Chlorophyll Fluorescence Spectrometer

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***Abstract* --- Plants are vital to human culture. They form the backbone of cultural ceremonies, social situations, medicines, decorations, food sources, oxygen production, and agriculture. By using a chlorophyll fluorescence spectrometer (CFS), a measure of relative plant health can be obtained by considering the intensity of a chlorophyll sample's spectrum in the 600 to 700nm waveband. The CFS was designed so that a casual user can afford to purchase it, use it without difficulty, and see a relative measure of plant health that they can easily understand. The demonstratable design parameters are mobile device interactivity via Bluetooth, conducting an analysis in less than one minute, and imaging at least the 600-700nm waveband.**

***Index Terms* --- Spectroscopy, fluorescence, optical devices, resonance light scattering, wireless communication**

I. INTRODUCTION

A chlorophyll fluorescence spectrometer (CFS) is a device that observes the spectral range and intensity of the light emitted when a sample of chlorophyll is fluorescing. Specifically, this group's CFS is designed to observe the 600 to 700nm emission spectra of various chlorophyll sample in acetone when illuminated by a laser source with a center wavelength at 405nm. By analyzing emission spectrum of the sample, it is possible to determine the peak wavelength of emission. Another device could use this information to analyze the emitted light at the measured peak wavelength to determine various parameters about the chlorophyll sample. These parameters, such as Fv/Fm, are used to determine overall plant health.

The reason chlorophyll is used as a sample metric is due to its close relationship with the photosystem II (PSII) protein complex. PSII is the first protein group chain involved in photosynthesis in plants. Chlorophyll fluorescence intensity reveals information about how well PSII is using light energy absorbed by chlorophyll to power photosynthesis, and since PSII's efficiency is a general indicator of photosynthetic performance, chlorophyll fluorescence is the fastest method of testing the state of PSII which itself is an indicator of plant health. A weakly fluorescing sample may be an indicator of plant stress, disease, or other anomalies which may be causing the plant to use light energy inefficiently. Once this has been established, the plant could then undergo more thorough tests to determine what exactly is affecting the plant. The CFS's purpose is not to determine the exact cause of the plant's ailment, but only to indicate whether the plant is experiencing weaker chlorophyll (lower PSII efficiency) than normal.

In order to communicate data that the device reads, a Bluetooth module has been implemented to relay the information to a mobile phone via an app. This demonstratable parameter enables the device to be used by casual user who might not understand how to utilize more complex spectrometry software.

It is important that the device provides results in an adequate time. If the device is too slow, the user will not want to use the device and would seek other products to suit their needs. The limit for analysis time for this device was set to be less than one minute, starting from when the user starts an analysis to when the analysis results are displayed. This demonstratable provides a realistic parameter for the CFS should the product ever become commercialized, since speed of analysis is a metric that many potential users will use to compare similar products.

The last demonstratable is the spectrum which the device images. The system should be able to image light which emits in the 600 – 700nm waveband at the minimum since this is where the Chlorophyll a protein fluoresces most intensely. This demonstratable shows that the device does not image any light in the system which may not be an indicator of plant health.

II. OPTICAL SYSTEM

There are two parts to the optical system: the pump source and the spectroscopy instruments.

A. The Pump Source

The first section containing the optics of our device is home to the laser module, chlorophyll sample, optical slit, and their respective mounts. This small optical section is where the chlorophyll sample undergoes the process of fluorescence.

Each chlorophyll sample was made to a standard to ensure reliable operation of our device. A leaf of a known species of plant was broken down and gram of the leaf was weighed out and added to a 150mL acetone solution. After mixing for one minute, the solution was poured through a coffee filter and a small portion was added to a quartz cuvette to be analyzed by our system.

The cuvette was placed inside a custom mount which was designed and 3D-printed by our team. The sample is then illuminated by a 405nm laser module also held in place by a custom 3D-printed mount. An optical slit 200um wide was placed against the side of the cuvette mount parallel to the direction of the light emitted by the laser source as can be seen in Fig. 1. The 405nm light is absorbed by the chlorophyll in the sample and is re-emitted as red light due to the process of fluorescence. This red light is emitted in every direction and propagates into the spectroscopy section of the device via the optical slit.

