Design of a leaf holder for real-time microscopy in the context of precision farming

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ABSTRACT

The study at hand provides a look into the world of microscopy in precision farming. A subject that focusses on improving crop yields, and thus with ever increasing necessity to the population as well as scientific community of the world. The goal is to design a leaf clip that will be mounted onto a microscope. The leaf needs to be preserved during the research and should therefore remain unaltered. The study discovers that light and airflow should be able to reach the leaf in ample amounts to accomplish this. Furthermore, prototyping towards the final design was done iteratively based on user tests, using a 3D resin printer. Accordingly, an optimised design with transparent materials and sufficient open space is chosen. The resulting product is largely functional but with some major flaws in form and structural integrity. A conclusion is drawn based on these works and recommendations are given.

Note: A short list of terms and abbreviations as used in the text is shown in figure 22 at the top of appendix B.

I. INTRODUCTION

1.1. Background Information

With more record high temperatures than ever being hit, and an increase in the amount of heat waves according to Perkins-Kirkpatrick & Lewis, droughts are an ever-growing threat for the future of agriculture [1]. As it becomes harder for farmers to grow their crops, food scarcity is becoming a serious problem, not only for third world countries. Within 28 years, in the year 2050, the global production could be down as much as 18% [2]. However, the global production of food is required to be approximately 50% higher by the same year if the global population is to be sustained [2]. This underlines the importance of techniques like crop breeding. Selectively picking crops has been happening since the domestication of plants and animals began several thousand years ago [3]. In the modern day, this technique has become a lot more refined and requires the use of microscopes and other advanced equipment to study the processes that are a part of photosynthesis in crops. This information can be used to assess the status of a plant and how it responds to different environmental factors, such as the amount of available water or nutrients. By monitoring plants more closely, it is possible to farm more precisely to the needs of the plants. This precision farming allows for optimisation of fertilisers, pesticides and water on the land and can be used to maximalise the efficiency of growing crops.

The objective of this paper is to develop a supporting item to make the use of a microscope setup measuring the photosynthesis of different crops as accurate as possible. The item that will have to be designed is used for clamping the leaf rigidly under the microscope, therefore preventing vibrations as these could ruin the images taken by the microscope. Several important design aspects were identified by the user alongside this initial goal and are underlined in the problem analysis in chapter II.

Van den Berg, the client that is being designed for, is active in the field of precision farming. The definition of precision farming as stated in the GP description is as follows: "*Precision farming is farming with input from smart data analytics of multisensory input, to use water, nutrients and pest-control measures more effectively.*" [4]. Research into this field is done through studying the gas exchange that happens in crops, which is a key driver of photosynthesis. This is done by taking images of the stomata of different crops, such as maize or Arabidopsis, and studying them for indicators such as size, density, and number. These stomata are so important as they form the means for a plant to affix carbon dioxide from the air, which is necessary in the process of photosynthesis as can be seen in figure 1. The processes shown here are how plants gain biomass, and therefore, they are key to understanding how plants react to differences in their environment.

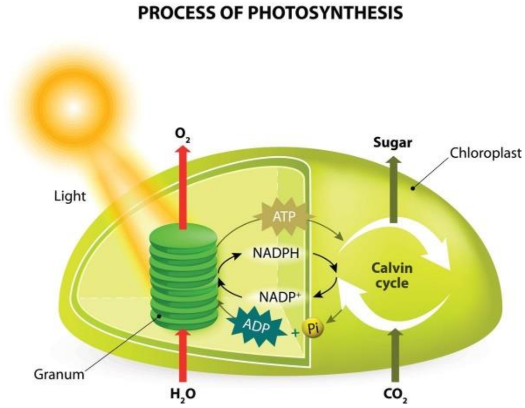

Figure 1: Schematic visualisation of photosynthesis [5].

In order to facilitate the intake of CO_2 and output of H_2O and O_2, the stomata of the leaf are necessary. This is because these are the way plants 'breathe', which is necessary in the process of photosynthesis, as is shown in figure 1. Therefore, it is key to this study to understand how these stomata, as well as the gas exchange work on a basic level. In figure 2, an overview is given of the gas exchange happening around the opening and closing of the stoma.

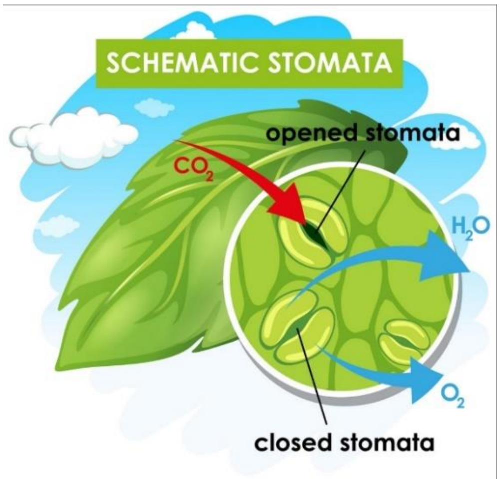

Figure 2: Schematic visualisation of the gas exchange in a leaf using stomata [6].

Next, to understand the basic workings of these stomata, it is important to zoom in a little bit on one of the stomas of the plant. The definition of a stoma is: "One of the minute pores in the epidermis of a leaf or stem through which gases and water vapor pass." These are what allow for the exchange of gasses with the air, making it possible to intake the necessary CO_2 when it is open. When closed, H_2O and O_2 can dissipate from the stoma. A more detailed view of both states in shown in figure 2, below.

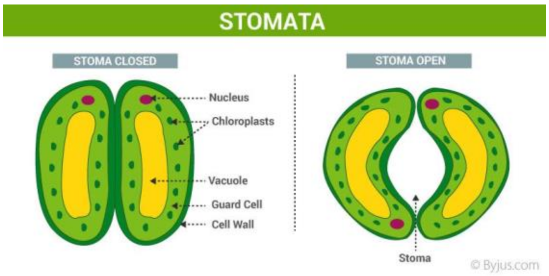

Figure 3: Schematic visualisation of the build-up of stomata [7].

Now that a basic understanding of the workings of photosynthesis and the stomata involved has been gathered, it is key to look at the design challenge at hand. To design the leaf clip, several design principles were formed. Based on these principles the problem statement was formulated as can be seen below. These criteria are based, mostly, on personal experience from researcher and expert van den Berg, as well as being the result of previous research done by Jacobs and Hartogsveld [8] [9].

II. PROBLEM STATEMENT

2.1. Problem Analysis

This paper focusses on identifying a design suited for the use in a microscope installation, for the use of studying stomata of different crops. As the setup has been revised from the previous generation of research, a new image presenting the current setup is provided for clarification in figure 4.

Figure 4: Image of the current microscope setup, provided by van den Berg. The black arrow points at one of the metal rods used for mounting. The leaf clamp will be mounted on the underside.

The current setup looks different and uses magnets embedded in a transparent Perspex plant with holes in it, to which the leaf is clamped with a metal ring. However, as this design is prone to damaging the plant or cutting of water flow, it is deemed suboptimal. Therefore, the challenge is to design a new solution that can be mounted to the current setup, shown in figure 4. This setup is mounted on different configurations of the metal rods that are visible in the image. This makes the construction very rigid, where multiple options for mounting can be identified. The setup is mounted on a tripod, such that it can be moved around freely in its entirety.

