WinControl-3

Windows Software for PAM Fluorometers: WATER-PAM Edition

Users Manual

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1 WinControl-3: Installation

The provided <Software & Manuals CD-ROM> contains the complete collection of the Walz Software & Manuals. The CD starts the default internet browser of your computer. (If automatic browser start fails, double-click on <index.html> in the root directory of the <Software & Manuals> CD-ROM.)

Choose <Fluorescence Products> → <WATER-PAM> → <PC software WinControl-3>. Clicking on <WinControl-3> will start software installation. Please follow instructions of the installation wizard.

The setup routine will create the folder <WinControl-3> containing WinControl-3 software in the <c:\Program Files> directory. Further, a USB serial converter driver will be installed, and shortcuts to the WinControl-3 software will be created in the <Program> section of the Windows <Start> menu and elsewhere, depending on your selection.

Using shortcuts in the <Start> menu, you can run two instances of WinControl-3 at the same time: one in the measuring mode (choose <WinControl-3>) and another one in the offline mode (choose WinControl-3 - Offline>). Note that <WinControl-3 - Network Mode> does not work with the WATER-PAM fluorometer but requires WinControl-3-compatible devices capable of RS485 communication (e.g., MONITORING PAM fluorometer). The measuring mode of WinControl-3 is also initiated by double-clicking on the WinControl-3 shortcut on the Windows desktop.

After launching the measuring mode of WinControl-3, the program scans for WinControl-3-compatible devices connected to the computer. With the WATER-PAM attached, the bottom of the opening window of WinControl-3 displays address number and name of your WATER-PAM, e.g., <#1: PAM-CONTROL #1>.

2 Operation: WinControl-3 WATER-PAM Edition

2.1 The Initial Window / Chart Window

WinControl-3 offers the standard options of Windows operating systems to manage program windows size.

Figure 2.1 depicts the initial window of the WinControl-3 software. The window is graphically divided into 11 sections: 10 boxes and the chart area. The graphical divisions will be used for orientation during the subsequent introduction to WinControl-3 functions.

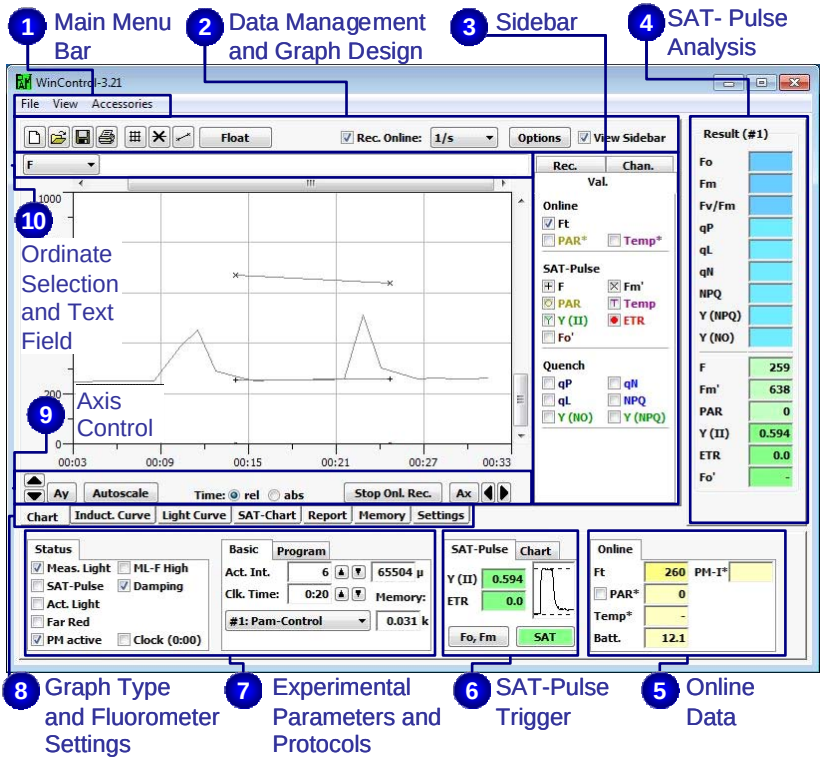


Figure 2.1: Initial Screen of WinControl-3

2.1.1 Box (1) - Main Menu Bar

<File>

<Load Data> Delete current data and load saved data. Note: loading data with <Rec. Online> checked (Box (2)) will display the currently recorded data rather than the loaded data. To view loaded data, select record <1> after selecting the <Rec.> tab in Box (3).

<Save Data> Save all data of the current experiment.

<Quit> Exit WinControl-3.

<View>

<Results Panel> Unchecking suppresses Box (5).

<Status Panel> Unchecking suppresses and Boxes (7) to (9).

<Warnings> Checking prompts the display of program starting time and non-critical errors. All changes are reversible.

<Batch Window>

Opens batch file window. Batch files are small programs for automated operation of WinControl-3 compatible devices.

<Accessories>

<Temperature Units>

Allows to toggle between °Celsius and °Fahrenheit as unit of Temp* in Box (7), but does not affect the chart temperature ordinate.

<Record File> The command prompts WinControl-3 to continuously save data and, thereby, minimize data loss in the case of program failure. The tick, <✔>, associated with <Record File> indicates that continuous saving of data is active. Data are saved

in the PAM data file format (filename.pam). If an existing file name is selected, a dialogue window appears which offers the options to delete the existing file or to append the new data to data of the existing file.

2.1.2 Box (2) -Data Management and Graph Design

<Delete All Data> Delete current data (all records and report data).

<Load> Delete the current data and load previously saved data. Same function as <Load Data> in the <File> menu.

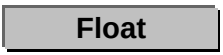
<Save> Save all records of the current experiment. Same function as <Save Data> in <File> menu.

<Print Chart> Print the current chart.

<Switch Grid on/off> Graph design command.

<View Symbols> Graph design command.

<View Lines> Graph design command.



<Float> Creates a floating chart window. Graph settings and data selection of the new chart are independent of the WinControl-3 main window.

<Rec. Online> Checking initiates continuous recording of Ft, PAR* and Temp*. <Rec. Online> does not affect feeding of the chard. For long-term measurements, <Rec. Online> may be turned off to keep data size reasonable.

<Sampling Frequency> The downward arrow gives access to three different sampling frequencies: <5/s>, <1/s>, and <1/10s>. The frequency setting affects only online data (Ft, PAR*, and Temp*, see below). The highest sampling frequency of <5/s> is available only for the Ft: sampling of PAR* and Temp* occurs with <1/s>

maximally. The actual intervals between measurements vary depending on communication between fluorometer and computer. The exact times points of measurements are recorded and, using the data export function, provides online data along with exact time values.

Options <Options> includes four menu items of which two (<Zoom to Selection> and <Export Selection>) are available only after having selected data in the chart. To select data, place mouse cursor in the <Chart> area, move mouse cursor with left mouse button pressed parallel to the x-axis across the data of interest: the selection will be highlighted. The options menu is also available by placing the cursor within the selected area and clicking the right mouse button. A single left-button click in the chart area removes an existing selection.

<Export All> exports all data currently graphed (online and saturating pulse analysis data) as CSV file which can be imported by most spread sheet or graphing programs. Exact ms time scales are provided for each measuring point.

<Select displayed record> selects data of the choosen record.

<Zoom to Selection> Choosing the command increases time axis resolution according to your selection. The action can be reversed by clicking in <Ax> icon (Box 11).

<Export Selection> exports selected data as CSV file similarly as described above (compare <Export All>).

☑ **View Sidebar** Controls display of Box (3)

2.1.3 Box (3) - Sidebar

Three Tabs (<Val.>, <Rec.> and <Chan.>) permit switching between different sidebars.

<Val.> sidebar

In the <Val.> sidebar, all types of fluorescence measurements and data calculated by WinControl-3 are represented by acronyms. The graphical presentation of data is controlled by checkboxes attached to the acronyms. In the sidebar, the data are distinguished into three groups, <Online>, <SAT-Pulse>, and <Quench>.

Note that Chapter 6, *Acronyms and Equations*, provides detailed information on fluorescence measurements and equation used by WinControl-3.

<Online> data are continuously reported with the frequency defined in Box (2) and the reservation reported above. The data group includes:

Ft Chlorophyll fluorescence yield (relative units).

PAR* Photosynthetic active radiation (µmol photons · m^{-2} · s^{-1}). With a quantum sensor connected to PAM-CONTROL unit and the <PAR*> in Box (5) checked, the PAR* shows data of the external sensor.

Temp* Temperature (°C). In the absence of an external sensor, temperature is not displayed.

<SAT-Pulse> data are recorded with light-exposed samples close to or during application of strong light pulses. Specifically:

F Actual fluorescence yield intensity at any time. The F used in saturating pulse analysis is measured shortly before onset of a strong light pulse (relative units).

Fm' Maximal chlorophyll fluorescence yield when photosystem II reaction centers are closed by a strong light pulse (relative units).

Fo' Minimum chlorophyll fluorescence yield in the state of open reaction centers. The Fo' is either calculated or measured in the presence of far red illumination with actinic light switched

off. Measuring of Fo' fluorescence is activated by ticking <Fo'-Mode> in Box (7) (Fo'-Mode will soon be available as batch file for WATER-PAM).

Y(II) Effective photochemical quantum yield of photosystem II; derived from F and Fm' measurements.

ETR Electron transport rate (µmol electrons/(m^2·s) derived from Y(II) and PAR.

PAR Photosynthetic active radiation (µmol photons/(m^2·s)). (= PPFD, photosynthetic photon flux density.)

