Dual-PAM Gas-Exchange Cuvette

(3010-Dual)

Manual

2nd edition

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1 Safety Instructions

1.1 General Safety Instructions

1. Read the safety instructions and the operating instructions first.
2. Pay attention to all the safety warnings.
3. Keep the device away from water or high moisture areas.
4. Keep the device away from dust, sand and dirt.
5. Always ensure there is sufficient ventilation.
6. Do not put the device anywhere near sources of heat.
7. Connect the device only to the power source indicated in the operating instructions or on the device.
8. Clean the device only according to the manufacturer's recommendations.
9. Ensure that no liquids or other foreign bodies can find their way inside the device.
10. The device should only be repaired by qualified personnel.

1.2 Special Safety Instructions

1. The GFS-3000, the Dual-PAM-100, and the Dual-PAM Gas-Exchange Cuvette are highly sensitive research instruments, which should be used only for research purposes, as specified in this manual. Please follow the instructions of this manual in order to avoid potential harm to the user and damage to the instrument.
2. The Dual-PAM-100 employs high intensity light sources, which may cause damage to the eye. Avoid looking directly into these light sources during continuous illumination, saturation pulses or single turnover pulses.

2 Introduction

The Dual-PAM Gas-Exchange Cuvette is designed to enable chlorophyll fluorescence and P700 measurements on leaf samples concurrently with gas exchange measurements in a climate controlled environment. Fig. 1 shows the complete set-up of the Dual-PAM Gas-Exchange Cuvette with the Dual-PAM-100 and the GFS-3000 connected. The GFS-3000 controls the gas exchange measurements including CO_2 and H_2O concentration. The temperature is controlled *via* the Electronics Box, which is connected to the GFS-3000. The Dual-PAM-100 controls the light and optical measurements, *via* the Dual-DB and the Dual-E Modules, which are connected to either side of the Dual-PAM Cuvette. A trigger line leads from the Dual-PAM to the Electronics Box.

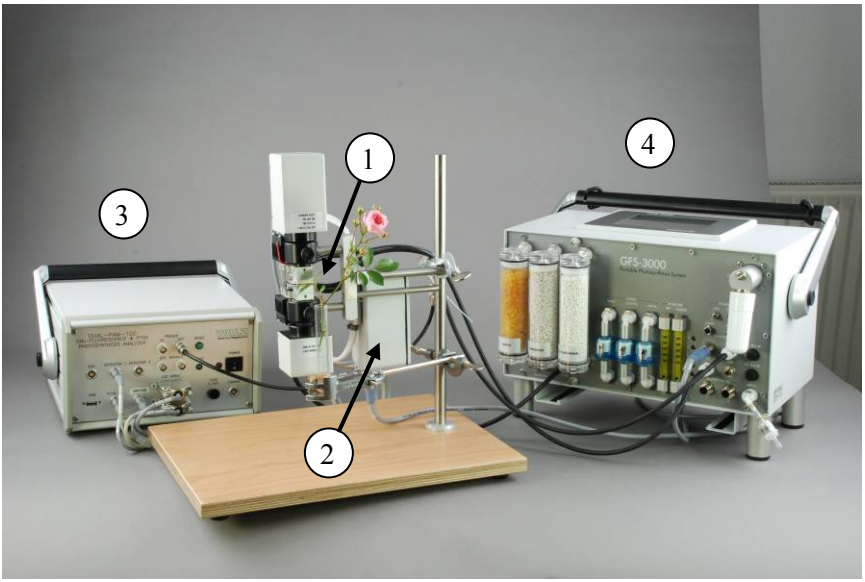

Fig. 1 Overview: Dual-PAM Cuvette (1) with Electronics Box (2), Dual-PAM (3) and GFS-3000 Central Unit (4).

Fig. 2 shows the Dual-PAM Gas-Exchange Cuvette without any parts belonging to the Dual-PAM-100 or the GFS-3000.

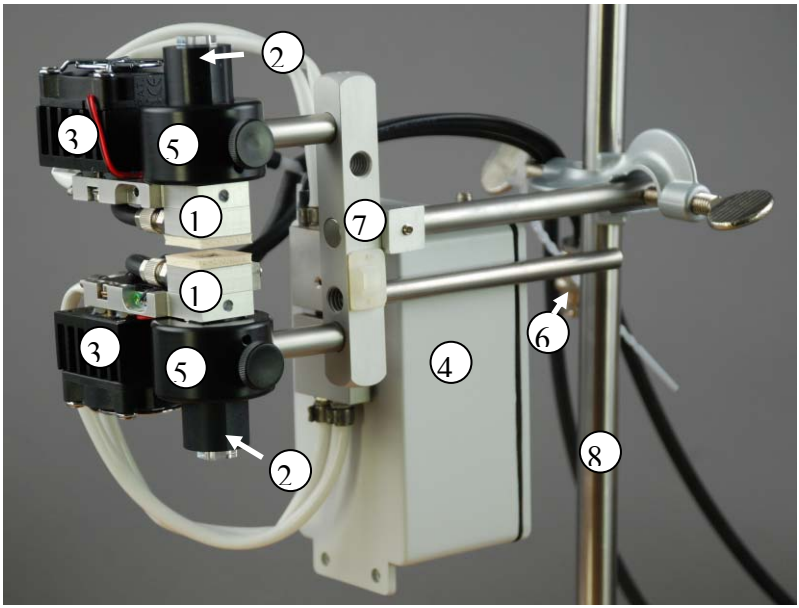

Fig. 2 Dual-PAM Cuvette

1. Upper and lower cuvette frame
2. 22 mm ∅ black metal tube enclosing a Perspex rod that serves as light guide
3. External heat exchanger attached to Peltier elements with fan and grid
4. Electronics Box controlling the Dual-PAM Cuvette
5. Ring fitting with knurled screw holding cuvette half.
6. Cable connecting to Trigger In
7. Support frame with countersunk socket screws at both ends
8. Pole of Mounting Stand ST-101

3 Operation

3.1 Setting up the system

3.1.1 Mounting the optical units

Fig. 3 Fastening Dual-DB

Mount the Dual-DB unit to the 22 mm ∅ black metal tube enclosing the Perspex rod of the upper frame of the Dual-PAM Cuvette. The original Perspex rod together with its metal tube needs to be removed from the Dual-DB unit beforehand.

Fasten the Dual-DB unit with the provided allen wrench (hex-key) as shown in Fig. 3.

Mount the Dual-E at the lower side in the same way. Within the optical units, there is a small central round Perspex rod protruding beyond the other optical parts, it will touch the squared Perspex rod of the Dual-PAM cuvette. Do not use to much force pushing this central rod back.

3.1.2 Opening and Closing of the cuvette

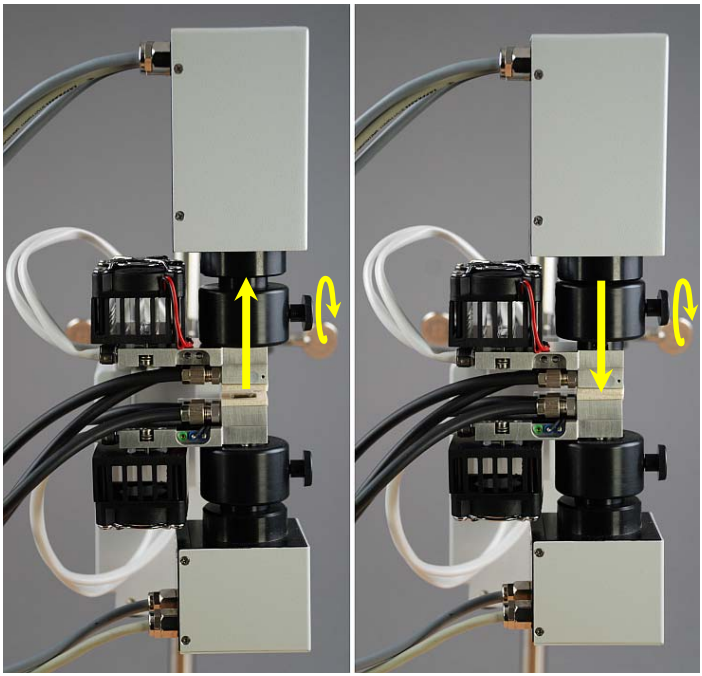

Fig. 4 Opening and closing of the cuvette

With the knurled screw of the ring-fittings, the height positions of the frames can be adjusted. Put the lower frame into its lowest possible position. Open and close the cuvette using the knurled screw of the upper ring fitting. If the pressure on the gaskets is too low even with the upper module being in its lowest position, slightly increase the height of the lower frame.

3.1.3 Connections

Connect the pneumatic connectors to the Central Unit of the GFS-3000. Connect one of them to the connector CUVETTE/FROM and the other to the connector CUVETTE/TO. Connect the cable of the Electronics Box to the connector CUVETTE of the GFS-3000. Connect the trigger cable from the Dual-PAM-100 (OUT) to the Electronics Box.

Connect the Dual-DB and Dual-E modules to the Dual PAM Control Unit. Connect power supplies and switch the instrument on.

3.2 Starting the Software

The concurrent operation of the Dual-PAM and GFS-3000 has been tested on a computer with Windows-XP operating system.

3.2.1 Regional and Language Options

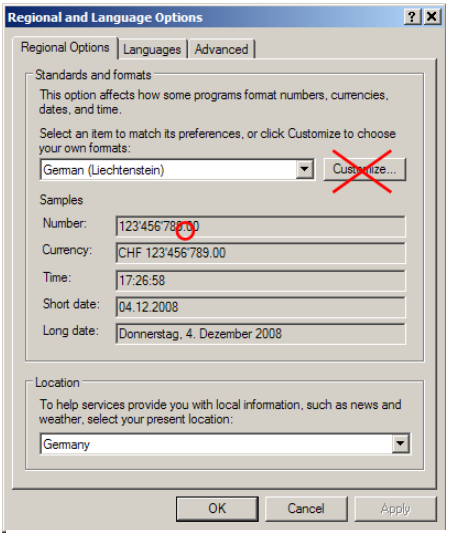

Fig. 5 Reg. and Language Options

Both software, the Dual-PAM program and the GFS-Win, require specific *Regional and Language Options*. GFS-Win requires a dot for the decimal symbol (red circle in Fig. 5), while the Dual-PAM does not allow any customized symbols (Red Cross in Fig. 5). Therefore, choose an option, which has a dot as decimal symbol (e.g. English (Trinidad) or German (Liechtenstein)). The given examples have a ";" as list-separator (see chapter 3.6.4)

In the Windows Operating System press *Start/ Settings/ Control Panel/ Regional and Language Options* to access these settings.

