Measuring spectral reflectance and transmittance (350-2500 nm) of large leaves using the Spectra Vista Corporation (SVC) DC-R/T Integrating Sphere Version 4

Etienne Laliberté,Raymond Soffer

Abstract

Here we describe the standardised protocol used by the Canadian Airborne Biodiversity Observatory (CABO) to measure leaf spectral reflectance and transmittance, using the Spectra Vista Corporation (SVC) DC-R/T Integrating Sphere fitted to a portable full-range SVC HR-1024i 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.

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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),
7. Leaves should be stored in a sealed, clear plastic bag (breathe into the bag before sealing it) and **brought immediately to the spectroscopist for measurements**.

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

Raymond Soffer 30 May 2018

Configure the SVC, ensuring that the system time, optics, and acquisition parameters are correctly set.

Raymond Soffer 30 May 2018

Decide if the results are recorded as radiance or raw DN. The benifit is that with Raw DN it is easy to view the signal levels to determine how signal strength is impacting the the noise levels. Ratios of raw produce the same results as ratios of radiance.

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.

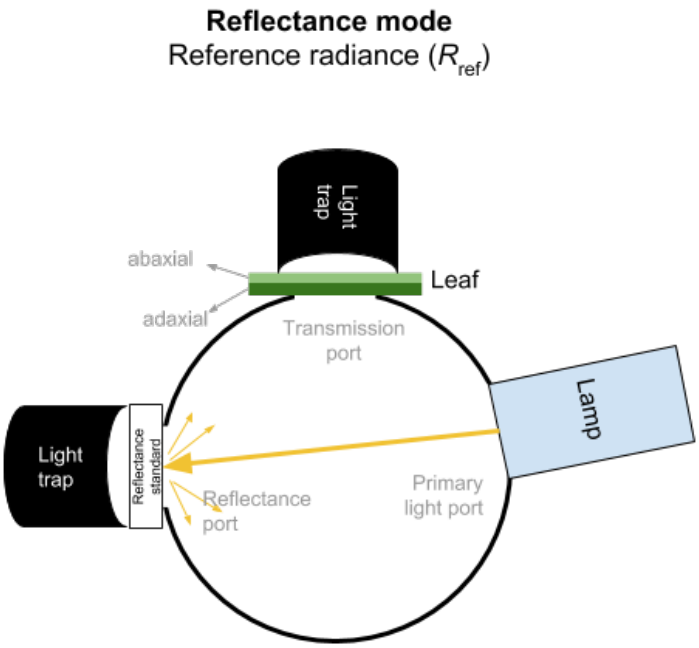
NOTES
Etienne Laliberté 26 Apr 2018

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.

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.

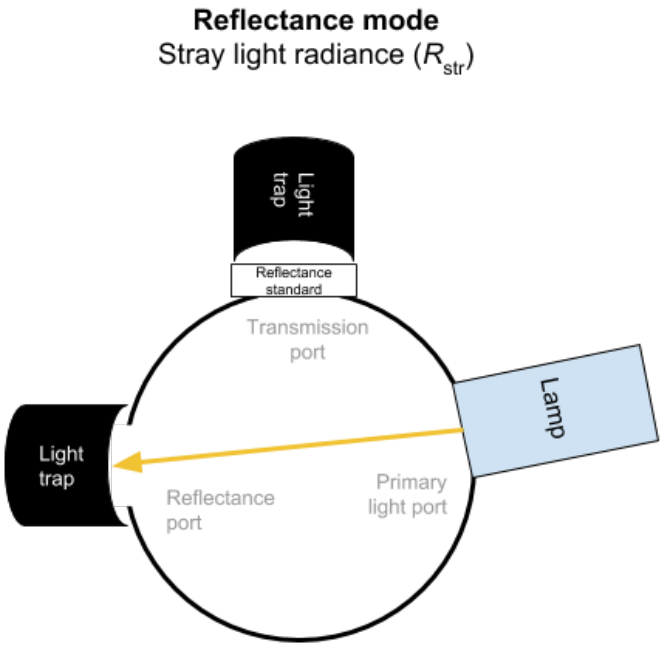
Place the tethered calibrated Spectralon® reflectance standard over the **transmission port** sample

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.

