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# Minimizing measurement uncertainties of coniferous needle-leaf optical properties, part I: methodological review

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Optical properties (OPs) of non-flat narrow plant leaves, i.e., coniferous needles, are extensively used by the remote sensing community, in particular for calibration and validation of radiative transfer models at leaf and canopy level. Optical measurements of such small living elements are, however, a technical challenge and only few studies attempted so far to investigate and quantify related measurement errors. In this paper we review current methods and developments measuring optical properties of narrow leaves. We discuss measurement shortcomings and knowledge gaps related to a particular case of non-flat nonbifacial coniferous needle leaves, e.g., needles of Norway spruce (Picea abies (L.) Karst.).

**Keywords**
Needles, optical properties, reflectance, transmittance, integrating sphere, leaf, conifers, gap fraction



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# Minimizing Measurement Uncertainties of Coniferous Needle-Leaf Optical Properties, Part I: Methodological Review

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***Abstract*—Optical properties (*OPs*) of non-flat narrow plant leaves, i.e. coniferous needles, are extensively used by the remote sensing community, in particular for calibration and validation of radiative transfer models at leaf and canopy level. Optical measurements of such small living elements are, however, a technical challenge and only few studies attempted so far to investigate and quantify related measurement errors. In this paper we review current methods and developments measuring optical properties of narrow leaves. We discuss measurement shortcomings and knowledge gaps related to a particular case of non-flat nonbifacial coniferous needle leaves, e.g., needles of Norway spruce (Picea abies (L.) Karst.).**

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## I. Introduction

ABSORPTION of visible and infrared light in plant leaves is an essential measurement for better understanding and modeling the photosynthetic process and energy balance that regulates global gas exchange with the atmosphere and consequently global terrestrial primary productivity [1]. Since leaves are the primary photosynthesizing organs, measurement of their optical properties (*OPs*) (i.e., absorption (*A)* complemented by the leaf reflectance (*R*) and transmittance (*T*)) is a crucial part of this puzzle. Direct measurement of the *in-vivo* optical absorption properties is still practically impossible [2], thus, efforts on measuring leaf *OPs* have been directed towards quantifying leaf *R* and *T*, from which *A* is derived through the following relationship: $1=A+R+T$. Despite an extensive history in measuring the directional-hemispherical (terminology following [3]) *R* and *T* of plant leaves [4], most of the methods have been designed for broad leaves. Measurement of narrow and small size leaves, as for instance coniferous needles or grasses, which represent a significant fraction of natural terrestrial ecosystems [5], is still a technical challenge. Even though *OPs* of coniferous needles are extensively used by the remote sensing community [6]-[10] only limited knowledge about their measurement related errors is available [11]. As a result of this, measurements with unknown accuracy and reliability are used for example for calibration and validation of radiative transfer models simulating reflectance factors of coniferous canopies [12]. The lack of needle *OPs* measurements and unknown measurement uncertainties have enforced modeling assumptions with a potentially negative impact on interpretation of remote sensing data of coniferous forests, as for instance the needle *T* being assumed to be equal to zero [13], or equal to the needle *R* [14]. This clearly demonstrates a need for a more robust and efficient measurement technique of narrow-leaf *OPs*.

In this paper we review the state of the art and recent developments in measurement methods for narrow leaf optical properties. We focus on methodological shortcomings and uncertainties, with special attention to non-flat nonbifacial coniferous needle-leaves (e.g., needles of Norway spruce). We conclude by recommending a set of potential improvements based on the existing methods. We continue to propose an experimental set-up for optimizing established needle-leaf *OPs* measurement approaches by systematically minimizing their uncertainties in a second part (this issue).

