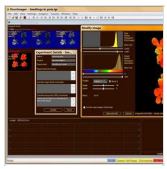

Version 2

Jun 03, 2021

Fluorescence analysis using CF imager V.2

Version 1 is forked from Fluorescence analysis using CF imager

Steven J Burgess[1], Lynn Doran[2]

[1]University of Illinois at Urbana-Champaign; [2]Realizing Increased Photosynthetic Efficiency (RIPE)

1 Works for me | Share | This protocol is published without a DOI.

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ABSTRACT

Measurement of photosynthesis parameters using the Chlorophyll Fluorescence Imager from Technologica http://www.technologica.co.uk/products/cfimager/index.html

PROTOCOL CITATION

Steven J Burgess, Lynn Doran 2021. Fluorescence analysis using CF imager. **protocols.io** https://protocols.io/view/fluorescence-analysis-using-cf-imager-bumqnu5w Version created by Lynn Doran

FORK NOTE

FORK FROM

Forked from Fluorescence analysis using CF imager, Steven Burgess

KEYWORDS

Chlorophyll fluorescence analysis, photosynthesis

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LAST MODIFIED

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OWNERSHIP HISTORY

Jun 02, 2021 Benjamin Haas

PROTOCOL INTEGER ID

49552

DISCLAIMER:

CF Technologica is no longer supported by the manufacturer.

Preparing plants

1 Dark adapt plants for at least 20 minutes prior to taking measurements.

This is done to ensure the photosynthetic electron transport chain is fully oxidized and reaction centres are open.

In an ideal situation plants are allowed to dark adapt overnight prior to measurement.

A properly adapted, healthy plant should give a Fv/Fm value of ~0.8. This has been shown to be highly stable between species. Significant deviation from this (e.g. <0.7) either suggests incomplete adaptation or stressed plant material.

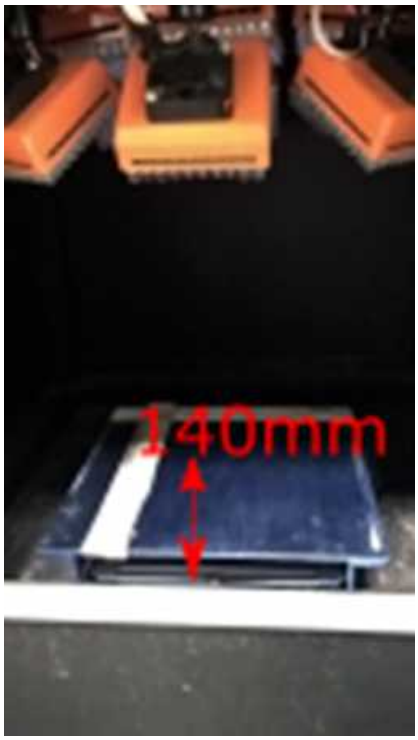
2 Turn on the cf imager and open the FluorImager software.

3 Start the FluorImager software

Set the focus

4 ⚠

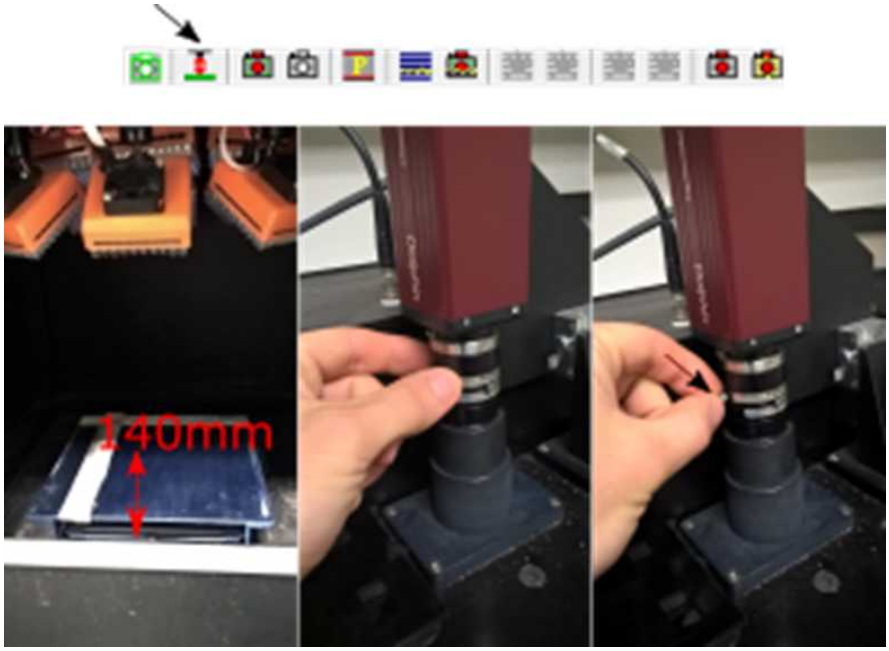
The surface of the leaf should be 140mm from the base of the imaging chamber, and can be adjusted by lowering or raising the plant under analysis using the knob on the left side of the scissor lift. Position plant/leaf in the chamber and measure the distance from the base of the chamber to the plant leaf using a ruler.



The high of the platform dramatically affects the amount of light the sample receives. Ensure the sample surface is at 140 mm. If exact light is necessary, measure using a PAR meter at the final adjusted height of the platform. Adjust the actinic light in the protocol accordingly.

