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Sampling leaf tissue for analysis of NPQ Relaxation using Technologica Chlorophyll Fluorescence Imager Data. V.3

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We use this protocol and it's working

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Abstract

Sampling leaf tissue for analysis of NPQ Relaxation using [Technologica Chlorophyll Fluorescence Imager Data](#).

Materials

- Water, tap.

Do not use distilled or MilliQ water for sampling or plating. Both cause unnecessary osmotic stress that may interfere with measurements.

- 24 Well Tissue Culture Plates (75/cs), ThermoFisher 142475
- #2 Humboldt Cork Bore, ThermoFisher S50166D.
- #7 Humboldt Cork Bore, ThermoFisher S50166D.
- Solid rubber stopper, ThermoFisher **14-130W**.
- 96 well microplate, Thermo Scientific™ Nunc™ MicroWell™, Nunclon Delta-Treated, Flat-Bottom
- 100-Pack of Premium Nasal Aspirator Hygiene Filters, Replacement for NoseFrida Nasal Aspirator Filters, BPA, Phthalate & Latex-Free: CUT IN HALF. Some brands autofluorescence. Test each new lot before using.
- Tweezers
- Parafilm
- Scissors
- Dark box or cabinet
- Long cotton swab
- Aluminum foil

Safety warnings

- ❗ Complete the required greenhouse or farm safety training before performing work in those locations.

Leaf Tissue Sampling

- 1 Cut #1 Whatman filter paper into small squares that are just bigger than the well on a 24-well culture plate.
- 2 Use the #7 cork borer to push two small squares of #1 Whatman filter paper into each well of the 24-well culture plate.



- 3 Wet the filter paper using tap water. The filter paper should be thoroughly soaked but there should not be standing water left in the well.

Note

Do not use milliQ or distilled water for this step. The ion-free water will cause undue osmotic stress on the cut leaf discs.

Alternatively, some species/cultivars can tolerate being directly sampled into standing water instead of wet filter paper. However, if initial F_v/F_m values are lower than 0.75 or if curve-fitting is failing on greater than 15% of leaf discs, the wet filter paper method should be used.

- 4 Label the lid of the plate and the side of the plate with the plots sampled.

Note

Leaf samples will be transferred out of this plate before analysis. Writing on this plate will not interfere with analysis.

- 5 Using a #2 Humboldt cork borer, take leaf tissue samples from each plot for each technical replicate. Hold the leaf flat against the rubber stopper, press the cork borer through the leaf into the stopper and twist. Try to use the most recently fully expanded leaf with mature chloroplasts and limited bug damage. Avoid sampling over the midrib.



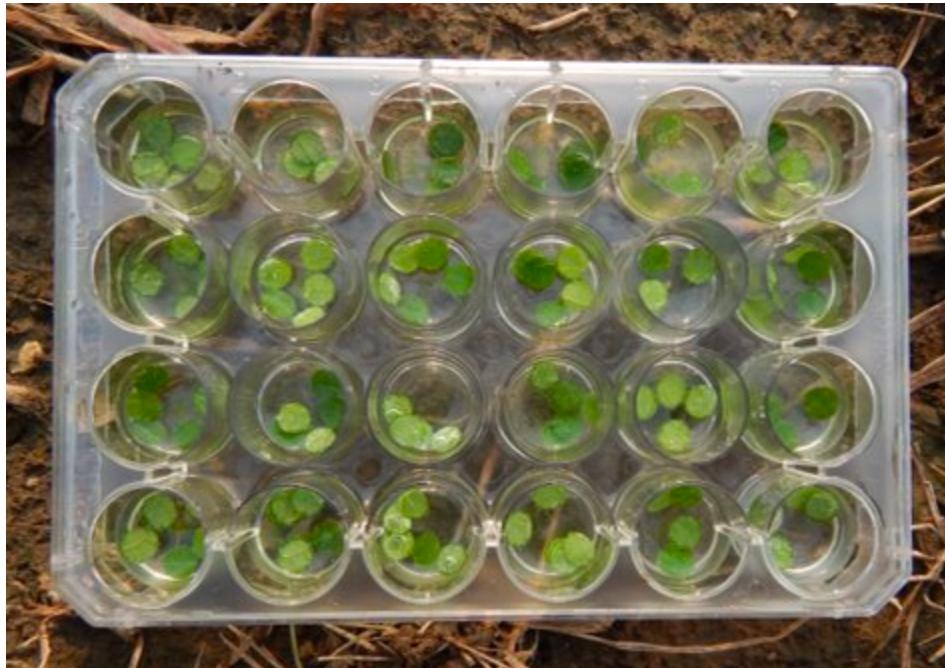
If taking multiple technical replicates from a plot, multiple leaf discs can be sampled simultaneously and will continue to move up the cork borer.