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## II. Needle-leaf optical properties

### A. Photon interactions with a needle-leaf

Photon interactions with a leaf result in a combination of scattering and absorption processes, which are driven by the spectral character and spatial distribution of the incoming collimated and diffuse light [15], [16] and by the leaf orientation and internal anatomy [17]-[20]. These attributes determine the degree of attenuation of the light flux passing through foliar tissues [21] and the spectral and spatial distribution of the outcoming photons [22]-[25]. The irregular

shape and orientation of the leaf cells, and also an uneven distribution of absorbers within the foliar tissue [26] makes the leaf a complex optical scattering microenvironment causing for instance sieve and detour effects [27]. Despite this complexity, light propagation within bifacial broad leaves has been successfully simulated [4], [20], [28], also using leaf radiative transfer (RT) models [29]. The leaf model PROSPECT approximates a bifacial leaf as an infinitely extending plate with distinct multiple layers of cells (Fig. 1(b)). In reality the inner layers of pigmented mesophyll cells are covered by epidermal layers, which are protected by outer cuticle layers [30]. When the light of a specific wavelength hits the leaf surface, a portion of the incoming photons is scattered outward by the waxy cuticle [17] and the complementary portion is transmitted through the leaf's surface layer into the mesophyll tissue. There, the interfaces between air spaces and cell walls cause multiple internal reflections and refractions of the light rays [31]. Multiple scattering redirects the light rays in multiple directions. Some photons encounter absorbers and are absorbed; some are scattered in an "upwards" direction, forming, together with the external surface scattering, the leaf *R*; and some are scattered out of the leaf in a "downwards" direction resulting in the leaf *T*.

RT models simulating light-leaf interactions in narrow needle leaves, such as in LIBERTY [32], are scarce and less accurate due to the higher geometrical complexity. First, the cross-section of coniferous needles is hardly similar to a plate configuration (Fig. 1(a)), but presents varying geometrical shapes with several facets (Fig. 1(c)). When compared to the broadleaf cross-section, these facets increase the number of possible incident angles of the interacting photons. Second, the inner layers are forming a set of dense irregular spherical microstructures rather than the flat regularly layered structure of a typical bifacial broad leaf [32] (Fig. 1(d)).

### *B. Conventional broad-leaf spectral measurements*

Conventional measurement of plant leaf *OPs* consists of directional-hemispherical *R* and *T* measurements performed with an integrating sphere coupled to a spectroradiometer [16], [30]. The leaf measuring integrating sphere, coated inside by a highly reflective material (e.g., barium sulfate), has several dedicated ports, where a collimated light source and the leaf sample can be placed during the measurements. The light beam is illuminating the leaf adaxial or abaxial side, which is covering the sample port (Fig. 2(a)). A portion of the incoming photons reaching the leaf surface is scattered (reflected/transmitted) in all directions from/through the leaf. The illuminated area is smaller than the sample port diameter, ensuring that the beam only interacts with leaf tissue. The integrating sphere is collecting and integrating the signal of scattered photons through the whole hemisphere, which is subsequently recorded by a spectroradiometer connected to the sphere with optical fibers. *T* measurement requires placing the leaf at an entry port of the sphere and illuminating it with direct collimated light from the external side of the leaf. The light enters the integrating sphere through the leaf (Fig. 2(c)), which means that the signal recorded by the sensor inside the sphere is the portion of light transmitted through leaf tissue. To measure *R*, a leaf is also mounted in a sphere entry port, but being illuminated by a collimated light placed in a port opposite to the sample (Fig. 2(b)). This way the collimated light beam passes through the sphere and interacts with the sample from the interior side resulting in a signal reflected back into the sphere. A correction for stray light is required for *R* measurements. Also correction of the so-called 'single-beam substitution error' must be considered to avoid producing lower *R* and higher *T* records occurring when the sample substitutes the portion of the sphere previously occupied by reference material of 100% reflectance[33]. Finally, *A* can be calculated from the *R* and *T* measurements through $A = 1-(R+T)$, where 1 is the total amount of light illuminating the sample leaf, and *R, T* and *A* are complementary fractional quantities.

### *C. Spectral measurements adapted for needle-leaves*

*R* and *T* measurements of narrow leaves require a specific adaptation of the conventional single beam integrating sphere measurement techniques due to the leaf size smaller than the illumination light beam. Reduction of the illuminated area to the dimensions of a single narrow needle would result in a too low signal-to-noise and would introduce potential errors of sample misplacements [34]. Placing the light beam-width-limiting slits at the entry port of the integrating sphere induces diffractive effects and does not allow for *T* measurements [35]. The only solution to increase the illuminated surface of very narrow leaves is to measure simultaneously a set of leaves collected from the same location (i.e. shoot). This approach requires an efficient and reproducible way of placing needle sets within the sampling port of an integrating sphere, ensuring that the *R* and *T* are recorded from the same sample leaf area in a time span short enough to prevent the biological degradation of detached leaves. This idea was implemented in three different approaches as described as follows.

