Creative Technology

Graduation Semester 2020-2

“We CreaTe Impact”

Designing a leaf holder for real-time microscopy

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Abstract

Currently, an experimental setup has been created to observe the opening and closing of stomata on the surface of a leaf. To observe this, the leaf needs to be held perfectly still above a microscope. To accomplish this, a leaf holder is made. The leaf is being clamped between a metal ring and a Perspex plate with embedded magnets. Due to its size, the metal ring needs to be placed with a pair of tweezers. Since the area above the microscope is also hard to reach, a new leaf clip needs to be designed that improves the user-friendliness while keeping optimal growth conditions for the leaf, regarding water, sunlight and air. The aim of this project is to design and build a new leaf holder that is more user-friendly, keeps optimal growth conditions and adds the possibility to measure with an ASD spectrometer simultaneously with the microscope. The ASD spectrometer measures the reflectance properties of vegetation, soils, rocks and bodies of water. In this setup, the ASD spectrometer plant probe will be used. The goal is to find a relation between the change in the spectrum in respect to the stomatal movement. After conducting a problem analysis and a state-of-the-art research, a new leaf clip was designed through 3d modelling and a rapid prototyping method. With the use of 3d printing technology, a new clip was built that improves on user-friendliness and adds the possibility to measure with the ASD spectrometer. The leaf can be clipped in the new leaf holder in a more natural body position while keeping optimal growth conditions with an open design and minimal interference. The ASD spectrometer probe can be placed onto a bracket that is connected to the leaf holder and features a second anchor point near the handle to support its weight. Initial user tests show that the new clip is easier in use and shows faster clipping procedure times.

Acknowledgement

I would like to thank my supervisor Cora Salm and my critical observer Tom van den Berg for their guidance, shared knowledge, motivational boosts and weekly meetings throughout this graduation project. Furthermore, I would like to thank Dominic and Sarah for their support and time at the WUR. Special thanks to my colleague Sem Jacobs for the great teamwork during the last few months. Finally, I would also like to thank my family for their continuous support during these difficult times.

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Chapter 1 – Introduction

Farmers have to deal with more extreme weather conditions every year. Our planet's climate will continue to change and our population will only increase. By 2050 the world's population is expected to grow to 9.7 billion people. [1] This asks for more sustainable and efficient ways of food production. To deal with these deteriorating conditions, we need to efficiently use our resources like water, space and energy. Vertical farming is the practice of growing crops in vertically stacked layers. This farming technique uses Controlled Environment Agriculture (CEA) technology, to artificially control light, humidity, temperature and gases. The goal is to maximize the crop yield per square meter. The energy cost can be reduced and the water usage is lowered by 95% [2].

To better understand what resources a plant specifically needs, it needs to be analyzed to check whether the plant is living in perfect conditions. Plants use a process called photosynthesis to create glucose($C_6H_{12}O_6$) out of light energy, carbon dioxide (CO_2) and water. Oxygen (O_2) is produced as a by-product of photosynthesis. This process can be seen in figure 1.

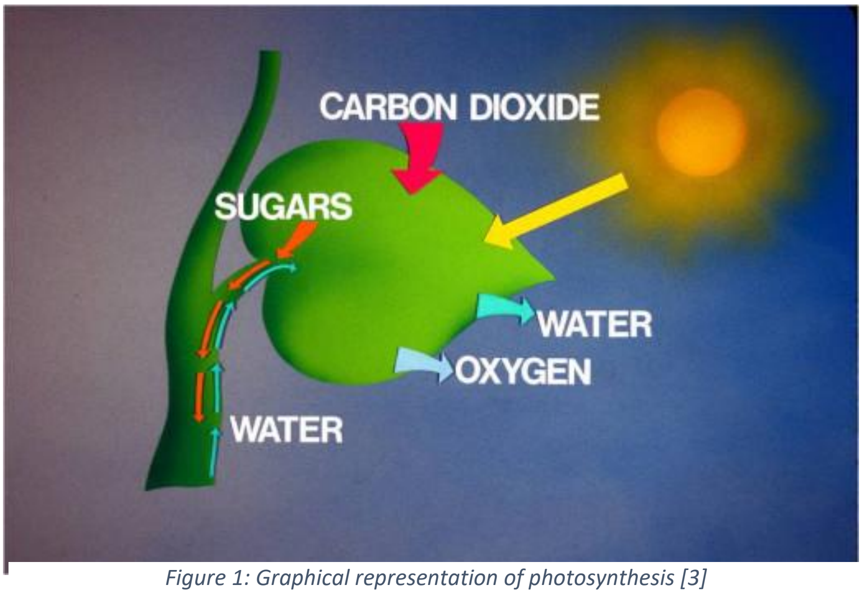

Figure 1: Graphical representation of photosynthesis [3]

The leaf of a plant has tiny pores in it that are called stomata. Stomata regulate the intake of CO_2 and its transpiration by opening and closing. A stoma consists of 2 guard cells and surrounding epidermal cells. In optimal conditions, the stomata are wide open which allows the exchange of gasses with the atmosphere. The 2 guard cells are contracting or expanding themselves which opens or closes the stomata. To open the stomata, water is rushed in through osmosis, which is dependent on the potassium concentration in the cells. This can be seen in figure 2. Potassium actively transports to the vacuoles which increases its concentration and thus driving water entry through osmosis. This will increase the cell size and turgency and open the pores. The opposite happens to close the stomata. Potassium is transported out, which attracts water to the exterior, effectively closing the pore.

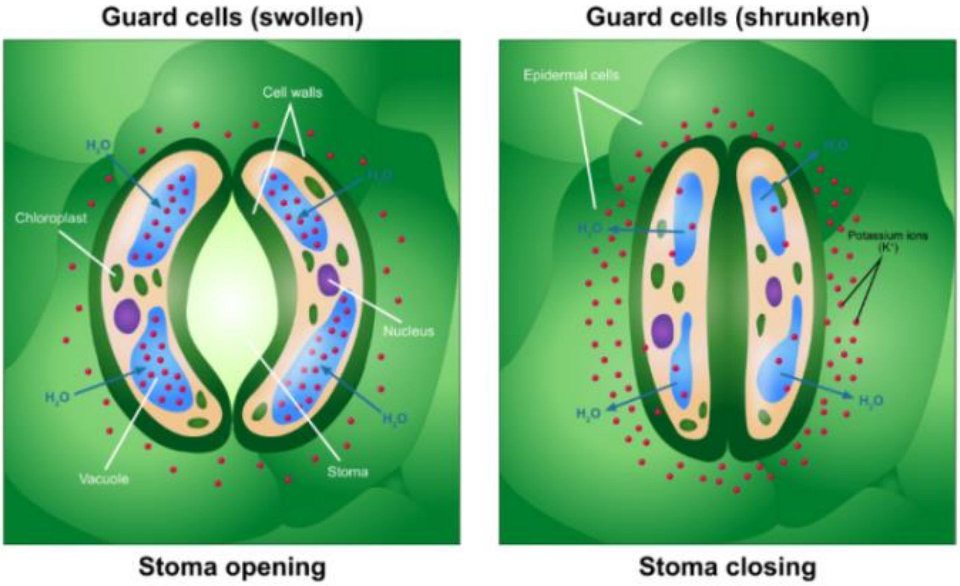

Figure 2: Opening and closing of the stomata [4]

With most plants, the stomata open during the day, allowing CO_2 to enter so photosynthesis can take place. Oxygen, the byproduct of photosynthesis can exit through the stomata. The uptake of water, its transportation and the release in the form of water vapor, is called transpiration. The exchange of gasses is depicted in figure 3. There is a constant trade-off happening between the intake of CO_2 and the release of water vapor. This is caused by internal and external cues. For example, high light intensity and high air humidity lead to stomatal opening. Transpiration of water vapor to the atmosphere drives exchange from the roots to the rest of the plant. Leaf transpiration also provides a cooling mechanism and it is

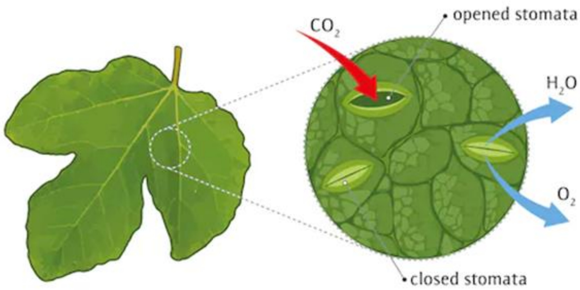

Figure 3: Exchange of gasses through the stomata [5]

essential for CO_2 uptake and plant growth. Upward movement of water through a plant is driven by a negative hydrostatic pressure in the leaves. This negative hydrostatic pressure is built up by the loss of water through transpiration.

This GP focuses on building a leaf holder for an experimental setup that is currently measuring this stomatal behavior. The experimental setup can be seen in figure 4.

Figure 4: The experimental setup

This setup uses a microscope to measure the kinetics of stomatal movement during a change in conditions e.g. change in humidity or light intensity. In order to look at the stomata error free, the plant must be held in place such that it can still receive its resources: water, air and light, but also in such a way that the leaf is not moving during the measurements. This is vital to success of the measurements, since a small movement of the leaf can cause the microscope to lose focus or miss the intended measuring area due to the magnification. The setup currently uses the leaf holder as seen in figure 5.

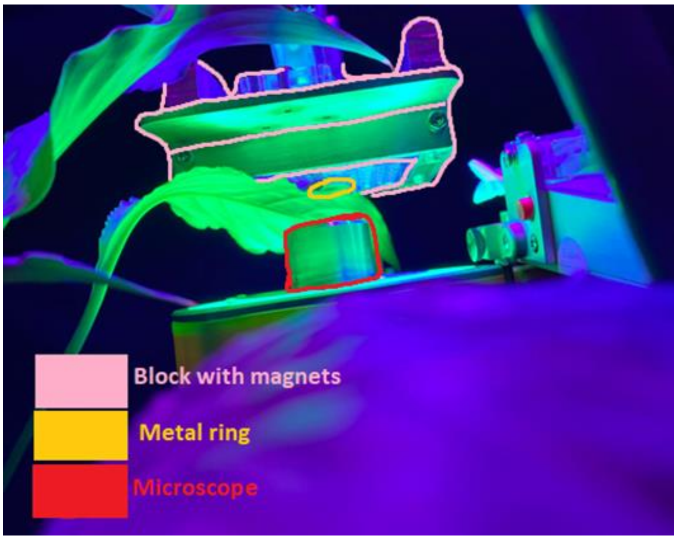

Figure 5: The current leaf holder with the most notable parts highlighted

The leaf is held in place above the microscope between a metal ring and magnets embedded in a Perspex plate. This design has some flaws regarding usability that will need to be improved on. First of all, the metal ring that holds the leaf between the microscope and the metal block, has to be placed with a pair of tweezers. The ring is too small to be able to place it using your fingers. Secondly, the area under the metal block is hard to reach. These inconveniences make the setup process before a measurement rather tedious and time consuming.

