Project Title:

Focus Stacking for Teaching and Publication in Plant Sciences

Report Title:

Progress in the project – July 2018:

The YouTube channel, the Documentation, and the Photographs.

Author:

Jennifer Deegan

Department of Plant Sciences,

University of Cambridge

Summary

Progress has been made in three main areas.

- On the advice of professional educators I aimed my materials at Honours students. I created complete and detailed teaching materials on how to duplicate my photography setup, and presented it both as YouTube videos and online text documentation.
- I have extended the photographic range of the set-up by adding three new microscope objectives.
- I have begun photographing the proposed range of photographic specimens. I have formed additional collaborations beyond the scope of the original grant proposal, and so am photographing a larger range of specimens even than initially envisaged.

Report and outcomes

We had three initial goals:

1) Develop written documentation for the construction of a £100 teaching tool for focus stacking in schools and universities.
2) Extend the x10 magnification focus stacking system to a much larger range of magnifications.
3) Use the system to photograph my fern gametophytes, and the *Arabidopsis* trichomes and *Utricularia gibba* traps of my collaborators.

Our modified goals:

We have stuck to these aims, with some slight modification and extension in response to feedback. Our actual progress:

1) I canvassed opinion among a wide range of educators and was given strong advice to aim my system at Honours undergraduate students. In the light of this, I modified my aim to:
    a. Create comprehensive video tutorials and written documentation to allow an Honours student to duplicate my system. In the end the cost calculated was closer to £250.
    b. A new website at Chlorophyllosophy.uk was created as a central hub for all of the materials.
    c. A simpler video was created, to explain focus stacking at secondary school and general undergraduate level.
2) The focus stacking system has been extended so that it now works from x5 to x50 magnification.
3) I am in the process of taking photos of fern gametophytes, *Arabidopsis* trichomes and *Utricularia gibba* traps.

    Extension : Additional collaborations have been formed at the Sainsbury Laboratory, and photos of *Marchantia* have also been taken. I hope to form further collaborations at the Sainbury Laboratory in future. They have a lot of interesting specimens there, and have space to accommodate my setup for days or weeks at a time when I bring it to visit. Ideally I would like to find a little niche there as a volunteer in the long run.

    Extension: The engineering of the system has been upgraded to improve camera shutter triggering in strong sunlight.

Delivered Results in detail.

Chlorophyllosophy.uk website was created as hub for teaching materials:

Chlorophyllosophy

A Plant Science Image Library

Focus Stacking Setup

Click this image to visit the YouTube videos, which will explain how the system works, and how any other group or lab can make their own working system.

What is it?

This page is about our focus stacking setup. I use it to take photographs of tiny plant specimens that are between about 0.2mm and 1cm wide.

Please feel free to copy it.

I am very happy for anyone to build a duplicate system, and have written detail instructions to help people and research labs to do this. If you would like to have a go, please watch the videos, or read the pages linked below. If you do try, I would love to hear from you about how you get on.

How can I do this for £100 if I am in a University or similar?

My hope in writing this is that an honours student in a University might be able to copy this system for only about £100, with a certain amount of borrowing and scavenging of equipment and parts. If you would like to know how to do this please see the parts and sourcing list.

Where are the building instructions?

OpenPlant Project 2018

GitHub write-up

Hackter write-up

YouTube Channel

Biomaker Challenge 2017

Hackster write-up

GitHub write-up

Focus Stacking

- Home
- About the library
- M.B. Wilkins
- J. Deegan
- Focus Stacking set up

Contact Form

Name

Email *

Message *

Send

To see some of the photos taken with this setup please visit my image library at http://www.chlorophyllosophy.co.uk/

The small versions of my images are available free for teaching purposes. For full sized versions of the images for teaching or commercial purposes, please contact me through the form at http://www.chlorophyllosophy.co.uk/.

Who did this work?

Who built it?

The system was put together in a collaborative effort between a large number of people. The people who worked together to build the system are shown on the GitHub and Hackster pages listed below.

A great deal of technical expertise was contributed by the members of the photomacrography.net discussion forum.

The bulk of the work was carried out on a volunteer basis, by me (Jennifer Deegan), and Tim Deegan, working at home, for fun. If you do try to duplicate our system, we hope you enjoy it as much as we do.

Which groups funded it?

Some of the work was funded by two OpenPlant grants, in collaboration with the University of Cambridge and the John Innes Centre. The OpenPlant grant was in turn funded by the BBSRC and EPSRC.

How do I give attribution if I copy it?

In any publications that may arise from duplicate systems, please include in your acknowledgements, our publication of the system method: Deegan, J., and Deegan, T., 2018, The Pteridologist Magazine.

Who inspired this work?

The Photography of Professor M.B. Wilkins

To read more about the photography of Professor M. B. Wilkins, please follow the link at the top right of this page.

The Bratcam by Chris Slaybaugh

To read more about Chris Slaybaugh's Bratcam, on which my system is based, please visit his write-ups on the photomacrography.net website. The first volume is here and the second is here.

