.5), which showed good homogeneity of variance and good normality.

In our Tukey test analysis, we found that the effect of treatment vs. date for both CFY Sensor and Mini-PAM had high significance for all parameters, and hence we analyse this data for F_s, F_m' and Φ_{PSII}.

The groupings indicate particular significance in early stages of plant stress in the F_m' fluorescence. The maximum LA fluorescence was significantly higher already one day after treatment application. Throughout the test, the consistency fluctuates, but is generally significant until the 5th day. Similarly, for F_s measurements, significant mean differences are found until day 5, but not significant until day 3. However, for Φ_{PSII}, we find significant means only for the two initial days after treatment application. Additionally, the statistical analysis on yield revealed that the effect of date, on its own, on the means was significant. The comparison of means on the effect of day shows that we obtained significantly different results from the second day, and continued until the 6th day with respect to day 1. After day 7, the means are insignificantly different with respect to day 1, and we conclude that the treatment group of plants had recovered.

These observations are important for the following reasons. We see that it is important to analyse not only yield, but also the individual fluorescence components. Additionally, these results demonstrate that early plant stress detection can be measured with statistical significance, even with a low-cost CF sensor.

3.6.2 Regression and Correlation

As we are interested in knowing how accurate the CFY Sensor is compared to the Mini-PAM, other interesting aspects are regression and correlation analyses. The yield data from the CFY Sensor and the Mini-PAM are scatterplotted, from which R^2 can be calculated. From this data, we can also calculate the Pearson and Spearman correlation. In Figure 13, we see two plots, each comparing one set of data (abaxial) from the CFY Sensor to each of two datasets (abaxial and adaxial) of the Mini-PAM.

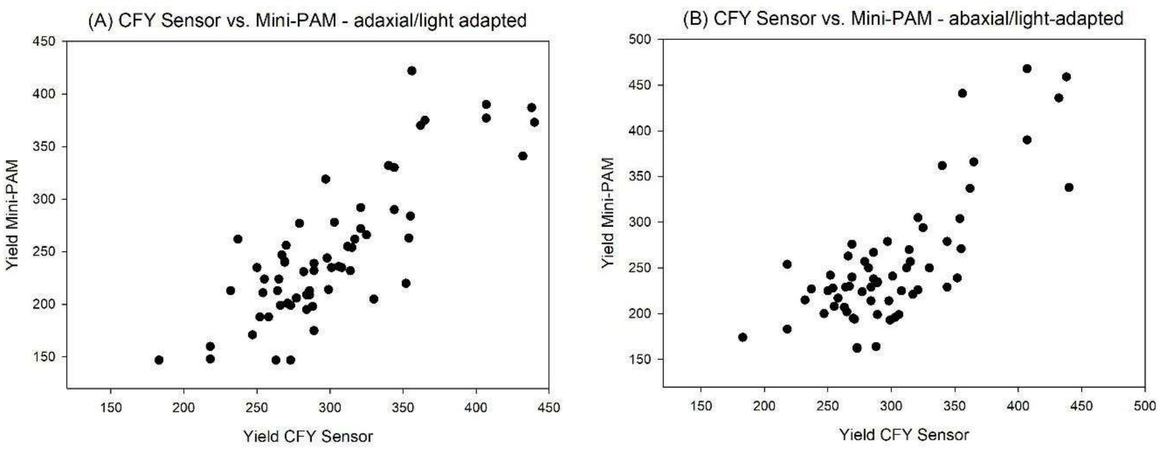

Figure 13: Scatter plots of yield calculations for each plant over the course of 9 days. Some outliers have been removed from the datasets that include both stress and control plant measurements. (A) CFY Sensor abaxial vs. Mini-PAM adaxial; (B) CFY Sensor abaxial vs. Mini-PAM abaxial.

From the data of Figure 13 (A), there is an R^2 of 0.669 (log-transformed), a Spearman correlation of 0.741, and a Pearson correlation of 0.818. From the data of Figure 13 (B), we obtain an R^2 of 0.543 (log-transformed), a Spearman correlation of 0.622 and a Pearson correlation of 0.781. We can observe that the correlation of the data from the CFY Sensor abaxial and Mini-PAM adaxial is notably higher than when compared to Mini-PAM abaxial, and may be attributed to higher accuracy in the measurements by the Mini-PAM in adaxial surfaces. In both cases, when removing some outliers, we obtain strong correlations for measurements in both adaxial and abaxial surfaces. The linear regression R^2 of each is lower, since the relationships do not appear linear, but monotonic nonetheless.

Additionally, given enough data, it is possible to plot the averages of data points sampled from the two groups per day, and obtain a somewhat linear relationship, as shown in Appendix C. This could also further improve Spearman and Pearson correlations.

3.6.3 Stress Detection

As early stress detection is frequently the aim of fluorescence measurement, we are particularly interested in our sensor's response to changes in CF. Naturally, the measurements done with the CFY Sensor are expected to be less sensitive to small changes than with the Mini-PAM. However, observable results were still obtained with both sensors only a few days into the treatment application. Of interest to us are F_v/F_m and Φ_{PSII} (DA and LA state quantum yield, respectively; see Section 3.3.3).

3.6.3.1 Laboratory test results

In Figure 14 (A) we can see the abaxial QY response using CFY Sensor Rev. A, and in Figure 14 (B-D), we can see the adaxial and abaxial responses using the Mini-PAM. Note that the large differences in QY at the beginning of the tests are due to a number of reasons. Both Mini-PAM and CFY Sensor had changes in light intensity calibrations carried out during the first few days, causing significant Φ_{PSII} decreases for CFY Sensor and increases for Mini-PAM. This affects the measurements proportionally, i.e. both stress and control QY change proportionally to one another with the calibration changes, and therefore does not invalidate the results. Another difference is due to the fact that not all measurements were possible to do simultaneously, meaning that changing light conditions and light adaptation state of the plants differed somewhat between individual measurements.

Based on the QY calculations, we can make a few important observations. We notice that in all LA measurements, the stressed plants started showing decreased photosynthetic efficiency within one day after treatments were applied. Subsequently, the QY of stressed plants began to recover, and eventually levelled out with the QY of the control plants. In the DA measurements taken by the Mini-PAM, we notice a stronger contrast and an undefined recovery. As the F_v/F_m (dark-adapted QY) is affected more significantly than Φ_{PSII} (light-adapted QY), this is to be expected.

