LED-Array/PAM-Fluorometer 3057-FL

Handbook of Operation

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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 LED-Array/PAM-Fluorometer 3057-FL is a high intensity light source (more than 6 times full sun light). It may cause damage to the eyes. Never look directly into this light source during continuous illumination or saturating pulses. Also make sure nobody else can look directly into the light source.

2 Introduction

Progress in LED and electronics technology led to the development of the new advanced LED-Array/PAM-Fluorometer 3057-FL. It has a higher saturating light pulse, free color mix of red and blue with even light distribution and a red measuring light. With its internal light sensors not only the intensity of the actinic light but also the intensity of the saturating pulses is measured and stored.

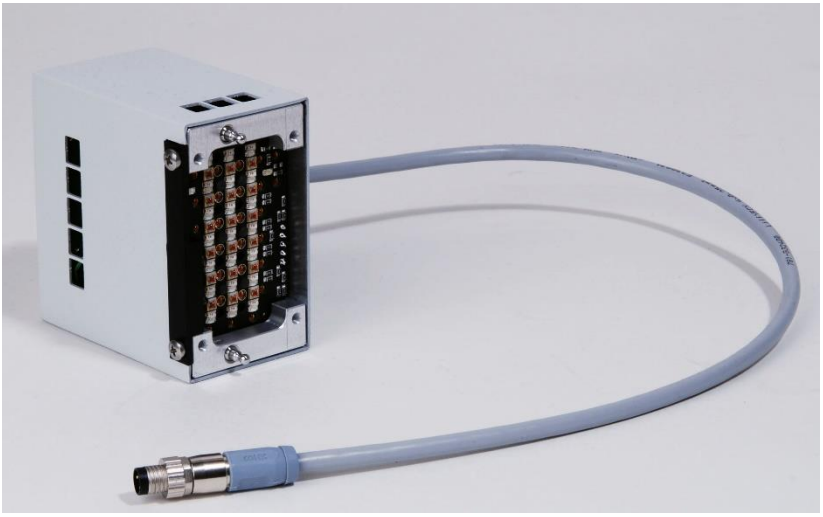


Fig. 1: LED-Array/PAM-Fluorometer 3057-FL

2.1 Product Application

The LED-Array/PAM-Fluorometer 3057-FL provides light to a leaf area of up to 8 cm² and in addition measures the chlorophyll *a* fluorescence, which is mainly emitted by the inner antennae of PSII. The fluorescence data are used to analyze the photochemistry of PSII. It uses pulse-modulated red measuring light to selectively detect the chlorophyll *a* fluorescence yield (F). The measurement of the photochemical yield of photosystem II (Y(II)) is performed by first recording the current fluorescence yield (F) and then applying a short, strong, saturating light pulse to completely suppress the

photochemical yield and induce maximum fluorescence yield (Fm′). Y(II) is calculated from these two fluorescence levels (Y(II) = 1 - F/Fm′). Numerous studies have shown a close correlation between the Y(II) parameter thus determined and the effective photochemical quantum yield of PSII in leaves, algae and isolated chloroplasts. In addition, the linear electron transport rate (ETR) can be determined from the Y(II) data if the light absorbed by PS II is known. When the saturating light pulse is applied in the dark, the fluorescence parameters are designated Fo and Fm instead of F and Fm′. In the dark acclimated state, the photochemical quantum yield of PSII is maximal (1 - Fo/Fm). Since this relationship was discovered and investigated long before the Y(II)-parameter, it has several names. For example, Fv/Fm is widely used, where Fv is the variable fluorescence with Fv = Fm – Fo. In formulating this definition, Fo was assumed to be constant, so that the other part was called variable. However, it soon turned out that in the light acclimated state, immediately after the light was switched off and far-red light was applied to excite only PSI and thus oxidize the plastoquinone pool, so that all PSII have an open acceptor side, a quenched Fo level could be observed. It was called Fo′, analogous to Fm′, the quenched Fm-level. The LED-Array/PAM Fluorometer 3057-FL allows the use of far-red light for recording Fo′. It also offers the possibility to obtain all relevant quenching coefficients (qP, qL, qN, NPQ, Y(NPQ) and Y(NO)). For a more detailed definition of the parameters and their equations see chapter 9. For further reading on chlorophyll fluorescence *see* Schreiber U (2004).

2.2 New Features of the LED-Array/PAMFluorometer 3057-FL

2.2.1 Saturating Light Pulse Intensity

The saturating light pulse can be chosen in steps up to 12000 µmol m^{-2} s^{-1} PAR. The intensity of the saturating light pulse is measured and indicated.

2.2.2 Free Color Mix

Another innovation of the LED-Array/PAM-Fluorometer 3057-FL are internal light sensors that can control the blue and red actinic light

independently of each other, so that the ratio of red (635 nm) and blue (470 nm) can be mixed freely. The blue range extends from 0 to 400 µmol m^{-2} s^{-1} PAR. Red ranges from 0 to 3000 µmol m^{-2} s^{-1} PAR. The total actinic light (red plus blue) is limited to 3000 µmol m^{-2} s^{-1} PAR.

Fig. 2: The color of the light can freely be mixed between red and blue

2.2.3 Red Measuring Light

In difference to its predecessor model, the LED-Array/PAM-Fluorometer 3057-FL uses red measuring light. Using red instead of blue measuring light has the advantage that it is similar to the actinic light, which usually is 90% red. In addition, the fluorescence signal of longer wavelength (> 700 nm) is detected. At these wavelengths, reabsorption of the fluorescence signal within the leaf is minimal, so that deeper layers also contribute to the signal. Since both the measuring light and the actinic light reach similar layers of the leaf, the gas exchange and fluorescence data become better comparable. The disadvantage of detecting the fluorescence signal at longer wavelength is that photosystem-I fluorescence (F(I)) has a higher contribution to the measured fluorescence signal than in the shorter wavelength range. The GFS-Win software now allows to enter a constant value for F(I) relative to Fo, which is

subtracted from each fluorescence value before a fluorescence parameter is calculated.

2.2.4 Zero-Offset

The zero-offset (Z-Offset) of the fluorescence signal is now more convenient. Not only the zero-offset of the momentarily set measuring light and gain is stored, but all offsets for all measuring light amplitudes and both gain options are stored. Therefore, it is now possible to change the settings after the sample has been inserted.

2.3 Definitions of Different Lights

2.3.1 Actinic Light

In chlorophyll-fluorescence terminology, actinic light is the photosynthetically active radiation given to drive photosynthesis. The term actinic light originates from photography, where it is the light having a photochemical effect on the film, making it black. With the LED-Array/PAM-Fluorometer 3057-FL, the actinic light can be blue (peak wavelength: 470 nm) or red (peak wavelength 635 nm) or a mixture of both.

2.3.2 Measuring Light

The measuring light (ML) has a weak intensity. It is pulse amplitude modulated and has the same color as the red actinic light (635 nm). Its purpose is to probe the chlorophyll *a* fluorescence yield. Strictly spoken also the measuring light has an actinic effect, meaning that also the measuring light causes charge separations in the photosystems and therefore causes electrons to move. However, if the measuring light is so weak that each photon hits an open photosystem II and the thylakoid lumen is not acidified, it will not change the fluorescence yield of a dark acclimatised sample.

2.3.3 Frequency of Measuring Light

The frequency of the measuring light (ML-Freq) is automatically controlled by the PAM-fluormeter. If the actinic light is off or low (up to 50 µmol m^{-2} s^{-1}) the frequency of the measuring light is between 5 and 100 Hz depending on the user settings. At 100 Hz and maximum amplitude the measuring light has almost 4 µmol m^{-2} s^{-1}. At 5 Hz the same amplitude results to 0.2 µmol m^{-2} s^{-1}. If the actinic light is switched on, and during saturating light pulses, the frequency of the measuring light is 100 Hz.

2.3.4 Saturating Light Pulse

The saturating light pulse is provided via the red LEDs also used for the actinic light. It reaches up to 12000 µmol m-2 s-1 at setting 12.

2.3.5 Far-Red Light

The far-red LEDs have an emission peak of 740 nm. At this wavelength, there is an almost selective excitation of photosystem I with the consequence of an enhanced reoxidation rate of photosystem II acceptors. This is most effective, if the acceptor side of PSI is activated, for example immediately after strong light is turned off.