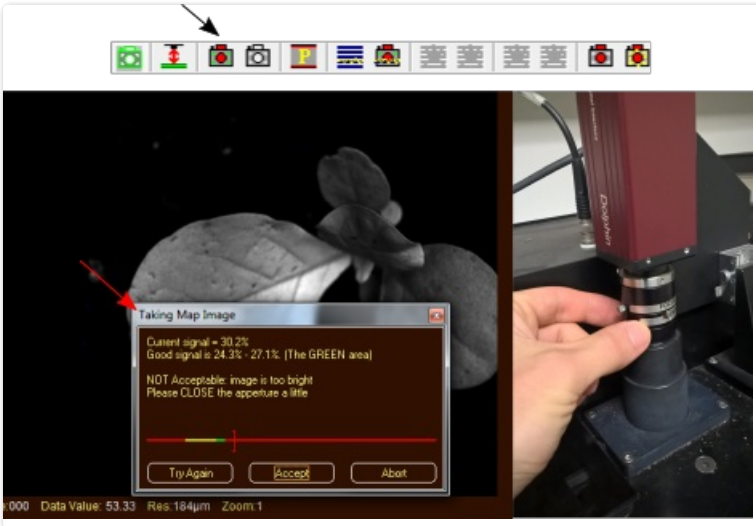
5 Use a plant or plate that is not intended for experimental measurement that day to set the focus. Push the double arrow button to turn on the camera's focus. Set the focus by adjusting the dial above the chamber, and lock in position by turning the screw on the side.

Setting the focus requires the camera to strobe light flash repeatedly. This will alter the response of any previously dark adapted plants. Do not use experimental plants or leaf punches to set the focus. If no other plants are available for setting the focus, re-dark adapt the plant used for focusing for a minimum of 30 minutes before analyzing.



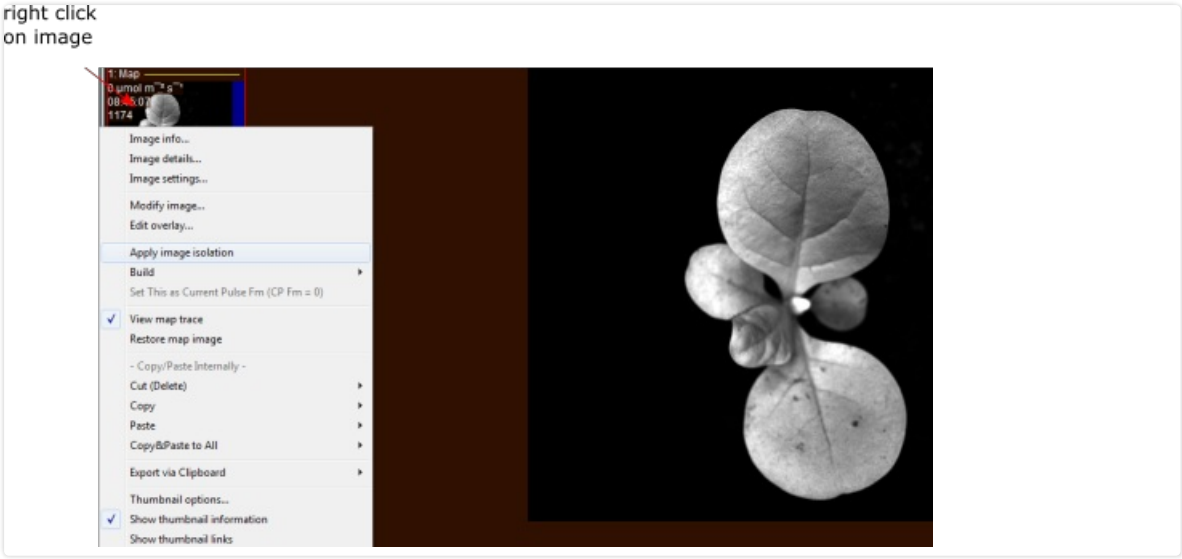
Set exposure

6 Manually adjust the aperture as shown on the right to allow the optimal amount of light into the imager so as not to overexpose measurements. After each adjustment of the aperture, click the try again button. When the aperture is correctly adjusted, the instrument will automatically take the photo.

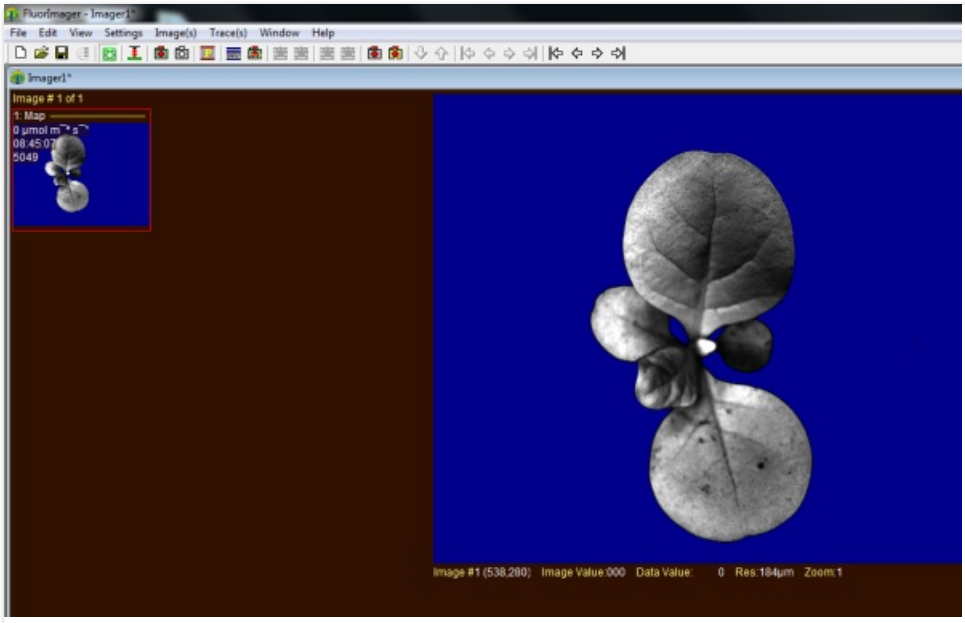


Isolate the Image

7 Isolate the plant or leaf of interest by selecting "Image' on the top tool bar and the "Apply image isolation.



After applying isolation background areas will be masked out in blue as shown below.

