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Theoretical algorithm and application of a double-integrating sphere system for measuring leaf transmittance and reflectance spectra

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We present the theoretical background and analytical equations for calculating spectral reflectance and transmittance factors for plant leaves from data collected by a field spectroradiometer attached to a double-integrating sphere. The sphere constants required for the calculations are derived from measurements of an empty sample port and a diffuse reflectance panel. The new method is applied in measuring the spectra of leaves belonging to 13 tree species. The greatest advantages of a double-sphere system compared with the conventional single-sphere substitution method is the speed of measurements, ease of operation, and increased portability and field-ruggedness. © 2017 Optical Society of America

OCIS codes: (120.3150) Integrating spheres; (120.5700) Reflection; (120.7000) Transmission.

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1. INTRODUCTION

Spectral libraries of terrestrial targets have a fundamental role in the development of interpretation methods for remote-sensing data. A spectral library that is used in Earth observation applications typically contains reflectance spectra of targets, and for vegetation preferably also transmittance spectra, and is used as a reference for material identification in remote-sensing images. Spectral libraries are increasingly important, as future remote-sensing techniques are rapidly moving toward the use of (hyper)spectral information both in active and passive remote sensing techniques; we can anticipate a rapid increase in the need for spectral libraries when new hyperspectral satellite missions are launched and as active remote-sensing techniques (lidar) also evolve toward utilization of spectral data.

Leaf reflectance and transmittance spectra convey information about the structure and biochemical constituents inside plant tissues and can be directly linked to the physiological processes of plants, and thus, to biomass production, harvest predictions, and health of plants. Integrating spheres have been actively used to measure leaf spectra since the 1960s [1]. However, a common challenge in using conventional, single-integrating sphere systems is that the measurement of leaf from both of its adaxial and abaxial sides, and for both reflectance and transmittance spectra, takes a fairly long time, during which the biological state of the leaf may change. Accurate determination of reflectance or transmittance with an integrating sphere using the comparison method [2–4] requires two measurements for each quantity, and the swapping of reference and sample in two ports of the sphere. The time-consuming nature of the measurement process has also hindered collecting large spectral libraries of vegetation.

Not only would a double-integrating sphere system, which measures reflectance and transmittance spectra nearly simultaneously, enable faster measurements of leaf spectra, but it would also enable collecting larger spectral databases urgently needed for many Earth observation applications. Theoretical equations for radiative power inside double spheres are available in the literature [5,6], but the published algorithms have limited applicability for beam incidence and require the system to be dismantled in order to retrieve the required parameters. In this paper, we present the theoretical background and analytical equations for calculating hemispherical reflectance and transmittance factor spectra for leaves under beam illumination from data collected by attaching a double-integrating sphere to a spectroradiometer. The method produces reflectance and transmittance factor values without moving the sample, and does not introduce significant substitution errors [2]. We present results...
from the validation of the equations. Finally, we demonstrate the use of the method in measuring spectra of leaves of 13 tree species.

2. THEORY

A. Double-Integrating Sphere

We start by assuming that the reflectance and transmittance factors of the leaf do not depend on which side the leaf is measured or measured. The consequences of this assumption will be discussed later. The double-sphere equations for radiative power received by an unabaffled sensor inside the reflectance \( P \) and transmittance \( P' \) spheres when the target is illuminated by a direct beam are now given as (Eqs. (34) and (35) in Ref. [5])

\[
P = \delta \frac{R_{cd} + mR_e + T_{sp} (T_{cd} + m'T_e)}{V(1 - T^2_{sp} \rho')} P_{in},
\]

\[
P' = \delta' \frac{T_{cd} + mT_e + T_{sp} (R_{cd} + mR_e)}{V'(1 - T^2_{sp} \rho')} P_{in},
\]

where unprimed symbols correspond to the reflectance and primed symbols to transmittance sphere,

\[
V = 1 - (\alpha + R_{ds} + rh)
\]

