

# Engineering a Chloroplast Movement Sensor

University of Twente – Bachelor Assignment Creative Technology

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03-07-2020

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## Abstract

To measure the chloroplast movement of mutants for virtual farming, a method is needed for in-field use. Mutation in chloroplast movement behaviour can have a great impact on the energy efficiency of a plant and therefore the growth process and biomass of that plant. This can be very useful in vertical farms where lighting conditions can be controlled. This study aims to engineer a chloroplast movement sensor for in-field use, with an accompanying data visualisation and user interface (UI). Chloroplast movement is the main subject of this project and is a protection mechanism of chloroplasts against harmful light which can damage the chloroplasts.

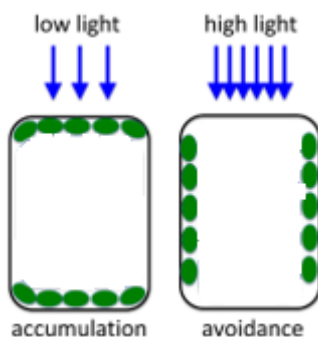
The designing process of the sensor is based on research into chloroplast movement and measuring methods. The sensor was tested and based on this testing a data visualisation and UI were designed. The test results from the sensor showed that the sensor can measure the expected behaviour in a controlled environment but not in-field. The data visualisation and UI were user tested which gave mostly positive results and are a good basis for further steps in the human centered design process. The results indicate that the sensor is mostly functional but further testing is needed and some adjustments have to be made to make it ready for in-field use. The data visualisation only needs small alterations and the UI needs further designing and testing. Overall the engineering of the sensor was somewhat successful and is a good first step into the right direction towards a final product.

# 1. Introduction

For as long as civilisation exists, man-kind has been cultivating the land and plants around them. This includes selecting plants to get the best yield, pest resistance and energy efficiency amongst other factors. Selection of plants still happens today. A next step is to use mutant plants for vertical farms. They can be selected on a mutation that can help grow more biomass in the controllable environments of vertical farms. The mutation that these plants can be selected on has to do with the photosynthesis process in their cells, in the chloroplasts to be specific. This introduction will explain how a specific behaviour of chloroplasts links to photosynthesis and the usability of mutations in this behaviour in vertical farms.

Chloroplasts are organelles of a plant cell where photosynthesis takes place. Organelles are subparts of a cell, they all have a specific function within the cell and are like organs for animals. Photosynthesis is the process where light energy is used to produce chemical energy which plants use to grow. [1] In order to produce enough energy, the chloroplasts need sufficient light. If chloroplasts absorb more light, they produce more energy, until there is enough light to saturate the photosynthesis.[2] Any extra light following saturation of photosynthesis is considered excessive light. If excessive light lasts for a longer period of time the chloroplasts might get damaged.[3] In order to prevent this damage, the chloroplasts can move around within the plant cell and avoid exposure to harmful light. Such an adaption mechanism is very useful because a plant in nature will encounter a varying pattern of light due to clouds and overhanging vegetation. [4]

The chloroplasts change the light intensity they experience by changing their location, as mentioned above. There are two characteristic positions described: the accumulative positions and the avoidance position. In the accumulative position, the chloroplast is located alongside the periclinal walls of the cell. These are the walls parallel to the top and bottom of the leaf. In this state, which occurs under low light, the chloroplasts are the most exposed to the light and in case of harsh light they might get damaged. In saturating light, chloroplasts move to the anticlinal walls. These anticlinal walls are perpendicular to the top and bottom of the leaf and in line with the incoming light. This way, chloroplasts avoid excessive light and prevent photodamage by using the shade of other chloroplasts, hence the term avoidance state. Photodamage is damage caused by too much energy from the light, it oxidises the chloroplast. [3] The chloroplast behaviour can be seen in figure 1.1.

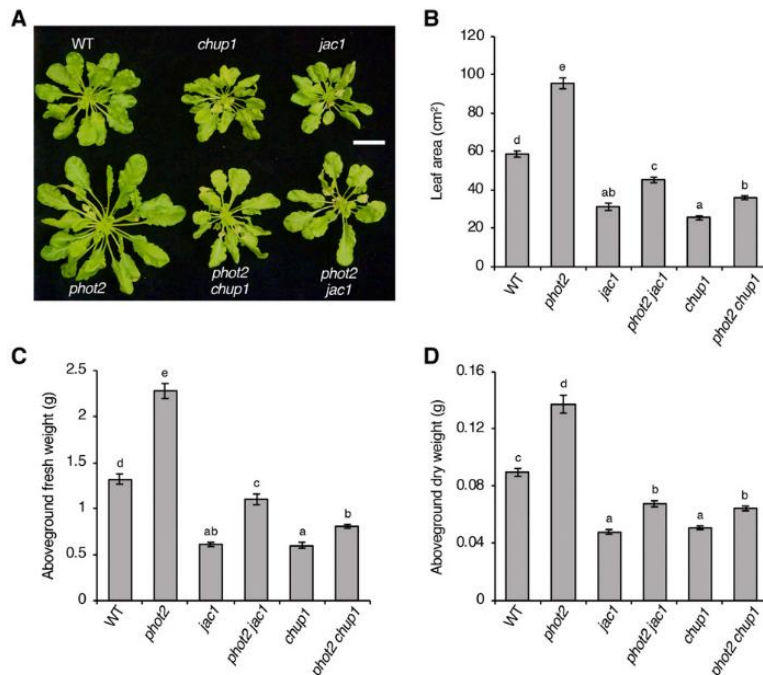


*Figure 1.1: A plant cell with the chloroplasts in two states: accumulation and avoidance state. The green dots are the chloroplasts. The position of the chloroplasts in the cell is dependent on the light intensity: Left, the accumulation state. The chloroplasts are located at the periclinal walls. Right, the avoidance state. The chloroplasts are located at the anticlinal walls. [3]*

This chloroplast movement mechanism is missing in some plants due to a genetic mutation. These mutations have a negative impact on the plant in a natural setting. In the case where the chloroplasts don't display any avoidance behaviour, they might get damaged and therefore cannot produce any energy anymore, which is needed for vital processes in the plant. However, these mutations might prove useful in a setting like a vertical farm. Here these mutated plants can, for example, have a constant production of energy under continuous strong light. The reason for this is that chloroplasts go into avoidance state at a relatively low light intensity, even though they would not experience photodamage at that level of light intensity. However, the photosynthetic capacity increases under higher levels of light intensity. Therefore mutants can stay in the accumulative state under a higher light intensity than non-mutant plants, thus have a higher photosynthetic capacity without getting damaged. [5] This project is part of the Plantenna project which will use a sensor in order to find crop plants that lack chloroplast movement. This way plants can be found that produce more leafy green biomass such as lettuce. To select these plants this sensor has to measure how large the chloroplast movements are compared to *Arabidopsis* for example, which is used in previous studies. [6]

When measuring chloroplast movement in crop plants the best place to do this is in the field. This generates the most accurate picture of how the plant will behave on the land or a vertical farm. Measurements will be most valuable if they are done in the most relevant place: in the field. Meaning either on a farm field, in a greenhouse, or on a vertical farm.

In the experiment done at the Biology Department of St. Mary's College of Maryland by Gotoh et al. it was proven that the mutant missing certain photoreceptors used to get the chloroplasts in avoidance state, had a greater biomass and leaf size. Photoreceptors are a group of sensory proteins in a cell that detect light. The control group was treated under the same circumstances, thus proving that this mutation has a positive effect on the photosynthetic capacity and therefore the growth of the plant. [5] In figure 1.2 the clear difference can be seen in weight and leaf size between the different mutants, where *phot2* is that mutant that could only stay in the accumulative state.



*Figure 1.2: The comparison of biomass and leaf size of a wild-type and different mutants of the Arabidopsis (Arabidopsis thaliana) where phot2 is the mutant which chloroplasts stay in the accumulative state. A: photographs of the plants. B: A graph of the leaf area of the different plants. C: A graph of the fresh weight of the different plants. D: A graph of the dry weight of the different plants. [5]*

In order to measure the movement of chloroplasts in the field, a sensor has to be designed and implemented in a measuring instrument. This instrument has to display data or transfer data to a laptop with subsequent visualisation. In order for the user to understand the data and to comfortably compare results of multiple measurements of one plant, for example, the data has to be visualised properly so the user can understand it. To design this data processing and the data visualisation research is needed on various ways of measuring chloroplast movement, what type of data comes out of those measurements, design requirements and user requirements. Literature studies and interviews will be used to gather the necessary information to

*Engineer a chloroplast movement sensor and the accompanying data visualisation and user interface.*

To answer this main research question some sub-questions will have to be answered first:

- *What is the mechanism behind chloroplast movement?*
- *How can the movement or the position of chloroplasts be measured?*
- *How can the movement or position be displayed and visualised?*
- *What is a fitting user interface design for this application?*

The first question has partly been answered in this introduction, it will be further discussed in the next chapter, together with the other questions.

One of the pre-set requirements, from the client, for the sensor is that it will have to use either transmittance or reflectance as a measurement technique, or preferably a combination of both. These techniques are widely used and not too expensive or complicated but still reliable and precise enough.[3] Both these techniques rely on exposing the leaf of a plant to a light source and measuring the difference in light intensity coming from the leaf. [7] Both methods work well, however, one might be more suitable for the application of this sensor. It can be dependent on plant species, measuring speed, or light intensity. Therefore, more literature research will be done on this topic and based on the findings, a preliminary experiment will be carried out. Findings will be filled in a pivot table, which can be seen in Table 1.

*Table 1.1: Pivot table to be filled in in chapter 2 based on additional research. The table will be filled in using a scale from 0,+,++,+++, depending on how much the measurement outcome using either transmittance or reflectance is influenced by one of the factors mentioned in the top row.*

	Plant species	Measuring speed	Light intensity
Transmittance			
Reflectance			



## 2. Literature research

Before starting the design process for a chloroplast movement sensor, some research has to be done. This research focusses on answering the sub-questions mentioned in chapter 1. This chapter is divided into sections that fit the sub-questions. Some choices will be made regarding measurement method, data visualisation and user interface. The choices will be explained based on why a certain technique best fits the scope of the project, available resources and goal of the project.

### 2.1. Chloroplast movement

The basic principle and use of the chloroplast movement have been explained in chapter 1. Further details will be discussed in this chapter.

The chloroplasts move to periclinal or anticlinal walls of the plant cell, the reason was elaborated before, the mechanism behind it will be explained here. It starts with the light that falls onto a leaf, photoreceptors absorb the light. Different types of photoreceptors exist, phototropin 1 (phot1) and phot2 are important for chloroplast movement. These receptors detect weak blue light and then send a “signal” to the chloroplasts. What this “signal” is exactly is not yet known. [3] This leads to the accumulation response, reacting to weak light. The avoidance response is triggered only by the phot2. A mechanism is set in place by the phototropin. This process uses different molecules that anchor the outside wall of the chloroplast to the membrane of the cell. This way the chloroplast is slowly pulled in one direction. With this mechanism, plants obtain a better balance between photo damage avoidance and light capture. [2]

The process is rather slow and it takes a few minutes to take effect, they move around  $1\text{ }\mu\text{m}/\text{min}$ . [3] Plant leaf cells are typically between 27 and  $7\text{ }\mu\text{m}$  in diameter and can differ depending on water content, pressure and plant species. [8] The chloroplasts can keep moving for an hour and the effect can get more intense during this time, it is important to take the duration of the effect into account when measuring chloroplast movement. [9]

### 2.2. Measurement methods

There is a wide range of different methods available for the measurement of chloroplast movement. There exist very sophisticated methods, using expensive equipment, which are especially well equipped to examine the exact movements of the chloroplasts. Microbeam, time lapse photographic analysis of movement, and confocal and TIRF microscopy are among these approaches. [3] These will not be discussed in any greater detail, because of their insignificance for this project because they can be ruled out as suitable methods for in field use.

On the other hand there are processes like the band method, where a strip of the leaf is exposed to light, this band will turn light green due to the absence of chloroplasts at the periclinal walls. Fixed-cell sectioning is a method where the cells are fixed with chemicals and then closely observed. These methods involve less costly equipment and are less sophisticated and precise. [3]

Another popular and relatively less expensive but effective way of measuring chloroplast movement is reflectance or transmittance of red light from the leaf. These approaches are both based on either red light reflectance or transmittance from the leaf and are interesting for this project because of the cost and accuracy ratio. [10]

### 2.2.1. Reflectance and transmittance

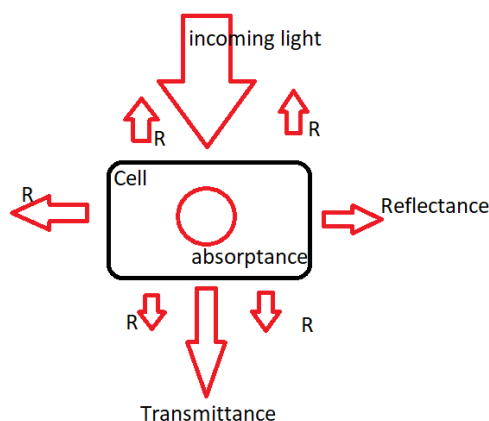
Both methods use light directed at a leaf and preferably a sphere to capture the light that is either reflecting off the leaf or transmitted through the leaf. For transmittance the leaf is exposed to light from all directions and the light that is transmitted will leave the leaf in all directions, hence the sphere for capturing light. In case of reflectance, the leaf is exposed to a beam of light and the reflected light will scatter. [9] The light that has to be used to measure reflectance or transmittance has to be red light. This will not affect the phot1 and phot2 receptors and therefore not trigger a change in the chloroplasts position. [11]

#### 2.2.1.1. Relation to chloroplast position

The reflectance and transmittance will help to find the absorbance of the leaf (A). Equation 1 shows how the absorbance can be calculated using the light used to illuminate the leaf, represented by the number 1, the transmittance (T), the reflectance (R), and the earlier mentioned absorbance (A). A, R, and T are all fractional to the light source. [9]

$$1 = T + R + A \quad (1)$$

This formula can also be visualised in a schematic, as seen in figure 2.1.



*Figure 2.1: A schematic of how incoming light is absorbed, transmitted, and reflected. The incoming light falls on the cell, part of the light is absorbed in the cell, a part is transmitted through the leaf, and another part is reflected in all directions.*

To link transmittance to the location of the chloroplasts we look at the effect the location of chloroplasts has on the transmittance. The transmittance decreases when the chloroplasts are in the accumulative position. [7] There are two different effects that cause either a decline or incline of absorptance and transmittance vice versa. The package effect is present in the avoidance state, causing the light to go through the leaf and being less obstructed by the chloroplasts. In this case the leaf works like a sieve and transmittance increases. The detour effect is caused by the light hitting the chloroplasts and is being scattered and absorbed, this means less transmittance. The detour effect is present during the accumulative state. [7]

Reflectance consists of internal reflectance ( $R_i$ ) and external reflectance ( $R_e$ ), this is due to some of the light remaining in the leaf and being scattered there. On both sides of the leaf the  $R_i$  and  $R_e$  will be different due to the different tissue in the leaf. [7] If measured at the same half of the leaf for every sample this will not differ too much and the difference can be negligible. [9] Reflectance increases during the avoidance response. [2]

Under diffuse light, meaning light coming from all sides using a sphere, the relation between reflectance and transmittance with absorptance as its function can be expressed as a rewritten version of equation 1. [7]

$$A = 1 - T - R \quad (2)$$

The transmittance and reflectance can be measured so the absorptance can be calculated and related to the position of the chloroplasts. There will be a gradient in the absorptance, depending on the plant species and the moment of measurement.

Previous research and projects often use either reflectance or transmittance. This is of course also possible. When T or R increases the other increase as well, however not in the same rate. This is because of the principles both methods are based on. The position of chloroplasts might have a different impact on transmittance and reflectance.

T and R do not scale exactly the same if A is changed. However, if an increase in either T or R is measured, the other will also have increased but in a different ratio. If one increases, the other cannot decrease because the cause of change for both is the different location of chloroplast. The location affects both T and R based on scattering and absorptance. This means T and R can be measured separately and are still good measures of A, even if used independently. In this project both will be used, this does make it different than most previous research. The main difference is measuring both T and R can give better insights in other processes that influence T and/or R by comparing the outcomes. This allows for a better assessment of the chloroplast movement.

#### 2.2.1.2. Comparison of external factors

As mentioned in chapter 1, both measurement methods can be influenced by a number of factors. Here Table 1.1 will be completed, creating Table 2.1. Depending on how much the outcome of the measurement is influenced by the corresponding factor the cell of the table will be filled with a 0,+,++,+++ accordingly. 0 meaning that the measurements are not influenced and +++ meaning that the

measurement will be greatly influenced by the factor. With this table we compare transmittance and reflectance based on their dependency. All comparisons discussed here are based on findings from earlier in this chapter.

Plant species have a very different response to light. Very thin leaves will have a higher transmittance, whereas very glossy leaves will have a higher reflectance. The thickness of leaves does influence transmittance more than reflectance. [10]

Measuring speed, as mentioned before, is very important. Chloroplast movement takes a few minutes to be visible and can still be in process after thirty minutes or even two hours. [4] Stopping too early can exclude a part of the behaviour in the measurement and therefore give an incomplete result. To make sure this does not happen, measurements should be done over a long period of time, start as early as possible and ending after there has been no significant change for a few minutes.

Light intensity of the red light should be consistent throughout the same measurement. If it is not consistent the measurements will be incorrect and cannot be used for determining chloroplast movement or location. This is because the measurements are described as a fraction of the initial light intensity. If the initial light intensity changes, the measurement has to be adjusted to fit the new initial value. This is very hard to do.

The light intensity can be altered for different measurement moments. This does not have an effect on the outcome because the transmittance, reflectance, and absorbance are always described as a fraction of the light intensity of the light source. However the intensity should be between the 5 and 1500  $\mu\text{mol}/\text{m}^2/\text{s}$ . In Table 2.1. the goal is to see differences in between measurements, therefore the first comment on change during a measurement will not be considered. The conclusion that it does not have an effect between different measurements will be used for this purpose.

*Table 2.1: Pivot table to be filled in in chapter 2 based on additional research. The table is filled in using a scale from 0,+,++,+++, depending on how much the measurement outcome using either transmittance or reflectance is influenced by one of the factors mentioned in the top row.*

	Plant species	Measuring speed	Light intensity
Transmittance	+++	+++	0
Reflectance	++	+++	0

The conclusion from this comparison table is that both methods do not differ on these measurement factors. However, as discussed in chapter 2.2.1.1. there are differences and advantages of using one or the other, or using both.

### 2.2.1.3. Challenges

Most experiments are being done under diffuse light. This requires a reflective sphere around the leaf with the sensor and light attached to it. It is an option to attempt to recreate such a sphere in order to

expose the leaf to the most optimal lighting conditions and to be able to measure all around, however, it will pose a challenge to do this at home. Something to keep in mind is that another solution has to be found or that the data gathered from the leaf might not be as accurate as expected. Inaccuracies in the measured data can be caused by errors in the measurement instruments or faults in calibration.

The light source might be influenced by temperature. [4] This can influence measurements because it can change during a measurement. This will have an impact on the outcome as discussed in chapter 2.2.1.2. Important to realise is that most factors that might influence the setup are kept the same. The graph with the specific behaviour for a 5 mm in diameter red LED from Vishay can be found in figure 2.2. The graph is specific for LED, however, the behaviour is general temperature related behaviour for simple LEDs.

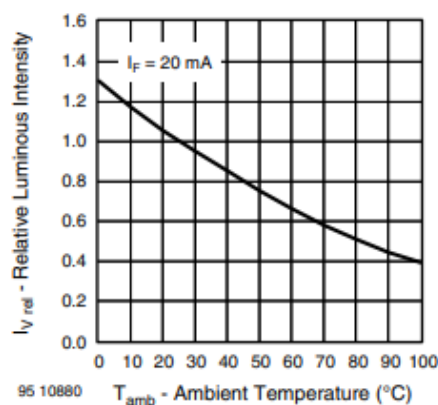


Figure 2.2: Table showing the relative light intensity related to the temperature. This behaviour is typical behaviour of a simple LED and even though this is specific for a 5 mm red LED from Vishay, the general behaviour is the same for simple LEDs. [12]

### 2.3. Data visualisation

The data that will be collected will be in the form of light intensity of the transmittance and a small part reflectance. Most of the reflectance will be internal or on the other side of the leaf. Subtract the T and R from 1 and the absorbance is left. The absorbance will be a fraction of 1 and will be easiest to display as an indication of the location of chloroplasts. This means that the absorbance has to be displayed as a fraction of 1 and can go from 0 to 1. In order to know the 0 and 1 some calibration will have to be done with different plants in different conditions (further discussed in method and instrumentation).

Once the data is acquired and put on the scale of 0 to 1 (0 being no absorbance so avoidance state, 1 being only absorbance so accumulative state), it has to be clear to the user what the data means. Due to there always being some absorbance and some reflectance and transmittance the absorbance will never truly be 0 or 1, looking at equation 2.

The target group of people using a measuring instrument and looking at the data will have at least some knowledge on chloroplast movement and the mechanics behind it. Higher absorbance means that the

chloroplasts will be in a more accumulative state. This will have to be made clear in the graph, either with words or pictures. To also make clear where the other light goes to other than absorptance, the graph can also display the transmittance and reflectance combined.

Another important component of data visualisation is the amount of information that is relevant for the target group and therefore what information is displayed. A scientist will be interested in purely the numbers, whereas a more practical user might need additional information for it to be useful. [13] An example is given in figure 2.3.

The application will also have an option to compare different data from different measuring times. This way people can compare light conditions and different plants. User should be able to compare: time of the measurement, absorptance (including reflectance and transmittance), initial light intensity and group. The group would be a category where the user can put a measurement in. This way all measurements can be classified under a certain plant or type of sunlight a plant received. Comparing groups can therefore mean comparing different plant types or different types of sunlight exposure.

Some research has been done into the accuracy people can read certain graphs and the amount of errors they make. For example, a graph is more clear and better to read when the components are different colours that stand out next to each other. [13] For mapping a gradient, for example our absorptance, one hue can be used going from light to dark. This helps establish that the data is in fact a gradient and does not exist of loose components. However, mapping a gradient using 3 colours is useful when there is a midway point that is very important, it will stand out more. [13]

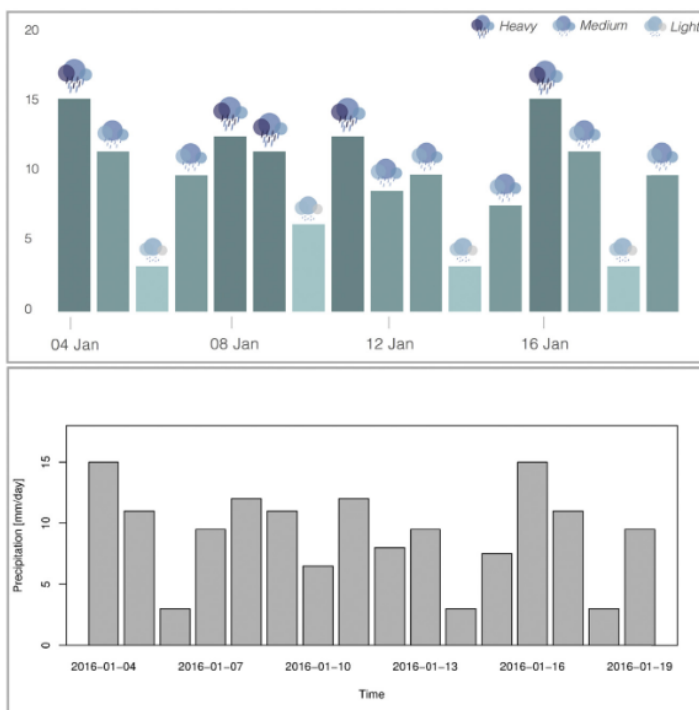


Figure 2.3: The top graph would be useful for farmers, knowing the weather and having a clear graph with colours and pictures. The bottom graph only displays the necessary numbers and is targeted towards scientists. [13]

This brings up the target group again. The application will be used for measuring something very specific which will mainly be used for research and application in vertical farms for example. The users will have knowledge about the mechanics and process that they will be measuring. The data can be used for research. Therefore, the design choice will be to use the version with pure data.

Lastly, the data coming in from the Arduino will be in volts. The range will be from 0 to 5 volt. The sensor setup will be calibrated using extreme environments. The lowest absorption will be calibrated using a leaf with the chloroplasts in avoidance state by exposing it to harsh light. Harsh light in this case has to cause the avoidance response, therefore the light intensity of 100 to 1000  $\mu\text{mol}/\text{m}^2/\text{s}$  will suffice. [4], [5], [14] To compare, at sea level on a sunny day in summer in Florida, USA, the intensity can be 1600  $\mu\text{mol}/\text{m}^2/\text{s}$  and in winter 1100  $\mu\text{mol}/\text{m}^2/\text{s}$ . [15] Accumulative state will be calibrated using the chloroplasts in accumulative state by having the leaf in the dark or purely red light.

## 2.4. User interface

The data comparison and the navigating through the application should be logical and intuitive. This cannot be achieved by doing just literature research but has to be done using user testing. A very popular form of user testing is using a paper prototype. It is low cost and can be changed in a short time without wasting a lot of resources. However, it is still very effective and can give the user a good idea of what the application will look like and how it will function. [16] Paper prototypes could be a challenge. There has to be contact between the interviewer and the interviewee while using the prototype.

Luckily, there are multiple online tools that allow the designer to make a representative prototype which you can navigate through but cannot actually use fully. Programs for this are Director, Flash and Visual Basics. [16]

To design a prototype and eventually an application some design choices will have to be made. Things like a back button, a loading icon and tick boxes for selection are all very important cues in a design. The choices will be made based on techniques like human centred design and activity theory. These techniques will lead the design path that will be taken in this project. [13], [16], [17] Most design decisions will be made based on user tests. Examples of suitable design choices are what type of graph, colour use in the graph, type of data on the axis. For the user interface a state-of-the-art example will be used to base the initial design of. Some of the design decisions have to be based on tests and measurements done with the sensor that will be build and what type of data comes out.

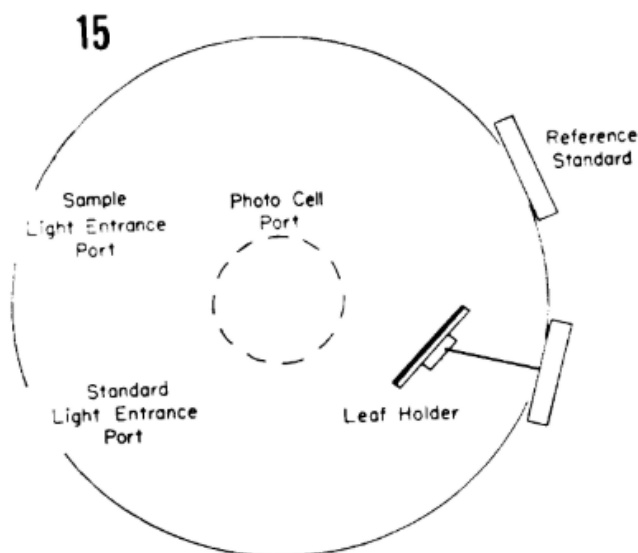
## 2.5. State of the art

The research stated in in this chapter will be the basis of the rest of this project. The measurements and biological aspects will be based on principles that are often used in measuring transmittance or reflectance. Technical aspects will mainly be based on a previous project on in field transmittance

measurements for chloroplast movements. The visualisation of the data will mainly be based on the type of data that will be collected and tests with users. Visualisation will not be based on earlier examples. The user interface will be based on an application that already exists. It will be used as a starting point and further developed to fit the needs of the users for this specific application. In the remainder of this chapter some examples and previous research will be discussed.

### 2.5.1. Diffuse light, transmittance, and reflectance

A plethora of studies use diffuse light to measure transmittance and reflectance. This is not a method that can be used for this project because of the timeframe, resources and costs. In addition the light source and measurements without a sphere are accurate enough for this study. As can be seen from other previously done research where direct light instead of diffuse light was used. [4] An example set up for this type of studies can be found in figure 2.4.



*Figure 2.4: An example set up of a diffuse light installation. [10]*

It uses a sphere with a reflective coat on the inside to expose the leaf to diffuse light. This situation is an ideal situation and gives more realistic measurements of transmittance and reflectance because it is also measured all around in the sphere. Multiple studies used this method which gave the information of chapter 2.2.1. [2], [7], [9], [10]

### 2.5.2. In field prototype



There has been research to in field prototypes for measuring chloroplast movement. One project is very applicable to this study and gives value insights on the technical aspects. [4] This prototype uses a set up as depicted in figure 2.5.

