WinControl
Windows Software
for PAM Fluorometers

Users Manual

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1   Introduction

WinControl is a dedicated software developed for **PAM fluorometers**. It is specialized to assess the photosynthetic performance of plant samples by chlorophyll fluorescence measurements. Depending on the properties of the investigated sample, different types of PAM fluorometers can be used, like e.g. the **MICROSCOPY-PAM** for single cells, the **MINI-PAM** for whole leaves and the **WATER-PAM** for phytoplankton suspensions. In all cases the basic features of the approach are the same:

- 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 (**P**ulse **A**mplitude **M**odulation, **PAM**)

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

- 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**)

- saturating pulses of light 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 at stepwise increasing light intensities in order to assess the light saturation properties of a sample (**Light Response Curves**).

The WinControl software originally was developed for PAM fluorometers of the **PAM-CONTROL** family (**MICROSCOPY-, MICROFIBER-, UNIVERSAL- and WATER-PAM).** Recently also the instrument software (EPROM) of the **MINI-** and **DIVING-PAM** was adapted for use in conjunction with WinControl. While all these instruments in principle also can be operated as stand-alone devices, i.e. carry out the above outlined basic measurements without the help of a PC, the WinControl software adds a new quality, as the powerful PC-facilities become part of the instrument:

- all commands can be given comfortably via the keyboard terminal

- instrument settings as well as recorded data are displayed on the monitor screen

- an almost infinite amount of data, including kinetic recordings and light response curves, can be stored on hard disc

- data can be further processed and analyzed using special program routines

- data can be exported into other programs, like Excel or Sigma Plot

Another important aspect is that of **remote control** of the fluorometer. The advantage of this feature is particularly evident in the case of the DIVING-PAM, which e.g. may be attached underwater to an investigated sample via the fiberoptics and connected to a a notebook PC on land or boot via an underwater cable. Remote control is also useful when highly sensitive

measurements (e.g. with MICROSCOPY- or MICROFIBER-PAM) are carried out in dim light or darkness.

Stand-alone and PC-operation are fully equivalent. All functions can be carried out either via the keyboard of the PAM fluorometer or the PC-keyboard. When e.g. a Saturation Pulse is triggered via START on the PAM-keyboard, the SAT-Pulse button on the PC-Windows-screen lights up and the measured fluorescence parameters are displayed on the LCD-display of the PAM fluorometer as well as on the Windows-screen. Also when the PAM is operated via PC, all data are stored in the instrument (MEMORY) as well as on PC hard disc.

This manual deals specifically with features of the WinControl software. Separate manuals are provided for the various instruments used (MINI-PAM, DIVING-PAM and PAM-CONTROL). Please consult these manuals for detailed descriptions of all instrument functions and for background information on chlorophyll fluorescence parameters. This WinControl manual is meant to serve as a quick reference guide. Actually, most users accustomed to Windows-programs will find that WinControl 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 touches the corresponding fields. The user is encouraged to try out all functions in a playful way; there is no risk of damaging the instrument. Even if a highly sensitive photomultiplier is involved, the instrument software takes care that this cannot be unintentionally damaged.

The WinControl program has been tested extensively with a variety of instruments and considerable efforts were undertaken to eliminate software errors. Nevertheless, experience tells us that still there may be hidden errors which will show only in specific

situations. We are grateful if such errors are pointed out to us and we are also open for suggestions of program extensions and improvements. Updated versions of WinControl will become available as free download of a self extracting EXE-file from the **Walz Internet Homepage** (www.walz.com) under **Support/Current Software Table**.
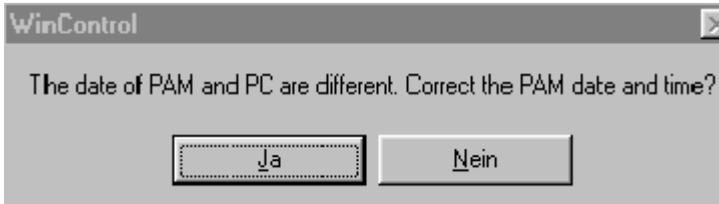
2   Installation of the WinControl software

The WinControl software is delivered in form of a **program set-up disc** (present version WinControl SetUp 1.48). In order to install the program on your PC, please start the file **Wcsu148.EXE** (or any higher number). Installation is carried out automatically. A new directory **WINCONT** and a corresponding start icon are created. The program is started either by clicking on this icon or via the **WINCONT.EXE** file, e.g. using the Windows Explorer. The program checks all available COM-ports for a connected PAM device:

> **Searching for connected PAM device.**
> **WinControl V1.48, 01.04.2000 by Friedemann**
> **Schlosser**
>
> Offline Mode [RETURN]

If no PAM fluorometer is connected, the user may select the **Offline Mode** by clicking RETURN. Using this mode it is possible to analyze data which are stored on floppy or PC hard discs. If a PAM is connected via RS 232 interface cable, this will be recognized by the program and it will be automatically switched on:

> **WinControl V1.48, 01.04.2000 by Friedemann**
> **Schlosser**
> **PAM found, please wait!**
>
> Offline Mode [RETURN]

The program automatically compares the time and date set via the instrument software (EPROM) with that of the PC and recognizes that there are differences. As it is important that stored data are characterized by the same time and date in instrument and PC memory, the suggestion to correct the PAM date and time (synchronize with PC) should be answered with Yes.

WinControl   ✕

The date of PAM and PC are different. Correct the PAM date and time?

[Ja]   [Nein]

Depending on the particular type of instrument connected via the RS232 interface cable to the PC, specialized versions of the WinControl software are started. The program recognizes which type of instrument is connected to the communication port of the PC. It can distinguish between MINI-PAM, DIVING-PAM and PAM-CONTROL. As the same PAM-CONTROL unit is used in conjunction with different optical systems to create the MICROSCOPY-, MICROFIBER-, UNIVERSAL- and WATER-PAM, before first use the user himself must define the particular type of fluorometer (see below). This information is stored, such that with future starts of WinControl the correct PAM-device is shown in the header line. For example, after program start with a MINI-PAM being connected to communication port 1 of the PC, the monitor screen shows the following user surface of the WinControl program. The header line also states the current number of the present instrument software version (V2.24) stored on EPROM.
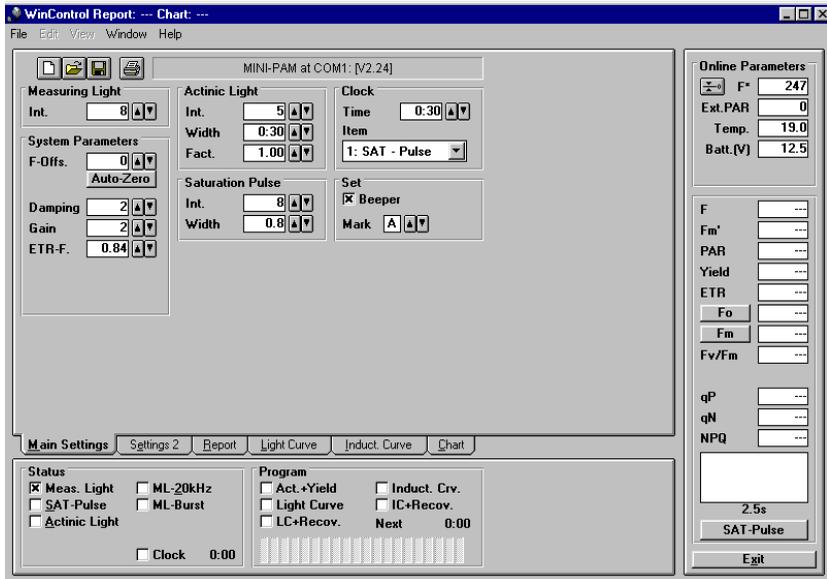
Fig. 1   WinControl user surface after first program start with a MINI-PAM being connected to the PC communication port 1.

3   WinControl user surface

The user surface of WinControl 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 online measured parameters, the most important of which is the momentary fluorescence yield. Below the values of all parameters are displayed which are measured in conjunction with a Saturation Pulse. These values are updated with each Saturation Pulse (started via the SAT-Pulse button), whereas the online parameters above are continuously updated.

2. The bottom area of the screen features two sections with check boxes (**Status** and **Program**), which serve as on/off switches, e.g. to turn on/off Actinic Light or to start the recording of a preprogrammed Induction Curve by mouse click. At the same time the check marks (x) indicate that a particular function is active.

3. The central window is defined by choosing one of 6 index fields **(Main settings, Settings 2, Report, Light Curve and Chart)**. **Main settings** are installed by default upon start of the program. The other windows can be called up by clicking on the corresponding index fields. The various windows will be explained in detail in separate chapters below.

It is possible to reduce the size of the WinControl user surface by pulling the lower right corner diagonally up, as usual under Windows. When this is done, the fluorescence parameters on the right are displayed in two groups under Yield and Quench, which can be selected via the corresponding radio button.
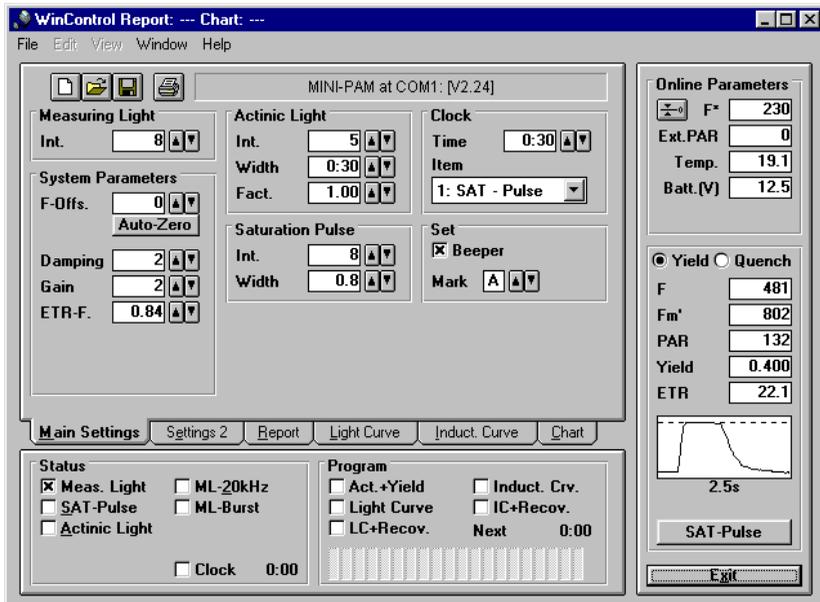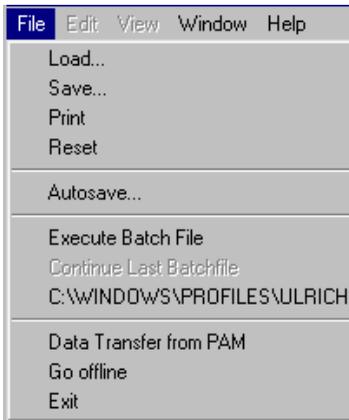
Fig. 2: WinControl user surface at reduced screen size featuring Yield and Quench selection buttons

