Jul 18, 2018

Fluorescence analysis using CF imager

DOI
dx.doi.org/10.17504/protocols.io.q4hdyt6

Steven J Burgess[1]

[1]University of Illinois at Urbana-Champaign

Steven J Burgess
University of Illinois at Urbana-Champaign

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.q4hdyt6

Protocol Citation: Steven J Burgess 2018. Fluorescence analysis using CF imager. **protocols.io https://dx.doi.org/10.17504/protocols.io.q4hdyt6**

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

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

Created: June 18, 2018

Last Modified: August 14, 2018

Protocol Integer ID: 13161

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.

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

cf imager
technologica
CE

3 Start the FluorImager software

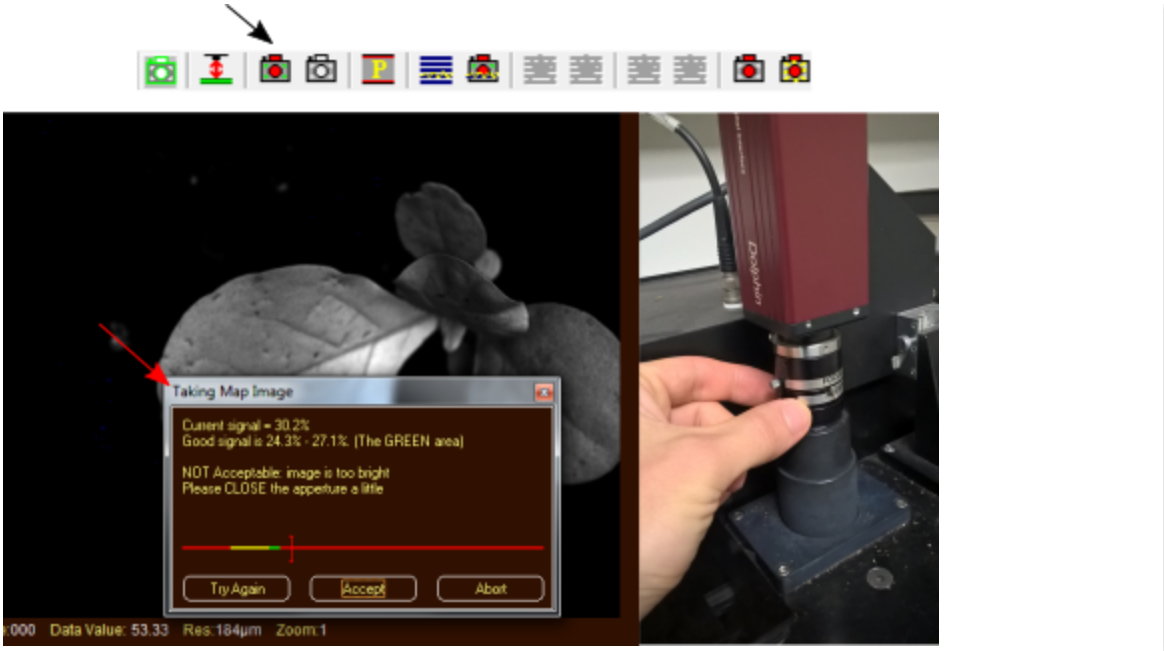
4 The surface of the leaf should be 140mm from the base of the imaging chamber, and can be adjusted by lowering or raising the plant under analysis. Position plant/leaf in the chamber

