Laser Induced Fluorescence Transient (LIFT) Method for Measuring Photosynthetic Performance and Primary Productivity in Terrestrial Ecosystems

Z. S. Kolber
Institute of Marine and Coastal Sciences, Rutgers University
71 Dudley Road, New Brunswick, NJ 08901

Abstract **The Laser Induced Fluorescence Transient (LIFT) method was developed to measure photosynthetic properties in terrestrial plants. In the LIFT method, a spatially and temporally modulated laser excitation signal is produced by an array of laser diodes to excite chlorophyll fluorescence, at a distance of 5 to 300 meters. The excitation signal, at energy levels of 30 to 50 W/m^2, saturates up to 80% of the photosynthetic electron transport, causing a transient increase in the measured fluorescence signal. Both the amplitude of the fluorescence transient and the rate of fluorescence saturation are controlled by the photosynthetic parameters, such as the efficiency of photosynthetic light utilization, the quantum yield of photochemical conversion, and the rate of photosynthetic electron transport. The LIFT method was implemented in both the stationary instrument, allowing to measure photosynthetic properties in vegetation remotely, within a 50 meter radius, and in the airborne instrument, operating at an altitude of 150 to 300 meters. The stationary instrument is controlled by a CAT5 cable, allowing continuous, unattended operation in a remote location. The excitation signal, produced by a temporally-modulated laser diode, produces a temporally-modulated emission signal, which is acquired by a red-sensitive avalanche photodiode. The airborne instrument, operating at an altitude of 150 to 300 meters, projects a spatially modulated excitation image on the ground. This excitation image, traveling along a flight path with a typical speed of 135 mph (60 m/s), produces a fluorescence image, with modulation characteristics controlled by photosynthetic parameters of the illuminated plants. This image is acquired by a red-sensitive CCD camera.**

INTRODUCTION

The fluorescence signal emitted by the green tissue of terrestrial plants represents one of the deactivation pathways for absorbed light, dissipating between 2 to 6 % of the excitation energy. The quantum yield of fluorescence is controlled, to a first degree, by the level of photosynthetic activity. Under low ambient irradiance, most of the absorbed light is utilized for photochemistry, resulting in a low fluorescence signal, F_o. At high irradiance levels, where the excitation rates exceed the photosynthetic capacity, a significant portion of the absorbed light cannot be utilized photosynthetically, and is dissipated both thermally and radiatively, increasing the fluorescence yield to its maximum level, F_m. The fluorescence signal can therefore be used as an indicator of the photosynthetic activity under ambient light. Additional information regarding the plants' photosynthetic performance can be acquired by stimulating the chlorophyll fluorescence with an artificial excitation source. Such an "actively stimulated" fluorescence signal was successfully used to measure of the functional status of the photosystem II (PSII) [1], to characterize the quantum efficiency of photochemistry in PSII reaction centers [2, 3], to estimate the number of primary and secondary electron-acceptors [4], to measure the functional (or effective) absorption cross section of PSII [5, 6], and to assess the kinetics of electron transport on the acceptor side of PSII [7, 8]. The Fast Repetition Rate Fluorescence (FRRF) technique [9] allows the measurements of all these parameters using a single, temporally modulated excitation signal.

The advantages of the FRRF method, such as instantaneous, non-invasive measurements, and the ability to completely characterize the photosynthetic performance, make it attractive in remote, Lidar-based applications. The Laser Induced Fluorescence (LIF) technique has been successfully used to measure spectrally resolved fluorescence of green plants [10, 11], to characterize the level of environmental stress [12, 13], and to assess photosynthetic yields [14, 15, 16]. Laser-induced chlorophyll fluorescence was also used to estimate changes in the photosynthetic yield under ambient irradiance [17, 18]. None of the existing LIF implementation, however, offer the level of detail in characterizing the photosynthetic performance comparable to the FRRF method. Unfortunately, the standard FRRF measurements require excitation energies of about of 0.5-1 kW/m^2 [9], much above the eye-safe level of laser radiation. Moreover, these energy levels would require powerful excitation sources, usually frequency-doubled YAG lasers operating in Q-switched mode, which cannot produce the excitation sequence required in FRRF operation. Here, we describe a novel method, called Laser Induced Fluorescence Transient (LIFT), where the photosynthetic performance of terrestrial plants can be measured remotely, using low excitation power of 30 to 50 W/m^2, which is within the range of ANSI Z-136.1 guidelines regarding eye-safe laser radiation.

