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Fluorescence Measuring System
XE-PAM

System Components
and
Principles of Operation

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1 Safety instructions

1.1 General safety instructions

To avoid the risk of electric shock, do not remove the casing or open the back. There are no user serviceable parts inside. Leave servicing to the experts! To prevent fire or the risk of electric shock, keep this device out of the rain and away from moisture. The lightning symbol with the arrow head inside an equilateral triangle means that there are live, uninsulated parts inside this device that may give you a dangerous electric shock if touched.

1. Instructions: Read all the safety instructions and all the operating instructions thoroughly before using the device for the first time. Keep these safety instructions and operating instructions somewhere safe in case you need to refer to them again in the future.

2. Safety warnings: In your own interest pay heed to all the safety warnings on the device and in the operating instructions. Follow the instructions on operation and use of the device in every respect.

3. Water and moisture: Never use the device near water, for example near a bath, a washing basin, a sink, a washing machine, in a damp cellar or near a swimming pool.

4. Ventilation: Wherever you put the device, always ensure there is sufficient ventilation. Never put the device on a bed, for example, or a sofa, a carpet or similar surface that might block the vents. Do not build it into furniture either, such as a bookcase or a cupboard.

5. Effect of heat: Do not put the device anywhere near sources of heat, such as radiators, hot-air shafts, ovens, etc.

6. Power source: Connect the device only to the power source indicated in the operating instructions or on the device.

7. Protecting the flex: Run the flex so that no one can step on it and nothing can rest on or against it. The flex is particularly at risk in the area of the plug, the socket and where it comes out of the device.

8. Cleaning: Follow the manufacturer's recommendations for cleaning the device.

9. Device not in use: If you are not going to use the device for some time, remove the plug from the socket.

10. Foreign bodies: Take great care to ensure that no liquids or other foreign bodies can find their way inside the device through the openings in the casing.

11. Repair in the event of damage: The device should only be repaired by qualified personnel. Never try to do more in the way of maintenance to your device than the operating instructions allow. Beyond that, always consult an expert for repair work.

12. Fixing to wall or ceiling: The device may only be fixed to a wall or ceiling in the manner specified by the manufacturer.

1.2 Special safety instructions

1. The XE-PAM Fluorometer is a highly sensitive research instrument 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 XE-PAM Fluorometer employs high intensity flash discharge lamps and continuous light sources, which may cause damage to the eye. Never look directly into these light sources or into the joint end of the fiberoptics on which these light sources are focused.

3. The Xenon flash lamps (XE-MF and XE-STC) produce high intensity UV-light which can be selected by UV-filters (like UG11 and DUG11). Please note that this UV-light can seriously harm the eye although it is not perceived as bright light. Therefore, never try to assess the UV-intensity by looking into the output window of the flash lamp, also when the light passes an UV-filter.

2 Introduction

The pulse-amplitude-modulation (PAM) measuring principle is based on selective amplification of a fluorescence signal which is measured with the help of intense, but very short probe pulses of measuring light. In the XE-PAM Fluorometer µsec probe flashes are generated by a Xenon-arc discharge lamp at relatively low repetition rates (2-64 Hz). While these flashes are orders of magnitude more intense than the LED pulses used in the other PAM Fluorometers (at frequencies up to 100 kHz), they are much weaker than the single turnover saturating flashes commonly used to induce total closure of PSII reaction centers. Therefore their "actinic" effect is weak and they can be used to monitor the yield of fluorescence, changes of which are induced by actinic illumination. As large signals are obtained with Xe-flash excitation, a satisfactory signal/noise ratio is given even at very low chlorophyll concentrations, as found in natural surface waters. In this way, the XE-PAM Fluorometer opens the way for chlorophyll fluorescence quenching analysis of phytoplankton at very low cell densities, in order to assess photosynthetic performance and primary biomass production. However, potential applications of the XE-PAM Fluorometer are not restricted to chlorophyll fluorescence quenching analysis. The following features shall be pointed out, which give rise to numerous further practical applications:

- Xe-flash light displays a broad spectrum extending from the UV to the NIR (near-infrared). Hence, any desired excitation wavelength may be selected with the help of appropriate optical filters. The unfiltered Xe-flash light is quite intense and appreciable intensities remain after passing through narrow band interference filters.

- The optical system is exceptionally compact and at the same time highly versatile with respect to the use of various types of illumination and parallel measurements of other signals, featuring adapters at five cuvette sides and the possibility to connect multibranched fiberoptics.
- Using UV-excitation and a quartz light guide it is possible to measure NAD(P)H fluorescence.
- By recording the UV-excited fluorescence of 9-aminoacridine, the transthylakoidal ΔpH can be monitored.
- By comparison of UV- and blue-light excited chlorophyll fluorescence, information on the UV-screening properties of the leaf epidermis can be obtained.
- The Xe-flash of measuring light (probe flash) can be applied after a defined delay time with μsec-resolution following a single-turnover saturating flash (pump flash), such that the rapid relaxation kinetics of chlorophyll fluorescence yield can be assessed (pump-and-probe technique).

3 Components of the XE-PAM Fluorometer

The basic operational system of the XE-PAM Fluorometer consists of:

a) the **Main Control Unit** (XE-PAM)

b) the **Optical Unit** (ED-101US/M) with standard 10 x 10 mm quartz-cuvette, which mounts on a **Stand with Base Plate** (ST-101) featuring a 15 mm mounting pole and heavy base plate

c) the **Xenon-Measuring Flash Lamp** (XE-MF), connecting via a 10 x 10 mm perspex or quartz light-guide to the cuvette

d) the **Photodiode Detector** (XE-PD), connecting via a 10 x 10 mm light-guide to the cuvette, featuring a 10 x 10 mm PIN-photodiode and a pulse signal amplifier.

For standard chlorophyll fluorescence measurements the following filter set is provided:

Excitation Filter: 5 mm BG39 (Schott)

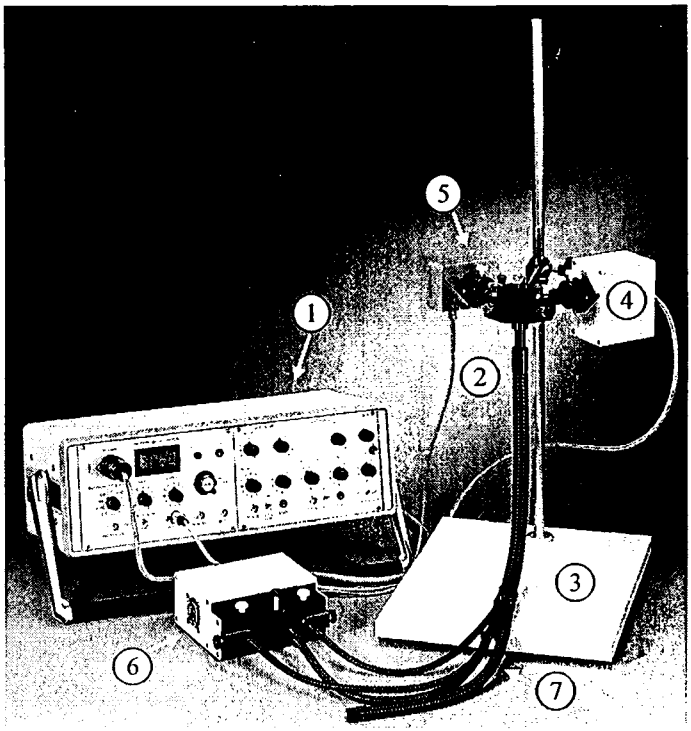
In addition, a set of 30 x 30 mm diaphragm plates with four different central hole sizes are provided for defined attenuation of the excitation light. These "attenuators", when pressed against the lamp opening, reduce maximal intensity to ca. 50, 25, 10 and 5 %.

Emission Filter: 1 mm R65 (Balzers) + 2 mm RG645 (Schott)

For special applications (see 3.4 and 3.5) an additional long-pass filter (1 mm RG9, Schott) is provided.

The system can be extended by a number of accessory components. For the study of light induced chlorophyll fluorescence changes and fluorescence quenching analysis by the saturation pulse method an **Actinic-/Saturation Light Unit** (XE-AL) is available, featuring two miniature halogen lamps (continuous light and

saturation pulse light) and a LED-cone (equipped with 7 red LEDs), which are powered by the **Main Control Unit**. For optical connection of the **Actinic-/Saturation Light Unit** with the cuvette in the Optical Unit, special **Fiberoptics** (101-F or 101-F5, with 4 or 5 arms, respectively) are required.



