Measuring spectral reflectance and transmittance (350-2500 nm) of large leaves using an integrating sphere Version 2

Etienne Laliberté

Abstract

Here we describe the standardised protocol used by the Canadian Airborne Biodiversity Observatory (CABO) to measure leaf spectral reflectance and transmittance, using an integrating sphere fitted to a portable full-range field spectroradiometer. This "standard version" of our protocol describes the common case where an individual leaf is large enough to entirely cover the reflectance or transmission port of the integrating sphere. Briefly, six mature, healthy and sunlit leaves from a canopy plant are selected for measurements of adaxial reflectance and transmittance. Leaf scans are referenced to a calibrated Spectralon® disk and corrected for stray light to yield NIST-traceable, high-fidelity leaf spectral reflectance and transmittance measurements. Our leaf spectroscopy protocol builds from that of the Carnegie Airborne Observatory, as well as from integrating sphere user manuals from two companies (ASD Inc., SVC).

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Guidelines

Handling Spectralon®

- **Do not touch Spectralon®** (e.g. sphere interior, reference disks, plugs) with your fingers.
- **Do not use canned air** to remove dust on the Spectralon® disk; canned air contains chemicals that can alter Spectralon®'s optical properties.
- **Do not attempt to clean Spectralon®** in the field, other than **blowing surface dust only on the Spectralon® reference disk or sphere plugs** using the Canless Air Duster System; cleaning Spectralon® requires a special procedure that should only done in the lab.
- **Never blow air inside of the integrating sphere, especially not when it is attached to the spectroradiometer**, as this will blow dust inside the instrument.

Equipment

- Spectra Vista Corporation HR-1024i full-range (350-2500 nm) field spectroradiometer
- Spectra Vista Corporation 3-inch Spectralon® DC-R/T Sphere
- Semi-rugged laptop or PDA running the SVC Scan software

- Canless Air Duster System O_2 Hurricane (**never use canned air**) to remove dust from the surface of the Spectralon® reference disk

- Plastic containers with lids to temporarily store leaf samples during measurements (optional)

Consumables

- Nitrile gloves for handling leaves

Before start

1. Consult the user manual of the spectroradiometer and the integrating sphere to set up the instrument.
2. The instrument should be set up in the shade, sheltered as much as possible from the elements.
3. All canopy plants selected for measurements should have already been tagged, identified, and georeferenced before spectroscopy measurements start.
4. The spectroscopist should be positionned as close as possible to the sampled plants to minimise time from collection to measurement.
5. The spectroscopist should be in a confortable position and have enough room around the instrument to spread leaf samples around without the risk of mixing up individual leaves during handling.
6. **Six mature, fully-developped, healthy-looking leaves from the sunlit (>3 h per day of direct sunlight) portion of the canopy are selected** for spectral measurements from the bulk leaf sample (often one of a few branches). Leaves should be collected from the uppermost surface of the branch (i.e. receiving the most direct sunlight).

Protocol

Instrument set-up

Step 1.

Install the integrating sphere onto the spectroradiometer.

NOTES

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Follow the SVC integrating sphere manual p. 9-14.

Instrument set-up

Step 2.

Power the spectroradiometer and integrating sphere lamp on and **warm up for >15 min**.

Reflectance (leaf adaxial side): Reference scan

Step 3.

Position the lamp over the sphere **primary light entrance port**.

SAFETY INFORMATION

The lamp can get very hot. Grab it by the slotted heat shield.

NOTES

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Make sure lamp is secured in locked position.

Reflectance (leaf adaxial side): Reference scan

Step 4.

Check lamp alignment.

NOTES

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Use a thin piece of paper at the exit of the reflectance sample port (empty port) the to ensure the light beam under-fills and is centered in the reflectance port. **If it is not, then proceed to lamp alignment** as described in the SVC integrating sphere user manual, p. 23-24.

Reflectance (leaf adaxial side): Reference scan

Step 5.

Screw the tethered light trap on the **reflectance port** sample holder.

