Fluorescence analysis using CF imager

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Abstract

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Protocol

Preparing plants

Step 1.

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

NOTES

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.

Step 2.

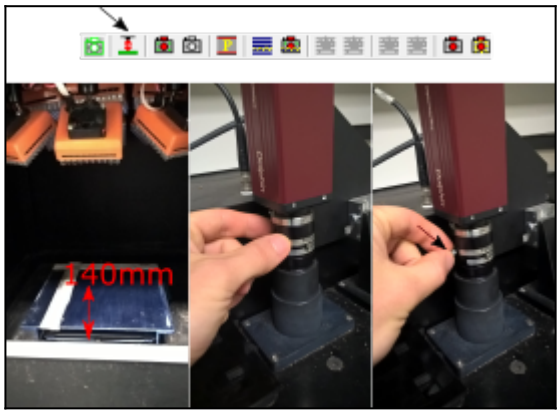
Turn on the cf imager and open the FluorImager software.

Step 3.

Start the FluorImager software

Step 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

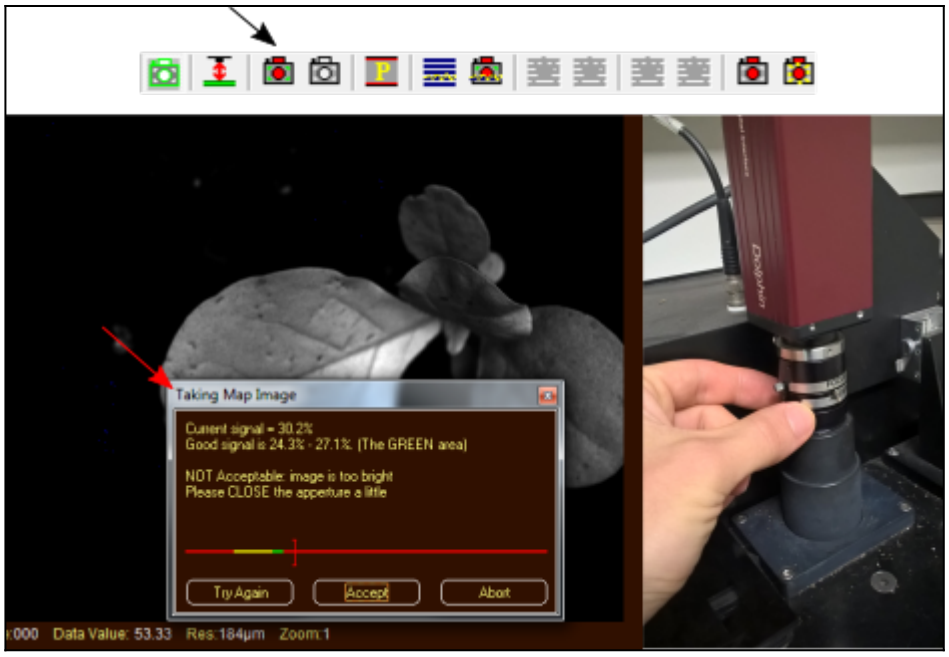
Step 5.