Based on this new setup and by talking with experts, that are actively working on the project, several criteria regarding the design were identified, as was also mentioned in the introduction. the design criteria for the leaf clip as issued by the client are listed below. These criteria include the need for:

- Stability of the leaf during the research to prevent blurry images from the microscope.
- Preservation of the leaf during the research, e.g., levels of photosynthesis and gas exchange in the leaf should remain stable and unaltered.
- Ease of installation of the leaf into the holder.
- The installation of certain sensors to accommodate the collection and monitoring of extra data during the research.
- The accommodation for leaves of multiple sizes, such that only one clip is needed for different sized leaves of various crops.

Based on these criteria the research questions were formed that are listed below.

2.2. Research questions

From these requirements the importance of stability for several different factors can be distinguished. The preliminary research this paper wishes to focus on, is the preservation of the leaf during use of the clip, and what the effects would be if fluctuations in the amount of light reaching the leaf would occur because of insufficient design. The resulting questions can be found below:

- What (existing) design best facilitates stability of the leaf clip, such that blurry images can be avoided as a result?
- What (existing) design best preserves a leaf while it is being clamped such that it remains in pristine condition?
- What (existing) design has the shortest time of installation for the leaf?
- How can the design best facilitate the addition of sensors for monitoring?
- What (existing) design best facilitates the installation of all different sizes of leaves such that the leaves are still intact after research?

Finally, the main research question is listed below:

- What design best facilitates all design criteria, such that optimal conditions can be guaranteed during research?

After the research questions had been identified it was key to investigate the existing solutions to identify important characteristics for the design. Many existing designs were already identified by Jacobs and Hartogsveld, thus their works have been used a source for finding relevant background materials as well [8] [9]. In the following part of this paper, these designs will be presented, and a look will be given into former research.

III. BACKGROUND RESEARCH

3.1. Former research

Former research that has been done into the exact topic has led to some interesting designs already. As last year two other students, now graduates, have already tackled the same problem, there is lots of former research to go through. This research was done by the aforementioned Jacobs and Hartogsveld, both of which presented a model for a leaf clip of their own. These clips, however, can be considered prototypes, and therefore the current paper will focus on the continuation of the design at hand.

Certain lessons can be learned from the previous projects thought, especially in the regard of carrying out the project. What is meant by this, is that the former students focused mostly on user satisfaction and things such as user completion times, etc. The client, however, indicated that this is not the final goal, and that the focus should be on the design and function of the product itself, placing user completion times second. This is mainly as studying the leaves in the research project generally takes multiple hours at least, as was indicated by van den Berg. This inadvertently means that it is key that the function of the product is guaranteed, as defects could lead to damaging the plant, and possibly also the research itself. This problem weighs much heavier in the evaluation of user satisfaction according to our expert and means that evaluation as is typically seen in the study of Creative Technology will not be sufficient for the project. Therefore, focus will lie on engineering a working product that obeys the chosen design criteria.

3.2. State of The Art

The state of the art consists of the designs currently available, as well as the results from the literature research that was performed. First, the literature research is talked through as it underlines the importance of one of the essential points of design criterium two: "Preservation of the leaf during the research, e.g., levels of photosynthesis and gas exchange in the leaf should remain stable and unaltered." Namely the importance of the stability of the factor light on the leaf. Literature research was performed on the influence of fluctuations in light, to evaluate whether the stated importance of this factor was justified. By quantifying the importance of one of the variables, and underlining why this is the case, it becomes possible to extend this line, also to other variables. As these are based on experience on the topic, from an expert. Thus, it becomes clear that all these criteria serve a righteous cause in the final design.

In the part 3.2.2 the existing leaf clips are listed and given slight evaluation. As to why not as much in-depth knowledge is given on these clips, it becomes clear that most of these existing designs were already broadly evaluated in both former papers by Hartogsveld and Jacobs. Therefore, focus is on the identification of useful traits that are of importance to fulfilling the design criteria. These parts or traits of the designs will then be presented in the ideation phase and an overview on the chosen solutions for prototype one will be given.

3.2.1 Literature research

The literature research was performed for the course of Academic Writing. The choice of topic was free and so a topic beneficial to the understanding of the graduation project was chosen. The study was on the effects of light fluctuations on certain key photosynthetic indicators. As stated in the introduction: "*The paper will explore the effects of light fluctuations on different processes in the plant leaves. The research will focus on highlighting the effects of a sudden change in the supply of light to a leaf. This is of major importance as one of the design aspects of the leaf clip is to make sure enough light can reach the leaf of the crop such that its g_s and A are not altered in a significant way. Therefore, it is key to understand the problems that may present themselves when light fluctuations occur.*"

These photosynthetic indicators are g_s, A, and ETR, and are defined as stomatal conductance, net photosynthetic rate, and electron transport (rate), respectively. Stomatal conductance can be seen as a measure of the gas exchange occurring in the leaf, thus giving direct insight into the activity of the stomata of a leaf [10]. Net photosynthetic rate is defined as “a gross measure of the rate at which a plant captures radiant energy and fixes it in organic carbon compounds” [11]. Lastly, Electron transport (rate) was taken as a measure of the average total electron flow around PSI and PSII [12].

The main question of the research was:

- Does a fluctuation in the amount of light on the leaf of a plant influence the indicators for active photosynthesis of the plant in a significant way?

And the sub-questions, based on the different indicators that were identified were:

- What values do the studies at hand present for the low light intensity and high light intensity of the fluctuating light?
- How do fluctuations in light intensity influence the stomatal conductance in leaves of different crops?
- How do fluctuations in light intensity influence the net photosynthetic rate in leaves of different crops?
- How do fluctuations in light intensity influence the average electron transport rate of PSI and PSII in leaves of different crops?

Following the PRISMA method, as illustrated in figure 5, a search was conducted on relevant literature. The database used for this purpose is called Scopus [13]. Scopus is a library that contains information on topics relevant to the research at hand, such as environmental sciences and engineering. Scopus also incorporates many features, such as great tools for filtering. This can help narrow down the initial search results into a manageable number of papers. As the database is mostly comprised of English literature, the search should yield many relevant articles, and translational errors will likely be avoided. Finally, the database is regarded as a source of high-quality literature and has many peer-reviewed papers. Therefore, the database can be regarded as an excellent choice for a source with many relevant papers [13].

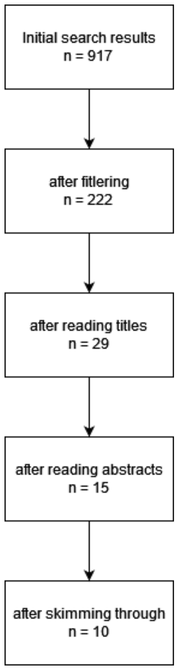

Figure 5: visualisation of the paper selection process [12].

This resulted in a total of ten papers, all of which were analysed to form a synthesis matrix, based on which the conclusion was drawn. To provide a clearer overview of the results gathered from constructing the synthesis matrix, a table with compacted results was made. These results can be seen in Figure 6 below. In here are the number of sources that reported a value, the value range encompassing the smallest and largest value in the array, and the average value for all results. As can be seen, there are quite significant outliers present in the sample. This is however not a problem as the different sources encompass research on different species of plants. This means that these values represent a wide variety of plants, which might feature large deviations in the average time it takes to, for example, reach 50% of the net photosynthetic rate after a change from LL to HL. The differences in plants that have been studied well represents the variety of different plants that will be researched using the clip that is being developed, and therefore these outliers in the sample were not omitted.

