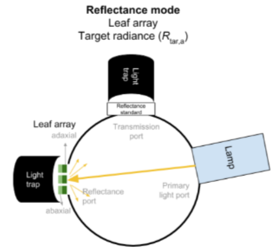
Apr 26, 2018 Version 1

Measuring spectral reflectance and transmittance (350-2500 nm) of small and/or narrow leaves using an integrating sphere V.1



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

DOI
dx.doi.org/10.17504/protocols.io.prkdm4w

Etienne Laliberté[1]

[1]Université de Montréal

Canadian Airborne Biodiversity Observatory
Tech. support email: **jocelyne.ayotte@umontreal.ca**

Etienne Laliberté
Université de Montréal

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.prkdm4w

External link: www.caboscience.org

Protocol Citation: Etienne Laliberté 2018. Measuring spectral reflectance and transmittance (350-2500 nm) of small and/or narrow leaves using an integrating sphere. **protocols.io https://dx.doi.org/10.17504/protocols.io.prkdm4w**

Manuscript citation:
Noda, H. M., T. Motohka, K. Murakami, H. Muraoka, and K. N. Nasahara. 2013. Accurate measurement of optical properties of narrow leaves and conifer needles with a typical integrating sphere and spectroradiometer. Plant, Cell & Environment 36:1903–1909. https://doi.org/10.1111/pce.12100

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

Created: April 25, 2018

Last Modified: May 31, 2018

Protocol Integer ID: 11788

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, for the special case where an individual **leaf is too small and/or too narrow** to entirely cover the reflectance or transmission port of the integrating sphere. Briefly, three arrays of mature, healthy and sunlit leaves from a canopy plant are arranged on a custom sample mount, and are then used for measurements of adaxial reflectance and transmittance. Leaf array scans are referenced to a calibrated Spectralon® disk and corrected for stray light to yield NIST-traceable, leaf spectral reflectance and transmittance measurements. Our leaf spectroscopy protocol builds from that of **Noda et al. (2013)**, as well as **Carnegie Airborne Observatory**'s **protocol** and integrating sphere user manuals from two companies (**SVC**, **ASD Inc.**).

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 arrays during measurements (optional)

Consumables

- Nitrile gloves for handling leaves
- Whatman No. 2 filter paper (110 mm diameter)
- Acetate sheets (to make thin plastic sample mounts)
- Manila file folders (to make thin cardbboard sample platforms)
- Scotch™ Magic Tape

Materials

MATERIALS

- Manila File Folders **Staples Catalog** #116723
- Write-On Transparency Film **Staples Catalog** #954144
- Whatman™ Qualitative Filter Paper: Grade 2 Circles (110 mm diameter) **Fisher Scientific Catalog** #09-810E
- Scotch™ Magic Tape **Staples Catalog** #14172

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.
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.
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).

Instrument set-up

1 Install the integrating sphere onto the spectroradiometer.

Note

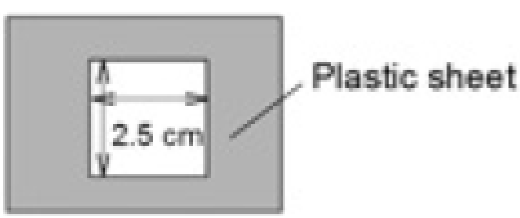
Follow the SVC integrating sphere manual p. 9-14.

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

00:15:00 Spectroradiometer and lamp warm-up time

Prepare sample mounts and sample holders

3 Build a few sample mounts from thin plastic (e.g. acetate sheets).



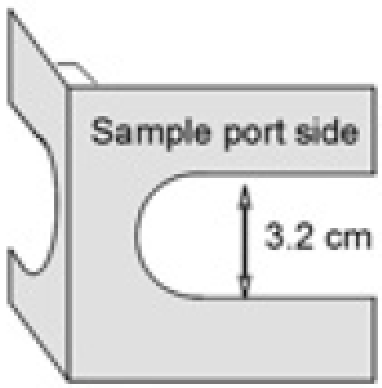
Note

The sample mount is made of a thin plastic sheet with a square window that is larger than the edge of the sample port lip (e.g. 2.5 cm × 2.5 cm).

Note

Fig. 3a from Noda et al. (2013). https://doi.org/10.1111/pce.12100

4 Build a few sample platforms from thin cardboard (e.g. file folders).



Note

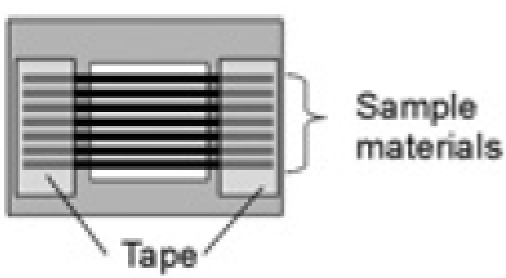
The sample platform is made of cardboard and will eventually attach to the integrating sphere to create a flat surface.

Note

Fig. 3c from Noda et al. (2013). https://doi.org/10.1111/pce.12100

Prepare leaf arrays

5 Fix leaves onto the sample mount with tape to make leaf array #1.



Note

Leaves should be separated by about 1 mm to avoid multiple scattering among them.

If leaves are shorter than the sample mount hole (i.e. <2.5 cm long), fix leaves by their petiole with tape in or two rows.

Note

Fig. 3b from Noda et al. (2013). https://doi.org/10.1111/pce.12100

6 Fix leaves onto the sample mount with tape to make leaf array #2.

Note

Leaves should be separated by about 1 mm to avoid multiple scattering among them.

If leaves are shorter than the sample mount hole (i.e. <2.5 cm long), fix leaves by their petiole with tape in or two rows.

