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Sampling leaf tissue for analysis of NPQ Relaxation using Technologica Chlorophyll Fluorescence Imager Data. V.1

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[1]Realizing Increased Photosynthetic Efficiency (RIPE)

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ABSTRACT

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KEYWORDS

Chlorophyll Fluorescence, CF Imaging, Technologica CF Imaging, Photoprotection, NPQ Relaxation, Non-Photochemical Quenching, Sampling

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MATERIALS TEXT

- Water, tap or distilled.
- 24 Well Tissue Culture Plates (75/cs), Thermofisher 142475
- #2 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**
- 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 Just cover the bottom of a each well in a 24 well plate with water.

2 Label the lid of the plate and the side of the plate with the plots sampled.

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

3 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.

> 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. It is recommended to take ~30% more technical replicate leaf discs for each plot then needed in case leaf tissue is damaged in transit or from boring.

4 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 it's own well.

Ensure that all leaf discs are floating in the water.

5 When a complete 24 well plate has been sampled, place the lid on and seal with parafilm. Store the plate out of direct sunlight at room temperature.

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

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6 When all plates have been collected, return to lab.

7 ⚠

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.

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 detector in the CF Technologica.

8 ⚠

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.

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.

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.

9 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.

10 Parafilm the lid to the plate.

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

12 Place in a dark box or cabinet for a minimum of 1 hour, but preferably up to 12 hours before imaging and analysizing per Fluorescence analysis using CF imagerV.2.

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