Set focus

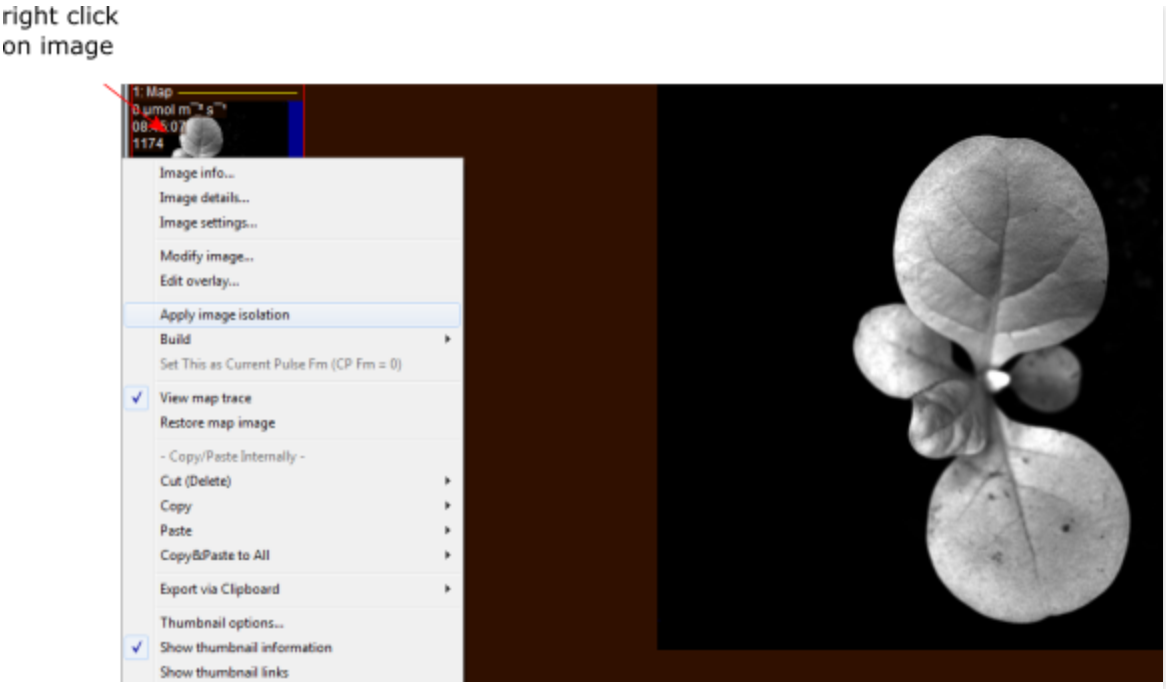
5 Set the focus by adjusting the dial above the chamber, and lock in position by turning the screw on the side.

Set exposure

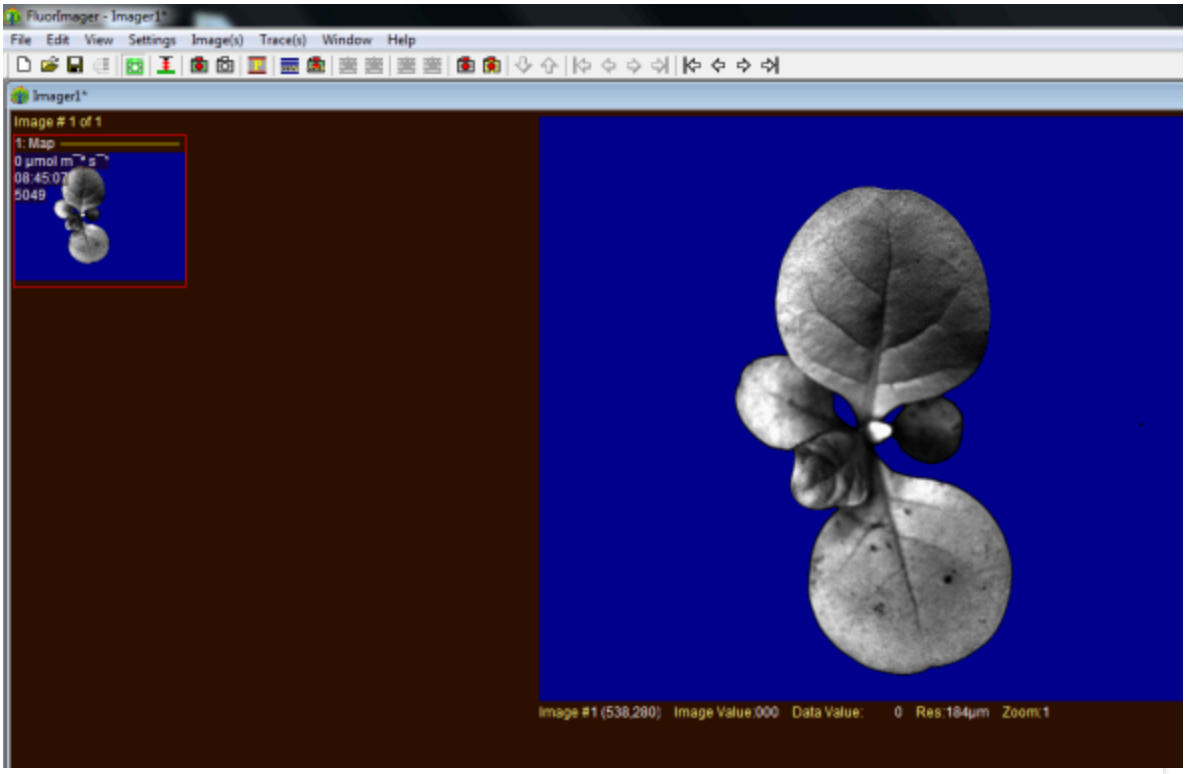
6 Maually adjust the apeture as shown on the right to allow and optimal amount of light into the imager so as not to overexpose measurements