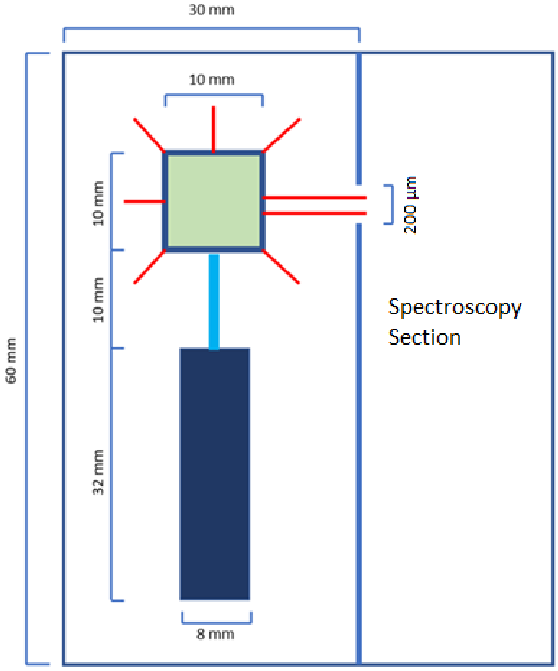


Fig. 1 Design for source and Chlorophyll sample housing

B. The Spectroscopy System

After the fluorescent light has passed through the slit, the light enters the spectroscopy section of the device. Housed within this section are two concave mirrors, a blazed rectangular reflective diffraction grating, and a CMOS sensor. It is here that the fluorescent light is separated into its component wavelengths and focused on to the sensor for spectrum analysis.

The components for the system were selected with great care. One of the engineering requirements for the CFS was that it be sized such that it is portable, can be carried, moved, or placed in any area that it needs to be placed in. As such, it was important to the design parameters that the system be made as small as possible while keeping costs below budget. It was discovered through discussing with faculty that lenses and mirrors commonly used in spectrometers have focal lengths of 40mm. For this reason, two curved mirrors with the nearest focal length, 50mm, were chosen for the project. The group went with silver-coated over aluminum-coated because, for the same cost, silver-coated provided a higher reflectivity in the 600-700nm waveband. The mirrors were chosen to have EFL=50mm and D=25.4mm (D is diameter), giving the system an f/2 setup. f/2 was chosen since it captures a great amount of light information and weak signal at the sensor was a chief concern during the primary stages of the project.

Since the device is in a Littrow configuration, a 300gr/mm reflective diffraction grating was chosen with a blaze angle of 4.3° (maximum efficiency at 500nm). The lower groove separation hinders spectral resolution, but due to the angle at which light is incident on the grating, it was decided that 300gr/mm was the best choice since, as grooves per millimeter increase, the allowable incident angle decreases as seen in Fig. 2. This grating was also the cheapest choice of those considered, fitting into the cost demonstratable rather nicely.

Light which is incident on a reflective grating from a non-normal angle is reflected at the angle θ_m as given by

$$\theta_m = \sin^{-1}\left(\frac{m\lambda}{a} + \sin(\theta_i)\right). \qquad (1)$$

For (1) and for the CFS, a is the groove spacing, λ=683nm, m=1 since the desired diffraction order is the first order, and θ_i is the angle of incidence. (1) is the equation which was used to find θ_m given certain values of a and θ_i.

	300 gr/mm	**600 gr/mm**	**900 gr/mm**
θ_m $(\theta_i = 15)$	27.64°	42.09°	60.87°
θ_m $(\theta_i = 30)$	44.84°	65.70°	OoR*
θ_m $(\theta_i = 45)$	65.81°	OoR	OoR

*OoR = out of (calculable) range

Fig. 2 Table of groove spacings vs incident angles

The final component in the spectroscopy section is the sensor. Most spectrometers use a linear sensor array to sample the spectrum seen from the diffraction grating. However, after researching other projects done by UCF students in the past, it was discovered that many projects struggled heavily with implementing and utilizing linear sensor arrays. It was suggested by faculty members that the CFS should incorporate a square array sensor and, in order to produce a spectrum, should have the pixels be summed column-wise until only one row of pixels remained. This would not only increase the amount of light sampled but would also be easier to interface with on the computer engineering side. Using a square array also allowed the group to interface with the array using basic camera software on personal computers for system calibrating and testing.