Components of the XE-PAM Fluorometer
1) Main Control Unit
2) Optical Unit (ED-101US/M)
3) Stand with Base Plate (ST-101)
4) Xenon-Measuring Flash Lamp (XE-MF)
5) Photodiode Detector (XE-PD)
6) Actinic-/Saturation Light Unit (XE-AL)
7) Four Armed Fiberoptics (101-F)

In order to extend the system for pump-and-probe measurements, a special pump flash system is required, for which purpose the new **Single Turnover Flash Unit** (XE-ST) can be recommended, which consists of the **Xe-Pump Flash Control Unit** (XE-STC) and the **Xe-Pump Flash Lamp** (XE-STL). The **Xe-Pump Flash Lamp** connects to the **Optical Unit** via the same **Fiberoptics** as the **Actinic-/Saturation Light Unit.**

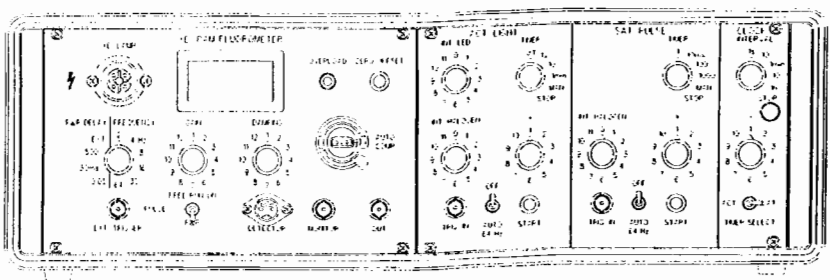
3.1 Main Control Unit XE-PAM

The **Main Control Unit** contains:

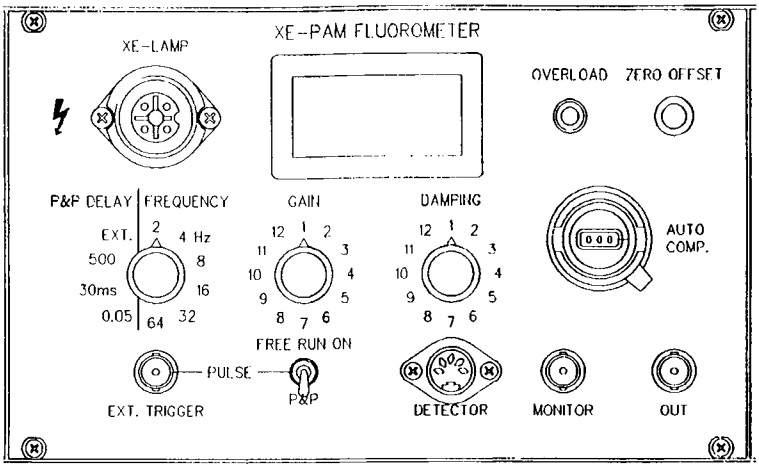
- Xe-flash lamp driver and pulse triggering circuitry
- Selective-window amplifier of pulse signal
- Actinic lamp drivers
- Lamp timers and clock

3.1.1 Front side panel

The front side panel is divided into four sections featuring the following controls and connectors:



3.1.1.1 XE-PAM Fluorometer, Xe-measuring flash (pulse) and pulse signal controls



- **XE-LAMP** connector, to connect the Xenon-Measuring Flash Lamp XE-MF.
- **Digital display**, display of the signal (0-10 V at 10 mV resolution) which was measured with the last pulse and is held until the next pulse.
- **OVERLOAD** indicator LED, lights up when signal exceeds 4 V.
- **ZERO OFFSET** pushbutton, to reset display to zero. Normally used if there is a signal after switching-on the instrument while measuring light is off (brief pressing of pushbutton is sufficient).
- **AUTO COMP.**, ten-turn potentiometer with 3-digit display, for adjustment of a small compensation signal which is effective only while measuring pulses are turned on. In phytoplankton fluorescence studies, for example, the signal obtained with a filtered water sample may be compensated, such that the remaining signal and its light induced changes are due to the phytoplankton only. The compensation signal increases with

GAIN-settings, in the same way as the measured signal. At gain 1 maximal compensation amounts to approximately 100 mV.

- **DAMPING**, dial-switch with 12 positions, for suppression of electronic noise at the cost of time resolution. The following list shows the relationship between DAMPING-settings and the time constants (time for 63 % of a rapid signal change):

DAMPING setting	1	2	3	4	5	6	7	8	9	10	11	12
Time constant, msec	9	16	26	47	85	155	263	470	850	1500	2600	4700

The choice of appropriate DAMPING depends primarily on the maximal expected rate of signal changes, including induction of maximal fluorescence yield, Fm, by a pulse of saturating light; for example, setting 6 is appropriate for assessment of Fm with a 600 ms saturation pulse. The DAMPING only applies to the OUT-signal and not to the original signal created in the pulse amplifier. Therefore, it does not affect the µsec-time-resolution of pump-and-probe measurements.

- **GAIN**, dial-switch with 12 positions, for amplification of signal output, as indicated on Digital display.
- **FREQUENCY**, dial-switch with positions 2, 4, 8, 16, 32 and 64 representing the frequency (in Hz) with which Xe-measuring-flashes are triggered when the PULSE-switch is in FREE RUN ON position.
- **P & P DELAY** dial-switch, with 4 positions provided for the following purposes:
  - **EXT.**, to trigger a measuring (probe) flash with the help of an external trigger signal which is applied via the EXT.TRIGGER

input socket; an external device may be used to set a defined delay between pump-flash (e.g. from XE-STC) and the trigger signal.

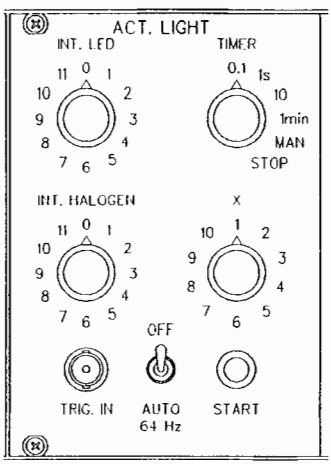
- **500 ms**, to trigger a probe flash 500 ms after Ext. Triggering, which may be synchronized with the onset of a saturation pulse of at least 500 ms length (assessment of Fm or P-level).
- **30 ms**, to trigger a probe flash 30 ms after Ext. Triggering, which may be synchronized with the onset of a multiple turnover saturating flash of at least 30 ms length (assessment of so-called I_2-level).
- **0.05 ms**, to trigger a probe flash 50 µs after Ext. Triggering, which normally is synchronized with triggering of a single-turnover saturating flash (pump-flash, e.g. from XE-STC) (assessment of the maximal fluorescence yield which can be induced by a single-turnover flash, equivalent to so-called I_1-level).

- **EXT.TRIGGER** input-socket, to trigger probe flashes by an external device, e.g. via the TRIG.OUT of the XE-Pump Flash Control Unit XE-STC. Triggering will occur only if the PULSE-switch is in the P & P (pump-and-probe position) and the P & P DELAY/FREQUENCY dial-switch is in one of the four P & P DELAY positions.
- **PULSE**-switch, allowing to choose between FREE RUN ON (measuring flashes automatically triggered with selected FREQUENCY of 2-64 Hz) and P & P (pump-and-probe, where flashes are triggered via the EXT.TRIGGER input, with selected P & P DELAY). When EXT. is selected by the P & P DELAY switch, upon switching to the FREE RUN ON position a single

probe flash is triggered. In standard applications, the PULSE-switch just serves the purpose of switching measuring light on/off.

- **DETECTOR** input socket, to connect cable from Photodiode Detector XE-PD
- **MONITOR** output socket, to view the original signal at the output of the preamplifier with the help of an oscilloscope at µsec time resolution. This feature can be helpful in pump-and-probe applications to monitor the position of the probe flash with respect to the pump flash, as well as the relative signal amplitudes.
- **OUT** socket, for output of signal measured with the last measuring (probe) flash. This signal is held until the next measuring flash is triggered.

3.1.1.2 ACT. LIGHT, control of actinic halogen lamp and LED source



- **INT.LED**, dial-switch with 12 intensity settings (0-11) pertaining to the output of the actinic LED source of the Actinic-/Saturation

Light Unit (XE-AL), consisting of a cone with 7 LEDs (standard version peaking at 660 nm). The table shows the quantum flux density of photosynthetically active radiation (PAR) within a water-filled standard cuvette as a function of INT.LED-settings. There is a linear increase with increasing intensity settings. The absolute values may vary between individual instruments and depend on the type of cuvette (e.g. mirrored), on the fiberoptics (active diameter) and on the particular entrance port.

Quantum flux density of photosynthetically active radiation (PAR) at different intensity-settings in µmol quanta m^{-2} s^{-1}

Setting	1	2	3	4	5	6	7	8	9	10	11
LED Lamp	15	30	45	60	75	90	105	120	135	150	165
Halogen Lamp	500	1000	1500	2000	2500	3000	3500	4000	4500	5000	5500

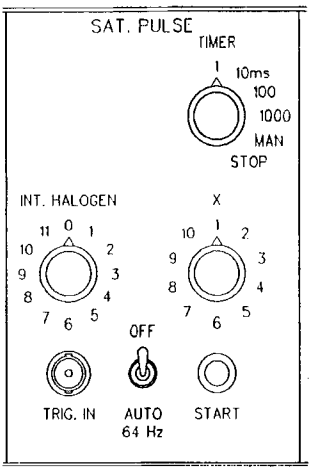
Note: These PAR-values were measured using standard Four Armed Fiberoptics (101-F) and bundles with 4.5 mm active diameter connected to the fiber adapters of the two lamps. Using the 5.5 mm bundle, PAR-values were ca. 10 % higher. The halogen light was filtered by a SP695 short-pass filter. The joint end of the fiberoptics was inserted via the bottom port of the Optical Unit. PAR was measured using the Spherical Micro Quantum Sensor US-SQS in conjunction with the light meter from LI-COR (Model LI-250). Absolute PAR-values may vary between individual instruments.

- **INT.HALOGEN**, dial-switch with 12 intensity settings (0-11) pertaining to the output of one of the two halogen lamps of the Actinic-/Saturation Light Unit (XE-AL). The table shows the quantum flux density of photosynthetically active radiation (PAR) at the cuvette site of the **Optical Unit** as a function of INT-HALOGEN-settings. There is a linear increase with increasing intensity settings. The absolute values may vary between

individual instruments and depend on the type of cuvette (e.g. mirrored), on the fiberoptics (active diameter), the particular entrance port and the applied optical filters.

- **TIMER/s or min**, dial switches, by the combination of which the actinic illumination time (ranging from 0.1 s to 10 min) is defined (LED- and/or HALOGEN-lamps switched on upon START). The time setting selected by the upper dial-switch, when multiplied by the position of the lower dial-switch, gives the illumination time. In the MAN-position, illumination is initiated by START and will continue until being manually terminated by selecting the STOP-position.
- **TRIG.IN** input socket, to trigger START of illumination period
- **AUTO 64 Hz** switch, when enabled the probe pulse frequency is automatically increased to 64 Hz for the duration of actinic illumination, in order to increase the signal/noise ratio and time resolution.
- **START** push-button, to trigger actinic illumination. As an alternative to the standard lamps in the Actinic-/Saturation Light Unit XE-AL, special High Power LED-Lamps (HPL-660, HPL-620) will become available which can be triggered via ACT.LIGHT TRIG.OUT ON/OFF (sockets at rear side of instrument).

