Measuring spectral reflectance and transmittance (350-2500 nm) of large leaves using an integrating sphere Version 3

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-looking 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. 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, <u>especially not when it is attached to the spectroradiometer</u>**, 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

Etienne Laliberté 24 Apr 2018

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**.

NOTES

Etienne Laliberté 26 Apr 2018

Record the **time of day** when the spectroradiometer was started.

ANNOTATIONS

Etienne Laliberté 01 May 2018

From Raymond Soffer 30/04/2018:

"A test can be performed to test the required startup time and system stability. Prior to pwering on the spectrometer, setup the experiment to acquire a reference measurement (reference disk in the reference port). Upon powering up the system , note the time, configure the instrument, and then take a SVC 'reference' measurment as quickly as possible. Configure the insturment to acquire 'target' measurements at an regular interval (i.e. 1 minute) over an extended period of time (i.e. 2 hours). This will allow not only the warm-up period to be determined, but the determination of the baseline noise level."

Review Protocol Summary Diagram

Step 3.

Review the document summarising the different sphere configurations (A−E), and the scans that need to be recorded in each configuration.

Configuration A: Reflectance Mode, Reference

Step 4.

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

Etienne Laliberté 24 Apr 2018

Make sure lamp is secured in locked position.

Configuration A: Reflectance Mode, Reference

Step 5.

Check lamp alignment.

NOTES

Etienne Laliberté 24 Apr 2018

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.

Configuration A: Reflectance Mode, Reference

Step 6.

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

NOTES
Etienne Laliberté 24 Apr 2018

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

Configuration A: Reflectance Mode, Reference
Step 7.

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

NOTES
Etienne Laliberté 26 Apr 2018

The light trap can stay on the sample holder for all measurements made in reflectance mode (configurations A−C).

Configuration A: Reflectance Mode, Reference
Step 8.

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

NOTES
Etienne Laliberté 26 Apr 2018

Place the standard over the reflectance port so that the light beam shines directly on its reflective surface (i.e facing into the sphere).

Configuration A: Reflectance Mode, Reference
Step 9.

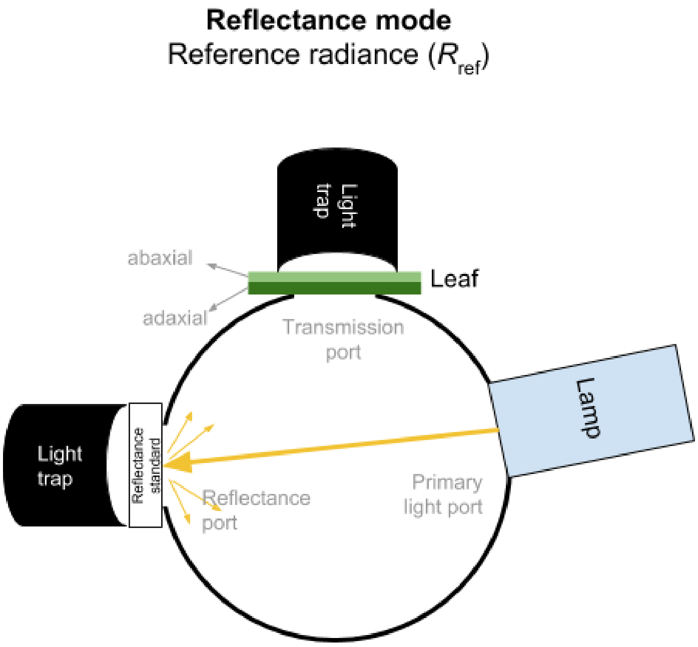
Position leaf #1 over the **transmission port** with its adaxial (upper) surface facing into the sphere.

NOTES
Etienne Laliberté 26 Apr 2018

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. 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.

Configuration A: Reflectance Mode, Reference
Step 10.

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



NOTES
Etienne Laliberté 25 Apr 2018

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.