3.2.2 Establishing the USB-connection to both systems on one computer

Both software use the same USB-driver, which can be installed by calling the program cdm_setup.exe supplied with the software CD of each instrument. It can be called directly, or it is installed automatically with the installation of the Dual-PAM program, or it can be called *via Start / Programs/ GFS 3000/ Install USB Port*

The latency time for the Dual-PAM needs to be set to 1 ms within the Windows Operating System. Use: *Start/ Settings/ Control Panel/ System/ Hardware/ Device Manager/ Ports/ USB Serial Port (COM..)/ Advanced/ Latency Time/ 1ms.* GFS-Win can run with any latency time.

Generally, you may plug in both USB-connections and start both programs, the Dual-PAM program (Version 1.8 or higher) and the GFS-Win program (Version 3.19 or higher).

If there is the error message from the Dual-PAM "FT_open, invalid handle", the Dual-PAM is checking the USB-port from the GFS-3000 and can not access it. This message can be ignored.

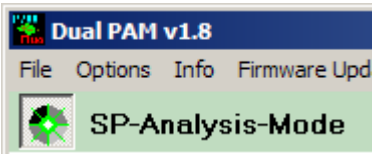

Fig. 6 Functional connection

The Dual-PAM program will show a green glowing LED symbol when the USB-connection is functional (Fig. 6).

In GFS-Win switch the Measure Mode on (System/ Measure Mode on). For the Measuring Head choose Dual-PAM Cuvette 3010-DUAL and press OK (see Fig. 7).

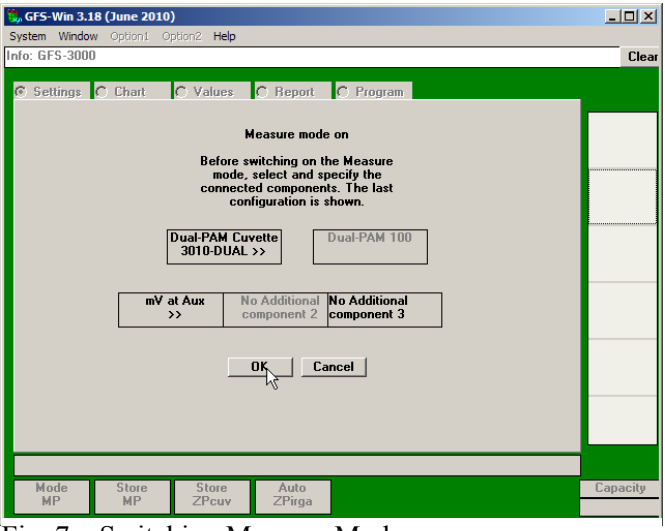

Fig. 7 Switching Measure Mode on

If there are any problems, when establishing the USB-connections, then unplug both USB-connections. Afterwards plug in the USB-connection to the Dual-PAM only and start the Dual-PAM program, next plug in the USB-connection of the GFS-3000 and switch the Measure Mode on. Unplugging a USB-connection generally resets the USB-driver. It takes a while until the driver is reset. Resetting the driver of one USB connection or device might disturb/interrupt any other USB-connection. For correct data calculation, it is important to choose Dual-PAM Cuvette 3010-Dual here (*see* chapter 4.13).

3.3 First steps with the Dual-PAM

3.3.1 Short overview on Settings

Here only the most novel or important settings of the Dual-PAM are briefly explained. For more detailed explanations, see Dual-PAM manual. When using the Dual-PAM for the first time, it is a good idea to start the system in the Single Channel Mode, with either the Fluores-

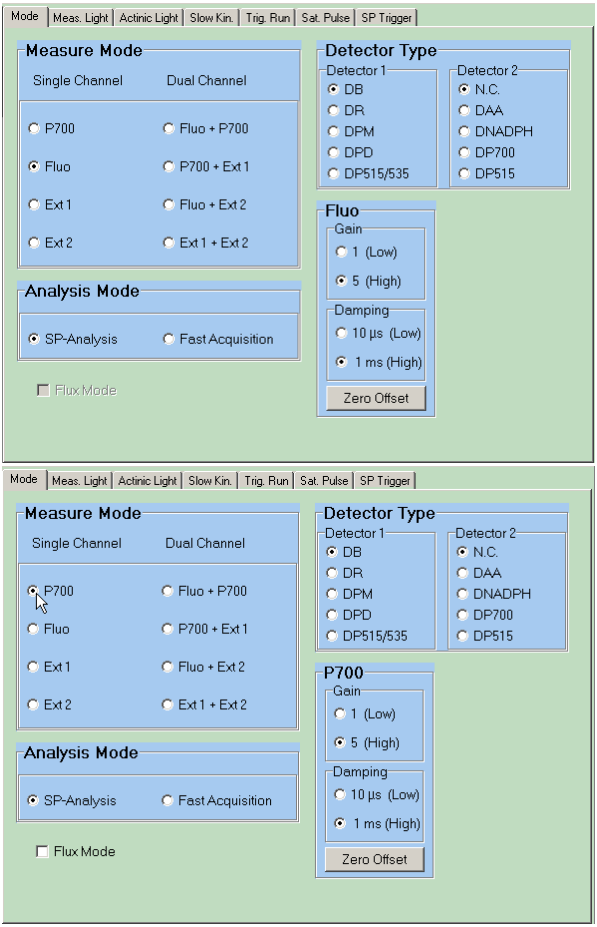

Fig. 8 Settings Measure Mode; top: Chlorophyll fluorescence; below: P700 measurement

cence or P700 channel activated (see Fig. 8). The Dual-PAM program will only show the parts belonging to the selected channel. The following figures show a comparison of the settings, when either the Mode **Fluo** or **P700** is selected. Each settings window has a button for default settings, which can be used in the beginning.

The Measuring Lights (see Fig. 9**)** are **P**ulse **A**mplitude **M**odulated. In the Dual-PAM this pulse modulation occurs in trains of several pulses, so called blocks. One complete block has 14 pulses and takes 35 µs (resulting in 400 kHz during each block). The **Block Frequency** is given in Hz. The time interval between blocks is varied. For fluorescence measurements in the dark a low frequency is required, so that the Measuring Light has no actinic effect. In the presence of Actinic Light a higher frequency can be chosen, to increase accuracy and time resolution. In the case of P700 measurements always maximal pulse frequency can be applied, as the near-infrared Measuring Light does not have any actinic effect.

The P700 signal is the difference between the transmission signals at two wavelengths (875 and 830 nm, seeFig. 9). Before a measurement the intensities of these two light beams need to be balanced for each sample, so that the difference signal is close to 0.00 V (further explanation, see chapter 3.3.2).

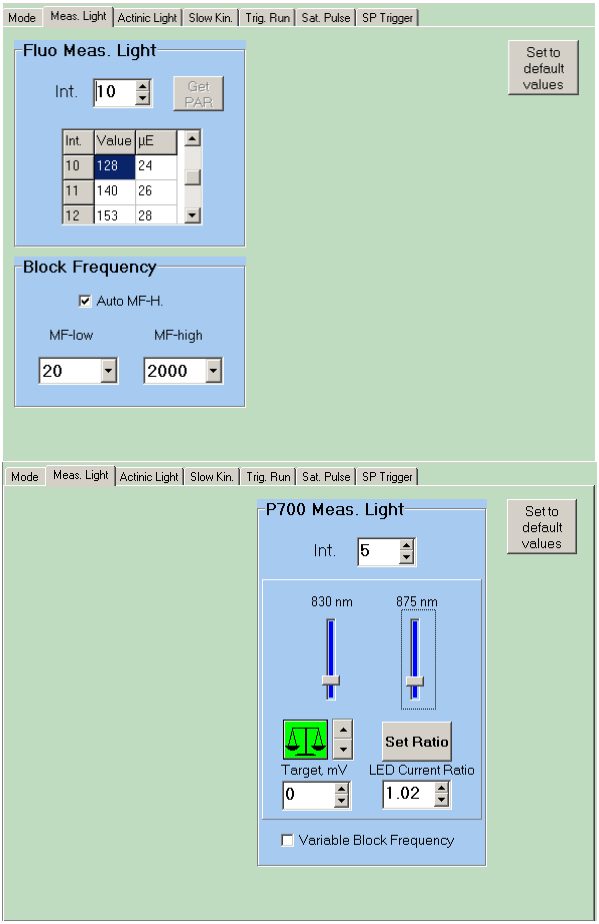

Fig. 9 Settings Measuring Light; top: Chlorophyll fluorescence; below: P700 measurement

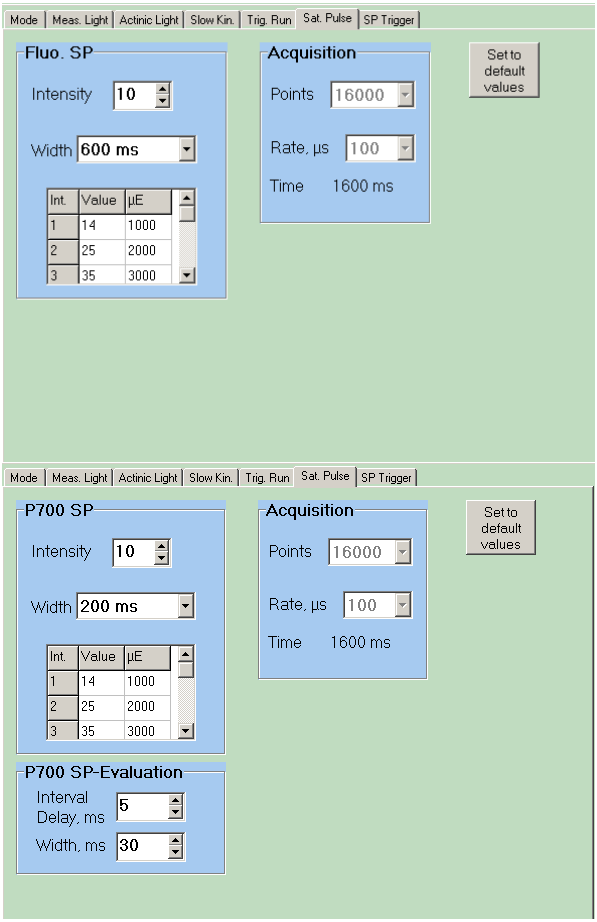

Fig. 10 Settings Saturation Pulse; top: Chlorophyll fluorescence, below: P700 measurement

As can be seen in **Fehler! Verweisquelle konnte nicht gefunden werden.** and Fig. 11, the default settings for the Saturation Pulses (**SP**) differ between the chlorophyll fluorescence (width 600 ms) and P700 (width 200 ms) measurements. For simultaneous Fluo and P700 measurements the default SP width would have been 300 ms.