As was stated in the problem statement, the design of the leaf clip calls for several different criteria, one of which was:

- The accommodation for leaves of multiple sizes, such that only one clip is needed for different sized leaves of various crops.

Here, the criterium clearly states that the clip should facilitate many different leaf sizes, and thus many different plants. Therefore, these results are of value to the research and were, instead of leaving them out, incorporated into the averages seen below in figure 6.

	Number of sources (out of ten)	Value range (smallest and largest value)	Average value
LL definition (PPFD in $\mu mol*m^{-2}*s^{-1}$)	10	0 - 100	42.8
HL definition (PPFD in $\mu mol*m^{-2}*s^{-1}$)	10	420 - 2000	1290.2
P_{50g} (s)	5	50 - 1854	534.97
P_{90g} (s)	5	50 - 3036	1146.23
P_{50A} (s)	8	39.5 - 810	278.55
P_{90A} (s)	8	227.5 - 2070	776.36
P_{50ETR} (s)	4	56 - 560	260.19
P_{90ETR} (s)	4	107 - 1692	774.25

Figure 6: Simplified model of all variables of the synthesis matrix [12].

The full conclusion from the review was that the preconceived notion that fluctuations in the amount of light on the surface of a plant could significantly influence the indicators for active photosynthesis, such as g_s, A and ETR, seems to be true. It was observed that after six hundred seconds, or ten minutes, on average the values for all indicators would have changed by more than 50% of the maximum they would attain. After twelve hundred seconds, or twenty minutes, on average these values would likely already have climbed to 90% or more of their final value. As the microscopic research that this clip is intended for, can easily take multiple hours, obstructions that block light falling on the leaf should be avoided as much as possible. Therefore, it can be concluded that it is indeed key for the design to obey at least this initial criterium and thus sufficient thought needs to be given into this part of the design.

On the other side, it was also observed that small, very short fluctuations (<10s) do not cause dramatic disturbances of g_s, A or ETR, even in outliers. Also, since these disturbances are only relatively small, indicators will likely return to their normal value before continuing research, Thus, worries about small temporary obstructions of the amount of light on the leaf, such as during the installation of the leaf in the clip, are not justified when it comes to the design process.

For the design goal, the conclusion of this research means that the initial criteria for design must be held up and should be considered an important part of the work that needs to be done. The result is that proper time will need to be allocated to ensure ample light will reach the leaf, even when placed in a small holder. For this purpose, the use of transparent materials could be a possible solution, allowing light to permeate from all sides, ensuring optimum conditions for the leaf to reside in. Another possibility could be to build a construction that utilises a porous design, as to let light shine through the holes in the material of the clip.

3.2.2 Existing leaf clips

A study was done into the existence of already commercially available leaf clips, to improve part 3.2, the state of the art. Leaf clips that have already been identified by the two previous students are the:

- Walz Mini-PAM leaf clips [14]
- Walz Mini-PAM II leaf clips [14]
- Walz Dual-PAM-100 linear positioning system [14]
- Walz Micro-PAM standard leaf clip [14]
- Walz (standard) leaf clip [14]
- ASD Spectrometer plant probe [15]
- Hansatech FMS/PTL leaf clip [16]
- As well as several, non-commercial, custom designed leaf clips [8] [9]

Alongside these clips that were already identified there was another manufacturer of leaf clips that has been discovered. The name of this manufacturer is Li-Cor, which is a brand that has specialised itself in making portable sensors and leaf clips. These portable sensors exist in many forms, but generally have in-common that they are large apparatuses used for remote work in the field and in green houses [17].

Although the identified leaf clips are generally well optimised for different plants, there is the problem that not all plants will fit under most of these clips. The mounting will also be a problem, as these cannot mount to the metal construction that is used for the current setup of the microscope. Therefore, to identify the characteristics of these clips, the clips were grouped based on series, and an overview of the useful qualities of each clip was made. For this purpose, the PAM series leaf clips were grouped together, as these have similar identifying traits. Apart from separating the two different Walz clip series, they were sorted on brand. The identified qualities will then be used for the further iterative design process, that will lead to the final product. The table is presented in figure 7, below.

Brand or series	Useful traits
Walz PAM series	Some models are portable. Many integrated sensors for different measurements such as PAR and fluorescence. Expandable through accessories, such as lamps or other sensors. Provides own source of light, intensity and wavelength can often be set. Machine specific, so functionality is guaranteed.

	Use of spring-clamping to provide sufficient force.
Walz standard clip	Very simple to use. Clothes pin-style clamping mechanism. Fits many different probes.
ASD	Provides own (low intensity) source of light. Can be integrated with ASD leaf clip for single-handed functionality Machine specific, so functionality is guaranteed. Use of spring-clamping to provide sufficient force.
Hansatech	Fits many different probes (with minimal adjustments). Tripod design benefits stability during research Clothes pin-style clamping mechanism.
Li-Cor	Portable models for research in the field. Many integrated sensors for different measurements such as gas exchange and fluorescence. Some models can measure aquatic samples. Some models have controlled environments in which the leaves can be places, in here values for temperature, light, CO_2 etc. can be set. Use of spring-clamping to provide sufficient force. Use of foam rings to avoid damaging leaf.
Custom clips	Custom made to fit many different probes. Often use 3D printing, so can be modified if necessary. Use of clear acrylics to allow sunlight to pass through. Often makes use of magnet clamping.

Figure 7: List of useful identified traits of several brands and series of leaf clamps.

In figure 7 not all traits of a series or brand of products are listed. This is because it is most beneficial to the study at hand to note down neutral or positive attributes. This is the case as based on these designs and different expert opinions, will the first iteration of the design be implemented. Later on, different aspects of these designs might be tried out, but for now the ones that seem to address the initial design criteria the best were chosen to be tested in the first prototype.

As it is clear there are not any designs yet that will be able to fulfil the role of clamp for the current setup of the microscope, it seems obvious that no answer to the final question can be provided yet. Therefore, through the identification of useful traits for the first prototype will the process of iterative design be started. This process will be explained in further detail below in the methods and techniques that form part four of the study at hand.

IV. METHODS AND TECHNIQUES

4.1 Design process

The design in question is heavily based on the idea of an iterative design process. This is a process where several iterations of prototypes are used to identify which characteristics of the design work well, and which not. This process is often used in product design and is therefore an essential part of the Creative Technology curriculum. This means that the designer in question is familiar with similar processes and will be able to improve upon it through user-feedback. In this case the user is also the expert, van den Berg. Others that work on the project will also function as test users and might provide useful information as experts in the field.

The successfulness of iterative design can be amplified with many iterations and the collection of ample feedback. For this purpose, the design will be done, using 3D-printing, as it allows for the production of fast, low-cost prototypes that can be tested out quickly. Next, the use of 3D modelling and printing will be explained, and an overview of the possibilities will be given

4.2 3D Modelling

Using the 3D model of the microscope setup, the prototypes were designed. This was done in the program SolidWorks 2021, where the clamp was constructed. A link to all SolidWorks files can be found in appendix B. Here, the differences between several iterations can be seen and exact measurements can be found. After finishing the designs, the models were converted to STL files and put into Chitubox 64 for placement on the printing surface, adding of support, and finally slicing of the model. This file was then saved and put onto a USB stick, with which the files were transferred to the printer.