is the average absorption (one minus reflectance factor) of the sphere area, \( \alpha \) the reflectance factor of sphere inner surface (wall coating), \( \alpha \) the fraction of the sphere not taken up by ports, \( r \) the average reflectance factor of sphere port surfaces (excluding sample port), \( \delta \) the fraction of the sphere surface taken up by the measurement port, \( h \) the fraction of the sphere surface taken up by ports (excluding sample port), \( s \) the fraction of sphere surface taken up by the sample, \( R_{cd} \) (\( T_{cd} \)) the diffuse reflectance (transmittance) factor of the sample under collimated incidence, \( R_e \) (\( T_e \)) the specular reflectance (beam transmittance) of the sample, \( R_{ds} \) (\( T_{ds} \)) the diffuse reflectance (transmittance) factor, \( P_{in} \) the radiative power entering the sphere and hitting the sample as a collimating beam, and \( \rho \) is the ratio

\[
\rho = \frac{s}{V}.
\]

Later, \( \rho \) will be shown to be proportional to the substitution error of the sphere. The fractional sphere surface areas in Eq. (1) are defined so that \( \alpha + s + h = 1 \) (i.e., \( \delta \) is included in \( h \)). The notation used here is generally similar to that used by Pickering et al. [5,6], with small modifications for brevity and clarity (e.g., use of the relative areas \( s, \delta, \) and \( h \)).

We assume that the spectrometer has a fiber-optical cable input, as is common in modern field spectroradiometers. Therefore, we need to adjust Eq. (1) to consider the limited field of view (FOV, 25°) of the optical cable acting as the receiving sensor inside the sphere. This is detailed in the work by Pickering et al. [5,6]: to obtain the power entering a sensor with a limited FOV, the right-hand sides of Eq. (1) need to be multiplied by \( f' ma \) (or, by \( f' m' a' \) for the transmittance sphere equation), where \( f \) is a sensor-specific coefficient depending on the FOV of the fiber.

Despite assuming the two sides of the sample identical, Eq. (1) contains three reflectance and two transmittance quantities for each sample: \( R_{cd}, T_{cd}, R_e, T_e, R_{ds}, T_{ds} \). It is evident that these cannot be determined from the two measurements of \( P \) and \( P' \).

As is customary in studies of leaf reflectance for biochemical constituent retrieval and in remote-sensing applications [7–10], we will only use a single value \( R \equiv R_{cd} \) for leaf spectral reflectance and \( T \equiv T_{cd} \) for leaf spectral transmittance and set \( R_e \equiv T_e \equiv 0 \). \( R \) and \( T \) characterize the sample side facing the reflectance sphere and serve as approximations to \( R' \) and \( T' \) (leaf properties when illuminated from the side facing the transmittance sphere) in Eq. (1).

Thus, we obtain from Eq. (1) the following equations for the spectral power registered by the spectroradiometer:

\[
P = \delta f ma \frac{R + \rho' T^2}{V(1 - T^2 \rho')} P_{in},
\]

\[
P' = \delta' f' m' a' \frac{T(1 + \rho R)}{V'(1 - T^2 \rho')} P_{in},
\]

The values of \( P \) and \( P' \) in Eq. (4) depend on \( T \) and \( R \) both directly and indirectly, via \( V', V, \rho \), and \( \rho' \). The indirect dependencies can be derived from the defining Eqs. (2) and (3) as

\[
\rho = \frac{\rho_0}{1 - R_{\rho_0}}
\]

and

\[
V = V_0 (1 - R_{\rho_0}),
\]

where

\[
\rho_0 = \frac{s}{V_0},
\]

\[
V_0 = 1 - (\alpha + mh)
\]

are the (theoretical) values of \( \rho \) and \( V \) for an absolutely black sample. Analogous expressions can be derived for \( \rho' \) and \( V' \).

B. Retrieving \( R \) and \( T \)

Sample reflectance and transmittance are relative quantities derived using reference measurements with predefined \( R \) and \( T \) values, \( R_{cd} \) and \( T_{cd} \). For reflectance, nonabsorbing Lambertian surfaces are used, represented by, e.g., Spectralon with \( R_{cd} = R_{SPC} \geq 0.95 \). For transmittance, we used the empty sample port with \( T_{cd} = 1 \). Thus, the set of spectra needed to calculate \( R \) and \( T \) include \( P \) and \( P' \) (the signals from the reflectance and transmittance spheres, respectively, with inserted sample), \( P(\text{SPC}) \) (the reflectance signal from Spectralon), and \( P'(0) \) (the transmittance signal for the empty clip).