3.1   Menu selection

 As general practice with Windows programs, WinControl features a number of routines which can be selected via the menu line at the top of the screen **(File, Edit, View, Window** and **Help).**

The menu point **File** contains the functions **Load, Save, Print and Clear** (or alternatively **Reset**), relating to the particular window presently opened (i.e. Main settings, Settings 2, Report, Light Curve, Induction Curve or Chart). The **Autosave** function relates to the **Report**-file only. **Execute Batch File** and **Continue Last Batchfile** are special routines for the preprogrammed control of various instrument parameters as well as of external devices (e.g. autosampler). **Go offline** cuts the connection between PC and PAM-device. In this case the windows with Online Parameters and Status/Program-check boxes disappear and stored data can be viewed and analyzed at higher resolution. Upon **Exit** the WinControl program is quit.

When the **Main Settings** or **Settings 2** window is active, **Load, Save, Print, Reset** relate to **instrument settings**. In practice, it is very convenient to save all instrument settings with which a particular experiment was carried out (**Save**). These can be re-installed at any time in order to repeat or continue the experiment with exactly the same settings (**Load**). The settings can be also printed out (**Print**). It is also possible to return to the default settings (**Reset**).

When the **Report, Light Curve, Induct. Curve** or **Chart** window is active, **Load, Save, Print, Clear** relate to the corresponding data files. The data are saved in two types of files: Text (*.txt) or PAM Chart File (*.pcf).

Alternatively **Clear/Reset, Load, Save** and **Print** can also be carried out by clicking the corresponding toolbutton.

Edit relates to the **Report**-window only. It contains the functions **(Cut, Copy, Paste and Clear)** which can also be reached by clicking on the central part of the screen with the right mouse key. The **Report** consists of a text file (*.txt), which can be edited by standard programs.

The **View** function relates to those windows which feature graphic displays (**Light Curve, Induct. Curve** and **Chart**). It allows to choose between displays with or without grid.
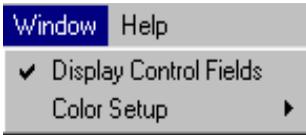
The menu-point **Window** contains two sub-menu functions. **Display Control Fields** relates to those windows which show recorded data (**Report, Light Curve, Induct. Curve** and **Chart**). When it is inactivated by mouse click, the windows featuring the Online Parameters (right hand side) and the Status/Program check boxes (bottom) disappear and the screen is filled with the central window displaying the recorded data. In this way the data can be viewed in more detail. This happens automatically when WinControl is started in the offline mode (no PAM connected).
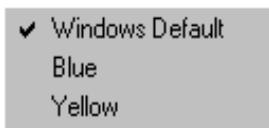
Color Setup allows to choose between various colors for the user surface. Besides the Windows Default version (Grey) also a Blue and a Yellow version can be selected.
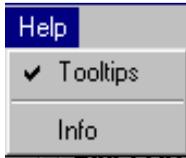
The **Help** function offers so-called Tooltips, which appear when the mouse cursor points to a toolbutton. The Tooltips give a brief description of the function of the particular tool. They can also be switched off. The **Info** gives details on the present WinControl-software version.

3.2   The Parameter Window

PAM fluorometers provide two types of measured values, continuously online measured parameters (like momentary fluorescence yield) and parameters, which are determined in conjunction with a saturation pulse and stored in the Report-file (like Fv/Fm, Yield and quenching coefficients).

3.2.1   Online Parameters
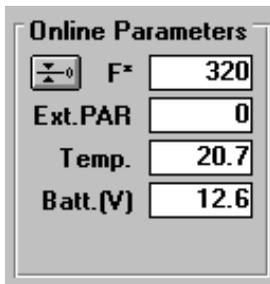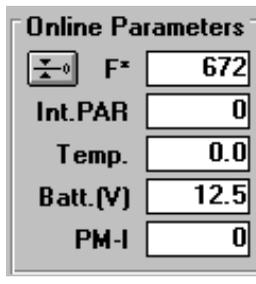
MINI-PAM   PAM-CONTROL

The **Online Parameters** are continuously measured, but not continuously stored in memory. A particular case is **Int.PAR** corresponding to the value of photosynthetically active radiation (PAR), which is derived from an Internal PAR-list. This list applies when no external light sensor is connected.

In **PAM-CONTROL** based instruments, which in contrast to **MINI-PAM** type instruments feature a photomultiplier instead of a photodiode detector, also the photomultiplier current **(PM-I)** is

displayed. Even at high PM-I values the photomultiplier is protected against damage. Before any harm can occur, the photomultiplier supply voltage automatically is regulated down. However, it should be noted that the signal/noise ratio drops with increasing PM-I values. At values exceeding 600 units there is also a decrease of signal amplitude. Then there is a warning: Display field of PM-I flashing red.

Note: In all PAM-CONTROL applications steps should be taken to minimize PM-I. Even at maximal PM-Gain the PM-I can be close to 0, if care is taken that no ambient light penetrates via the optics to the PM.

F*	Momentary fluorescence yield corrected for the background signal by the value of F-Offset. It can assume values between 0 and 4000 units. Between 3500 and 4000 the signal becomes nonlinear and there is an OVERFLOW warning (F* box flashing red).
	Auto-Zero Offset button. One of two ways to determine the background signal **(F-Offs.)** which is stored under **System Parameters (Main Settings window)**. Alternatively, F-Offs. can be also determined under Main Settings. This function is particularly important at high PM-Gain, when there is also a significant signal in the absence of a plant sample (e.g. originating from stray measuring light reaching the PM or from non-plant fluorescence). Any background signal will cause an apparent decrease of variable fluorescence and of quantum yield. Please note, that any change in Gain, Measuring Light Amplitude and optical conditions will cause a corresponding change in F-Offs., which then has to be redetermined (see below).

Ext.PAR   **Photosynthetically active radiation**, which is measured externally with special micro quantum sensors (e.g. Leaf-Clip Holder 2030-B with MINI-PAM and pin-hole detector MC-MQS with MICROSCOPY-PAM) the output signal of which is processed online. The momentary PAR-values displayed at the time of a saturation pulse are stored in the Report-file in the data set associated with this particular saturation pulse.

Int. PAR   Photosynthetically active radiation derived from an **Internal PAR-list** which applies whenever no external PAR-sensor is connected to the instrument. For measurement of the Internal PAR-list special micro quantum sensors (e.g. MC-MQS) and special WinControl routines (see pp. 35-36) are available. The momentary PAR-values displayed at the time of a saturation pulse are stored in the Report-file in the data set associated with this particular saturation pulse.

Temp.   **Momentary temperature** at the measuring site which is displayed if a suitable external temperature sensor is connected to the instrument (via AUX Input with PAM-CONTROL type instruments and LEAF CLIP Input with MINI-PAM). The value displayed at the time of a saturation pulse is stored in the Report-file in the data set of this particular saturation pulse.

Batt.(V)   **Internal Battery Voltage.** There is a warning (display box flashing red), if Batt.(V) drops below 11.2 V. In this case the battery should be recharged.

PM-I   Photomultiplier current (only with PAM-CONTOL based instruments) (see above).

3.2.2 Parameters determined in conjunction with a saturation pulse

F	378
Fm'	619
PAR	129
Yield	0.389
ETR	21.0
Fo	244
Fm	976
Fv/Fm	0.750
qP	0.642
qN	0.487
NPQ	0.576

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, and Fm',** 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 MINI-PAM, DIVING-PAM or PAM-CONTROL. Here just a brief description is given:

F   Fluorescence yield measured briefly before onset of last saturation pulse

Fm'   Fluorescence yield reached during last saturation pulse; normally measured in the presence of actinic light

PAR   Photosynthetically active radiation displayed under Online Parameters at the time shortly preceding the last saturation pulse

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

$$\textbf{Yield = (Fm'-F)/Fm'}\text{ (Genty-parameter)}$$

normally measured in the presence of actinic light

ETR   Relative Electron Transport Rate calculated from the equation:

$$\textbf{ETR = Yield} * \textbf{PAR} * \textbf{0.5} * \textbf{ETR-factor}$$

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.

| Fo | Button for determination of dark-level fluorescence yield, Fo. The displayed value of Fo is also updated upon Fv/Fm determination using the Fm-button.

| Fm | Button for determination of maximal fluorescence yield of a dark-adapted sample. Not only Fm but also Fo is determined and the quantum yield Fv/Fm is calculated.

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).

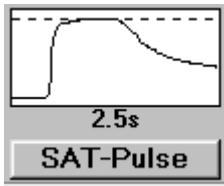**qP**   Coefficient of photochemical quenching defined by the equation:

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

qN   Coefficient of nonphotochemical quenching defined by the equation:

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

NPQ   Parameter describing nonphotochemical quenching defined by the equation:

NPQ = (Fm-Fm')/Fm'

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 determined Fm (or Fm') value. Based on this information **Saturation Pulse Intensity** and **Width** can be optimized (under Main Settings). The intensity and width should be adjusted such that a plateau is formed and the broken line describes the peak value.

A saturation pulse can be started either via the **SAT-Pulse button** or **via the SAT-pulse check box** (under Status). These are equivalent to the START-key on the PAM fluorometer. Saturation pulses are also triggered upon determination of Fm and Fv/Fm, as well as in conjunction with the **Program runs (Act+Yield, Light Curve, LC+Recov., Induct. Crv., IC+Rec.).**

3.3   The Status/Program window

The Status/Program window features two groups of check boxes which serve at the same time as on/off switches and status indicators of the corresponding functions. While the general layout of this window is the same for MINI-PAM type and PAM-CONTROL based instruments, some of the Status check boxes differ between the two types of instruments.

