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Measuring photosynthetic parameters at a distance: laser induced fluorescence transient (LIFT) method for remote measurements of photosynthesis in terrestrial vegetation

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Abstract

We have developed a laser induced fluorescence transient (LIFT) technique and instrumentation to remotely measure photosynthetic properties in terrestrial vegetation at a distance of up to 50 m. The LIFT method uses a 665 nm laser to project a collimated, 100 mm diameter excitation beam onto leaves of the targeted plant. Fluorescence emission at 690 nm is collected by a 250 mm reflective telescope and processed in real time to calculate the efficiency of photosynthetic light utilization, quantum efficiency of PS II, and the kinetics of photosynthetic electron transport. Operating with peak excitation power of 125 W m^{-2}, and duty cycle of 10–50%, the instrument conforms to laser safety regulations. The LIFT instrument is controlled via an Internet connection, allowing it to operate from remote locations or platforms. Here we describe the theoretical basis of the LIFT methodology, and demonstrate its applications in remote measurements of photosynthetic properties in the canopy of cottonwood and oak trees, and in the rosette of *Arabidopsis* mutants.

Introduction

Actively induced chlorophyll fluorescence is the most widely used indicator of the functional status of Photosystem II (PS II) (Krause and Weis 1991). Norio Murata developed early chlorophyll fluorescence technologies (Murata et al. 1966), and he pioneered their application in research on the response of PS II to environmental stress (Satoh and Murata 1999). We are pleased to honor Norio's contributions in these fields by introducing a novel remote sensing methodology that will expand the scale and range of fluorescence measurements *in situ*.

Leaf level chlorophyll fluorescence techniques are widely used in plant physiology to characterize the quantum efficiency of PS II and to estimate photosynthetic electron transport (ETR). The most common is the 'pulse amplitude modulation' (PAM) approach (Schreiber et al. 1986; Schreiber and Bilger 1993). ETR measured by the PAM technique (Genty et al. 1989) has proven to be a robust indicator of leaf photosynthesis under a variety of experimental conditions (Maxwell and

Johnson 2000). However, it remains impractical to deliver saturating flashes at the canopy scale. The growing demand for large scale ecosystem monitoring in times of global change has led to the development of laser-based techniques to measure photosynthetic properties. To date the most common approach employs band ratios of 445, 530, 685, and 735 nm induced by a short (5–100 ns) laser pulse at 512 nm (Gunther et al. 1991), or 355 nm (Jeffrey et al. 2003) as indicators of plant response to environmental stress. The quantum efficiency of photosynthesis has been measured by a laser-PAM instrument at a distance of 1 m (Ounis et al. 2001), and the Lidar induced fluorescence (LIF) technique has extended these measurements to longer distances (Moya et al. 1995; Chekalyuk et al. 2001).

More extensive characterization of photosynthetic properties, such as the efficiency of photosynthetic light utilization, the quantum yield of photosynthesis, and the kinetics of the photosynthetic electron transport, might be achieved remotely by the laser induced fluorescence transient (LIFT) technique. Based on the principles of fast repetition rate fluorescence (FRRF) (Kolber et al. 1998), this method uses a laser beam to project the excitation signal onto a target at a distance of 5–50 m. The LIFT technique detailed here operates with peak excitation power of 125 W m^{-2} (684 μM quanta m^{-2} s^{-1}) and a 10–50% duty cycle, satisfying the ANSI Z-136.1-2000 guidelines regarding eye-safe laser radiation. Here we describe the instrument, and present the preliminary test results from the Biosphere 2 Laboratory (B2L).

Materials and methods

Laser induced fluorescence transient (LIFT): general approach and model

In the LIFT method, the pulsed laser excitation signal with a variable duty cycle is used to both manipulate the level of photosynthetic activity and to measure the corresponding changes in the chlorophyll fluorescence yield. At high duty cycle the rate of stable charge separation (Q_A reduction) exceeds the rate of photosynthetic electron transport, and Q_A becomes progressively reduced causing a transient increase of the fluorescence yield (Figure 1a). At low duty cycle the rate of stable charge separation is lower than the rate of photosynthetic electron transport, Q_A reoxidizes, and the fluorescence yield relaxes with kinetics controlled by photosynthetic turnover time. The dynamics of Q_A reduction is controlled by the imbalance between the rate of charge separation, R_{cs}, and the rate of the photosynthetic electron transport R_{etr}:

$$\frac{\partial[Q_A^-]}{\partial t} = R_{cs} - R_{etr}. \tag{1}$$

The R_{cs} is a function of the excitation power, $i(t)$, the ambient irradiance, E, the concentration of PS II reaction centers, n, the functional absorption cross-section of PS II, $\sigma_{PS\ II}$, and the level of $[Q_A^-]$. In the absence of energy transfer between PS II reaction centers, R_{cs} can be expressed as

$$R_{cs} = (i(t) + E)\sigma_{PSII}\left(n - [Q_A^-]\right), \tag{2}$$

while R_{etr} is controlled by the kinetics of photosynthetic electron transport, k_{etr}:

$$R_{etr} = [Q_A^-]k_{etr} = [Q_A^-]\frac{1}{\tau_{Q_A}}, \tag{3}$$

where τ_{Q_A} is the time constant of Q_A^- reoxidation. The product $(i(t) + E) \times \sigma_{PS\ II}$ represents the rate of stable charge separation in open reaction centers. Transient changes in the level of Q_A reduction can be therefore described as

$$\frac{\partial}{\partial t}[Q_A^-] = (i(t) + E)\sigma_{PSII}\left(n - [Q_A^-]\right) - \frac{[Q_A^-]}{\tau_{Q_A}}. \tag{4a}$$

Dividing both sides by the concentration of the PS II reaction centers

$$\frac{\partial}{\partial t}\frac{[Q_A^-]}{n} = (i(t)+E)\sigma_{PSII}\left(1 - \frac{[Q_A^-]}{n}\right) - \frac{[Q_A^-]}{n}\frac{1}{\tau_{Q_A}}, \tag{4b}$$

and expressing $[Q_A^-]$/n as the level of PS II reduction, C, leads to the following:

$$\frac{\partial}{\partial t}C = (i(t) + E)\sigma_{PSII}(1 - C) - C\frac{1}{\tau_{Q_A}}. \tag{5}$$

The dynamics of C is controlled by both, the actual ambient irradiance E, and by the excitation signal $i(t)$. In the absence of the LIFT excitation signal ($i(t) = 0$), C attains a steady-state level C_E

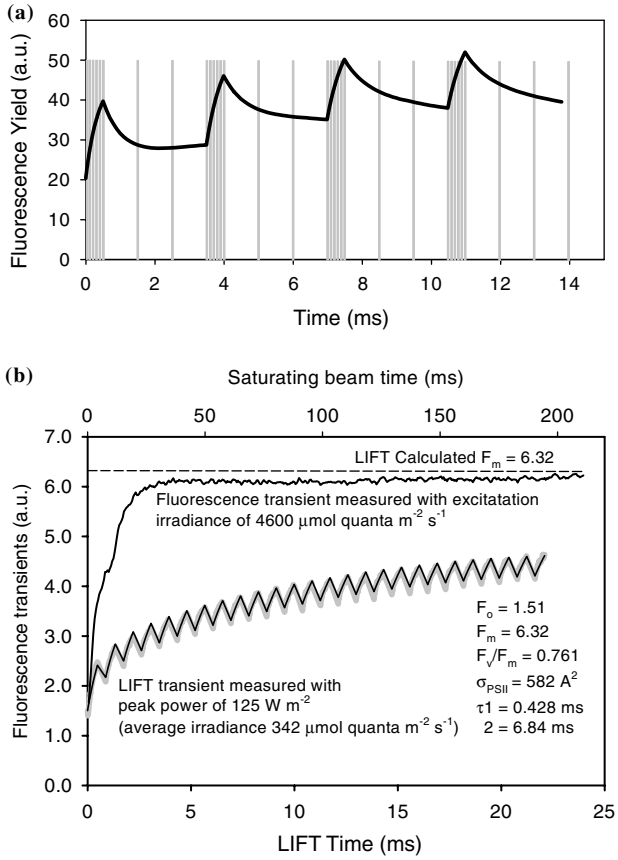

Figure 1. (a) Principle of the LIFT method. The excitation power is modulated by changing the frequency of the excitation flashes (grey bars). The fluorescence signal (black line) transiently increases when the rate of stable charge separation exceeds the capacity of the photosynthetic electron transport (high frequency flashes), and decreases when the rate of stable charge separation is lower than the rate of photosynthetic electron transport. (b) Example of the measured fluorescence transient (grey points), and the numerical fit (dark trace) to the model described by Equations (1–8) (dark line) measured on *Malva parviflora* L. leaves in the dark. The results of numerical fit are displayed in the inset. A fluorescence transient measured with 4600 µmol quanta m^{-2} s^{-1} excitation signal, normalized with the first data point of the LIFT transient is shown in the upper trace.

