# Remote Sensing of Time-Resolved Chlorophyll Fluorescence and Back-Scattering of the Laser Excitation by Vegetation

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## ABSTRACT

Chlorophyll fluorescence is widely used to monitor the physiological state of plants. Their photosynthetic activity greatly depends on the presence of environmental constraints such as water or nutrients availability. Under laboratory conditions, the photosynthetic activity can be assessed by measuring the quantum yield (f) of chlorophyll fluorescence. We present a new method to access the parameter f under outdoor conditions, by measuring the mean fluorescence lifetime (t). We also present a prototype of a fluorescence LIDAR with sub-nanosecond time resolution, together with the specific mathematical algorithms for retrieving the lifetime parameter in the case of complex targets. With this tool the monitoring of vegetation can be done at large spatial scale by a remote sensor for photosynthesis.

## INTRODUCTION

The biosphere, whether continental or oceanic, plays a major role in the change of our planet's climate. Continental vegetation, in particular, is one of the main components of the hydrological cycle that regulates water and carbon dioxide fluxes between surface and atmosphere. To discriminate and identify spectral signatures associated with the response of vegetation to various stresses, it becomes necessary an extended spectral coverage, together with higher spectral resolution. All these necessities are covered by a new generation of optical sensors, the so-called imaging spectrometers.

Besides these new sensors, a new technique for remote sensing of the physiological state of plants focuses the interest for large scale monitoring, the detection of laser-induced chlorophyll fluorescence. The aim of this paper is to describe a new method to determine the fluorescence quantum yield, based on the measurement of the chlorophyll fluorescence lifetime. Since the chlorophyll fluorescence is only unique for green vegetation, it can be considered complementary to the existing techniques. Besides this complementarity, the direct link of the fluorescence to the process of photosynthesis can be regarded as a major advantage.

## I. ORIGIN OF FLUORESCENCE

During the photosynthetic process, light energy is absorbed by large arrays of light-harvesting chlorophyll-protein complexes and transferred to the reaction centers of photosystem I (PSI) and photosystem II (PSII), where charge separation and stabilization take place. During the energy transfer the excited state may undergo a fluorescence emission in the red region (from 660 nm to 800 nm) due to chlorophyll a. In a full functioning photosynthetic system the major part of the absorbed light energy is used for photosynthesis. The remainder is lost as heat or re-emitted as fluorescence [1].

The quantum yield of the chlorophyll fluorescence shows an inverse dependency on the capability of the photosynthetic apparatus to photochemically convert the absorbed light energy [2]. Owing to this inverse relationship, the measurement of the chlorophyll fluorescence can be used to describe the physiological state of vegetation. However, the study of fluorescence in intact leaves at room temperature has shown that photosynthesis, together with fluorescence yield, can be also lowered by processes not directly related to the efficiency of photochemistry. These mechanisms mainly due to excess of light, denoted as non-photochemical quenching, are of general occurrence in outdoor conditions [3]. They must be understood as regulatory or protective

deactivation pathways, developed by plants to prevent gross destruction of the photosynthetic apparatus.

Over the past decades the study of several parameters of the chlorophyll fluorescence emission has become a rapid, sensitive and non-destructive laboratory method to investigate numerous aspects of the photosynthetic function [4, 5]. When the possibility of airborne detection of laser-induced fluorescence emitted from trees, bushes and grasses has been demonstrated [6, 7], the application of one of these fluorescence techniques in remote sensing is under discussion [8]. In addition to chlorophyll fluorescence, recent developments take into account the additional emission bands at 440 nm and 520 nm, which appear upon excitation with UV lasers [9]. Within the frame of the EUREKA project LASFLEUR (EU 380), several institutes and companies are investigating the use of different fluorescence parameters for the remote detection of vegetation stress [10].

However the intensity of the fluorescence signal depends on several factors including ambient light, chlorophyll content, fluorescence re-absorption and fluorescence quantum yield. The latter parameter is essential to define the physiological state of the plant, as it can be related to photochemistry and carbon assimilation [11].

## II. CHLOROPHYLL FLUORESCENCE LIFETIME IN VIVO

It is well documented from a large number of reports that chlorophyll fluorescence *in vivo* is a heterogeneous emission [12-19], containing at least 3 lifetime components, the origin of which is still a matter of discussion. However, it has also been shown in previous works [14, 20, 22] that the mean chlorophyll fluorescence lifetime stays almost proportional to the fluorescence quantum yield in most of experimental conditions in which the fluorescence quantum yield is affected (Figure 1). Thus, this relationship is observed by decreasing the efficiency of the photochemistry upon reaction center's closure (Figure 1A) or in presence of increasing levels of non-photochemical quenching (Figure 1B). Therefore, from lifetime measurements, a direct estimation of the quantum yield could be obtained through the relation:

$$\Phi = \tau/\tau_0,$$

where $\Phi$ is the quantum yield, $\tau$ is the mean lifetime and $\tau_0$ is the lifetime of fluorescence in the absence of any other deactivation process ($\tau_0 \approx$ 15-18 ns). Laboratory chlorophyll fluorescence lifetime measurements on healthy leaves showed that $\tau$ ranges from 0.3 to 0.5 ns under moderate daylight conditions, when fluorescence has reached a stationary level ($F_s$). However, during a saturating light pulse ($F_m$) $\tau$ increases up to 2 ns [14, 15]. Several methods are known to measure fluorescence lifetimes, among them phase fluorometry [21], time-correlated single-photon-counting (TCSPC) and direct decay measurements after a picosecond excitation. Fluorescence measurements under daylight conditions require to maximize the ratio of the fluorescence emission to the ambient light. This can be done by using a pulsed laser source in the sub-nanosecond time domain [22]. Since t is determined from the fluorescence decay within a few nanoseconds, this method is fast and well adapted to outdoor measurements under daylight conditions. Thanks to recent improvements on laser sources and detectors, lifetime measurements in the sub-nanosecond time range are now routine investigation.

In addition, the lifetime parameter has also the advantage to be hardly affected by re-absorption. This phenomenon is due to the overlap between the chlorophyll fluorescence

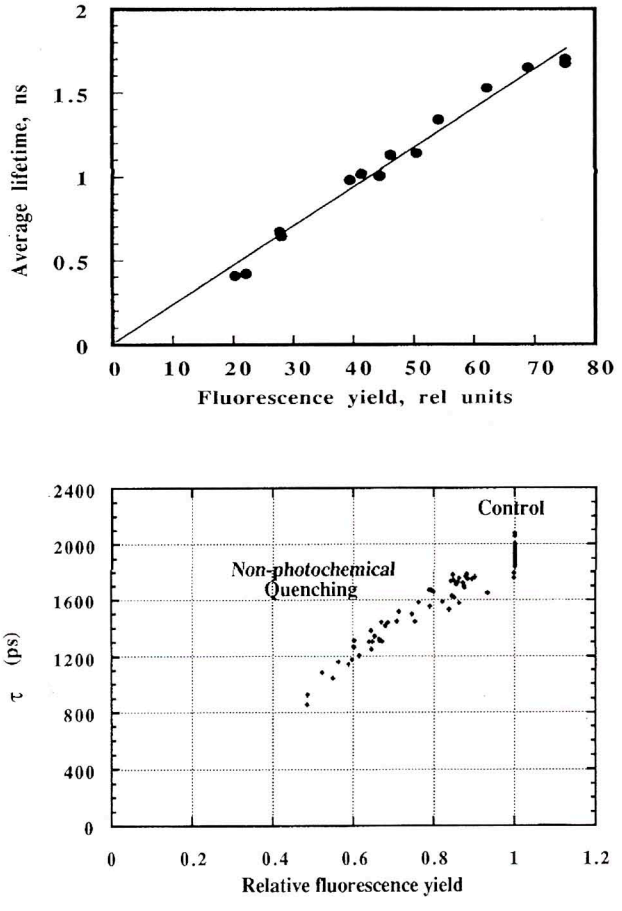


*Fig. 1* - **Top:** *Raltionship between mean fluorescence lifetime and fluorescence yield of Chl_a in vivo upon reaction center's closure by light (After Moya et al., [14]).*

**Bottom:** *Relationship between mean fluorescence of Chl_a and fluorescence yield during non-photochemical quenching in an attached leaf of barley. The fluorescence yield is changed by varying the pre-illumination (After Y. Goulas [22]).*

emission and absorption spectra for wavelengths Ø nm. Chlorophyll concentration of green leaves ranges from 30 to 70 nmol/cm$^2$ of leaf area, depending on species and age. This concentration is synthesised by plants to maximize light absorption. However, such a concentration is too high for correct spectroscopic measurements. In fact, fluorescence re-absorption mostly determines the actual form of the emission spectrum and strongly decreases the apparent fluorescence yield of green leaves. Interestingly, calculations of the effect of re-absorption on t predict a negligible lengthening. This has been confirmed by a direct comparison between leaves and a suspension of diluted isolated chloroplasts [15, 18]. As a consequence, the lifetime parameter appears to be the most significant for monitoring the fluorescence properties of intact leaves.

### Early detection of water stress by lifetime measurements

The sensitivity of the lifetime parameter to water stress has been studied during the first joint Lasfleur campaign of measurements held at the JRC, Ispra, Italy in October 1990. Two weeks old maize plants were submitted to water stress by withholding water from the soil for 3, 4 and 5 days. The loss of water was 20% after 5 days but no change in chlorophyll content was observed. Lifetime measurements were performed by the TCSPC technique using a laser diode for excitation ($\lambda$ = 670 nm). Fluorescence was detected at $\lambda$ = 695 nm [23]. The measurements were performed on attached leaves after 4 min of pre-illumination at 150 µE/m$^2$/s on pre-darkened samples. This treatment induces a level of chlorophyll fluorescence intermediate between $F_m$ and $F_s$ (not shown). From Figures 2 A and 2B it can be seen that the mean lifetime decreases by a factor of 2, after 4 days of water stress. Figure 2 C shows the effect of infiltration by DCMU $10^{-4}$ M on a control maize leaf. As a consequence of the inhibition of photosynthetic electron transport, the mean lifetime increases to 2.2 ns ($F_m$). Parallel measurements using a pulsed fluorometer (PAM fluorometer, H. Walz, Effeltrich, FRG) showed that the decrease of $\tau$ can be accounted for by an increase in non-photochemical quenching (not shown). Similar experiments with wheat plants show no effect on $\tau$. Thus, the response to water stress seems to be specific of $C_4$ plants. It is concluded from this work that chlorophyll fluorescence lifetime measurements appear to be a promising tool for early detection of water stress effects on $C_4$ plants.