Temp Temperature (°C) of an external sensor

<Quench> data quantify fluorescence quenching caused by photochemical energy use or non-photochemical energy dissipation. All fluorescence parameters related to non-photochemical quenching require both, saturating pulse analysis of the dark-acclimated and light-exposed leaf. Coefficients of photochemical fluorescence (qP and qL) require fluorescence measurements with dark-acclimated material only if Fo´ is calculated (see 3.1.2).

qP Coefficient of photochemical fluorescence quenching.

qL Coefficient of photochemical fluorescence quenching assuming that many photosystem reaction centers share a light-harvesting antenna (lake model). In comparison, the qP is more consistent with separated photosystems (puddle model).

qN Coefficient of non-photochemical fluorescence quenching.

NPQ Non-photochemical fluorescence quenching: quantification of non-photochemical quenching alternative to qN calculations. The extent of NPQ has been suggested to be associated with the number of quenching centers in the light-harvesting antenna.

Y(NPQ) Quantum yield of non-photochemical fluorescence quenching due to down-regulation of the light-harvesting function.

Y(NO) Quantum yield of non-photochemical fluorescence quenching other than that caused by down-regulation of the light-harvesting function.

Note that Y(II)+ Y(NPQ)+ Y(NO)=1

<Rec.> sidebar

Clicking the icon <New Record> starts a new record which is added to the currently loaded data. The <Rec.> sidebar lists, as consecutive numbers, each experiment recorded since the program start or since the last time the command <Delete All Data> was carried out. Each record can be selected and, thus, displayed in the chart by left-clicking on the record number.

Note: when induction and light curves are displayed, the <Rec.> sidebar lists the presently loaded induction curves (IC) and light curves (LC), respectively. In the latter display modes, new induction or light curves are initiated by starting the corresponding automated exposure program (Box (7)). Several induction or light curves can be carried out during a single record.

<Chan.> sidebar

The <Chan.> sidebar lists all WinControl-3 compatible devices connected *via* USB to the computer. Note: at any one time, only one PAM-CONTROL based PAM can be operated by WinControl-3, but the combination of 1 PAM-CONTROL fluorometer and other WinControl-3 compatible devices (higher-order PAM fluorometers or light meters) is possible.

2.1.4 Box (4) - SAT Pulse Analysis

The headline of Box (4) displays the number of the device connected. With the WATER-PAM connected, the headline reads <Results (#1)>. Also, three groups of saturating pulse data are displayed in Box (4). Group 1 (Fo, Fm, Fv/Fm) includes data measured with a dark-acclimated sample before light-exposure but group 3 (F to Fo') represent data obtained during illumination. Group 2 represents fluorescence parameters characterizing the state of photosynthesis in the light. Some of the group 3 data, however, require fluorescence measurements with the dark-acclimated sample that is Group 1 data.

Group 1 Fluorescence data from a dark-acclimated sample with actinic light switched off.

Fo Basic fluorescence yield (relative units) recorded with low measuring light intensities.

Fm Maximal chlorophyll fluorescence yield when photosystem II reaction centers are closed by a strong light pulse (relative units).

Fv/Fm = (Fm-Fo)/Fm; maximum photochemical quantum yield of photosystem II.

Group 2 The group includes data of qP, qL, qN, NPQ, Y(NPQ), and Y(NO) and, thus, matches the group of <Quench> data in the <Val.> sidebar (see above). If Fo' is calculated (see 3.1.2), calculations of all 6 types of data require both, initial fluorescence determinations with the dark-acclimated sample and saturating pulse measurements during exposure. If Fo'-Mode is checked, Fo or Fm fluorescence data are not considered in calculations of qP of qL (see chapter 3).

Group 3 The group corresponds to the <SAT-pulse> data in the <Val.> sidebar (see above) and includes F, Fo' and Fm' of the last saturating pulse analysis during light exposure but also data of

Y(II), PAR and ETR. Note: a tilde (~) as prefix to the value of Fo' indicates that the Fo' is calculated as described in 3.1.2 and not measured during a post-pulse interval with only the far red illumination switched on (Fo'-Mode, Box (7)).

2.1.5 Box (5) - Online Data

Online data in Box (5) represent digital values of current measurements of the <Online> data. Checking the PAR* will activate the PAR measured by an external quantum. PM-I* displays the photomultiplier current, Temp* the measured temperature of an external temperature sensor and Batt. indicates the voltage of the internal battery of PAM-CONTROL.

2.1.6 Box (6) - SAT-Pulse Trigger

In Box (6) includes the two tabs SAT-Pulse and Chart. In the SAT-Pulse tab, both, the <Fo, Fm> and the <SAT> icon, manually releases a saturating light flash of interval and intensity defined in <Settings>. Clicking the <Fo, Fm> determines the fluorescence yields <Fo> and <Fm>. By definition, the <Fo> and <Fm> levels are properties of dark-acclimated photosynthesis. With light-exposed samples, saturating pulse analysis is initiated by the <SAT> icon to determine F, Fo' and Fm'. In total, WinControl-3 derives 9 different fluorescence quotients from the 5 types of fluorescence yield (see Box (3) and Box (4)). The current value of one quotient, the Y(II)=(Fm'-F)/Fm', is displayed in Box (6) together with the corresponding fluorescence trace during saturating pulse analysis. In the graph, dashed lines indicate the levels of Fm' and F used for Y(II) calculation. The graph is viewed enlarged after clicking the <Chart> tab.

2.1.7 Box (7) - Experimental Parameters and Procedure

Box (7) includes 3 groups of commands which determine different aspects of experimental procedures: the <Status>, <Basics> and <Program> field: tabs allow switching between the latter two fields.

<Status>

The <Status> field provides access to the 8 central functions of the WATER-PAM fluorometer. A checkbox is associated with each function listed. Checkboxes represent on-off switches but they also report the status of measuring and actinic light when the WinControl-3 software runs the WATER-PAM automatically. Settings of all 8 fluorometer functions are stored on a microcontroller in WATER-PAM fluorometer and kept until power supply is disconnected.

The fluorometer functions are:

<Meas. Light> Low frequency measuring light.

<ML-F high> High frequency measuring light. To become active, <Meas. Light> needs to be checked. Note that measuring light changes automatically to high frequencies at actinic light intensities higher than level 3 and during saturating light pulses.

<SAT-Pulse> Saturating light pulse initiates a saturating pulse analysis to determine F, Fo' and Fm' and, hence, the <SAT-Pulse> corresponds to the <SAT> in Box (6).

<Act. Light> Actinic light.

<Far Red> Far Red Light.

<PM-active> Photomultiplier status

<Damping> Signal Damping of factor 10. When Damping is active, it is automatically inactivated for 2 seconds when rapid signal changes occur at low frequency of measuring light (following a saturation

pulse, following actinic illumination, after switching photomultiplier on and off).

<Clock> Triggers a process with the interval defined as <Clk. Width> in the <Basic> field (this Box) or in <Settings> (see below). Also, the event to be triggered is defined in <Settings>.

<Fo'-Mode > Follows up a saturating pulse with an interval with actinic light switched off and far red illumination on, and takes the fluorescence yield measured at the end of this interval as Fo' fluorescence. This command will soon be available as Batch File for WATER-PAM.

<Basic>

The <Basic> window permits adjustments of the level of PAR (<Act. Int>) and the clock interval (Clk. Width). Similarly as settings in the <Status> window, the <Basic> adjustments are stored in the WATER-PAM fluorometer. Also, the lower-most line of the <Basic> window states the WinControl-3 compatible instruments connected to the computer. If the Water-PAM is the sole WinControl-3 compatible instrument, the instrument display is invariable. Further, the <Memory:> window (lower-most line) reports the number of data sets recorded divided by 1000. Data set numbers greater than 100 k might compromise the efficiency of WinControl-3, depending on the computer used. The accumulation of huge data set numbers during long-term measurements can be avoided by switching off the <Rec. Online> (Box (2)).

The adjustable parameters of the <Basic> window are:

<Act. Int> 12 levels of PAR can be selected by clicking the up or down arrow in the <Act. Int> line. In the same line, the photon flux density (in µmol photons/($m^2 \cdot s$)) of the PAR level chosen is displayed. Note

that these photon flux densities represent values of the internal PAR list.

<Clk. Width> Clock widths from 10 seconds to 50 minutes can be selected by clicking the arrow icons in the line of <Clk. Width>.

<Program>

The commands in the program field start various exposure regimes. The exact program sequence is by defined in the <Settings> window (see chapter 2.9). Except batch runs, all programs can be clock-triggered and their exact behavior is defined in the <Settings> window (see chapter 2.9). Short descriptions of the routines are described below.

<Act.+Yield> Illuminate with actinic light and perform a saturating pulse analysis at the end of illumination.

<Induct. Curve> Determine Fo and Fm and, subsequently, carry out saturating pulse analyses during sample illumination with constant light intensity.

<IC +Recov.> Same as <Induct. Curve> but followed by a dark phase with saturating pulse analysis performed at increasing pulse intervals.

<Light Curve> Determine Fo and Fm and illuminate sample with 8 increasing light intensities. Perform a saturating pulse analysis at the end of each light step.

<LC +Recov.> Same as <Light Curve> but followed by a dark phase with saturating pulse analysis performed at increasing pulse intervals.

<Batch> Run exposure program as defined by a batch file (chapter 4).

2.1.8 Box (8) - Graph Type and Fluorometer Settings

The graph type and fluorometer setting windows will be explained in detail in 2.2 - 2.9.

<Chart> Graphic presentation of the measured data

<Induct. Curve>, <Light Curve> Clicking the latter tabs gives access to alternative graphic representations of slow kinetics data; namely, the <Induct. Curve> and <Light Curve> screens. Display of data in either of the two latter screens requires the execution of an induction curve or light curve program.