2.3.6 Fluorescence

The PIN-photodiode detectors for detecting the chlorophyll *a* fluorescence excited by the pulse amplitude modulated measuring light are protected by long-pass filters with $\lambda > 700$ nm. Since only the fluorescence excited by the measuring light is detected, the recorded signal is the fluorescence yield. The fluorescence caused by the other lights has no influence on the detected signal. It is therefore possible to observe directly how the other lights change the state of PSII and thus the fluorescence yield.

3 Start of Measurement

This chapter gives a short overview on the procedures and considerations, each time a series of experiments is started, please read the complete manual before returning to this chapter.

- When the LED-Array/PAM-Fluorometer 3057-FL is used with another measuring head or if the geometrical setup has been changed, e.g. the leaf area adapter plates are changed, the internal light sensors of the LED-Array/PAM-Fluorometer 3057-FL and the PARtop or PARbot sensor of the measureing head must be matched with an external reference sensor at leaf distance (*see* chapter 10.3).
- Before inserting a sample, insert a dark non-fluorescent foam into the measuring head and measure the *Z-Offset* (*see* chapter 4.2). Repeat this procedure, when starting a new series of experiments or if the geometrical setup has been changed (e.g. leaf area adapter plates). The zero-offset values for all ML and Gain settings are stored.
- Adjust parameters for fluorescence measurements. You may adjust the settings with a sample that can be discarded, before starting a series of experiments with the same settings.

Table 1: Recommended settings for fluorescence

parameter	**recommended value**
Gain	low, unless the ML cannot be increased to reach an Fo value higher than 340.
ML-Ampl	8 or change to obtain Fo between between 500 and 680, so that the maximum fluorescence will not over-saturate the detector. Switch on before taking measurements, atleast 1s before measuring Fo.
ML-Freq	test with a discardable sample, whether the ML has an actinic effect. Reduce frequency, if necessary, or switch ML only on for 1s before measureing Fo.
Far-Red	12 off
FR Time	3-5s, as long as required to reach minimal values for Fo′.
F(I)/Fo	0.20 to 0.39 for C_3 plants or 0.48 to 0.57 for C_4 plants (*see* Pfündel et al. 2013).

ETR-Fac	0.84 or other value for light absorption of chloroplasts.
Sat-Int	8 to 12 depending on whether shade-adapted or sun-adapted samples are used.
SatWidth	0.35 - 0.4 s, check whether plateau is reached for 0.05 seconds.
Mode	SP or SP+Fo′

- If Fo and Fm shall be determined, a dark acclimated sample is required. 10 min is often applied as a rule of thumb for dark acclimation. Nevertheless, photoinhibition does not recover during such a short time period. Since Fv/Fm is the maximal photochemical quantum yield, the conditions under which the maximum Fv/Fm can be obtained are optimal for its determination. Consider that after dark acclimation, it will take a while for a leaf to reach a steady state in photosynthesis (12 min to 1 h). To avoid a closure of stomates, it may help to use low CO_2 concentrations like 200 ppm during dark acclimation. To measure Fv/Fm, press *Store MP Fv/Fm* or use a user-program with the command *Fv/Fm*. Since also gas exchange data will be stored together with Fv/Fm, during dark acclimation a dCO2ZP and dH2OZP should be stored, so that the true respiration will be recorded. Often, photosynthetic rates are best in the morning. If only Y(II) shall be deterimened, Fv/Fm is less important, so that the dark acclimation can be skipped, but note that F(I) requires the determination of Fo.
- After switching the light on, give saturating pulses to measure fluorescence parameters. E.g. use *Store MP+Yield*, or give several saturating pulses by adjusting the timing with *Interval* and *1Yield / y*MP* and then press *Start storing*, or use a user-program.

4 Operation

4.1 Enable during Measure Mode On

In the GFS-Win software, press *Menu* → *Measure Mode ON*, to enable the components and switch the instrument on.

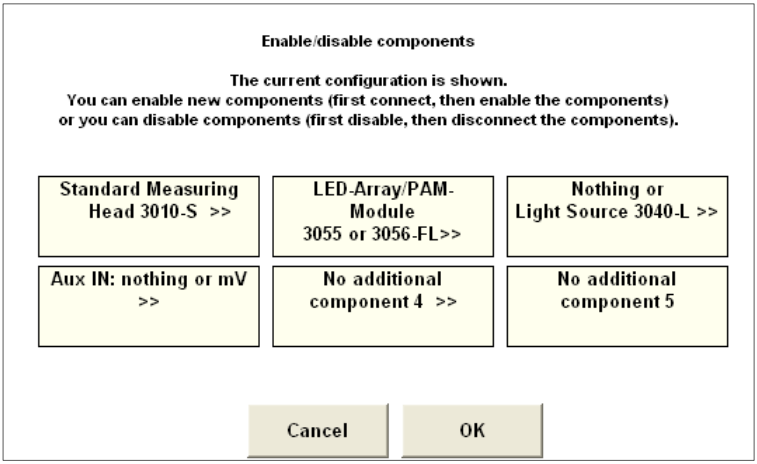


Fig. 3: Enable components when switching the measure mode on

The LED-Array/PAM-Fluorometer 3057-FL is enabled with the same option as the LED-Array/PAM-Fluorometer 3055-FL or 3056-FL.

From the software point of view, it is also possible to use the LED-Array/PAM Fluorometer 3057-FL without any measuring head or even without the GFS-3000. To do this, use the menu item *Measuring Head only ON* instead of *Measure Mode ON*. From the hadware point of view, there is no connection for data transfer to GFS-Win and no power supply for the LED-Array/PAM-Fluorometer 3057-FL.

4.2 Settings

The buttons for the settings of the LED-Array/PAM-Fluorometer 3057-FL are in the section for the fluorescence module, and in the section for the measuring head. The LED-Array/PAM-Fluorometer 3057-FL now controls the light intensity by itself, but previous versions did not. As a result, the buttons concerning the actinic light intensity are still located in the measuring head section, although the measuring head is not controlling the light. The other buttons, concerning the measurement of fluorescence are in the fluorescence module section.

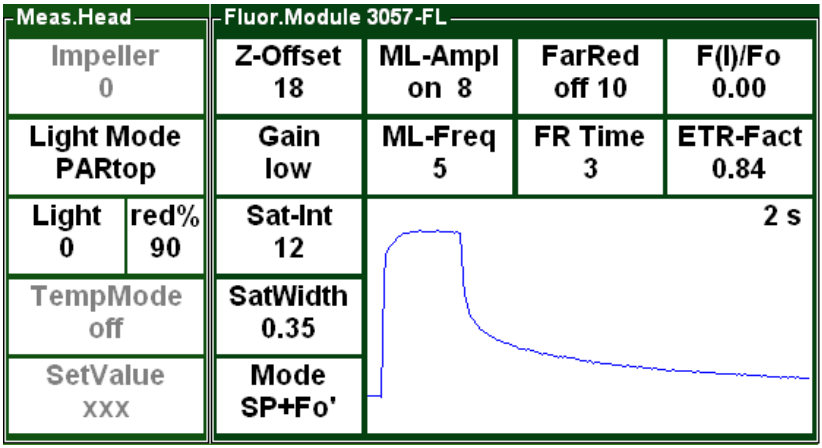


Fig. 4: Settings of the LED-array/PAM fluorometer 3057-FL partly located in the *Measuring Head* section

Light Mode PARtop

The measuring head has two internal light sensors called PARtop and PARbot. While with old fluorescence and light modules the *Light Mode* determined, which sensor shall be used for the light regulation, it now only gives the information to the measuring head, which of its internal sensors is facing towards the LED-array/PAM fluorometer 3057-FL. The light-source factor (*see* chapter 10.3.5.) is applied to the PAR measurement of the selected light sensor. The light regulation itself is not dependent on this value.

Light 0

The light intensity can be chosen between 0 to 3000 µmol m^{-2} s^{-1} red light, or 0 to 400 µmol m^{-2} s^{-1} blue light or any mixture of them. The maximum is 3000 µmol m^{-2} s^{-1} mixed light.

red% 90

Beside the intensity, also the percentage of red light can be chosen. The standard value for red light is 90%. If this is the set-value, the background of the button is white. If any other value is chosen and the light is turned on, the button color turns into violet, red or blue. The actual percentage of red light indicated in the *Values Window* and stored in the *Report* may be different from the set-value. For example, if only measuring light is shining on the sample, it is 100% red. The same, if 0% red light is chosen, still there is measuring light in addition to the blue light. In the dark or with low measuring light (< 1 µmol m^{-2} s^{-1}), the set-value will be stored.

Z-Offset 21 **wait... 3**

Z-Offset sets the fluorescence signal to zero. The zero value of the fluorescence signal consists of a small preset electronic offset and a background signal. The background signal can be caused by fluorescent materials or a small interference of reflected measuring light.