3.1.1.3 SAT.PULSE, control of saturation pulse halogen lamp



- **TIMER/ms**, dial-switches, by the combination of which the saturation pulse length (in the range from 1 ms to 10 s) is defined. The time setting selected by the upper dial-switch, when multiplied by the position of the lower dial-switch, gives the saturation pulse length. In the MAN-position, illumination by the saturation lamp is initiated by START and will continue until being manually terminated by selecting the STOP-position.

- **INT.HALOGEN**, dial switch with 12 intensity settings (0-11) pertaining to the output of one of the two halogen lamps of the Actinic-/Saturation Light Unit (XE-AL). The same table of intensities applies as shown above (see 3.1.1.2) for the ACT. LIGHT. These intensities are increased by ca. 10 % when a fiber bundle with 5.5 instead of 4.5 active diameter is applied.

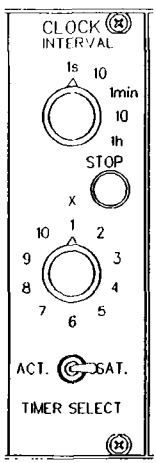
- **TRIG.IN** input socket, to trigger START of saturation pulse illumination

- **AUTO 64 Hz** switch, when enabled the probe pulse frequency is automatically increased to 64 Hz for the duration of the saturation

pulse illumination, in order to increase the signal/noise ratio and time resolution. This function is particularly essential when short saturation pulses are applied.

- **START** push-button, to trigger saturation pulse. As an alternative to the standard halogen lamp in the Actinic Illumination Unit, special HIGH-POWER LED-Lamps (HPL-660, HPL-620) will become available which feature steep on/off characteristics, such that short saturation pulses (less then 100 ms) are possible (making use of SAT.PULSES TRIG.OUT ON/OFF at rear side of instrument).

3.1.1.4 CLOCK, for repetitive triggering of ACT.LIGHT or SAT.PULSE illumination



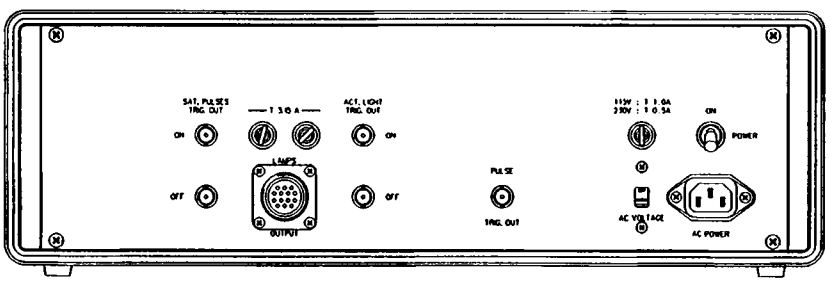
- **INTERVAL** dial-switches, by the combination of which the time interval (1 s to 10 h) between two consecutive trigger signals is defined. The time setting selected by the upper dial-switch, when multiplied by the position of the lower dial-switch, gives the interval. When the STOP-position is selected, a running CLOCK

will be stopped and thereafter the CLOCK-function is disabled, i.e. it cannot be started via START of ACT.LIGHT or SAT.PULSE.

- **STOP**-pushbutton, to stop a running CLOCK, without preventing renewed start of the CLOCK via START of ACT.LIGHT or SAT.PULSE. The pushbutton-lamp lights up red when the CLOCK is running.
- **TIMER SELECT** switch, to select between ACT. (ACT.LIGHT source) and SAT. (SAT.PULSE source) to be repetively triggered by the CLOCK.

3.1.2 Rear side panel

At the rear side of the Main Control Unit the following items are located:



- **SAT.PULSES TRIG.OUT ON/OFF** sockets

The ON-TRIG.OUT signal is synchronous with the START-trigger (given with the START-pushbutton or a TRIG.IN signal of the SAT.PULSE unit). The OFF-TRIG.OUT signal is synchronous with the termination of the saturation pulse illumination (defined by the TIMER of the SAT.PULSE unit).

- **ACT.LIGHT TRIG.OUT ON/OFF** sockets

  The ON-TRIG.OUT signal is synchronous with the START-trigger (given with the START-pushbutton or a TRIG.IN signal of the ACT.LIGHT unit). The OFF-TRIG.OUT signal is synchronous with the termination of the actinic illumination (defined by the TIMER of the ACT.LIGHT unit).

- **LAMPS OUTPUT** socket, to connect the special Actinic-/Saturation Light Unit (XE-AL), featuring two halogen lamps and a LED source (7 LEDs).

- **T 3.15 A**, fuses for the two halogen lamp circuits

- **PULSE TRIG.OUT** socket, trigger output signal which is synchronous with probe flash triggering; in the FREE RUN ON position at 2-64 Hz and in the P & P position with a defined delay (selected via P & P DELAY switch) relative to an external trigger signal (applied via the EXT.TRIGGER input socket).

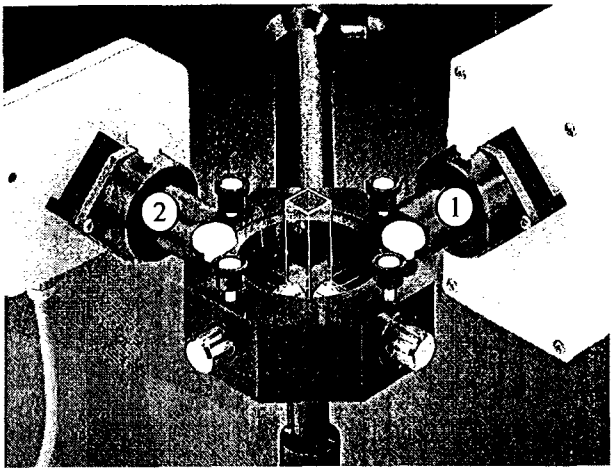
- **115 V: T 1.0 A**, mains fuse at 115 V line voltage
  230 V: T 0.5 A, mains fuse at 230 V line voltage

- **AC VOLTAGE** selector switch for 230 or 115 V line voltage

- **POWER** switch, to turn the Main Control Unit ON/OFF. The instrument must be installed in such a way that this switch is always accessible (safety instruction).

- **AC POWER** socket, to connect the main-line cable. Please note that only cables may be used which are certified for the country where the instrument is used. The instrument is grounded via the ground conductor of the power cable. Before connection of the cable to the power line, it should be made sure that the AC VOLTAGE selector switch is in the proper position and that the proper fuse is installed in the mains fuse holder.

3.2 Optical Unit ED-101US/M



Optical Unit with excitation port (1) and emission port (2) and fiberoptics for actinic illumination connected at the bottom.

The basic **Optical Unit** is identical to that of the ED-101US Emitter-Detector Unit which was previously introduced for ultrasensitive measurements using the standard PAM Fluorometer. This has the advantage that the same accessories, like fiberoptics, Miniature Magnetic Stirrer and High Power LED-Lamps, can be used. The design of the Optical Unit has been optimized for the following purposes:

- efficient use of excitation light
- homogenous illumination
- efficient collection of fluorescence
- low background signal caused by scattered light
- high flexibility with respect to the application of various light sources and parallel measurements of other signals.

The **Optical Unit** consists of a solid aluminum holder (black anodized) with an octagonal body, in the center of which a 10 x 10 x 45 mm glass- or quartz-cuvette can be positioned. Two 10 x 10 x 100 perspex- or quartz-rods, which slide in two tube-ports mounted sidewise at 90° angle in the holder, can be moved to two adjacent sides of the cuvette. In this way, optimal optical coupling of excitation- (port 1) and emission- (port 2) pathways to the cuvette is achieved. Furthermore, the amount of excitation light is minimized which may enter the emission port and cause a background signal at the detector (e.g. via filter fluorescence). Two 15 mm Ø metal rods with highly reflecting front surfaces are provided to be positioned against the free vertical sides of the cuvette. They serve the purpose of fixing the cuvette and at the same time increasing the signal by reflecting transmitted excitation light back into the cuvette and reflecting fluorescence via the emission port to the detector. Alternatively, also cuvettes with mirrored side walls (1 or 2) are available, with which signal amplitudes can be further improved.

Another adapter port with a 15 mm Ø hole is provided at the bottom of the cuvette which can be used for connecting the standard fiberoptics (101-F or 101-F5). In this way, effective optical connections to the **Actinic-/Saturation Light Unit** (XE-AL) and to optional additional light sources, like the new Single Turnover Flash Unit (XE-ST, for pump-and-probe measurements), can be readily achieved. In principle, by introducing further multibranched fiberoptics via the 15 mm Ø side openings (replacing the metal rods), an almost unlimited amount of optical inputs and outputs can be created. This is, for example, useful for the parallel study of the transthylakoidal ΔpH (via 9-aminoacridine fluorescence, measured with XE-PAM Fluorometer) and of electron transport rate (via chlorophyll fluorescence, measured with a standard PAM Fluorometer).

In high sensitivity applications (like measurement of natural phytoplankton fluorescence) the fiberoptics should be preferentially introduced via the bottom port, in order to minimize potential excitation of fiber fluorescence. Also great care must be taken to keep all optical components (light guiding rods, cuvette, cuvette holder, metal rod fronts, filters) clean and free of potentially fluorescent substances. This is particularly important when working with UV-excitation, in which case quartz rods and a quartz cuvette must be used. Cuvettes tend to accumulate fluorescing debris in their corners which can be most effectively removed by ultrasonic treatment.