MINI-PAM

PAM-CONTROL

3.3.1   Status Check Boxes

The following check boxes serve for switching on/off the corresponding functions. The check marks (x) indicate which functions presently are active.

Meas. Light   Measuring Light for fluorescence excitation

SAT-Pulse   Saturation Pulse; serving primarily as status indicator; start of Saturation Pulse preferentially via the SAT-Pulse button

Actinic Light   Actinic Light; manual on/off or start of a fixed illumination period which is defined under Main Settings

Far-Red LED   Far-Red LED Light (with PAM-CONTROL); manual on/off or start of a fixed illumination period which is defined under Main Settings

Damping   Signal Damping (with PAM-CONTROL). When Damping is active, it is automatically inactivated for 2 sec when rapid signal changes occur at low frequency of measuring light (following a saturation pulse, following actinic illumination, after switching photomultiplier on and off).

ML-20kHz   Switch between the standard measuring light frequency of 0.6 kHz and 20 kHz (with MINI-PAM). When ML-20kHz is inactive, it is automatically activated for the duration of a saturation pulse as well as during actinic/far-red illumination.

ML-Burst   Switch between normal signal detection (continuously pulsed measuring light) and signal detection by short bursts of measuring light (with MINI-PAM). When ML-Burst is active, the pulse trains are 0.2 sec with dark-intervals of 0.8 sec, resulting in a reduction of integrated measuring light of a factor of 5.

ML-F. High   Switch between the Measuring Light Frequency defined under Main Settings and the maximal

Measuring Light Frequency corresponding to 688 kHz (PAM-CONTROL).

Photo-Multipl. Photomultiplier power supply (PAM-CONTROL). It normally may remain switched on without risk of damage, as high voltage is automatically regulated down when the Photomultiplier current (PM-I) becomes excessive.

Clock Repetition Clock which serves to trigger Saturation Pulses or some other functions defined under Clock Item (Main Settings). Beside the Clock check box the time remaining until the next triggering event is shown.

3.3.2   Program Check Boxes

The following check boxes serve for switching on/off the corresponding functions. The check marks (x) indicate which functions momentarily are active.

Act+Yield Start of an actinic illumination period, the duration of which is defined under Main Settings, with application of a Saturation Pulse at the end of illumination for assessment of effective quantum yield (Yield)

Light Curve Start/stop of light response curve recording which involves an initial Fv/Fm measurement followed by 8 consecutive Yield-measurements at increasing light intensities. The Light Curve parameters are defined under Settings 2. The recorded data can be viewed on the **Light Curve** and **Report** windows. In addition, it is also possible to synchronize the start of Light Curve

recording with the start of a Chart recording, in order to analyze the kinetic responses.

LC+Recov. Start/stop of light response curve recording followed by assessment of dark recovery of Yield and other fluorescence parameters by 6 consecutive Saturation Pulses applied at 10 sec, 30 sec, 1 min, 2 min, 5 min and 10 min after termination of illumination.

Induct. Crv. Start/stop of the recording of standardized dark-to-light Induction Curve with Saturation Pulse Quenching Analysis.The Induction Curve Parameters are defined under Settings 2. The recorded data can be viewed on the **Induct. Curve** and **Report** windows. In addition, it is also possible to synchronize the start of Induction Curve recording with the start of a Chart recording, in order to analyze the kinetic responses.

IC+Recov. Start/stop of the recording of standardized dark-to-light Induction Curve with Saturation Pulse Quenching Analysis followed by assessment of dark recovery of Yield and other fluorescence parameters by 6 consecutive Saturation Pulses applied at 10 sec, 30 sec, 1 min, 2 min, 5 min and 10 min after termination of illumination.

Next 0:10 Remaining time until the next measurement, i.e. application of a Saturation Pulse.

 Status box giving information on the progress of a running Program. Each of the 20 bars corresponds to a Saturation Pulse (i.e. a single measurement). The total number of measurements involved in a particular program is reflected by the

grey bars. The colored bars represent completed measurements and the blinking bar points to the next measurement which will take place after the time displayed under **Next**.

3.4   Main Settings
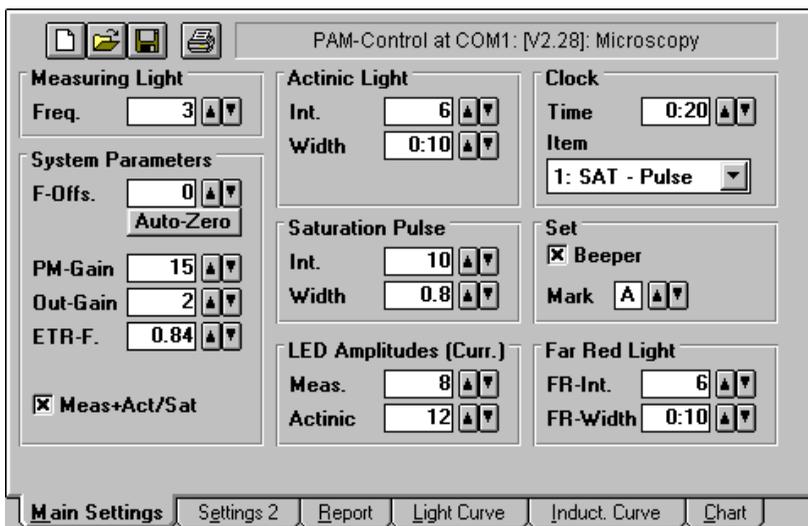
Fig. 3: Main Settings window in conjunction with PAM-CONTROL and MICROSCOPY-PAM

The Main Settings window features the most essential instrument parameters. There are a few differences between PAM-CONTROL and MINI-PAM type instruments, which relate to the fact that with PAM-CONTROL based instruments not only the Measuring Light but also the Actinic Light is pulse modulated and that a photomultiplier is used instead of a photodiode. Fig. 3 shows the Main Settings for the MICROSCOPY-PAM (for the corresponding window in the case of MINI-PAM type instruments see Fig. 1).

While the WinControl software does recognize whether a PAM-CONTROL or MINI-PAM type instrument is connected, the user has to define himself which particular instrument (e.g. MICROSCOPY-, MICROFIBER- or WATER-PAM) is applied. This can be done via the **Reset**-function.

When the **Reset-Icon** in the upper left corner is clicked, a dialogue window is opened, which allows to define the selected device. Once this selection is made via Ok, it will remain valid with every new start of the program. At the same time, there is a Reset to the **Standard Settings** applying to the selected device. The Reset not only applies to the Main Settings, but to the Settings 2 as well. While all settings can be modified by the user with the help of the arrow keys, it is always possible to return to Standard Settings via the Reset-function.

It is also possible to save a particular set of instrument setting, which has proven useful for a particular type of experiment, in a so-called **psf-file**, and to reload this file at a later time in order to reproduce the experiment with exactly the same instrument settings. The settings can be also printed out. These functions can be carried out most easily clicking the corresponding icons (**Load, Save and Print**).

The various settings are arranged in functional groups which correspond to the functional blocks in the Mode Menus of PAM-

CONTROL and MINI-PAM. They will be only briefly explained here. Reference will be made to the Main Settings for the most complex instrument, i.e. the MICROSCOPY-PAM. For more extensive explanations please consult the Handbooks of the corresponding instruments.

Measuring Light In PAM-CONTROL based instruments, like the MICROSCOPY-PAM, the effective intensity of the **Measuring Light** is varied primarily via the **frequency of the µsec-excitation pulses**. A total of 12 settings is available, with the setting 3 being standard. At higher settings the measuring light may become actinic, i.e. sufficiently high to induce variable fluorescence. In the case of MINI-PAM type instruments, the Measuring Light Intensity is mainly determined by the amplitude of the individual excitation pulses and only two different pulse frequencies are provided (600 Hz and 20 Khz). In principle, also with PAM-CONTROL the amplitude of the excitation pulses can be changed (see under LED Amplitudes on p. 27).

System Parameters Under **System Parameters** a number of settings is listed which are relevant for the signal output.

F-Offs. Via **Auto-Zero** the **Zero Offset** signal is determined, which can be manually modified with the help of the arrow keys.

Damping **Signal Damping**. The three settings correspond to the following time constants

(defined by 63.2 % of a signal change): 1: 0.05 sec, 2: 0.2 sec, 3: 1 sec (MINI-PAM devices).

PM-Gain   **Photomultiplier Gain** which can be varied in 30 steps with the default setting being 15 (PAM-CONTROL based devices)

Out-Gain   **Output Gain** which can be varied in 12 steps with the default setting being 2.

ETR-F.   **ETR-factor**, with the standard value of 0.84 corresponding to the fraction of incident light absorbed by a standard leaf. While it proves difficult to determine this factor in many practical cases, it is not important to enter the correct value, as long as changes in <u>relative</u> electron transport rate are of primary interest.

Meas.+Act/Sat Switch between two fundamentally different programs defining pulse pattern of measuring light (PAM-CONTROL). Meas+Act/Sat must be active in order to apply Actinic Light and Saturation Pulses with the same LED which provides Measuring Light. This is the case with the MICROSCOPY- and MICROFIBER-PAM. Meas+Act/Sat is automatically activated upon Reset (File-menu or tool button) and definition of one of these instruments.

Actinic Light Under **Actinic Light** a number of parameters is listed which relate to the properties of actinic illumination.

Int.   **Actinic Light Intensity**, which can be varied between 12 settings. In PAM-CONTROL devices, Actinic Intensity is varied primarily via the frequency of actinic LED pulses. In principle, it can be also varied via the amplitude of actinic LED pulses (see under LED Amplitudes on p. 27).

Width   **Actinic illumination time** which can be varied between 5 sec and 5 min (MINI-PAM) or 10 min (PAM-CONTROL type instruments). In the latter case, at the end of the scale also 10 min 5 sec can be set. In this case, however, actinic illumination time is indefinite and has to be terminated manually.

Fact.   **Actinic Light Factor** by which the range of actinic intensities can be shifted up and down. This applies to MINI-PAM devices only which feature a halogen lamp as actinic light source. In analogy, with PAM-CONTROL type instruments the range of actinic intensities can be shifted via the LED Amplitude setting (see on p. 27).