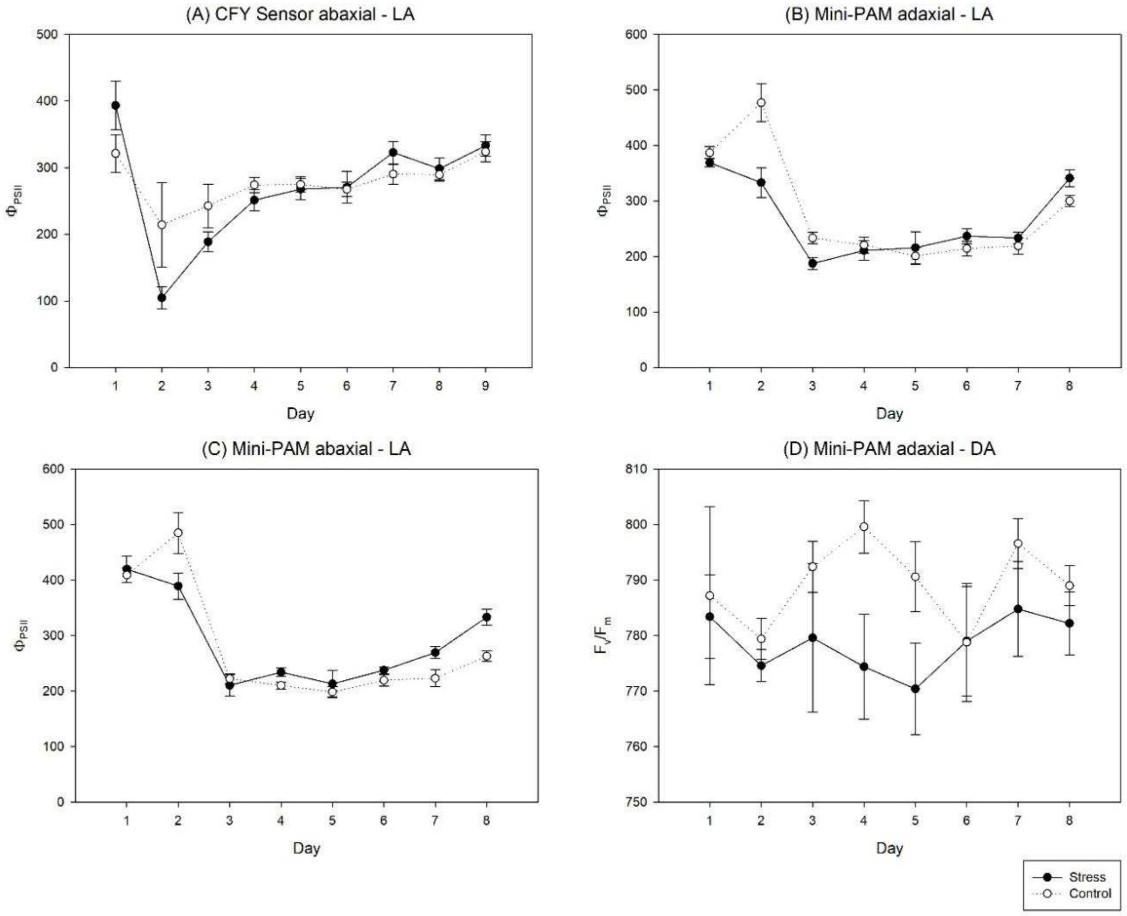

Figure 14: QY measurements plotted using average values and standard error. Note that we have one more data point (day) for the CFY Sensor. (A) CFY Sensor measurements indicate an immediate fall in quantum yield, thus photochemical efficiency, upon applying acid rain treatment to stress group. (B), (C) Measurements from Mini-PAM for light-adapted adaxial and abaxial measurements also indicate an immediate decrease in efficiency. (D) Dark-adapted adaxial measurements indicate a more pronounced difference between stressed and control plants.

In order to confirm the quantum yield results, we analyse these using confusion matrices. A confusion matrix has four possible outcomes: true positive (TP), false positive (FP), true negative

(TN), and false negative (FN). We carry out a confusion matrix analysis on all four datasets above in the following manner: complete datasets, i.e. day 1-final, and for the datasets in which we know that stress affected the plants (displayed in parenthesis), where subsequent data, i.e. plant recovery, and day 1, i.e. prior to acid rain application, can be excluded. Note that data points for one day is missing from the Mini-PAM measurements. The matrices are based on comparisons between raw QY and the average QY of the opposite group, for example: day 1 stress QY data point 1 vs. day 1 control QY average. Table 2 – Table 5 show the four confusion matrices corresponding to Figure 14.

Table 2: Confusion matrix for CFY Sensor – abaxial LA for day 1-9 (day 2-5).

n = 88 (n = 50)	N’ [predicted]	P’ [predicted]
N [actual]	**TN = 24 (17)**	FP = 21 (8)
P [actual]	FN = 18 (8)	**TP = 25 (17)**

Table 3: Confusion matrix for Mini-PAM – adaxial LA for day 1-8 (day 2-4).

n = 80 (n = 40)	N’ [predicted]	P’ [predicted]
N [actual]	**TN = 19 (12)**	FP = 21 (8)
P [actual]	FN = 18 (5)	**TP = 22 (15)**

Table 4: Confusion matrix for Mini-PAM – abaxial LA for day 1-8 (day 2-4).

n = 80 (n = 40)	N’ [predicted]	P’ [predicted]
N [actual]	**TN = 11 (8)**	FP = 29 (12)
P [actual]	FN = 27 (9)	**TP = 13 (11)**

Table 5: Confusion matrix for Mini-PAM – adaxial DA for day 1-8 (day 2-4).

n = 80 (n = 40)	N’ [predicted]	P’ [predicted]
N [actual]	**TN = 31 (17)**	FP = 9 (3)
P [actual]	FN = 13 (5)	**TP = 27 (15)**

3.6.3.2 Field test results

Limited field tests (in a greenhouse) have been carried out using CFY Sensor Rev. B (the only experiment carried out with this revision). Since the current availability of sensor nodes is restricted to only one unit, the tests could not be carried out simultaneously. Nonetheless, it is important to note that the differences in plant activity are still observable between the two plants, particularly during the night when both plants were dark-adapted. In Figure 15 is a graph with the control and the stressed plant, where it is possible to observe that for both light and dark-adapted measurements, the healthy plant generally exhibited a higher quantum yield (photosynthetic efficiency in LA state, or potential maximum efficiency in DA state).

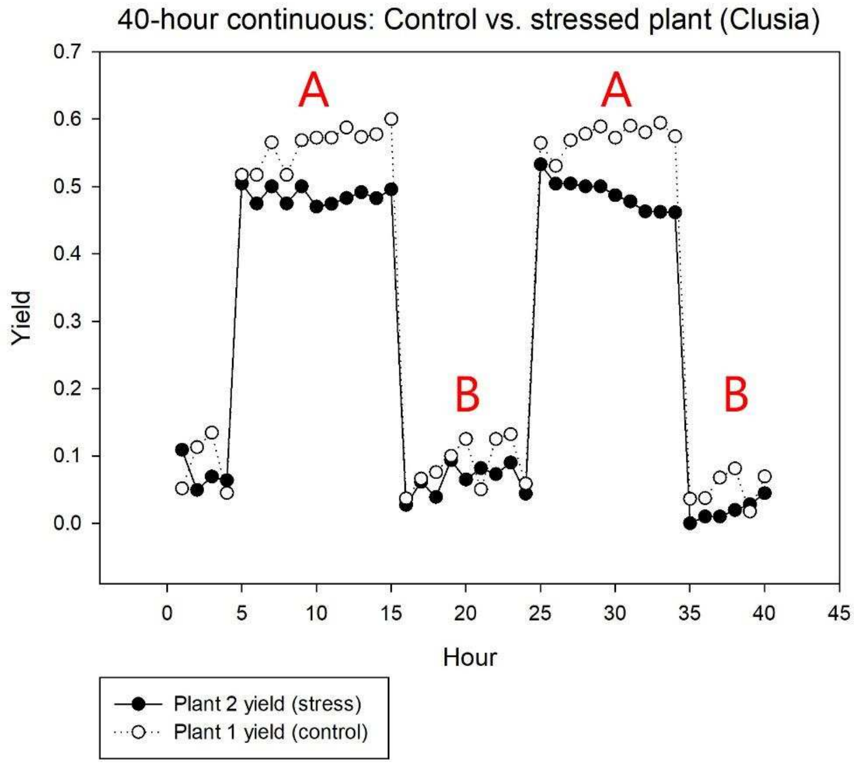

Figure 15: Field test using CFY Sensor Rev. B on two plants with different photosynthetic activity. Measurements by the system were automatically taken every hour, although some measurements are absent due to technical problems with the prototype. Due to stress, it is possible to observe a decrease in both F_v/F_m (A) as well as Φ_{PSII} (B), relative to the healthy plant, throughout the monitoring process.