For setting the zero-offset, place the PAM-fluorometer on the measuring head, remove the sample, insert a non-fluorescent foam and press the Z-Offset button. Now the PAM-fluorometer step by step changes the amplitude of the measuring light (*ML-Ampl*) and the *Gain* and stores the Z-Offset for each setting. During this process the *Z-Offset* button turns yellow and displays *wait*…. Always when the geometrical set-up is changed (leaf area adapter plates, distance) the zero offset must be determined again.

Gain low

Pressing the *Gain* key changes the sensitivity of the fluorescence detection by a factor of 2 between low and high. The signal saturates around 4000 (4095 minus Z-Offset). The *Gain* should be set to high, if the Fo signal is below 340. But first check whether the measuring light can still be increased to get a higher signal.

ML-Ampl 8 on

ML-Ampl changes the intensity of the measuring light. With the same button, the measuring light can also be switched on or off. The higher the *ML-Ampl* level, the better the signal-to-noise ratio. However, if it is too high, this can lead to an actinic effect or to an overload of the fluorescence signal during Fm determination. To avoid overload, the fluorescence value of the dark acclimated sample should remain below 650. To check whether the measuring light causes an actinic effect, observe the fluorescence in a dark-acclimated sample after switching on the measuring light. If the measuring light itself causes a transient change of the Fo-level, it is too high. However, if only the Y(II) parameter is of interest, not the Fo-level or any parameter requiring the exact Fo determination, an actinic effect of the measuring light can be tolerated. The default value for *ML-Ampl* is 8.

ML-Freq 10

ML-Freq changes the frequency of the measuring light. If the actinic light is off or low (below 50 µmol m^{-2} s^{-1}) the frequency is the set value, which can be 5, 10, 15, 20, 25 or 100 Hz. The higher the frequency, the better the signal to noise ratio, but to avoid an actinic effect a low frequency is necessary. At 50 µmol m^{-2} s^{-1} and above, the frequeny is automatically changed to 100 Hz. At 100 Hz with maximum amplitude, the measuring light has almost 4 µmol m^{-2} s^{-1} at standard distance. At 5 Hz the same amplitude results to less than 0.2 µmol m^{-2} s^{-1}.

FarRed 10 on

Far-Red light can be switched on and off or changed in steps up to 12. The setting 1 corresponds to about 50 µmol m^{-2} s^{-1} far-red light, 12 corresponds to about 300 µmol m^{-2} s^{-1} at standard distance.

FR Time 4

FR Time is the duration of the far-red illumination for Fo′ determination; either after a saturating light pulse in Mode SP+Fo′ (*see Mode* this chapter) or if only Fo′ is determined.

F(I)/Fo 0.00

Photosystem I contribute to the chlorophyll *a* fluorescence signal. Especcially, if the fluorescence signal is measured at longer wavelengths above 710 nm. In order to take this

contribution into account an estimate can be entered under (*F(I)/Fo*). This value can also be concluded from fluorescence measurements, when there is no photoinhibition, no chloroplast movement and no state transition (*see* Pfündel et al. 2013). Such a calculation is performed, and the result is suggested, when the *F(I)/Fo* button is pressed. But only if, no blue light is used, and dark-acclimated as well as light-acclimated fluorescence data have been measured. The fluorescence paramters can be recalculated with a new estimate of F(I)/Fo in the *Report* window *(see* Chapter 7).

ETR-Fac 0.84

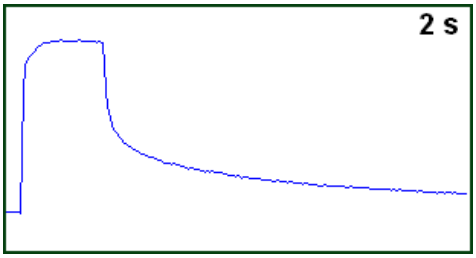
The *ETR-Factor* refers to the fraction of the incident light absorbed by both phtosystems. It is used to calculate *ETR*. It is not measured by the PAM-fluorometer but must be entered by the user. An average value for green leaves in moderate climate is 0.84, which may be used, if the *ETR-Factor* cannot be determined.

Sat Int 12

Sat-Int changes the intensity of the saturating light pulse in steps from 1 to 12, roughly corresponding to 1000 µmol m^{-2} s^{-1} to 12000 µmol m^{-2} s^{-1}.

SatWidth 0.35

SatWidth changes the duration of the saturating pulse in steps of 0.05 s within the range from 0.05 to 2 s. The recommended setting is 0.35 s. A fluorescence plateau shall be reached for 0.05 s.



The saturating light pulse serves to completely reduce the acceptor side of photosystem II, so that charge separation within photosystem II is stopped or becomes very slow. This state of photosystem II is called closed. It is triggered with the *Store MP + Fv/Fm* button or the *Store MP + Yield* button at the bottom of the screen. *Fv/Fm* is used with a dark-acclimated sample. *Yield* is measured in the light. After each saturating light pulse, the fluorescence kinetics are displayed in a small graph. The fluorescence value

before and during the flash is stored. In the raw data also the light intensity before and during the flash is stored (*see* chapter 7.1). During the flash, the values are separated into 50 ms intervals and averaged within each interval. Fm and Fm′ are determined as the maximum of those averages. The intensity and duration of the saturating light pulse should be adjusted, so that the plateau lasts for 50 ms. If no plateau is reached, the intensity of the saturating pulse is too low, or it is too short. If the fluorescence is quenched during the pulse, the saturating pulse is too high or too long. For sun-adapted plants application of maximal pulse intensity is recommended. For shade-adapted plants the intensity of the saturating pulse can be decreased.

The *Mode* button allows to chose between *Mode SP* and *Mode SP + Fo′* . With the Mode *SP*, only a saturating pulse is given. With the Mode *SP + Fo′* after the saturating pulse, the light is switched off, far-red light is switched on and the minimal fluorescence *Fo′* is determined. Afterwards the light is switched on again. If *Fo* has been measured and *Fo′* is not determined, it will be calculated.

When the LED-Array/PAM-Fluorometer 3057-FL is enabled, there are three additional buttons on the lower bar with *frequently used command*s.

Pressing one of those buttons saves a record set (MP) with gas exchange and fluorescence data. The fluorescence measurement is only triggered after the gas exchange date have been recorded for storage. During the saturating light pulse and the *Fo′* determination, only data from the PAM-fluorometer are received by the GFS-Win software. The data of the GFS-3000 or measuring head are not updated, the previously measured values are continuously displayed instead.

Store MP +Fv/Fm

Pressing *Store MP + Fv/Fm triggers a saturating light* pulse. The fluorescence data are assigned *Fo*, *Fm* and *Fv/Fm*. For an *Fv/Fm* measurement, the leaf must be acclimated to darkness. A healthy dark acclimated leaf reaches values above 0.8 for *Fv/Fm*. Normally, *Fv/Fm* of a dark-acclimated sample is measured before a series of *Yield* measurements. The calculation of *qP*, *qN* and *NPQ* require the values *Fo´, Fo* and *Fm*. For each sample and after changing the fluorescence settings (*ML-Ampl, Gain* or *Z-Offset*) the specific Fo and Fm values are required. Especially with gas exchange measurements it is often counterproductive to expose a sample to darkness as it leads to a closure of the stomata. A method to avoid this could be to expose the leaf to a low CO_2 concentration of e.g. 150-200 ppm during dark acclimatization. Another possibility could be to measure or estimate Fo and Fm after the end of the measurement and recalculate the fluorescence data with these estimates. For example, the change in Fo and Fm caused by the measurement could be estimated in a few samples only and used for all samples.

Store MP +Yield

Pressing *Store MP + Yield* triggers a saturating ligh pulse. The fluorescence data are assigned *F, Fm'* ,*Yield* and *ETR*. If an *Fv/Fm* measurement has been performed, also the quenching parameters (qP, qL, NPQ…) are calculated. Note that *Fo'* is calculated and not measured, if the *Mode SP* is chosen.

Store MP +Yield,Fo'

If the *Mode SP + Fo'* is chosen the title of the button is changes from *Store MP + Yield* into *Store MP + Yield, Fo'*. Now after each saturation light pulse the actinic light is switched off and the far-red light is switched on to perform the *Fo'* determination. The duration of far-red light for the *Fo'* determination is set with *FR Time*.