Besides the improvements regarding usability, the experimental setup needs to be able to use the ASD spectrometer together with the microscope. The ASD spectrometer measures the reflectance properties of vegetation, soils, rocks and bodies of water. In this setup, the ASD spectrometer plant probe will be used. This device can be seen in figure 6. The goal is to find a relation between the change in the spectrum in respect to the stomatal movement.

Plant tissues are made up of numerous bio-compounds - such as carbohydrates, sugars, starch, amino acids, proteins, pigments, minerals, etc. - all of which have a different spectral signature [6]. The content of, for example, chlorophyll, can be an indication of how much carbon dioxide a plant takes in which can be used to estimate crop productivity. Another use of this device is to analyze plant water content through leaf spectroscopy. The reflectance of the leaf will change depending on the water content.

The usability needs to be improved and the ASD needs to be integrated. However, one aspect of the current design needs to remain unchanged. It is important that the plant remains under optimal growth conditions during the measurements. The leaf still needs access to water, light and air. When the leaf is clamped too tight, it could potentially stop the water flowing into the leaf. The leaf needs proper air circulation around it and light needs to be able to hit the leaf.

Figure 6: ASD spectrometer plant probe

A set of 4 challenges can be defined to reach the goal of this graduation project:

1. Holding the leaf in place
2. Keeping optimal growth conditions
3. Making the leaf holder more user-friendly
4. Integrate the ASD spectrometer

These challenges all have their own sub challenges or problems that will be encountered during the design process of the improved leaf holder and are listed in chapter 2.1.

This leads to the following research question:

How can a leaf be held such that a microscope and an ASD spectrometer can both be used simultaneously while keeping optimal conditions regarding sunlight, water and air?

Chapter 2 – Background Research

This chapter contains all research needed prior to designing the leaf holder. This also encompasses a problem analysis and a state-of-the-art review.

2.1 Problem analysis

For the problem analysis, the 4 challenges as defined earlier, are broken down in separate problems that can be encountered during the design process. The first 2 challenges: ‘holding the leaf in place’ and ‘keeping optimal growth conditions’ are already accomplished in the current leaf holder. However, that design was made by someone else and the problems that might have occurred during that particular design process, could also be encountered here. That is why it is important to not lose sight of these challenges.

3 different problems are defined for the challenge ‘holding the leaf in place’. The leaf needs be swappable. For the research it is important to be able to measure more than one leaf. This is because the leaves of a plant can provide different results, not all stomata on all leaves show the same behavior. This is the most time-consuming step during the setup phase before measurements with the microscope are done. This problem is therefore closely related to challenge 3: ‘making the leaf holder more user-friendly’.

The leaf holder needs to be able to hold different sized leaves. Multiple plant species are used in plant research and therefore this leaf holder needs to be able to hold at least some of them. Tomato plants and cucumber plants have for example more hand-sized leaves, while the Arabidopsis thaliana has coin sized leaves. The leaf holder either needs to be adjustable to all these sizes, or separate clips need to be designed to fit the most used plants. This is described in more detail in section 2.2.

The leaf holder should not put too much pressure on the leaf. If the leaf clip exerts a certain pressure threshold, which is different for each plant, it could potentially cut off the water transport into the leaf. The stomata need water to open and close, without his mechanism, it could stop the leaf from growing, making it useless for the measurements. This is a problem that is closely related to the first problem of challenge 2: ‘keeping optimal growth conditions’.

The optimal growth conditions point to water, light and air. The three main things that are of use for the leaves. Light needs to be able to reach the leaves that are being held. The current design accomplished this by using a transparent Perspex plate with holes. The air circulation is in the current design accomplished by having holes above the leaf and nothing but the metal ring on the bottom.

The third challenge is defined as: ‘making the leaf holder more user-friendly’. The area where the leaf is held is in the current leaf holder design hard to reach. On top of that, the metal ring needs to be placed with tweezers. The process of setting up before a measurement is therefore very tedious and time consuming. Avoiding the use of small objects that need to be placed above the microscope is key here. The metal block that houses the Perspex plate with the magnets, is able to rotate on the X-bar and is adjustable in height. These are features that are useful and are favorable to keep in the new design.

The fourth challenge is defined as: ‘integrate the ASD spectrometer’. This will undoubtedly be the hardest challenge. The ASD spectrometer or ASD for short, is big and heavy. The probe needs to be very close to the leaf to conduct the proper measurements. Ideally, the probe is in direct contact, but it can be placed within 15mm if measured in a dark room This can also be seen in figure 6. For the ASD to work properly, it needs to measure a white reference or spectralon panel. This action needs to be every few measurements and therefore it is an important thing to keep in mind during the design phase. Making the ASD swappable or the object under it could be a viable solution. Figure 7 shows a picture of two differently sized spectralon panels.

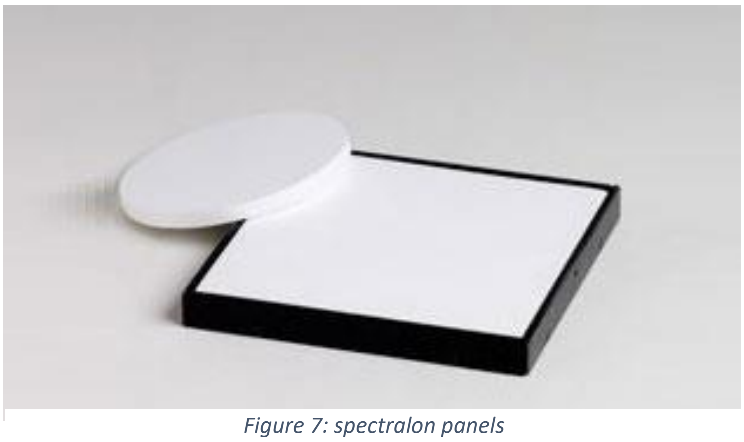

Figure 7: spectralon panels

2.2 different types of plants

In theory, the experimental setup can be used to measure every plant. However, some plants are of more interest. This section briefly describes 4 plants that would preferably fit in the new design of the leaf holder. Cucumber (figure 8), tomato (figure 9) and corn (figure 10) are 3 plants that would benefit from increased crop yield or precision farming in general since they are in high demand. To put this in perspective, a production level of 1.09 billion metric tons of corn was achieved in 2018/19, where the US alone produced 345 million metric tons [7].

The leaves of a cucumber plant average between 10-20cm in diameter for regular cucumbers and 20-40cm in the seedless variant [8]. Tomato leaves can grow up to 25cm, however the leaves consist of five to nine smaller leaflets which can grow up to 8cm [9]. Corn generally grows up to 3m in height. The stem commonly consists of 20 internodes of 18cm. The leaves, which grow from each node are 9cm in width and 120cm in length.

The Arabidopsis thaliana is also an interesting plant. Not because it produces crops, but because it is the first plant to have its genome sequenced. It is a popular tool to better understand the molecular biology of many plant traits. The average A. Thaliana leaf is 1.5-5cm long and 2-10mm wide.

At this point, the cucumber plant looks like the biggest challenge. The leaves are big and there are no pointy lobes sticking out. The other leaves have more sharp and pointy edges which makes them easier to put under the ASD measuring area. The ASD only measures in a 2 cm diameter. During the ideation phase, it might be wise to also consider multiple clip designs to fit all the leaves, instead of a one sized clip.

Figure 8: Cucumber and tomato plant

Figure 9: Corn and Arabidopsis Thaliana

2.3 State of the art

The state-of-the-art review consists of an analysis of the already available leaf clips. The clips are categorized in 3 sections. The state-of-the-art review is key to determine if an answer already exists for the stated research question. Reviewing existing leaf clips could lead to new insights, which could lead to better design choices.

2.3.1 machine specific leaf clips

The first category in the state-of-the-art review is described as machine specific leaf clips. These clips/holders are pre-attached to the measuring probe and are designed to work perfectly for their intended use. The way they are designed makes the probe usable with the clip, but the clip itself is useless without the probe. The first machine specific clip is the one from the MINI-PAM as seen in figure 12. This design houses the probe and sensors for temperature and humidity. The part that actually clamps the leaf, is donut shaped. This allows the probe to measure the leaf directly without interference. The design itself is useful, but there are many bulky and obsolete parts.

Figure 10: MINI-PAM leaf clip

The second leaf clip in this section is the clip mechanism attached to the ASD spectrometer plant probe as seen in figure 13. The metal bar is spring-loaded which causes the leaf to be clamped in between the end of the probe and the leaf clip. The leaf needs to be very close to the probe and this leaf clip design accomplishes that. This design also makes it portable and easy to swap leaves in between measurements.

Figure 11: ASD spectrometer plant probe

2.3.2 universal leaf clips

The second category in the state-of-the-art review is described as universal leaf clips. These designs can be tweaked slightly to work with more than 1 probe size. The first leaf clip is an accessory for the MINI-PAM however, the holder for the probe on top of it, could be made slightly bigger to also fit different probes. The design is very minimal and clamps the leaf with a similar mechanism to that of a clothespin. It is not possible to look at the leaf using a microscope, but it is one of the most convenient and user-

Figure 12: Walz leaf clip

friendly designs.

The second clip in this section is the FMS/PTL from Hansatech instruments. This clip also uses a clamping mechanism similar to a clothespin. The difference here is that the whole clip is attached to a tripod. This clip is categorized as universal because this clip can also be used with other probes or devices with minimal adjustments. The tripod is a good design choice to hold the leaf perfectly still during measurements, but the tripod also prevents a microscope from being used underneath the leaf.

Figure 13: FMS/PTL leaf clip from Hansatech instruments

2.3.3 custom leaf clip

The leaf clip in this section is made for microscopy research by Purwar &Lee [10]. This leaf clip is made with custom acrylic and 3d printed parts. This design is optimized for microscopy measurements. The acrylic parts are clear, which allows for the sunlight to reach the leaf. The specific way in which the acrylic is cut allows for proper air circulation around the leaf. The way the leaf is clamped between magnets is similar to the leaf clip on the current experimental setup.

