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Measuring spectral reflectance and transmittance (350-2500 nm) of large leaves using the Spectra Vista Corporation (SVC) DC-R/T Integrating Sphere V.5

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Protocol status: In development
We are still developing and optimizing this protocol.

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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. Reflectance measurements are referenced to a calibrated Spectralon® disk and corrected for stray light. Our leaf spectroscopy protocol builds from **that** of the **Carnegie Airborne Observatory**.

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.

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

Troubleshooting

Safety warnings

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

Before start

Consult the user manual of the spectroradiometer and the integrating sphere to set up the instrument.

The instrument should be set up in the shade, sheltered as much as possible from the elements (definitely not exposed to rain).

All canopy plants selected for measurements should have already been tagged, identified, and georeferenced before spectroscopy measurements start.

The spectroscopist should be positionned as close as possible to the sampled plants to minimise time from collection to measurement.

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.

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),

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**.

Instrument Set-Up

1 Install the integrating sphere onto the spectroradiometer.

Note

Follow the SVC integrating sphere manual p. 6-16.

2 Power the spectroradiometer and **warm up for >15 min**.

00:15:00 Spectroradiometer warm-up period

Note

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

3 Power the integrating sphere lamp and **warm up for >5 min**.

00:05:00 Integrating sphere warm-up period

Software Set-Up

4 Ensure that the spectroradiometer is connected to the computer via Bluetooth.

Note

The default Bluetooth password for SVC HR-1024i spectroradiometers is **hr1024i**.

5 Launch SVC Scan.

6 Select **auto-save mode** using *File* > *Data Options* > *Auto Save.*

7 Select *File* > *New* to create a new working folder in your shared '**spectra**' Google Drive CABO folder.

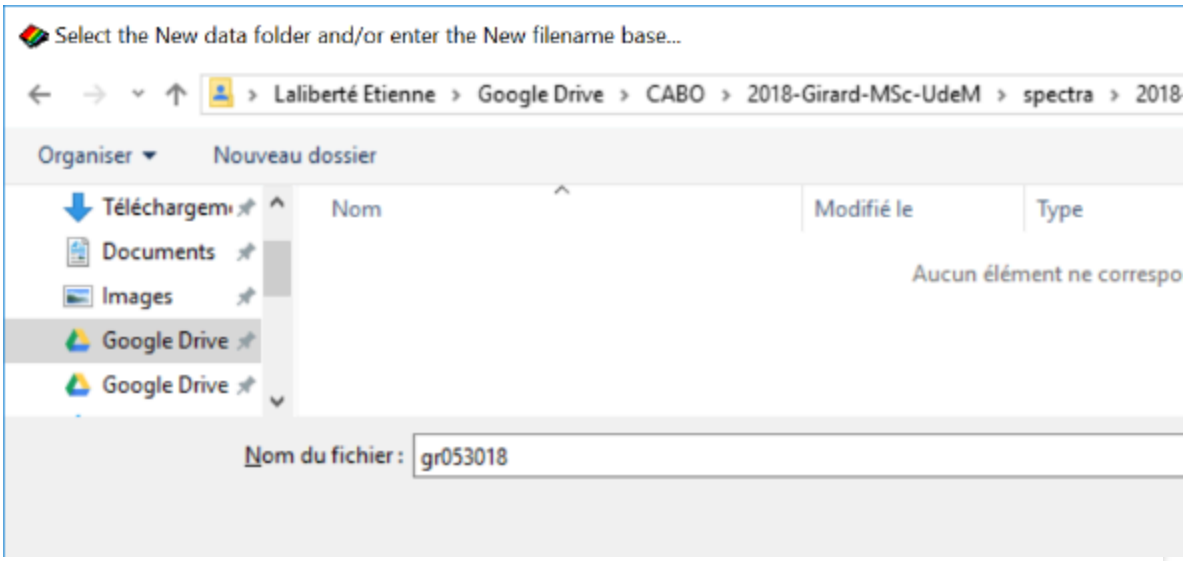
Note

If you do not yet have a shared Google Drive folder for your project, contact the CABO data manager to create one (**etienne.laliberte@umontreal.ca** or **jeremy.goimard@umontreal.ca**).

Note

Your shared CABO Google Drive folder should be named *YYYYstarted-YourLastName-Degree-University*, e.g. 2018-Girard-MSc-UdeM.