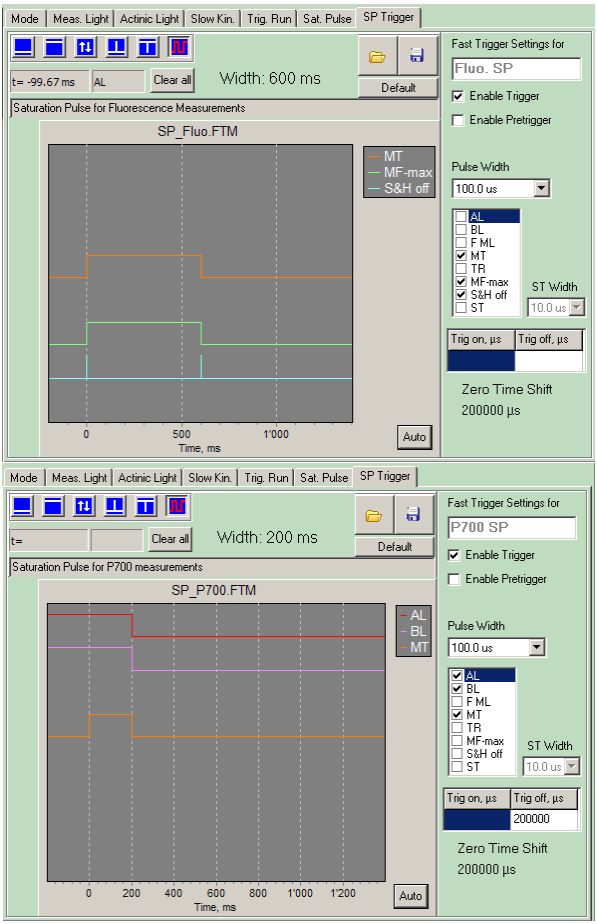

Fig. 11 Trigger Schedule for Saturation Pulse; top chart: Chlorophyll fluorescence; chart below: P700 measurement

In Fig. 11 the trigger schedule for the Saturation Pulse procedure is shown. The saturation pulse itself is asigned **MT** (multiple-turnover with regard to single-turnover **ST)**, its line is in the middle of both charts. Its width (compare upper and lower chart of Fig. 11) can be shorter for P700 measurments than for chlorophyll fluorescence, since full oxidation of PSI occurs in the very beginning of the pulse, when

there are few electrons in the electron transport chain, while full reduction of PSII is achieved later during a saturation pulse, when the electron transport *via* the intersystem chain becomes limiting.

The two upper lines in the lower chart of Fig. 11 show the schedule for the Actinic Light (**AL**) and Blue Light (**BL**/**FR**). They are transiently switched off after each Saturation Pulse, in order to assess the P700 signal in the fully reduced state.

The time before the Saturation Pulse is used to measure the signal level of the current state. In the dark adapted state this is Fo for the fluoresence, with PSII fully oxidized and no non-photochemical quenching (heat dissipation down-regulated). For P700 the current state is only meaningful, when Actinic or Far-red Light is applied.

The two lower lines in the SP trigger schedule for fluorescence (upper chart Fig. 11) have technical reasons: The frequency of the Measuring Light is transiently switched to maximum (**MF-max**) during the Saturation Pulse in order to obtain maximal time resolution. MF-max is higher in the Single-Channel Mode (400 kHz) than in the Dual-Channel mode (blocks of pulses alternating between the two channels). The lowest line, anotated with **S&H,** refers to a sample-and-hold amplifier in the system. It is switched off for a short period of time (default: 100 µs) during strong fast changes in light, which otherwise would disturbe the pulse modulated measurement.

Settings for Actinic Light sources are shown in Fig. 12. Since Far-red light normally is given in the dark, it shares a driver with Blue light. Therefore, Far-red or Blue can be given alternatively only. In the Script file, shown in chapter 3.6.2, the driver will be toggled between Far-red and Blue.

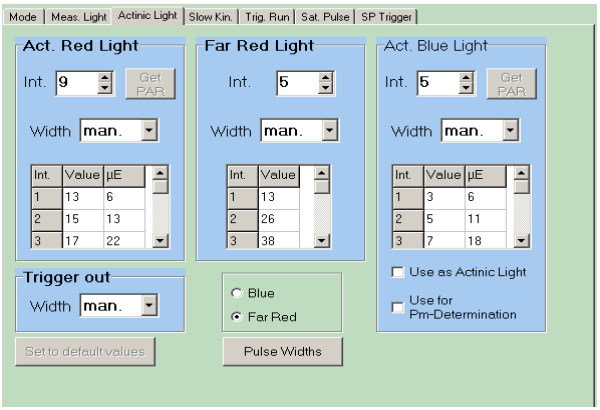

Fig. 12 Settings for Actinic Light

Settings for the recording of Slow Kinetics recordings are shown in Fig. 13. Since gas exchange changes are slow and may be recorded over long time periods, we recommend the maximal number of points and a slow recording rate.

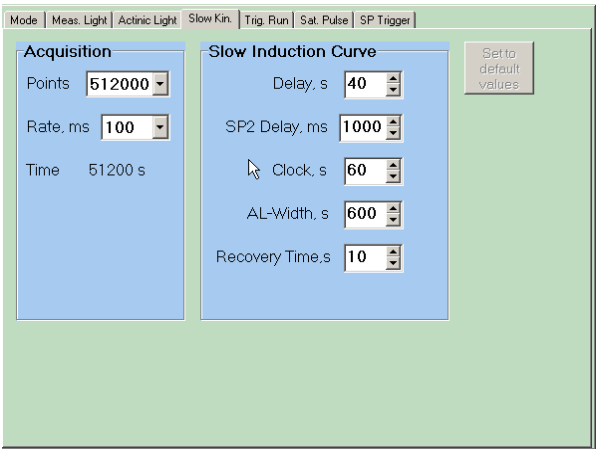

Fig. 13 Slow Kinetics Settings

3.3.2 Chlorophyll fluorescence and P700 measurements in the dark adapted state

For a full analysis of the chlorophyll fluorescence signal, a measurement in the dark adapted state is required. We recommed to use a typical sample for a system trial, which can be discarded afterwards.

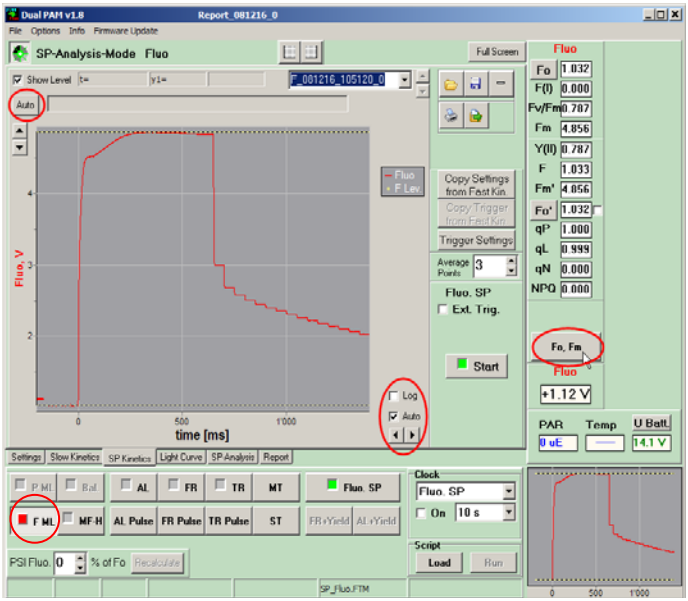

Fig. 14 Saturation Pulse kinetics during Fm determination

For fluorescence measurements, choose the Mode **Fluo** (see Fig. 8) and switch the Measuring Light on using the **F ML button.** Then press the button **Fo, Fm** (see Fig. 14), which triggers a Saturation Pulse according to the trigger schedule shown in Fig. 11. To see the complete kinetics of the rapid SP-induced fluorescence changes open the window **SP Kinetics**. To autoscale the curve, use the buttons **Auto** (Fig. 14).

For P700 measurements, choose the Mode P700 (see Fig. 8). When the Measuring Lights are switched on using the **P ML button,** an automatic Balance of the difference signal is performed (see Fig. 15).

The two beams are balanced best, when the P700 signal (red circle at the right side of Fig. 15) is close to 0.00 V. Fine adjustment can be done with the two arrow keys beside it. Please note that the absolute level of the difference signal has no effect on the observed signal changes, as long as the difference signal does not saturate (at +6.5 and -2.8 V).