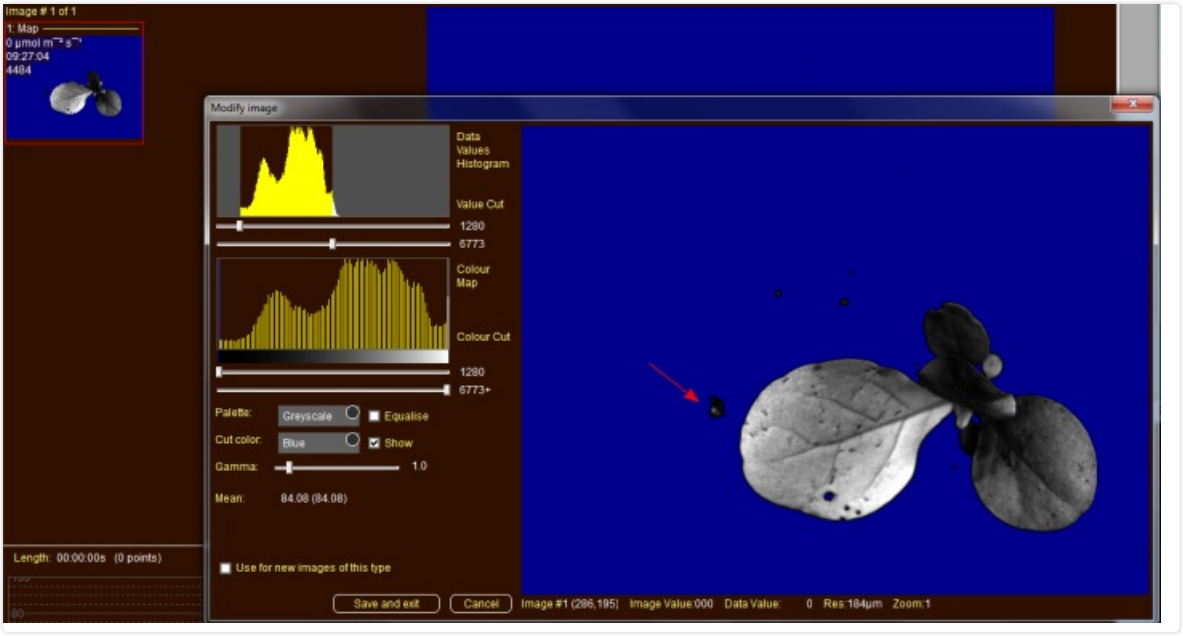


If you forget to apply isolation and start the protocol, the image can be isolated prior to exporting the files for processing.

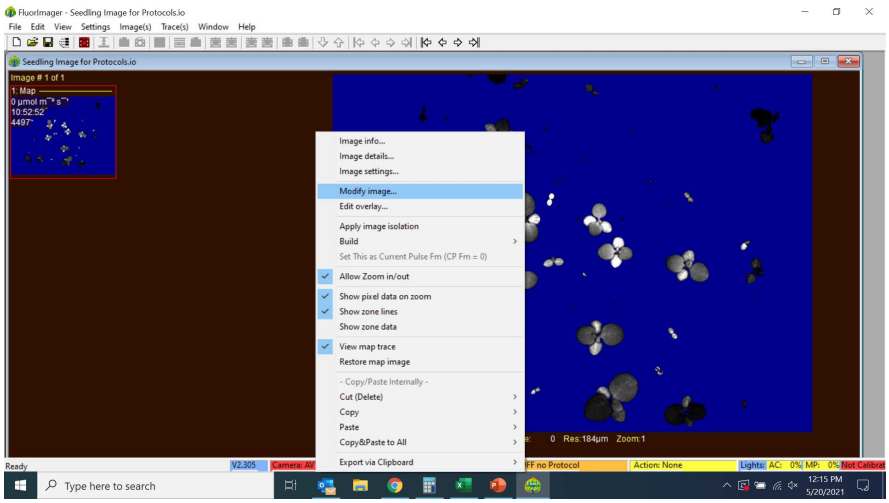
Leaf tissue on filter paper or leaves or plants grown in media will typically have pretty good isolation. Plants grown in soil will have significant remaining noise.

If plants grown in soil have a soil surface coating of algae, it will make isolation easier to physically remove the algae using a scoopula.