The chosen sensor was a monochromatic CMOS image sensor from the AR0130CS series by ON Semiconductor. The sensor has an active pixel array area of 4.86 x 3.66 mm2, comes with an on-board 12-bit ADC, has a peak spectral efficiency at 570nm, and a required supply voltage of 1.8 or 2.8 V depending on the application (digital or analog operation). A monochromatic sensor was chosen since all pixels share the exact same quantum efficiency curve, making it easier to correct for pixel QE in the signal processing stage.

The spectroscopic system was designed in a Littrow configuration. The original design can be seen below in Fig. 3.

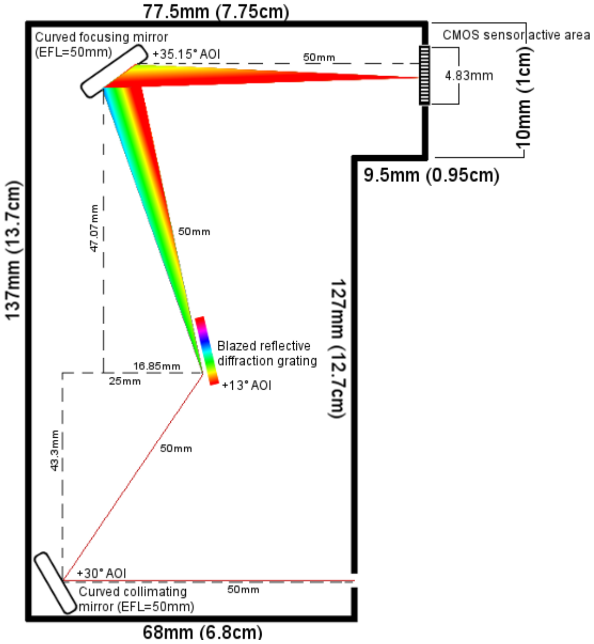


Fig. 3 Original spectroscopy section design

As can be seen in the above figure, in the original design, a distance of 50mm was between every optical component—that is, from slit to collimating mirror, from collimating mirror to grating, from grating to focusing mirror, and from focusing mirror to sensor interface. The difficulty encountered with this setup was that the system did not account for acute astigmatism which led to severe focusing errors at the sensor interface. There was also light signal clipping due to the intense angles in the original design, with the grating receiving light from the collimating mirror at a very off-normal angle of incidence. In order to remedy the problem, the system was redesigned into the design shown below. Note the changed location of the diffraction grating and sensor but the stationary positions of the collimating and focusing mirrors. This was the simplest change that could be made in order to eliminate astigmatism and clipping. The final spectroscopy system is shown in Fig. 4.