ANNOTATIONS
Etienne Laliberté 01 May 2018

From Raymond Soffer 30/04/2018:

"If the multiple leaves tested in this sequence have similar reflectance/transmittance, then acquiring a single reference radiance measurment should be adequate. However, the sensitivity of the Reference radiance in reflectance mode to the leaf reflectance levels can be tested with relative ease."

Configuration A: Reflectance Mode, Reference
Step 11.

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

Configuration B: Reflectance Mode, Stray light

Step 12.

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

Configuration B: Reflectance Mode, Stray light

Step 13.

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

Configuration B: Reflectance Mode, Stray light

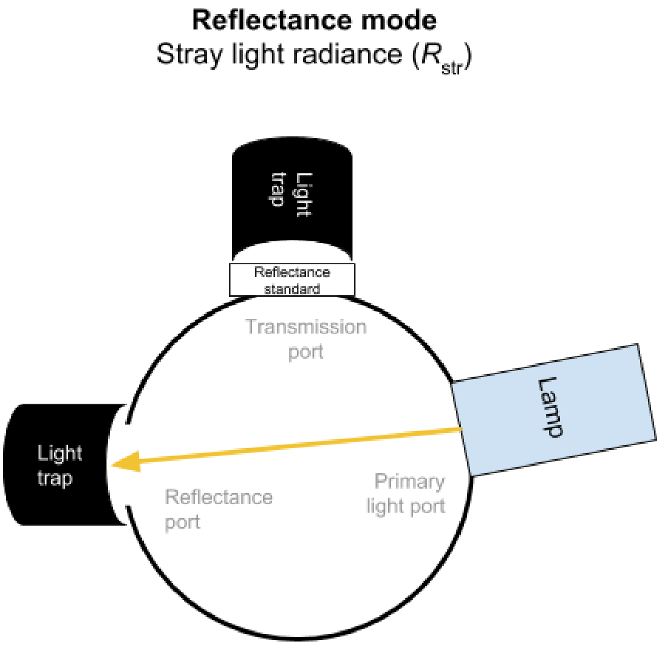
Step 14.

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

Configuration B: Reflectance Mode, Stray light

Step 15.

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



NOTES

Etienne Laliberté 25 Apr 2018

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

Configuration C: Reflectance Mode, Target

Step 16.

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

NOTES

Etienne Laliberté 24 Apr 2018

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.

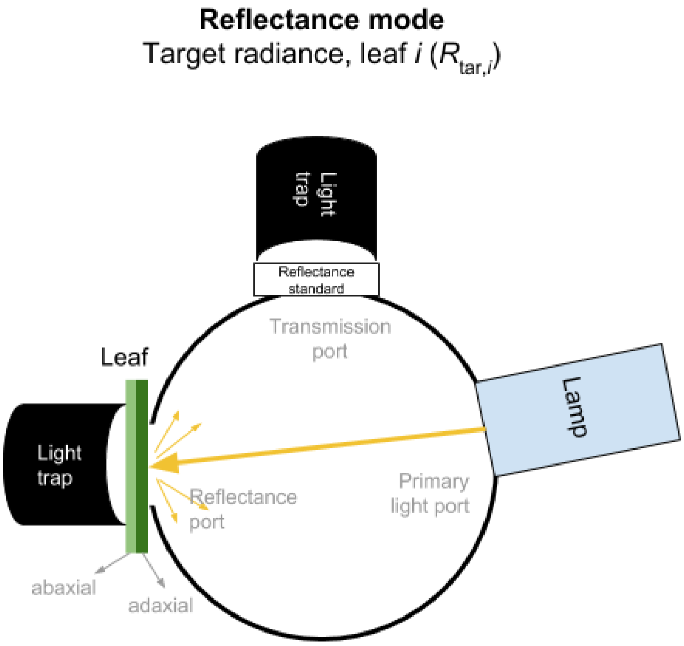
Etienne Laliberté 24 Apr 2018

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

Configuration C: Reflectance Mode, Target

Step 17.

