ASSESSING PHOTOSYNTHESIS IN VIVO: A NEW HIGHLY SENSITIVE INSTRUMENT TO MEASURE ABSORPTION AND FLUORESCENCE CHANGES.

This new photometer is designed along the lines of the so called 'Joliot-type' spectrophotometers which allows probing photo-induced absorption changes by short detecting pulses. These pulses, of 15 µs duration, are provided by broad-band Light Emitting Diodes (LED), filtered through interference filters (FWHM 3-10 nm) to select wavelengths of interest. The optical design of this set-up is suitable to study highly scattering and/or strongly absorbing objects with high sensitivity and time resolution. It can easily accommodate various samples including liquid suspension and whole leaves.

The basic version includes several LED arrays of various wavelength range covering the spectral region of carotenoids band-shifts, the alpha-band of cytochrome, the Q_X region of chlorophylls etc …Three different continuous actinic sources are included (520 nm, 620nm and 720 nm). Yet, additional continuohs or pulsed sources can be easily connected for specific requirement.

The same set-up allows fluorescence measurements. Here again two actinic light sources may be used: a continuous source, specially designed to be homogeneously distributed on the sample, or a flash lamp source. The set-up is thus suited for most fluorescence measurement usually performed to asses the photosynthetic activity such as NPQ measurement, kinetics of light-induced fluorescence yield change, flash-induced fluorescence relaxtion kinetics etc … The set-up is computer-controlled. Data can be acquired, visualized and treated by a user-friendly program.

Advantages:

- A single set-up with two applications: a photometer and a fluorimeter
- A pump probe design using LED as probe pulses.
- An unequalled, quantum noise limited, sensitivity: 10^{-5} O.D. combined to an excellent time resolution (15 µs).
- Suitable for samples up to 2 O.D. and highly scattering samples.
- The time between two successive probe pulses may be as short as 100 µs.
- Probe pulses may be distributed at will, to follow kinetics of absorption changes occurring in the 20 µs to several minute time-range.
- Exchangeable sample holders allowing accomodation of various sample geometry extending from liquid suspension to thin solid layer.
- A library of pre-defined pump/probe sequence suited for most of the 'must-do' experiments.

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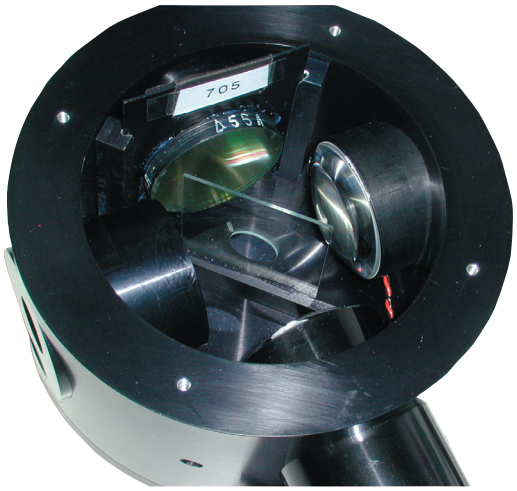
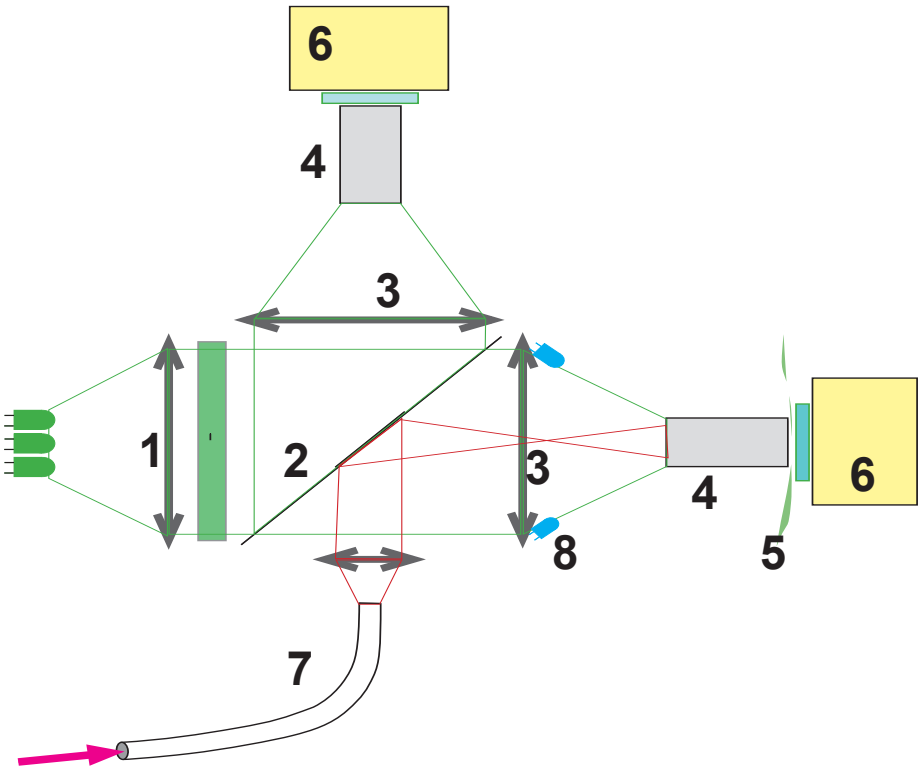
Optical design.



