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Fluorescence analysis of leaf discs using CF imager

Forked from Fluorescence analysis using CF imager

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Protocol status: Working
We use this protocol and it's working

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Last Modified: September 01, 2018

Protocol Integer ID: 13912

Prepare leaf discs

1 After each plate is full of leaf dics in the field, the plate should be wrapped in tin foil to prevent exposure to the sun, this dark adapts them. When inside and in the dark, unwrap the foil and take the lid off the plate. Make sure that each leaf disc is horizontal. This can be done using a small tool (such as tweezers) which do not have a sharp edge. Double check that the underside of the leaf is facing down. Ideally, the leaf discs will all be at the same height, but due to the difficulty of creating sponges of the same size, the leaf discs may be at different heights within the plate.

Dark adapt plants

2 Wrap the plates up in tin foil (to prevent exposure to light) for an hour. This can be done overnight but increases the risk of the plants going into stress.

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.

Begin using the Software

3 Turn on the computer and scree. Then turn on the cf imager using the switch on the top right of the machiene. Open the FluorImager software which can be found on the desktop.


cf imager
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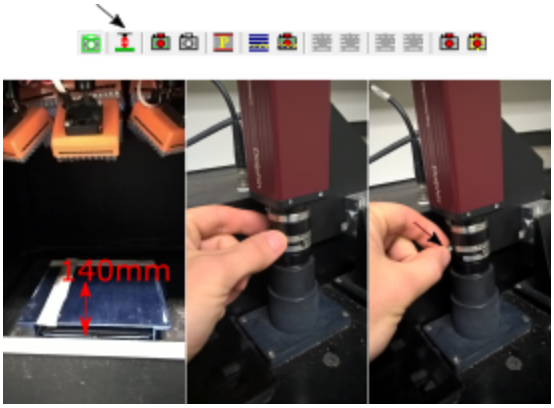
4 Start the FluorImager software by clicing the RED camera button. This will then activate many other options (buttons), as can be seen below.

5 Position the raised platform in the chamber, this can be done by rotating the height controller knob.

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. As there are many leaf discs in each plate, and they may be at different heights. Estimate an average height for the leaf discs and use this.



Put the plate into the chamber

6 Unwrap the plate and take the lid off. Put the plate into the chamber. Position the plate so that it is in the centre. Use tape so that it is easy to precicesly locate where the middle of the raised platform is.

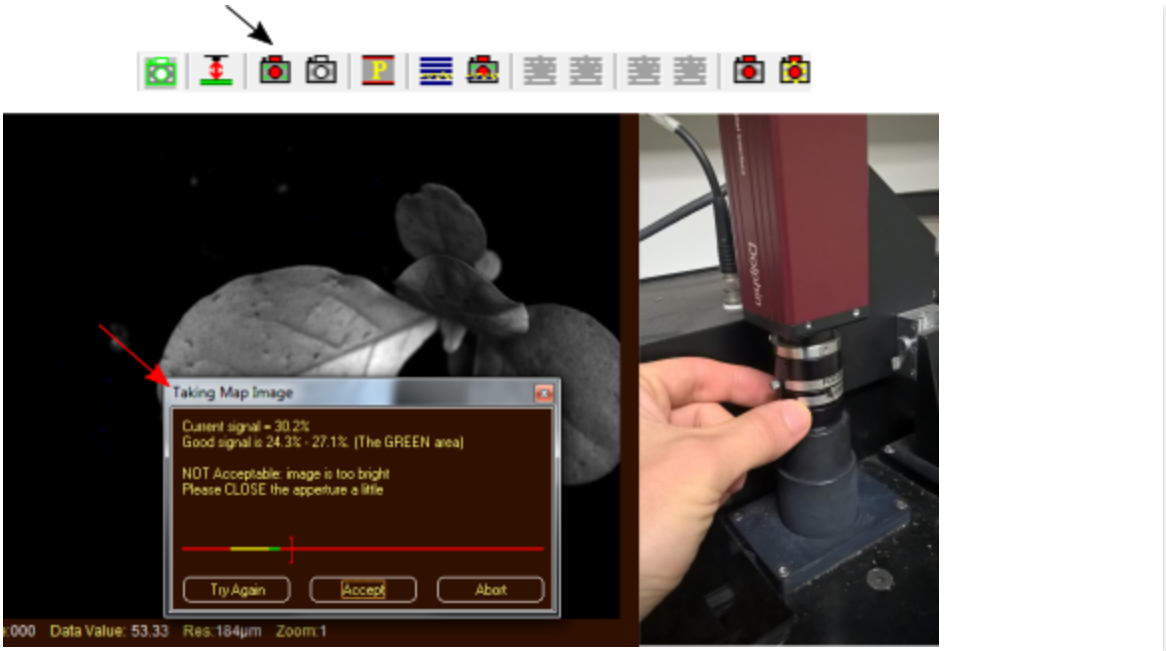
Use a dummy plate with which has leaf discs as a test for the height of the plate, the