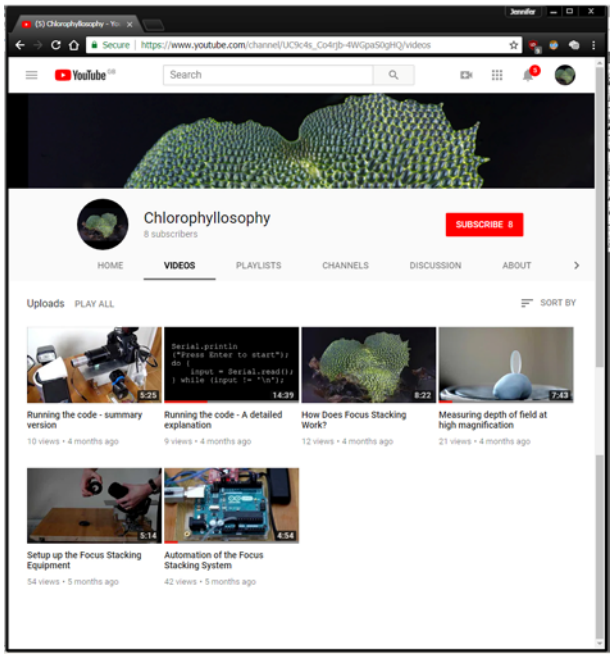
The Photomacrography.net Forum

In designing and building my system, I received a great deal of advice and help from the members of the photomacrography.net forum. To see the discussions that led to the building of my system please see the thread here.

This work was carried out by Jennifer Deegan in Cambridge. She was working especially with Tim Deegan, but also with the help of many collaborators, who are all listed on the GitHub and Hackster write-ups listed above.

Youtube “Chlorophyllosophy” channel was created. (https://tinyurl.com/yde6pg54)

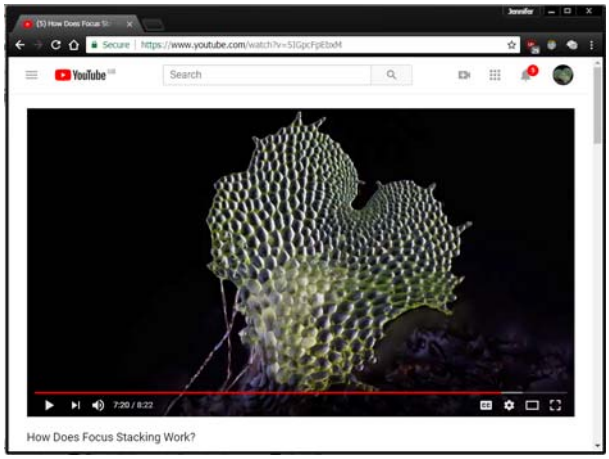
The channel is the home of all of the videos that I created to walk Honours students through the process of building a complete duplicate of my system from scratch. A list of the videos and their contents is shown on the following pages.



Videos (All at https://tinyurl.com/yayuptso)**:**

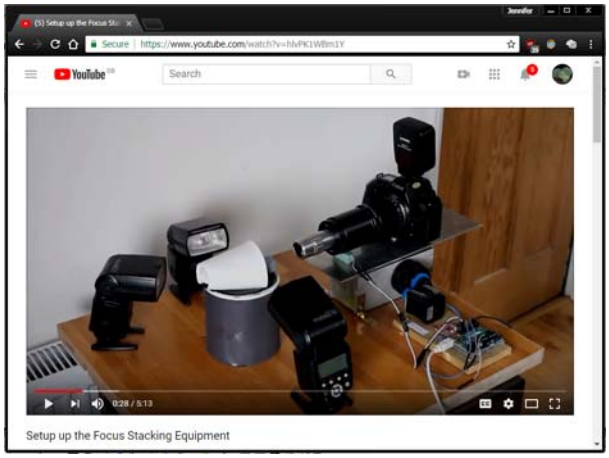
1) How does focus stacking work? (8 mins)

This is aimed at a general audience and explains depth of field, and the use of multiple images with shallow depth of field to create a single composite image that is fully focussed.



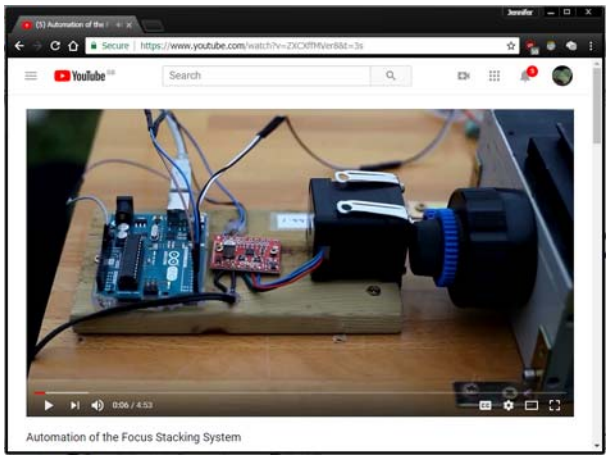
2) Setting up the focus stacking equipment. (5 mins)

This video shows how to build the physical structure of the focus stacking setup using various scavenged parts.