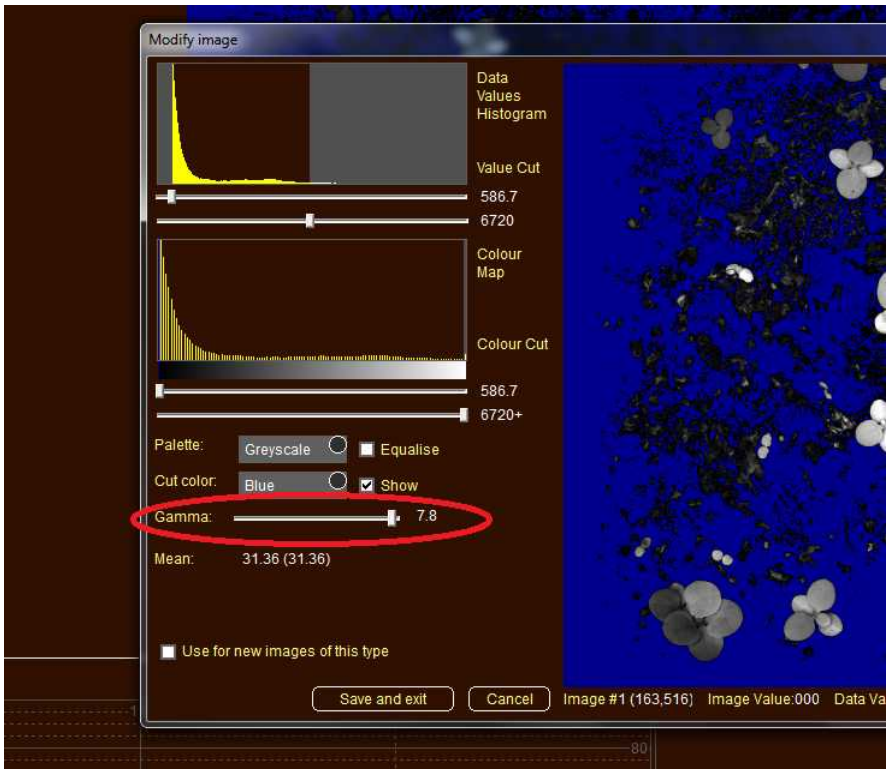
8 Sometimes the software picks up background noise as real signals, shown below as black dots on the blue background. This is frequently vermiculite or algae on the soil. It is advisable to remove the noise, otherwise each individual instance will be counted as a separate 'colony' during analysis and measurements will be recorded for each of these spots in the final data sheet.



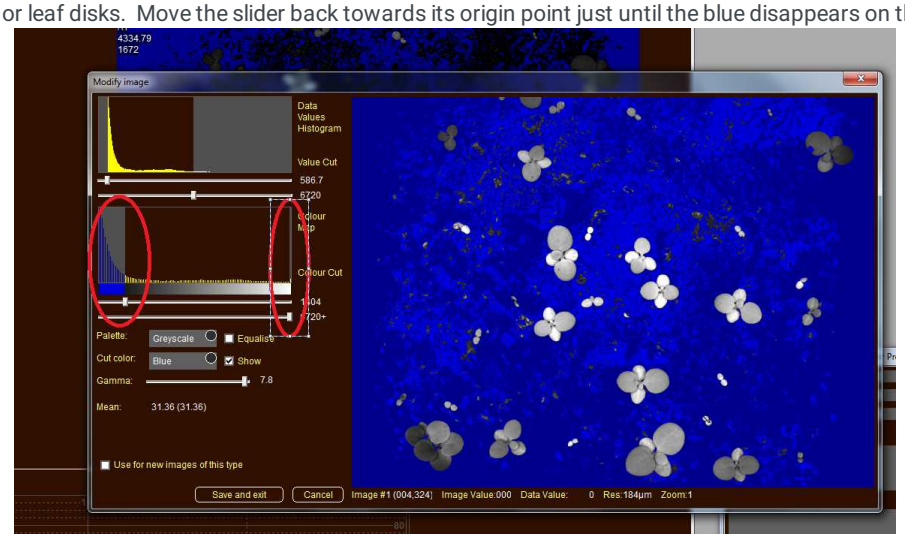
9 Modify the image isolation by right clicking on the image and selecting "Modify image..."



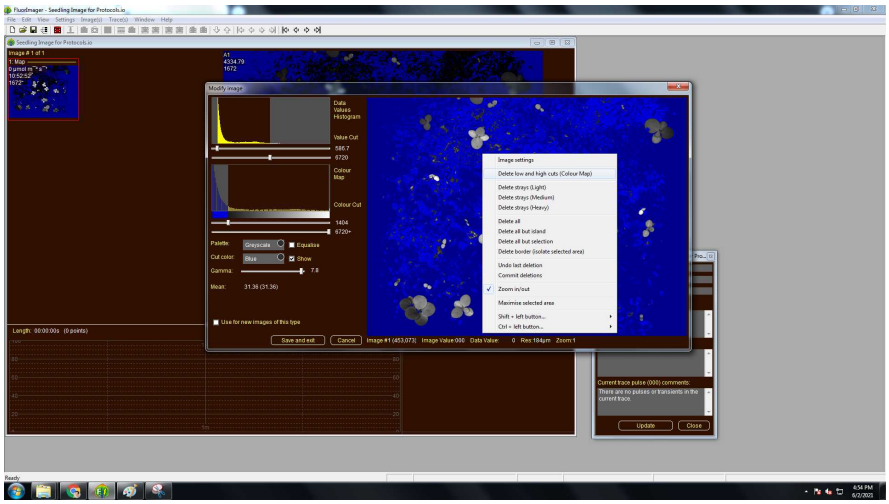
10 Use the "gamma" slider bar to lighten or darken the image to make it easier to identify plants or leaf disks.



11 Slide the two sliders below the color cut graph towards the center until you see blue spots begin to appear on the plants or leaf disks. Move the slider back towards its origin point just until the blue disappears on the leaves or leaf disks.

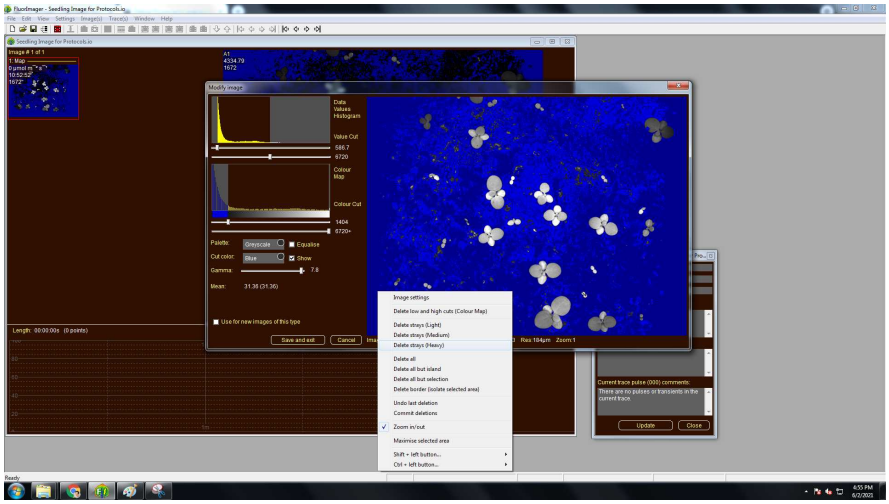


12 Right click on the image and select "Delete high and low cuts (Colour Map)" to delete the highlighted area. This will remove anything that is colored too light or dark to be plant material.