$$C_E = \frac{E\sigma_{\mathrm{PSII}}}{E\sigma_{\mathrm{PSII}} + \frac{1}{\tau_{Q_\mathrm{A}}}}. \tag{6}$$

The additional change in Q_A induced by the excitation light $i(t)$ can be calculated as

$$\frac{\partial}{\partial t} C_i = \left[i(t)\sigma_{\mathrm{PSII}}(1 - C_E)(1 - C_i) - \frac{C_i}{(1 - C_E)\tau_{Q_\mathrm{A}}} \right], \tag{7}$$

allowing the LIFT-stimulated fluorescence signal to be expressed as

$$f(t) = F + \left(F_\mathrm{m}' - F\right) C_i, \tag{8}$$

where F_m' is the fluorescence signal corresponding to fully reduced Q_A, and F is the fluorescence under background irradiance ($F = F_\mathrm{o}$ in the darkness). By numerically integrating Equation (7) over the length of the excitation protocol, substituting the integrated C_i into Equation (8), and fitting this equation into the measured fluorescence transient, all the photosynthetic parameters, including F, F_m', (F_o and F_m, when measured in darkness), $\sigma_{\mathrm{PS\ II}}$, and $\tau_{Q\mathrm{A}}$, can be calculated. Usually $\tau_{Q\mathrm{A}}$ has to be expressed as a sum of two exponential decays.

In situations where energy transfer between PS II reaction centers cannot be ignored, Equation (7) can be expressed as follows:

$$\frac{\partial}{\partial t} C := \left[(i(t) + E)\sigma_{\mathrm{PSII}} \frac{1 - C}{1 - pC} - \frac{C}{\tau_{Q_\mathrm{A}}} \right], \tag{9}$$

where p is the extent of energy transfer between PS II units (Paillotin 1976). When measured in darkness, the LIFT controlled reduction of Q_A is

$$\frac{\partial}{\partial t} C_i = \left[i(t)\sigma_{PSII} \frac{1 - C_i}{1 - pC_i} - \frac{C_i}{\tau_{Q_A}} \right], \quad (10)$$

allowing expression of the measured fluorescence transient as

$$f(t) = F + \left(F_m' - F\right) \frac{C_i(1-p)}{1 - pC_i}. \quad (11)$$

Usually, we measure p in a range of 0.1–0.4 in the darkness, but very close to 0 in the light.

Technical description of the LIFT instrument

The LIFT instrument described and tested here differs from the first prototype (Ananyev et al. 2005) in that it conforms to ANSI Z-136.1-2000 regulations regarding eye-safety standards. The excitation signal is generated by a single 665 nm laser diode (Boston Laser 1F5257D, Binghamton, NY) operating at 1 W optical power, expanded into a collimated 100 mm diameter beam, of uniform peak power density of 125 W m^{-2}. The laser diode operates in a pulsed mode, producing up to 2000 flashes with individually controlled length and time intervals. The fluorescence emission signal is collected by a 250 mm Cassegrian telescope (Meade 200 LX GPS, Meade Corporation, Irvine, CA), filtered with a 690 nm, 10 nm bandwidth interference filter, and detected by a cooled, large-area avalanche photodiode (LAPD 639-70-72-631 Advanced Photonix, Inc., Camarillo, CA). The detected signal is amplified by a variable, 0–96 dB gain preamplifier (AD 675, Analog Devices, Norwood, MA), digitized at 4 MHz, and numerically fitted into the theoretical model described by Equations (1–11).

The excitation laser diode is mounted on the body of the telescope, with remotely controlled pan (360°), and tilt (−45 to +60°). The instrument is equipped with a video camera (W500FL, Sony Corporation, NY) accessible via a video server (Axis 2400, Axis Communications Corporation, Chelmsford, MA) and is controlled by a dedicated PC (Optiplex GX260, Dell, Inc., Round Rock, TX) running a web server application. Control of the instrument, including selection of the excitation protocol, selection of the target, measurement, and data processing, are all performed remotely from any terminal connected to the Internet. In this configuration, the instrument, located in the B2L, Columbia University, AZ, was operated from Rutgers University, NJ, and from the Monterey Bay Aquarium Research Institute, CA, over extended periods of time.