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 reflectance mode for leaf #1 ($R_{tar,1}$).

Configuration C: Reflectance Mode, Target

Step 18.

Carefully replace leaf #1 by leaf #2.

NOTES

Etienne Laliberté 24 Apr 2018

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.

Configuration C: Reflectance Mode, Target

Step 19.

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 ($R_{tar,2}$).

Configuration C: Reflectance Mode, Target

Step 20.

Carefully replace leaf #2 by leaf #3.

NOTES

Etienne Laliberté 24 Apr 2018

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.

Configuration C: Reflectance Mode, Target

Step 21.

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

NOTES

Etienne Laliberté 24 Apr 2018

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

Configuration C: Reflectance Mode, Target

Step 22.

Carefully replace leaf #3 by leaf #4.

NOTES

Etienne Laliberté 24 Apr 2018

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.

Configuration C: Reflectance Mode, Target

Step 23.

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 ($R_{tar,4}$).

Configuration C: Reflectance Mode, Target

Step 24.

Carefully replace leaf #4 by leaf #5.

NOTES

Etienne Laliberté 24 Apr 2018

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.

Configuration C: Reflectance Mode, Target

Step 25.

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

NOTES

Etienne Laliberté 24 Apr 2018

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

Configuration C: Reflectance Mode, Target

Step 26.

Carefully replace leaf #5 by leaf #6.

NOTES

Etienne Laliberté 24 Apr 2018

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.

Configuration C: Reflectance Mode, Target

Step 27.

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

NOTES

Etienne Laliberté 24 Apr 2018

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

Configuration C: Reflectance Mode, Target

Step 28.

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

NOTES

Etienne Laliberté 24 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.

ANNOTATIONS

Etienne Laliberté 04 May 2018

We should add a second reference measurement in reflectance mode after all leaves are measured (see step 10) before moving into transmission mode, at least until we're confident in the stability of our system.

Configuration D: Transmission Mode, Reference

Step 29.

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

Configuration D: Transmission Mode, Reference

Step 30.

Remove the light trap from the transmission port sample holder.

Configuration D: Transmission Mode, Reference

Step 31.

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

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

NOTES
Etienne Laliberté 24 Apr 2018

Make sure lamp is secured in locked position.

Configuration D: Transmission Mode, Reference
Step 32.

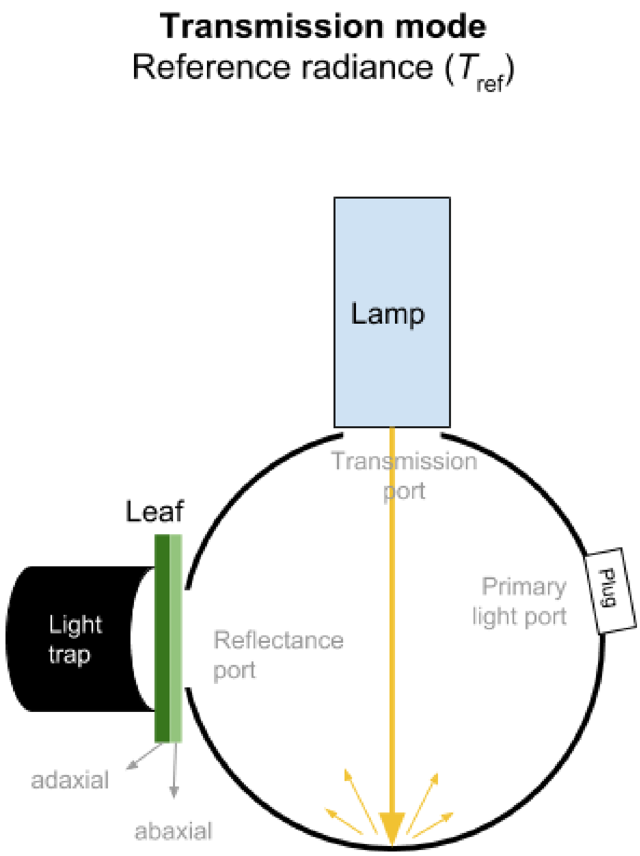
Install the Spectralon® plug over the **primary light port**.