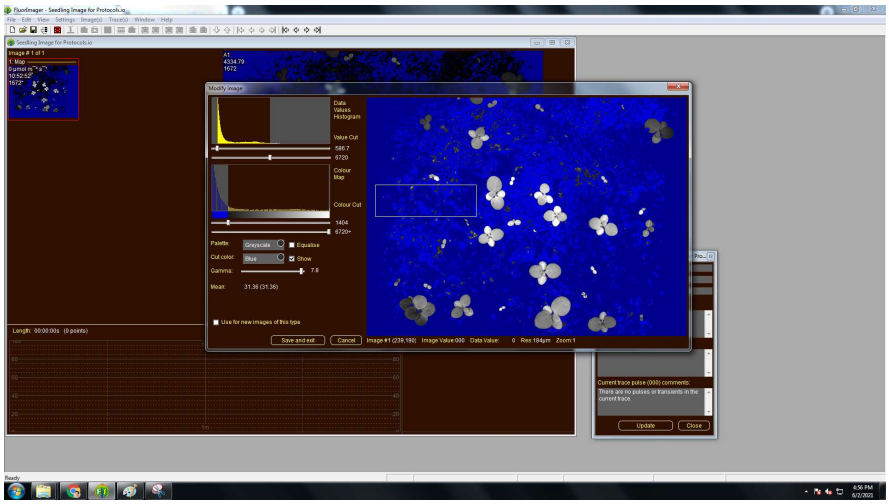


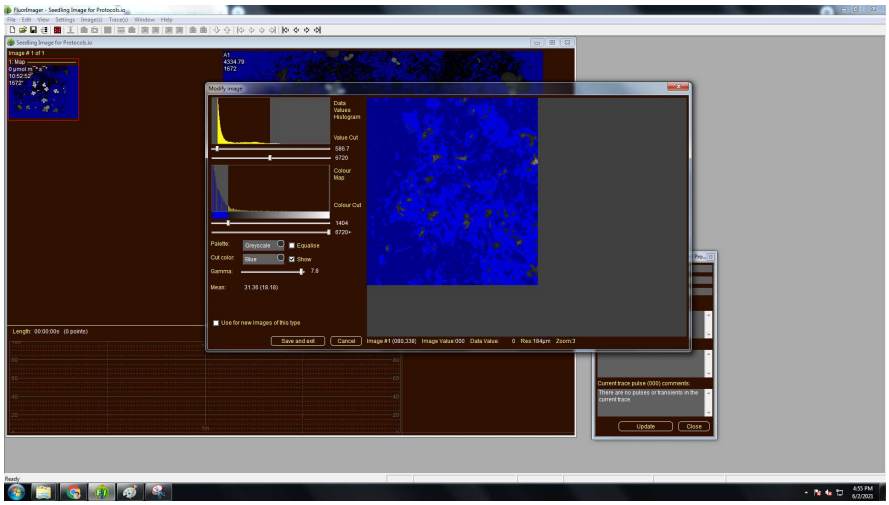
Delete low and high cuts must be selected after moving the sliders to save the isolation. If you only move the sliders and then press save and exit, the isolated area in a lighter blue will still be measured in your image. Only the dark blue areas are isolated.

13 Right click on the image and select "Delete strays (Heavy)". This will delete any pixels that are not touching at least 3 other pixels.



14 To zoom, hold shift and control simultaneously and select the area you want to zoom to using the left click of the mouse and the yellow box that will appear. Highlight and zoom into a large square of the image that does not contain leaf tissue.