7 Fix leaves onto the sample mount with tape to make leaf array #3.

Note

Leaves should be separated by about 1 mm to avoid multiple scattering among them.

If leaves are shorter than the sample mount hole (i.e. <2.5 cm long), fix leaves by their petiole with tape in or two rows.

8 Fix sample mount holding leaf array #1 onto the sample platform with tape.

Note

The sample mount holding the leaf array should be sandwiched between the two sides of the cardboard sample platform.

9 Fix sample mount holding leaf array #2 onto the sample platform with tape.

Note

The sample mount holding the leaf array should be sandwiched between the two sides of the cardboard sample platform.

10 Fix sample mount holding leaf array #3 onto the sample platform with tape.

Note

The sample mount holding the leaf array should be sandwiched between the two sides of the cardboard sample platform.

Reflectance: Reference scan set-up

11 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.

12 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. 23-24.

13 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.

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

Note

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

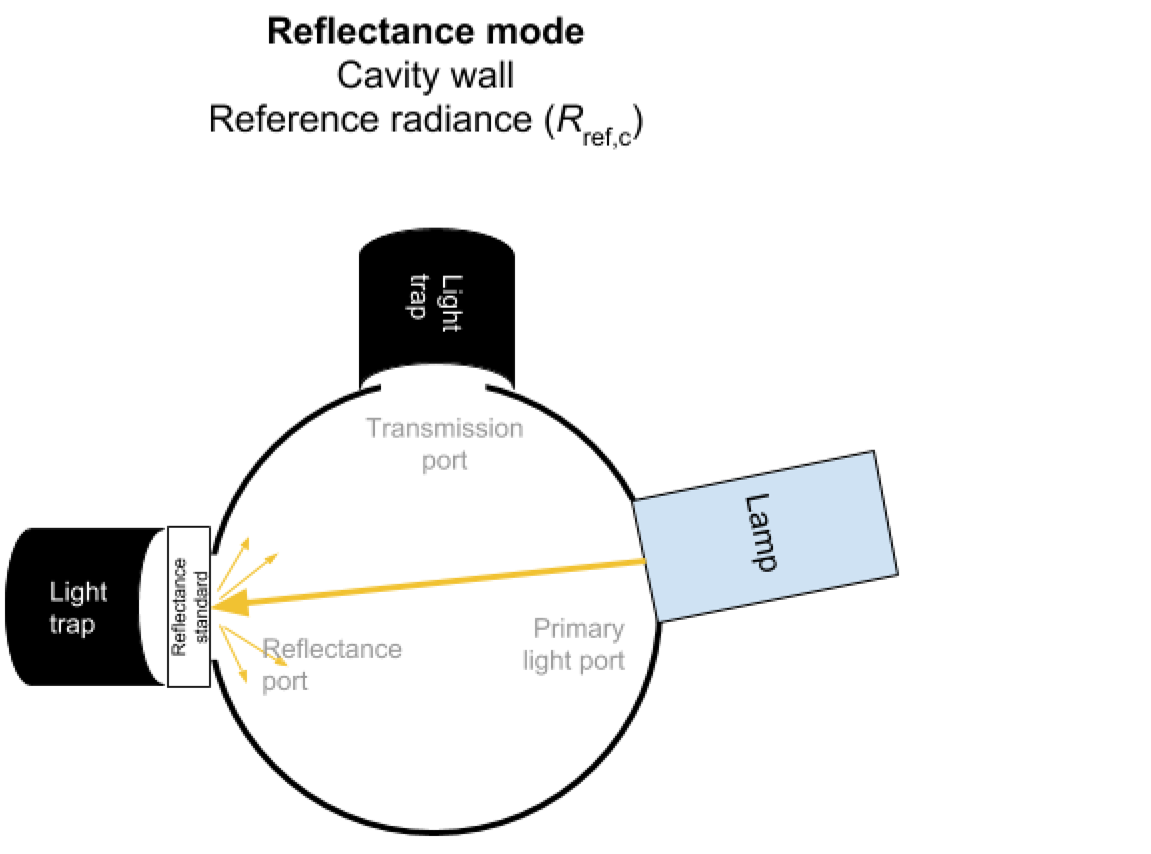
15 Place the tethered calibrated Spectralon® reflectance standard over the **reflectance port**.

Note

Place the standard over the reflectance port so that the light beam shines directly on its reflective surface (= facing inside of the sphere).

Reflectance (cavity wall): Reference scan

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



Note

The **transmission port** should be **empty** (but with the **light trap on**).