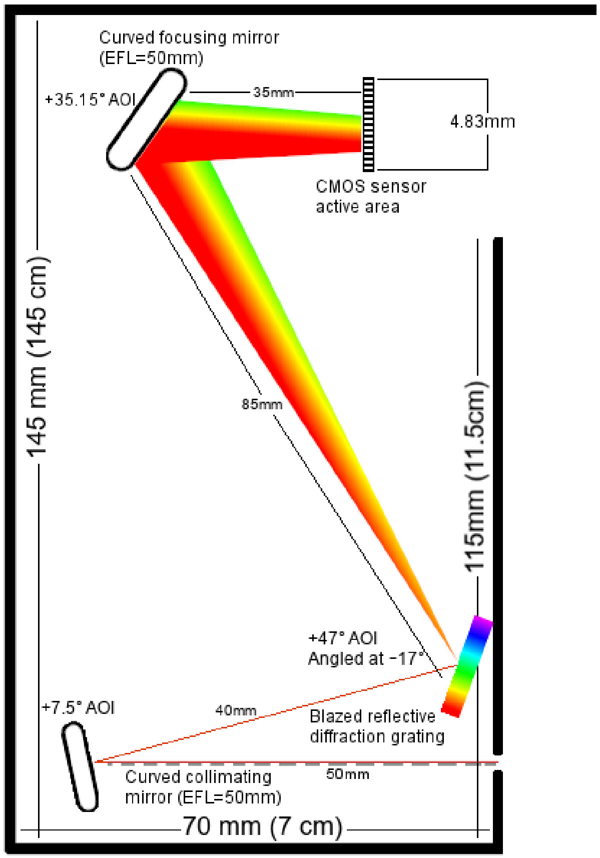


Fig.4 New spectroscopy section

III. ELECTRICAL SYSTEM

The electrical system consists of the STM32F407VE SOC which is a low power ARM M4 processor, the control for the laser device, the RN4020 Bluetooth low energy module, the Samsung R25 18650 lithium battery, the charging circuit and microUSB port, the AR0130 CMOS sensor from ON Semiconductor, and a pair of PCBs to facilitate each of the components, connected by a ribbon cable connector. On each of our printed circuit boards is the power supply circuitry for each component. The processor and Bluetooth module share a 3.3 V regulator in the AMS 1117 package. The laser module is powered by a 3.0 Volt regulator. The CMOS sensor receives 1.8 Volts and 2.8 Volts from a TPS62231DRY and a TLV71328PDBV. Multiple regulators are required to prevent interference, as well as an analog ground isolated from the digital ground. The electrical system in its entirety is shown on the next page in Fig. 5.

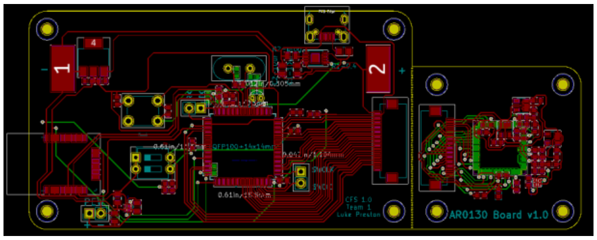


Fig. 5 Main PCB, left; image sensor board, right.

The PCBs are manufactured as a single unit, separated by a perforation. This is done to reduce costs in attaining the PCBs from the board house. The Bluetooth module overlaps the edge of the printed circuit board to minimize Bluetooth signal attenuation. The main PCB is equipped with a reset button, a power switch, debugging pins and the pinout for power to the laser module. An 8 MHz oscillator is installed as well as a 32,768 Hz crystal oscillator.

The passive components on the PCB (resistors and inductors) were designed in the 0805 and 0603 sizes. This was done to facilitate soldering by hand with minimal complications. The pinouts for the laser module power, SWCLK, SWDIO, UART_RX and UART_TX were implemented with 2.56 mm spacing to make the connecting our components and debugging equipment easier with standard jumper cables.

A. Microcontroller

The microcontroller which we selected for the Chlorophyll Fluorescence Spectrometer is the STM32F407VE. The purpose of this microcontroller is to control the image sensor, the laser module and the bluetooth module, to sum the pixels vertically to create a spectrum and to control the dataflow from the CFS to an android device. The reason we selected this part is that it is a low cost, low power component which has enough GPIO pins to interface with each of the components in our project as well as schematics available for the development board. The STM32F407VE is a more modern processor than some of the other options we looked at, including the MSP430FR2672. The STM32F407VE is a SoC which is based on the ARM M4 architecture.

B. Bluetooth Module

The bluetooth module which we selected is the RN4020 which is manufactured by Microchip. Our reasons for selecting this part are that it is an economical option that supports bluetooth low energy. This allows us to keep the power consumption low while still transmitting the necessary data from the Chlorophyll Fluorescence Spectrometer to the phone for use with our android app. The bluetooth module is also capable of communicating over UART, this means only 4 connections are required between the microcontroller and the bluetooth module. This allows us to reduce the complexity required of the PCB. The RN4020 fit well into our budget costing about $15 per part. Additionally, the footprint for the PCB is relatively compact compared to other parts we looked at. The space which the RN4020 takes up is 11.5x19.5, which is less than half the size of the ESP32.

C. CMOS Sensor Array

The CMOS sensor which we have chosen is the AR0130CSSC00SPBA0-DR. The reason for selecting this part is that it is a monochromatic sensor with an integrated ADC, which makes interfacing much simpler. While our microcontroller does have an ADC, it is less work to choose a sensor that does that for us. The AR0130CSSC00SPBA0-DR also operates in the correct waveband to measure data from our plant sample. Other options we looked at were a few linear arrays, but we were aware of other senior design teams which had faced complications with linear arrays so elected to avoid those. The other CMOS sensor which we considered was a unit from Renesas which was more than twice the price at $20.

D. Laser Module

The purpose of the laser module is to cause the chlorophyll sample to fluoresce, and this spectrum produced is what we measure in order to determine the relative health of the plant sample. The laser device which we decided to use for our project is the MZH8340550D-AL01A which is manufactured by Zhuhai MZlaser Tech Co. The reeason we chose this part is that the frequency of light produced is appropriate at 405 nm, with a beam size and intensity that will work for our application. The power draw is acceptable at about 50 mW and the price is lower than the other modules we looked at. At $9.00 per unit the MZH8340550D-AL01A fits well within our budget.