7 Isolate the plant or leaf of interest

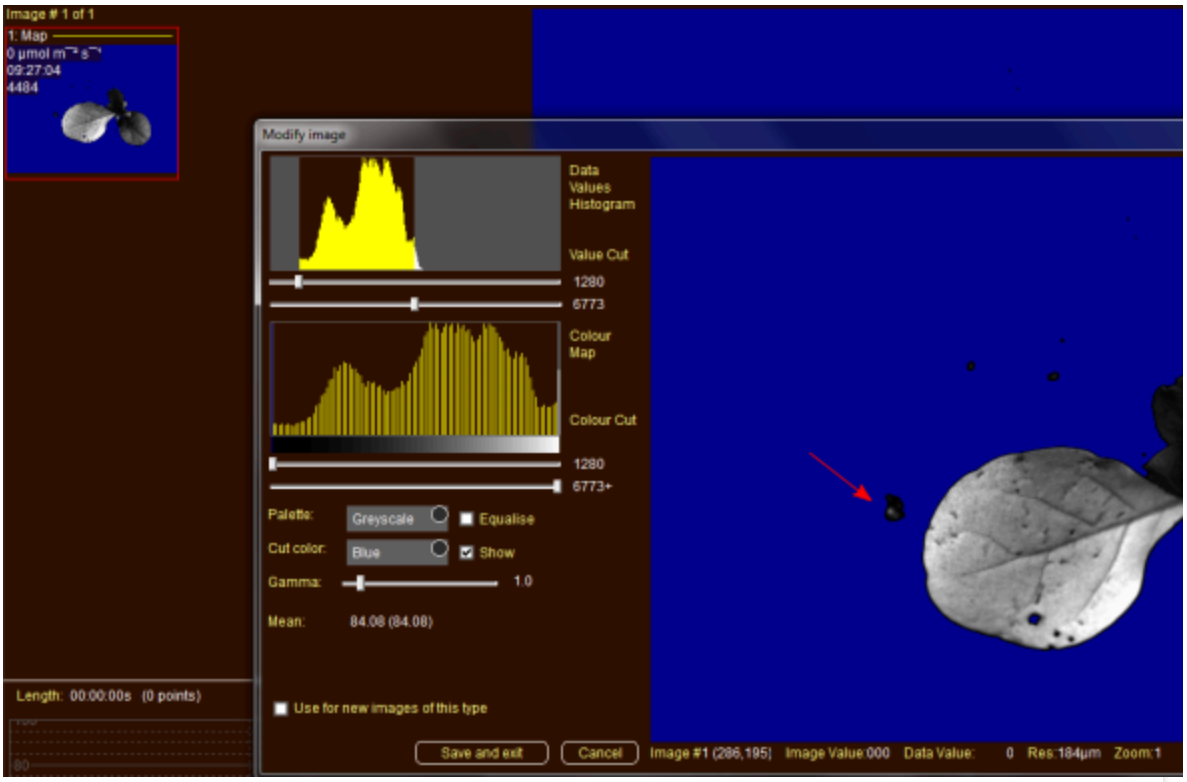


After applying isolation background areas will be masked out in blue as shown below.