NOTES
Etienne Laliberté 24 Apr 2018

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

Configuration D: Transmission Mode, Reference
Step 33.

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



NOTES

Etienne Laliberté 26 Apr 2018

This corresponds to the **reference radiance** in **transmission** mode (T_{ref}).

ANNOTATIONS

Etienne Laliberté 01 May 2018

Question for Raymond Soffer:

For transmittance, in the SVC manual it is suggested (p. 19) to use (see also step 52 below)

τleaf,i = (Ttar,i / Tref) * ρref

where

τleaf,i is the transmittance of leaf i (adaxial or upper side of leaf),
Ttar,i is the target radiance of leaf i in transmission mode (in appendix A of SVC manual, top right corner),
Tref is the reference radiance in transmission mode (this step #33 diagram; also SVC manual appendix A, top left corner)
ρref is the absolute reflectance of the calibrated Spectralon® reflectance standard.

However, as mentionned in the SVC manual, this assumes that the reflectance of the sphere cavity wall (actually what is being measured by Tref) is the same as the calibrated Spectralon standard. This is probably a reasonable assumption since the sphere wall is also made of Spectralon, but does not give a NIST-traceable measurement since it cannot be traced back to an actual calibrated standard.

Technically shouldn't we have:

τleaf,i = (Ttar,i / Tref) * ρcav

where

ρcav is the absolute reflectance of the sphere cavity wall that the light beam directly hits in Tref mode?

But this would require ρcav to be estimated...

Alternatively, could/should we use the same approach than the Li-Cor 1800 sphere (in which the reference and transmittance modes use two different lamp positions, see Fig. 2a and 2c from Noda et al. 2013 Plant Cell Env), and use (eqn. 5 of Noda et al. 2013):

τleaf,i = [Ttar,i / (Rref - Rstr)] * ρref where

Rref is the reference radiance of the leaf abaxial (lower) side in reflectance mode (SVC manual Appendix A, bottom left corner)
Rstr is the stray light radiance in reflectance mode.

Configuration D: Transmission Mode, Reference

Step 34.

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

Configuration E: Transmission Mode, Target

Step 35.

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

NOTES

Etienne Laliberté 24 Apr 2018

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

Configuration E: Transmission Mode, Target

Step 36.

Gently pull lamp away from the sphere.

SAFETY INFORMATION

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

Configuration E: Transmission Mode, Target

Step 37.

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

NOTES

Etienne Laliberté 26 Apr 2018

Position the leaf to target the same area measured for the reflectance radiance, with the exception that its abaxial surface now faces into 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.

