Leaf Optical Properties EXperiment 93 (LOPEX93)

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1. Introduction

The estimation of leaf biochemistry and leaf water status with remote sensing data is a challenge for the years to come. It also has an important potential in agriculture to follow crop development and yield predictions. The biochemical constituents of interest in this experiment were lignin, proteins (nitrogen), cellulose and starch, as well as chlorophyll and foliar water. The major processes involved in the terrestrial ecosystem such as photosynthesis, primary production, or foliar decomposition can be related to these constituents. As leaves are the most important surfaces of a plant canopy, relating their optical properties to these constituents is a priority (Jacquemoud et al., 1994).
The overall objective of the experiment was to investigate the use of high resolution visible and near infrared reflectance spectroscopy for the retrieval of chlorophylls, water, protein, cellulose, lignin, and starch both on fresh and dry material, on individual leaves and on optically thick samples (stacked leaves + needles or powders).

2. The Experiment

In order to have a wide range of variation of leaf internal structure, pigmentation, water content and biochemical components, plant species with different types of leaves were collected during two separate periods during the summer of 1993. About 70 leaf samples representative of more than 50 species were obtained from trees, crops and plants in the area of the JRC. In addition, various substances such as powdered starch or proteins and vegetative material such as stems or bark were also included in the data set to increase its variability.

About 800g of leaves were required for each sample which normally yielded about 80g of dry material.

3. Spectral measurements

A Perkin Elmer Lambda 19 double-beam spectrophotometer equipped with a BaSo4 integrating sphere was used for the measurement of the reflectance (R) and transmittance (T) of the upper faces of leaves. In addition, the reflectance of optically thick samples (R∞) was measured by stacking leaves in order to magnify the radiometric signal and minimize the leaf to leaf variability or, in the case of needles or powders, by placing them in a quartz cuvette.
Spectra were scanned over the 400-2500 nm wavelength interval with 1 nm step starting at 2500 nm and ending at 400 nm. The spectral resolution varied from 1 to 2 nm

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in the visible / near infrared (400-1000 nm) and from 4 to 5 nm in the middle infrared (1000-2500 nm). The calibration of the instrument was performed using Spectralon™ reflectance and wavelength calibration standards. For each sample, measurements were made on 5 different areas in order to quantify the small but not negligible leaf to leaf variability. The scan time required for each sample was about 4 minutes. In the case of needles and powdered material, the quartz cuvette was positioned vertically against the side of the BaSo4 integrating sphere. The reflectance spectra made in this mode have been corrected for the effect of the quartz plate in front of the sample taking into account the reflectance and transmittance of a single quartz plate of the same thickness, as described below.
All the above procedure was repeated some days later on dried leaves and needles to analyse the influence of water which is known to obscure the biochemical information in the middle infrared region.

3.1 Instrumental corrections

The integrating sphere is 60 mm in diameter with a ratio aperture/internal surface of 8 %. In the VIS/NIR, the detector is a photomultiplier; in the IR region , a PbS detector is used. The transition between the two detectors occurs at 860.8 nm.
First the full scale was set by running the instrument with two white diffusing reflectors positioned on the sample and reference ports of the sphere. The instrument stores this measurement and uses it to automatically correct the following measurements. The diffusing reflectors should be calibrated standards; however, at the time of the experiment, these were not available and two uncalibrated spectralon samples (sample A on the sample port, sample B on the reference port) were used. The reflectance of these two samples was later measured with reference to a SR99 diffuse reflectance standard. Let r_B be the measured ratio of sample B to SR99 standard reflectances ($r_B=R_B/R_{SR99}$). A background measurement was also performed by positioning a light trap (reflectance < 10^{-4}) on the sample port. The apparent measured background reflectance (r_0) has its origin in the small fraction of the sample beam not incident on the light trap but on the surface of the sphere.
The reflectance measurements were then performed by placing the sample on the sample port, leaving the diffuse reflector B on the reference port.
Transmittance measurements were performed with diffuse reflector B positioned on the sample port while the sample itself intercepted the sample beam at its entrance in the sphere. Diffuse reflector B was always used on the reference port.

If r_S and t_S denote the raw reflectance and transmittance measurements, the absolute reflectance (R_S) and transmittance (T_S) can be approximated with the following formulæ:

$$R_s = \frac{(r_s - r_0).r_B.R_{SR99}}{(1.-r_0)} \qquad (1)$$

$$T_s = \frac{t_s.r_B.R_{SR99}}{(1.-r_0)} \qquad (2)$$

where R_{SR99} is provided by the certified calibration of the standard.

