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Copy of Fluorescence analysis using CF imager-v2

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Steven J Burgess[1]

[1]University of Illinois at Urbana-Champaign

Steven J Burgess
University of Illinois at Urbana-Champaign

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Protocol status: In development
We are still developing and optimizing this protocol

Created: June 27, 2019

Last Modified: June 27, 2019

Protocol Integer ID: 25185

Keywords: copy of fluorescence analysis, fluorescence analysis, fluorescence, using cf

Troubleshooting

Preparing plants

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

Note

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 properlly 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.

Prepare an extra plate with few leaf disks(or have extra plants/leaf disks) to adjust the focus (on step 5). During the focus adjustment, the light flashes inside the chamber, so you should not use the plates that are with yourdark-adapted samples.

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

cf imager
technologica
CE

3 Start the FluorImager software

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. Position plant/leaf in the chamber

Set focus

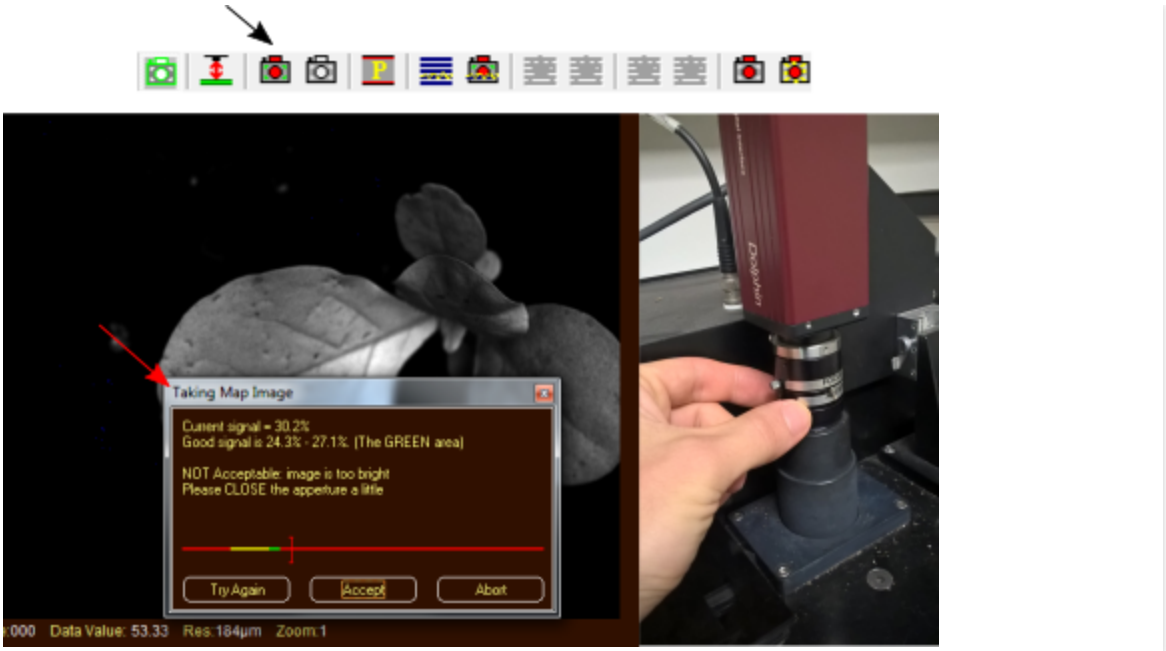
5 Before initiating this step, place the extra plate/plants inside the chamber.

Set the focus by adjusting the dial above the chamber, and lock in position by turning the screw on the side.