Alternatively to the fiberoptics, the Miniature Magnetic Stirrer US-MS may be introduced via the bottom port. The user should make sure that the applied stirring bar does not fluoresce.

A short cable is fixed to the metal body of the **Optical Unit**, which can be plugged into the ground-socket at the bottom of the **Photodiode Detector XE-PD**. This may result in a lowering of electronic noise picked up by the antenna system of the various metal parts of the **Optical Unit**.

3.3 Xenon-Measuring Flash Lamp XE-MF

The **Xenon-Measuring Flash Lamp** features a HAMAMATSU Xenon Discharge Lamp (Type L4641) with 1.5 mm arc. A special filter holder (V-shaped) is provided, which accepts filters of variable sizes with a total thickness of up to 15 mm. It mounts on one of the two tube-ports of the **Optical Unit** in such a way that always a minimal distance between flash lamp and light guiding rod can be achieved, as the filters are gently pressed against the Flash Lamp opening by the light-guide. For standard chlorophyll fluorescence

measurements a 5 mm BG39 blue-glass filter (Schott) is provided. At high signal levels and with light sensitive samples, the excitation intensity can be lowered by use of additional filters (interference filters narrowing down excitation bandwidth or neutral density filters) or by introducing thin metal plates with suitable central holes. For this purpose a set of 30 x 30 mm plates is provided with 2.4, 3.4, 5.1 and 6.9 mm Ø holes. When put directly against the Flash Lamp opening, these "attenuators" decrease flash intensity to ca. 5, 10, 25 and 50 %.

3.4 Photodiode Detector XE-PD

The **Detector Unit** consists of a 10 x 10 mm PIN-photodiode (Hamamatsu), a pulse preamplifier and a special filter holder (V-shaped), which accepts filters of variable sizes with a total thickness of up to 15 mm. The whole unit can be mounted on one of the two tube-ports of the **Optical Unit** in such a way that always a minimal distance between photodiode and the signal-guiding rod can be achieved, as the filters are gently pressed against the photodiode opening by the light-guide. For standard chlorophyll fluorescence measurements a filter combination consisting of 3 mm red-glass filter (RG645, Schott) and a 1 mm long-pass dichroic filter (R65, Balzers) are provided. The dichroic filter serves the purpose of reflecting scattered blue excitation light, thus preventing excitation of fluorescence in the RG645 filter. Therefore, it is essential that the dichroic filter is facing towards the light-guide (CUV. SIDE). When very strong white light is applied, as e.g. in pump-and-probe measurements using the SP695 filter with the XE-ST flash lamp, an additional long-pass filter should be placed in front of the detector, to avoid saturation of the detector by 650-700 nm light. For this purpose use of a 1 mm RG9 filter (λ>710 nm) is recommended, which should

be protected by the R65/RG645 filter from scattered blue excitation light, in order to avoid filter fluorescence. A ground socket is provided at the bottom of the Detector Unit, into which the cable can be plugged which is fixed to the Optical Unit (see 3.2).

3.5 Actinic-/Saturation Light Unit XE-AL

The **Actinic-/Saturation Light Unit** features two miniature 8 V/20 W halogen lamps (Bellaphot, Osram) and an array of 7 LEDs (HLMP 8103, Hewlett-Packard, selected for peak emission around 660 nm). A lucite cone funnels the light of the LEDs into one of the endpieces of the **Fiberoptics** (101-F or 101-F5), the joint end of which connects to the **Optical Unit** (ED-101US/M) (see 3.2). The light of the two halogen lamps is focused on two other fiberoptics endpieces. It first passes IR-reflecting filters (permanently installed) and then the filter boxes, which provide room for up to 15 mm additional filters. Two dichroic short-pass filters (λ<695 nm, SP695) are provided for standard illumination with white light (see table in section 3.1.1.2 for intensities). As part of this light (650-700 nm) passes the standard filter combination (R65/RG645) in front of the detector, under unfavourable conditions (e.g. fiberoptics pointing towards detector) strong white light may cause transient artefacts. These can be avoided by adding a longpass detector filter opening at λ>700 nm. For this purpose a RG9 filter (1 mm) is recommended which should be placed behind the R65/RG645, in order to avoid filter fluorescence.

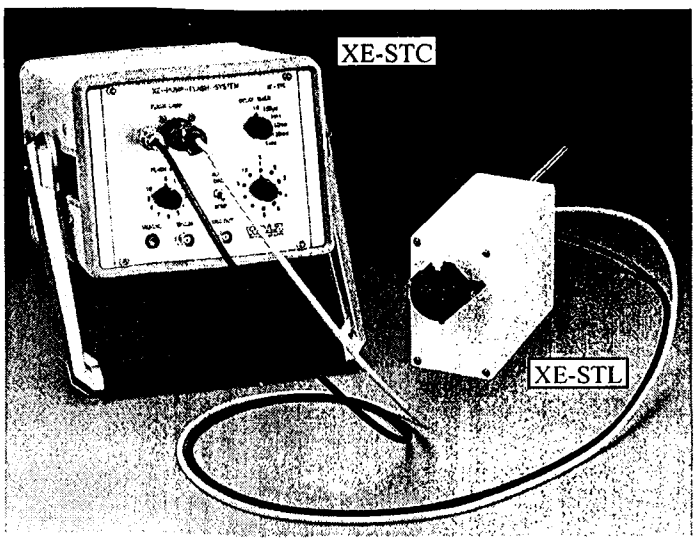
The intensities and illumination times of the three actinic light sources are controlled by the **Main Control Unit** (see 3.1.1.2-3.1.1.4), with which the **Actinic-/Saturation Light Unit** is connected. The ACT.LIGHT and SAT.PULSE modules apply to the left and right halogen lamps, respectively. The CLOCK may trigger

either the timer of ACT-LIGHT or of SAT.PULSE (choice via TIMER SELECT-switch). The same TIMER applies for the actinic LED- and halogen sources. If only one of these two sources shall be used, the intensity of the other source should be set to zero.

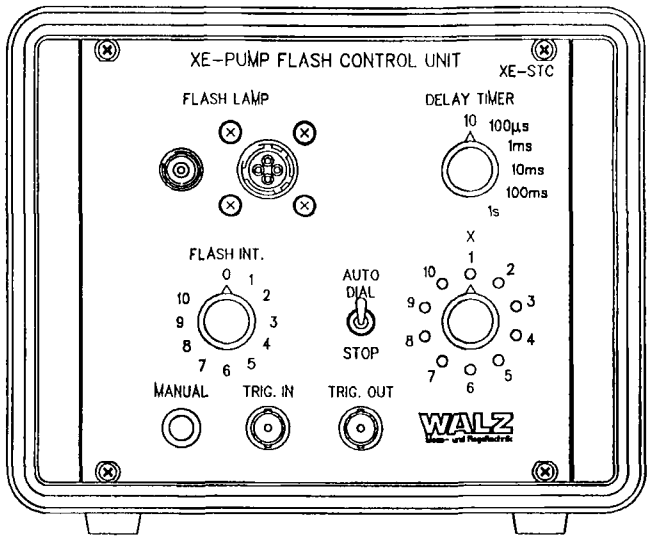
The **Actinic-/Saturation Light Unit** is equipped with a cooling fan. In this way excessive heating of the unit is avoided.

3.6 Single Turnover Flash Unit

The Single Turnover Flash Unit (XE-ST) consists of the **Xe-Pump Flash Control Unit (XE-STC)** and the actual **Xe-Pump Flash Lamp (XE-STL)**. This system provides saturating single turnover flashes. It displays a number of outstanding features which render it well suited for pump-and-probe measurements in conjunction with the XE-PAM Fluorometer (see 4.3).



3.6.1 Xe-Pump Flash Control Unit XE-STC



The various items on the front side panel of the **Xe-Pump Flash Control Unit XE-STC** serve the following purposes:

- **FLASH LAMP** sockets, to connect the two cables of the Xe-Pump Flash Lamp (XE-STL). The right hand socket carries high voltage (up to 900 V).
- **FLASH INT.**, dial switch with 12 positions for choice of pump flash intensity. Settings 1-10 correspond to voltages between 285 and 900 V, with a close to linear increase of voltage. In setting 0 (provided twice between settings 10 and 1) the energy storing capacitor is discharged. In many applications, FLASH INT. setting 3 or 4 already may be saturating. In this case, in order to evaluate the flash saturation characteristics, flash intensity can be lowered by neutral density filters or by "attenuators" (metal plates with defined central holes).

- **DELAY TIMER**, dial switches, by the combination of which the delay time (ranging from 10 µs to 10 s) between pump and probe flashes is defined. The time selected by the upper dial-switch, when multiplied by the position of the lower dial-switch, gives the delay time. The positions of the lower dial switch serve an additional function in the AUTO DIAL mode of operation (see below). Each position features a status-LED which lights up when a particular position is selected.

- **AUTO DIAL/STOP** switch, to select between AUTO DIAL mode and the fixed-position mode. When AUTO DIAL is selected, after each triggering of a pump flash (via MANUAL push button of TRIG.IN) the DELAY TIMER position (lower dial switch) is advanced one step, as indicated by the status-LED. In this way, flash relaxation kinetics can be automatically recorded (see 4.3). In order to start an AUTO DIAL recording in position 1, the dial switch first has to be manually moved to position 1, while the selection switch is still in the STOP-position. If in this way another dial position (e.g. position 3) is chosen, the preceding positions will be excluded from the dial cycle. For example, it may be advantageous to exclude positions 1 and 2 when the DELAY TIMER is set to 10 µs (upper dial switch), as in many applications ca. 30 µs are required for full recovery of detector sensitivity after a pump flash (see 4.3). When repetitively triggered (e.g. via the CLOCK of the Main Control Unit), AUTO DIAL cycles continue until the STOP-position is selected.