Measurements

Most of the LIFT measurements described in this manuscript were performed in B2L in the Fall of 2003. Extended time series measurements (data points every 1 min for 3 days) were performed on a free-standing oak tree (*Quercus* sp.) growing outdoors at B2L, at a distance of about 40 m. Fluorescence quenching measurements were performed in ambient [CO_2] using wild-type, and the mutants L5 (PsbS protein over expressed), and npq4-1 (PsbS protein absent) of *Arabidopsis* (Li et al. 2002) at a distance of 14 m, with actinic illumination of 1200 μmol quanta m^{-2} s^{-1} from a video projector. Single rosettes of wild type and mutants (maintained in a growth chamber at 100 μmol quanta m^{-2} s^{-1}) were dark adapted for 30 min prior to brief exposures to actinic light, and examined with LIFT at 2-s intervals.

Comparisons with the saturating flash method (Mini-PAM)

The comparative LIFT and Mini-PAM measurements using the conventional pulse-modulated, saturating flash method were performed on cottonwood trees (*Populus deltoides*) in an agriforest mesocosm maintained at 400 ppm [CO_2] in B2L (Baron-Gafford et al. 2005). LIFT measurements were performed on one side of large cottonwood leaves targeted through the glass structure of B2L at a distance of 10 m, and PAM measurements were performed on the other half of the large target leaves using a Mini-PAM (H. Walz, Effeltrich, Germany) attached to a Licor gas analyzer (model Licor-6400, Licor Inc., Lincoln, NE). Light intensity was measured using the quantum sensor on the gas exchange system. The optimal quantum yield of PS II (F_v/F_m) of the leaf in the dark was calculated as $F_v/F_m = (F_m - F_o)/F_m$, with F_m being the maximum fluorescence measured when a

saturating light pulse of 800 ms duration (intensity $\approx$ 4000 μmol quanta m^{-2} s^{-1}) was applied. The effective quantum yield of PS II ($\Delta F/F_m'$) of the light adapted leaf was calculated as $(F_m' - F)/F_m'$, where F is fluorescence yield of the leaf in actinic light and F_m' is the maximum fluorescence yield when the saturating light pulse is applied in the actinic light (Genty et al. 1989; Schreiber and Bilger 1993). Non-photochemical quenching (NPQ) was calculated as $(F_m - F_m')/F_m'$ (Bilger and Bjorkman 1990). The apparent rate of photosynthetic electron transport of PS II (ETR) was obtained as $ETR = \Delta F/F_m' \times PFD \times 0.5 \times 0.84$, where the factor 0.5 assumes equal excitation of both PS II and PS I; and the factor 0.84 accounts for surface reflection.

Results

We measured F_m' at high laser excitation power (4600 μmol photons m^{-2} s^{-1}) and calculated F_m' from the fluorescent transient at normal operating laser intensity. A reasonable agreement between the experimental, and the theoretical fluorescence transient (Figure 1b) indicates that our model satisfactorily describes the measured fluorescence responses. We compared the LIFT measurements of $\Delta F/F_m$ against the PAM measurements of $\Delta F/F_m$ over the course of a day. Figure 2a shows the nearly identical data acquired by LIFT and Mini-PAM measurements on a cottonwood tree at low irradiance (0–150 μmol quanta m^{-2} s^{-1}). However, at high irradiance the LIFT-based ETR values are much noisier than the Mini-PAM data (Figure 2b). We attribute this behavior to a low signal-to-noise ratio under high irradiance levels. We also acknowledge about 9% difference between the LIFT and PAM measurements of F_v/F_m that may produce up to 9% systematic deviation between the PAM and LIFT-based estimates of ETR.

The LIFT method was developed primarily for continuous monitoring of the photosynthetic properties in the relatively inaccessible outer canopy of trees over long time periods (Osmond et al. 2004; Ananyev et al. 2005). The three-day time series of measurements on the upper part of an oak tree canopy shows an asymmetric daily cycle of $\Delta F/F_m'$, declining after sunrise, reaching a minimum level at about 10:00 a.m., and then