Note

Number of biological replicates (repeating plots) and number of technical replicates (repeating leaf tissue samples from the same plot) will vary depending on experimental design. Typically about 15% of leaf discs do not successfully curve fit. Increase number of technical reps plated to account for failed curve fitting. It is recommended to take ~30% more technical replicate leaf discs for each plot than needed in case leaf tissue is damaged in transit or from boring.

6 technical replicates (leaf punches) analyzed has worked well for soybean and tobacco.

- 6 Using a long cotton swab, or spatula, push the leaf discs out of the cork borer into the appropriate well of the 24 well plate. All technical replicates can be in the same well but each plot (biological replicate) must go in its own well.



- 7 When a complete 24 well plate has been sampled, place the lid on. Store the plate out of direct sunlight at room temperature.

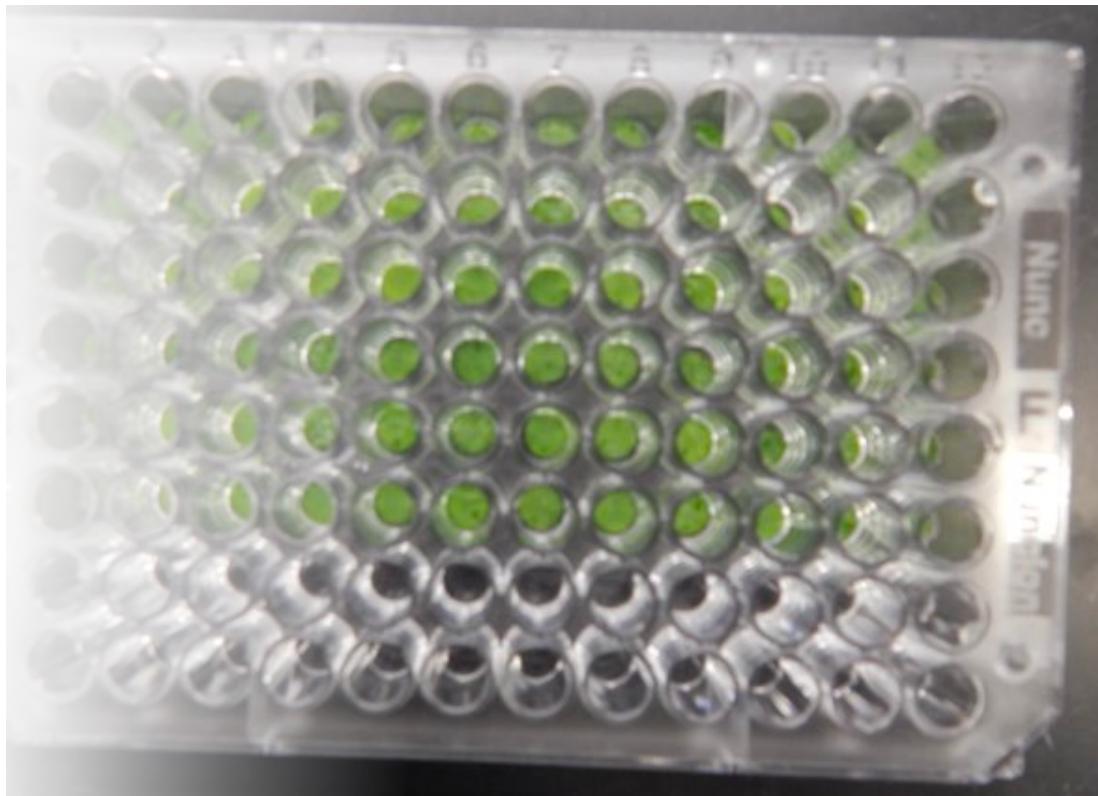
Typically a bag, box, or empty cooler (no ice) works well.