LIFT METHOD

In the LIFT method, the laser excitation signal is used to both manipulate the level of photosynthetic activity, and to measure the corresponding changes in the fluorescence yield. In the stationary version of the method, the excitation power is modulated temporally, to produce a periodically varying fluorescence signal (Fig. 1). At periods when the excitation

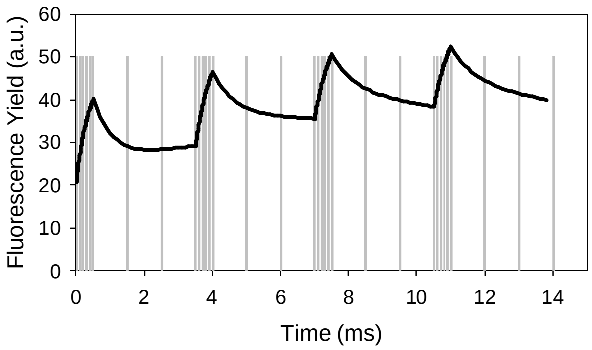

Fig. 1. Principle of the LIFT method. The excitation power is modulated by changing the frequency of the excitation flashes (grey bars). The fluorescence signal (black line) transiently increases when the excitation power exceeds the capacity of the photosynthetic electron transport, and decreases at low excitation power. The average excitation power is kept at about 30 W/m^2 to satisfy ANSI Z-136.1 guidelines regarding eye-safe laser radiation.

power exceeds the rates of the photosynthetic electron transport, the fluorescence signal transiently increases due to saturation of the photosynthetic capacity. When the excitation energy is lower than the rate of the photosynthetic electron transport, the fluorescence signal decreases with the kinetics proportional to the rate of photosynthetic electron transport.

The measured fluorescence transient, f(t), can be formally expressed as:

$$f(t) = F_o + \left(F_m - F_o\right)\left(C(t)\frac{1-p}{1-C(t)p}\right), \tag{1}$$

where C(t) is the level of photosynthetic activity, 0<C(t <1, and p is the extent of energy transfer between photosynthetic reaction centers. C(t) is controlled by the equilibrium between the rate of absorption of the excitation energy, r_{abs}, and the rate of photosynthetic electron transport, r_{tr}:

$$\frac{\partial C(t)}{\partial t} = r_{abs}(t) - r_{tr}(t). \tag{2}$$

The r_{abs} is a function of the excitation power, i(t), and the functional absorption cross section of photosystem II, σ_{PSII}:

$$r_{abs}(t) = \sigma_{PSII} i(t)\frac{1-C(t)}{1-pC(t)}, \tag{3}$$

and r_{tr} is controlled by the time constant of the photosynthetic electron transport, τ_p:

$$r_{tr}(t) = C(t)\exp(-t/\tau_p). \tag{4}$$

The most important photosynthetic parameter, the quantum yield of photosynthesis, Φp, can be calculated as

$$\Phi_p = \left(F_m - F_o\right)/F_m. \tag{5}$$

Φp, σ_{PSII}, p, and τp varies among different species of terrestrial vegetation, and are affected by the environmental conditions such as nutrient status, water and temperature stress, and prolonged exposure to high irradiance. In addition, τ_p is a function of the photosynthetic activity, varying from about 0.5 ms when C = 0 (i.e., in the darkness), to 10 ms when C approaches 1 (under supersaturating irradiances).

All the photosynthetic parameters (Φp, σ_{PSII}, p, and τ_p) can be calculated by fitting the measured fluorescence transients to (1-4).