The first approach, introduced by Hosgood *et al*. [36] within the LOPEX project, consists of measuring an infinite *R* of needles contained in a glass cuvette positioned at the sample port of an integrating sphere. These *R* spectra were subsequently corrected for the effect of the cuvette.

As opposed to the above, the other two approaches substitute the cuvette by a flat sample holder that presents only a single layer of needles at the entry port of an integrating sphere. These needles are placed side-by-side at an even distance and fixed between two holder plates, which are tightened and positioned at the sample port (Fig. 3(d)). However, different sample holders and subsequently required corrections are applied in both approaches.

The second approach by Harron *et al*. [37], [38] is used in several studies of coniferous species [39]-[43]. They employ a sample holder made of two black anodized plates with narrow hollow slots. The needles placed inside the slots are closing them completely ensuring that the light can only pass through the leaf tissue (Fig. 3(c)). The approach requires a correction removing the spectral contribution of the holder itself, which

is also illuminated during the measurements. A similar approach, but applicable only to leaves of at least 5 mm in width (which is considerably wider than needles of most coniferous species), was proposed by [35].

In the third approach by Daughtry *et al*. [34] and further improved by Mesarch *et al*. [11] the sample holder has a hollow central aperture bigger than the illuminated area. The needles presented at this aperture are separated by air gaps in-between them (Fig. 3 (a) and (b)). Therefore, an accurate removal of the air gap fraction (*GF*) between the needles is needed to correct the recorded *R* and *T* signal [44]-[46].

## III. Benefits and Shortcoming of Needle-Leaf *OPs* Methods

Hosgood *et al*. [36] used for the *OPs* measurements nonportable devices requiring reallocation of the foliar material from field to the laboratory. The use of portable devices is more efficient and provides higher flexibility and lower transportation costs especially during measuring campaigns taking place at remote locations. Moreover, the possibility to acquire *OPs* in-situ ensures that the measurements are done in **a** time frame short enough to prevent biological degradation of the leaf samples. Apart from this, no detailed information was found about the positioning of the needles inside the cuvettes, how their position in relation to the light source was affecting the recorded signal or if the signal was averaged based on the specific number of needles measured in each sample. Due to the highly varying size and shape of the needles inside the cuvette, these issues are expected to affect multiple scattering processes within the cuvette. A standardized and reproducible way of positioning the needles is crucial to ensure that *R* and *T* are recorded from the same sample area. Finally, a direct *T* measurement cannot be achieved with this technique.

The approach by Harron *et al*. [38] is highly systematic and based on portable measuring devices, but a major drawback are the narrow needle slots of the sample holder. As they are fixed in width and length, the sample holders are species-specific, which requires manufacturing many sample holders with different slot sizes. Moreover, twisted and/or strongly arced needles (e.g., Norway spruce needles) are not properly filling the slots, enforcing measurements of straight needles with a certain width only. Finally, since the holder presents only the needle core (typically the thickest part) to the sphere, the *T* measurement might potentially be underestimated [11].

The Daughtry *et al.* approach [34] is using portable equipment [11], it is not species specific, and it does not require manufacturing a highly advanced sample holder as those used in [38]. However, its weak point is the necessity to retrieve the area of air spaces between the measured needles, also termed gap fraction (*GF*). Authors suggested that the *GF* correction factor can be estimated as the ratio of the transmission recorded from a mat of evenly spaced needles painted in black to a 100% transmission measurement (i.e. empty sample port) at 680 nm. The even distance between needles of approximately one-needle width results in a *GF* of about 0.5. Unfortunately, the requirement to paint the needles in black color is time consuming, and more importantly, the $GF = 0.5$ appeared to underestimate *T* and overestimate *R*. A strong reduction of the gap size by using more needles still caused a certain overestimation of the *R* values, which was attributed to multiple scattering occurring between adjacent needles. Therefore, a modified approach by calculating *GF* directly through the acquisition of a sample digital image and the subsequent digital extraction of its gap area was proposed by Mesarch *et al.* [11]. On one hand, this reduced the number of measurements required and further eliminated the needle painting. On the other hand, it added the need to use an imaging system; however, economically feasible adaptations have already been developed [47]. The method can be applied to narrow leaves of several plant species including grasses [48] and all sorts of coniferous needles [47], [49], [50].