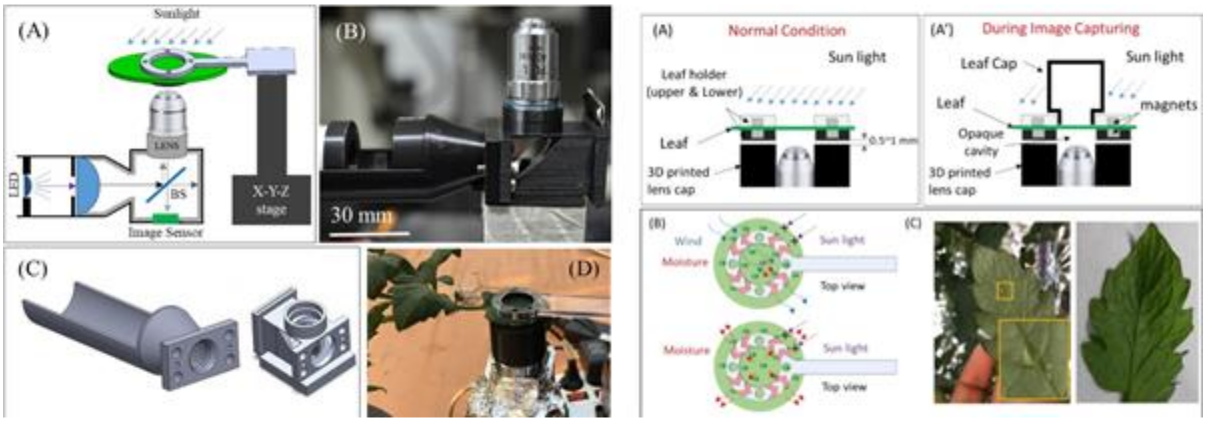

Figure 14: microscopy leaf clip

2.3.4 conclusion

Several designs have been discussed in this state-of-the-art review. There are 3 main clamping mechanisms: linear spring-loaded, clamping (similar to a clothespin) and clamping between magnets. None of the designs would allow for the usage of both a microscope and ASD spectrometer probe. This leads to the conclusion that, to my knowledge, there is no existing answer to my research question. However, multiple design choices could be to combined to form a new design that fits the criteria. The Advantages from t the leaf clip by Purwar & Lee are that it works well with a microscope and it keeps optimal growth conditions. This design lacks the ability to house the ASD spectrometer probe and it is not as easy to set up as the Walz leaf clip or the standard ASD plant probe leaf clip. If these design choices can be combined, a viable leaf clip could be made.

Chapter 3 – Methods and Techniques

This chapter touches upon the various methods that are used throughout the whole graduation project. Each of the methods and techniques will be explained and their uses are discussed.

3.1 Creative technology design process

Figure 15 depicts the Creative Technology design process. There are four phases in this process. The first phase is called *Ideation*, which is about identifying the problem and exploring various ideas. The Ideation phase in this report will be focussed on making sketches to explore the possible solution to the problem analysis. The next phase is called *Specification*, where the project requirements are defined and these can then be implemented in the explorative sketches done in the Ideation phase. This phase includes making small prototypes based on the explorative designs [11]. These prototypes are evaluated by the researcher and changes can be made through multiple iterations. All the specified components come together in the third phase called *Realisation*. This is where the building of the envisioned end product takes place. The product is evaluated again, to see if it meets the requirements. The specifications can be altered if the product does not meet the requirements. Once the prototype meets the requirements, the fourth phase can be started, the *Evaluation* phase. This is where user tests are done with a concluding critical reflection [11].

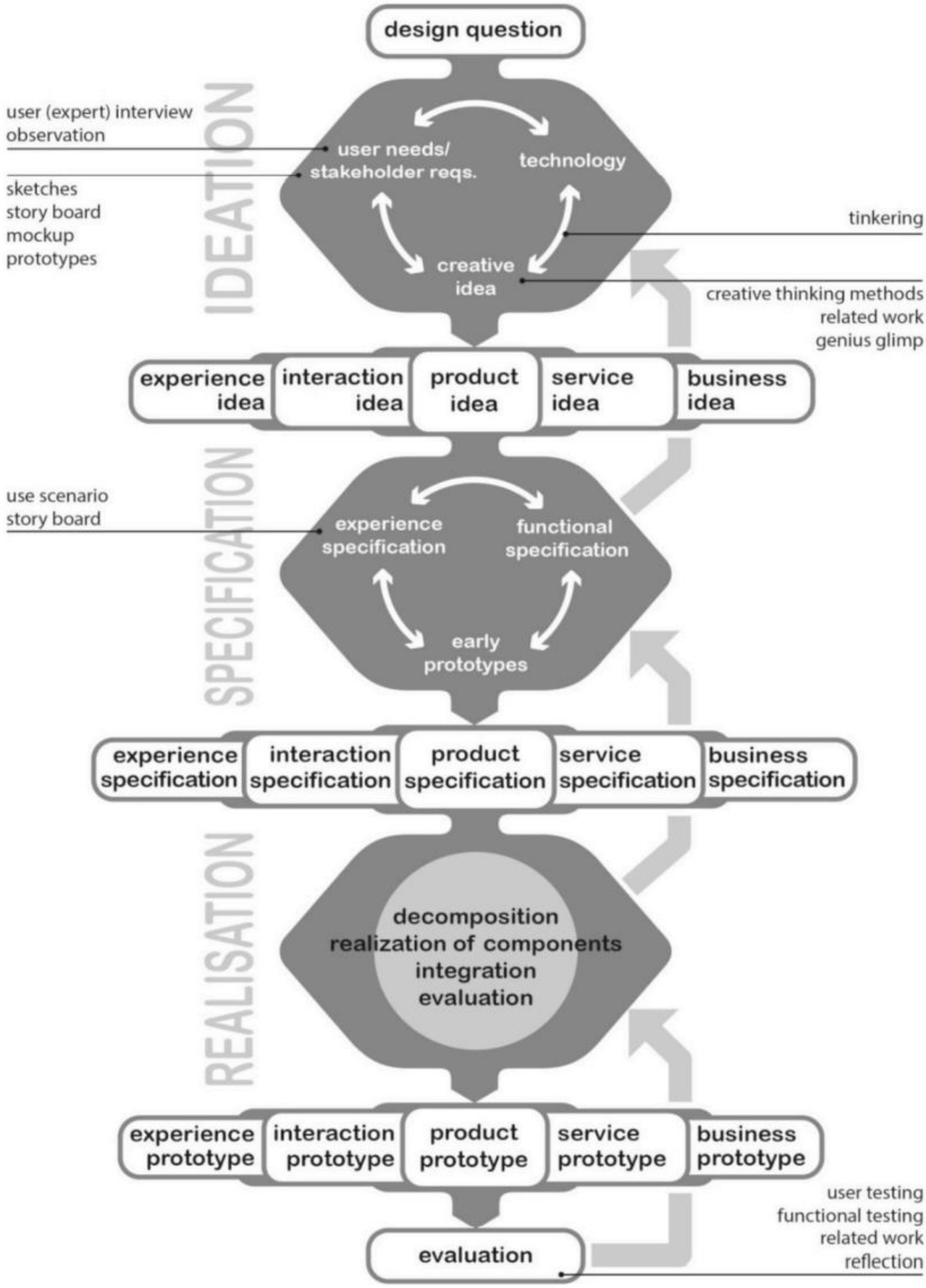

Figure 15: Creative technology design process

3.2 Photogrammetry

The final product that will be delivered after the graduation project has to be able to house the ASD spectrometer as seen in Figure 11. The plant probe has certain curves in its design that are hard to model which makes it hard to 3d print something that holds the probe above a leaf. Photogrammetry was used to help with this problem. This is a method that can convert pictures of an object to a 3d model of that object. The user takes around 30 to 500 pictures of an object from all angles. The number of pictures needed depends on several factors including: colour/shade of the object, its size and the preferred detail in the model. The photogrammetry software recognises the angle that the picture was taken from and then compares all the points of interests to form a mesh. A mesh consists of triangles that resemble the original object. Optionally, the software can also add texture to the mesh to give its original colour, for example.

The photogrammetry software that is used during this graduation project is Meshroom from the AliceVision framework. This software is opensource and makes use of the AliceVision AI. The features that make this software appealing are live modelling and step-by-step calculation. Live modelling allows for the user to add or remove pictures during computation when the user sees irregularities in the early stages of the computation. If for example, the handle of the ASD probe is computed with holes or too little detail, the user can add pictures without having to redo the entire computation, which can take up to 8 or more hours, depending on the number of pictures. The three main steps in photogrammetry are: converting to a point cloud, meshing the object, and texturing the object. There are other software programs that calculate all the steps in one go, which takes a long time. Meshroom allows the user to select which steps are calculated and also allows for pauses in between. Lets say that the point cloud is computed and the user notices that there might not be enough pictures as input. The user can pause the computation and add more pictures. Another example where the step-by-step calculation is useful is when the user is not interested in one or more steps that take place at the end of the computation process. The user can simply select a point in the timeline where the program can stop the computation, which speeds up the photogrammetry process.

3.3 3d Modelling

The process of creating shapes in 3D on a computer is called 3d modelling. The software that is used during this graduation project is Autodesk Fusion360 or F360 for short. All 3d modelling programs work with different modelling principles and F360 uses sketches and extrusions.

First, a 2D sketch is drawn on a plane (XY, XZ, YZ). This sketch can be drawn using geometric shapes like rectangles, circles or triangles, but also lines, curves or freehand drawings. Dimensions and constraints can be added to these sketches to specify the design. Once the sketch is complete, the user can extrude this 2D sketch into a 3D object. These 3D objects can then be altered using other operations.

To make a hole in a cube, for example, the user can select a side of the cube, sketch a circle and use the extrude command with the 'cut' operation facing inwards to not create a solid cylinder but instead cut that cylinder out of the cube, which essentially makes a hole. This process can be seen in figure 17. Fusion360 has way more advanced features but sketching and extruding is basically what this program is all about.

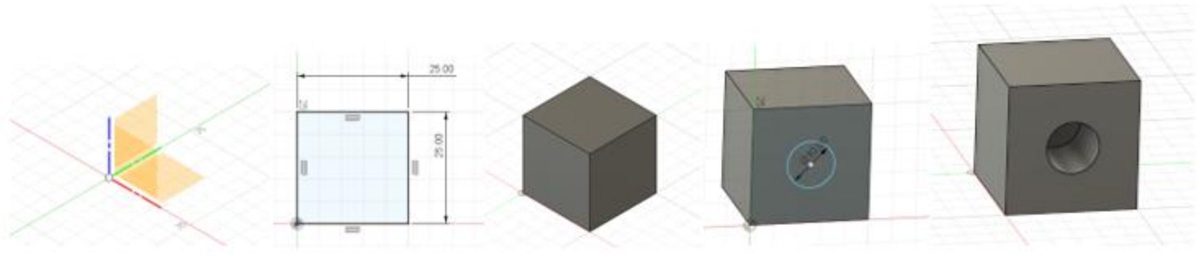

Figure 16: Fusion360 design process of making a cube with a hole

3.4 3D printing

3D printing a form of additive manufacturing and is the main method that is used to realize the design of the leaf holder. This is an upcoming technique and a great way for rapid prototyping solutions. Currently, the 2 consumer grade methods in 3D printing are SLA and FDM 3D printing. Both methods have their advantages and disadvantages, as well as their own choice of materials.