Adjust the image focus

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

Set exposure

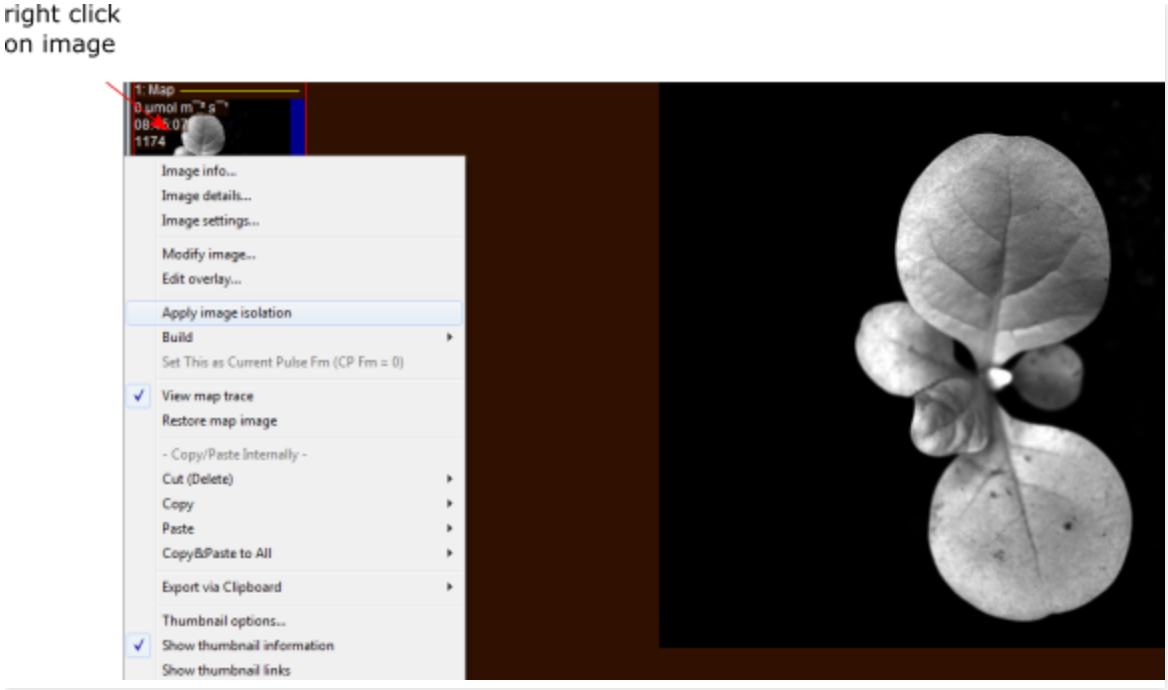
8 Maually adjust the apeture as shown on the right to allow and optimal amount of light into the imager so as not to overexpose measurements