<SAT Graph> The window displays graphs of fast fluorescence kinetics during saturating pulse analysis. Specific regions in slow kinetics graphs (<Chart>, <Induct. Curve> or <Light Curve>) can be linked to fast fluorescence changes: selecting slow kinetics data, using the mouse with left button pressed, will highlight the corresponding fast kinetics in the <SAT Graph> window.

<Report> The window displays numerically the results of saturating pulses analyses. Analogously, as described for <SAT Graph>, highlighting data in the chart will highlight the corresponding lines in the report data.

<Memory> The window provides convenient handling of the PAM-CONTROL memory data.

<Batch> Loading of pre-programmed batch files is carried out using this window.

<Settings> The <Settings> window provides the commands required for full control of WATER-PAM performance.

2.1.9 Box (9) - Axis Control

 <Manual Ordinate Scaling> Scaling of the ordinate (y-axis) can be manually adjusted by clicking the upward or downward pointing arrows in Box (9). Manual adjustment affects only the currently selected ordinate (see subsequent comments to Box (10)). This means that various ordinates can be individually scaled. Manual scaling, in combination with the vertical scroll bar at the right chart border, permits flexible positioning of data.

 <Automatic Ordinate Scaling> The command scales all data which are selected in Box (3) so that they fit on the chart.

 <Manual Abscissa Scaling> Clicking the horizontally pointing arrows changes scaling of the abscissa (time axis). At increased time axis resolution, experimental data can be scrolled using the scroll bar on top of the chart area, or by placing the mouse pointer inside the chart and turning the mouse wheel.

 Normally, the chart ordinate is a relative time scale starting at 0. Checking <abs> prompts the display of absolute data including data and time of the day. Both, relative and absolute time data are saved when chart data are exported.

 <Automatic Abscissa Scaling> Scales the time axis so that all the time intervals of the record fits into the chart.

 <Automatic Ordinate and Abscissa Scaling> Clicking the <Autoscale> icon displays all graphed data on the chart.

Table 2.1: Chart Ordinate Types

Ordinate	Parameter
F, counts	Fo, Fm, Ft, Fo', F, Fm'
PAR, µmol / (m^2 · s)	PAR*, PAR
Yield, dimensionless ratio	Fv/Fm, Y(II), Y(NO), Y(NPQ)
ETR, µmol / (m^2 · s)	ETR
Quench, dimensionless ratio	qN, qP, qL
NPQ, dimensionless ratio	NPQ
Temp., °C	Temp*, Temp

2.1.10 Box (10) - Ordinate Selection and Text Field

 Clicking the downward arrow in Box (10) displays 7 different ordinate scales used for the various categories of data available. An ordinate can be picked by left-clicking with the mouse on one of the list items. Table 2.1 provides a summary of ordinates and associated data. Also, Box (10) provides a text field to note down key information of your experiment. Each record can tagged differently, and additional text fields are available for each induction or light curve.

2.2 CHART

The chart window displays the measured data with functions chosen in the sidebar (Box(3)).

To select a section of graphed data, click with the left mouse button on the left border of the target data, and move the mouse with the left button pressed down to the right border of target data. Releasing the mouse button will highlight the selection made.

Selection in the chart window highlights the corresponding data in the <Induct. Curve>, <Light Curve>, <SAT Graph> and <Report Window>. Similarly, data selected in one of the latter windows (except <SAT Graph>) will also be valid for the other windows. Therefore, data selection is able to connect different representations of the same data and, thus, facilitates linking between primary fluorescence data and derived fluorescence coefficients.

2.3 Induction Curve Window

Generally, control icons and checkboxes of the <Induct. Curve> window work as described for the <Chart> window.

The <Induct. Curve> window displays fluorescence induction curves, and combinations between fluorescence induction and recovery curves provided that the curves were automatically recorded by execution of <Induct. Curve> or <IC+Recov.> programs, respectively. The <Rec.> panel of the sidebar (Box (3)), lists the induction curves present as ascending numbers with prefix IC. You select an induction curve by left-clicking on the curve number. Thereafter, sequential viewing of "IC curves" is possible by using the up/down arrows keys on your computer keyboard. The parameters of <Induct. Curve> or <IC+Recov.> programs can be configured in the <Settings> window.

2.4 Light Curve Window

Time PAR With <PAR> checked, the <Light Curve> window displays "Rapid Light Curves" recorded by the <Induct. Curve> or <IC+Recov.> programs (Box 7) using PAR (photosynthetic active radiation, µmoles photons/($m^2 \cdot s$)) as the abscissa data.

The Rapid Light Curve function of WinControl-3 employs 8 increasing light levels which correspond to 8 neighboring levels of the 12-partite

internal PAR list displayed in the <Settings> window. The intensity range of the light curve and the time interval allotted to each intensity level can be adjusted in the <Settings> window. The <Rec.> panel of the sidebar (Box (3)), lists the light curves present as numbers with prefix LC. The <Online> data <Ft>, <PAR*>, and <Temp*> are not available in a light curve diagram but can be displayed by choosing experimental time as abscissa data by checking <Time>.

The <Sidebar> in the <Light Curve> window offers the control functions introduced before. Additionally, the <Val.> panel in Box (3) permits the display of two different graphs denoted <REG1> and <REG2>. These graphs result from fitting two different empirical functions to data of ETR versus PAR: the <REG1> function can exhibit lower ETR values at high, compared to intermediate, PAR data but the <REG2> function is always monotonically non-decreasing (see 3.4).

2.5 SAT Chart Window

The SAT chart window displays all fluorescence transients recorded since start of WinControl-3 or since <Clear All Data> was executed represented by a protocol panel and a graphic window..

The protocol panel lists the exact time point of the saturating pulse analysis, as well as its numerical order in the current experiment and its line number in the report table (<Nr:> and <Rep. Nr:>, respectively). A single fluorescence trace can be selected by a double-click into the protocol window. Selection highlights the graph and shifts it to the top of the Sat Graph panel. Also, the respective data line in the report data is accentuated.

Individual graphs depict fluorescence traces during saturating pulses. Each record start 150 ms before pulse begin and lasts for 2.4 s. Fluorescence traces are automatically scaled to fit into the coordinate systems. Also, values of F and Fm' are shown as dashed horizontal lines.

A series of fluorescence transients can be quickly looked over by using window's scroll bar or, with the mouse pointer located inside the SAT graph panel, by turning the mouse wheel.

Figure 2.2 depicts the SAT-Chart screen divided into eight numbered boxes and the SAT Graph Panel. Numbering and functionality of boxes is identical to Figure 2.1 except boxes (2) and (3).

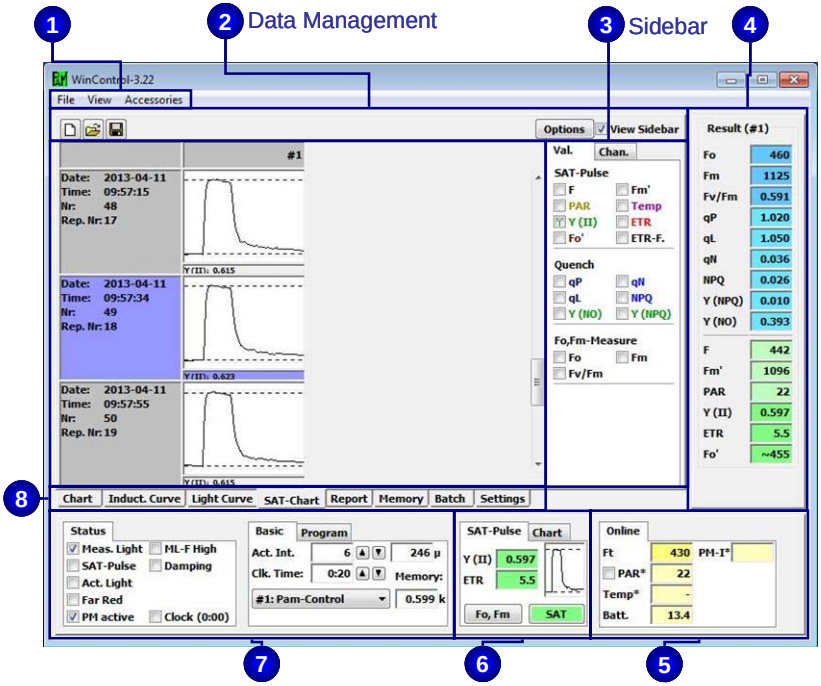


Figure 2.2: SAT- Chart Screen

2.5.1 SAT Chart– Data Management Box (2)

Options The <Options> icon includes three menu items of which two (<Jump to Selection> and <Delete Selected Data>) are only available if saturating pulses have been selected in one of the graphics screens (Chart, Induction or Light Curve) or in the report data. In the SAT graph window, selected fluorescence curves are shown with blue background color (see Figure 2.2). The three menu items are:

<Follow Selection> When <Follow Selection> is checked, the SAT Graph window automatically displays the fluorescence transients selected in a graph window or report data.

<Jump to Selection> displays the previously selected saturating pulse data.

<Delete Selected Data> Delete saturating pulse and online data associated with the highlighted graphs.

2.5.2 SAT Chart – Sidebar Box (3)

View Sidebar The checkbox <View Sidebar> controls the display of the sidebar. Similar as described in chapter 2.1.3, checkboxes on the <Val.> sidebar affects the display of data: checking of boxes results in the display of the data type associated with the box as numerical values below each fluorescence transient in the SAT graph panel. The <Chan.> sidebar lists all WinControl-3-compatible instruments connected to the computer.