Store MP + Fo'

With the LED-Array/PAM-Fluorometer 3057-FL it is also possible to only determine *Fo'* without giving a flash beforehand. The light will be switched off, far-red light will be switched on, the frequency of the emasureing light will be set to the set value (*see ML-Freq)* and the minimal fluorescence will be determined and stored as *Fo'*. To

determine the minimum, 5 values are averaged, which is 1 average per second at 5 Hz but more at 1 higher frequency. Afterwards the actinic light will be switched back on. The thus determined *Fo'* will not be assigned to any other values and not be used for the calculation of any fluorescence parameters. If the actinic light is off, when *Store MP + Fo'* is pressed, no far-red light will be given, but only the minimal fluorescence will be determined and stored.

5 Chart

The fluorescence values can be indicated in the *Chart* window. The Fm and Fm′ values are marked with a square, the F value with a circle, and the Fo′ value with a cross. The mark is set at the time under which those values are stored in the report file together with the gas exchange data, although the saturating light pulse is measured after the gas exchange data have been recorded and *Fo′* even later.

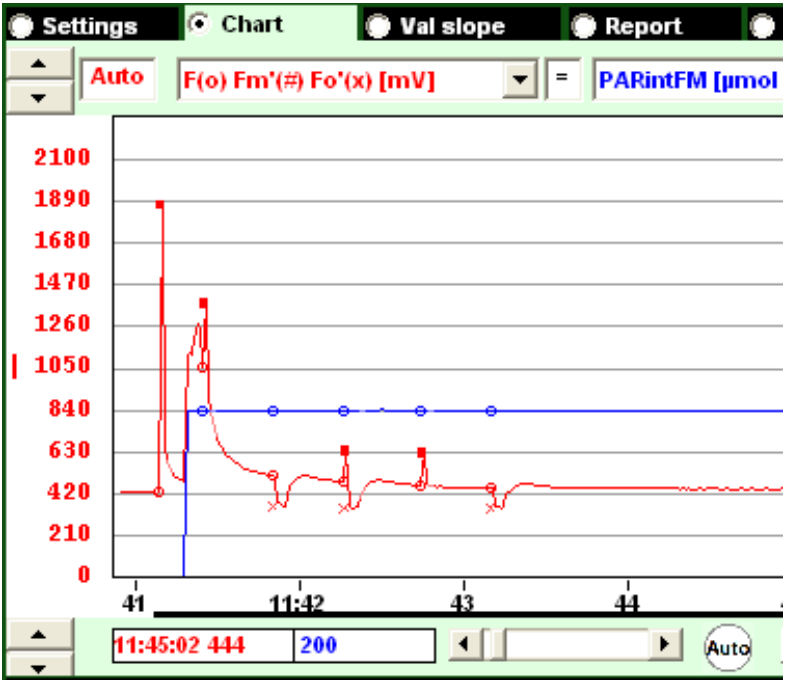


Fig. 5: *Chart* displaying fluorescence (red), square: Fm, Fm′, circle: F, cross: Fo′, and PAR measured with internal sensor (blue)

6 Values

The *Values* window of the GFS-Win software now has three rows with values from the LED-Array/PAM-Fluorometer 3057-FL. To see the last row, scroll down in the *Values* window.

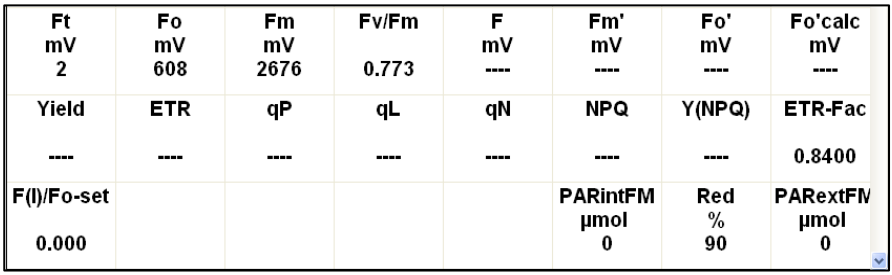


Fig. 6: Data of the LED-Array/PAM-Fluorometer in the *Values* Window

All values are explained in Table 2, for the equations of the fluorescence parameters see chapter 9.

Table 2: Values with definition and ranges

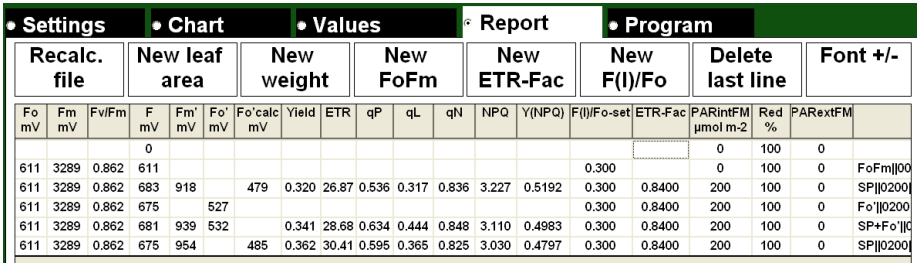
Value	Definition	Range, options	Unit
Ft	Currently measured Fluorescence value (t meaning transient)	0…ca. 4000	digits
Fo	Fluorescence of the dark-acclimated sample	0…ca. 4000, usually up to 650	digits*
Fm	Fluorescence of the dark-adapted leaf during a saturating light pulse	0…ca. 4000	digits
Fv/Fm	Maximal quantum efficiency of photosystem II (Kitajima and Butler 1975 and Butler and Kitajima 1975)	0…0.84	
F	Fluorescence	0…ca. 4000	digits
Fm	Fluorescence of the illuminated leaf during a saturating light pulse	0…ca. 4000	digits
Fo′	Fluorescence of reoxidized photosystem II illuminated with	0…ca. 4000	digits

	only far-red illumination and measuring light.		
Fo′calc	Calculated Fo′ value (see equations).	0…ca. 4000	digits
Yield	Quantum yield of photosynthetic electron transport (Genty et al. 1989, for a mathematical derivation see Schreiber et al. 1995)	0..0.85	
ETR	Electron transport rate		µmol m^{-2} s^{-1}
qP	photochemical quenching if no connectivity (Schreiber et al. 1986)	0..1	
qL	photochemical quenching if infinite connectivity (Kramer et al. 2004)	0..1	
qN	Non-photochemical quenching (Schreiber et al. 1986)	0..1	
NPQ	Non-photochemical quenching (Bilger and Björkman, 1990)	0..10	
Y(NPQ)	Quantum yield of the NPQ related energy loss	0..1	
Y(NO)	Quantum yield of the intrinsic energy loss (only calculated on user-request)	0..1	
ETR-Fac	Factor used to calculate the electron transport. Corresponds to the proportion of light absorbed by both photosystems. The value must be entered by the user	0..1	
F(I)/Fo	Proportion of photosystem I fluorescence contributiong to the Fo signal, it must be entered by the user.	0..0.9	
PARintFM	Photosynthetically active radiation (PAR) applied to the sample, measured internally by the LED-Array/PAM-Fluorometer 3057-FL	0..25000	µmol m^{-2} s^{-1}
Red%	Proportion of red actinic light, rest is blue	0-100	%
PARextFM	Photosynthetically active radiation (PAR) measured with the quantum sensor connected externally to the LED-Array/PAM-Fluorometer 3057-FL	0..25000	µmol m^{-2} s^{-1}

*The software indicates mV, but the value is acutal a digital value coming from an analog digital converter and is not a mV reading.

7 Report

The *Report* window indicates the stored values and allows data recalculation. The values are the same as described in the previous chapter, except for the last column, which contains raw data.