The ratio \( P/P(\text{SPC}) \) can be calculated from Eqs. (4), (5), and (6) using the Spectralon reflectance and transmittance factors \( R_{SPC} \) and \( T_{SPC} \equiv 0 \), respectively, as

\[
P \frac{P(\text{SPC})}{P(\text{SPC})} = \frac{R + \rho_0 (T^2 - R^2)}{(1 - R_{\rho_0}) (1 - R_{\rho_0}) - T^2 \rho_0^2} \frac{1 - R_{SPC} \rho_0}{R_{SPC}}.
\]

Evidently, Eq. (9) cannot be solved without knowledge of \( T \). To overcome this, \( R \) and \( T \) may be determined iteratively. For the first iteration, we use an approximation of Eq. (9) without \( T \). To a reasonable accuracy (better than 0.05 reflectance units for leaves with \( R \approx T \)), Eq. (9) can be simplified as

\[
\frac{P}{P(\text{SPC})} = \frac{R}{(1 - R_{\rho_0}) (1 - R_{\rho_0})} \frac{1 - R_{SPC} \rho_0}{R_{SPC}}.
\]
Next, Eq. (10) can be solved for \( R \) to yield
\[
R = \frac{\rho_0^2 Q_R + 1 - \sqrt{(\rho_0 - \rho_0')^2 Q_R^2 + 2 \rho_0^2 Q_R + 1}}{2 \rho_0 \rho_0' Q_R},
\]
(11)

where
\[
\rho_0^n = \rho_0 + \rho' = \frac{s}{V_0} + \frac{s'}{V_0}, \quad Q_R = \frac{P}{P(\text{SPC})} R_{\text{SPC}}\frac{1}{1 - R_{\text{SPC}} P_0}. \]
(13)

Derivation of Eq. (11) involved solving a quadratic equation. We only retained the solution yielding physical values \( 0 \leq R \leq 1 \). The first estimate of sample transmittance can now be calculated from the ratio
\[
\frac{P'}{P(0)} = \frac{T(1 - \rho_0 \rho_0')}{(1 - R \rho_0)(1 - R \rho_0') - T^2 \rho_0 \rho_0'}, \quad \text{(14)}
\]
which gives
\[
T = \rho_0 \rho_0' - 1 + \sqrt{4(1 - R \rho_0)(1 - R \rho_0') \rho_0 \rho_0' Q_T^2 + (1 - \rho_0 \rho_0')^2}, \quad \text{(15)}
\]
where
\[
Q_T = \frac{P'}{P(0)}. \quad \text{(16)}
\]

As before, we ignored the nonphysical solution of Eq. (14).

In the second iteration, the value of \( T \) from Eq. (15) can be inserted into Eq. (9), which can then be solved analytically to yield (ignoring the nonphysical solution)
\[
R = \frac{\rho_0^2 Q_R + 1 - \sqrt{4 \rho_0^2 (\rho_0 Q_R + 1)T^2 + [(\rho_0 - \rho_0') Q_R + 1]^2}}{2 \rho_0' (\rho_0 Q_R + 1)}, \quad \text{(17)}
\]

The value of \( T \) can again be calculated from Eq. (15) using the updated \( R \). We have found two iterations to yield sufficient accuracy compared with the overall uncertainties involved in the measurements. If needed, the cycle can be repeated until required accuracy is achieved.

**C. Sphere Constants**

Calculation of \( R \) and \( T \) from Eqs. (11), (15), and (17) requires the knowledge of the sphere constants \( \rho_0 \) and \( \rho_0' \). These constants, characterizing the reflectance and transmittance sphere, respectively, are functions of relative port sizes and sphere material. They are equal to the reflectance factor of the sample port surface. If the spheres can be detached from the clip assembly, \( \rho_0 \) and \( \rho_0' \) can be measured directly. Unfortunately, this is not always the case. Nevertheless, the constants can be determined with a simplifying assumption from simple radiation measurements. The ratio of the signals of the two spheres in an empty double sphere is
\[
P(0) = \frac{\delta f ma}{\delta f'm'a'} P_0'. \quad \text{(18)}
\]