NOTES

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The light trap can stay on the sample holder for the entire measurement session.

Reflectance (leaf adaxial side): Reference scan

Step 6.

Screw the tethered light trap on the **transmission port** sample holder.

NOTES

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The light trap can stay on the sample holder for the entire measurement session.

Reflectance (leaf adaxial side): Reference scan

Step 7.

Place the tethered calibrated Spectralon® reflectance standard over the **reflectance port**.

NOTES

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Place the standard over the reflectance port so that the light beam shines directly on its reflective

surface (= facing inside of the sphere).

Reflectance (leaf adaxial side): Reference scan

Step 8.

Position leaf #1 over the **transmission port** with its adaxial (upper) surface facing the inside of the sphere.

NOTES

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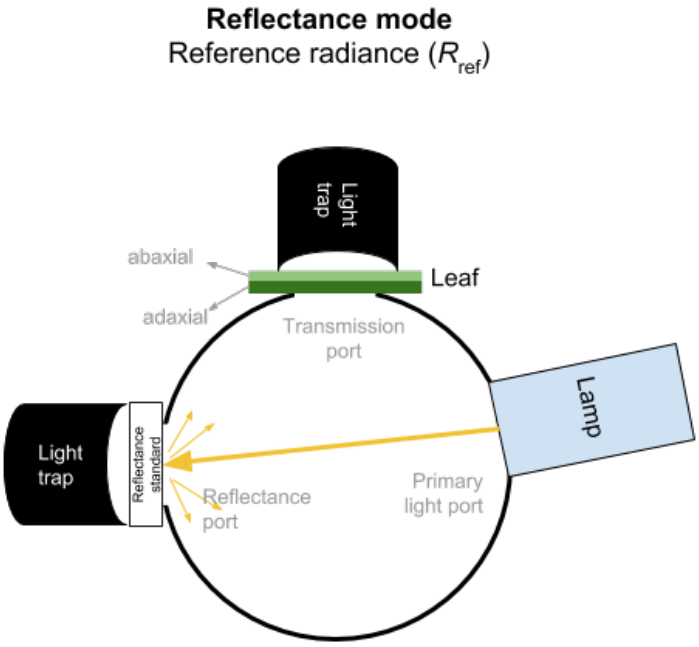
Position the leaf so that the amount of leaf and vein material over the port is roughly proportional to the area of leaf and vein found throughout the leaf, while avoiding the large midrib vein. Try to to position the leaf so that it is approximately halfway between the mid-rib vein and the leaf margin, and halfway between the tip and the base of the leaf lamina.

Reflectance (leaf adaxial side): Reference scan

Step 9.

Collect a '**Reference Scan**' in this configuration.



NOTES

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This corresponds to the **reference radiance** in reflectance mode (R_{ref}). The reference data will be automatically saved in all successive target scan files until a new 'Reference Scan' is made.

Reflectance: Stray light

Step 10.
Carefully remove leaf #1 from the transmission port sample holder.

Reflectance: Stray light
Step 11.

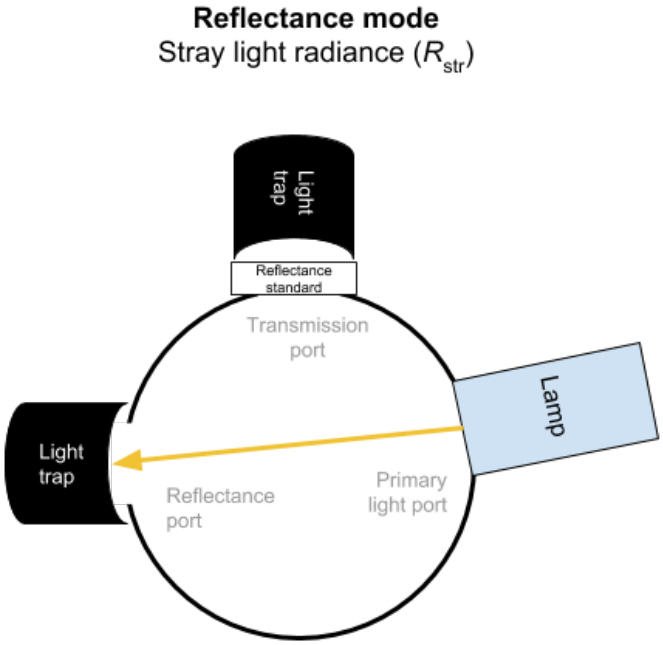
Remove the tethered calibrated Spectralon® reflectance standard from the reflectance port.