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

Int.   **Saturation Pulse Intensity**, which can be varied between 12 settings. In PAM-CONTROL devices, Saturation Pulse Intensity is varied primarily via the frequency of actinic LED pulses. In principle, it can be also varied

	via the amplitude of actinic LED pulses (see under LED Amplitudes on p. 27).
Width	**Saturation Pulse Width**, which can be varied between 0.2 and 3 sec in PAM-CONTROL based devices as compared to 0.4 and 3 sec in MINI-PAM devices.
LED Amplitudes	These settings relate to **PAM-CONTROL based devices only**, in which not only the Measuring Light but also the Actinic Light and the Saturation Pulses are based on pulse modulated LED light, with the consequence that the overall intensity is determined by both the amplitude and the frequency of the LED pulses. In most practical situations it is advantageous to modify the effective intensities of Measuring Light, Actinic Light and Saturation Pulses via the frequency (see Measuring Light Frequency and Actinic Light Intensity above). Changing the preset amplitude values will lead to corresponding changes of the **Internal PAR List**. Furthermore, also the Fo-Offset will be affected. Therefore, if the user tries to change the preset values of LED Amplitudes, there is a corresponding warning. Please note that for technical reasons the Actinic LED Amplitude not only has an effect on the effective intensity of actinic illumination, but on that of far red illumination as well (see below).
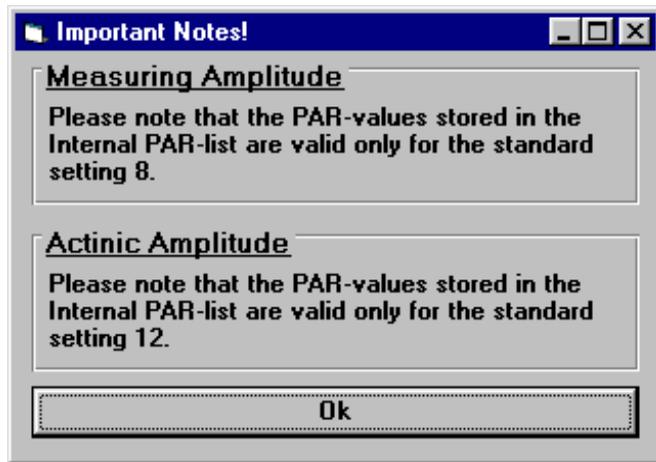
Meas.   **Measuring Light Amplitude**, which is defined by the LED current during individual Measuring LED pulses. Please note that the Measuring Light Amplitude also determines the effective Actinic and Saturation Pulse Intensities when the function **Meas+Act/Sat** is active (see p. 25).

Actinic   **Actinic Light Amplitude**, which is defined by the LED current during individual Actinic LED pulses. Please note that Actinic Light Amplitude does apply only when a **separate Actinic LED Light Source** is applied (e.g. Actinic LED Array with WATER-PAM), i.e. it is <u>not</u> effective when **Meas+Act/Sat** is activated (see p. 25).

Clock   Under **Clock** the parameters of the **Repetition Clock** can be defined. The Clock relates to a variety of repetitive measuring routines which

always involve the application of Saturation Pulses.

Time   **Time Interval** between two consecutive measuring routines, i.e. in the most simple case between two Saturation Pulses. When more complex routines are involved (as e.g. Light Curve recordings) the Time Interval corresponds to the time between the starts of two consecutive routines (e.g. Light Curves).

Item   **Clock Item** corresponding to one of 6 different measuring routines. Using this function it is possible to monitor photosynthetic parameters of a sample over extended periods of time, e.g. in the case of field investigations with the MINI- or DIVING-PAM during the course of a day or even longer times.

1: SAT - Pulse ▼
1: SAT - Pulse
2: Act.+Yield
3: Light Curve
4: LC+Recov.
5: Induct. Crv.
6: IC+Recov.
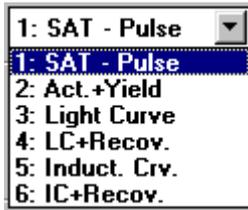
Set   **Set** boxes

Beeper   Check box for enabling (x) and disabling the **Beeper**-function. When enabled, a beep is given upon switching on/off of Actinic and Far Red Light, at the start of a Saturation Pulse, at start/termination of a Program-routine (e.g. Light Curve), upon Reset and in conjunction with Error Messages, like NEW OFFSET or SIGNAL LOW.

Mark   Definition of a **Mark** attached to each data set saved in the **Report**-file. This can be useful for work involving different types of samples,

e.g. field studies covering various plant species.

Far Red Light   Under **Far Red Light** two parameters relating to illumination with far red light are listed. This function applies to PAM-CONTROL devices only. Please note that the use of far red light may cause artifacts, if too much of this light reaches the photomultiplier (see PAM-CONTROL Handbook of Operation).

FR-Int.   **Far red light intensity**, which can be varied between 12 settings. It is recommended to apply moderate intensities in order to avoid high photomultiplier currents (**PM-I**) and the resulting decrease in signal/noise. Please note that for technical reasons the effective intensity of far red illumination is not only determined by FR-Int., but by Actinic LED Amplitude as well (see p. 27).

FR-Width   **Far red illumination time** which can be varied between 5 sec and 10 min. At the end of the scale also 10 min 5 sec can be set. In this case, however, far red illumination time is indefinite and has to be terminated manually.
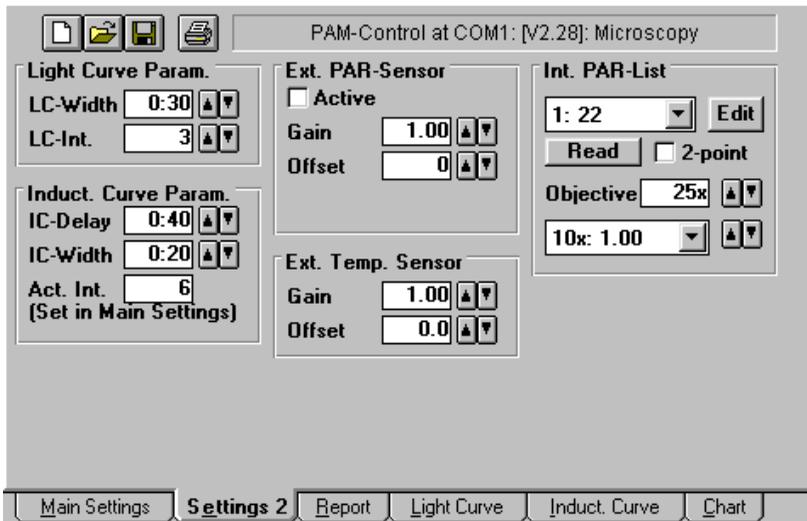
3.5   The Settings 2 window

Fig. 4: Settings 2 window in conjunction with PAM-CONTROL
and MICROSCOPY-PAM
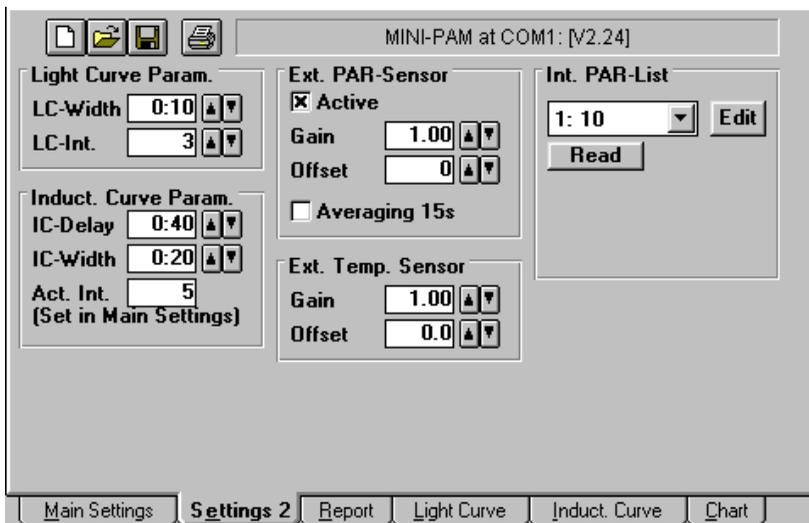
Fig. 5: Settings 2 window in conjunction with MINI-PAM

The Settings 2 window features the instrument parameters defining Light Curve and Induction Curve Recordings, as well as the calibration factors of external light and temperature sensors.

The Reset, Load, Save and Print icons provide quick access to the corresponding functions, as described above for Main Settings. Please note that these functions always relate to both Main Settings and Settings 2.

Light Curve Param. The **Light Curve Parameters** define details of recordings of light response curves as measured via the Light Curve Program.

LC-Width Duration of actinic illumination (**Actinic Width**) for each of the 8 illumination periods.

LC-Int. Setting of **Actinic Intensity** defined for the first of the 8 consecutive illumination periods.

Induct. Curve Param. The **Induction Curve Parameters** define details of recordings of induction curves as measured via the Induct. Crv. Program.

IC-Delay **Delay time** between the first Saturation Pulse applied for Fo-Fm (Fv/Fm) determination and onset of Actinic Light.

IC-Width **Time interval** between two consecutive Saturation Pulses during actinic illumination.

Act. Int. Setting of **Actinic Intensity** applied in the course of Induction Curve. The setting is displayed only. It can be changed via Main Settings.

Ext. PAR-Sensor The **External PAR-Sensor** parameters relate to the function of an optional external Micro

Quantum Sensor, like the sensor integrated in the Leaf-Clip Holder 2030-B for the MINI-PAM, the Pin-hole sensor MC-MQS for the MICROSCOPY-PAM and the Spherical Micro Quantum Sensor US-SQS for the WATER-PAM. When these sensors are connected to the corresponding devices via the Leaf Clip (MINI-PAM) or AUX Input (PAM-CONTROL) sockets, the PAR can be measured online. WinControl supports the adjustment of sensor **Gain** and **Offset**.

X Active   Checkbox for activating the display of externally measured PAR-values. When **Ext. PAR-Sensor** is inactive, the displayed PAR-values are derived from an Internal PAR-List (see p. 35).

Gain   **Gain calibration factor** serving for adjustment of PAR-reading by comparison with calibrated device. Please note that this calibration factor is saved together with the other settings in a psf-file (see p. 23).

