A new instrument for passive remote sensing
1. Measurements of sunlight-induced chlorophyll fluorescence

I. Moya\textsuperscript{a}, L. Camenen\textsuperscript{b}, S. Evain\textsuperscript{a}, Y. Goulas\textsuperscript{a}, Z.G. Cerovic\textsuperscript{a},
G. Latouche\textsuperscript{a}, J. Flexas\textsuperscript{c,}\textsuperscript{*}, A. Ounis\textsuperscript{a}

\textsuperscript{a}Laboratoire pour l’Utilisation du Rayonnement Electromagnetique (LURE)-CNRS, Univ. Paris-Sud, BP34, 91898 Orsay, France
\textsuperscript{b}Horiba Jobin-Yvon, 16-18 rue du canal, 91165 Longjumeau, France
\textsuperscript{c}Laboratori de Fisiologia Vegetal, Universitat de les Illes Balears, Carretera de Valldemossa Km 7.5, 07122 Palma de Mallorca, Balears, Spain

Received 10 June 2003; received in revised form 25 February 2004; accepted 28 February 2004

Abstract

Under natural sunlight illumination, the chlorophyll fluorescence emitted by the vegetation represents less than 3\% of the reflected light in the near infrared part of the spectrum. This small amount is difficult to quantify except at certain wavelengths, where the solar spectrum is attenuated (Fraunhofer lines). An instrument measuring the in-filling of the atmospheric oxygen absorption band at 760 nm by chlorophyll fluorescence has been designed and constructed at the “Laboratoire pour l’Utilisation du Rayonnement Electromagnetique” in Orsay, France. The system was calibrated against a pulsed fluorimeter (FIPAM), especially developed for monitoring chlorophyll fluorescence at distance. The penetration of diuron, a herbicide acting on photosynthesis, was monitored by the passive instrument for several days on a corn canopy. A good agreement was found between gas exchange and variable chlorophyll fluorescence at the canopy level and variable fluorescence at the leaf level. The potential application of the passive chlorophyll fluorescence measurements for long range vegetation remote sensing is discussed.

© 2004 Elsevier Inc. All rights reserved.

Keywords: Remote sensing; Chlorophyll fluorescence; Fraunhofer line principle; Passive method; Terrestrial vegetation

1. Introduction

In the last decades, several spectroscopic methods have been considered for assessing the status of plant canopies and assessing their biomass and production. Among these methods, the use of the fluorescence signal emitted by plants under laser or daylight excitation has been the object of an intense research activity. Sun-induced chlorophyll fluorescence in the upwelling at sea surface has been considered since more than 20 years (Gower & Borstad, 1989; Neville & Gower, 1976). Following the successful application of airborne remote sensing (Carter et al., 1996; Doerfler, 1993; Gower & Borstad, 1989), ESA and NASA began development on ocean colour sensors, which include appropriate channels for the detection of chlorophyll fluorescence.

In order to extend this approach to monitoring continental vegetation and to map photosynthetic activity at large scales, two experimental missions have been proposed in the past: FLEX (ESA) in 1998 and FLEXSAT (NASA) in 1999 (Stoll et al., 1999). FLEX intends to explore the possibility to use the Fraunhofer lines of the solar spectrum for passive measurements of natural sunlight-induced fluorescence, with the aim of improving estimates of vegetation photosynthetic activity and its implications for surface carbon fluxes estimation. To support the FLEX project, ESA has funded a measuring campaign to observe the solar-induced fluorescence signal over a northern boreal coniferous forest site (SIFLEX-2002 campaign, April–June 2002, Sodankyla, Finland). In addition, an ESA study with the objective of developing a canopy fluorescence model has also been undertaken.

The increasing interest for remote sensing of fluorescence from terrestrial vegetation has stimulated the exploration of new methods, such as detection from derivative reflectance and double-peak red-edge effects (Zarco-Tejada et al., 2002). Radiative transfer models at the leaf level have also been developed to account for reflectance, transmit-
tance and also fluorescence of green leaves. In the SLOP model (Maier, 2000; Maier et al., 1999), diffuse and transmitted light are introduced with weighted probabilities according to the theory of homogenous Markov chains. The reflectance, transmittance and fluorescence properties are simulated by introducing the specific absorption spectra of pigments in vivo. Besides this work, the effect of chlorophyll fluorescence emission on the apparent reflectance has been recently investigated using a double-illumination radiometer (Zarco-Tejada et al., 2002). These authors developed their fluorescence, reflectance and transmittance model (FRT), based on the matrix formulation of Yamada and Fujimura (1991). The FRT model takes into account the existence of differentiated leaf tissues by introducing a stack of three layers: top epidermal layer, compact inner layer containing the chloroplasts and cellular material, and lower epidermal layer. It allows the simulation of the apparent reflectance with the superimposed effects of fluorescence.