Reflectance: Stray light
Step 12.

Place the tethered calibrated Spectralon® reflectance standard over the **transmission port** sample holder.

Reflectance: Stray light
Step 13.

Collect a '**Target Scan**' in this configuration and **save the file**.



NOTES
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This corresponds to the **stray light radiance** in reflectance mode (R_{str}).

Reflectance (leaf adaxial side): Leaf scans
Step 14.

Position leaf #1 over the **reflectance port** with its adaxial (upper) surface facing the inside of the sphere.

NOTES

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Position the leaf to target the same area measured for the reference radiance. Position it so that the amount of leaf and vein material over the port is roughly proportional to the area of leaf and vein found throughout the leaf, while avoiding the large midrib vein. Position the leaf so that it is approximately halfway between the mid-rib vein and the leaf margin, and halfway between the tip and the base of the leaf lamina.

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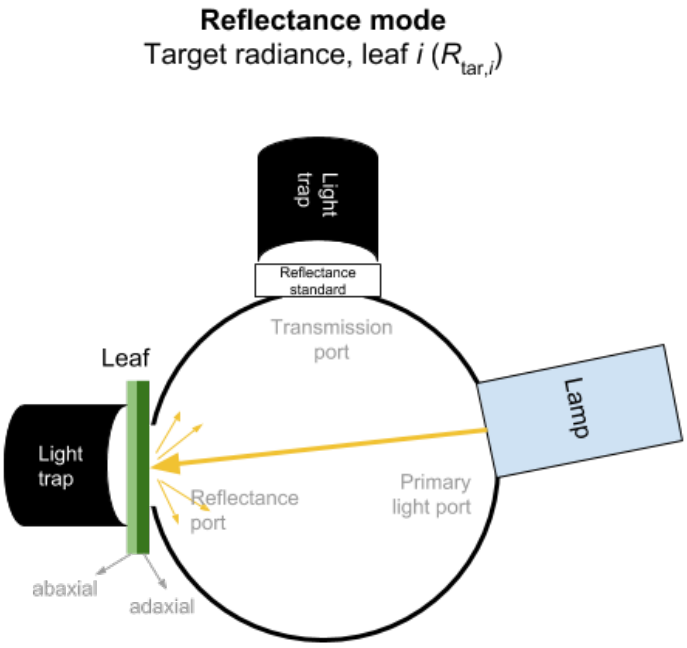
The light trap should remain on the reflectance port sample holder.

Reflectance (leaf adaxial side): Leaf scans

Step 15.

Collect a '**Target Scan**' for leaf #1 in this configuration and **save the file**.



NOTES

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This corresponds to the **target radiance** in reflectance mode for leaf #1 ($R_{tar,1}$).

Reflectance (leaf adaxial side): Leaf scans

Step 16.

Carefully replace leaf #1 by leaf #2.

NOTES

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Position the leaf to target the same area measured for the reference radiance. Position it so that the amount of leaf and vein material over the port is roughly proportional to the area of leaf and vein found throughout the leaf, while avoiding the large midrib vein. Position the leaf so that it is approximately halfway between the mid-rib vein and the leaf margin, and halfway between the tip and the base of the leaf lamina.

Reflectance (leaf adaxial side): Leaf scans

Step 17.

Collect a '**Target Scan**' for leaf #2 in this configuration and **save the file**.

NOTES

Etienne Laliberté 24 Apr 2018

This corresponds to the **target radiance** in reflectance mode for leaf #2 ($R_{tar,2}$).