Offset   **Manual Offset** to adjust PAR-reading to zero when the quantum sensor is completely darkened. Please note that this offset value is saved together with the other settings in a psf-file (see p. 23).

☒ Averaging 15s **Averaging** function which serves to obtain an overall PAR-reading integrated over 15 sec. This applies to MINI-PAM type instruments only, which are used under field conditions. In particular, this function is useful for underwater measurements with the DIVING-PAM.

Ext. Temp. Sensor The **External Temperature-Sensor** parameters relate to the function of an optional external temperature sensor, like the one integrated in the Leaf-Clip Holder 2030-B for the MINI-PAM. When this sensor is connected via the Leaf Clip (MINI-PAM) socket, the Temperature can be measured online. WinControl supports the adjustment of sensor **Gain** and **Offset**.

   Gain   **Gain calibration factor** serving for adjustment of Temperature-reading by comparison with calibrated device. Please note that this calibration factor is saved together with the other settings in a psf-file (see p. 23).
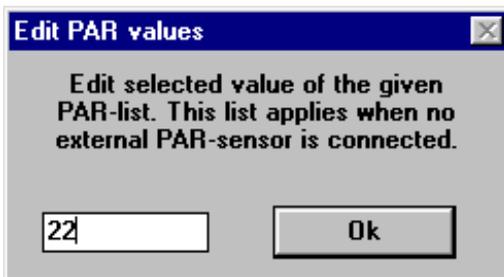
   Offset   **Manuel Offset** to adjust Temperature-reading to zero when the sensor is in contact with ice water. Please note that this offset value is saved together with the other settings in a psf-file (see p. 23).

Int. PAR-List

1: 11	▼
2: 16	▲
3: 24	
4: 34	
5: 52	
6: 77	
7: 118	
8: 176	
9: 251	▼

Edit

The **Internal PAR-List** applies when no external PAR-sensor is connected to the PAM and the **Ext. PAR-Sensor** check box is **not activated**. This is particularly relevant when simultaneous fluorescence and PAR-measurements are not possible, as e.g. with the MICROSCOPY-PAM. Then the PAR normally is measured under identical optical conditions beforehand (reading of Internal PAR-list) and the PAR-values of the Internal List are treated **as if PAR were measured simultaneously**, i.e. they are used for calculating ETR-values and plotting Light Curves (for possibilities to redefine the PAR-list in the Report-file, see pp. 41-44). The list contains 12 values corresponding to the 12 settings of Actinic Intensity. At setting 0 the actinic light is off and the sample is illuminated by measuring light only. The complete list can be viewed in a pull down window after clicking the selection box.

Edit PAR values ✕

Edit selected value of the given PAR-list. This list applies when no external PAR-sensor is connected.

22	**Ok**

The PAR-value at a selected setting can be edited. In this way, it is also possible to enter a complete PAR-list manually, e.g. if a suitable micro quantum sensor for reading the list is not available.

Read ☐ **2-point** For PAM-CONTROL based instruments, two different modes are provided for reading the

Internal PAR-List. Either the PAR consecutively is measured at all settings of Actinic Light or it is just measured at two defined settings and the other values are calculated (2-point calibration). The latter applies with instruments employing LEDs as actinic light sources, the pulse frequency of which determines the actinic intensity in a precisely predictable manner.

Change PAR-Reading Mode ✕

Upon Ok the mode will be changed and a new PAR-list will be determined using the selected mode.

OK	Abbrechen
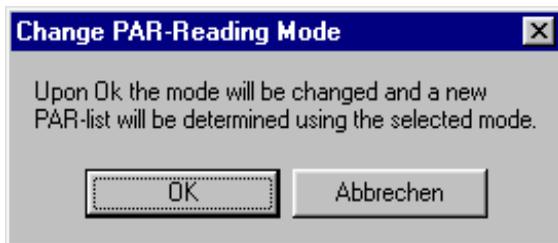
Changing the PAR-Reading mode via the **2-point check box** is linked with the reading of a new Internal PAR-List using the selected mode.

Two-point PAR Calibration ✕

This function reads two PAR-values, one for a defined Measuring Light setting and another one for a defined Actinic Light setting.. For this purpose a suitable Micro Quantum Sensor must be used. Alternatively the two values may be also entered manually. Upon OK the Int. PAR-List is determined on the basis of these values.

ML-Value [15]   **AL-Value** [158]

Read	Ok	Cancel

3.6   The Report window

The **Report window** shows the data stored in the **Report file**. This data file is identical to the MEMORY of the PAM fluorometer. When the PAM is connected via RS232 interface cable to the PC, the same data sets are automatically saved in the PAM and in the Report file. When the Report window is active, the user can observe directly the addition of a new line (one data set) to the Report with every new measurement (i.e. Saturation Pulse). It is also possible to transfer data from the **PAM MEMORY** to the Report file, e.g. after experiments in the field (see **Data Transfer from PAM**, under **Options** on pp. 45-46).

ID	No.	Mark	H:M:S	D.M.Y	F	Fm'	Yield	ETR	PAR	1
						Options				
	PAM-CONTROL (Universal) SETTINGS 09:47 22/JUL/98									
	MF=6;SI=8;SW=0.4;AI=9;AW=0:20;AF=1.00;PG=13;FI=12;FW=5:00;MA=5;EF=0.84;FO=24									
	266	A	09:44:11	22.07.98	2	476	0.995	0.0	0	
	MF=7;SI=8;SW=0.4;AI=9;AW=0:20;AF=1.00;PG=19;FI=12;FW=5:00;MA=5;EF=0.84;FO=25									
	268	A	09:48:02	22.07.98	307	1100	0.720	0.0	0	
L1	269	I	09:48:39	22.07.98	320	1076	0.702	0.0	0	
	270	A	09:48:48	22.07.98	372	1015	0.633	7.8	30	
	271	A	09:48:59	22.07.98	386	1005	0.615	11.3	44	
	272	A	09:49:09	22.07.98	410	993	0.587	16.2	66	
	273	A	09:49:19	22.07.98	438	984	0.554	22.2	96	
	274	A	09:49:30	22.07.98	481	972	0.505	30.7	145	
	275	A	09:49:40	22.07.98	536	959	0.441	40.1	217	
	276	A	09:49:50	22.07.98	604	947	0.362	46.7	308	
	277	A	09:50:00	22.07.98	683	936	0.270	48.7	430	
I1	278	q	09:51:33	22.07.98	310	1029	0.698	0.0	0	
	279	A	09:51:51	22.07.98	501	966	0.481	62.1	308	
	280	A	09:52:11	22.07.98	605	927	0.347	44.8	308	
	281	A	09:52:32	22.07.98	588	918	0.359	46.3	308	

Main Settings | Settings 2 | **Report** | Light Curve | Induct. Curve | Chart

Fig. 6: Report window

The Report file features a total of **16 columns** which are specified in the header line. In the column **Mark** the beginning of a Light Curve is marked by an l and the beginning of an Induction Curve by a q. In this way, Light and Induction Curves are

distinguished from other measurements and given consecutive numbers, which are displayed in the first column (ID, Identification). An Fm determination (including Fo and Fv/Fm) is marked by an f. After every program start, a new Report file is opened. In conjunction with the first measurement the momentary instrument settings are listed. As soon as a setting is changed, with the next measurement the updated settings will be listed again. In this way, a detailed record on all changes in instrument parameters is obtained.

The icon boxes in the upper left corner feature the usual Windows symbols for **Clear** file (Delete), **Load** file (Open) and **Save** file (Store). The Report files are saved in form of ***.txt files** and are compatible with all common text editor programs as well as spreadsheet and database programs. Data are separated by a **comma**, whereas setting are separated by a **semicolon**. In this way, it is up to the user whether the settings are entered into the spreadsheet colums or not, depending on whether the semicolon is allowed or not.

The Report window displays properties similar to common **spreadsheet** programs, as e.g. **Excel**.. The width of a column can be manipulated by pulling the mouse over the vertical line separating the column names within the header line, while keeping the left key pressed (drag and drop). In this way, also whole columns can be narrowed down to the point that they are hidden.

Using the **Print** command the complete Report file or parts of it can be printed out using a standard printer. Parts of the file can be marked in the usual way by pulling the mouse with the left key kept pressed. Single lines can be marked by double click with the left mouse key. Alternatively, parts of the file can be also marked by pressing the shift key and moving with the cursor keys up/down.

After being properly marked, data sets can be further processed using the common **Cut**, **Copy** and **Paste** commands. In this way the data can be also transferred into other programs like **Excel** or **Word**.

Cut
Copy
Paste
Clear
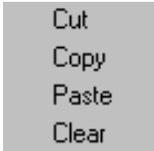
The same commands can be also given via the Edit submenu (see above) or even more readily after clicking the right mouse key.
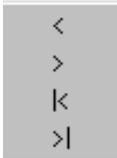
This button operates as a switch between the standard column order (**Force Standard Column Order**) and user defined column order (symbol dark). In the latter case, when the header of the column is clicked, a selection menu with 4 different possibilities for shifting the corresponding column is offered. The column can be shifted stepwise to the left/right or moved to the left or right edge position. Please note that these manipulations only concern the Report window. The actual Report file (i.e. also the file exported to e.g. Excel) is not affected.

When this button is pressed (symbol light), with every start of a Light or Induction Curve a **Comment Line** automatically is inserted into the Report file and above the graphic (shown in Light Curve and Induction Curve windows). In its standard version this line informs about Curve Number, Date and Time. It can be freely edited by the user. In this way, it is e.g. possible to attach a specific note to a particular measurement.

Using this button it is possible to activate the **Average** function. For this purpose the columns which are going to be averaged have to be marked (see above for Print).