Within this context, the aims of the present work are: (i) to present a new passive Chl fluorescence remote sensing instrument based on the in-filling of the atmospheric oxygen absorption band at 760 nm, and (ii) to demonstrate the capability of this new instrument to accurately track sunlight- and stress-induced Chl fluorescence variations from the leaf to the canopy level.

2. Materials and methods

2.1. Principle of the FLD method

The amount of chlorophyll fluorescence emitted by a leaf under natural sunlight (which may represent up to 1% of the absorbed light in the visible part of the spectrum) is difficult to quantify because the signal is obscured by the reflected light. However, at certain wavelengths where the solar spectrum is attenuated (Fraunhofer lines), the fluorescence signal can be quantified. Solar irradiance exhibits three main absorption bands in the red and near infrared part: the Hα line at 656.3 nm is due to the hydrogen absorption by the solar atmosphere whereas two bands at 687 and 760 nm are due to the molecular oxygen absorption by the terrestrial atmosphere. These bands largely overlap the chlorophyll fluorescence emission spectrum of leaves. Therefore, they have a potential use to monitor the chlorophyll fluorescence emission under daylight excitation by the method of the Fraunhofer lines in-filling. Applications using the Hα line method have been successful (Plascyk, 1975; Plascyk & Gabriel, 1975).

However, the position of the Hα line is far from the maximum of the chlorophyll fluorescence emission. The oxygen absorption bands, situated closer to the chlorophyll fluorescence peaks, are better candidates. The potential gain in the signal-to-noise ratio also depends on the shapes of these bands. Furthermore, it is also necessary to take reflectance into account, which is 5–10 times higher at 760 than at 656 or 687 nm. Fig. 1 shows a low-resolution emission spectrum of the solar radiation at sea level superimposed on the fluorescence emission and reflectance spectra of a green leaf. It can be observed that the oxygen-B band coincides almost totally with the red peak of the Chl fluorescence spectrum (687 nm), but its depth is ca. 70% lower than that of the oxygen-A band (Fig. 1, inset). The latter band does not coincide with the far-red peak of the Chl fluorescence emission, but at 760 nm the Chl fluorescence emission still accounts for ca. 50% of the peak. In addition, the width of the oxygen-A band is larger (Fig. 1, inset). Because of all these features, the oxygen-A band at 760 nm appears to be the most suitable to evaluate the possibilities of passive measurements for detection of chlorophyll fluorescence of leaves, and therefore has been chosen for the present instrument. A preliminary version of the instrument has already been presented (Moya et al., 1999).

Fig. 2 summarizes the principle of the approach and shows related equations. The radiance of the target (plant) is compared to that of a reference panel situated in the same light climate. a and b represent the detected radiance from the reference panel in and out of the oxygen absorption band at 760 nm.
feature, respectively. Similarly, \( c \) and \( d \) represent the detected radiance from the target at the border and at the bottom of the band. The output parameters are the reflectance coefficient \( (R) \) and the fluorescence flux \( (f) \) contributions to the total target radiance. \( R \) is defined as the ratio between the energy flux reflected by the sample in a given solid angle and the energy flux reflected by the reference panel, for the same solid angle. Assuming that the \( R \) and \( f \) are constant in the vicinity of the band, they can be derived from the measurement of \( a, b, c \) and \( d \). According Plascyk and Gabriel (1975):

\[
c = Ra + f
\]

\[
d = Rb + f
\]

It follows that \( R = (c - d)/(a - b) \). It should be noted that \( R \) is obtained free of any fluorescence contribution. Once \( R \) is calculated, \( f \) can be deduced by subtracting the reflectance component to the total radiance of the target.

### 2.2. Description of the instrument

The system is designed to follow fluorescence and reflectance changes as a function of natural daylight variations. Commercially available interference filters and photodiode detectors were chosen for the sake of simplicity. Fig. 3 shows a photograph of the optical head of the instrument and a diagram of the main components. A beam splitter (microscope cover) is used to measure simultaneously the \( a \) and \( b \) signals by two identical detectors (amplified photodiodes HUV 2000, EGG). As the \( a \) channel is detected on the reflection side, the difference between reflection and transmission in the beam splitter approximates the differences between the border and bottom signals. A possible drawback of the glass beam splitter is its strong polarising effect on the reflected beam. This effect has been removed by inserting a polariser (polarising cube 03PBS057, Melles Griot) in front of the beam splitter, in order to transmit only polarised light parallel to the beam splitter. The disadvantage is a decrease of 50% of the available light flux. In order to focus the instrument on a target, a small sighting telescope is placed in front of the beam, which receives light reflected at 90° by the polarising cube.

