**PamWin
Windows Software for
PAM-2100 Chlorophyll Fluorometer**

Software V 1.17

Users Manual

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1   Introduction

PamWin is a dedicated windows software developed for the **PAM-2100 Chlorophyll Fluorometer**. Special versions of this software for the precursor model **PAM-2000** and the **PAM-210** (Teaching-PAM) are in preparation. The PAM-2100 can be operated either in the stand-alone mode making use of the internal panel PC and the **DA-2100** software, or under the control of an external PC using **PamWin**. Data collected in the stand-alone mode can be transferred with the help of the **Trans-2100** windows software to a PC, in order be analyzed under PamWin or to be exported into a spread sheet program, like Excel.

PamWin supports the most essential functions of chlorophyll fluorescence measurements by **PAM fluorometry** and quenching analysis by the **Saturation Pulse method**. Basic features of this approach are:

- chlorophyll fluorescence is excited by μsec-pulses of measuring light (normally derived from light-emitting-diodes, LEDs) and the yield of pulse modulated fluorescence is selectively measured (Pulse Amplitude Modulation, PAM)

- actinic light is applied to drive photosynthesis; before reaching a steady state, characteristic changes in the yield of chlorophyll fluorescence are induced, which are due to changes in the yields of photochemical energy conversion and of heat dissipation

- dark/light induction curves (**Kautsky effect**) are recorded under defined conditions in order to obtain detailed information on various partial reactions of photosynthesis (**Induction Curves**); the rapid kinetics reflect the light induced closure of photosystem II reaction centers, whereas the slow transients reflect the

complexity of overall photosynthesis, including dark enzymatic reactions and regulation)

- saturating pulses of light (**Saturation Pulses**) are applied to induce a transient block of photochemical energy conversion and a maximal yield of chlorophyll fluorescence; in this way in any given state the effective quantum yield of energy conversion (**Y**) and the coefficients of so-called photochemical quenching (**qP**) and nonphotochemical quenching (**qN** and **NPQ**) can be determined

- the effective quantum yield, Y, is measured after defined periods of illumination at stepwise increasing light intensities in order to obtain light response curves (**Light Curves**), which give insight into the photosynthetic capacity and the current light adaptation state of a sample

The PamWin software can be used either for measurements (**MEASURE-mode**) or for analyzing previously measured data (**VIEW-mode**). When used in the MEASURE-mode, the panel PC integrated in the PAM-2100 is switched off, so that all instrument functions are under the full control of the external PC. This is the most comfortable mode of operation under laboratory conditions. On the other hand, for field work it is more practicable to operate the PAM-2100 in the stand-alone mode, without the burden of an external PC. In both cases, the same PamWin software can be used for data analysis in the VIEW-mode.

This manual deals specifically with features of the PamWin software. A separate manual is provided for the PAM-2100 and its operation in the stand-alone mode using the Data Acquisition system DA-2100. Please consult the DA-2100 Manual for detailed

description of all instrument functions and for further background information on chlorophyll fluorescence parameters. This PamWin manual is meant to serve as a quick reference guide. Actually, most users accustomed to Windows-programs will find that PamWin is mostly self-explanatory. A brief description of each functional field on the screen is given by so-called Tooltips, which appear when the mouse-cursor approaches the corresponding fields. The user is encouraged to try out all functions in a playful way; there is no risk of damaging the instrument.

The PamWin program has been tested extensively and considerable efforts were taken to eliminate software errors. Nevertheless, experience tells us that there are still hidden errors, which will show only in particular situations. We appreciate when such errors are pointed out to us and are also open for suggestions of program extensions and improvements. Updated versions of PamWin will become available as free downloads from our Internet Homepage (www.walz.com under Support/Downloads).

2   Installation of the PamWin software

Together with your instrument you receive a **CD** with the software that has to be installed on the PC which is going to be used in conjunction with the PAM-2100. You can also download the software from our homepage (see above). At the end of the guided installation procedure a **PamWin folder** is created on the PC, which contains the **Data-directory,** the **PamWin.exe** file and the **Trans-2100.exe** file (for data transfer from the PAM-2100 to an external PC). Into the Data-directory all measured data will be written.

Please note that for proper display of the PamWin user surface on the PC screen, the screen resolution should be set under Windows to **1024x768 dots** and the DPI-setting should be **96 dpi** (normal size, small letter size).

Steps of the PamWin installation

If an older version of PamWin is already installed on the PC, this first has to be deinstalled under Windows via Start/System Control/Software. Then the new PamWin software can be installed:

- Put CD into drive D of your PC

- Call up "My Computer" and select drive D

- Double click the file Setup.exe
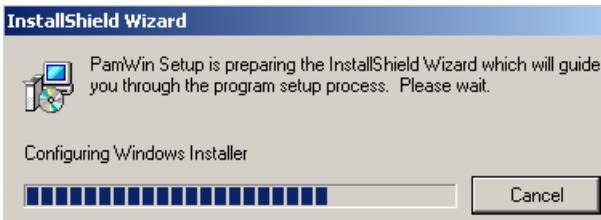
After start of **Setup.exe** the Install Wizard is called up, which guides you through the installation, at the end of which the PamWin folder will be installed on your PC (normally on drive C), with links to **PamWin Folder** and to **PamWin.exe** created on the desktop. In order to start the program, the PamWin icon has to be double-clicked.

When in the future updates of PamWin.exe become available, the previous PamWin.exe file can be simply overwritten.

3   PAM-2100 operation under PamWin

In order to operate the PAM-2100 under PamWin, i.e. under the control of an external PC, the internal panel PC must be switched off. If the instrument is switched on in the stand-alone mode (controlled by DA-2100 program), the DA-2100 program is quit via the menu point Quit Program ALT-X in the Com-menu. Alternatively, the instrument can be also turned off by pressing the POWER ON switch for longer than about 2 sec. In this case, however, the instrument settings are not saved in the INI-file.
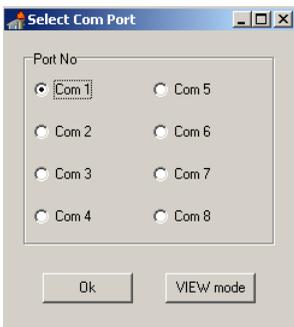
When PamWin is started for the first time on a particular PC, the **serial interface communication port** on the PC has to be defined. This information is stored by the program and in the future the program does not ask for this information again, unless there is something wrong with the communication between instrument and PC. When the PAM-2100 is connected via a **RS-232 cable** to a PC, the instrument is automatically switched on upon start of PamWin, as indicated by the green Status LED. No serial connection is required for the analysis of previously recorded data in the **VIEW-mode** (see section 4).
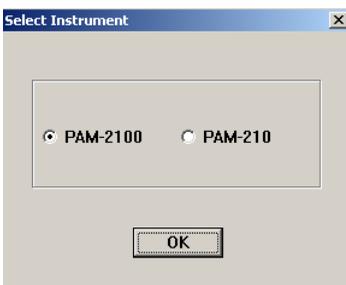
Once the communication between instrument and PC is established, the program asks whether the PAM-2100 or the PAM-210 is going to be used. After confirmation of the instrument choice, the program is started and the PamWin user surface is opened showing the **Settings-window**, which is one of 5 windows.

3.1   PamWin user surface
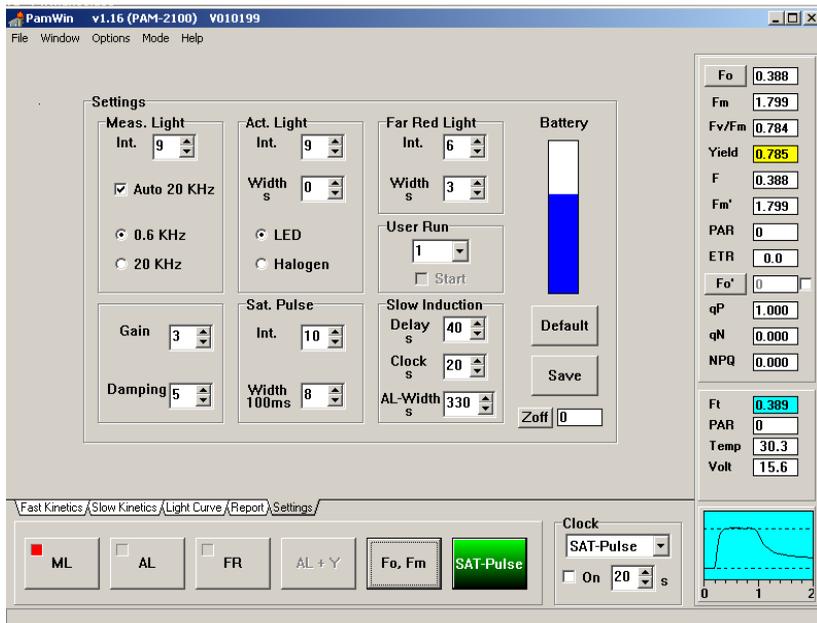
Fig. 1   User surface of PamWin with display of Settings-window

The user surface of PamWin is divided into three main sections (see Fig. 1), which are briefly explained before details on the various functions will be outlined in the following sections.