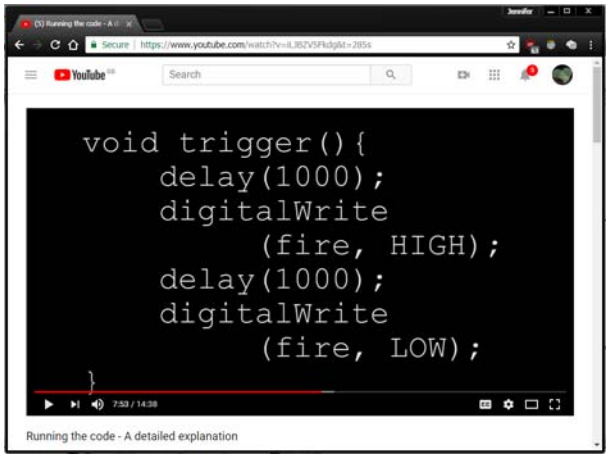
3) Automation of the focus stacking equipment. (5 mins)

This video shows how to add the electronic parts of the setup so that the movement of the focus stacking platform will automatically more forward in tiny increments, and so that the camera shutter will fire automatically.



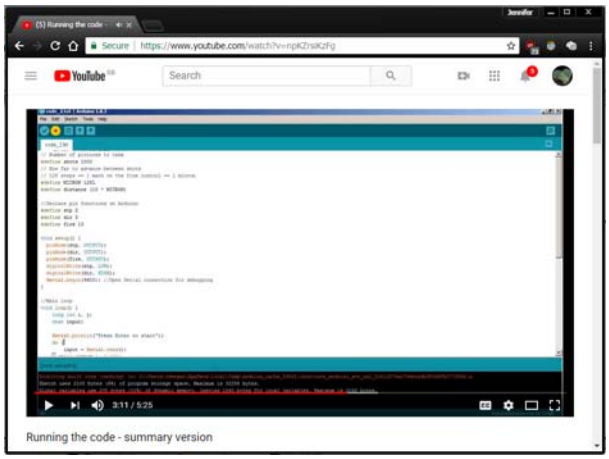
4) Running the code – A detailed explanation. (14 mins)

This video shows the computer program that drives the platform forward and fires the shutter. It explains in detail how the program works so that users can modify the code to improve the results of their photography.



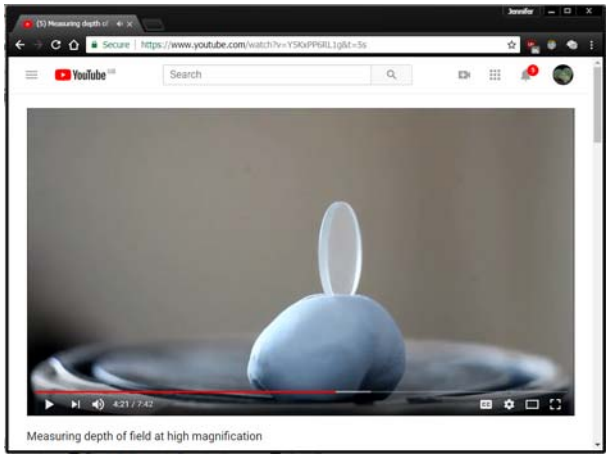
5) Running the code – summary version. (5 mins)

This is a summary version of the video above. It shows how to run the computer program, in a way that enables people to use the system without necessarily needing to understand in detail how the program works internally.



6) Measuring depth of field at high magnification. (8 mins)

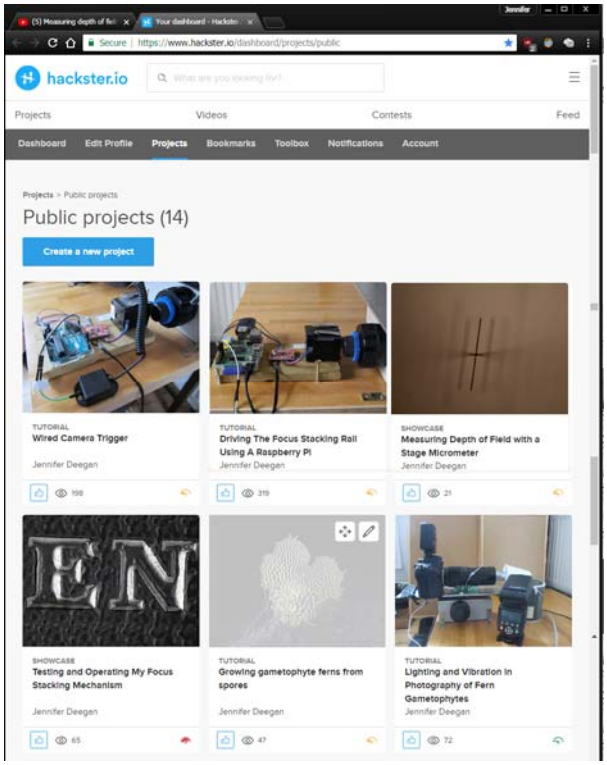
This video explains how to measure the depth of field of a given lens setup. This is essential as the platform must not move forward in increments that are longer than the depth of field of the lens setup, otherwise blurred stripes will appear in the final photograph.