Note

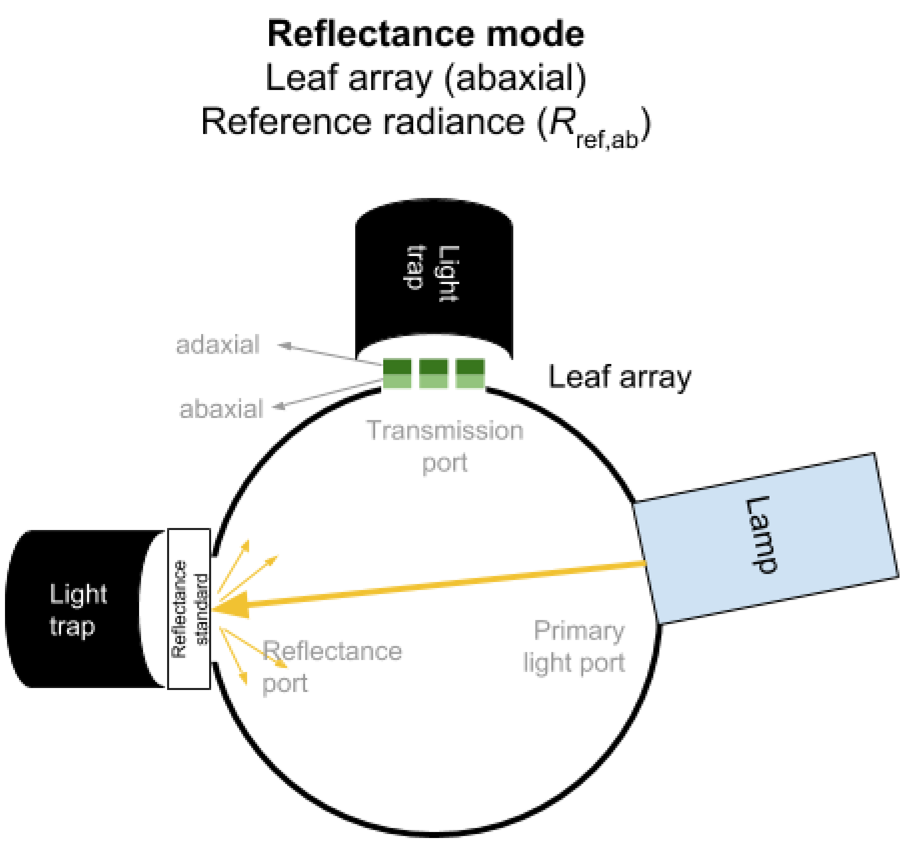
This corresponds the the **reference radiance** of the **cavity wall** (i.e. sphere wall) in reflectance mode ($R_{ref,c}$). It will be used in the transmittance calculation.

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

Reflectance (leaf array, abaxial): Reference scan

18 Position the sample platform containing leaf array #1 over the **transmission port** with the **abaxial** (lower) surface of the leaves facing the inside of the sphere.

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



Note

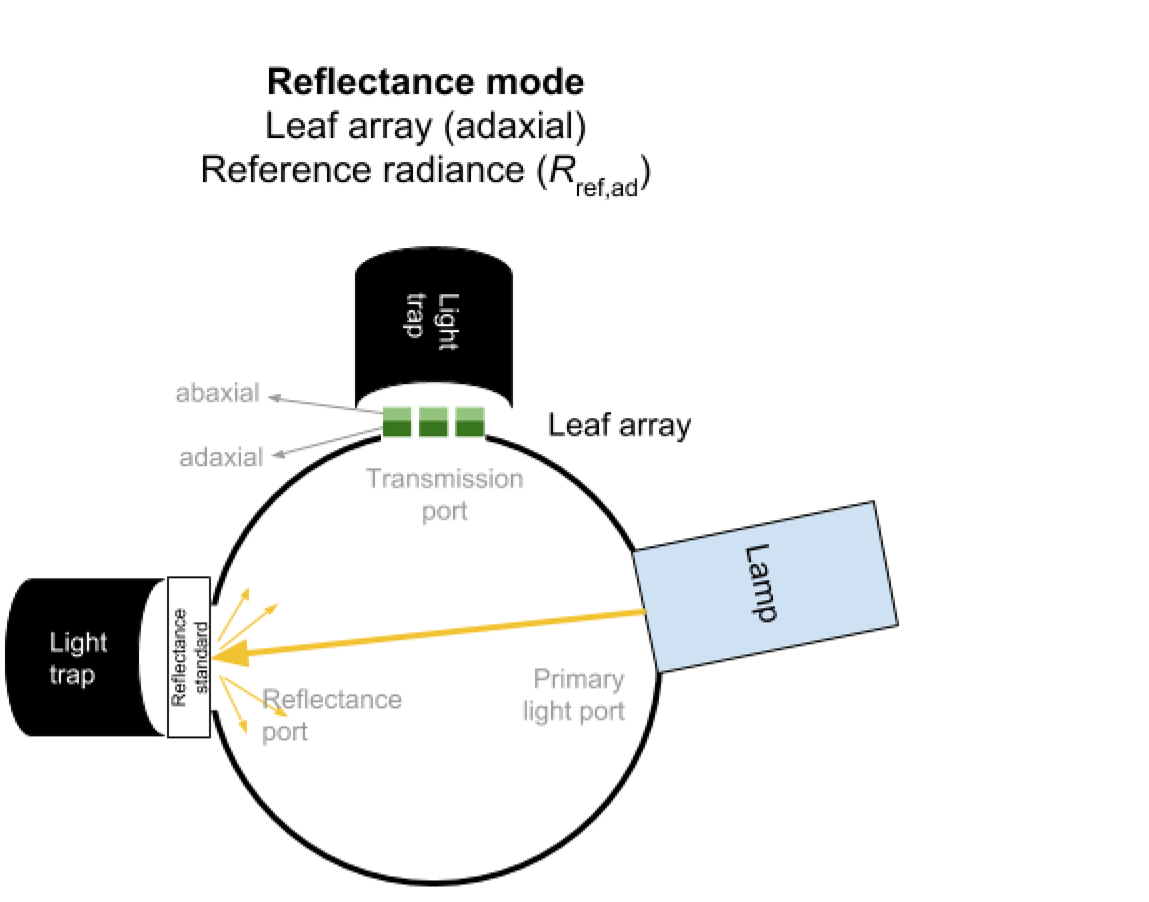
This corresponds to the **reference radiance** for the **leaf array** (**adaxial**) in reflectance mode ($R_{ref,ad}$).

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

Reflectance (leaf array, adaxial): Reference scan

21 Flip leaf array #1 around the transmission port so that the **adaxial** (upper) surface of the leaves is now facing the inside of the sphere.

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



Note

This corresponds to the **reference radiance** for the **leaf array** (**adaxial**) in reflectance mode ($R_{ref,ad}$).