2.6 Report Window

The report window logs all data associated with saturating pulse analyses. The boxes (11) to (13) drawn in Figure 2.3 include specific functions for data handling explained below.

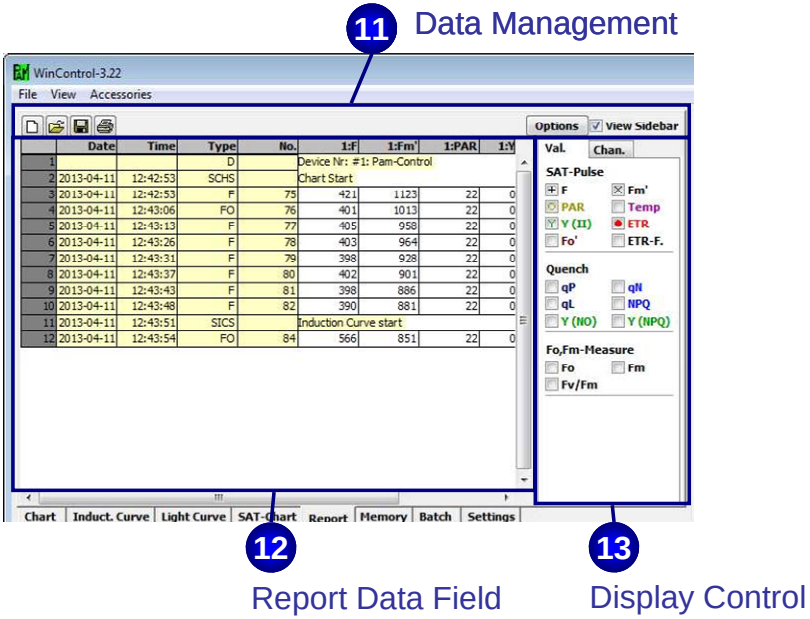


Figure 2.3: Report Window

2.6.1 Report - Data Management Box (11)

Clicking the <Print Report> icon opens the printer dialogue window from which printing of all parameters present in the <Report Data Field> is initiated. The parameters displayed and printed can be controlled by check boxes in the <Val.> sidebar. In Box (11), the icons for data handling (<Clear all Data>, <Load> and <Save>) retain their usual function.

Options

Clicking on the <Options> icon or right-clicking in the <Report Data Field> opens the <Options> menu.

<Follow Selection> in the <Options> menu automatically displays selected data in the <Report Data Field>. Data can be selected in graphics charts as described above (see 2.2) or in the <Report Data Field> by moving the mouse cursor with left mouse button pressed across data lines.

<Show Mark> opens up a column 1:Mark in the <Report Data Field> Marks can be set in PAM-CONTROl by menu point 55 of PAM-CONTROL or manually in the <Report Data Field>.

<Export All> exports all data in CSV (comma separated values) format which can be imported into most spread sheet programs. The parameters exported can be selected by the checkboxes in the <Val.> sidebar.

<Delete all measured Data> clears all measured data from report sheet but not from PAM-CONTROL memory.

Print commands: Three print commands are available. As described for Data-Export the printed parameters can be selected by the checkboxes in the <Val.> sidebar.

<Page Setup for Printing> permits adjustment of page design settings.

<Preview Printing> shows the appearance of the print-out.

<Print Report> prints all data shown in the Report.

Provided that data have been selected five more commands are available.

<Export Selected Lines> exports only the selected data.

<Jump to Selection> command is available which moves selected data into the <Report Data Field>

<Delete Selected Data> clears selected data from report sheet but not from PAM-CONTROL memory.

<Preview Print Selection> shows the print-out appearance of selected data.

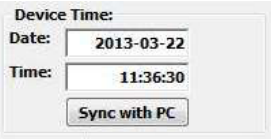
<Print selected Data> prints only the selected data.

2.6.2 Report - Data Field Box (12)

In addition to the parameters chosen from <Display Control> (Box (13)), the report data documents time of action and type of data. Table 2.2 summarizes the abbreviations used to log data types.

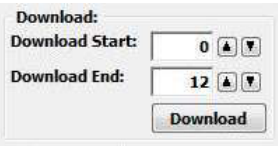
2.7 Memory Window

The <Memory> Window offers a convenient handling of data stored in PAM-CONTROL.



Device Time: shows Date and Time stored in the PAM-CONTROL Unit. The Sync with PC button adjusts Date and Time according to the connected PC.

Memory: Shows datasets stored in the PAM-CONTROL unit indicating start and end line. The Clear Memory button deletes all Datasets in the memory of PAM-CONTROL.



Download: Defines the start and end line for data download. The lower button starts the download of data sets from PAM-CONTROL to the report window.

The data sets are displayed underneath these control buttons.

2.8 Batch Window

WinControl-3 batch files consist of a series of commands which can be executed by the WinControl-3 software. Using batch files, the WATER-PAM can be automatically operated, thus experimental protocols or fluorescence analysis can be standardized and routinely executed. For detailed information see chapter 4.

 Loads a batch file for use in WinControl-3.

2.9 Settings Window

The <Settings> window controls the function of the WATER-PAM's LEDs as well as the performance of preprogrammed fluorescence experiments (see chapter 2.1.7).

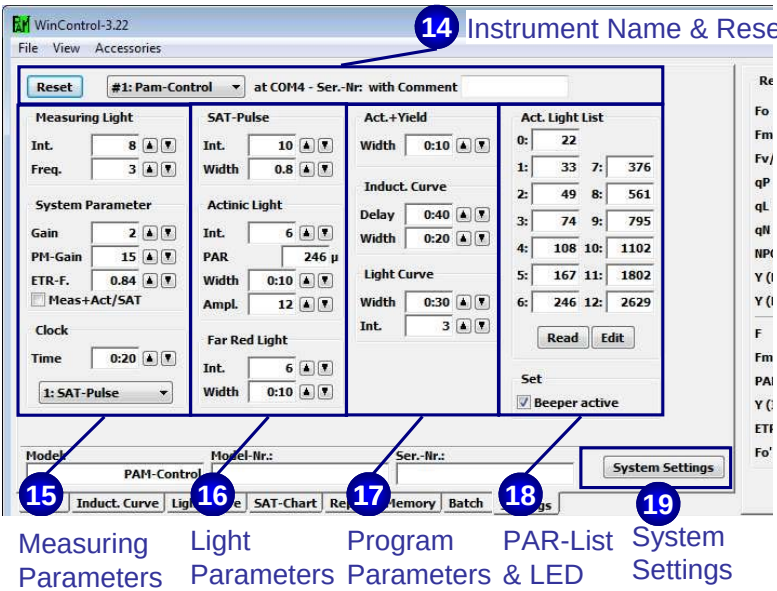


Figure 2.5: Settings Window

2.9.1 Box (14) - Instrument Name & Reset

Reset <Reset> deletes all changes made in the settings window and restores factory default settings (Act. Light List remains unchanged). Box (14) also includes a text window for comments of up to 20 characters. The Instrument Name and the content of the text window are written in the first line of each record file.

Table 2.2: Report Data - Abbreviations

D	Identification of device: number, type and user annotation
F	SAT Normal operation and saturating flash
FO	Fo, Fm determination
REG1	Parameters of regression 1 (see chapter 3)
REG1	Parameters of regression 2 (see chapter 3)
SCHS	Start of new record/Chart start
SICE	End of induction curve
SICS	Start of induction curve
SLCE	End of rapid light curve
SLCS	Start of rapid light curve

2.9.2 Box (15) - Measuring Parameters

<Measuring Light>

The 5 µs lasting Measuring "pulses" are repeated with frequencies ranging between 8 and 688 Hz according to the Measuring Light Freq. settings 1-12 (standard setting 3 corresponds to 18 Hz).

The measuring light intensity equals PAM-CONTROL´s Menu point #49: Meas.Ampl. and is alterable in 12 steps. Note: This setting normally remains fixed (standard setting 8) and the measuring light is modified via measuring light frequency changes.

<System Parameters>

The <Gain> field equals PAM-CONTROL´s OUT-GAIN (menu point # 53). PM-Gain represents the photomultiplier gain. The settings can be varied in 12 steps for <Gain>, respectively in 30 steps for <PM-Gain>. An increase in either gain function results not only in an increase in signal but also proportional in an increase in noise. Note: Any change in either gain function requires a new determination of the unavoidable background signal (System Sett. / F-Offset see chapter 2.10).

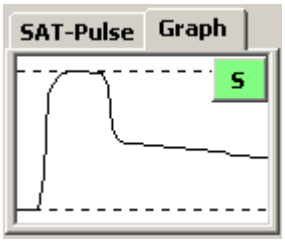
The <ETR-F.> is used for calculations of electron transfer rates (see chapter 3.3). The preset value of 0.84 corresponds to the fraction of incident light absorbed by a leaf. Note: In many practical cases this value is far from realistic and the calculated ETR can be only considered a relative measure of electron transport activity.

<Clock>

The clock utility executes repetitively one of 6 different functions: saturating pulse analysis and 5 pre-programmed sequences (<Act.+Yield>, <Induct. Curve>, <IC +Recov.>, <Light Curve> and <LC +Recov.>). The interval time can be adjusted between 10 s and 50 min depending on the action triggered and experimental demands.

2.9.3 Box (16) – Light Parameters

<SAT-Pulse>



12 different intensity levels are available. Pulse width can be adjusted from 0.2 to 2 seconds. The <SAT-Pulse> defaults (intensity level 10 and 0.8 seconds pulse width) work well with most samples. The fluorescence transient reaches a plateau and does not drop at the end of the saturating pulse interval (Graph interval set value 2.5 s).