Under high-quality manufacturing conditions that can be expected for a commercial system or modern laboratory setup, we can assume \( \delta = \delta' \) and \( m = m' \). As the two spheres only differ by the presence of the illumination port (collimating lens), the error in assuming \( a'/a' \) approximately equals the fractional sphere area taken up by the lens and can be considered small. Finally, if the optical pathways (fibers and connectors) from the two spheres to the spectroradiometer are identical, \( f = f' \). The last condition can be checked by swapping the cables connected to the two spheres without dismantling the clip. Thus, for a double-sphere system where the two spheres were manufactured identically, we can write
\[
\rho_0 = \frac{P(0)}{P'(0)}. \quad \text{(19)}
\]

To derive the expression for \( \rho_0' \), we start with the ratio
\[
\frac{P(0)}{P(\text{SPC})} = \frac{\rho_0'}{V_0(1 - \rho_0 \rho_0') R_{\text{SPC}}}, \quad \text{(20)}
\]
where
\[
V_{\text{SPC}} = 1 - (\alpha a + R_{\text{SPC}} + \gamma b). \quad \text{(21)}
\]

Next, we use the identity \( V_{\text{SPC}} = V_0(1 - R_{\text{SPC}} \rho_0) \) and solve Eq. (20) for \( \rho_0' \)
\[
\rho_0' = \frac{Q_{R0} R_{\text{SPC}}}{R_{\text{SPC}} \rho_0(Q_{R0} - 1) + 1}, \quad \text{(22)}
\]
where
\[
Q_{R0} = \frac{P(0)}{P(\text{SPC})}. \quad \text{(23)}
\]

**D. Stray-Light Correction**

The collimating lens at the entrance port of the sphere inevitably scattered some small fraction of incident radiation in directions other than toward the sample. A part of this noncollimated light is received by the spectrometer before it hits the target. This stray-light signal \( S \) is measured in units of the radiative power \( P \). It is expected to remain constant if lamp output power and illumination port characteristics do not change and depend smoothly on wavelength. For a normal laboratory measurement with an integrating sphere, the fraction of stray light would be determined by placing a photon trap at the sample port or just measuring the signal with an open port in a dark room. This stray light \( S \) could later simply be subtracted from \( P \) measured under identical incident power.

Because the reflectance sphere cannot be detached from SpectroClip, we could not measure stray light directly. However, a stray-light signal can be retrieved from measurements of a reflectance standard which has zero transmittance. By setting \( T = 0 \) and substituting Eq. (19) into Eq. (9) we obtain
\[
R \left\{ \left[ P(\text{SPC}) - R_{\text{SPC}} \frac{P(0)}{P'(0)} \right] - R_{\text{SPC}} P(0) \frac{P(0)}{P'(0)} \right\} = -P R_{\text{SPC}}. \quad \text{(24)}
\]

Because the \( P, P(0), \) and \( P(\text{SPC}) \) in Eq. (24) do not include stray light, we add it by setting \( P = P - S, P(0) = P(0) - S, \) and \( P(\text{SPC}) = P(\text{SPC}) - S \). This gives
\[
R \left\{ \left[ P(\text{SPC}) - S \right] - \left[ R_{\text{SPC}} P(0) - S \right] \right\} - R_{\text{SPC}} (P - S) \frac{P(0) - S}{P'(0)} = -(P - S) R_{\text{SPC}}. \quad \text{(25)}
\]
which can be solved for \(S\)

\[
S = \frac{P[P(0)R - P'(0)]R_{SPC} + P(SP)R[P'(0) - P(0)R_{SPC}]}{R_{SPC}[P - P(SP)] + P'(0)[R - R_{SPC}]}
\]  

(26)

where \(P\) is the spectrometer reading obtained from a known reflectance standard.