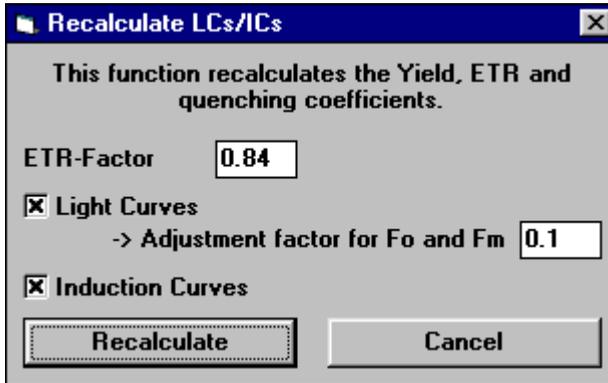| ID	No.	Mark	H:M:S	D.M.Y	F	Fm'	Yield	ETR	▲
	50	A	09:59:35	16.02.00	452	1180	0.616	22.4	
	51	A	09:59:57	16.02.00	447	1169	0.617	22.0	
	52	A	10:00:19	16.02.00	441	1165	0.621	21.8	
	53	A	10:00:40	16.02.00	439	1160	0.621	21.5	
	54	A	10:01:02	16.02.00	436	1152	0.621	21.3	
	55	A	10:01:23	16.02.00	433	1145	0.621	21.0	
	56	A	10:01:45	16.02.00	431	1141	0.622	20.8	
	57	A	10:02:06	16.02.00	429	1135	0.622	20.5	
	58	A	10:02:28	16.02.00	426	1129	0.622	20.5	
	52	a	00:02:09	16.02.00	434	1147	0.621	21.1	
Avg:	52	a	00:02:09	16.02.00	434	1147	0.621	21.1	▼
Ins ◀									▶

When Average is activated, at the bottom of the Report window a new line appears in which the averaged data of the marked columns are listed. In the H:M:S column the time span over which was averaged is shown. By clicking the **Insert** button (**Ins** symbol) the **Avg. line** is inserted to the Report file at the end of the marked block. In the Mark column it is characterized by an "a".

Options

Recalculate LCs/ICs
PAR - Recalculation

Use these settings
Insert/Renumber Comments
Data Transfer from PAM
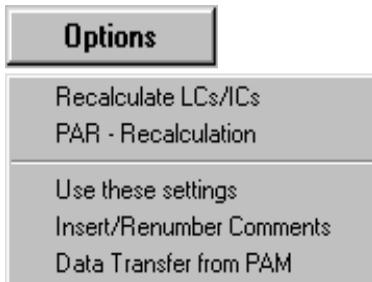
Under **Options** two groups of helpful functions are available. The first group relates to the **recalculation of data** stored in the **Report file** by manipulation of the basic fluorescence parameters and correction of the effective light intensity. The second group serves to facilitate experiments with the PAM and the data transfer from the PAM to the PC. It should be noted that the use of these options is not required for basic operation of PAM fluorometers in conjunction with the WinControl software. They were developed for special applications following suggestions of users.

Recalculate LCs/ICs ✕

This function recalculates the Yield, ETR and
quenching coefficients.

ETR-Factor   `0.84`

☒ **Light Curves**
        -> Adjustment factor for Fo and Fm `0.1`

☒ **Induction Curves**

[**Recalculate**   ]   [**Cancel**   ]

Recalculate LCs/ICs allows to recalculate all data stored in Light Curves (LC) and Induction Curves (IC) after manipulation of the underlying values of fluorescence and PAR in the Report file. The values can be modified manually. Upon **Recalculate** the Report file is recalculated on the basis of the new values. The Report-file features the quenching coefficients qP, qN and NPQ, calculation of which requires knowledge of Fo and/or Fm. But in many practical situations the true Fo and Fm values are not exactly known, e.g. when Light Curves are recorded in the field and not sufficient time is given for dark adaption of the sample. Still, it is desirable to obtain information on the trends followed by these fluorescence parameters. With Light Curves as well as with Induction Curves before actinic illumination a Saturation Pulse is given for determination of the apparent Fo, Fm and Fv/Fm. Whether or not these values correspond to the real Fo, Fm and Fv/Fm values depends on previous dark adaptation. While dark adaptation is desirable for Induction Curves, it is problematic with Light Curves, particularly when the illumination periods are short (Rapid Light Curves). In this case, in order to obtain information on the trends of quenching coefficients during a light curve, the apparent Fo and Fm values can be corrected

under the assumption that the true Fo would be somewhat lower and the true Fm somewhat higher An empirical „**Adjustment factor“** between 0 and 0.2 can be defined. For example, when the factor is 0.1 this means that Fo is decreased by 10 % and Fm is increased by 10 %. This function is also useful, when due to strong nonphotochemical quenching the Fo-value of an illuminated sample (Fo') is significantly lower than the original Fo. In this case, momentary fluorescence yield, F, may decrease even below the original dark level fluorescence, Fo, such that qP, if calculated on the basis of the original Fo, would reach values above 1, which is not possible. Furthermore, once values above 1 are reached, it is not possible anymore to follow the trend of changes. By decreasing the Fo-value artificially via the „Adjustment factor“ qP values above 1 can be avoided. For reliable information on quenching coefficients, the Fo' has to be measured shortly after turning off the actinic light, preferentially with application of far red light.

Another practical problem is that in most cases it is not known how much of the incident light is absorbed by the sample, at least not at the time when an experiment is carried out. For **calculation of ETR**, WinControl proposes a standard **ETR-factor** of 0.84, which corresponds to the average light absorption of leaves. WinControl not only offers the possibility to redefine the ETR-factor for current measurements (under Main Settings), but also to **recalculate ETR** values previously stored in a Report file on the basis of a newly defined ETR-factor.

PAR - Recalculation   ✕

Light Curve PAR - Adjustment

This function inserts (custom) light values to Light Curves,
recalculates ETR and Yield, and compensates the
decrease of halogen light intensity.

Reference PAR Vals   **Apply on**

1 ▼	5.Value	155		1
1.Value	0	6.Value	242	2
2.Value	37	7.Value	340	
3.Value	67	8.Value	534	
4.Value	107	9.Value	797	

ETR-Fact.   **Light Decrease Compensation**

0.84   ☒ Active   Factor 0.997

Calculate

Light Sensor Correction

This function recalculates all PAR- and ETR-values of the
report on the basis of corrected Offset and Gain factors.

Offset 0   Gain 1.00

Recalculate

Under **PAR-Recalculation** it is possible to modify the PAR
values stored in the Report-file and to recalculate the derived ETR-
values and Light Response Curves. PAR values for all 9 settings can
be manually entered or the complete set of PAR values of one Light
Curve (**Reference PAR Values**) can be copied into the data set of
another Light Curve (or several other Light Curves which can be
marked as a block). At the same time also the ETR-factor may be
redefined (see also above). The Reference PAR Values can also
originate from a simulated Light Curve recording in which a Micro
Quantum Sensor occupies the same position as the plant sample in a
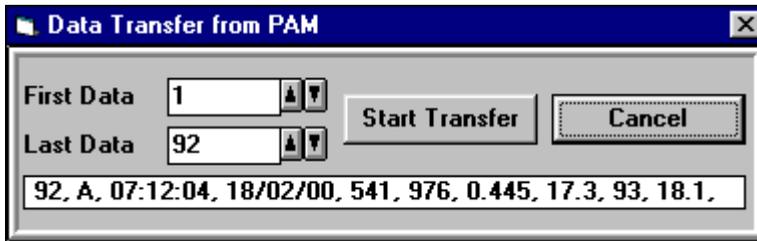real recording. This procedure is quite useful in all cases, when it is

difficult to have the sensor and the sample in the same position at the measuring site during measurements, as e.g. with the DIVING-PAM or the MICROSCOPY-PAM. The Reference PAR Values can be recorded either before or after a series of Light Curve recordings. The **Light Decrease Compensation** allows to correct for the unavoidable drop in battery voltage in the course of consecutive Light Curve recordings which consume a substantial amount of battery power. Empirically, there is an approximately 0.3 % decrease of light intensity of the internal halogen lamp (MINI- and DIVING-PAM) for each consecutively recorded Light Curve. The suggested Compensation Factor of 0.997 can be modified by the user. When the Light Decrease Compensation check box is activated, upon **Calculate** the corrected PAR-values and the derived ETR-values are determined. It should be noted that for correct use of this function the recording of consecutive Light Curves should not be interrupted by any other measurements using actinic illumination (i.e. involving a drop in battery power). The **Light Sensor Correction** provides the possibility to first correct the Gain and Zero Offset of the Micro Quantum Sensor and then to recalculate all PAR- and ETR-values of the Report-file.

Use these settings   Upon program start and after every change of **Main Settings** or **Settings 2** a new line is entered into the Report file listing the momentary instrument settings (**Settings Line**). When a Report file is saved and reloaded at a later time, a particular Settings Line can be marked by double click (left mouse key). With the help of the „**Use these settings**" command the settings corresponding to the selected Settings Line are installed in

the PAM and will be valid in further measurements.

Insert/Renumber Comments In conjunction with the start of a **Light Curve** or **Induction Curve** automatically a **Comment Line** is inserted into the **Report file**. This line shows the current number of the corresponding curve as well as time and date at the start of the first measurement (i.e. Saturation Pulse). The same line also shows at the top of the Light Curve and Induction Curve windows (see below). It can be edited anytime by inserting comments both at the Report file and the Light/Induction Curve levels. The **Insert/Renumber Comments** command applies to the special situation that Light/Induction Curves were erased and the current curve numbers differ from the numbers in the Comment Lines. In particular, this function is also useful after downloading of data from the PAM to the PC via the **Data Transfer** routine.

Data Transfer When the PAM is connected via RS232 interface with a PC, the data automatically are transferred online from the PAM to the PC and stored in the Report file.

Data Transfer from PAM

First Data   1

Last Data   92

Start Transfer   **Cancel**

92, A, 07:12:04, 18/02/00, 541, 976, 0.445, 17.3, 93, 18.1,

The **Data Transfer** function applies to cases when the PAM is operated in the **stand alone mode**, as e.g. in **field investigations**. Then the data stored in the **MEMORY** of the PAM can be transferred at any later time from the PAM to a PC. The first and last data lines to be transferred can be defined. After clicking **Start Transfer**, the selected part of the MEMORY is transferred to the Report file and can be viewed in the Report window. The thus created file contains the same information as if created online. It can be given a name and stored on disc. As described avove, with the help of the Insert/Renumber Comments command comments can be added. The WinControl Data Transfer routine substitutes the PamTrans Data Transfer Software originally delivered together with the MINI- and DIVING-PAM. The Data Transfer routine is not only accessible via Options but via the **File-menu** as well.