• Settings • Chart • Values ◦ Report • Program

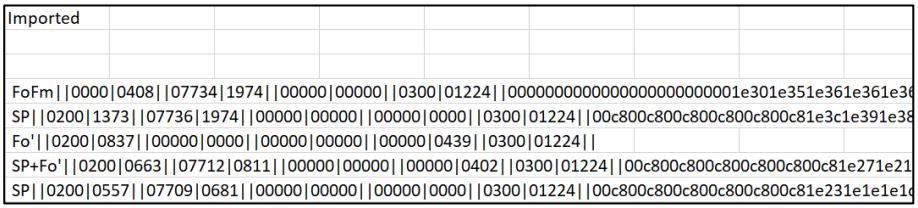
Recalc. file	New leaf area	New weight	New FoFm	New ETR-Fac	New F(I)/Fo	Delete last line	Font +/-

Fo mV	Fm mV	Fv/Fm	F mV	Fm' mV	Fo' mV	Fo'calc mV	Yield	ETR	qP	qL	qN	NPQ	Y(NPQ)	F(I)/Fo-set	ETR-Fac	PARintFM µmol m-2	Red %	PARextFM	
			0													0	100	0	
611	3289	0.862	611											0.300		0	100	0	FoFm||00
611	3289	0.862	683	918		479	0.320	26.87	0.536	0.317	0.836	3.227	0.5192	0.300	0.8400	200	100	0	SP||0200|
611	3289	0.862	675		527									0.300	0.8400	200	100	0	Fo'||0200
611	3289	0.862	681	939	532		0.341	28.68	0.634	0.444	0.848	3.110	0.4983	0.300	0.8400	200	100	0	SP+Fo'||0
611	3289	0.862	675	954		485	0.362	30.41	0.595	0.365	0.825	3.030	0.4797	0.300	0.8400	200	100	0	SP||0200|

Fig. 7: Stored fluorescence data and paramter in the *Report* window

7.1 Raw Data, Last Column of Report

The last column of the report contains the raw data of the fluorescence measurements. It has the heading *Imported*.



Imported

FoFm||0000|0408||07734|1974||00000|00000||0300|01224||00000000000000000000000001e301e351e361e361e36
SP||0200|1373||07736|1974||00000|00000||00000|0000||0300|01224||00c800c800c800c800c800c81e3c1e391e38
Fo'||0200|0837||00000|0000||00000|00000||00000|0439||0300|01224||
SP+Fo'||0200|0663||07712|0811||00000|00000||00000|0402||0300|01224||00c800c800c800c800c800c81e271e21
SP||0200|0557||07709|0681||00000|00000||00000|0000||0300|01224||00c800c800c800c800c800c81e231e1e1e1c

Fig. 8: Raw data in the last column of the *Report*

The raw data have the following format: First there is the annotation, what kind of fluorescence measurement was performed followed by a double bar(||). The following values are in pairs separated by a single bar (|). The first value of each pair is usually the PAR value, the second value the corresponding fluorescence. The pairs are separated from each other with double bars (||). The format for an Fv/Fm measurement is: Annotation FoFm || PAR during Fo determination (should be 0) | Fo || PAR during SP | Fm || reserve (0) | reserve (0) || F(I)set/Fo x 1000 | F(I) || time-course of PAR data

(hexadecimal-values) || time-course of fluorescence data (hexadecimal-values).

The raw format for the other measurements is similar, except that there is an additional pair for the Fo′ measurement: Annotation || PAR during F determination | F || PAR during SP | Fm′ || reserve (0) | reserve (0) || PAR during Fo′ determination (0, far-red light is not measured) | Fo′ || F(I)set/Fo x 1000 | F(I) || time-course of PAR data (hexadecimal-values) || time-course of fluorescence data (hexadecimal-values).

7.2 Data Recalculation

The *Report* window offers three different options for the recalculation of fluorescence parameter.

New FoFm

If the Fo and Fm values were not determined before the measurement, but afterwards or if those values have only been estimated, they can be entered, and the fluorescence parameter will be recalculated for the object number, which can be specified during this process.

New ETR-Fac

This button serves to recalculate the electron transport rate with a new ETR-factor. The ETR factor is the PAR absorbed by the chloroplasts (*see* …)

New F(I)/Fo

The fluorescence parameters can be recalculated with a new estimate for the fluorescence of photosystem I.

8 Programming

Table 3: Command List

	LED-Array/PAM-fluorometer 3057-FL
*1Yield/y*MP =*	Determines the repetition rate of saturating light pulses for fluorescence measurements. A yield is measured every specific number (y) of MPs.
Fv/Fm	Triggers an Fv/Fm measurement: In an Fv/Fm measurement, a saturation light pulse is applied to determine Fo and Fm. The sample should be dark acclimated for this measurement. Gas exchange data are recorded and averaged before the saturating flash.
Yield	Trigger a Yield measurement: In a yield measurement a saturation light pulse is applied to determine F and Fm′. If the *Mode* is *SP+Fo′*, Fo′ is determined immediately afterwards. Gas exchange data are recorded and averaged before the yield measurement.
Default F	Sets all user-settings of the fluorescence module to default values (default values may change with firmware changes).
Fo'-Mode FL =	Switches the Mode between *SP+Fo′* and *SP*: Either only a saturating pulse is given or also Fo′ is determined with every *Yield* measurement.
Gain FL =	Sets the gain (high/low).
M-Light FL =	Switch the modulated measuring light on or off.
ML-Amp FL =	Sets the amplitude (intensity) of the modulated measuring light (ML). Note, readjust the zero-offset of the fluorescence module after changing this setting.
SatWidth FL =	Sets the duration (s) of the saturating light pulse.
Sat-Int FL =	Sets the light intensity (steps) of the saturating light pulse.

Set Z-Offset FL	Adjusts the zero-offset of the fluorescence module: This command pauses execution and prompts the user to insert black, non-fluorescent foam into the cuvette. The user can bypass this measurement by selecting "Cancel". After OK or Cancel, the user program resumes execution.
ETR-Fact FL=	Sets the ETR-Factor with which the electron transport rate (ETR) is calculated from yield measurements and PAR. The factor corresponds to the proportion of light absorbed by the leaf.
FR-Int FL=	Sets the intensity of the far-red light without switching it on (use command *"FarRed FL ="*).
FarRed FL =	Switches far-red light on or off
New commands:	
Fo'	Measures only Fo′
Red% =	Sets the percentage of red light, rest is blue.
FR-Time =	Sets the duration of far-red light and actinic light off for Fo′ determination
SatPulse Mode =	equivalent to *Fo'-Mode FL =*
Commands listed under Measuring Head:	
Set PARtop =	Sets the intensity of the actinic light and switches it on. The fluorescence module controls the light. The light-source factor is applied to the *PARtop* sensor of the measuring head.
Set PARbot =	Sets the intensity of the actinic light and switches it on. The fluorescence module controls the light. The light-source factor is applied to the *PARbot* sensor of the measureing head.

9 Equations for Fluorescence Parameters

The following parameters are calculated from the fluorescence values Fo, Fm, F, Fo′, Fm′, F(I)/Fo, ETR-Fac and the photosynthetically active radiation measuered internally (PARintFM). For the derivation of the equations see Klughammer and Schreiber 2008:

First the fluorescence of photosystem I (F(I)) is calculated, so that it can be subtracted from every fluorescence value. It is set in relation to Fo. Therefore, Fo is required to calculate F(I), if F(I)/Fo is above 0.

(1)

$$F(\mathrm{I}) = \left(\frac{F(\mathrm{I})}{Fo}\right)_{\text{user setting}} \cdot Fo$$

The photosynthetic yield (Y(II)) indicates the quantum efficiency of photosystem II. It is the fraction of PAR that the PSII use for water splitting (Genty et al. 1989). For a mathematical derivation see Schreiber et al. 1995 or Schreiber 2004). The equation here also includes F(I).

(2)

$$\mathrm{Y(II)} = \frac{\mathrm{Fm}' - \mathrm{F}}{\mathrm{Fm}' - \mathrm{F(I)}}$$

The electron transport rate through PSII is calculated by multiplying the yield of PSII with the amount of PAR absorbed by PSII:

(3)

$$\mathrm{ETR} = \mathrm{Y(II)} \cdot \frac{\mathrm{PAR}}{2} \cdot \mathrm{ETRFac}$$

whereby:

PAR: photosynthetically active radiation is divided by two, because it is reasonable to assume, that the absorbed light is equally distributed between photosystem I and II.

ETRFac: PAR absorbed by the sample or more pricise by the photosystems, of the sample excluding the epidermis. The value needs to be given by the user.

Fo′ is either measured, if the mode for the saturating pulse is SP+Fo′, or calculated with the relationship published by Oxborough and Baker (1997), but taking the F(I) setting into account.

(4)

$$\mathrm{Fo}' = \frac{1}{\frac{1}{\mathrm{Fo} - \mathrm{F(I)}} - \frac{1}{\mathrm{Fm} - \mathrm{F(I)}} + \frac{1}{\mathrm{Fm}' - \mathrm{F(I)}}} + \mathrm{F(I)}$$

If Fo′ has been measured, this equation can be resolved to F(I) and under restricted circumstances used to get an estimation of F(I) as shown by Pfündel et al. (2013).