**E. Substitution Error**

The value of \(R\) retrieved using Eq. (17) is largely determined by the reflectance factor of the leaf side facing the reflectance sphere. Use of the same \(R\) for the leaf side facing the transmittance sphere leads to an incompletely corrected substitution error in the transmittance sphere. To estimate the magnitude of this substitution error, we use the equivalent of Eq. (9) for a single sphere [11]

\[
\frac{P}{P(SP)} = \frac{R}{R_{SPC}} \frac{V_{SPC}}{V}.
\]  

(27)

Without restricting generality, we can assume \(R_{SPC} = 1\). The substitution error is defined as the difference between the uncorrected reflectance factor estimate \(r = P/P(SP)\) and the actual reflectance factor \(R\). For the relative substitution error, we get from Eq. (27)

\[
\frac{r - R}{R} = \frac{r}{R} - 1 = \frac{V_{SPC}}{V} - 1 = \frac{V_{SPC} - V}{V}.
\]  

(28)

Using the defining equation for \(V\), Eq. (2), we obtain that \(V_{SPC} - V = s(R - R_{SPC})\), and

\[
\frac{r - R}{R} = \left(\frac{R_{SPC} - R}{R}\right) s = \left(\frac{R_{SPC} - R}{R}\right) \rho.
\]  

(29)

According to Eq. (29), the substitution error expressed as a fraction of true reflectance (or transmittance) is the product of the change of the reflectance factor of the sample port and the parameter \(\rho\) defined by Eq. (3).

**3. VALIDATION TEST**

**A. Instrumentation and Methods**

The commercially available instrument used in the study is the SpectroClip-TR manufactured by Ocean Optics. The system is a simple double-sphere system, and thus the results presented here are general and can be easily replicated by a custom-built instrument.

The SpectroClip consists of two integrating spheres, both 20 mm in diameter. The reflectance sphere has three ports: an illumination port containing a collimating lens (diameter 4 mm), a measurement port with a SMA-905 fiber attachment (diameter 4 mm), and a sample port (diameter 6 mm). Besides the absence of the illumination port, the transmittance sphere is identical to the reflectance sphere. The measurement port is located so that a standard fiber with a FOV of 25° only sees the coated wall surface. The two spheres are installed in a fixture that guarantees that the sample ports of the two spheres remain aligned while the spheres are moved away from each other, regardless of sample thickness.

The measurement started by opening the SpectroClip—moving the two spheres apart to the maximum distance of approximately 15 mm. The sample was placed between the spheres, and the clip was closed. The collimating lens at the illumination port projected the radiation arriving through an SMA-905 terminated optical fiber from a tungsten halogen lamp (HL-2000-HP-FHSA, Ocean Optics, power 20 W) onto the sample. The radiation hit the sample as a collimated beam at a standard 8° angle from sample normal. According to the manufacturer, the illuminated area was 5 mm in diameter. The fibers from the measurement ports were connected to an optical switch (model FOS-2X2-TTL, Ocean Optics), which blocked the optical pathway from one sphere at a time (Fig. 1). However, the actual switching was done manually by reconnecting the input fiber of ASD FieldSpec 4 spectroradiometer (350–2500 nm wavelength range) to either of the two output ports of the switch; use of a bifurcated cable led to unreasonable signal attenuation. The uncertainties related to reconnecting the cables were assessed by repeated measurements of empty reflectance and transmittance spheres without opening the clip and were found negligible.

The integration time in the measurements was set to 2 s and an average of 30 spectra was stored from each measurement. One measurement therefore took 1 min. Due to limitations in lamp spectral power and the sensitivity of the spectroradiometer, the usable wavelength region was limited to 400–2300 nm. Dark current was measured manually each time after the spectroradiometer settings were changed (instrument optimization). Dark current was subtracted from each measurement of \(P_0\), \(P'\), \(P(0)\), \(P'(0)\), and \(P(SP)\) before using them in any calculations.

An uncalibrated Spectralon panel was used as white reference. Its reflectance spectrum \((R_{SPC})\) was later determined by comparing it to a calibrated Spectralon

\[
R_{SPC} = \frac{P_{uncalib}}{P_{calib}} \times R_{SPC, calib}.
\]  

(30)

where \(P_{uncalib}\) is the reflectance reading obtained from the uncalibrated Spectralon, \(P_{calib}\) the reflectance reading obtained from the calibrated Spectralon, and \(R_{SPC, calib}\) the reflectance spectrum given in the calibration certificate of the calibrated Spectralon. Ten readings of \(P_{uncalib}\) and five readings of \(P_{calib}\) were averaged in order to determine an average \(R_{SPC}\) using Eq. (30). It was filtered with a Savitzky–Goaly filter, and the filtered version was used in further calculations. Figure 2 shows 10 individual measurements as well as the average of \(R_{SPC}\) and comparison to \(R_{SPC, calib}\).
B. Stray-Light Correction