We confirm the results above by analysing the data using confusion matrix for the complete dataset for the 40-hour experiments (in total 80 data points). Again, we compare between raw QY and the average QY of the opposite group, but separated into LA and DA, for example: LA/day 1 stress QY data point 1 vs. LA/day 1 control QY average, or DA/day 1 stress QY data point 6 vs. DA/day 1 control QY average. The confusion matrix in Table 6 corresponds to the data presented in Figure 15.

Table 6: Confusion matrix on 40-hour experiment with CFY Sensor – adaxial LA and DA using two plants: control (no stress, 40h) and stressed (40h).

n = 80	N' [predicted]	P' [predicted]
N [actual]	**TN = 34**	FP = 6
P [actual]	FN = 3	**TP = 37**

3.7 Plant-Stress Detection Algorithm

In this section, we outline a high-level algorithm able to distinguish between data from stressed and non-stressed plants. Further details are reserved for future work.

There are two methods in which we could detect issues with plants. One would be to have an established base-level for healthy plants, which may be difficult to establish due to the CFY Sensor design, as CF signal selectivity is lower than that of general fluorometers. The other method would be to form a cluster-oriented algorithm for recognising when one or more plants, whether individually (cluster from historical data), in sub-groups or large clusters, begin exhibiting changes in QY (or other parameters) to that of the general population. The possibility also exists that the entire population might undergo changes due to pollution, which should also be taken into account. Given pre-defined clusters of sensor nodes (using various local sink nodes with their own satellite nodes), the proposed method could be simplified. Figure 16 presents an overview of the proposed algorithm for recognising plants undergoing stress, reflecting that of the results in this paper.

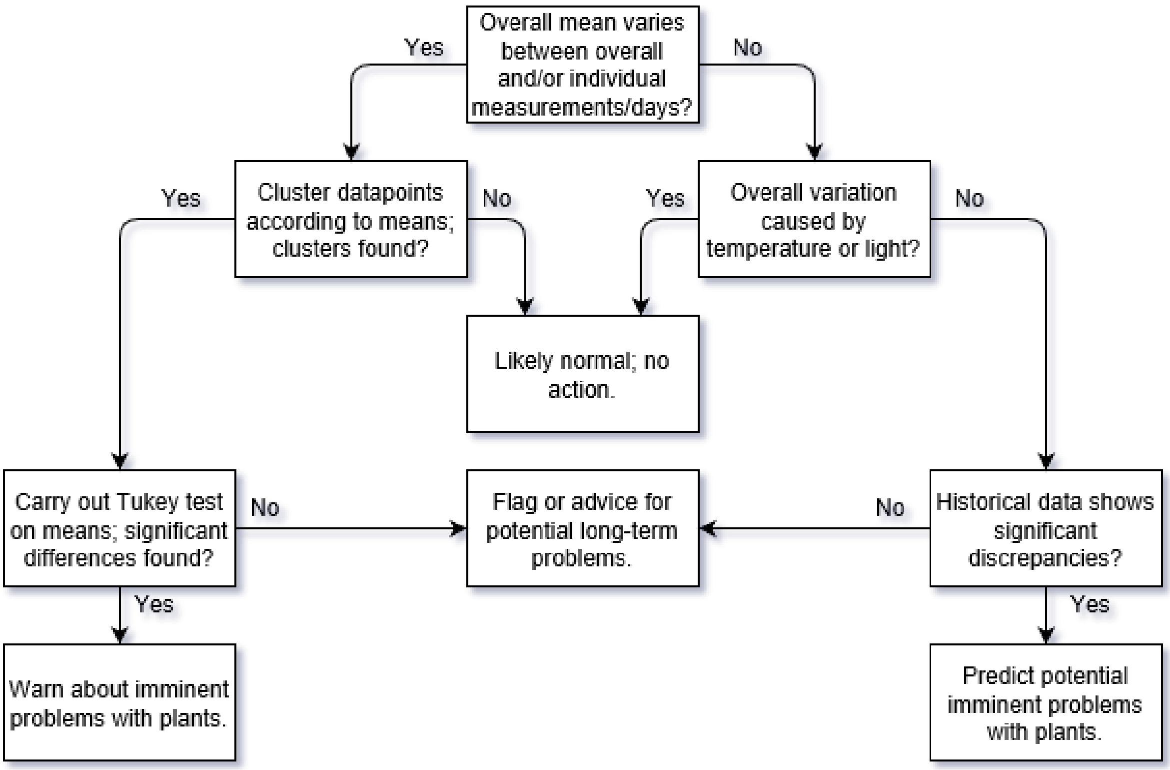

Figure 16: Proposed algorithm for evaluating plant stress using raw or processed data. The data from all plants and recent individual historical data is clustered to form potential physical areas that can be identified as affected by pollution or other stress factors. Data may also be treated as one large cluster changing over time to indicate large-scale effect of pollution. Four different outcomes are present: no action, flag for potential problems, predict potential imminent problems, and take immediate action.

3.8 Discussion

The results confirm that our low-cost sensor effectively measures CF. In addition, the statistical analyses have proven that the CFY Sensor not only is able to discriminate between stressed and non-stressed plants, but also do so before the onset of any visual symptoms. The confusion matrices confirm the reliability of the sensor system, showing majority true positives and true negatives for the CFY Sensor, both for lab and field experiments. In fact, the results obtained by the

CFY Sensor were better than that of the Mini-PAM, according to our experiments. The feasibility of a low-cost sensor has therefore been proven successful, with results consistent with that of other authors using similar stress factors and methodologies (Cetner *et al.*, 2014; Kancheva *et al.*, 2008).

However, contrary to the work of other authors, we do not employ state-of-the-art technology. A lot of existing research on the topic of monitoring chlorophyll fluorescence focus on maximising accuracy and precision to marginally improve the systems. Among the technologies used in the strive for increasingly improved measuring techniques we find charge-coupled device (CCD) cameras for laser-induced fluorescence (LIF) (Lichtenthaler & Miehé, 1997) and hyperspectral imaging using Fraunhofer lines (Meroni *et al.*, 2008). However, using a multi-spectral CCD camera (coupled with a laser) requires computational power for analysis, complicating wireless and remote deployment. Other relatively complex setups require frequency-induced pulse-amplitude modulation (FIPAM) using laser technology (Flexas *et al.*, 2000) or field-programmable gate arrays (FPGA) (Jaramillo-Fernandez *et al.*, 2015), of which the latter is also not suitable for actual monitoring of plant health, being aimed solely at the research of best-practices of measurement methodologies.

The authors of these papers use high-precision or experimental equipment to further improve both measurements and measurement methodology, which, in turn, becomes costly and something that requires trained personnel to use and analyse. This is not optimal for an average user who may have little knowledge in this field. The implementation most similar to ours is that of a Japanese team. Since 2009, Kameoka *et al.* (2017) has been using WSNs in 'smart cultivation management practices'. Their use of networked sensors certainly confirms that such technology has potential, although the sensors used by the team included a range of sensor types, from X-ray fluorescence to thermal imaging. Similarly, they also provide a wireless infrastructure with a database and management.