4.3 3D Printing

3D printing was done with the use of the 3D printing device that is present in the lab of the expert. The printing was done using a resin printer, where a basin of resin is lit up with UV light, such that it hardens out to become a new layer. This process is repeated until it forms a 3D print. The printer that was used for this purpose is the Phrozen Sonix Mini 4K 3D printer. It has a high resolution which benefits both the strength and form of the shape one wishes to print. It can print using several different materials (resins) among which are also clear, colourless UV resins.

Therefore, the initial material that has been chosen is the Liqcreate Clear Impact resin as this resin was left over from previous projects. It being clear will allow for the permeation of light onto the leaf, even on parts that would normally have been excluded from natural or supplied light. The importance of sustaining the levels of natural light is underlined in part 3.2.1, which encompasses the literature research. Therefore, the use of a clear resin for

3D printing was chosen to suit the relevant design criterium.

Further iterations, apart from the last one, were done with a different resin as there was only a limited amount of the Liqcreate available. Therefore, in the meantime Phrozen Aqua Ivory 4K resin was used. This resin features excellent adhesion and resolution, which allowed for easy printing in fine details for the intermittent prototypes.

Generally normal printer profiles for the respective resins were used, although (initial) exposure times were sometimes increased by up to twenty percent to compensate for bad adhesion to the plate. Apart from this the default profiles were left unchanged for other prints. In Chitubox anti-aliasing was enabled and set to 4 for all prints. All other settings were left at default values.

V. IDEATION

5.1 Design Criteria

The different design criteria as stated by the expert were already identified in part 2.1 of the current study. Here the different criteria were outlined and the importance of these was underlined. Based on these criteria the research questions were formed. Later in part 3.2, where the state of the art was presented, the importance of these criteria was stated again, as the conclusion to the literature review. The criteria have once again been provided below:

- Stability of the leaf during the research to prevent blurry images from the microscope.
- Preservation of the leaf during the research, e.g., levels of photosynthesis and gas exchange in the leaf should remain stable and unaltered.
- Ease of installation of the leaf into the holder.
- The installation of certain sensors to accommodate the collection and monitoring of extra data during the research.
- The accommodation for leaves of multiple sizes, such that only one clip is needed for different sized leaves of various crops.

These criteria form the basis of the first iteration of the design that will be done. This iteration will be based on the useful traits that have been identified in part 3.2.2 on the existing leaf clips and the ideas and feedback from the experts van den Berg and Sanders. The current ideas for the design will be presented in part 5.2, fulfilling the design criteria, below.

5.2 Fulfilling the design criteria

To fulfil the design criteria presented above it is of key importance to consider the time and cost involved with manufacturing a prototype. This is mostly the case for the first iterations for reasons provided above. Next, a list of the proposed identified solutions to address the design challenge will be presented and a short explanation for each choice will be given below. The chosen preliminary solutions to the design criteria are as follows:

- Mounting to the frame of the microscope setup, to provide stability to the clip and prevent the blurring of images during long exposure shots.
- Inclusion of inert foam rings on the surfaces where the clip meets the plant, to provide ample support and to avoid damaging the leaf.
- Use of see-through resin, to facilitate natural light to the plant, while it is clamped.
- Use of magnetic rings of different sizes, embedded with magnets that can snap to a backplate. These rings will be coated with the inert foam.
- Open top rings such that light can reach the top of the leaf, and gas exchange in the leaf is still possible.
- Easy quick-snap mechanism using magnets to provide ease of installation and removal.

The use of foam rings was inspired by the apparatuses from Li-Cor as these foam rings were identified to work well in protecting the leaf by expert van den Berg. These rings are provided by the expert as they are spare parts of currently owned Li-Cor models. Mounting to the frame will be done using two or three of the metal beams of the model, depending on the stability of each. This idea was discussed with expert Sanders and the least number of beams will be occupied as possible, depending on the outcome of the tests. The use of magnetic rings was inspired by expert van den Berg as well and is based on the current setup. Using these rings, the ease of installation is also immediately tackled, as magnetic rings inherently provide a quick snap mechanism to the final user. For this purpose, the magnetic rings will have to fit the backplate well and space will have to be allocated to magnets for different sizes of rings. These rings will have open tops as to provide air as well as light to the plant in question.

As this is the first iteration it can only be seen as a preliminary conclusion to the research question. For now, however, the current answer to the main research question will be used to create a first prototype and after running it through a user test on the effectiveness of the design will the prototype be revised, and the next iteration begin.

VI. REALISATION

6.1 Design of the model

The design of the model was done in SolidWorks 2021. This program was used to construct the backplates, rings and a holder for the PAM module that make up the design. The design was digitally made to fit the already existing 3D model of the microscope setup. As this setup was also being developed physically during the duration of this project it was possible to test out the prototypes and evaluate their usefulness in terms of the project.

A few tests were done with the help of van den Berg, who tested out the basic functionalities of the clamp and suggested improvements for the new design. Using this new information, further iterations could be made, allowing for improved functionality of the leaf clip. In total four prototypes were constructed before the design was finalised. These prototypes can be found below, as well as the evaluation process for each of them. Furthermore, different aspects of the design are highlighted in each part, explaining what the aim of the test was and how it improved the design.

6.2 Prototype I

The first prototype consists of a basic backplate and two rings. Its aim was to test out the magnetic quick-snap system that resulted from the ideation phase, as well as the feasibility of printing in clear resin. Something of lesser importance that was tested here was the stability of the mount using only two mounting points and whether this would work sufficiently.

Backplate

For this purpose, first, a backplate was designed that could hold four magnets for both the smaller and larger ring as can be seen in figure 8. It also features two holes on opposite sides of the plate to facilitate mounting on the metal beams of the setup. The magnets for the first prototype had been used in the lab before and were provided by expert Sanders. The holes for every design had 0.1 millimetres of extra wiggle room in every dimension, to allow for ease of installation. This extra 0.1 millimetres of room was incorporated into every dimension of every feature of the design to ensure everything would fit.

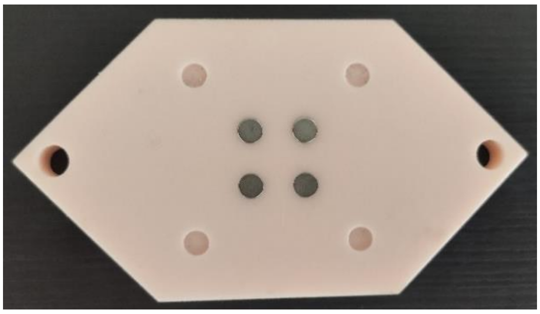

Figure 8: Backplate prototype 1, the holes in the middle are filled with the round magnets, whereas the outer holes were left empty. The hole in the middle is not present yet.

The two holes for mounting were designed with the size of the metal beams in mind. Thus, these were made to be 6 millimetres in diameter.