The probe beam is collimated by a lens (1) onto an interference filter and split into two beams of unequal intensity by a glass plate (2). These two beams are then focused by a lens (3) onto light guides (4) in order to even the light intensity on the surface of the sample. The measuring beam passes through the sample (5). The intensity of both beams are measured by photodiodes (6), protected from the actinic light by broad band cut-off filters. A light pipe (7) is used to shine the actinic light onto a small mirror, in the center of the separating glass plate. Most types of actinic source may be easily plugged in the instrument. A LED array (8) located at the entrance of the light guide in the measuring line may be used as an alternative actinic light source. When detecting fluorescence change, a light source made of green leds may be plugget in a position (1) and may be used either under a continuous or pulsed mode. These various light sources are triggered by the computer.



Probing light sources

Various LED arrays are available: white, blue, green red or near infra-red.

Pumping light sources

LED, continuous or pulsed Lasers, Laser diodes

Sample holders

- Liquid sample: the set-up may accommodate temperature controlled cuvettes with a path length of 2 or 10 mm and a circular surface with a diameter of 12 mm.
- Leaf: the leaf may be placed in a gas controlled and/or temperature controlled chamber.

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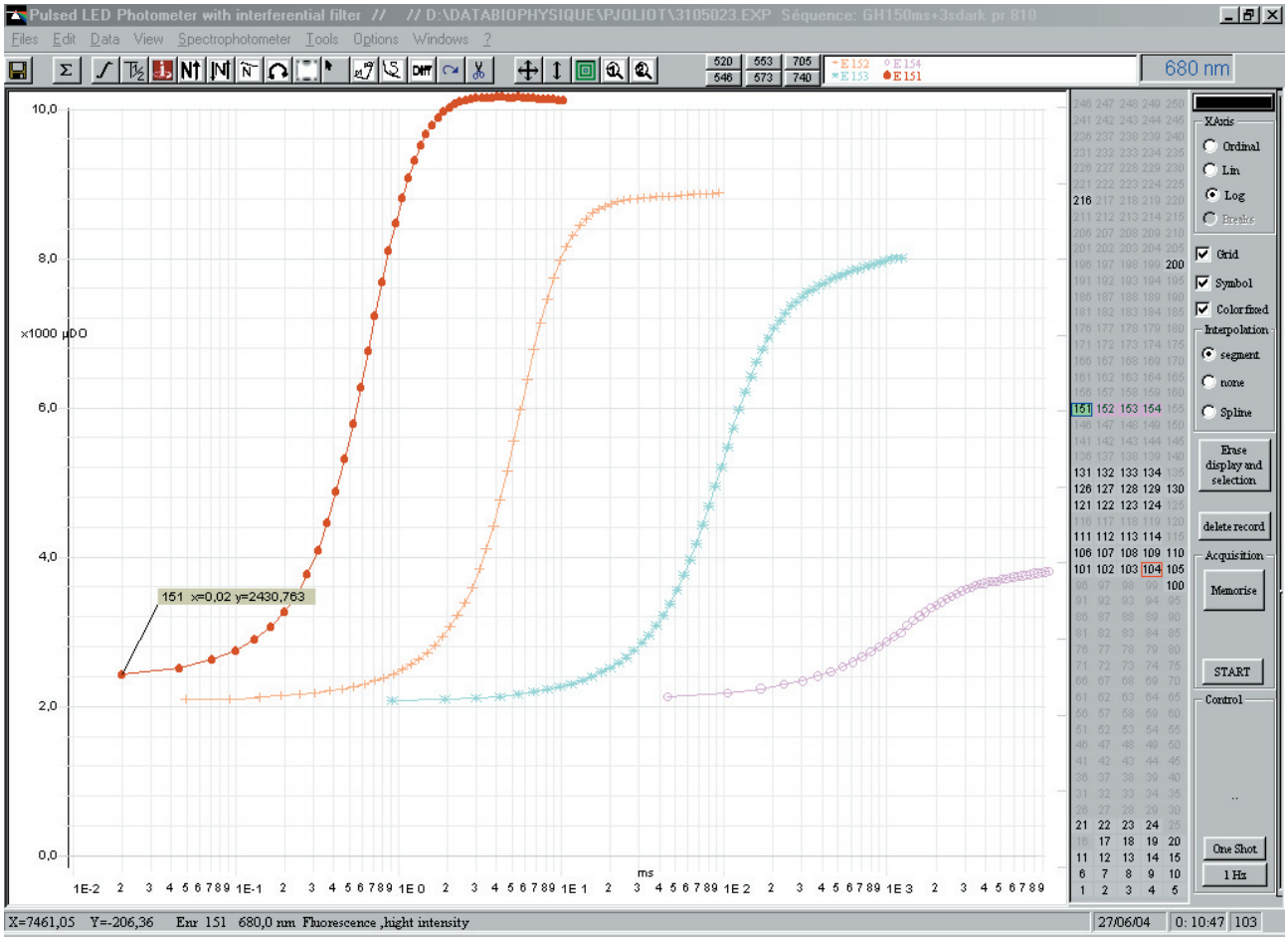
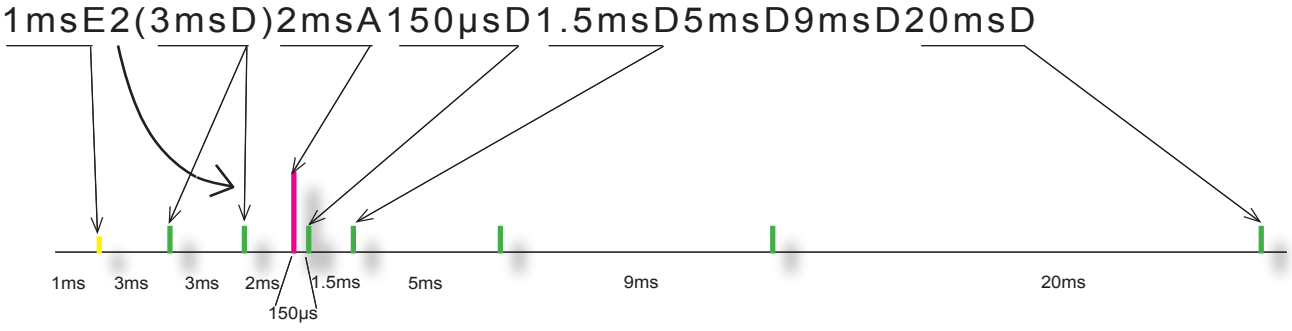
A User friendly Software.