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

Set exposure

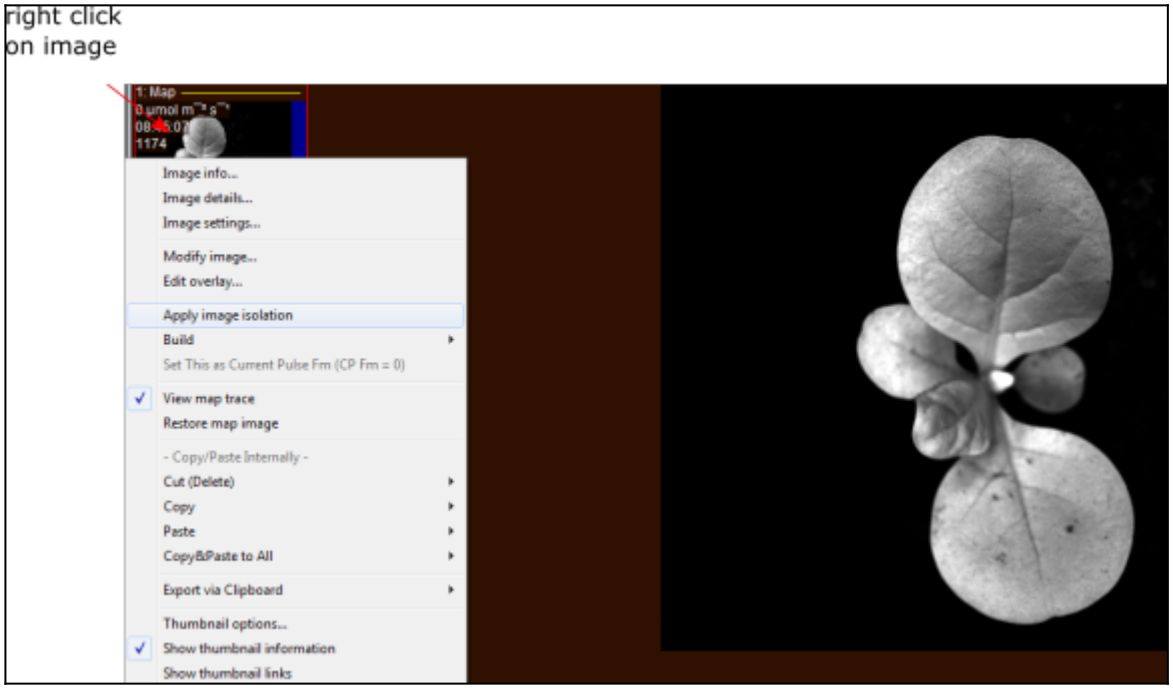
Step 6.