- 8 When all plates have been collected, return to lab.

- 9 Using tweezers, transfer each leaf disc to an individual well in a 96 well plate. The top of the leaf disc should be facing down on to the bottom of the 96 well plate.

Handle the leaf discs gently. Damage to the tissue, i.e. being poked with tweezers, will affect chlorophyll fluorescence analysis.



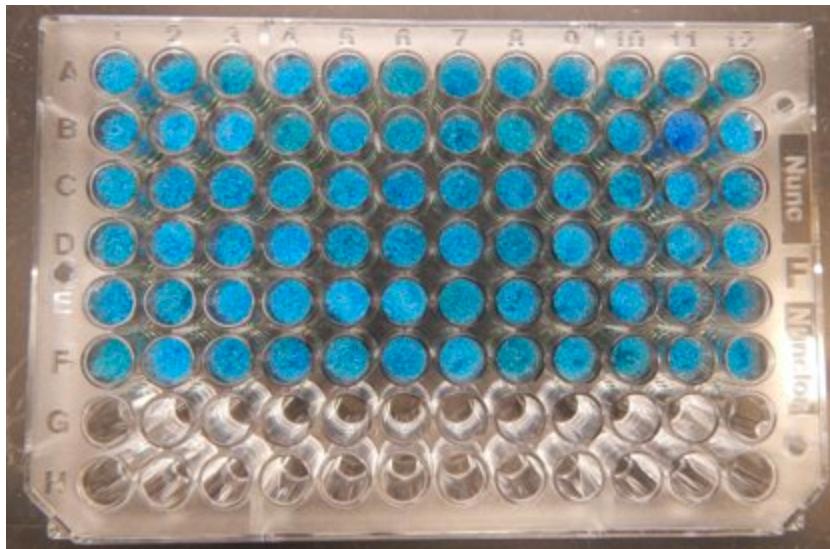


Note

Lay the leaf discs as flatly as possible on the bottom of the 96 well plate. Any angle at all will affect the reflectance and detection of chlorophyll fluorescence back to the fluorescence detector.

- 10 Dip a nasal aspirator filter halfway into water and then immediately dab on a paper towel. Insert filter in the well on top of the leaf disc. Gently press the nasal aspirator filter all the way to the bottom of the well, ensure that the leaf disc is flat on the bottom of the 96-well plate and that the damp filter is touching the leaf disc.