Set-up control, data acquisition and treatment are run by a unique home made software. Similarly switching from absorption to fluoresence measurement is computer controlled. A genuine programming language has been developped to



Main graphic window displaying four kinetics of fluorescence yield changes observed in the presence of DCMU with four different light intensities. The time scale ranges from 20µs to 10s.

define the sequence of the detecting and or actinic pulses. The software is multi-user: several user profiles with specific configuration and sequences are allowed. Data visualization and treatment is easily achievable through icons, windows, scrolling menus etc ....



A schematic representation of a user defined pulse sequence

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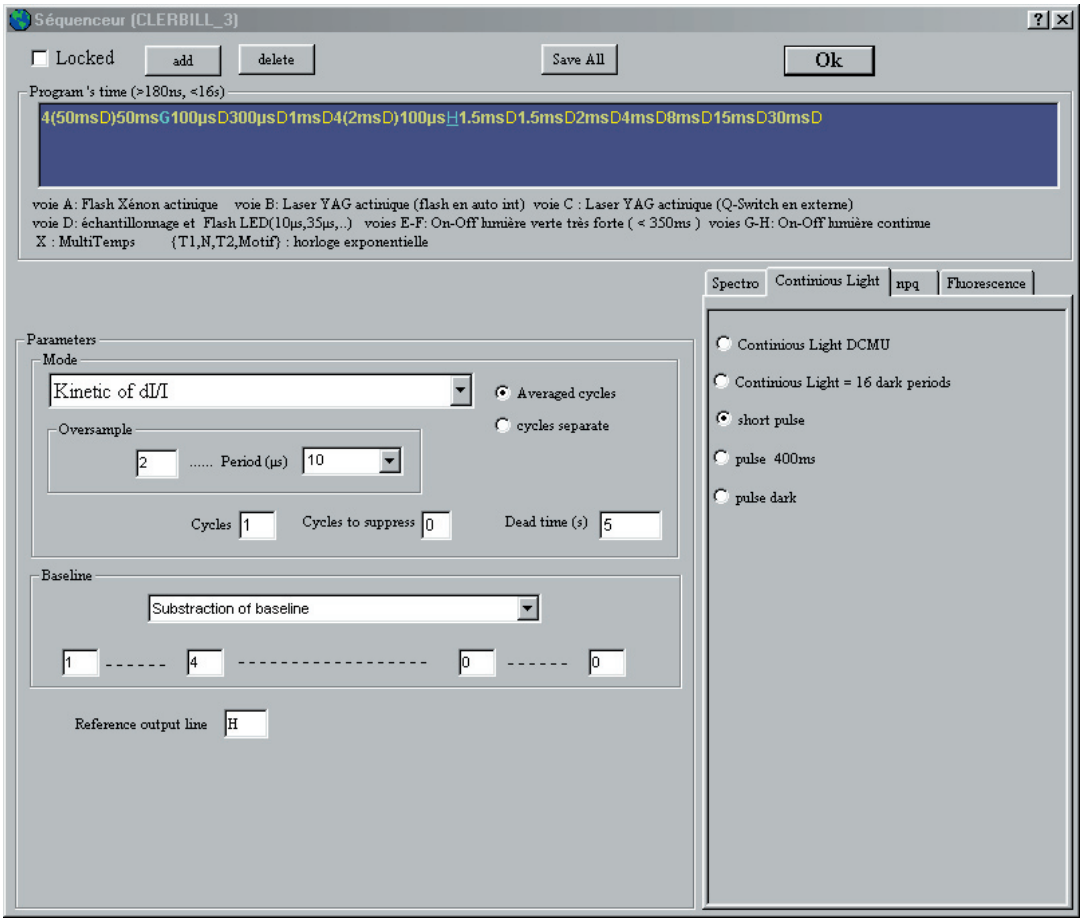
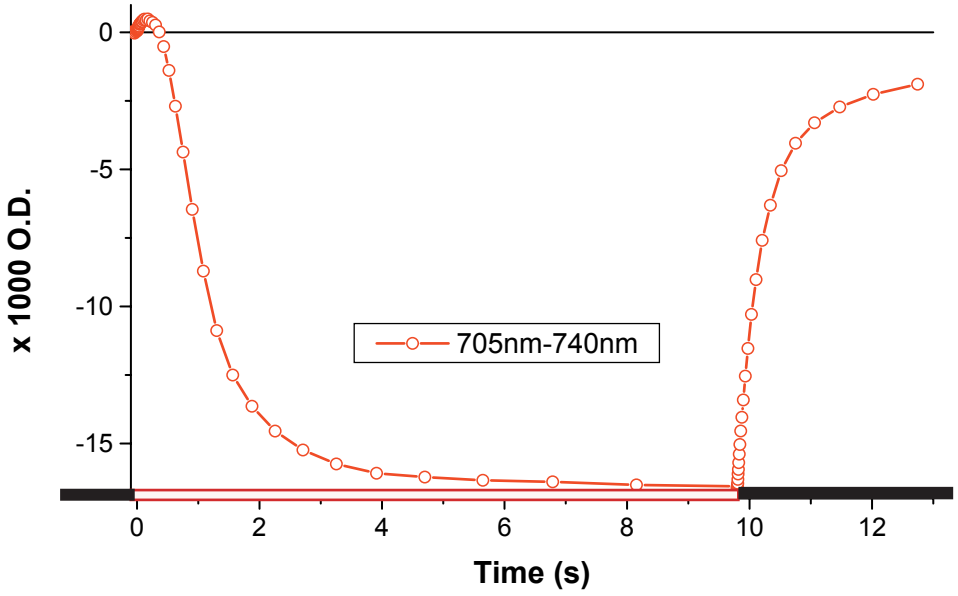