1) On the right hand side the fluorescence data and the values of some other relevant parameters are shown. At the top are the parameters Fo, Fm and Fv/Fm, which are normally

measured with a dark-adapted sample at the start of an experiment (Fo, Fm-measurement). Then the parameters Yield, F, Fm', PAR, Fo', qp, qN and NPQ are displayed. These values are updated with each Saturation Pulse (started via the SAT-Pulse button). Below that the on-line parameters Ft, PAR, Temp and Volt are displayed, which are continuously updated. The Ft value is particularly important, as it reflects the on-line measured fluorescence yield. At the bottom there is a small graphics field in which the rapid kinetics during a Saturation Pulse are depicted (see section 3.2.2).

2) The bottom part of the screen features **on/off switches** for Measuring Light (**ML**), Actinic Light (**AL**) and Far-Red Light (**FR**) as well as Start-buttons for Actinic illumination with terminal Yield determination (**AL+Y**), for **Fo, Fm-determination** and for a single Saturation Pulse (**SAT-Pulse**). With the help of the **Clock** a sequence of Saturation Pulses at a defined interval time can be switched on/off (On checkbox).

3) The central part of the screen is defined by choosing one of 5 register cards **(Fast Kinetics, Slow Kinetics, Light Curve, Report, Settings)**. The Settings-window is installed by default upon start of the program. The other windows can be called up by clicking the corresponding register cards. The various windows will be explained in detail in separate chapters below.

3.2   Measured parameters

3.1.1   On-line monitored parameters

Ft	0.396
PAR	724
Temp	31.0
Volt	15.8

While the **on-line parameters** are continuously measured, they are <u>not</u> stored, unless a Saturation Pulse is given.

Ft   Momentary value of the **fluorescence yield**; this value is saved in the parameter F in conjunction with a Saturation Pulse.

PAR   Current value of **Photosynthetically Active Radiation**, as measured externally with the Leaf-Clip Holder 2030-B. If the latter is not connected, the PAR-value is derived from a previously defined "Internal PAR-list".

Temp   Current value of **temperature** in degree centigrade at the measuring site, as measured with the Leaf-Clip Holder 2030-B.

Volt   Current **voltage** value of the internal **Li-ion battery.**

3.1.2   Parameters determined in conjunction with a Saturation Pulse

Fo	0.367
Fm	1.846
Fv/Fm	0.801
Yield	0.154
F	0.445
Fm'	0.526
PAR	728
ETR	47.2
Fo'	0.285 ☑
qP	0.337
qN	0.845
NPQ	2.508

Saturation pulses play a central role in chlorophyll fluorescence quenching analysis with PAM fluorometers. The chlorophyll fluorescence signal as such depends on numerous often uncontrolled factors (e.g. distance to sample, chlorophyll concentration, sample size, output of measuring light LED etc.) and, therefore, can serve only as a qualitative indicator. On the other hand, on the basis of ratio terms featuring the closely associated values of **Fo, Fm, F, Fm' and Fo',** which are measured in conjunction with a Saturation Pulse, quantitative information on photosynthetic parameters of a sample can be obtained, which is expressed in the fluorescence parameters **Fv/Fm, Yield** and **ETR**, as well as the **quenching coefficients qP, qN and NPQ**. These are the actual fluorescence data, which together with the correlated information on **PAR** and **Temperature** (if appropriate sensors are connected) are stored in the **Report**-file. For a detailed explanation of these parameters and background information on the Saturation Pulse method, please consult the separate Handbooks for the PAM-2100. Here just a brief description is given:

Fo   **Minimal fluorescence yield** of a dark-adapted sample measured by pressing the Fo-button; this parameter is also determined in conjunction with an Fm-determination using the Fo, Fm-button.

Fm   **Maximal fluorescence yield** of a dark-adapted sample measured by pressing the Fo, Fm-button.

Fv/Fm   Quantum yield of dark-adapted sample, with **Fv** being defined as **Fm-Fo**. Fv/Fm is a measure of the **potential quantum yield** of a sample, which normally is lowered in the light by partial closure of reaction centers and dissipation of light energy by processes associated with nonphotochemical quenching. (Hence, the effective quantum yield, Yield, of an illuminated sample generally is lower than Fv/Fm).

Yield   **Effective quantum yield** of photochemical energy conversion at PS II reaction centers calculated according to the equation:

Yield = (Fm'-F)/Fm' (Genty-parameter)

normally measured in the presence of actinic light.

F   **Fluorescence yield** measured briefly before application of the last Saturation Pulse; normally measured in the presence of actinic light.

Fm'   **Maximal fluorescence yield** reached during last Saturation Pulse; normally measured in the presence of actinic light.

PAR   **Photosynthetically active radiation** displayed in the on-line PAR field briefly before the last Saturation Pulse.

ETR   Relative Electron Transport Rate calculated from the equation:

ETR = Yield ∗ PAR ∗ 0.5 ∗ ETR Factor

with the factor 0.5 taking into account that roughly 50% of all absorbed quanta reach PS II, and the ETR Factor

defining the fraction of incident light estimated to be absorbed by the sample (default value 0.84).

Fo'   **Minimal fluorescence yield** of a preilluminated sample, determined briefly after turning off the actinic light and after several seconds of far-red illumination to assure full oxidation of PS II acceptors. This parameter is measured only when the **Fo'-check-box** is activated (measurement with every Saturation Pulse) or when the **Fo'-button** is pressed (measurement with next Saturation Pulse).

qP   Coefficient of photochemical quenching defined by the equation:

qP = (Fm'-F)/(Fm'-Fo')

If Fo'-determination is not activated, qP is determined on the basis of Fo.

qN   Coefficient of nonphotochemical quenching defined by the equation:

qN = (Fm-Fm')/(Fm-Fo')

If Fo'-determination is not activated, qN is determined on the basis of Fo.

NPQ   Parameter describing nonphotochemical quenching defined by the equation:

NPQ = (Fm-Fm')/Fm'

Please note that this parameter is not affected by a lowering of Fo' with respect to Fo.
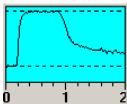
After every Saturation Pulse the **kinetics** of the induced fluorescence change is displayed in a little graph. In this

way the user can judge whether a distinct plateau of maximal fluorescence yield is reached during the Saturation Pulse. The broken line corresponds to the Fm value.
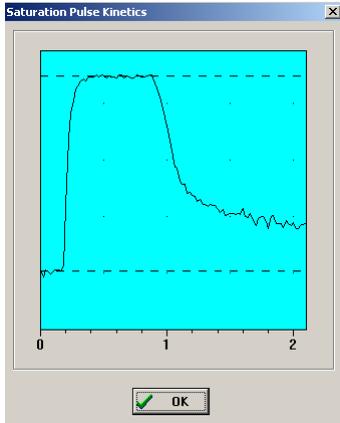

Fig. 2

Kinetics of fluorescence changes induced by a 800 ms Saturation Pulse in conjunction with an Fo, Fm-determination. These kinetics reflect the Saturation Pulse induced reduction of the PS II acceptor pool and its reoxidation in the following dark period. The dotted lines show the measured Fo and Fm levels.

The **Saturation Pulse Kinetics** can be displayed in enlarged form in a separate window by **double click** with the **left mouse-button**.