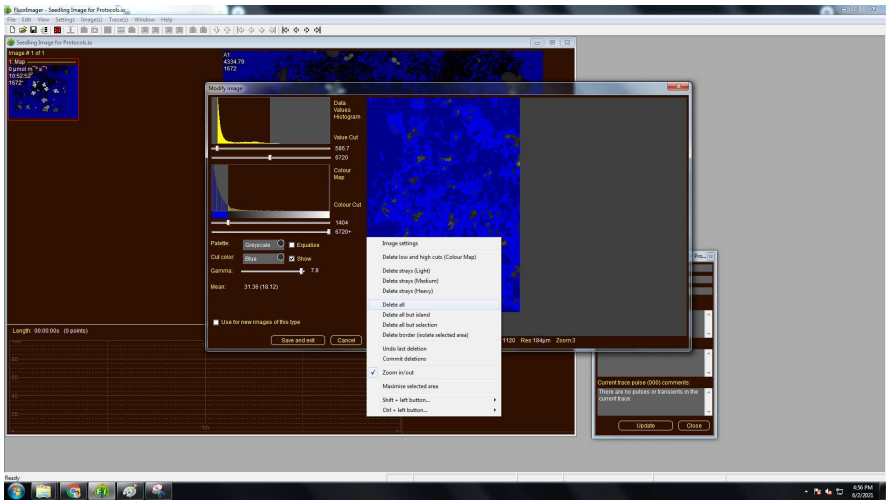




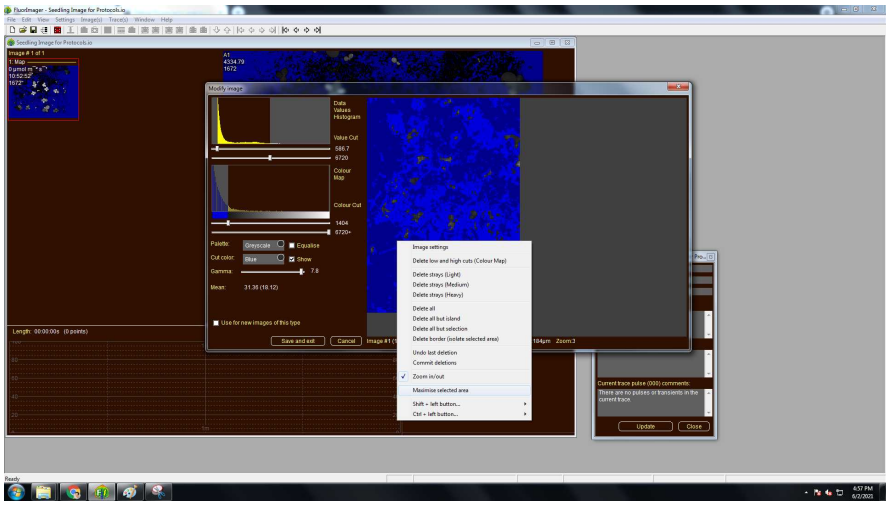
If at any time you accidentally delete a sample, right click on the image and select "Undo last deletion".

The program can be glitchy when initially learning it. Save and exit to your primary FluorImager screen frequently. Reopen editing window using right click and selecting "Modify image".

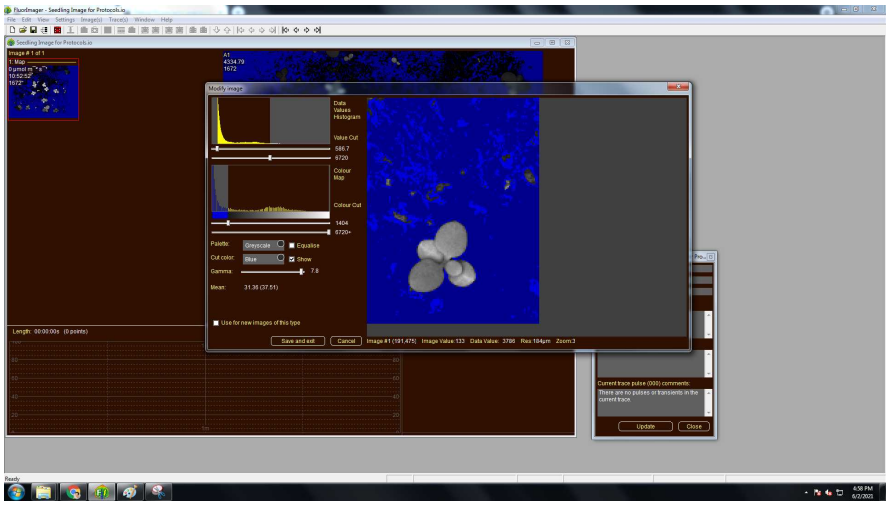
15 Once zoomed in, right click on the image and select "Delete all". This will only delete everything highlighted in the zoomed window.



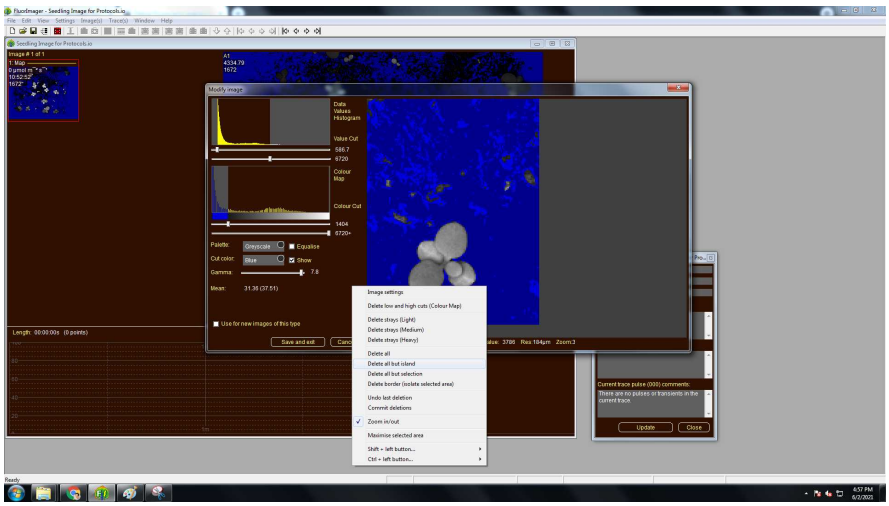
16 Hold shift and control simultaneously and left click with the mouse to zoom back out. Alternatively, right click the image and select "Maximize the selected area" to return to the full image for editing. If an area is only highlighted and not being zoomed, right click on the image and verify that "zoom in/out" has a check mark next to it in the dropdown menu.



17 Once the noise in the open spaces has been removed, noise near and attached to the plant or leaf disks will need to be removed. Select and zoom into an area that contains only a single sample (plant or leaf disk) using Shift + Ctrl + Left Mouse Click. If more than one sample is selected, zoom again until only a single sample is in the image window.



18 Right click directly on the plant or leaf disk, select "Delete all but island" to remove all pixels that aren't contiguous with the pixel you selected in the currently selected zoom window.



19 If there is noise touching the sample, hold shift while using the left mouse click to remove individual pixels. Draw a blue isolation border around your sample using the left mouse click while holding shift. After an isolation border has been drawn, right click the sample and "Delete all but island". If the noise does not disappear, there is still a pixel touching the sample. It may be a diagonal pixel.