Set exposure

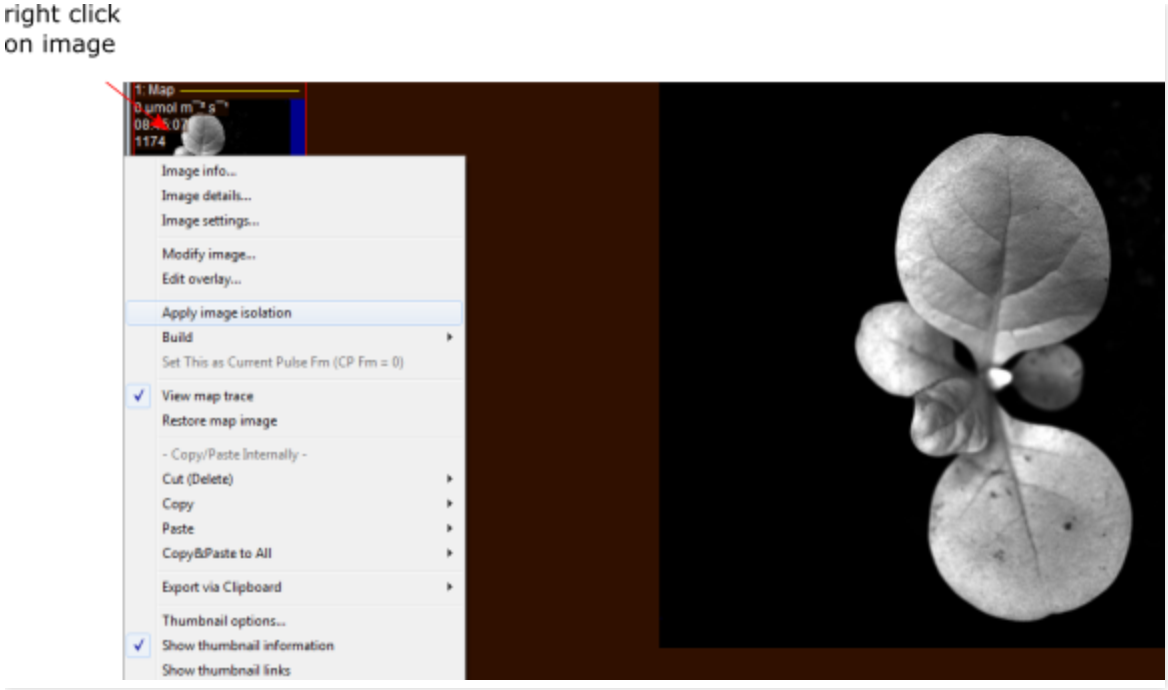
6 Maually adjust the apeture as shown on the right to allow and optimal amount of light into the imager so as not to overexpose measurements.

After adjusting the focus and press the "map image" button, change the plate/plant to the one you are going to read. If you don't press "map image", the light will be still flashing when you open the chamber.