Based on this kinetic information the **Saturation Pulse Intensity** and **Width** can be optimized (under Main Settings). The intensity and width should be adjusted such that a plateau is formed. Please note that higher intensities are required with preilluminated samples (as compared to dark-adapted samples), as photosynthetic electron transport is light-activated and quantitative reduction of the PS II acceptor side becomes more difficult. In most applications, maximal Saturation Pulse intensity (setting 10) and minimal pulse width (400 ms) can be recommended.

3.2   Light Controls

ML   On/off switch of **Measuring Light** for fluorescence excitation.

AL   On/off switch of **Actinic Light** for driving photosynthetic electron transport.

FR   On/off switch of **Far-Red Light** for preferential illumination of Photosystem I, in order to stimulate oxidation of PS II acceptor pool.

AL + Y   Start-button of actinic illumination, the duration of which is defined under Settings, with terminal application of a Saturation Pulse for assessment of the effective quantum yield (Yield); this function is activated only, when an actinic illumination time of at least 3 s is defined (under Settings).

Fo, Fm   Start-button for **Fo, Fm-determination** by application of a Saturation Pulse, which should be carried out after dark-adaptation or adaptation to low light intensity (up to ca. 40 µmol quanta m^{-2} s^{-1} PAR); the Fo and Fm values are the basis for calculation of the potential PS II quantum yield, Fv/Fm, and of the coefficients of photochemical and nonphotochemical quenching, qP and qN (or NPQ), respectively.

SAT-Pulse Start-button for a single **Saturation Pulse,** with which the fluorescence yields F and Fm' are measured, on the basis of which the effective quantum yield ,Yield = (Fm'-F)/Fm', as well as the coefficients of

photochemical and nonphotochemical quenching are calculated.
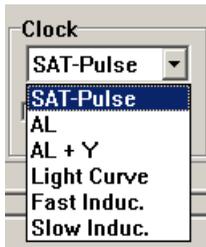
The **Repetition Clock** serves for the repetitive start of a sequence of Saturation Pulses or of various other types of illumination. A selection menu is provided for definition of the Clock Item. The time interval between two consecutive starts can be set with the help of the arrow keys. The Clock sequence is switched on/off via the On checkbox.

3.3 Settings window
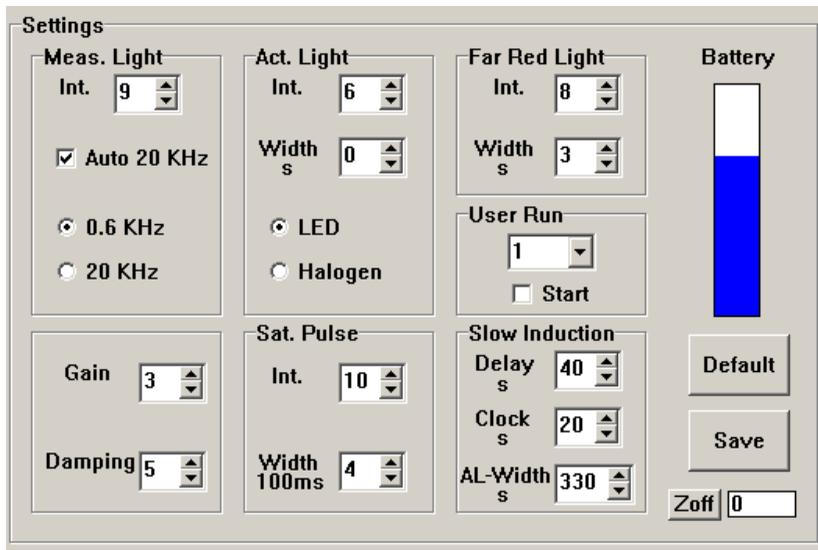
Fig. 3   Settings window

The Settings-window is installed upon start of the PamWin program. It features the controls of the variable instrument settings, i.e. primarily of the various light sources. In the following, the

various controls and functions depicted on the Settings-window are outlined:

Meas. Light   The intensity of the **Measuring Light** (Meas. Light Int.) can be varied by 10 settings, with setting 9 being standard. Meas. Light Int. relates to the **amplitude** of the individual μsec-pulses of the Measuring Light. When the **Auto 20 KHz** check-box is activated, the frequency of Measuring Light pulses is increased automatically from **0.6 KHz** to **20 KHz**. This leads to increased time resolution and enhanced signal/noise. It is also possible to select 0.6 or 20 KHz manually with the help of the corresponding radio buttons. While at 0.6 KHz the Measuring Light does not change the illumination state of a sample (no actinic effect), at 20 KHz the Measuring Light pulses sum up to an overall intensity equivalent to ca. 10 µmol quanta m^{-2} s^{-1} PAR (at default setting 9 of Meas. Light Int.), which has a distinct actinic effect.

Act. Light

   Int.   The **Actinic Light Intensity** (Act. Light Int.) can be varied between 12 settings. A special role is played by setting 0, which relates to the actinic illumination due to the measuring Light at 20 KHz (see above). Largely different irradiance levels are obtained at the various settings of Act. Light Int. depending of the

choice between the LED-array or the Halogen actinic lamp.

Width **Actinic illumination time** which can be varied between 1 sec and 15 min. At setting 0 the illumination time is indefinite, which means that Actinic illumination has to be switched off manually.

LED Radiobutton for selection of **LED-array actinic lamp**, with which red light (655 nm) at intensities of up to ca. 600 µmol quanta $m^{-2} s^{-1}$ PAR can be applied. This lamp is particularly important for the recording of Fast Kinetics. When Fast Kinetics are started, the LED lamp is switched on, irrespectively of whether it is selected under Settings or not.

Halogen Radiobutton for selection of **Halogen actinic lamp**, with which white light (<700 nm) at intensities of up to ca. 5000 µmol quanta $m^{-2} s^{-1}$ PAR can be applied. This lamp is required for light saturation of plants adapted to high intensities. It cannot be used for Fast Kinetics, because of the slow light-on characteristics.

Sat. Pulse Under **Saturation Pulse** the two parameters defining the intensity and width of Saturation Pulses are listed.

Int. **Saturation Pulse Intensity** can be varied between 10 settings. In most applications the maximal setting 10 is feasible.

Width   **Saturation Pulse Width** can be varied between 0.4 and 2 sec. In many applications a clear-cut plateau is already reached within 0.4 sec, as can be ascertained via the Saturation Pulse Kinetics (see Fig. 3). For the sake of noninvasiveness of the method an excessive Saturation Pulse Width should be avoided.

Far Red Light   Under **Far Red Light** two parameters relating to illumination with far red light are listed.

Int.   **Far red light intensity**, which can be varied between 10 settings.

Width   **Far red illumination time** which can be varied between 1 and 10 sec. At setting 0 the illumination time is indefinite, which means that Far Red illumination has to be switched off manually.

A Run is a preprogrammed sequence of commands. **User Runs** can be programmed by the researcher. Presently (i.e. September 2003), however, this function is not yet supported by the PamWin software.

The **Gain** relates to the amplification of the fluorescence signal. It can be changed between 9 settings, with the standard setting 3. Please note that the signal/noise ratio is not changed by the Gain setting. For optimal results it is recommended to choose a high setting of Measuring Light intensity (e.g. standard setting 9) and then adjust the signal amplitude to ca. 400 units.

The **Damping** serves for increasing the time constant of a signal change, thus leading to the elimination of rapid noise. It can be

changed between 8 settings, with the standard setting 5. For the recording of Fast Kinetics (see 3.8), this Damping is too high and Damping 2 is recommended.
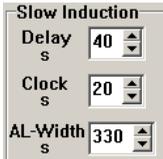
Under **Slow Induction** three different parameters can be set, for definition of a Slow Induction Curve recording with repetitive application of Saturation Pulses (see 3.7). The **Delay** defines the time between the initial Fo, Fm-determination and the onset of Actinic Illumination. The **Clock** time determines the interval between two consecutive Saturation Pulses. The **AL-Width** defines the overall length of the Induction Curve.
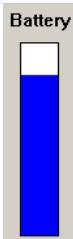
The Battery-status is indicated by a blue bar, the height of which represents the remaining capacity. When the Battery Charger 2120-N is connected, high capacity is indicated even when the battery is only partially charged. hence, the true Battery-status should be evaluated with the charger being disconnected. Please note that the life time of the Li-ion battery is increased when the charger is not connected during measurements. For the sake of battery lifetime it is best to charge the battery over night. Normally a fully charged battery will give power for a whole working day.