To allow for a rigid, non-deformable backplate a thickness of 7 millimetres total was chosen. This choice was based on a previous short test with the clear Liqcreate Clear Impact resin, with which a print of about 2 millimetres thick was made. This print was supposed to be thicker, but unfortunately did not stick to the printer surface and fell into the resin bath, resulting in the final thickness of about 2 millimetres. This thickness still had quite some flexibility to it, so just to be safe with the first prototypes a high thickness of seven millimetres was chosen.

Another choice that came from this test with the clear resin was that there was not much of it left, and for now, it seemed that there would be no problem printing with it, as long proper adhesion to the printing surface could be guaranteed. As there are multiple options to ensure this, such as sanding the printing surface, or having longer initial exposure times, to harden out the first layers of resin better, this was deemed an easy fix.

Furthermore, the problem regarding the limited amount of clear resin was tackled. It would have been possible to order some new resin, but since this would take a long time, a different resin would have to be used in the meantime. Thus, it was decided that another resin, namely the Phrozen Aqua ivory 4K, would be used as this was a leftover resin from another project. Because the transparency of the resin was of no doubt anymore, the choice was made to continue with this non-transparent resin for the other prototypes as well, and to do the final print with the Clear Impact resin.

Rings

Next, the rings as shown in figure 9 below, were designed with their placement on the backplate in mind. Here, a communication error resulted in the rings being one centimetre and four centimetres in diameter, for the small and big ring, respectively. This was based on the supposed sizes of the foam rings that were provided. These sizes turned out to differ slightly from the sizes of the rings, as they were industry-grade foam rings, used in existing leaf clamps from the company Li-cor. For the magnetic strength test, however, the difference in ring sizes from the supplied foam rings did not prove to be a problem. Therefore, testing was commenced, and sizes would be adjusted in the next prototype. During the test, the magnetic pull on the rings felt weak. Thus, it was also decided to incorporate stronger magnets into the next design and to test out which magnet strength would be optimal. Further information on the foam rings from Li-Cor can be found in appendix B.

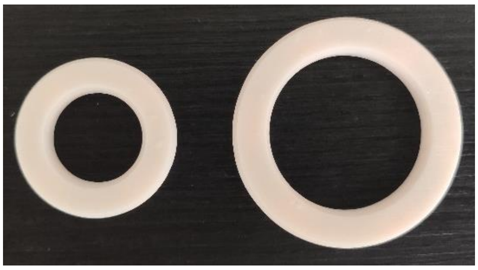

Figure 9: An overview of the top of both the small and large ring.

6.3 Prototype II

For the second prototype a few design changes were made based on feedback and tests. First, a few modifications were made to the backplate based on the information gained during the last iteration. These modifications included making a hole in the middle, inclusion of a raster at the top side of the hole and modifications to the magnet placement. Next, a test with magnets was performed to see which would suit the new design the best. Based on this choice the rings were modified and the hole sizes were adjusted to fit the new magnets. The rings were also adjusted in size to fit the supplied foam rings, as mentioned previously.

Backplate

A hole was added in the middle of the plate. This hole was always intended to be there, but since it was unclear if this design would be rigid enough with the chosen resin it was left out in the first prototype. This was partially because no real tests were to be performed with it yet. In this iteration, however, it was included, and a supporting raster was added on the topside of this hole, as can be seen in figure 10.

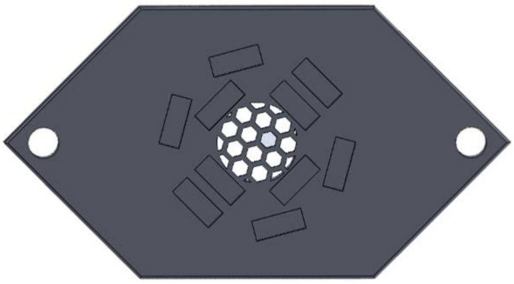

Figure 10: SolidWorks model of backplate prototype II. The original raster is visible in the middle.

This hexagonal raster was added to incorporate some support for the leaf after it would be clamped between the foam ring and the surface of the plate. This idea was quickly abandoned, however, as the raster was found to be obstructing the microscope, thus resulting in a useless image. Another reason for the removal of the raster was the idea of putting the foam rings on both sides of the leaf for better protection, which would make the raster obsolete. This was done to prevent squishing larger leaf veins to the hard backplate and damaging them. Hence, the raster was removed during the user test by drilling it out as can be seen in figure 11.

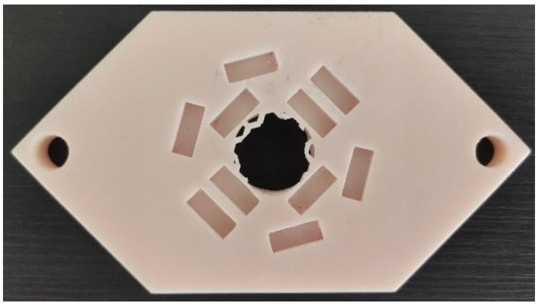

Figure 11: Backplate prototype II: With newly designed rectangular holes for larger, stronger magnets. The middle has been drilled out.

After it had been removed it was possible to get an image with the camera from the microscope and the extra foam was added for underside support of the leaf. This setup was left for approximately twenty-four hours and no defects of the leaf could be detected with the naked eye. Before and after can be seen in figure 12A (before) and 12B (after), where there was no difference to the naked eye, nor on camera. Therefore, the clamping mechanism of this prototype was deemed satisfactory.

Figure 12A and 12B: Approx. twenty-four-hour leaf clamp test. No new visible defects could be found on the leaf.

Rings

To see which strength would be optimal, three different strengths of magnets were ordered, each with a significant increase in carrying capacity from the original magnets. The original magnets had 350 grams of carrying capacity, whereas the new ones had 640, 900 and 1100 grams, respectively. A test was done where two magnets were put on opposing sides of a piece of the foam that was to be used. Then, based on the rigidness of the clamping and the amount of time the magnets could be left on before permanently deforming the foam a choice was made. The result was that after approximately six hours there were no visible signs of permanent deformation on the foam for any of the magnets. Logically, the strongest magnets were the firmest in place, which is why the choice was made for these magnets. The exact details of these magnets can be found in Appendix B.

After the magnets were chosen, the holes were adjusted for the size of these magnets and were also adjusted to fit the size of the foam rings, and thus the new printed rings. For extra rigidity of the larger ring, six holes instead of four were added to both the backplate and the ring in a hexagonal pattern. Changes to the rings can be seen in figure 13.

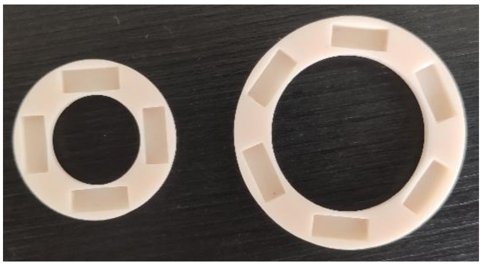

Figure 13: An overview of the bottom of both the small and large ring. The holes for the magnets have been adjusted to fit the larger ones.

To sum up, some of the improvements that were to be made in the following prototype were discussed. Among others, a thinner backplate, improved mounting mechanism, removal of the raster, optimised magnet placement and incorporation of more wiggle room were agreed upon.