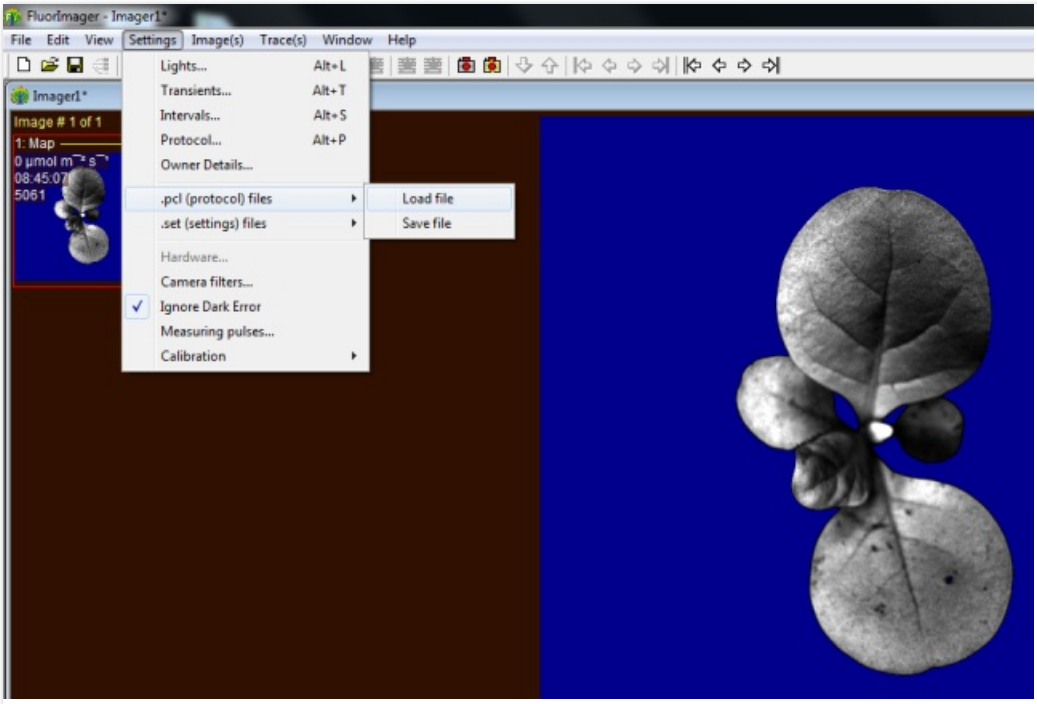
20 If there is noise around the edges of the image that cannot be highlighted using the zoom and delete all features, hold control and left mouse click on the noise to delete a noise group, the pixel you clicked and any contiguous pixels.

21 Right click anywhere on the image and highlight "Delete strays (Heavy)" again to remove any stray pixels that may have been created during noise deletion. At this point only the desired samples should remain in the image. Select "Save and Exit".

Once the protocol begins, the camera will image the entire photo. As long as "Image 1: Map" shows the properly isolated samples, the additional captured data will not be processed.

Load the Protocol

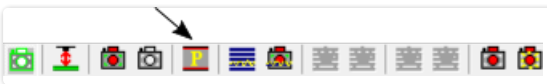
22 Load protocol



23 Highlight any steps that require changing and use the display on the left to change the desired parameters.

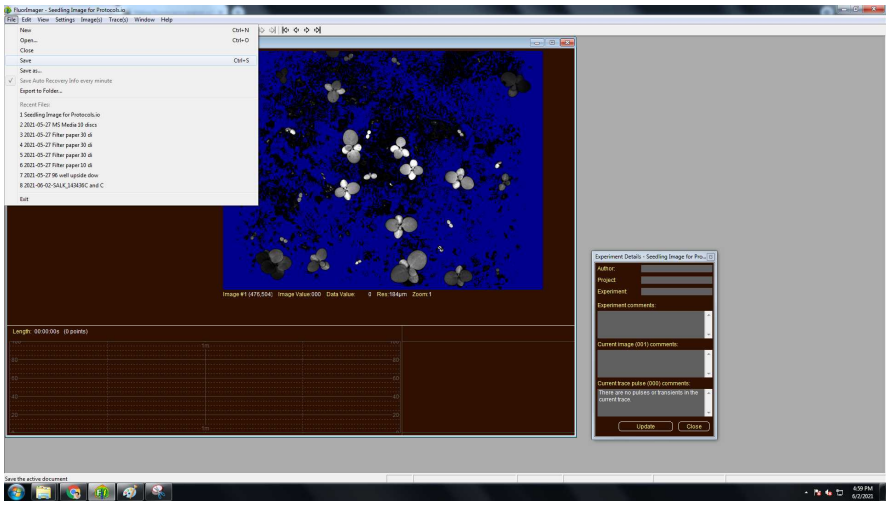
23.1 "Change actinic" adjusts the amount of light that is output by the lamp.
"Simple loop" repeats the protocol from a designated step back to the command simple loop a prescribed number of times.
"Apply pulse" adjusts the frequency and intensity of the saturating light.