A. Stationary LIFT instrument

In the stationary LIFT instrument the excitation signal is generated by a single 660 nm laser diode, 1F5257D from Boston Laser, operating at 1 W optical power. The excitation signal is expanded into a collimated, 200 mm diameter beam, of uniform power density of about 30 Wt/m^2. The excitation beam is projected at 5 to 50 meters distance, at a selected target. The emission signal is collected by a 250 mm telescope, filtered with a 690 nm, 10 nm bandwidth interference filter, and detected by a cooled, large-area avalanche photodiode, 639-70-72-631 from Advanced Photonics. The detected signal is amplified by a variable, 0 – 96 dB gain amplifier, utilizing a double operational amplifier, AD 605, from Analog Devices. The fluorescence signal is digitized at 4 MHz, and the digitized fluorescence transients are numerically fitted into the model described by (1-4)

The excitation laser diode is mounted coaxially with the telescope, allowing remote control of the pan and tilt. The instrument is equipped with a video-camera (Sony W500FL), and is controlled by a dedicated PC running a web server application. In such configuration, the instrument control, including selection of the excitation protocol, selection of the target, measurement, and data processing, all are performed remotely, from any terminal connected to the web. One of such instruments is operated at the Biosphere 2 Center (B2C), Columbia University, to continuously monitor photosynthetic properties of selected plant species. A typical fluorescence transient, remotely measured in one of the B2C biomes, is shown in Fig. 2. This instrument is used to characterize the photosynthetic response of cottonwood trees to elevated CO_2 concentrations, and to identify the patterns of photosynthetic response to water stress. Daily patterns of the measured fluorescence transients (Figs. 3a, 3b) indicate an increase of about 20% in the quantum yield of photosynthesis, and about 30% faster rates of photosynthetic electron transport in plants grown at high (1200 ppm) concentration of CO_2, compared to ambient (400 ppm) CO_2 concentration.

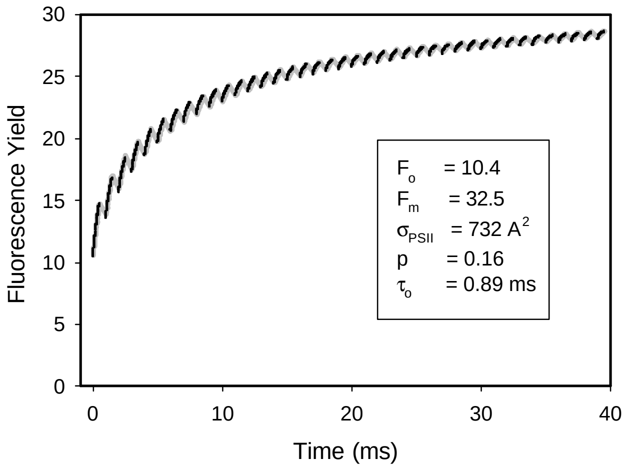

Fig. 2 Typical fluorescence transient measured remotely at B2C in cottonwood trees, exposed to 1200 ppm CO_2 concentration. Black dots: experimental data points; grey line: numerical fit to a model described by (1-4), with photosynthetic parameters as shown in the inset.

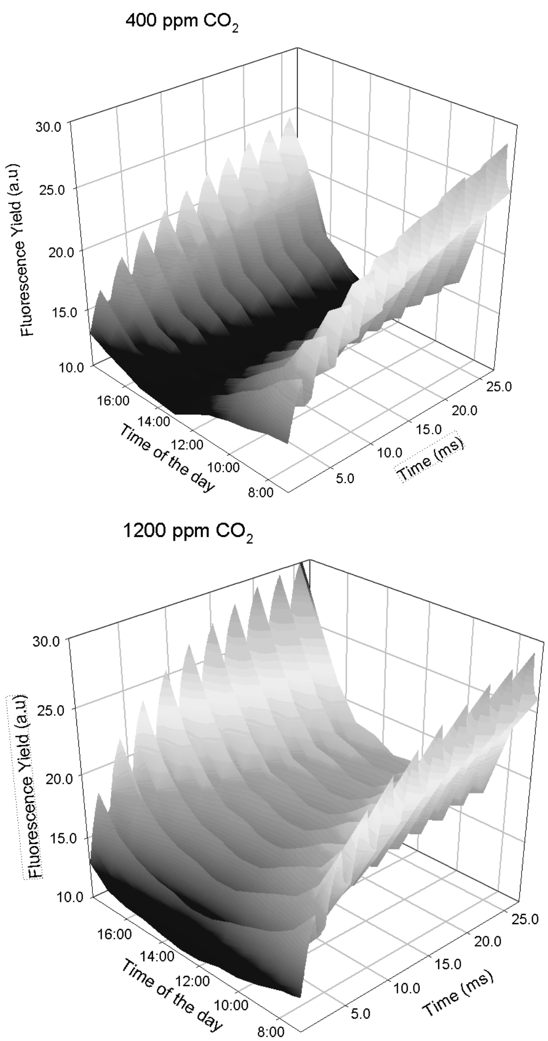

Fig. 3. Daily pattern of fluorescence transients measured in 1200 ppm CO_2 biome (upper panel) and in 400 ppm CO_2 biome (lower panel).