In the transmittance formula, the background r_0 is not subtracted, as the fraction of the sample beam not incident on the sample port is part of the signal (being transmitted through the sample). The denominator of the formulæ takes into account the effect of r_0 on the instrumental full scale value.
These correction formulæ were tested in various ways.

(i) Results on diffuse reflectance standards

Grey standard diffusers (reflectance of 80, 60, 40 ,20 ,10, 5 and 2%) were measured and their corrected reflectance was found to lie within the calibration specifications (std. dev. ± 0.005). The same was done for a number of coloured standard diffusers.

(ii) Results on transmittance samples

Two diffusing transmittance samples were measured both for reflectance and transmittance (SDM-200-DU and SDM-200-DM). These samples are made of a film (~300 μm) of Spectralon™ and the sum of their reflectance and transmittance should be very close to 1 (almost negligible absorption). In some cases, summing the raw r and t measurements, the result is >1. After correction, however, the result is acceptable.

3.2 Correction for samples measured in a cuvette.

Since the spectrophotometer does not allow to position the sample horizontally, some material (needles, uncompressed powders) had to be contained in a glass cuvette. The reflectance (R_g) and transmittance (T_g) of the cuvette wall was measured and the reflectance of the studied material (R_S) retrieved using the following formula:

$$R_s = \frac{R_{s+c} - R_g}{R_g.(R_{s+c} - R_g) + T_g^2} \qquad (3)$$

where R_{S+C} is the corrected (with formula (1)) reflectance measurement on the sample in the cuvette. The formula takes into account the multiple reflections.
The validity of this correction was checked by measuring a black painted aluminium plate both inside and outside the cuvette.

In most of the spectra, a small disturbance can be observed at the 860 nm point due to the automatic change from Pbs detector to photomultiplier. In the case of some optically thick samples such as stalks, this disturbance may increase noticeably since the instrument slit width also changes at this point and thus the geometry of the target surface observed may be altered.

4. Auxiliary measurements

In parallel with the spectral measurements, many physical and biological measurements were performed on the samples. Leaf blade thickness was measured with a calliper rule (5 measurements per leaf). The fresh weight of a 4.10 cm² disk taken on each leaf using a cork borer was then immediately measured. The disk was then placed in a drying oven at 85°C for 48 hours and reweighed to determine the water content (WC = water

mass over fresh mass), the equivalent water thickness or water depth (EWT = water mass per unit leaf area), and the specific leaf area (SLA = dry weight per unit leaf area).

With regard to the other **biochemical constituents**, about 250 g of fresh material were partially dried in an oven and then sent to two independent and specialized laboratories in France and Belgium which performed the measurements of total proteins, cellulose, lignin, and starch using standard wet chemical analyses. The comparison between the concentration values (g/g) provided by the two laboratories gives an idea of the precision of these analyses: protein and cellulose measurements were quite consistent while lignin and starch measurements differed significantly. These discrepancies are probably mainly due to the different methods of chemical extraction.

Extraction methods:	Protein: Kjeldahl Cellulose: Weende (B) / Van Soest (F) Lignin : Van Soest Starch : Ewerts (B)

A total of 120 samples was sent to each laboratory in 2 batches. The first batch, collected in July, contained 70 samples and the second batch, collected in September, contained 50 samples. Each batch contained a number of double samples which allows an estimation to be made of the repeatability of the chemical analyses. Furthermore, some of the vegetation types contained in batch 1 were repeated in batch 2 in order to be able to assess the natural variation of the biochemical concentrations during the period of maximum phenological activity of the vegetation.

Part of the remaining leaf samples was frozen for later biochemical analysis: the photometric determination of **photosynthetic pigments** (chlorophyll a, b and total carotenoids) was performed with a UV-2001 PC spectrophotometer in 100% acetone using the equations of Lichtenthaler (1987) at the University of Karlsruhe (Botanical Institute II).

1. Chlorophyll a : $c[chl\ a] = 11.24*A_{661.6} - 2.04*A_{644.8}$

2. Chlorophyll b : $c[chl\ b] = 20.13*A_{644.8} - 4.19*A_{661.6}$

3. Total chlorophylls a + b : $c[chl\ a] = 7.05*A_{661.6} + 18.09*A_{644.8}$

4. Total carotenoids : $c[x+c] = (1000*A_{470} - 1.9*c[Chl\ a] - 63.14\ [Chl\ b]\ ) / 214$

where A = absorption coefficient

Another part of the remaining samples was ground to a fine powder using a Retsch ZM1 grinder equipped with a 10µm filter. Part of the **powders** was then compressed under a pressure of 20 tons and formed into pastilles in aluminium and plastic cups (∅ = 30 mm).