3.4.1 SLA

SLA stands for stereolithography apparatus. This is also known as: photo-solidification or resin printing. In short, this method turns photosensitive liquid (resin) into a solid 3D plastic object. A laser photopolymerises the resin layer-by-layer. An SLA printer generally consists of 4 primary sections:

- A tank filled with resin
- A platform that can be lowered into the tank and can move up and down according to the stage in the process
- An ultraviolet laser
- A computer interface that controls the platform and the laser

A schematical drawing of an SLA 3d printer with all of its components is shown in Figure 17

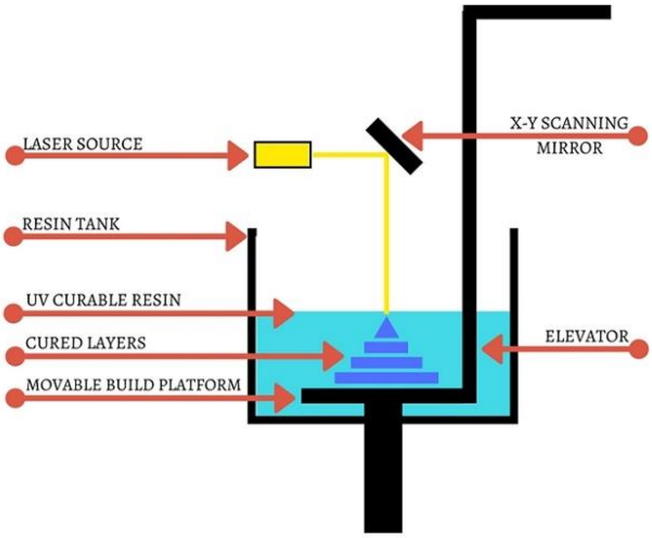

Figure 17: schematic view of the components of a SLA 3d printer

The process starts with the laser aiming at the platform and solidifying the first layer of resin. The platform will then move up by 1 layer height, which is usually between 0.05mm and 0.15mm. This process is repeated until the entire object is solidified. After the object is removed from the platform, it needs to be post-processed. This is done by placing the printed object in a UV oven to strengthen the object.

3.4.2 FDM

FDM stands for Fused Deposition Modeling. Another common name for this technique is fused filament fabrication (FFF). This method uses a spool of thermoplastic material, which is heated to its melting points through a hotend. The hotend moves over the printing surface, layer-by-layer, while extruding the material. An FDM printer generally consists of the following components:

- An extruder, which pushes the plastic forward
- A hotend, which heats the plastic to its melting point
- A printhead, which houses the hotend and the cooling fans (this can also include the extruder, depending on which extrusion type is used: Bowden extrusion or direct extrusion in which case the extruder is mounted on the printhead)
- Printing surface, this is where the plastic sticks to after it has been extruded. Printing surfaces are sometimes heated for better adhesion.

An FDM 3d printer with all of its components is shown in Figure 18.

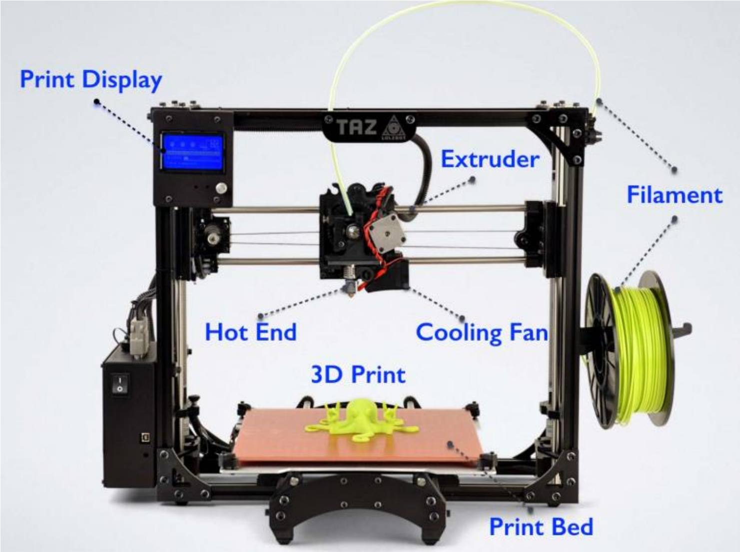

Figure 18: An FDM 3d printer with its components

One of the biggest advantages of FDM printing is its wide variety of materials which you can choose from. Some exotic materials that are worth noting are flexible filaments, water-soluble filaments and infused filaments. These filaments offer edge case solutions where the user needs these very specific filaments.

Water-soluble filaments are sometimes used as support material. This is sometimes needed when the object you want to print exceeds a certain overhang angle where the printer has trouble sticking the new layer the old layer. Support material can be used to fill the space under the overhang and the new layer can be printed on the support material. Usually, the support material is made of the same material as the staring material, however, this is sometimes hard to remove. When using Water-soluble filament, the whole object can be put in water and only the starting material will remain.

Infused filaments are base materials infused with tiny pieces of other material. Common materials that are used to infuse filaments are wood, metal (copper, bronze, magnetic material) and carbon fiber. The most commonly used filaments are PLA, ABS, nylon and PETG. Each material has varying strength, flexibility and prices. PLA is cheap and ecofriendly, bit lacks strength. ABS combines flexibility and strength, but comes with a higher printing difficulty. Nylon is strong and flexible, but highly water intolerant and expensive. PETG is easy to print, chemically resistant endless expensive than nylon, but low on durability.

There are a lot of filaments on the market with new ones coming out almost every week. Each filament has advantages and disadvantages and therefore it is always a good idea to take a moment and decide which material you should use for a project before you start printing.

3.5 Slicing

Slicing is done by a slicer, this is a program that convert a 3d model to print instructions. The 3d printer works on G-code, which essentially are just small operations that a printer does. An example of such an operation is “move to coordinate X,Y” or “move Z-axis by 0.1mm”. The cube from the previous section is put into the slicing software and can be seen in Figure 19.

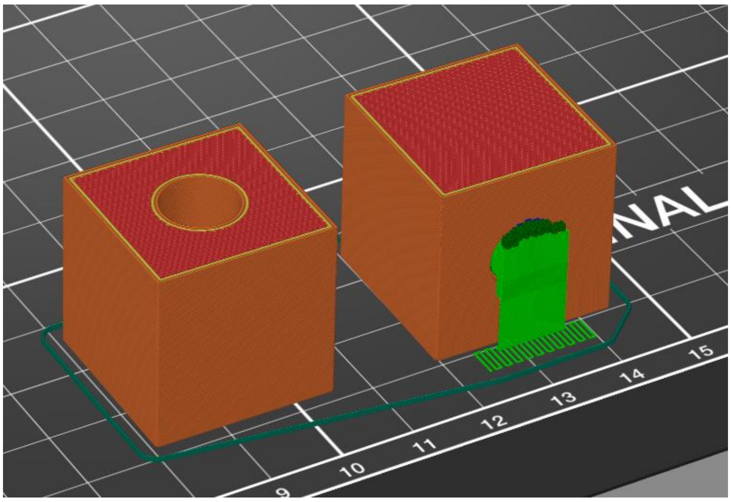

Figure 19: 2 identical cubes with a different orientation in the slicing software

When slicing a model, it is important to keep a few things in mind. A 3d printer cannot print mid-air, meaning that the new layer must be placed on a layer below it, excluding the first layer, which is printed on the print bed. A printer can print on steep overhangs, depending on the printer, these overhangs could reach 70 degrees. If the software thinks that it will exceed that overhang, it will suggest the user to use support material that creates a temporary layer. This layer is shown in green in the figure above. This material can easily be removed after the print is finished. The downsides to support material is that it increases the print time and material costs and it can leave some residue behind.

Printing orientation is therefore an important factor to keep in mind when slicing a model. In the figure above, the left cube is printed upright, while the right one is printed on its side. The left model has its hole facing upwards, which does not need support material. The right cube will eventually reach an overhang and the software decided to fill that gap with support material.

Furthermore, all print settings will need to be filled in before starting the print. There are many settings available, but the most important ones are listed below.

1. Filament settings: this includes the required temperature to melt the filament, this is different for each material; the print bed temperature, which impact the adhesion to the print bed; and the costs of the material can be set to estimate the costs of the print.

2. Printer settings: these settings are usually unchanged and are supplied by the manufacturer of the printer. The minimum and maximum values that a printer can tolerate are examples of these settings.
3. Print settings: these settings encompass how the material in constructed.
    a. Layer height determines the thickness of every layer, usually between 0.15mm and 0.3mm. This can affect the printing speed and print strength
    b. Printing speeds determine how fast the print head moves around. This directly affects the total print time, but this can also affect print quality and bed adhesion.
    c. Infill patterns and density is what happens inside the model. If the user wants to make a part that is not solid, these settings will need adjustments. The patterns and density affect the strength of the print but can also affect the print time. In figure 20, two infill patterns are shown. The left cube uses roster and the right cube uses the gyroid infill pattern.

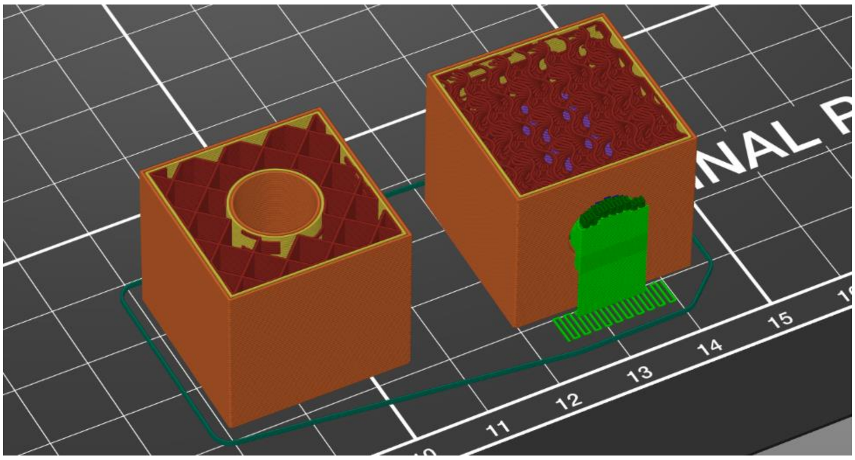

Figure 20: 2 cubes with different infill patterns. Roster on the left cube and Gyroid in the right cube

All of the above-mentioned settings and the printing orientation of the parts need to be taken into account when 3d printing, since they have a great impact on the outcome of the print. Holes and printing orientation are things that should be taken into account when 3d modeling, so the user knows what to expect when starting the slicing process.