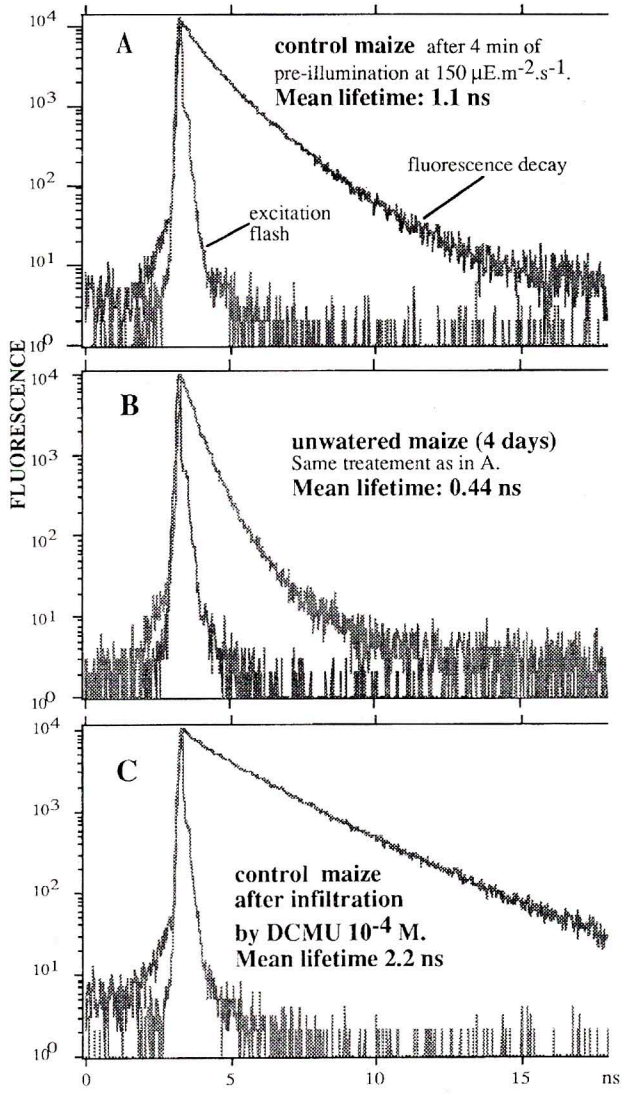


*Fig. 2 - Effect of water stress on maize. Picosecond fluorescence decay were acquired after 4 min of pre-illumination (see text). A: control plant, B: similar plant measured with the same protocol after identical pre-treatment and C: similar plant measured after DCMU infiltration.*

## III. DESCRIPTION OF A LIDAR SYSTEM FOR REMOTE TIME-RESOLVED FLUORESCENCE DECAY MEASUREMENTS

Eco-physiological applications of fluorescence lifetime measurements must satisfy specific requirements to be useful for monitoring the state of the plant. The distance from the detector to the target together with the presence of a daylight background are the major difficulties to overcome. Laboratory techniques for measuring sub-nanosecond fluorescence lifetimes can be hardly extrapolated to field conditions. Remote fluorescence lifetime measurements are better done by recording both fluorescence decay and elastic back-scattering signals from the same laser shot (or from

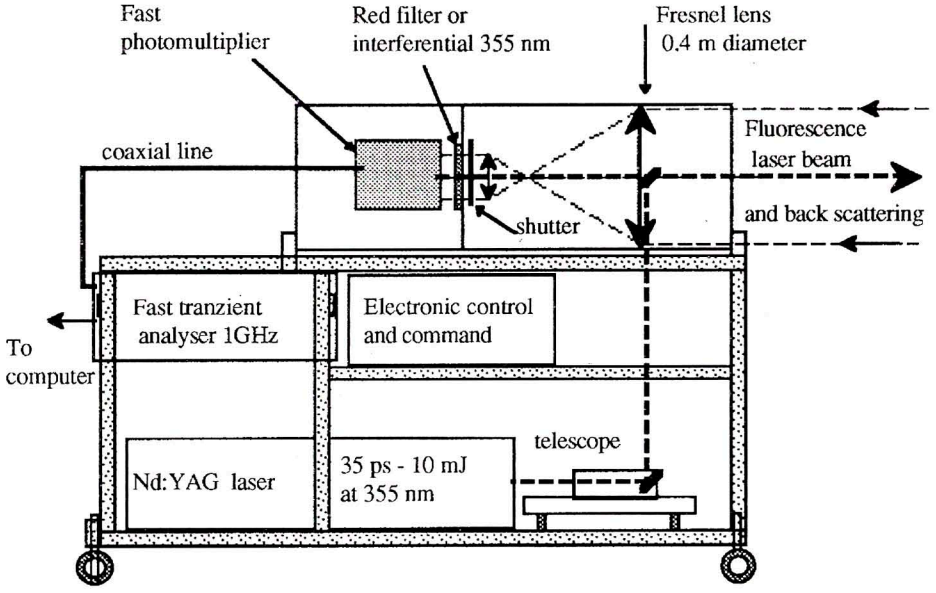


*Fig. 3 - Mobile picosecond LIDAR system of the LURE laboratory for vegetation monitoring via chlorophyll fluorescence.*