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

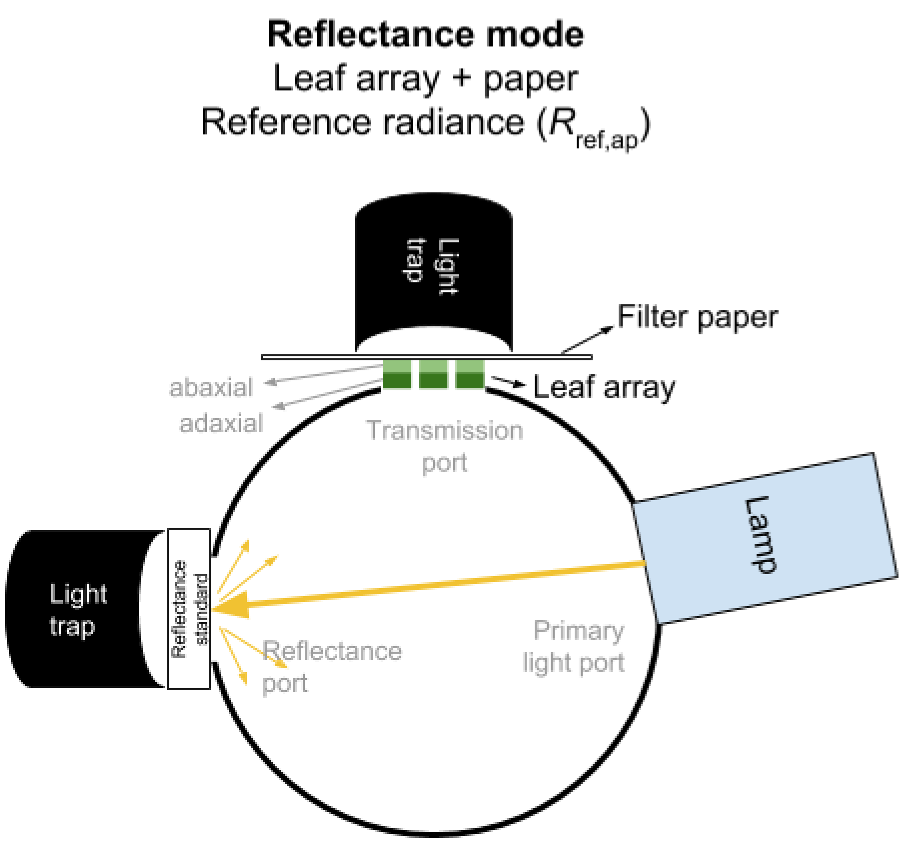
Reflectance (leaf array, adaxial + filter paper): Reference scan

24 Place a filter paper directly behind the sample platform holding leaf array #1.

Note

The leaf array #1 should remain in the same position.

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



Note

This corresponds to the **reference radiance** for the **leaf array** (**adaxial**) **+ paper** in reflectance mode ($R_{ref,ap}$).

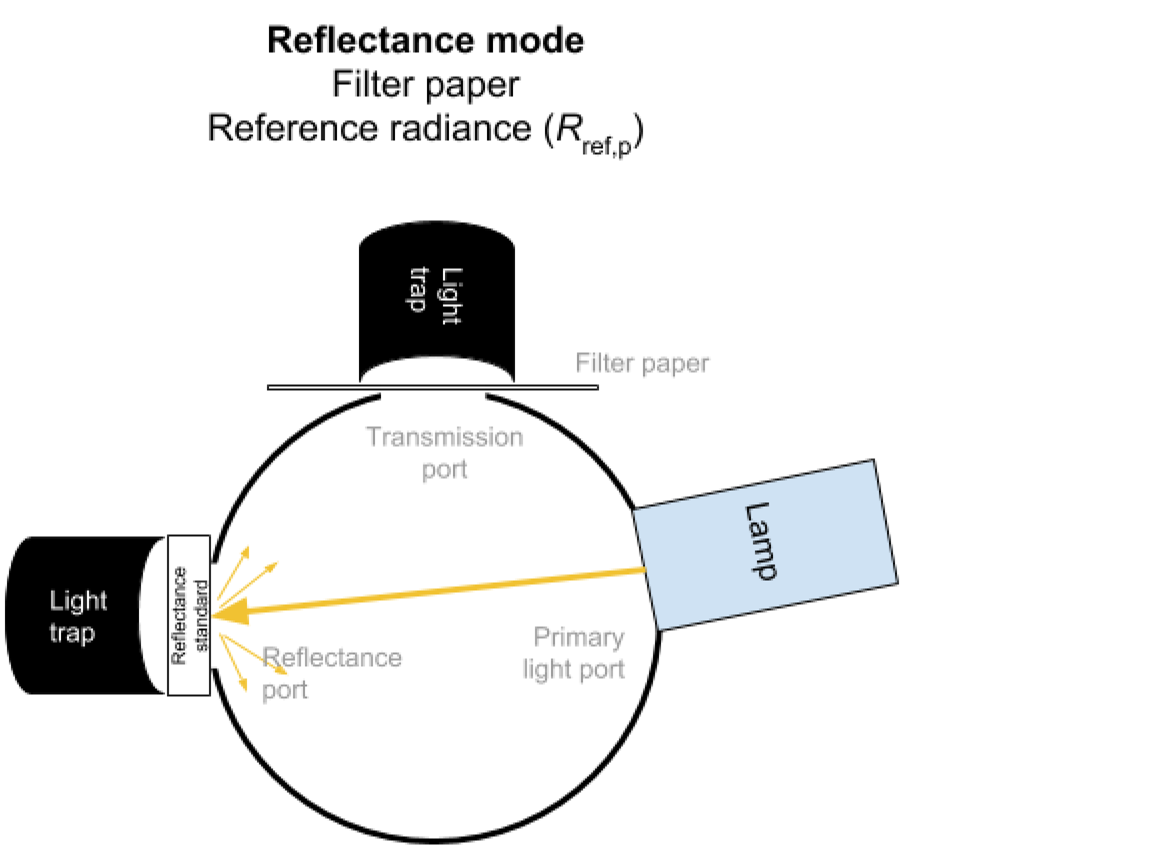
26 Collect a '**Target Scan**' in the same configuration and **save the file**.