2) Written documentation:

a. Hackster (https://www.hackster.io/dashboard/projects/public)

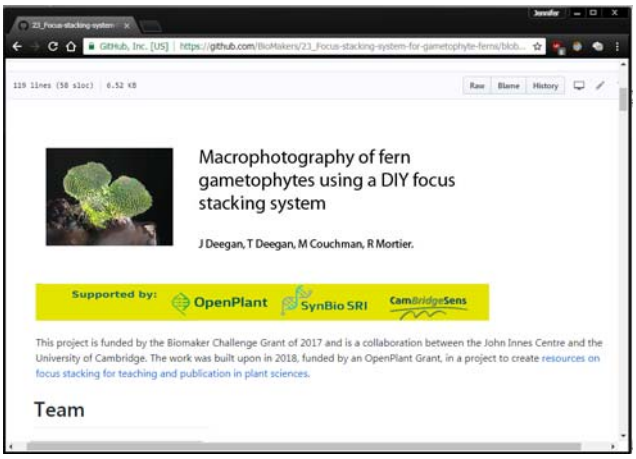
This documentation gives full written instructions on how to build a duplicate copy of my focus stacking system. The written documentation shows how to drive the system using either a Raspberry Pi computer or an Arduino computer. There are also instructions on how to grow gametophyte ferns.



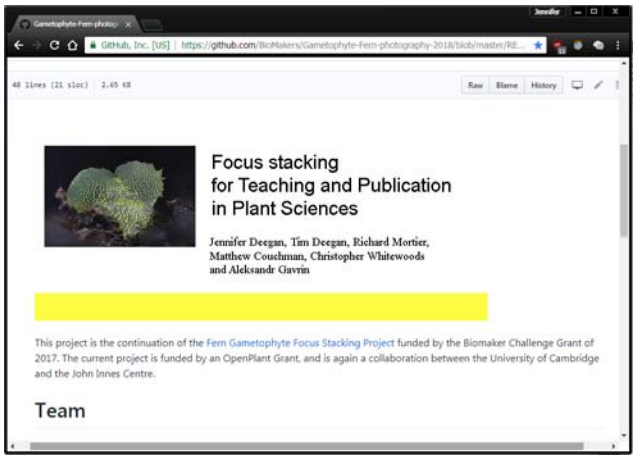
b. GitHub:

The GitHub pages contain full written instructions for building a duplicate copy of my focus stacking setup. Once again, instructions are given on how to drive the system using either a Raspberry Pi or an Arduino computer, and information is given about the culture and life cycle of gametophyte ferns.

i. 2017 write-up: https://tinyurl.com/yasjehrx



ii. 2018 write-up: https://tinyurl.com/y8qr3lak



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B) Completed microscope setup.

Additional microscope objectives have been purchased and are working well. The setup now photographs subjects at between 5x and 50 magnifications.

C) Photographs of plant subjects.

This part of the project is still in progress as the video making really took a long time. I only started on the photography quite recently.

From my point of view, the most satisfying part of this project was providing photographs for other scientists. A big part of my work was in listening to them to understand what they needed.

Often their needs were very different from my plans, and I had to learn to change tack to do what they wanted me to do. This meant even allowing them to be the only ones to handle their specimens in my photography setup as the plants were so tiny and so precious to them. This was quite complex to accommodate as the photography setup has to be adjusted to micron-scale degrees of accuracy.

Generally I was aiming for beauty in the photography, even if that might take months. Conversely my collaborators needed scientific accuracy and speed in getting results. I was able to accommodate that once I understood, because my system has the flexibility to handle that too.

My favourite part of the work was meeting the scientists, and hearing about their projects, and seeing their passion for their experimental subjects. I would like to do a lot more of that. I love learning about their tiny photographic subjects and seeing their enthusiasm for the structures that they study. I hope that with my photographs, they will be able to spread their enthusiasm more widely, as they will then have great photos to illustrate their stories.

Here is some feedback from my collaborators on why the images are useful:

Christopher Whitewoods (John Innes Centre):

[The photos] will be really useful to get an idea of how cell shape changes across the trap, and to see differences between mutant and wild type plants. The traps are really curved which makes standard still images almost useless. Your setup does a lot better job than our lab light microscope.

