ABSTRACT:

A prototype instrument called RAMIS has been designed to non-destructively measure the biochemical properties of plant leaves such as water, dry matter, and total chlorophyll content. The spectral distribution of light transmitted through the leaf is closely related to the concentration of these constituents. In consequence, their retrieval from in situ optical measurements is possible by selecting the appropriate wavelengths. In RAMIS, the adaxial face of the leaf is alternately illuminated by five light-emitting diodes (LED) centred at 656, 721, 843, 937 and 1550 nm and the amount of light transmitted through the leaf blade is measured by a double layer Si-Ge photodiode sensor. A measurement of the raw signal when the leaf is removed and a calibration factor allow us to derive the hemispherical leaf transmittance. The PROSPECT model which physically relates the leaf optical properties to foliage anatomical structure and biochemical composition is then inverted to estimate the water content by using a method based of neural networks. Results show a reasonable agreement between optical and gravimetric measurements.

RESUME:

RAMIS est le prototype d'un instrument de mesure non destructif des propriétés biophysiques des feuilles comme la teneur en eau, en matière sèche et en chlorophylle. La distribution spectrale du rayonnement électronique transmis à travers la feuille est fortement liée à la concentration de ces constituants. Par conséquent, il est possible de les déterminer grâce à des mesures optiques effectuées in situ à des longueurs d'onde convenablement choisies. Dans RAMIS, la face supérieure de la feuille est alternativement illuminée par cinq diodes électroluminescentes centrées sur 656, 721, 843, 937 et 1550 nm, la quantité de lumière qui traverse la feuille est mesurée par une photodiode bicouche Si-Ge. A partir des mesures du signal brut sans feuille et avec feuille, on déduit la transmittance hémisphérique après avoir appliqué un terme correctif. Le modèle PROSPECT qui relie les propriétés optiques d'une feuille avec sa structure anatomique et sa composition chimique est alors inversé pour déterminer la teneur en eau en utilisant une méthode basée sur des réseaux neuronaux. Les résultats montrent une concordance raisonnable entre les mesures optiques et gravimétriques.

1. INTRODUCTION

For various application domains such as precision agriculture, global-scale ecology, or for the validation of satellite remote sensing products, it is very important to assess the leaf biochemical composition: mainly the total chlorophyll (C_ab), water (C_w also called equivalent water thickness), and dry matter (C_m also called leaf mass per area) content. The measurement of leaf chlorophyll content involves analytical chemical techniques which are time consuming and expensive. A precision balance associated with a drying oven allows one to simply determine C_w and C_m, but these procedures are difficult to carry out when the experiment field is far away from the laboratory. Moreover, once detached from the plant, leaves lose water by evaporation and chlorophylls begin to degrade. These constraints limit the availability of such measurement techniques to a limited number of samples, in areas close to the analytical facilities.

In order to overcome these limits, we designed a hand-held biophotonic instrument called RAMIS (Radiomètre portatif de Mesure In Situ) which is based on the interaction of electromagnetic radiation (EMR) with plant leaves (Frangi et al., 2003). It non-destructively measures the leaf transmittance at five judiciously selected wavebands sensitive to chlorophyll, water, and dry matter. This work involves two research activities: instrumentation and modelling. Indeed we have to solve technical issues (the measurement of the fraction of light transmitted through a plant leaf at several wavelengths) and algorithmic issues (the modelling of this transmittance as a function of the leaf biochemical constituents).

This paper is organized in four sections. After describing the physical principles of the instrument, its functioning and the processing of the output signal are detailed.

2. PHYSICAL PRINCIPLES

The interaction of EMR with leaves can be computed from knowledge of the spectral variation of the complex refractive index: the real part explains the multiple reflections of light at the cell-air interfaces and the imaginary part the absorption of light by the leaf biochemical compounds. RAMIS is based on knowledge of these absorption properties in the solar domain from 400 nm to 2500 nm. Figure 1 presents the specific absorption coefficients of chlorophyll, water, and dry matter used in PROSPECT, a radiative transfer model which simulates the directional-hemispherical reflectance and transmittance of plant leaves (Jacquemoud and Baret, 1990) with C_ab, C_w, and C_m as input parameters.
One can notice that the pigment absorption spectrum is distinct from the others, but that water and dry matter overlap each other, which may complicate the deconvolution of the measured signal.

3. DESCRIPTION OF RAMIS

In RAMIS, the leaf to be measured is illuminated by five light-emitting diodes (LED) (Figure 2). Their wavebands have been selected in such a way that they correspond to the specific absorption lines of the leaf biochemical compounds. The LEDs are centred on 656, 721, 843, 937, and 1550 nm (Figure 3). Today, manufactured LEDs are available at almost any wavelength in the visible (VIS) and near-infrared (NIR), while the choice is very limited in the shortwave-infrared (SWIR).

The amount of light transmitted through the blade is measured by a double layer Si-Ge photodiode also called the “sandwich” detector (Figure 4).