The photochemical quenching (qP) is a measure of the fraction of open photosystems. It is calculated on the assumption that PSII centers are not interconnected and cannot transfer energy between them (Schreiber et al. 1986 as formulated by van Kooten and Snel, 1990):

(5)

$$\mathrm{qP} = \frac{\mathrm{Fm}' - \mathrm{F}}{\mathrm{Fm}' - \mathrm{Fo}'}$$

The coefficient of photochemical fluorescence quenching can also be calculated assuming infinite interconnection between PSII antennae (lake model, Lavergne and Trissl 1995). It is calculated according to Kramer et al, 2004, but including F(I):

(6)

$$\mathrm{qL} = \mathrm{qP} \cdot \frac{\mathrm{Fo}' - \mathrm{F(I)}}{\mathrm{F} - \mathrm{F(I)}}$$

The non-photochemical quenching is defined as the fluorescence quenched by processes other than photochemistry. There are three different approaches in the literature. qN It , where the non-photochemical fluorescence quenching is determined in relation to the maximal variable fluorescence (Schreiber 1986; van Kooten and Snel 1990); NPQ, where the equation is formulated such that NPQ is expected to be proportional to the quenching agent (Bilger and Björkman 1990); and Y(NPQ) which is the fraction of PAR that is dissipated in PSII via the non-photochemical quenching mechanisms (Genty et al. 1996). NPQ is the only quenching coefficient, which can be greater than 1, it ranges to around 4. qN is the only quenching coefficient not affected by F(I).

(7)

$$\mathrm{qN} = 1 - \frac{\mathrm{Fm}' - \mathrm{Fo}'}{\mathrm{Fm} - \mathrm{Fo}}$$

(8)

$$\mathrm{NPQ} = \frac{\mathrm{Fm} - \mathrm{F(I)}}{\mathrm{Fm}' - \mathrm{F(I)}} - 1$$

(9)

$$\mathrm{Y(NPQ)} = \frac{\mathrm{F} - \mathrm{F(I)}}{\mathrm{Fm}' - \mathrm{F(I)}} - \frac{\mathrm{F} - \mathrm{F(I)}}{\mathrm{Fm} - \mathrm{F(I)}}$$

(10)

$$\mathrm{Y(NO)} = \frac{\mathrm{F} - \mathrm{F(I)}}{\mathrm{Fm} - \mathrm{F(I)}}$$

Note, that older versions of the GFS-3000 and GFS-Win used slightly different equations. Y(NO) is only calculated on user request (no extra costs).

10 Adjustment

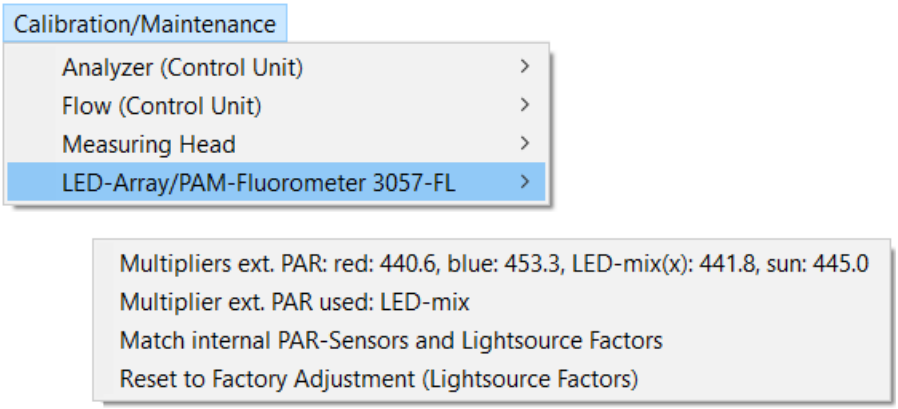


Fig. 9: Menu for adjustments of the LED-array/PAM fluorometer 3057-FL

10.1 Multipliers for external PAR Sensor (PARextFM)

The LED-Array/PAM-Fluorometer 3057-FL has its own connector for connecting a PAR sensor for example the Mini-Quantum Sensor MQS-B/GFS.

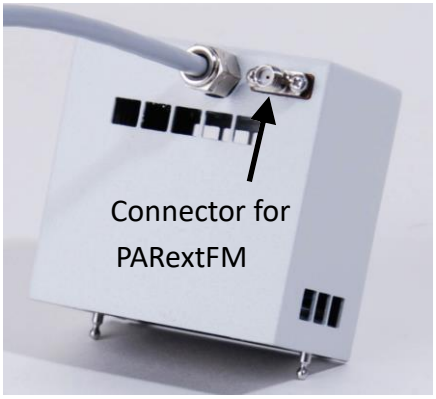


Fig. 10: Connector for the Mini-Quantum Sensor MQS-B/GFS measuring external PAR (PARextFM) used as reference for matching the internal light sensor and PARtop or PARbot to the PAR applied to the sample.

The multipliers for the PAR measurement must be correctly entered. Since the sensitivity of the sensor is slightly wavelength dependent, three different values must be entered with the menu item *Multipliers ext. PAR: red: x, blue: y, LED-mix(x): f(x,y), sun: z.* The cross behind the LED-mix indicates that currently this multiplier is chosen (see next chapter).

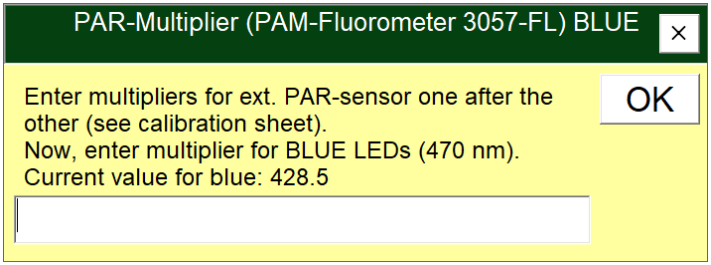


Fig. 11: Input box for entering the PAR-multiplier for blue light

The multipliers for specific wavelength can be found in the calibration sheet of the Mini-Quantum Sensor MQS-B/GFS. After the menu item *Multipliers ext. PAR red:… blue:… sun:…* has been pressed, the value for the blue LEDs is prompted first. If the current value is already correct, press OK or X to proceed to the next prompts, which are the multipliers for the red LEDs and then the sunlight.

10.2 Multiplier used for external PAR (PARextFM)

The menu item *Multiplier ext. PAR used…* offers the choice between two multipliers: sun or LED-mixture.

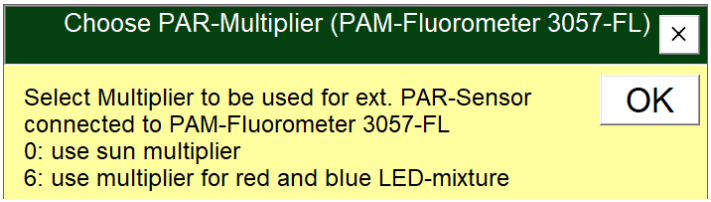


Fig. 12: Input box selecting the applied PAR-multipler

The multiplier for the LED mixture is calculated from the multipliers for blue and red LEDs and the currently set red-percentage value. The result is indicated in the menu item above under LED-mix(x). The resultant PAR value

is indicated in the GFS-Win software under the name PARextFM (external PAR monitored by PAM-fluorometer).

10.3 Match internal PAR-Sensors and Lightsource Factors

When the LED-Array/PAM-Fluorometer 3057-FL is connected to a Standard Measuring Head 3010-S, there are two separate PAR-measurements that indicate the PAR applied to the sample. One from the internal sensors of the PAM-fluorometer (PARintFM) and the other from the PARtop or PARbot sensor of the measuring head. The menu item *Match internal PAR-Sensors and Lightsource Factors* starts a procedure that matches these sensors to the external reference, a PAR sensor (MQS-B/GFS) that must be connected to the external connector of the PAM-fluorometer (see Fig. 10). Since the LED-Array/PAM-Fluorometer 3057-FL performs the measurements and adjustment itself, all the values for its own internal sensors are stored immediately. The procedure is now described step by step.

10.3.1 Before Starting

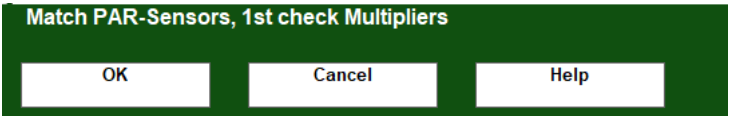


Fig. 13: Prompt to check multipliers of external PAR sensor

The Mini-Quantum Sensor MQS-B/GFS must be separated from the measuring head and connected to the LED-Array/PAM-Fluorometer 3057-FL. The multipliers from the calibration sheet must be correctly entered into the LED-Array/PAM-Fluorometer 3057-FL (see chapter 10.1). Afterwards place the mini-quantum sensor in the location of the sample, so that it measures the radiation that the sample would receive. Avoid any light penetrating into this setup and start the adjustment process with *OK*.