B. Airborne LIFT instrument

To increase the spatial coverage of the LIFT measurements, the LIFT method was implemented in an airborne instrument. In this implementation, the excitation signal is generated by an array of sixty laser diodes, with total excitation power of about 40 W. The excitation image, in the form of twelve stripes, is projected on the ground over an area of 1 x 1.5 meters. This excitation pattern, moving at a speed of about 60 m/s, induces a spatially-modulated fluorescence image, with modulation characteristics similar to that produced by the stationary instrument (Fig. 4). The fluorescence image is collected by a custom-built, 250 mm refractive telescope, and acquired by a cooled CCD detector (ANDOR DV420BV camera with an EEV30-11 CCD from Andor Technology). The photosynthetic parameters are calculated by fitting the fluorescence image to a theoretical model (1-4), where time is expressed as a function of the airplane speed, and the geometrical size of the excitation pattern.

The laser excitation array and the emission telescope are designed as a single mechanical package (Fig. 5). Twelve laser modules (Fig. 6), each containing 5 laser diodes, are mounted in twelve pockets around the telescope. Each module contains the necessary electronic (power supply, laser drivers, and optical power stabilization circuitry), allowing them to operate independently. Such a design allows convenient servicing of the excitation source, and possible replacement of the red (660 nm) excitation signal with other color laser diodes. In order to stabilize the laser diodes

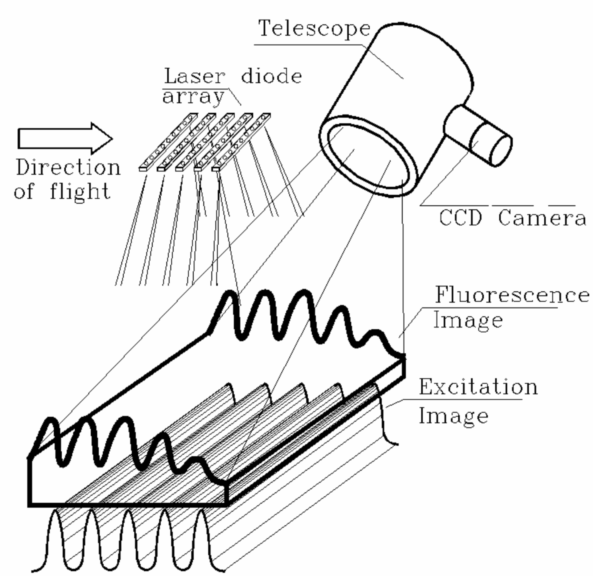

Fig. 4. Airborne implementation of the LIFT method. The excitation signal, in a form of several stripes, is produced by an array of laser diodes. The excitation pattern, traveling at the speed of the airplane along the flight path, subjects the ground target to a temporally-modulated excitation signal, similar to that shown in Fig. 1. The resulting fluorescence image is collected by a 250 mm telescope, and acquired by a CCD camera.

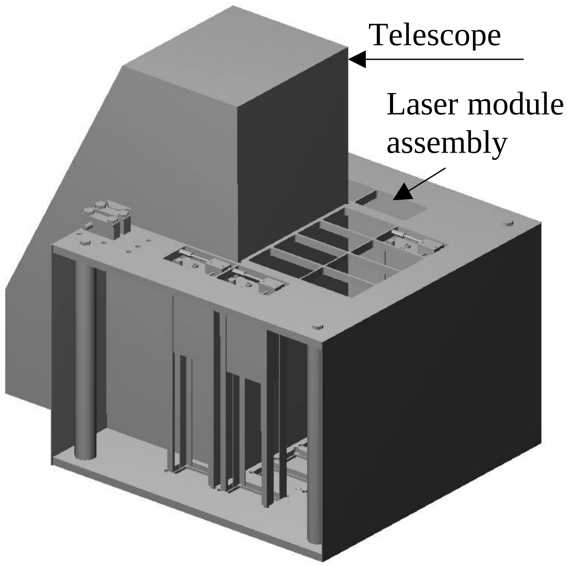

Fig. 5. Mechanical design of the excitation source and the emission telescope in the airborne version of the LIFT instrument**.**

temperature, laser modules are actively cooled using a custom-built refrigeration unit based on Peltier devices.