Note

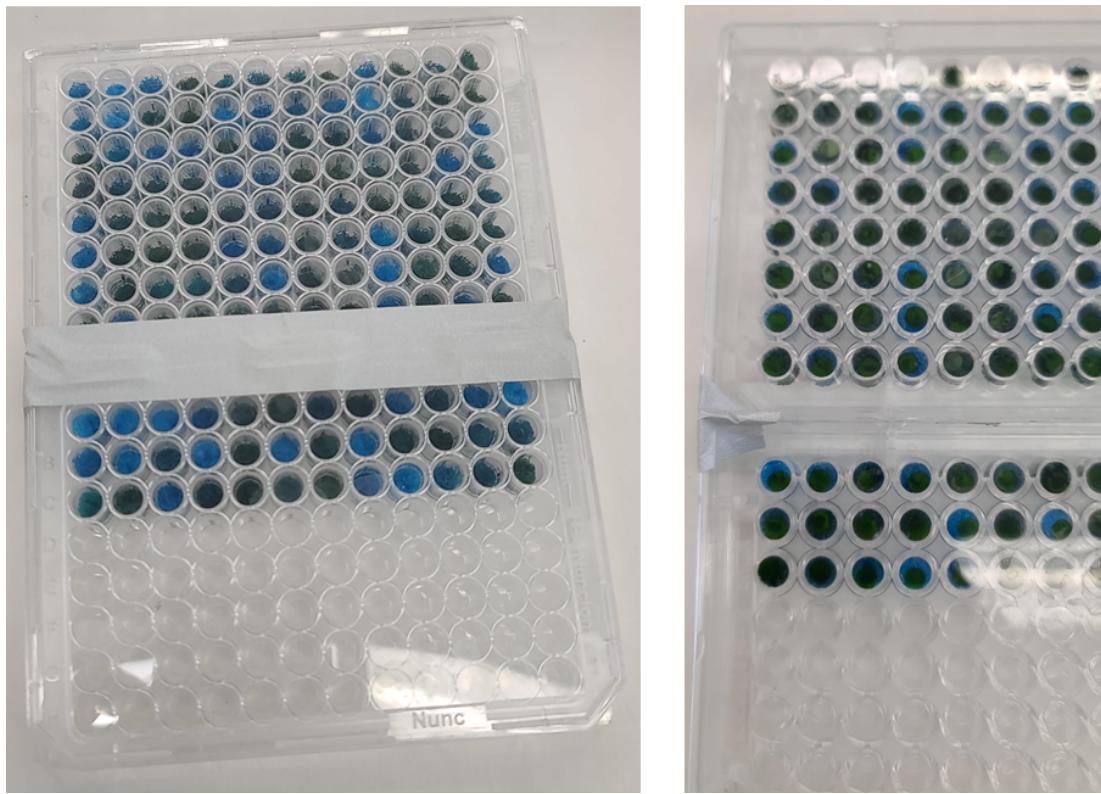
Do not over wet the filters. If the leaf tissue can't get oxygen through the sponge, it will suffer damage that will affect chlorophyll fluorescence analysis. If the filters are too dry or are not pushed down until they are touching the leaf, the humidity will be too low in the well and the leaf disc will dry out and that will affect chlorophyll fluorescence analysis.

Cut the original nasal aspirator filters in half. Too thick of filter will trap too much water and cause damage to the tissue.

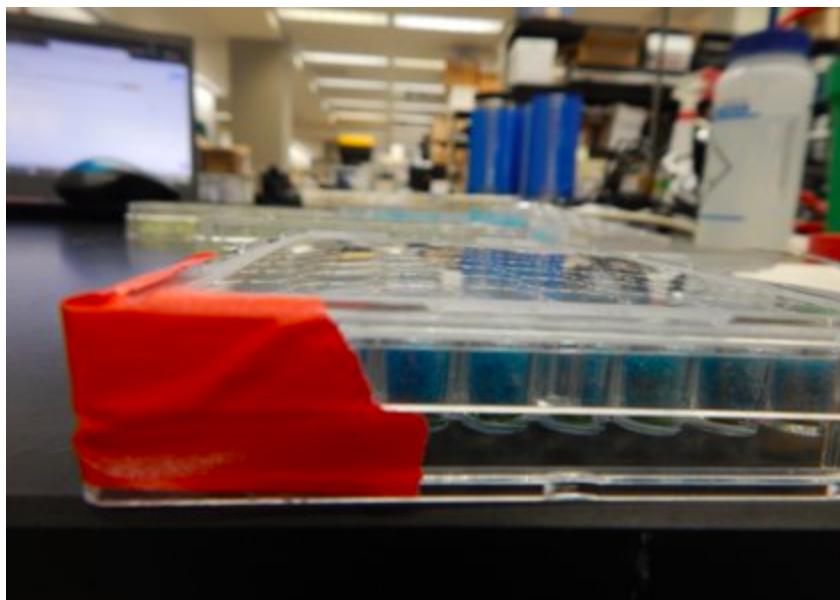
Use all of the same brand of filter for an experiment and make sure to use the same filter on the fake plate used for focusing the CF Imager in [**Fluorescence analysis using CF imagerV.2**](#). Density changes and auto-fluorescence of the sponge material can create variation in sample results if sponge brand, density, and width are not consistent throughout the experiment.

It is advisable to do a test run on any new batch of filters ordered against old filters. At least one brand was found to contain a dye that led to such high auto-fluorescence that measurement of leaf was impossible.