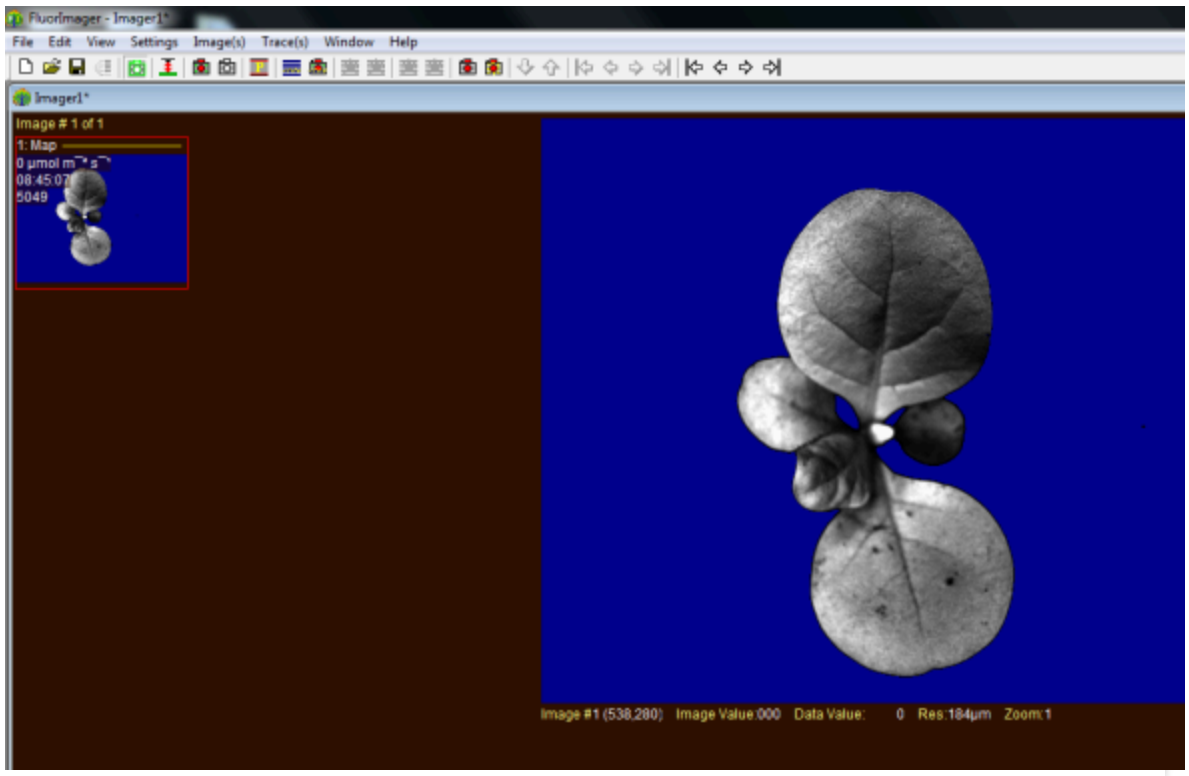


7 Isolate the plant or leaf of interest

Applying isolation background - it can be done more than once if the image is still with some background

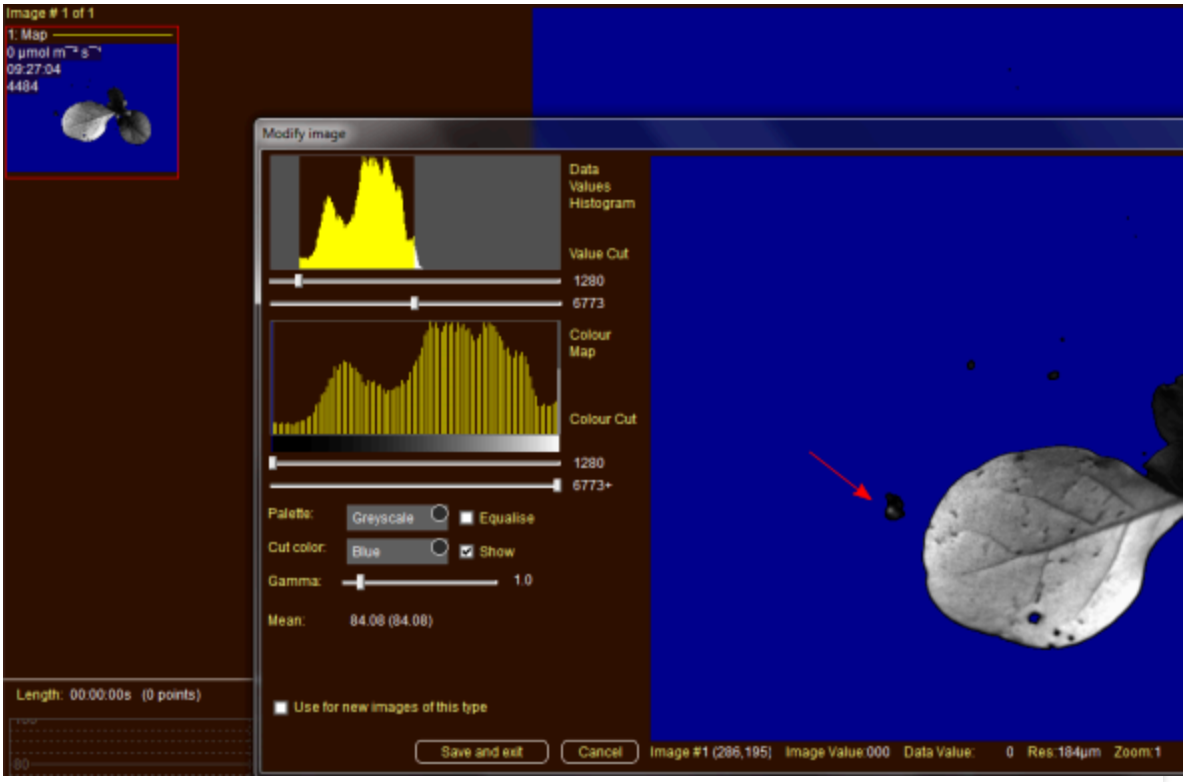


After applying isolation background areas will be masked out in blue as shown below.