When the **Default**-button is pressed, the standard instrument settings are installed.

Upon pressing the **Save**-button, the current instrument settings are written into the **Report-file** (see 3.5).

When the **Zoff**-button is pressed, the current fluorescence signal is zeroed (**Zero Offset**). In this way a background signal can be suppressed. This function is

particularly useful at high Gain setting, when a non-chlorophyll background signal contributes significantly to the overall signal.

3.4   The Report window


```
 STANDARD.RPT [████        12%                    ]          📂 💾

; Helianthus annuus, sun leaf
07AUG2003 LC   ML   Tmp.   PAR   Fo           Fv/Fm              Em
17:31:11       206  27.8     0  0.354         0.775           1.571
  Time    No.  ML   Tmp.   PAR   Ft    ETR    Yield  qP    qN    Fm'   Fo'
17:31:31    2  206  28.0     0  0.407   0.0   0.738 0.955 0.014 1.554
17:31:51    3  202  28.1    76  0.445  21.0   0.659 0.904 0.220 1.304
17:32:11    4  200  28.5   126  0.494  32.3   0.610 0.847 0.249 1.268
17:32:31    5  200  28.4   199  0.471  52.5   0.629 0.872 0.248 1.269
17:32:51    6  201  28.8   312  0.451  81.3   0.621 0.883 0.313 1.190
17:33:11    7  200  28.9   482  0.434 114.3   0.565 0.875 0.472 0.996
17:33:31    8  200  29.1   676  0.414 141.1   0.497 0.872 0.615 0.823
17:33:51    9  199  29.5  1081  0.395 172.2   0.379 0.854 0.768 0.636
17:34:11   10  198  29.8  1651  0.389 191.9   0.277 0.810 0.849 0.537
; kinetics saved in helia01.PWS


;ivy, shade leaf
07AUG2003 LC   ML   Tmp.   PAR   Fo           Fv/Fm              Em
17:50:44       205  29.9     0  0.425         0.775           1.889
  Time    No.  ML   Tmp.   PAR   Ft    ETR    Yield  qP    qN    Fm'   Fo'
17:51:04    2  205  30.0     0  0.444   0.0   0.763 0.987 0.011 1.873
17:51:24    3  201  30.1    79  0.660  14.7   0.443 0.691 0.481 1.185
17:51:44    4  200  30.3   130  0.618  19.0   0.347 0.631 0.644 0.946
```
\Fast Kinetics ʌSlow Kinetics ʌLight Curve ʌReport ʌSettings /

Fig. 4   The Report-window in the MEASURING-Mode showing two Light Curve recordings

The **Report window** shows all measurements that involve the application of Saturation Pulses. The Report is organized into **Records**, each of which is started by an **Fo, Fm-determination**. Light Curves (see 3.6) and Slow Kinetics (Induction Curves, see 3.7) are separate Records. The comment written on top of a particular Light Curve or Induction Curve recording is automatically written at the start of the corresponding part of the Report (see Fig. 4). Text lines in the Report are started with a **semicolon** and thus can be distinguished by the program from data lines (no semicolon). If the kinetics of a Light Curve or Slow Kinetics recording is saved, this is

documented by a corresponding text line at the end of this Record (see Fig. 4). While the user may enter further comment lines into the Report, which always have to start with a semicolon, it should be noted, that these comment lines are <u>not</u> shown in the VIEW-mode and, hence, will be lost when the Report is saved, as only the current Report-file (**STANDARD.RPT**) can be viewed in the MEASURE-mode. The Report under PamWin is compatible with the Report recorded in the stand-alone mode under DA-2100.

The horizontal **indicator bar** above the Report-window provides information on the extent to which the **storage capacity** of the Report-file currently is occupied. Before 100 % is reached, the current Report-file (STANDARD.RPT) should be first saved and then cleared.

File
    Open Report
    Save Report
    Clear Report
    Printer Setup
    Print Report
    Exit
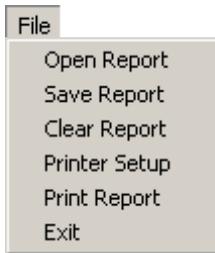
The **Save Report** and **Clear Report** commands are part of the **File**-menu, which also features commands for opening a Report-file in the VIEW-mode (**Open Report**), for setting up a printer (**Printer Setup**) and for print-out of the Report (**Print Report**).

For opening a Report-file in the VIEW-mode and for saving a Report file also corresponding **speed-buttons** are provided.

When under Settings the **Save**-button is pressed, a text line (starting with a semicolon) is inserted in the Report which documents the current **instrument settings**. The following abbreviations are used: MI=Measuring Light Intensity, G=Gain, D=Damping, AI=Actinic Light Intensity, AW=Actinic Illumination Width, FI=Far Red Intensity, FW=Far red Illumination Width, SI=Saturation Pulse Intensity, SW=Saturation Pulse Width, ID=Induction Curve Delay

Time, II=Induction Curve Clock Interval and IS=Induction Curve Seconds (total length).

`; MI=9, G=3, D=5, AI=5, AW=0, FI=8, FW=3, SI=8, SW=8, ID=40, II=20, IS=330`

Read Settings
Copy
Cut
Paste
Print

Settings saved in the Report can be reinstalled at any later time by marking the corresponding text line and the **Read Settings** command, which is contained in the **pull-down menu** that opens with a **right-mouse click.** This menu also features the **Copy, Cut, Paste** and **Print** commands. The parts of the Report, for which these commands shall apply, have to be marked by pressing the left mouse-button and pulling over the corresponding part.

The Report-file plays a particular role in the VIEW-mode (see section 4), where the current Report (STANDARD.RPT) as well as previously saved Report-files can be viewed. A Report-file recorded in the stand-alone mode using the DA-2100 Data Acquisition System can be transferred with the help of the Trans-2100 program (see DA-2100 Manual) to a PC and then be analyzed under PamWin in the VIEW-mode.

3.5   Light Curve window

The recording of a **Light Curve** consists of a series of illumination steps, with a Saturation Pulse being applied at the end of each step for assessment of chlorophyll fluorescence parameters, from which the effective PS II quantum yield (Yield), the relative electron transport rate (ETR) and the coefficients of photochemical and nonphotochemical quenching are calculated. The measured (or calculated) parameters are plotted against the incident PAR (photosynthetically active radiation in µmol quanta m^{-2} s^{-1}).

Preferentially the PAR is measured on-line with the help of the Leaf-Clip Holder 2030-B. If this is not available, one of two internal PAR-lists applies, which were measured at the factory under standard conditions using the LED or the Halogen-lamp. These lists can be recalibrated by the user under Options/Light Calibration). The program automatically recognizes whether the LED- or Halogen-lamp is used.

Fig. 5   Light Curve window with ETR-display

Typical Light Curve recordings are shown in Fig. 5 (ETR) and Fig. 6 (Fluorescence). The ETR-Light Curve is fitted on the basis of the photosynthesis model of Eilers and Peeters (1988) (see below). In the given example, an illumination step width of 20 s was chosen, which is too short to reach a true steady-state. It should be pointed out that such "Rapid Light Curves" provide somewhat different information than conventional P-I Light Response Curves obtained via gas exchange measurements. The same leaf can show under in situ conditions quite different Rapid Light Curves during the course

of a day, thus reflecting the light adaptation and light regulation state of a plant.
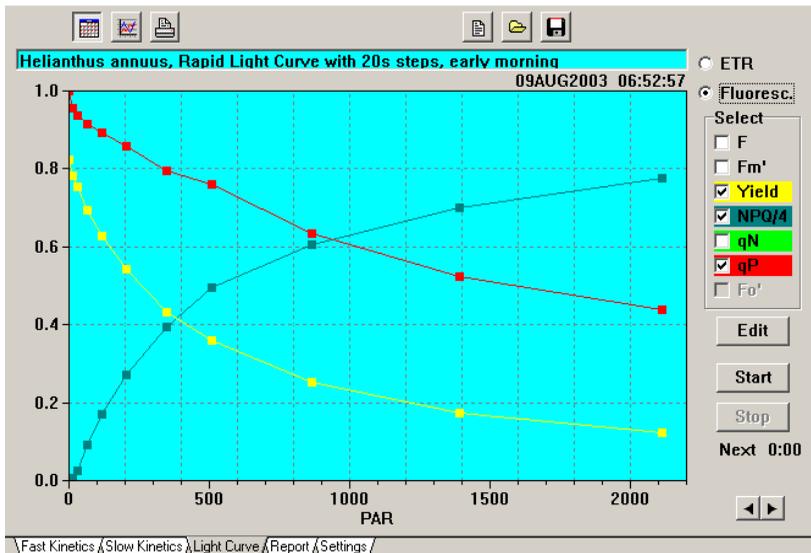
Fig. 6   Light Curve window with Fluorescence-display and selection of the parameters Yield, NPQ/4 and qP