Chapter 4 – Ideation

This chapter focusses on exploring the needs of the user and finding ways to implement these needs into the final product. This is mainly done by brainstorming and discussing the needs with the critical observer. Additionally, explorative sketches are made in Fusion360 to envision the end product.

4.1 Improve user friendliness

The first problem that was identified in chapter 2 was the user friendliness that needed to be improved on. The key elements that make the old clipping procedure not user friendly are the tedious work with small metal rings, the use of tweezers and the hard-to-reach area above the microscope. The idea to improve on all of these points is to take that clipping procedure out of the grow cabinet or away from the measuring setup and being able to perform the clipping procedure with a more natural body posture without the need of extra tools.

Initial ideas are clamping the leaf between two plates or some other magnet incorporating plastic pieces and clamping the leaf in a clothespin-like mechanism. The clamped leaf can then be inserted in the entire leaf holder that is mounted to the X-frame, above the microscope. The first iteration of the leaf clip is shown below in Figure 21. This design features rattling teeth that hold the two plates together and it has rails that can slide into the part that holds the clip. This is an open design that allows light to reach the leaf surface and lets air flow freely around the leaf.

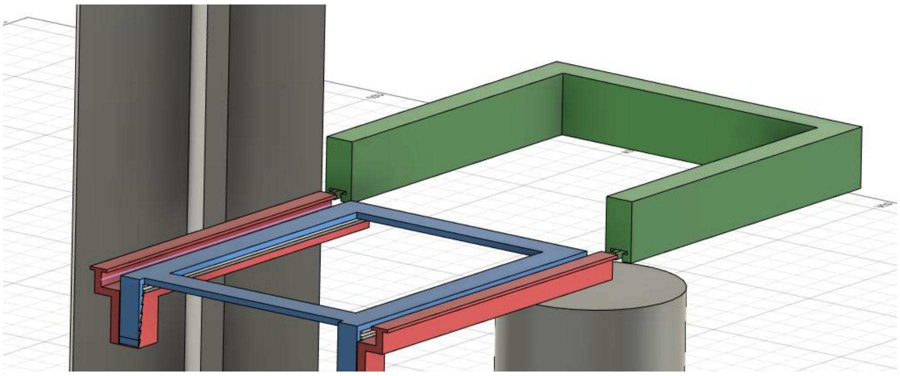

Figure 21: initial leaf clip design

4.2 Adding the functionality of the ASD

To be to house the ASD spectrometer plant probe, a clamp or holder will need to be designed. The two problems that are encountered are the weight of the probe and the unusual shape of the head of the probe. To cope with the weight of the probe, two anchor points are used to mount the probe to the X-frame. One clamp will be attached to the handle of the probe and a place to let the probe head rest on above the leaf will need to be designed.

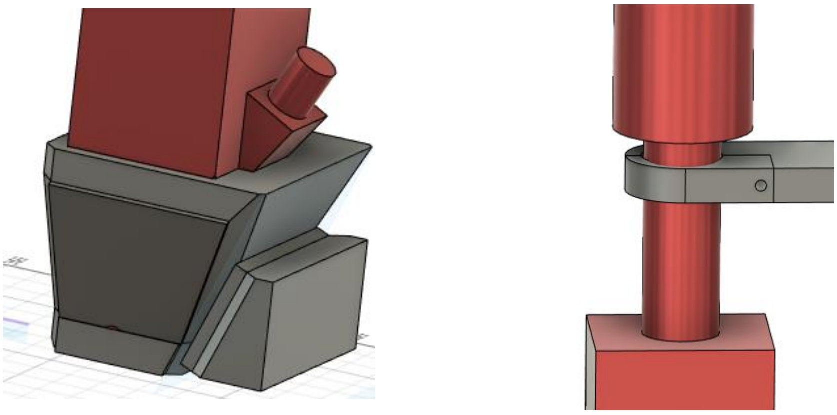

Figure 22:initial ASD head rest and holder

Another aspect to keep in mind when working with the ASD probe is that it needs a spectralon panel (Figure 7) as a reference material in between measurements. This can be done in 2 ways:

- Moving the probe towards the spectralon panel
- Moving the spectralon panel to the probe

In the early stages of the design phase, it seems more logical to move the spectralon panel towards the probe because it would be more time consuming to unscrew the probe and moving it than moving the small panel towards the probe. Also, if the probe is removed, it might not be aligned perfectly with its last location above the leaf. This could potentially result in discrepancies between the measurements. The first design iteration will therefore have space under the probe and above the leaf to either place or slide the spectralon panel in front of the head of the probe.

4.3 Identifying the necessary parts

After brainstorming and explorative sketches, several parts have been identified that will be necessary to accommodate all the user needs.

The first and foremost part is the leaf clip. This will be the part that holds the leaf that will be observed through the microscope. For this part, it is essential that the force exerted onto the leaf does not exceed a yet unknown number that will damage the leaf or cut off its water supply. To improve the user-friendliness of the final product as supposed to the original leaf clip design and as already described earlier, the clipping procedure should be able to be done in a comfortable body position and without the need of extra tools.

The next two parts that are identified are the parts that hold this leaf clip above the microscope and the part that connects to the X-frame of the setup. Ideally, there are no extra parts or tools needed to mount everything together. This will speed up the mounting process and also slightly increases user-friendliness.

In the process of examining the problem analysis and explorative sketching, it became clear that adding the functionality of the ASD requires a minimum of two parts. One part to let the ASD head rest on and one part needs to be designed as a second anchor point to accommodate the weight of the ASD. Both these parts might also need some kind of connection to the X-frame or the part that holds the leaf clip.

4.4 Materials

As described in the methods & techniques section, it is essential to explore the 3d printing process upfront to eliminate potential failed prints or useless prototypes. After brainstorming on the use of 3d printing filaments, a number of materials that could be useful, were found. PLA, PETG, ABS and TPE are the four materials that would be of use in the specification and realisation phase. These materials are selected based on a few characteristics. PLA, PETG and ABS are tough materials, while TPE is a flexible, rubbery material. TPE could be used for example, as soft material in the leaf clip so the leaf won’t be damaged as much as it would be with a tougher material. It could also be used as a connection piece if one of the parts needs a more flexible connection.

PLA, PETG and ABS are strong materials that are also easily accessible and cheap. This will make them useful when making rapid prototypes. A big part of 3d printing involves finding the right tolerances. Tolerances are the space between two objects. This can be between two 3d printed objects, but also between a 3d printed part and a non 3d printed part. Tolerances can be higher when two parts are moving or lower when parts need be fixed. These tolerances deviate between materials, printers, printer settings and even environmental factors could be at play (shrinking and expanding objects). Therefore, it is wise to start the rapid prototyping with an easily accessible and cheaper material. A more detailed choice of materials can be made during the specification phase.

Chapter 5 – Specification

This section is about specifying the design. The previous explorative designs will be evaluated and the designs are improved. Another look is taken at the problem analysis and the user's needs. How will everything come together? What is the right material for the application? Does this new solution work? and does it fit the requirements? Those are the questions that will be answered in this chapter.

5.1 Rapid prototyping

In order to test multiple iterations, without losing too much manufacturing time, the rapid prototyping technique is used. 3d printing is a great tool for this since 3d printers are on hand and can print while the user is doing something else. There is no need for paper prototyping or woodworking to make proper prototypes. Several ideas can be printed in the same print which makes it possible to compare the designs more easily.

The material that is used for this rapid prototyping is PETG. This material is on hand and has very good print adhesion. With a better print adhesion, there is less chance of print failure and therefore more iteration can be printed in less time, compared to other materials that are prone to failure.

5.2 Specifying the requirements for the leaf clip

The initial sketch for the leaf clip that was introduced in the ideation phase does not work. This is due to the fact that the teeth offset is too big for the thickness of the leaf. The idea was that the teeth allow the user to select different amounts of clamping pressure. However, a leaf is so thin that only the last tooth would be of use. This idea is off the table, so a new design is made using magnets. This new design is shown below in Figure 23. This design was tested and it was clear that the actual clipping of the leaf went great. The requirement of keeping optimal growth conditions is kept by keeping the design open for light and air around the leaf.

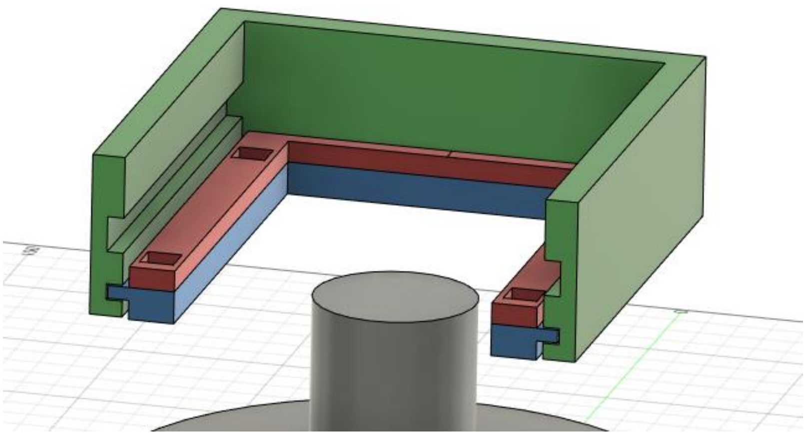

Figure 23: second leaf clip iteration with magnets. held in place in the leaf clip holder(green)

A design requirement was overlooked in the process of making this new clip design. This new design can only clip leaves that fit in the green part, since the rail is in the inside of it. As described in chapter 2, the clip needs to able to hold banana, corn and cucumber plants, which are far bigger than this. For the clip to be able to hold bigger leaves, the rail needs to be on the bottom. Figure 24 shows the final iteration of the rails. By moving the rails to the bottom of the yellow part, bigger leaves are able to be observed above the microscope.

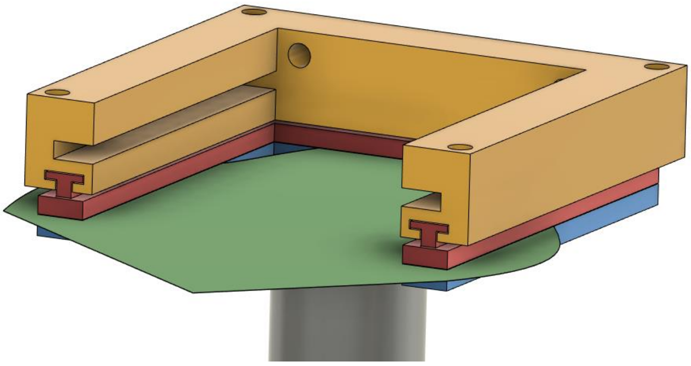

Figure 24: Last iteration of the leaf clip rail which can hold larger leaves

The next thing that was tested several times was the configuration of the magnets. The following configurations were tested:

1. Magnet on magnet
2. Magnet on magnet with separation layer
3. Magnet on metal
4. Magnet on metal with separation layer

Configurations 1 and 2 were too strong, meaning they would either destroy the leaf or make the clipping procedure uncomfortable. configuration 4 was too weak; the bottom clip would fall off with the slightest movement. From this point onwards, configuration 3 is used in every iteration of the leaf clip.

the final version that will be used during the user tests is shown in figure 25. The user test will be done with the Arabidopsis thaliana plant. This is the plant that is available and to not waste the opportunity to test the user-friendliness, a new clip is designed specifically for this test. The leaves of the Arabidopsis plant are very small and therefore the gap is made smaller.