Reflectance (leaf adaxial side): Leaf scans

Step 18.

Carefully replace leaf #2 by leaf #3.

NOTES

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Position the leaf to target the same area measured for the reference radiance. Position it so that the amount of leaf and vein material over the port is roughly proportional to the area of leaf and vein found throughout the leaf, while avoiding the large midrib vein. Position the leaf so that it is approximately halfway between the mid-rib vein and the leaf margin, and halfway between the tip and the base of the leaf lamina.

Reflectance (leaf adaxial side): Leaf scans

Step 19.

Collect a '**Target Scan**' for leaf #3 in this configuration and **save the file**.

NOTES

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This corresponds to the **target radiance** in reflectance mode for leaf #3 ($R_{tar,3}$).

Reflectance (leaf adaxial side): Leaf scans

Step 20.

Carefully replace leaf #3 by leaf #4.

NOTES

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Position the leaf to target the same area measured for the reference radiance. Position it so that the amount of leaf and vein material over the port is roughly proportional to the area of leaf and vein found throughout the leaf, while avoiding the large midrib vein. Position the leaf so that it is approximately halfway between the mid-rib vein and the leaf margin, and halfway between the tip and the base of the leaf lamina.

Reflectance (leaf adaxial side): Leaf scans

Step 21.

Collect a '**Target Scan**' for leaf #4 in this configuration and **save the file**.

NOTES

Etienne Laliberté 24 Apr 2018

This corresponds to the **target radiance** in reflectance mode for leaf #4 ($R_{tar,4}$).

Reflectance (leaf adaxial side): Leaf scans

Step 22.

Carefully replace leaf #4 by leaf #5.

NOTES

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Position the leaf to target the same area measured for the reference radiance. Position it so that the amount of leaf and vein material over the port is roughly proportional to the area of leaf and vein found throughout the leaf, while avoiding the large midrib vein. Position the leaf so that it is approximately halfway between the mid-rib vein and the leaf margin, and halfway between the tip and the base of the leaf lamina.

Reflectance (leaf adaxial side): Leaf scans

Step 23.

Collect a '**Target Scan**' for leaf #5 in this configuration and **save the file**.

NOTES

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This corresponds to the **target radiance** in reflectance mode for leaf #5 ($R_{tar,5}$).

Reflectance (leaf adaxial side): Leaf scans

Step 24.

Carefully replace leaf #5 by leaf #6.

NOTES

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Position the leaf to target the same area measured for the reference radiance. Position it so that the amount of leaf and vein material over the port is roughly proportional to the area of leaf and vein found throughout the leaf, while avoiding the large midrib vein. Position the leaf so that it is approximately halfway between the mid-rib vein and the leaf margin, and halfway between the tip and the base of the leaf lamina.

Reflectance (leaf adaxial side): Leaf scans

Step 25.

Collect a '**Target Scan**' for leaf #6 in this configuration and **save the file**.

NOTES

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This corresponds to the **target radiance** in reflectance mode for leaf #6 ($R_{tar,6}$).

Transmittance (leaf adaxial side): Reference scan

Step 26.

Flip leaf #6 around on the **reflectance port** so that its abaxial (lower) side is now facing the inside of the sphere.

NOTES

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Position the leaf to target the same area measured for the reflectance radiance, with the exception that its abaxial surface now faces the inside of the sphere. Position it so that the amount of leaf and vein material over the port is roughly proportional to the area of leaf and vein found throughout the leaf, while avoiding the large midrib vein. Position the leaf so that it is approximately halfway between the mid-rib vein and the leaf margin, and halfway between the tip and the base of the leaf lamina.

Transmittance (leaf adaxial side): Reference scan

Step 27.

Remove the tethered calibrated Spectralon® reflectance standard from the sphere transmission port.

Transmittance (leaf adaxial side): Reference scan

Step 28.

Remove the light trap from the transmission port sample holder.