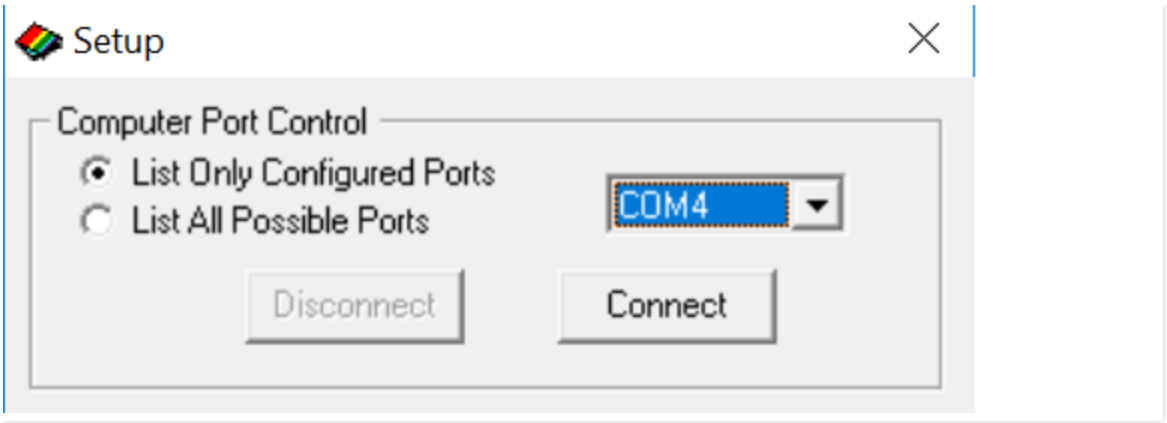
8 Create a new folder named *YYYY-MM-DD-SiteID-SpectroradiometerSerialNumber* (without spaces) within that '**spectra**' folder, and keep the default file base name (= *grMMDDYY*).



Note

The base file name is the prefix for all files that will be saved within that folder. In auto-save mode, each file will be added an incremental step, e.g., *gr053018_0000.sig*, *gr053018_0001.sig*, etc.