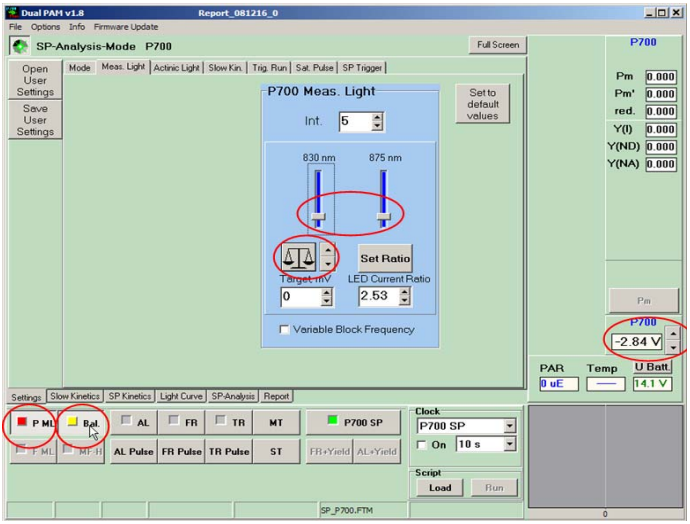

Fig. 15 Balancing of the P700 Measuring Light

The aim of the **Pm** determination is to define the maximal signal change observed between the states of P700 being fully oxidized and reduced. To reach full oxidation in a dark-adapted leaf, a preillumination with Far-red light is required. Choose the window **Slow Kinetics**, Select the Mode **Manua**l and press **Start** (see Fig. 16). Switch the Far-red Light on (**FR** as indicated in Fig. 16) and watch the signal rise.

When oxidation is at a high constant level, press **Pm**. The time of Far-red preillumination required varies with plant material and Far-Red intensity. It should be determined with a typical sample and used for a set of experiments. Automatically there is a 10 s preillumination with

Far Red light, when Pm is pressed. But this time might be to short for long-term dark-adapted leaves.

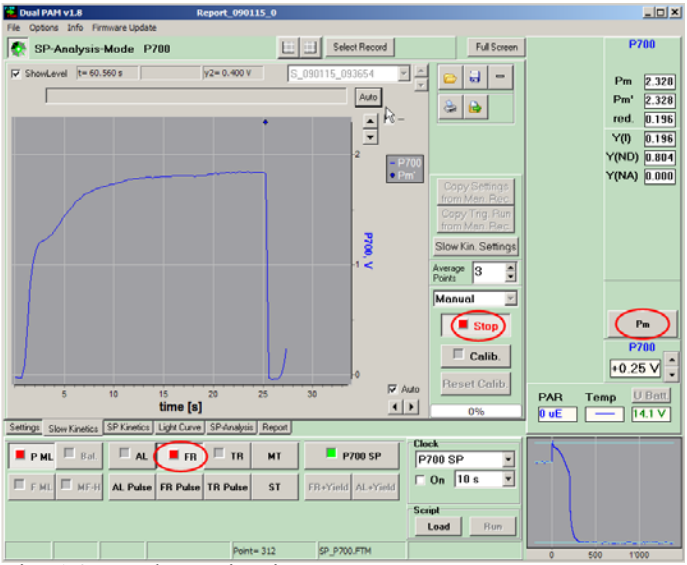

Fig. 16 Pm determination

After this short introduction, the Dual-PAM can be used in the Dual Channel-mode with a fresh sample. Users are encouraged to make frequent use of the help texts written for the various functional elements of the user surface. Help texts can be called up for any element (e.g. Pm button) showing a tooltip when selected by the cursor by pressing the F1 key.

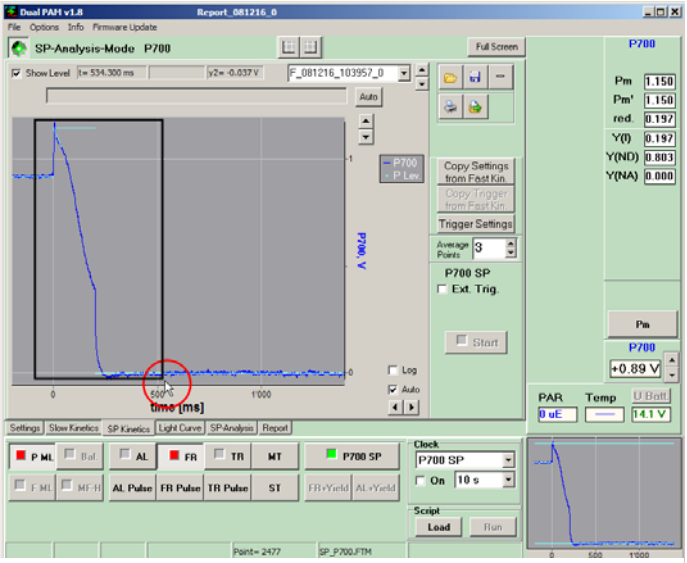

Fig. 17 Saturation Pulse kinetics of P700 signal. To magnify any part of the curve, draw a rectangle around it with the mouse as indicated.

3.4 Starting the Gas Exchange

3.4.1 Checks before Gas Exchange Measurement

Read chapter 6 of the GFS-3000 manual for a short reminder on the checks and calibrations that might be required before a gas exchange measurement can be started.

You may want to confirm the PAR list of the Dual-PAM program before doing any serious measurements (see chapter 5.3). In its standard version, the Dual-PAM 100 gives actinic light from both sides. To shut off the actinic light to the lower sample side, an optional connector is required, which is called Connector without Actinic Light Dual-E/WAL.

3.4.2 Setting-Up and Starting the Gas Exchange Measurement

Decide on the flow, the standard value for the Dual-PAM Gas-Exchange Cuvette is 400 µmol/s (ignore any indication that the flow should be between 600 and 900). With this flow, adjust the valves of the

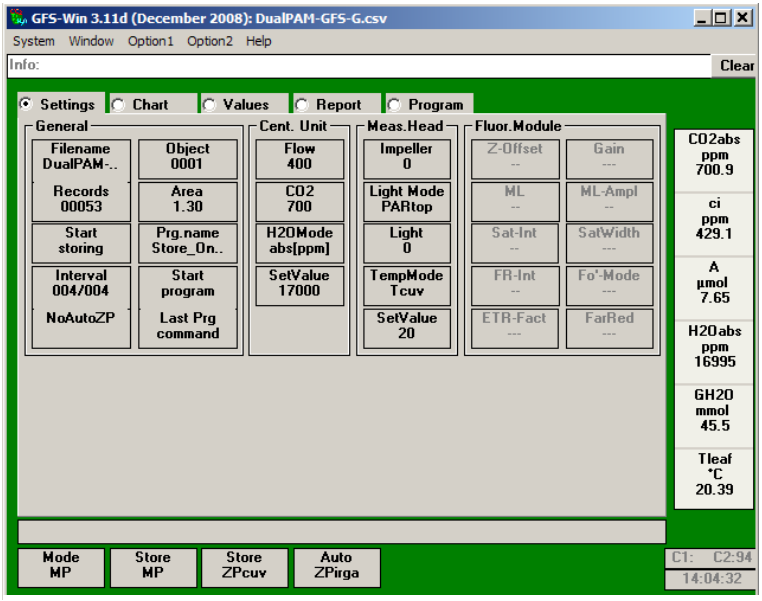

Fig. 18 Window Settings

GFS-3000 with a small screw driver. To do that, press *Option1/Calibration/Central Unit/Valve Adjustment* in GFS-Win and follow the given instructions. Usually, the valve adjustment only needs to be performed, if the cuvette or the flow has been changed between experiments.

In the Window Settings of the GFS-Win software (see Fig. 18), switch on the flow, choose the CO_2 and H_2O concentration in ppm, enter the cuvette temperature and the leaf area, and finally enter a filename. Ignore the fields for impeller speed and light. They are not functional, when the Dual-PAM Gas-Exchange Cuvette is connected.

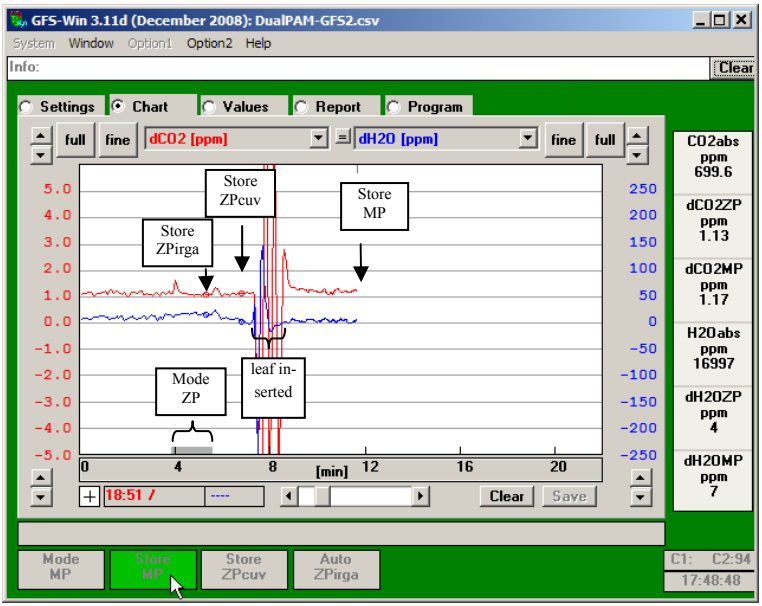

Fig. 19 Differential

For exact gas exchange measurements, it is essential to measure the zero for the differential CO_2 and H_2O concentration before the CO_2 assimilation or H_2O evaporation can be measured. This can be done using the ZP Mode or with an empty cuvette. Fig. 19 shows an example, where the Zero Point has been determined with both methods, in ZP Mode (ZPirga) as well as in MP Mode with an empty cuvette (ZPcuv).

Further information on MP and ZP Mode can be found in the GFS-3000 manual.