Transmittance (leaf adaxial side): Reference scan

Step 29.

Position the lamp over the sphere **transmission port**.

SAFETY INFORMATION

The lamp can get very hot. Grab it by the slotted heat shield.

NOTES

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Make sure lamp is secured in locked position.

Transmittance (leaf adaxial side): Reference scan

Step 30.

Install the Spectralon® plug over the **primary light port**.

NOTES

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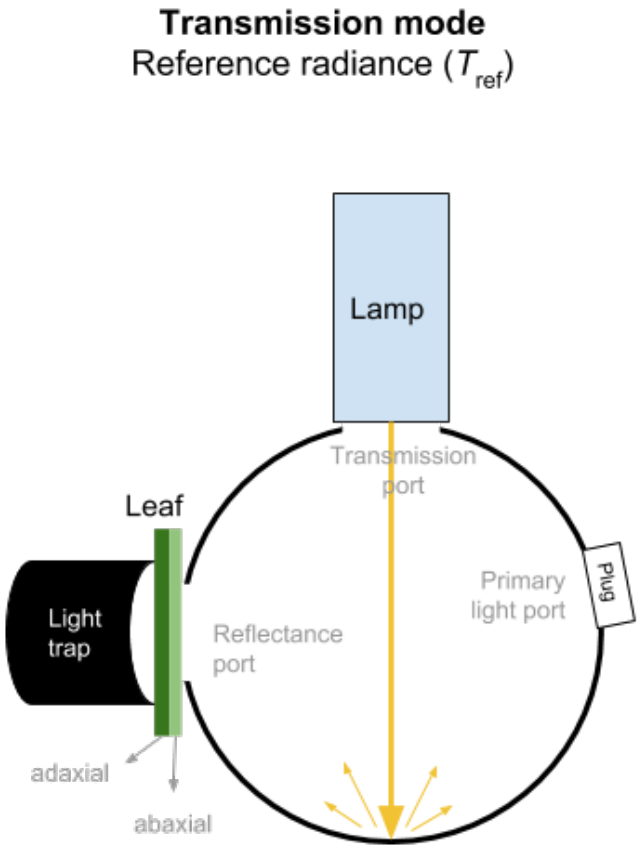
Ensure that the curved plug is placed the correct way to match the curvature of the sphere.

Transmittance (leaf adaxial side): Reference scan

Step 31.

Collect a '**Reference Scan**' in this configuration.



NOTES

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This corresponds to the **reference radiance** in transmittance mode (T_{ref}). The reference data will be automatically saved in all successive target scan files until a new 'Reference Scan' is made.

Transmittance (leaf adaxial side): Leaf scans

Step 32.

Carefully remove leaf #6 from the reflectance port sample holder.

NOTES

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The reflectance port should now be **empty** (but with **light trap on**).

Transmittance (leaf adaxial side): Leaf scans

Step 33.

Gently pull lamp away from the sphere.

SAFETY INFORMATION

The lamp can get very hot. Grab it by the slotted heat shield.

Transmittance (leaf adaxial side): Leaf scans

Step 34.

Place leaf #1 over the **transmission port** with its abaxial (lower) surface facing the inside of the sphere.

NOTES

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Position the leaf to target the same area measured for the reflectance radiance, with the exception that its abaxial surface now faces the inside of the sphere. Position it so that the amount of leaf and vein material over the port is roughly proportional to the area of leaf and vein found throughout the leaf, while avoiding the large midrib vein. Position the leaf so that it is approximately halfway between the mid-rib vein and the leaf margin, and halfway between the tip and the base of the leaf lamina.