10.3.2 Adjustment of Zero-Offset of external PAR-Sensor

First the electronic zero of the external PAR-measurement is adjusted. The mini-quantum sensor must be kept dark, which should be the case it it is placed as described in the chapter before.

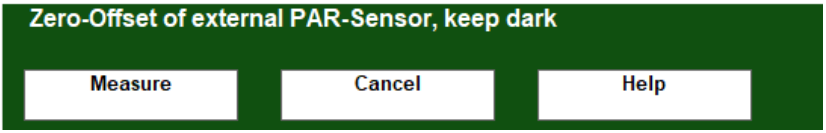


Fig. 14: Prompt to adjust the zero-offset of the external PAR measurement

After *Measure* has been pressed, the zero-offset will be averaged, stored and indicated.

Fig. 15: Result of zero-offset determination for *PARextFM*

10.3.3 Adjustment of Zero-Offset of internal PAR-Sensors

The internal PAR sensors are used for actinic blue light, actinic red light and saturating pulse determination. All three measurements have a zero-offset. The LED-array, where the internal sensors are located must be kept dark. *Measure* must be pressed, and the three zero-offsets are determined and stored. Afterwards all 4 zero-offsets will be indicated.

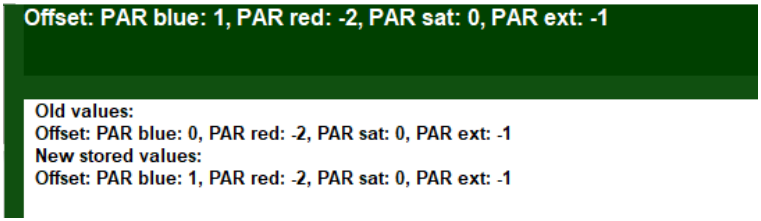


Fig. 16: Result of zero-offset determination for all PAR-sensors of the PAM-fluorometer

10.3.4 Determination of internal Light-Lists and Sensor-Sensitivities

In this step, the LED-Array/PAM-Fluorometer 3057-FL determines the sensitivity of the internal sensors for the red and the blue LEDs at a list of settings. The external PAR sensor is used as reference and must be placed so

that it receives the light instead of the sample. First, the intensity of the measuring light at the sample distance is determined and stored. Next, the PAM-Fluorometer determines, which DAC values (DAC means digital analog conversion and is a value that drives the LED intensity) are used to match the internal PAR sensors. The sensitivity values (CAL) for the internal light sensors will be measured for each color and DAC value. Afterwards the new sensitivity values are used to determine the PAR value at each step. All light lists (DAC-, PAR- and CAL lists) are displayed before and after the measurement.

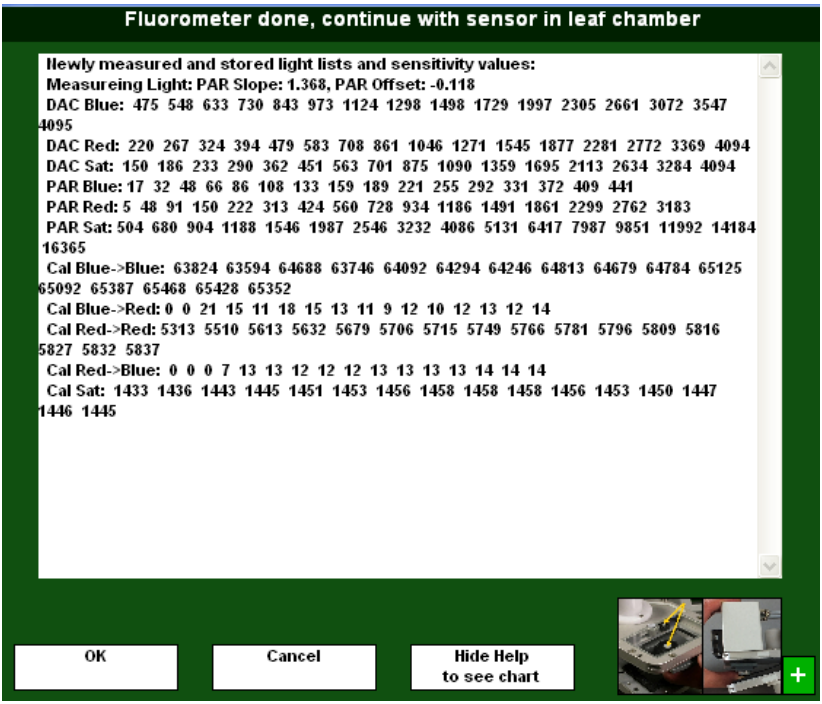


Fig. 17: Result of matching procedure for internal PAR sensors

Look at the values displayed. If, for example, the *CAL Blue->Blue* values are very different for low light intensities (first values), some light may have penetrated during zero-offset determination. The values are logged in the file *"My Documents\ GFS-3000\ ini\ calibrat.rpt"*.

10.3.5 Determination of Lightsource Factors

After the LED-Array/PAM-Fluorometer 3057-FL has adjusted its internal sensors to the external sensor, in the next step, the light-source factors for PARtop or PARbot of the used measuring head will be determined. They will be stored in the PAM-fluorometer together with the serial number of the matched measureing head.

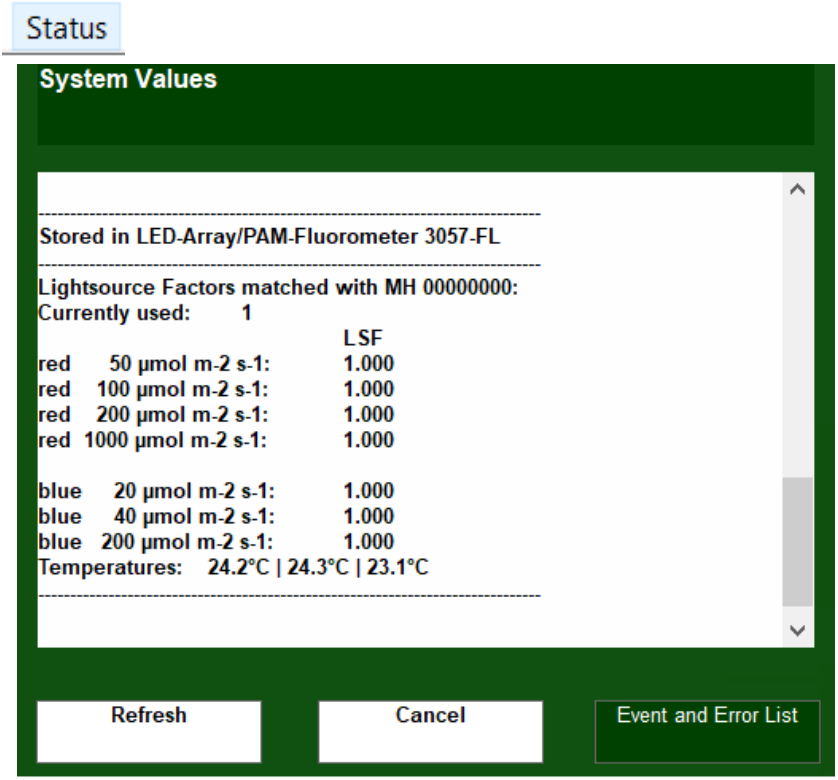


Fig. 18: Light source factors for PARtop or PARbot, if they have never been matched with any measureing head

Under the menu item *Status-> System Values*, the light-source factors (LSF) of the LED-array for the PARtop or PARbot sensor of the measuring head are listed. If the light-source factors have never been matched to any measuring head, they are 1.000 and the value indicated by the measuring head for PARtop or PARbot will not match the PAR received by the leaf. To adjust

the LSF for PARtop or PARbot the matching procedure must be proceeded while the external mini-quantum sensor is still connected to the PAM-Fluorometer and located at the position of the sample.

The LSF will be determined at several light levels with blue or red light.

Afterwards the newly determined lightsource factors can be stored.