3.7   The Light Curve window

The **Light Curve window** shows graphical plots of all fluorescence parameters measured in the course of an automatically recorded Light Curve. The underlying data are listed in the Report file, where all Light Curves are consecutively numbered. The **header line** on top of the display window corresponds to the Comment Line in the Report window. It can be edited by the user.
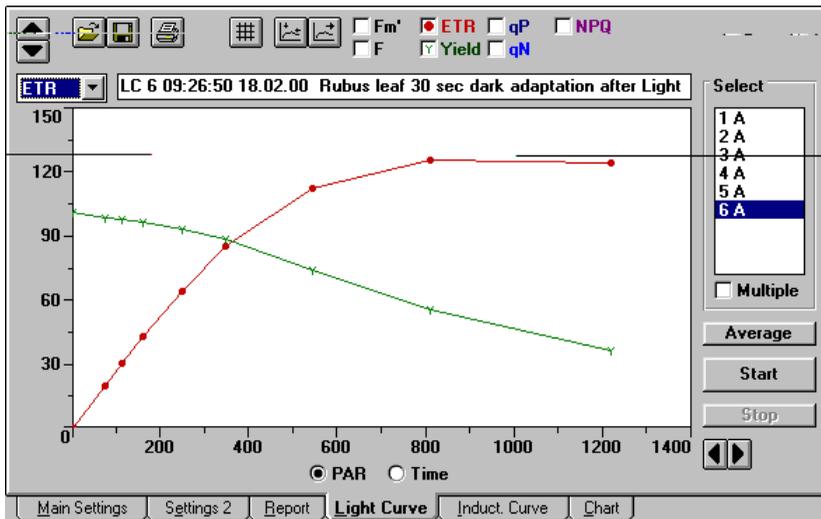

Fig. 7:   Light Curve window

With the help of the **vertical arrow keys** the scaling of the **Y-axis** can be modified within wide ranges. The **selection box** located underneath the arrow keys allows to choose between the scales of different parameters (F and Fm', ETR, Y, qP and qN, as well as NPQ). Please note that the vertical arrow keys are not functional when the Y,qP,qN scale is selected,

as in this case the scale is always ranging from 0 to 1.

With the help of the **horizontal arrow keys** the scaling of the **X-axis** can be modified. While normally the X-axis features a **PAR Scale**, it is also possible to switch to a **Time Scale** by applying the corresponding radio button. The latter scale is useful for display of Light Curves including the consequent recovery phase.

These icon boxes feature the usual Windows symbols for **Load** file (Open) and **Save** file (Store). Loading and Saving always relates to **complete Report files**, i.e. not only the Light Curve which is on display.

A **Printout** of the Light Curve window can be obtained using the **Print** icon. When the print format is defined as upright (vertical, portrait), a printout in DIN A5 is carried out. Otherwise (horizontal, landscape format) the printout will be in DIN A4.

This icon serves to switch on/off the **Grid** display.

With the help of the **Auto Scale** function a curve automatically is displayed at maximal size.

The **Auto Chart** function serves to start **Chart Registration** simultaneously with the start of a **Light** or **Induction Curve**. While Light/Induction Curves only feature data associated with application of a Saturation Pulse, Chart Registration involves the storage of continuously measured fluorescence yield, F, which means a very large number of stored data

points. This should be considered when Auto Chart is active during recording of Light/Induction Curves.

 Check boxes are provided for every **fluorescence parameter** which can be displayed. They are activated by mouse click and then show the corresponding symbol of the selected parameter.
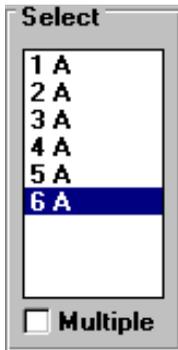
 The **Select** box shows a list of the Light Curves contained in the Report file. A particular curve can be selected by mouse click and then will be displayed. Alternatively also the Page Up and Down keys can be used to move in the list up and down. When keeping one of these keys pressed, the Light Curves are scanned like in a movie. With the keys Pos.1 and End one can jump to the first and last curve, respectively. It is also possible to display several curves on top of each other. For this purpose, the **Multiple** check box must be activated (x). In practice, it is particularly useful to compare consecutively recorded Light Curves in order to obtain information on the process of light adaptation of a sample. When Multiple is active, one can e.g. select the last recorded curve and then record the next curve on top of it (see Fig. 8).
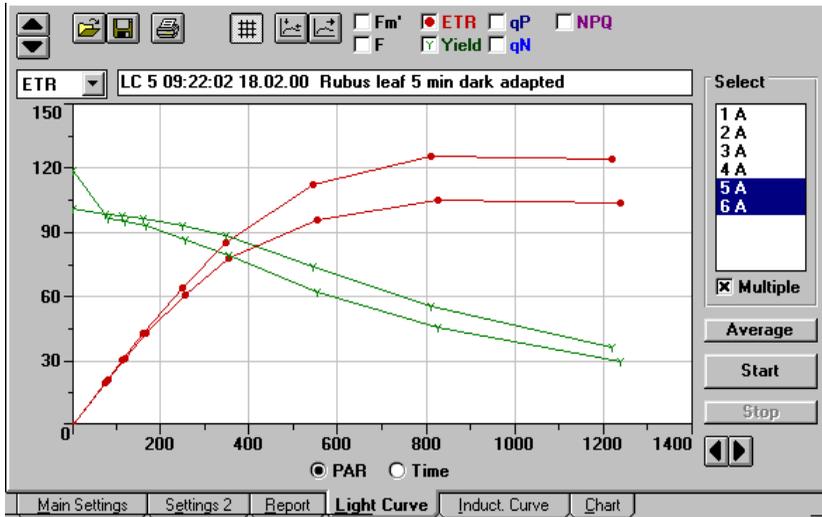
Fig. 8:   Display of two consecutively recorded Light Curves with the
          Multiple function being active.

Average   Using the Average function it is possible to average the data points of several selected Light Curves in order to obtain one representative curve. The new curve is added with the following number to list and is also saved in the Report file, with a header line listing the numbers of the averaged curves (see below).

ID	No.	Mark	H:M:S	D.M.Y	F	Fm'	Yield	ETR
	Averaged Curves: 5, 6							
L7	149	I	09:22:02	18.02.00	342	1349	0.733	0.0
	150	A	09:22:34	18.02.00	456	1298	0.649	20.4
	151	A	09:23:06	18.02.00	451	1264	0.643	30.8
	152	A	09:23:38	18.02.00	454	1231	0.630	42.8
	153	A	09:24:10	18.02.00	461	1151	0.598	62.6
	154	A	09:24:42	18.02.00	469	1065	0.558	81.8
	155	A	09:25:14	18.02.00	497	910	0.454	104.1
	156	A	09:25:46	18.02.00	520	782	0.337	115.4
	157	A	09:26:18	18.02.00	532	683	0.222	114.0

Start	The **Start** and **Stop** buttons can be used to start and
Stop	stop a Light Curve. Alternatively also the Light Curve Status check box can be used for this

purpose.

3.8 The Induction Curve window

The **Induction Curve window** shows graphical plots of all fluorescence parameters measured in the course of an automatically recorded Induction Curve. The underlying data are listed in the Report file, where all Induction Curves are consecutively numbered. The **header line** on top of the display window corresponds to the Comment Line in the Report window. It can be edited by the user.
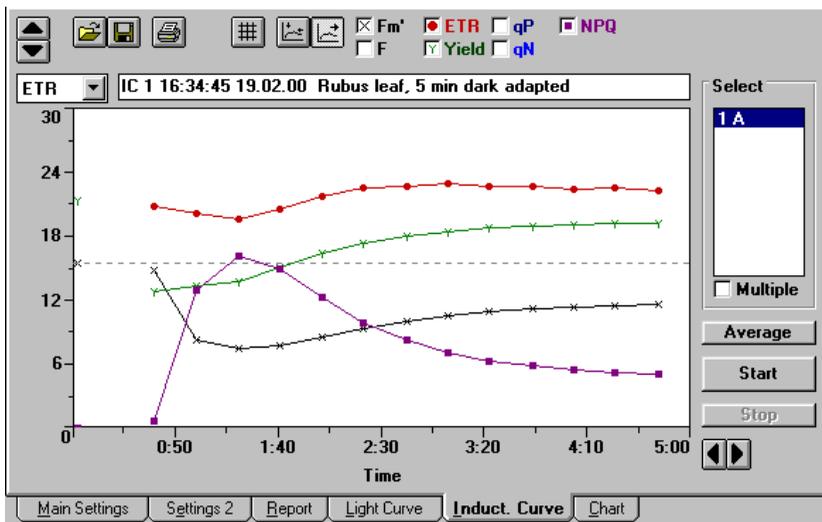

Fig. 9:   Induction Curve window

	With the help of the **vertical arrow keys** the
ETR	scaling of the **Y-axis** can be modified within

wide ranges. The **selection box** located underneath the arrow keys allows to choose between the scales of different parameters (F and Fm', ETR, Y, qP,and qN, as well as NPQ). Please note that the vertical arrow keys are not functional when the Y, qP, qN scale is selected, as in this case the scale is always ranging from 0 to 1.

With the help of the **horizontal arrow keys** the scaling of the **Time-axis** can be modified.

These icon boxes feature the usual Windows symbols for **Load** file (Open) and **Save** file (Store). Loading and Saving always relates to **complete Report files**, i.e. not only the Induction Curve which is on display.

A **Printout** of the Induction Curve window can be obtained using the **Print** icon. When the print format is defined as upright (vertical, portrait), a printout in DIN A5 is carried out. Otherwise (horizontal, landscape format) the printout will be in DIN A4.

This icon serves to switch on/off the **Grid** display.

With the help of the **Auto Scale** function a curve automatically is displayed at maximal size.

The **Auto Chart** function serves to start **Chart Registration** simultaneously with the start of an **Induction Curve** (in analogy to Light Curve). While Induction Curves feature data only which are associated with application of a Saturation Pulse, Chart Registration involves the storage of **continuously measured fluorescence yield, F**, which means

a very large number of stored data points. This should be considered when Auto Chart is active during recording of Induction Curves.