6.4 Prototype III

In this iteration more modifications were made to the backplate, while the rings remained mostly untouched. Except for the addition of a new slit-ring, that was the size of the smaller ring, to incorporate a mounting possibility for blades of grass and other similarly shaped plant leaves. The backplate was improved by reducing its thickness and adding a chamfer to the underside of the hole. These additions were made to improve light penetration, as well as airflow in the final design. Furthermore, magnet placement was optimised to improve airflow around the leaf and mounting points were added for alternative mounting to the inside shafts of the setup. Lastly, an experiment was done to test the inclusion of screw-in nuts which would be used to tighten down the plate to the mounting poles. The amount of wiggle room was also adjusted for several areas to better fit the magnets and to make mounting go smoother.

Backplate

First, the thickness of the plate was reduced from seven to four millimetres, as the previous backplates had seemed excessive in size. Thus, the thickness was cut down to improve light penetration in the final design, and to allow for as little room between the lens and leaf as possible with this mount. This was important as certain high-resolution lenses have a relatively short working distance of 5.2 millimetres [18]. This distance would only be achieved by cutting down on the thickness and allowing the lens to sit close to the plate. Furthermore, the decrease in thickness would also result in a smaller number of layers in the sliced 3D model, thus resulting in less material use and shorter printing times. Rigidity of the plate did not seem to be affected by this change and no further problems were encountered.

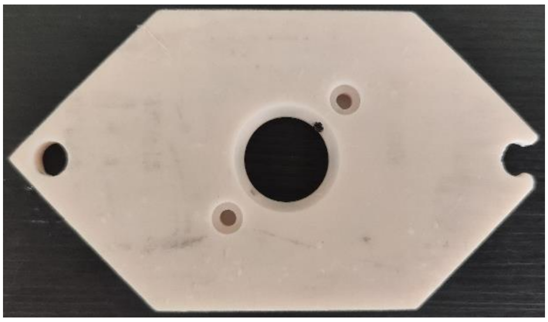

Figure 14: Backplate prototype III: Bottom, with new inner mounting holes and chamfered middle hole. One side broke off during mounting due to tight tolerances.

Additionally, the hole in the middle of the backplate now included a chamfer on the bottom side after it was deemed to optimise airflow around the leaf, as can be seen in figure 14 above. To further improve the airflow around the leaf on the topside, it was decided upon that the foam rings would, instead of being used whole, be cut into smaller pieces, only covering the magnets in the plate. Therefore, improving airflow around the leaf, and possibly waterflow inside the leaf, as fewer parts were actively being clamped down. The way the foam was arranged is visible in figure 17 of prototype IV. Incidentally, this also led to a change to the angle of the inner magnet ring, with respect to the outer one. The decision was made to rotate it by forty-five degrees, to form a centre groove allowing for leaves with larger veins to be compressed less while being studied, and to always ensure a flow of air on the underside of the leaf. These changes can be seen in figure 15.

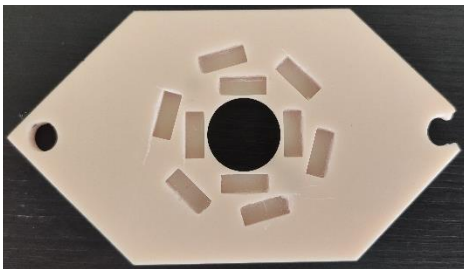

Figure 15: Backplate prototype III: Top, with adjusted positions for inner magnet ring.

Another problem that had to be tackled was the prototype hanging loosely from the mounting poles during the previous user test. The other components that are in the setup come from ThorLabs, a company that specialises in this type of equipment. These components used inbus screws to mount everything to the central metal poles holding it all together. Therefore, these were incorporated into the prototype as well, as it was a proven way of securely mounting to the setup.

Since this backplate is only four millimetres in thickness, the smallest available screws were used, which was M2. A hole was pre-drilled for these screws, several millimetres smaller than their two-millimetre diameter. Next, they were screwed into these holes, allowing them to thread themselves into the material. In a test it was revealed that this addressed the mounting problem well and the only problem here was with the decay of the screw thread over time. As the screws had been screwed directly into the backplate it was deemed of concern that after rigorous use the threading in the brittle resin would break, thus making the screws useless. Therefore, it was settled that a metal screw-in thread would have to be installed into the plate in the next iteration. This would solve the problem of wear on the threading, but meant the plate had to be thicker around the mount holes. Thus, the new design would opt to increase thickness in certain parts, while decreasing it as much as possible overall.

Rings

The rings remained mostly unchanged for this prototype. The only major change that was applied here is the integration of a new type of ring. The existing prototype rings were donut-shaped, making it possible to clamp multiple sizes of leaves onto the backplate while obstructing as little contact with the light and air as possible. For smaller blades of grass, that are sometimes studied by the client as well, it is not ideal to use these types of rings though. This is mostly because of the long and thin shape of grass and leaves like it. Therefore, to prevent the blades from moving in the existing rings another type of ring was introduced. The ring is shaped like the blades themselves, by including a two-millimetre-wide slit instead of a donut hole. As can also be seen in figure 16 below. The size was chosen based on a small number of samples that were taken from a garden and measured for their approximate sizes. Another benefit of this ring is that it blocks the light that is not falling onto the leaf from reaching the lens, such that over-exposure to light will not occur. Otherwise, this might have been a problem due to the nature of the setup, where a bright light is placed over top of it during use.

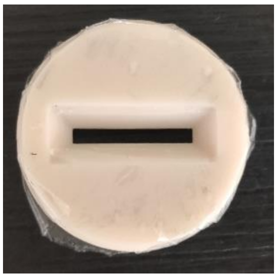

Figure 16: Top view of the new slit ring. It is the same size as the smaller existing ring. A chamfer is added to the top side to allow for more light and better airflow around the leaf.

During the user tests a lot of suggestions were made. The general idea of this prototype was deemed all right but needed some modifications to make it work. For the final iteration, the importance of being able to facilitate the HR lens was underlined. Therefore, a new approach was taken to the problem, and it was settled upon that a second backplate would have to be created. Condensation had also been a problem when using the HR lens with large (cucumber) leaves before, thus ample airflow had to be ensured. In the end, it seemed like a total redesign of the prototype would be necessary to make it work, with modifications to all parts of the design. Finally, the option to have a removable mount for a PAM measuring device, that is used by the researchers, was discussed, and incorporated into the ideas.

6.5 Prototype IV

As this was the last iteration that could be designed before the project came to an end many changes were implemented. First, a second backplate was implemented, that saw a lot of material removed to make airflow around the leaf as much as possible. Thus, essentially creating a 'default' and a 'HR' backplate. This new plate saw the removal of the option to mount to the inner poles of the setup, as well as the removal of the smallest ring, by enlarging the middle hole. The slit ring was upgraded in size to fit the same mounting as the larger ring so it could be used with the HR plate. The number of magnets for the larger ring mount was reduced back to four, allowing for more room, and thus airflow, around the clamped leaf. Material thickness was increased around the mounting points to be six millimetres, where the rest of the plate was decreased to three. Further revisions were made to incorporate mounting for a PAM holder on both backplates, as well as an adapted design from the previous works of Jacobs and Hartogsveld [8] [9].