3.5 Trigger connection between Dual-PAM-100 and GFS-3000

The trigger leading from the Dual-PAM-100 to the GFS-3000 *via* the Electronics Box has the same effect as pressing **Store MP** (in **MP Mode**) or **Store ZP** (in **ZP Mode**) (see solid arrow in Fig. 20). The trigger effect might be changed in future versions of GFS-Win into **Start program** (see dotted arrow in Fig. 20). This change will depend on user feedback.

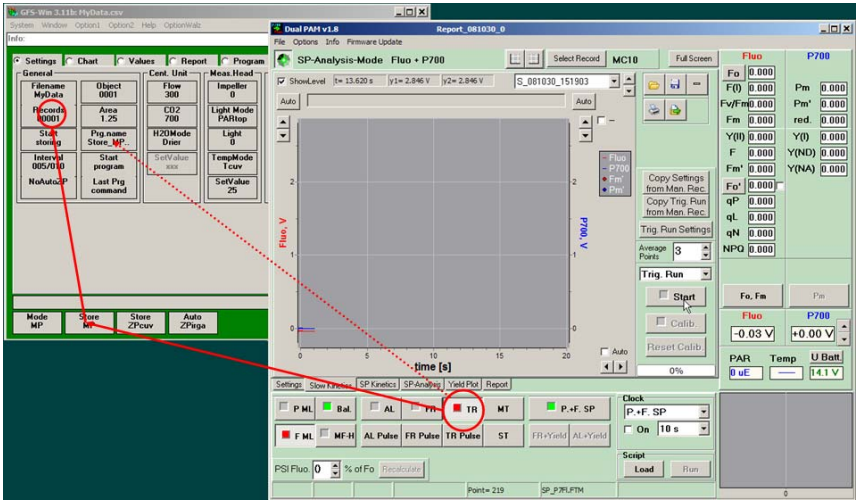

Fig. 20 Trigger Effect, solid arrow: current GFS-Win Version, dotted arrow: possible future GFS-Win version.

Check the trigger connection by pressing and releasing **TR** at the Dual-PAM program. The GFS-3000 reacts to the release of the trigger. The averaging-time required for storing the data is determind with the settings given under **Interval**. (e.g. 4 s compare Fig. 20). A **Filename** needs to be entered in the GFS-Win program before any data can be recorded.

3.6 Example: Dark-light Induction Curves with concurrent Dual-PAM and GFS-3000 measurements

3.6.1 Before starting the automatic Induction Curve

Concurrent measurements of gas exchange, fluorescence and P700 are best performed using script files. Here we show an example of an Induction Curve. Start the Dual-PAM in the Dual Channel Mode.

Before starting any optical measurements, set-up the settings of the GFS-3000 (see chapter 3.4) and measure the zero point (ZP). In mode MP, wait until the values are in a steady state.

In the Dual-PAM program, check the settings for the **Report** (Fig. 22). Tick or untick the box determining the saving of SP Kinetics.

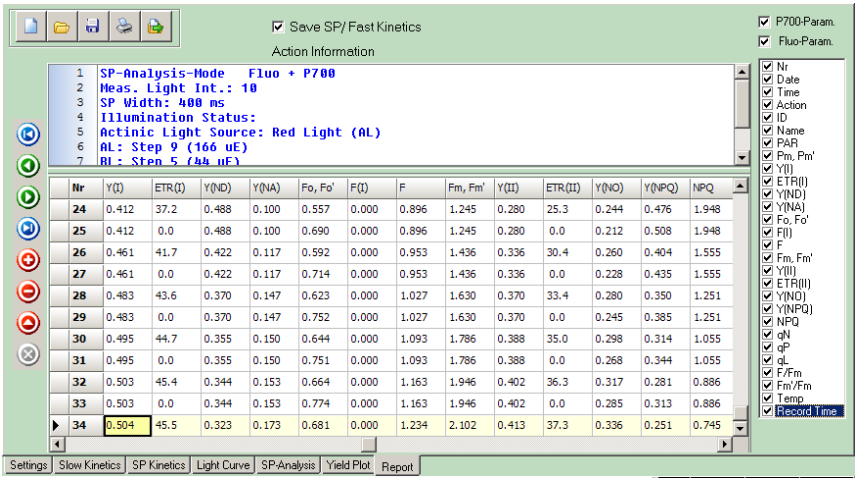

Fig. 21 Report of Dual-PAM program

In the settings window for the SP trigger, check the procedure for the Saturation Pulse (see Fig. 22). The width of the pulse will be adjusted automatically during the Script-run.

After these checks the Script file for the Dual-PAM shown in **Table 1** to **Table 3** can be loaded, (name: With_Gas_exchange.prg). Press button **Script:Load** in the lower right corner of the Dual-PAM program: (Fig. 23).

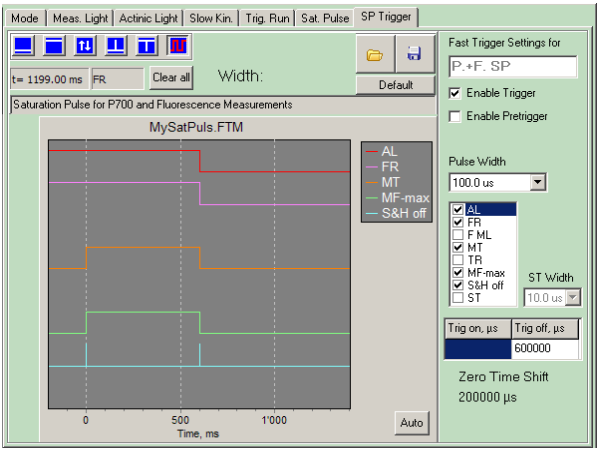

Fig. 22 Saturation Pulse triggering schedule for Induction Curve recording in the Dual Channel mode

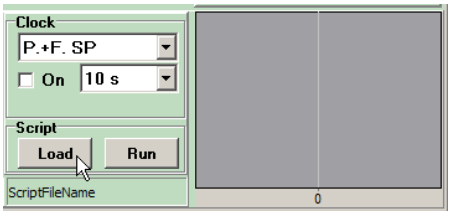

Fig. 23 Script buttons in lower right corner of Dual-PAM program user surface

3.6.2 Script file for Induction Curve

Table 1 Script file (Dual-PAM program): Settings

Save Report as	Saves previous Report with automatically generated name.
New Report	Deletes all data in Report
	Settings Mode (Fig. 8)
Measure Mode = Fluo + P700	
Analysis Mode = SP-Analysis	
Gain Ch1 = 5 (High)	
Damping Ch1 = 1 ms (High)	
Gain Ch2 = 5 (High)	
Damping Ch2 = 1 ms (High)	
	Settings Measuring Light (Fig. 9)
MF-H = 2000	
MF-L = 20	
AutoMF_H On	
F ML-Int. = =10	
P ML-Int. = =5	
	Settings Light (Fig. 12)
AL-Int.= 14	**adjust for your experiment**
AL Width = man.	
BL-Int. =5	**adjust for your experiment**
BL Width = man.	
FR-Int. =5	**adjust for your samples**
FR Width = man.	
SP-Int. =10	**adjust for your samples**
SP Width = 400 ms	**adjust for your samples**
Select FR/BL = Far Red	
FR/BL Off	
TR ms-Pulse Width = 20 ms	Setting for Trigger Signal to Electronics Box (Pulse Widths Fig. 12)
Slow Kin. Acquisition Points = 512000	Settings Slow Kinetics Acquisition, (Fig. 13)
Slow Kin. Acquisition Rate = 100	

Table 2 Script file continuation: Start of Induction curve, tuning the Measuring Light and measurements (Fo, Fm, Pm) in the dark adapted state.

Recording Mode = Manual	Slow Kinetics: Mode (Fig. 24)
Slow Kinetics On	Start of Slow Kinetics Recorder
F ML On	Switch Measuring Light on
P ML On	
TimeStep(s) =5	
Balance	Balance Ratio of P700 Measuring Light
TimeStep(s) =5	Wait 5 s (time since last Time Step command)
Begin of Repetition Block P700_Adj1	Loops Fine adjusting P700
P700 Sig. Fine Down	Measuring Light
TimeStep(s) =5	
End of Rep. Block; Repeat until Sig.2(P700)< 0.150	
Begin of Repetition Block |P700_Adj2	
P700 Sig. Fine Up	
TimeStep(s) =5	
End of Rep. Block; Repeat until Sig.2(P700)> 0.100	
TR Pulse	Trigger to the GFS-3000
TimeStep(s) =6	Wait for gas exchange data to be averaged and stored
Fo,Fm	Determination of Fo and Fm
FR/BL On	Switch Far Red Light On
TimeStep(s) =60	Wait 60 s, **adjust for your samples**
Pm	Determination of Pm
TimeStep(s) =2	Wait

Table 3 Script file continuation: Induction curve and loop for recording Saturation Pulse responses and Fo'. Before each pulse a trigger signal is sent to the GFS-3000 *via* the electronics box,

Begin of Repetition Block Induction1	
AL On	Switch Actinic Light On
Select FR/BL = Blue	Select Blue
FR/BL On	Switch Blue Light On
Save Report as	Use the next waiting time to save Report with an automatically generated name
TimeStep(s) =30	Wait 30s (**adjust for your experiment**)
TR Pulse	Trigger to the GFS-300
TimeStep(s) 6	Wait for gas exchange data to be averaged and stored (**match with Interval in GFS-Win**)
Sat-Pulse/Fast Kin.	Give a Saturating Light pulse
FR/BL Off	Switch Blue Light Off
Select FR/BL = Far Red	Select Far Red
FR/BL On	Switch Far Red Light On
TimeStep(s) =2	Wait
Fo'	Determine Fo'
FR/BL Off	Switch Far Red Light Off
End of Repetition Block; Loops = 6	Repeat 6 times
Begin of Repetition Block Induction2	The next Repition Blocks have the same Commands
……	
TimeStep(s) =120	The loop has the same content, except for the Time step(s), which are chosen longer.
End of Repetition Block; Loops = 6	
Save Report as	Save Report in the End

3.6.3 Example of Induction Curve recording

The following figures show a typical example of an Induction Curve recorded on a *Hedera helix* leaf. Fig. 24 shows the Dual-PAM program running the Script file. In the Slow Kinetics window the current signals are displayed.