Check boxes are provided for every **fluorescence parameter** which can be displayed. They are activated by mouse click and then show the corresponding symbol of the selected parameter.
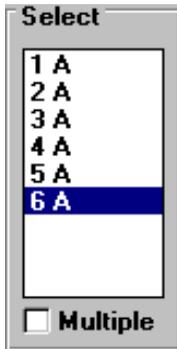
The **Select** box shows a **list of the Induction Curves** contained in the **Report file**. A particular curve can be selected by mouse click and then will be displayed. Alternatively also the **Page Up** and **Page Down** keys can be used to move in the list up and down. When keeping one of these keys pressed, the Induction Curves are scanned like in a movie. Using the keys Pos.1 and End one can jump to the first and last curve, respectively. It is also possible to display several curves on top of each other. For this purpose, the **Multiple** check box must be activated (x). In practice, it is particularly useful to compare consecutively recorded Induction Curves in order to obtain information on the properties of light adaptation of a sample. When Multiple is active, one can e.g. first select the last recorded curve and then record the next curve on top of it.

Using the **Average function** it is possible to average the data points of several selected Induction Curves in order to obtain one representative curve. The new curve is added with the following number to the list and is also saved in the **Report file**, with a **header**

Average

line listing the numbers of the averaged curves (see analogous example of Light Curve averaging on p. 50).

Start

Stop

The **Start** and **Stop** buttons can be used to start and stop an Induction Curve. Alternatively also the Induction Curve Status check box can be used for this purpose.

3.9   The Chart window

The **Chart window** basically simulates the function of a **chart recorder**. In addition it also allows to plot the fluorescence parameters determined in conjunction with Saturation Pulses. Furthermore, under **Options** also an **Averaging** function and two different **Data Export** routines are offered. Fluorescence yield, F, is sampled at a maximal rate of 100 points/sec and written into a buffer memory (capacity of 32000 data points). At maximal sampling rate the buffer length is 5 min. A Chart recording is started either via the **Start** button or simultaneously with the recording of a Light or Induction Curve after activation of the **Auto Start/Stop** function (see p. 48).

Note: The Chart recording occupies a substantial amount of computation time and, hence, slows down other computer controlled functions, including those of the PAM, which may be slowed down by ca. 10 %. Therefore, the Chart function should be activated only if it really is of interest and should not just run in the background.

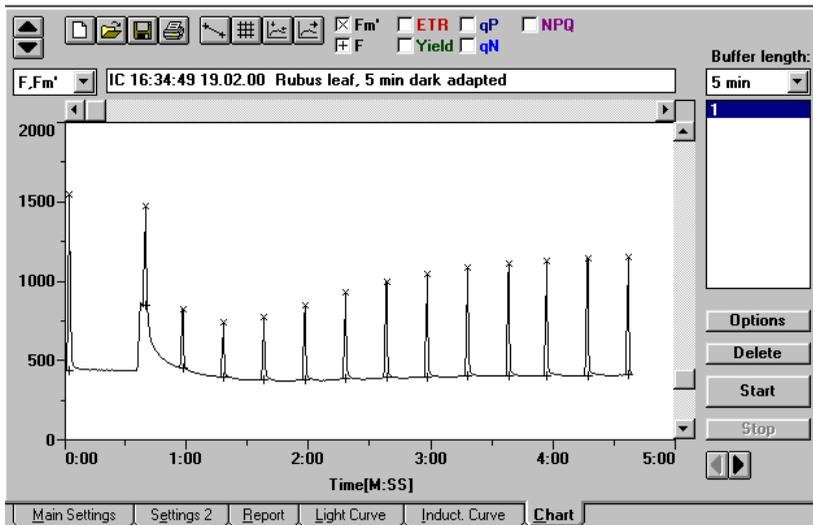Please note, that the Chart continues running when you switch to another window.

Fig. 10: Chart window showing a standard Induction Curve recording with F and Fm' plotting being active

With the help of the **vertical arrow keys** the scaling of the **Y-axis** can be modified within wide ranges. The **selection box** located underneath the arrow keys allows to choose between the scales of different parameters (F and Fm', ETR, Y, qP, and qN, as well as NPQ). Please note that the vertical arrow keys are not functional when the Y, qP, qN scale is selected, as in this case the scale is always ranging from 0 to 1.

With the help of the **horizontal arrow keys** the scaling of the **Time-axis** can be modified. The right arrow key serves for **Zooming**. At a maximal sampling rate of 100 data

points/sec, even the fluorescence kinetics during a single Saturation Pulse can be zoomed out of a 5 min recording. As shown in Fig. 11, with the help of the mouse cursor the coordinates of a particular data point can be displayed. Using the roll bar it is possible to move the selected window along the curve.
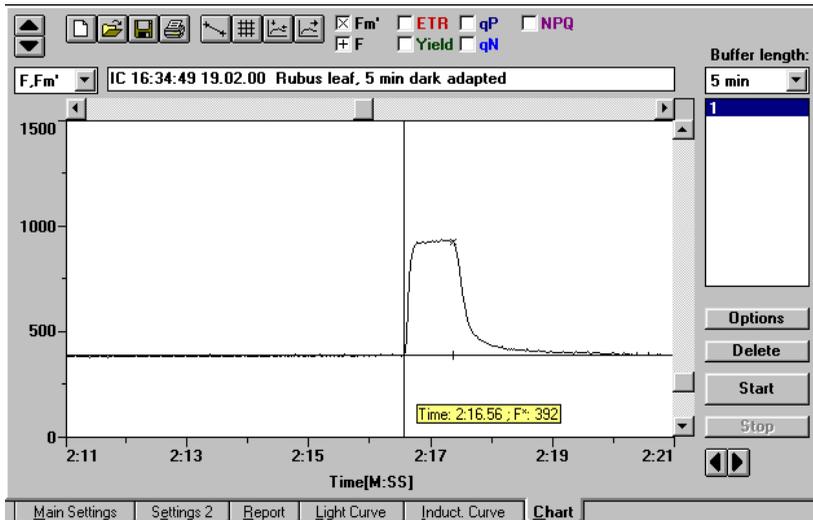

Fig. 11: Chart window after horizontal zoom

Using this icon it is possible to clear all chart recordings with a single command. This for example is helpful when a number of recordings was accumulated unintentially, e.g. because the Auto Chart function was activated on the Light Curve or Induction Curve Window. Before the command is

carried out, one has to reconfirm that really all chart data shall be deleted. It is also possible to delete individual recordings using the **Delete button** (see below).

These icon boxes feature the usual Windows symbols for **Load** file (Open) and **Save** file (Store). Loading and Saving always relate to **complete Chart files**, i.e. not only to the Chart Curve on display. Data are stored in so called **pcf-files**, which also contains information on the parameters F, Fm', Yield, PAR, Yield, qP, qN and NPQ, if Saturation Pulses were involved, as e.g. with Light Curves or Induction Curves. However, data storage in pcf-files is independent from data storage in Report-files. This means that the user himself is responsible for keeping a record on the link between such files, e.g. by giving identical file names and adding corresponding comments in the Report file.

Note: Due to the large number of data points in a complete chart recording (32000 points) only those recordings should be saved which are important. Those which are of minor interest can be cleared via **Delete**.

A **Printout** of the Chart Curve window can be obtained using the **Print** icon. When the print format is defined as upright (vertical, portrait), a printout in DIN A5 is carried out. Otherwise (i.e. horizontal, landscape) the printout will be in DIN A4.

With the **Connect points** command the points of each fluorescence parameter are connected by line segments. In this way quasi-continuous traces are created

This icon serves to switch on/off the **Grid** display.

Display front moves the horizontal roll bar towards the right edge, such that the last recorded data points (front of the memory) is displayed.

With the help of the **Auto Scale** function a curve automatically is displayed at maximal size.

Check boxes are provided for every **fluorescence parameter** which can be displayed. When activated by mouse click, they show the corresponding symbol of the selected parameter.

The **Buffer length** corresponds to the time of a full recording which involves 32000 points of fluorescence yield. At the standard buffer length of 5 min this is equivalent to a sampling rate of ca. 100 points/sec.

One can choose between 8 different buffer lengths, ranging from 5 min to 40 min. With increasing buffer length, the time resolution declines, as the total number of sampled points remains constant.
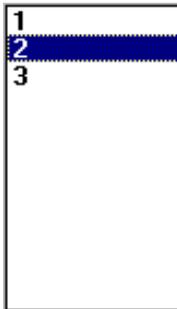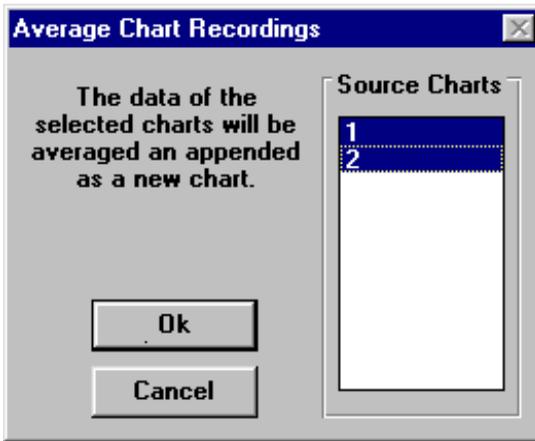
The **Select** box shows a list of the recorded Chart Curves. A particular curve can be selected by mouse click and then will be displayed. With the keys Pos.1 and End one can jump to the first and last curve, respectively.

Options

Average
Export as ASCII
Export pulse data to report

Under **Options** a number of functions are offered which can serve to further process the recorded chart data.

Average Chart Recordings

The data of the selected charts will be averaged an appended as a new chart.

Source Charts

1
2

Ok

Cancel

The **Average** function offers the possibility to average the data points of several Chart Curves, which were selected by mouse click, in order to obtain one representative curve. The new curve is automatically appended to the end of the list. Averaging is applied on the points of continuously measured fluorescence yield as well as on the fluorescence parameters determined in conjunction with a Saturation Pulse. The Averaging function is not only useful to increase the signal/noise ratio, e.g. in experiments using samples with very low chlorophyll content, but also to obtain one representative curve from heterogeneous samples. Heterogeneity may relate e.g. to differences between various areas of the same object or to differences between various samples.

Delete

Using the **Delete** button, particular **Chart Curves** can be removed from the buffer list after being

marked by mouse click.

Start

Stop

The **Start** and **Stop** buttons can be used to start and stop a Chart recording. Alternatively a Chart recording can also be started simultaneously with a Light or Induction Curve, when the **Auto Start** function is active (see above).

Restart

Using the **Restart** button a running recording can be stopped and at the same time a new recording will be started. Please note that an interupted recording is listed as a file in the buffer list. It can be cleared via the **Delete** button.