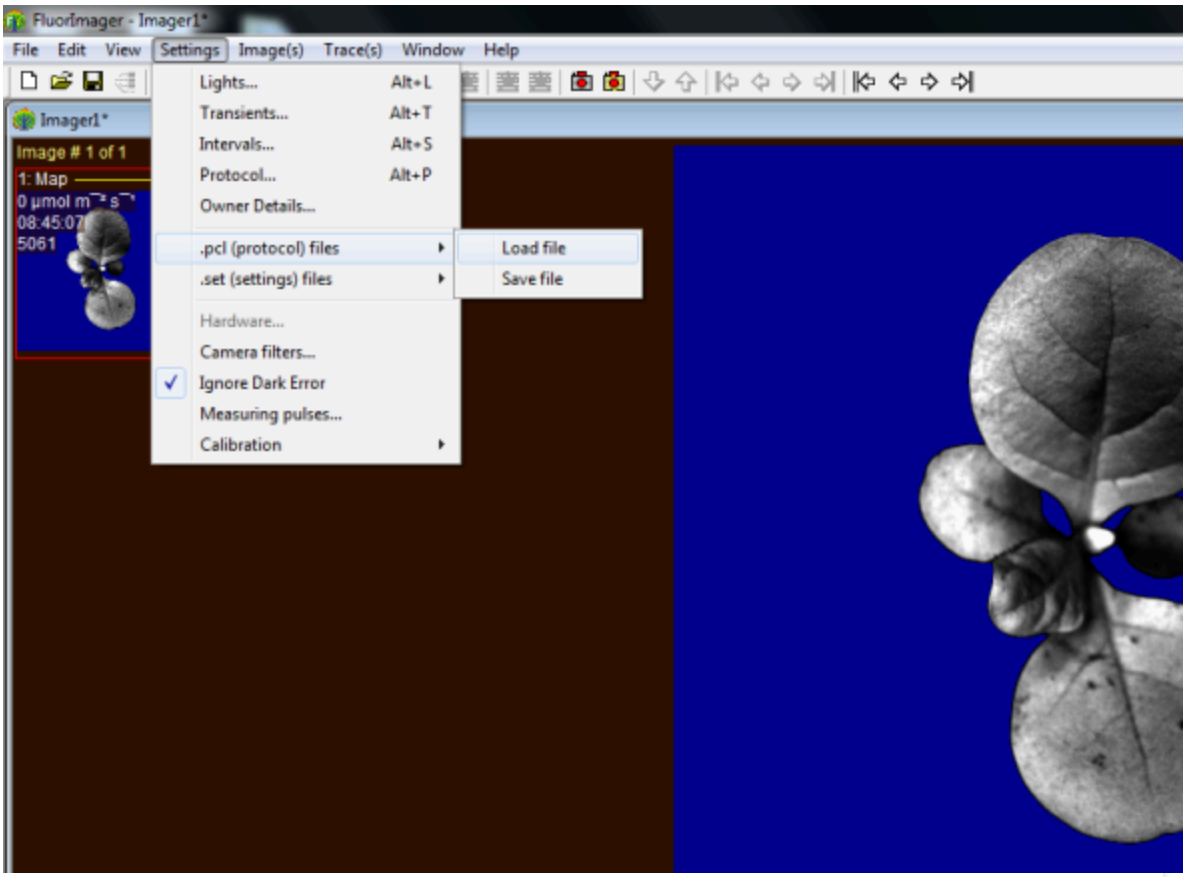


8 Remove residual noise from image

Sometimes the software picks up background noise as real signals, shown below as black dots on the blue background. It is advisable to mask these, otherwise they will be counted as a separate 'colony' during analysis and measurements will be recorded for each of these spots in the final data sheet. Noise can be masked by moving the cursor over the dot, pressing CTRL+left click simultaneously.