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

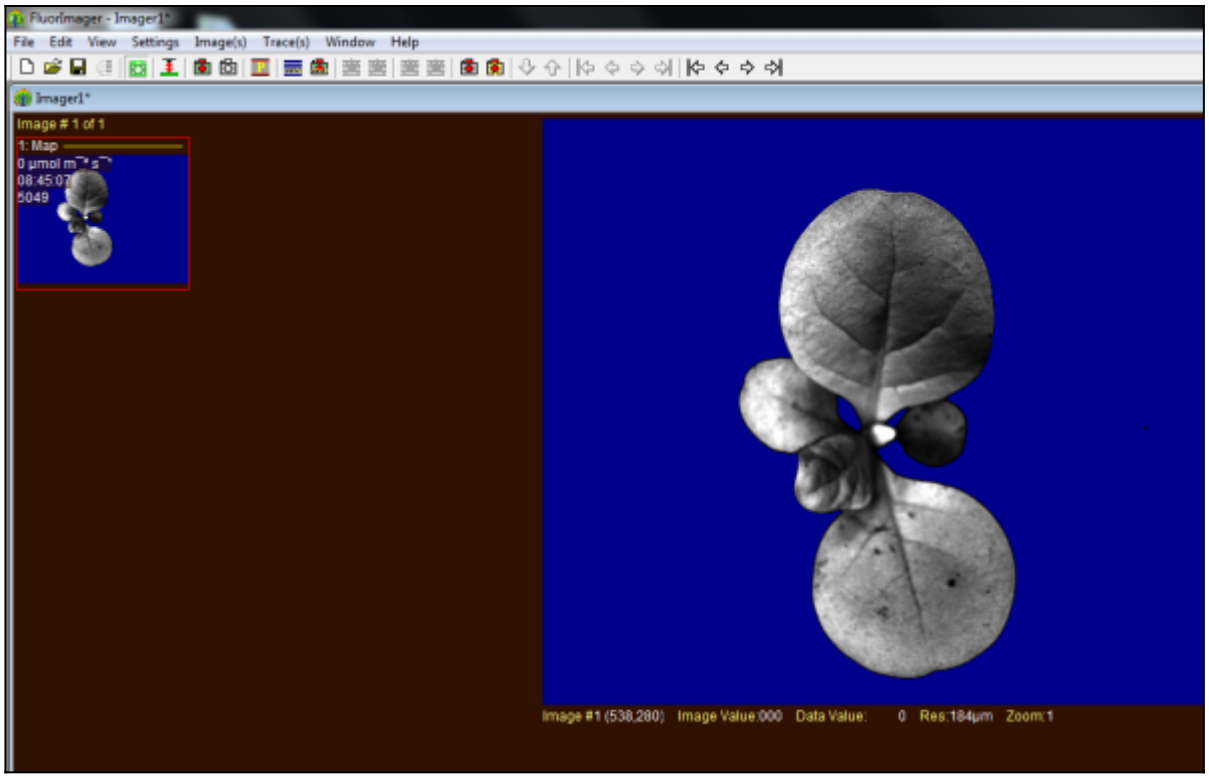


Step 7.

Isolate the plant or leaf of interest



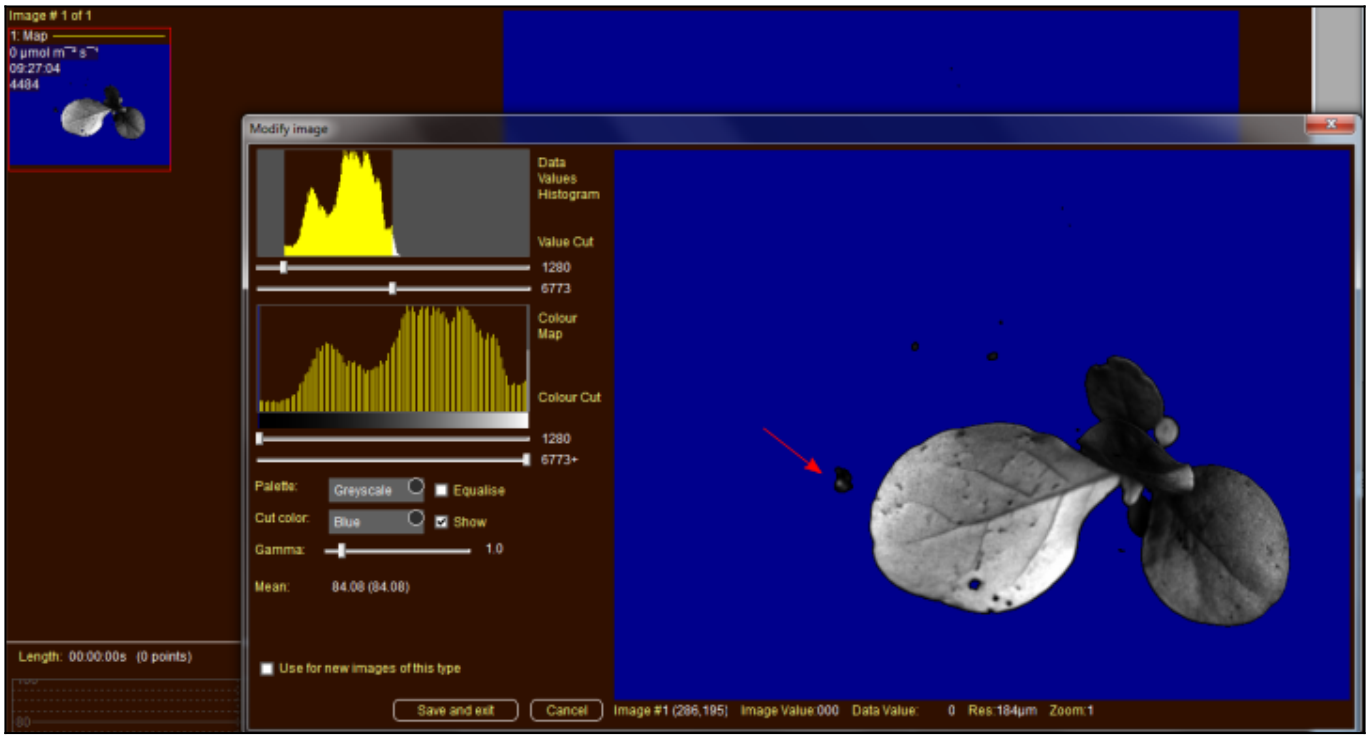
After applying isolation background areas will be masked out in blue as shown below.