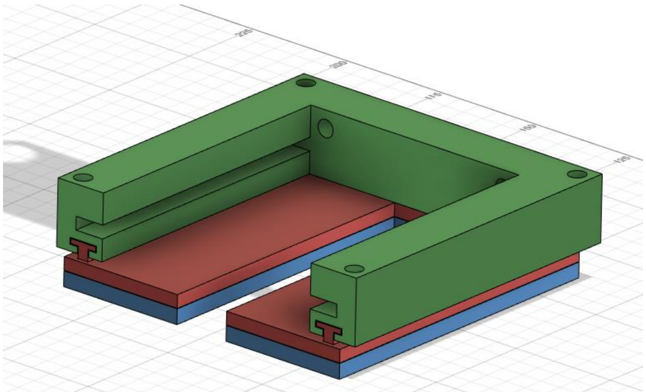

Figure 25: Leaf clip used for the first user test with small Arabidopsis leaves

5.3 Specifying the ASD spectrometer integration

To be able to slide in the spectralon panels, a cutout is modelled in the leaf clip holder. The spectralon panels will be able to slide in under the ASD spectrometer.

The initial idea was to cut out the head of the ASD from a solid block that had the same shape of the ASD but slightly bigger. The hypothesis was that this would create a holder for the ASD that fits perfectly above the leaf. The ASD in all the 3d models is an estimation of the shape based on measurements of the probe, since the actual shape is nearly impossible to model in Fusion360. In Chapter 3 Methods & Techniques, photogrammetry was explained. This method would give the correct dimensions of the ASD in a 3d model; however, this method was not precise enough. This idea is therefore discarded. In figure 26, a new design for the ASD head rest is shown. This design has a ring on which the ASD head can rest and four legs that keep the ring raised above the leaf. The legs have holes that are used to screw this part to the leaf clip holder (the green part). One of the problems mentioned in the problem analysis was the temperature of the ASD head. The light that shines out of the ASD head is produced by a Halogen lamp. This type of lamp can produce significant amounts of heat. A simple test was conducted to see if this would be an issue. The ASD was turned on warmed up for 15 minutes to reach its peak temperature. The glass temperature of PETG is around 80°C. The glass temperature is the temperature after which the material starts do deform. After the warmup time, the ASD did not reach this temperature and therefore the parts passed the test.

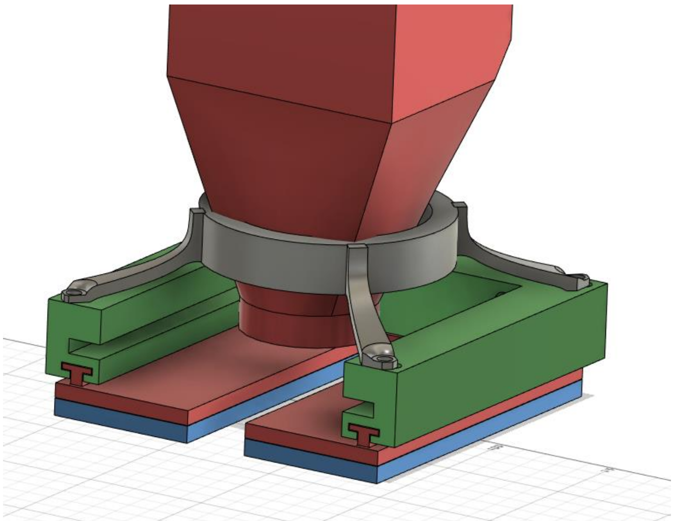

Figure 26: New ASD head rest design

The initial idea for the ASD holder that connects to the handle fits well and works as intended, no further changes are made to this design. The entire holder is shown in figure 27. The holder is designed in three pieces. The part that connects to the ASD handle consists of a small detachable clip that can be connected to the long part with a screw. This then connects to a sleeve that fits over the X-frame.

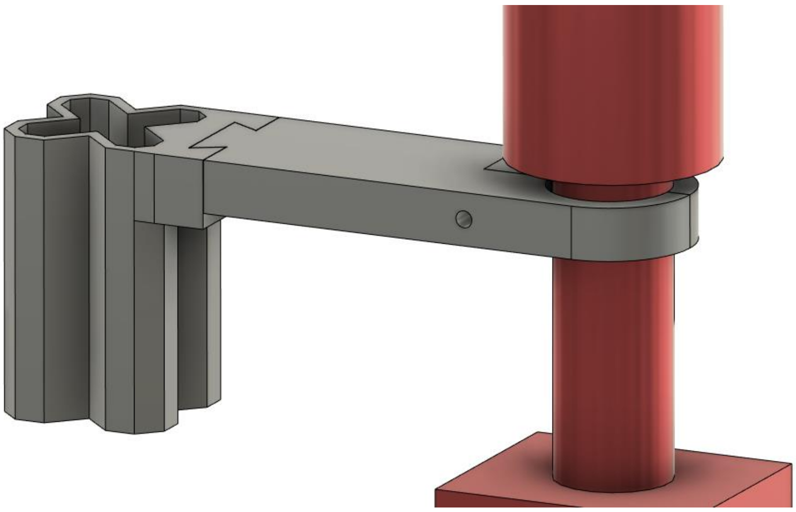

Figure 27: part that connects the top of the ASD to the X-frame

5.4 Connection of the leaf clip to the frame

To keep everything steady and centred above the microscope, another sleeve is designed that connects to the leaf clip holder. This part is shown in figure 28. This part has holes cut out, through which screws can be inserted that hold the parts together.

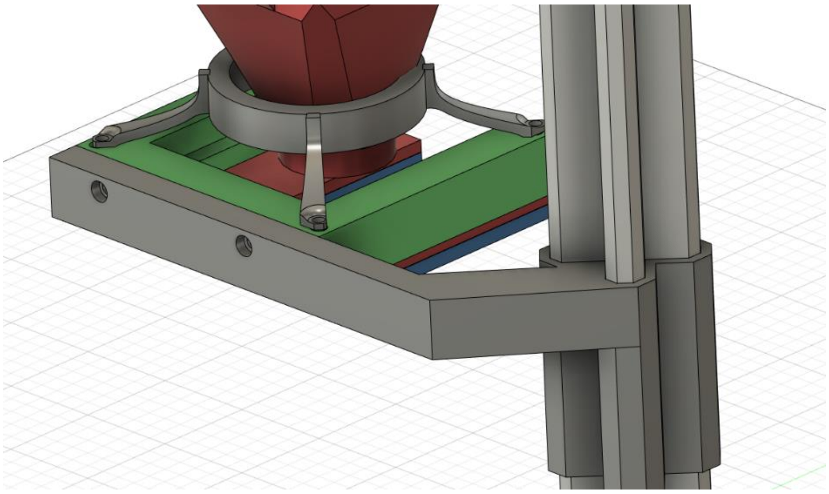

Figure 28: part that connects the leaf clip holder to the X-frame

Most of the parts can be screwed onto each other. This has several reasons behind it. The first reason is that this design choice makes sure that there are minimal parts and tools needed to connect everything to the existing setup. Only two sleeves are needed that connect everything together and these can be 3d printed. Furthermore, all parts can be separated for easy replacement and or stowing away during transport. The result of this design is a clip that can be used in a more natural body position as opposed to the current leaf holder and this clip has easy to manufacture parts.

Chapter 6 – Realisation part 1

This chapter focusses on building all the components in the design. This includes the details in the 3d printing process, the used materials and making all the components ready for use. Chapter 7 – evaluation will evaluate this design and then there will be a small realisation part 2 that focusses on incorporating the feedback from the user evaluation.

6.1 3d printing materials

The 3d printing materials that are used to make all the components are 1.75mm black PETG and 1.75mm clear PETG. This material is used because of the durability and the ease at which it prints. Two printers were available to print all the components, an SLA resin printer and an FDM printer. More information on these printer types can be found in chapter 3 – methods & techniques. The FDM printer was nearby and easily accessible and besides that, more prior knowledge was available on this type of printer. The only advantage of the SLA resin printer would have been an increased print quality of 15 microns, but the parts in this design do not require this amount of detail.

The FDM printer that was used is the Prusa MK3s with a smooth PEI print bed. All parts were sliced in the PrusaSlicer with almost the same settings. The only difference between some of the parts is the layer height. The printing temperature was set to 230 °C and the print bed temperature was set to 85 °C.

6.2 Non 3d printed materials

The first non 3d printed parts in this design are the magnets and metal plates in the leaf clip. These parts are glued in place in the leaf clip as shown below, in figure 29. The magnets are circular neodynium magnets with a 5mm diameter and are 2 mm thick. The metal plates are 6x12mm and are 0.4mm thick.