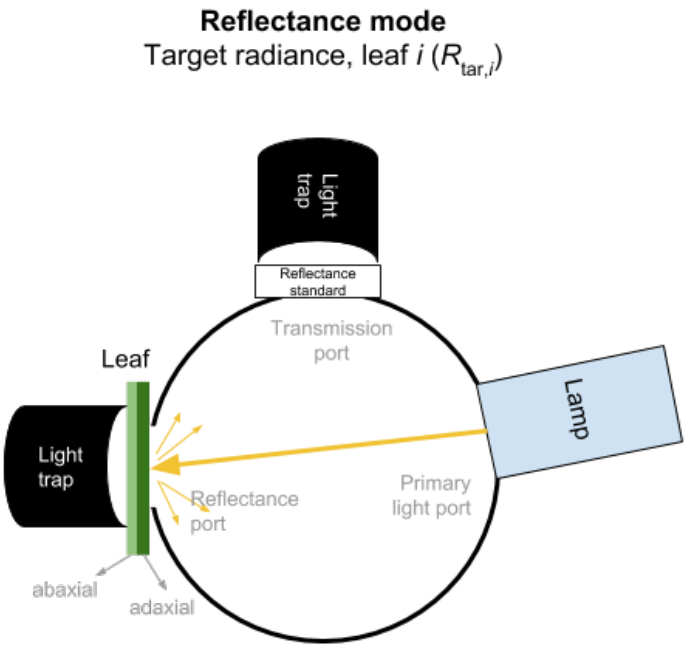
Etienne Laliberté 24 Apr 2018

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 A: Reflectance Mode, Reference

Step 28.

Remove leaf #6 from the reflectance sample port holder.

Configuration A: Reflectance Mode, Reference

Step 29.

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 30.

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

NOTES

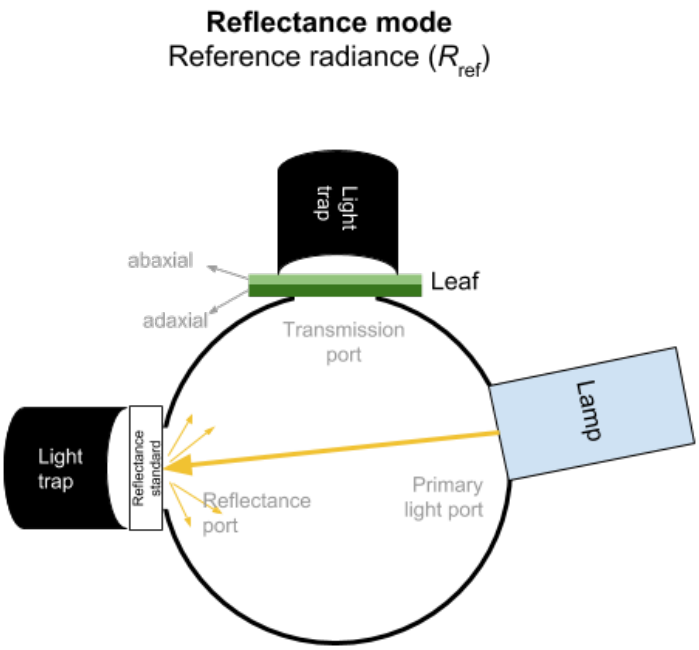
Etienne Laliberté 26 Apr 2018

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 31.

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



NOTES

Etienne Laliberté 08 May 2018

This second reference radiance scan is only used to assess the stability of the system in reflectance mode.

Configuration D: Transmission Mode, Reference

Step 32.

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

Configuration D: Transmission Mode, Reference

Step 33.

Remove leaf #1 from the transmission port sample holder.

Configuration D: Transmission Mode, Reference

Step 34.

Position leaf #1 over 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.

Configuration D: Transmission Mode, Reference

Step 35.

Remove the light trap from the transmission port sample holder.

Configuration D: Transmission Mode, Reference

Step 36.

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 37.

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

NOTES

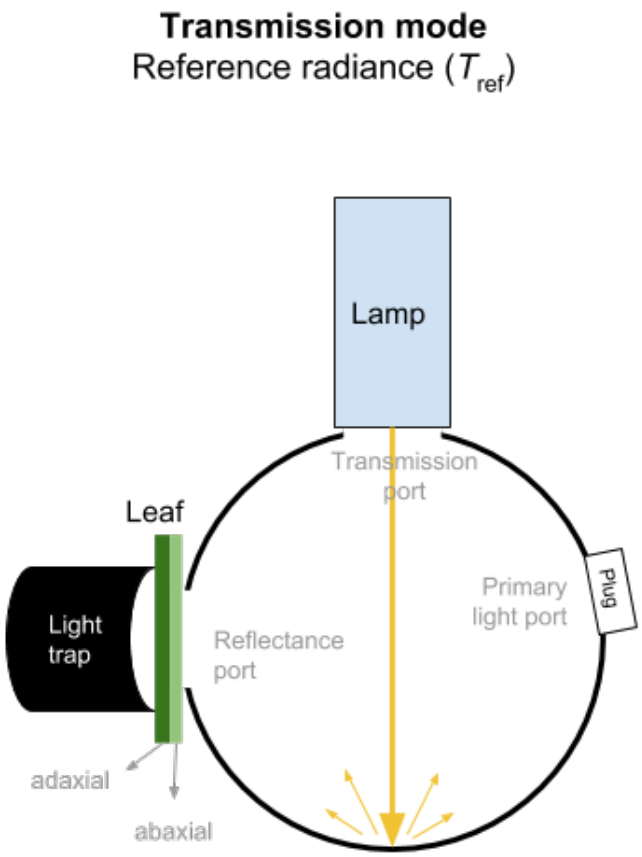
Etienne Laliberté 24 Apr 2018

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

Configuration D: Transmission Mode, Reference

Step 38.