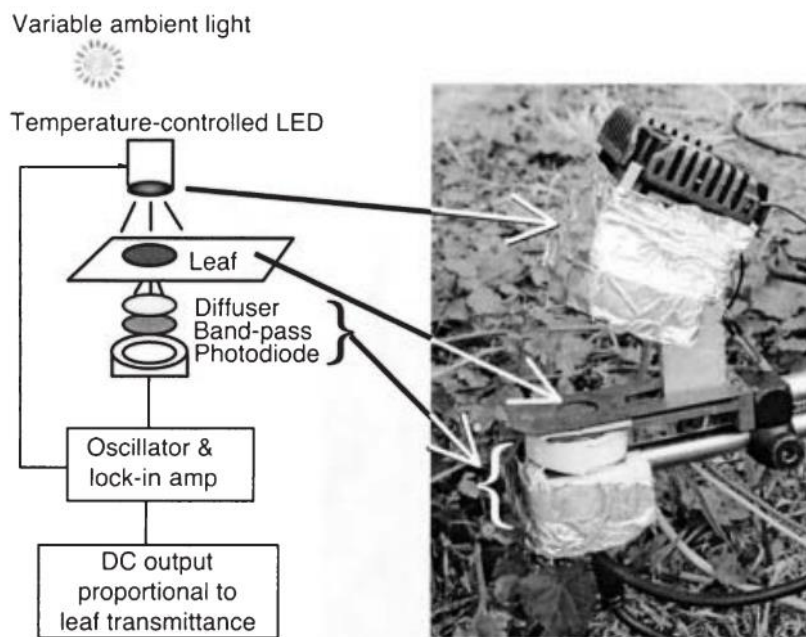


Figure 2.5: Set up of a previous project. Depicted is a schematic and photograph of the set up. [4]

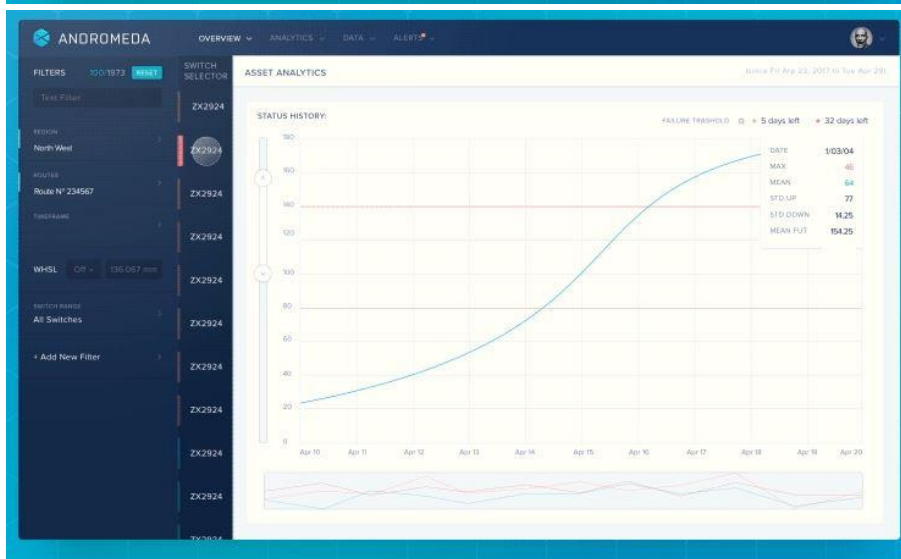
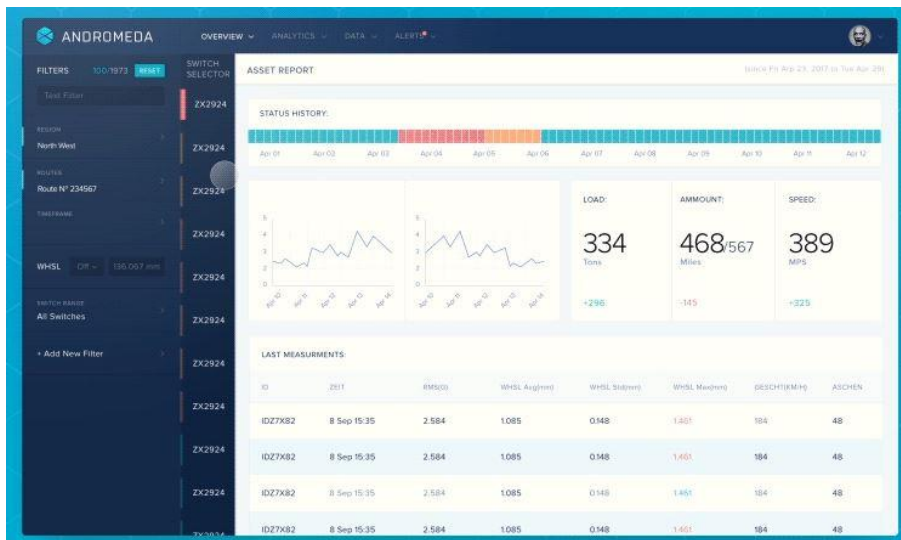
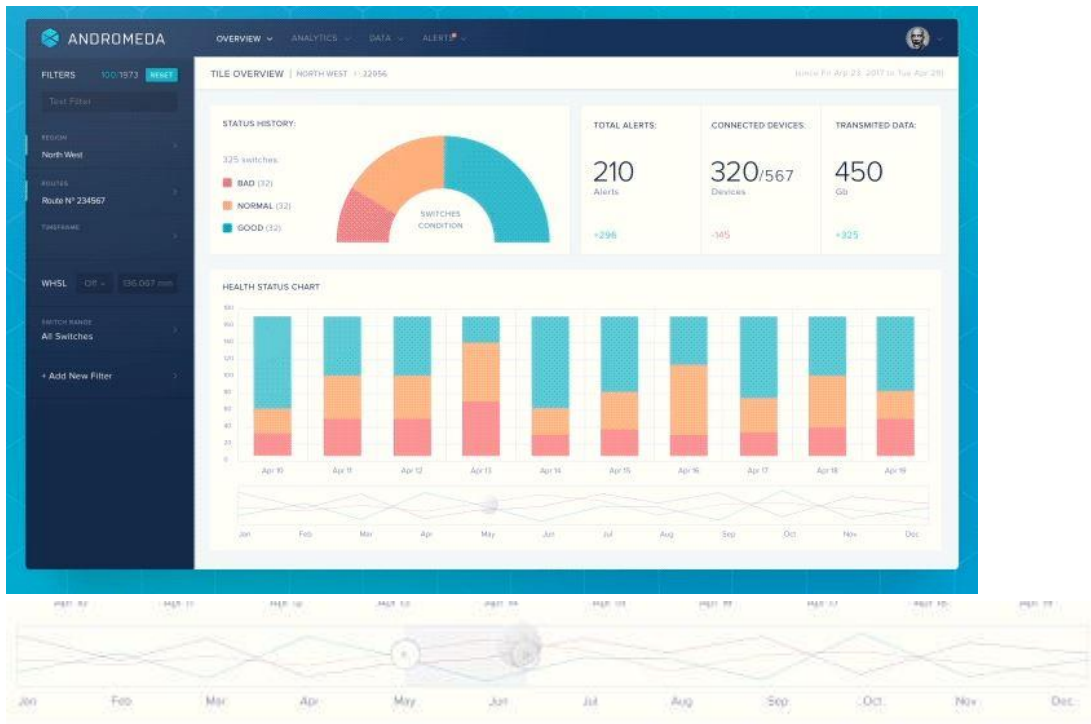
As can be seen in 2.4, this set up uses a similar set up as in this project: an LED, leaf, and photodiode. Next to that it also uses a diffuser, red band-pass filter, and lock-in amplifier. The first two will be optional for this project if it turns out that it is needed for better measurements. The lock-in amplifier will be emulated in software in the Arduino instead of being a physical version.

This example set up also indicated the problems an LED can give in combination with temperature. They isolated the LED in a solid block where temperature could be regulated. [4] This will not be an issue in this project, because this project only focusses on the sensor working with transmittance and reflectance and not about the whole set up being field ready. An option to cover this problem is to have a second photodiode measure the LED light directly. This way any changes in the light intensity can be measured directly and the gathered data can be processed accordingly.

### 2.5.3. User interface

As stated before in chapter 2.4, the first version of a user interface will be based on an existing one. This version will then be changed according to the feedback the users give.

The user interface this application will be based on is a user interface designed by Igor Pavlinski. His design is an animation showing several uses of an interface which is used to analyse data. As can be seen in figure 2.6, the animation shows several uses of the interface to compare data in different ways. The flexibility of the interface is something that is of great value in this project because the user has to be able to compare data based on all types of information. [18]



*Figure 2.6: Four example images of an user interface animation. The top image displays different kinds of graphs. The second image shows the bottom graph of image 1 with a selection tool to select a specific point in the graph. The third image shows more data visualisation options with graphs and tables. The fourth image shows graphs and a menu on the left side to select different graphs.*

The user interface example in figure 2.6. is a rough base for designing the application for this project. The main objective is to use different types of graphs like in the example. This should give a clear overview of differences in different data sets. The selection tool mentioned would be very effective to zoom in on a specific time period of a measurement. Measurements will be done over the duration of multiple minutes to an hour so it is useful to be able to select a certain period. As shown in the third image of 2.5 it might be functional to show graphs and tables next to each other. Some correlations can be spotted sooner in a table than a graph or vice versa. The last image of 2.5 shows the option to easily navigate through multiple graphs. This is of value because this way the user can easily navigate all the measurement moments and search based on vision what they are looking for. [18]

This example that will be used is not an existing application. This choice was made because of the limited access to already existing applications and trouble in finding them online. After consideration this animation which has multiple good examples of usability was picked to be the example for the application. As said before, the user interface will be designed with the user in mind and in a very iterative process. The starting point is important but will not be the last version of the application, merely an inspiration.

## 3. Method and instrumentation

Now that all background information has been gathered, the setup for this project can be designed. First the electrical components will be discussed in greater detail. This includes what type of sensor will be used, what type of light source, what controller, and also why these choices have been made. After that the measurement system setup will be discussed. From the designing phase to the fabrication of the setup. Lastly some basic experiment will be described. These calibrating experiments will make sure the setup works and that the output is linear in an optimal case.

### 3.1. Electronic components

The core of the setup will consist of a couple of electronic components. These components such as an LED and a sensor will have to be coordinated by a (mini)computer. Data coming into this computer has to be processed on a laptop where the visual interface will be constructed around the incoming data. In this chapter the requirements for all components will be discussed and several options will be considered.

#### 3.1.1. Arduino

The electronic part of the setup will be run on a Arduino Uno connected to a laptop. This Arduino Uno is a minicomputer which allows you to connect all types of sensors and actuators to it. It has 6 analogue pins and 14 digital pins of which some can send out a PWM signal.

Arduino is accompanied by the Arduino software in which code can be written to command the Arduino and its components and it will also read any incoming data from the Arduino. Using this software a program will be written to turn on the LED and to read out the sensor information.

Figure 3.1. contains a picture of an Arduino. The microcomputer contains multiple in/output pins, a cable to connect to a computer using USB, and a power source that can be used as an option in case the setup requires more power than the computer USB can put out.



*Figure 3.1: Picture of an Arduino Uno, showing the pins and electronics on top.*

Other options were a raspberry pie, a computer of the same category. However, these are pricier and more elaborate which is not necessary for the scope of this study.

Considering a test phase in which some components will have to be tested and compared a few different components were acquired.

### 3.1.2. Sensors

The sensor needed has to be a photosensor which can measure the light intensity of the transmittance and reflectance of the leaf but also the direct LED intensity. For this task a few sensors have been selected.

The Luna Optoelectronics NSL-5112 photodiode is the first sensor. This photodiode has a spectral peak at 550 nm, is sensitive to visible light, and displays the light intensity in the form of how much Volt goes through the diode. A higher intensity will cause a greater resistance and therefore a greater voltage over the diode. The datasheet for this sensor contains little information and no performance specifics. [19]

The second sensor is the Kodenshi ST-1CL3H. This is a photodiode. It shows the light intensity through voltage between 0.15 and 0.4 Volt. It is sensitive to light from 400 to 1000 nm and has its peak at 800 nm, meaning it is most sensitive to infrared light. The datasheet contains the most important information and some tables on the performance of the sensor. [20]

The last sensor is a more elaborate sensor, adafruit TSL-2591. It consists of two parts: a infrared photodiode and a broadband photodiode (visible and infrared) in an integrated circuit. The infrared sensor has its peak in about 800 nm, the broadband sensor has its peak in 650nm. It outputs light sensitivity between -0.5 and 3.8 V. Next to the advantage of having two photodiodes (infrared and visible light) the sensor is also highly accurate and can supply light intensity in Lux accurate to 188 $\mu$ Lux and can measure up to the value of 88.000 Lux. This range is useful because of the range of tests that will have to be done. Testing in full light can give numbers in the thousands of Lux where the transmittance or reflectance can go into the microlux. The sensor is accompanied by a very elaborate datasheet giving many specifics on the performance of the sensor. [21]

Above mentioned photodiodes are also temperature dependent, a higher temperature can influence their performance. Not only the temperature dependence but also above mentioned light spectrum peaks, accuracy, and intensity to volt conversion is important to base a decision on. In order to decide which sensor is most suited the specifics have been organised in table 3.1. Costs have been gathered from conrad.nl, a regular supplier to the University of Twente. [22]

For reference in figure 3.2. a graphical representation of the light spectrum has been included. wavelength in nm is displayed in the figure to match the specification of the sensors.

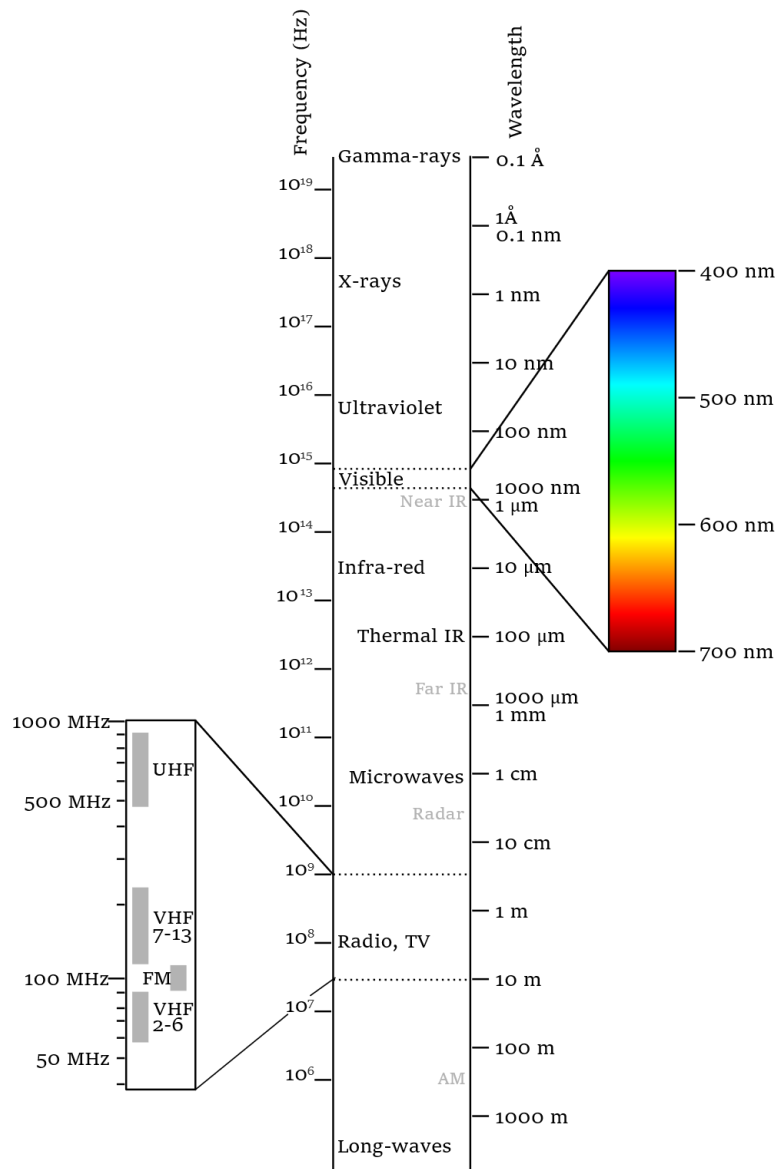


Figure 3.2: graphical representation of the light spectrum including wavelength indicators in nm. [23]

Table 3.1: Table containing the specifics of the three sensors. Luna optoelectronics NSL-5112, Kodenshi ST-1CL3H, and Adafruit TSL-2591. [19]–[21]

Sensor	Spectrum range (nm)	Spectrum peak (nm)	Operating temperature (°C)	Output voltage (V)	Light resistance (KΩ)	Cost per sensor including taxes (21%) (euros)
Luna NSL-5112	430-620	550	-60 - +75	N.A.	6 - 14	1.16
Kodenshi ST-1CL3H	480-1000	800	-20 - +70	0.15 – 0.4	N.A.	5.70
Adafruit TSL-2591	400/500-1100	650 and 800	-40 - +85	-0.5 – 3.8	N.A.	14.50

Considering the information given in table 3.1. and the text above the Adafruit sensor seems the best suitable for this project. It has a wider range of output voltage, a wider measurement spectrum, has an elaborate datasheet, and is highly accurate. The only downside is the cost which is three times more expensive than the Kodenshi sensor and ten times more expensive than the Luna sensor. However, this cost does cover the quality of the sensor and the advantages outweigh the higher cost.

### 3.1.3. LEDs

The red light needed to measure transmittance or reflectance needs a light source. This light source needs to be pure red light in order to not trigger a chloroplast movement as explained in chapter 2. In addition is also needs to be powerful enough to ensure that the sensor can pick up the transmittance and the part of the reflectance, which is a certain part of the light source. For the light source purpose a few LEDs have been selected and ordered.

The 8034R1C-CSE-D red LED is a LED with an 8mm casing. [24] The second and third LEDs both have a 5mm casing and are both red. One is from Huiyuan opto-electronics [25], the other from Vishay semiconductors. [12] The final LED is an LED spot from Signal Construct called a LED-spot Minostar. [26] These LEDs have different peak wavelengths, radiation angels, intensity and power. All this info is gathered in table 3.2. Almost all LED datasheets gave light intensitiy in microcandela and have been converted to Luminous Flux in lumens (lm) because the Minostar LED-spot only gave light intensity in lumens.

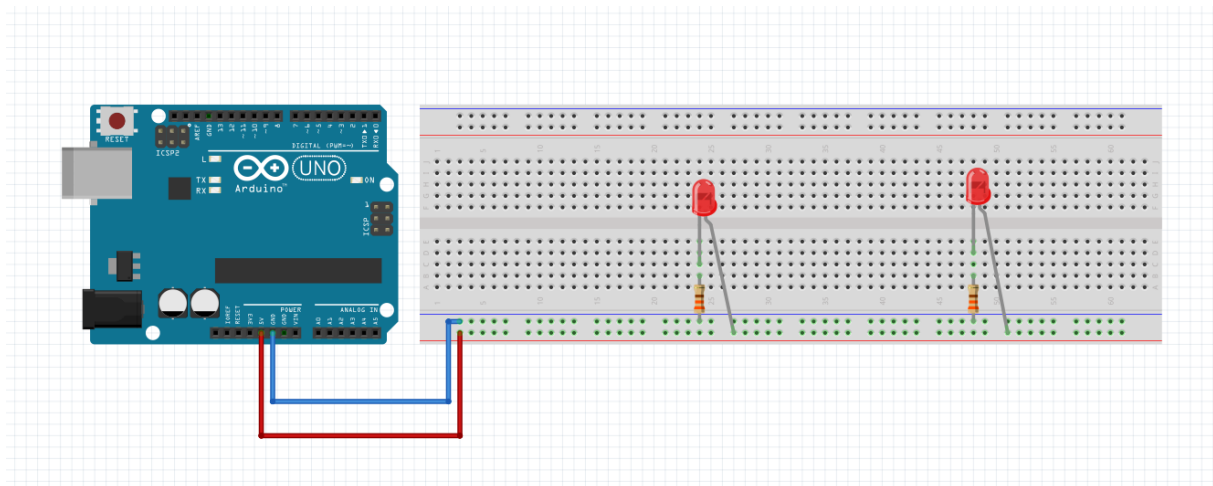
*Table 3.2: four different LEDs with accompanying specification information.*

LED	Wavelength (nm)	viewing angle (°)	Intensity Iv (mcd)	Luminous Flux (lm)	Current (mA)	Voltage (V)	Cost including taxes (21%) (euros)
<b>8mm LED</b>	620 - 635	25	8000 - 9000	1.19 - 1.34	20	1.9 - 2.5	0.59
<b>Huiyan 5mm LED</b>	580 - 595	25	1500 - 2500	0.22 - 0.37	10	1.9 - 2.3	0.15
<b>Vishay 5mm LED</b>	630	8	1000 - 5500	0.015 – 0.084	20	1.9 – 2.6	0.25
<b>LED-spot Minostar</b>	Not given - Red	30	327000	70	350	2.3	17.99

Looking at the LED specifications the minostar spot seems to be a great option due to its light intensity. However, the exact wavelength is not given in the datasheet. It does state it is pure red light. The second best option is the 8mm LED which is also cheaper.

After connecting both the minostar and the 8mm LED to the Arduino and tested on the sensor the 8mm LED gave a higher Lux than the minostar. In figure 3.3 this experimental setup can be found.

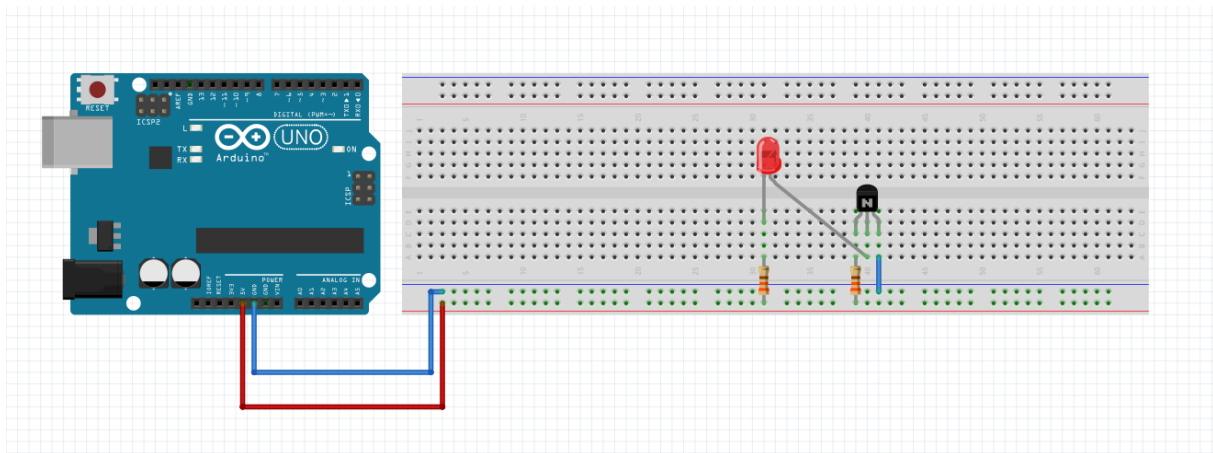




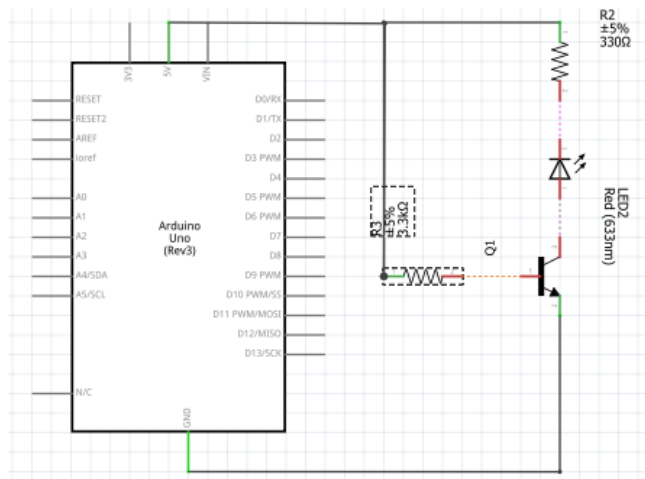
3.3: experimental setup to test which LED has a higher light intensity. The most left LED is the 8mm LED connected to the 5V supply of the Arduino and connected to the ground of the Arduino using a 330Ω resistor. The most right LED is depicting the minostar LED spot (due to a lock in the Fritzing program of a schematic picture of the spot this was use). The minostar is connected in the same manner as the 8mm LED. The sketch was made with Fritzing.

The light intensity is important because the transmittance and reflectance will be a fraction of the incoming light. The higher the light intensity is of the incoming light, the higher the light intensity of the T and R are which makes them easier to measure.

The minostar LED spot had the highest light intensity and is therefore the best option to pick for the setup. The transistor setup can be found in figure 3.4. Resistors used in this set up were 330Ω to ensure enough current could go through the LED but it would still be limited to not damage it.







*Figure 3.4: Test setup of the minostar spot with a transistor. Het minostar is depicted as a red LED. The transistor is an NPN transistor connected with the Base to the 5V with a 3k.3Ω resistor. Connected with the Collector to the minostar which is in turn connected to the 5V with a 330Ω resistor. The Emitter is connected to the ground. The schematics can be seen on the bottom of this figure.*

Next to the red LED, blue LEDs are also required. When the testing on the setup is finished and it can be established that the setup works, the setup will be tested on actual plants. To trigger chloroplast movement it is important to have sufficient environment light. This can be achieved using additional blue LEDs. After a successful setup with the Minostar red LED, the same one was purchased in the colour blue and added to the setup. In addition, five smaller blue LEDs were added to the setup. (Their brand remains unknown because they were not purchased for this project specifically but were already in possession.)

### 3.1.4. other components

Next to the sensors and LEDs some other components were acquired to get a complete set of tools. This set of tools allowed for experimenting and freedom. This is very important because prototyping this setup can take a number of different tries and types of setups. Included here is a list of other components and accessories that were acquired:

- resistors ranging from 10Ω to 1MΩ
- 3 types of cables
- Transistors BD13910STM
- Amplifiers AD620ANZ Lineaire IC - instrumentation amplifier PDIP-8
- Multimeter BaseTech BT-22
- Plug-in power supply 3-12VDC // max 1500 mA
- Protection bag (anti-static)

All components are ordered to ensure a certain flexibility when prototyping and minimise the delay if any new parts should be ordered. Note that not all components might be used in the final setup.

## 3.2. Measurement system setup

Using all the previously mentioned equipment and components are used to put together the electronic part of the measurement system setup. The goals of this setup is to measure the transmittance and reflectance of a leaf. Before this goals can be reached some tests have to be done as well. This includes calibration tests and durability tests. More information and process descriptions on the testing will be discussed in chapter 3.3. Method. This chapter will focus on the ideation and final result of the system setup, including the requirements, limitations, and availability of materials. Two parts will describe this process, one about the electronic part and the other about the mechanical part.

### 3.2.1. Electronic schematics

As stated in chapter 3.1 where electronic components were discussed, the main components of this setup will be the light source, the sensor, the Arduino, and a computer. The computer is in this case an ASUS laptop which suffices for the programmes it has to run: Arduino, Processing, Excel etc. The Arduino Uno will be connected to the laptop which will supply it with information and power. Next is to determine the setup of the sensor and the LED.

#### 3.2.1.1. Ideation

The electronics of this setup will all be connected using an Arduino Uno. This Arduino has multiple analogue and digital in/out-puts. Next to these in and out puts it also has a 5V, 3V and GND (ground) pin. The sensor requires a 5V and GND connection to the Arduino for power and needs to be connected to two analogue pins to send data. The example from the Arduino library gives analogue pin 4 and 5 as example, these pins will also be used in this setup.

The LED will also be connected to the same GND pin and to a digital PWM pin. A PWM pin can alter its duty cycle causing the output to go between 0 and 5V. This is very useful in this setup because it allows the LED to be set to different intensities. A PWM can go from 0 to 255 bit in the Arduino code, which is the same as going from 0 to 100% light intensity. This way the LED intensity can easily be controlled. A resistor will also be put between the LED and the GND, otherwise the LED will ask too much current from the Arduino which it cannot supply and it will shut down. The resistor will reduce the current and make sure the Arduino does not shut down.

The schematics for this setup can be found in figure 3.5.

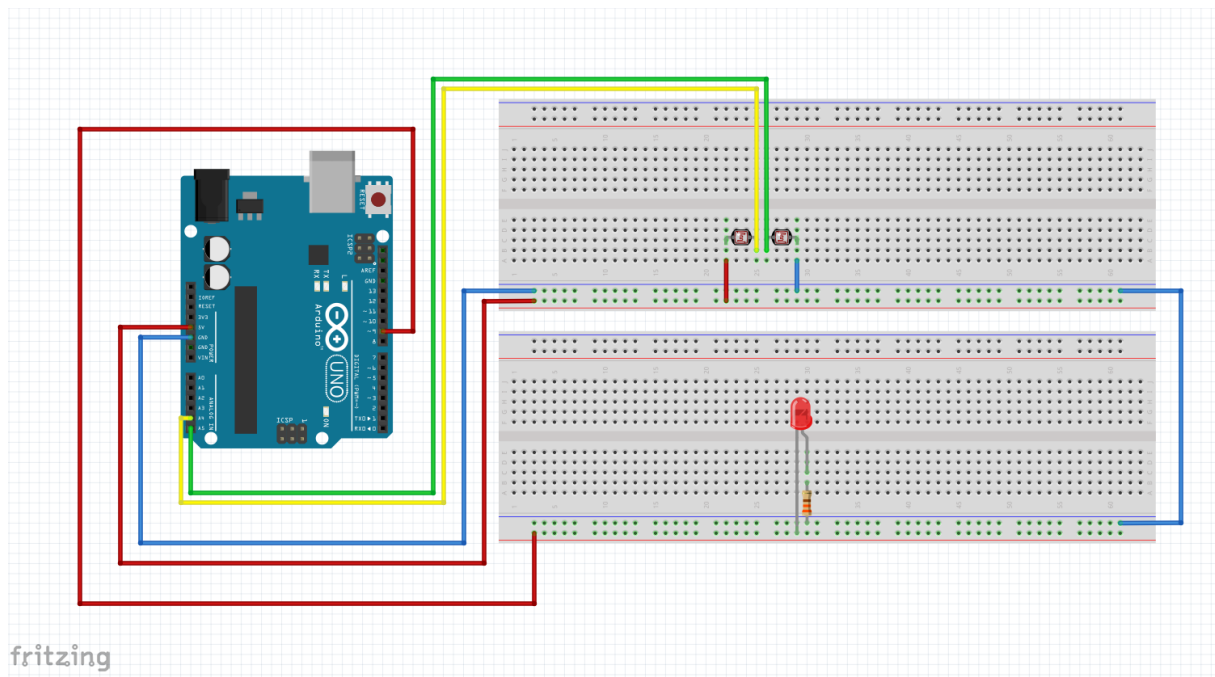


Figure 3.5: Fritzing of the ideation of the electrical part of the setup. There is no Fritzing object to represent the sensor cell accurately, therefore 2 photodiodes have been used, together they have 4 pins, one for 5V, one for GND, and two for data (green and yellow wire).

### 3.2.1.2. Result

There were no set backs on this part of the setup, everything worked according to the plan based on figure 3.5. Pictures of the result can be found in figure 3.6.

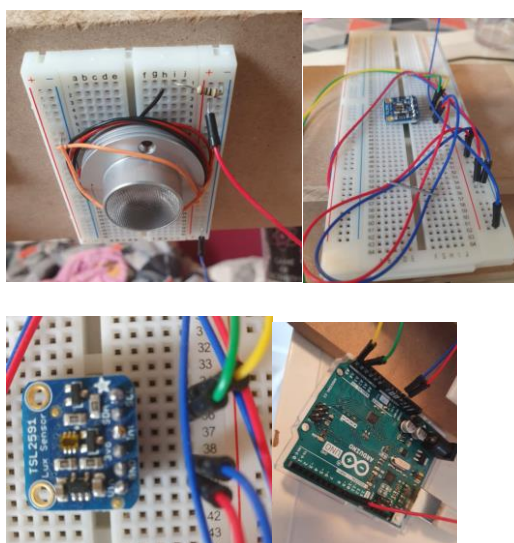


Figure 3.6: Photos of electrical part of the finished setup. Top left: LED setup. Top right: sensor setup. Bottom left: close up sensor. Bottom right: Arduino setup.

## 3.2.2. Measurement system design

The second part of the setup is the mechanical part, the physical design of the setup. In this project this entails a construction that is used to hold up the Arduino and its accompanying sensor and LED. There are some requirements which can be taken from literature on earlier studies done on chloroplast movement.

### 3.2.2.1. Ideation

While creating concepts and ideas for the physical design some requirements had to be thought of. The first requirement was the distance between the LED and the sensor. A leaf has to fit in between the sensor and the LED and in order to test multiple setups with one device the distance between LED, leaf, and sensor has to be adjustable. Talking to the client revealed that the distance used for a already working setup is around 1.7cm from LED to leaf. Literature gave the insight that the distance would indeed be a few centimetres, not more than 5. [4]

The LED, leaf, and sensor had to be movable in the vertical direction but also need to be able to be turned. This can give the optimal conditions so as much light as desirable can hit the leaf in a certain angle. In previous chapters the importance of the angle became clear because of the different effects it can have on the transmittance and reflectance of the leaf. [10]

The physical setup also has to be sturdy, this can prevent all components from shifting around which can cause differences in outcome. The angle is very important and if it is easy to accidentally change it during a measurement it can have great influence on the results. The setup should therefore be heavy enough to not be knocked over accidentally and movable parts should be able to be set into place by for example tightening a screw.

Being movable is also a requirement, mostly important for the flexibility in testing. A movable setup can give more options on which plants can easily be tested on, which leaves of this plants can reach the setup, and where it can be tested (outside or inside for example). To accommodate movability the setup should be not too big and light weight.

Given all these requirements a plan was drawn for a wooden structure. Wood is easily workable with a (figure)saw, can be sturdy if the right materials are used, and is lightweight. The wooden structure would be supported by metal corner supports. The movable parts can be held up in a rails using bolts and nuts which are easily tightened and untightened. The thickness of the wood should be enough to support the insertion of dowels for sturdiness. However, it should not be too thick otherwise it could take up a lot of space within the setup giving the wooden slabs less space to move around. A thickness of 12mm would be good as most dowels come in the size of 6mm. Leaving enough space to work with them.