IV. SOFTWARE SYSTEM

The software for the CFS consists of embedded software, which powers the measurement device, and a mobile application, which allows users to interact with the measurement device. The two separate programs interact via the Bluetooth Low Energy wireless communication standard.

A. Embedded Software

The embedded software for the CFS is the firmware that executes on the microcontroller on the measurement device. Due to the low-level nature of this software, it has been written using the C Programming Language.

The development has been performed using the System Workbench for STM32 Integrated Development Environment, due to its many features geared towards ARM firmware development. The main purpose of this software is to coordinate communication between the sensors on the physical device, and to facilitate the transfer of sensor data to the mobile application for further processing and visualization.

Ensuring that our microcontroller properly interacts with the CMOS image sensor is pivotal to the successful function of the CFS. The image sensor measures the amount of light hitting each individual pixel on the sensor, and reports that data to the Microcontroller. Interaction with the image sensor is achieved via 2 separate digital connections. To configure the image sensor, we use an I2C connection to write the desired configuration values to the different configuration registers on the senor. Unlike the sensor configuration, the pixel data is not transmitted over an I2C connection. Instead, the sensor sends the raw pixel data over a parallel data connection line, which is connected to the microcontroller.

Because we have a limited amount of RAM memory, we will not be able to store all pixels in memory at the same time; to get around this, we decided that we only need to store the accumulation of all pixels in each column instead of the individual value of each pixel. This means that in our algorithm, once a pixel comes in, the value of that pixel will be added to a variable holding the sum of all the pixels with that same X-axis, which is stored in an array. With this method, we will only need to store one array with an integer for each column, which is much less than storing an integer for each pixel. Once all pixels have been iterated over, we send the values of the accumulators over Bluetooth to the mobile application for further processing.

The embedded software is also in charge of communicating with the RN4020 Bluetooth module. This module features an ASCII command API that allows us to communicate with the module easily over a UART connection. When interacting with the Bluetooth module, the software must accept connections, keep track of the connection state, receive signals from the mobile application, and send data to the mobile application.

B. Mobile Application

The CFS' Mobile Application will serve as the main user interface for the device. This software consists of a mobile application which configures the CFS to prepare it for sample analysis, as well as initiates any analyses and receives the sensor data resulting from that analysis. The mobile application is be tasked with storing all analysis results and providing the end-user with the results in a multitude of graphics and result summaries which ensure that the results of the analysis are both simple enough for a layman to get value out of the results, but also detailed enough for expert analysis. Throughout the initial design process for the mobile application, the team decided to limit the target operating system for the mobile application to Android. This choice was made due to Android being easier to develop for and test on, due to Apple having more restrictions on application development and publishing for iOS.

The graphical user interface of our mobile application has been designed to make sure that the product can be used by non-technical users. When the user opens the app a Bluetooth Low Energy scan is automatically initiated, and a list of nearby Bluetooth devices is given to the user to choose from. After selecting their CFS device from the list of Bluetooth devices, a user can choose from a list of analysis options which include starting a new analysis, viewing stored analysis results, and comparing current analysis results to a reference sample.

To allow the user to store and retrieve analysis results, the application uses a database stored locally on the phone to store data. For our application, we have chosen SQlite3 as our database management system because it is lightweight and does not require an extra program to be running on the mobile device. The SQLite database will mainly be used to store 2 types of data: CFS device data and analysis result data.

C. Wireless Communication

When designing the CFS, we knew we wanted a mobile application to be a component of the product so that the product could be more portable and easier to use. Because of this, it was important for us to find a wireless communication protocol that would allow us to connect the CFS with a mobile application, while keeping the development difficulty and power consumption of the device relatively low. Throughout our research we found that, out of the wireless communication technologies analyzed, Bluetooth stood out due to its overall low power consumption, low implementation difficulty, and high device compatibility. This left us needing to decide between Bluetooth Low Energy and Bluetooth Classic; at the end of the research phase of our development, we decided that Bluetooth Low energy was the best fit for this product. The main deciding factor was the significant reduction in power consumption for Bluetooth Low Energy. Although Bluetooth Low Energy is compatible with slightly less devices, there are still enough mobile phones which support Bluetooth Low Energy to make it a viable choice.