- **MANUAL**, pushbutton for manual triggering of single pump flash

- **TRIG.IN**, input socket for triggering pump flashes externally. For repetitive triggering, it is recommended to use the CLOCK of the **Main Control Unit** (see 3.1.1.4). For this purpose, a BNC-cable is connected to the TRIG.OUT ON socket of the SAT.PULSE or

ACT.LIGHT unit at the rear side of the Main Control Unit. Normally, if one of the two units is just used for triggering pump flashes, interference of the actinic lamps should be prevented by selecting INT.-position 0 and by switching the AUTO 64 Hz-function OFF.

- **TRIG.OUT**, output socket for trigger signal which is delayed with respect to a pump flash (triggered via MANUAL push-button or TRIG.IN) by the time determined by the DELAY TIMER (see above). In pump-and-probe applications, TRIG.OUT normally is connected to the EXT.TRIGGER socket (front side of Main Control Unit) via a BNC-cable. In order to trigger a probe flash, the P & P DELAY switch of the XE-PAM Fluorometer then must be in the EXT.-position.

The various items on the rear side panel of the **Xe-Pump Flash Control Unit XE-STC** serve the following purposes:

- **115 V: T 1.0 A**, mains fuse at 115 V line voltage
  230 V: T 0.5 A, mains fuse at 230 V line voltage
- **M 1 A**: fuse for secondary circuitry
- **AC VOLTAGE** selector switch for 230 or 115 V line voltage
- **POWER** switch, to turn power of the Main Control Unit ON/OFF. The instrument should be installed in such a way that the ON/OFF switch is always accessible (safety instruction).
- **AC POWER** socket, to connect the main-line cable. Please note that only cables may be used which are certified for the country where the instrument is used. The instrument is grounded via the ground conductor of the power cable. Before connection of the cable to the power line, it should be made sure that the AC

VOLTAGE selector switch is in the proper position and that the proper fuse is installed in the mains fuse holder.

3.6.2 Xe-Pump Flash Lamp XE-STL

The Xe-Pump Flash Lamp XE-STL features a High-Power type Xenon Discharge Lamp (Hamamatsu L4633) with 1.5 mm arc which is equipped with a built-in elliptical mirror. Due to this mirror the flash light is converging behind the lamp output window. A special filter holder (V-shaped) is provided, which accepts filters of variable sizes with a total thickness of up to 15 mm. For standard applications, a 5 mm BG39 blue-glass filter (Schott) is provided. For higher intensities an optional SP695 short-pass filter may be used, which passes almost all photosynthetically active radiation. However, it should be noted that part of this light (ca. 650-700 nm) will reach the detector when the standard R65/RG645 detector filter is used. This may lead to signal lowering in pump-and-probe applications when the delay between pump and probe flashes is small (see 4.3). In this case the detector should be protected by an optional, additional RG9 filter (Schott), placed behind the R65/RG645, in order to prevent RG9 filter fluorescence.

Normally the pump flash intensity can be conveniently adjusted via the FLASH INT. dial switch at the front side of the XE-STC control unit. There is also the possibility to lower the range of available intensities by placing appropiate neutral density filters or "attenuator plates" (see 3.3) into the filter box.

A mounting rod is provided to fix the Xe-Pump Flash Lamp XE-STL to the Stand with Base Plate (ST-101). Connection with the XE-STC control unit is via two cables. Optical connection with the cuvette in the Optical Unit is achieved via the fiberoptics (see 4.1.3).

4 Principles of Operation

4.1 How to get set up

4.1.1 Setup of the standard configuration

First the two perspex rods (or optionally quartz rods) are installed in the two light-guide ports of the **Optical Unit** (ED-101US/M), such that they gently push against the walls of the cuvette placed in the center. The two metal rods are pushed against the two other walls. After the **Optical Unit** has been mounted on the **Stand with Base Plate** (ST-101), the **Xenon-Measuring Flash Lamp** (XE-MF) and the **Photodiode Detector** (XE-PD) can be connected to the two light-guide ports and the appropriate filters are installed. For chlorophyll fluorescence measurements, these are the BG39 blue-glass filter (at **Xenon-Measuring Flash Lamp**) and the R65/RG645 red filter combination (at the **Photodiode Detector**). It is important that the dichroic R65 filter (reflecting surface) is faced towards the cuvette. Please make sure that the whole set-up has a safe stand and that all components are securely fixed at their appropriate sites (safety instruction). Then the cables of **Xenon-Measuring Flash Lamp** and **Photodiode Detector** must be connected to the respective sockets at the front side of the **Main Control Unit**. The **Main Control Unit** is connected via the main-line cable to the power line. Please note that only cables may be used which are certified for the country where the instrument is used. The instrument is grounded via the ground conductor of the power cable. Before connection of the cable to the power line, it should be made sure that the AC VOLTAGE selector switch is in the proper position and that the proper fuse is installed in the mains fuse holder. The **Main Control Unit** must be installed in such a way, that the Power-switch at its rear side is always accessible (safety instruction). When the POWER-switch (rear side) is turned ON, the PULSE-switch (front side) should be in the P & P position

(i.e. there will be no measuring light pulses unless an external trigger signal is applied). If in this state a signal is displayed, this should be suppressed by briefly pressing ZERO OFFSET.

4.1.2 Setup of the Actinic-/Saturation Light Unit XE-AL

The cable of the **Actinic-/Saturation Light Unit** is connected to the corresponding socket at the rear side of the **Main Control Unit**. The optical connection is made via the **Fiberoptics 101-F or 101-F5**. The joint end of the Fiberoptics preferentially is connected via the bottom port of the **Optical Unit,** unless the Miniature Magnetic Stirrer US-MS occupies this port. Fiberarms with 10 mm endpiece outer diameter can be connected to the three different light sources in the **Actinic-/Saturation Light Unit**. At the side with the cooling fan a free space of 20 cm should be kept. Depending on specific applications, suitable filters can be placed into the boxes at the output of the halogen lamp sources. The intensities of the three light sources are controlled by the corresponding dial switches of the ACT.LIGHT and SAT.PULSE control units (front side of the Main Control Unit, see 3.1.1.2 and 3.1.1.3).

4.1.3 Setup of the Single Turnover Flash Unit XE-ST

The two cables of the Xe-Pump Flash Lamp (**XE-STL**) are connected to the corresponding sockets at the front side fo the Xe-Pump Flash Control Unit (**XE-STC**). At the rear-side of the Flash Lamp there is a threaded opening, which accepts a small mounting rod, which is provided for fixing the Flash Lamp on the Stand with Base Plate (**ST-101**). Please make sure that all components are reliably fixed on the stand (safety instruction). Optical connection of the Flash Lamp to the cuvette in the **Optical Unit** is achieved via the

Fiberoptics (101-F or 101-F5), with the joint end of the Fiberoptics preferentially being connected to the bottom port of the Optical Unit, unless the Miniature Magnetic Stirrer US-MS occupies this port. There is the choice of connecting either a bundle with 10 or 15 mm outer diameter to the Flash Lamp. Appropiate PVC adapters are provided. The Xe-Pump Flash Control Unit (XE-STC) is connected via the main-line cable to the power line. Please note that only cables may be used which are certified for the country where the instrument is used. The instrument is grounded via the ground conductor of the power cable. Before connection of the cable to the power line, it should be made sure that the AC VOLTAGE selector switch is in the proper position and that the proper fuse is installed in the mains fuse holder. The instrument must be installed in such a way that the ON/OFF switch at its rear side is always accessible (safety instruction). Depending on applications, appropriate connections between TRIG.IN and TRIG.OUT sockets and the MAIN CONTROL UNIT have to be made (see 3.6.1).

4.1.4 Connection of additional devices

Using the polyfurcated fiberoptics, other measuring devices (like PAM-101/102/103 Chlorophyll Fluorometer) can be combined with the XE-PAM Fluorometer and other light sources (like the FL-101/E Fiber Illuminator) can be connected to the Optical Unit. In the latter case, care should be taken that the corresponding fiberoptics endpieces are protected by appropiate filters against excessive heat.

Triggering of alternative light sources is accomplished via the ACT.LIGHT TRIG. OUT ON/OFF and SAT.PULSE TRIG. OUT ON/OFF sockets at the rear side of the Main Control Unit. Specifications of trigger signals are given under Technical Data (see 6.2.1).

Further trigger signals can be fed into the EXT. TRIGGER and TRIG. IN sockets of the Main Control Unit and the TRIG. IN socket of the Xe-Pump Flash Control Unit. Please consult the corresponding sections under Technical Data (6.2.1 and 6.3.2) for details concerning the trigger signals.

Finally, peripheral analog recording devices can be connected to the Main Control Unit via the BNC-sockets OUT and MONITOR (see 3.1.1.1). Please consult the corresponding sections under Technical Data (6.2.1 and 6.3.2) for details concerning the output signals.

4.1.5 Miniature Magnetic Stirrer US-MS

The Miniature Magnetic Stirrer US-MS is inserted via the bottom port of the Optical Unit. For optimal performance, please make sure that it is all the way pushed in. In order to avoid stirring noise and background signal, proper non-fluorescing magnetic flees should be used. When the bottom port is occupied by the US-MS, the fiberoptics carrying the actinic light preferentially should be inserted into the side port facing the flash-lamp and not the detector.

The stirring rate is controlled by a potentiometer located on the power supply unit. For further information, please consult the separate manual for this device.