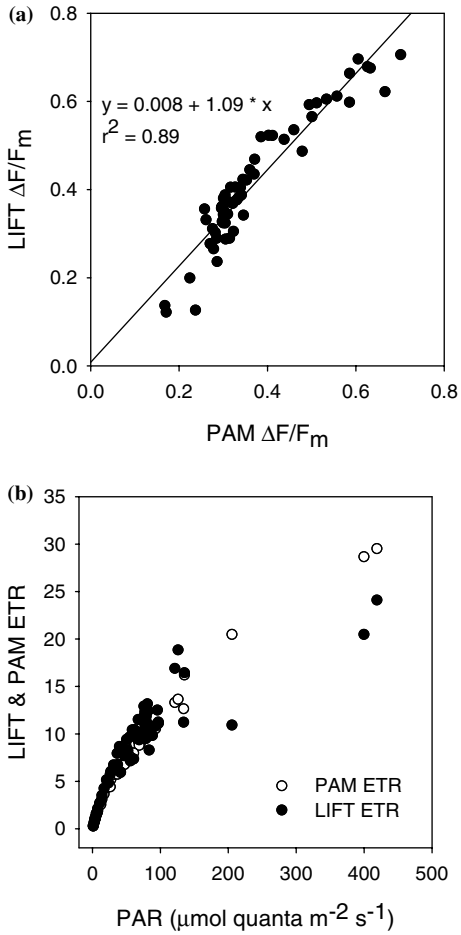

Figure 2. Comparison between the LIFT and Mini-PAM assessments of photosynthetic parameters. (a) Results from a cottonwood tree (*Populus deltoides*) in an agriforest mesocosm at 400 ppm CO_2 in B2L. (b) Calculation of photosynthetic electron transport rates based on the LIFT and Mini-PAM measurements of $\Delta F/F_m$.

recovering slowly towards sunset (Figure 3). The F_m' signal also displayed a strong daily modulation, with the maximum of the NPQ of about 2.8, not necessarily coincident with the minimum $\Delta F/F_m'$ signal. Interestingly, the observed F_m' signal displayed a transient increase following sunrise, and decreased to its minimal value (maximal NPQ) only after $\Delta F/F_m'$ signal decreased to its minimum level. The functional absorption cross section ($\sigma_{PS\ II}$), on the other hand, decreased immediately following sunrise, and increased toward sunset, in a highly symmetric pattern around noon. The amplitude of the $\sigma_{PS\ II}$ change (about 30% of the total) was much smaller than

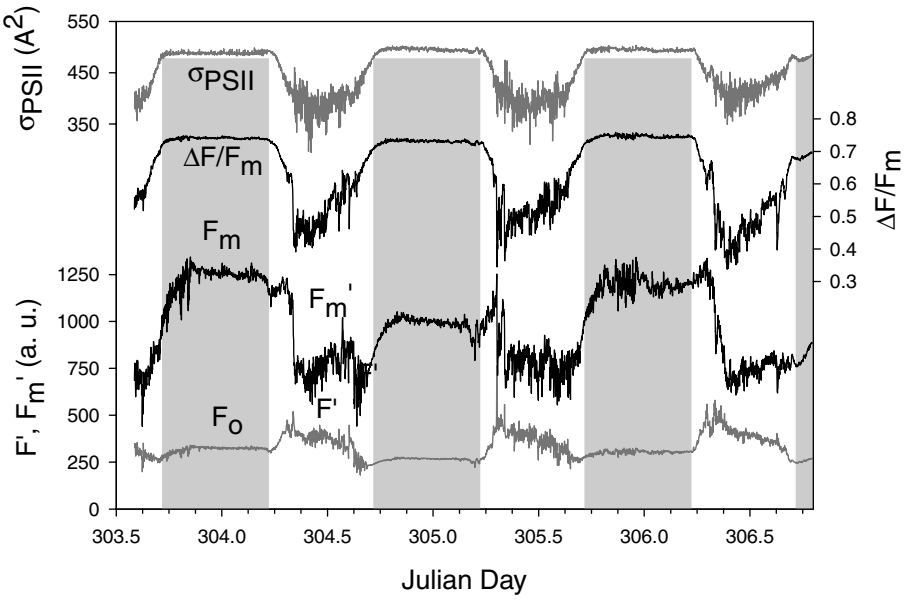

Figure 3. Example of long-term, autonomous LIFT measurement on a free-standing oak tree (*Quercus* sp.) growing outdoors at B2L, at a distance of about 40 m. The shaded areas indicate night periods. F' and F_m' are the initial, and the maximal levels of variable fluorescence (F_o and F_m in the darkness), σ_{PSII} is the functional absorption cross section of PSII.

the amplitude of the F_m' changes (up to 75% of the total).