Reflectance (filter paper): Reference scan

27 Carefully remove the sample platform holding leaf array #1 from the transmission port.

> Note
>
> The filter paper should remain in the same position.

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



> Note
>
> This corresponds to the **reference radiance** for the **filter paper** in reflectance mode ($R_{ref,p}$).

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

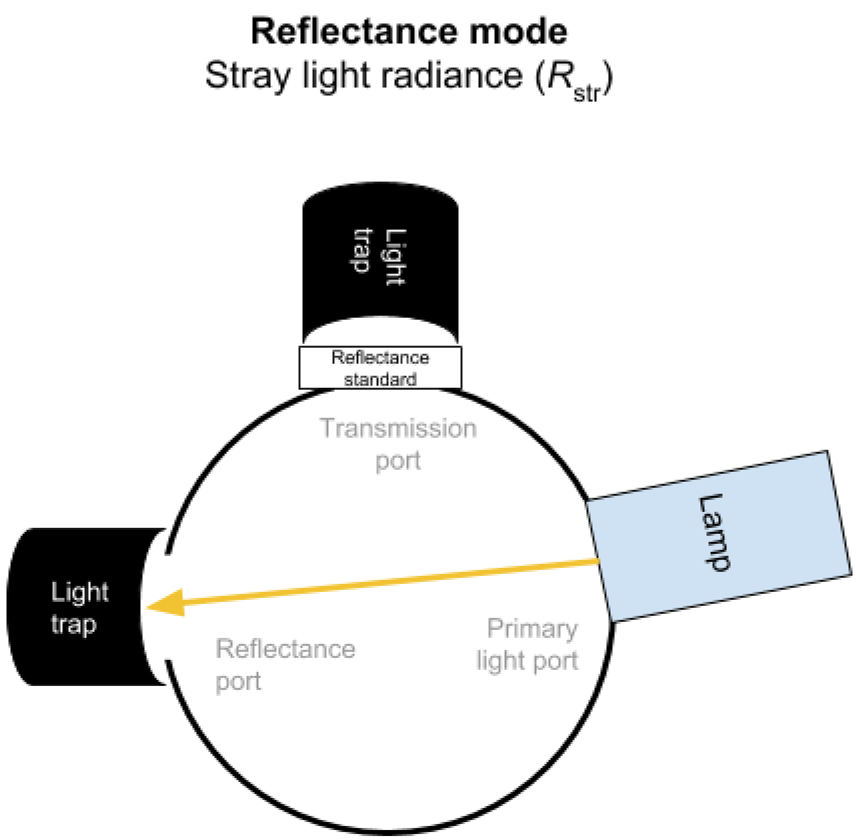
Reflectance: Stray light

30 Remove the filter paper from the transmission port sample holder.

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

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

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