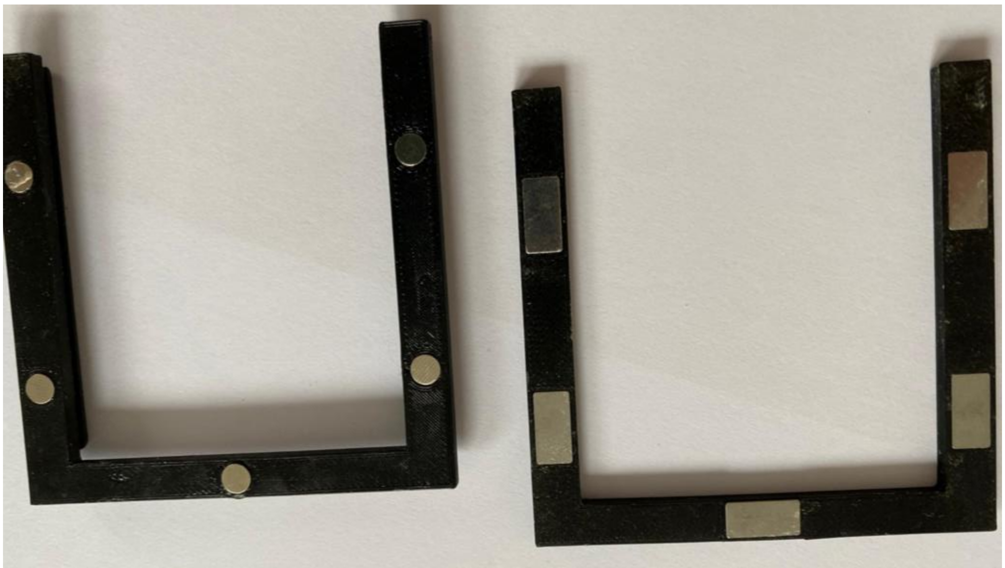

Figure 29: magnets and metal plates glued onto the leaf clip

Threads are needed in some of the parts so they can be connected to each other. There are two ways in which threads can be made in 3d printed parts. The first way is to 3d model them in the parts and print them. This comes with some disadvantages; for a thread to be properly 3d printed on an FDM printer, they need to be printed upright or else the threads will bend inwards resulting in a undesireable hole diameter. Since the parts will need threads in the vertical and horizontal direction, 3d printing the threads is not possible. Therefore the second way of making threads in 3d printed parts was used, threaded brass inserts. The inserts that are used for this projects are 3mm inserts by Ruthex, as shown in figure30. These brass inserts can be melted into the 3d printed parts by using a small tipped soldering iron. By applying heat for a few seconds, the brass inserts melts into the pre modeled hole. The advantages of this method are increased torque and pull out resistance on the screws and a more solid connection. The disadvantage is that these inserts cost money, when the 3d printed threads could be made for free. The screws that are used in the final prototype are standard M3 screws.

Figure 30: Ruthex M3 threaded brass inserts

6.3 3d printing the parts

All the parts except for the leaf clip, are printed with 0.3mm layer height. There is no need to add more detail and is saves 30% on print times. In figure 31, the slicer file is shown and the final result of those parts connected to a sample piece of the X-frame. The orientation of the parts on the print bed is chosen to minimise the need of support material. The ASD bracket was printed in this orientation for added strength to the fragile legs.

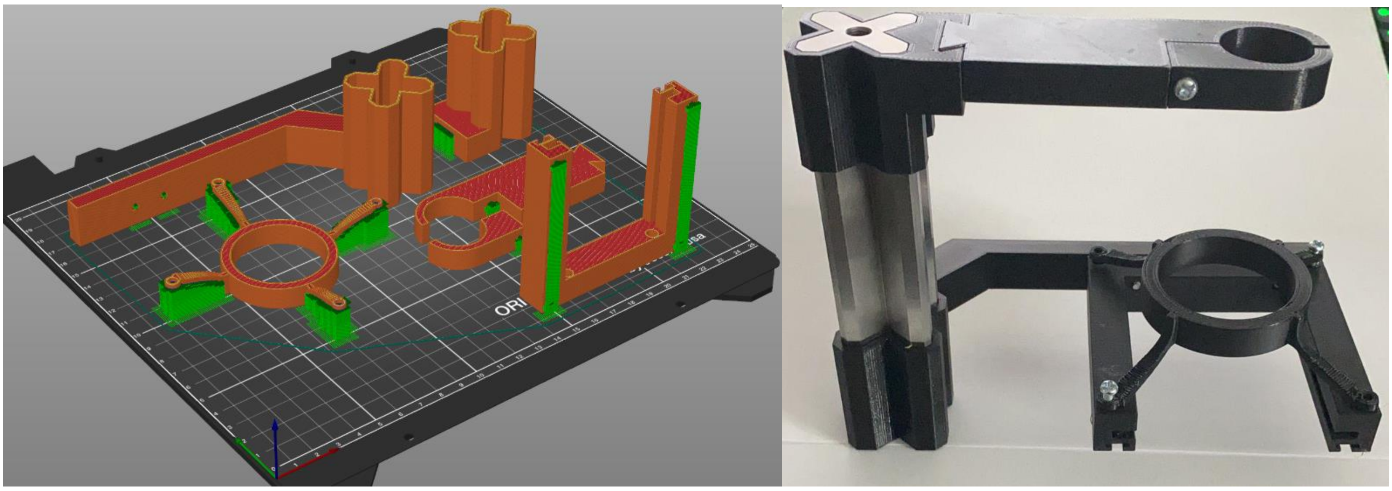

Figure 31: parts in the slicing software and the finished parts

The leaf clip is printed with a 0.2mm layer height. This is done because the metal plate that fits in the rectangular cut out is 0.4mm thick. To make the metal plate flush with the top of the clip, a 0.4mm deep cut is made, which can easily be printed with 2 layers at 0.2mm layer height. If the printer was set to 0.3mm layer height, like the other parts, the hole would be too deep or not deep enough.

Printing the rails on the leaf clip was tough since the rails needed support material. This extra material could not be fully removed, which resulted in the rail not sliding in properly. A new design was

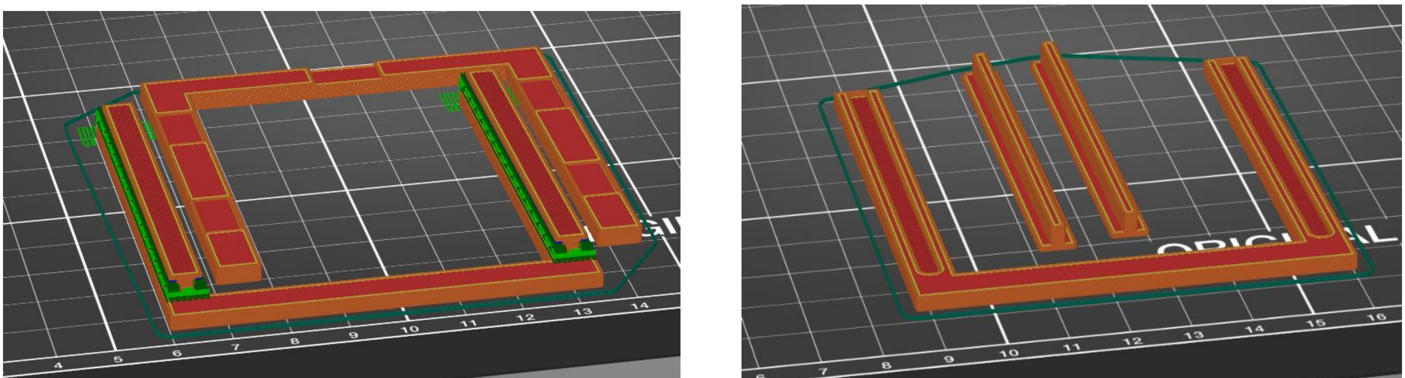

Figure 32: rails with support material (left) and seperately printed rails (right)

made to tackle this problem. The rails were printed separately and late glued in place on the clip. Both designs can be seen in figure 32.

Chapter 7 – Evaluation

A user test has been conducted to test the user-friendliness of the newly designed leaf holder. A separate test has been conducted on the ASD bracket, since both machines could not be in the same place at the same time during the tests. An ethical checklist needs to be filed with the ethics committee of the university prior to the user test. This ethical checklist can be found in appendix A. The plan for the user test can be found in appendix B.

7.1 User test

The user test is conducted with 3 participants. All 3 participants had prior knowledge of the experimental setup and has used the current leaf clip prior to the user test. Each participant started by clipping a leaf in the current leaf holder as a zero measurement. The time it took for the participant to clip the leaf was noted. This is later compared to the clipping times of the new clip to see if there is an improvement. Each clipping procedure was repeated 2 times to be able to get an average time and to observe the learning curve of the new clip. After using the new clip, the participants were asked how they perceived the physical strain on the body and on the mind. The answers to these questions indicate the difficulty of using the clip. Furthermore, the participants were given a chance to give some final remarks about the newly designed leaf clip

7.2 Results

Participant 1 had zero measurements of 18.7s and 14.1s. The clipping procedure with the new leaf clip took 16.4s and 15.1s. This participant noted that the new clip has about the same cognitive load as the current leaf clip but perceived less physical strain while using the new clip. A final remark was that the new clip would be easier to use with bigger leaves.

Participant 2 had zero measurements of 24.6s and 19.4s. The clipping procedure with the new leaf clip took 11.9s and 9.8s. This participant noted that the use of the new leaf clip is less intensive as the current leaf clip and has about the same difficulty of understanding as the current leaf clip. As a final remark, this participant noted that the new clip work smooth and is easy to use.

Participant 3 had zero measurements of 22.5s and 9.2s. The clipping procedure with the new leaf clip took 56s and 8.1s. The participant perceived slightly less physical strain compared to the current leaf clip and noted that the new design was easier to understand. As a final remark, this participant noted that the open end of the design made it harder to clip the leaf and that it works better with bigger leaves.

7.3 Conclusion

The clipping times were on average faster with the new design when compared to the current design. There is one outlier of 56s, but during this measurement, the participant made a mistake and had to start over. The perceived physical strain on the body was either the same or better while using the new clip and the new clip was not harder to understand. This shows that there is an improvement with the new clip compared to the current clip. A learning curve can be seen, which could potentially decrease the clipping times with the new clip even further.

7.4 ASD fitting test

The ASD bracket was tested with the ASD plant probe to see if the bracket was able to hold the ASD correctly. The test showed that the ASD was being held steadily and centered. The distance towards the leaf was not correct. This distance was 28mm and this needs to be reduced by at least 14mm.

7.5 Changes based on feedback – realisation part 2

With the feedback from the user test and the ASD test, a new, final design was made and 3d printed. The changes to the leaf clip include a closed end, a cut out for the nerve of the leaf and two bars that can hold smaller leaves in the middle, but room for extension to the outside for bigger leaves. The material was changed to clear PETG so more light can reach the leaf surface. The final iteration of the leaf clip is shown in figure 33.

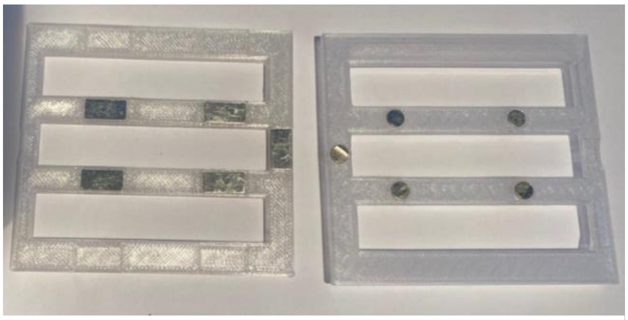

Figure 33: final iteration of the leaf clip

The ASD bracket was completely redesigned to lower the ASD head by 14mm. The ring diameter is not changed. Furthermore, the leaf clip holder is now also printed in clear PETG, to allow for more light at the leaf surface. Figure 34 shows the final prototype with all changes.