The potential of LIFT to measure transient processes was assessed using wild type, L5, and npq4-1 mutants of *Arabidopsis* on transfers from 0–1200 μmol quanta m^{-2} s^{-1}, observed at 2 s intervals. The L5 mutant over expresses PsbS proteins, while the npq4-1 mutant is PsbS deficient, producing different patterns of NPQ development in strong light (Li et al. 2002). Again, we observed transient increase in the F_m' signal lasting about 5–10 s following dark/light transitions in wild type and both mutants. Because of the transient increase in F_m' on illumination, the dark F_m referenced NPQ traces in Figure 4 show initially negative values. However, the trends with illumination over periods of minutes show the expected patterns of NPQ dynamics, with lower level of NPQ in mutant npq4-1, and higher NPQ in mutant L5, compared to the wild type. The wild type (Figure 4a) displayed two characteristic components in the NPQ response; a fast component, with a $t_{1/2}$ of about 60 s, and a slow one, with $t_{1/2}$ of about 15–30 min. The L5 mutant had the highest amplitude of the fast phase (Figure 4b) and this was scarcely detectable in npq4-1 (Figure 4c).

Discussion

The results with *Arabidopsis* mutants are generally similar to those obtained using PAM techniques (Figure 4) (Li et al. 2002). They confirm interpretations of the components of NPQ associated with the levels of expression of the ΔpH sensing small protein subunit PsbS in the super complex of PS II. Our experiments with cottonwood leaves described here show better agreement between LIFT and Mini-PAM measurements than was obtained using the first prototype LIFT (Ananyev et al. 2005). Together these studies establish the feasibility of long term observation of photosynthetic responses to varying environmental conditions such as temperature, drought, and elevated atmospheric [CO_2], as well as the diversity of these responses in complex canopies (Osmond et al. 2004; Ananyev et al. 2005).

The LIFT theory and instrumentation described here facilitates remote measurements of important photosynthetic properties in terrestrial vegetation at a distance of up to 50 m. Although it is not possible to directly measure the absolute F_m signal under such conditions, we have demonstrated that this signal can be reliably derived from fluorescence transients induced by low-intensity,