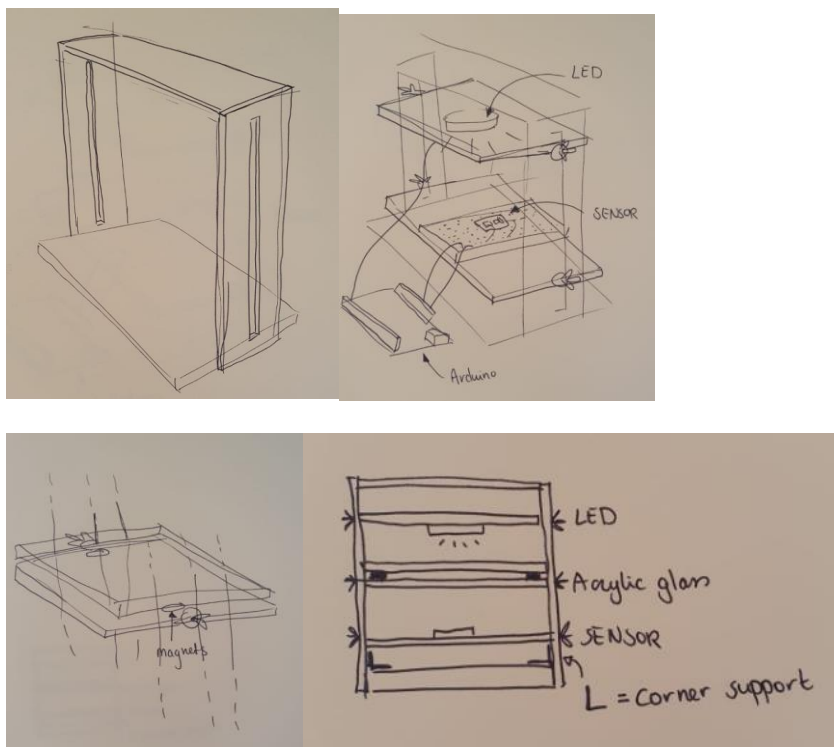
Another requirement of the setup is to hold a leaf in place with enough spare room for fresh air to reach the leaf. The holder would be some kind of clip holding the leaf into place so the results are not changed. If another part of the leaf is used all of a sudden this can greatly influence the outcome. On the other

hand the leaf should still be able to get air and moist. Completely closing off the leaf can alter the physiological properties of the leaf and also alter measurements. The goal is to keep all factors constant except the environment light during a measurement. The acrylic glass should be consistent in thickness so light won't be bent in another direction. The thickness would not matter too much for the way the light behaves, of course a thinner slice will give less light obstruction but too thin might make the acrylic too fragile. A good thickness would be about 3 or 4mm thick. This thickness is not too thin to be fragile but not too thick so the clip still has room to move around.

The clip should also be transparent. This way no wavelengths will be blocked and little light intensity will be lost. A few options here are glass, plastic, or acrylic glass. Glass is very fragile, can break easily and is quite expensive. Regular plastic, think about a lid or container, is easily accessible and cheap but can often bend easily changing the direction of the light which can alter the results. Lastly, acrylic glass is sturdy, easily accessible and can be cut to the desired sizes well. The best option therefore is to go with acrylic glass.

The clip has to hold the leaf as well but leave enough room. A method to do this is to use magnets on either side of the leaf the clip together and hold the leaf without damaging it. The best way to ensure the magnets don't damage the leaf is to put a little bit of fabric between the magnets and the leaf. The magnets should not block any light from the LED to the sensor and should therefore be located to the side of the setup.

Sketches for the setup can be found in figure 3.7. The sketches are based on previously mentioned requirements.



*Figure 3.7: sketches for the physical setup. Top left is the supporting frame of the setup. Consisting of 4 parts: a bottom part and a top part and the two side parts which have a long rectangular hole cut out. This*

*slit is where the bolts and nuts will go in and move around. Op right explains the positions of the LED, sensor, and Arduino. The LED will be attached to the top platform, a wooden slab with a breadboard on its bottom. The breadboard is where the LED will be attached to. The slab can be moved around by unscrewing the bolt and tightening it again when it is in the desired position. The sensor slab works the exact same way. The Arduino will be placed next to the setup because it has to reach both the LED and sensor even when either parts are moved around, making it easier for the Arduino to not be attached to anything. Bottom left displays the two acrylic glass plates. One will be attached to bolts and nuts and therefore movable. The other will be attached to the first plate using magnets, it does not need bolts and nuts because it will be attached to the first plate and move around with it. Bottom right gives a simple schematic of the front view of the setup, displaying the position of the LED, the acrylic, and sensor. Next to that it also gives the position of the corner support which will help keeping the setup sturdy.*

### 3.2.2.2. Result

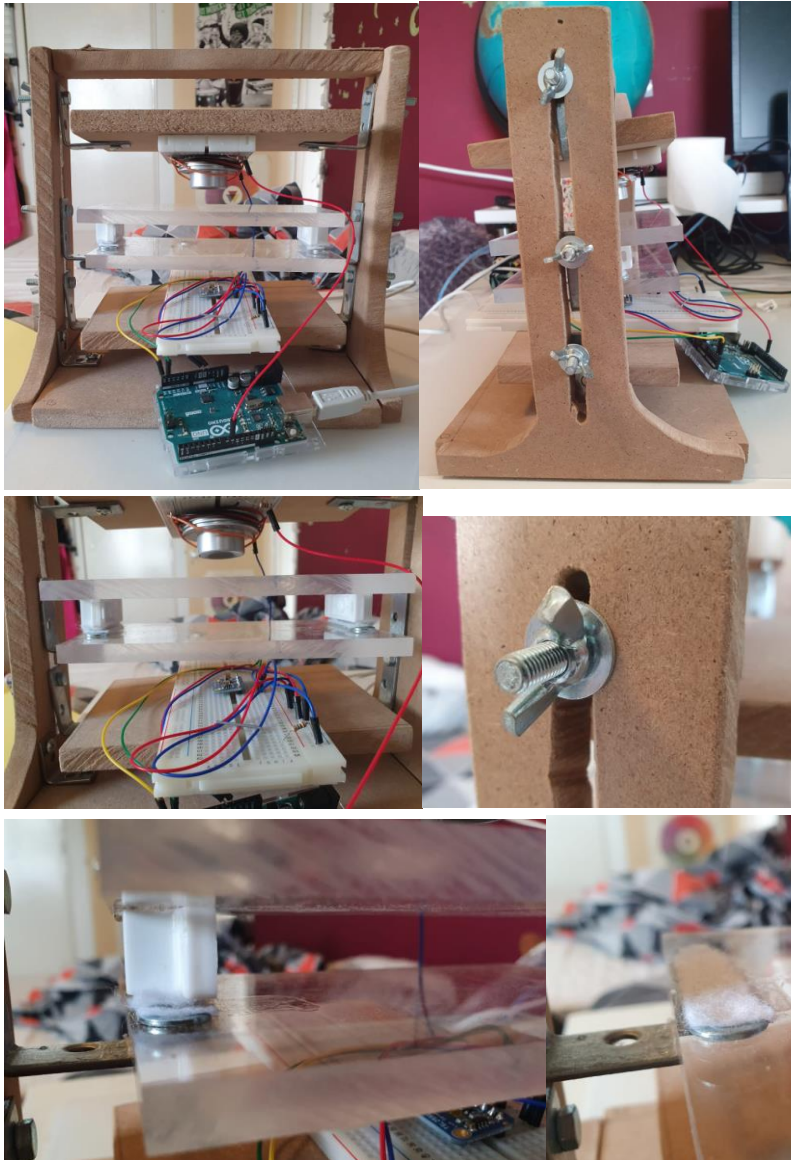
The realisation of the plan started with a visit to the Gamma, a hardware store. Most components on the list were available, however some setbacks were experienced in this step of the realisation.

Magnets were not as widely available there as expected and they were thicker than desirable. This means the acrylic clip has less space to be moved around. The other set back was the availability of acrylic glass. A lot of businesses like supermarkets use acrylic for COVID-19 measures meaning the most accessible sizes were not available anymore. This meant a 8mm thick slab will be used for this setup. Double the thickness that was desired, giving the two slabs in the clip less room to move around. However, after assembly this turned out to be a minor inconvenience and would not obstruct measurements.

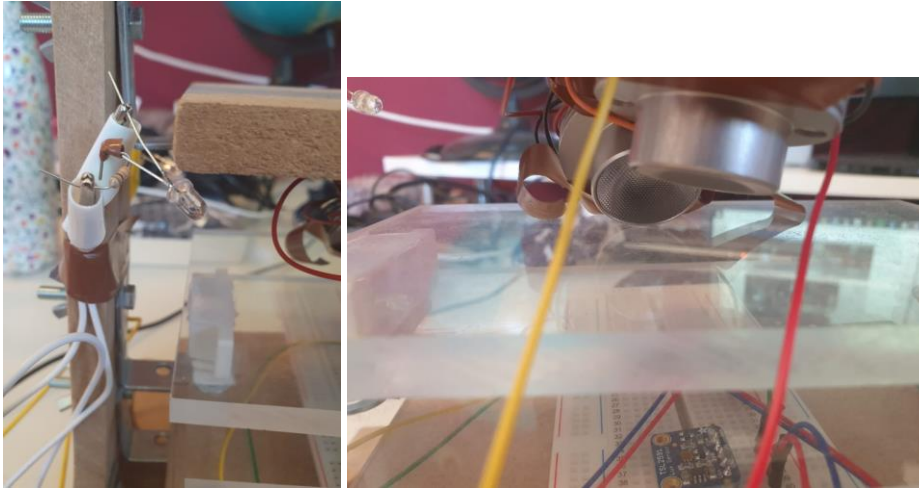
The blue LEDs were added at a later stage in the project. The Minostar LED spot can be controlled with one pin, the other five LEDs are also controlled with one pin. The blue spot is added right next to the red LED spot and turned towards the red LED. This is to ensure that both LEDs illuminate the same point on the leaf, giving the best results.

The final setup is very similar to the plan. Next to the magnets and thickness of the acrylic one other alteration was made. The wooden and acrylic slabs are not directly attached to the frame with the bolts but first to corner supports which are attached to the bolts. This way some space is bridged between the frame and the slab. Pictures of the final setup can be found in figure 3.8, pictures of the additional blue LEDs can be found in figure 3.9.





*Figure 3.8: The final setup. Top left: front view of the setup. Top right: side view of the setup. Middle left: close up of the 3 slabs: bottom sensor, top LED, middle clip. Middle right: the bolt and nut mechanism. Bottom left: the two acrylic plates attached. Bottom right: only the bottom acrylic plate. Note the piece of fabric on the metal part to protect the leaf. The white block on the top slab is the magnet.*



*Figure 3.9: The two kinds of blue LEDs added to the setup. On the left one of the five smaller LEDs, all connected to the same pin. On the right the minostar LED spot, this is added right next to the red spot and turned towards the red LED. This ensures that the blue and red LED both illuminate the same spot of the leaf, giving the best results.*

### 3.3. Software setup

Both physical parts of the setup are finished. The last step to complete the setup and make it test ready is the software. Two software development programmes are being used. The first is Arduino, this is the software that accompanies the Arduino Uno console. The other program is Processing 3. This software program is very visually focussed and allows the user to create visuals on screen and make them interactive.

Both codes will be shared in appendix A. The Arduino code is based on the example that Adafruit supplies, it comes with the sensor that is used in the setup. Most of the code has been copied to ensure that the sensor functions. However, some of the code has been deleted in the final version of the Arduino code because it did not add any value to the tests and only made copying the data harder.

The processing code is a rough version and allows the user to set the intensity of the LEDs on the setup. Processing communicates with Arduino and vice versa. This way Processing can receive data from the Arduino and send data to adjust intensity for example.

In total 3 different codes were used for this project:

- Arduino code to communicate with Processing
- Processing code to communicate with Arduino
- Arduino code to carry out the actual tests on the plants.

For the tests on plants only Arduino code was used. The reason is that getting the data from Arduino was less complicated and no data could be lost in the process of transferring data to processing.



## 4. Method of testing

The finished setup also has to be tested. Some important factors here are if tests are repeatable, is there any drift, how dependent is the setup on temperature etc. Starting with the most basic tests and working up to the more specific tests ensures that any problems are noted at the right moment. There are many options for what to test on, some more valuable than others. Therefore it is important to think about what kind of tests there are and which ones are more appropriate to do in the time frame of the project.

### 4.1.1. Test ideation

To ensure all valuable tests are carried out it is important to think off all options. All possible tests can be found in table 4.1.

*Table 4.1: A collection of all possible tests with specifications.*

Name	Variables	Goal	Duratoin [min]	Repeated
Drift type A	Constant distance, constant intensity, no leaf, no clip, no environment light	Is there any drift?	60	3
Drift type B	Constant distance, constant intensity, no leaf, clip, no environment light	Is there any drift with the clip?	30	3
Vibration	Everything constant, no clip, no leaf, shake the setup, no environment light	Does movement have any impact on the results?	10	1
Leaf	Everything constant, with leaf and clip, no environment light	What is the drift with leaf and clip?	60	2
Leaf and intensity	Distance constant, with leaf and clip, alter the intensity towards the plant, no environment light	What is the effect of a lower intensity, at what intensity does the sensor not pick up any light from the LED?	10	5
Initial values	Everything constant, no environment light	Are the initial values the same every test?	5	5
Temperature	Thermometer at a constant distance under the LED (where the sensor usually is)	Does the LED heat up itself or the sensor during a test?	120	1

<b>High temperature</b>	LED and sensor on, constant distance and intensity, point a hairdryer at the setup	How does a higher temperature effect the results	10	1
<b>Leaf temperature</b>	Turn on the setup with red LED, measure temperature right next to the leaf	How does the LED influence the temperature	60	1
<b>Sun test</b>	Put the setup in the sun, constant LED intensity and distance	What is the effect of natural sunlight in the setup, does it heat up over time?	60	2
<b>Dark test</b>	Only blue LED on, dark room	To see behaviour going from dark to blue LED	60	3
<b>Leaf movement</b>	Constant intensity, constant distance, with clip and leaf, move the leaf around every few minutes	Does moving the leaf effect the results?	30	5
<b>Final test</b>	How it would be used in the field	How does the setup function in its intended environment?	60	5

All test options are mentioned in table 4.1. Some tests are more valuable than others and the times some tests are repeated can also alter. The goal is now to select some tests to do, how many times to do it, in what order and which ones can be dropped if the time schedule does not allow for any more tests.

### 4.1.2. Test plan

Based on all possible tests from table 4.1 a test plan has been made. This includes the name of the test, the repeated times minimum, the priority (1 is high priority, 3 is low priority), and is put in chronological order. Some tests are important to do at the beginning so adjustments can be made or things like drift can be taken into account. The complete plan can be found in table 4.2.

*Table 4.2: The complete test plan including the name of every test, referring to table 4.1, with the minimum repeated times, priority, and in chronological order. Note that some tests indeed have to be done before each other, however, some 'groups' of test can be done in any order. The three leaf tests is best to be done in order but could all be done after the high temperature and sun test.*

Name	Repeated times minimum	Priority (1=high, 3=low)
<b>Drift type A</b>	5	1
<b>Drift type B</b>	2	1
<b>Temperature</b>	1	2
<b>Initial values</b>	3	3

<b>Vibration</b>	1	2
<b>Leaf</b>	2	1
<b>Leaf and intensity</b>	1	2
<b>Leaf and movement</b>	5	3
<b>High temperature</b>	1	2
<b>Leaf temperature</b>	1	1
<b>Dark test</b>	3	1
<b>Sun test</b>	1	1
<b>Final test</b>	5	1

The priority as can be seen in table 4.2 is based on how important it is for a test to be done. It is very important to know if the setup shows any drift, how it interacts with a leaf and the sun. Tests like temperature, which have priority 2, are less important. They do contribute to a clear view of how the setup works but are not absolutely necessary. The initial value and leaf movement tests are marked with priority 3, the reason for this is that the initial value does not matter that much. The ultimate goal is to measure transmittance which will always be a relative difference between the initial value and the end value, the absolute initial value is therefore not important. The leaf movement test is not that important because from theory it can be seen that movement would influence results depending on the environment light source. If the light used to induce chloroplast movement is focussed on one part of the leaf, moving the leaf will alter results because a different part will now be exposed to the light. If the light source effects the leaf as a whole it would have less of an impact. However, the setup is made as such that the leaf is not moved at all. It will be clipped between the two magnets, therefore unable to move.

### 4.1.3. Test plants

For the testing of the setup and performing the actual experiments on plants some plants have to be selected. The selected plants will fit in either of two categories: sun or shadow plants. This is because they both have different thresholds when they show chloroplast movement, which is an interesting difference and something that can be tested on. A few candidates were selected, they can be found in table 4.3. Some of the plants were purchased for testing. The way the plants are selected is because of their appearance in literature. This way the tests results can be compared to literature. Next to that they were also selected and purchased based on availability.

*Table 4.3: Table of selected plants that can be used for testing. Behind them it will say in what category they fall and if they were purchased for testing.*

<b>Name</b>	<b>Scientific name</b>	<b>Category (sun or shadow)</b>	<b>Purchased</b>
<b>Strelitzia</b>	Strelitzia	Sun	No
<b>Peas</b>	Pisum sativum	Sun	Yes
<b>Corn</b>	Zea Mays	Sun	Yes
<b>Wandering jew</b>	Traadecentia albiflora	Shadow	Yes, not albiflora but zebrina

<b>Peace Lily</b>	<i>Spathiphyllum walissii</i>	Shadow	Yes
<b>Hosta sieboldiana</b>	<i>Hosta sieboldiana</i>	Shadow	No
<b>Trachystemon orientalis</b>	<i>Trachystemon orientalis</i>	Shadow	No
<b>Alocasia</b>	<i>Alocasia cucullata</i>	shadow	Yes

## 5. Test results and discussion

In the previous chapter the test plan has been discussed. The order and test titles can be found in table 4.2. and in the subchapters of chapter 4. In the first half of this chapter the results will be shown, accompanied by the actual duration, times a test was repeated, changed variables etc. The second half of this chapter will discuss the results, showing any connection between results, what some results mean for the setup or further use in the field. During the test period some new insights were gained. This means that some tests have been added, these are mentioned in the test plan, but this can also mean that some tests are not carried out.

### 5.1. Test results

This first half contains the results of the tests and how the actual test was carried out. This can sometimes differ from the plan described in chapter 4, due to a change in possible available materials, or because previous tests showed that the test became obsolete or part of the test is not applicable anymore.

The first step is to carry out drift tests. This is done by only switching on the red LED and the sensor and leaving it on for about three hours. This time is picked to ensure a complete image of any drift that can occur during an actual test with plants, which will take up to 2 hours. The first drift tests were carried out without Plexiglas in between, in the previous chapter marked as drift test type A. The second through sixth drift test are all normalised using the average and the standard deviation. The graphs corresponding to drift test type A are graphs 5.1 through 5.6.

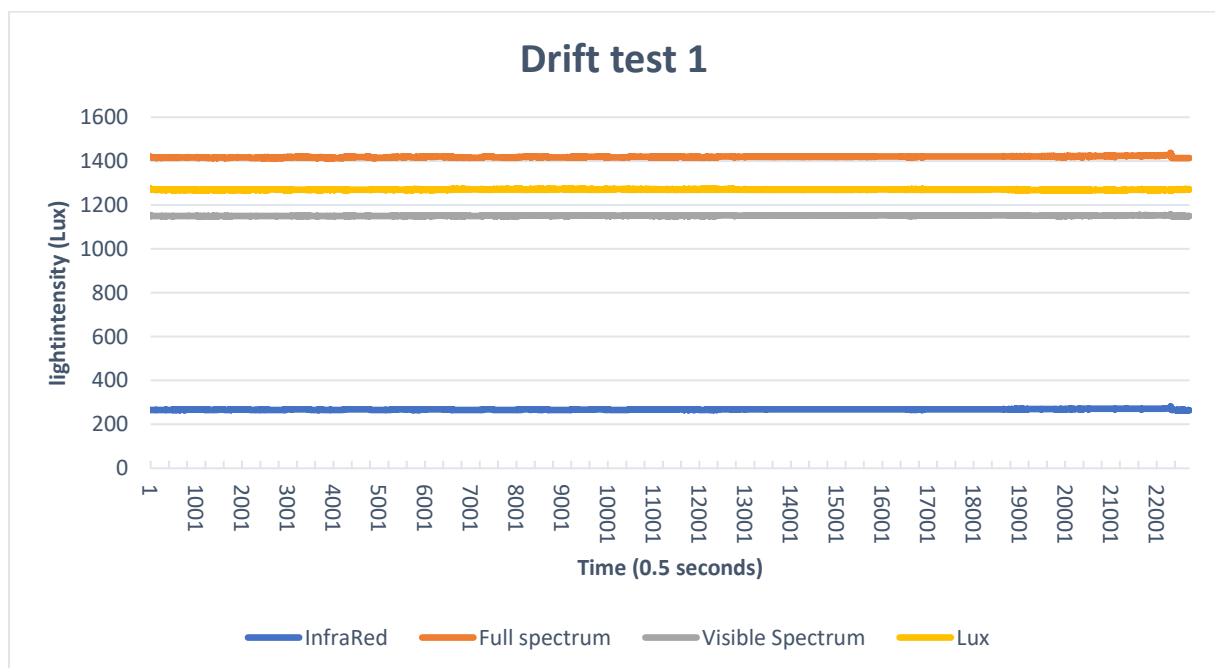


Figure 5.1: The first drift test, all four data sets from the sensor are depicted. The setup was turned on for three hours. On the x-axis the time is depicted in half seconds because the sensor measured the intensity every half a second.

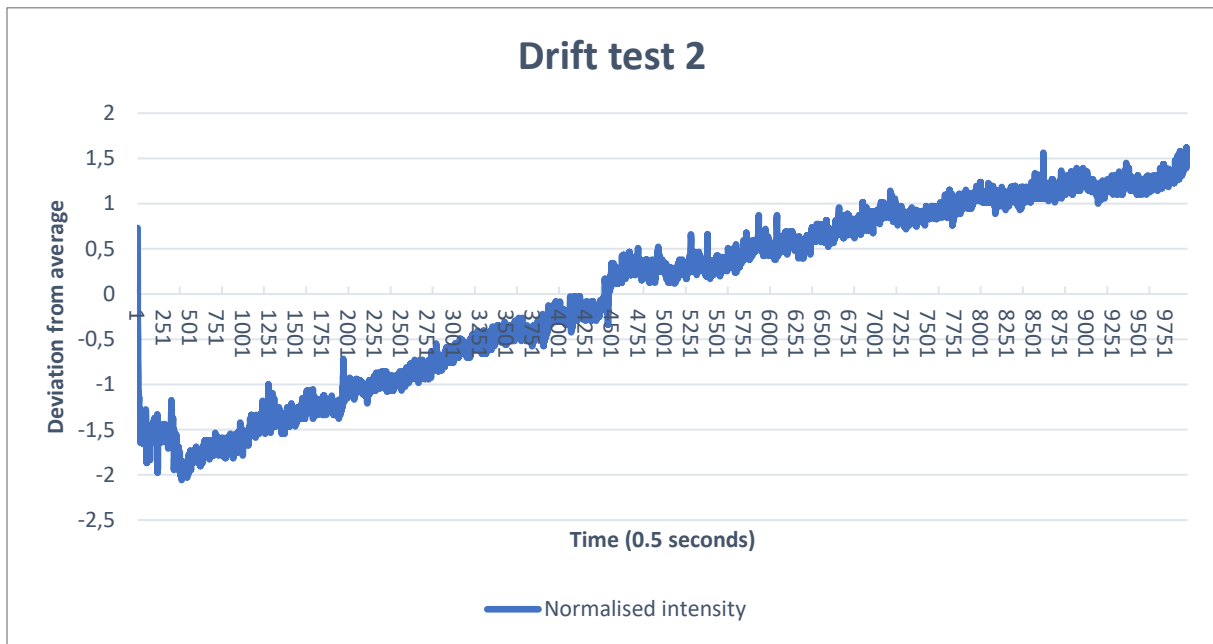


Figure 5.2: The second drift test. Only the lux data set from the sensor is used and normalised on its average. In contrast to drift test 1, where all four data sets were used. On the x-axis the time is depicted in half seconds because the sensor measured the intensity every half a second.

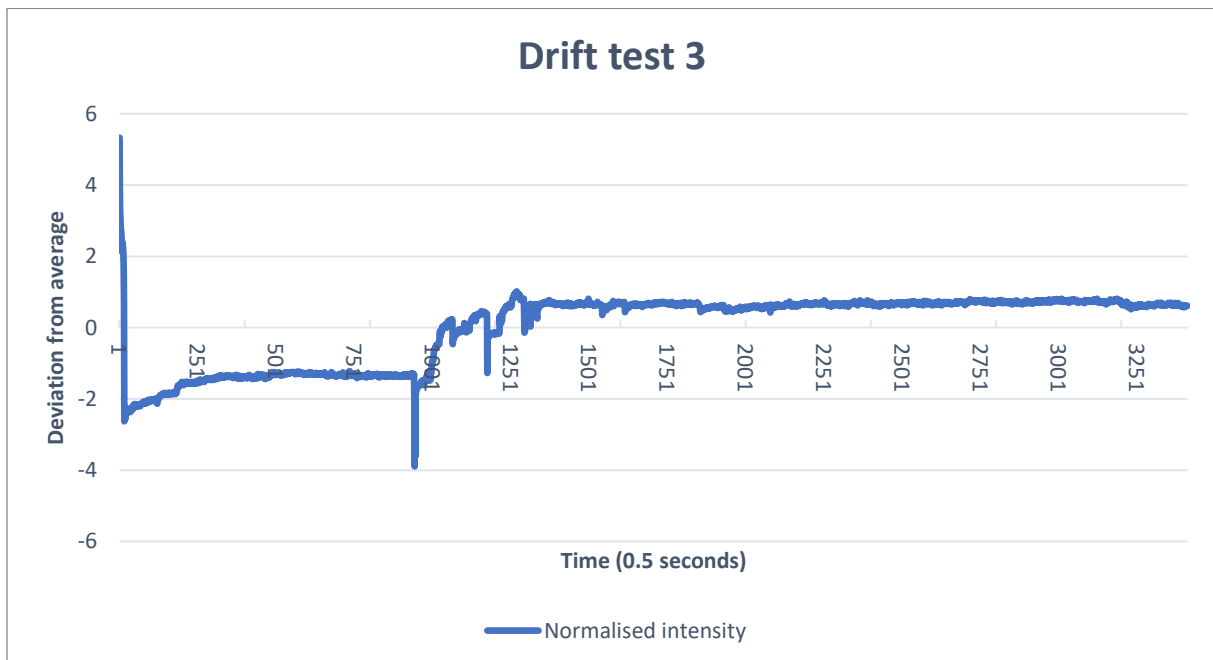


Figure 5.3: The third drift test. Only the lux data set from the sensor is used and normalised on its average. In contrast to drift test 1, where all four data sets were used. On the x-axis the time is depicted in half seconds because the sensor measured the intensity every half a second.

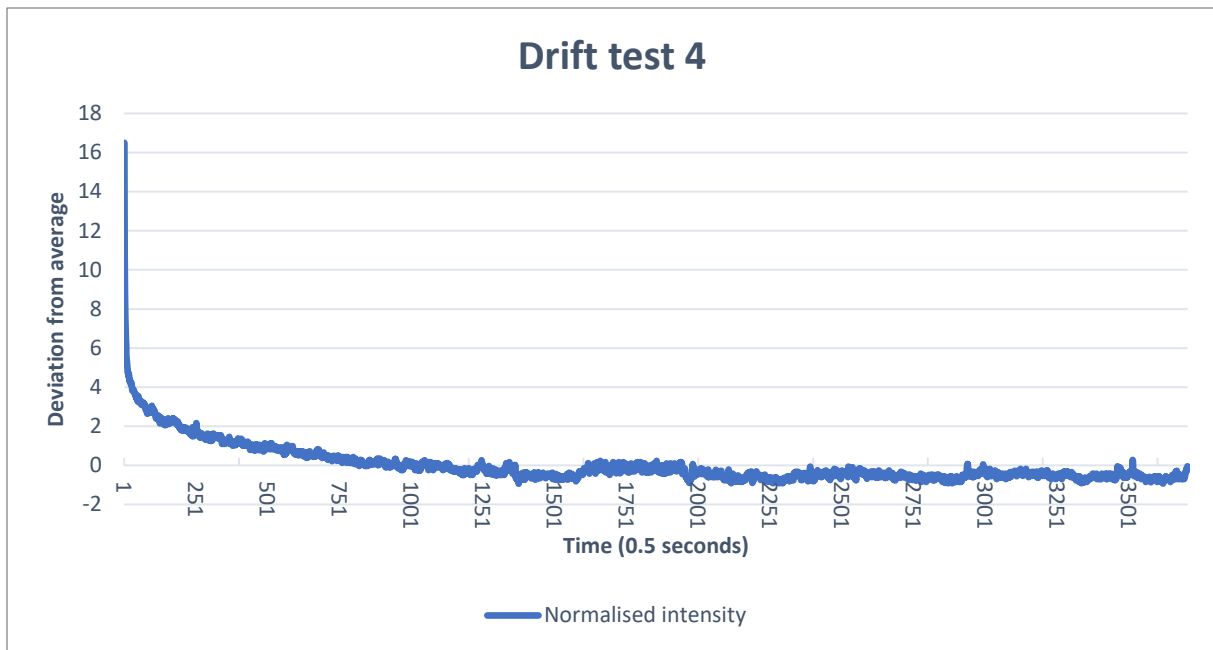


Figure 5.4: The fourth drift test. Only the lux data set from the sensor is used and normalised on its average. In contrast to drift test 1, where all four data sets were used. On the x-axis the time is depicted in half seconds because the sensor measured the intensity every half a second.

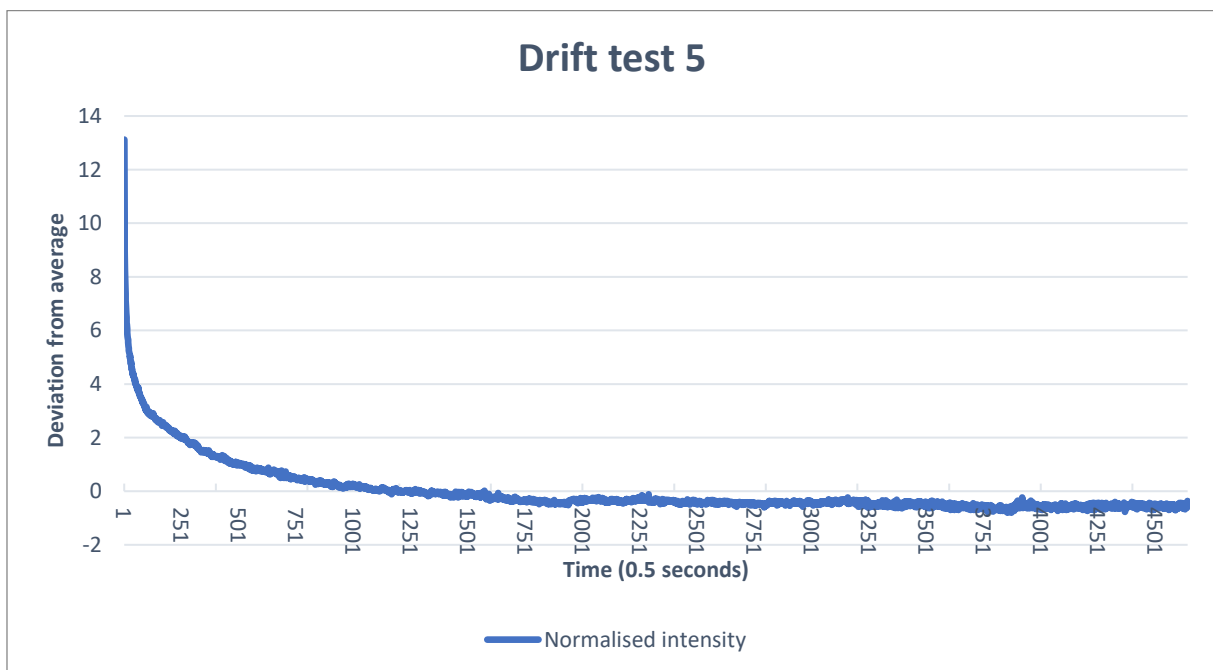
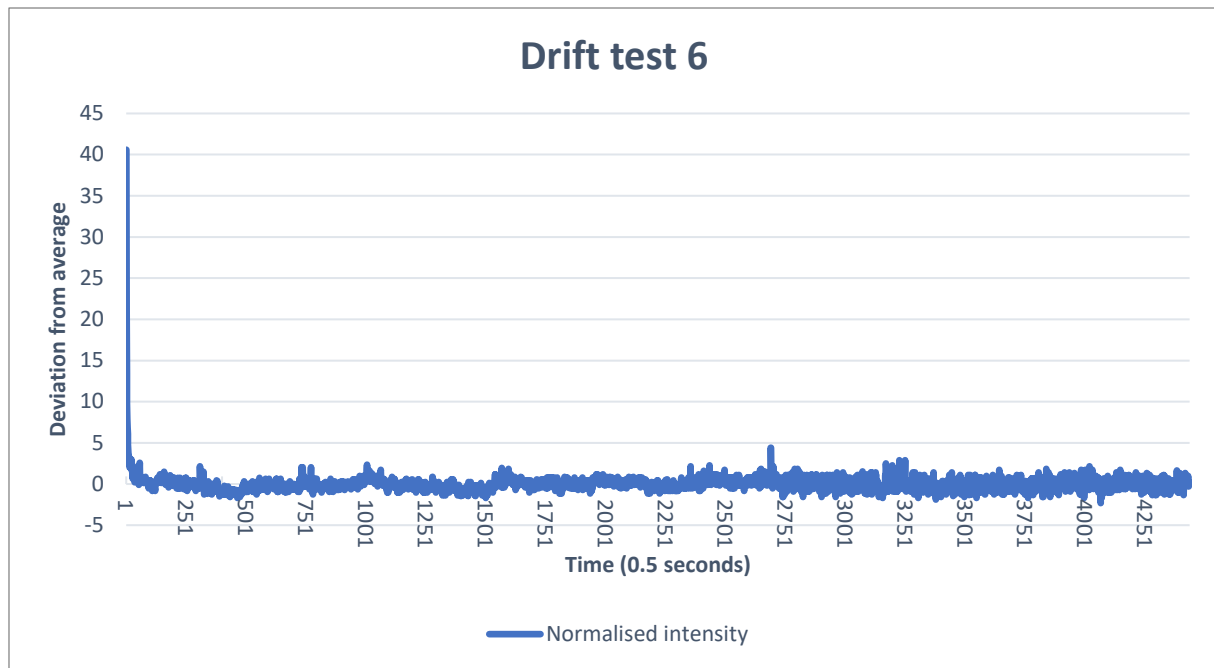


Figure 5.5: The fifth drift test. Only the lux data set from the sensor is used and normalised on its average. In contrast to drift test 1, where all four data sets were used. On the x-axis the time is depicted in half seconds because the sensor measured the intensity every half a second.