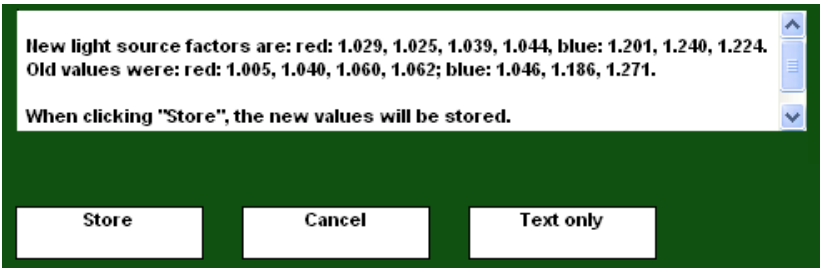


Fig. 19: Prompt to store newly determined light-source factors for PARtop or PARbot

10.3.6 End of Match Process

In the end of the matching process, the user is encouraged to check the result. PARextFM, PARintFM and PARtop or PARbot, should now show the same value, if PARextFM is still in the correct location and the multiplier for the external PAR sensor is still set to LED-mix.

When the adjustment is complete, the multiplier that was set before the matching process is activated again (sun or LED-mix).

10.4 Reset to Factory Settings

The menu point *Reset to Factory Settings* restores the light lists and sensitivity values to the state of delivery. There are additional values like far-red adjustment, ML-adjustment, which also belong to the factory settings.

10.5 Light-Source Factor

In the menu point *Measuring Head →Light-Source Factor 3057-FL→PAM-Fluorometer 3057-FL*, the currently used light-source factor for the PARtop or PARbot measurement of the measuring head is indicated.

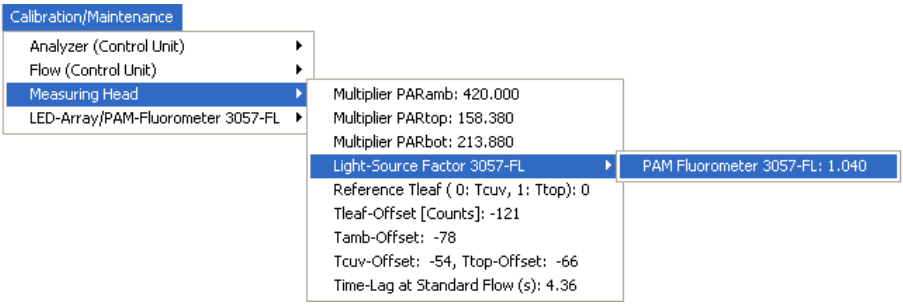


Fig. 20: Currently used light-source factor for PARtop or PARbot in the menu of the measuring head.

Pressing this menu point starts the same matching procedure as the menu point *Calibration/Maintenance→LED-Array/PAM-Fluorometer 3057-FL-→Match Internal PAR-Sensors and Lightsource Factors* (*see* chapter10.3).

10.6 Updating Firmware

Stop GFS-Win, so that another software can take control over the 3010-I/Box. Start the Software *SPanelUpdater.exe* by clicking on it in the Explorer.

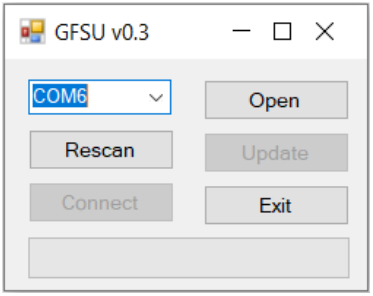


Fig. 21: *SPanelUpdater.exe* before updating

Usually the correct COM-port is automatically found. But if the instrument is not connected or was still under the control of GFS-Win, the *Rescan* button can be used to find the correct COM-port.

Press the *Open* button and find the new firmware for the LED-Array/PAM-Fluorometer 3057-FL, for example called gfspam-r38-2022-02-02.cbi. The firmware will be loaded, meaning it is ready to be sent to the PAM-fluormeter.

Afterwards press *Update* to send the new software to the PAM-fluorometer. During the update process, the LED of the PAM-Fluorometer will be permanently green.

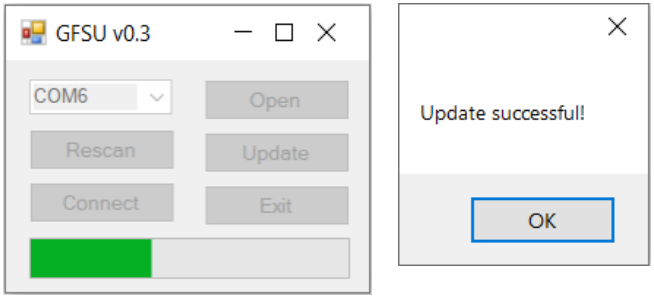


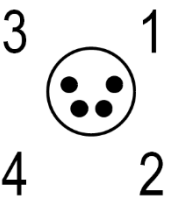
Fig. 22: *SPanelUpdater.exe* during updating and notification afterwards

A successful update will be indicated in a separate window. Press *Exit* or *X* to end the update-software.

11 Appendix

11.1 Pin Assignments

Connector:

		1: RS485/A 2: input (+ 16 V) 3: RS485/B 4: GND

11.2 Status LED

LED	**Mode**
red blinking	WinControl Mode
green blinking	GFS-Win Mode
green permanent	updating

12 Technical Specifications

Design: Combined PAM chlorophyll fluorometer and LED light source comprising an LED array with red LEDs (for actinic illumination and saturation pulses), blue LEDs (for additional actinic illumination), far-red LEDs, additional red LEDs (for measuring light) and 6 photodiodes (for chlorophyll fluorescence detection)

Measuring light: Red LEDs (635 nm), modulation frequency 5 to 100 Hz

Actinic light: Blue LEDs (470 nm) and red LEDs (635 nm), range for blue: 0 to 400 µmol m^{-2} s^{-1} PAR, range for red: 0 to 3000 µmol m^{-2} s^{-1} PAR, maximal range for mixed illumination : 0 to 3000 µmol m^{-2} s^{-1} PAR at 25°C

Saturation light: Red LEDs (635 nm), up to 12000 µmol m^{-2} s^{-1} PAR at 25°C

Far-red light: Far-red LEDs (peak: 740 nm)

Signal detection: PIN-photodiode protected by long-pass filter (> 700 nm), selective window amplifier

External PAR Sensor: 0 to 22000 µmol m^{-2} s^{-1}

Internal PAR Sensors: detection of blue, red, saturating light pulse

Leaf area: 8 cm^2

Power consumption: 48 W max. (during saturating light pulse), power supply via Standard Measuring Head 3010-S

Operating temperature: -5 to +50°C

Dimension without cable: 7.5 cm x 6 cm x 8 cm (L x W x H)

Weight: 225 g

13 Literature

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14 Manufacturer's Guarantee

Under this Manufacturer′s Guarantee ("Guarantee"), subject to the Conditions and Instructions below, Heinz Walz GmbH, Germany ("Manufacturer"), guarantees (§443 BGB) to the end customer and user ("Customer") that all products supplied by it shall substantially conform in material respects to the Specifications for 24 months from the delivery date (date on invoice). In this Guarantee, "Specifications" means the product′s features (as may be amended by Manufacturer from time to time), which are set out under the headings "specifications" and/or "technical specifications" within the product′s respective brochure, data sheet, or respective tab on the Manufacturer′s website for such product, and which may be included with the documents for the product when delivered. In case of an eligible guarantee claim, this Guarantee entitles the Customer to repair or replacement, at the Manufacturer′s option, and this Guarantee does not include any other rights or remedies.

14.1 Conditions

This Guarantee 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.
- Any product supplied by the Heinz Walz GmbH, Germany which has been subjected to misuse, abuse, abnormal use, negligence, alteration or accident.
- Damage caused from improper packaging during shipment or any acts of God.
- Batteries, cables, calibrations, fiberoptics, fuses, gas filters, lamps, thermocouples, and underwater cables.
- Defects that could reasonably have been detected upon inspection of the product when received by the Customer and not promptly noticed within ten (10) days to Heinz Walz GmbH.
- Submersible parts of the DIVING-PAM or the underwater version of the MONITORING-PAM have been tested to be watertight down to the maximum

operating depth indicated in the respective manual. Guarantee shall not apply for diving depths exceeding the maximum operating depth. Further, guarantee shall not apply for damage resulting from improper operation of devices, in particular, the failure to properly seal ports or sockets.

14.2 Instructions

- To obtain guarantee service, please follow the instructions below:
- The Walz Service Information Form available at https://www.walz.com/support/repair_service.html must be completed and returned to Heinz Walz GmbH, Germany.
- 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, and/or shipping costs incurred in returning equipment for guarantee service are at customer expense. Duty and taxes are covered by Walz.
- All products being returned for guarantee 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.

14.3 Applicable law

- This Guarantee is governed by German law. Place of jurisdiction is Bamberg, Germany.

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