Step 8.

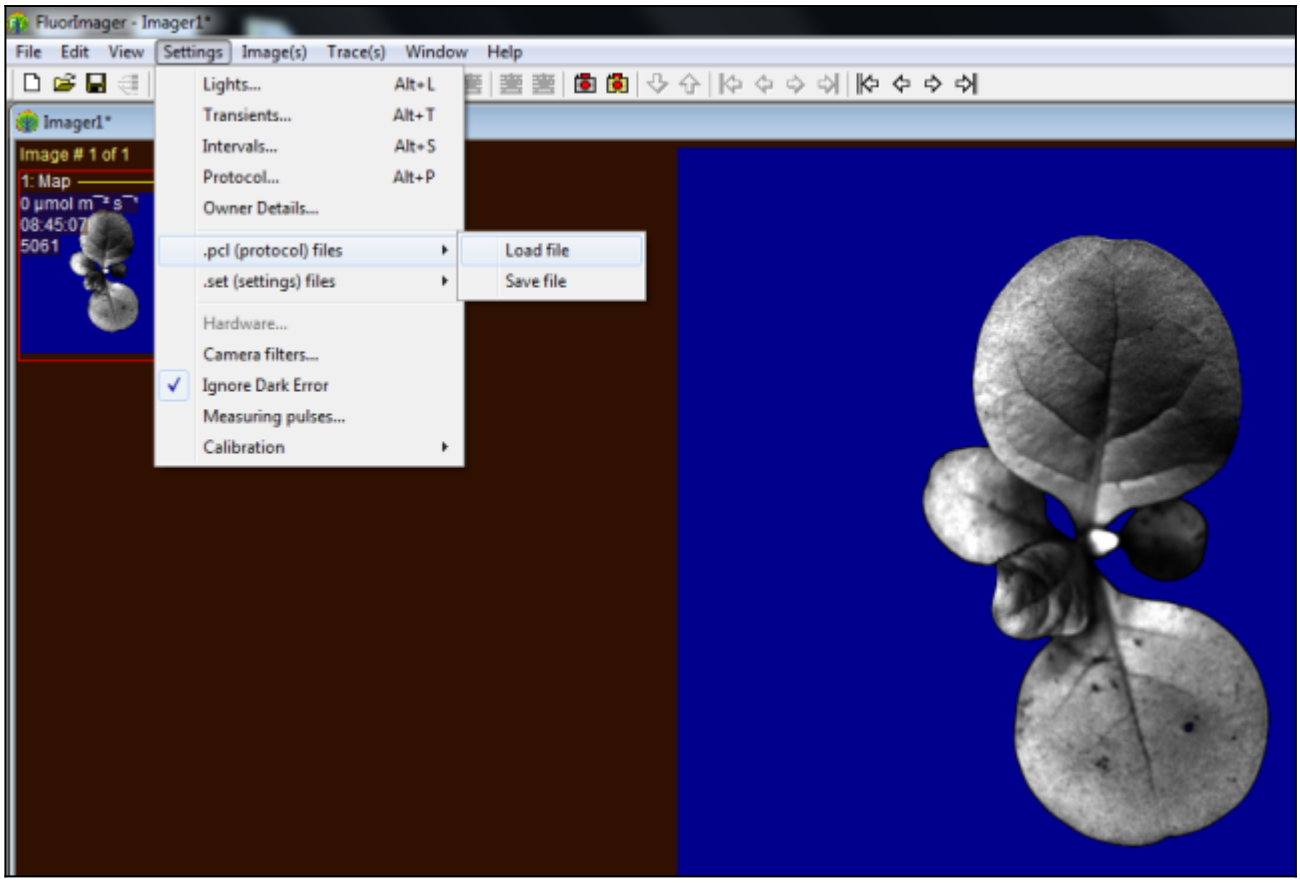
Remove residual noise from image

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.



Step 9.

Load protocol



Step 10.

Step 11.

Step 12.

Once you are happy with the scheduled program click on the protocol icon in the toolbar (black arrow

below) to start the run

Step 13.
Export data