We have solved the problems of ambient light interference in a much simpler and practical manner than that of other authors, who have e.g. turned to technologies such as nanometre-scale spectrometers to separate CF signals from other light sources. Through non-conventional measurement methodology using abaxial leaf surfaces, we avoid the need for intricate systems while augmenting the possibilities for early detection of stress. This solution, therefore, sets our work apart from that of many existing works, using a non-competing approach, where we do not attempt to completely overcome the ambient interference, and instead attenuate it to a level that the sensor system can handle. The fact that we have been able to simplify existing methods while obtaining useful data proves that simpler systems like ours may find a use as an alternative method aiming to continuously and autonomously, and with little prior knowledge of the system, provide useful information to a user.

As a result, our findings open up possibilities for employing many sensor nodes in the field, using wireless sensor networks. WSNs have the strength of multiple simultaneous sampling points, enabling us to potentially do statistical analysis and data mining on a multitude of plants in order to discover deviating individual or population behaviour over short or long-term. By using individual plant data that we can relate to other plants and over time, we do not necessarily need to know the exact chlorophyll content or carbon assimilation of plants in order to make conclusions about plant stress, as we have proven in this paper using one sensor on multiple plants. This is a stark contrast with traditional methods, where plant health has a well-known definition, i.e. can we see visual symptoms of pollution on leaves, and do the measurements on a specific plant indicate a lower-than-normal chlorophyll content? An interesting benefit of this approach could be mapping soil health and pollution around or in polluted areas. In addition to environmental monitoring, this could be used in determining the suitability of an area for crop growth. We therefore believe that the possibilities for a low-power, low-cost sensor has a lasting potential in an array of scenarios including pollution monitoring and agricultural activities.

In Table 7, is a compiled list of high-level differences between the CFY Sensor and related systems. As our contributions focus on low-cost, low-power, simplicity and scalability, it is possible to observe strengths, especially in terms of cost, where we expect each sensor node to cost less than US$100, whereas common fluorometers may cost several thousand dollars per unit, making acquiring of multiple devices a costly proposition. This leads to poor scalability and non-real-time measurement capability. The energy consumption of the CFY Sensor is expected (after optimisations) to be low enough to operate for several months on a Li-Po battery, or indefinitely with energy harvesting technology.

Table 7: Comparison between CFY Sensor and common or state-of-the-art solutions, such as Mini-PAM and discussed work by other authors. This table estimates factors based on types of technology used, available information, and empirical data. Accuracy is based on established methods, and is loosely comparable for the CFY Sensor, since methodologies differ.

	CFY Sensor	**Related (general)**
Cost	Low	High – very high
Power	Very low	Medium – very high
Accuracy	Medium	Very high
Complexity	Low – medium	High – very high
Scalability	Very high	Low – medium
Real-time	Very high	Very low – low

3.9 Conclusion

Our sensor system prototype, the CFY Sensor, is the main contribution of this paper. It is designed to provide easy and independent operation for extended periods of time. We achieved this through focussing on the essentials of fluorometry, while adopting new, low-powered technology and methods not used or uncommon to the field. Contrast this with commercial fluorometers, which are not only expensive, but generally also require that a user take manual measurements in a field, which can be time-consuming and costly. We believe that our solution can resolve the weak points of commercial fluorometers, and provide real-time monitoring with the ability to detect pollution and plant stress before the onset of damage, using low-cost methods.

In order to verify our sensor prototype, we set up an *in vivo* experiment with the *Paspalum densum*, in which we compared results from the CFY Sensor to those of the Mini-PAM. Using the two sensors on multiple plants over several days (and in a controlled environment) – essentially emulating a sensor network – we verified our sensor prototype's capability of distinguishing stressed plants from non-stressed ones. This has been demonstrated by means of statistical analyses, where both sensors were able to identify stresses and plant recovery in a similar fashion and with similar significance.

Naturally, our approach comes with its limitations as well, due to its low-cost nature and inherent accuracy restrictions. However, as we aim for different types of measurements and a different *modus operandi*, there are interesting possibilities in this approach. For example, the fact that we can potentially gather a multitude of simultaneously collected data points from a network of these sensors, means that large data and data mining is possible – an implementation which is largely non-existent with traditional methods of measuring, using handheld fluorometers.

3.10 Acknowledgements

We thank the research agencies FAPEMIG, CAPES, CNPq and GAPSO for their financial support.

4. General Conclusion

In this dissertation, we set out to find solutions to problems that are inherent to traditional techniques of measuring plant and environmental health. Among these, we identified cost and complexity, which ultimately lead to productivity and economic losses from inefficiencies and problems stemming from pollution. In the search for new ideas and concepts, we were faced with challenges that had to be met with unconventional solutions. Among the challenges were CF selectivity, dealing with limitations of low-cost components, low-power requirements, and accuracy. Throughout the development, we focussed on means of achieving similar performance to that of commercial equipment, at a fraction of the cost at an estimated $100 per unit. Due to the non-state-of-the-art approach, our ideas had to be based around acceptable compromises, such as lowered, but sufficient, sensitivity to CF, and measuring this in non-standard areas (abaxial) of leaves. The contributions of our work allow for new methods of meeting demands in an increasingly demanding world of environmental monitoring and agricultural production.

Our proposed sensor system makes monitoring of large areas possible in real-time, since the system relies on low-powered, wireless and automated measurements that do not interfere with plant growth. This is of special interest to those who directly rely on natural resources for production, such as farmers. Using traditional fluorometers (such as Mini-PAM-type devices) is not only expensive per unit, but require a user to manually gauge the photosynthetic efficiency one plant at a time. The use of these devices as real-time and large-scale monitoring systems becomes unfeasible, as they provide mere 'snapshots' of small areas with data that must be analysed in non-real-time, causing further inaccuracies. While commercial equipment like these provide very precise and accurate measurements, it also becomes clear that these, in turn, possess limitations that cannot be solved with further improvements in precision.

The results obtained throughout the evolution of the CFY Sensor show that not only can we measure chlorophyll fluorescence, but also do so with statistical significance. In both laboratory and real-world tests, the results show that there is a real potential, even in the methods that we have devised, which do not follow common approaches, such as adaxial measurements and the use of red wavelength excitation light. While our sensor system may not be able to obtain an exhaustive list of parameters, it can, however, provide a high-level understanding of changes in plant physiology.

The added advantage of our work is that all of this was achieved using completely off-the-shelf components and concrete methods. This means that this solution is not a futuristic concept, but rather something which is achievable today and with minimal additional efforts. As such, we believe that this system can achieve its large-scale goal of monitoring and even detecting early signs of pollution contamination in soils in and around regions susceptible to this.

4.1 Final Considerations

The monitoring of plant chlorophyll fluorescence and environmental pollution will always be challenging. Since there are so many factors playing a role, solutions to accuracy in both time and plant physiology measurements must be met with their respective trade-offs. We know that in order to accurately measure plant physiology, we need precision equipment, but this renders it difficult to use as real-time monitoring systems on a large scale. Solutions to this problem must, on the other hand, forego the high-precision technology and instead rely on large data from many low-precision sensors.