Transmittance (leaf adaxial side): Leaf scans

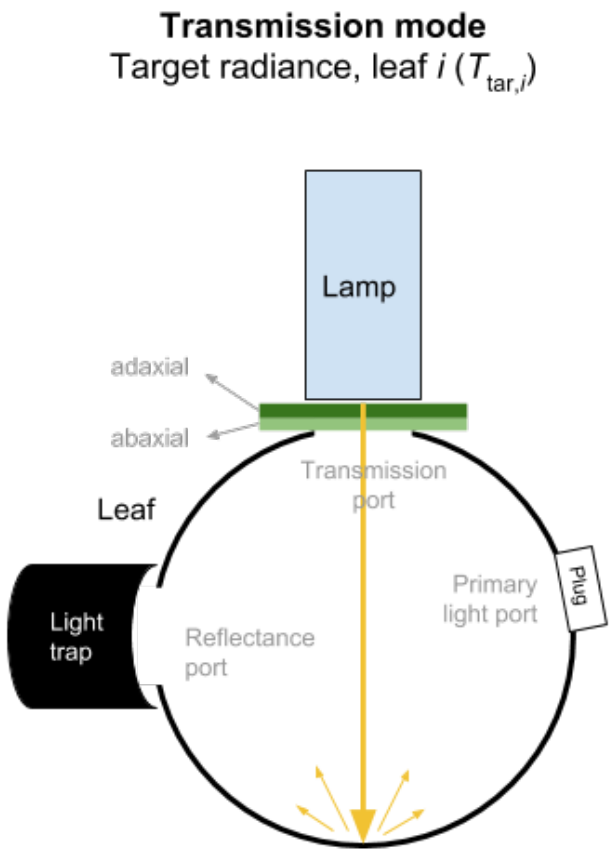
Step 35.

Release the transmission sample holder and move lamp back to its locked position.

Transmittance (leaf adaxial side): Leaf scans

Step 36.

Collect a '**Target Scan**' for leaf #1 in this configuration and **save the file**.



NOTES

Etienne Laliberté 25 Apr 2018

This corresponds to the **target radiance** in transmittance mode for leaf #1 ($T_{tar,1}$).

Transmittance (leaf adaxial side): Leaf scans

Step 37.

Carefully replace leaf #1 by leaf #2.

NOTES

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Position the leaf to target the same area measured for the reflectance radiance, with the exception that its abaxial surface now faces the inside of the sphere. Position it so that the amount of leaf and vein material over the port is roughly proportional to the area of leaf and vein found throughout the leaf, while avoiding the large midrib vein. Position the leaf so that it is approximately halfway between the mid-rib vein and the leaf margin, and halfway between the tip and the base of the leaf lamina.

Transmittance (leaf adaxial side): Leaf scans

Step 38.

Collect a '**Target Scan**' for leaf #2 in this configuration and **save the file**.

NOTES
Etienne Laliberté 25 Apr 2018

This corresponds to the **target radiance** in transmittance mode for leaf #2 ($T_{tar,2}$).

Transmittance (leaf adaxial side): Leaf scans
Step 39.

Carefully replace leaf #2 by leaf #3.

NOTES
Etienne Laliberté 25 Apr 2018

Position the leaf to target the same area measured for the reflectance radiance, with the exception that its abaxial surface now faces the inside of the sphere. Position it so that the amount of leaf and vein material over the port is roughly proportional to the area of leaf and vein found throughout the leaf, while avoiding the large midrib vein. Position the leaf so that it is approximately halfway between the mid-rib vein and the leaf margin, and halfway between the tip and the base of the leaf lamina.

Transmittance (leaf adaxial side): Leaf scans
Step 40.

Collect a '**Target Scan**' for leaf #3 in this configuration and **save the file**.

NOTES
Etienne Laliberté 25 Apr 2018

This corresponds to the **target radiance** in transmittance mode for leaf #3 ($T_{tar,3}$).

Transmittance (leaf adaxial side): Leaf scans
Step 41.

Carefully replace leaf #3 by leaf #4.

NOTES
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Position the leaf to target the same area measured for the reflectance radiance, with the exception that its abaxial surface now faces the inside of the sphere. Position it so that the amount of leaf and vein material over the port is roughly proportional to the area of leaf and vein found throughout the leaf, while avoiding the large midrib vein. Position the leaf so that it is approximately halfway between the mid-rib vein and the leaf margin, and halfway between the tip and the base of the leaf lamina.