two consecutive shots). The light source must satisfy several requirements, including directivity, pulse duration d ps as required for sub-nanosecond lifetime measurements, wavelength matching to the absorption of photosynthetic pigments, eye safety and energy [8]. An excitation wavelength near (but below) 400 nm is the best compromise between eye-safety requirements and the efficiency of chlorophyll excitation. an additional limitation appears when using short light pulses. Non-linear effects (singlet-singlet annihilation) occur when the energy density exceeds 2 x $10^{13}$ photons/pulse/cm$^2$ (i.e., 10μJ/cm$^2$/pulse at 355 nm) [24]. A telescope with a large aperture is necessary to collect efficiently both fluorescence and back-scattering responses. We describe in the following a new picosecond LIDAR system developed by LURE for remote fluorescence lifetime measurements of plant canopies [25].

Figure 3 shows a scheme of the system. The excitation unit consists in a flash-pumped frequency-tripled mode-locked Nd-Yag laser (Quanta Systems, Milan). The repetition rate was set to 10 Hz, the pulse duration was approximately 50 ps (FWHM) and the energy up to 10 mJ per pulse. This laser was kindly lent to us from the IRSA laboratory of the JRC (Ispra, Italy). Depending on the target distance, the laser beam was expanded by two lenses in order to illuminate a suitable area. It was, for instance, approximately 40 cm$^2$ for a target distance of 4.5 m. This area is the result of a compromise: it must be imaged on the photocathode of the detector and large enough to maintain the energy density below the threshold level for non-linear effects. The collecting optics consist in a Fresnel lens of 380 mm diameter with a focal length of 400 mm. This solution is cheap and efficient. The optical system focuses the fluorescence into the detector through a set of filters. The fluorescence ($670 < \lambda < 750$ nm) and the back-scattering (interference filter at 355 nm) signals are measured alternatively. The laser beam is made coaxial with the optical axis of the fresnel lens by two mirrors. The detector consists of a Sylvania 502 crossed-field photomultiplier with a rise and fall time of <150 ps. It provides a signal of a few hundreds mV, when loaded with 50 Ohms. This signal is directly fed to a Tektronix SCD 1000 transient digitizer (which has an analog bandwidth of 1 GHz and an encoding resolution of 11 bits for 100 mV full scale) and further transferred to an HP 9816 computer through an IEEE 488 interface. The whole system has a bandwidth of 1 GHz. The detector is protected from the ambient light by a mechanical shutter that opens during a time window of approximately 3 ms synchronized with the laser shot.

## IV. EXPERIMENTAL RESULTS

To test the ability of our LIDAR system to detect changes in the fluorescence quantum yield, experiments have been carried out investigating the effect of iron deficiency on this photosynthetic parameter.

Figure 4 shows the fluorescence decay of a single sugar beet leaf as a function of time after excitation with a single-shot laser pulse. It also shows the instrumental response function recorded by looking at the back-reflected light, at the same wavelength as the excitation pulse. Experimental conditions were designed to simulate a detection from 50 m by reducing the field of view of the optics (actual distance of detection 4.5 m). One can see that the fluorescence signal is delayed