9 Go to *Control* > *Setup Instrument...* to connect the spectroradiometer.

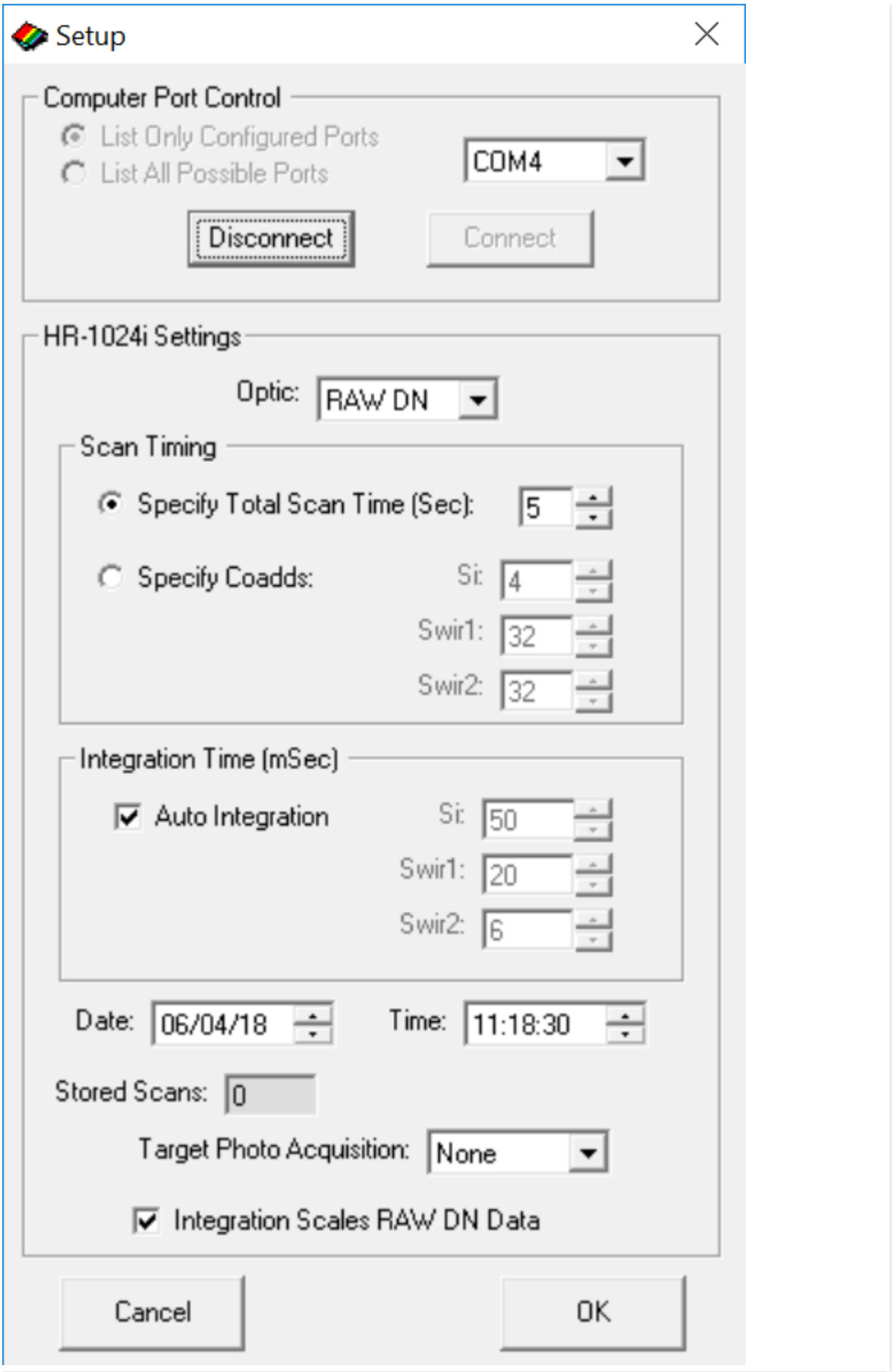


Note

Different instruments may be configured under different virtual COM ports.

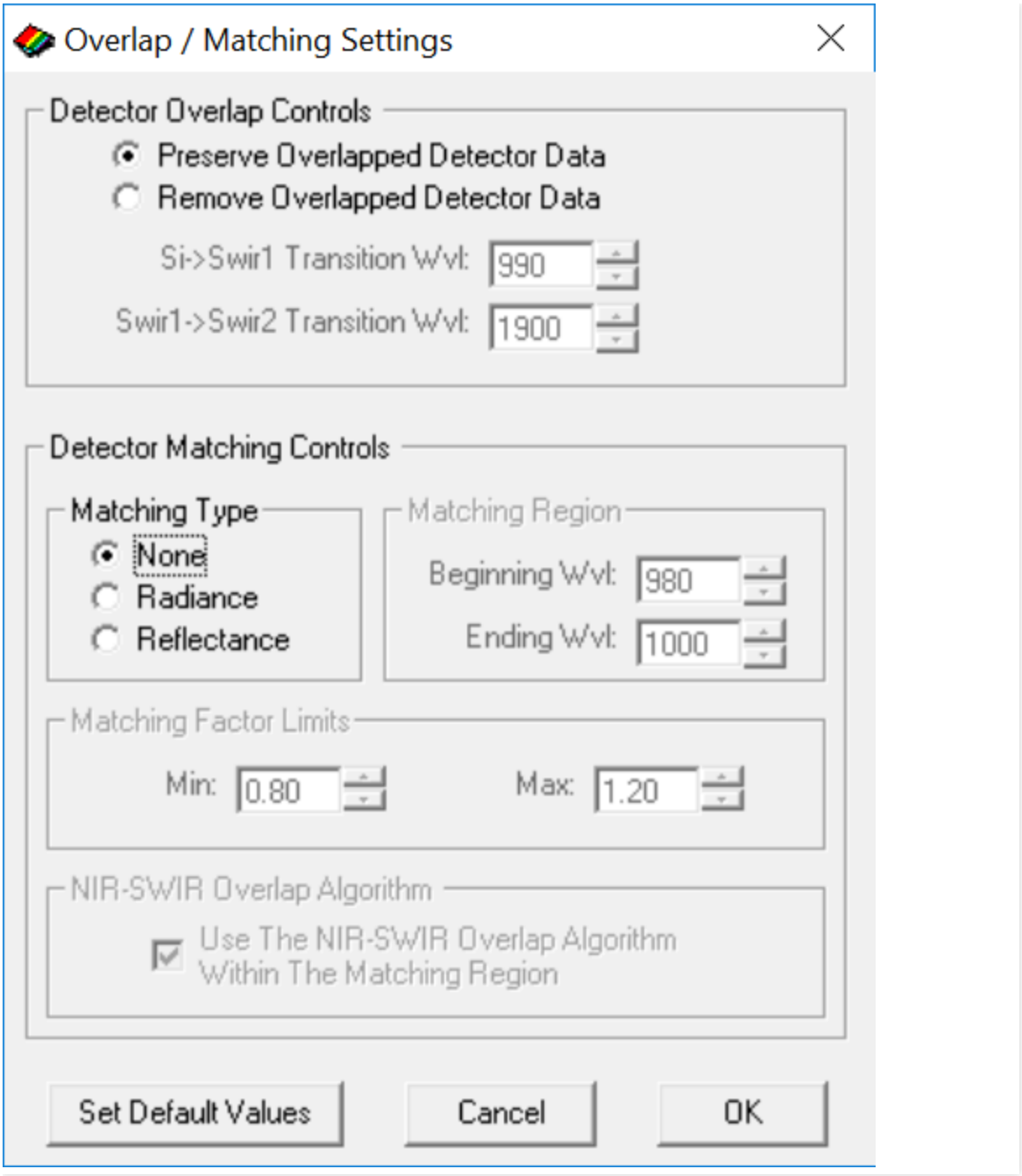
10 1. Choose the *RAW DN* optic

2. Select 5 sec scan time
3. Select *Auto Integration*
4. Ensure the date and time are correct
5. Check *Integration Scales Raw DN Data*