## IV. Methological Uncertainties in *OPs* Measurements

Recognizing the above universality requirements, we focus on Mesarch *et al.* [11] and use this method as a basis for our recommendations to improve its methodological approach and to minimize the uncertainties of this technique.

The initial Mesarch *et al.* [11] method can be summarized with the following five sequential measurement steps: (a) needles are placed in a sample holder with evenly spaced air gaps in between them; (b) the sample *R* and *T* signals are recorded using a spectroradiometer coupled with an integrating optical sphere; (c) a digital image of the masked sample holder aperture is acquired (the mask for the central aperture reproduces the size and position of the light beam illuminating the sphere sample port); (d) the *GF* of the sample is retrieved using computer-based image processing; (e) the measured spectra and *GF* are introduced in (1) and (2) to compute the spectrally dependent directional-hemispherical *R* ($R_{needle}$) and *T* ($T_{needle}$) of needles as follows:

$$R_{needle} = \frac{R_{TOTAL}}{(1 - GF)} \tag{1}$$

and

$$T_{needle} = \left[T_{TOTAL} - R_w \cdot GF\right] \cdot \frac{1}{(1 - GF)}, \tag{2}$$

where $R_{needle}$ is the *R* of individual needles, $T_{needle}$ is the *T* of individual needles, and $R_w$ is the *R* of the integrating sphere wall (assumed to be close to 100%). Consequently, the $R_{TOTAL}$ and $T_{TOTAL}$ are computed as:

$$R_{TOTAL} = \frac{R_{needles+gaps} - STR}{REF - STR} \tag{3}$$

and

$$T_{TOTAL} = \frac{T_{needles+gaps}}{REF - STR}, \tag{4}$$

where $R_{needles+gaps}$ is the radiation reflected from the sample, including the photons lost through the air gaps; $T_{needles+gaps}$ is the radiation transmitted through the sample, including the photons passing through the air gaps; *STR* is the stray light radiation and *REF* is the reference reflectance of a white panel.

To validate the method and to test the effect of the air gaps on the final signal, Mesarch *et al.* [11] proposed the concept of using the so-called *true GF*. They extracted the *GF* from (3), as the *true GF* that the sample should have in order to estimate the recorded signal for $T_{needle}$:

$$TrueGF = \left[T_{TOTAL} - T_{needle}\right] \cdot \frac{1}{\left(1 - T_{needle}\right)}. \quad (5)$$

They measured the *OPs* of an optically stable material (a film paper) to simulate broad leaves and narrow needle leaves (i.e. the film paper was cut in narrow strips). Since the *OPs* are inherent to the material irrespective to their shape and size, they substituted $T_{needle}$ in (5) by the *T* of a broad leaf assuming $T_{needle}=T_{broad\text{-}leaf}$. Subsequently they analyzed samples with *GF* ranging between 0.05 and 0.6 and computed the deviation of the digital *GF* from the *true GF* as the error attributable to their approach. Their results showed inherent errors connected to the *GF* image analysis. A relative error up to 40% was attributed to insufficient camera resolution and misalignment of the mask for the sample illumination beam. When identifying the optimal gap size they found errors being larger in samples having large *GF*s (0.3-0.6) than in samples of small *GF*s (0.05-0.15). The large size *GF*s were affecting the *T* signal more negatively than the *R* signal. They also measured *OPs* of flat mesquite leaflets and found them to vary in the same way as the *OPs* obtained from the film paper measurements. Contrary to this, measurements conducted with fir needles, i.e. leaves having a non-flat cross section, showed an increase in *R* with decreasing *GF*. Authors attributed this phenomenon to multiple scattering effects occurring between measured needles [34]. The non-flat cross-section (e.g., circular or rhomboidal) of the evenly spaced needle layer forming the sample allows the collimated light rays to hit the needle surface in a direction different from the normal to the sample front plane. This increases the probability of photons being scattered sidewise and interacting with the neighboring needles, especially if needles are placed too close to each other (i.e. in case of small *GF*). The scattered light can consequently escape from or be introduced into the integrating sphere during the *R* and *T* measurements, subtracting or adding a certain amount of photons to the recorded optical signals. According to published results [11], authors managed to optimize the method for flat narrow leaves, but not for non-flat needle-shaped leaves, which are in general represented by most of the coniferous species.