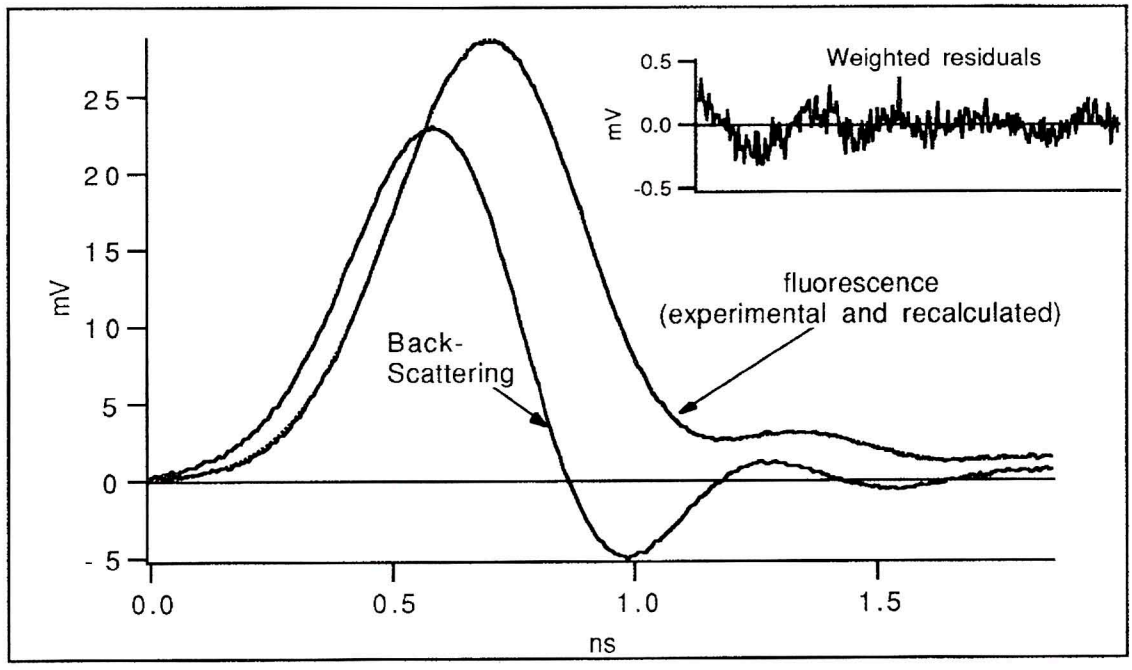


*Fig. 4 - Chlorophyll fluorescence and back-scattered excitation signal from a sugar beet leaf, measured with the LIDAR system of LURE, after a single laser pulse. The experimental decay Fex(t) has been modelized by:*

$F(t) = A_1 \exp\left(-\frac{t}{\tau_1}\right) + A_2 \exp\left(-\frac{t}{\tau_2}\right)$. *Mean lifetime:* $\tau = 0.35$ *ns.*

$A_1 = 85$, $A_2 = 25$, $\tau_1 = 0.17$ *ns and* $\tau_2 = 0.6$ *ns. With these parameters, the recalculated fluorescence decay is almost identical to the experimental decay, as it can be seen by the weighted residuals (see insert).*

compared to the back-scattered signal. The fluorescence lifetime can be computed if we consider that the measured fluorescence decay Fex(t) is the convolution product of the instrumental function Dex(t) with the actual fluorescence decay F(t):

$$Fcal(t) \otimes Dex(t)$$

F(t) is modeled as a sum of m exponential components

$$F(t) = \sum_{j=1}^{m} A_j \exp\left(-\frac{t}{\tau_j}\right)$$

Deconvolution of the former expression, or the determination of parameters $A_j$ and $\tau_j$, is performed with a least squares method using the Marquardt search algorithm for non-linear parameters [12, 24]. The insert of Figure 4 shows the weighted difference:

$$\frac{Fex(t) - Fcal(t)}{\sqrt{Fex(t)}},$$

between the experimental decay Fex(t) and the re-calculated decay Fcal(t). With the parameters indicated, the weighted residual function is near zero. The mean lifetime of fluorescence can be computed by:

$$\tau = \frac{\Sigma A_j \tau_j^2}{\Sigma A_j \tau_j} = 0.35\text{ns}$$

**Effect of iron deficiency on fluorescence lifetime**

Iron deficiency mainly affects the photosynthetic electron transport system in leaves, due to the location of iron in the three main photosynthetic complexes (PSII, cyt $b_6f$, and PSI). Iron-deficient leaves have a relative increase in carotenoids (mostly in lutein and violaxanthin cycle pigments), together with a major depletion in chlorophyll [28]. As a consequence of these changes, iron-deficient leaves show an impaired PSII quantum yield [28, 29].

Figure 5 shows the increase of the mean fluorescence lifetime as long as iron deficiency, and its concomitant depletion in chlorophyll, develops. Measurements have been performed at steady-state photosynthesis ($F_s$) after 15 min of actinic illumination at 270 $\mu E/m^2/s$, that corresponds to natural conditions under moderate light. Control leaves showed a mean fluorescence lifetime of approximately 0.4 ns compared to iron-stressed leaves which showed a fluorescence lifetime of approximately 1.0 ns (Figure 5). In contrast to these data, Figure 2 shows that water stress in

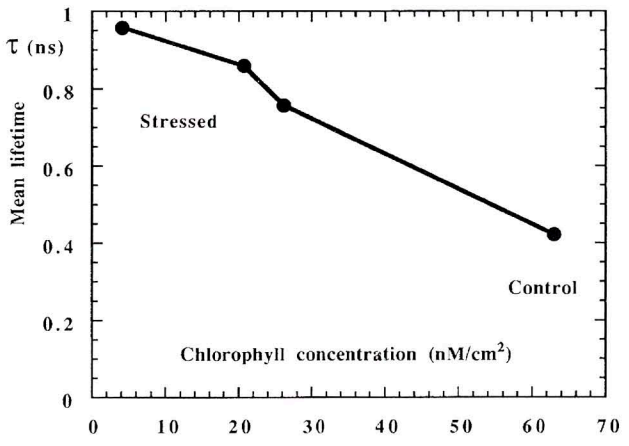


*Fig. 5 - Variation of the mean lifetime of fluorescence of sugar beet leaves in iron-deficient crops. The measurements have been performed at steady-state photosynthesis after 15 min of actinic illumination at 270 μ $E.m^{-2}.s^{-1}$, which corresponds to natural conditions under moderate light.*

maize leaves decreased the mean fluorescence lifetime from 1.1 ns to 0.44 ns (measured after 4 min of actinic illumination at 150 $\mu E/m^2/s$). From these data it can be concluded that submitting the leaves to a different combination of stress and pre-illumination conditions may lead to changes in the mean fluorescence lifetime which go in opposite directions. Concerning iron deficiency, the increase in the lifetime of the chlorophyll fluorescence evidences an impaired electron transport, confirming data obtained previously [30]. Further works are in progress at LURE to understand the origin of such impaired electron transport.