Note

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

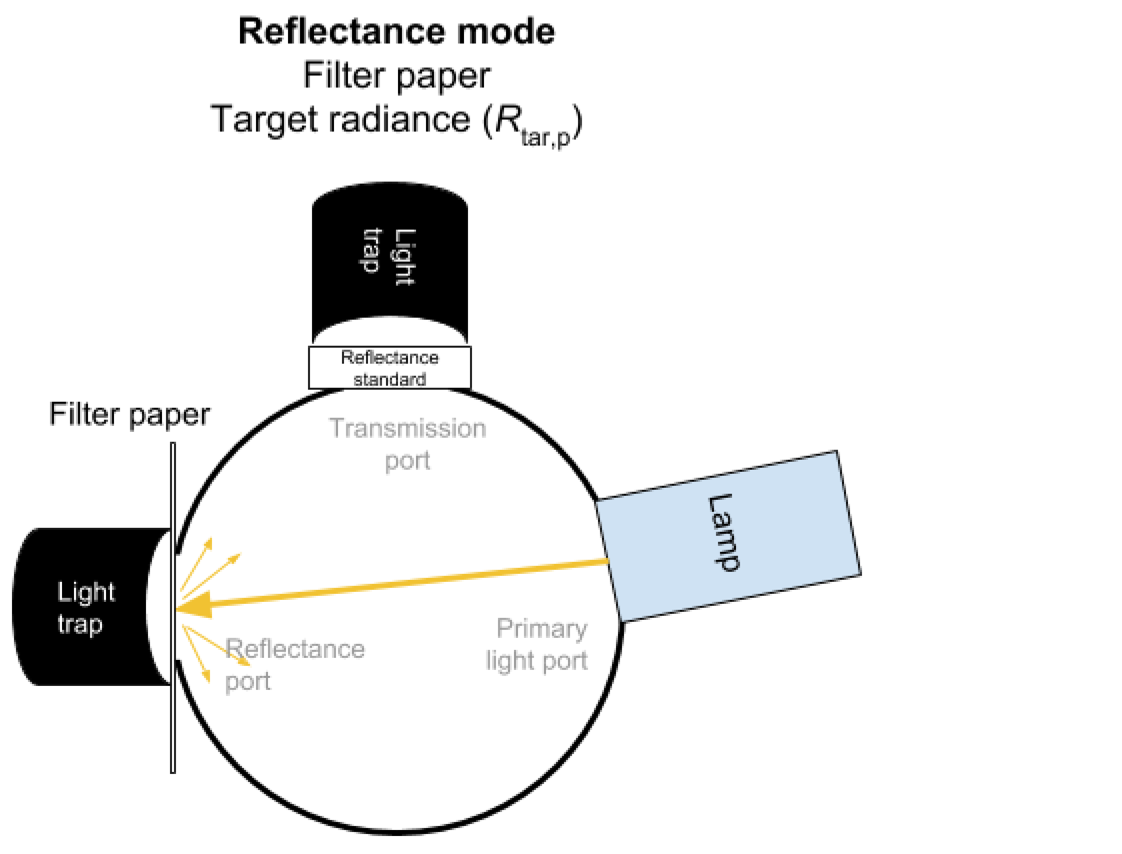
Reflectance (filter paper): Target scan

34 Place the filter paper over the **reflectance port**.

Note

Focus the measurements on the same area of the paper used in previous measurements.

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



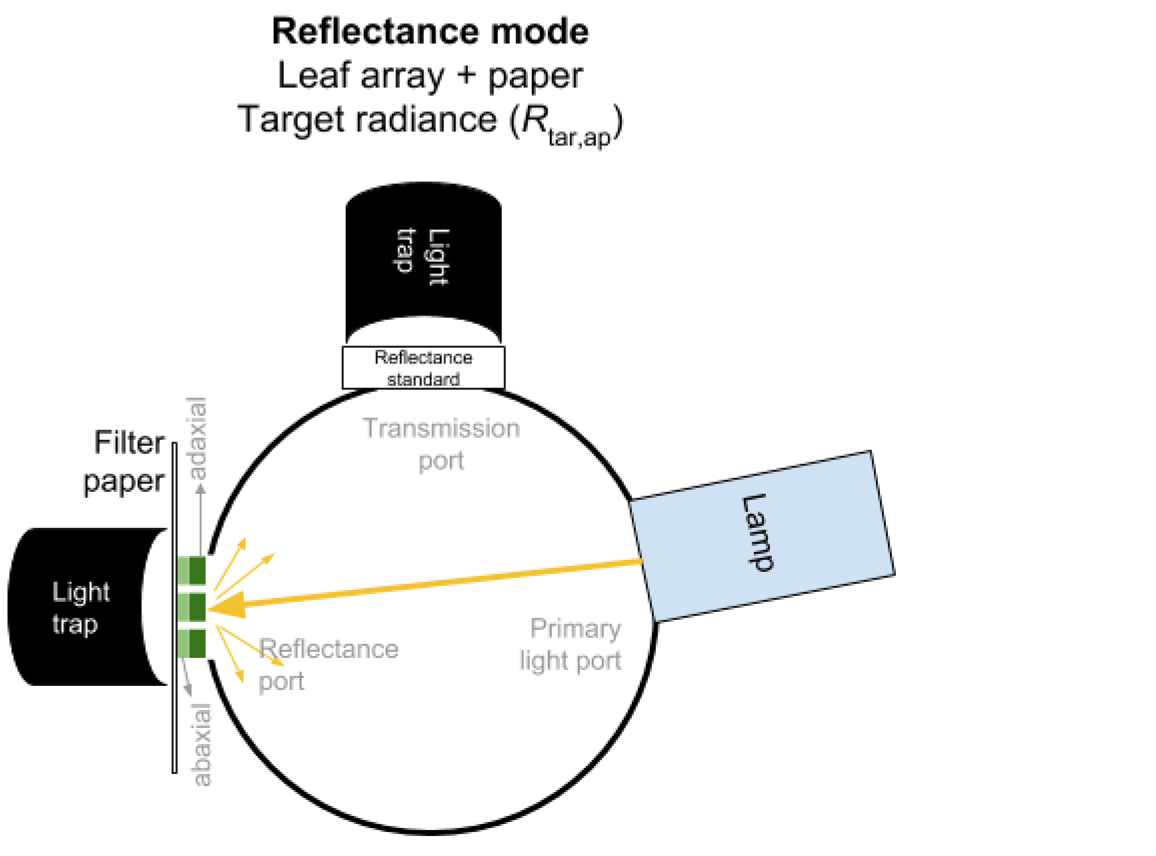
Note

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

Reflectance (leaf array, adaxial +/- filter paper): Target scans

36 Position the sample platform containing leaf array #1 in front the filter paper over the **reflectance port** with the adaxial (upper) surface of the leaves facing the inside of the sphere.