Two similar interference filters (Omega Optical, Brattleboro, VT, USA, FWHM = 1 nm, transmission 70%, 25 mm of diameter) are used to select \( a \) (758.5 nm) and \( b \) (760.5 nm) signals. A protective window constituted of a 3-mm Schott RG 9 long-pass filter is placed in front of the optical components. The peak wavelengths and the widths of the interference filters are chosen to maximise the \( a/b \) ratio, but as close as possible. In order to minimise incidence effects on the interference filters, the field of view of the instrument has been limited to 4° by a field stop situated at the tube entrance.

To account for the variation of the depth of the oxygen absorption band, a flip-flop mirror alternates the field of view between the target and the reference. The reference is a panel of Spectralon (Labsphere, USA) situated near the target, as shown in Fig. 2. The period of the mirror, which fixes the time resolution of the measurement, is limited to 1 s for mechanical reasons. \( c \) and \( d \) signals are linearly interpolated and scaled in the time base of the \( a \) and \( b \) signals. For kinetic measurements under stable sun illumination, the flip-flop mirror is not moved, allowing a time resolution of \( \approx 100 \) ms.

The interference filters are a critical issue in the design of the instrument. Interference filters are known to red shift the center wavelength by 0.2 nm when the temperature increases by 10 °C, as indicated by the manufacturer. On the other hand, a blue shift of the center wavelength occurs when the incident angle is increased, according to the relation:

\[
\lambda_\theta = \lambda_0 (1 - \sin^2 \theta/n^2)^{1/2}
\]

where \( \lambda_\theta \) is the shift with incident angle \( \theta \) (\( \theta < 10^\circ \)), \( \lambda_0 \) is the central wavelength of the band-pass filter and \( n \) is the
refractive index of the spacer layers (generally \( \approx 2 \)). We have used these two antagonistic properties to carefully adjust the center wavelength at the bottom of the oxygen absorption band. Both filters and detectors are enclosed in a heating jacket maintained at a constant temperature of 35 °C \( \pm 0.1 \) which guarantees long-term stability. Higher temperatures can be used if the tilt of the filters in their holder is increased.

The gain of each channel (which includes the transmission of all the optical components and the responsiveness of the photodiode) was determined by measuring the emission of a spectrally calibrated black body (Li-Cor 1800-02, NE, USA). Also, the linearity of the photodiode response was checked by a comparison with the photon-counting instrument described in Goulas et al. (1990). Two identical RC filters, having a time constant of 100 ms, were placed in the output circuit of each photodiode to exactly match their response time constant within \( \pm 10^{-4} \). A laboratory-made program developed with HP BASIC (HP E2060, Hewlett Packard, Les Ulis, France) allowed for on-line control and display of measured signals. Two digital voltmeters (HP 34401, Hewlett Packard) were used to digitise the signals from the photodiodes with a resolution of six digits. The voltmeters were controlled by a laptop PC computer through a GPIB bus by means of a GPIB-PCMCI interface board (INES GPIB-PCM-NT+, Bourbaki, Tournon, France). In the absence of light, the noise was \( < 5 \) μV whereas up to 130 mV was measured in full sunlight for the lowest \((d)\) signal. However, there is a greater amount of noise in the presence of light. The statistics of the reference signals measured on a sunny day under stable solar illumination around noon yielded a signal-to-noise ratio S/N\(>1500\). The time interval was restricted to 100 s in order to minimize atmosphere transmission variations. Similar values were obtained at the leaf level since the reflectance is about 50%. In such conditions, the statistics of the retrieved fluorescence signal shows a S/N\(>50\). This is roughly in agreement with the estimated errors calculated according to Plascyk and Gabriel (1975).

The voltmeters, power supply and the laptop computer are placed in a watertight aluminium box and connected to

![Fig. 3. Top: Components of the Chl fluorescence detector for passive fluorescence measurements in the oxygen absorption band, at 760 nm. 1, reference; 2, target; 3, chopped mirror; 4, field stop; 5, high pass filter; 6, polarization cube; 7, view finder; 8, beam splitter; 9, interference filter (758.5 nm); 10 and 13, lenses; 11 and 14, photodiodes; 12, interference filter (760.5 nm). Bottom: Photograph of the instrument. The length is about 90 cm and the weight 3 kg.](image-url)
the optical head by a single multiwire watertight cable. The head is about 90 cm long and weighs 3 kg. The weight of the box including the computer and electronics is about 20 kg.