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



NOTES
Etienne Laliberté 26 Apr 2018

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

Configuration D: Transmission Mode, Reference

Step 39.

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

Configuration E: Transmission Mode, Target

Step 40.
Carefully remove leaf #1 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 41.

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 42.

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

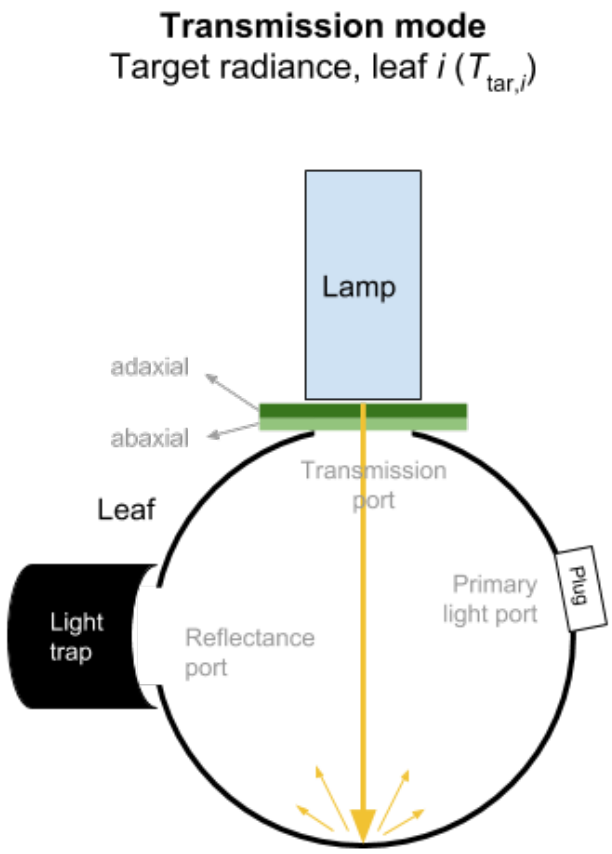
Step 43.

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

Configuration E: Transmission Mode, Target

Step 44.

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 ($T_{tar,1}$).

Configuration E: Transmission Mode, Target

Step 45.

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 46.

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 47.

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 48.

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 49.

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 50.

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 51.

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 52.

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 53.

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 54.

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 ($T_{tar,6}$).

Configuration D: Transmission Mode, Target

Step 55.

Remove leaf #6 from the transmission sample port holder.

Configuration D: Transmission Mode, Reference

Step 56.

Position leaf #1 over the **reflectance port** so that its abaxial (lower) side is now facing the inside of the sphere.

NOTES

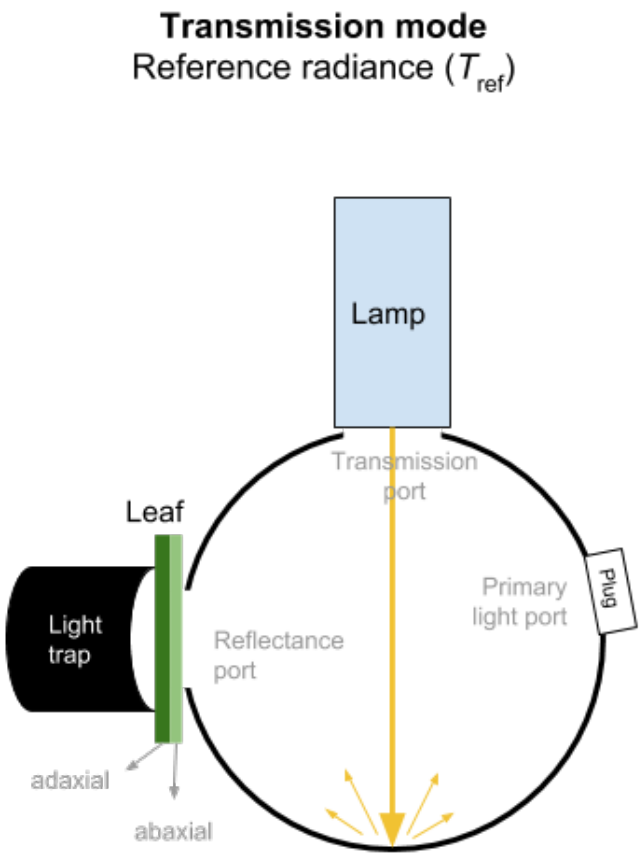
Etienne Laliberté 24 Apr 2018

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.

Configuration D: Transmission Mode, Reference

Step 57.

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



NOTES

Etienne Laliberté 08 May 2018

This second reference radiance scan is only used to assess the stability of the system in transmission mode.

Calculating Leaf Reflectance (Adaxial Surface)

Step 58.

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

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

where

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

Calculating Leaf Transmittance (Adaxial Surface)

Step 59.

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

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

Warnings

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