The airborne instrument is designed to fit a 20'' photographic mount, commonly found in small airplanes outfitted for aerial photography. In collaboration with NOAA, the instrument mount is designed to fit the Twin Otter airplane. This platform offers the stability required for the LIFT operation, is relatively economical, and can be operated at 150 meters altitude, preferable for LIFT measurements.

C. Conclusions

The LIFT method allows the characterization of photosynthetic properties, and estimation of photosynthetic rates in terrestrial vegetation nondestructively, remotely, and in real-time. The stationary LIFT instrument can operate autonomously, performing continuous measurements within 50 meters radius. It is controlled from a remote terminal via the existing computer network. This instruments will be deployed at AmeriFlux tower sites, where it will operate continuously to measure photosynthetic performance and the primary production, allowing to constrain terrestrial CO_2 fluxes measured by the Eddy Correlation towers. The instrument is equipped with a dedicated web server, allowing multi-user access to the instrument control and data. Such a remote access allows a group of collaborators to operate the instruments remotely, and will provide easy, on-line access to the raw data for modeling of carbon fluxes. We plan to deploy and maintain an array of such instruments in several facilities involved in the global carbon research.

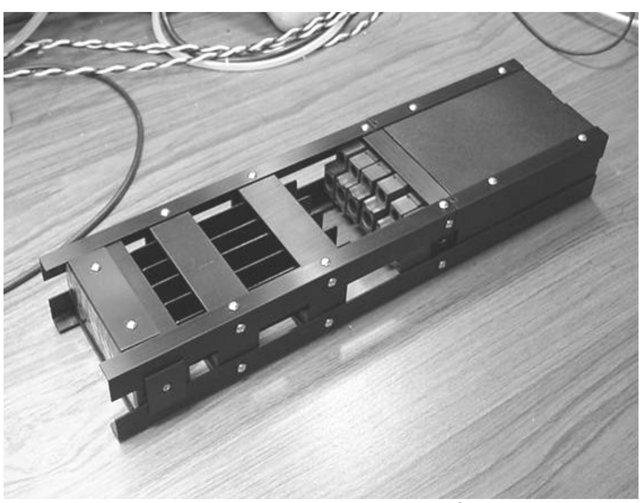

Fig. 6. Laser module with six laser diodes. Twelve of such modules are used in the airborne LIFT instrument.

The airborne instrument will be used for large-spatial scale measurements of the photosynthetic properties. Within the next few years we will design an airborne observation campaign, to fly the instrument over different types of vegetation, and to characterize the photosynthetic signature of different vegetation types. Next, we will identify the photosynthetic response to changes in the environmental conditions (nutrient availability, water and temperature stress, pollutants), and we will use this information to quantify these conditions from the airborne-measured fluorescence signal.

In conjunction with the fluorescence measurements, we will measure the spectral reflectance of the vegetation to identify the functional relationship between the photosynthetic performance and the spectral quality of the reflected light. We will use this information to derive empirical and functional relationships between photosynthesis and the spectral reflectance signal measured by the satellite sensors. Such a relationship will allow the extrapolation of photosynthetic measurements performed by both the stationary and airborne LIFT instruments, over global scales.

ACKNOWLEDGMENT

This work was supported by the NASA Instrument Incubator Program (IIP).

REFERENCES

[1] G. H. Krause and E. Weis, "Chlorophyll fluorescence and photosynthesis - the basics," *Ann. Rev. Plant Physiol. Plant Mol. Biol.*, vol. 42, pp. 313-349, 1991.

[2] W. L. Butler and M. Kitajima, "Energy transfer between PSII and PSI in chloroplasts," *Biochim. Biophys. Acta*, vol. 396, pp. 72-85, 1975.