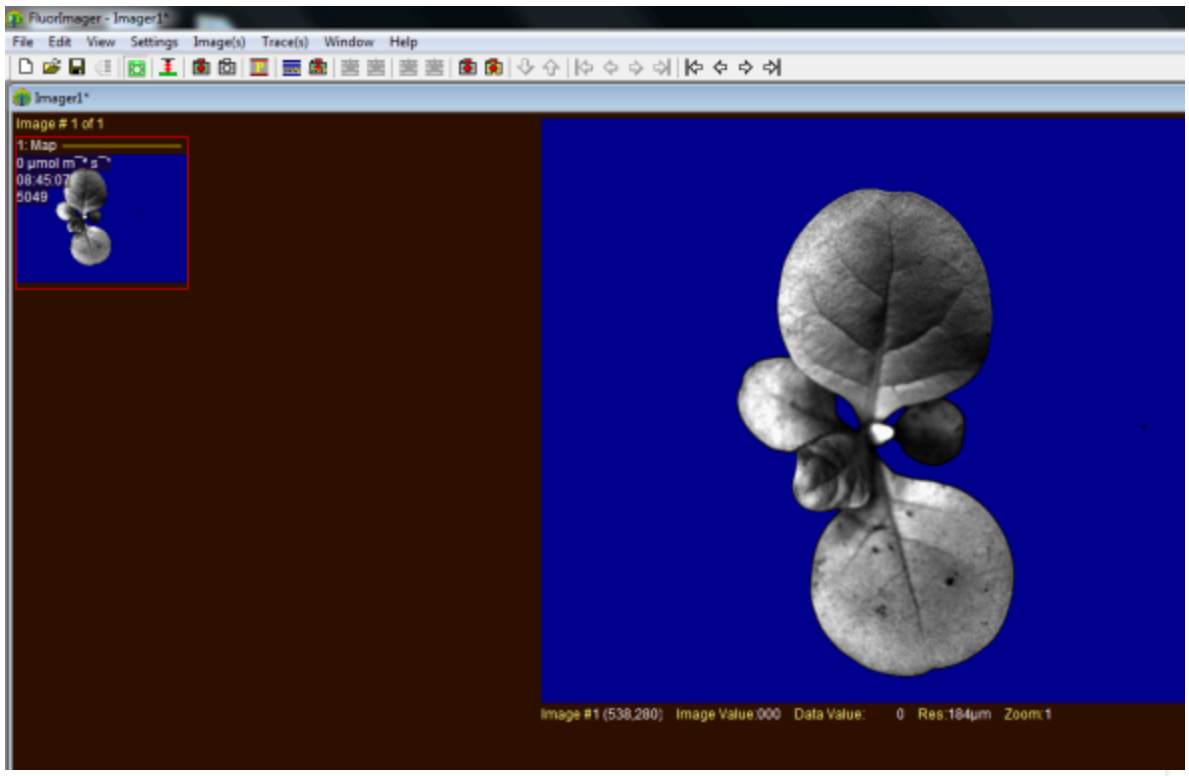
Isolate each leaf disc as a seperate image

9 Isolate the plant or leaf of interest.

Click 'image' and the 'apply image isolation'. This will produce an image where each leaf disc is distinct