Setup
Computer Port Control
List Only Configured Ports
List All Possible Ports
COM4
Disconnect
Connect
HR-1024i Settings
Optic:
RAW DN
Scan Timing
Specify Total Scan Time (Sec):
5
Specify Coadds:
Si:
4
Swir1:
32
Swir2:
32
Integration Time (mSec)
Auto Integration
Si:
50
Swir1:
20
Swir2:
6
Date:
06/04/18
Time:
11:18:30
Stored Scans:
0
Target Photo Acquisition:
None
Integration Scales RAW DN Data
Cancel
OK

11 In *Control* > Overlap / *Matching Settings:*

1. Select *Preserve Overlapped Detector Data*
2. Select *Matching Type* > *None*



Review Protocol Summary Diagram

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

spectrostandard-5.pdf

Configuration A: Reflectance Mode, Reference

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

Note

Make sure lamp is secured in locked position.

Safety information

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

14 Check lamp alignment.

Note

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. 29-32.

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

Note

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

16 Screw the untethered light trap on the **transmission port** sample holder.

Note

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

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

Note

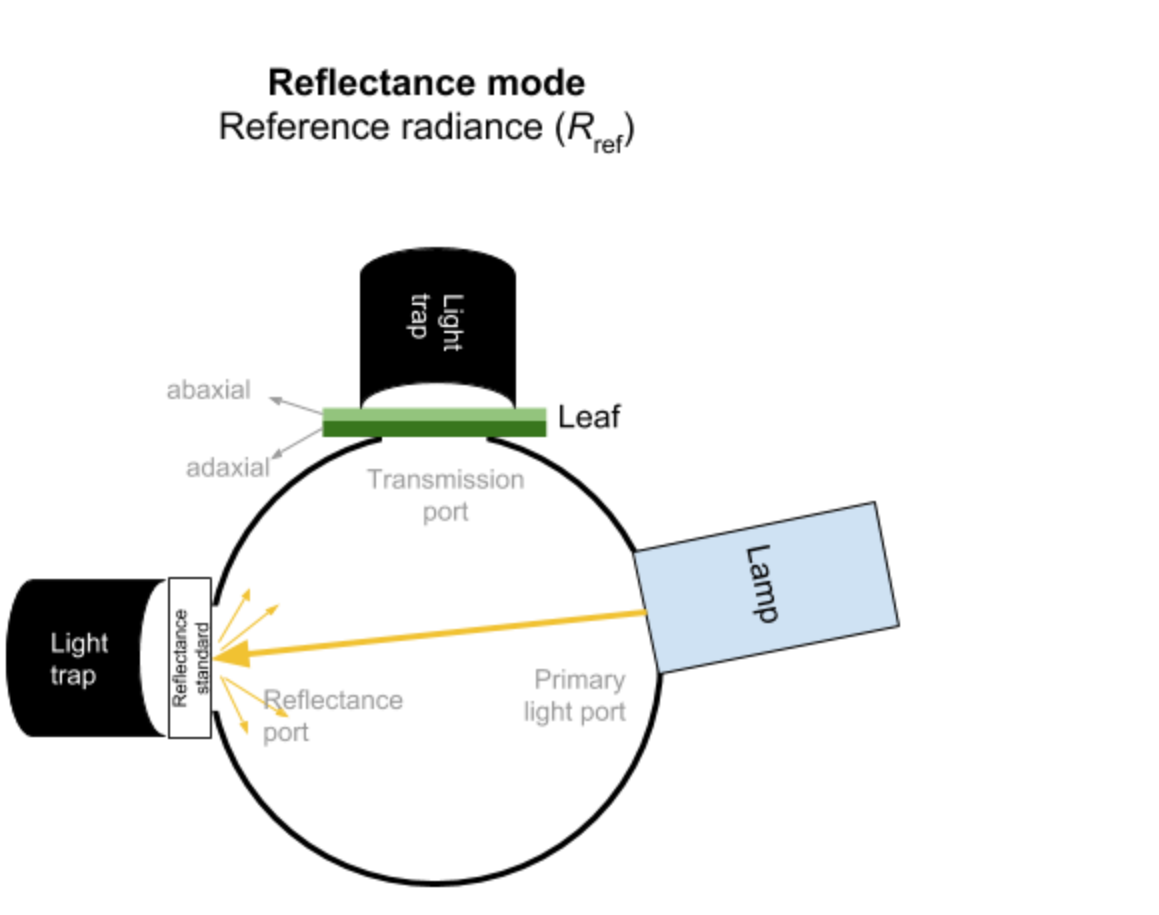
Place the standard over the reflectance port so that the light beam shines directly on its reflective surface (i.e facing into the sphere).