Aleksandr Gavrin (Sainsbury Laboratory):

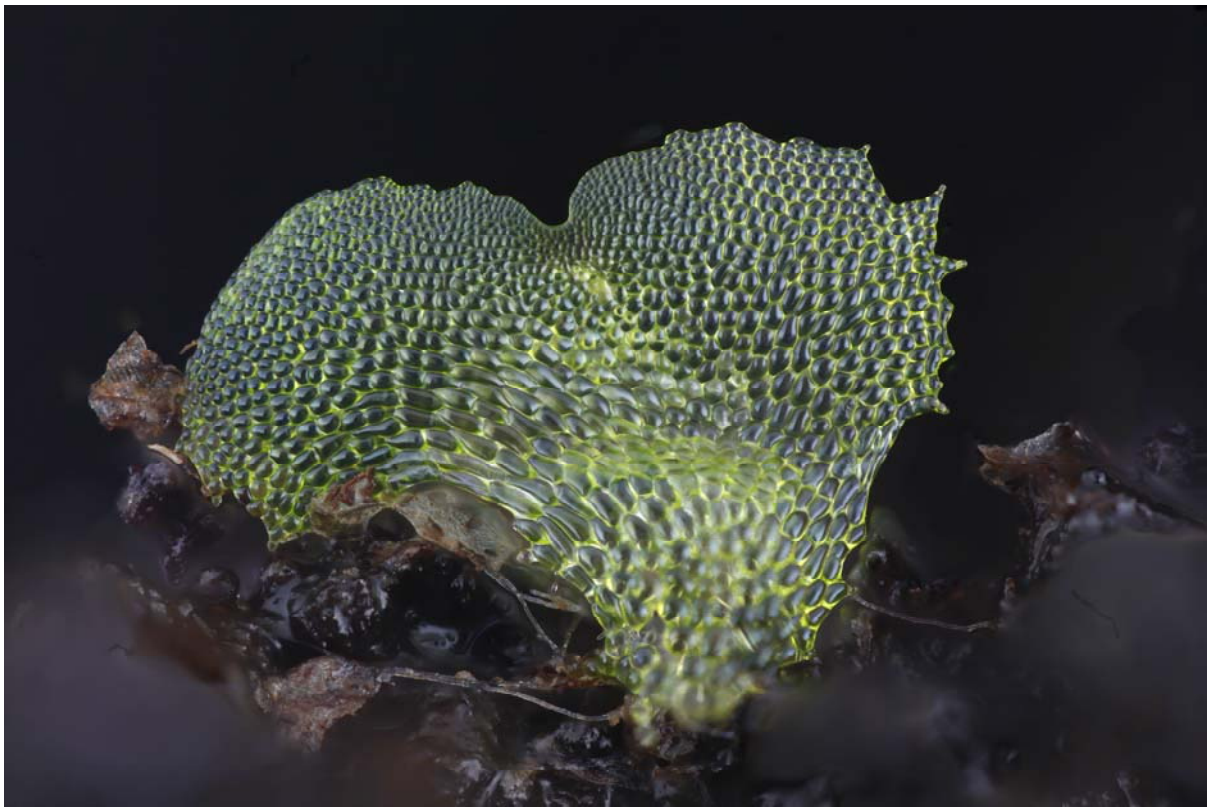
Related [to] trichrome imaging, this project is about phenotyping of plant structures which are too small for fotocamera but too big for a microscope. This can seriously slow down any project like this. Your setup allows us to obtain deep focus picture and to fill this gap. I think it's a very useful phenotyping tool for researchers who work with this type of object.

On the following pages are some of the first few photographs that have been taken with my system.

***Asplenium scolopendrium* gametophytes (ferns)**

Jennifer Deegan, Department of Plant Sciences, Cambridge.

X10 magnification. (Taken before OpenPlant grant began)