*Figure 5.6: The sixth drift test. Only the lux data set from the sensor is used and normalised on its average. In contrast to drift test 1, where all four data sets were used. On the x-axis the time is depicted in half seconds because the sensor measured the intensity every half a second.*

Figure 5.1, the first test, contains all four different data sets from the sensor. The code that came with the sensor was not very clear on the unit in which the full spectrum, infra-red, and visible spectrum were. The Lux data set was clear, this is the intensity in Lux. Therefore that dataset will be used from then on. Drift tests 2 to 6 are all done in the same way. The sensor and red LED are both turned on, the red LED on full power, and the setup is left in a dark environment for about an hour. The dark environment was created with a sweater and a leather jacket, for every test the sensor gave a value of zero intensity if the LED was turned off, meaning that any potential drift could not come from environmental light.

Test two and three have very different graphs from four, five, and six. The majority of tests do show the same behaviour as the last three tests, giving a reason to believe that the second and third tests do not represent the regular behaviour of the setup. For that reason only test four, five, and six will be used. As can be seen the intensity starts off with a big deviation from the average. This can be due to the setup starting up, either the sensor or the LED. In test four and five the setup was turned on for the first time that day. For test six the setup had been turned on before, that was test five, and the graph settles sooner in that test.

After type A the next drift tests were carried out, type B, with Plexiglas in between. The results can be seen in figure 5.7.



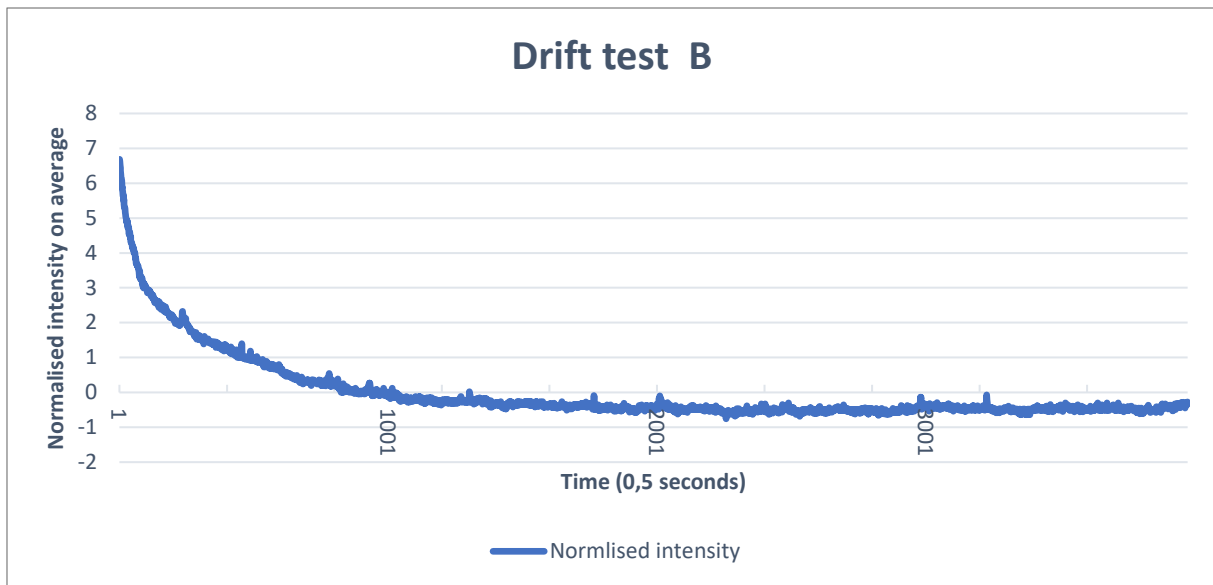


Figure 5.7: Drift test 1 B, the setup was turned on in a dark environments with the Plexiglas in between the sensor and the LED. The intensity was normalised on the average.

This test was only carried out once. There is a lot of noise on the signal but it deviates between 0 and -1 where the intensity in lux was about 13000, making the deviation a small part of the actual intensity. In this test a commonly seen graph is presented. This behaviour looks like the behaviour without the Plexiglas.

Next a vibration test was done. Here the setup was place in a dark environment, was turned on and then shaken a few times. The shaking was light shaking and the turning was about 20 degrees maximum. The outcome of this test can be found in figure 5.8.

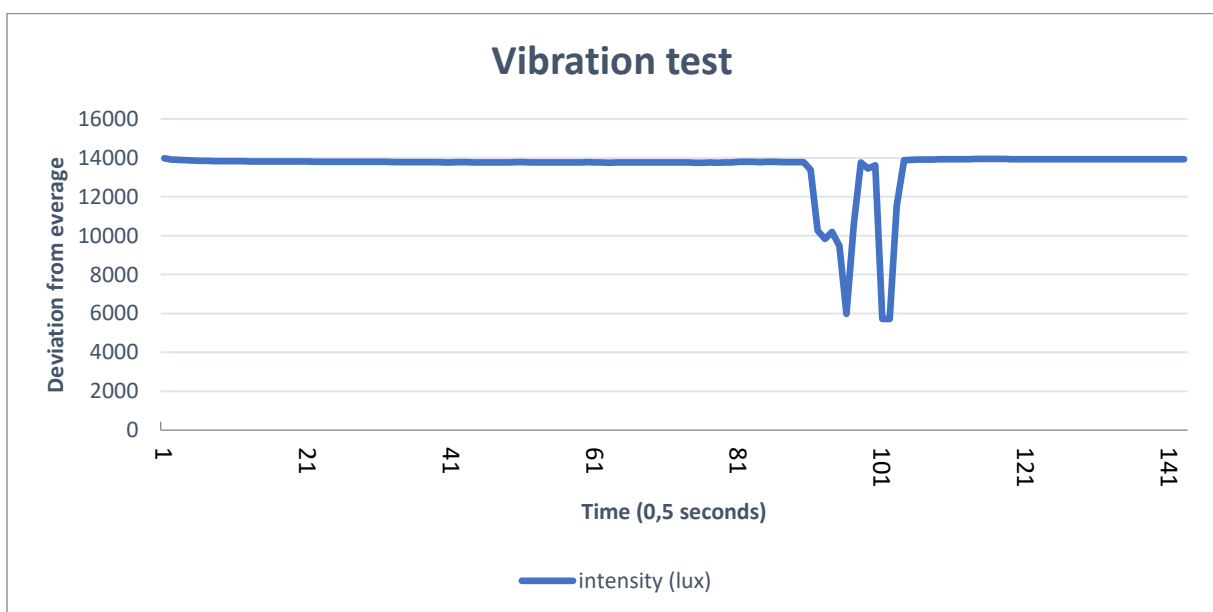


Figure 5.8: The vibration test. First the setup was only shaken, then the LED platform was touched to turn.

During the time that the setup was only shaken there is no big difference in intensity. It stays around the 14000 lux without any big deviation meaning the shaking has almost no impact. Where the LED platform was turned there are big deviations where the LED is not directly pointed at the sensor anymore. This can be seen in the drastic decrease in intensity around the 100 mark.

After this vibration test the temperature test was done where the sensor was taken out of the setup and instead a thermometer was placed and monitored. This was done over the course of two hours. The measurement was done in a room in an apartment building. The room is facing south which makes it hard to control the temperature. However, windows and curtains were kept closed to make sure the temperature would not rise due to sun light. The test was carried out between 12.00 and 14.00 which is a warm time of day, but with a stable sunlight intensity. The schematics of the setup can be seen in figure 5.9 and the results can be found in figure 5.10.

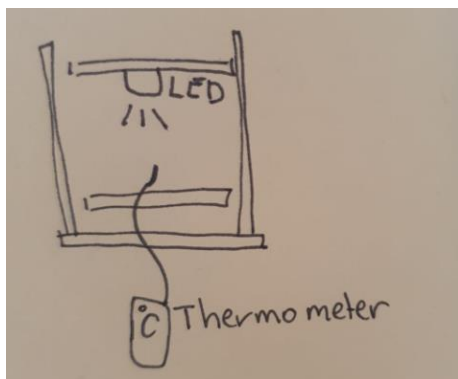


Figure 5.9: the setup for this experiment with the thermometer on the location of the sensor.

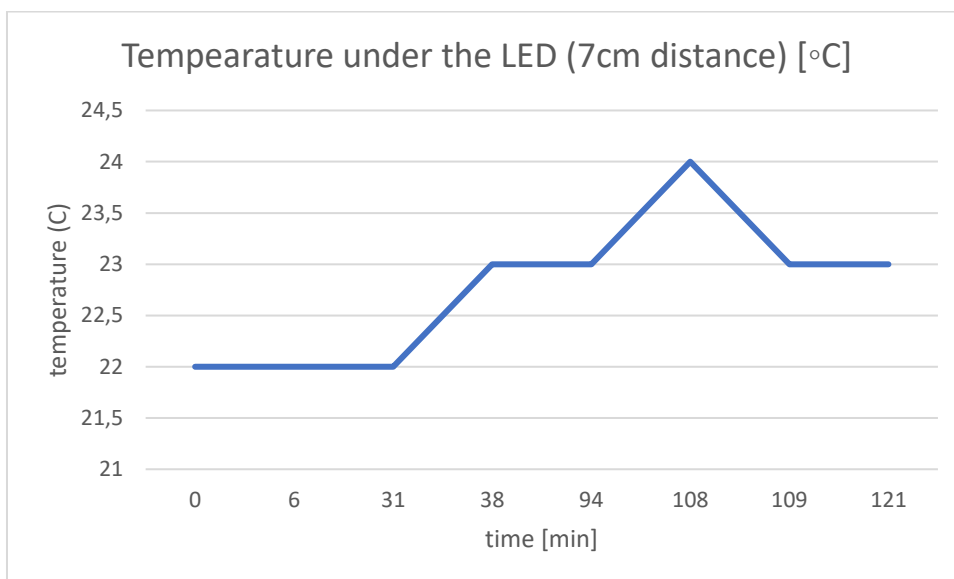


Figure 5.10: the temperature test. The sensor was replaced with a thermometer and monitored for two hours.

The temperature rises with 2 degrees over the course of about 1.5 hours. The temperature of the room and under the LED could not be measured at the same time because there was only one thermometer available.

The test done after this one is the high temperature test. This test used the setup with LED and sensor both turned on, and the thermometer in the middle. A hairdryer was used to increase the temperature around the setup. The setup for the experiment can be seen in figure 5.11 and the results can be found in figure 5.12.

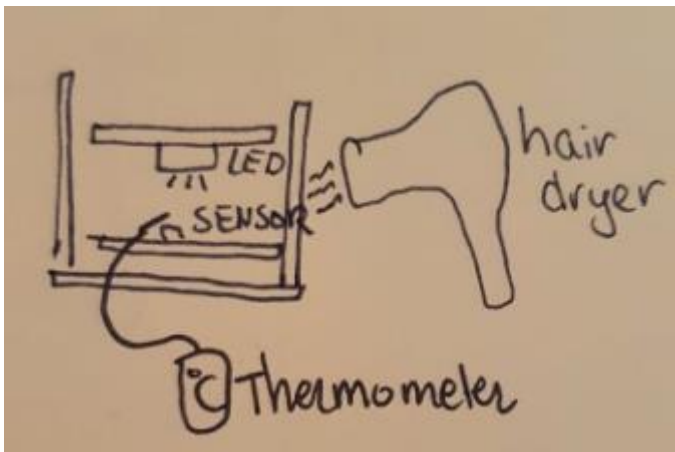


Figure 5.11: The setup for the experiment where a hairdryer was pointed at the setup when it was on to see the effect of high temperatures on the setup.

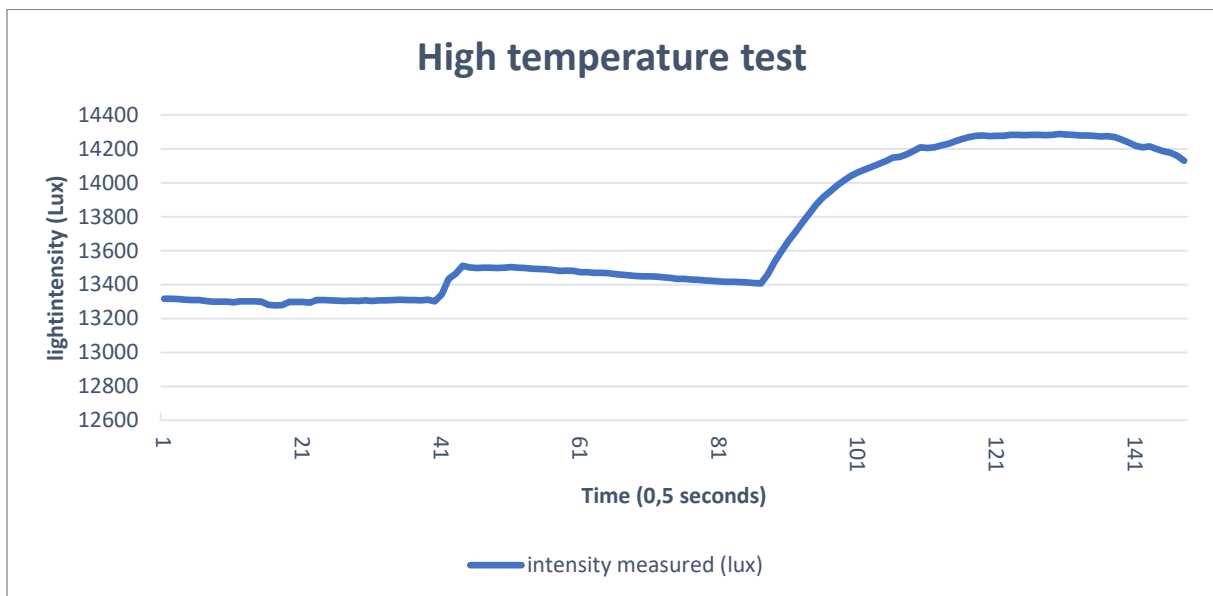


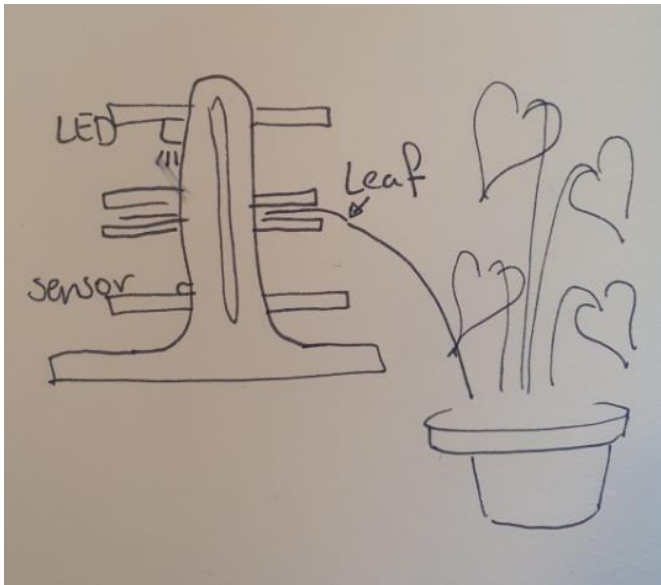
Figure 5.12: the high temperature test. A hairdryer was used to increase the temperature. The temperature started at 20 °C and was 50 °C at the highest point.

The temperature increased from 20 C to 50 C at the highest point. Whenever the dryer was pointed at the setup the measured intensity increased even though the intensity did not change in real life because the setup was kept in stable lighting conditions.

At this point all the setup specific tests were done. The initial values test has not been done because this differs per test but the behaviour is always the same. To counter the initial peak, which settles after about 15 minutes, the setup should be on before the measurements are going to be done. This way the setup has settled already when the measurements start so it does not pose a problem.

The next step is to do some tests on leaves. The Alocasia was picked for these tests because there is a lot of literature on this plant which would make testing and comparing easier. The next tests were done in a dark environment, the Alocasia was taken from a moderately lit room and put in a dark environment. The goal for this tests is to see how the signal changes as to when there is no leaf between the LED and the sensor. Therefore these tests were only carried out for 10 minutes at a time to see how the signal would behave, what kind of drift we see, how big is the noise?

The setup for this experiment can be seen in figure 13. In figure 5.14 and 5.15 the leaf tests are shown.



*Figure 5.13: Schematics of the experiments where leaves were used in the setup.*

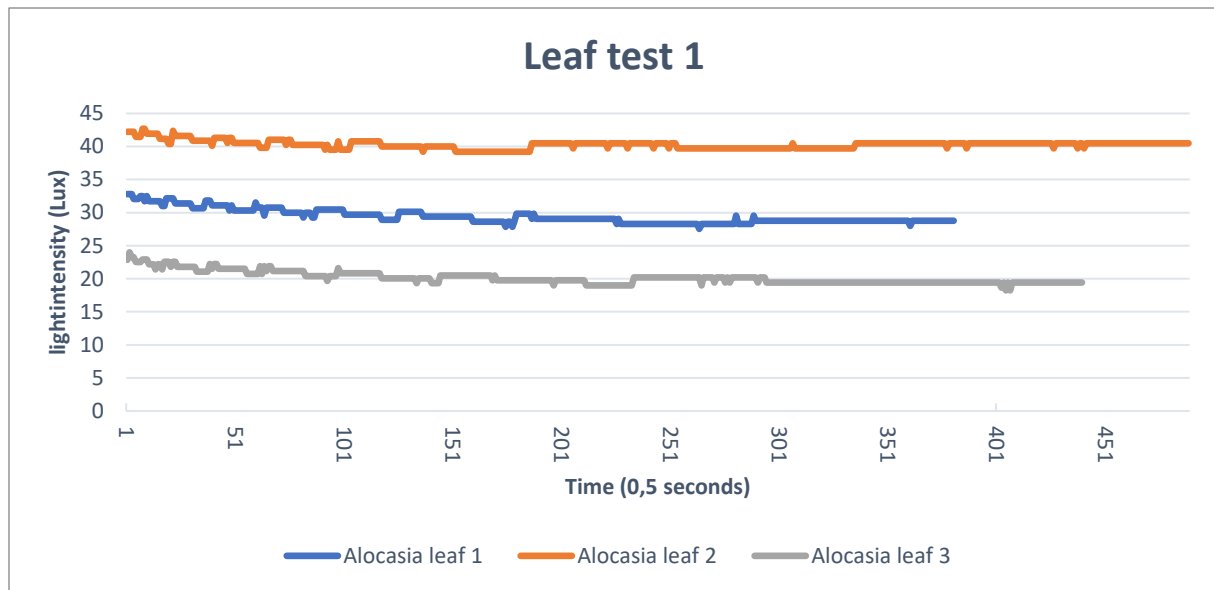


Figure 5.14: The first test on three different leaves of the Alocasia. All leaves were taken from a moderately lit room and places in a dark environment. Every leaf stayed in the setup for about 10 minutes.

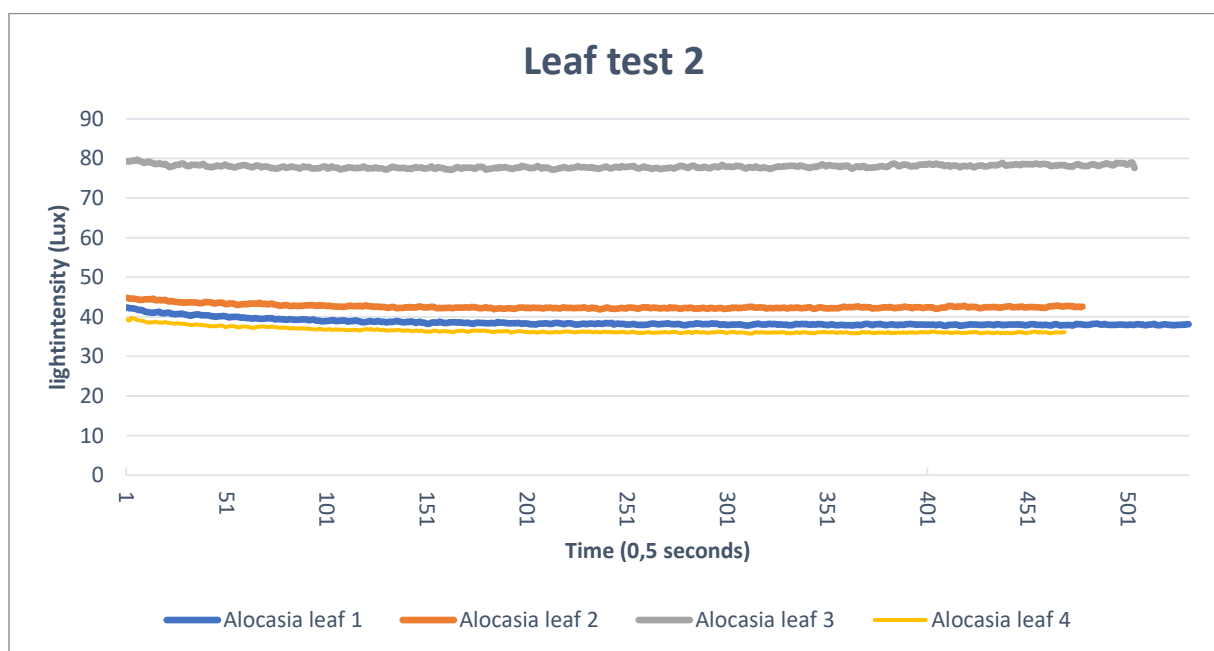


Figure 5.15: the second leaf test with four different leaves from the Alocasia. All leaves were taken from a moderately lit room and places in a dark environment. Every leaf stayed in the setup for about 10 minutes. The third leaf was a younger leaf than the other three.

At the start, the graphs show a small fall off. The intensity of the red LED has gone down by 12900 lux compared to when only the Plexiglas was between the LED and the sensor resulting in a intensity of around the 45 lux.

All tests up until now were done in the dark to ensure a stable lighting environment that would not influence the measurements. However, for measuring in the field the setup has to be able to measure transmittance when there is environment light. In previous research, a lock-in amplifier was used. [4] This

amplifier filters out the environment light to ensure the measurement is only the red light. For this project a similar principle was made and had to be tested.

The next step after this is starting the lock-in test. This means that the red LED will be on for a few seconds, then it will turn off for the same amount of seconds. From both measurements the average will be taken. Then the average when the LED is off will be deducted from when it was on and that will be the intensity of the light that will come in from the red LED through the leaf. This is an alternative from an actual lock-in amplifier which are expensive and is called synchronous detection. The code for this mechanism can be found in the second Arduino code in appendix A. The code was constructed and now has to be tested. A series of tests will be done, these were not stated in the test plan but we made it part of constructing the code and ensuring it was functioning properly.

For the five lock-in tests, these were the conditions:

1. Dark environment, no blue LED added, no leaf
2. Dark environment, no blue LED, with leaf
3. Dark environment, blue LED, with leaf
4. Dark environment, blue LED, with leaf
5. Light environment, blue LED, with leaf

For every test with a leaf a different leaf was used.

The results from the five tests can be found in figure 5.16 – 5.20.

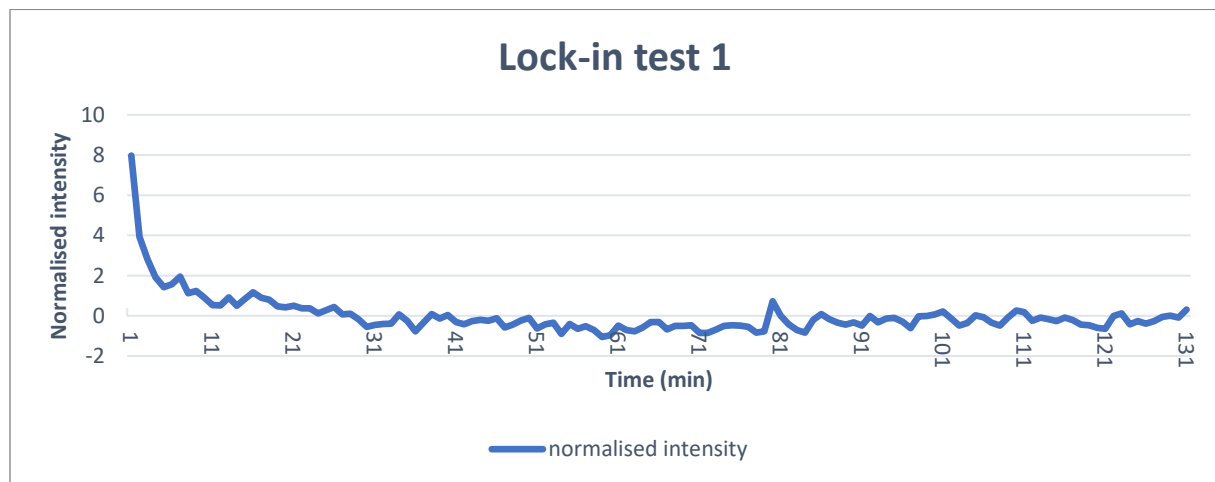


Figure 5.16: Lock-in test 1, Dark environment, no blue LED added. The average was 13176,99 lux.

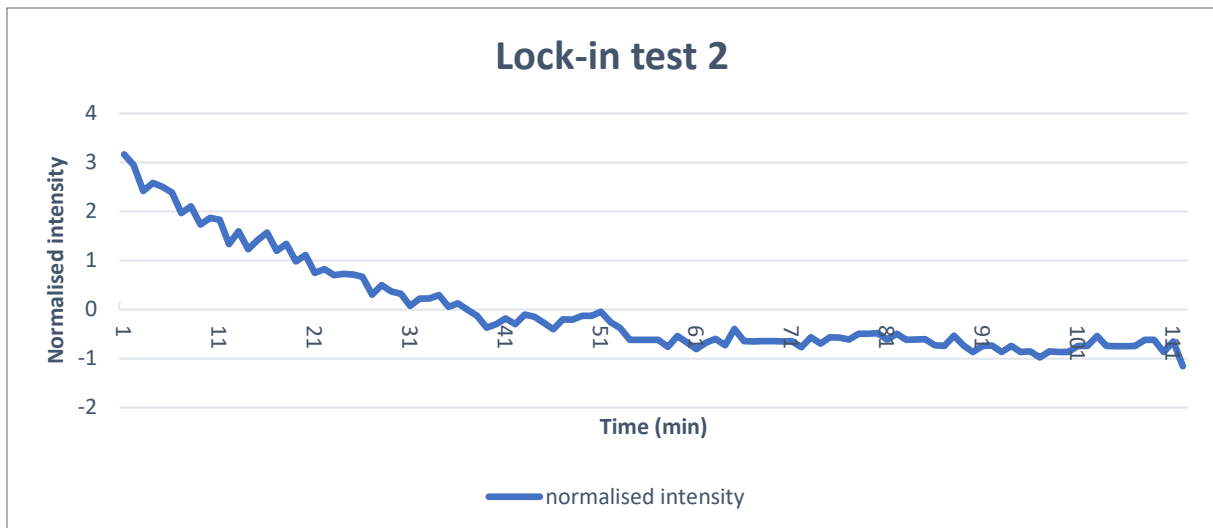


Figure 5.17: Lock-in test 2, Dark environment, no blue LED, with leaf. The average was 47,33 lux.

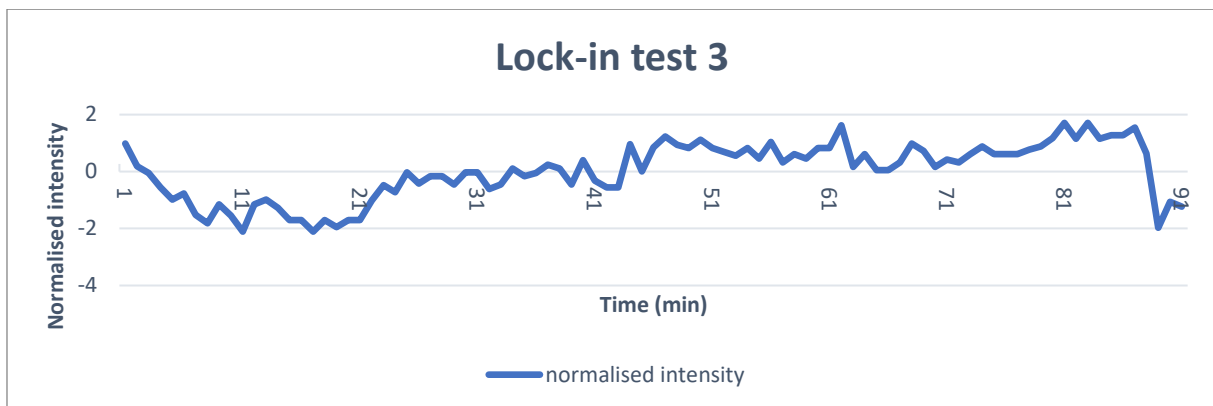


Figure 5.18: Lock-in test 3, Dark environment, blue LED, with leaf. The average was 46,89 lux.

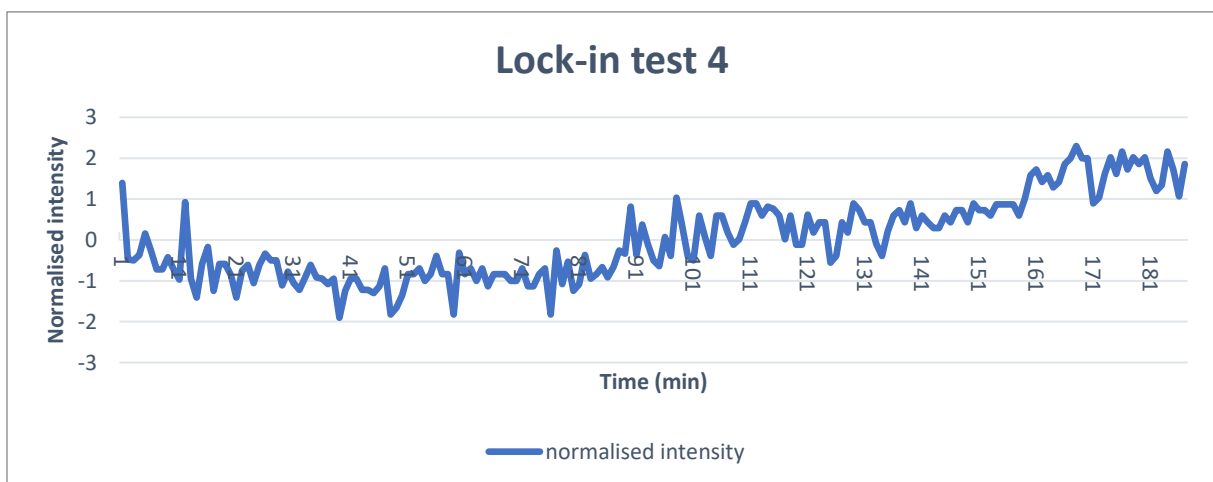


Figure 5.19: Lock-in test 4, Dark environment, blue LED, with leaf. The average was 36,35 lux.