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

Note

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.

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



Note

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.

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

Note

This reference 'target' scan, immediately collected after the reference, should give relative reflectance around 1 (+ noise) for all wavelengths. It is saved in a separate file to facilitate post-processing of spectra.

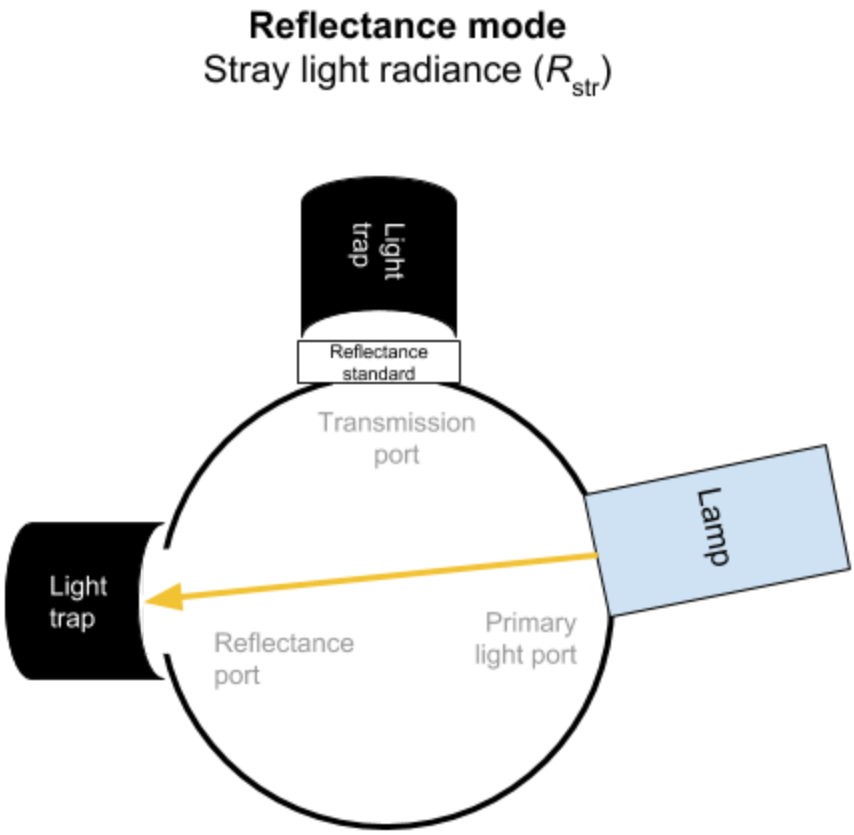
Configuration B: Reflectance Mode, Stray light