The CFY Sensor provides an answer to this, but being of a low-cost nature, we must understand its limitation. As a single sensor unit, it will not be useful, because each sensor node is dependent on other nodes in a (wireless) sensor network. As discussed in Chapter 2, the reason for this is because we do not know the exact state of a single plant (absolute conditions), but must instead rely on comparing and analysing statistical differences between plants over a large area and over time, and discover discrepancies between plants (relative conditions), or temporal differences on the population in its entirety. Hence, the strength of our solution lies in the quantity, and not necessarily in the quality of the measurements.

4.2 Future Work

Future work on the sensor solution will need to address issues with sensitivity and CF selectivity. While the sensor system is able to function in non-controlled environments, it is still susceptible to issues with distinguishing background radiation from the fluorescence signals, especially under intense light conditions. Improvements to the electronic and physical design of the sensor, as well as optical filtering, will further enhance the sensor without significant cost addition.

Finding a baseline fluorescence for healthy plants could also be interesting, as it may aid in more rapid stress detection. For this, it may be necessary to combine data from other sensors, such as temperature, light intensity and humidity sensors. Future work also needs to include real-time measurements from multiple sensor nodes mounted on several plants, which we were unable to carry out. To accompany this, appropriate machine learning or automated statistical analysis must be implemented, as data has been hitherto analysed manually and offline.

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A. Detailed View of the Electronic Circuit

A.1 CFY Sensor Main Board

The PCB design is dual-layer, with all components except the sensor mounted on the same side. The CFY Sensor uses two 150mA ultra-low dropout voltage regulators, an instrumentation amplifier, a constant-current LED driver, the Texas Instruments MSP430G2553 microcontroller and circuit for Spy-Bi-Wire programming. The system operates on a fixed 3.3V. Figure 17 shows both sides of the main PCB. Figure 18 and Figure 19 show the schematics of the CFY Sensor main PCB.

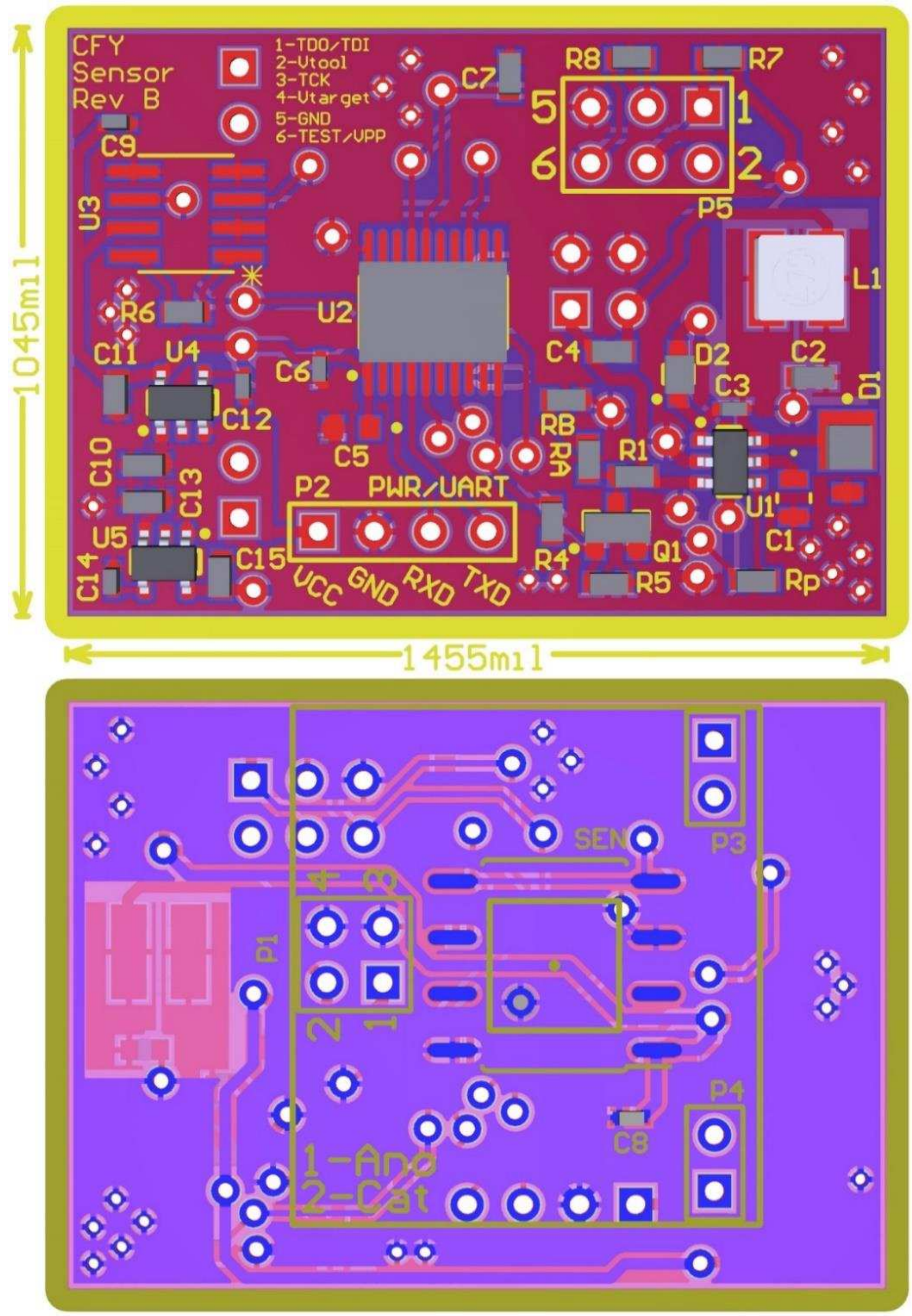


Figure 17: Top and bottom view of CAD-rendered main PCB.