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



Note

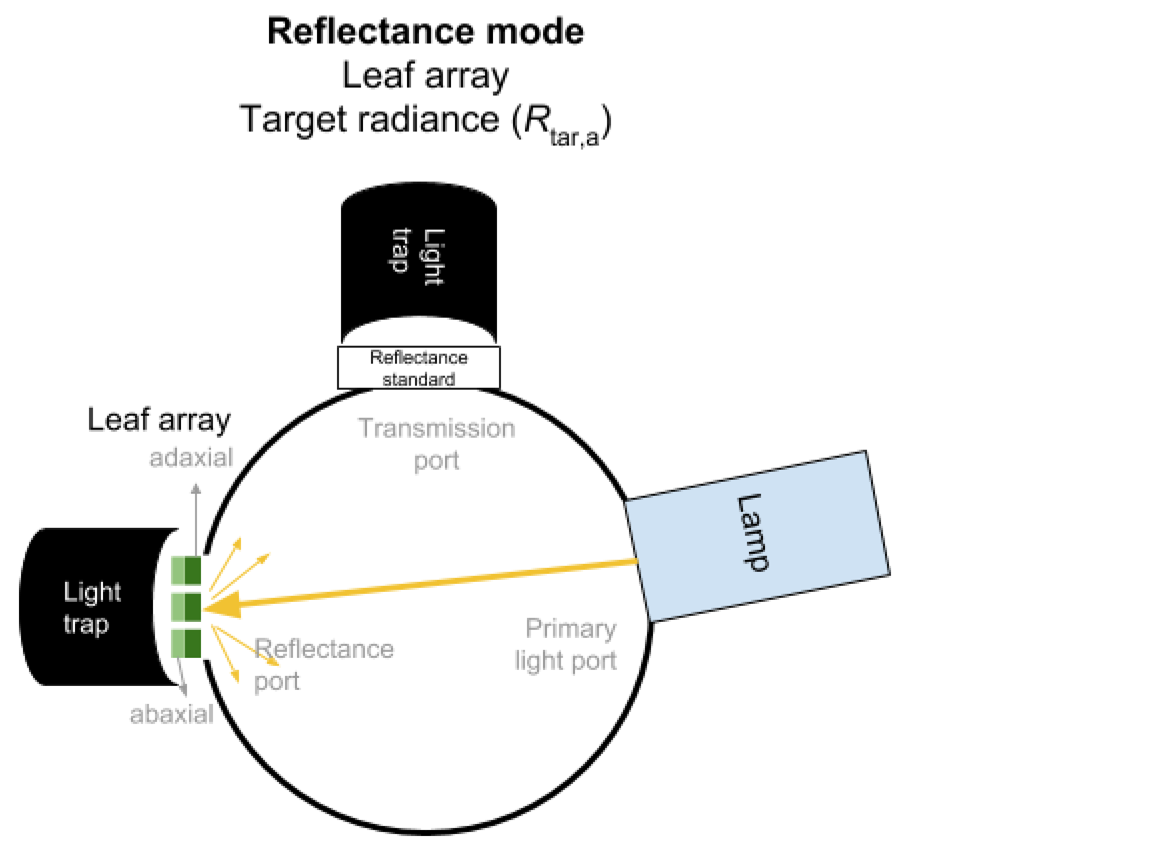
This corresponds to the **target radiance** of **leaf array** #1 (**adaxial**) in reflectance mode ($R_{tar,a,1}$).

38 Remove the filter paper from the reflectance port.

Note

The sample platform holding leaf array #3 should remain in the same position.

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



Note

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

40 Carefully replace leaf array #1 by leaf array #2.

41 Collect a '**Target Scan**' for leaf #array 2 in this configuration ($R_{tar,a,2}$) and **save the file**.

42 Place the filter paper directly behind leaf array #2 over the **reflectance port**.

Note

The sample platform holding leaf array #2 should remain in the same position.

43 Collect a '**Target Scan**' for leaf array #2 + filter paper in this configuration ($R_{tar,ap,2}$) and **save the file**.

44 Carefully replace leaf array #2 by leaf array #3.

Note

The filter paper should remain in the same position.

45 Collect a '**Target Scan**' for leaf array #3 + filter paper in this configuration ($R_{tar,ap,3}$) and **save the file**.

46 Remove the filter paper from the reflectance port.

Note

The sample platform holding leaf array #3 should remain in the same position.

47 Collect a '**Target Scan**' for leaf array #3 in this configuration ($R_{tar,a,3}$) and **save the file**.

Transmittance (cavity wall): Target scan

48 Carefully remove leaf array #3 from the **reflectance port**.

Note

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

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

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

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

Note

Make sure lamp is secured in locked position.

Safety information

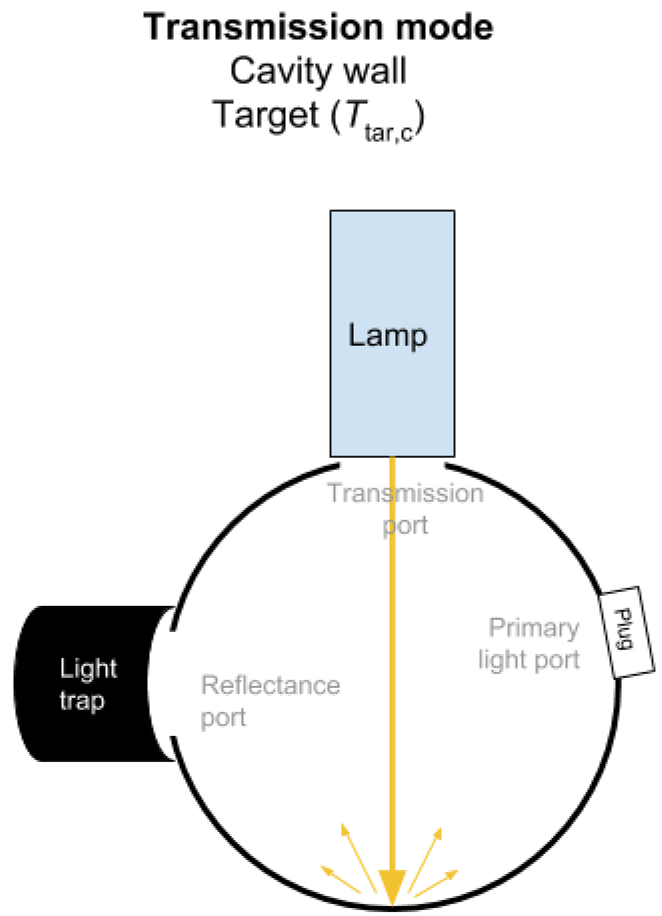
The lamp can get very hot. Grab it by the slotted heat shield.

52 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.

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



Note

This corresponds to the **target radiance** of the cavity wall in transmission mode ($T_{tar,c}$).