[3] E. Weis and J. A. Berry, "Quantum efficiency of photosystem II in relation to 'energy '- quenching of chlorophyll fluorescence," *Biochim. Biophys. Acta*, vol. 894, pp. 198-208, 1990.

[4] S. Malkin and B. Kok, "Fluorescence induction studies in isolated chloroplasts. I. Number of components involved in the reaction and quantum yields.," *Biochim. Biophys. Acta*, vol. 126, pp. 413-432, 1966.

[5] C. Bonaventura and J. Myers, "Fluorescence and oxygen evolution from *Chlorella pyrenoidosa*.," *Biochim. Biophys. Acta*, vol. 189, pp. 366-383, 1969.

[6] A. C. Ley and D. C. Mauzerall, "Absolute absorption cross-sections for photosystem II and the minimum quantum requirement for photosynthesis in *Chlorella vulgaris*," *Biochim. Biophys. Acta*, vol. 680, pp. 95-106, 1982.

[7] B. Bouges-Bocquet, "Electron transfer between the two photosystems in spinach chloroplasts," *Biochim. Biophys. Acta*, vol. 314, pp. 250-256, 1973.

[8] J. Bowes and A. R. Crofts, "Binary oscillations in the rate of reoxidation of the primary acceptro of Photosystem II," *Biochim. Biophys. Acta*, vol. 590, pp. 373-384, 1980.

[9] Z. S. Kolber, O. Prasil, and P. G. Falkowski, "Measurements of variable chlorophyll fluorescence using fast repetition rate techniques: defining methodology and experimental protocols," *Biochim. Biophys. Acta*, vol. 1367, pp. 88-106, 1998.

[10] E. M. Chappelle, F. M. Wood, W. W. Newcomb, and J. E. McMurtrey III, "Laser induced fluorescence of green plants. 3: LIF spectral signatures of five major plant types," *Applied Optics*, vol. 24, pp. 74-80, 1985.

[11] E. W. Chappelle, J. E. McMurtrey III, and M. S. Kim, "Identification of the Pigment Responsible for the Blue Fluorescence Band in the Laser Induced Fluorescence (LIF) Spectra of Green Plants, and the Potential Use of This Band in Remotely Estimating Rates of Photosynthesis," *Remote Sens. Environ.*, vol. 36, pp. 213-218, 1991.

[12] H. K. Lichtenthaler, "Applications of Chlorophyll Fluorescence in Stress Physiology and Remote Sensing.," in *In: Applications of Remote Sensing in Agriculture.*, M. STEVEN and J. A. CLARK, Eds. London: Butterworths Scientific Ltd., 1990, pp. 287-305.

[13] H. G. Dahn, K. P. Gunther, and W. Ludeker, "Characterization of Drought Stress of Maize and Wheat Canopies by Means of Spectral Resolved Laser Induced Fluorescence.," *EARSeL Advances in Remote Sensing*, vol. 1, pp. 12-19, 1992.

[14] A. M. Chekalyuk and M. Y. Gorbunov, "Development of the pump-and-probe lidar technique for remote measuring the efficiency of primary photochemical reactions in leaves of green plants.," *EARSeL Adv. Remote Sens.*, vol. 3, pp. 42-56, 1995.

[15] A. M. Chekalyuk, F. E. Hoge, C. W. Wright, and R. N. Swift, "Short-pulse pump-and-probe technique for airborne laser assessment of Photosystem II photochemical characteristics.," *Photosynt. Res.*, vol. 66, pp. 33-44, 2001.

[16] A. M. Chekalyuk, F. E. Hoge, C. W. Wright, R. N. Swift, and J. K. Yungel, "Airborne test of laser pump-and-probe technique for assessment of phytoplankton photochemical characteristics.,", vol. 66, pp. 45-56, 2001.

[17] A. Rosema and H. Zahn, "Laser pulse energy requirements for remote sensing fo chlorophyll fluorescence.," *Remote Sens. Environ.*, vol. 62, pp. 101-108, 1997.

[18] A. Rosema, J. F. H. Snel, H. Zahn, W. F. Buurmeijer, and L. W. A. Van Hove, "The relation between laser-induced chlorophyll fluorescence and photosynthesis," *Remote Sens. Environ.*, vol. 65, pp. 143-154, 1998.