Normally, stray light is determined using a photon trap in the sample port. As this was not possible for Spectroclip, we used measurements of the darkest diffuse reflectance standard available (Avian Technologies, 2% nominal reflectance), $P_{0.0133}/0.0134/0.0132$, as the signal produced by a known standard in Eq. (26). To eliminate noise, we applied the Savitzky–Golay filter to smooth the observations below 2000 nm region, fitted a linear polynomial to observations in 1500–2000 nm, and used the polynomial to extrapolate to the noisy region above 2000 nm to obtain the spectral stray-light signal $S$ (Fig. 3). As the 2% standard was measured only once, we expressed $S$ as proportion of $P_{0.0133}/0.0134$. This way $S$ could be calculated independent of spectrometer settings, provided that $P_{0.0133}/0.0134$ and the sphere properties were unaltered between the determination of $S$ and the measurement of $P_{0.0133}/0.0134$. The stray-light signal was later subtracted from all measurements of $P$, $P_{0.0133}0.0134$, and $P_{0.0133}/0.0134$.

C. Sphere Constants

The variables needed for calculation of sphere constants ($P_{0.0133}0.0134$, $P_{0.0133}0.0134$, $P_{0.0133}/0.01340.0134$) were continuously monitored over a measurement campaign in summer 2016. Therefore, we had 80 individual estimates of sphere constants available. We calculated the average of these estimates [Fig. 4(a)] and applied them to Eqs. (11), (15), and (17) to determine the reflectance and transmittance spectra of the measured samples. The sphere constants varied most in blue and shortwave infrared (SWIR) regions, due to measurement noise in these regions (Fig. 3). There were no notable trends in the sphere constants over time.

D. Measurement of Calibrated Reflectance Targets

Performance of the equations was tested by measuring calibrated diffuse reflectance standards manufactured by Avian Technologies. The standards had nominal reflectance values of 2%, 5%, 20%, and 50%. For each reflectance standard, we took measurements as follows:

i. $P_{0.0133}/0.0134$

ii. $P_{0.0133}0.0134$

iii. Five times $P$

In (iii), one measurement was made at the center of the panel, and four measurements a few millimeters away from the center, in all four cardinal directions. The filtered sphere constants were then applied to calculate five estimates of reflectance, using (i) and (ii), and each of the five measurements of (iii). Zero transmittance was assumed. The five reflectance spectra obtained were then averaged, and the average was compared to the reference spectra given in the calibration certificates of the standards (Fig. 5). Finally, root mean square errors (RMSEs) between the measured average and the reference spectra were calculated as

$$\text{RMSE} = \sqrt{\frac{\sum_{\lambda=400}^{2500} (R(\lambda) - R_{\text{ref}}(\lambda))^2}{1900}} \quad (31)$$
The calculated reflectance spectra matched well with the reference according to the RMSE values given in Table 1.

### 4. CASE STUDY

Finally, we tested the new methodology in practice in measuring reflectance and transmittance spectra of 13 tree species (Alnus glutinosa, Alnus incana, Acer platanoides, Betula papyrifera, Betula pendula, Populus balsamifera, Populus tremula, Populus tremuloides, Prunus padus, Quercus robur, Salix caprea, Sorbus aucuparia, Tilia cordata). The purpose of the case study was to demonstrate that the methodology described in this paper produces realistic leaf spectra [i.e., reflectance and transmittance spectra present their natural (large) variation and do not overlap].

Data for the case study were collected between 4 July and 25 August 2016 in the Helsinki area (Finland): from Otaniemi campus (24°48′39″, 60°11′24″), Viikki arboretum (25°00′30″, 60°13′11″), Kumpula botanical garden (24°57′19″, 60°12′08″), and Ruotsinkylä research forest (24°59′15″, 60°21′37″). For each tree species, branches were detached from two trees, and from two locations (sun-exposed and shaded) in each tree crown (sensu [8]). The detached branches were stored in water, and kept in a cool and dark location. Leaves were detached from the branches only immediately before spectral measurements, and both the adaxial and abaxial sides of a total of six leaves (three shaded, three sun-exposed) per tree was measured. This yields a total of 12 sampled leaves per tree species. For two species (Populus balsamifera, Populus tremuloides) only one tree was available, and the number of samples per tree was doubled in order to obtain 12 samples. Only healthy leaves (based on visual inspection) were used in the measurements. All samples were measured within 0–8 h after the branch had been cut from a tree. The instrumentation was the same as used in the validation test (Section 3), but in order to obtain feasible measurement time, the integration time was reduced to 544 ms. Measuring one leaf (from both sides, including also reference standard measurements for every third leaf) lasted approximately 4 min.