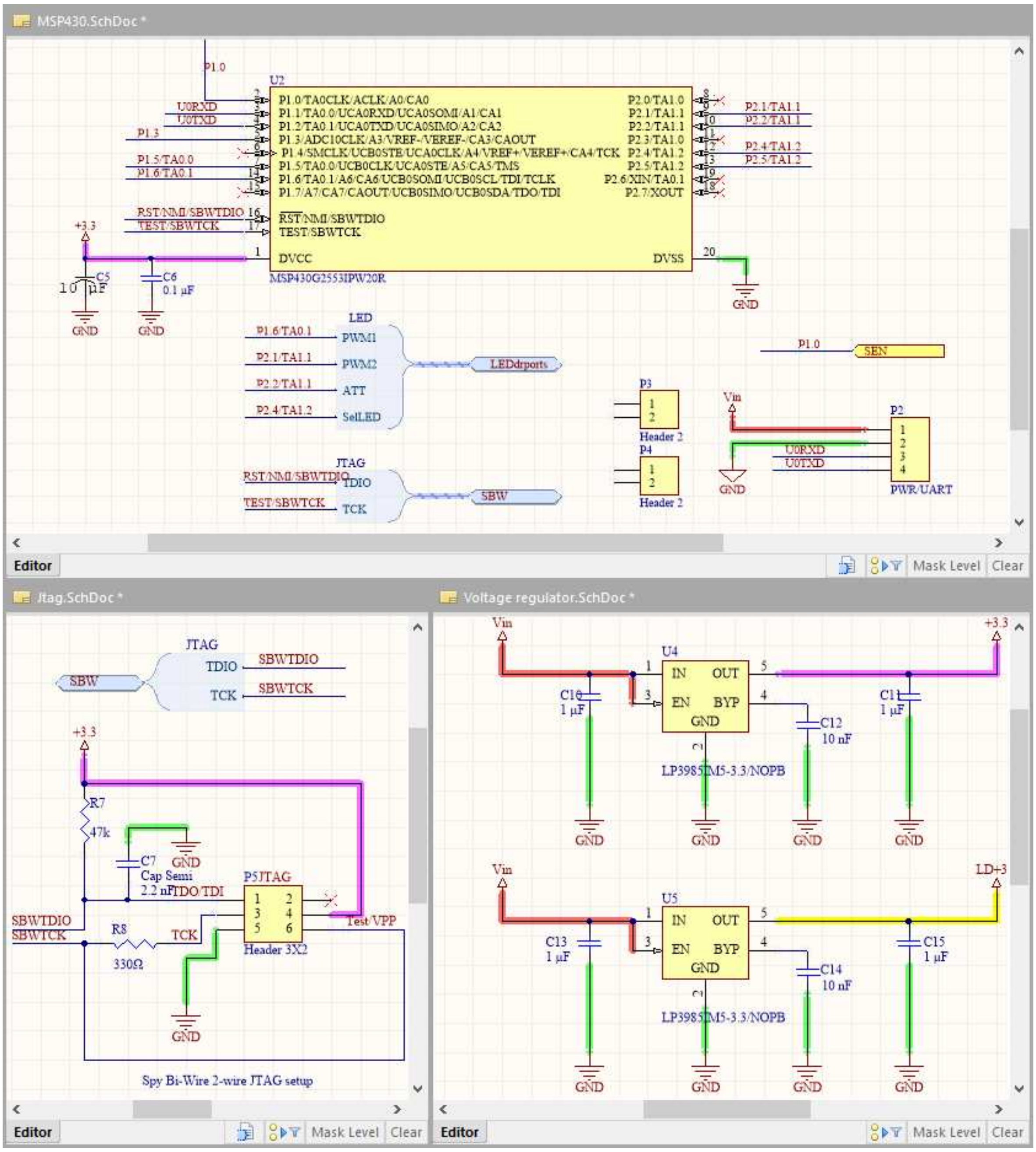


Figure 18: CFY Sensor main PCB schematics.

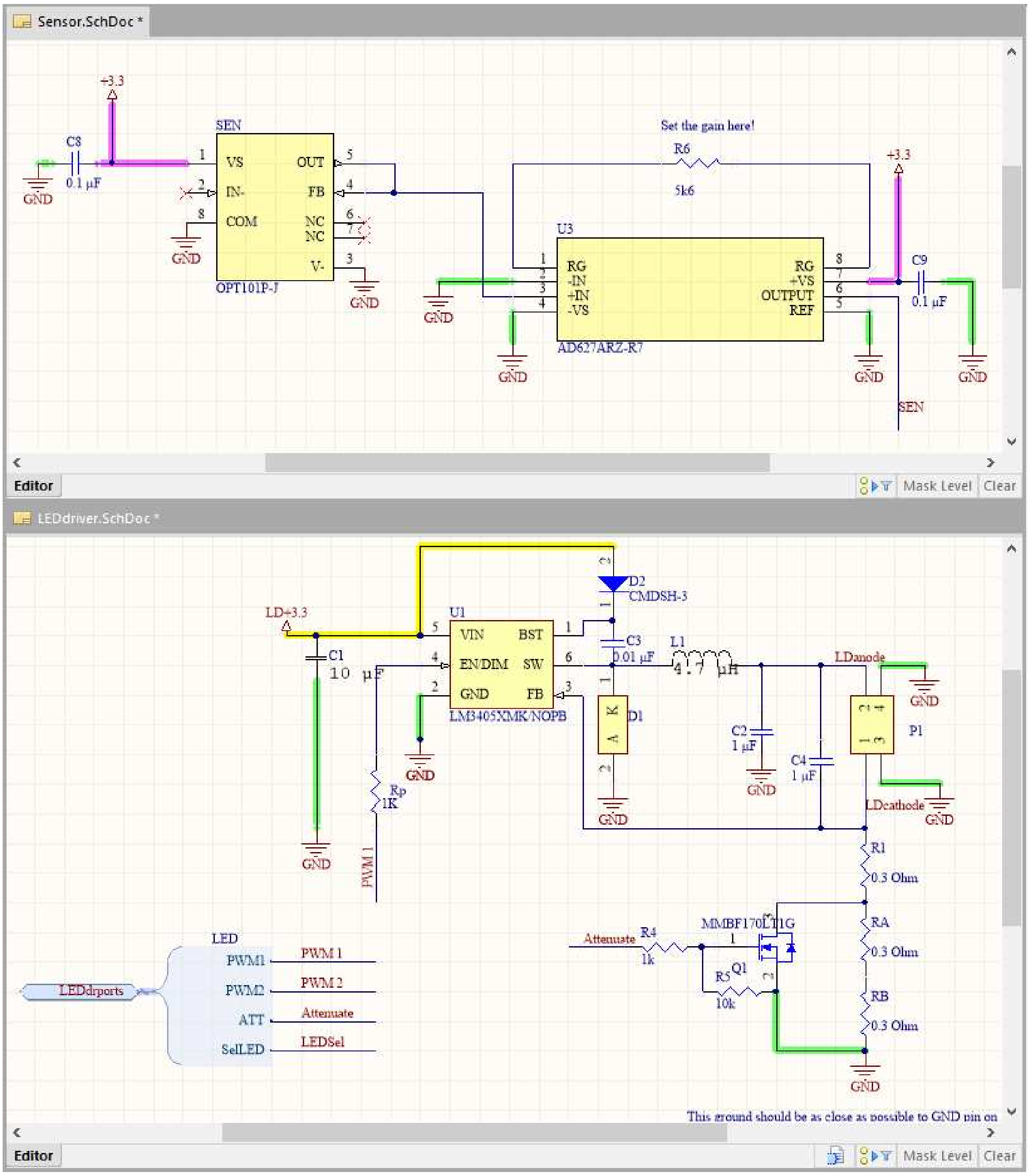


Figure 19: CFY Sensor main PCB schematics (continued).

A.2 CFY Sensor LED Board

The LED PCB is dual-layer with all components mounted on the top-side. It holds two high-powered Luxeon Rebel Blue LEDs that flank the PCB cutout that aligns above the sensor of the main PCB. The LEDs have custom heat-dissipating elements to protect from overheating. Figure 20 shows CAD renders of the LED PCB. Figure 21 shows the schematics.