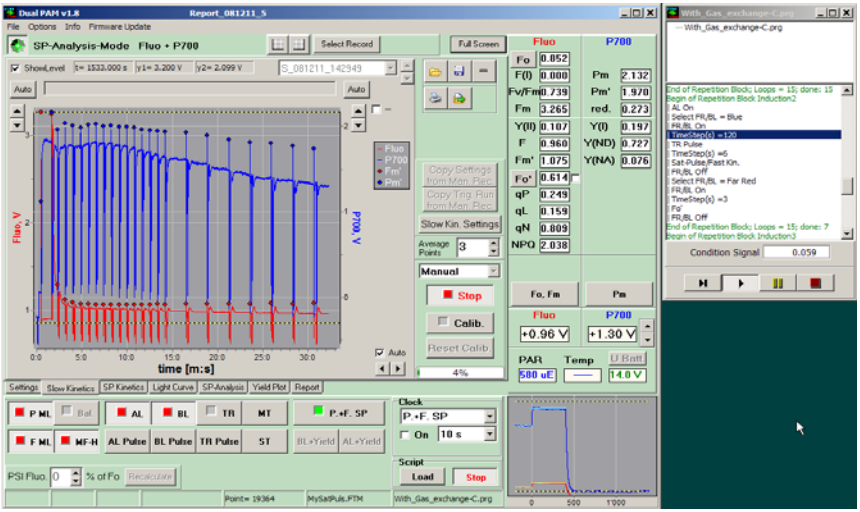

Fig. 24 Induction Curve of *Hedera helix*, chlorophyll fluorescence (red) and P700 signal (blue)

On the right side of Fig. 24 the Script-file is shown. The current position refers to the measuring loop waiting for two minutes. After this time period a trigger signal will be given to the GFS-3000, which will average and store the gas exchange data.

In Fig. 25 the curves for the CO_2 assimilation rate and the stomatal H_2O conductance are displayed. Each circle corresponds to one data set, stored in the Report. The Script-file has a time step of 6s to wait until the GFS-3000 has stored the data before a Saturation Pulse is given.

The relatively low Fm' values in the fluorescence signal in Fig. 24 indicate that the actinic light was excessive for a shade-leaf.

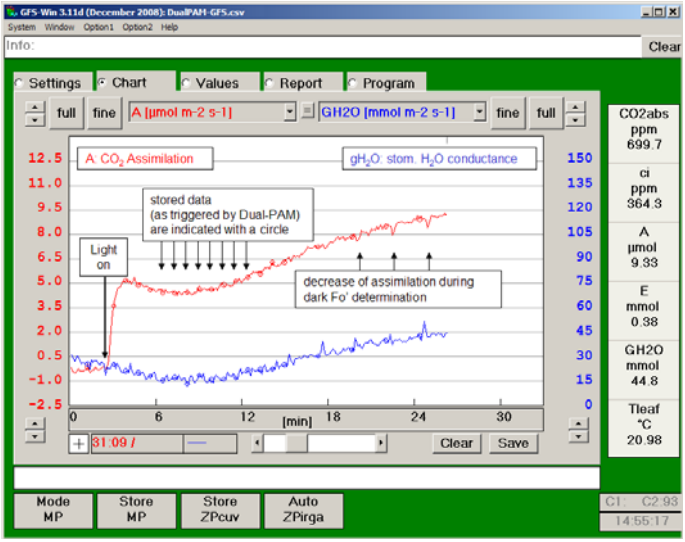

Fig. 25 Induction Curve of *Hedera helix*, CO_2 assimilation and stomatal H_2O conductance

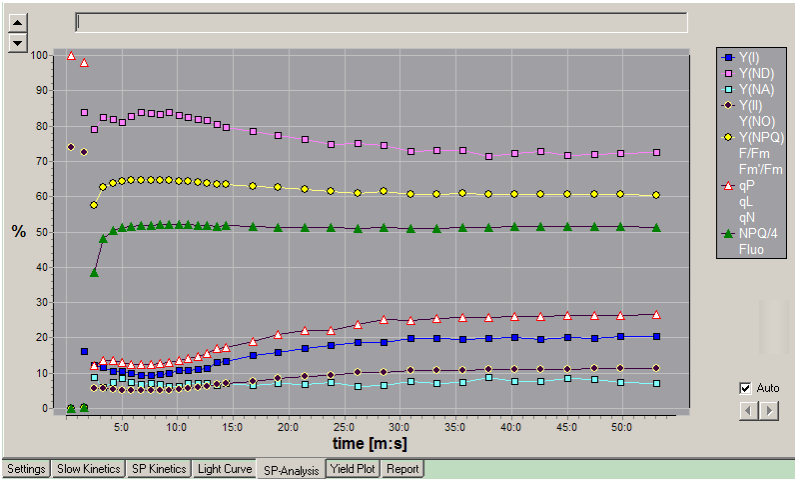

Fig. 26 Induction Curve, Saturation Pulse Analysis

In the SP-Analysis window of the Dual-PAM (Fig. 26) the automatic data analysis of the Saturation Pulse responses is displayed. The rise in CO_2-Assimilation measured with the GFS-3000 corresponded to the rise in Y(I), Y(II) and qP and a drop in Y(ND) and Y(NPQ) measured with the Dual-PAM.

3.6.4 Reading Data into Excel 2003 and higher

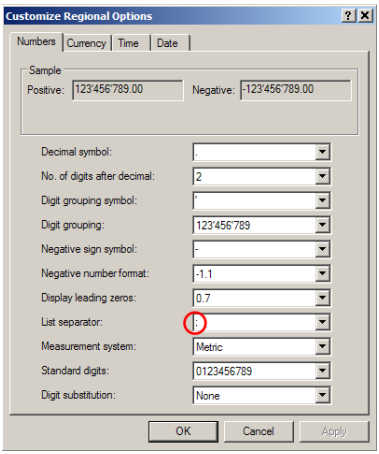

Fig. 27 Customize Reg. Opt.

The data are stored in the character-separated-value format (.csv-format). The character separating the values is either a comma or a semicolon. If the symbol used in the character-separated-value format matches the symbol given in Customize Regional Options, then the .csv-file will open up in Excel without problems and data will be automatically separated into columns. If this is not the case, the list separator can be changed with *Start/ Settings/ Control Panel/ Regional and Language Options/ Customize.*

Furthermore, in Excel it might be required to choose the appropriate decimal symbol. This can be done within Excel with: *Extra/ Option/ International/ Decimal Symbol*: "."

If these general changes shall not be changed, there is also the following option: Select the complete column A and then choose: *Data/ Text into Columns/ Delimited/ Other ";" / Next/ Advanced/ Decimal Symbol "."/ Next/ Finish.*

4 Calculations

4.1 Introduction

The equations, which will be described in the following paragraphs, are used, when the Dual-PAM Gas Exchange Cuvette 3010-Dual is connected and enabled (see Fig. 7).

Table 4: Parameters used for calculation

Given parameter or measured value	**Description**	**Unit**
CO2delay	Time difference between the gas arriving in the sample or reference side of the gas analyzer	s
Area/Weight	Reference value of the sample used for calculations	cm^2/mg
CO2abs	CO_2 mole fraction in reference cell of analyzer, equal to CO_2 concentration at inlet of cuvette.	ppm*
CO2sam	CO_2 mole fraction in sample cell of analyzer. Not corrected for dCO2ZP	ppm
dCO2ZP	= CO2sam - CO2abs (in Mode ZP or in Mode MP with an empty chamber)	ppm
H2Oabs	H_2O mole fraction in reference cell of analyzer, equal to H_2O concentration at inlet of cuvette.	ppm
H2Osam	H_2O mole fraction in sample cell of analyzer. Not corrected for dCO2ZP	ppm
dH2OZP	= H2Osample - H2Oabs (in Mode ZP or in Mode MP with an empty chamber)	ppm
Pamb	Ambient barometric pressure	kPa
Flow	Gas flow entering cuvette	µmol/s
Tcuv	Air temperature within cuvette	°C
Tleaf	Leaf temperature	°C
rH	Relative humidity within cuvette	rh %

*ppm= µmol mol^{-1}

Also when data are recalculated with GFS-Win, these equations will be used; if the status string indicates that the Dual-PAM Gas Exchange Cuvette 3010-Dual was used during the measurement (*see* chapter 4.13).

Table 4 lists the parameters integrated in the formulas and directly assessed by the GFS-3000 during the measurement.

4.2 Calculation of the Differential CO_2 Mole Fraction in Measure Mode MP – dCO2MP and dCO2ZP

dCO2MP is calculated from the CO_2 mole fraction at the sample side of the analyzer and CO_2 mole fraction on the reference side of the analyzer, while the system is in MP Mode.

(1) $$dCO2MP = \mathrm{CO2sam}_{(t)} - \mathrm{CO2abs}_{(\text{t-CO2delay})}$$

Whereby CO2delay is the time lag caused by the difference in tube length between the reference side and measuring side of the pneumatics. It depends on the actual flow rate.

With GFS-Win Version 3.15 and higher the "Time-Lag at standard flow (s)" is stored in the electronics of Measuring Head. The stored standard value is always the value for the flow rate 750 µmol s^{-1}. It will be recalculated for the chosen flow rate. Adjusting of the stored value can be done with *Option1/Calibration/Measuring Head/ Time-Lag at standard flow (s)*. The actual value is shown in *Option1/Advanced setting.*

The differential zero is either measured in ZP Mode (dCO2ZPi), with no delay or in MP Mode with an empty cuvette (dCO2ZPcuv) with delay taken into account.