4.1.6 Spherical Micro-Quantum-Sensor US-SQS

The Spherical Micro-Quantum-Sensor US-SQS is available for assessment of the photosynthetically active radiation (PAR) within the cuvette. This sensor can be connected to the display unit of the widely-distributed light meter from LI-COR (Model LI-250), also

available from Heinz Walz GmbH. The spherical sensor consists of a 3 mm Ø highly scattering plastic sphere which receives light from all sides and which on one side is connected to a 1 mm Ø plastic fiber, guiding the scattered light to the detector. This features a blue-enhanced silicon photodiode and a filter combination selecting the photosynthetically active radiation between 380 and 710 nm. In conjunction with the light meter from LI-COR (LI-250), the micro-quantum-sensor measures incident photosynthetically active radiation in µmol quanta $m^{-2}s^{-1}$, i.e. in units of flux density. The Spherical Micro-Quantum-Sensor US-SQS is unique in its reliable assessment of all light which may enter the cuvette from the various ports of the Optical Unit and which may be reflected from the mirrored surfaces of cuvette and metal bars. It may be assumed, that the PAR sensed by the Spherical Micro-Quantum-Sensor US-SQS corresponds to the mean PAR sensed by microscopic samples suspended in the cuvette.

The Spherical Micro-Quantum-Sensor was calibrated against a LI-COR Quantum Sensor (Type LI-190) in air, using a distant light source illuminating the spherical sensor from the bottom side. A correction factor of 1.5 was applied to obtain proper readings underwater. A potentiometer is provided on the detector unit of the spherical sensor to recalibrate the device, if necessary.

The spherical sensor should be treated with great care, particularly at the delicate connection between the scattering sphere and the fiberoptics. Excessive bending of the fiber should be avoided. After use, the sphere should be rinsed with clean water. Organic solvents should be avoided. An ethanol moistened tissue may be used to gently clean the surface of the sphere. In applications when fluorescence is recorded with high sensitivity, the spherical sensor may contribute to the signal and, hence, should be removed from the cuvette. When not in use, the sensor head may be covered by the protecting hood provided with the device.

Besides the BNC-output at the detector unit connecting to the light meter from LI-COR (LI-250), a special LEMO-output socket is provided for display of the signal in conjunction with other PAM Fluorometers (like PHYTO-PAM and WATER-PAM). The output signal of the BNC adaptor is adjusted to -10 µA/1000 µmol quanta $m^{-2}s^{-1}$, e.g. a factor of -100 must be entered in the light meter LI-250 from LI-COR.

4.2 How to measure fluorescence yield

When the PULSE-switch at the front side of the Main Control Unit is moved into the FREE RUN ON-position, Xe-flashes are triggered at a rate determined by the FREQUENCY dial-switch. Normally the lowest frequency (2 Hz) is appropriate for assessment of steady-state fluorescence yield. Higher frequencies may cause some increase of chlorophyll fluorescence in dark-adapted samples. Furthermore, for *in vivo* chlorophyll fluorescence measurements it is recommended to attenuate the Xe-flash intensity (to ca. 10 % of maximal intensity) by use of neutral density filters or of one of the "attenuators" (metal plates with small central holes) provided with the XE-PAM Fluorometer (see 3.3). In order to measure the minimal (quasi-dark) fluorescence yield of a sample (Fo-level), the fraction of PSII reaction centers closed by a probe flash should be small. This can be judged by assessment of ΔF/Fm, i.e. the relative increase of fluorescence yield, which can be induced upon total closure of PSII centers (increase to Fm-level), e.g. by application of a saturating light pulse. The more reaction centers are already closed by the probe flash, the smaller the relative increase of fluorescence upon total center closure. For assessment of Fm by a saturation pulse, the **Actinic-/Saturation Light Unit** must be connected (see 4.1.2). In order to apply single saturation pulses manually, the CLOCK

INTERVAL switch should be in the STOP-position. Standard settings of SAT.PULSES are:

TIMER: 600 ms (100 ms x 6)

INT.HALOGEN: 6 (wavelengths <700 nm; SP695 filter)

AUTO 64 Hz: enabled (switch down)

Although in principle the fluorescence yield signal can be followed via the Digital display of the **Main Control Unit**, it is more practical to monitor the signal with a chart recorder connected to the analog output (OUT-socket at the front side of the **Main Control Unit**). At moderate intensity of the excitation light (ca. 10 % of maximal intensity) the Fo-level can be continuously monitored. Even at very low excitation intensities, there may be a slow rise of fluorescence yield caused by so-called "inactive" PSII centers, which are characterized by extremely slow kinetics of acceptor oxidation. If this is observed, the original Fo-level can be easily assessed from a kinetic recording by extrapolating to time zero, i.e. to the moment when the measuring light was switched on after a longer period of darkness. When a saturation pulse is applied by pressing the START-pushbutton of the SAT.PULSE unit, the Xe-flash frequency automatically is increased to 64 Hz for the 600 ms pulse duration, if the AUTO 64 Hz function is enabled. The increase to Fm is recorded as a spike. The ratio (Fm-Fo)/Fm is equivalent to the potential quantum yield of PSII (all centers open) and amounts to values of 0.6 - 0.8 in healthy green algae. It is lowered, for example, by photoinhibition, water pollution and various other natural and man-made stress factors. As outlined above, for correct assessment of Fo the intensity of the probe flashes must be appropiately attenuated.

In the presence of continuous background light, as e.g. provided via the fiberoptics by the **Actinic-/Saturation Light Unit**, the

quantum yield of PSII is decreased (by PSII centers being partially closed and by relative increase of energy dissipation into heat). This can be assessed by monitoring the changes of fluorescence yield and by repetitive application of saturation pulses to determine maximal fluorescence yield, Fm'. For this purpose, the CLOCK can be used (e.g. set to 10 s x 2 = 20 s, with the TIMER SELECT switch in the SAT.-position). It can be observed that with increasing actinic intensity (INT.LED- or INT.HALOGEN-position) the fluorescence yield (F) is increased, the maximal yield induced by a saturation pulse (Fm') is decreased and, hence, the ratio (Fm'-F)/Fm'=ΔF/Fm' is also decreased. ΔF/Fm' has been shown to be a good measure of the effective quantum yield of PSII in green algae and higher plants. The product of ΔF/Fm' and the photon flux density of photosynthetically active radiation (PAR), gives a relative measure of photosynthetic electron transport rate. Plots of ΔF/Fm' x PAR versus PAR are equivalent to so-called PI-curves, i.e. light response curves of photosynthetic activity.

4.3 How to make use of the pump-and-probe mode of operation

When the PULSE-switch on the front side of the Main Control Unit is in the P & P position, this normally means that Xe-flash illumination is off and, hence, no repetitive signal is measured. To start measuring, an external trigger signal must be applied (via EXT.TRIGGER socket). The essence of the pump-and-probe mode of operation is that defined delay times may be applied between the external trigger signal and the firing of the Xe-probe flash. Hence, when the external trigger signal is synchronized at defined delay times with a single-turnover saturating flash (pump flash), the much weaker probe flash of the XE-PAM Fluorometer measures the increase in fluorescence yield induced by the saturating flash and its

dark relaxation. In the 0.05 ms position of the P & P DELAY selector the probe flash is fired 50 µs after the pump flash, which is at the peak of fluorescence yield. At longer delay times, fluorescence yield decays due to reoxidation of the primary PSII acceptor.

In the EXT.-position of the P & P DELAY selector the delay is zero and an external device is required to establish a defined delay between the pump-flash and the EXT.TRIGGER input. When the new **Single Turnover Flash Unit XE-ST** of the XE-PAM Fluorometer is used, delay times between 10 µs and 10 s can be chosen using the DELAY TIMER dial switches of the XE-STC unit (see 3.6).

The positions 30 ms and 500 ms are provided for assessment of the fluorescence yield at two characteristic levels of the fluorescence yield after onset of continuous saturating light. In order to make use of the 30 ms position, a saturation lamp with steep onset characteristics must be used (e.g. High Power LED-Lamp HPL-620 or Multiple Turnover Flashlamp XMT-103). After 30 ms the so-called I_2- (or J-) level is reached. The 500 ms position applies to the standard saturation pulses, i.e. 600 ms illumination with white light, using the **Actinic-/Saturation Light Unit**. In this case the trigger signal is obtained from the TRIG.OUT ON-output of the SAT.PULSE unit. At 500 ms the P-level of fluorescence yield is assessed (corresponding to the conventional Fm). Of course, a 600 ms saturation light pulse can be also given with a High Power LED-Lamp (e.g. HPL-620).

It is important to note that the pump-and-probe mode of operation of the XE-PAM Fluorometer offers the unique possibility to assess the maximal fluorescence yield after a single turnover saturating flash ($F_{50\mu s}$), as well as the characteristic levels of I_2 and P in the same sample under identical conditions. The question, which of these three

levels is best suited to derive the PSII quantum yield, $\Delta F/Fm'$, is still under debate.

The pump-and-probe method allows to measure the rate of electron transfer between the primary PS II acceptor Q_A and the secondary acceptor Q_B with µsec-time resolution using a simple chart-recorder. This information is obtained by repetitive measurements with increasing delay times between pump and probe flashes. For this purpose, the **Xe-Pump Flash Control Unit XE-STC** provides a so-called AUTO DIAL-function, by which with every consecutive pump-flash-triggering the delay time is increased by one step (see 3.6).

In control samples, most of the pump flash induced increase of fluorescence yield relaxes within 500 µs. The relaxation kinetics are dramatically slowed down by PSII inhibitors, like Atrazin or DCMU (herbicide diuron), which block electron transfer between Q_A and Q_B.

It should be noted that a reliable assessment of fluorescence yield at very short delay times (10-40 µs after pump flash) is complicated by the perturbance of the detector/amplifier system caused by the pump flash. Generally, this perturbance results in an underestimation of fluorescence yield by the probe flash. For optimal results, the following aspects should be taken into consideration:

(1) The "flash tail" extending into the 10-40 µs time range after triggering of a pump flash is considerably more pronounced in the red than in the blue part of the flash light. Therefore, use of a blue filter (e.g. BG39) is recommended.