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

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

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

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



Note

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

Configuration C: Reflectance Mode, Target

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

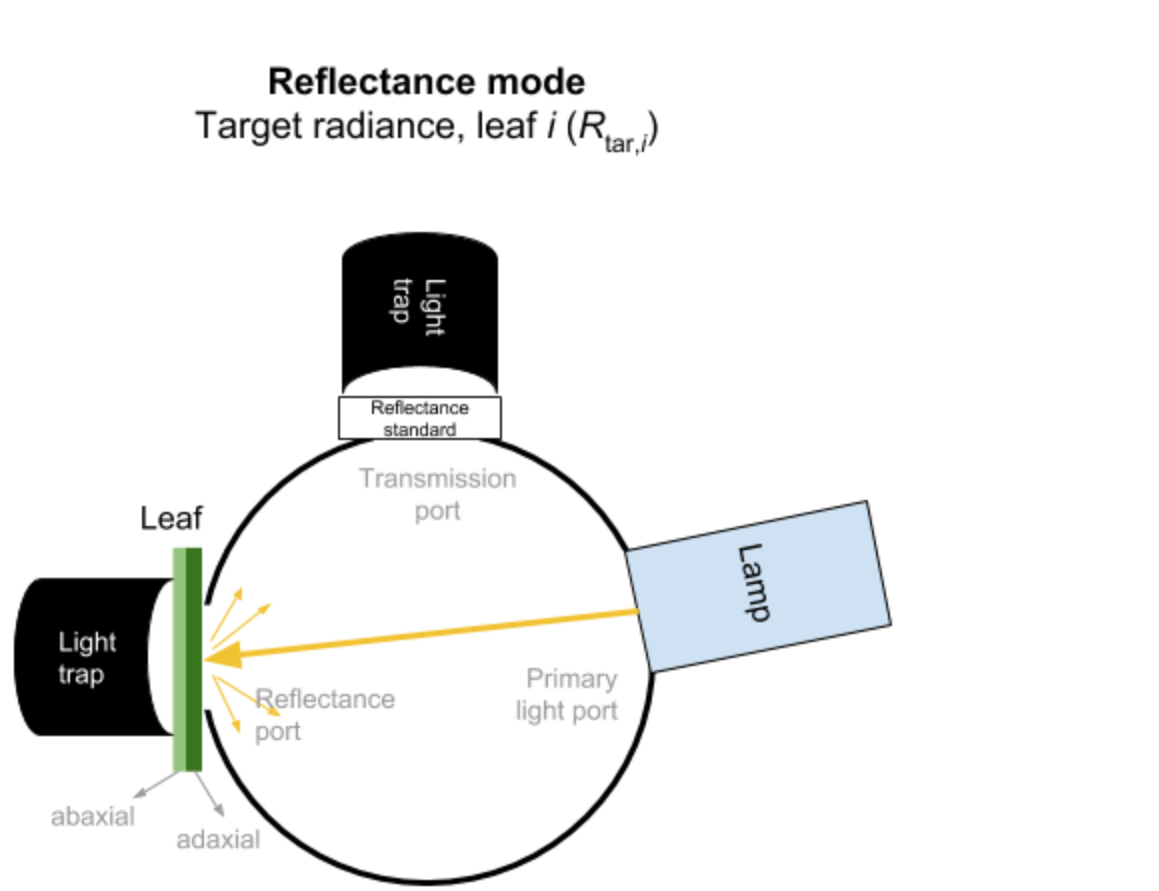
Note

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.

Note

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

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



Note

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

27 Carefully replace leaf #1 by leaf #2.

Note

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.

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

Note

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

29 Carefully replace leaf #2 by leaf #3.

Note

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.

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

Note

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

31 Carefully replace leaf #3 by leaf #4.

Note

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.

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

Note

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

33 Carefully replace leaf #4 by leaf #5.

Note

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.

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

Note

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

35 Carefully replace leaf #5 by leaf #6.

Note

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.

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

Note

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

Configuration A: Reflectance Mode, Reference

37 Remove leaf #6 from the reflectance sample port holder.

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

Note

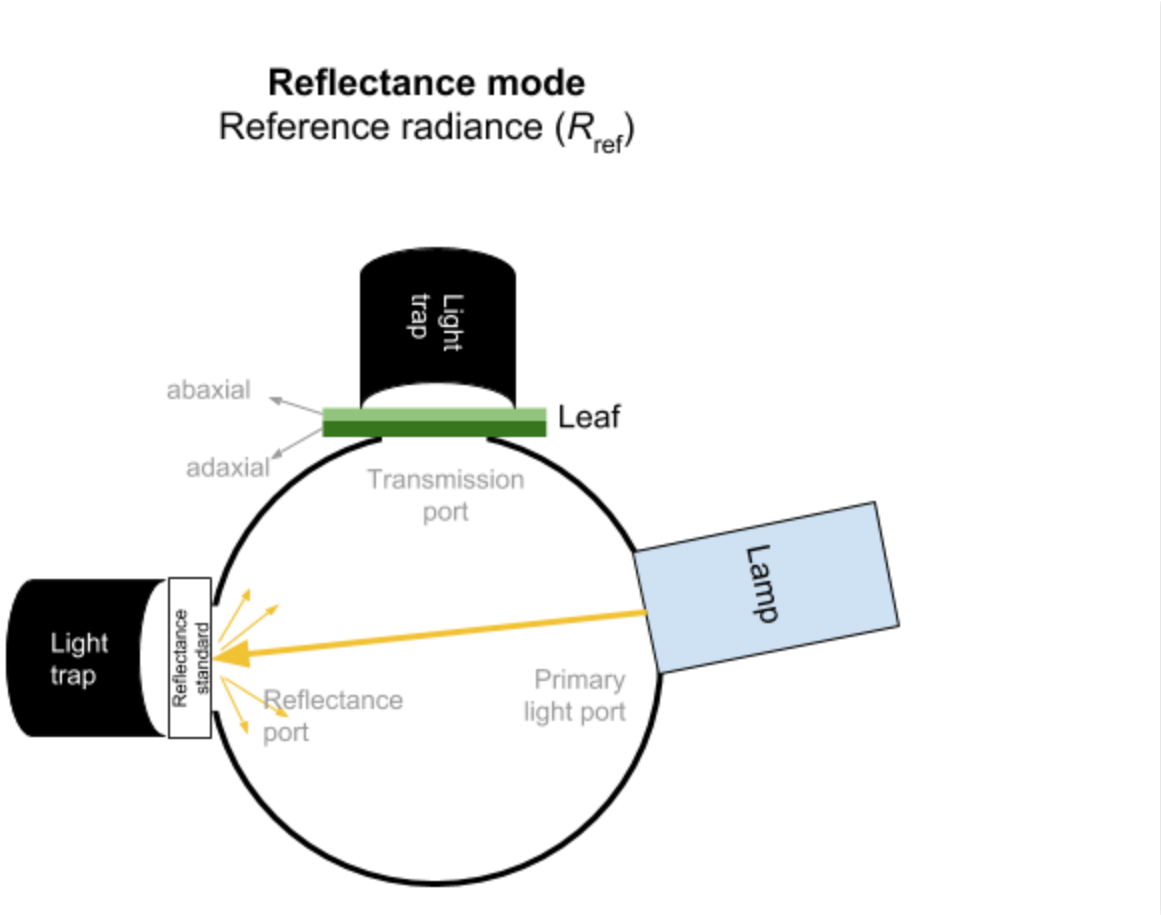
Place the standard over the reflectance port so that the light beam shines directly on its reflective surface (i.e facing into the sphere).