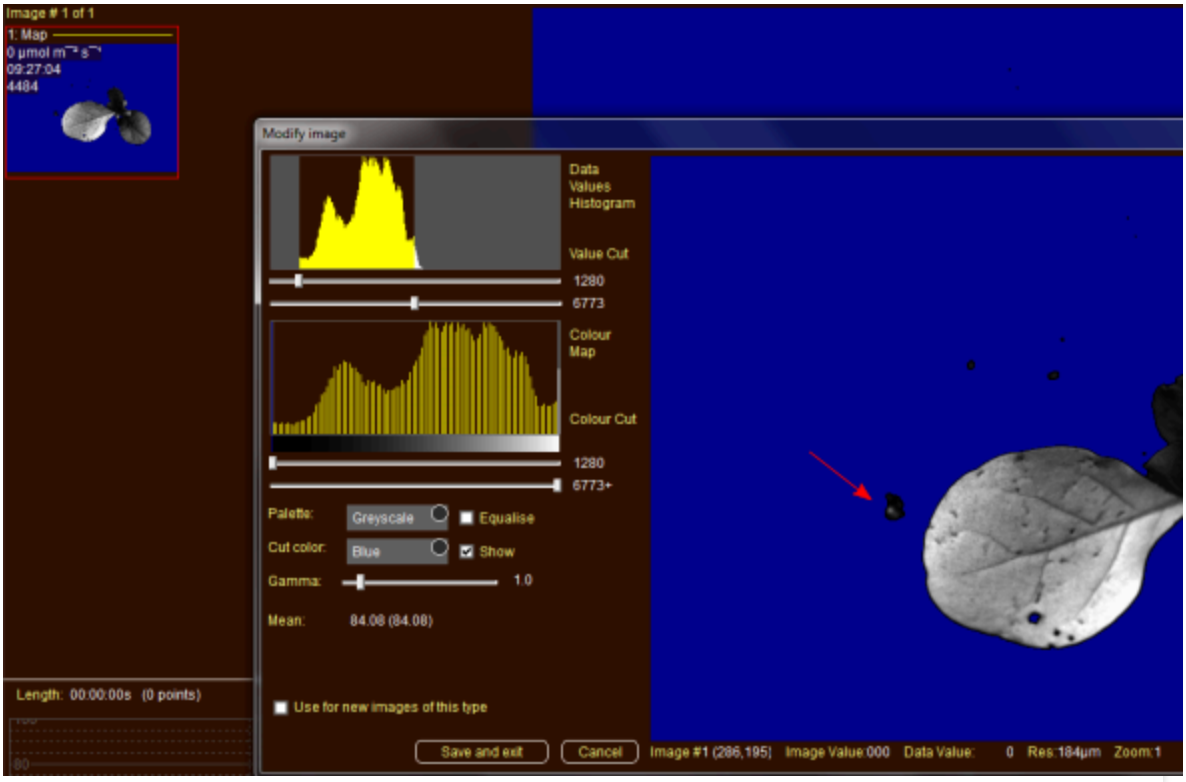


After applying isolation background areas will be masked out in blue as shown below.



10 Remove residual noise from image

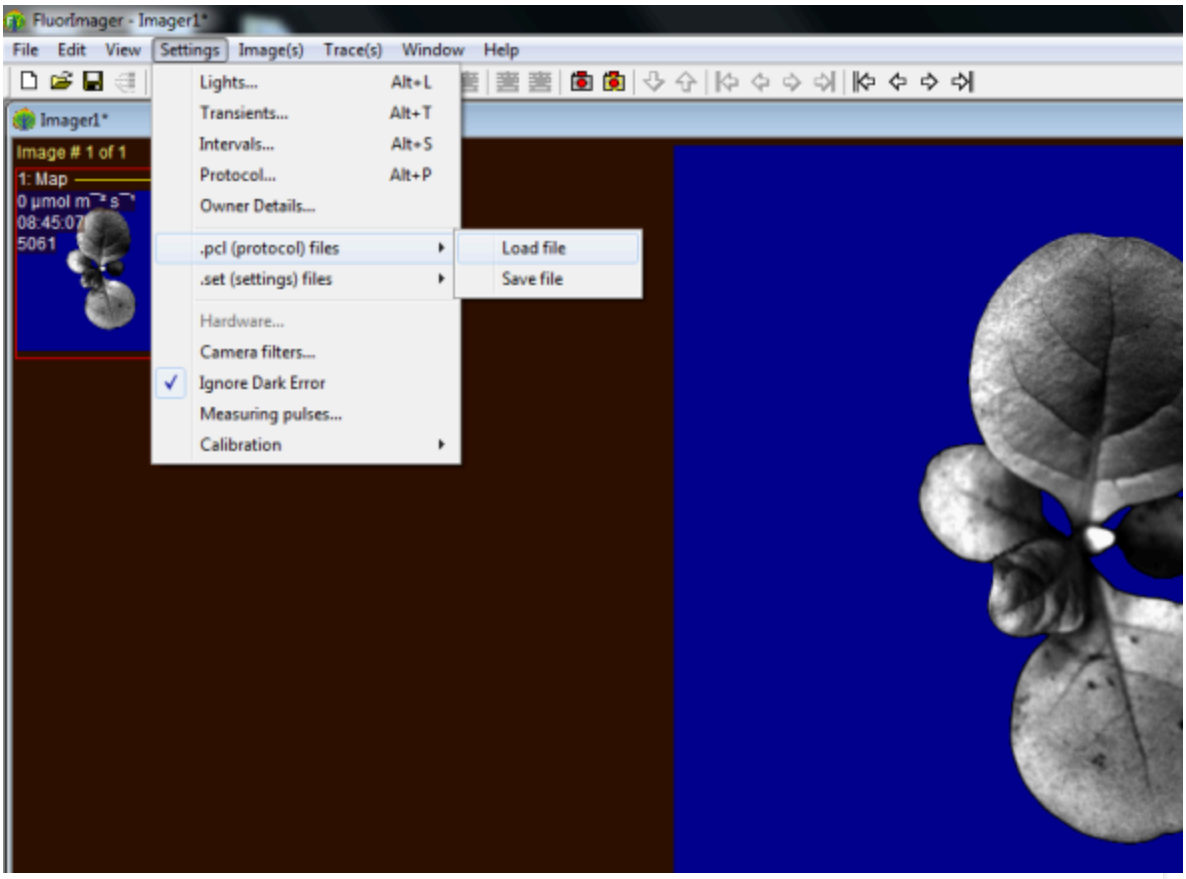
Sometimes the software picks up background noise as real signals, shown below as black dots on the blue background. It is advisable to mask these, otherwise they will be counted as a separate 'colony' during analysis and measurements will be recorded for each of these spots in the final data sheet. Noise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



Begin the protocol

11 Load protocol by clocking the RED 'P' button. This will come up with an automatically loaded protocol, click 'Yes' when it asks for approval, and the protocol will begin.