(2) If the pump-flash-induced signal does cause amplifier saturation, recovery to full sensitivity may require ca. 100 µs. Hence excessive pump flash intensities should be avoided and the signal amplitudes measured at GAIN-setting 1 of the **XE-PAM**

Fluorometer should be moderately low (in pump-and-probe applications). In certain applications, the latter aspect may even mean, that a sample should be diluted or the fluorescence reaching the detector should be attenuated (by a neutral density filter or "attenuator plate"). If a storage oscilloscope is available, the pulse signals induced by pump-and-probe flashes can be assessed via the MONITOR-output of the **XE-PAM Fluorometer** (see 3.1.1.1).

(3) Even when detector/amplifier saturation is avoided, the probe flash signal is somewhat attenuated by a preceding pump flash as the „baseline" is sloping down due to an unavoidable „tail" in Xe-discharge flashes. This effect increases with the ratio of pump/probe flash intensities. Therefore, in order to obtain reliable information in the 10-40 µs range, relatively high probe flash intensities are recommended. On the other hand, it should be kept in mind, that high intensities of probe flashes lead to an overestimation of the minimal fluorescence yield, Fo. Hence, in pump-and-probe applications optimal conditions for correct Fo- and Fm-determinations cannot be easily reconciled with each other.

(4) The reliability of fluorescence yield determination at short delay times following a pump flash can be assessed by substituting the investigated sample against a „dead" sample, e.g. an appropriately diluted chlorophyll extract. The concentration of such a sample should be adjusted to give the same signal as the investigated photosynthetically active sample (at 50 µsec following pump flash) under identical conditions of pump/probe flash intensities, sample geometry, detector sensitivity and GAIN. Actually, in this way the possible error can be quantified and applied for correction of the *in vivo* data.

Even after proper correction, the maximal yield of fluorescence ($F_{30\ \mu sec}$) determined by the pump-and-probe method tends to be lower than the Fm determined by the saturation pulse method. The most likely reason for this deviation is a particular type of nonphotochemical fluorescence quenching which is induced by the pump flash and which requires at least 50 µsec to disappear. While it would be out of scope to go into further details, it may be mentioned that this type of quenching, which can be studied by comparative PAM and pump-and-probe measurements, appears to be caused by increased dissipation of excitation energy at the reaction center level (see recent publication by Schreiber and Krieger, 1996, Two fundamentally different types of variable chlorophyll fluorescence in vivo. FEBS Letters 397: 131-135).

4.4 How to assess UV-screening properties of the leaf epidermis

The XE-PAM Fluorometer provides high levels of measuring light in the UV-A and UV-B spectral regions. Such UV-light is also contained in natural sun light and plants have evolved special mechanisms to protect themselves against its detrimental effects. Due to the observed shrinkage of the UV-absorbing ozone layer in the earth-atmosphere, in certain regions of the earth UV-radiation levels have been dramatically increasing. Consequently also research on UV-effects on plants has become more and more relevant.

Recently, Bilger et al. (Physiologia Plantarum 101: 754-763, 1997) have introduced a method to assess epidermal UV-absorption with the help of the XE-PAM Fluorometer. The rationale of this method is as follows: UV-light is absorbed by chlorophyll and, hence, will induce chlorophyll fluorescence, if it is allowed to penetrate via the epidermis into the chlorophyll containing mesophyll of the leaves. Leaves adapted to high UV-levels, which have accumulated

UV-absorbing pigments (like flavonoids) in their epidermis, will show much weaker UV-excited fluorescence than shade leaves. The relative extent of UV-attenuation can be quantified by comparison of UV-excited fluorescence (F_{UV}) with the fluorescence excited by blue-green light (F_{BG}). It has been shown by Bilger et al. that the ratio F_{BG}/F_{UV} is a reliable measure of epidermal UV-absorbance. This ratio may be normalized to one unit for a leaf from which the epidermis has been removed.

In practice, such measurements are carried out as follows:

(1) A special **UV-Filterset** (FS-UV) is available for measurements of UV-excited chlorophyll fluorescence in comparison to blue-green excited fluorescence. Filters are provided for excitation by broad band UV (UV-A + UV-B), only UV-A, only UV-B and UV-free blue green light. In combination with the standard R65/RG645 detector filter a very low background signal is observed, when these excitation filters are used. For the various modes of excitation the following filter combinations apply:

Broad band UV (UV-A + UV-B): DUG11 + UG11

UV-A: DUG11 + UG11 + BG39

UV-B: DUG11 + UG11 + GG19

Blue-green: BG39 + UV-BLOCKING.

(2) A 10x10x100 mm quartz rod must replace the standard perspex rod in the excitation port of the **Optical Unit**.

(3) The intensity of the measuring light should be sufficiently attenuated (preferentially by "attenuator plates" provided with the **XE-PAM Fluorometer**) to make sure that the minimal fluorescence level, Fo, is monitored. Actually, very weak

measuring light intensities are feasible, as intact leaves give very large signals due to their high chlorophyll content.

(4) It is recommended to adjust the ratio F_{BG}/F_{UV} to unity by appropriate attenuation of the blue green excitation beam, using a leaf from which the epidermis was removed (e.g. leaf of *Vicia faba*).

(5) The sample (normally a leaf disc) should be placed at 45° angle with respect to both excitation and emission pathway. For this purpose a special **UV-Sample Holder** (XE-SH-UV) for UV-excitation with triangular cross-section (replacing the standard 10x10x45 mm cuvette in the **Optical Unit**) is available.

Additional information on specific substances responsible for UV-absorption in the epidermis may be gained by narrowing down UV-excitation bands in the UV-A and UV-B range with the help of additional narrow-band interference filters. In this case, it is recommended to add such filters to the basic DUG11/UG11 filter combination, which assures a low background signal.

4.5 How to measure NAD(P)H-fluorescence

Due to its high content of UV-radiation and its high sensitivity, the XE-PAM Fluorometer is ideally suited for measurements of UV-excited NAD(P)H-fluorescence. In practice, such measurements are relatively simple when the following requirements are fulfilled:

(1) A special **NADPH-Filterset** (FS-NADPH) is available for separation of UV-excitation light (UG11) and selective detection of NAD(P)H-fluorescence in the blue-green spectral region (BG18 + KV418)

(2) A 10x10x100 mm quartz rod must replace the standard perspex rod in the excitation port of the **Optical Unit**.

(3) A quartz cuvette should be used.

(4) Particular care should be taken that all parts which are exposed to direct or scattered measuring light are clean, in order to avoid an excessive background signal.

(5) Particular attention should be paid to the choice of buffer. Standard buffer substances, like TRICINE or HEPES, may display substantial UV-excited blue-green fluorescence, the extent of which may vary between various batches from different manufacturers. In any case, the AUTO COMP.-function should be applied for electronic suppression of any background signal (see 3.1.1.1).

4.6 How to measure 9-aminoacridine fluorescence

For measurements of 9-aminoacridine fluorescence (**9-AA Filterset** FS-9AA) essentially the same requirements are given as for measurements of NAD(P)H-fluorescence (see 4.5). However, we recommend to narrow down the UV-excitation band to UV-A by adding 2 mm BG18 filter in the excitation path. In this way, the background signal can be decreased. The other filters are identical. The quenching of 9-AA fluorescence can be quantified as follows:

First the signal obtained with chloroplasts in buffer is assessed in the absence of 9-AA. This signal can be suppressed via AUTO COMP (see 3.1.1.1). Then ca. 1 µmol 9-AA is added and some time is given for signal stabilization under quasi-dark conditions. The obtained signal (100 %) is quenched when actinic light illumination is applied (e.g. with the help of the **Actinic-/Saturation Light Unit** or of a **High Power LED-Lamp**). If available, a chart recorder

should be connected (via OUT-socket) to follow the quenching kinetics.

Preferentially such measurements should be carried out with stirred samples. For this purpose the Miniature Magnetic Stirrer US-MS is available, which can be introduced via the bottom port of the **Optical Unit**. Then the fiberoptics, which carry actinic illumination, are introduced from one of the side ports, if possible avoiding the port facing the detector.

9-AA fluorescence quenching reflects the transthylakoidal proton gradient in chloroplasts. A linear relationship applies between the logarithm of quenching (percent of total signal) and the ΔpH. Strong quenching is favored by a high buffer pH (8.0-8.5).

5 Service and Cleaning

5.1 General service instructions

- All electronic components of the XE-PAM Fluorometer should be handled with care. For cleaning a soft, moist cloth may be applied. Do not use abrasive substances.
- The optical components (perspex and quartz light guides, quartz cuvettes, fiberoptics end pieces, optical filters) should be regularly cleaned using soft, non-abrasive tissues and some ethanol. It should be noted that the cuvettes tend to accumulate fluorescing debris in their corners which can be best removed in an ultrasonic bath.
- All electrical cables and connectors should be regularly inspected for potential damage.
- All parts mounted on the stand should be regularly inspected for being securely fixed at their appropiate sites.

5.2 Replacement of halogen lamp in the Actinic-/Saturation Light Unit XE-AL

First remove the cable which connects the Actinic-/Saturation Light Unit XE-AL with the Main Control Unit XE-PAM. Then the four screws at the top of the XE-AL unit can be removed and the cover lifted up. Now the halogen lamp is accessible. Please note that a heat reflecting filter is located between lamp frame and the housing. After disconnecting the lamp from its socket, it can be replaced by a new lamp. First the heat filter is inserted. The smaller opening of the filter holder should point towards the front side of the housing. When inserting the new lamp, the red spot on the frame

should point upwards. After replacing the cover, and making sure that it properly connects with the housing, the four screws can be fixed again.