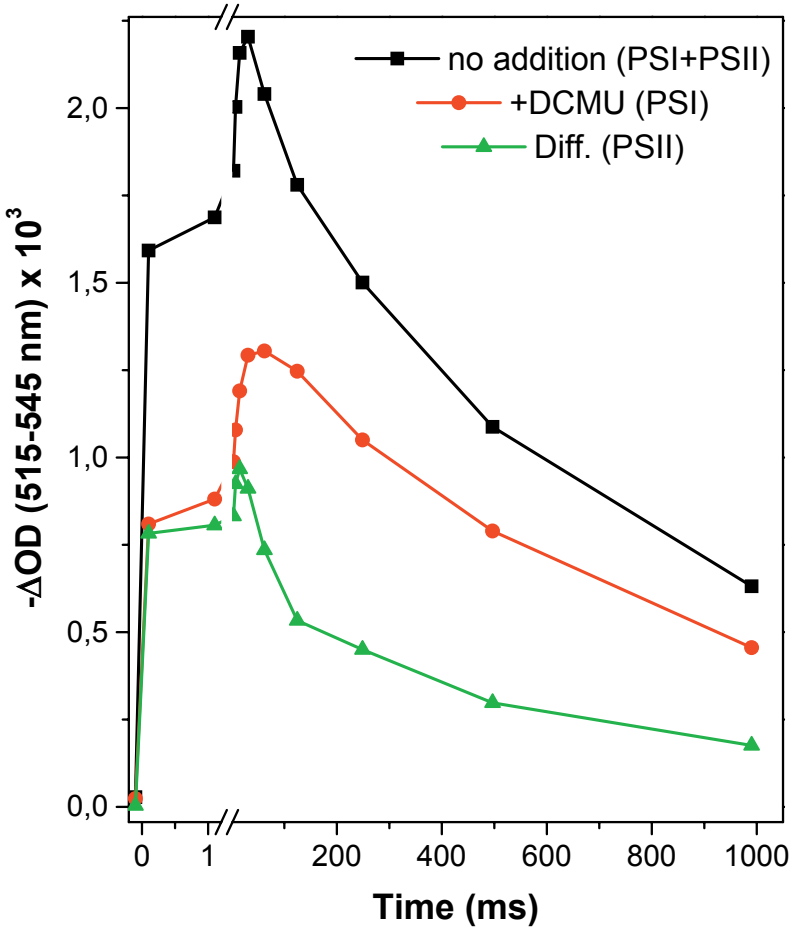
The various detecting or actinic light sources are triggered by TTL pulses. Eight BNC cables (noted A-H), corresponding to eight different possible light sources, feed the detecting LED or actinic sources with ON/OFF pulses.
The time delays between successive pulses is programmed and converted into a pulse sequence profile by the sequencing device. The delays between pulses (which can vary from 1 µs to 16 s) are defined with three significant digits. Alternatively a geometrical series may be used to define successive time delays in a sequence.
A library of predifined sequences is provided. These may be modified at will.