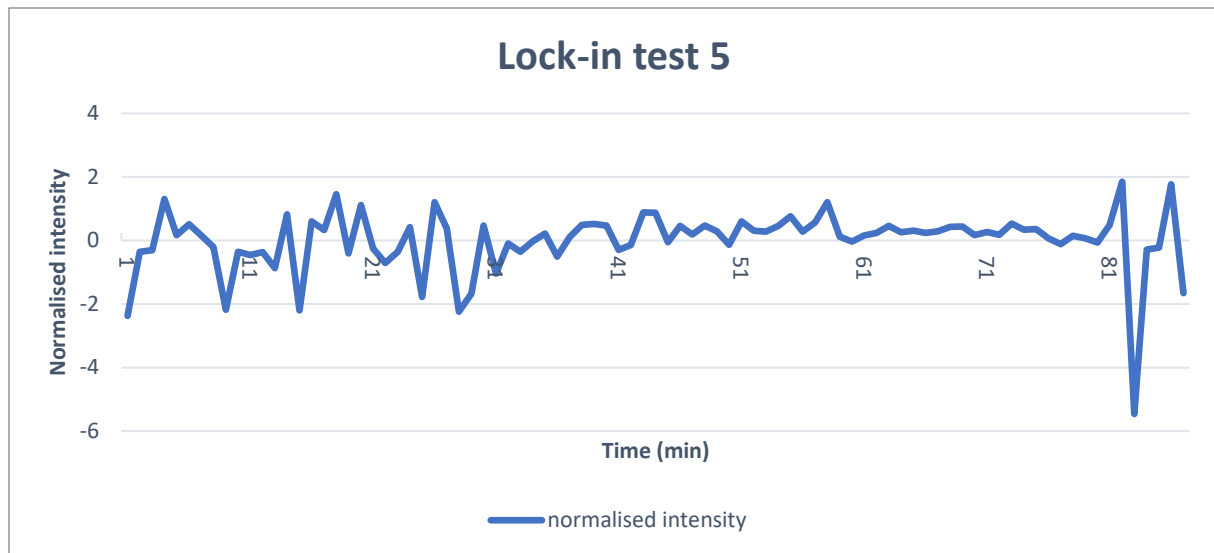


Figure 5.20: Lock-in test 5, Light environment, blue LED, with leaf. The average was 32,71 lux.

For all the lock-in tests, these were the accompanied actual intensity averages:

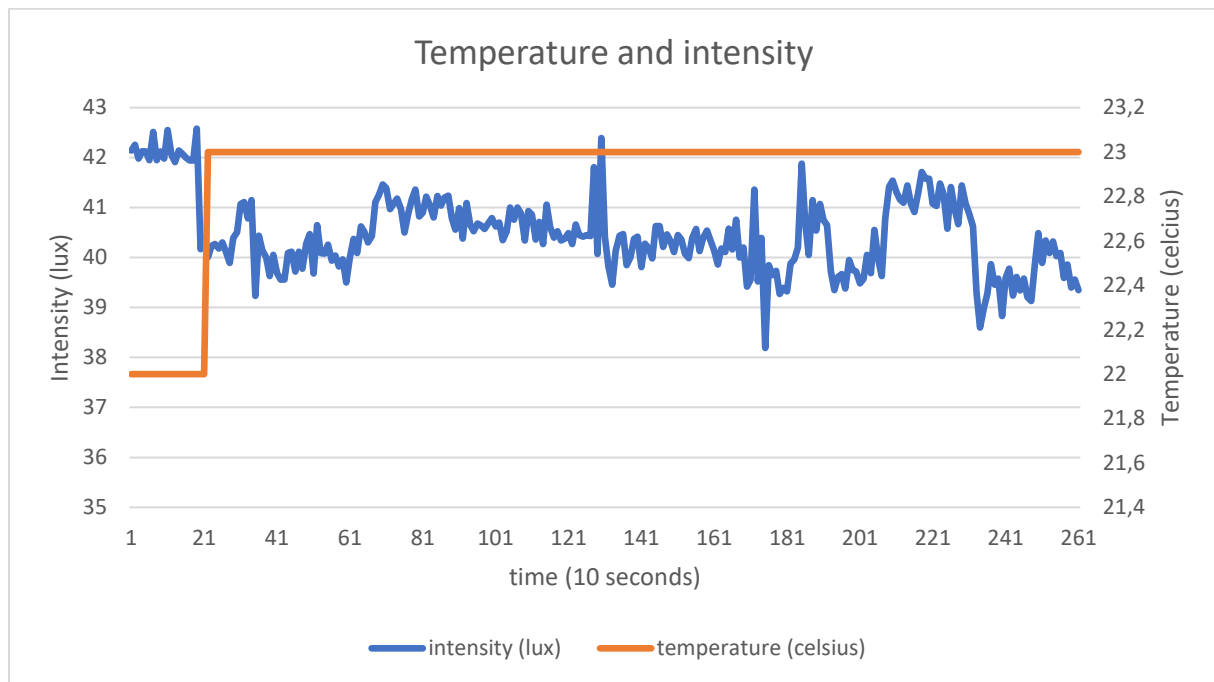
1. 13176,99 lux
2. 47,33 lux
3. 46,89 lux
4. 36,35 lux
5. 32,71 lux

The lock-in mechanism worked as follows. First the red LED would go on, every second the sensor would measure the intensity and it would do that for five seconds, so five measurements. These five measurements would be added together, divided by five, and give the average intensity during those five seconds. Then the red LED would turn off, the sensor would measure five seconds, or five measurements, again. The program would also add these measurements, divide by five, and give the average of the measurements when the red light was off. Then the program would subtract the average when the light was off, from the average from when the light was on. This results in the program giving the intensity of the red light the sensor would pick up. This means that the program can detect the red light that is transmitted and reflected by the leaf.

The question arose what influence the LED would have on the leaf. In earlier stages this was tested for the sensor but the influence on the leaf had not been tested. This could of course be different because the leaf might absorb more energy than the sensor and this would mean the leaf could heat up. Heating up of the leaf will influence the kinetics and therefore can influence the behaviour of chloroplasts and the measurements. [27]

This influence of heat of the LED was tested by sticking the thermometer under the Plexiglas, right next the spot on the leaf where the red LED would shine. This gave the results as in figure 5.21. During the test, the red LED would turn on every ten seconds and after 200 seconds the blue LED would also be turned on to see the effect that has on the temperature.





*Figure 5.21: the measurement of the leaf temperature and the measured intensity of the red LED using the lock-in principle.*

The temperature of the room was 21 °C, the leaf was 22 °C and after 200 seconds this rose to 23 °C and then stayed stable after that. This is also at the same time the blue LED was turned on. There could be a connection between the two but it is unlikely that the switching on of the blue LED triggered the temperature difference. The temperature change would have happened in a few seconds, where as the heating up of a leaf of 1 degree would take a longer time. Therefore the blue LED most likely did not trigger the temperature change. However, to ensure that temperature would be influence as little as possible the red LED frequency would be altered.

Now the red LED would still be on for five seconds, measure, turn off, and measure again for five seconds but there was a delay added of 50 seconds where the sensor would not measure and led would be off. This meant that the LED would only be on for five seconds every minute, thus not exposing the leaf to an energy burst every ten seconds but rather every minute. This time was chosen to give the leaf enough time to cool off again if it had been heated up by the red LED.

Another effect that can be seen is the drop in intensity when the blue LED was turned on. This required another test to see if this was consistent. The setup was turned on, there was no leaf in the clip, and the red LED intensity was measured using the new version of the lock-in system. Results can be seen in figure 5.22.

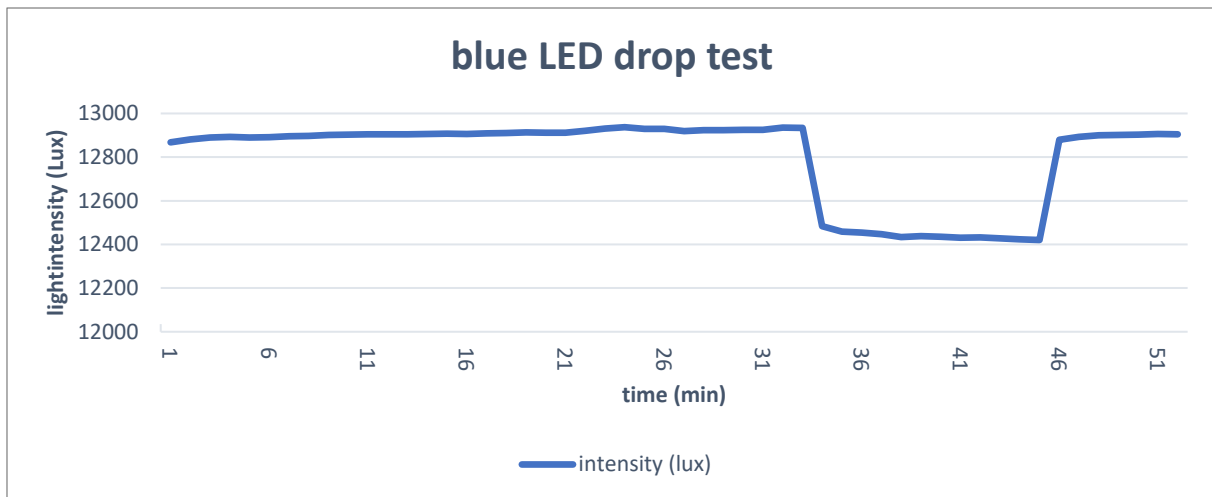


Figure 5.22: the blue LED was turned on, turned off at minute 33 and on again at minute 46. The effect can be seen in the drop and rise of the intensity.

The graph in figure 5.22 shows how the change in intensity measured happens when the blue LED is switched on or off. However, it does seem to be consistent. The test was repeated to see if it was really consistent. The second test can be seen in figure 5.23.

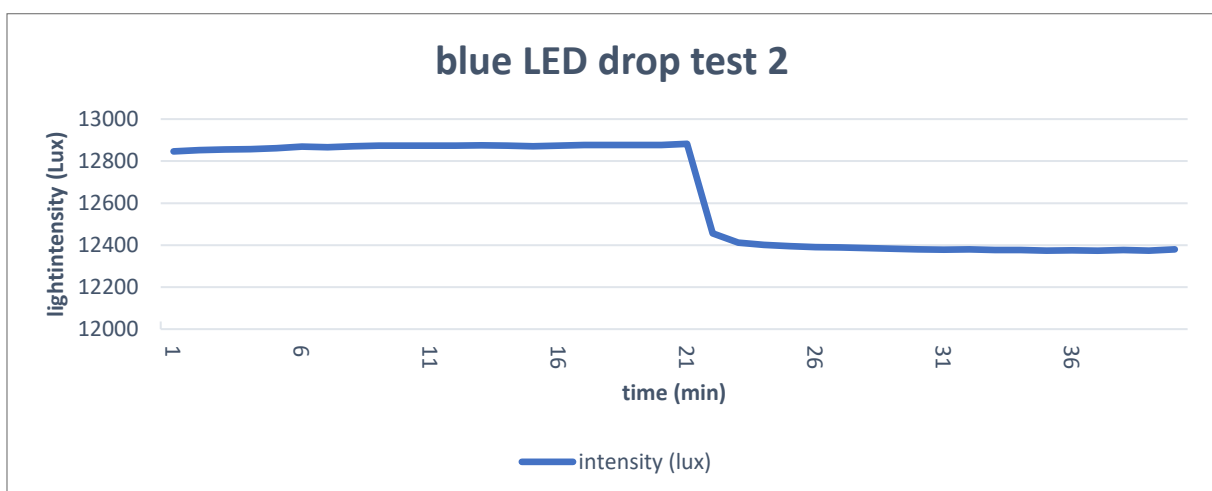


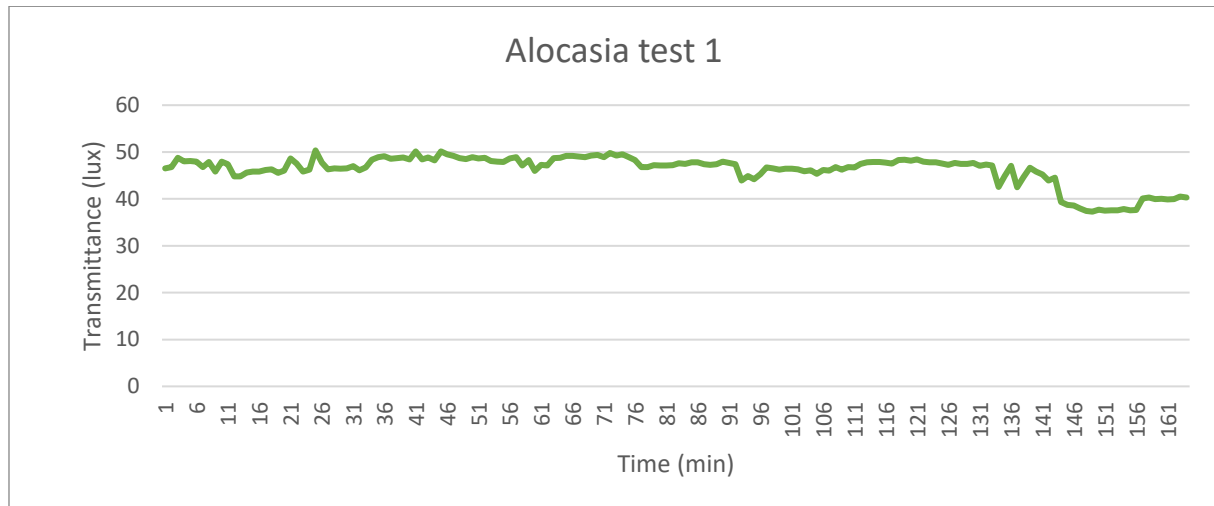
Figure 5.23: the second blue LED drop test that was carried out. At minute 21 the blue LED was turned off, a drop in intensity measured can be seen there.

The same behaviour happened in the second test.

The next step is to test the whole setup with the lock-in mechanism, a leaf, and an increase of environmental light. First the Alocasia will be tested. The Alocasia was left in the dark for two hours. Then the setup was turned on and after 15 minutes the Alocasia was placed into the setup. This would counter the initial peak or start-up drift which was observed earlier. The blue LED was also turned on, giving a blue

environment light of about  $633 \mu\text{mol}/\text{m}^2/\text{s}$  or about 6000 lux. For all tests to come the unit of  $\mu\text{mol}/\text{m}^2/\text{s}$  will be used for environmental light, because this is widely used and common in already existing research, making it easier to compare.

The schematics are the same as in figure 5.13 for all tests with plants inside. Results to this test can be found in figure 5.24.



*Figure 5.24: the first alocasia test, the plant was adapted to darkness for two hours, then placed in the setup with the blue LED on giving  $633 \mu\text{mol}/\text{m}^2/\text{s}$  of environment blue light. The shown measurement starts when the blue LED was just turned on.*

In the graph from figure 5.24 there is no clear increase of T and R, which would be expected. The graph even goes down at the end, which is the opposite of what was expected. This asked for the same experiment but with different plants. The corn and the lily were chosen for the next attempt for this experiment. The same conditions were kept. Results can be found in figure 5.25 and 5.26.

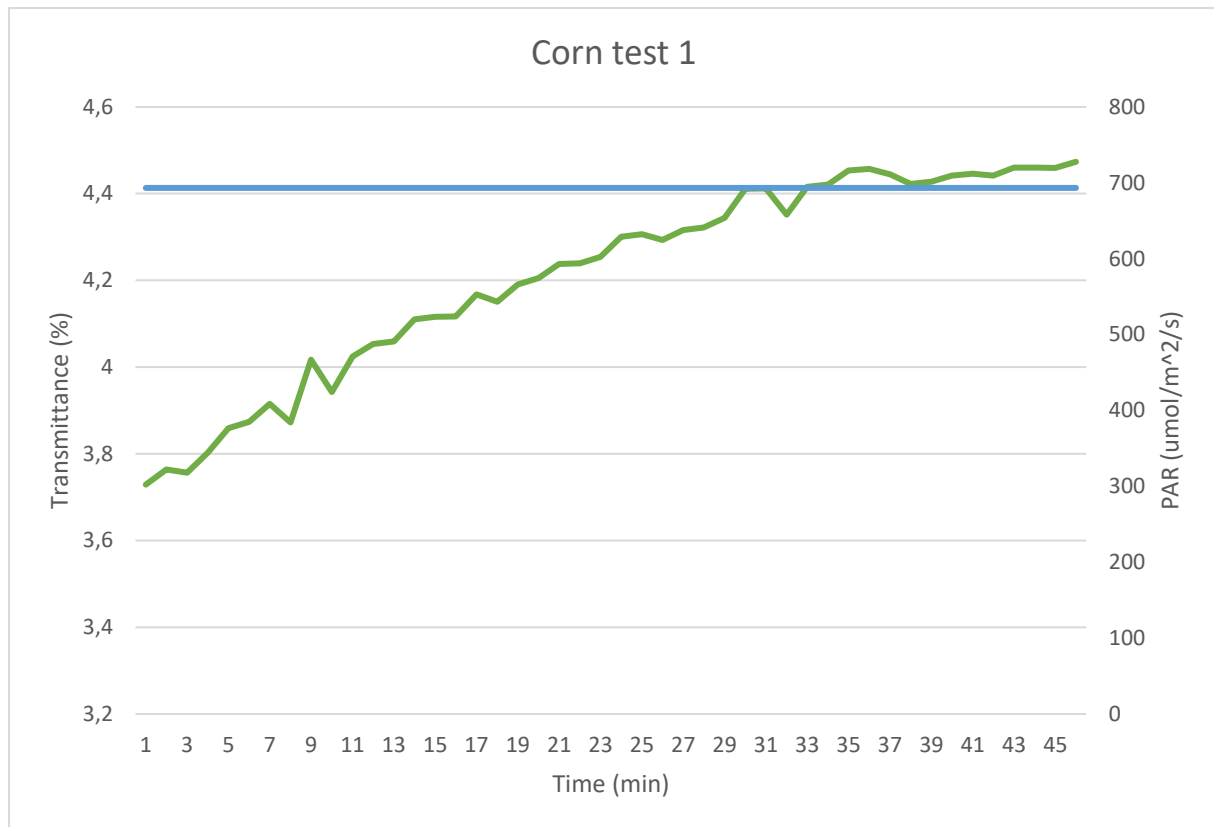


Figure 5.25: the first test on corn. The corn was first adapted to the dark for two hours, then placed in the setup with the blue LED turned on. The shown measurement starts when the blue LED was just turned on.

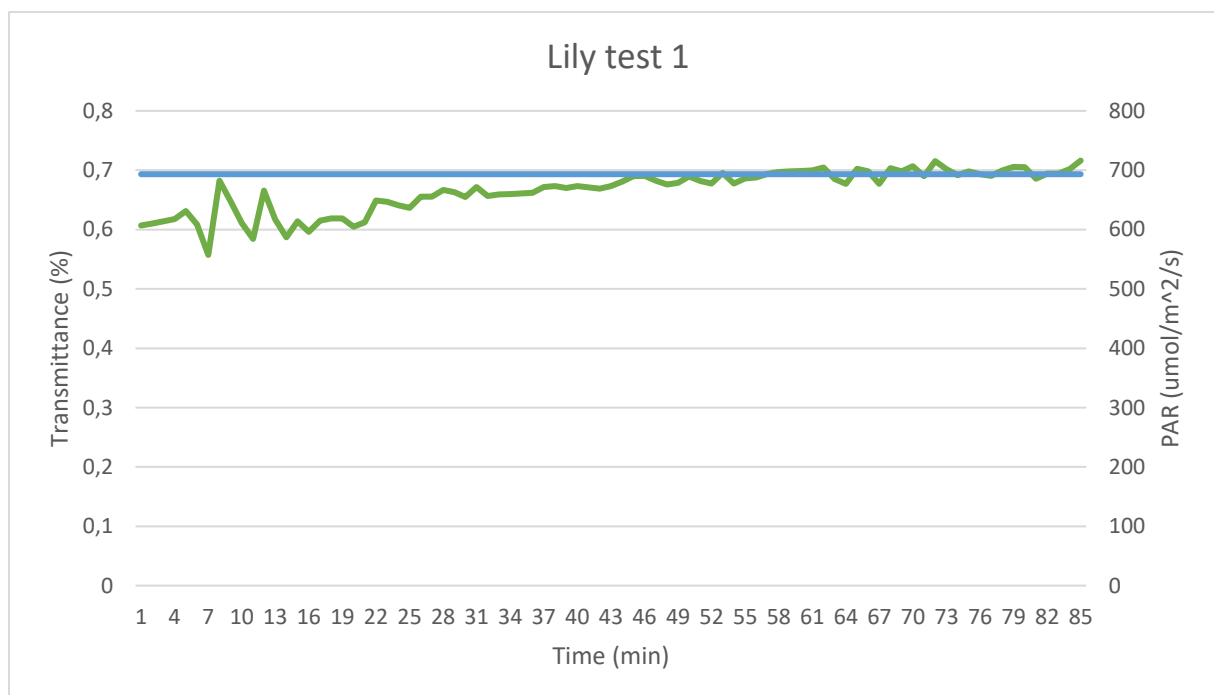


Figure 5.26: the first test on the lily. The lily was first adapted to the dark for two hours, then placed in the setup with the blue LED turned on. The shown measurement starts when the blue LED was just turned on.

After the first test round with the three plants another test round was done with the same plants and the exact same execution. This was done to see if the results are reproduceable. The graphs for these tests can be seen in figure 5.27, 5.28, 5.29 and 5.30.

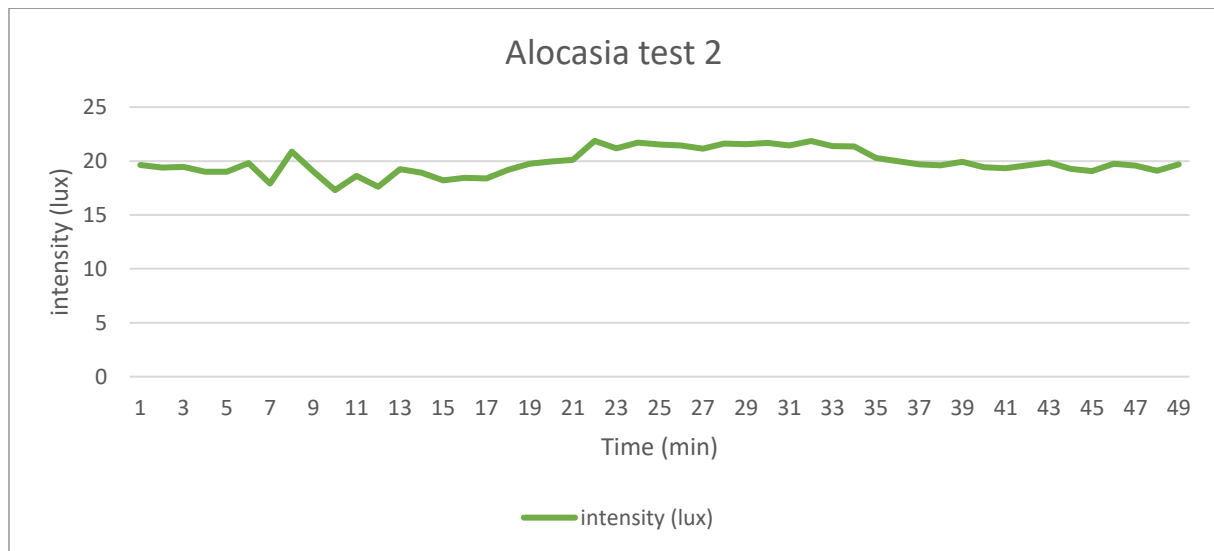


Figure 5.27: the second alocasia test. The plant was adapted to darkness for five hours, then placed in the setup with the blue LED on giving  $633 \mu\text{mol}/\text{m}^2/\text{s}$  of environment blue light. The shown measurement starts when the blue LED was just turned on.

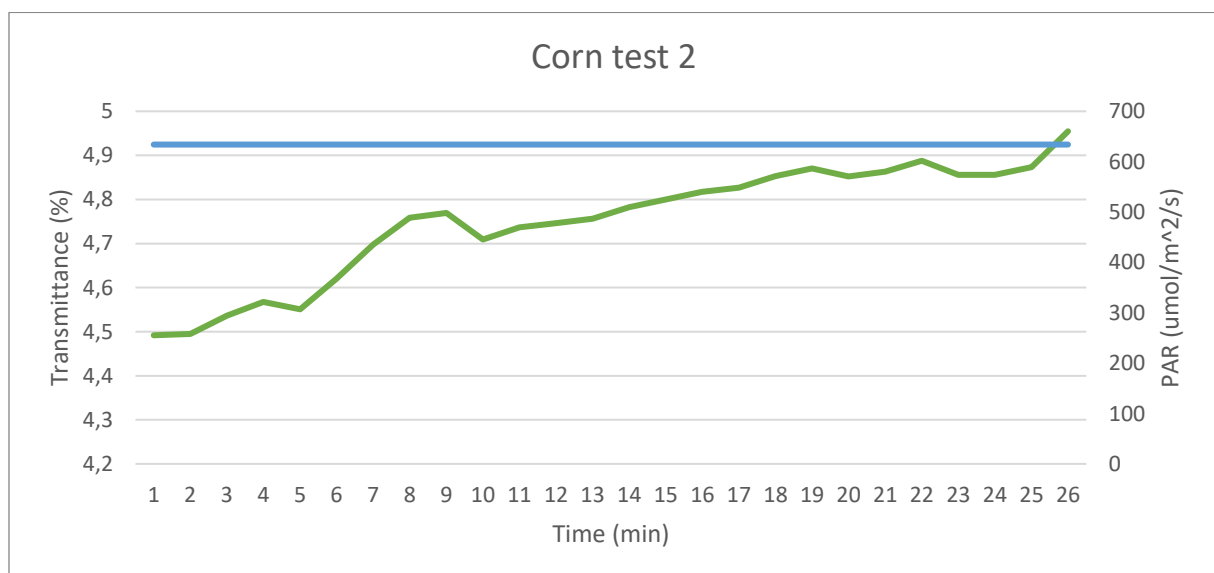


Figure 5.28: The second corn test. The corn was first adapted to the dark for two hours, then placed in the setup with the blue LED turned on. The shown measurement starts when the blue LED was just turned on.

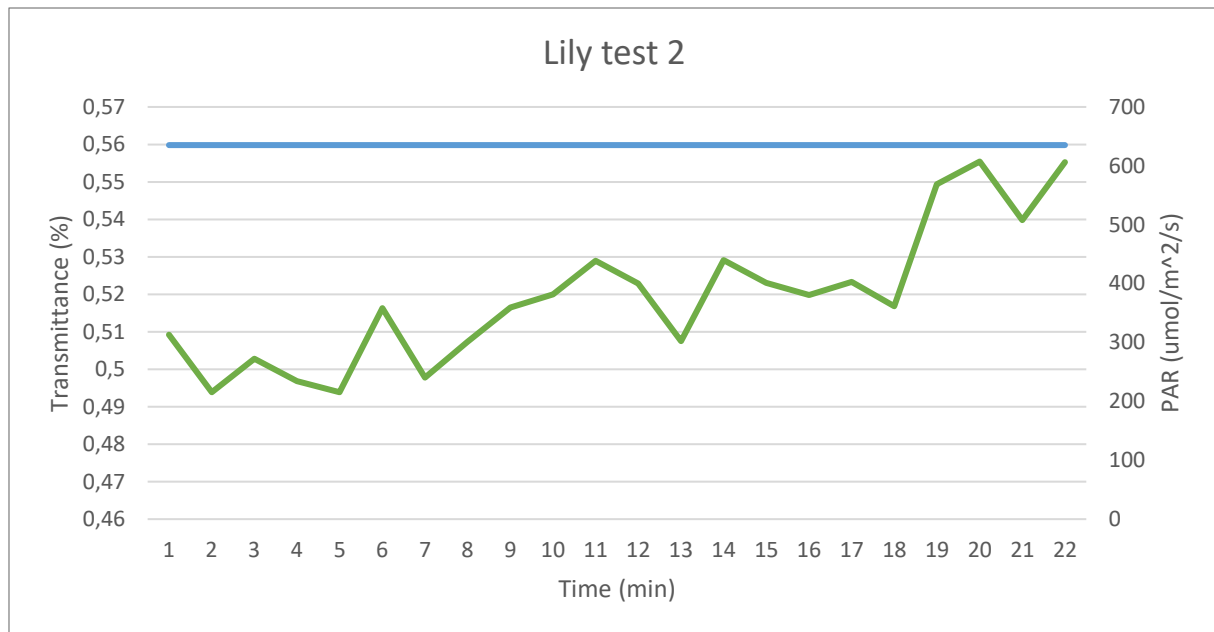


Figure 2.29: The second lily test. The lily was first adapted to the dark for two hours, then placed in the setup with the blue LED turned on. The shown measurement starts when the blue LED was just turned on.

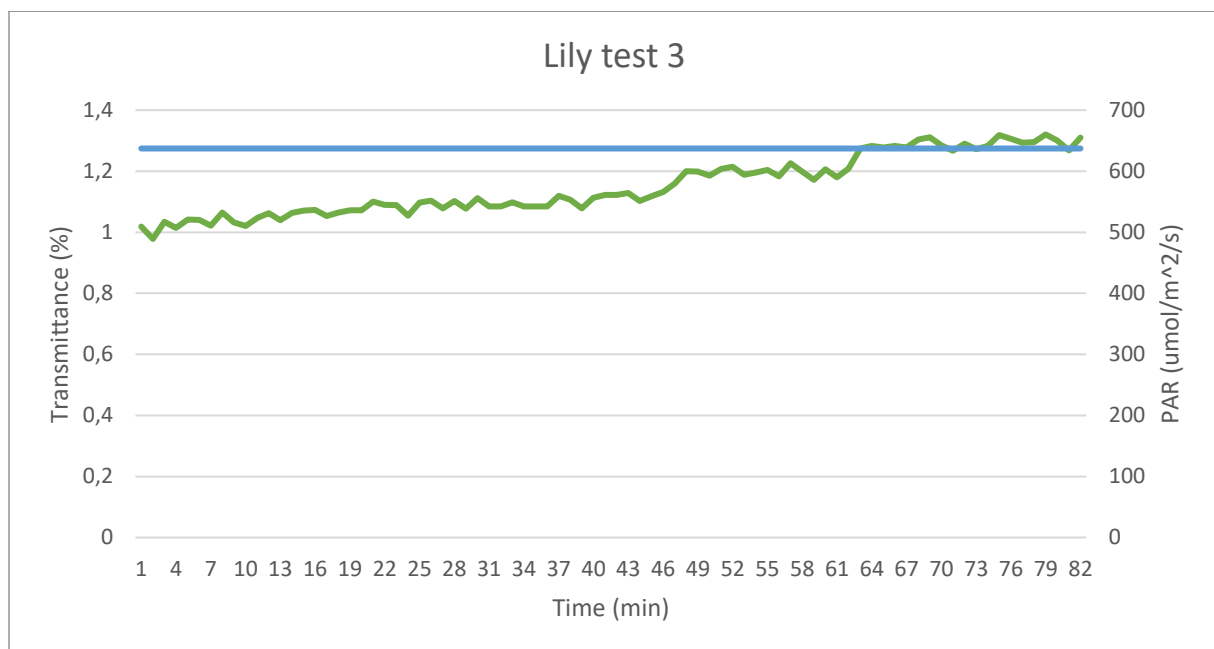


Figure 2.30: The third lily test. The lily was first adapted to the dark for two hours, then placed in the setup with the blue LED turned on. The shown measurement starts when the blue LED was just turned on.