Kebabian et al. (1999) described another passive instrument intended for monitoring vegetation fluorescence within the atmospheric oxygen absorption bands. It makes use of an original method, based on oxygen luminescence. The system can measure alternatively at 687 nm (oxygen-B band) or at 760 nm (oxygen-A band) and does not need a reference. Discrimination against scattered sunlight is achieved by a design that utilizes the absorption spectrum of atmospheric oxygen in combination with absorption and fluorescence in a bulb filled with low-pressure oxygen. The bulb is contained in a high-reflectivity integrating sphere. Two precision, electronically synchronized mechanical choppers allow, alternatively, excitation of the bulb oxygen luminescence or analyses of the luminescence by a cooled red-sensitive photomultiplier tube operating in the photon counting mode. In spite of the impressive technical complexity of the Kebabian sensor, an integration time of 30 s is required to reach a S/N of 30–50 at 760 nm and of 2–10 at 687 nm (Kebabian et al., 1999). In another application of this instrument, Freedman et al. (2002) reported integration times up to 600 s. Contrary to the instrument presented here, this very slow response time precludes kinetic application of the Kebabian sensor.

2.3. The equatorial mirror mount system

For calibration purposes some experiments were performed indoors. The sunlight radiation was directed to the target by means of two mirrors. One mirror was fixed on an equatorial mount and oriented in order to reflect the light in a direction parallel to the terrestrial pole line. The diurnal movement of the earth was counterbalanced by the motion of the motorised equatorial mount (one rotation/24 h) in such a way that the reflected direction was maintained constant. The second mirror redirected the sunlight to the plant. The equatorial mirror mount system allowed an almost constant solar illumination indoors during a few tens of minutes, limited by the accuracy of the motor and of the mechanism.

Laboratory measurements under controlled light conditions during a sunny day (August 14th 1998) were performed using the equatorial mirror mount illuminator. The passive detector was directed towards a fully expanded leaf of a bean plant at a distance of one meter. A metal grid was used to temporally decrease the light intensity at the plant level by 10-fold. A quantum-meter and the reference panel were situated beside the target in the same light climate. Fluorescence flux variations were calculated according to the equations shown in Fig. 2. A relative fluorescence yield was computed by dividing the fluorescence flux by the PPFD.

2.4. Variability of the depth of the atmospheric oxygen absorption lines

The illumination of a horizontal target by solar radiation at ground level may be decomposed into three components: (i) direct radiation, (ii) diffuse radiation by the atmosphere and (iii) radiation diffused by the environment. The path length through the atmosphere is known as the air mass (m). By definition, m = 1 for the quantity of air seen along the zenith sight, which corresponds to the absorption of a uniform air layer of ~8.4 km at the sea level pressure. The depth and the shape of the oxygen absorption bands depend of the path length of solar radiation. The atmosphere path length is mainly determined by the sun zenith angle but is also affected by other effects including refraction, curvature of the atmosphere and variations of the air density with height, atmospheric pressure, etc. Fig. 4 shows the variation of the apparent depth of the 760 nm atmospheric oxygen absorption band with time during a sunny day in Avignon, France (45°56′N, 4°49′E) on October 4th 2001. The sun zenith angle was 48°24′ at midday. The a/b ratio has been calculated from the reference data of a regular experiment. Although direct radiation account for up to 85% of the total irradiance during a sunny day at noon, the diffuse radiation cannot be neglected. For example, up to 45% of the solar radiation is diffuse when the sky contains 4/8 of cumulus. In the case of completely cloudy sky, all solar radiation is diffuse. The decrease of light intensity induced by clouds is accompanied by an important increase of the oxygen absorption band depth as a result of the longer path length of diffuse radiation compared to the direct one (Fig. 5). To ensure valid measurement, attention should be paid to provide identical illumination to the target and the reference. In addition to the spatial constraints, there are also temporal constraints, because the depth of the oxygen absorption bands depend of the path length of solar radiation.

![Fig. 4. Variation of the apparent depth of the 760 nm atmospheric oxygen absorption band with time during a sunny day at Avignon, France (45°56′N, 4°49′E) on October 4th 2001. The sun zenith angle was 48°24′ at midday. The measurement was done with the passive instrument by considering only the data of the reference panel.](image-url)
absorption band may change suddenly due to light intensity variations (clouds, see Fig. 5). Therefore the luminance signals \( a, b, c \) and \( d \) mentioned in Fig. 2 should be acquired simultaneously.

2.5. Plant material and treatments

Three types of green leaves from higher plants were used in this study:

(i) For measurements at the leaf level, we used a single bean leaf attached to the plant. Bean plants (Phaseolus vulgaris L., cv. Carioca) were grown for 2 weeks in a growth chamber under a photosynthetic photon flux density (PPFD) of 350 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) with a 16-h photoperiod. The temperature was 20 and 16 °C, during the day and night periods, respectively (80% relative humidity). Fluorescence measurements were performed on fully expanded leaves attached to the plant. The concentration of Chl per unit leaf area was estimated using a SPAD 502 (Minolta Japan). Values between 35 and 45 \( \mu \text{g cm}^{-2} \) of Chl were usually observed. The passive fluorescence measurements were compared to the measurements using the FIPAM active fluorescence instrument.