A few examples of time resolved absorption changes.



Absorption changes reflecting P_{700} oxidation induced by the continuous illumination of an *Arabidopsis thaliana* leaf with a LED source peaking at 720 nm; followed by its reduction in the dark.

Determining the relative amount of PSI and PSII in whole leaves: transient absorption changes induced at 520 nm by a saturating laser flash. The first detecting pulse is 100 µs after the actinic flash and yields a signalproportional to the amount of active photosystem. PSII may be inhibited by addition of DCMU and hydroxylamine. Under these conditions the signal is proportional to the sole amount of active PSI.



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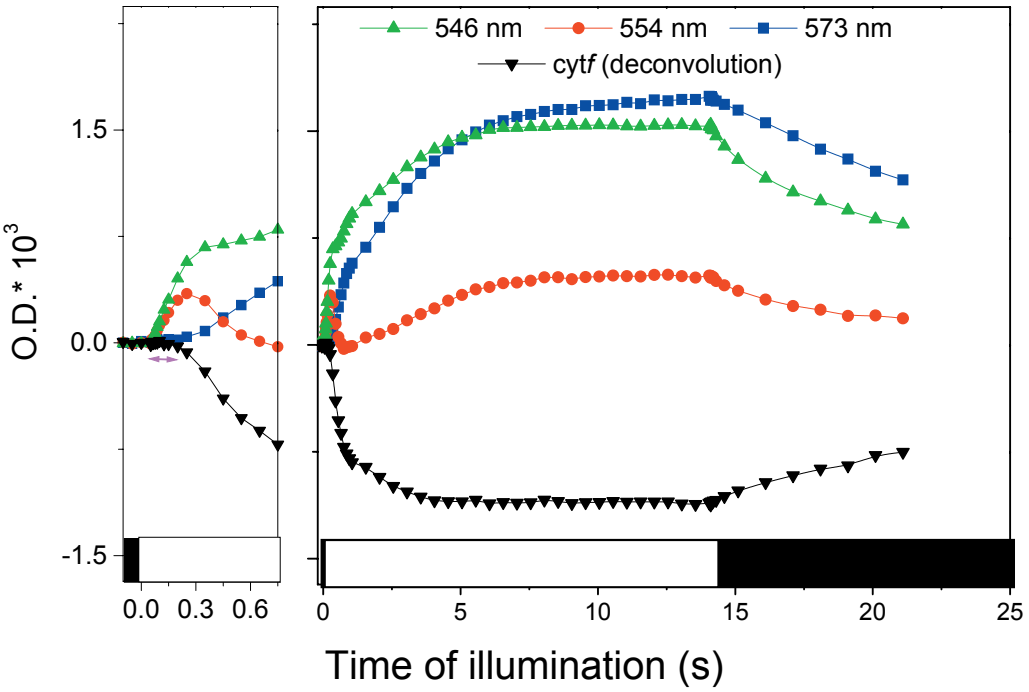
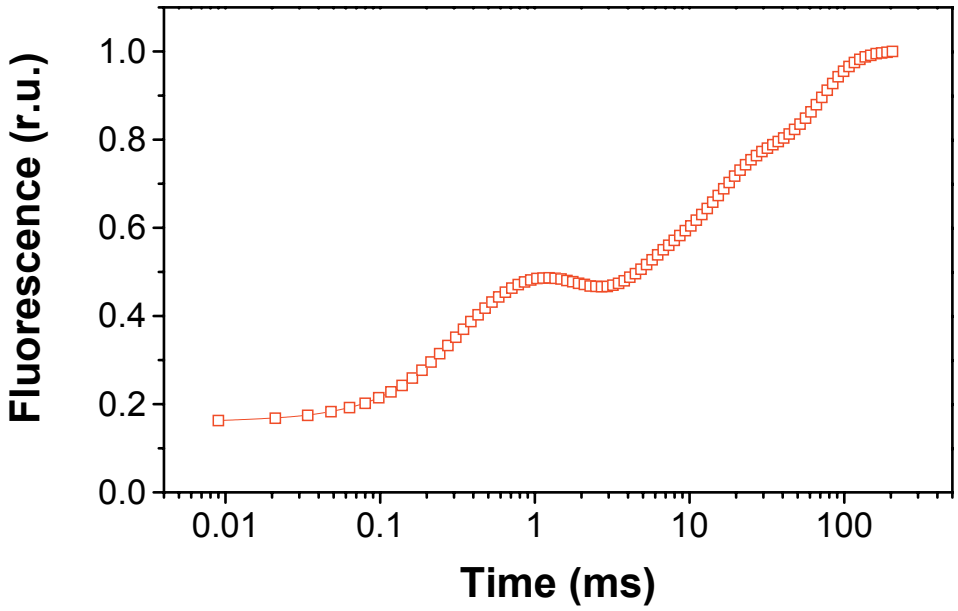
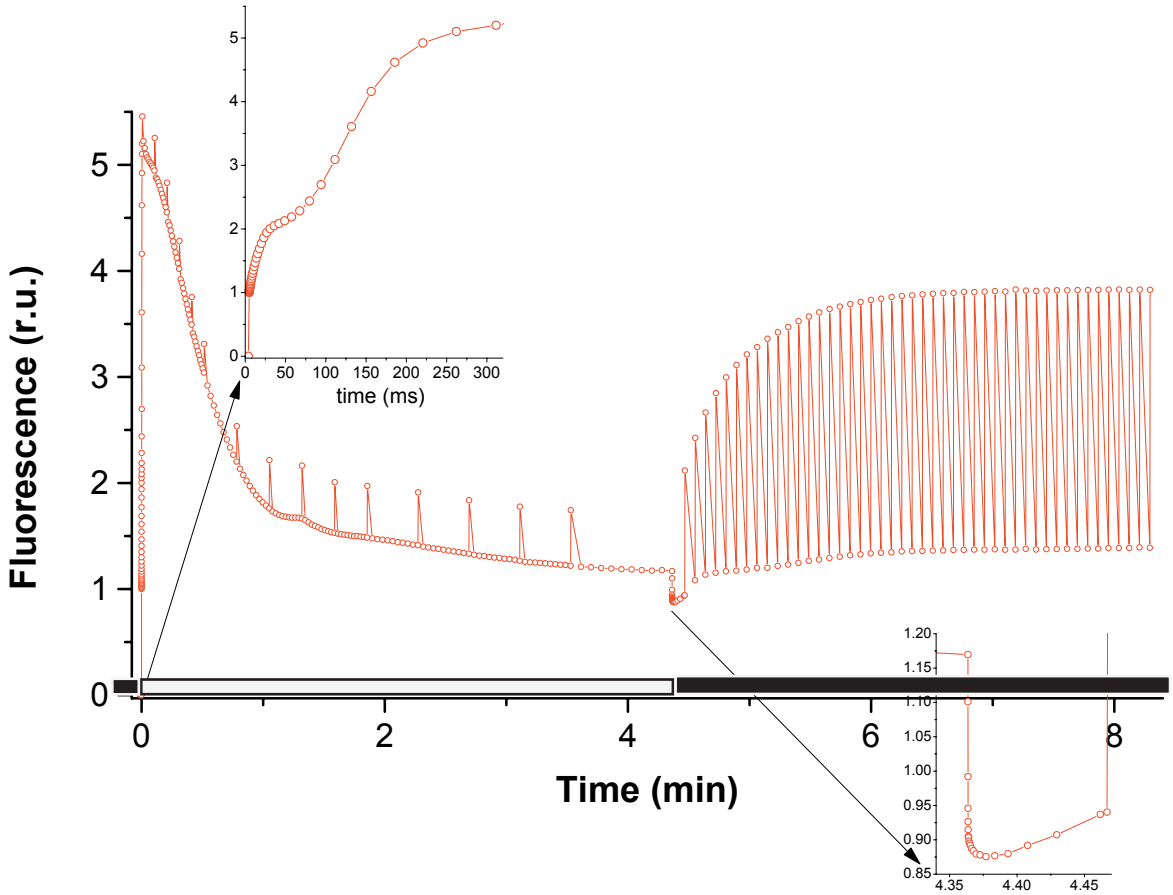