9 Load protocol



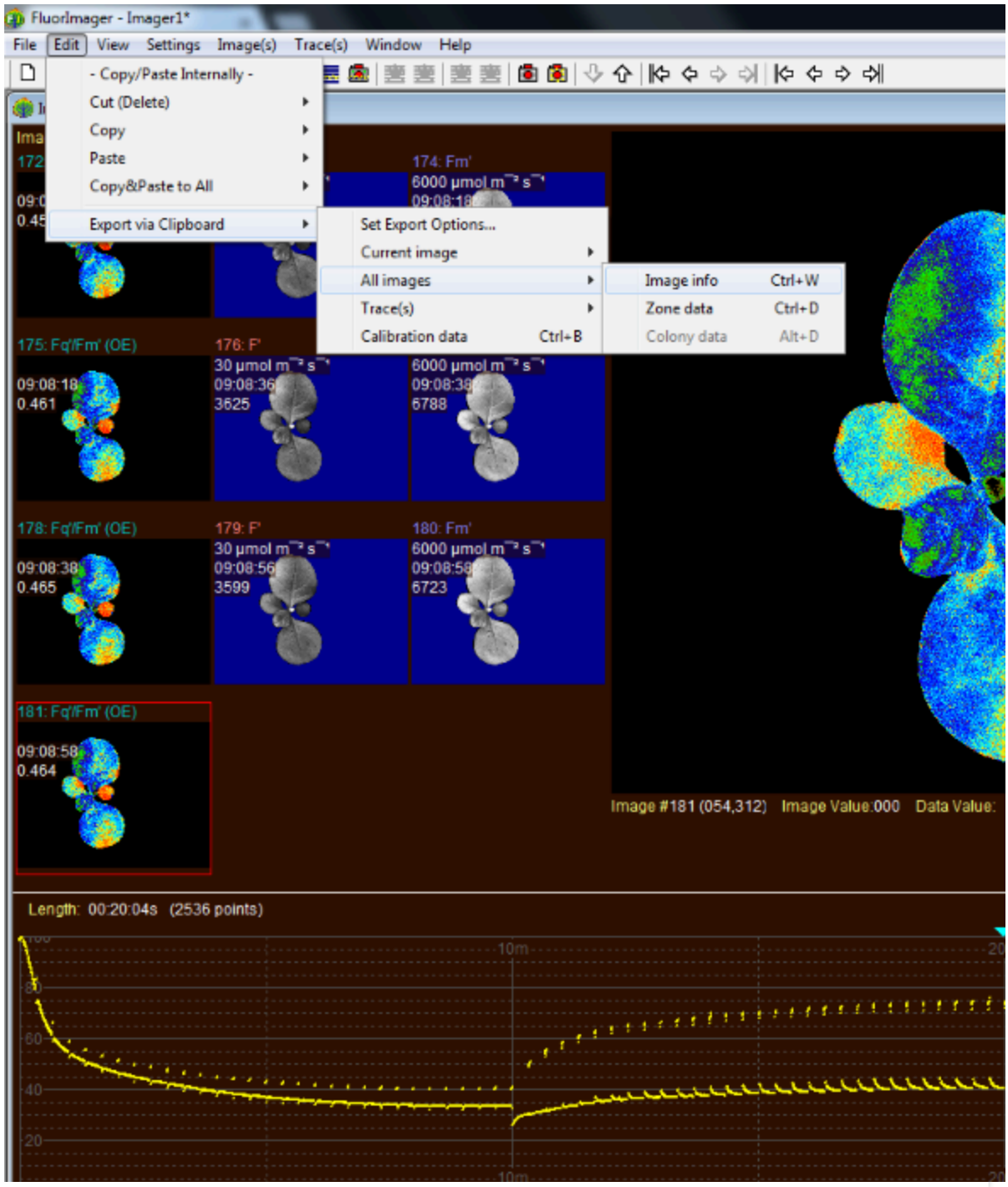
10 If the protocol needs change, press Settings > Protocol. Use the left menu to adjust the parameters you want to.

11

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

13 Export data

After the reading is complete, save the images (File > Save As).
To obtain the files to process the images, go to File > Export to Folder. It will save in the folder that is opened.


FluorImager - Imager1*
File Edit View Settings Image(s) Trace(s) Window Help
- Copy/Paste Internally -
Cut (Delete)
Copy
Paste
Copy&Paste to All
Export via Clipboard
Set Export Options...
Current image
All images
Trace(s)
Calibration data Ctrl+B
Image info Ctrl+W
Zone data Ctrl+D
Colony data Alt+D
174: Fm'
6000 µmol m⁻² s⁻¹
175: Fq'/Fm' (OE)
09:08:18
0.461
176: F'
30 µmol m⁻² s⁻¹
09:08:36
3625
6000 µmol m⁻² s⁻¹
09:08:38
6788
178: Fq'/Fm' (OE)
09:08:38
0.465
179: F'
30 µmol m⁻² s⁻¹
09:08:56
3599
180: Fm'
6000 µmol m⁻² s⁻¹
09:08:58
6723
181: Fq'/Fm' (OE)
09:08:58
0.464
Image #181 (054,312) Image Value:000 Data Value:
Length: 00:20:04s (2536 points)
10m
60
40
20