For the sake of clarity, in the example of Fig. 6 only part of the available fluorescence parameters are displayed. Please note the close correspondence of Yield and NPQ/4, which suggests that under the given experimental conditions with increasing PAR the effective quantum yield decreases to the same extent as excessive light energy is dissipated into heat, thus reflecting a close to perfect regulatory system.

With every Light Curve recording automatically also a recording of the **Slow Kinetics** is started (see 3.7). While the Light Curve is always saved in the STANDARD.RPT (see 3.5), the Slow Kinetics have to be saved separately in form of a PamWinSlow (**PWS**)-file (see below).

The various functional elements of the Light Curve window are outlined as follows, starting at the upper left corner and ending at the lower left corner of the screen:

Vertical scale adjustment (for ETR-display only)

Activate/deactivate **grid**

Horizontal and vertical **Autoscale**

Printout of displayed Light Curve

Speedbutton for opening **Comment-file**, into which information on a Light Curve recording can be written, provided this was saved in form of a PWS-file.

Speedbutton for **opening** a stored **PWS-file**, which means moving from the MEASURE- to the **VIEW-mode**. Please note that the previously measured Slow Kinetics will be lost unless saved in a PWS-file. This is not true for the Light Curve recording displayed in the Light Curve window, which is saved in the STANDARD.RPT.

Speedbutton for **saving** the **Slow Kinetics** of a Light Curve recording in form of a **PWS-file.**
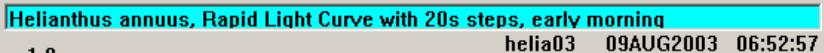
Helianthus annuus, Rapid Light Curve with 20s steps, early morning
1.8   helia03   09AUG2003 06:52:57

Above the display of a Light Curve automatically the date and time of the recording are written. If the Slow Kinetics were saved in a **PWS-file**, the corresponding file name is shown. In addition, the user may also write a short comment into a **text line** on top. Furthermore, longer comments may also written into a **Comment-file** (see above).

⊙ ETR
○ Fluoresc.

The user may choose between display of ETR or of various Fluorescence parameters. A selection menu for the Fluorescence parameters is provided (see Fig. 6). Various such parameters can be displayed on top of each other by marking the corresponding check-boxes.

When the **Fit-button** is clicked, a routine is started for fitting a displayed ETR-Light Curve with a theoretical light response function according to the photosynthesis model of Eilers and Peeters (1988):

Fit

$$ETR = \frac{PAR}{a * PAR^2 + b * PAR + c}$$

Light Curve Fit

alpha   0.343

ETRmax   109.9

Ik   320.9

✓ OK
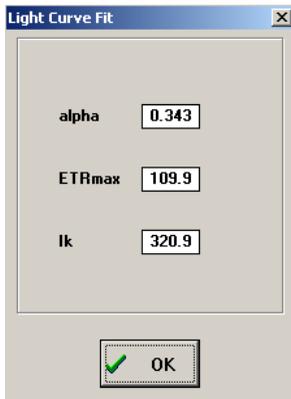
with the coefficients a, b and c being fitted for least square deviation. The fitted curves are displayed as continuous lines superimposed on the data points of the ETR-Light Curves (see Fig. 5).

The calculated **Light Curve Fit Parameters** are displayed after clicking the speedbutton located above the Fit-button. The parameters alpha (α), ETR_{max} and I_k describe the photosynthetic performance of a leaf. The numerical value of α is equivalent to the maximal Yield multiplied by a PS II absorptivity term, which is equivalent to the product of PAR absorptivity and the fraction of absorbed quanta distributed to PS II. A default value of 0.84 is assumed for PAR absorptivity (ETR factor), which may be modified by the user under Options/ETR factor. It is further assumed that 50% of the absorbed quanta are distributed to PS II. Hence, normally ETR is described by the equation:

$$ETR = 0.42 \times PAR \times Yield$$

In the given example, the maximal quantum yield, as determined by a Fo, Fm-determination at the start of the Light Curve, was $0.343/0.42 = 0.82$

ETR_{max} represents maximal electron transport rate calculated by the fitting routine. Please note, that based on the applied model this parameter can be calculated, even if the applied PAR is not sufficient to reach the maximal rate.

The parameter I_k corresponds to the particular PAR-value at the crossing point of the line defined by the initial slope (equivalent to α) and a horizontal line through ETR_{max} (ceiling of the Light Curve). The numerical value of I_k, which corresponds to the expression α/ETR_{max}, is characteristic for onset of light saturation. ETR_{max} as well as I_k increase with the photosynthetic capacity of a leaf.

When the **Edit-button** is pressed, a separate window is opened, which shows the current **Light Curve parameters**, which are different depending on the choice of LED- or Halogen-lamp. The **Intensity**-settings as well as the **Time** interval for each step can be edited by the user. For changing a setting, the corresponding field first has to be marked by the cursor (left mouse click) and then the desired setting can be typed in. The PAR value is updated, as soon as the cursor is clicked into another field. Up to **20 Intensity Steps** can be freely defined. Please note that it is also possible to define "up&down" Light Curves, with
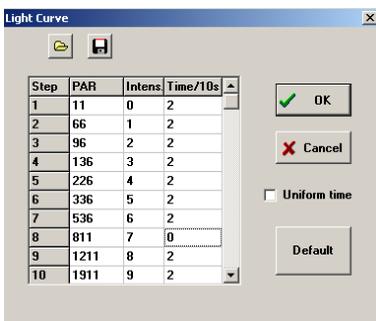
intensities first increasing and then decreasing again. In this way the effect of preillumination can be assessed. A Light Curve can be terminated after less than 20 steps by entering a 0 into the corresponding Time/10s field.

When **Uniform time** is activated, the same time will be entered for all steps, when first a new value is entered and then the cursor is clicked into another field.

The edited Light Curve parameter become effective after being confirmed by **OK**. If the changes shall not become effective, the Edit-window can be quit via **Cancel**.

The standard Light Curve parameters defined at the factory can be restored by pressing the **Default-button**. Separate Light Curve parameters are defined for use of the LED- and Halogen-lamps.

The Light Curve parameters can be saved in an **lcp-file**, which can be opened at any later time, in order to install the same parameters again.

When the Start-button is pressed a Light Curve recording is started by an Fo, Fm-determination. It can be terminated at any time by pressing the Stop-button.
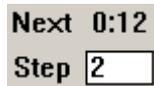
Under **Next** the remaining time until the next measurement is shown. The number of the current **Illumination Step** is displayed.

The PAR-scale can be modified using the horizontal arrow-buttons.

A number of different Light Curves can be superimposed and compared in the **VIEW-mode** (see section 4).

