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Fluorescence analysis using CF imager

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Protocol status: In development

We are still developing and optimizing this protocol

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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 properly adapted, healthy plant should give a F_v/F_m 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

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 Manually adjust the aperture as shown on the right to allow an optimal amount of light into the imager so as not to overexpose measurements

- 7 Isolate the plant or leaf of interest

right click
on image

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

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