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

Note

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.

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



Note

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

Configuration D: Transmittance Mode, Reference

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

42 Remove leaf #1 from the transmission port sample holder.

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

Note

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.

44 Remove the light trap from the transmission port sample holder.

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

Note

Make sure lamp is secured in locked position.

Safety information

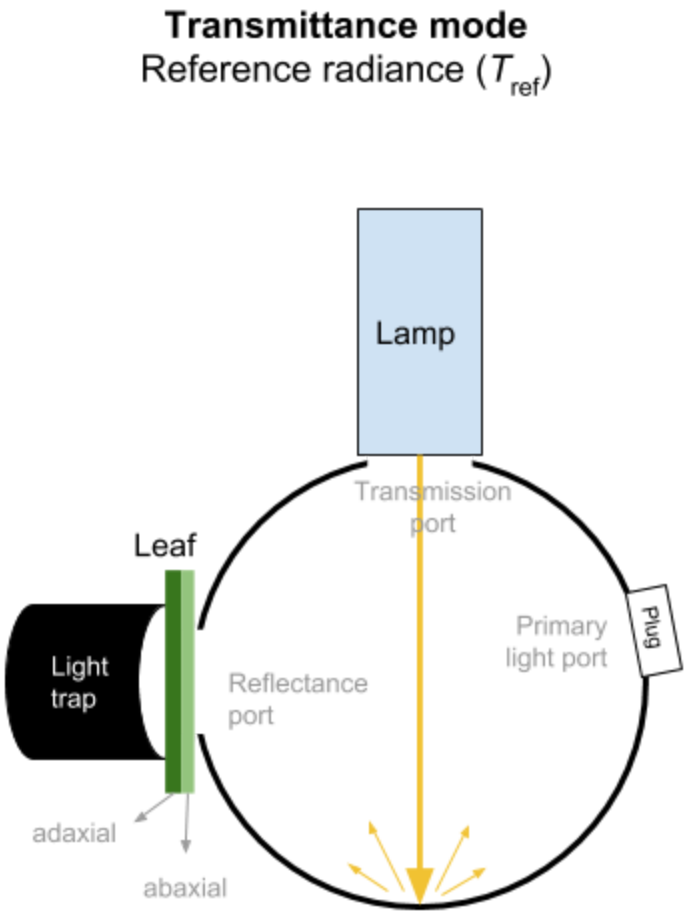
The lamp can get very hot. Grab it by the slotted heat shield.

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

Note

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

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



Note

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

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

Note

This reference 'target' scan, immediately collected after the reference, should give relative reflectance around 1 (+ noise) for all wavelengths. It is saved in a separate file to facilitate post-processing of spectra.