3.6   Slow Kinetics window
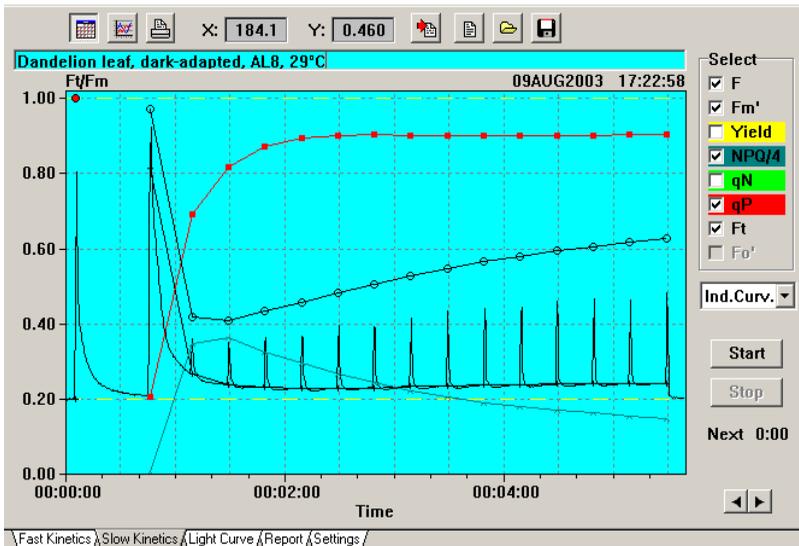
Fig. 7   Slow Kinetics window showing recording of Induction Curve

On the **Slow Kinetics** window light-induced changes of fluorescence parameters are plotted versus time. A standard **dark-light Induction Curve** is displayed in Fig. 7. For the sake of clarity only part of the available fluorescence parameters are displayed. After Start of an Induction Curve first an **Fo, Fm-determination** is triggered. The Fo, Fm information is essential for scaling the Y-axis in units of Ft/Fm and for determination of the coefficients of photochemical and nonphotochemical quenching (qP, q and NPQ). Actinic light is switched on after a delay defined by the Slow Induction Delay parameter (see 3.4). Saturation Pulses are applied repetitively with intervals defined by the Slow Induction Clock. The duration of the actinic illumination is determined by the Slow Induction AL-Width (see 3.4).

After Start of an Induction Curve, the fluorescence yield **Ft** is continuously monitored and stored in a buffer memory. All data measured in conjunction with Saturation Pulses are written in the Report-file (see 3.5). While the latter data are automatically saved with the STANDARD-RPT, the Ft-data have to be saved separately in a **PWS**-file (**P**am**W**in**S**low).

A Slow Kinetics recording is started automatically together with a **Light Curve** recording (see 3.6). If these kinetics are of interest, they can be saved in a PWS-file as well.

The various functional elements of the Slow Induction window are described as follows, starting from the upper left corner and ending in the lower right corner:

On/off switch of **Grid**

Horizontal **Autoscale**

Start-button for **Printout**
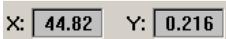
X: 44.82   Y: 0.216   **x-y coordinates** of Ft, as marked by cursor

Speedbutton for import of CMP- or CMN-files that originally were recorded on-line under DA-2100 and then transferred to the PamWin Folder using Trans-2100.

Speedbutton for opening **Comment-file**, into which information on a Slow Induction recording can be written, provided this was saved in form of a PWS-file.

Speedbutton for **opening** a stored **PWS-file**, which means moving from the MEASURE- to the **VIEW-mode**. Please note that the previously measured Slow Kinetics will be lost unless saved in a PWS-file.

Speedbutton for **saving** the **Slow Kinetics** in form of a **PWS-file.**

Dandelion leaf, 130 s illumination at AL8, 25°C
    Ft/Fm   dande06 10AUG2003   08:36:20

Above the display of a Slow Kinetics recording automatically the date and time of the recording are written. If the Slow Kinetics were saved in a **PWS-file**, the corresponding file name is shown. In addition, the user may also write a short comment into a **text line** on top. Furthermore, longer comments may also written into a **Comment-file** (see above).

Eight check boxes are provided for selection of the displayed fluorescence parameters. Normally it is advantageous to display only part of the overall information for the sake of clarity.

The Fo' parameter can be displayed only, if the Fo-checkbox under Settings (see 3.4) was activated during measurement of the Induction Curve. It should be pointed out, however, that measurement of Fo' is problematic at the beginning of a dark-light induction, as the PS I acceptor side may not be sufficiently activated yet to support rapid oxidation of the intersystem electron transport chain by Far-Red illumination.

A selection menu is provided for choosing between three different types of Slow Kinetics recordings, the standard **Induction Curve**, the **Manual** recording and the recording of an Induction Curve plus Recovery (**Ind.+Rec**.). In all three cases, first an Fo, Fm-determination is carried out after Start, for scaling the Y-axis in Ft/Fm units and as a prerequisite for calculation of fluorescence

quenching parameters. The latter can be correct only, if the sample was sufficiently dark-adapted, to allow reoxidation of the PS II acceptor side and relaxation of nonphotochemical quenching.

After the initial Fo, Fm-determination a **Manual** recording is completely controlled by the user. An example is given in Fig. 8-Fig. 9 where ca. 2 min after Start Actinic Light (LED-lamp) at intensity setting 1 is switched on and then the effect of three consecutive 10 s Far-Red illumination periods is demonstrated. This experiment shows the antagonistic effect of short-wavelength (655 nm) and long-wavelength (730 nm) light on the reduction state of the PS II acceptor side.
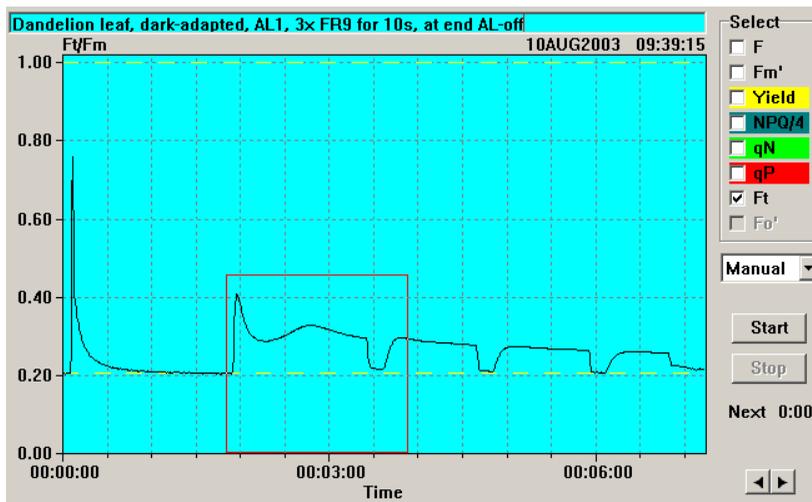
Fig. 8   Example of Manual recording of Slow Kinetics with a part of the recording being marked for Zoom-display
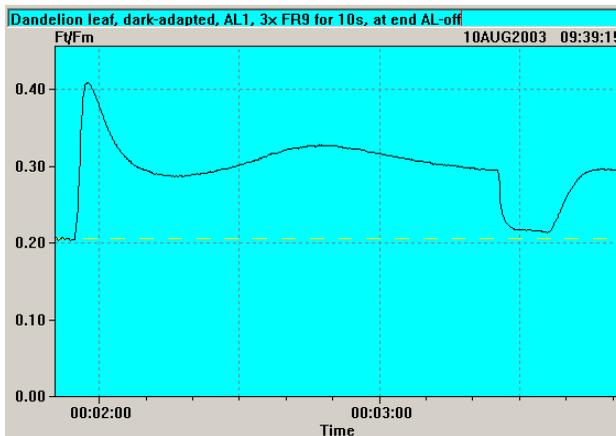
Fig. 9   Zoom display of Slow Kinetics part marked in Fig. 8

For closer inspection of the fluorescence transients, a part of the Slow Kinetics can enlarged by the **Zoom**-function. For this purpose, with the help of the **cursor** and **left mouse-button** a rectangle can be defined, which corresponds to the zoomed part of the overall display. After defining one corner of this rectangle by **left mouse click**, the left mouse-button has to be kept pressed, while the mouse is pulling the cursor to a second corner for final definition of the rectangle. The original full scale display can be restored via **Autoscale** (speedbutton in the upper left corner).