## V. TIME-RESOLVED MEASUREMENTS ON COMPLEX TARGETS

It has been shown in the previous section (Figure 4) that the time response of the instrument (0.35 ns) is of the same order of magnitude as the mean lifetime to be determined. The deconvolution of the fluorescence decay by taking into account the back-scattered signal becomes necessary to retrieve the actual fluorescence components. However, measurements in the time domain in the case of complex target may introduce new difficulties, among them the major one is the depth of field of the illuminated area.

When trees or bushes are illuminated by a laser spot of approximately 7 cm of diameter, as it is the case with our instrument, fluorescence is usually emitted by several layers of leaves. If L is the distance between two leaves in the direction of the laser beam, the increase of the optical path-length for the leaf located behind is 2L. In the time domain this corresponds to a delay $\Delta T = 2L/30$ (where L is in cm and $\Delta T$ in ns, Figure 6), as a consequence of the finite speed of light. Let us suppose that all the leaves fluoresce with the same decay law F(t). One can suppose that the fluorescence signal is still expressed as the convolution product of F(t) by the complex back-scattered signal Dex(t). In fact, this situation is rarely observed, since the laser beam may be intercepted by non-fluorescent materials, like stems or the ground. These parts may contribute noticeably to the back-scattered signal without any contribution to the fluorescence signal. Even when only leaves are intercepted, we have also to consider that back-reflectance may contain an important specular component. As the fluorescence emission is isotropic, an amplitude decorrelation between Fex(t) and Dex(t) is still introduced by the different inclinations of leaves inside the canopy.

To solve this problem we have developed a new approach for the deconvolution of the fluorescence and the back-scattering signals in the case of complex targets. In a first step we have decomposed the Dex(t) into a sum of identical components corresponding to the individual contribution of each leaf. The shape of the elementary component D(t) is obtained by measuring for every set of measurements an auxiliary signal, generated by the back-scattered response of a flat target. If the measurements spend more than one hour a new auxiliary signal is measured. In a second step we used these components to fit the fluorescence signal Fex(t).

### Model of the back-scattered signal

Let us model the back-scattered signal Dex(t) by a sum of elementary signals D(t). This is expressed by the equation:

$$Dcal(t) = \sum_{i=1}^{n} a_i .D(t - t_i)$$

where $a_i$ is the relative amplitude parameter for $D(t-t_i)$ and $t_i$ is the time delay generated by the actual position of the emitting part of the target. The relative amplitude ($a_i$) and the time origin ($t_i$) of each elementary flash are both fitted, using a Marquardt search algorithm. The number of components (n) is increased until the fit doesn't improve. In the majority of situations tested, the best fit corresponds to the actual number of hitted surfaces.

### Model of the fluorescence decay

In a second step we introduced Dcal(t) instead of Dex(t) in a modified version of our deconvolution program. Fex(t), the experimental fluorescence decay, is then fitted by a sum of m exponential components, F(t).