V. TESTING

Testing for the CFS took place in two phases: component testing and integration testing.

A. Component Testing

Component testing for the device involved checking that all components selected and purchased worked as specified by the manufacturer. Component testing also included making sure that the mobile application for our demonstratable parameter worked as planned. Fig. 6 is a table of components which were tested and the results of those tests.

Component	**What Tests**	**Pass? (Y/N)**
Laser module	Spot size, current-power curve	Y
Diffraction grating	Reflectivity, creating spectrums	Y
AR0130 Sensor	Pixel data parsing, communication with MCU	Y
STM32 CPU	IDE connectivity, deployment of software	Y
Bluetooth module	Connectivity to MCU, expected output of data	Y

Fig 6. Table of tested components for CFS

B. Integration Testing

Integration testing involved building the device in its entirety and testing every component as was intended in the original design of the device. Due to the COVID-19 pandemic in the Spring 2020 semester, full integration testing with all planned parts could not be completed. Demo parts were used in the final demonstration, specifically with the power system, PCB, and wireless communication interfaces. The CFS was tested with these demo parts and the following results were compiled from those integration tests. The CFS was also not fully machined. Instead of being able to operate even in brightly lit environments, the device could only operate in a completely dark room where there was no light noise.

The CFS was calibrated using a red laser source and a green laser source with known wavelengths. Pictures were generated using our spectrum software and images captured using the default Camera app on a Windows laptop. Fig. 7 and 8 show the side-by-side comparison of the Camera imagery (left) and the spectrum produced by our software (right).

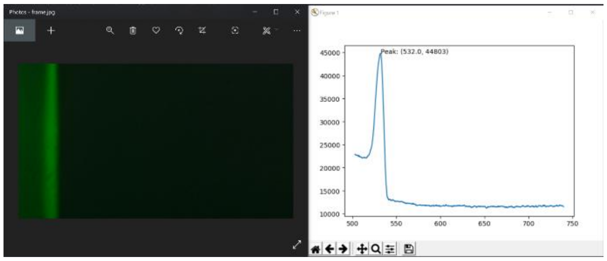


Fig. 7 Green laser source showing a spectrum on our spectrum software on the computer.

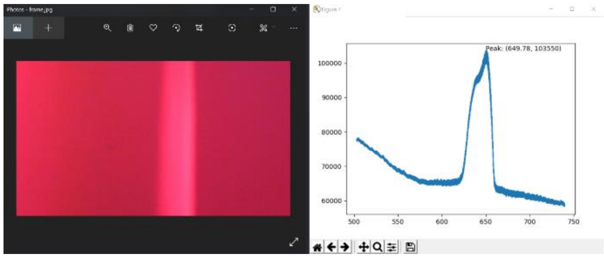
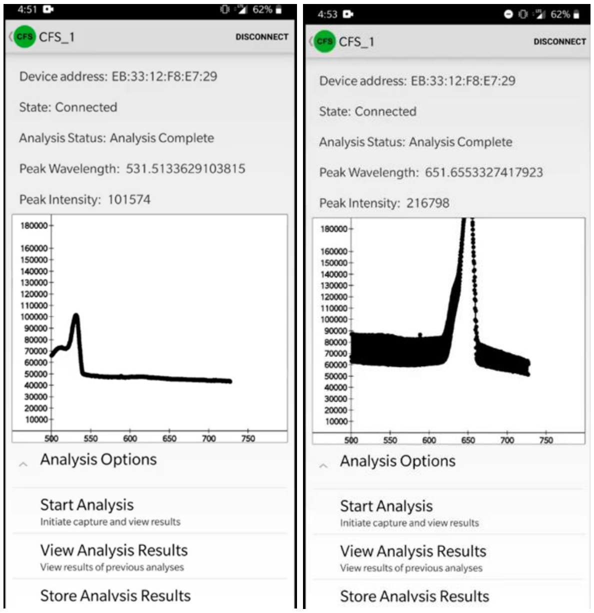


Fig. 8 Red laser showing a spectrum on our spectrum software on the computer.

Once the system was confirmed to be working on the computer and producing a spectrum that matched the peaks of our laser sources, the next step was to confirm that the system was working on the mobile app. The following two pictures in Fig 9. are from the same two laser sources at the same positions with spectrums produced by the CFS mobile app.