The recording of **Ind.+Rec**. is fully software controlled, similarly to Ind. Curve. In addition to an Induction Curve recording, as defined by the **Slow Induction parameters** under Settings, after termination of Actinic Illumination the dark recovery of the sample is investigated with the help of repetitive Saturation Pulses, which are applied with exponentially increasing time intervals between consecutive measurements: 10s, 12s, 14s etc. according to the function

$$y = 10 \text{ x } 1.2^{n-1}$$
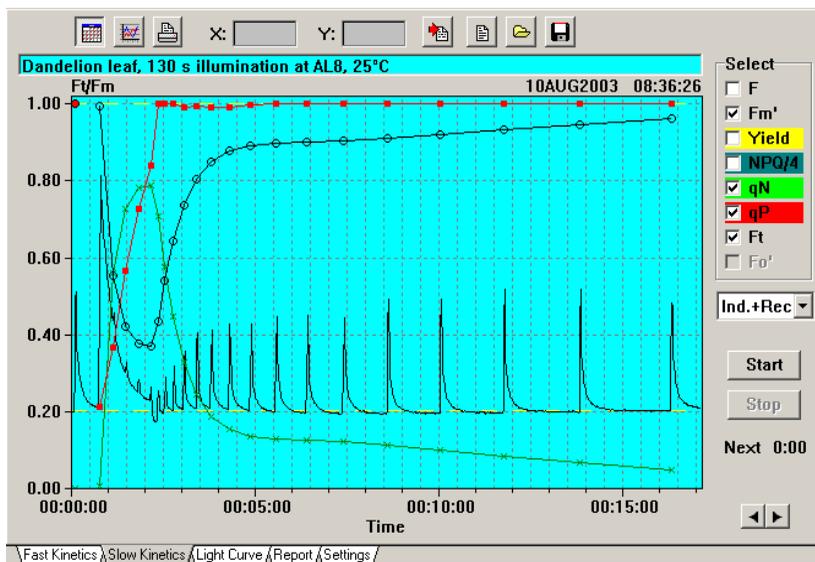
Fig. 10   Example of Induction Curve plus Recovery with display of the fluorescence parameters Fm', qN, qP and Ft

In the Ind.+Rec. recording shown in Fig. 10, relatively short actinic illumination (130 s) was applied, before the dark-recovery of fluorescence parameters was measured. It is apparent that photochemical quenching recovered completely within the first 20 s, whereas relaxation of nonphotochemical quenching proceeded in two steps, with the major first step showing a half-time of ca. 1 min and the second step a half-time of ca. 13 min.

When the Start-button is pressed a Slow Kinetics recording is started by an Fo, Fm-determination. It can be terminated at any time by pressing the Stop-button.

Under **Next** the remaining time until the next measurement is shown.

The **time scale** can be modified using the horizontal arrow-buttons.

Slow Kinetics recordings that were saved in PWS-files can be viewed at a later time in the VIEW-mode, from where they can be also exported into other programs, like Excel for further analysis.

3.7   Fast Kinetics window
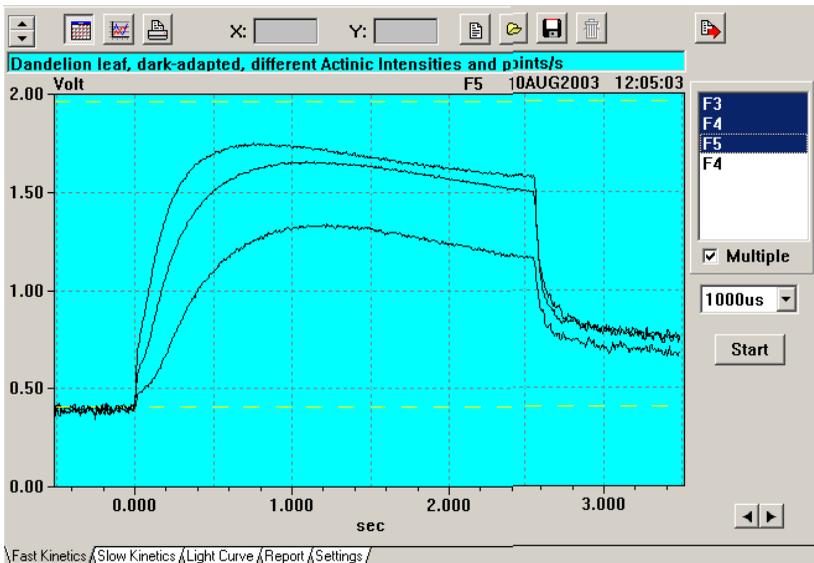
Fig. 11   Fast Kinetics window showing recordings of rapid dark-light induction curves

Rapid dark-light fluorescence induction curves (**Kautsky effect**) can be recorded on the Fast Kinetics window. When the **Start-button** is clicked, a kinetic recording with defined time resolution (μs/point) and illumination time

is triggered. During the recording no fluorescence data are displayed and a window is opened just stating that the system is occupied with "Measuring".

When the measurement is terminated, the recorded Fast Induction Curve is displayed.

The time scale depends on the selected **time resolution (µs/point).** Always a **total of 4000 points** are recorded. Hence, if time resolution is set to 1000 µs/p, the total recording takes 4 s (see Fig. 11). Before onset of Actinic Illumination the **baseline** (normally corresponding to the Fo-level) is recorded with 500 points. Unless an **Actinic Light Width** is defined under Settings (see 3.4), Actinic Illumination is terminated after 3000 points, so that during the last 1000 points the **dark-relaxation kinetics** of fluorescence yield are recorded. The user may choose to set manually an Actinic Light Width that corresponds to less or more data points at the selected time resolution. In this case, the dark-relaxation kinetics will be longer or shorter, respectively, or will be not recorded at all.

The **ordinate scale** corresponds to the fluorescence yield, **Ft**, measured in **Volt** at the output of the signal amplifier, which saturates at 2.5 Volt. The default scale reaches from 0 to 2 Volt. If an **Fo, Fm-determination** was carried out before the Fast Kinetics recording, the Fo and Fm levels are displayed in form of broken yellow lines. In this case, the Fm line is decisive for Autoscaling (see below).

The various functional elements of the Slow Induction window are described as follows, starting from the upper left corner and ending in the lower right corner:

Arrow buttons for changing the **ordinate scale**

On/off switch of **Grid**

Vertical and horizontal **Autoscale**

Start-button for **Printout**

X: 44.82   Y: 0.216   **x-y coordinates** of Ft, as marked by cursor

Speedbutton for opening **Comment-file**, into which information on a Fast Kinetics recording can be written, provided this was saved in form of a PWF-file.

Open file button for writing the recordings of a stored **PWF-file** into the Selection Box (see below).

Store file button for saving **Fast Kinetics** in form of a **PWF-file.** All recordings are saved the names of which are marked in the **Selection Box** (see below).

Delete button for erasing a selected Fast Kinetics recording. This function does <u>not</u> work when the Multiple display (see below) is activated.

Speedbutton for the **export** of a selected Fast Kinetics recording in form of a **CSV-** (comma separated values) file. The latter is stored in the Data directory of the PamWin Folder under the name **EXPORT.CSV.**

> **Information**
> ⓘ Kinetics data written to file EXPORT.CSV!
> OK

When this file is double clicked, it will be opened in **Excel**, provided this program is installed on the used PC. Using Excel the data can be further processed and analyzed.

> **Dandelion leaf, dark-adapted, different Actinic Intensities and points/s**
> Volt   dande01-3   11AUG2003 04:46:52

Above the display of a Fast Kinetics recording automatically the date and time of the recording are written. If the Fast Kinetics were

saved in a **PWF-file**, the corresponding file name is shown. In addition, the user may also write a short comment into a **text line** on top. Furthermore, longer comments may also written into a **Comment-file** (see above).

A **Selection Box** is provided for definition of the recordings that shall be displayed. Into this box new recordings are written, which are first named F1, F2 etc. The recordings are renamed name-1, name-2 etc. after saving of the Fast Kinetics in a PWF-file. Using the Open file button (see above), also the recordings of previously saved PWF-files can be loaded. In this way, it is possible to compare the recordings of different files. A recording is displayed in the Fast Kinetics window after marking its name by left mouse click.

When the **Multiple** checkbox is activated, several names can be marked, so that the corresponding curves are superimposed. It is also possible to superimpose curves recorded at different time resolution (µs/point). Then the time scale is determined by that curve, the name of which was clicked last (marked by dotted box). The same is true for the curve decisive for Autoscale.

Time resolution and length of a recording are defined by the **Sampling Time (µs/point).** The user may choose between 150, 300, 1000, 3000 and 10000 µs/point. As a recording contains a total of 4000 points, the corresponding recording times are 0.6, 1.2, 4, 12 and 40 s.