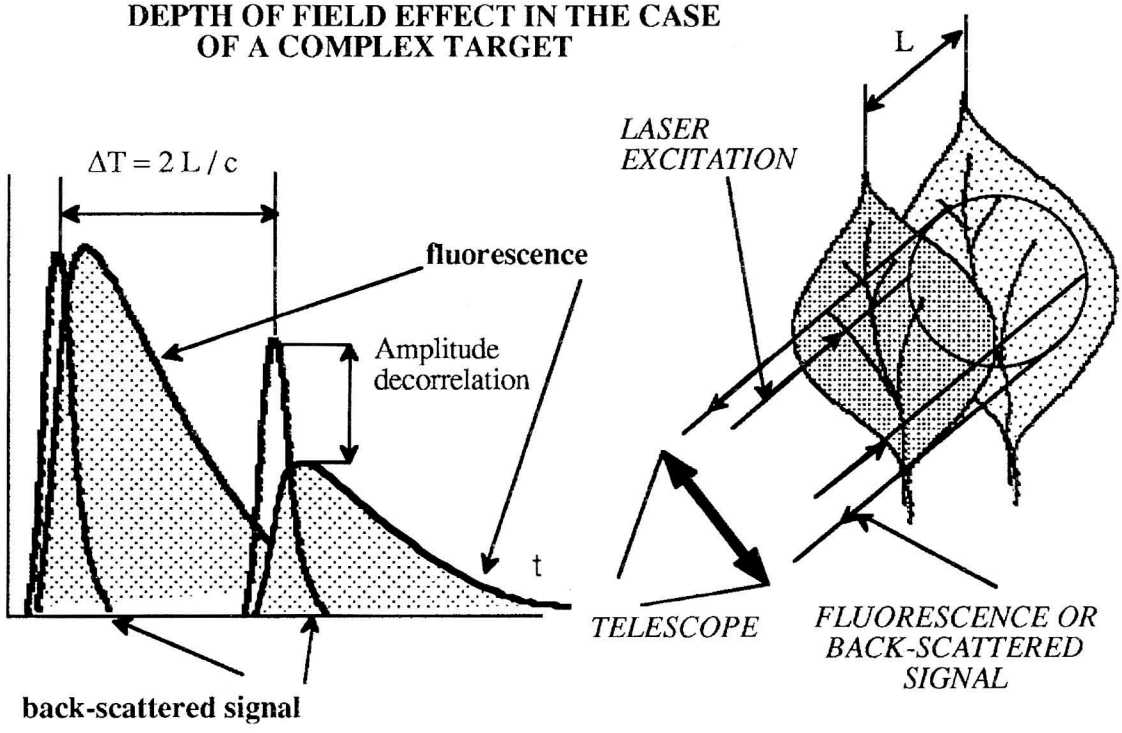


*Fig. 6 - The partially specular character of the back-scattered reflectance from leaves produces an amplitude decorrelation when compared with the fluorescence signal.*

$$F(t) = \sum_{j=1}^{m} F_j \exp\left(-\frac{t}{\tau_j}\right)$$

F(t) is then convoluted with each elementary flash $D(t-t_i)$ of the scattering signal determined in the previous step, obtaining Fcal(t). Thus,

$$Fcal(t) = \left[\sum_{i=1}^{n} b_i .D(t - t_i) \otimes F(t)\right],$$

where $\otimes$ denotes the convolution product and $b_i$ are weighting factors. These factors are required to account for the differences in the fluorescence intensity produced by each elementary flash as a result of the different properties of Dex(t) and Fex(t). In this second step, the fit is done on $b_i$, $F_j$ and $t_j$ parameters by a marquardt search algorithm.

To test the validity of our new deconvolution approach we have adapted a time-correlated single- photon-counting experiment described in [27] to measure the chlorophyll fluorescence decay of several types of complex targets. Measurements were done at 2 m of distance on wheat, sorghum and soybean canopies of variable height (30 to 60 cm). The light was applied from the top of the canopy and the cross-section of the beam was approximately 5x5 cm. The result of the decomposition of the back-scattering signal is shown in Figure 7. The shape of the elementary component, which looks like a gaussian band, was recorded independently by measuring the back-scattered response of a flat target. As it can be judged by the distribution of the weighted residuals:

$$\frac{Dex(t) - Dcal(t)}{\sqrt{Dex(t)}},$$

a correct fit was obtained for n=7. Less components lead to an unacceptable distortion for the weighted residuals, while more components introduce two bands at the same position. Using the components of Dcal(t), we applied the new deconvolution to the fluorescence decay. The computations were done with an Apple Powerbook 170 computer. The program was written in Pascal. In spite of the greater number of parameters to be adjusted, our program was able to find

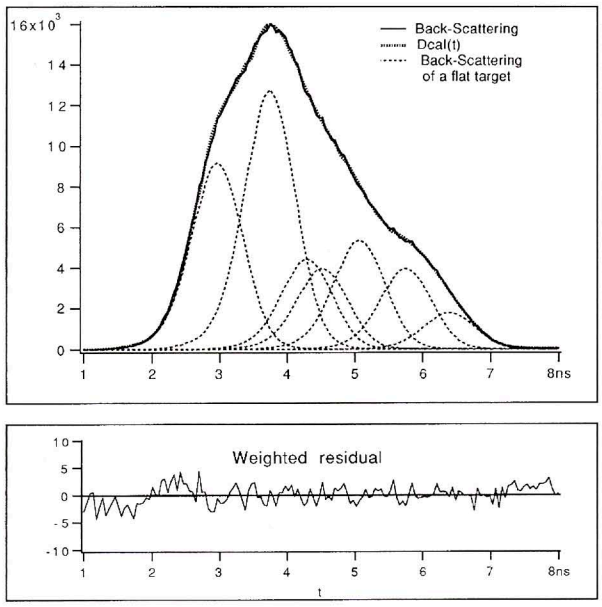


*Fig. 7 - Fit of the back-scattered excitation signal of a wheat canopy by a sum: Dcal(t), of elementary components. The shape of the elementary components results from an independent measurement with a flat target. The measurements have been done using the time-correlated single-photon-counting techinique. The goodness of the fit can be judged by the flat distribution of the weighted residuals.*