After the three plants had been through the same experiment again, the same test was done on the lily but in reverse. The lily was exposed to the blue light of around  $640 \mu\text{mol}/\text{m}^2/\text{s}$  for two hours. Then it was placed in the dark where the input blue light was about  $33 \mu\text{mol}/\text{m}^2/\text{s}$ . The results can be seen in figure 5.31.

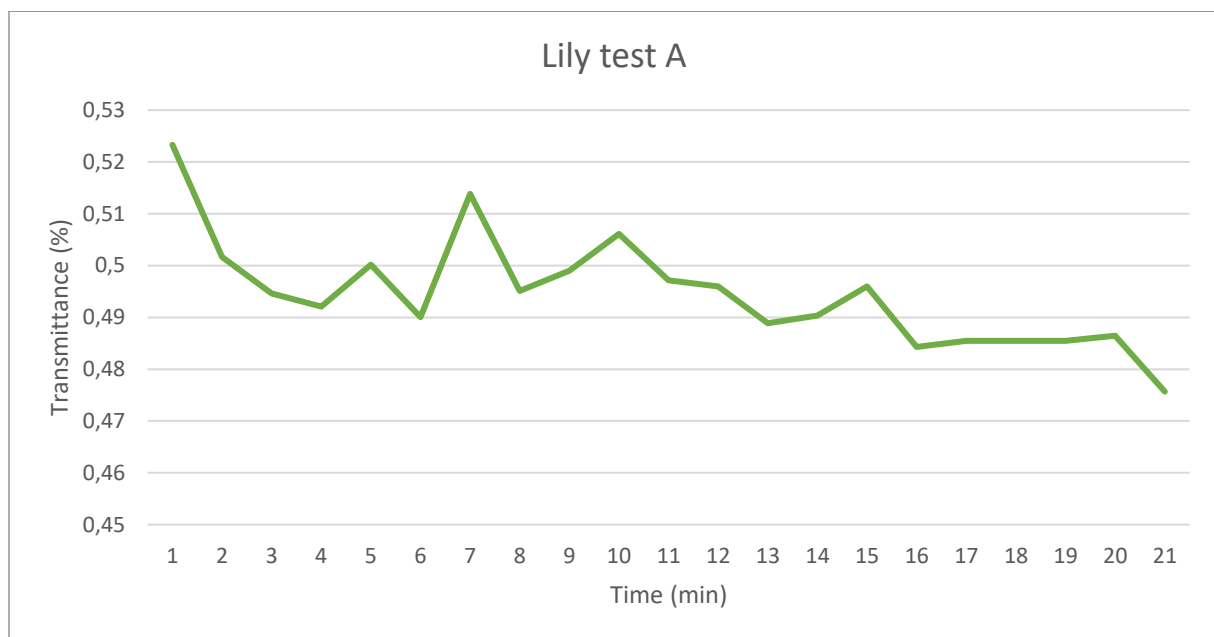
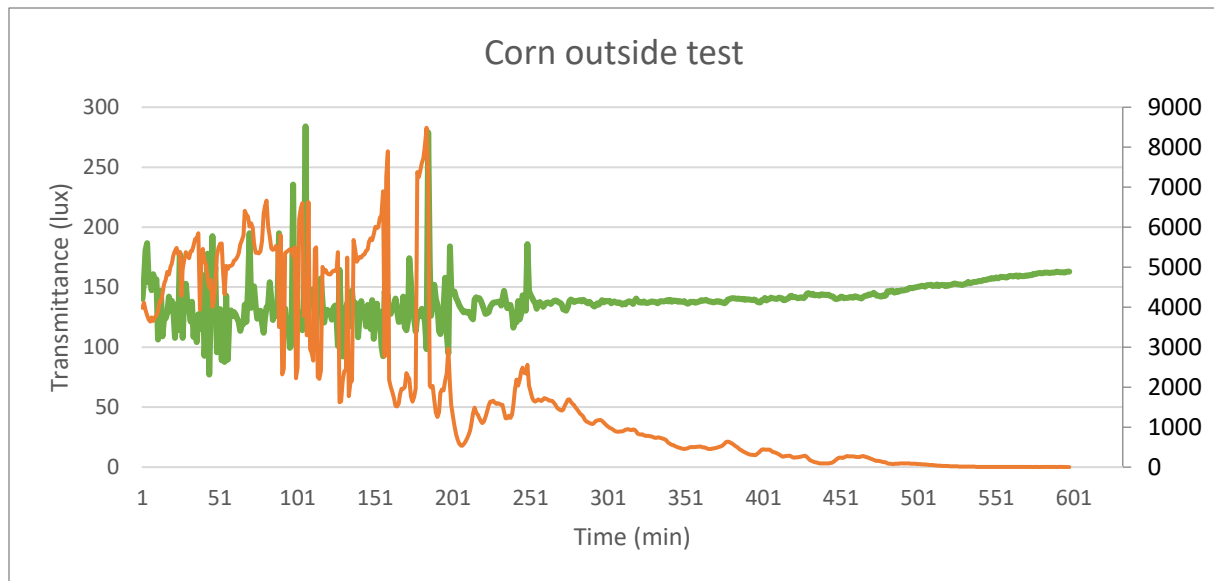


Figure 5.31: The test on the lily where it was first exposed to  $640 \mu\text{mol}/\text{m}^2/\text{s}$  for two hours and then to  $33 \mu\text{mol}/\text{m}^2/\text{s}$ .

Another test that had to be done was the final test, this test was done with corn on a south facing balcony. The test was started at 11:00 when the corn plant was still located in the shade. At 12:30 the corn was in the direct sunlight until 20:14 when the sun lowered behind buildings to block the sunlight. The red LED light going through the leaf was measured next to the environment light which is measured every minute as well. The results can be found in figure 5.32.



*Figure 5.32: the results of the corn test outside. Until minute 90 the plant was located in the shadow, from then to minute 556 it was exposed to direct sunlight, after that the sun lowered so the plant was in the shadow again.*

These were all the tests that were done in this project. When comparing to all the tests from the test plan some tests were skipped. The Initial values, leaf and intensity, sun test, and leaf movement were not carried out. The reason for this is because they were not necessary or useful anymore or had low priority so were taken out of the realisation of the tests to save time.

## 5.2. Discussion

Here all the results from the previous subchapter will be discussed. Some reasoning will also be given as to why some tests were done after the other, or why tests were done less or more times than planned.

Starting with the drift tests. As can be seen in the corresponding figures the setup has a settling time of about 10 to 15 minutes where the initial value is quite high and then the measured intensity settles, often with an error of 40 on 13000 lux or 0,3%. The setup seems stable over time.

Something that could influence the measurement of light intensity is the temperature. This can either influence the LED performance or the sensor performance. The performance related to temperature can be found in figure 5.33. The left picture is the functionality of the sensor compared to temperature. The right picture is that of the Vishay LED. Unfortunately the datasheet of the minostar spot does not contain any information on the behaviour of the LED in relation to temperature. The data might not be completely correct, but the behaviour will be similar. Therefore the assumption is made that when the temperature increases the LED will shine less bright and the sensor will give different values depending on



which of the two channels is used most for the measurement. These two channels are the two sensors used in the Adafruit sensor, one for infrared and one for the visible spectrum of light.

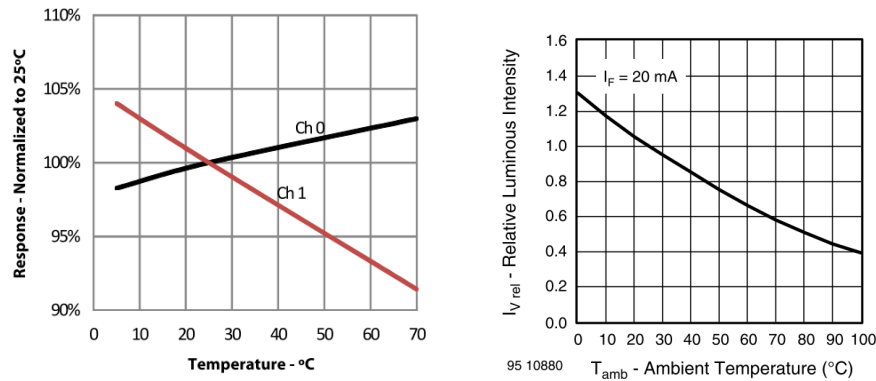


Figure 5.33: showing the functionality of the sensor (left) [21] and a Vishay red LED (right) [12] compared to temperature.

When comparing the two first temperature tests (figures 5.10 and 5.12) it can be seen that the LED does not warm the sensor up significantly for the functionality to be effected. However, when temperatures rise drastically, as seen in figure 5.12, the measured intensity is greatly affected. A temperature of 50 °C was reached in the second test which had a clear impact on the sensor. A difference from 21 °C to 50 °C gives a difference in intensity from 13200 lux to 14200 lux. This means that the setup should not get too hot or the measurements can be greatly affected. When a leaf was inserted in this setup it would have also warmed up, changing the kinetics of the leaf. Extreme heat will change the behaviour of chlorophyll fluorescence for example. Chlorophyll are molecules in chloroplasts and will re-emit more or less light depending on the temperature and light intensity. [27] This can greatly influence the measurements.

Another factor that could have influenced the setup was vibrations. Looking at structure of the setup it is unlikely that shaking the setup will change much in the measurements, depending on how heavily the setup is shaken. The factor that will have the most influence is the turning of the LED, this will result in the most light dense part being shifted away from the sensor and therefore the sensor will measure a lower intensity. Comparing this to results the conclusion can be drawn that light shaking will not affect the measurements but the turning of the LED will. Turning the LED by 20 degrees will result in a drop of approximately 8000 lux in intensity on an intensity of 14000 lux. This test shows it is very important to not turn the setup, or shake it hard enough for the LED to move.

The tests done after that were the tests with the leaves inside the setup. These results (figures 5.14 and 5.15) show the same initial peak in the measurements, which is to be expected. What can also be seen is that the intensity of red LED light getting through the leaf is between 40 and 50 compared to the 13000 lux when there is no leaf in between. 40 is only 0,3% of 13000, meaning a big percentage of light does not go through the leaf. The plant used here was an Alocasia.

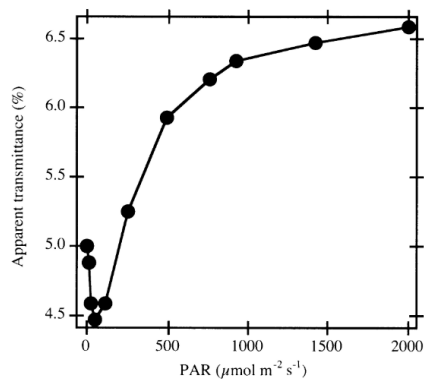


Figure 5.34: The behaviour of the chloroplasts in the *alocasia* plant compared to incoming sun light. [4]

The environmental light in both tests was 0, expecting a transmittance percentage of around 4,5%. As can be seen in figure 5.34.

However, the results from the tests give 0,3% of the incoming red light. The difference is that the red light used in this specific paper is much lower, 40 lux, compared to 13000 lux. In the measurements for figure 5.34, they measured transmittance of red light (wavelength 660 nm) with an avoidance response induced by white light. [4] If this setup would use such a low intensity it would not detect enough light going through. The intensity of the red LED can be brought down, which will be done in the dark tests later on.

Another noteworthy aspect of these tests is that the younger leaf from figure 5.15 has a lower absorbance percentage which is interesting to keep track of when doing experiments and comparing to literature.

To actually start the measurements where the behaviour of the chloroplasts can be seen using the blue trigger light the lock-in system had to be made. The code is already explained in the previous subchapter and can be found in appendix A. The three tests associated with the lock-in were carried out to see if any abnormal measurements were taken. The code could have obstructed the normal functioning of the sensor or give a larger drift. It was expected that the noise on the signal would become smaller than before because of the extra averaging which happens when the measurements are added and divided by five.

Compared to the earlier drift tests the initial peak in the lock-in tests were smaller but the noise level stayed around the same. When a leaf was added to the setup the deviation from the average was between 2 and -2 lux for both the leaf tests and the lock-in tests.

After the lock-in tests, the tests with the blue LED were done. When the blue LED was turned on or off there was a jump in the data. However, this jump is consistent so it can be countered in the data processing part of an experiment. All data after the jump will get a correction by adding or subtracting the amount of lux the jump was. This means the jump is undesirable but it can be countered.

The tests after this are the most interesting tests. Here the leaves were first adjusted to darkness for a few hours and then put in the setup with the blue LED giving a light intensity of around  $640 \mu\text{mol/m}^2/\text{s}$ . The results from these tests should clarify if the setup is functional.

Starting with the global behaviour of the Spathiphyllum and the corn. In both cases, in all the tests, the Transmittance increases when the environment light increases. This is what is expected. For the Spathiphyllum, no previous research could be found. Unfortunately the tests with the Alocasia were unsuccessful because there exists a plethora of previous researches about this plant type so comparing to previous research would have been more accessible. However, to compare results of the Spathiphyllum the results will be compared to other shadow plants. For comparison the Cyrtomium Fortunei will be used. This is also a shadow plant.

For the Cyrtomium the transmittance will increase from 5,5% to 6,8% when exposed to enough light to saturate the avoidance response. The plant was first adapted to darkness and then exposed to light. The graph for this previously done research can be seen in figure 5.35.

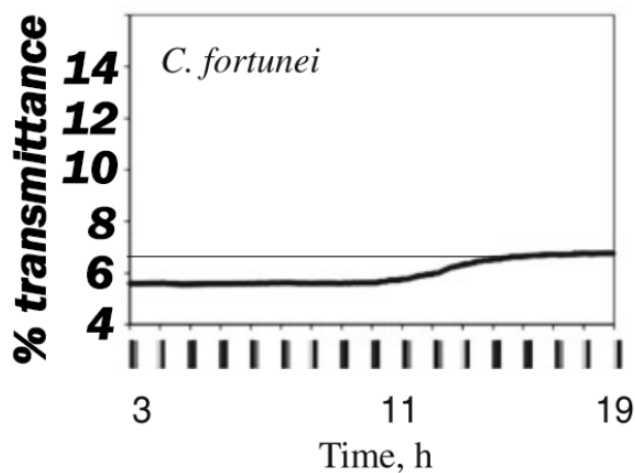


Figure 5.35: the behaviour of the cyrtomium fortune when first being exposed to darkness and to enough light to saturate it afterwards. An increase of 5,5% to 6,8% can be seen in the graph. [28]

In table 5.1 the specific results from the lily tests can be seen. This includes the starting and ending value and the difference.

Table 5.1: The starting and ending percentage of transmittance + reflectance of the lily.

Name	Starting %	Ending %	Difference
Lily test 1	0,61	0,71	0,1
Lily test 2	0,50	0,56	0,06
Lily test 3	1,16	1,31	0,15
Mean	0,76	0,86	0,10
Standard deviation	0,35	0,40	0,05
Variance	0,13	0,16	0,00

First difference that stands out is that the percentages are a lot lower than the ones from the Cyrtomium. This could be explained due to it being different plants, however, as mentioned before for the first Alocasia tests the percentage was also lower than was expected. The increase is also smaller, around 10 times smaller.

For the corn a research gives a change of 0,9% in absorptance when going from dark to light. These results can be seen in figure 5.36.

Sample	$D$	$+ \Delta D_{\max}$ (%)	$I_w$ (ergs/cm <sup>2</sup> sec)	$- \Delta D_{\max}$ (%)	$I_o$ (ergs/cm <sup>2</sup> sec)
<i>Monocotyledonae</i>					
green foxtail	0.89	0.12 (14.0)	720	0.11 (12.0)	3,800
corn	1.21	0.11 ( 9.1)	1,800	0.12 ( 9.9)	7,300
large crabgrass	0.98	0.05 ( 5.1)	80	0.14 (14.0)	1,800
goosegrass	0.87	0.05 ( 5.8)	1,100	0.04 ( 4.6)	3,800
Job's-tears	1.19	0.04 ( 3.4)	960	0.05 ( 4.2)	3,800
silver hairgrass	0.97	0.02 ( 2.1)	270	0.02 ( 2.1)	1,800
dayflower	1.45	0.01 ( 0.7)	470	0.07 ( 4.8)	1,000
rice	1.15	0.01 ( 1.0)	1,100	0	—
<i>Dicotyledonae</i>					
begonia	0.94	0.16 (17.0)	80	0.24 (26.0)	1,800
dandelion	1.49	0.20 (13.0)	900	0.30 (20.0)	1,800
annual fleabane	1.45	0.14 ( 9.7)	470	0.14 ( 9.6)	1,000
pokeweed	1.25	0.10 ( 8.0)	900	0.10 ( 8.0)	3,800
erigeron sp.	1.49	0.09 ( 6.1)	1,300	0.03 ( 2.0)	10,000
lettuce sp.	1.04	0.05 ( 4.8)	2,600	0.01 ( 1.0)	10,000
milk smartweed	1.35	0.04 ( 3.0)	460	0.04 ( 3.0)	1,800
curly dock	1.41	0.03 ( 2.1)	1,300	0.03 ( 2.1)	9,200
spinach	1.34	0.02 ( 1.5)	270	0.08 ( 6.0)	900

Figure 5.36: results of different plants, one of which is corn. Showing a difference in absorptance. The +D is for increase and the -D for decrease of absorptance. [29]

For comparing to the corn tests the value of -D will be used. This is because the figure uses absorptance and the results use transmittance + reflectance. An increase in absorptance is a decrease in T and R. Here it starts off at 1,21 % and can be decreased with a maximum of 0,12%. In table 5.2 the results of the corn tests will be shown for comparing.

Table 5.2: the starting and ending percentages and the difference between them

Name	Starting %	Ending %	Difference
Corn test 1	3,73	4,47	0,74
Corn test 2	4,50	4,95	0,45
Mean	4,12	4,71	0,60
Standard deviation	0,54	0,34	0,21
Variance	0,30	0,12	0,04

Again the difference between these tests and the previously done research is the starting percentage, which is a lot higher for this projects tests. The increase in percentage is also not the same, however, the previous research gave a maximum decrease which does not exclude lower decreases for this plant.

Even though the starting and ending percentages do not match previous research, the time it took to saturate the avoidance response can also be compared and does match expectations. In table 5.3 the times it took for the avoidance response to be saturated.

*Table 5.3: The times it took for the avoidance response to be saturated in the corn and lily experiments. The cells represent the time it took in minutes, the top row represents which tests are being compared. Lily test 1,2, and 3 and corn test 1 and 2.*

Time [min]	1	2	3
Lily	85	22	82
Corn	45	30	-

As can be seen from table 5.3 the times from the lily test 1 and 3 are almost the same and also match the expected time of around 2 hours for other shadow plants like the Alocasia. [4], [14] The corn has two saturation times that are not that close but do fit the expectations of about 30 minutes. [29] More testing should be done to make this a trusted comparing method but these first tests give promising results.

The last test mentioned in the previous subchapter is the test done outside with the corn leaves. However, there is no clear correlation between the values of transmittance + reflectance and the environmental light. It is even counter intuitive. The T+R should go down when the environmental light goes down, however, it goes up. Explanations here could be that the light was varying too much. The Arduino code only works if the environmental light is stable enough due to averaging of multiple measurements.

Another factor that might have influenced the outside setup is the temperature. In previous research the LED was cooled to ensure a stable red input light. [4] This was not done in this setup and might need to be added to make the setup field ready.

On an important note, a different species of plants can influence the outcome because of numerous factors like thickness of the leaves, structure of the leaves, surface reflectance, and water content. [10]

## 5.3. Conclusion

All setup tests were tools to reach the final few tests. The discussion on these initial tests mainly showed that, next to an initial peak, the setup was functional. It registered data and the final lock-in system worked. The most important part, the final tests with corn and the lily, however did show different results than the literature. Initial and ending percentages which were a lot higher or lower than expected, and

the differences from start to end which were lower than expected. However, the transmittance + reflectance did always increase when tests were done and the mean is very low from all the tests which suggests a repeatable experiment with similar outcomes each time it is carried out.

This still leaves a difference between the results and the literature but there are countless factors that have influence on such an experiment. Even a slightly different species or mutations of a plant can give different results. Not all literature experiments could be copied and therefore these tests are likely to find different values.

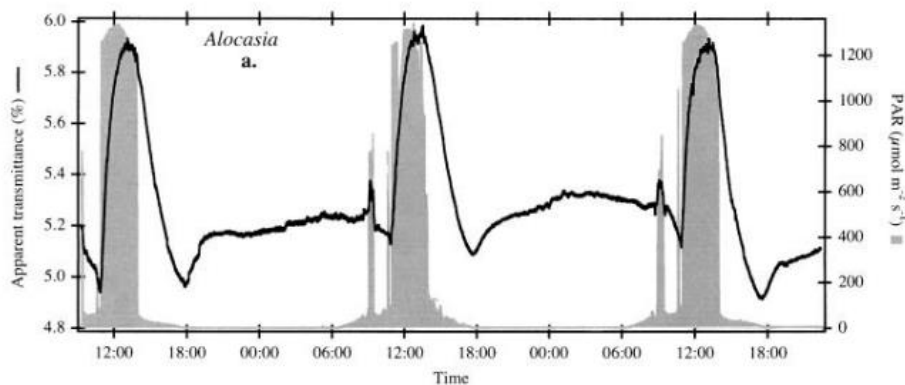
Concluding, the setup can give similar results for repeated experiments, suggesting it is functional, however, the values from these tests are different than results found in literature. It is recommended to do further testing to improve the sample size of the tests, giving a more complete picture of the functionality of the setup.

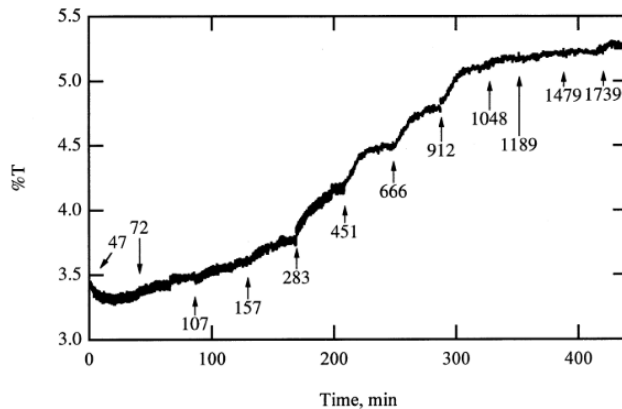
## 6. Data Visualisation

This chapter will discuss the ideation of the data visualisation. Mainly based on what is important for the user and existing programmes that are used for a similar purpose. After the ideation phase the data visualisation will be made in Adobe XD, a program to simulate user interfaces. This program will also be used to design the user interface, which makes it more efficient to also use it to design the data visualisation. The data visualisation will be user tested together with the user interface. Both of these will be designed with the target group in mind. These are people who do research on this topic of chloroplast movement and therefore have background knowledge on the subject.

### 6.1. Ideation

As said in chapter 2, the data visualisation of the transmittance and reflectance will be between 0 and 1, the information will contain the core information but can also show some extras. The main element of the information is the graph of the transmittance and reflectance shown as a fraction of the input red light. It will be presented the same way most papers present their data. The transmittance and reflectance in percentage, not from 0 to 1, on the y-axis and the time on the x-axis. In addition the light intensity of the environment light can also be shown on either the right y-axis, or as points within the graph. A few examples of the graph representation can be found in figure 6.1.





*Figure 6.1: Examples of the graph representation of the data. Transmittance and reflectance will be shown on the left y-axis, time on the x-axis. The top graph also represents the environment light on the right y-axis. [4] The bottom graph shows the same information on the y- and x-axis but shows the environment light as point in the graph. [14]*

Adjustments to the first idea as presented in chapter 2 are that the T and R will not be presented on a scale from 0 to 1 but rather as a percentage of the input light. This is also the most common way of presenting this type of data in previous papers.

For the environment light the first option will be used, it will be displayed on the right y-axis. This gives a more clear look to the graph and makes it less crowded in the graph.

## 6.2. Realisation

The graphs were made in excel. This program is supplied for free to students and is a great tool to gather data and display. Excel has an abundance of options to display data and as said in ideation a graph will be used with time on the x-axis and a double y-axis. On the left the percentage of transmittance and reflectance and on the right the environment light so it can be seen what the behaviour is compared to the environment. The graphs can be seen in figure 6.2. Both graphs were also used in the data visualisation part of the user test.



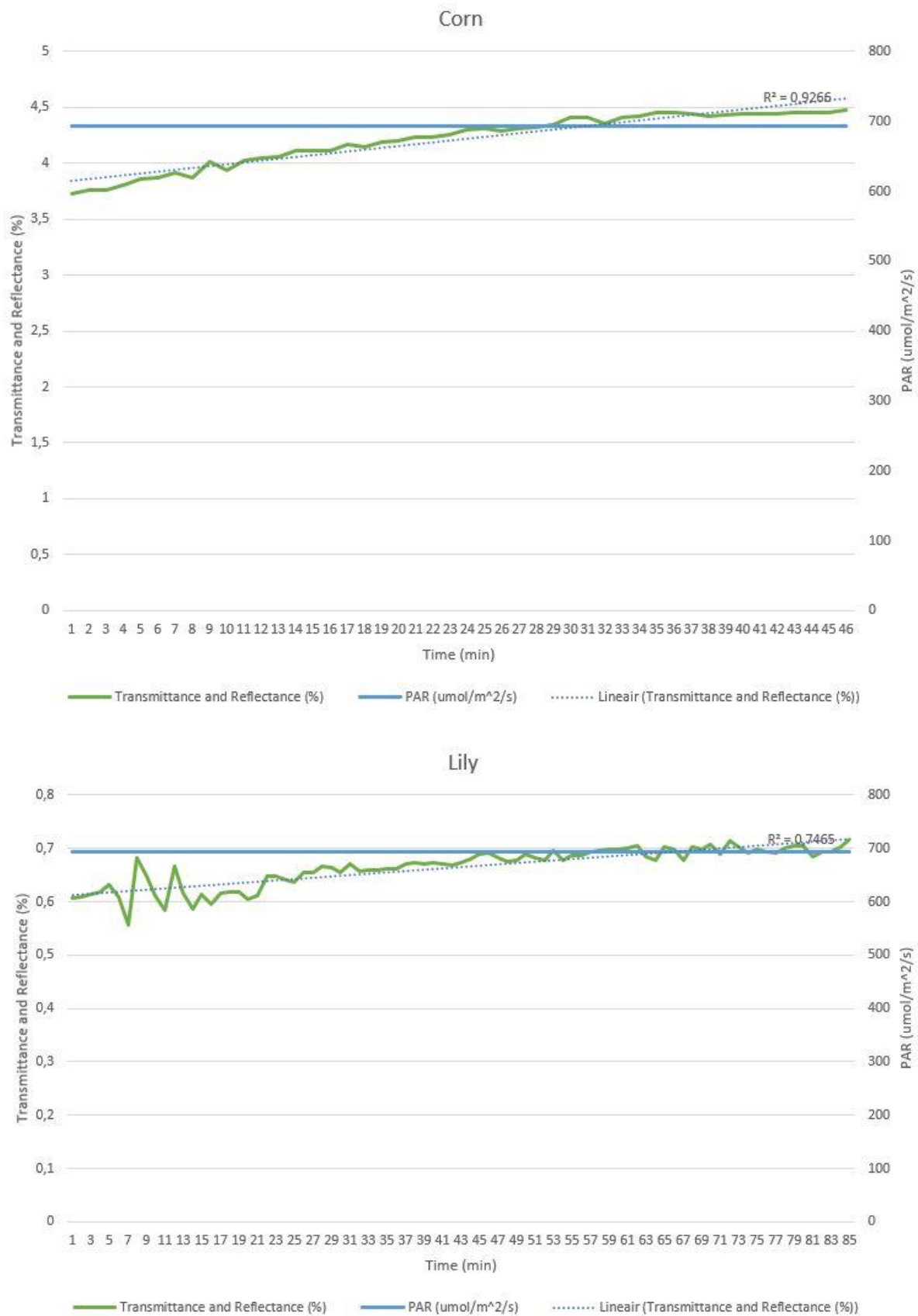


Figure 6.2: The two graphs from tests on plants. The top one of corn, the bottom one of the peace lily.

## 7. User Interface

The data visualisation will be added to the user interface (UI). The UI will be made in Adobe XD which is a commonly used program to design and test UIs. This program also allows the designer to do user tests online. Adobe XD generates a link which the testers can use to test the UI. The first step in designing the UI is the ideation phase. Here existing programmes will be analysed and used as source of inspiration for this program. After the ideation phase the prototype will be constructed in Adobe XD. When the prototype is made it will be shared with potential users of the Plantenna project (the overarching project). These users will navigate through the prototype and fill in a questionnaire about the prototype providing feedback. This part is called user testing and will take place online. Once the testing has been done an evaluation will be made for improvement of the prototype.

### 7.1. Ideation

The ideation consist of three parts. First a concept for the prototype will be thought off, after which a user scenario will be played out to observe needed adjustments, and lastly the final prototype will be made.

#### 7.1.1. First prototype

Before starting an analysis with the persona a concept has to be thought up. There are a few requirements set by the client and a few that are a logical step for a data analysis program. The requirements are as follows:

- The software has to be able to measure data and store it
- The data should be kept in a logical place, with a logical name
- The data should be accompanied by valuable meta data like a name, date, place, plant type etc.
- The data should be comparable, so multiple graphs should be able to be shown on screen
- The software should support the import and export of data to compare to previously done research
- There should be some type of data management present, like selecting, moving, and deleting data.

For this project these requirements are the must haves. Besides this there are a lot of other options like the movability of data from one folder to another, the ease with which one can delete data, the amount of graphs someone can compare at the same time etc. However, these are functionalities that are 'could' features. This project is focussed on the data from the constructed sensor and not on the entire program with which one can compare all the data. Therefore only the necessary functions will be discussed and

implemented. However, the 'could' functions will be mentioned as recommendations and more 'must' functions can come forward during the user scenario or the user tests.

Looking at the requirements stated above three main components are very important: being able to measure, being able to compare, being able to import and export data. Because these are the main must have functionalities they will be implemented in the home or starting screen. This is to anticipate the intended use which will be either of the three. After that there will be a menu in which the user can make a new measurement, import, export, or delete data.

The first function will be the new measurement. In this part of the program the user can fill in some meta data which needs to accompany the measurements. This is necessary to store the data correctly and to give the user enough information when returning to the data to know which measurement it concerns. Data that should be filled in is: name, category, day, starting time, location, plant type, and sub type. Most information is basic labelling information: name, day, time, location. The category, plant, and sub type are specifically important for this type of data. The category is the folder under which the measurement will be put to keep the measurements organised. This way the user can have multiple experiments or clients at the same time without the data storage becoming confusing. The plant type and sub type are necessary so the user can easily indicate the plant and sub type of the test subject. This means the user does not have to save this in the name of the data which can be forgotten. Another benefit is that users can quickly find data for the plants they intend to test and compare.

An important feature for filling in this information is that in the menu to make a new measurement most data should have a drop down menu of already existing categories, locations, plant types, and sub types but when the user wants to make a new one they should be able to do it in the same menu to simplify the process. The day and time will be generated automatically, set to the information delivered in the data by the used device. There is no reason to manually provide this information when starting a measurement and filling it in automatically will make the process easier.

When making a new measurement it should also be possible to save the settings for the measurement and store them in a queue. This way the user can already prepare measurements, store them, and start them at a later point, giving more flexibility in the field.

The next function is the view measurements function, here the user can compare data. Being able to add graphs, delete graphs, see details about the graphs are all must have functions. The user can navigate through the categories and add more graphs to the screen, see details from the graphs and select parts they want to compare for certain graphs. Note here that Adobe XD is meant for basic prototyping and does not allow for complicated functions. Comparing the graphs in more detail in this prototype will therefore not be possible.