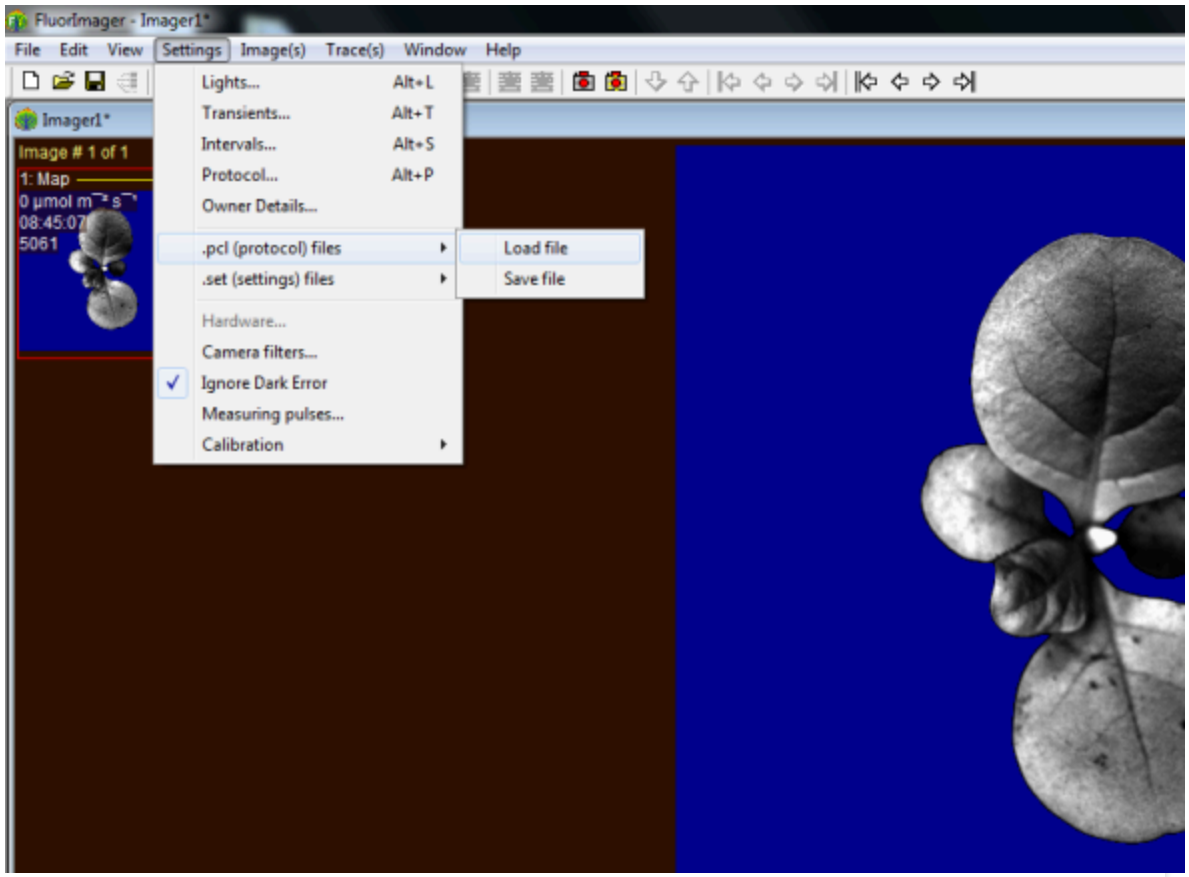


8 Remove residual noise from image

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


FluorImager - Imager1*
File Edit View Settings Image(s) Trace(s) Window Help
Lights... Alt+L
Transients... Alt+T
Intervals... Alt+S
Protocol... Alt+P
Owner Details...
.pcl (protocol) files
.set (settings) files
Load file
Save file
Hardware...
Camera filters...
Ignore Dark Error
Measuring pulses...
Calibration
Imager1*
Image # 1 of 1
1: Map