Backplates

Many changes were made to the design of the original backplate. First, the original default backplate was completely redesigned from scratch based on the basic model of the previous iterations. Along the increase in resin thickness around the mounting, new holes were made to fit the metal threading. The inbus screws were now M3 instead of M2 as these parts were more readily available and size was not really of any concern anymore. With the added benefit of being easier to handle because of the increased size as well. The metal threads were glued into place, which worked well. Unfortunately, the plate was largely destroyed in the process as the holes needed to be drilled out because of narrow printing tolerances and the material being very brittle. Apart from this, the inner mounting was adjusted to be the right size, as it was slightly off before. Threading was installed for this as well and the product can be seen in figure 17.

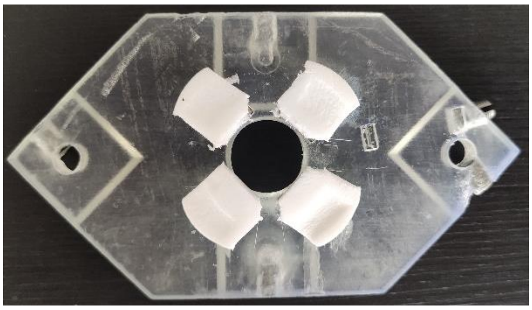

Figure 17: Backplate prototype IV: Top, printed in clear resin. The foam in the middle is spaced out to allow for additional airflow under the leaf.

The plate now also incorporated holes for a mount which fits a Walz Mini-PAM. This tool was also discussed in chapter III: Background research, and a mount for this had already been created previously. Dimensions and general mounting principle were copied form the works of Jacobs and Hartogsveld and were adjusted to fit the backplates and to be removable [8] [9]. Here, mounting into the plate was done with a fork, on top of which the holder sits, as can be seen in figure 18. This fork then goes into the two holes on the plates and can be secured in the back with screw-ins as well. The same mounting applies to both backplates. Furthermore, some dimensions were tweaked and larger margins for wiggle room were introduced as previous models had problems fitting the metal rods into the mounting holes.

Figure 18: PAM mount: Printed in clear resin. PAM device goes into top where arrow points. Bottom legs go into the backplate and are screwed tight.

For the new HR plate only outside mounting was available, as mounting to the inner poles would interfere with the intention of the plate, which is visible in figure 19. Since the HR lens would have to be placed right next to the plate to be in working distance, it was important to make the centre hole as large as possible. Therefore, mounting options for the smaller ring were removed and the hole was made to fit the inner diameter of the larger ring. A chamfer was added on the bottom to fit the edge of the lens, such that it could essentially be slotted in there. To prevent condensation from forming on the lens during research, the plate was made to be as open as possible, and only parts important to its structural integrity were kept. This made the plate somewhat less rigid but this is likely fine if used carefully.

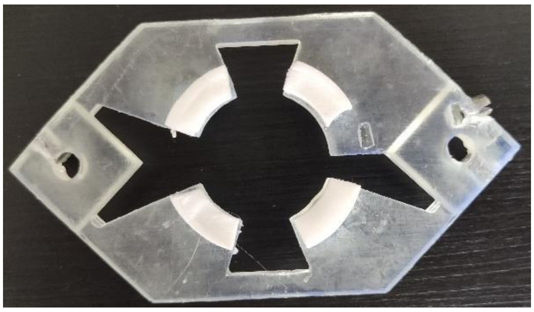

Figure 19: HR-Backplate prototype I: Top, printed in clear resin. Additional room for airflow was made by cutting away large chunks of material where possible.

Rings

The smaller ring remained the same, while the larger and slit ring were modified. The larger ring was reduced to four mounting magnets again, as its clamping force was deemed sufficient even while removing two magnets. The choice was made as it would decrease the number of clamped points on the leaf even further. This allows for better airflow around it and might also improve flow of water inside the leaf. These things have not been professionally measured, however, but serve to optimise circumstances.

Next, the slit ring was upscaled in size, which also allowed the slit to be longer. This did not affect the ability of the ring to clamp smaller grasses, as its diameter was still only forty-one millimetres. Therefore, virtually all samples would be able to fit. It also featured the inclusion of a row of foam-tape along the underside of the slit to make clamping of the grasses more stable. The new rings can all be seen in figure 20.

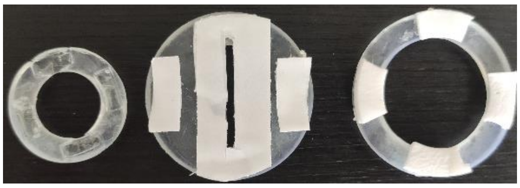

Figure 20: An overview of all final rings, printed in clear resin. The magnets on the large rings have been reduced to four and the slit ring is now the size of the large ring.

6.6 Final design

The final design consists of prototype IV, which was printed in the Liqcreate clear resin. Some slight modifications were made to the model before handing it over to the client and some recommendations were made. One of the recommendations from the final prototype was that it served its function but should preferably be laser cut or CNC milled out of a block of e.g., clear acrylic. This would improve its structural integrity, would allow for better margins, and improve form of the product. Function-wise it seemed to perform well, so no major improvements were suggested there, except for possible optimisations after a larger number of user-tests. Furthermore, it would be recommended to investigate the inclusion of mounting for more sensors and to possibly upgrade the mounting mechanism for the PAM sensor. In conclusion, the recommendations for a final design are to use custom manufacturing techniques to improve the final product to a usable level, while ironing out existing flaws in the long term.

VII. EVALUATION

7.1 Final user test

During the final user test a look was takes at the final product and whether the design could fulfil its purpose. Apart from the recommendations that were done and the flaws in form that were observed, the product seemed to fulfil its function on a very basic level. It was possible to, at least with the normal lens, generate a picture of the stomata, and the result of the final test can be seen in figure X. Therefore, it has been proven that the product is largely functional, even in a non-complete state. That said, it is impossible to evaluate beforehand how a final product would perform non-theoretically, and to what extend custom manufacturing would improve the current problems. Hence, further experimenting is needed, and implications, limitations, future research, and recommendations will be discussion in chapter VIII.

7.2 Results

To answer the main question of this research paper, it is important to see whether an answer was found to each of the sub-questions and to assess whether this answer presents a sufficient solution to the goals. As was stated in the problem statement that forms chapter II of this paper, the main question asked what design would best be able to facilitate all criteria, such that optimal conditions could be guaranteed during the research. This question was based on five sub-questions which will be evaluated briefly to be able to form a conclusion.

What (existing) design best facilitates stability of the leaf clip, such that blurry images can be avoided as a result?

The result of this question is a mounting mechanism that is very similar to the existing way of mounting to the microscope. This was imitated by making holes that go through the backplates and are tightened down with inbusscrews. This mechanism seems to be a rigid way of mounting to the microscope and it was possible to generate sharp images with it. Such an image can be seen in figure 21 below.

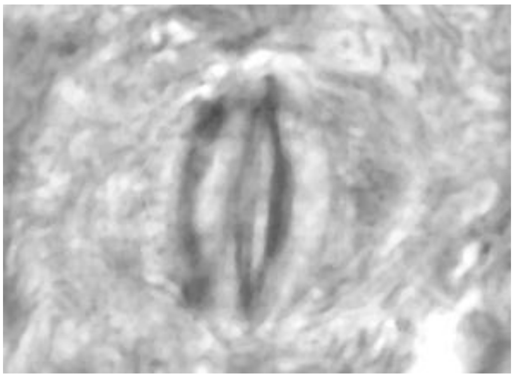

Figure 21: Photo of a stoma, taken with the final setup. The general outline of the stoma and the stomatal pore can be seen in the middle.