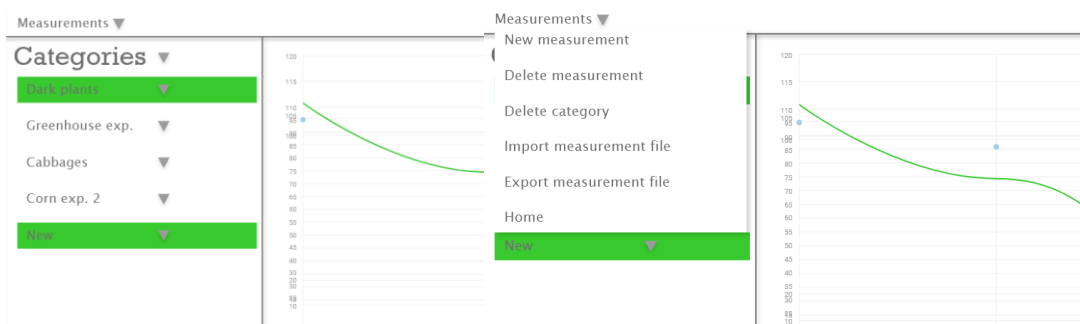
The third main function is the import and export of data. This part is not as complicated as the other two but does need to be one of the main three functionalities. The user can import data, assign the meta data to it as with a new measurement and can then save it in a category.

Lastly the interface should have one basic layout, menu bar at the top, on the left a menu in which the user can switch between the queue and the categories and on the right room for the details that accompany a graph. This is standard layout that is used in wide variety of programmes. Ranging from Microsoft Word to Adobe Photoshop to 20-Sim.

## 7.1.2. User scenario

The user interface will be based on requirements thought of through a user scenario, a commonly used method in Human Centred Design. A user scenario is based on a concept or prototype and the observations that are done while navigation through. For example, a designer will take the role of a user and handle the prototype the way a user would. This method is often used during the paper prototype phase of the design. A paper prototype is a life size prototype of the project, it is a low-fi prototype, which means it is low quality compared to the eventual design but is easily adjustable. Is there an option in your paper prototype that is not logical to user? Then it is easy and cheap to alter that part. After low-fi prototypes the high-fi prototypes start to play a big role, most big decisions have been made and only little and easily adjustable things need to change. [30]

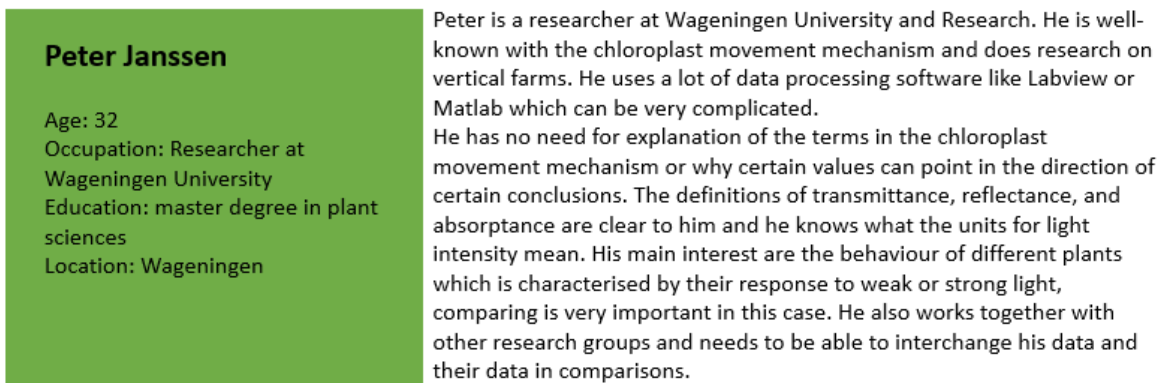
In this project we will use a digital paper prototype. This means that the prototype will not be functional and will be papers stacked onto each other. Usually for applications and programmes the paper prototype will be sheets of paper with the interface drawn on them. The user tester can navigate through the papers by flipping them. In this project the prototype will be digitally made in Adobe XD but will work the same way. It will not be fully functional but instead consist of different pages which all have small variations depending on what shape gets clicked. [30] An example can be seen in figure 7.1.



*Figure 7.1: An example of a digital paper prototype. This is a drop down menu that appears when the drop down arrow is clicked. To get from one state (no menu) to the other (with menu) Adobe XD has switched from one page to another.*

Benefits for using Adobe XD is that the prototype is easy to change and the prototype can be made into a link which anyone can click and test online on their own device.

To construct a user interface a user scenario will be made up. For this a potential user profile is needed. This user profile, or persona, includes the basic information like age and gender but also some background information. This makes the character more accessible and real. The goal of this persona is the act as a user to see what a user would need in the program. [30] The persona profile can be found in figure 7.2.



*Figure 7.2: the persona profile for the scenario test for the ideation of the chloroplast movement sensor interface.*

The persona used in this project, Peter Janssen, is a relatively short persona. Usually a lot of social information is included but as will be discussed in the chapter user tests, this is not of importance for this study. This project focusses more on the compatibility and usability between the sensor, the data, and the UI and not on the marketability of the program.

Now the user scenario can be played out. For this the first prototype will be made in Adobe XD as described in chapter 6.1.1. The scenario will be fully typed out in appendix B.

Concluding the user scenario a few things became apparent. There is a need for a setting to set the end of the measurement. The three options that will be added here are a set amount of minutes, a relative difference between the first measurement and the final measurement, and a manual stop. The graph will appear live on screen and a stop button can be pressed to stop the measurement. Another requirement that came forward from the scenario is the ability to zoom in on the graphs. This is important for comparing, however, Adobe XD does not support his kind of action. The zoom option will be taken into account for recommendations.

Lastly, during the testing phase of the setup another setting was observed to be very useful. This setting would be the option to turn on a 'trigger' light. In the setup a blue LED spot is used to trigger an avoidance response. Even though the setup can be used to see the influence of sunlight on the chloroplast movement, it can then also be used to see the behaviour of the chloroplasts under laboratory conditions. This gives a more controlled test environment which is a good additional option.

## 7.2. Prototype

The prototype is constructed in Adobe XD and consist of a lot of different panels. These panels are all design elements, like squares, circles, lines and text which can be made interactive. Adobe XD gives the options to make something clickable and send the user to another panel. Important to take into account

here is that the prototype cannot be made completely functional. This would take hundreds of panels and interactions and in addition a lot of time. Next to that, not all kinds of actions can be added in Adobe XD. The main actions are clickable, typing on the keyboard, dragging an object, or voice command.

The prototype was mainly explained in chapter 6.1. For a complete version of the prototype see appendix C. Every figure in the appendix is accompanied with a small explanation. Next to that a link is included which will open the prototype in an internet browser.

## 7.3. User Tests

The user test, specifically the questionnaire, is based on the unified theory of acceptance and use of technology (UTAUT) model. This model is based on multiple older models and is based on the 4 key determinants. These determine how likely it is for the technology to be accepted and used. [31]

The key determinants of the UTAUT are performance expectancy, effort expectancy, social influence, and facilitating conditions. Performance expectancy is how much the user believes that using the system will help them get to their goal. Is the system useful, does it contribute to the current situation, etc. The second one, effort expectancy, is about the ease of using the system. Is the system easy to use, it intuitive, are the buttons in the expected locations. The social influence is about what the user thinks other people's opinions are about using the system. Do your peers or colleagues find the system good enough, is it socially acceptable to use this system, does it give you status when you use the system. The last one, facilitating conditions, is the only determinant that is not influencing the intended behaviour but is directly influencing the behaviour. Does the user believe their employer will support this system, are there organisational and technical facilities to enable the use of the system. [31] The influence of the determinants on the intended behaviour and actual behaviour can be seen in figure 7.3.

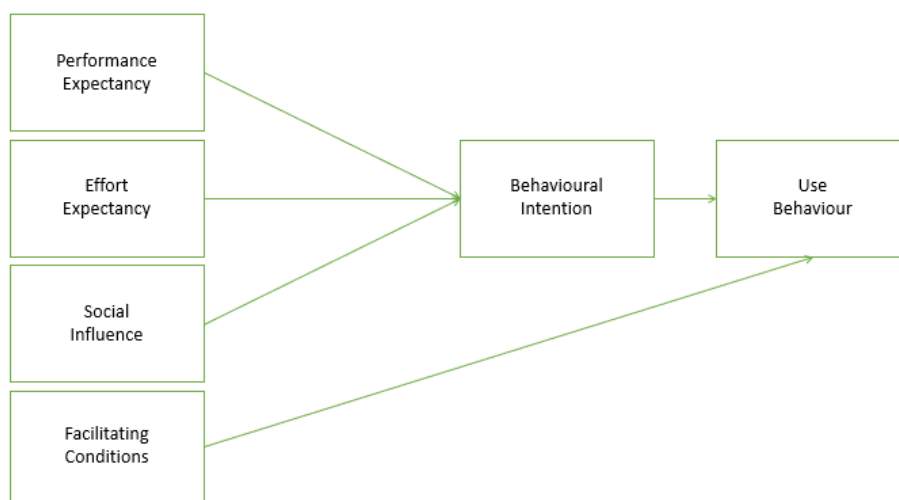


Figure 7.3: The UTAUT model and how the determinants influence the behaviour of the user. [31]

Originally the UTAUT model also takes into account gender, age, experience, and voluntariness of use. However, this is outside the scope of this study. These factors influence the key determinants but are arbitrary in this specific case. The system and UI that will be designed is not targeted towards a specific group of gender, age, experience, or voluntariness of use. This system is designed for a specific academic project for a specific use but not for a specific marketable target group.

The UTAUT will be used in the questionnaire for the user tests. This questionnaire will be made in Google forms on the University of Twente account and will not be shared with anyone outside of the project. The data will only be used for this project and will be anonymous. The questions for the survey can be found in appendix D.

The questions are based on the UTAUT model, specifically asking for the ease of use, usability, and logic. The social influence and facilitating conditions are less relevant and will not be brought forward as much as the other two key determinants. The first two are targeted mainly to the usability of the program which is the main question for this project: What is a fitting UI for this project. This is targeted at the compatibility between the data from the sensor and the UI and the functionality inside the Plantenna project. It is not targeted towards the social and marketable aspects of a product.

Other options for user tests would have been an interview or a test where the tester speaks out loud why they make certain choices in the use of the system. However, these methods consume more time and need more planning since the observer/interviewer and the tester/interviewee have to be available at the same time and place. Next to that, it also asks for a more functional prototype for which it is still too early in the design process.

### 7.3.1. Protocol

For user testing a protocol is required. Here all the tasks of the test will be described, the context will be explained, and all ethical considerations will be clarified. This protocol will be based on the EEMCS faculty ethical protocol checklist. [32] Some information will also be taken from the protocol guidelines. [33] This contains all regulations regarding ethical implications for research in the EEMCS faculty of the University of Twente. The completely filled in protocol can be found in appendix E.

### 7.3.2. Results and discussion

The user test was completed by three individuals. All contacted through the Plantenna project to ensure they have background knowledge on the topic. Specific results can be found in appendix F.

First, the data visualisation will be discussed. The first couple of questions asked had the purpose to check if the graphs were readable. Everyone answered them as expected which is a sign that the graphs are

clear enough to read. However, some recommendations were made. The time scale should be less dense, transmittance and reflectance should be changed to transmittance plus reflectance to be accurate, and instead of showing transmittance and reflectance it might be better to only show absorptance. One person recommended to show both, however showing both would give two graphs inversed because  $\text{absorptance} + \text{transmittance} + \text{reflectance} = 1$ , as can be seen in equation 2 in chapter 2.

The user test consisted of four parts, namely, three tasks and some general questions. For the first task, person number 1 had problems during subtask 2 which is filling in the information in the new measurement menu. This problem can arise when a tester does not understand how to fill in the information in a prototype that is not fully functional. This is a big downside of doing such a prototype. However, The answers to the functionality and expectancy questions were positive.

The second person had problems during more subtasks (2,3,5,6,7, and 8) and had some comments. There are four comments, the first suggesting that 'additional lighting' does not clearly describe that it is the blue light trigger. This is a useful comment that can be taken into account when redesigning. The other three comments were less useful because they are all part of this prototype. The problems were that one could only type in 'plant' as a name, which was intentional and that the entries could not be edited anymore after being completed. This is also typical for a prototype and in the beginning of the survey it had been made clear that the prototype was not fully functional. The last comment said there was no button to stop the measurement. However, when looking at the other answers from this test person later in the survey they say they are missing some features which are there and were found by the other people. However, this problem should not have appeared. The survey tells the people to set the prototype to full screen, otherwise one would not see the bottom of the screen. Looking at how this person commented often that the buttons that are supposed to be on the bottom of the screen are missing it is very plausible that this person failed to make their prototype full screen. This also argues if the results are reliable but they will be used because only three people filled in the survey so every data set has value. The functionality and expectancy questions were answered rather positive.

The third person had trouble with almost all subtasks. They comment that the prototype did not respond and crashed. This is an unknown problem. Later in the survey this person does not encounter this problem again. Presumably the information input part is what made this difficult. The other questions were answered positively.

The second task was a shorter task. No one had any problems with this task and all questions were answered very positively.

The third task was again a longer task. The first person had no problems and was positive on all other questions. The second person got stuck in the prototype, it is unclear how this happened, however it is something to look into. The third person said they got an empty screen at one point which meant they could not complete the task and was very negative on the functionality and expectancy questions.

The last part contained some general questions. Person one was positive but had one comment about the functionality of the prototype. This problem of low functionality would not have existed in a fully functional version of the program. Person two was generally neutral to negative about the design, functionality, and expectancy. As said before this person likely had problems with navigating the prototype. Person three also had some problems with the prototype causing the questions about functionality to be answered negatively. However, they did find the functions presented useful.



### 7.3.3. Conclusion

A clear conclusion can be drawn from the results of the data visualisation test. It would be better to only show absorptance and to have a less dense time scale. Other aspects can be considered to be put in, like the slope of the graph.

For the user interface part the answers were diverse. The person having no trouble with the prototype was very positive about the functionality and expectancy, this was the main focus of the user interface as referred to in this chapter. The other two has some problems running the prototype which resulted in generally less positive answers. The conclusion here would be that the user interface has some unexpected parts for some users that need adjusting but some functions were clear and logic to others. Making the prototype a first step but something that has to be approved upon.

It is recommended to do another user test with a more functional prototype or functional program. This is also a natural step in the UI design process.

## 8. Conclusion

This chapter deals with the overall conclusion of the whole project. Here the connection between all the different parts will be discussed.

When looking at the results of all the sub projects the setup is likely to be functional. The results it gave for the final tests did not have the same numbers as the literature, however, the behaviour is very similar which indicates it works well enough. The transmittance increased when an avoidance response was triggered, which is as expected based on literature. Next to that, the most important thing in measuring chloroplast movement is the absolute difference between the starting and ending percentage. This difference had a very low variance which does imply that the setup is functional enough to reproduce experiments.

For the data visualisation the user testing gave clear insights in what can be improved on the graphs. The graphs were already clear but could be improved in that the time scale should be less dense and instead of using transmittance and reflection % it could say transmittance + reflection %. However, others argued it could also show the absorptance % instead. This would not be completely correct because the sensor mainly measures the transmittance, so  $1-T$  could be displayed instead. Overall, the data visualisation was perceived as clear but not perfect.

The user interface had some trouble while testing. The use of the adobe XD prototype, which was not fully functional, gave some problems and made the test unclear. On the other hand, the functions and options in the prototype were perceived as useful and most things behaved as expected. For the functionality and expectancy parts of the UTAUT model the interface was seen as useful.

At the start of this project some tasks and questions were posed:

*Engineer a chloroplast movement sensor and the accompanying data visualisation and user interface.*

To answer this main research task some sub-questions had to be answered first:

- *What is the mechanism behind chloroplast movement?*
- *How can the movement or the position of chloroplasts be measured?*
- *How can the movement or position be displayed and visualised?*
- *What is a fitting user interface design for this application?*

Looking back at these questions, it can be concluded that this project did engineer a chloroplast movement sensor including data visualisation and user interface. The mechanism behind the chloroplast movement was researched in chapter 2, this included how it can be measured. The sensor was designed and tested, giving data to visualise and a prototype for a user interface. Even though the tasks were completed and questions were answered this is only the start. First prototypes were made but the process of designing either a machine to sense the world, or a data visualisation and interface, is complex and lengthy. To ensure the best final product a lot of steps have to be taken, and this project was a first step.

## 9. Recommendation

Based on the discussion and conclusion there are some recommendations that can be done. These recommendations include aspects for a continuation of this project and for a repetition of (part of) this project.

For the setup it is recommended to carry out more experiments to prove the stability and functionality of the setup. Next to that, if the setup is functional, it could be turned into a more sophisticated device which can actually be carried and used on other locations. Meaning it should have battery, casing, buttons, a stand for in-field use etc. Other parts of the setup should be improved like the synchronous detection to lock-in amplifier and a cooler for the LED and possible for the sensor.

The data visualisation can be adjusted and fine-tuned to different types of experiments. For the user interface a new prototype could be made, using the recommendations that were done in the survey, and tested again. If developed further this could be turned into a functioning program to support the sensor, or any other chloroplast movement focussed equipment.

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## Appendix A – Arduino and Processing 3 code

### Processing code:

```
int widthSlide = 20;
int heightSlide = 80;
int widthTrack = 256;
int heightTrack = 10;
int lengthLine = 20;
int xLocSlide = 600/2;
int yLocSlide = 500/4;
int LedSetting = 0;
int offSetUpper = 5;
int offSetLower = 2;
int heightscreen = (500/4) * 3;
int xPosGraph = 1;
int graphData = 12000;
int inGraph;
int index = 0;
int graphTime = 0;

int expectedLux = 12000;

String inString;

int[] data = new int[graphData];

import processing.serial.*; // use serial port libraries
Serial myPort;

void setup() {
  size(600, 500);

  println("Available serial ports:");
  for (int i = 0; i<Serial.list().length; i++)
  {
    print("[ " + i + " ] ");
    println(Serial.list()[i]);
  }
  myPort = new Serial(this, Serial.list()[0], 9600); // open port 0
  in the list at 9600 Baud

  myPort.bufferUntil('\n');
}

void draw() {

  int measurement;

  background(150);

  drawTrack();
  drawSlide();
  drawDisplay();
  drawGraphScreen();

  if (inString != null) {
    measurement = Integer.parseInt(trim(inString));
    // println(measurement);
    //if ((millis() % 100) == 0) {
      data[index] = measurement;

      println(data[index]);
      stroke(0, 255, 0);
      line(100+graphTime, heightscreen+100, 100+graphTime,
height-map(data[index], 0, expectedLux, 0, 200));
      if (graphTime <= 400) {
        graphTime++;

      } else {
        graphTime=0;
      }
      index++;
    //}
  }

  //drawGraph();

  LedSetting = xLocSlide-170;
  myPort.write(LedSetting);
}

boolean mouseLocation(int xLocSlide, int yLocSlide) {
  if (mouseX >= xLocSlide-(widthSlide/2) && mouseX <=
xLocSlide+(widthSlide/2) ) {
    if (mouseY >= yLocSlide-(heightSlide/2) && mouseY <=
yLocSlide+(heightSlide/2)) {
      return true;
    } else {
      return false;
    }
  } else {
    return false;
  }
}

void drawDisplay() {
  fill(0);
  rect((width/2), 0, 100, 50);
  fill(255);
  textSize(20);
  text(LedSetting, (width/2)-15, 20);
}

void drawSlide() {

  if (mouseLocation(xLocSlide, yLocSlide) == true) {
    fill(#42D847);
  } else {
    fill (255);
  }
  rectMode(CENTER);
  noStroke();
  rect(xLocSlide, yLocSlide, widthSlide, heightSlide);
}

void drawTrack () {

  rectMode(CENTER);
  noStroke();
  fill(0);
  rect(width/2, height/4, widthTrack, heightTrack);

  for (int j = 0; j<11; j++) {
    textSize(10);
    int digitTrack = 0 + (j*10);
    text(digitTrack, ((width/2)-(widthTrack/2))+(j*25.6),
(height/4)+heightTrack*2+lengthLine*2);
    stroke(5);
  }
}
```

```

    line(((width/2)-(widthTrack/2))+(j*25.6),
(height/4)+heightTrack*2, ((width/2)-(widthTrack/2))+(j*25.6),
(height/4)+(heightTrack*2)+lengthLine);
}
}

void drawGraphScreen() {
  rectMode(CENTER);
  fill(0);
  rect(width/2, heightscreen, 400, 200);
}

void serialEvent (Serial myPort) {
  inString = myPort.readString();
}

```

## Arduino code to communicate with Processing:

```

#include <Wire.h>
#include <Adafruit_Sensor.h>
#include "Adafruit_TSL2591.h"
Adafruit_TSL2591 tsl = Adafruit_TSL2591(2591); // pass in a
number for the sensor identifier (for your use later)
int led = 9;
int incomingByte; // initiate if not connected to processing
int blue = 5;
int setBlue = 255; //intensity of the blue leds

// connect SCL to I2C Clock
// connect SDA to I2C Data
// connect Vin to 3.3-5V DC
// connect GROUND to common ground

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

  // Serial.println(F("Starting Adafruit TSL2591 Test!"));

  if (tsl.begin())
  {
    // Serial.println(F("Found a TSL2591 sensor"));
  }
  else
  {
    // Serial.println(F("No sensor found ... check your wiring?"));
    while (1);
  }

  /* Display some basic information on this sensor */
  //displaySensorDetails();

  /* Configure the sensor */
  configureSensor();

  pinMode(led, OUTPUT);
  pinMode(blue, OUTPUT);
}

void loop() {

  // see if there's incoming serial data:
  if (Serial.available() > 0) { // remove if not connected to
processing
    // read the oldest byte in the serial buffer:
    incomingByte = Serial.read();
    analogWrite(led, incomingByte);

  }
  analogWrite(led, setBlue);

```

```

void mouseDragged() {
  if (pmouseX <= mouseX && xLocSlide <= width-((width/2)-
(widthTrack/2))-offsetUpper ) {
    xLocSlide += mouseX-pmouseX;
  }
  if (xLocSlide >= width-((width/2)-(widthTrack/2))) {
    xLocSlide = width-((width/2)-(widthTrack/2))-offsetUpper;
  }
  if (pmouseX >= mouseX && xLocSlide >= (width/2)-
(widthTrack/2)-offsetLower ) {
    xLocSlide -= pmouseX-mouseX;
  }
  if (xLocSlide <= (width/2)-(widthTrack/2) ) {
    xLocSlide = (width/2)-(widthTrack/2)-offsetLower;
  }
}

```

```

analogWrite(blue, setBlue);

```

```

if (millis()%500 == 0 ){
  advancedRead();
}

```

```

}

```

```

/*void displaySensorDetails(void)
{
  sensor_t sensor;
  tsl.getSensor(&sensor);
  Serial.println(F("-----"));
  Serial.print (F("Sensor:   ")); Serial.println(sensor.name);
  Serial.print (F("Driver Ver:  ")); Serial.println(sensor.version);
  Serial.print (F("Unique ID:  "));
  Serial.println(sensor.sensor_id);
  Serial.print (F("Max Value:  "));
  Serial.print(sensor.max_value); Serial.println(F(" lux"));
  Serial.print (F("Min Value:  "));
  Serial.print(sensor.min_value); Serial.println(F(" lux"));
  Serial.print (F("Resolution:  ")); Serial.print(sensor.resolution,
4); Serial.println(F(" lux"));
  Serial.println(F("-----"));
  Serial.println(F(""));
  delay(500);
}*/

```

```

void configureSensor(void)
{
  // You can change the gain on the fly, to adapt to
brighter/dimmer light situations
  tsl.setGain(TSL2591_GAIN_LOW); // 1x gain (bright light)
  //tsl.setGain(TSL2591_GAIN_MED); // 25x gain
  //tsl.setGain(TSL2591_GAIN_HIGH); // 428x gain

  // Changing the integration time gives you a longer time over
which to sense light
  // longer timelines are slower, but are good in very low light
situations!
  //tsl.setTiming(TSL2591_INTEGRATIONTIME_100MS); //
shortest integration time (bright light)
  // tsl.setTiming(TSL2591_INTEGRATIONTIME_200MS);
  // tsl.setTiming(TSL2591_INTEGRATIONTIME_300MS);
  // tsl.setTiming(TSL2591_INTEGRATIONTIME_400MS);
  tsl.setTiming(TSL2591_INTEGRATIONTIME_500MS);
  // tsl.setTiming(TSL2591_INTEGRATIONTIME_600MS); //
longest integration time (dim light)

  /* Display the gain and integration time for reference sake */
  /* Serial.println(F("-----"));
  Serial.print (F("Gain:   "));
  tsl2591Gain_t gain = tsl.getGain();

```

```

switch(gain)
{
  case TSL2591_GAIN_LOW:
    Serial.println(F("1x (Low)"));
    break;
  case TSL2591_GAIN_MED:
    Serial.println(F("25x (Medium)"));
    break;
  case TSL2591_GAIN_HIGH:
    Serial.println(F("428x (High)"));
    break;
  case TSL2591_GAIN_MAX:
    Serial.println(F("9876x (Max)"));
    break;
}
Serial.print (F("Timing:  "));
Serial.print((tsl.getTiming() + 1) * 100, DEC);
Serial.println(F(" ms"));
Serial.println(F("-----"));
Serial.println(F("")); */
}

void advancedRead(void)
{
  // More advanced data read example. Read 32 bits with top 16
  bits IR, bottom 16 bits full spectrum
  // That way you can do whatever math and comparisons you
  want!
  uint32_t lum = tsl.getFullLuminosity();
  uint16_t ir, full;
  ir = lum >> 16;
  full = lum & 0xFFFF;
  // Serial.print(F("[ ")); Serial.print(millis()); Serial.print(F(" ms ]
  "));
  // Serial.print(F("IR: ")); Serial.print(ir); Serial.print(F(" "));
  // Serial.print(F("Full: ")); Serial.print(full); Serial.print(F(" "));

  // Serial.print(F("Visible: ")); Serial.print(full - ir); Serial.print(F("
  "));
  // Serial.print(F("Lux: ")); Serial.println(tsl.calculateLux(full, ir),
  6);

  // Serial.print(F(" ")); Serial.print(ir); Serial.print(F(", "));
  // Serial.print(F(" ")); Serial.print(full); Serial.print(F(", "));
  // Serial.print(F(" ")); Serial.print(full - ir); Serial.print(F(", "));
  Serial.print(round(tsl.calculateLux(full, ir))); Serial.print('\n');
}

void unifiedSensorAPIRead(void)
{
  /* Get a new sensor event */
  sensors_event_t event;
  tsl.getEvent(&event);

  /* Display the results (light is measured in lux) */
  //Serial.print(F("[ ")); Serial.print(event.timestamp);
  Serial.print(F(" ms ] "));
  if ((event.light == 0) |
      (event.light > 4294966000.0) |
      (event.light < -4294966000.0))
  {
    /* If event.light = 0 lux the sensor is probably saturated */
    /* and no reliable data could be generated! */
    /* if event.light is +/- 4294967040 there was a float
    over/underflow */
    // Serial.println(F("Invalid data (adjust gain or timing)"));
  }
  else
  {
    // Serial.print(event.light); Serial.println(F(" lux"));
  }
}
}

```

### Arduino code for testing on plants:

```

#include <Wire.h>
#include <Adafruit_Sensor.h>
#include "Adafruit_TSL2591.h"
Adafruit_TSL2591 tsl = Adafruit_TSL2591(2591);
int led = 9;
int blue = 3;
int blueH = 5;
int i = 0;
int j = 0;
float value;
boolean ledOn = true;
float onArray[10];
float offArray[10];
int ledShine;
float TandR = 0;
boolean ledSwitch = false;
boolean calculateTandR = false;
float averageOn;
float averageOff;
int startM = 0;
int blueOn = 0;
int experiment = 0;

void setup() {

  Serial.begin(9600);

  configureSensor();

  pinMode(led, OUTPUT);
  pinMode(blue, OUTPUT);
  pinMode(blueH, OUTPUT);

  // Serial.print(F("Visible: ")); Serial.print(full - ir); Serial.print(F("
  "));
  // Serial.print(F("Lux: ")); Serial.println(tsl.calculateLux(full, ir),
  6);

  // Serial.print(F(" ")); Serial.print(ir); Serial.print(F(", "));
  // Serial.print(F(" ")); Serial.print(full); Serial.print(F(", "));
  // Serial.print(F(" ")); Serial.print(full - ir); Serial.print(F(", "));
  Serial.print(round(tsl.calculateLux(full, ir))); Serial.print('\n');
}

void loop() {
  uint32_t lum = tsl.getFullLuminosity();
  uint16_t ir, full;
  ir = lum >> 16;
  full = lum & 0xFFFF;
  // Serial.println(tsl.calculateLux(full, ir), 6);
  value = (tsl.calculateLux(full, ir));

  if (ledOn == true && startM == 0) {
    onArray[i] = value;
    if (i == 4) {
      averageOn = onArray[0] + onArray[1] + onArray[2] +
onArray[3] + onArray[4]; // + onArray[5] + onArray[6] +
onArray[7] + onArray[8] + onArray[9];
      averageOn = averageOn / 5;
      i = 0;
      ledSwitch = true;
    } else {
      i++;
    }
  }

  if (ledOn == false && startM == 0) {
    offArray[j] = value;
    //Serial.println(offArray[j]);
    if (j == 4) {

```



```

    averageOff = offArray[0] + offArray[1] + offArray[2] +
offArray[3] + offArray[4]; // offArray[5] + offArray[6] +
offArray[7] + offArray[8] + offArray[9];
    //Serial.print(averageOff);
    averageOff = averageOff / 5;
    j = 0;
    ledSwitch = true;
} else {
    j ++;
}
}

if (ledOn == true && ledSwitch == true) {
    ledOn = false;
    ledSwitch = false;
    tsl.setGain(TSL2591_GAIN_LOW);
    ledShine = 0;
}
if (ledOn == false && ledSwitch == true) {
    startM ++;
    if (startM == 50) {
        ledOn = true;
        ledSwitch = false;
        tsl.setGain(TSL2591_GAIN_LOW);
        ledShine = 150;
        calculateTandR = true;
        startM = 0;
    }
}

if (calculateTandR == true) {
    TandR = averageOn - averageOff;
    calculateTandR = false;
    Serial.println(TandR);
    experiment ++;
}

```

```

}

/*if (experiment <= 20) {
    blueOn = 10;
}*/
if (experiment >= 0) {
    blueOn = 255;
}
analogWrite(led, ledShine);
analogWrite(blueH, blueOn);
analogWrite(blue, blueOn);
delay(500);
}

void configureSensor(void)
{
    // You can change the gain on the fly, to adapt to
    brighter/dimmer light situations
    //tsl.setGain(TSL2591_GAIN_LOW); // 1x gain (bright light)
    // tsl.setGain(TSL2591_GAIN_MED); // 25x gain
    //tsl.setGain(TSL2591_GAIN_HIGH); // 428x gain

    // Changing the integration time gives you a longer time over
    which to sense light
    // longer timelines are slower, but are good in very low light
    situations!
    // tsl.setTiming(TSL2591_INTEGRATIONTIME_100MS); //
    shortest integration time (bright light)
    // tsl.setTiming(TSL2591_INTEGRATIONTIME_200MS);
    //tsl.setTiming(TSL2591_INTEGRATIONTIME_300MS);
    // tsl.setTiming(TSL2591_INTEGRATIONTIME_400MS);
    tsl.setTiming(TSL2591_INTEGRATIONTIME_500MS);
    // tsl.setTiming(TSL2591_INTEGRATIONTIME_600MS); //
    longest integration time (dim light)
}

```

## Appendix B – user scenario

The user scenario will use the persona of Peter Janssen as described in figure 7.2.