(ii) The first tests of our passive instrument at the canopy level were done outdoors in Avignon (France). A natural grassland field containing approximately 80% clover and 20% rye grass of 4–8 cm height was chosen. A scaffolding of about 4 m height was placed in the middle of the field in order to avoid any shading produced by the surrounding. The passive instrument was fixed at the top of the scaffolding and oriented to the north. The green part of the target was estimated to be 80%, as ground and yellow leaves were also present. The soil was slightly moist.

(iii) Maize was chosen as a representative of crops, with the aim to apply controlled stress conditions. Maize (Zea mays L.) was grown in pots, two plants per pot in 30 l of soil, under outdoor conditions, but watered daily during the summer 2001 in Avignon (France). Growing in pots was chosen to allow controlled additions of herbicides. Twelve pots were put together to form a small canopy with 24 plants. Plants reached 1.5 m height at the beginning of the experiment. A Li-6400 (Li-Cor) portable photosynthesis analyser was used to monitor CO2 assimilation \( (A) \) and stomatal conductance \( (g) \) at a constant PPFD of 1600 \( \mu \text{mol photons m}^{-2} \text{s}^{-1} \). Gas exchange measurements were made every day around midday (1300–1400 local time) on several representative leaves. At the same time, the stationary Chl fluorescence yield was measured with a portable fluorometer (PAM2000, Walz, Effeltrich, Germany), and the Chl concentration estimated using a SPAD 502 (Minolta, Ramsey, NJ) on the same leaves.

2.6. The use of the FIPAM fluorometer for the comparison of active and passive measurements of kinetic transients at the leaf level

The FIPAM is a PAM-like fluorometer measuring the pulse-modulated Chl fluorescence in plants, from a distance of up to 4 m (Apostol et al., 2001; Flexas et al., 2000). The excitation beam was produced by a pulsed laser diode (635 nm, 2 μs pulse width, 15 mW, Philips, Issy-les-Moulineaux, France). The frequency of modulation can be varied from 0.5 to 120 kHz. The signal was processed by specially designed electronics, which make the detector insensitive to continuous illumination. Chl fluorescence was detected by a photodiode after it passed through a wide-band interference filter transmitting from 670 to 750 nm (710DF80, Omega optical, Brattleboro, VT, USA) in addition to a long-pass 665 nm filter (RG 665, 3 mm, Schott, Clichy, France). The maximum Chl fluorescence level (Fm) is obtained by increasing the frequency of repetition up to the 120 kHz Chl fluorescence saturation level. This scenario has not been performed in the present work.

Fig. 6 shows the setup for concomitant measurement of a fluorescence induction curve with the passive instrument and the FIPAM fluorometer. To avoid interferences or shading between the two instruments, the distance was set to 1 m. A sunny, cloud-free day was chosen. A bean leaf attached to the plant was maintained in the shade for several minutes behind the reference plate used by the passive detector (Fig. 6). In addition to the two voltmeters used for passive measurements, a third digital voltmeter (HP 34401) was used to record the active fluorescence signal. The software program triggered the three voltmeters simultaneously. After several minutes of dark adaptation during which the reference signal was continuously acquired, the reference plate was rapidly removed, and the fluorescence variation produced by the sudden illumination was recorded. The time resolution of both instruments was set to 100 ms.
2.7. The scaffolding and experiments at the canopy level

For continuous measurements under outdoors conditions the passive detector was fixed on top of a 4.25-m-high scaffolding, and oriented approximately due north. This orientation was chosen in order to maximise the luminance signals from the target. The angle of the viewing direction with the horizontal was set to about 38°, in order to avoid the shade of the instrument on the target during spring and summer in Orsay, France (48°42′ N, 2°10′ E). The actual distance of measurement was 6.9 m and the resulting target size on ground was 0.48 × 0.78 m.

For the DCMU experiment, the passive fluorescence detector was recording continuously from the top of the scaffolding over several days. DCMU is a widely used inhibitor of the photosynthetic electron flow acting at the quinone binding site of PSII. After infiltration by DCMU, Q₂ accumulates even under low light and Chl fluorescence increased up to a level close to Fm but slightly lower due to the remaining quenching by oxidised plastoquinones (Vernotte et al., 1979). DCMU was applied at a saturating concentration 2 days after the onset of the experiment.