<Actinic Light>

12 actinic light levels are available. The PAR (µmoles/(m^2·s)) are annotated according to the <Act. Light List>. The width of the Actinic Light exposure can be adjusted from 0:05 to 5:00 min.

The Actinic Light <Ampl.> can be changed in 12 steps but normally remains fixed at standard setting 12 and the intensity of actinic illumination is changed via <Int.>.

<Far Red Light>

Far red light is adjustable in 12 far red intensity levels with a width between 0:05 and 10:00 min. The default values for intensity and far red illumination are level 6 and 10 seconds, respectively. Note: Use of far-red light may cause artifacts, if too much of this light reaches the photomultiplier.

2.9.4 Box (17) - Program Parameters

<Act. +Yield>

<Width> determines the time interval of sample illumination with actinic light of the intensity selected under <Actinic Light>. Always, a saturating pulse analysis is carried out at the end of actinic illumination.

<Induct. Curve>

Time courses of fluorescence induction curves are determined by three parameters: <Delay> indicates the dark time interval between determination of Fo and Fm with dark-acclimated material, and onset of actinic illumination. The delay time can be adjusted between 5 seconds and 10 minutes. The default value is 40 seconds. <Width> is the time interval between saturating pulse analysis during fluorescence induction. Fluorescence recovery measurements under dark conditions can be appended to the induction curve by choosing <IC + Recov> (see 2.1.7). During fluorescence recovery, time points of saturating pulse analyses are set automatically.

<Light Curve>

Always, 8 levels of consecutively increasing actinic light intensities are applied when a light curve program is carried out (see 2.1.7). Therefore, the time course of a light curve is determined by only two parameters: <Width> determines the time interval (5 seconds to 10 minutes) of each light step, and <Int.> defines the initial intensity level of actinic illumination. In the case of a subsequent recovery curve, time points of saturating pulse analyses are chosen automatically.

2.9.5 Box (18) - Act. Light List & Beeper

Box (18) lists the photon flux data of actinic light levels. The internal PAR list can be modified in two ways. The Read button starts the rou-

tine for light calibration thus compilation of a new internal PAR-list. The Edit button allows manual alterations of the PAR values.

Calibration requires the application of special quantum sensor US-SQS/WB. The external quantum sensor needs to be activated by ticking the box in the online data (see Figure 2.6) The vertical depth of the US-SQS/WB sensor (ca. 5 mm from the cuvette bottom) is critical for correct PAR-readings. It can be optimized by maximizing the PAR-reading while actinic illumination is switched on. The Read button starts the calibration routine involving illumination at the 12 settings of ACT-INT and storage of the PAR-readings in the internal PAR-list.

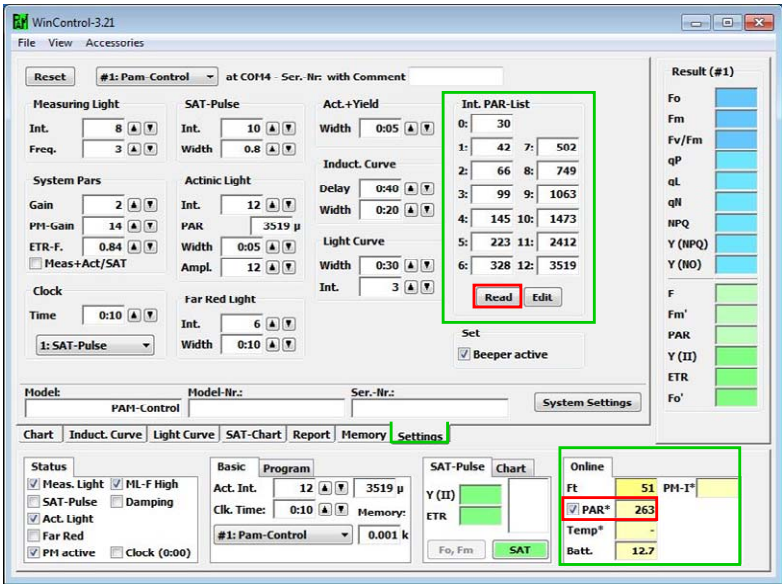


Figure 2.6: Calibration of the Internal PAR-list

The internal PAR list can be modified by entering the PAR values manually in the Int. PAR-List Edit Window Figure 2.7.

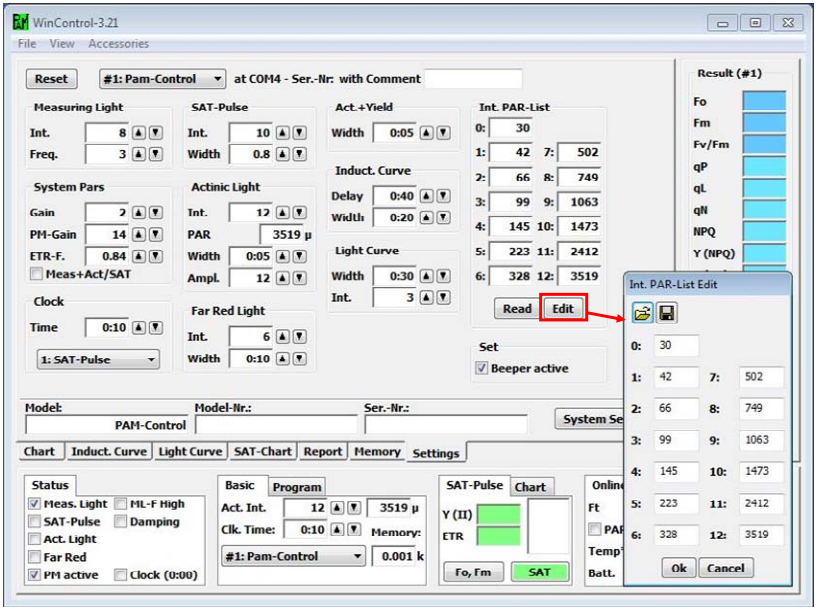


Figure 2.7: Edit internal PAR-List

The Beeper is an acknowledge signal of the PAM-CONTROL Unit and can be enabled by ticking off the box.

2.10 Box (19) - System Settings

If system settings have been altered, default setting can be restored using the icon <Reset> in the left upper corner.

<Ext. PAR-Sensor>

Ticking the box activates an external quantum sensor analogous to the box in the Online Data (Box 5).

If a quantum sensor is connected to the WATER-PAM, data input can be adjusted *via* <Offset> and <Calib> settings.

< Ext. Temp. Sensor >

If a temperature sensor is connected to the WATER-PAM, data input can be adjusted *via* <Offset> and <Gain> settings.

<F-Offset>

The Auto-Zero button starts the determination if the signal in absence of sample (F-Offset) which is subtracted from all fluorescence signals.

F-Offset remains effective for all following measurements until being deliberately changed.

Note: The F-Offset value has to be newly determined whenever measuring Light Amplitude, Output-Gain or PM-Gain are modified.

3 Acronyms and Equations

3.1 Relative Fluorescence Yields

Typically, five different types of fluorescence levels are acquired by saturating pulse analyses. In most cases, the PAM fluorescence signal is proportionally related the yield for chlorophyll fluorescence. Therefore, differences between these five fluorescence levels reflect variations in chlorophyll fluorescence yields.

Two of these levels (Fo and Fm) need to be determined with the dark-acclimated sample. The three remaining levels (Fo', F, and Fm') are repeatedly measured during subsequent sample treatments (e.g., exposure to actinic light).

3.1.1 Measurements with dark-acclimated samples

Fo Minimum fluorescence level excited by very low intensity of measuring light to keep PS II reaction centers open.

Fm Maximum fluorescence level elicited by a strong light pulse which closes all PS II reaction centers.

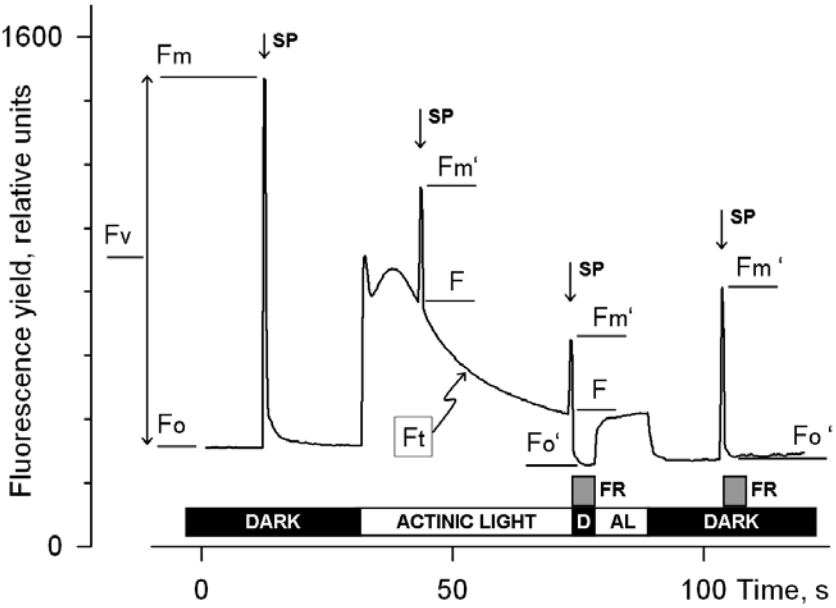


Figure 3.1: Measurements for Saturating Pulse Analysis. AL, actinic light; D, dark; SP, saturating pulse; FR, far-red illumination.