If a particular or different protocol is needed to the one already on the system, you can upload a protocol by clicking 'Settings', ',pcl (protocol) files', then 'load files' and select the one you require. This is shown in the image below:

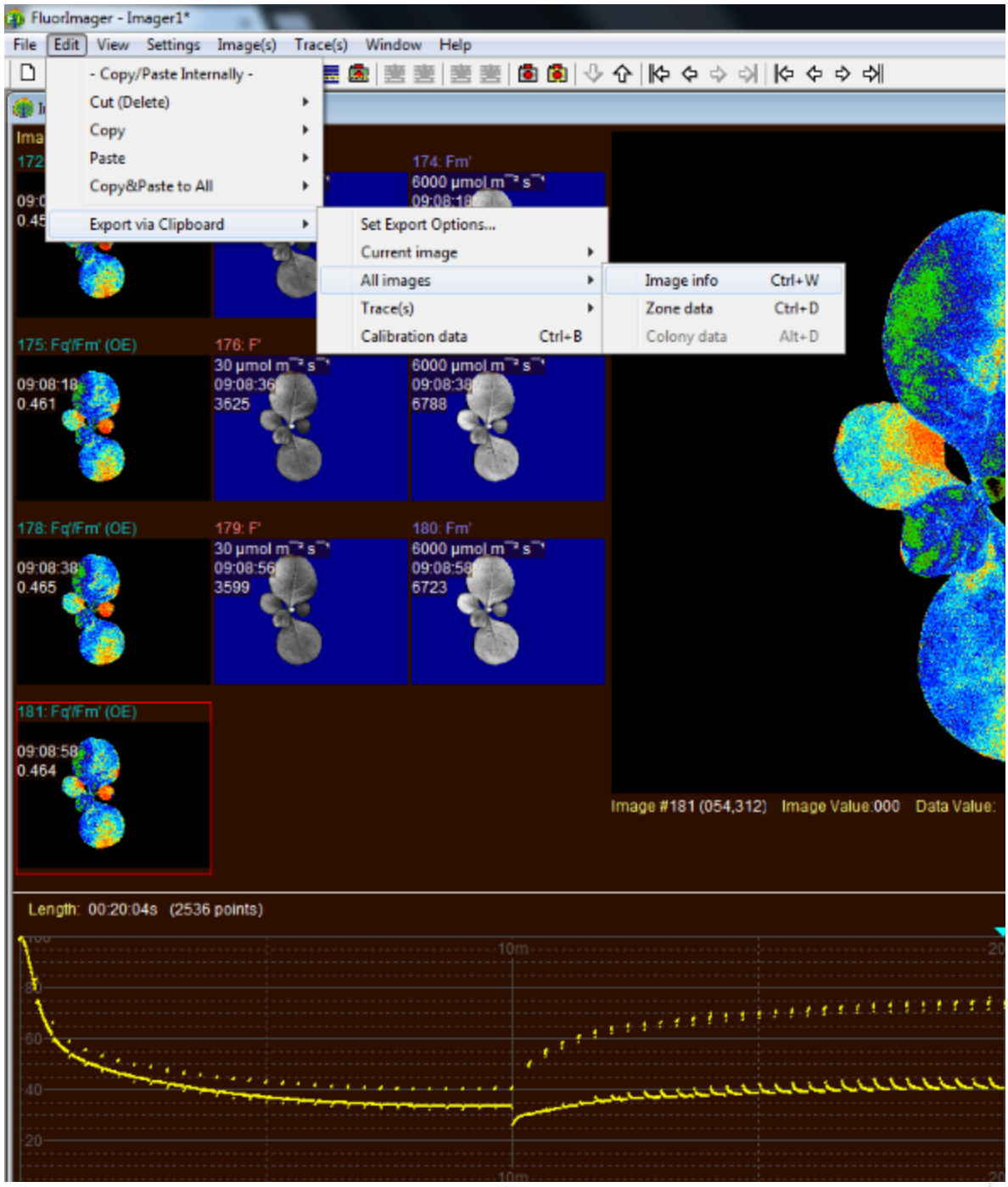


12 The image below shows the progress of the protocol as it runs thorugh. A green line indicates that the protocol is still running, a red line indicates that it has stopped.

Export the data

13 Export data by clicking 'File', 'Save As', and selecting the folder and a name for the data entry.

The image below shows an alternative way of saving the file.


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