Transient absorption changes induced by a continuous illumination with a 720 nm LED at 546 nm and 554 nm. The difference between these two transients is indicative of the kinetics of the cytochrome f redox changes.

A few examples of fluorescence yield measurement



Fluorescence yield changes induced by a continuous illumination (@520 nm, 5500 µE/m^2/s) with an *Arabidopsis thaliana* leaf.



Fluoresence yield changes induced by the illumination of a dark adapted *Arabidopsis thaliana* leaf by a 520 nm contiuous light (200µE/m^2/s). The maximum fluorescence yiedl is assesed by the superimposition of short (200 ms) intense pulses 5500µE/m^2/s. The insets are zoomed on the transient changes observed upon the onset and the offset of illumination.

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TECHNICAL SPECIFICATION

OPTICS

Wavelength selection	Interferential filter	
Optical resolution	6 nm ou 10 nm (< 6 nm with less sensitivity)	
Wavelength range	380nm-980nm	
Sensitivity	10^{-5} O.D. for O.D. less than 2, without averaging	
Time resolution	15µs	
Time range	20µs to 1 minute	
Cuvette	12mm diameter x 2 mm or x10 mm for solutions 2x6 mm^2 for leaf of thin object	
Detecting light source	white leds	(510nm-630nm)
	Near ir leds	(700nm-750nm), (800nm-900nm)
	Blue leds	(380nm-480nm) Option
	Cyan leds	(490nm-500nm) Option
Actinic light source	green Leds	525nm
	orange Leds	620nm
	Near ir leds	720nm
	Options: leds blues, red..... Lasers continuous or pulsed Xenon flash	
Size (mm) :	~ 400(l)x300(w)x100(h)	

CONTROL UNIT

A/D Converter	two ADC 18 bits, 3 µs
Gain	differential gain of 8
Trigger output	12 TTL
Sync input	1 rising edge triggered TTL input
Number of detecting flashes	Maximum 1000
One delay time	1 µs to 16 s (3 digits)
Jitter	1ns max
Size	~ 400(l)x300(w)x200(h)

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