3.1.2 Measurements with illuminated samples

Fo' Minimum fluorescence level of illuminated sample which is lowered with respect to Fo by non-photochemical quenching. When the measuring routine for Fo' is active, the Fo' level is determined during a dark interval following the Saturation Pulse. In the dark interval, far-red light is applied to selectively drive PS I and to quickly remove electrons accumulated in the intersystem electron transport chain, thus reopening PS II reaction centers (see Figure 3.1, time 75 s). Alternatively, the Fo' can be estimated according to Oxborough and Baker (1997):

$$Fo' = \frac{1}{1/Fo - 1/Fm + 1/Fm'}$$

Fm' Maximum fluorescence level of illuminated sample as induced by saturating pulses which temporarily close all PS II reactions centers. Fm' is decreased with respect to Fm by non-photochemical quenching.

F The F corresponds to the momentary fluorescence yield (Ft) of an illuminated sample shortly before application of a saturating pulse.

3.2 Fluorescence Ratio Parameters

To quantify photochemical use and non-photochemical losses of absorbed light energy, fluorescence ratio expressions have been derived which are based on the relative fluorescence yield introduced above. Table 3.1 compiles the fluorescence ratio parameters available in WinControl-3. Subsequently, these parameters will be briefly explained.

Table 3.1: Fluorescence Ratio Parameters.

Source	Equation
Maximum photochemical quantum yield of PS II (Kitajima and Butler, 1975)	$\frac{Fv}{Fm} = \frac{Fm - Fo}{Fm}$
Effective photochemical quantum yield of PS II (Genty *et al.*, 1989)	$Y(II) = \frac{Fm' - F}{Fm'}$
Coefficient of photochemical fluorescence quenching (Schreiber *et al.* 1986 as formulated by van Kooten and Snel, 1990)	$q_P = \frac{Fm' - F}{Fm' - Fo'}$
Coefficient of photochemical fluorescence quenching assuming interconnected PS II antennae (Kramer *et al.* 2004)	$q_L = q_P \cdot \frac{Fo'}{F}$
Coefficient of non-photochemical fluorescence quenching (Schreiber *et al.* 1986 as formulated by van Kooten and Snel, 1990)	$q_N = 1 - \frac{Fm' - Fo'}{Fm - Fo}$
Stern-Volmer type non-photochemical fluorescence quenching (Bilger and Björkman, 1990)	$NPQ = \frac{Fm}{Fm'} - 1$
Quantum yield of non-light induced (basal or dark) non-photochemical fluorescence quenching: this quenching type does not require the presence of a trans-thylakoid ΔpH and zeaxanthin (Kramer *et al.* 2004)	$Y(NO) = \frac{1}{NPQ + 1 + q_L \cdot (\frac{Fm}{Fo} - 1)}$
Quantum yield of light-induced (ΔpH- and zeaxanthin-dependent) non-photochemical fluorescence quenching (Kramer *et al.* 2004)	$Y(NPQ) = 1 - Y(II) - Y(NO)$

Fv/Fm and Y(II) Maximum and effective photochemical quantum yield of PS II

Both fluorescence quotients estimate the fraction of absorbed quanta used for PS II photochemistry, (i.e., for stable charge separation in the PS II reaction center). For measurements of Fv/Fm, it is important that samples are acclimated to darkness or dim light so that all reactions centers are in the open state and non-photochemical dissipation of excitation energy is minimal. In algae and cyanobacteria, the dark-acclimated state often is not showing maximal PS II quantum yield, as the PS II acceptor pool may be reduced in the dark by stromal reductants and consequently the so-called state 2 is formed exhibiting low PS II quantum yield. In this case, preillumination with moderate far-red light should precede determinations of Fo and Fm. The far-red preilluminated quasi-dark state normally serves as reference state with maximal PS II quantum yield for assessment of the functional absorption cross-section of PS II.

The Y(II) value estimates the photochemical use of excitation energy in the light. It is lowered with respect to Fv/Fm by partial closure of PS II centers and various types of nonphotochemical energy losses induced by illumination. To derive from the Y(II) information on the overall state of photosynthesis, knowledge of the absorbed PAR is essential, as a sample e.g. may be severely damaged in Calvin cycle activity and still show a high value of Y(II) in weakly absorbed light. This aspect is particularly important in the study of algae and cyanobacteria, which display large wavelength-dependent differences in light absorption. Therefore, photosynthetic performance should be assessed during steady state illumination at a photon flux density which is somewhat below saturation in a control sample.

q_P and q_L Coefficients of photochemical fluorescence quenching.

Both parameters estimate the fraction of open PS II reaction centers. The q_P is based on concept of separated PS II antennae (puddle model) puddle model but the q_L assumes interconnected PS II antennae (lake model) which appears to be the more realistic situation in leaves (cf. Kramer *et al.*, 2004). Determinations of q_P an q_L do not require fluorescence measurements with the dark-acclimated sample except when Fo' is not measured but calculated (<Fo'-Mode> unchecked) according to Oxborough and Baker (1997) (see 7.1).

Coefficients of photochemical quenching qP is defined in PAM-CONTROL by the equations qP=(M-F)/(M-Fo). M here represents the maximal fluorescence measured by a saturation pulse in any given light state (normally denoted Fm'), whereas Fm and Fo are the particular values sampled via menu point 27 after dark-adaptation.

q_N and NPQ Parameters of non-photochemical quenching

Both parameters are associated with non-photochemical quenching of excitation energy, mainly involving a low thylakoid lumen pH- and a zeaxanthin-dependent quenching mechanism. In contrast to Y(II), qP and qL, calculations of the qN and the NPQ parameters always require fluorescence measurements with the sample in the dark-acclimated and in the light-exposed state (see Table 7.1). Calculation of NPQ (or SV_N; Gilmore and Yamamoto, 1991) corresponds to the Stern-Volmer equation for fluorescence quenching which predicts proportionality between fluorescence quenching (NPQ) and the concentration of fluorescence-quenching centers in the photosynthetic antennae (e.g. zeaxanthin).

Coefficients non-photochemical quenching qN is defined in PAM-CONTROL by the equations qN=(Fm-M)/(Fm-Fo). M here represents the maximal fluorescence measured by a saturation pulse in any given

light state (normally denoted Fm'), whereas Fm and Fo are the particular values sampled via menu point 27 after dark-adaptation.

Y(NO) and Y(NPQ) and Y(II) Complementary PSII quantum yields

Genty et al. (1996) first presented expressions based on basic fluorescence parameters that describe the partitioning of absorbed excitation energy in PS II between three fundamental pathways, which were further investigated by Klughammer and Schreiber (2004) and expressed in terms of the complementary quantum yields of

Y(NO) sum of non-regulated losses of excitation energy including heat dissipation and fluorescence emission,

Y(NPQ) regulated energy losses of excitation energy via heat dissipation involving ΔpH- and zeaxanthin-dependent mechanisms, and

Y(II) use of excitation energy for charge separation.

The yields of photochemical energy conversion and non-photochemical losses sum up to 1:

$$Y(II) + Y(NPQ) + Y(NO) = 1$$

This concept of "complementary PS II quantum yields" is useful to analyze the partitioning of absorbed light energy in photosynthetic organisms. For instance, under high light-conditions, a much higher Y(NPQ) than Y(NO) indicates that excess excitation energy is safely dissipated at the antenna level, that is, photosynthetic energy fluxes are well-regulated. In variance, high values of Y(NO) would signify that excess excitation energy is reaching the reaction centers, resulting in strong reduction of PS II acceptors and photodamage, e.g. via formation of reactive oxygen species.

3.3 Relative Electron Transfer Rate (ETR)

Relative electron transfer rates are calculated according to:

$ETR = PAR \cdot ETR\text{-}Factor \cdot P_{PS2}/P_{PPS} \cdot Y(II)$.

The basic idea of the ETR equation is to multiply Y(II), the effective photochemical quantum yield of PS II, by an estimate for the photon flux density absorbed by PS II. The latter approximation uses three parameters which are explained below:

PAR Photosynthetically active radiation

Depending on settings, the PAR values of the active light list or measured data of an external quantum sensor are used.

ETR-Factor Absorptance of photons by photosynthetic pigments

The ETR-Factor corresponds to the fraction of incident photons absorbed by photosynthetic pigments. The WinControl-3 default value for the ETR-Factor is 0.84 which is a reasonable match to the average absorption in the visible range (400-700 nm) of many green leaves. Hence, the ETR-Factor is variable and this variability needs to be considered when different samples are compared.

P_{PS2}/P_{PPS} Photons absorbed by PS II relative to photons absorbed by photosynthetic pigments.

WinControl-3 uses 0.5 as default value for P_{PS2}/P_{PPS}. The value of 0.5 is reasonable if one assumes the presence of only linear electron transport and, hence, equal electron transfer rates in PS I and PS II, and comparable photochemical quantum yields of PS I and PS II under strongly light-limiting conditions.