The tree leaf spectra were comparable to those previously reported in the literature [8,10,12–15]. The mean reflectance varied from 0.05 to 0.45, and the mean transmittance from 0.00 to 0.47 (Fig. 6). The reflectance and transmittance spectra did not overlap; the smallest mean absorptance was observed at approximately 1100 nm (0.08) and the largest at approximately 400 nm (0.95). Among the studied species, the smallest absorption in near-infrared (NIR) was recorded for Acer platanoides and Tilia cordata (0.06); the absorption for Betula pendula was 0.07. The largest relative variation in reflectance between samples was observed in the visible domain (400–700 nm) and the smallest relative variation in NIR (700–1400 nm). For transmittance, the relative variation showed a similar pattern, but shortwave-infrared (particularly the water absorption peak at 1930 nm) showed almost as large relative variation as the visible domain.

The optical properties of the abaxial and adaxial leaf sides are somewhat different (Fig. 7). Generally, absolute differences are

<table>
<thead>
<tr>
<th>Reflectance Standard</th>
<th>2%</th>
<th>5%</th>
<th>20%</th>
<th>50%</th>
</tr>
</thead>
<tbody>
<tr>
<td>No correction</td>
<td>0.003</td>
<td>0.012</td>
<td>0.038</td>
<td>0.053</td>
</tr>
<tr>
<td>Corrected</td>
<td>0.002</td>
<td>0.005</td>
<td>0.005</td>
<td>0.004</td>
</tr>
</tbody>
</table>
larger for $R$ compared with $T$. In the visible wavelengths, differences in $R$ can be up to 0.1 reflectance units, while $T$ is almost independent of which leaf side was measured. In NIR and SWIR, the absolute differences in $R$ and $T$ have similar magnitudes.

5. DISCUSSION

Overall, the results obtained with the Spectroclip are very encouraging. Use of the power ratios $P/P(\text{SPC})$ and $P'/P'(0)$ as estimates of $R$ and $T'$, respectively, would lead to errors of up to 0.05; the algorithm presented here reduced the error to below 0.01. Considering the generality of the algorithm and small computational requirements, we find it surprising that no such corrections were suggested by the manufacturer. We were not able to determine the uncertainty more accurately, as the limit achieved by the Spectroclip approached the variation in the surface properties of the reflectance standards. No transmittance validations were performed, as we could not devise a transmittance standard that could be fit into the SpectroClip. Liquid samples of various concentrations were used by Pickering et al. [6], but such an approach is not possible with the Spectroclip, designed for field measurement of leaves.

The retrieved $R$ and $T$ are neither bihemispherical nor directional-hemispherical [16], but rather a mixture of the two. The fraction of diffuse irradiance depends on the reflectance of the sample at each particular wavelength and the sphere constants. Thus, these quantities are not exactly equivalent to the directional-hemispherical reflectance factor (DHR) and directional-hemispherical transmittance factor (DHT) measured using a substitution sphere. The uncertainty in the measured quantity is increasingly relevant the more a sample deviates from a Lambertian sample. For the reflectance standards, the issue of the measured reflectance quantity is therefore irrelevant. For leaves, the directionality of incident light can be relevant [17]. Nevertheless, in remote-sensing studies, scattering directionality is ignored, and the quantity related to both the measured signal and leaf biochemical composition is the leaf single-scattering albedo [18,19], defined as the probability that a photon hitting the leaf will not be absorbed. It equals the sum of leaf reflectance and transmittance under natural illumination conditions. The natural conditions, which should be simulated in laboratory for accurate determination of the single-scattering albedo, are more similar to the semidiffuse field inside the sphere with the diffuse fraction increasing with leaf reflectance than $8^\circ$ beam incidence. Also, all techniques for retrieving leaf biochemical and structural properties from leaf optical data [7,9], as well as leaf optical models, make use of a single $R$ and $T$ value; leaf-scattering anisotropy is nearly universally ignored.