Three more problematic issues can be additionally identified from these results, opening space for a methodological revision. First, although this method does not allow for any direct interaction between the illumination beam and the sample holder, it might potentially suffer from an indirect influence of the holder presence (e.g., second order interaction with sample scattered light), as the holder of significant thickness is placed at the sample port of an integrating sphere. The multiple scattering enhanced by the non-flat cross section of the needles can potentially redirect some of the photons towards the sample holder plates. The increase of the optical path length from the light source to the sample surface and presence of holder edges can induce extra photon recollisions resulting in an unwanted but nonnegligible additional absorption [51].

Secondly, the identified deviation from the *true GF* was attributed to the complex inherent error of the technique as a whole. No sensitivity analysis of the *GF* to the specific factors involved in the image acquisition and digital image processing (e.g., threshold selection criteria applied for separating the air-needle interface during the digital *GF* estimation) has been performed.

Finally, the samples are expected to fit in a range of optimal *GF* values; however, the calculation of *GF* prior to the measurement in not straightforward or visually feasible. The *GF*, defined as the ratio of the total gap area between needles to the total measurement area, needs to be measured from irregularly shaped areas. This will have a significant and practical impact on timing and arrangement of a field campaign. On the one hand, there might be extra time needed to calculate the desired GF during sample preparation, when the leaves are already cut and attached to a sample holder. This elongation may cause further biological degradation of the sample before the *OPs* measurement is finished. On the other hand, if the samples are measured without knowing their *GF* value, a significant number of *OPs* might potentially be discarded after the processing due to an unacceptable high uncertainty caused by too large or too small *GF*s. This further delay, including also potential additional physiological investigations (e.g., carbon assimilation or water potential measurements) that are usually performed in parallel to *OPs* measurements [50], can lead to a substantial reduction of overall usable data.

## V. Conclusion

Progress has been achieved in systematically measuring *OPs* over the past decades. However, when considering the global ecological relevance of coniferous species with predominantly non-flat needle-shaped leaves, progress is considered relatively slow. When analyzing *OPs* measurement approaches used in literature, we were able to group them into three predominantly used approaches. These were those suggested by Hosgood *et al.* [36], Harron *et al.* [38], and Daughtry *et al.* [34] (with improvements by Mesarch *et al.* [11]).

Revisiting the limitations of the *Mesarch* method revealed further potential for improvements. Given the increasing importance of scaling based approaches [52]-[54] in combination with the ecological importance of ecosystems dominated by non-flat needle- shaped leaves [55], improvements to the error-prone Mesarch *et al.* [11] method are over-due.

## VI. Outlook

To further reduce parts of the above uncertainties addressed, we propose an experimental set-up improving the original method of Mesarch *et al*. [11]. Our experiment has three main objectives: 1) to investigate the potential of indirect influence of the sample holder presence on the measured leaf *R* and *T*, 2) to evaluate the errors introduced by image acquisition and processing settings applied to compute the sample *GF*, and 3) to investigate the possible occurrence of multiple scattering induced by the non-flat profile of the conifer needles, focusing on: a) the influence of the needle cross-section shape and b) the particular distance between the needles in the sample, instead of in the *GF* size itself. A detailed methodological description and final outcomes of this experiment are presented in Part II of this paper (this issue).