Please note that the time resolution is also determined by the **Damping**-setting (see 3.4). The Default-setting 5, which is well suited for normal Saturation Pulse quenching analysis and Slow Kinetics recordings, is too high for Fast Kinetics recordings. The

proper choice of Damping depends on the fastest fluorescence changes which are of interest. With most Fast Kinetic recordings Damping 2 is appropriate.

Start   When the **Start**-button is pressed, a new recording is started, which is defined by the Sampling Time and the Actinic Light parameters (see 3.4). Always the LED-lamp is used because of the fast light on/off characteristics. Unless the illumination time is defined by Actinic Light Width under Settings, it is automatically adjusted to the overall recording time, which depends on the Sampling Time (see above).

◄ ►   Arrow-buttons for adjustment of **Horizontal Scale**

Details of a Fast Kinetics recording can be displayed in enlarged form using the **Zoom**-function already outlined for Slow Kinetics (see Fig. 8 and Fig. 9).

4   VIEW-mode

The PamWin software can be used in two different modes, the **MEASURE-mode** and the **VIEW-mode.** While the MEASURE-mode requires connection of the PAM-2100 via an RS-232 interface cable with the PC, for the VIEW-mode only the PC is required. In the MEASURE-mode, all data involving the application of Saturation Pulses are automatically written into the current Report (STANDARD.RPT, see 3.5), which can be permanently saved as an RPT-file after renaming and then reloaded at any later time for inspection in the VIEW-mode.
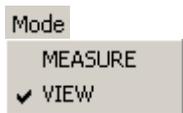
From the MEASURE-mode one can change over to the VIEW-mode at any time via the **Mode-menu**, which is part of the Windows Main Menu accessible in the upper left corner of the screen. Alternatively, the VIEW-mode is also entered by opening a **Report-** or **PWS**-file via the **Open file** button. Please note that upon loading a PWS-file the Ft-information of the last measured Slow Kinetics recording will be lost, unless it is saved in a PWS-file. Before this can happen, there is a corresponding warning.

For returning to the MEASURE-mode the **MEASURE-mode** button has to be pressed. This button is active only when instrument and PC are connected via a RS-232 cable.

Fig. 12   Report-window in the VIEW-mode with a previously saved
          Report-file loaded and the data of a Light Curve recording being
          selected for display

Fig. 12 shows the Report-window in the VIEW-mode after loading a
previously saved **Report-file** and selection of a particular **Record**
featuring a Light Curve recording. Report-file selection is via the
File-menu or using the **Open file** speedbutton. When the VIEW-
mode was entered coming from the MEASURE-mode (instrument
and PC connected via RS-232), the **STANDARD-RPT** automatically
is installed.

In the VIEW-mode the Report is organized into **Records** that
are displayed in spreadsheet format. It can be exported in form
of a CSV-file into a Spreadsheet program, like Excel using the
Export speedbutton.

Also **Copy and Paste to Clipboard** (like Wordpad or Editor) is possible.

In the **Select Record** box the user may choose either generally from the various Records of the Report (by use of the horizontal arrow-buttons) or specifically from those Records, for which also **PWS-files** were saved. Arrow buttons for selection of the first and last Records are provided, as well as arrow buttons for leafing forward and backward in the Report. The current number and the number of lines (measurements) of the selected Record as well as the total number of Records in the Report are shown. If a PWS-file was saved in conjunction with a particular Record, its name is displayed. A speedbutton for opening another Report-file is provided.

If a selected Record consists of a Light Curve or Slow Kinetics recordings it can be further viewed in the Light Curve or Slow Induction windows, respectively. A particular feature is provided for the display of Light Curves, which can be superimposed over each other. An example is shown in Fig. 13.
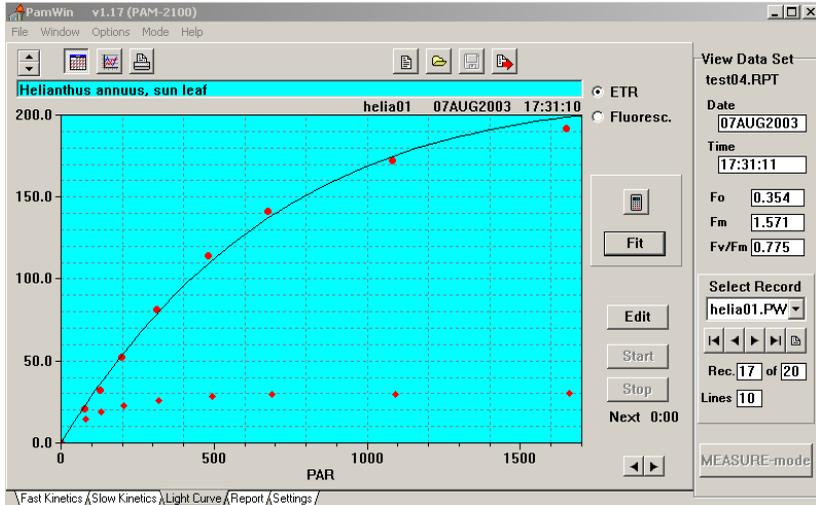
Fig. 13   Light Curve window in the VIEW-mode with display of two superimposed Light Curves
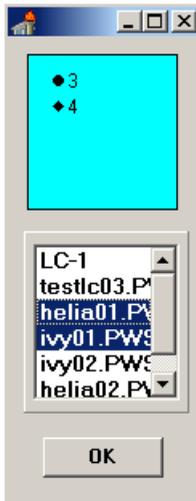
A **Light Curve Selection** box is provided, which shows the names of all Light Curve recordings of the currently loaded Report-file. If the Slow Kinetics of a Light Curve were not separately saved in a PWS-file, it is just called LC1, LC2 etc.

A Light Curve is selected for display by left mouse click. Several Light Curves can be marked in this way and, hence, will be displayed on top of each other. The symbols corresponding to the various curves are displayed in the **Symbol** box on top of the **Name** box. The **scaling** of the Light Curve display is determined by the curve, the name of which was last marked (dotted frame). It is also this particular curve, for which the Light Curve **Fit Parameters** are calculated.
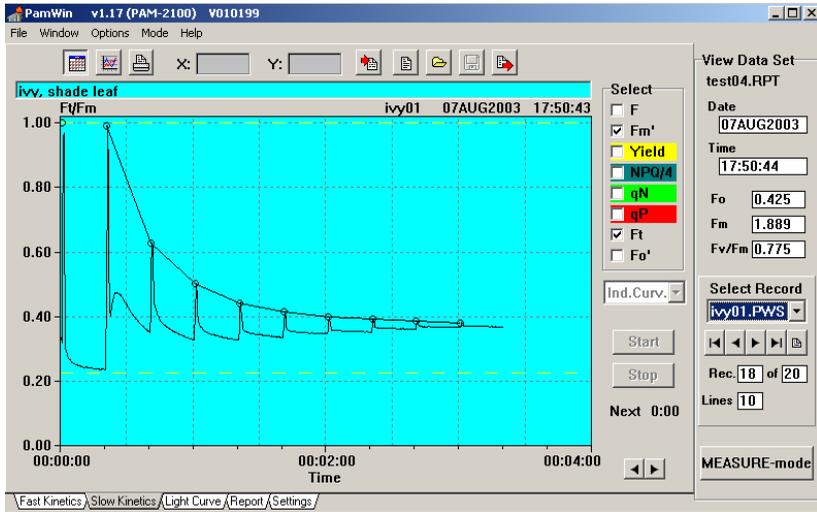
Fig. 14   Slow Kinetics window in the VIEW-mode showing the kinetic
         information saved in the PWS-file which was recorded in
         conjunction with one of the Light Curves displayed in Fig. 13

After selection of a **PWS-file** in the **Select Record** box, the
corresponding recording is displayed in the Slow Kinetics window.
In Fig. 14 an example of a PWS-file recorded in conjunction with a
Light Curve is shown. For the sake of clarity only the parameters Ft
and Fm' were selected for display.

Treatment of **Fast Kinetics**, which is identical in the
MEASURE- and VIEW-mode, was already outlined in section 3.8.