Abaxial and adaxial sides of the leaves are not identical (Fig. 7). The uncorrected substitution error caused by this affects mostly transmittance measurements. The magnitude of this error depends on the difference between the reflectance factors of the two leaf sides and the characteristics of the measurement system. Based on Eq. (29), and Figs. 3, 6, and 7, the relative uncorrected substitution error is largest in the visible region with values up to 0.025. This can be significant for some
applications: for example, when measuring leaf samples with a three-inch ASD integrating sphere (Analytical Spectral Devices Inc., Boulder, Colorado, USA), a reflectance difference of less than 20% is sufficient [20]. Although the general optical properties of leaves of most species are similar on both sides (low reflectance in blue and red, high reflectance in NIR with notable water and organic compound absorption bands in shortwave infrared), this limit can be exceeded in the visible region.

To further reduce measurement error, the appropriate value of $\tilde{R}$ can be used in Eq. (15) during postprocessing. However, this requires adequate sampling of the two leaf sides, as leaf optical properties vary due to internal structure (e.g., veins). Alternatively, correction for substitution inaccuracy, as well as other corrections to the measured signals presented above, can be derived based on the double-sphere theory. For example, Pickering et al. [6] present a stray-light correction algorithm that accounts for multiple scattering between the spheres, but consider it normally not significant.

The biggest shortcoming of a double-sphere system is the inevitable lateral loss through the sample of finite thickness. The loss depends on sample (leaf) thickness. According to the manufacturer, the loss for a port difference of 1 mm is 3% at 620 nm. It is best quantified in NIR, where the absorption of thin green leaves is very low. Data for the same species growing under similar conditions are sparse; hence, we compared our results with samples from the same genus. The LOPEX [13] and BOREAS [14] data sets include NIR reflectance values of approximately 0.05 for species of Betula and Alnus genus while Knapp and Carter [15] found low overall variation in minimum NIR absorption among 26 herbaceous and broadleaf tree understory species with an average of 0.03. More recently, even lower values have been reported for Betula, from almost no absorption up to 0.03 [8,10,12], with absorption increasing with the growing season [10] and light availability [8]. Based on these data, we estimate that leaf absorption measured by the SpectroClip is underestimated by approximately 0.03 (absolute units). This estimate is not affected by the uncorrected part of substitution error, as the substitution corrections for different leaf sides have different sides and therefore cancel out.

Although the commercial system available to us, the Spectroclip, allowed for a robust and quick determination of leaf reflectance and transmittance, its construction could be improved. The biggest shortcoming was the lack of the possibility to inspect and measure the two spheres separately: determine stray light, sphere constants, or visually inspect the sphere interior for debris or contamination. A further enhancement would be the inclusion of an illumination port in the transmittance sphere. Together with appropriate optical switches, it would allow the measurement of leaf adaxial and abaxial surface reflectance and transmittance factors of exactly the same areas within minutes. It would also allow an immediate correction for the substitution error in transmittance measurements.

The greatest advantages of a double-sphere system compared with the single-sphere substitution method is the speed of measurements, ease of operation, increased portability, and field-ruggedness. The supremacy is most evident in situations where absolutely exact illumination geometry is not critical and the samples have large spatial and temporal variability, as is the case for green vegetation. However, in laboratory measurements of stable samples, the increased uncertainty [6] and the ambiguity of the reflectance and transmittance parameters may cause a double-sphere system not to be the optimal option.

6. CONCLUSIONS

The theoretical background presented here allowed us to reliably calculate hemispherical reflectance and transmittance factor spectra for plant leaves under beam illumination from data measured by a relatively new, commercially available double-integrating sphere system (SpectroClip) that was attached to a spectroradiometer. The correction algorithm presented here is mathematically and computationally simple. SpectroClip, weighing just 500 g, demonstrates well the increased portability and field-ruggedness of double-sphere systems. In measurements of live plant material, the portability of the system, speed of measurements, and the ability to sample the reflectance and transmittance of the same leaf area compensate for the absorption measurement inaccuracy. The measured data are suitable for retrieving leaf structural and biochemical properties as well as for applications in environmental imaging spectroscopy.

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