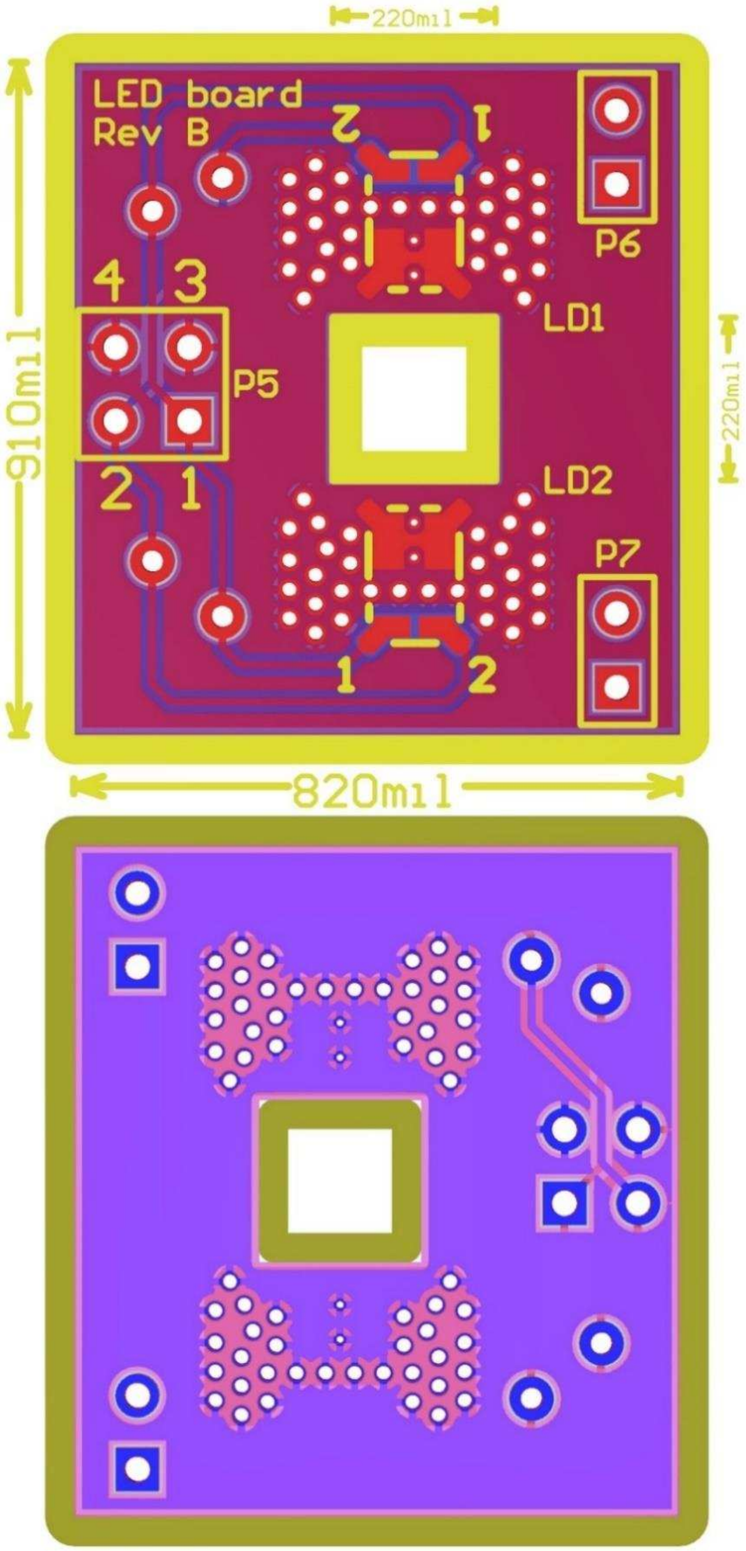


Figure 20: Top and bottom view of CAD-rendered LED PCB.

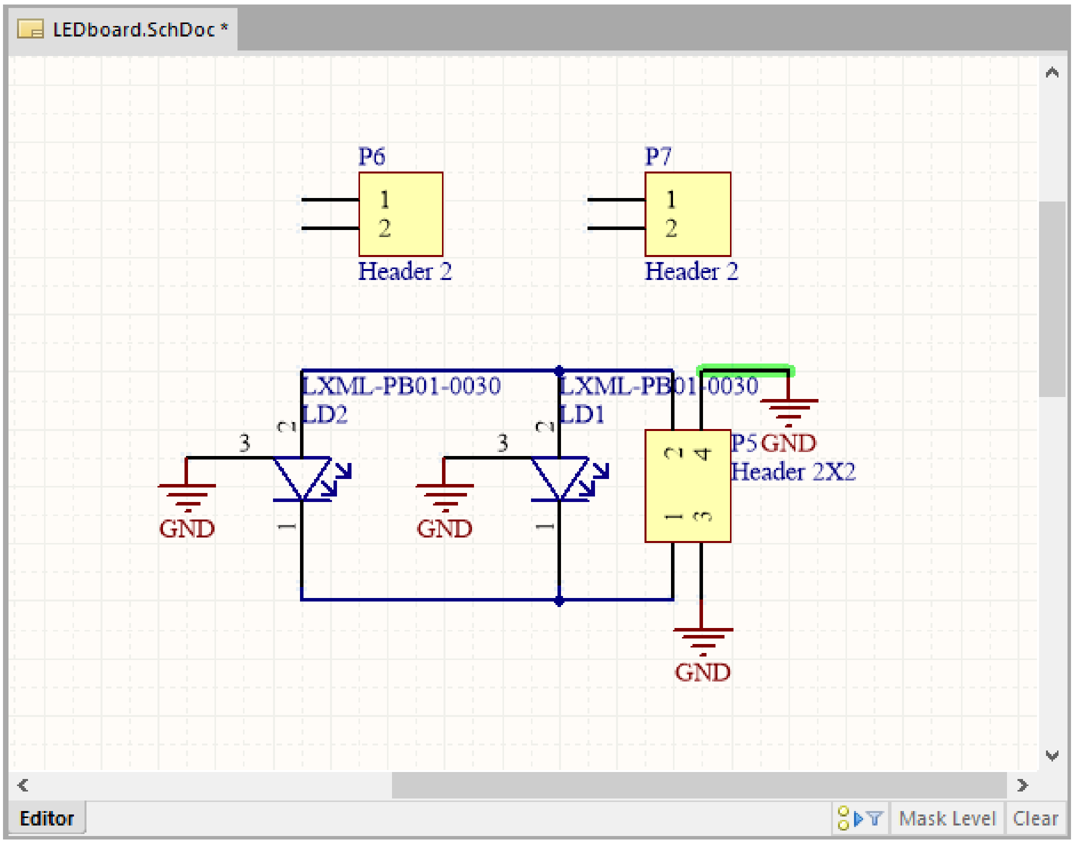


Figure 21: CFY Sensor LED PCB schematics.

B. Tables of Statistics – Detailed Analysis

In these statistical analyses, we only show the results for the CFY Sensor (Rev. A).

B.1 Homogeneity of Variance: Cochran's Q and Bartlett

The Cochran tests are significant, with the critical values of chi-square at 1% and 5% probability being much larger than the test statistics (see Table 8). This suggests that the parameters are homogenous in their variances (i.e. contain no outliers), thus rejecting the null hypothesis, and is therefore fit for Tukey tests. The parameters were, however, not significant according to the Bartlett tests. All critical chi-square values were lower than the corresponding calculated values, thus failing to disprove the null hypothesis, indicating an unequal variance among the samples.

Table 8: Cochran and Bartlett statistical tests on homogeneity of variance with date (degrees of freedom=8) as the effect, using data from CFY Sensor.

Parameter	**Test**	**Test statistic**	**Crit.Val. (α=0.05)**	**Crit.Val. (α=0.01)**
Fm'	Cochran	0.3877	≫	≫
Fm'	Bartlett	35.7724	15.507	20.090
Fs	Cochran	0.4346	≫	≫
Fs	Bartlett	41.0772	15.507	20.090
QY	Cochran	0.4372	≫	≫
QY	Bartlett	38.2634	15.507	20.090

B.2 Normality Test: Lilliefors

Fm' is a significant parameter according to the Lilliefors test, but Fs and QY are not significant for α=0.05. For α=0.01, the values are very close, and we therefore assume significance of all values at this critical value of chi-square, as there are a few outliers present in the data. These outliers were likely caused by measuring errors or plant response that could be attributed to factors such as temperature or position in greenhouse with respect to light source, and to the fact that plants can respond differently under the same circumstances. (See Table 9.)