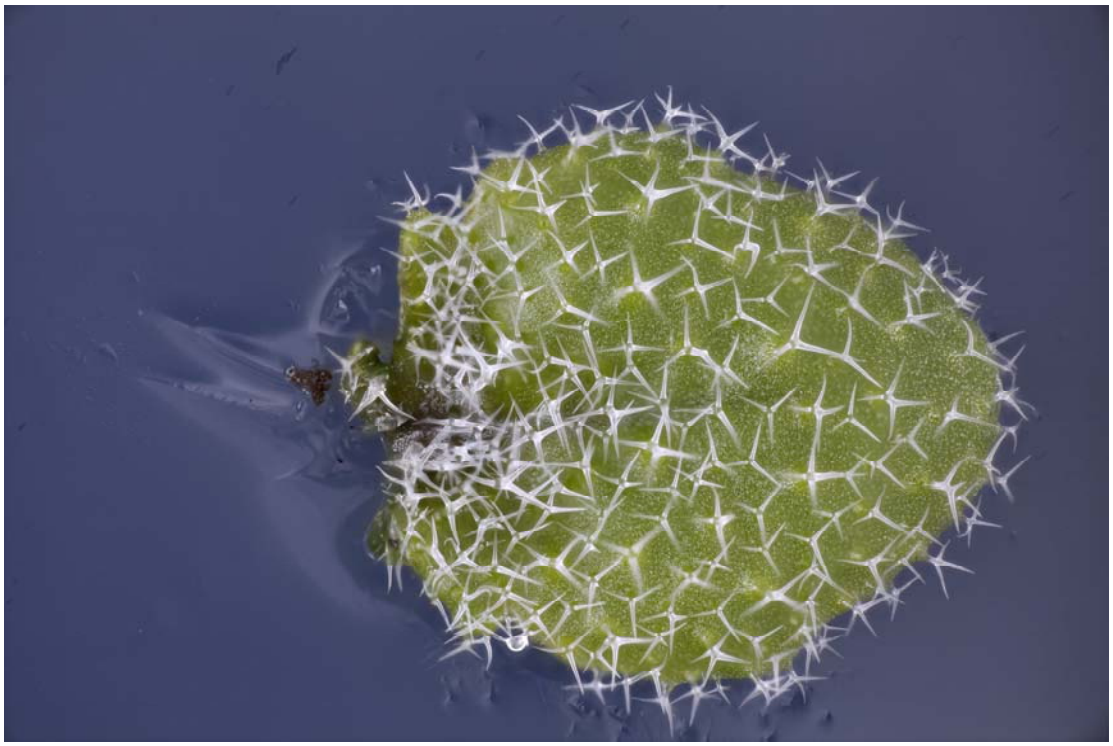


***Arabidopsis* trichomes**

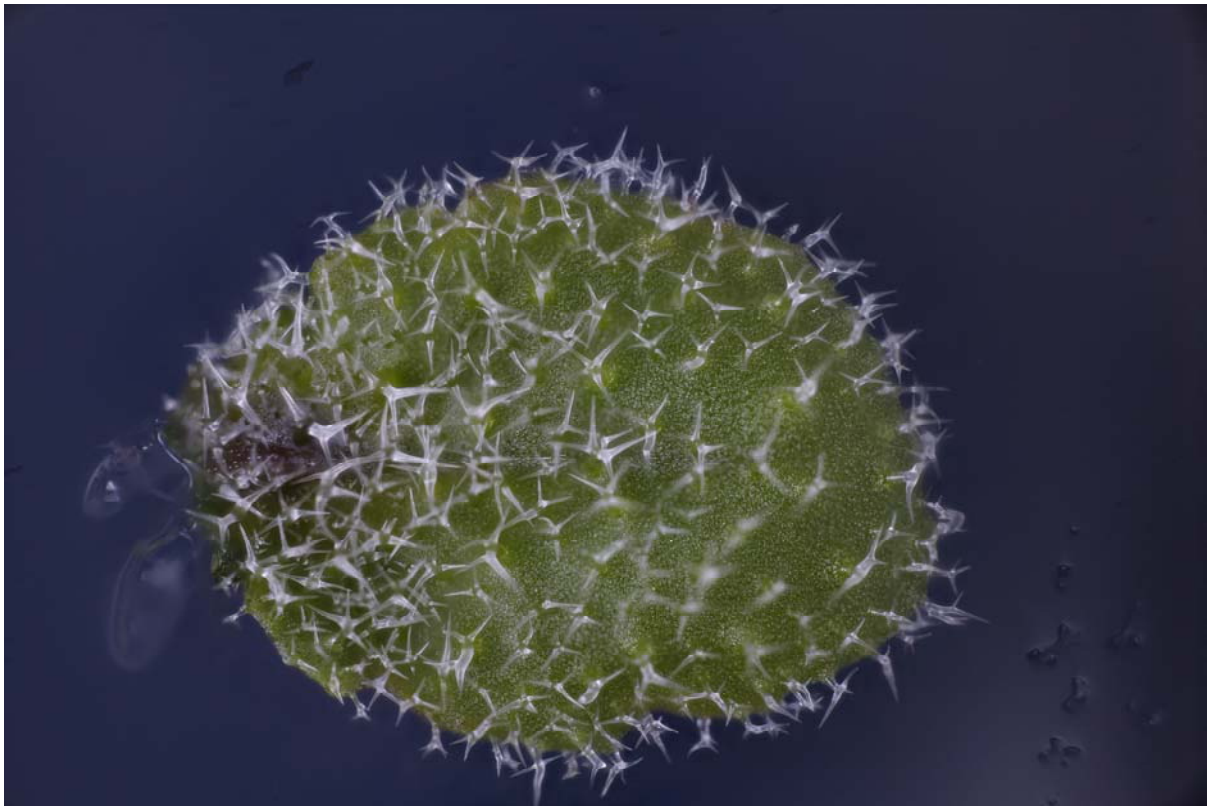
Aleksandr Gavrin, Sainsbury Laboratory, Cambridge.

X10 magnification, taken 2018.

Wild type:



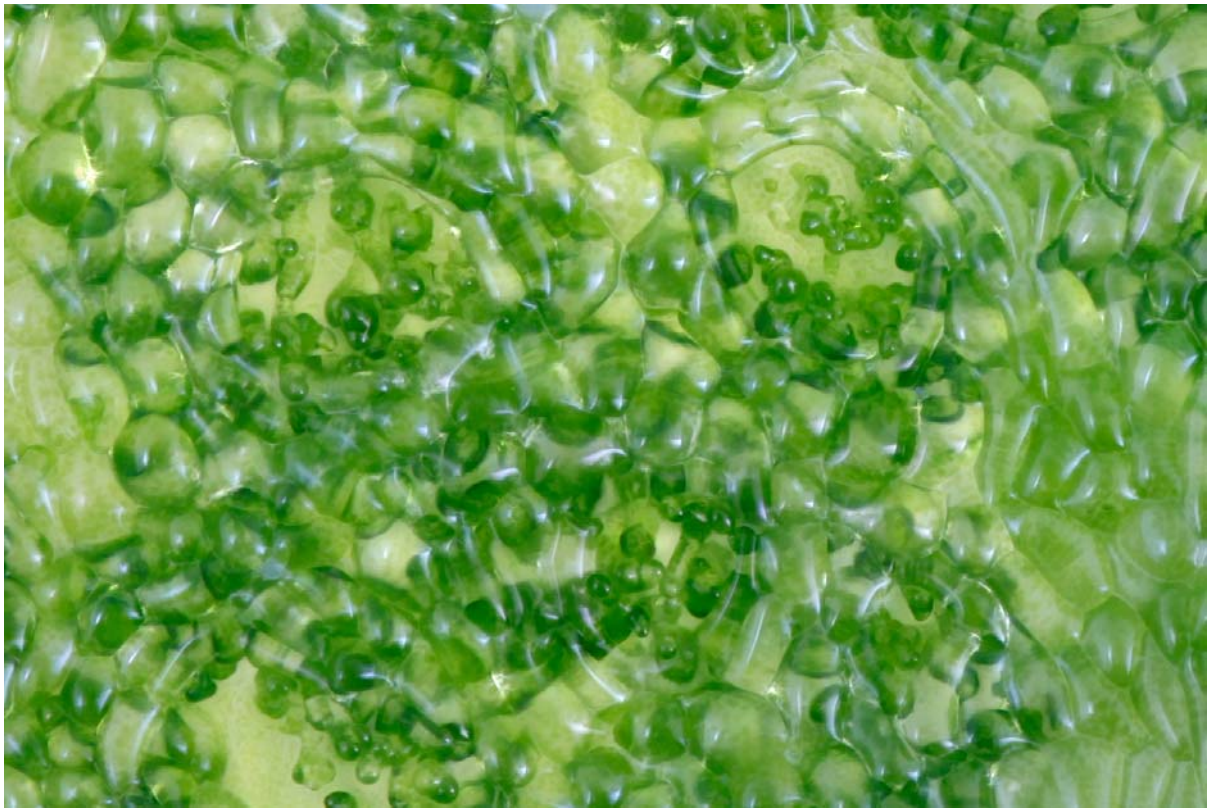
dis3-4 mutant



***Marchantia* mutant**

Philip Carella, Sainsbury Laboratory, Cambridge

Photographed at x10 and cropped. Taken 2018.