Peter wants to do an experiment on an Alocasia. He already has data from another measurement from half a year ago. His goal is to do a new measurement, import the old measurement, and compare the two. First he wants to import his old measurement. He finds the option immediately in the start up menu, he imports it, gives it an appropriate name and attaches all the information to it. Once his old measurement is imported he wants to start a new measurement. He looks at the measurement menu and selects: new measurement. Here he can fill in all relevant information. When starting a measurement you want to know when it has to stop. This can be done manually while looking at the live data, after a certain time, or after a specific difference in measured transmittance/reflectance. He can choose either option and start his measurement. When it is done he can add the saved imported measurement and compare. He can select either to read the specifications of the measurement and zoom in on the graphs. He completed his task.

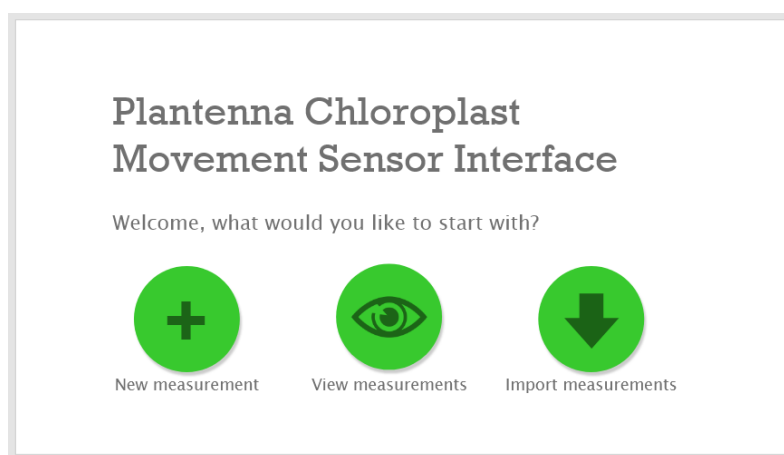
## Appendix C – Adobe XD prototype

The link to the prototype:

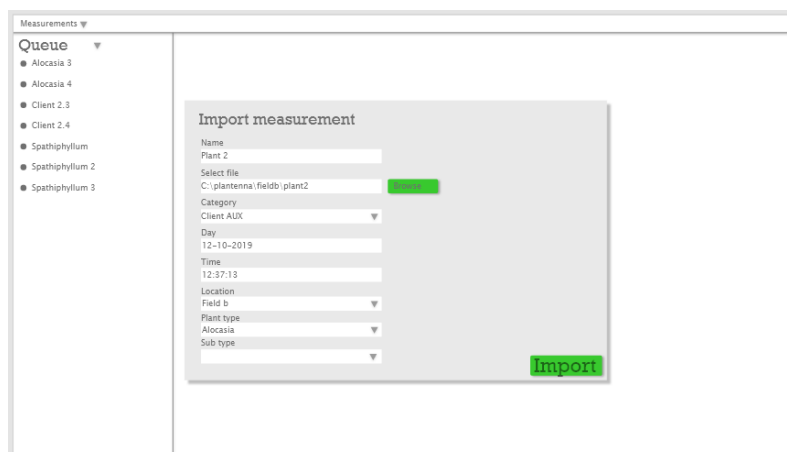
<https://xd.adobe.com/view/c2c5bf94-4b5f-46b9-46a9-4c27f1256ba7-b4f4/?fullscreen&hints=off>

*As also stated in the user test, the prototype can be viewed best using the F11 key to make it full screen.*

Most important panels of the prototype and a graph of the interactions can be found here.



*Figure B.1: The main menu and start screen of the application.*



*Figure B.2: The import measurement screen. Here the user can select a file and add the necessary information.*

Figure B.3: The new measurement screen. Here the user can fill in the necessary information and select a measurement method / duration. This can either be manual, a certain time, or a difference in transmittance/reflectance.

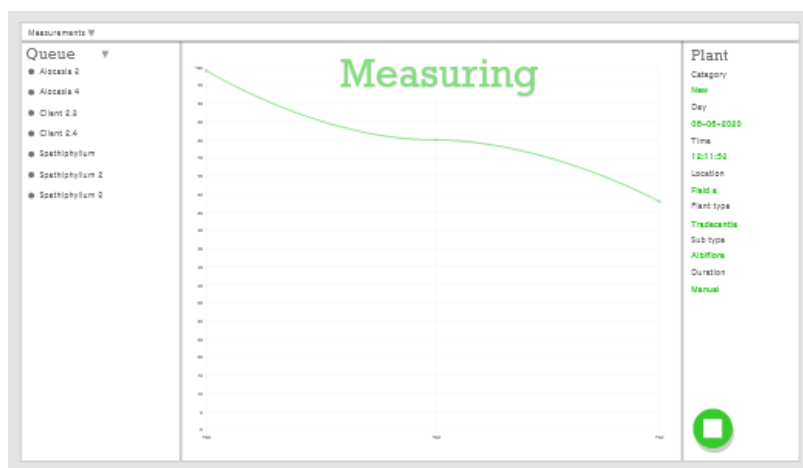


Figure B.4: The measuring screen, here the measurement is ongoing and the duration is set to manual. The stop button on the bottom is to stop the measurement whenever the user wants to.

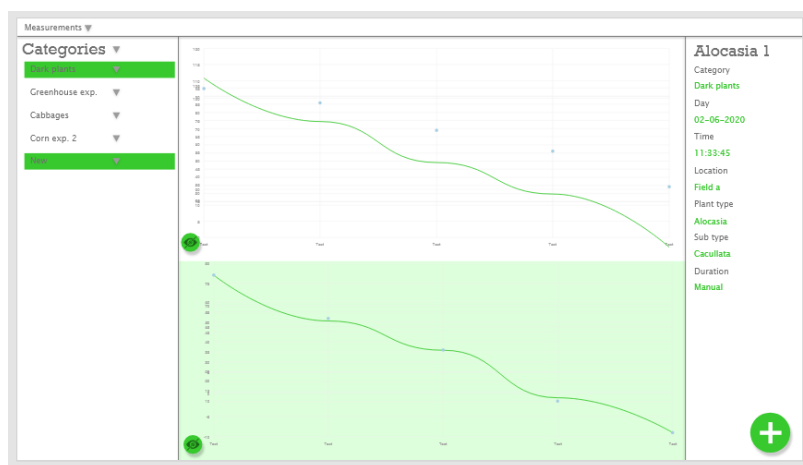


Figure B.5: The measurement comparing screen. Here two measurements are being compared, one in category new and one in category dark plants. Now the Alocasia 1 is selected, information can be seen on

the right side. Graphs can be taken from the screen with the hide button, more graphs can be added with the plus button.

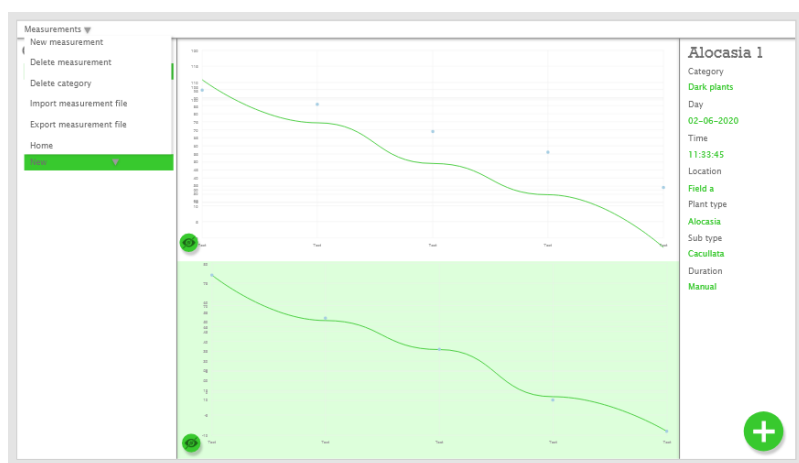


Figure B.6: The comparing screen but the measurement menu is opened. Here the user can select multiple action like making a new measurement, importing another measurement, or deleting one. The home / start screen can also be reached from this menu.

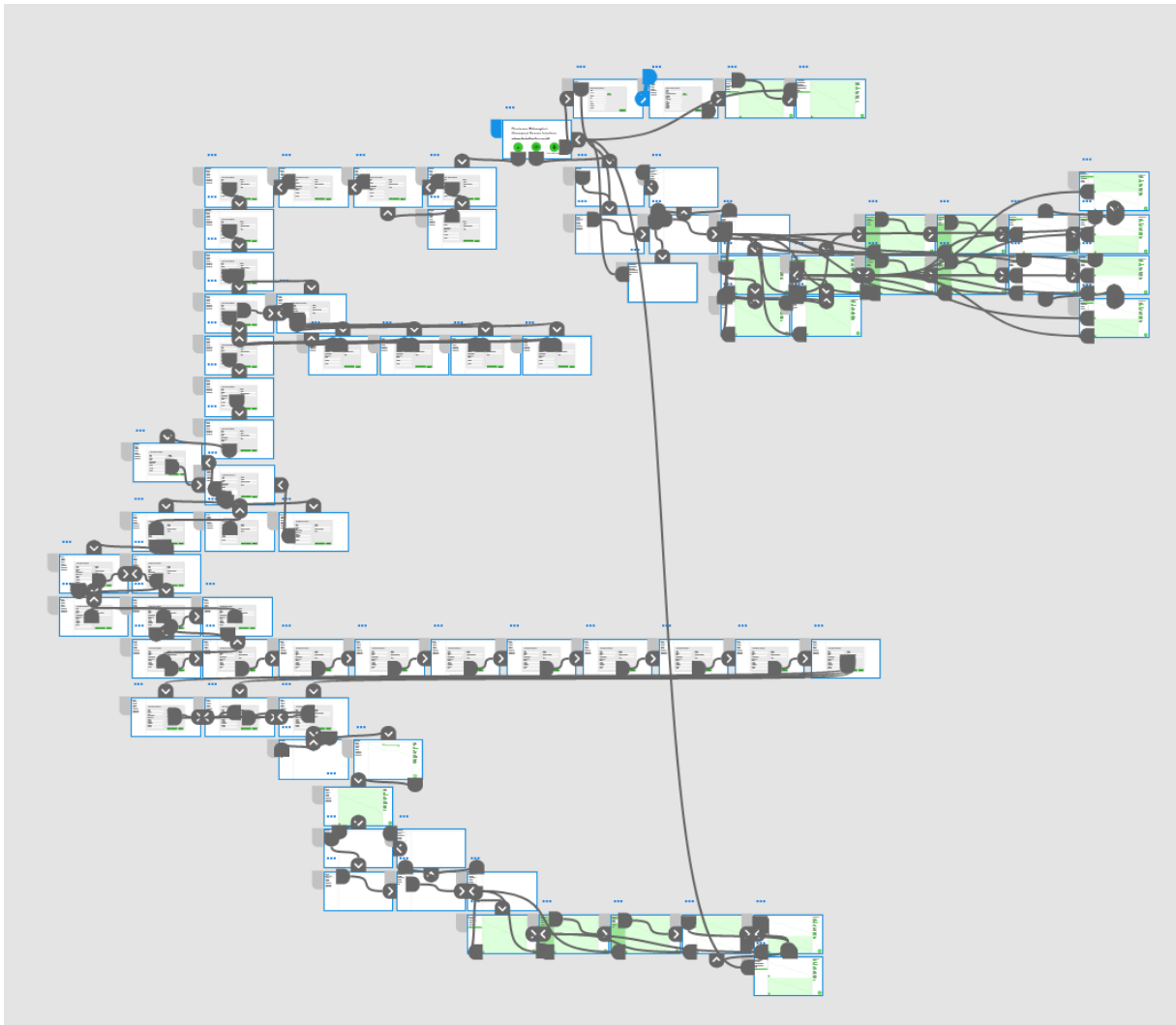


Figure B.7: The complete graph of the interactions. All arrows are interactions, for a example when the new measurement button is clicked it takes the user to the next panel.

## Appendix D – User test survey questions

Here the whole survey is written out. Bold text is titles, regular text is explanatory text, and italics are the questions with their answer options below.

Section 1:

### **Chloroplast movement measurement software**

This project is focused on designing a chloroplast movement sensor and accompanying data visualisation and user interface. The sensor is constructed in such a way it can measure chloroplast movement in the field. It measures transmittance and reflectance of red light from a leaf. With this, the location or behaviour of the chloroplasts can be determined.

In this questionnaire, you will be guided through a user interface. This user interface is a prototype of software in which you can measure, import, and compare chloroplast movement measurements. This questionnaire and the tasks will take approximately 20 minutes. It is recommended you do this questionnaire on a computer, then you can view the prototype more easily and switch between questionnaire and prototype better.

The data gathered in this questionnaire is anonymous and will only be used for this specific project. By answering yes to the next question you agree to the use of the gathered data for this project and that your answer will be added to the report of this project anonymously. This project is part of the graduation semester of the bachelor Creative Technology at the University of Twente, Faculty EEMCS.

If you have any questions or concerns you can contact the bachelor student working on this project through this email address:

p.y.kemper@student.utwente.nl

*I have read and understood the explanation of this questionnaire and agree to the use of the gathered data in the report of this project anonymously.*

- Yes
- No

Section 2:

### **The data visualisation**

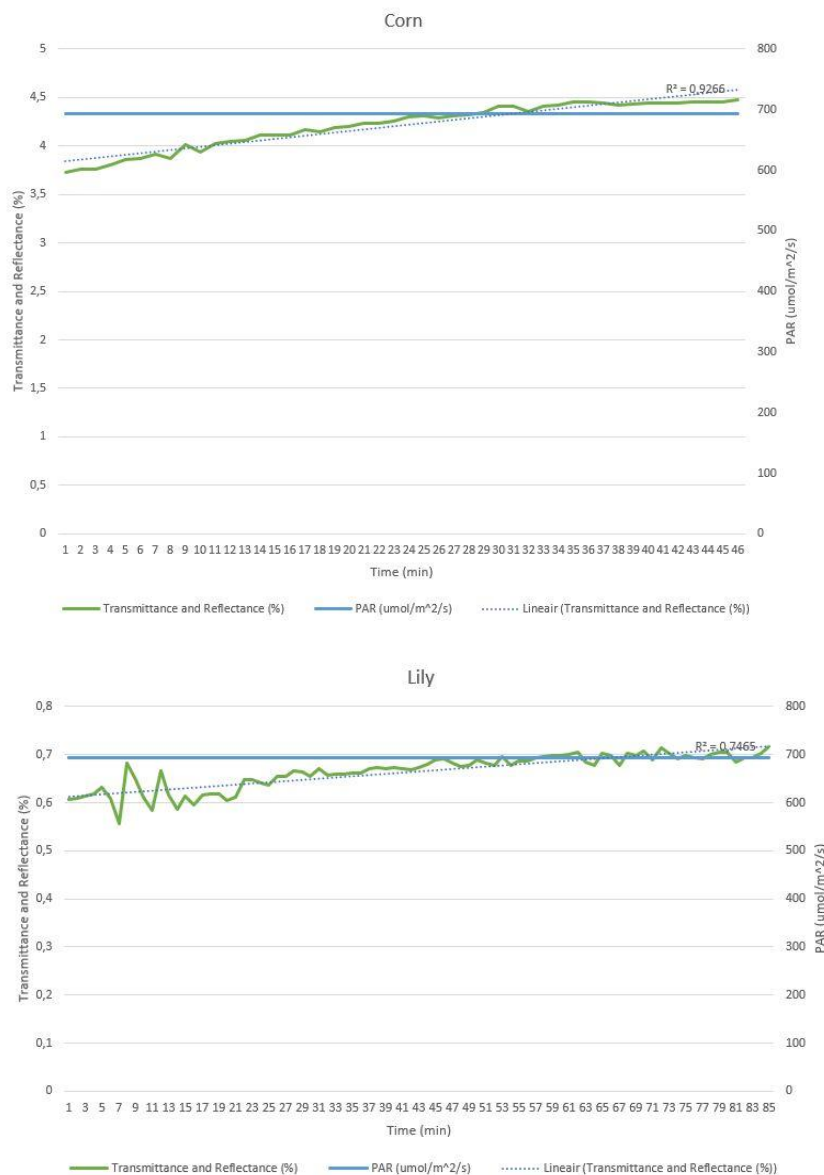
The first part of this questionnaire is about the data visualisation. As said before this project consists of three parts: the sensor, the data visualisation and the user interface. The sensor already has been constructed and is functional. The data that will be presented are actual data measured with this sensor.

The data will be presented to you in a graph. Important is to know that to determine the location of chloroplasts in a plant cell you can measure absorbance. Absorbance is the amount of light that is absorbed by the plant cells. The transmittance and reflectance is the light that is not absorbed but either

transmitted or reflected by the leaf. Absorptance, transmittance, and reflectance are always a fraction of the input light because this input light will either be absorbed, transmitted, or reflected.

Next to the input light, which is red, there is also environment light which can trigger the movement of the chloroplasts. The intensity of this environmental light is also shown in the graph.

You will see two graphs of two different plants, the questions below the graphs will be focussed on the clarity of the graphs and if the data is represented in a logical way.



*Does absorption increase or decrease in both graphs?*

- Increase
- Decrease
- Constant

*Which plant has a greater increase in transmittance and reflectance?*

- Corn



- Lily
- Equal

*What is the approximate intensity of environment light? (you do not have to write down the unit)*

- Open answer

*The graph is clear*

- Agree 1 – 2 – 3 – 4 – 5 Disagree

*The information in the graph is useful*

- Agree 1 – 2 – 3 – 4 – 5 Disagree

*I'm missing information in the graph*

- Open answer

*There is information in the graph that is not useful*

- Open answer

*Do you have any other recommendations or suggestions?*

- Open answer

Section 3:

### **The User Interface**

For the user interface you will be asked to complete a number of tasks. Keep in mind that this prototype is not fully functional and only allows for the asked to be completed. Also take your time when typing, the prototype cannot respond to fast typing. When using the prototype use the F11 key to go in and out of full screen mode, this way you can see the prototype without having to scroll.

You will be guided through 3 tasks, each accompanied by questions. If you get stuck or want to redo the task you can reload by clicking the link again and starting over.

The link to the prototype:

<https://xd.adobe.com/view/c2c5bf94-4b5f-46b9-46a9-4c27f1256ba7-b4f4/?fullscreen&hints=off>

### **Task 1: create a new measurement**

In this task you will create a new measurement, execute the measuring, and compare it to an already existing measurement. As said before, the prototype is not fully functional and will allow you only to do the task. If you get stuck you can reload the page by clicking on the link again and please type slowly. The steps to this task are as follows:

1. create a new measurement

2. fill in the info, you either have to select it in the dropdown menu, or type it yourself to create a new entry:

name: plant

category: new

location: field 1a

plant type: tradecentia

sub type: albiflora

duration: manual

you don't need a blue trigger light so do not select that option (you will use natural light to observe the chloroplast movement)

3. add your measurement to the queue

4. find your new measurement, click on it and start the measurement

5. stop the measurement manually

6. Remove the graph from the screen

7. switch from queue mode to category mode, find your measurement and click on it

8. add another graph: dark plants > alocasia 1

9: find the home option in the measurement menu

You can leave the prototype in the home screen.

*Did you have any trouble during the task?*

- *During subtask 1*
- *During subtask 2*
- *During subtask 3*
- *During subtask 4*
- *During subtask 5*
- *During subtask 6*
- *During subtask 7*
- *During subtask 8*
- *During subtask 9*
- *No*

*Can you elaborate on what went wrong? (example: I couldn't find a button or function, the layout is unclear, the prototype did not respond)*

- *Open answer*

*The functions were easy to find*

- Agree 1 – 2 – 3 – 4 – 5 Disagree

*The functions and buttons had logical names*

- Agree 1 – 2 – 3 – 4 – 5 Disagree

*The tasks I did were logical*

- Agree 1 – 2 – 3 – 4 – 5 Disagree

## **Task 2: import an existing measurement**

In this task you will import an already existing measurement. As said before, the prototype is not fully functional and will allow you only to do the task. If you get stuck you can reload the page by clicking on the link again and please type slowly. The steps to this task are as follows:

1. import a measurement
2. press 'p' to fill in the information
3. import your measurement
4. find the home option in the measurements menu

You can leave the prototype in the home screen.

*Did you have any trouble during the task?*

- During subtask 1
- During subtask 2
- During subtask 3
- During subtask 4
- No

*Can you elaborate on what went wrong? (example: I couldn't find a button or function, the layout is unclear, the prototype did not respond)*

- Open answer

*The functions were easy to find*

- Agree 1 – 2 – 3 – 4 – 5 Disagree

*The functions and buttons had logical names*

- Agree 1 – 2 – 3 – 4 – 5 Disagree

*The tasks I did were logical*

- Agree 1 – 2 – 3 – 4 – 5 Disagree

### Task 3: comparing measurements

In this task you will compare two existing measurements. As said before, the prototype is not fully functional and will allow you only to do the task. If you get stuck you can reload the page by clicking on the link again and please type slowly. The steps to this task are as follows:

1. view measurements
2. go from the queue to the category mode
3. select one of the two dark plants measurements
4. add the other dark plants measurement
5. get the details of the top graph
6. get the details of the bottom graph
7. remove one of the graphs from the screen
8. remove the other graph from the screen
9. find the home option in the measurements menu

*Did you have any trouble during the task?*

- *During subtask 1*
- *During subtask 2*
- *During subtask 3*
- *During subtask 4*
- *During subtask 5*
- *During subtask 6*
- *During subtask 7*
- *During subtask 8*
- *During subtask 9*
- *No*

*Can you elaborate on what went wrong? (example: I couldn't find a button or function, the layout is unclear, the prototype did not respond)*

- *Open answer*

*The functions were easy to find*

- *Agree 1 – 2 – 3 – 4 – 5 Disagree*

*The functions and buttons had logical names*

- *Agree 1 – 2 – 3 – 4 – 5 Disagree*

*The tasks I did were logical*

- Agree 1 – 2 – 3 – 4 – 5 Disagree

*Do you have any comments ore recommendations for this part?*

- Open answer

### **General questions**

You have completed the tasks and accompanying questions. There are a few general questions left about the design and purpose of the software.

*The design is aesthetically pleasing*

- Agree 1 – 2 – 3 – 4 – 5 Disagree

*The program has a clear layout*

- Agree 1 – 2 – 3 – 4 – 5 Disagree

*The functions are useful*

- Agree 1 – 2 – 3 – 4 – 5 Disagree

*The functions and option are easy to navigate and find*

- Agree 1 – 2 – 3 – 4 – 5 Disagree

*All options do what I expect them to do*

- Agree 1 – 2 – 3 – 4 – 5 Disagree

*The program is a useful addition to the sensor that was designed*

- Agree 1 – 2 – 3 – 4 – 5 Disagree

*Are there any functions or options that you are missing in this program?*

- Open answer

*Do you have any comments or recommendations on the program in general?*

- Open answer

Section 4:

### **Complete**

Thank you for finishing this questionnaire. If you want your answers send to you please fill in your email address below.

*Your email address*

- Open answer

## Appendix E - Protocol

### General

1. Title: Engineering a chloroplast movement sensor
2. Context: BSc thesis
3. Researcher: Puck Kemper
4. Supervisor: Cora Salm
5. Other people involved: Tom van den Berg (critical observer), Remco Sanders (technical support)
6. Department responsible for research: Integrated Devices and Systems (IDS)
7. Location where research will be conducted: Home of the researcher, online user tests
8. Short description of the project:
  - a. Chloroplasts in plant cells move in either of two positions. These positions have influence on for example the photosynthesis of the plant and therefore it's growth. The goal is to design a sensor which can indicate the position of the chloroplasts, which can be used in the field. This sensor will have to use 1 of 2 measuring techniques, or a combination of both: reflectance and transmittance. This data will be displayed in a user interface. A prototype will be made of this UI and it will be tested by people who are involved in the Plantenna project.
9. Expected duration of the project and research period: February 3<sup>rd</sup> 2020 – July 17<sup>th</sup> 2020: 6,5 months
10. Number of experimental participants: 3
11. EC member of the department: Cora Salm

### Questions about fulfilled general requirements and conditions

1. Has this research or similar research by the department been previously submitted to the EC?
  - a. No?
2. Is the research proposal to be considered as medical research?
  - a. No
3. Are adult, competent participants selected?
  - a. Yes, participants are contacted through the Plantenna project, all employees of either University of Twente or the University of Wageningen.
4. Name all characterises participants must possess in order to be included in the research, such as gender, age, membership of a specific organisation.
  - a. Gender and age do not matter. They do need background information on chloroplast movements and measurements on plants. Therefore they will be selected within the Plantenna project.
5. Are the participants completely free to participate in the research, and to withdraw from participation whenever they wish and for whatever reason?
  - a. Yes
6. Is there a risk for adverse effects on the research for certain participants?
  - a. No, there is no such risk because it does not involve any personal information.
7. Does the method used allow for the possibility of making an accidental diagnostic finding which the experimental participant should be informed about?
  - a. No, the method does not allow for this possibility.

8. Are participants briefed before participation and do they sign an informed consent beforehand in accordance with the general conditions?
- This project is focused on designing a chloroplast movement sensor and accompanying data visualisation and user interface. The sensor is constructed in such a way it can measure chloroplast movement in the field. It measures transmittance and reflectance of red light from a leaf. With this, the location or behaviour of the chloroplasts can be determined.

In this questionnaire, you will be guided through a user interface. This user interface is a prototype of software in which you can measure, import, and compare chloroplast movement measurements. This questionnaire and the tasks will take approximately 20 minutes. It is recommended you do this questionnaire on a computer, then you can view the prototype more easily and switch between questionnaire and prototype better.

The data gathered in this questionnaire is anonymous and will only be used for this specific project. By answering yes to the next question you agree to the use of the gathered data for this project and that your answer will be added to the report of this project anonymously. This project is part of the graduation semester of the bachelor Creative Technology at the University of Twente, Faculty EEMCS.

If you have any questions or concerns you can contact the bachelor student working on this project through this email address:

p.y.kemper@student.utwente.nl

9. Are the requirements with regard to anonymity and privacy satisfied as stipulated in (§3.8)?
- Yes
10. If any deception should take place, does the procedure comply with the general terms and conditions (no deception regarding risks, accurate debriefing) (§3.10)?
- No deception takes place
11. Is it possible that after the recruitment of experimental participants, a substantial number will withdraw from participating because, for one reason or another, the research is unpleasant?
- No

#### Questions regarding specific types of standard research

12. Does the research fall entirely under one of the descriptions of standard research as set out in the described standard research of the department?
- Yes.
13. If yes, what type of research is it? Give a more detailed specification of parts of the research which are not mentioned by name in this description (for example: What precisely are the stimuli? Or: What precisely is the task? OR: What is the nature of the measurement/interview questions?)
- Test of user interface, software only
14. Not applicable.

Why is your work COVID-19 proof?

15. Do you add additional face-to-face contact?
  - a. No, only work in disturbed fashion over an online survey.
16. Not applicable
17. Not applicable
18. Give a thorough explanation, why you consider your research can be considered COVID-19 proof include any considerations you discussed with your supervisor to address contingency of any additional risks you identified. Explanatory notes: The will be no physical contact between the researcher and the test participants. Everything will be done online therefore there is no risk connected to COVID-19.



## Appendix F – user test results

This appendix includes all the answers the testers gave on the user test survey. They will be categorised per person and presented in a table. The last question where the email is asked is not included in the results to protect the privacy of the tester.

Test person	1	2	3
I have read and understood the explanation of this questionnaire and agree to the use of the gathered data in the report of this project anonymously	Yes	Yes	Yes
Does absorption increase or decrease in both graphs?	Decrease	Decrease	Decrease
Which plant has a greater increase in transmittance and reflectance?	Corn	Corn	Corn
What is the approximate intensity of the environment light? (you do not have to write down the unit)	700	675 units for Corn and 695 units for Lily	700
This graph is clear	2	3	4
The information in the graph is useful	1	2	3
I'm missing information in the graph	slope of T+R change	Depends on what question you want to answer using the graphs. See my recommendations below.	Absorptance
There is information in the graph that is not useful	too dense time scale	none	The values on the x-axis are spaced too closely
Do you have any other recommendations or suggestions?	for comparison, particularly of rates, same timescale should be used	1. The label for the left Y-axis is a bit vague. A better phrase would be "Transmittance plus reflectance". A value of 4% on the y-axis cannot be both transmittance and reflectance. 2. One should be careful in comparing the "decrease" in absorbance of the two	Intuitively, it would be much better to replace info on transmittance+reflectance by absorptance, as this information correlates positively with chloroplast movement behaviour. If there's an accumulation response by the chloroplasts, absorptance will increase. If there's an avoidance response, absorptance will decrease.

		<p>leaf samples, based on the graphs. This is because any change in absorbance within a fixed time interval is directly proportional to the absorbance at the desired instant (blame the exponential decay). In the case of Corn, the absolute change is 0.008 while the same is 0.001 for Lily from time = 1 to 45 minutes. If you want relative change, we need to do normalize this with respect to the initial values at <math>t=1</math> min. The relative change will only depend on the time-dependent pigment concentration and optical path length inside the leaf.</p>		
<b>Did you have any trouble during this task?</b>	During subtask 2	During subtask 2, During subtask 3, During subtask 5, During subtask 6, During subtask 7, During subtask 8	During subtask 2, During subtask 3, During subtask 4, During subtask 5, During subtask 6, During subtask 7, During subtask 8, During subtask 9	
<b>Can you elaborate on what went wrong? (example: I couldn't find a button or function, the layout is unclear, the prototype did not respond)</b>	the menu for new measurement seems inactive (safari, latest version)	<p>1. It is not clear that the option for additional lighting means "blue light trigger".</p> <p>2. I was forced to write "Plant" as the new measurement name. It would not accept any other keypress on the keyboard.</p> <p>3. I could not edit any entry anymore after adding it to queue and selecting it again.</p> <p>4. There was no button on the screen to stop the measurement. Also no button to remove the graph.</p>	The software hardly responds, and at some point seems to crash completely. So I couldn't actually get a measurement done	
<b>The functions were easy to find</b>	2	4	1	
<b>The functions and buttons had logical names</b>	2	2	2	
<b>The tasks I did were logical</b>	2	2	1	
<b>Did you have any trouble during this task?</b>	No	No	No	

<b>Can you elaborate on what went wrong? (example: I couldn't find a button or function, the layout is unclear, the prototype did not respond)</b>	it worked	this task worked as described.	This went relatively OK, though I find it hard to see where the cursor is in the menu where I'm supposed to specify what to import
<b>The functions were easy to find</b>	1	2	1
<b>The functions and buttons had logical names</b>	2	1	1
<b>The tasks I did were logical</b>	3	1	1
<b>Did you have any trouble during this task?</b>	No	During subtask 4, During subtask 7, During subtask 8	During subtask 2, During subtask 3, During subtask 4, During subtask 5, During subtask 6, During subtask 7, During subtask 8, During subtask 9
<b>Can you elaborate on what went wrong? (example: I couldn't find a button or function, the layout is unclear, the prototype did not respond)</b>	it worked fine	When I select one measurement, the other one disappears automatically from the graph. So, I cannot "add" a graph on top of the other. There is no button to remove a graph.	After I clicked on "View measurements", it did open a new window but did not work beyond that (empty screen). So, I couldnt do anything after that
<b>The functions were easy to find</b>	1	3	5
<b>The functions and buttons had logical names</b>	1	2	5
<b>The buttons have logical locations</b>	1	3	5
<b>Do you have any comments or recommendations for this part?</b>			
<b>The design is aesthetically pleasing</b>	1	3	3
<b>The program has a clear layout</b>	1	4	3
<b>The functions are useful</b>	2	3	2
<b>The functions and options are easy to navigate and find</b>	2	3	5
<b>All options do what I expect them to do</b>	1	4	5
<b>The program is a useful addition to the sensor that was designed</b>	3	3	3

<b>Are there any functions or options that you are missing in this program?</b>	not knowledgable enough to tell	Cannot give an answer here as I do not know what sensor was designed and which information is intended to be extracted.	Not per se (I guess), but I would have to have the chance to actually try out the program well. This wasnt possible because of abovementioned issues with running the program
<b>Do you have any comments or recommendations on the program in general?</b>	names, not only tiny triangles, should be clickable		