3. Results and discussion

3.1. Measurements at the leaf level

3.1.1. Preliminary measurements

Fig. 7A shows the variations of the a and b parameters and the depth (a/b) of the atmospheric absorption band determined on the reference panel during a time period of 20 min starting at 10:30 local time. While the maximum radiation stays almost constant, we observe an overall monotonous decrease in the depth of the oxygen band, which is obviously due to the decrease of the air mass as a result of the increase of the solar zenith angle at this moment in the day. Superimposed to this variation, we observe a small but reproducible increase of the depth when the solar flux is reduced by the grid situated between the sun and the target. This effect is the consequence of a modification of the diffuse/direct radiation ratio, which favours diffuse radiation when the grid is present. Fig. 7B shows similar effects are also present in the signals from the target (c and d) under the same light climate. In addition, we observe induction kinetics of small amplitude on the d signal as a result of the transition from low to high light. This induction is also present on the c signal although less pronounced. These transients are due to the contribution of the fluorescence emission, which is superimposed to the reflectance signal. From the amplitude of these transients, it can be directly deduced that the fluorescence emission at 760 nm accounts for 1–2% of the luminance signal of the target outside the oxygen absorption band and 6–8% of the luminance signal at the bottom of the absorption band. The effect of the fluorescence contribution is even more evident on the depth of the band (c/d ratio), which exhibits a negative peak when the light increases or a positive peak when the light decreases. Fig. 7C shows the relative fluorescence yield computed according to the relations shown in Fig. 2. The time resolution of the experiment was 1 s. During the high light period, we can observe an initial increase of the fluorescence yield, followed by a decrease probably as a consequence of the development of a strong NPQ mechanism (Flexas et al., 2000). The rapid transition to low light conditions induces an initial decrease
of the fluorescence yield followed within a few tens of seconds by an increase, due to the relaxation of the NPQ under low light, to finally attain a steady state, which is slightly lower than the stationary level attained in high light. This generates a conspicuous undershot at the onset of the low light period. The opposite behaviour is observed at the beginning of the high light period. Note the good signal to noise ratio, which is obtained without the use of a smoothing algorithm.

3.1.2. Fluorescence during a dark-to-light transition

One of the most striking features of Chl fluorescence in vivo, known for more than 70 years, is its variation following a low to high light transition (Kautsky & Hirsch, 1931; Krause & Weis, 1991). The extent of this variation has been used extensively to derive information on the functioning of the photosynthetic apparatus (Genty et al., 1989; Govindjee, 1995; Renger & Schreiber, 1986). Recently, we developed a new laser-based fluorometer (FIPAM) to accurately and non-invasively measure variable Chl fluorescence from leaves from a distance. Using the FIPAM, we aimed to compare the fluorescence variations induced by changes in PPFD with a signal-to-noise ratio equivalent or even better than the active instrument. This is remarkable considering that, depending on the Chl concentration and on the leaf structure, the fluorescence flux at 760 nm represented only 50–60% of the FRF fluorescence peak. Moreover, since PSI fluorescence yield is known to be independent of light variations (Moise and Moya, submitted for publication), the similarity of light-induced changes in both signals (active and passive at 760 nm) strongly suggests that the contribution of PSI fluorescence is similar in both conditions, although it cannot be ignored (Agati et al., 2000).

We repeated the measurements at the leaf level using two FIPAM fluorometers, one equipped with a Schott RG9, >700 filter and another one with an interference filter centred at 760 nm (Omega 760 NB 7, Δλ = 7 nm). We obtained the following values, averaged over 21 independent measurements: (Fv/Fm)λ>700 = 0.779 ± 0.021 and (Fv/Fm)λ<700 = 0.809 ± 0.024. It is concluded that the difference is within the experimental error.

In contrast to suggestions by Freedman et al. (2002), the present results support that fluorescence detected at 760 nm may be used to monitor the PSII electron flow.

3.1.3. Response of a bean leaf to different light regimes

Once we have established that passive fluorescence measurement reflects the same variations as typical active fluorescence measurements, we decided to confirm its accuracy by analysing the well-known dependence of stationary fluorescence on light regime (Cerovic et al., 1996; Flexas et al., 2000). For this purpose, we measured Chl fluorescence of a single bean leaf attached to the plant continuously over several hours. Due to cloud movement, solar radiation often changes between direct and diffuse, generating important PAR variations (200–1900 μmol photon m⁻² s⁻¹). Fig. 9A shows a typical recording after exposure to moderate light. The relative stationary fluorescence yield (Fs) is obtained by dividing f by a. The latter can
be considered as a measurement of PPFD. It is worth noting that Fs is positively correlated with PPFD in this range of light intensity. This tight correlation means that photochemical quenching (QP) is the dominant mechanism that determines the actual fluorescence level: an increase in the light intensity modifies the equilibrium of the electron transport chain in the direction of reduction, which is accompanied by an increase of Chl fluorescence. After several hours of high light exposure, Fs becomes negatively correlated with PPFD (Fig. 9B). Antiparallel correlation between Fs and PPFD has been already reported by Cerovic et al. (1996) and Flexas et al. (2000). It is interpreted as an accumulation of non-photochemical quenching (NPQ), under high irradiance. NPQ relaxes when the light decreases. The threshold at which the antiparallel variations are observed is dependent of the type of plant, light acclimation and stomata aperture (Cerovic et al., 1996).