24 Once you are happy with the scheduled program click on the protocol icon in the toolbar (black arrow below) to start the run



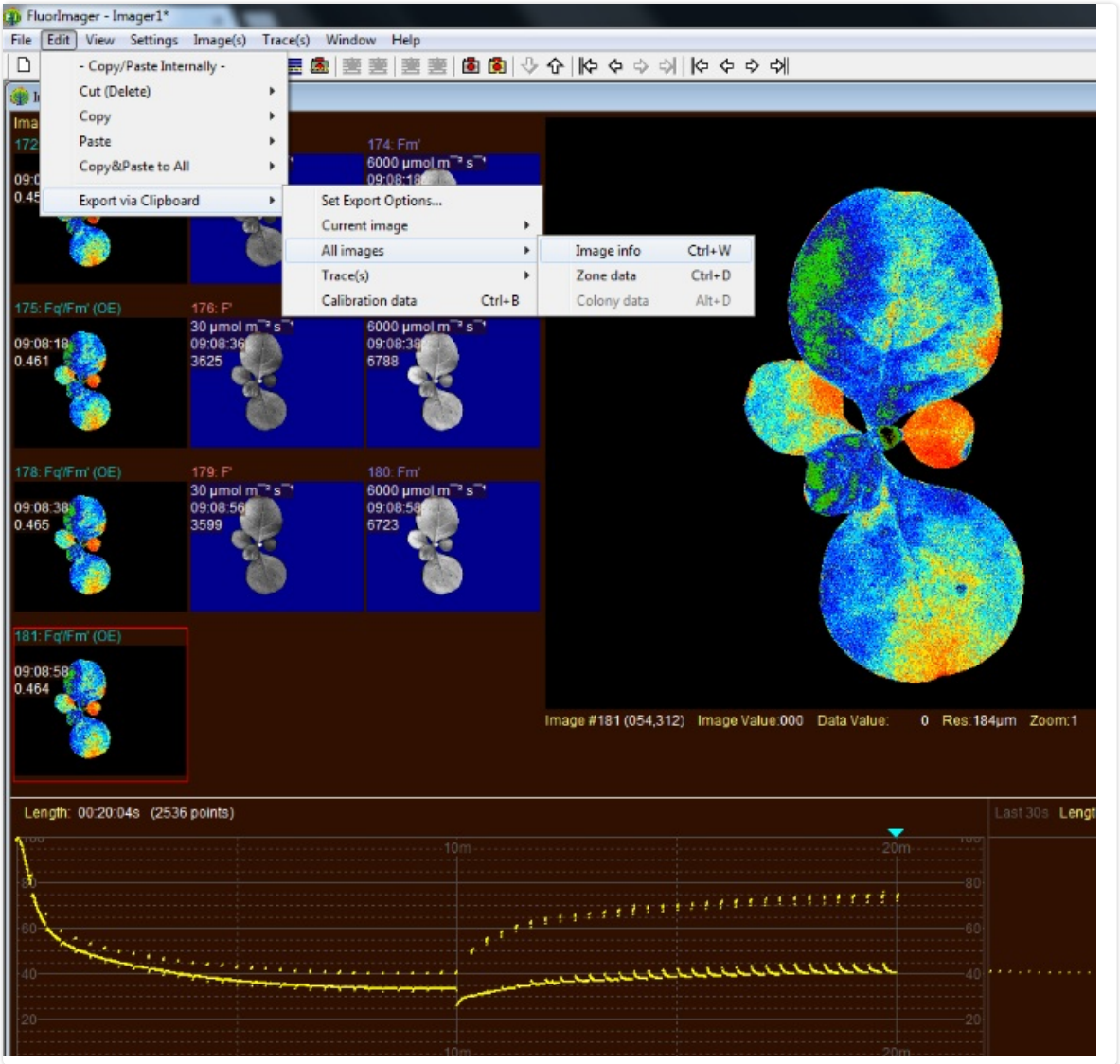
25 Save the data after each sample analyzed.

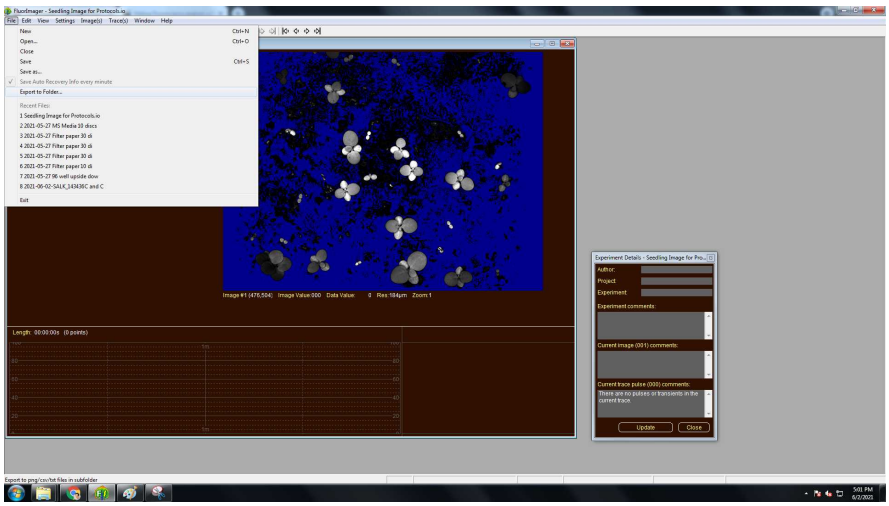
The imager can only hold ~450 images. If more images are collected on a file, the protocol will automatically abort.



If you forgot to apply isolation prior to starting the protocol, isolation can be applied to the captured images using the same instructions as above. Apply isolation prior to exporting the data for processing.

26 Export data via "Edit > Export via Clipboard > All Images > Image Info":. Alternatively, export the data via "File > Export to Folder"





27 After saving and exporting the set of data for that sample, close out of the current file using the small red X in the right hand corner of the display window. Open a new file for the next sample by selecting "File > New" or by pressing the white paper in the upper left of the toolbar. Always save the data and open a new data file for each sample.

The imager does not delineate between samples. It will just continue adding images on sequentially to your current data file which makes processing the data difficult. It also maxes out around 450 images. If you run multiple samples in the same .igr data file, the machine may run out of storage and abort the protocol.

It is best practice to save each samples images generated from a single protocol as a single .igr file and then open a new individual .igr file for each new individual sample.