(2) $$dCO2ZPi = \mathrm{CO2sam}_{(t)} - \mathrm{CO2abs}_{(\text{t})}$$

(3) $$dCO2ZPcuv = \mathrm{CO2sam}_{(t)} - \mathrm{CO2abs}_{(\text{t-CO2delay})}$$

4.3 Calculation of the Differential H_2O Mole Fraction in Measure Mode MP – dH2OMP and dH2OZP

For the differential H_2O measurement, no delay is taken into account:

(4) $$dH2OMP = \mathrm{H2}O\mathrm{sam}_{(t)} - \mathrm{H2}O\mathrm{abs}_{(t)}$$

4.4 Calculation of Transpiration Rate – E,

According to Caemmerer and Farquhar (1981) the transpiration rate is calculated as follows:

(5) $$E = \frac{u_e * (w_o - w_e)}{LA * (1 - w_o)}$$

Whereby:

E = transpiration rate [mmol m^{-2} s^{-1}],
u_e = molar flow rate at the inlet of the cuvette [µmol s^{-1}],
w_o = H_2O mole fraction at the outlet of the cuvette [ppm],
w_e = H_2O mole fraction at the inlet of the cuvette [ppm],
LA = leaf area [m^2].

The terms in equation (4) relate to the values provided by the GFS-3000 as follows:

(6) $$u_e = Flow$$

(7) $$w_o - w_e = dH2OMP - dH2OZP$$

(8) $$w_o = H2Oabs + dH2OMP - dH2OZP$$

(9) $$LA = Area$$

Using the values provided by the GFS-3000 and equations (4) - (8) the transpiration rate E can be calculated as follows:

(10) $$E = \frac{Flow * (dH2OMP - dH2OZP)}{Area * (1 - H2Oabs - dH2OMP + dH2OZP)}$$

4.5 Calculation of Assimilation Rate - A

According to Caemmerer and Farquhar (1981) the assimilation rate A is calculated as follows:

$$A = \frac{u_e * (c_e - c_o)}{LA} - E * c_o \quad (11)$$

where

A = assimilation rate [µmol m^{-2} s^{-1}],
u_e = molar flow rate at the inlet of the cuvette [µmol s^{-1}],
c_o = CO_2 mole fraction at the outlet of the cuvette [ppm],
c_e = CO_2 mole fraction at the inlet of the cuvette [ppm].
LA = leaf area [m^2],
E = transpiration rate [mmol m^{-2} s^{-1}],

The terms in equation (11) relate to the values provided by the GFS-3000 as follows:

$$u_e = Flow \quad (12)$$

$$c_e - c_o = dCO2ZP - dCO2MP \quad (13)$$

$$c_o = CO2abs + dCO2MP - dCO2ZP \quad (14)$$

$$LA = Area \quad (15)$$

Using the values provided by the GFS-3000, equations (11) - (15) and the result of equation (10), the assimilation rate A can be calculated as follows:

$$A = \frac{Flow \cdot (dCO2ZP - dCO2MP)}{Area} - E * (CO2abs + dCO2MP - dCO2ZP) \quad (16)$$

4.6 Calculation of Water Vapor Conductance - GH2O and wa

Since the Dual-PAM Gas-Exchange Cuvette 3010-Dual has no internal fan, the water vapor around the leaf (w_a) is not equal to the water vapor at the exit of the cuvette (w_o), but w_a increases, while the air flows along the leaf surface. The change of w_a depends on the H_2O-conductance (GH2O) and on the water vapor inside and outside of the leaf. GH2O is calculated from the integral of the following differential equation:

$$(17) \qquad \frac{dw_a(x)}{dx} = GH2O \cdot \frac{w_i(x) - w_a(x)}{1 - \frac{w_i(x) + w_a(x)}{2}} \cdot \frac{Area}{X} \cdot \frac{(1 - w_a(x))^2}{Flow \cdot (1 - w_a(0))}$$

X: is the complete path length of the air over the leaf [m]

Area: is the leaf area [m^2]

$w_a(x)$: water vapor at the position x , with x= 0 at entrance [ppm]

$w_i(x)$: intercellular water vapor at the position x [ppm]

GH2O = total water vapor conductance [mmol m^{-2} s^{-1}]

Flow= Flow entering the chamber

w_i is calculated from the temperature of the leaf, which is measured with a thermocouple. For simplification, any temperature gradients along the leaf-sample are neglected:

$$(18) \qquad w_i(x) \approx w_i = \frac{SVP(Tleaf)}{Pcuv}$$

Whereby:

SVP (Tleaf) = saturation vapor pressure at Tleaf calculated according to Goff-Gratch [kPa] (*see* manual of the GFS-3000),

Pcuv = total pressure in the cuvette [kPa].

In the GFS-3000 the ambient pressure Pamb is measured. Only a small overpressure exists in the cuvette, it is neglected. Therefore:

(19) $Pcuv = Pamb$

With the given simplifications, the integral of equation (17) is:

(20) $$GH2O = \frac{Flow}{Area} \cdot \left(\frac{1}{2} \cdot \left(1 - \frac{(1 - w_e)}{(1 - w_{out})} \right) + \frac{(1 - w_e)}{(1 - w_i)} \cdot \ln \left(\frac{(w_i - w_e)(1 - w_{out})}{(w_i - w_{out})(1 - w_e)} \right) \right)$$

4.7 Calculation of average water around the leaf – wa and Vapor-Pressure-Deficit - VPD

According to Caemmerer and Farquhar (1981) the total water vapor conductance GH2O is calculated as follows:

(21) $$GH2O = \frac{E}{VPD}$$

Whereby:

GH2O = total water vapor conductance [mmol m^{-2} s^{-1}],

E = transpiration rate [mmol m^{-2} s^{-1}],

VPD = (Air-to-Leaf-)Vapor-Pressure-Deficit [Pa/kPa].

(22) $$VPD = \frac{(w_i - w_a)}{1 - \frac{(w_i + w_a)}{2}}$$

These equations can be rearranged, so that the average concentration of water vapor around the leaf (wa) can be calculated from E and GH2O, obtained in chapter 4.4 and 4.6.

(23) $$wa = w_a = \left(\frac{2 \cdot GH2O + E}{2 \cdot GH2O - E} \right) \cdot w_i - \frac{2 \cdot E}{2 \cdot GH2O - E}$$

VPD can be calculated with equation (23) and (18).

4.8 Relative Humidity (rh %)

The relative humidity is the ratio of the actual vapor pressure of the air to the saturation vapor pressure. The relative humidity is usually expressed in percent.

$$(24) \qquad rh = \frac{Actual\ Vapor\ \Pr essure}{Saturation\ Vapor\ \Pr essure}$$

Using the values provided by the GFS-3000, the relative humidity rh in the Standard Measuring Head 3010-S can be calculated as follows:

$$(25) \qquad rh = \frac{wa * Pamb}{SVP(Tcuv)}$$

Whereby:

SVP(Tcuv) = saturation vapor pressure at Tcuv calculated according to Goff-Gratch [kPa] (*see* manual of GFS-3000).

wa: *see* chapter 4.7

The relative humidity is calculated from the cuvette temperature and the H_2O concentration in the cuvette, measured with the infra-red gas analyzer. Since the cuvette temperature of the Dual-PAM Gas-Exchange Cuvette is not measured inside the cuvette but in the frame, the value given for relative humidity is only approximate.

4.9 Calculation of CO_2 Mole Fraction in the Cuvette - ca

Since the change of the CO_2 concentration is small in the Dual-PAM Cuvette 3010-Dual, the CO_2 mole fraction in the cuvette ca can be calculated from the average between the CO_2 mole fraction at the inlet and outlet. Using the values provided by the GFS-3000, the CO_2 mole fraction in the cuvette (ca) can be calculated as follows:

$$ca = CO2abs + \frac{(dCO2MP - dCO2ZP)}{2} \quad (26)$$

4.10 Calculation of Intercellular CO_2 Mole Fraction

According to Caemmerer and Farquhar (1981) the intercellular CO_2 mole fraction ci is calculated as follows - ci:

$$ci = \frac{(GCO2 - \frac{E}{2}) * ca - A}{GCO2 + \frac{E}{2}} \quad (27)$$

Whereby:

ci = intercellular CO_2 mole fraction [ppm],
GCO2 = conductance for CO_2 [mmol m^{-2} s^{-1}],
E = transpiration rate [mmol m^{-2} s^{-1}],
ca = CO_2 mole fraction in the cuvette [ppm],
A = assimilation rate [µmol m^{-2} s^{-1}].

The conductance for CO_2 depends on the conductance for H_2O as follows (simplified equation):

$$GCO2 = \frac{GH2O}{1.56} \quad (28)$$

Using the values provided by the GFS-3000, the intercellular CO_2 mole fraction ci can be calculated as follows:

$$ci = \frac{(\frac{GH2O}{1.56} - \frac{E}{2}) * ca - A}{\frac{GH2O}{1.56} + \frac{E}{2}} \quad (29)$$

4.11 Recalculation of Data

The gas exchange data can be recalculated with a new leaf area. The button **New leaf area** in the report window (Fig. 28) allows to enter a new leaf area for each object number and automatically recalculate the data.

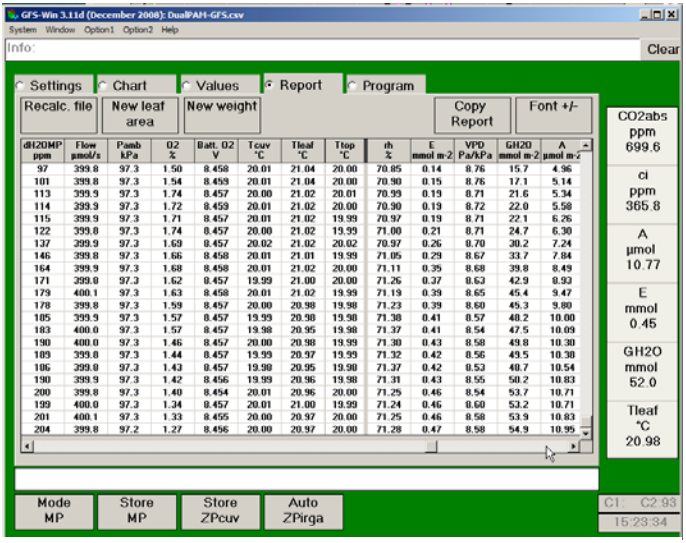

Fig. 28 GFS-Win: Window Report

As stated above, for the accuracy of the gas exchange data, it is important to determine an exact zero point for the differential CO_2 and H_2O concentration (dCO2ZP and dH2OZP). To get an idea on the zero during a measurement, the experimental protocol should be repeated with an empty cuvette, or better with a sheet of black anodized metal inside the cuvette. To recalculate the data with a more exactly determined ZP, the values for dCO2ZP and dH2OZP can be changed within Excel and then recalculated with GFS-Win. In Excel they need to be saved in .csv-format (separate the values with a comma or semicolon). For the recalculation, press the button **Recalc file** in the Report window (Fig. 28).