What (existing) design best preserves a leaf while it is being clamped such that it remains in pristine condition?

The final design uses magnetic clamping with minimal pressure points. It also incorporates foam on both sides to even out pressure on the leaf and reduce the possibilities of damaging the leaf. In tests where the leaf was left in the clamp for a prolonged period of time (approximately 24 hours) no sings of stress in the leaf were visible to the naked eye. It should be noted, however, that important variables such as water flow and gas exchange cannot be measured without using the right equipment. These measurements were not performed in this test.

What (existing) design has the shortest time of installation for the leaf?

The final prototype has a notably short installation time when it comes to installing the leaf itself. However, adjusting the backplate and tightening it back down again can take slightly longer, especially as the screws are relatively small. Simple user tests during which feedback was given indicated that there were no major problems with installation times, and rather installation rigidness was of larger concerns. Therefore, given that installation should not take more than a few minutes maximum the current design could be considered as fulfilling this criterium.

How can the design best facilitate the addition of sensors for monitoring?

This is a part where the final result fails to answer a sub-questions nearly completely. Apart from the possibility to mount the PAM sensor to the clamp there are no additional sensors for monitoring present, nor have precautions been taken to accomodate these.

What (existing) design best facilitates the installation of all different sizes of leaves such that the leaves are still intact after research?

The current design fits leaves of most sizes, due to the inclusion of rings with multiple diametres and designs. There are two donut-shaped rings, which have a large open hole in the middle to accomodate general smaller and larger types of leaves. Then there is also the slit ring, which was designed with a different type of leaf in mind. This ring fits blades of grass, and other narrower looking leaves. There are likely still leaves that are either too large or too small for the plate, but in general it should be possible to clamp down at least a part of most leaves that are being researched by the client.

7.3 Conclusion

After investigating the results and evaluating the answers to all five different sub-questions a conclusion can be drawn. As the answers to the sub-questions differ quite a lot, it is not possible to outright state that the results completely satisfy every aspect of the design. For example, even though it can be said that the leaf does most like remain in a pristine condition, even during prolonged periods of being clamped there is no sensory evidence to back this up. It can be said however, that a large change in any important variables would likely be noticed by comparing the before and after pictures, however, there is no guarantee. Therefore, this criterium has not been fully satisfied, and should probably be investigated more thoroughly in the future. That said, it is not very likely to be a problem for the end user, thus it could be said that the ends justify the means here.

Another point that remains unsatisfied is the integration of sensors into the project. The initial plan was to have these be present, or at least accommodate them in some shape or form. Nevertheless, they are not present in the current design, and therefore the final design cannot be said to fulfil this criterium. This also leaves the overall research question to be partially unanswered as the answer to this sub-question remains incomplete.

In the end, it could be noted that several of the design criteria seem to have been fulfilled. Therefore, the answer to the research question is that the current design does indeed best facilitate the design criteria, out of the investigated and iterated designs. Hence, the design could be regarded as the most complete version of the iterations, where the final design mostly suits the initial design challenge. However, if it were possible to extend the research further into the future with more user test, refining could be done, resulting in a more polished result. This is also visible in the last prototype, as it still looks very rough.

VIII. DISCUSSION

8.1 Implications

The research at hand has proven a mostly effective method for rigidly clamping a leaf for microscopic research. The aim of this project was to design a functional leaf clamp that could help the client with their research. Assuming that the clamp is mostly functional, it would ultimately be used to develop new technologies that might aid development in the field of precision farming. Since this field focuses on more efficient farming in the future, it might help alleviate some food scarcity.

Even if this is not a realistic assumption, the research done can still be a steppingstone towards a fully functional microscope setup that will eventually be used for this kind of research. Therefore, the implications of this research are likely positive, and the importance of the works have been underlined.

8.2 Limitations

The presented work has several limitations, a few of which are important to discuss. First, the design challenge was not completely fulfilled, leaving the conclusion to be slightly negative or incomplete. Therefore, it could be argued that the main research question has not been fully answered as this is not the most optimal design for the given task. This would mean that the product might not actually be useful making a good part of this paper obsolete.

Another problem that occurred is the incomplete final product. The aim of the research was to present the client with a final working product in the end, but this was not fully achieved. While the main functions of the backplates worked, some were rendered useless by bending of the materials while they were hardening out. This resulted in the PAM mount not being able to mount properly into the plate, as the very precise positioning was now off. This was also true for the screw-in threads as their holes had to be drilled out because of tolerance differences in the printer. Therefore, it was impossible to glue in the threading without drilling out the pre-made holes first. Other problems occurred where the resin did not adhere to itself properly, leaving holes in the sides of the plates, and ruining the rings in some cases. From this it can be concluded that the resolution of this Liqcreate clear resin is largely inferior to that of the Phrozen Aqua resin. In a perfect world, there would be one final iteration where the result would be custom manufactured to deal with all these problems.

Some minor problems also occurred in the communication between both parties. This meant that sometimes not all parties were completely up to date of the progress or expectations of the other party. This mostly happened during the busier weeks where the planning was more chaotic. This resulted in several occasions with bad communication between both parties, sometimes even resulting in miscommunications. This issue was also a result of the high degree of freedom that was associated with this project, which was inherently accompanied by a bigger responsibility. In the end, these problems were slight bottlenecks in the project, but also proved to be a great learning experience.

8.3 Future research and recommendations

Future research could expand upon this paper, as it did on the foundations that were laid by Jacobs and Hartogsveld. A recommendation would be to investigate the implementation of sensors and to finalise the design of the clamp. It could also be interesting to see a broader type of research where more of the total scope of the works performed by the client would be assessed. This would be able to provide insight into their works and an assessment might be made on the positive or negative influence of this research.

Some recommendations include the use of custom tooling, through CNC milling or laser cutting to custom manufacture a final product. This would drastically increase the structural integrity and form of the product, possibly allowing things such as the PAM mount to work as well. It would also be advised to do a longer-term user test with the product and to assess what might still be improved based on this. If this were to be done, it would allow the final kinks to be worked out and thereby a final design for a leaf clamp to be created.

APPENDIX A

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APPENDIX B

A short list of terms and abbreviations as used in the text is shown below.

Term/abbreviation	Meaning
LL	Low light
HL	High light
g_s	Stomatal conductance
A	Net photosynthetic rate
ETR	Electron transport (rate)
PSI	Photosystem I
PSII	Photosystem II
PPFD	Photosynthetic photon flux density
CO_2	Carbon dioxide
H_2O	Water
O_2	Oxygen
HR	High resolution
$P_{50X/90X}$	An indicator of the amount of time that has progressed before 50/90% of the maximum value of variable X is reached after going from a LL to HL situation.

Figure 22: A short list of abbreviations.

The foam rings are provided by Li-Cor [19].

The magnets were bought from Supermagnete, they have a carrying capacity of 640, 900, and 1100 grams respectively [20] [21] [22].

The SolidWorks files can be found on OneDrive. You need to have a UT email address to access the files [23].