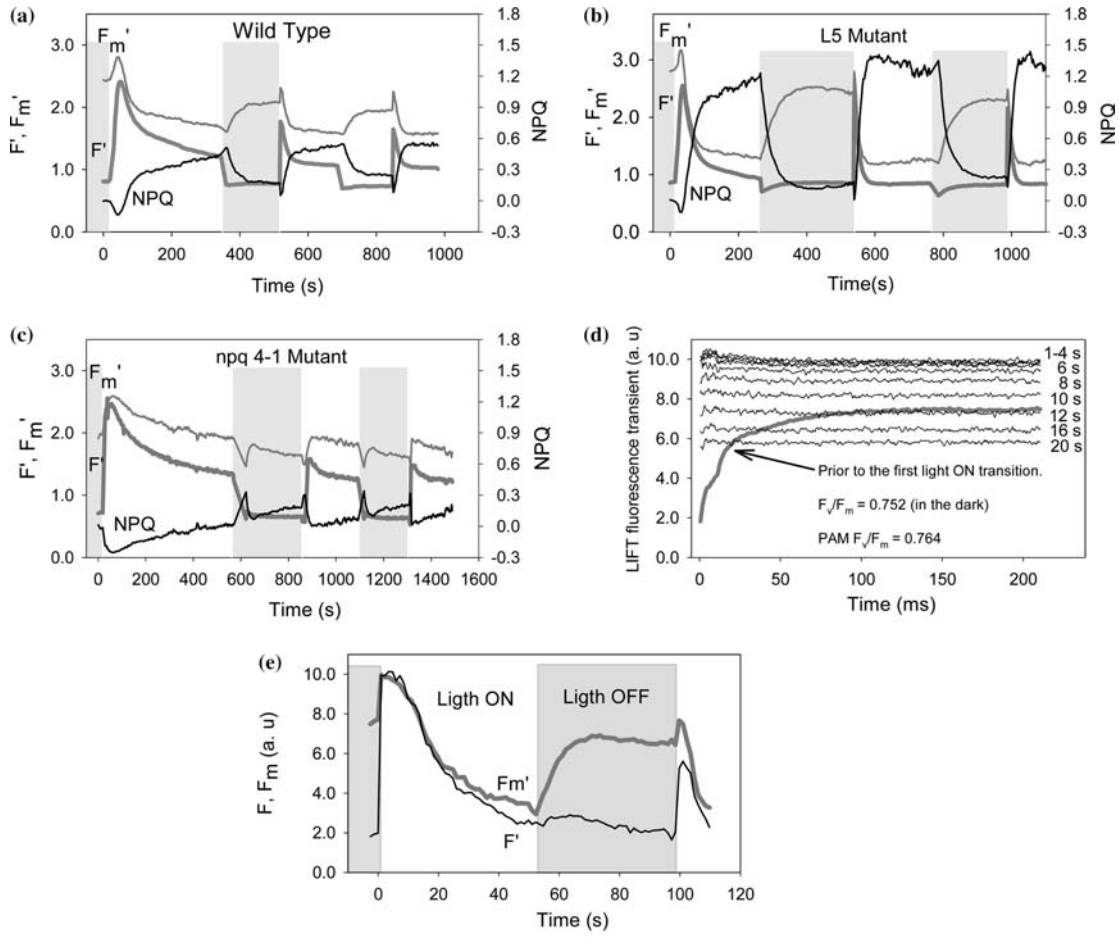

Figure 4. Dynamics of NPQ observed in the wild-type (a), the L5 mutant (b), and the npq4-1 mutant (c) of *Arabidopsis* exposed to a transient illumination of 1200 μmol quanta m^{-2} s^{-1} measured using LIFT protocol. The shaded areas indicate periods of darkness. No significant changes in dark F_v/F_m signal were detected over the course of the experiment (data not shown); (d) fluorescence transients measured directly on *Malva parviflora* L. leaves using 210 ms long excitation sequences at 4600 μmol quanta m^{-2} s^{-1} excitation power in the darkness (grey thick trace), and 1–20 s following exposure to 1200 μmol quanta m^{-2} s^{-1} ambient illumination (horizontal black traces); (e) corresponding F' and F_m' traces assessed from the first, and the last flash of the fluorescence transient measured during exposure to 1200 μmol quanta m^{-2} s^{-1} illumination, at one second time intervals.

high frequency, laser excitation (Figure 1b). We continue to experience some difficulties in estimating $\Delta F/F_m$ under high irradiances, where LIFT estimates of $\Delta F/F_m$ are usually noisier than those measured by Mini-PAM (Figure 2). The main problems are the relatively small amplitude of the measured fluorescence transients due to high levels of Q_A reduction at high irradiances, and large noise due to background fluorescence produced by the ambient light. These problems can be partially alleviated by extended signal averaging, and by employing excitation protocols utilizing periodically variable excitation power.

The delayed development of NPQ reported in Figure 3, and the transient negative NPQ reported in Figure 4 were the most unexpected observations. To examine the possibility that calculation artifacts were responsible for these results, we measured F_m directly, using a 210 ms long, 4600 μmol quanta m^{-2} s^{-1} excitation pulses (Figure 4d). The measured F_v/F_m was 0.752, almost identical to that of the corresponding PAM F_v/F_m (=0.764), indicating a level of Q_A reduction close to that produced by the PAM excitation signal. Both the F' and F_m' measured with high excitation power displayed a transient increase

above the dark-level F_m during the first light–ON transition (Figure 4e), similar to that measured with low excitation power LIFT (Figure 4a–c). We could not confirm these observations with the PAM method (Schreiber and Bilger 1993). The integrated excitation energy used to induce the F_m signal in the PAM method exceeds the excitation energy used in our protocol by about five times, which may eliminate the observed positive F_m transient within the first flash of the PAM protocol. The ability of LIFT to record NPQ transients in natural ecosystems, over long time scales, should reveal the patterns of environmental control over this phenomenon.

The LIFT instrument is designed to either be controlled remotely, from any terminal connected to the Internet, or to operate automatically to execute a set of preprogrammed measurements. The acquired data, in raw format and in processed format are immediately available by logging into the LIFT server. As demonstrated in Figure 3, this mode of operation allows systematic monitoring of photosynthetic parameters over extended time periods. Another possible application of the LIFT technique is demonstrated in Figure 4. Because the instrument can be programmed to perform rapid measurements on an array of targets, it can be used for fast screening of plant mutants, in natural or controlled, high security environments, over the whole cycle of plant growth, and with minimal sample manipulation.

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