4.12 Calculated Values

The GFS-Win software automatically calculates photosynthetic parameters. They are correct only under certain conditions.

The value given for the intercellular CO_2 concentration (ci) generally has no meaning with closed stomates, since a division by zero or close to zero takes place.

4.13 Status String

The status string indicates the type of the measuring head. For the Dual-PAM Gas-Exchange Cuvette 3010-Dual, the type is 5.

The calculation of gas exchange data is performed as described above, if the status string contains a 5 for the type of the measuring head. If the type was 3 during the measurement, it can be changed into 5 (in an Excel .csv file) and the data can be recalculated with GFS-Win.

The status FF means ok, otherwise the error-number is indicated.

Table 5: Status String

Area/ Weight	Battery Control	Central Unit			Measuring Head 5: Dual-PAM cuvette		
	Status	Type	Status	CO_2	Type	Version	Status
A/W	FF/err	1	FF/err	0-9	**5**	2	FF/err

…

PAM-Fluorometer		Additional Temperature Sens.		Oxygen Sens.	Cold-Trap	
Type	Status	Type	Status	Type	Type	Status
1-3	FF/err	1	FF/err	1	1	FF/err

5 Maintenance

5.1 Demounting a cuvette half and exchanging gaskets

Fig. 29 Demounting

The support frame has countersunk socket screws at both ends (see arrows in Fig. 29). Use the provided allen wrench (hex-key) to loosen them.

The rod of the ring fitting can now be pulled out, so that the cuvette half is detached (see Fig. 29) or it can be pushed further in, so that the cuvette frames are offset to each other (see Fig. 31). The gaskets are now accessible.

Remove the old gaskets. Before sticking down the new gaskets, regard the orientation. The gaskets are not square, but the inside is 1.1 x 1.2 mm in size.

The rods have a flat side, which needs to face towards the countersunk socket screws after mounting. If this is not the case, the ring fittings are mixed up between the upper and lower cuvette frame.

5.2 Exchanging the Thermocouple

First switch power off. The thermocouple for measuring the leaf temperature is mounted in the lower cuvette frame.

For replacement, remove the gasket (see chapter 5.1), unscrew the protection shield (1) and the fixing screws (2). Now the thermocouple can be pulled out. Thread the new thermocouple through the hole in the cuvette frame, push in the metal square and plug in the plug (3). Mount a new gasket around the thermocouple.

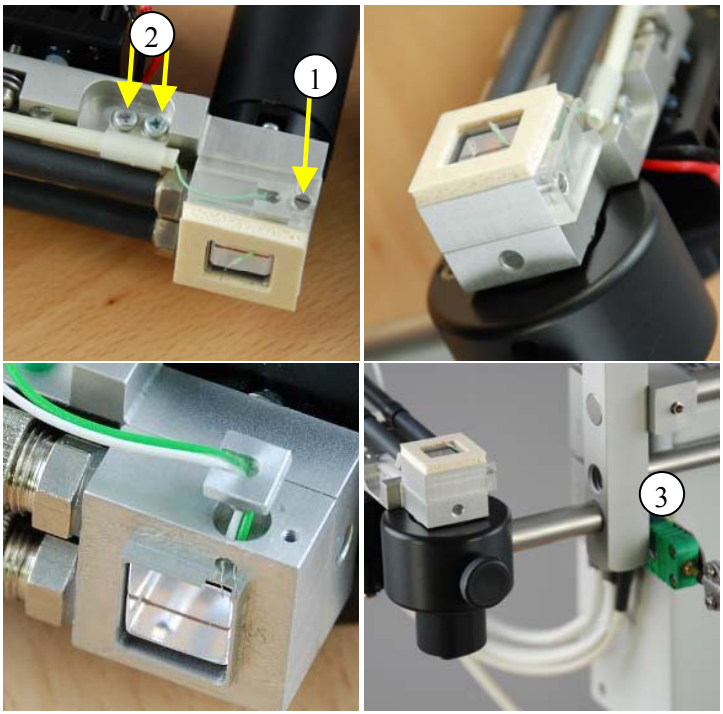

Fig. 30 Exchange of thermocouple

5.3 Adjusting the Offset for leaf temperature probe

To adjust the offset for the leaf temperature measurement, switch any cooling or heating function of the Dual-PAM cuvette off. Wait until the Dual-PAM cuvette (Tcuv) has no temperature drift. Place a calibrated thermometer near the tip of Tleaf. Adjust the Offset of Tleaf with *Option1/Calibration/Measusuring Head/Tleaf Offset[Counts]* until the indicated leaf temperature matches the temperature of the calibrated thermometer.

5.4 PAR Sensor MQS-B/GFS

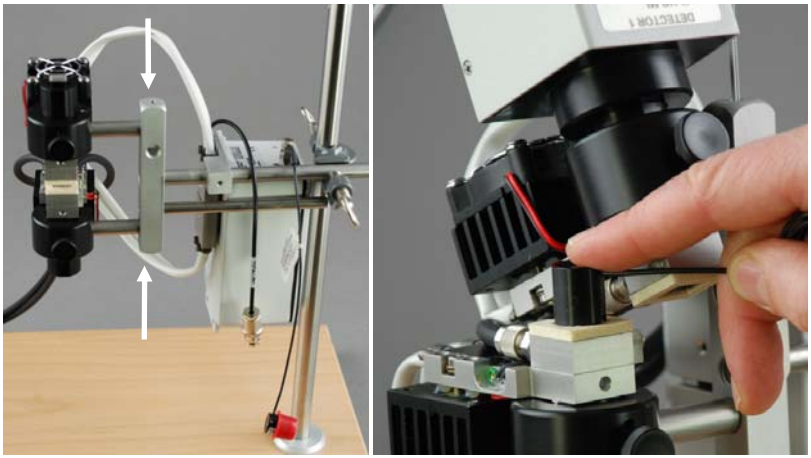

Fig. 31 PAR Sensor

To measure the photosynthetic photon flux density at the leaf surface, an external PAR sensor (MQS-B/GFS) can be connected to the Electronics Box. Loosen one cuvette half as described in chapter 5.1, so that it becomes possible to hold the PAR sensor in place of the leaf to measure the value.

6 Technical Data

Design: Dual-PAM Gas-Exchange Cuvette designed for the combination of the GFS-3000 with optical instruments like the Dual-PAM-100 or the KLAS-100, the upper and lower cuvette frame enclosing a light guide (Perspex rod) for the connection of optical modules like the Dual-DB Module or the Dual-E Module (connection of Dual DR Module not possible!), featuring a wide range of temperature control, pneumatically separated upper and lower cuvette halves, controlled by an Electronics Box with sockets for cable connections to the Control Unit 3000-C.

Cuvette temperature, measured within the aluminum frame outside of the cuvette: Pt 100 type A, range -10 to +50 °C, accuracy ±0.1 °C

Temperature control: Set point value: cuvette temperature, which is measured in the cuvette-frame ranging from 10 degree below ambient to +50 °C

Leaf temperature. measurement: Thermocouple, range -10 to +50 °C, accuracy ±0.2 °C, reference: cuvette temperature, which is measured in the cuvette-frame.

External miniature quantum sensor: Selective PAR measurement, range 0 to 2500 µmol m^{-2} s^{-1} PAR, accuracy ±5 %, cosine corrected

Leaf area: 1.3 cm^2

Trigger in: triggers a signal to the GFS-3000 at 5V→0V signal change.

Distance between light guide (Perspex rod) and leaf: ca. 1 mm on each side

Operating temperature: -5 to +45 °C

Dimensions:

Cuvette itself: 10 cm x 4 cm x 12 cm (L x W x H)
Electronics Box : 7 cm x 7 cm x 15 cm (L x W x H)

Weight: 1 kg (incl. cable and tubes)

7 Warranty Conditions

All products supplied by the Heinz Walz GmbH, Germany, are warranted by Heinz Walz GmbH, Germany to be free from defects in material and workmanship for one (1) year from the shipping date (date on invoice).

The warranty is subject to the following conditions:

1. This warranty applies, if the defects are called to the attention of Heinz Walz GmbH, Germany, in writing within one year (1) of the shipping date of the product.
2. This warranty shall not apply to any defects or damage directly or indirectly caused by or resulting from the use of unauthorized replacement parts and/or service performed by unauthorized personnel.
3. This warranty shall not apply to any product supplied by the Heinz Walz GmbH, Germany which has been subjected to misuse, abuse, abnormal use, negligence, alteration or accident.
4. This warranty does not apply to damage caused from improper packaging during shipment or any natural acts of God.
5. This warranty does not apply to underwater cables, connectors, batteries, fiberoptic cables, lamps, gas filters, thermocouples, fuses or calibrations.

To obtain warranty service, please follow the instructions below:

1. The Warranty Registration form must be completed and returned to Heinz Walz GmbH, Germany.
2. The product must be returned to Heinz Walz GmbH, Germany, within 30 days after Heinz Walz GmbH, Germany has received written notice of the defect. Postage, insurance, custom duties, and/or shipping costs incurred in returning equipment for warranty service are at customer expense.
3. All products being returned for warranty service must be carefully packed and sent freight prepaid.

Heinz Walz GmbH, Germany is not responsible or liable, for missing components or damage to the unit caused by handling during shipping. All claims or damage should be directed to the shipping carrier.