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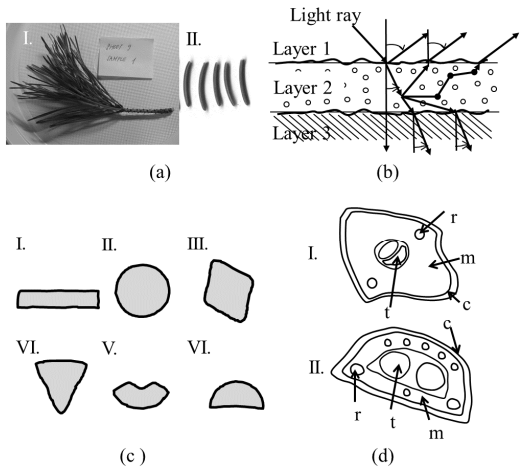


Fig. 1. (a) *Pinus nigra* shoot (I) and *Picea abies* needles detached from shoot (II); (b) geometry of the light interactions within a typical broad leaf (adapted from [56]); (c) overview of cross-sectional shapes of conifer needles (adapted from [57]) and a broad leaf (representing the majority of deciduous species): (I) flat leaf; (II) *Pinus monophylla* (Torr. & F&m.); (III) *Picea asperata* Master; (IV) *Pinus cemhra* L.; (V) *Abies nordmanniana* Spach; (VI) *Pinus sylvestris* L.; (d) sketch (modified from [58]) of cross-sections of (I) spruce (*Picea abies*) and (II) pine (*Pinus nigra*) needle (r=resin channel; t=transfer channel; m=mesophyllum; c=cuticle).

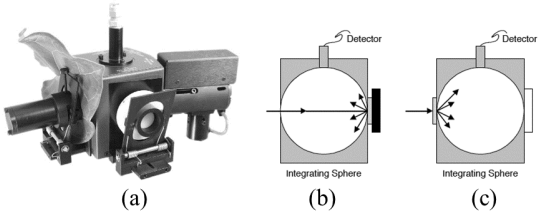


Fig. 2. (a) Example of a commercial integrating sphere designed for measuring broad leaves (ASD 190 RTS-3ZC) [59]; (b) Directional hemispherical measurements of leaf reflectance; and (c) Transmittance measurements (adapted from [4]).

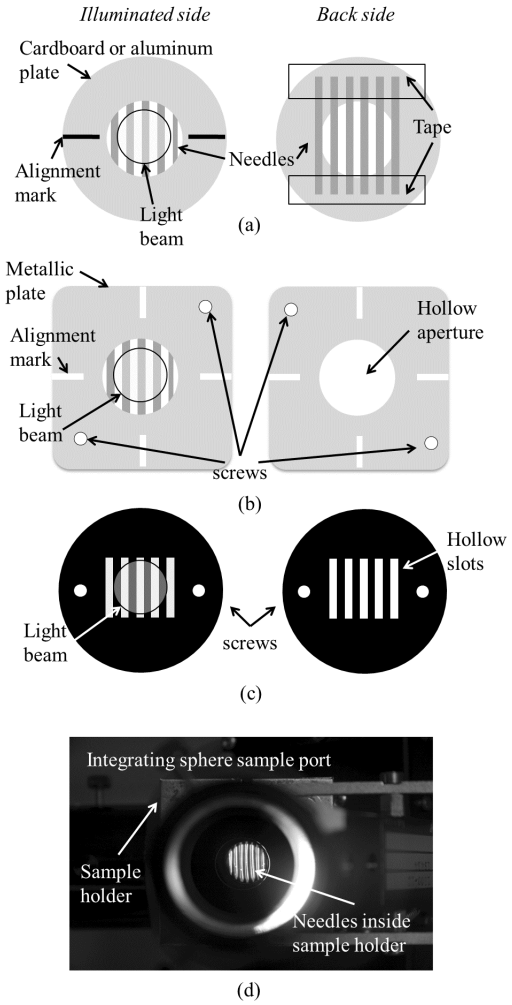


Fig. 3. Example of needle-leaf sample holders: (a) sample holder used in [34, 11] (Thickness is approximately half of the needle thickness ≈ 0.7 mm); (b) sample holder used by [47], which is an adaptation of [11] (Approximate holder thickness ≈ 1 mm; (c) sample holder from [37]-[38] (Approximate thickness ≈ 1.5 mm). In all cases, the needle sample holders are placed in the same position as the broad leaf sample in Fig. 2; (d) Sample holder placed at the sample port of the integrating sphere [47], [60].