Transmittance (leaf adaxial side): Leaf scans

Step 42.

Collect a '**Target Scan**' for leaf #4 in this configuration and **save the file**.

NOTES
Etienne Laliberté 25 Apr 2018

This corresponds to the **target radiance** in transmittance mode for leaf #4 ($T_{tar,4}$).

Transmittance (leaf adaxial side): Leaf scans
Step 43.

Carefully replace leaf #4 by leaf #5.

NOTES
Etienne Laliberté 25 Apr 2018

Position the leaf to target the same area measured for the reflectance radiance, with the exception that its abaxial surface now faces the inside of the sphere. Position it so that the amount of leaf and vein material over the port is roughly proportional to the area of leaf and vein found throughout the leaf, while avoiding the large midrib vein. Position the leaf so that it is approximately halfway between the mid-rib vein and the leaf margin, and halfway between the tip and the base of the leaf lamina.

Transmittance (leaf adaxial side): Leaf scans
Step 44.

Collect a '**Target Scan**' for leaf #5 in this configuration and **save the file**.

NOTES
Etienne Laliberté 25 Apr 2018

This corresponds to the **target radiance** in transmittance mode for leaf #5 ($T_{tar,5}$).

Transmittance (leaf adaxial side): Leaf scans
Step 45.

Carefully replace leaf #5 by leaf #6.

NOTES
Etienne Laliberté 25 Apr 2018

Position the leaf to target the same area measured for the reflectance radiance, with the exception that its abaxial surface now faces the inside of the sphere. Position it so that the amount of leaf and vein material over the port is roughly proportional to the area of leaf and vein found throughout the leaf, while avoiding the large midrib vein. Position the leaf so that it is approximately halfway between the mid-rib vein and the leaf margin, and halfway between the tip and the base of the leaf lamina.

Transmittance (leaf adaxial side): Leaf scans

Step 46.

Collect a '**Target Scan**' for leaf #6 in this configuration and **save the file**.

NOTES

Etienne Laliberté 25 Apr 2018

This corresponds to the **target radiance** in transmittance mode for leaf #6 ($T_{tar,6}$).

Transmittance (leaf adaxial side): Leaf scans

Step 47.

Remove leaf #6 from the transmission sample port holder.

Calculating leaf absolute reflectance (adaxial side)

Step 48.

The equation for NIST-traceable **adaxial reflectance** of leaf i, $\rho_{leaf,i}$ is

$$\rho_{leaf,i} = (R_{tar,i} - R_{str}) / (R_{ref} - R_{str}) * \rho_{ref}$$

where ρ_{ref} is the absolute reflectance of the calibrated Spectralon® reflectance standard.

Calculating leaf absolute transmittance (adaxial side)

Step 49.

The equation for NIST-traceable **adaxial transmittance** of leaf i, $\tau_{leaf,i}$ is

$$\tau_{leaf,i} = (T_{tar,i} / T_{ref}) * \rho_{ref}$$

where ρ_{ref} is the absolute reflectance of the calibrated Spectralon® reflectance standard.

ANNOTATIONS

Etienne Laliberté 26 Apr 2018

Perhaps we should not call the transmittance measurement 'NIST-traceable' when done this way since ρ of sphere cavity wall is not calibrated against ρ of Spectralon® reference standard (the SVC manual just assumes the wall is the same absolute reflectance as the standard)? Should we instead use the reference radiance of leaf abaxial side in reflectance mode minus stray light radiance at the denominator, similar to what the Li-Cor 1800 sphere does? e.g. τ_leaf = [T_tar,i / (R_ref,abax − R_str)] × ρ_ref, as in eqn. 5 of Noda et al. (2013)? This would mean removing

measurement of T_ref (step 31) and adding measurement of R_ref,abax (just after step 9, where R_ref,adax) is measured.

Warnings

The lamp of the integrating sphere can get **very hot** and should handled from its slotted base to avoid burns.