Table 9: Lilliefors tests on normality of data with sample size n=90, using data from CFY Sensor.

Parameter	**Test statistic**	**$D_{n,a}$ (α=0.05)**	**$D_{n,a}$ (α=0.01)**
Fm'	0.0801	0.093	0.109
Fs	0.1159	0.093	0.109
QY	0.1139	0.093	0.109

B.3 Tukey Test

The groupings indicate particular significance in early stages of plant stress in the Fm' fluorescence. The maximum LA fluorescence was significantly higher already one day after treatment application. Throughout the test, the consistency fluctuates, but is generally significant until the 5th day. Similarly, for Fs measurements, significant mean differences are found until day 5, but not significant before day 3. However, for ΦPSII, we find significant means only for the two initial days after treatment application. (See Table 10.)

Table 10: Tukey test on individual parameter grouping with respect to day and treatment, using 95% confidence to test for significant differences of means on data from CFY Sensor.

Day	Fm'-C	Fm'-T	Fs-C	Fs-T	ΦPSII-C	ΦPSII-T
1	354.4	492.8	238.4	313.6	321.4	393.4
	B	A	A	A	B	A
2	358.0	374.8	282.2	335.2	214.2	104.6
	A	A	A	A	A	B
3	528.6	337.6	402.4	272.8	242.6	188.8
	A	B	A	B	A	A
4	499.2	460.4	362.8	343.8	274.0	251.2
	A	A	A	A	A	A
5	497.6	374.8	361.2	272.8	274.8	267.8
	A	B	A	B	A	A
6	445.8	365.8	326.8	264.4	267.4	270.6
	A	A	A	A	A	A
7	420.4	401.8	298.2	270.4	290.2	322.8
	A	A	A	A	A	A
8	452.8	444.4	321.4	310.4	289.4	298.4
	A	A	A	A	A	A
9	476.8	452.0	321.6	299.4	323.8	333.20
	A	A	A	A	A	A

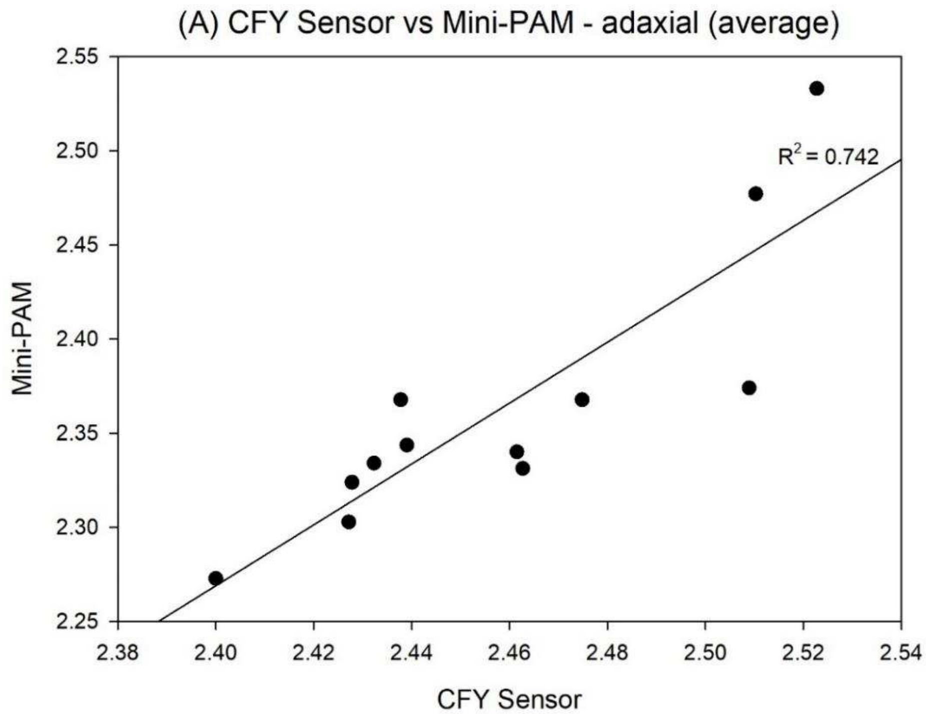
Additionally, the statistical analysis on yield revealed that the effect of date, on its own, on the means was significant. In Table 11, we see the grouping information on 10 measurements per day using 95% confidence interval.

Table 11: Tukey test on daily ΦPSII means, using CFY Sensor data.

Day	Mean	Grouping
1	357.4	A
9	328.5	AB
7	306.5	ABC
8	293.9	ABC
5	271.3	BCD
6	269.0	BCD
4	262.6	CD
3	215.7	DE
2	159.4	E

C. Regression Analysis on Average Fluorescence Values

In Figure 22 are the regression analyses using the average log values instead of the individual data points. Using average values, the correlations have improved by 15.4% and 15.8%, respectively, compared to plotting and calculating R^2 with raw values.



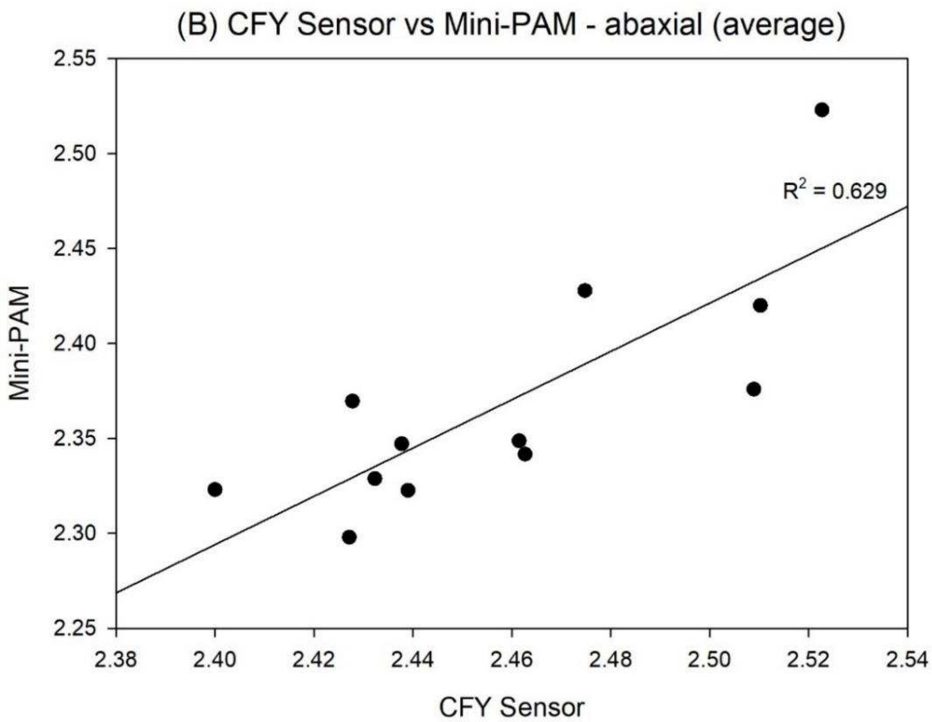


Figure 22: Log-transformed average yield for all plants over the course of nine days. (A) CFY Sensor abaxial vs. Mini-PAM adaxial; (B) CFY Sensor abaxial vs. Mini-PAM abaxial.