Configuration E: Transmission Mode, Target

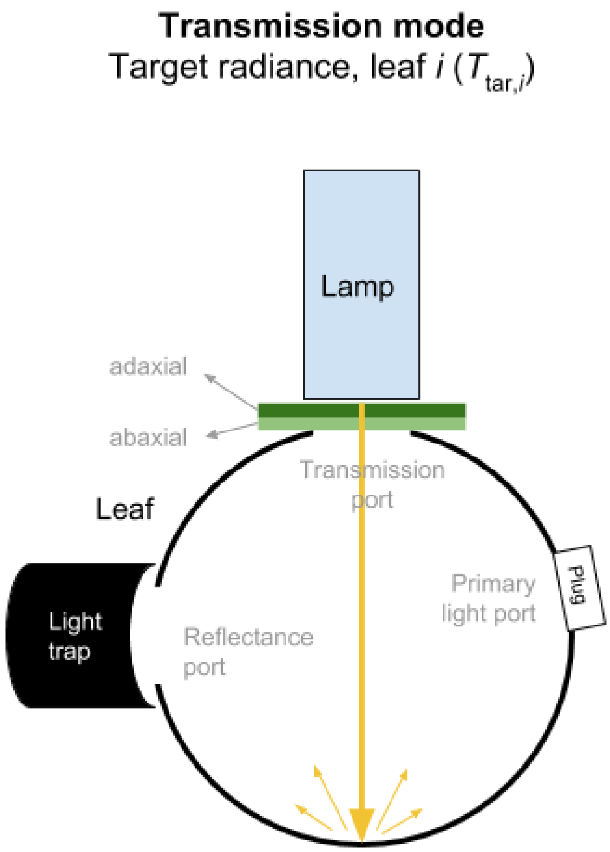
Step 38.

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

Configuration E: Transmission Mode, Target

Step 39.

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



NOTES

Etienne Laliberté 26 Apr 2018

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

ANNOTATIONS

Etienne Laliberté 01 May 2018

From Raymond Soffer 30/04/2018:

"When the leaf is placed in the transmissin port for the Transmission mode measurments, it is likely that it will be subject ot heat form the lamp. This heat can lead to changes at the cellular level that could impact the measured transmission. The time that the leaf is positioned in this configuration should be minimized. Measuring the temperature at this port and designing a destructive test to evaluate the impact of the lamp heat on the leaf is recommended."

Configuration E: Transmission Mode, Target

Step 40.

Carefully replace leaf #1 by leaf #2.

NOTES

Etienne Laliberté 26 Apr 2018

Position the leaf to target the same area measured for the reflectance radiance, with the exception that its abaxial surface now faces into 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.

Configuration E: Transmission Mode, Target

Step 41.

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

NOTES

Etienne Laliberté 26 Apr 2018

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

Configuration E: Transmission Mode, Target

Step 42.

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.

Configuration E: Transmission Mode, Target

Step 43.

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

NOTES

Etienne Laliberté 26 Apr 2018

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

Configuration E: Transmission Mode, Target

Step 44.

Carefully replace leaf #3 by leaf #4.

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.

Configuration E: Transmission Mode, Target

Step 45.

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

NOTES

Etienne Laliberté 26 Apr 2018

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

Configuration E: Transmission Mode, Target

Step 46.

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.

Configuration E: Transmission Mode, Target

Step 47.

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

NOTES

Etienne Laliberté 26 Apr 2018

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

Configuration E: Transmission Mode, Target

Step 48.

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.

Configuration E: Transmission Mode, Target

Step 49.

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

NOTES

Etienne Laliberté 26 Apr 2018

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

Configuration E: Transmission Mode, Target

Step 50.

Remove leaf #6 from the transmission sample port holder.

Calculating Leaf Reflectance (Adaxial Surface)

Step 51.

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

$$\rho_{\mathrm{leaf},i} = (R_{\mathrm{tar},i} - R_{\mathrm{str}}) \div (R_{\mathrm{ref}} - R_{\mathrm{str}}) \times \rho_{\mathrm{ref}}$$

where

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

Calculating Leaf Transmittance (Adaxial Surface)

Step 52.

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

$$\tau_{\text{leaf},i} = (T_{\text{tar},i} \div T_{\text{ref}}) \times \rho_{\text{c}}$$

where

ρ_{c} is the absolute reflectance of the cavity wall, i.e. the region of the sphere that the light beam directly hits in configuration D (i.e. transmission mode, reference).

Because the SVC sphere interior is made of Spectralon®, if ρ_{c} is unknown, it can be reasonably approximated by ρ_{ref}, leading to

$$\tau_{\text{leaf},i} = (T_{\text{tar},i} \div T_{\text{ref}}) \times \rho_{\text{ref}}$$

where

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

Warnings

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