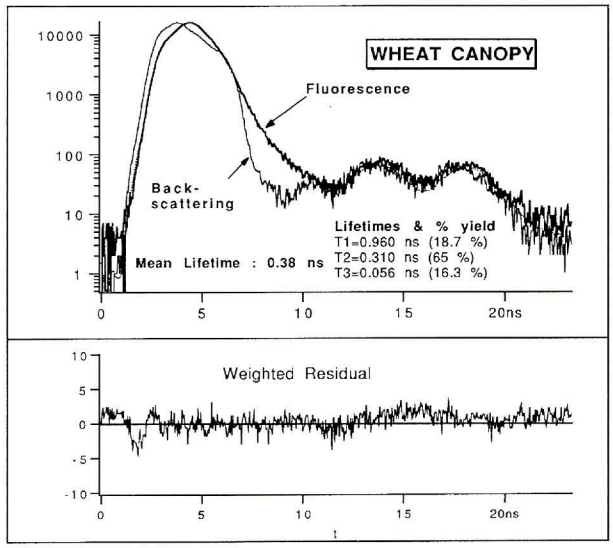


*Fig. 8 - Deconvolution of the fluorescence decay of a wheat canopy after decomposition of the back-scattering into its elementary components. Same experiment as in Fig. 7. The back-scattering signal is presented with its companion fluorescence decay in a log.scale.*

a stable solution in all cases within a few seconds of computation time. The results are shown in Figure 8. F(t) has been adjusted using 3 exponential components. Both the lifetime of the elementary components ($t_j$) and its relative yield are in good agreement with measurements performed in contact (not shown). The reduced $Ki^2$ of approximately 1.8 together with the flat distribution of the weighted residuals were found good, specially when taking into account the double fit operated. It is worth noting that the direct deconcolution of Fex(t) by Dex(t) cannot be applied in that case. Figure 9 shows the fluorescence and back-scattered signals emitted by the different leaf levels intercepted by the excitation beam. The higher level (51 cm) has no fluorescence associated with it, as it was expected for a signal corresponding to the ground. Similar treatment has been done with soybean and sorghum canopies (data not shown). The new deconvolution method has been further applied with success to fluorescence decay data obtained with our LIDAR system. Figure 10 shows the signals obtained on a sorghum canopy at an equivalent distance of 50 m. Due to some ringing noise at the level of the photomultiplier, 16 laser shots have been averaged. As a consequence of the lower sensitivity of the LIDAR system, the fluorescence signal has been fitted with only two exponential decay components instead of three as obtained by the photon-counting method. This has no effect on the mean lifetime, the principal parameter we want to determine. For the measurement of figure 10 a mean lifetime of 0.32 ns was computed, as expected considering the low light conditions (fluorescence level near $F_0$).

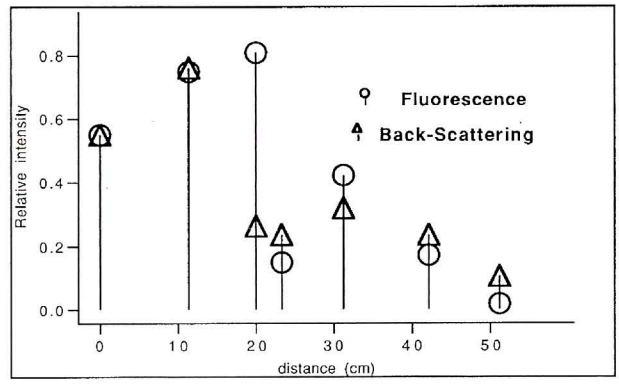


*Fig. 9 - Intensity of the back-scattered signal (triangles) and the associated chlorophyll fluorescence signal (circles). Same experiments as in Fig. 7 and 8. Note the absence of a fluorescence signal for the last peak (51 cm): it corresponds to the ground level.*

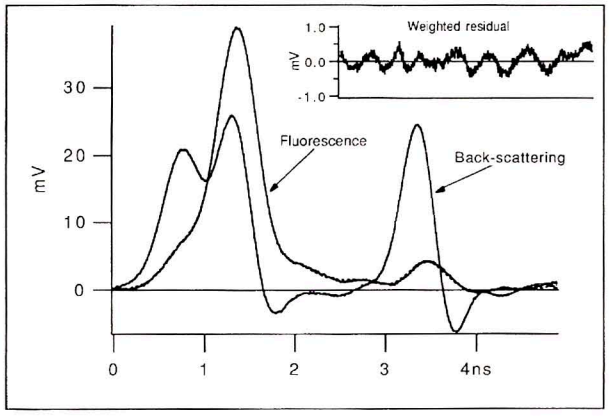


*Fig. 10 - Fluorescence and back-scattered signals from a sorghum canopy accumulated 16 times. The figure shows the complex pattern due to the particular fluorescence and reflectance properties of each layer. The recalculated fluorescence (mean lifetime of 0.32 ns) is close to the experimental decay, as indicated by the residual function.*

## VI. CONCLUSION

The results presented here show that, with a special method of deconvolution, remote sensing of the fluorescence lifetime can be performed on complex plant canopies with a laser instrumentation. They also show that information about the relative positions of the leaves inside the canopy can be extracted from the back-scattered signal. This information would provide helpful data to canopy models developed for the understanding of interactions between vegetation and its environment. Fluorescence lifetime measurement on canopies offers new perspectives for vegetation monitoring on a large spatial scale, because it is closely related to photosynthetic activity. Experiments are now being performed with this new type of picosecond LIDAR and are mainly focused on developing methods for assessing the physiological state of the plant and for detecting stress conditions.

## ACKNOWLEDGEMENTS

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