- 11 If using the PSI Fluorcam, the camera field of view from the third shelf can accommodate one and a third 96 well plates for maximum throughput. Continue plating leaf discs through row C of the second 96 well plate. Replace both lids and use lab tape on the top to attach the two plates in the correct order. Ensure that none of the tape covers the wells on the bottom.



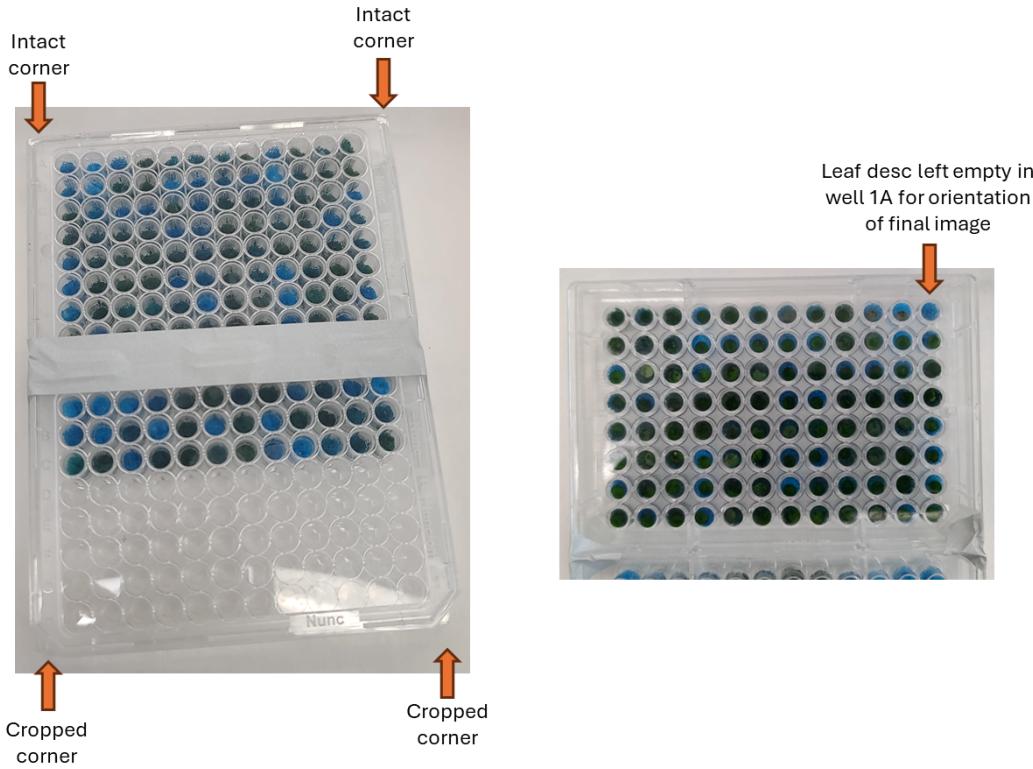
- 12 When all wells have leaf discs and sponges inserted, place the lid on the plate. Tape the top right corner to help orient the plate in the dark for imaging.