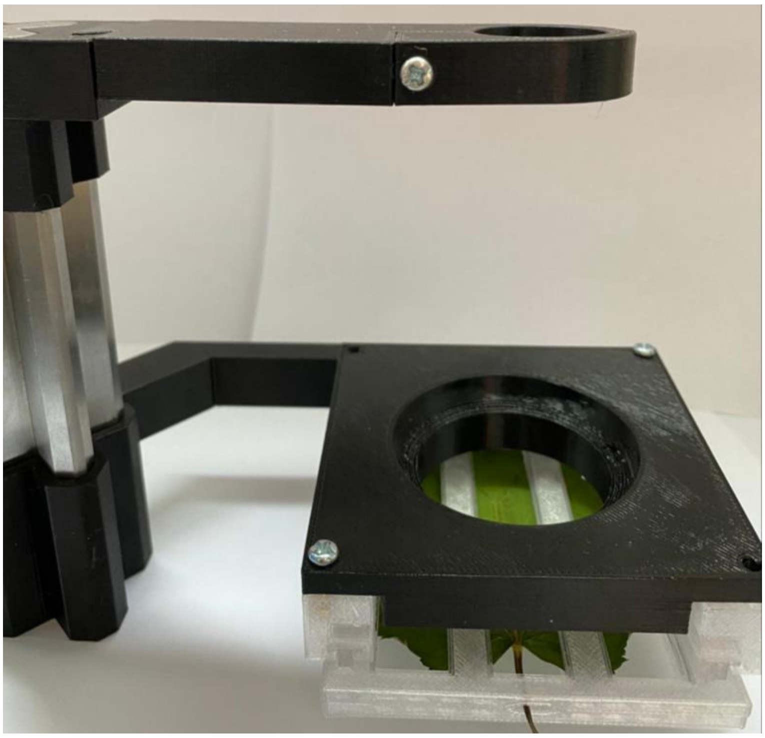

Figure 34: final prototype after feedback from testing

Chapter 8 – Conclusion

The research question was:

How can a leaf be held such that a microscope and an ASD spectrometer can both be used simultaneously while keeping optimal conditions regarding sunlight, water and air?

The first iteration from the ideation phase was already based around keeping optimal growth conditions. The design was open on both sides of the leaf, allowing light to reach the surface and allowing air to flow freely around the leaf. Several iterations were made to fit all the design requirements in one leaf clip. The final clip is able to hold different sized leaves and keeps the optimal growth conditions, while being more user-friendly. The user test showed that slightly faster, but could become even faster after using it for a while. Being able to clip the leaf in a more comfortable body position adds to the user-friendliness. An added benefit of this design is that all parts are easily replaceable and are easily assembled and disassembled without the need for extra tools.

Both the microscope and the ASD can be used by using a set of holder that keep the ASD above the leaf and the leaf above the microscope. This way, both machines that do measurements on the same leaf and at approximately the same spot. The weight of the ASD was coped with by using two anchor points; one anchor points was made near the handle and a second was made as a rest for the ASD head. The conclusion is that the product that was designed during this graduation project is the answer to the research question.

Chapter 9 – Future Work

The user evaluation is conducted with 3 people and only a few clipping times were measured. To fully grasp the added benefit of the new leaf clip, more tests need to be done with more participants. This could also lead to more feedback which could improve the design even further. It satisfies the requirements, but is it the best possible solution?

There are still some unknowns that need further research. First of all, the ASD spectrometer was not available for live measurements so the working of the machine is not tested. The fit is tested and looks promising but there is no certainty that it works. Furthermore, the ASD needs to be in contact with the leaf for a measurement or it needs to be in close proximity, given that it measures in a dark room. This leads to the next option for future research. Different configurations of materials can be tested to see if the black or clear PETG influences the measurements of the ASD. Will the material of the ASD bracket influence the measurement? Will the material of the leaf clip holder influence the measurement? These are question that require further research.

Another experiment can be conducted on the light that is able to pass through the bottom of the leaf clip to the surface of the leaf. How much light is able to pass through the clear PETG? Another interesting approach would be to look at the infill patterns in the 3d printed leaf clip. The Roster infill pattern has more open gaps than the gyroid infill pattern. Is there a difference in the amount of light that reaches the surface of the leaf?

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Appendices

Appendix A: ethical assessment form

1. General

1. Title: Designing a leaf clip for real-time microscopy
2. Context: BSc thesis
3. Researcher(s): Lars Hartogsveld
4. Supervisor: Cora Salm
5. Other people involved: Tom van den Berg (Critical Observer)
6. Department responsible for the research: EEMCS/IDS
7. Location where research will be conducted: University of Wageningen, Unifarm
8. Short description of the project:
    - This project aims to build a leaf holder that holds a leaf between a microscope and a spectrometer. This leaf holder will be used in an experimental setup that measures the stomatal behaviour of plants. With the gathered data, a relation could be established between the stomatal behaviour and the reflectance properties of the leaf. This could give insights in the plants' health, which could in turn optimize plant growth
9. Expected duration of the project and research period: February 1st 2021 – July 2nd 2021: 6 months
10. Number of experimental participants: 3
11. EC member of the department: Cora Salm

2. Questions about the research

1. Has this research or similar research by the department been previously submitted to the EC?
    1. No, but a second student is independently also conducting this research at the same time

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 members are from the University of Twente or Wageningen University.

4. Name all characteristics participants must possess in order to be included in the research, such as gender, age, membership of a specific organization, etc:

a. the only requirement for a participant to enter is to have previous experience with the microscopy setup.

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 of the research for certain participants?

a. No, no personal information will be gathered, only the performance and opinions of participants during user tests.

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?

a. Yes

b. This project is focused on designing a leaf clip for real-time microscopy. The current setup makes use of a clip that makes use of small magnets and a metal ring. This is an unoptimized method and will be improved during this project. a new clip is designed and 3D printed in order to find out whether the new clip is better than the current one.

> The participant is asked to place a leaf in the current leaf holder and in the new leaf holder. The time it takes to do these procedures are noted. After each procedure, the participant is asked to answer 2 questions regarding the difficulty of the procedure

The data gathered in this questionnaire and user test 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 answers will anonymously be used in the report. 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:
l.e.hartogsveld@student.utwente.nl

9. Are the requirements with regard to anonymity and privacy satisfied as stipulated in (§3.8)?
a. Yes

10. If any deception should take place, does the procedure comply with the general terms and conditions?
a. 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?
a. No

12. Give a detailed description of the research. Ensure all data relevant for an ethics consideration is given or, if necessary, attach the research protocol.
a. Test on user experience, the user will be asked to attach a leaf in the clip. This task will be timed in order to find out if this clip is more user friendly and less difficult in use than the current clip.

3. Why is your work COVID-19 proof?

13. Do you add additional face-to-face contact?
a. Yes, the observer and the participant are in the same room, to time the procedure and to answer any questions. This can all be done at 1.5m distance
14. Do you add indirect physical contact? For instance, sharing a tangible device, please explain why and what actions will be done with the device.

. The leaf holders that are tested will be touched by multiple people, but these can be cleaned after each use.

15. Do you put additional burden on people from the care sector that are under pressure?

a. No, the participants are not from the medical field. The participants work for the Plantenna project.

16. 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.

. The observer and participant can be at 1.5m distance throughout the whole user test and all objects can be cleaned in between measurements to ensure that it is covid19-proof

Appendix B: plan for experimental research study

Plan for Experimental Research Study

Evaluation plans

Introduction

The problem in the earlier phases of this graduation project revealed that the current leaf holder has some flaws regarding the usability. The placement of the metal ring and the fact that the magnets are hard to reach, makes the process of clipping a leaf rather difficult and time consuming. The newly designed leaf holder is focussed on bringing the clipping of the leaf outside of the measuring setup to remove physical strain on the body. Furthermore, the new leaf holder has the ability to house an ASD spectrometer, together with the microscope.

Research questions

In order to test the validity of the newly designed leaf holder, a research question and its sub-research questions will need to be answered. These questions are as following:

RQ: to what extent is the new leaf holder better in terms of usability?

Sub1: What are elements to still improve on?

Sub2: How does the new leaf holder influence the pace of the setup i.e all the steps needed before a measurement?

Participants

Ideally the participants consist of regular users of the experimental setup as described in the introduction of this report (XX). The participants will be recruited through the critical observer of this graduation project, Tom van den Berg. Tom uses the experimental setup at Wageningen University with master students and thus knows who uses the setup regularly. There are not many possible participants. However, this user test does not need more than 2-3 participants.

Materials

The materials that will be used during this test include the newly designed leaf holder, a device that can be used as a stopwatch, the experimental setup in Wageningen and a plant that can be used for a measurement. The participant will be standing close to the setup during the test and the observer will be standing somewhere with a visual on the setup and the actions of the participant. The positions are subject to change depending on the available space at Wageningen University.

Experimental design

The independent variable in this experiment will be the clipping of a leaf into the experimental setup. The Dependent variable will be the times and perceived difficulty of the clipping procedure. The experiment will be between-subject to reduce the learning effect. The participants are first asked to do a zero measurement with the current leaf holder. This way, the results from the newly designed leaf holder can be compared to the current leaf holder. The perceived difficulty is measured after each measurement using a set of questions. It consists of the physical strain on the body during the clipping procedure and the cognitive load.

The experimental setup has to be altered when the newly designed leaf holder is installed. For this reason, all participants have to do a zero measurement with the current leaf holder first. The Setup will then be changed to the new leaf holder, after which the participants can continue with the second measurement. The measurements are done in the order as indicated by the table below:

Participant 1	Zero measurement
Participant 2	Zero measurement
Participant 3	Zero measurement
--	Changing leaf holder
Participant 1	Timing the new leaf holder
Participant 2	Timing the new leaf holder
Participant 3	Timing the new leaf holder

Measures

Different aspects that will be measured are listed below. However, measuring performance based on only one clipping procedure is difficult. Results would be more accurate if the participants would do these tests multiple times on multiple occasions. A big part of learning a skill is repetition, which cannot be measured with one test. Therefore repeating the test of this chapter would benefit the research.

Clipping time (s)	average time it takes to complete one clipping procedure measured with a stopwatch in seconds
Perceived difficulty	The perceived difficulty will be measured by asking the following questions: How much physical strain did you experience, more or less than the old leaf holder? Is the clipping procedure with the new leaf holder easier to understand compared to the old leaf holder?

Furthermore, questions, facial expressions and spoken reactions will be noted to get a more complete picture of the participant's experience during the clipping procedure.

Analysis

The hypothesis of this user test is that the newly designed leaf holder will decrease the time and difficulty of the clipping procedure. To determine if this is the case, the clipping times and perceived difficulty questions are analysed. Lower clipping times and lower perceived difficulty measures are favorable.