Transmittance (leaf array, adaxial): Target scans

54 Gently pull lamp away from the sphere.

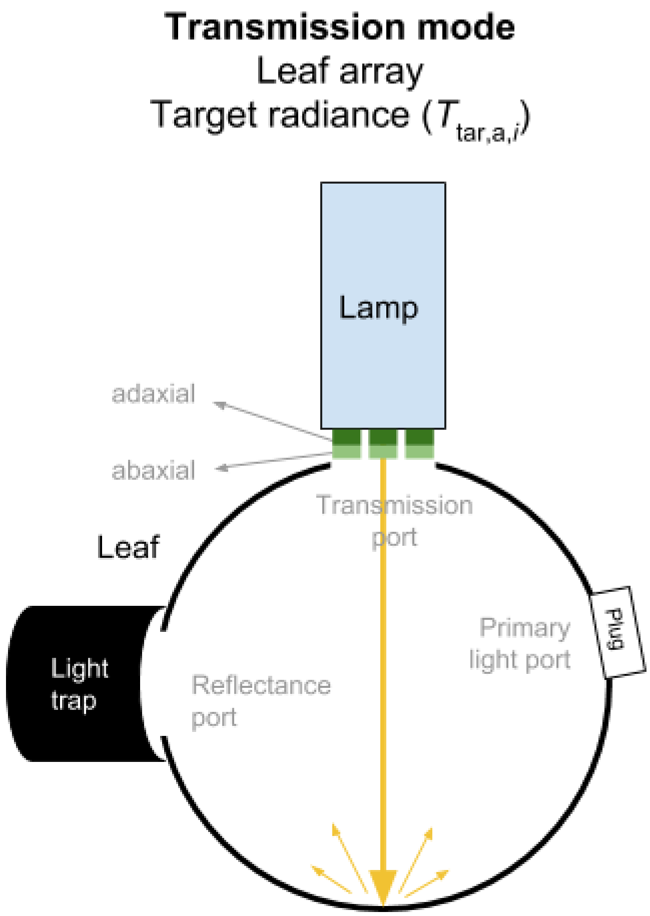
Safety information

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

55 Place the sample platform holding leaf array #1 over the **transmission port** with the abaxial (lower) surface of the leaves facing the inside of the sphere.

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

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



Note

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

58 Carefully replace leaf array #1 by leaf array #2.

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

Note

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

60 Carefully replace leaf array #2 by leaf array #3.

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

Note

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

62 Remove leaf array #3 from the transmission sample port holder.

Calculating absolute reflectance of leaf array (adaxial side)

63 The equation (Noda et al. 2013; eqn. 9) for **adaxial reflectance** of leaf array i, $\rho_{a,i}$ is

$$\rho_{a,i} = [(R_{tar,a,i} - R_{str}) \div (R_{ref,ad} - R_{str})] \times \rho_{ref} \times [1 \div (1 - G_{r,i})]$$

where

$R_{tar,a,i}$ is the target radiance of leaf array i (adaxial side) in reflectance mode,
$R_{ref,ad}$ is the reference radiance used for all leaf arrays (**adaxial** side) in reflectance mode,
R_{str} is the stray light radiance in reflectance mode,
ρ_{ref} is the absolute reflectance of the calibrated Spectralon® reflectance standard, and
$G_{r,i}$ is the gap fraction in reflectance mode for leaf array i, which is calculated **at 400 nm** (Noda et al. 2013; eqn. 13) by

$G_{r,i}$ = [(($R_{tar,ap,i}$ − R_{str}) ÷ ($R_{ref,ap}$ − R_{str})) − (($R_{tar,a,i}$ − R_{str}) ÷ ($R_{ref,ad}$ − R_{str}))] × (ρ_{ref} ÷ ρ_p)

where

$R_{tar,ap,i}$ is the target radiance of leaf array i (adaxial side) + filter paper in reflectance mode,
$R_{ref,ap}$ is the reference radiance used for all leaf arrays (adaxial side) + filter paper in reflectance mode, and
ρ_p is the absolute reflectance of the filter paper, which is calculated (Noda et al. 2013; eqn. 3) by

ρ_p = [($R_{tar,p}$ − R_{str}) ÷ ($R_{ref,p}$ − R_{str})] × ρ_{ref}

where

$R_{tar,p}$ is the target radiance of the filter paper in reflectance mode, and
$R_{ref,p}$ is the reference radiance of the filter paper in reflectance mode.

Calculating absolute transmittance of leaf array (adaxial side)

64 The equation (Noda et al. 2013; eqn. 15) for **adaxial transmittance** of leaf array i, $\tau_{a,i}$ is

$\tau_{a,i}$ = [(($T_{tar,a,i}$ × ρ_{ref}) ÷ ($R_{ref,ab}$ − R_{str})) − $G_{t,i}$ × ρ_c] × [1 ÷ (1 − $G_{t,i}$)]

where

$T_{tar,a,i}$ is the target radiance of leaf array i in transmission mode,
ρ_{ref} is the absolute reflectance of the calibrated Spectralon® reflectance standard,

$R_{ref,ab}$ is the reference radiance used for all leaf arrays (**abaxial** side) in reflectance mode,
R_{str} is the stray light radiance in reflectance mode,
ρ_c is the absolute reflectance of the cavity wall (i.e. sphere wall interior), calculated as (Noda et al. 2013; eqn. 5)

ρ_c = [$T_{tar,c}$ ÷ ($R_{ref,c}$ − R_{str})] × ρ_{ref},

$G_{t,i}$ is the gap fraction in transmission mode for leaf array i, which is calculated **at 400 nm** (Noda et al. 2013; eqn. 16) by

$G_{t,i}$ = [$T_{tar,a,i}$ ÷ ($R_{ref,ab}$ − R_{str})] × (ρ_{ref} ÷ ρ_c).