3.4 Light Curves

The measuring program <Light Curve> exposes the sample to increasing intensities of actinic illumination. Usually, the time interval of each intensity level is too short for full equilibration of photosynthetic reactions. Therefore, these so-called "Rapid Light Curves" (RLC) provide information on the present state of photosynthesis and are not to be con-

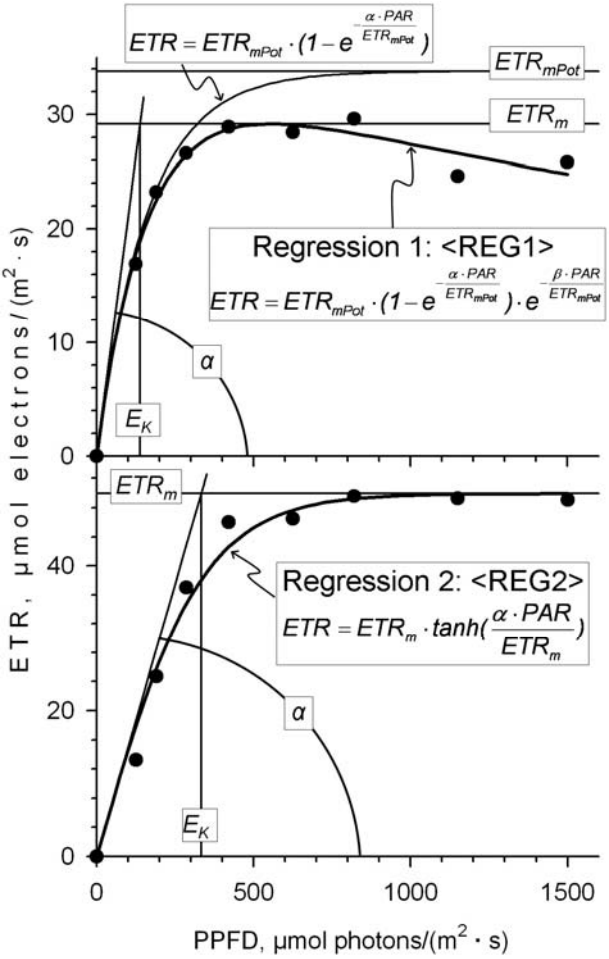


Figure 3.2: Model Functions of Rapid Light Curves

fused with classical photosynthetic light response curves in which photosynthetic rates under steady state conditions are plotted against light intensities. By plotting ETR versus PAR (see previous section), Rapid Light Curves provide subsequent key parameters:

- α, electrons/photons: Initial slope of RLC which is related to quantum efficiency of photosynthesis.
- ETR_m, µmol electrons/(m^2 · s): Maximum electron transport rate.
- E_K, µmol photons/(m^2 · s): Minimum saturating irradiance.

WinControl-3 uses two empirical functions to estimate these cardinal data: the functions <REG1> and <REG2> which have been introduced by Platt *et al.* (1980) and Jassby and Platt (1976), respectively, to describe classical light response curves of photosynthesis (Figure 3.2).

<REG1> In case of <REG1>, the α results from fitting

$$ETR = ETR_{mPot} \cdot (1 - e^{-\frac{\alpha \cdot PAR}{ETR_{mPot}}}) \cdot e^{-\frac{\beta \cdot PAR}{ETR_{mPot}}}$$

to the light curve data using the Levenberg-Marquardt algorithm. The latter equation considers photoinhibition of photosynthesis by high light intensities. Therefore, the fitting procedure yields estimates for β, the "photoinhibition parameter"(Platt *et al.*, 1980), and for ETR_{mPot}, the maximum potential light-saturated electron transport rate which would be observed if photoinhibition was absent.

Platt *et al.* (1980) introduced the "Photoinhibition Index" (I_b) to quantify photoinhibition. The authors defined I_b as the PAR value required to photoinhibit ETR_{mPot} by the factor of 1/e according to:

$$I_b = ETR_{mPot} / \beta$$

With the results from curve fitting, WinControl-3 computes the ETR_m and E_K according to:

$$ETR_m = ETR_{mPot} \cdot (\frac{\alpha}{\alpha+\beta}) \cdot (\frac{\beta}{\alpha+\beta})^{\frac{\beta}{\alpha}} \quad \text{and} \quad E_K = \frac{ETR_m}{\alpha} \quad .$$

<REG2> The function <REG2> is monotonically nondecreasing and, hence, does not allow for photoinhibition:

$$ETR = ETR_m \cdot \tanh(\frac{\alpha \cdot PPFD}{ETR_m})$$

Here, the α and ETR_m are estimated by the fitting procedure. With the latter two parameters, the E_K is calculated as described above.

3.4.1 Some Papers on Rapid Light Curves

Fouqueray M, Mouget J-L, Morant-Manceau A, Tremblin AG (2007) Dynamics of short-term acclimation to UV radiation in marine diatoms. J Photochem Photobiol B: Biology 89: 1–8

Perkins RG, Mouget J-L, Lefebvre S, Lavaud J (2006) Light response curve methodology and possible implications in the application of chlorophyll fluorescence to benthic diatoms. Marine Biol 149: 703-712

Ralph PJ, Gademann R (2005) Rapid light curves: A powerful tool to assess photosynthetic activity. Aquat Bot 82: 222-237

Rascher U, Liebig M, Lüttge U (2000) Evaluation of instant light-response curves of chlorophyll fluorescence parameters obtained with a portable chlorophyll fluorometer on site in the field. Plant Cell Environ 23: 1397-1405

Serôdio J, Vieira S, Cruz S, Coelho H (2006) Rapid light-response curves of chlorophyll fluorescence in microalgae: relationship to steady-state light curves and non-photochemical quenching in benthic diatom-dominated assemblages. Photosynth Res 90: 29-43

Serôdio J, Vieira1 S, Cruz S, Barroso F (2005) Short-term variability in the photosynthetic activity of microphytobenthos as detected by measur-

ing rapid light curves using variable fluorescence. Marine Biol 146: 903-914

White AJ, Critchley C (2005) Rapid light curves: A new fluorescence method to assess the state of the photosynthetic. Photosynth Res 59: 63-72

3.5 Literature Cited in Chapter 3

Bilger W, Björkman O (1990) Role of the xanthophyll cycle in photoprotection elucidated by measurements of light-induced absorbance changes, fluorescence and photosynthesis in leaves of *Hedera canariensis*. Photosynth Res 25:173-185

Genty B, Briantais J-M, Baker NR (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim Biophys Acta 990: 87–92

Gilmore AM, Yamamoto HY (1991) Zeaxanthin formation and energy-dependent fluorescence quenching in pea chloroplasts under artificially mediated linear and cyclic electron transport. Plant Physiol 96: 635–643

Jassby AD, Platt T (1976) Mathematical formulation of the relationship between photosynthesis and light for phytoplankton. Limnol Oceanogr 21: 540-547

Kitajima M, Butler WL (1975) Quenching of chlorophyll fluorescence and primary photochemistry in chloroplasts by dibromothymoquinone. Biochim Biophys Acta 376:105-115

Kramer DM, Johnson G., Kiirats O, Edwards GE (2004) New flux parameters for the determination of Q_A redox state and excitation fluxes. Photosynthesis Res 79: 209-218

Oxborough K, Baker NR (1997) Resolving chlorophyll a fluorescence images of photosynthetic efficiency into photochemical and non-

photochemical components - calculation of qP and Fv'/Fm' without measuring Fo'. Photosynth Res Volume 54 135-142

Pfündel EE, Ben Ghozlen N, Meyer S, Cerovic ZG (2007) Investigating UV screening in leaves by two different types of portable UV fluorimeters reveals in vivo screening by anthocyanins and carotenoids. Photosynth Res 93: 205-221

Platt T, Gallegos CL, Harrison WG (1980) Photoinhibition of photosynthesis in natural assemblages of marine phytoplankton. J Mar Res 38: 687-701

Schreiber U, Schliwa U, Bilger W (1986) Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. Photosynth Res 10: 51–62

van Kooten O, Snel J (1990) The use of chlorophyll fluorescence nomenclature in plant stress physiology. Photosynth Res 25: 147–150

4 WinControl-3 Batch Files

WinControl-3 batch files enable automation of experimental protocols and fluorescence analysis.

Batch files are created and modified using an ordinary text editor program. Subsequently, an introduction to batch file writing is provided which includes example batch files and a complete list of commands and variables. Generally, to be recognized by the WinControl-3 program, a batch file has to be saved with the filename extension <**wcb**> (**W**in**C**ontrol **B**atch).

4.1 Sample Batch Files

4.1.1 Kautsky Fluorescence Induction Kinetics

Dark-light transitions of leaves induce the classical variations in fluorescence emission described by Kautsky and Hirsch in 1931. Under control of a suitable batch file, the WATER-PAM automatically carries out fluorescence induction experiments. The subsequent sample file consists of for command lines:

Line 1

```
$AI = 10
```

The "$AI" stands for "Actinic Light Intensity". The "$AI" belongs to the group of "variables" defined for WinControl-3 batch files. All variables start with the "$"-sign (see chapter 0). In the case of the variable $AI, a numerical value of 10 sets the actinic light intensity to level 10. Thus, the first line of our batch file sets the actinic light intensity to level 10.

Line 2

```
$A = 1
```

The "$A" also belongs to the group of variables and represents an on/off switch for the actinic light source. Owing to its switch function, the $A variable can assume only two values: 0 corresponding to the actinic light switched off, and 1 for actinic light switched on. Hence, line 2 of the present batch file switches actinic light on.

Line 3

```
delay 420
```

The "delay" represents a "command" of the WinControl-3 batch file language (see 8.2.4). The delay command introduces a waiting period (defined in seconds) between the two neighboring command. In the present case, the state of actinic light at intensity level 10 switched on will remain for 420 seconds.

Line 4

```
$A = 0
```

After the delay period, actinic light is switched off.

Run the batch file

- Save your text file using ASCII- or ANSI-code.
- Start WinControl-3, turn measuring low frequency measuring light on, locate fluorescence signal on the chart.
- In the Main Menu Bar of WinControl-3, open the <View> menu and click on <Batch Window>.
- In the batch file window, click on icon <Load Batch File> and select batch file.
- Click on icon <Run Batch> to execute the current batch file.

- Hint: use the <Float> command in the chart window of WinControl-3 to open a new chart window, return to the batch file window, and simultaneously watch fluorescence measurements and execution of the batch file.

4.2 WinControl-3 Language Reference

4.2.1 Syntax Rules

- Commands and variables of WinControl-3 batch files are case sensitive.
- Commands are typed in lower cases (see 0).#
- Commands must be followed by a SPACE.
- First character of all variables is the dollar sign: "$"(see 4.2.6).