The **pastilles** were then dried in an oven for one week at 40°C before their spectral characteristics were measured again in the Lambda 19 spectrophotometer. Each spectral measurement was made on three different points of the pastille. In all, 94 pastilles were measured in this way. (See data files OPEX2---)

A small part of the powders (~ 8g.) was put aside for analysis of the elemental composition of the samples.

The elemental analyses were made at the bioclimatology laboratory of INRA Clermont Ferrand (F) using a microanalyser ERBA. The elements of interest were Carbon (C), Hydrogen (H), Nitrogen (N) and Oxygen (O). The elements were not analysed simultaneously. The composition in C, H, and N was estimated using the Dumas and Pregl method. Samples and standards are weighted into tin containers and sealed. The sample is dropped into the combustion furnace. A fixed volume of oxygen is flushed in by the heluim gas carrier. The tin oxydizes immediately and temperature rises to 1800° C. Combustion gases pass on a first catalyst (CR2O3) to produce CO2, H2O, SO2/SO3 and NOx and on a second catalyst (pure copper) to reduce NOx, sulphur and residual oxygen. Gases are then separated in a chromatographic column and quantified using a thermal conductivity detector.
The composition in oxygen was determined using the Unterzaucher method. The method is similar to the Dumas and Pregl method except that the catalyst is nickel and combustion gases are transformed in NO. Similary, gases are separated in a chromatographic column and quantified in the same way.
Results are expressed in % of dry matter. 2 or 3 repetitions were made for each sample analysis.

5. Classification of the experimental results

The experimental results have been classified and archived for future use in a series of ASCII files in the main directory **lopex93.**
The bulk of the data files is constituted by the reflectance and transmittance spectra. A total of 1938 files have been generated with the root name **OPEX** contained in the sub-directory **spectra**. Each file has been radiometrically corrected and is expressed in terms of absolute reflectance (as a fraction of 1). The corresponding wavelengths which are identical for all spectra are contained in the file **OPEX.WVL** and are expressed in nanometres (integer values ranging from 400 to 2500).

All auxiliary measurements are contained in a separate sub-directory (**auxmeas**).
The complete list of samples is given in Latin (where possible) and English in Tables 1 and 2 respectively. These names are also contained in the files **SAM_LNAM** and **SAM_ENAM.LST**.

A key element in this classification is the association between the **spectrum number** and the relative **auxiliary measurements.** This is the file **SPEC_AUX.DAT**. An explanation of the code employed in this file is given in Table 3.

The association between the **sample number** and the relative **biochemical analyses** is contained in the file **SAM_BIO.DAT**. This file also contains the code indicating the type of sample in question (ie. monocotyledon, dicotyledon etc). An explanation of the code employed is given in Table 4.

The association between the **sample number** and the relative **spectra** is contained in the file **SAM_SPEC.DAT**. An explanation of the code employed in this file is given in Table 5.

The association between the sample number and the spectrum number can thus be obtained in 2 ways:
1. Indirectly, by means of the spectrum block number in the SAM_BIO and SPEC_AUX files
2. Directly, by means of the SAM_SPEC.DAT file.

The results of the chlorophyll and total carotenoids analyses can be found in the file **SAM_PIG.DAT**. An explanation of the code employed in this file is given in Table 6.

The results of the elemental analyses can be found in the file **SAM_ELE.DAT**. An explanation of the code employed in this file is given in Table 7.

6. Conclusion

An important and valuable data set has been put together with these measurements. The preparation of the leaf samples was particularly time-consuming especially in the case of plants with small leaves. The spectral measurements were made with the best equipment available and can be considered to be very precise. The fact that the samples were also powdered and compressed means that they will also be available in the future for further measurements or comparison. Preliminary analyses of the data show many promising results but there are many other analyses of correlation which still remain to be made at the time of writing. The authors hope that these data can be used by other researchers in this field and that the results will contribute to a better understanding of the relationship between the spectral characteristics of vegetation and its biochemical components for application in Remote Sensing.

For further information regarding the structure and content of the data set please contact: B. Hosgood at JRC, Ispra.
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See also: http://www.sigu7.jussieu.fr/Led/LED_lopex.htm

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8. References

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