6 Technical Data

6.1 General environmental conditions

The general environmental conditions are valid for all instruments outlined in chapters 6.2 and 6.3. The values referring to the mains voltage apply only if the instrument features a mains connector.

Permissible environmental temperature

during operation: -5 °C to +45 °C

in resting state: -30 °C to +60 °C

Environmental humidity: up to 31 °C ≤ 80%, linearly decreasing to 50 % at 40 °C

Maximal altitude

during operation: 4000 m

in resting state: 15000 m

Mains voltage fluctuations: max. ±10 %

Overvoltage category: II

Contamination level: 1

6.2 Basic System

6.2.1 Main Control Unit XE-PAM

Control Unit for Xenon-measuring flash lamp, photodiode detector and signal processing:

Display:	LED-display (red), 3½ digits, 14 mm character size
Measuring flash frequency:	2, 4, 8, 16, 32 and 64 Hz
P&P-delay:	0.05 ms, 30 ms and 500 ms or defined by external trigger signal
Trigger input:	external trigger signal 5 to 12 V DC, max. 35 mA via BNC-input
Trigger output:	synchronized with each measuring flash via BNC-output, 12 V DC
Gain:	12 settings
Damping:	12 settings featuring time constants from 9 ms to 4.7 s
Analog output:	-0.5 to +10 V
Monitor output:	max. 10 V
Special functions:	ZERO OFFSET- and AUTO COMP.-function

Control unit for actinic illumination:

Intensity settings:	12, for red LED-array as well as for halogen lamp
Timer:	illumination times adjustable from 0.1 s to 10 min in 4 x 10 steps, and continuous light
Trigger input:	external trigger signal (5 to 12 V DC, max. 3 mA) via BNC-input

Trigger outputs:	start- and stop trigger pulses (5 V DC) synchronous with start and stop of illumination, via BNC-outputs
Special function:	AUTO 64 Hz-function

Control unit for saturation pulses:

Intensity settings:	12
Timer:	illumination times adjustable between 1 ms and 10 s in 4 x 10 steps, and continuous light
Trigger input:	external trigger signal (5 to 12 V DC, max. 3 mA) via BNC-input
Trigger outputs:	start- and stop trigger pulses (12 V DC) synchronous with start and stop of illumination, via BNC-outputs
Special function:	AUTO 64 Hz-function

Clock for Actinic Light and Saturation Pulses:

Clock timer:	intervals adjustable between 1 s and 10 h in 5 x 10 steps
Special function:	switch selecting between clock triggering of actinic light or saturation pulses

General specifications:

Power supply:	selectable 115/230 V AC, 50/60 Hz, 0.64/0.32 A
Fuses:	T1A for 115 V AC or T0.5A for 230 V AC as well as two times T3.15A for halogen lamps
Dimensions (with carrying-handle):	53 cm x 15 cm x 28 cm (W x H x D)
Weight:	7.9 kg

6.2.2 Optical Unit ED-101US/M

Design:	black anodized aluminum body featuring two light-guides at right angle to each other, for attachment of emitter and detector unit; three additional optical ports provided for fiberoptics and optional light sources, for illumination of central 10 x 10 mm cuvette; light-tide hood with injection hole
Mounting:	stand with Ø 15 mm bar (e.g. ST-101)
Dimensions:	Ø 26 cm
Weight:	1 kg

6.2.3 Xenon-Measuring Flash Lamp XE-MF

Flash lamp:	Hamamatsu Type L4641
Filter holder:	for optical filters (standard 30 x 30 mm), up to 15 mm thick
Connecting cable:	1.4 m long
Dimensions:	140 mm x 105 mm x 65 mm (L x W x H)
Weight:	750 g

6.2.4 Photodiode Detector XE-PD

Signal detection:	PIN-photodiode 10 x 10 mm
Filter holder:	for optical filters (standard 30 x 30 mm), up to 15 mm thick
Connecting cable:	1.4 m long
Dimensions:	97 mm x 71 mm x 78 mm (L x W x H)
Weight:	350 g

6.2.5 Stand with Base Plate ST-101

Base plate:	39.5 cm x 30 cm
Height:	76.5 cm
Stand bar:	Ø 15 mm
Weight:	2.8 kg

6.3 Accessories to Basic System

6.3.1 Actinic-/Saturation Light Unit XE-AL

Design:	compact unit featuring two separate halogen lamps (for actinic light and saturation pulses) and a red LED-array
Halogen lamps:	12 V/20 W, type Bellaphot (Osram), blue-enriched
Wavelengths:	ranging from UV-A to NIR
Intensity at exit of fiberoptics:	max. 8500 µmol quanta m^{-2} s^{-1} PAR (without added filters)
	max. 6500 µmol quanta m^{-2} s^{-1} PAR (with SP695 filter)
IR-blocking filter:	Calflex 3000, permanently installed
Filter holders:	for optical filters (standard 50 x 50 mm), up to 15 mm thick
Red light LED-array:	featuring 7 LEDs, type HLMP 8103, and collimating lucite cone
Emission peak:	665 nm
Light intensity at exit of fiberoptics:	max. 180 µmol quanta m^{-2} s^{-1} PAR

General specifications:

Connecting cable:	1.4 m long
Dimensions:	180 mm x 80 mm x 160 mm (W x H x D)
Weight:	1.35 kg

6.3.2 Single Turnover Flash Unit XE-ST

Xe-Pump Flash Control Unit XE-STC

Intensity settings:	10
Flash width:	1.5 µs at half-maximal intensity
Flash frequency:	max. 1 Hz
Trigger input:	external trigger signal (5 to 12 V DC, max. 3 mA) via BNC-input
Trigger output:	5 V DC pulse via BNC-output
Delay time for trigger output:	interval adjustable between 10 µs and 10 s in 6 x 10 steps
AUTO DIAL function:	automatic advancement of delay times (up to 10 in dial cycle)
Power supply:	selectable 115/230 V AC, 50/60 Hz, 0.1/0.05 A
Mains fuse:	M0.2A for 115 V AC or M0.1A for 230 V AC
Fuse for secondary circuitry:	M1A
Dimensions (with carrying-handle):	24 cm x 15 cm x 28 cm (W x H x D)
Weight:	3.7 kg

Xe-Pump Flash Lamp XE-STL

Flash lamp:	type Hamamatsu L4633

Filter holder:	for optical filters (standard 30 x 30 mm), max. 15 mm thick
Connecting cable:	1.5 m long
Dimensions:	65 mm x 105 mm x 182 mm (W x H x D)
Weight:	1 kg

6.3.3 Four Armed Fiberoptics 101-F

Design:	flexible, plastic-covered special fiberoptics featuring four arms, the fibers of which are thoroughly randomized at the joint end facing the measuring site
Total length:	930 mm
Single fiber arms:	1 x 350 mm, active Ø 4.5 mm, outer Ø 10 mm 1 x 350 mm, active Ø 8.5 mm, outer Ø 15 mm 1 x 500 mm, active Ø 4.5 mm, outer Ø 10 mm 1 x 500 mm, active Ø 5.5 mm, outer Ø 10 mm
Measuring site:	active Ø 12 mm, outer Ø 14 mm
Mixing length:	400 mm
Weight:	580 g

6.3.4 Five Armed Fiberoptics 101-F5

Design:	flexible, plastic-covered special fiberoptics featuring five arms, the fibers of which are thoroughly randomized at the joint end facing the measuring site
Total length:	930 mm
Single fiber arms:	1 x 350 mm, active Ø 4.5 mm, outer Ø 10 mm

	1 x 350 mm, active Ø 7.5 mm, outer Ø 15 mm
	1 x 500 mm, active Ø 4.0 mm, outer Ø 10 mm
	1 x 500 mm, active Ø 4.5 mm, outer Ø 10 mm
	1 x 500 mm, active Ø 5.5 mm, outer Ø 10 mm
Measuring site:	active Ø 12.5 mm, outer Ø 14 mm
Mixing length:	400 mm
Weight:	660 g

6.3.5 Quartz Glass Cuvette US-K0

Material:	quartz glass SUPRASIL, transmitting 200 to 2500 nm
Cross section:	10 x 10 mm
Window thickness:	1.25 mm
Design:	optically polished at all sides, top open, unmirrored
Dimensions:	45 mm x 12.5 mm x 12.5 mm (H x W x D)

6.3.6 Quartz Glass Cuvette US-K1

Design as US-K0, but featuring one mirrored side.

6.3.7 Quartz Glass Cuvette US-K2

Design as US-K0, but featuring two mirrored sides at 90° angle.

6.3.8 9AA-Filterset FS-9AA

UG11: Ø 20 mm, 1 mm thick

BG18: Ø 20 mm, 2 mm thick

The filters UG11 and BG18 are combined in an aluminum filter holder with an active Ø of 15 mm.

KV418: Ø 20 mm, 3 mm thick

BG18: Ø 20 mm, 2 mm thick

The filters KV418 and BG18 are combined in an aluminum filter holder with an active Ø of 15 mm.

6.3.9 NADPH-Filterset FS-NADPH

UG11: Ø 20 mm, 1 mm thick, in aluminum filter holder with an active Ø of 15 mm

KV418: Ø 20 mm, 3 mm thick

BG18: Ø 20 mm, 2 mm thick

The filters KV418 and BG18 are combined in an aluminum filter holder with an active Ø of 15 mm.

6.3.10 UV-Filterset FS-UV

DUG11: Ø 15 mm, 2 mm thick

UG11: Ø 15 mm, 3 mm thick

The filters DUG11 and UG11 are combined in an aluminum filter holder with an active Ø of 12 mm.

GG19: 12.5 x 12.5 mm, 1 mm thick, in aluminum filter holder with an active Ø of 10 mm

UV-BLOCKING: Ø 15 mm, 1 mm thick, in aluminum filter holder with an active Ø of 12 mm

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, 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.

4. 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.

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