Device address: EB:33:12:F8:E7:29

State: Connected

Analysis Status: Analysis Complete

Peak Wavelength: 531.5133629103815

Peak Intensity: 101574

Device address: EB:33:12:F8:E7:29

State: Connected

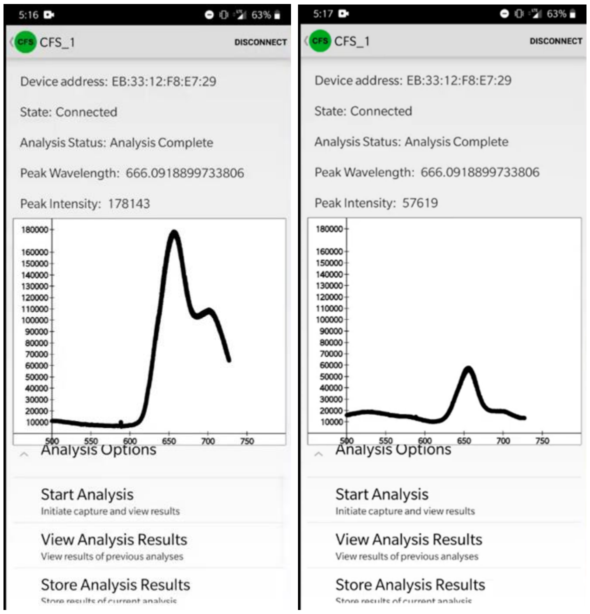
Analysis Status: Analysis Complete

Peak Wavelength: 651.6553327417923

Peak Intensity: 216798

Fig. 9 Green laser spectrum as seen on the CFS mobile app, left; red laser spectrum as seen on the CFS mobile app, right.

With the system confirmed to be calibrated, fluorescence testing commenced. A sample of chlorophyll dissolved in acetone was inserted into the device and illuminated by the blue laser. The mobile app started the analysis and produced the spectrum graph shown at the left in Fig. 10.

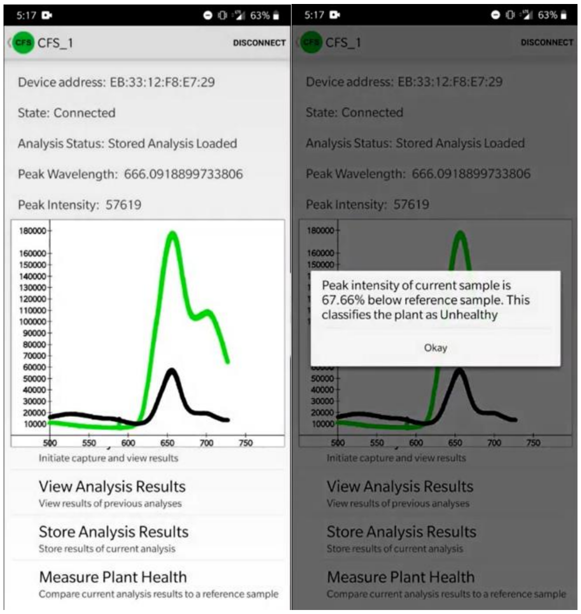

Device address: EB:33:12:F8:E7:29
State: Connected
Analysis Status: Analysis Complete
Peak Wavelength: 666.0918899733806
Peak Intensity: 178143

Device address: EB:33:12:F8:E7:29
State: Connected
Analysis Status: Analysis Complete
Peak Wavelength: 666.0918899733806
Peak Intensity: 57619

Fig. 10 Fluorescence spectrum test of chlorophyll a using chlorophyll freshly dissolved in acetone (left) and chlorophyll dissolved in acetone several weeks prior (right).

This test shows a peak wavelength of 666 nm, which is close to generally accepted chlorophyll *a* peak wavelengths. [1] [2] [3] Another notable feature of the graph is the secondary bump on the right side of the spectrum. This closely matches other chlorophyll *a* spectrum characteristics since there should be a small plateau at the edge of the spectrum which trends from 700nm toward the low-mid 700s. [4] It can then be said that the CFS successfully imaged the chlorophyll fluorescence response of a chlorophyll sample excited by a blue laser centered about 405nm.

A second test was performed with an older chlorophyll sample. This spectrum is seen in Fig 10. Note that the measured wavelength of the second sample is identical to that of the first sample. Though more difficult to see, the graph shown in Fig. 10 does also carry the characteristic second bump on the right side of the spectrum. The mobile app for the CFS then compares the peak intensity of the chosen sample to the reference sample and determines whether relative health. If the intensity of the chosen sample's peak is less than 40% of that of the reference sample, the chosen sample is said to be unhealthy. This can be seen in Fig. 11 using the spectrums from Fig. 10.