4.2.2 Comments

Always, a double-slash "`//`" precedes a <comment>.

A <comment> represents a non-executable character sequence which usually contains remarks to comment or structure a batch file. <Comments> can be inserted at any part of a batch file, either as an extra line or in a command line.

Examples

```
// This is a comment
$a = 5 // Set variable "a" to value "5"
```

4.2.3 Expressions

WinControl-3 batch file language permits simple arithmetic, comparative, and Boolean operations. Table 4.1 compiles the available operators and results. Examples of operations are given next:

`$a + 5`	Results in content of variable $a plus 5
`$a = 5`	Results in 1 (yes) if $a equals 5
`$a = 5 || $a > 7`	Results in 1 if a equals 5 or is greater than 7
`$a = 3 * ($b + 5)`	Assigns the expression "3 * ($b + 5)" to $a.

Table 4.1: Operators

Category	Operator		Results
Basic arithmetic	`+`	addition	Numerical values
	`-`	subtraction	
	`*`	multiplication	
	`/`	division	
Comparative	`=`	equality	`0` (false) or `1` (true)
	`>`	greater than	
	`>=`	greater than or equal	
	`<`	less than	
	`<=`	less than or equal to	
	`!=`	not equal	
Boolean	`&&`	logical AND	`0` if one or both operands = 0 `1` if both operands != 0
	`||`	logical OR	`0` if both operands = 0 `1` if one or both operands != 0

Operator precedence: (*Parentheses override the normal precedence as expected.*)	1. `*`, `/`. 2. `+`, `-`. 3. `=`, `>`, `>=`, `<`, `<=`, `!=`. 4. `&&` 5. `||`

4.2.4 Commands

Commands are instructions which tell the computer to carry out particular operations. WinControl-3 includes 4 types of commands: Commands for repeated operations (Table 4.2), commands affecting the user interface (

Table 4.3), commands controlling timing of operations (Table 4.4) and other commands (Table 4.5).

Table 4.2: Commands - Repeated Operations

Command/Example	**Comment**
Conditional Commands	
Command: `if`	Execute if condition is TRUE.
Example: `if $a = 5`	Condition is: `"$a = 5"`.
Command: `else`	Execute if condition is FALSE.
Example: `if $a = 5 else`	
Command: `endif`	End of conditional block.
Conditional Loop	
Command: `while`	If condition is TRUE execute next commands. If condition is FALSE jump to command after "wend", that is, leave loop. Condition is "`$b < 10`".
Example: `while $b < 10`	

Command/Example	Comment
Command: `wend`	End of conditional loop and returns to the conditional statement of loop.
Repeated Loop	
Command: `for` Example: `for $a = 1 to 8`	Execute block 8 times. Count variable ”a” from 1 to 8.
Command: `for` Example: `for $b = 10 downto 2`	Execute block 9 times. Count variable ”b” from 10 to 2.
Command: `next`	Marks the end of the repeated loop and returns to the loop’s start.
Subroutine	
Command: `gosub <label>` Example: `gosub OffMinor`	Branches out into subprogram `”OffMinor”`.
Command: `<label>:` Example: `OffMinor:`	Marks begin of subroutine ” `OffMinor`”.
Command: `return`	End of subroutine: return to main program.

Table 4.3: Commands - User Interface

Command: `input OK` Example: `input OK “Please attach leaf clip to leaf and click Ok“`	Show character string in quotation marks and wait until OK is keyed.
Command: `input YN` Example: `input YN $a “New sample?“`	Show character string in quotation marks, wait for YES/NO response and assign 1/0 to variable.
Command: `input <variable>` Example: `input $a [1]-`	Show character string in quotation marks, wait for a numeric value in a given range (1 to 8), or use default value

`[8]:[5] "How many Sat-Pulses?"`	(5).
Command: `print`	Print a value in the Warnings card
Example: `print $a`	which is especially useful for debugging a batch file.
Command: `card`	Switch view to specified card.
Example: `card "Chart"`	

Table 4.4: Commands - Time instructions

Command: `delay`	Halt batch execution for 20 seconds.
Example: `delay 20`	
Command: `wait <condition>`	Wait until condition "SAT-Pulse completed" becomes true.
Example: `wait $S = 0`	
Command: `at <time of day>`	Wait until time of day (10:00).
Example: `at 10:00`	

Table 4.5: Commands - Miscellaneous

Command: `inc, dec`	Increment or decrement a variable by
Example: `inc $a, dec $a`	one.
Command: `end`	End batch execution.

4.2.5 Reuse Subroutines of Existing Batch Files

A currently running batch file can use a subroutine of an external batch file.

Example. It is assumed that:

(1) a previously created batch file named <SAT_analysis.wcb> is located in the same directory as the currently executed batch file.

(2) the file <SAT_analysis.wcb> containes the subroutine <DoSatPulse>.

To execute the subroutine <DoSatPulse>, two command lines are required:

Line 1 registers the external batch file

```
include "SAT_analysis"
```

Line 2 executes the external subroutine

```
gosub DoSatPulse
```

Note that the first command line should be part of the header. The header corresponds to the first line(s) of a batch file.

4.2.6 WinControl-3 Language - Variables

All instrument settings and measured values are accessed using built-in variables (see Table 4.6). As a rule, all labels of build-in variables contain at least one capital letter. The use of uncapitalized user-defined variables is recommended to distinguish the latter from build-in variables.

When measuring values are accessed, the data of the currently selected device channel is retrieved. If several instruments are running, specific data channels can be selected by appending the device number to the variable. Example. It is assumed that devices #1, #2, and #3 are operated by WinControl-3 and that device #1 is currently selected. Then:

`$cFt` returns the Ft-value of the device #1, but

`$cFt(#3)` returns the Ft-value of device #3.

Table 4.6: Predefined Variables of WinControl-3 Batch Files
Devise Parameters

Device Parameter	Variable	Settings	Significance
Act. + Yield switch	`$AY`	`0/1`	start/stop

Device Parameter	Variable	Settings	Significance
Act. + Yield. Width of actinic light.	$AW	5 to 300	time in seconds
Actinic light intensity	$AI	1 to 12	intensity level
Actinic light intensity at light curve start	$LCI	1 to 5	intensity level
Actinic light switch	$A	0/1	off/on
Clock width	$CW	10 to 3000	time in seconds
Clock-triggered event	$CI	1 to 6	1:SAT-pulse 2:Act+Yield 3:Light Curve 4:LC+Rec 5:Induct.Curve 6:IC+Rec.
ETR-Factor	$EF	0.1 to 1	fraction of absorbed light
F offset	$FZ	0 to 4096	relative values
Far red light intensity	$FRI	1 to 12	intensity level
Far red light width of the Fo' determination	$FRW	2 to 30	time in seconds
Far-red light switch	$FR	0/1	off/on
Fo' mode switch	$FOM	0/1	off/on
Fo/Fm-determination	$FOFM	0/1	start/stop
Gain	$G	1 or 2	gain level
Induction curve + recovery switch	$ICR	0/1	start/stop
Induction curve delay	$ICD	5 to 600	time in seconds
Induction curve length	$ICL	8 to 50	number of SAT analyses
Induction curve width	$ICW	5 to 600	time in seconds
Induction curve switch	$IC	0/1	start/stop
Light curve + recovery switch	$LCR	0/1	start/stop

Device Parameter	Variable	Settings	Significance
Light curve width	$LCW	5 to 600	seconds
Light curve switch	$LC	0/1	start/stop
Light sensor (external) switch	$EPAR	0/1	off/on
Measuring light frequency	$MLF	0/1	low/high
Measuring light flash/pulse number	$MI	1 to 5	number of flashes
Measuring light intensity	$MEA	1 to 12	intensity level
Measuring light switch	$M	0/1	off/on
SAT pulse	$S	0/1	1 initiates a SAT pulse
SAT pulse intensity	$SI	1 to 12	intensity level
SAT pulse width	$SW	0.2 to 2	time in seconds

Table 4.7: Predefined Variables of WinControl-3 Batch Files. Data parameters

Data Parameter and Significance		Variable
-	**Current measurement**	
Ft	fluorescence level, relative	$cFt
PAR	photosynthetically active radiation, µmol/(m^2·s)	$cPar
Temp.	temperature, °C	$cTemp
-	**Measurements of previous SAT pulse analysis**	
Fo	Fo fluorescence level	$Fo
Fm	Fm fluorescence level	$Fm
F	F fluorescence	$FPrime
Fm'	Fm' fluorescence level	$FmPrime
Fo'	Fo' fluorescence level, (calculated or, in Fo'-Mode, measured).	$FoPrime
PAR	photosynthetically active radiation, µmol/(m^2·s)	$PAR

Data Parameter and Significance		Variable
Temp.	of previous SAT pulse analysis	$Temp
-	**Fluorescence ratio parameters of previous SAT pulse analysis**	
ETR	Electron transport rate	$ETR
Fv/Fm	Maximum photochemical quantum yield of PS II	$Fv
NPQ	Stern-Volmer type non-photochemical fluorescence quenching	$NPQ
qL	Coefficient of photochemical fluorescence quenching assuming (lake model)	$qL
q_N	Coefficient of non-photochemical fluorescence quenching	$qN
q_P	Coefficient of photochemical fluorescence quenching (puddle model)	$qP
Y(II)	Effective photochemical quantum yield of PS II	$YII
Y(NO)	Quantum yield of basal non-photochemical fluorescence quenching	$YNO
Y(NPQ)	Quantum yield of light-induced non-photochemical fluorescence quenching	$YNPQ

5 Some Reviews on Chlorophyll Fluorescence

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