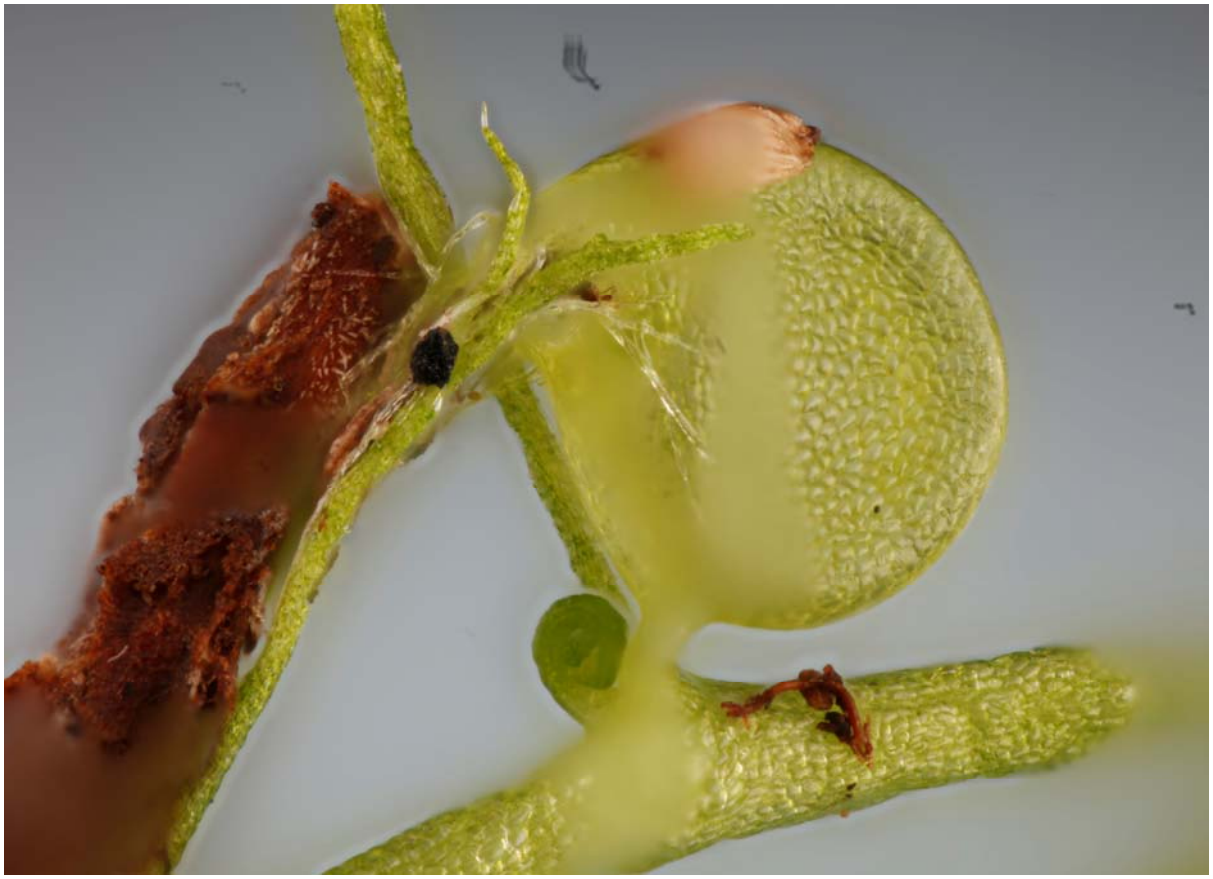


***Utricularia gibba* traps**

Christopher Whitewoods, John Innes Centre, Norwich.

These are more difficult because they are pond plants and have to be removed from the water before photographing. I am still working on how to produce the best result for this specimen.

I have some ideas of how to do this, but have run out of time to do the work before submitting this report, so I include my initial attempt at a photograph below. Christopher has helpfully explained to me which part of the plant he is most interested in imaging, and I hope that that will help me to target my effort better.



What were the problems?

When the OpenPlant grant was given to me, the judges asked that I should provide my documentation in video form, rather than as written material, which had been my proposed plan.

This was hugely challenging, but very enjoyable. I had never made videos before, and did not have the equipment to do it.

One of the challenges of making a video about photography is that the images in the video have to be taken very carefully, with very good composition, optics and lighting. There isn't much mileage in making a badly photographed video about photography. I took a great deal of care over this aspect the project, and I hope that the videos are attractive to look at, as a result.

I figured out the following workarounds for some of the challenges:

- My DSLR camera recorded the video footage.
- In videos where the DSLR appears, I used my film SLR as a body double for the DSLR.
- My old copy of Adobe Premiere Pro video editing software was brought back to life with help directly from the Adobe CEO's help address (after many days of effort.)
- My laptop is too slow to run the videos in the video editor, but they can be replayed after uploading to youtube. Uploading takes 2 hours each time, so this slowed me down a lot each time I needed to view an edited video.
- I borrowed a good microphone to capture sound, and enjoyed layering the recordings of my voice over the recordings of the camera shutter. The film camera does not fire its shutter, so all shutter sounds are artificially added in the videos.
- In the end I made 43 minutes of video footage. I'm really quite proud of having managed that. I hope the judges enjoy them too. If they only watch one, this is the best one: https://tinyurl.com/ybewyfzo

Expenditure

Almost all the grant was spent on microscope objectives. We bought the following:

Objectives

Mitutoyo 5X M Plan Apo Objective £615.30
Mitutoyo 20X M Plan Apo SL (30.5mm WD) £1,396.50
Mitutoyo 50x M Plan Apo £1,879.50

Total cost = £3891.30

Insurance

The remaining money was spent on insurance, so that the photography setup could be taken to visit my collaborators at the Sainsbury Laboratory. They have GM specimens that could not be brought to me, so I went to them.

Insurance = £176.92

Total

£4068.22 (I paid the £68.22 overspend myself)

Feedback from Outreach work

Part of the objective of this project was to enthuse other people about deep focus photography of fern gametophytes and other tiny specimens.

Two people who have seen my teaching materials and enjoyed them, wrote to say that they are also going to start gametophyte photography. Below are emails that they sent.

Contact 1)

From:	Pau
To:	jsp
Posted:	Fri Jan 12, 2018 10:10 am
Subject:	growing gametophytes

Hello Jennifer,
Photomacrography.net group

Now I've just retired as teacher and have time to recover long time parked projects.
I have some ferns, including a very nice *Polypodium cambricum* and I often have photographed their sporangia but never have been able to see live gametopytes.

I recall your site explaining how to grow them, I plan to follow your method

What's the good time to collect the spores? Right now they begin to be mature and drop off the sporangia, is better to collect them just fresh or to let them to dry?

Any other advice, please?

Thanks for your attention

Best

Pau Renard

Contact 2)

Helicon Focus – Facebook group

Michael Rose Jennifer, We have many ferns in our garden & I just never realised the complexity of their life cycle! Thank you for educating me, I will have to look out for the gametophytes, are they found near the base of the sporophytes or do they spread further afield?

Follow on Plans

For context – I am a stay-at-home Mum, and I work part time, from home, as a volunteer.

In the future, I would like to carry on taking deep focus photographs of tiny plant specimens using the equipment that I have built.

Working from home can be quite isolating, and as part of this project I really enjoyed meeting my new collaborators. I would like to find more collaborators in the future, and work more with the ones that I have found through this project.

I very much enjoyed my visit to the Sainsbury Laboratory and would be very interested in visiting there again to take further photos of other specimens.

If further funding is provided I would like to spend the money on a faster computer as this project has been constantly hamstrung by the slowness of my 10 year old laptop.

Thank you for giving me the grant. I really very much enjoyed doing the work.