3.2. Measurements at the canopy level

3.2.1. Fluorescence of natural grassland

After fluorescence measurements at the leaf level the next step was to test the instrument at the canopy level, under outdoors conditions. For this test we used the scaffolding and natural grassland described in Materials and methods. The reference was a non-fluorescent white PVC board of 1 × 1 m. To avoid the effects of specular reflectance, the surface of the board was pumiced with fine sandpaper. The reflectance coefficient of the PVC board was calibrated against the Spectralon reference. A cloudy day was chosen for the presence of very sudden light transitions. Fig. 10 shows the fluorescence flux variations over 1 h. It is obvious that fluorescence flux increases much more than PPFD during light transitions, and then rapidly decreases while the PPFD stays almost constant. This behaviour is very similar to what has been observed in Fig. 7 at the leaf level. The variations of the relative fluorescence yield (Fig. 10C) are very similar to what has been shown in Fig. 7C (f/PPFD). One can observe, beginning with periods of constant illumination, a rapid increase of the Chl fluorescence yield followed by a decrease to a constant level. So, it is concluded that the fluorescence flux signal was detectable with high sensitivity in spite of heterogeneity of the target.

However, while the a signal can be used as an estimation of PPFD at the leaf level, at the canopy level the f/a “relative yield” does not show the same variations as the f/PPFD. The f/c ratio has the same response as f/a (not shown). This fact poses a question regarding the reliability of fluorescence yield estimations at the canopy level. In contrast with active fluorescence methods, in which the excitation energy can be known, the exact excitation of sunlight-induced fluorescence depends of the radiation intercepted by the target, which is difficult to estimate. At the leaf level, the problem can be solved using a quantum-meter or using the light reflected by the reference panel (a). In most cases, also the leaf radiance (c) may be an acceptable estimation of the incoming light. However, this
is not the case when measuring at the canopy level. Both the PPFD and the reflectance of the reference panel are measured from surfaces, which may not reflect the heterogeneity of a tridimensional canopy structure, thus leading to only rough estimates of the light absorbed by the latter. To define a fluorescence yield, this problem should be properly addressed in the future.

3.2.2. Detection of the effect of a herbicide on a maize canopy

Changes of Chl fluorescence induced by natural stress conditions may occur after a long time. Aiming to check whether our passive fluorescence instrument was applicable for plant stress detection, we designed an experiment based on a small canopy of maize plants grown in pots and measurements were taken simultaneously with active measurements in fully developed, top leaves of the canopy, using a PAM-2000 instrument. Fig. 11A shows that net CO2 assimilation (A) and stomatal conductance (g) maintained high values during the first 4 days. Two days after DCMU application both A and g started to decrease steadily. Five days after DCMU application A and g became close to zero. Fig. 11B shows the evolution of both passive and active Chl fluorescence parameters. Comparing Fig 11A and B, one can observe an antiparallel behaviour consisting of a low fluorescence yield during 4 days and a steady increase to a value two to three times higher at the end of the measurement. Note the similar variation of both passive and active Chl fluorescence measurements. It is concluded that the information automatically obtained by our passive Chl fluorescence instrument at the level of the canopy and several metres above the canopy is similar to that obtained by multiple sampling at contact by the PAM-2000 fluorometer.

4. Concluding remarks and prospects

The present work shows a new method for passive Chl fluorescence remote sensing that opens new possibilities of application in ecophysiology, agriculture and forestry. The accuracy and simplicity of the method makes tower-based Chl fluorescence measurement a valuable tool for continuous monitoring over extended periods of time. New insight on the plant status and on stress detection at the canopy level would be gained. Further development of this instrument should be performed in the following areas:

(1) Improving the scale of measurement. Up to now, the passive instrument has been used at distances of up to 50 m. Based on the same methodology, it would be possible to design a new instrument for measuring from a low altitude aircraft. This would increase both the distance of measurement and the target size, thus allowing for a landscape or regional perspective. In order to obtain a target size of about 35 m at ground, which is a reasonable size for agricultural applications, the field of view should be reduced to about 2° (half angle of 1°). As a result, the detected radiance flux will be reduced by fourfold. It would be possible, after some engineering effort, to compensate this effect in several ways: (i) increasing the entrance pupil from 21 mm (filter size of 25 mm of diameter) to 46 mm (filter size of 50 mm of diameter) would increase the flux by 4.8-fold; (ii) using a small telescope to match the field of view (half angle of 1°) to the maximum acceptance angle of the interference filters, which is about 3°, a gain of 9-fold is expected; (iii) in addition, the 1 s integration time of the instrument, which is mainly due to the commutation of the flip-flop mirror, can be reduced to the electronic time constant of 100 ms. Assuming a speed of 50 m/s this would represent a target blur of 5 m. A rough estimation using the MODTRAN 4 atmospheric transmission program predicts that measuring from an aircraft at an altitude of 1 km looking in the Nadir direction would result in a signal attenuation of only about 40% in the absorption band and 8% out of the absorption band, which would not hinder the possibility of making measurements. In conclusion, the improvements mentioned above largely compensates the reduction of field of view and a low altitude aircraft application of the method seems feasible.

In a second step, it would be useful to consider the possible application of the method to satellite observations once an accurate atmospheric oxygen absorption model was developed. Two aspects of this model are
important: (i) under clear atmospheric conditions it should predict the actual depth of the oxygen band versus time and spatial localisation; (ii) it should predict the attenuation factor versus the air mass between the detector and the target. As the molecular mechanisms of oxygen absorption are well known, this seems feasible. A simulation based on the actual bandwidth of our filters and on the maximum altitude allowed by MODTRAN 4 (100 km) indicates that upwelling radiance at 760 nm would be 20% within the oxygen-absorbing band and by 75% out of the band. Since most of the absorption of the atmosphere takes place in the first 100 km, higher altitudes would not modify the radiance fluxes. Although this attenuation is important, the radiance fluxes compare favorably with those expected by measuring in the H α band at 656.3 nm (Plascyk, 1975) or in other narrower bands at 685 or 739 nm (Theisen, 2002). Work towards this goal is in progress.

(2) Estimation of absorbed photosynthetic active radiation. Under favourable conditions (non-stressed plants), the variations in $\phi_s$ are small (Flexas et al., 2000) and can be estimated. If $\phi_s$ is known, Chl fluorescence flux contains direct information about absorbed photosynthetically active radiation. This parameter could become a quantitative measure of absorbed PAR, if measured at a wavelength not influenced by re-absorption, which is the case for the oxygen-A band. Chl fluorescence emission is generated after light absorption, within the photosynthetic apparatus, as a deactivation pathway directly in competition with the photochemical conversion. In addition, Chl fluorescence is an isotropic emission. It follows that Chl fluorescence should be a better estimation of absorbed PPFD than reflectance, which refers to light simply reflected by the leaf surface and subjected to directional effects. To our knowledge, Chl fluorescence has not yet been used towards this goal.

(3) Measurement of vegetation reflectance indices. The first prototype presented here opens the way to the monitoring of other biophysical parameters related to the photosynthetic activity of plant canopies. An example is given in the companion paper concerning the PRI index, which can be monitored with the same instrument by simply changing the interference filters. Furthermore, we aim to build a new detector integrating the measurement of the fluorescence and the reflectance at 760 nm together with the fluorescence and the reflectance at 687 nm (using the atmospheric oxygen absorption band at 687 nm) and the PRI index. A very complete signature of the vegetation would be obtained this way.

**Abbreviations and symbols**

$a$ border of the reference signal  
$b$ bottom of the reference signal  
$c$ border of the target signal  
$d$ bottom of the target signal  
Chl chlorophyll  
DCMU (3,4-dichlorophenyl)-1,1-dimethylurea  
f fluorescence flux  
FIPAM frequency-induced pulse amplitude modulation  
FLD Fraunhofer line discriminator  
FLIDAR fluorescence LIDAR  
FRF far-red fluorescence  
Fs stationary Chl fluorescence flux  
LIDAR light detection and ranging  
m airmass  
NPQ non-photochemical quenching  
PAM pulse amplitude modulation  
PPFD photosynthetic photon flux density  
PSI photosystem I  
PSII photosystem II  
QA primary quinone acceptor of PSII  
QP primary quinone acceptor of PSI  
RF red fluorescence  
$\phi_s$ stationary Chl fluorescence yield  
$\phi_f$ relative fluorescence yield  
$\phi_s$ stationary Chl fluorescence yield

**Acknowledgements**

We wish to acknowledge the support of the CNRS through the GDR 1536 “FLUOVEG”. LURE projects BF 026-98 and BF 024-99 are also acknowledged. S. Evain was granted a PhD fellowship by CNES, and J. Flexas was granted a Beca d’Investigació from UIB. Max Henkle is gratefully acknowledged for his helpful comments and grammar corrections.

**References**