Device address: EB:33:12:F8:E7:29
State: Connected
Analysis Status: Stored Analysis Loaded
Peak Wavelength: 666.0918899733806
Peak Intensity: 57619

Peak intensity of current sample is 67.66% below reference sample. This classifies the plant as Unhealthy

Fig. 11 Fluorescence spectrum comparison of chlorophyll a using chlorophyll freshly dissolved in acetone (green) and chlorophyll dissolved in acetone several weeks prior (black).

VI. CONCLUSION

The CFS has seven engineering requirement specifications: build cost, device volume, power delivery, Bluetooth communication, radio power consumption, analysis time, and spectrum. Three of these (spectrum, Bluetooth communication, and analysis time) were chosen as demonstratable for the project. The build cost was 400 USD which was less than the 500 USD specified by the group, so this requirement was met. The device volume was 1958.4 cm^3 which was less than the 4000 cm^3 specified by the group, so this requirement was met. The power delivery for the device was 3.61 Watts which was less than the 5 Watts specified by the group, so this requirement was met. The device used Bluetooth Low Energy to communicate with a mobile app as specified, so this requirement was met. The device had a radio power consumption of 39.6 mW which was less than the 50 mW specified by the group, so this requirement was met. The device analyzed a sample in 40 to 45 seconds which was less than the 60 seconds chosen by the group, so this requirement was met. The device was able to image a spectrum from 500 nm to 740 nm which was greater than the 600 to 700 nm spectrum specified by the group, so this requirement was met. All seven engineering requirements are met and three of these have been successfully demonstrated.

During construction of the physical housing of the device, the COVID-19 pandemic struck, and the housing was unable to be completed since the machine shop was closed along with UCF's campus. As such, the device is only able to be operated in a completely dark room, defeating the ideal goal of having this device usable by a casual user in any amount of light. The sensor is not currently mounted into the baseboard and into its wall, making calibration difficult but not impossible to complete. The laser module is powered by a DC power supply since the PCB and battery source designed to power the device is not integrated into the system. The chlorophyll samples being tested had to be made by the team outside of a laboratory environment, but this is similar to what the team would expect a casual user to have access to and is not considered a positive or negative characteristic of the testing procedure.

Since the CFS determines both peak emission wavelength and peak fluorescence intensity (Fm), two future metrics that the CFS could measure are maximum quantum yield (Fv/Fm) and the fluorescence decrease ratio (Rfd). The maximum quantum yield is used to determine if a sample is healthy based on the variable fluorescence (Fv) of chlorophyll when the sample is pulsed with a red LED and a saturating white light source. If Fv/Fm is between 0.74 and 0.85, the sample is healthy. The fluorescence decrease ratio $R_{F_d} = (F_m/F_s) - 1$, where Fs is the steady state fluorescence after the sample has been illuminated for several minutes, is used to determine if a sample is getting enough sunlight. If Rfd is greater than 2.5, the sample is receiving enough light and is healthy. With these metrics, it would be possible to see both the efficiency of the energy exchange between chlorophyll and the PSII protein and whether the plant is getting enough sunlight in the first place.

ACKNOWLEDGEMENTS

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BIOGRAPHIES

Robert Bernson is a current senior at CREOL studying to earn his Bachelor's of Science in Photonic Science and Engineering with a Minor in Mathematics. Robert currently plans on joining the Photonics Packaging Team at the Tyndall National Institute while earning his PhD at University College Cork.

Samuel Knight is a current senior at CREOL studying Photonic Science & Engineering. He will obtain his bachelor's from UCF in May 2020. After graduation, he plans to enter the work force and dedicate time to expanding his professional network, his photonics career, and his writing career.

David Maria is currently a senior at the University of Central Florida and will receive his Bachelor's of Science in Computer Engineering in May of 2020. After graduation, David will be joining the Cisco Talos Incident Response Team as an incident response consultant.

Luke Preston is currently a senior at the University of Central Florida and will receive his Bachelor's of Science in Electrical Engineering in the Summer of 2020. He has plans to join the work force and continue to improve his technical skills as he advances his career in the electronics industry.

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