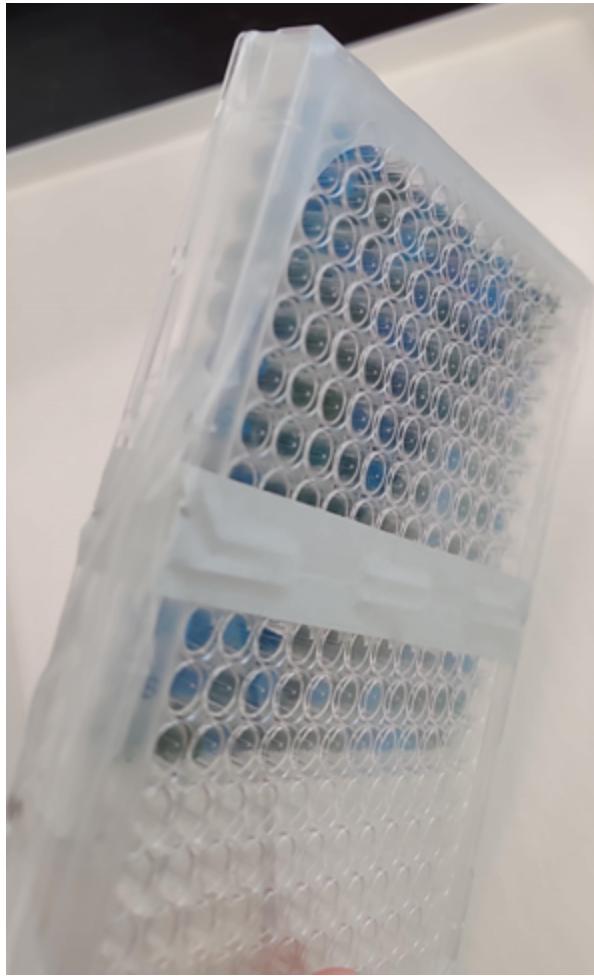


Note

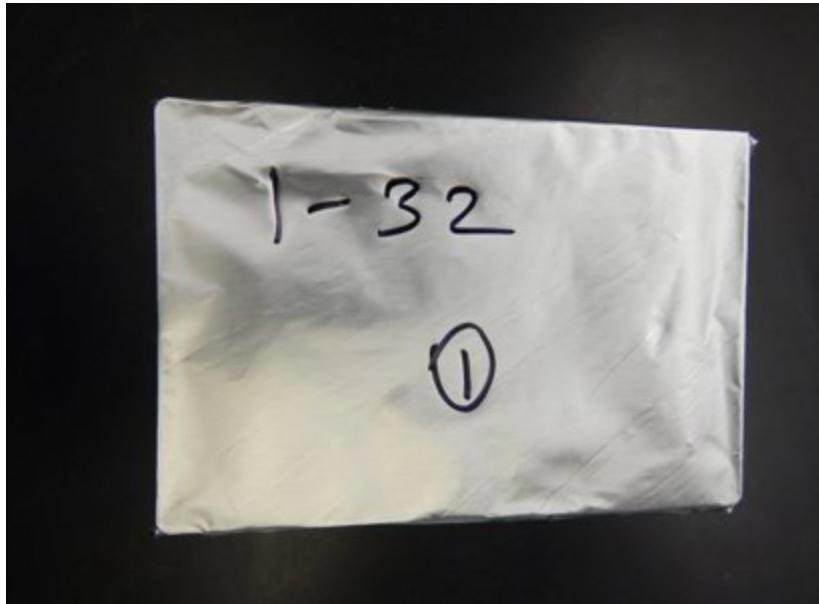
The 96 well plates have intact corners on the top and cropped corners on the bottom that can be used to help orient the plate in the dark for chlorophyll fluorescence analysis. If plate orientation is a major concern, a single technical rep can be sacrificed from the first or last sample (Plate 1 well 1A or Plate 2 12C) so that orientation can be determined in final images.



- 13 Parafilm the lid to the plate by wrapping the edges with parafilm.



14 Wrap the plate in aluminum foil and write the plot IDs and plate ID on the aluminum foil.



- 15 Place in a dark box or cabinet for a minimum of 1 hour, but preferably overnight before imaging and analyzing for chlorophyll fluorescence.

Note

Field grown high light adapted soy will take longer to fully dark adapt while growth chamber grown Arabidopsis may only require 30 minutes to an hour. Successful dark adaptation on healthy plants will yield initial F_v/F_m values > 0.75 and the length of dark adaptation for a particular growing condition or species may need to be determined empirically.

Overnight dark adaptation also provides more flexibility in time required for sampling, plating, and imager analysis.

Protocol references

Gotarkar, D., Doran, L., Burns, M., Hinkle, A., Kromdijk, J., Burgess, S. J. High-Throughput Analysis of Non-Photochemical Quenching in Crops Using Pulse Amplitude Modulated Chlorophyll Fluorometry. *J. Vis. Exp.* (185), e63485, doi:10.3791/63485 (2022).