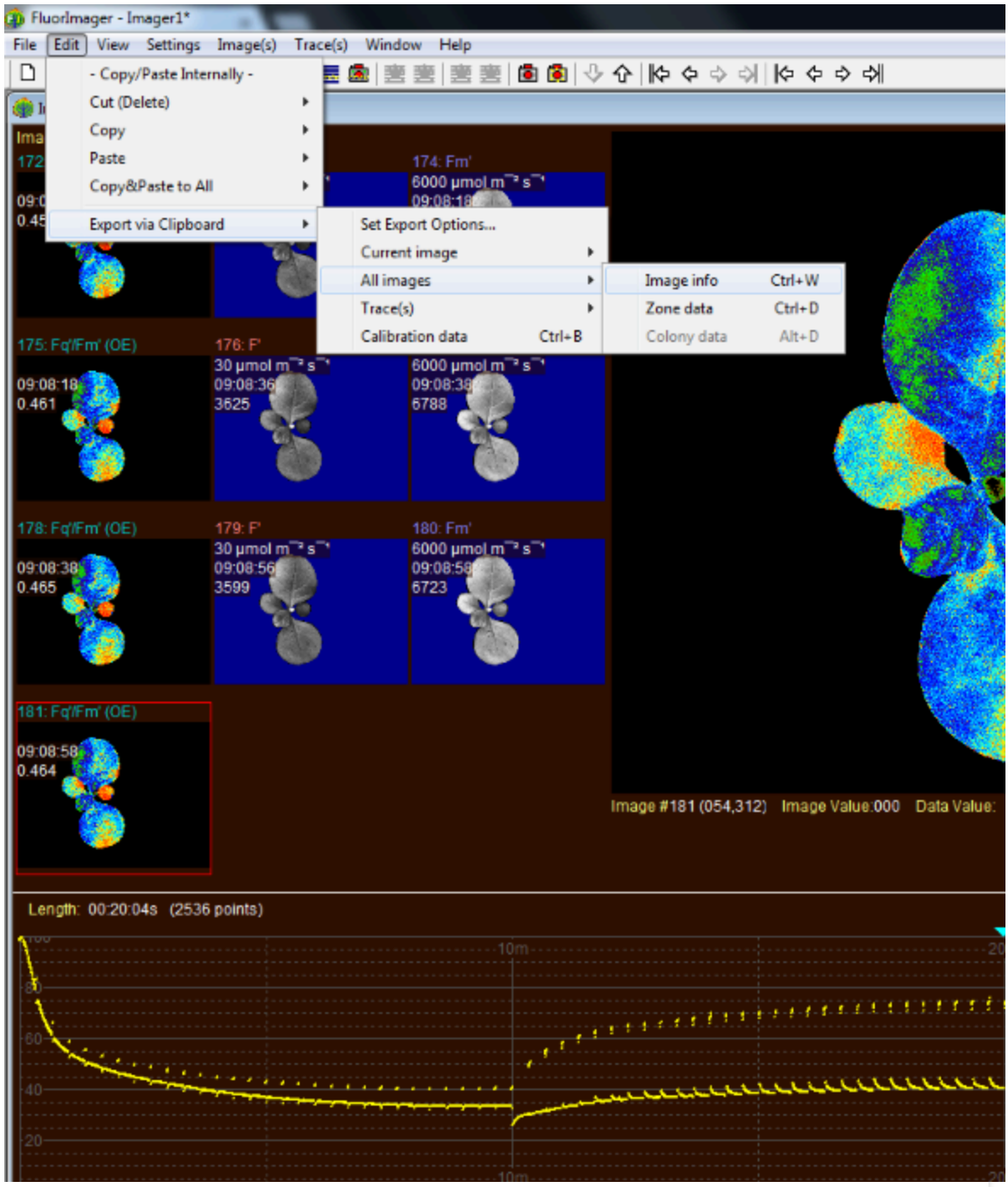
10

11

Protocol
Line 1/4 - Stopped
After a delay of
min
s
Perform the following action:
Change actinic
Apply pulse
Apply transient
Auto pulse
Simple Loop
Details:
% max.
Actinic PPFD
1000
16.3
Update This
Add to End
Insert After
Delete All
Cut
Copy
Paste
Line
Delay
Action
Cycles
1 0 s Change Actinic -
2 20 s Apply Pulse 30
3 0 s Change Actinic -
4 20 s Apply Pulse 30
Line 1/4 - Stopped
Value:000 Data Value: 0 Res:184µm Zoom:1

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


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