Configuration E: Transmittance Mode, Target

49 Carefully remove leaf #1 from the reflectance port sample holder.

Note

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

50 Gently pull lamp and transmission sample holder away from the sphere.

Safety information

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

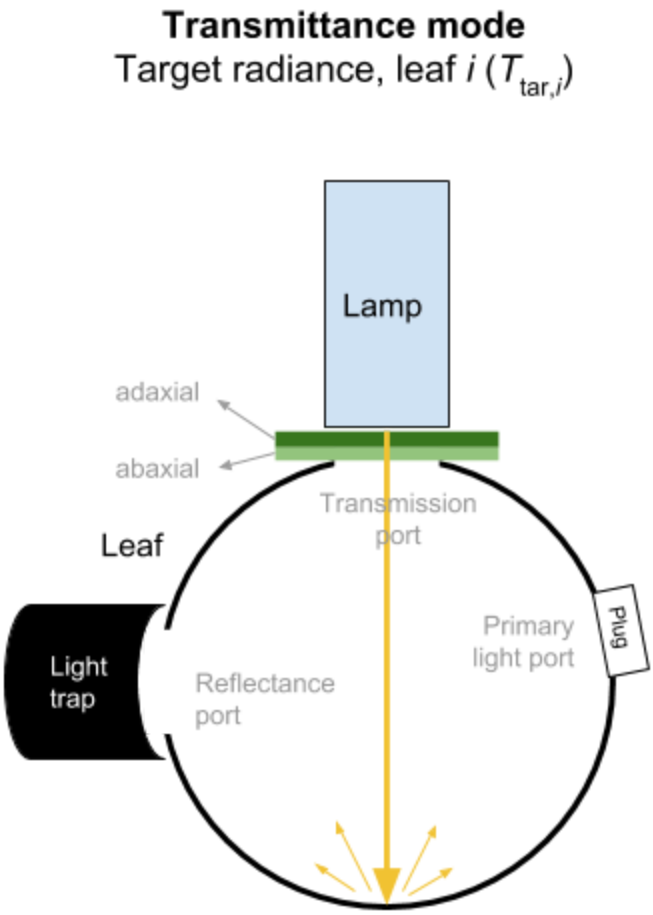
51 Place leaf #1 over the **transmission port** with its **abaxial** (lower) surface facing into the sphere.

Note

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.

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

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



Note

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

54 Carefully replace leaf #1 by leaf #2.

Note

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.

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

Note

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

56 Carefully replace leaf #2 by leaf #3.

Note

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.

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

Note

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

58 Carefully replace leaf #3 by leaf #4.

Note

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.

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

Note

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

60 Carefully replace leaf #4 by leaf #5.

Note

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.

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

Note

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

62 Carefully replace leaf #5 by leaf #6.

Note

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.

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

Note

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

Configuration D: Transmittance Mode, Reference

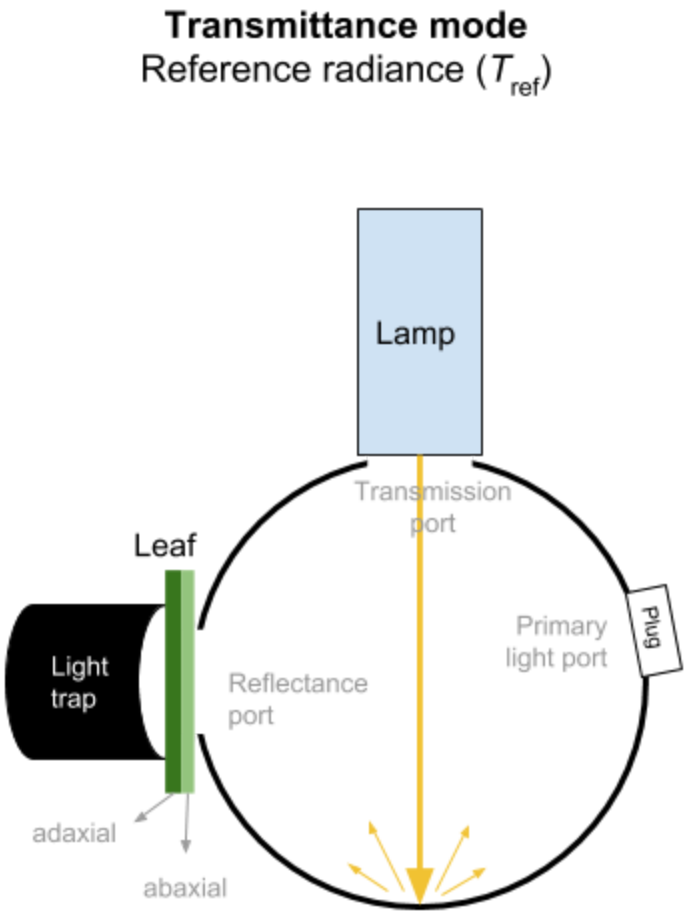
64 Remove leaf #6 from the transmission sample port holder.

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

Note

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.

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



Note

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

Calculating Leaf Reflectance (Adaxial Surface)

67 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)

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

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