The lighting of the LEDs is controlled and the acquisitions of the output signal is achieved using a data acquisition card (Labview, National Instrument) connected to a laptop. One must emphasize that the photodiode works as a mono-channel band detector although the LEDs are sequentially powered.

The transmittance of a semi-transparent object is defined as the ratio of the transmitted flux ($\Phi$) to the incident flux ($\Phi_0$). To determine the transmittance of a plant leaf with RAMIS, we measure the detector output signal in the presence of the leaf and after removing it (Figure 6).

The diodes are successively powered, with the leaf inserted or not. The response of the photodiode is recorded as an electrical current $I$ which is assumed to be proportional to the light flux $\Phi$.
falling on the photodiode, so long as its response to $\Phi$ is linear:

$$\Phi(\lambda) = aI(\lambda) \quad \text{and} \quad \Phi_0(\lambda) = aI_0(\lambda)$$

then $\Phi(\lambda)/\Phi_0(\lambda) = I(\lambda)/I_0(\lambda)$. The calibration of RAMIS is performed with the help of three Lambertian diffusers (SphereOptics) of thickness 1 mm, 250 $\mu$m, and 100 $\mu$m, spectrally calibrated by the manufacturer and corresponding to average transmittances of 0.08, 0.25, and 0.50, respectively.

4. PROCESSING OF THE RAMIS OUTPUT SIGNAL

We first tried to determine simple relationships between the RAMIS output signal and the leaf biochemical compounds. For instance, we looked at the variation of the output signal as a function of the leaf water content $C_w$ by slowly drying a detached leaf of *Tradescantia fluminensis* in natural conditions. The leaf was periodically weighed and, at the end of the experiment, totally dried in an oven to determine the equivalent water thickness at each stage. Figure 7 shows that the ratio $I(\lambda)/I_0(\lambda)$ calculated at 1550 nm (band 5 in Figure 3) is well related to $C_w$, that it decreases with $C_w$, and that the signal output is sensitive enough to estimate water content.

![Figure 7. Relationship between the RAMIS output signal at 1550 nm and water content](image)

Unfortunately, this relationship is not unique when trying to repeat the experiment with different leaf species showing a wide range of leaf internal structures and dry matter contents. Then we decided to calculate the ratio of the near-infrared to the short-wave infrared transmittances. The SPAD-502 (Minolta) works on the same principle: it calculates the ratio of the near-infrared to the red transmittances to retrieve $C_{ab}$ (we actually found out that the two devices had very similar characteristics for chlorophyll estimation). However, the same kind of semi-empirical approach applied to water and dry matter was unsuccessful because of their overlapping absorption spectra, as mentioned earlier.

To overcome this problem, we introduced PROSPECT, a radiative transfer model which calculates the leaf hemispherical transmittance $T(\lambda)$ every nanometer in the NIR and SWIR with the leaf structure, the leaf water and dry matter contents as input parameters (Jacquemoud and Baret, 1990). PROSPECT can be run in direct mode to compute the RAMIS output signal $I(\lambda)/I_0(\lambda)$, as long as the emission spectrum $E(\lambda, \lambda_c)$ of each LED (Figures 3 and 8) and the responsivity spectrum $S(\lambda)$ of the Si and Ge sensors (Figure 5) are well characterized. Equations 1 and 2 respectively give the expressions of the electrical currents $I_0(\lambda_c)$ and $I(\lambda_c)$ measured for each LED without a leaf and when a leaf is clamped.

$$I_0(\lambda_c) = \int_0^\infty E(\lambda, \lambda_c)S(\lambda)d\lambda \quad (1)$$

$$I(\lambda_c) = \int_0^\infty E(\lambda, \lambda_c)T(\lambda)S(\lambda)d\lambda \quad (2)$$

A « feedforward » neural network where the neurons are arranged in two layers was then implemented to relate the RAMIS output signal $I(\lambda)/I_0(\lambda)$ to the leaf water content $C_w$. The training dataset was built using PROSPECT simulations. This technique has been already used to estimate the leaf biochemistry from leaf reflectance or transmittance spectra (de Rosny et al., 1995; Dawson et al., 1998; Le Maire et al., 2004). In this work, inversions were conducted on the five RAMIS transmittances measured over 130 leaf samples representing fifteen plant species collected outdoors in the spring of 2006.

Figure 9 presents the estimation of leaf water content using RAMIS compared to laboratory analytical measurements. One can see a good agreement between the two methods, specially for high values of $C_w$, and some difficulties to estimate low values.

![Figure 9. Validation of leaf equivalent water thickness estimation using RAMIS](image)
5. CONCLUSION

In this paper, we demonstrate the ability of RAMIS to collect radiometric measurements in the field and to provide accurate and non-invasive estimates of leaf water content. The use of PROSPECT in the retrieval process implemented into RAMIS is essential to the success of this instrument. Similar studies performed by Pavan et al. (2004) to estimate chlorophyll content also led to good results.

Some improvements of the prototype are under development. For instance, the measurements of the leaf transmittance at appropriate wavelengths in the vicinity of 1600 nm should permit reliable estimates of leaf dry matter content, and therefore better estimates of water content.

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REFERENCES


