

Flow-through WATER-PAM Chlorophyll Fluorometer

General description:

The **Flow-through WATER-PAM Chlorophyll Fluorometer** is a highly sensitive device for continuous monitoring of phytoplankton content and photosynthetic activity in surface waters, like rivers, lakes, oceans and drinking water reservoirs. It consists of a **water-proof measuring-head** covered by a **flow-through chamber** and is operated in conjunction with the standard **PAM-Control** unit, either in the stand-alone mode or via a PC under the **WinControl** software. The chamber can be connected to a common pump, with which samples are drawn from the investigated surface water.



Fig. 1 Measuring-head covered with flow-through chamber, with the water inlets/outlets at the left side and the electrical cables connecting to the PAM-Control unit at the right side. Depicted scale showing centimeters.



Fig. 2 For cleaning and maintenance the cover of the cuvette (“TOP”) can be removed from the optical and electronic part.

To remove the cover, please remove the four metal screws (red arrows) with a left turn, then turn the plastic screws right and the cover will be pushed off. The cover is sealed with an o-ring so that it is held quite tight



Fig. 3 Optical part of measuring-head after removing cover of flow-through chamber; the excitation window (right hand side) and detector window (left hand side) form a right angle with each other and are in vertical position in order to minimize deposit of phytoplankton.

Outstanding features of the Flow-through WATER-PAM

- Extremely high sensitivity
- Low background signal
- Compact and robust design
- Operation via standard PAM-Control unit
- Special set of commands for user specific measuring protocols
- Assessment of photosynthetic activity by quenching analysis
- Easy to handle
- Different excitation wavelengths available

Like the other types of WATER-PAM (featuring the Emitter-Detector units WATER-ED or WATER-EDF) the Flow-through WATER-PAM applies **LED light** for pulse-modulated excitation of chlorophyll fluorescence and a highly sensitive **photomultiplier** module for signal detection. The optical design was optimized for **low background signal**, so that measurements can be carried out at extremely low chlorophyll contents down to **0.1 µg Chl/L**. Saturating light pulses can be applied for assessment of maximal fluorescence yield and standard routines of **quenching analysis**. Measured parameters are:

- dark fluorescence yield, **F₀**, as a linear measure of chlorophyll content
- maximum fluorescence yield, **F_m**, of dark-adapted sample
- **F_v/F_m** as a measure of PS II quantum yield of dark-adapted sample
- fluorescence yield, **F**, of illuminated sample
- maximum fluorescence yield, **F_m'**, of illuminated sample
- **(F_m'-F)/F_m'** as a measure of **effective PS II quantum yield** of illuminated sample

Using the WinControl software **Light Response Curves** and **Induction Curves** can be measured for assessment of the photosynthetic capacity of the phytoplankton.

The standard version of the Flow-through measuring-head features orange-red excitation, with which all types of phytoplankton (including cyanobacteria) can be assessed. For applications, in which suppression of cyanobacteria signals is advantageous, blue excitation is recommended. This is e.g. the case when assessment of the photosynthetic activity of green algae/diatoms in drinking water reservoirs is of main interest. Besides red-orange and blue a wide range of other excitation wavelengths (including white light) are available for special applications.

The Flow-through measuring-head is very compact (see Figs. 1 and 2) and robust. After removing the cover of the flow-through chamber the optical windows can be readily cleaned.

In the standard position the optical windows are oriented vertically so that phytoplankton deposit is minimized.

Besides operation via the dedicated WinControl software the instrument can be also controlled via RS-232 interface using a special set of commands, so that users may write their own software for specific measuring protocols.

Major fields of application

- Limnology
- Oceanography
- Water management
- Drinking water monitoring
- Phytoplankton research

Instructions for users

- 1) The Flow-through WATER-PAM is a modified version of the WATER-PAM, for which a manual has been issued by the Heinz Walz GmbH, which is included in the shipment, just like the manuals for the PAM-Control unit and the WinControl software. Presently no separate manual is available for the Flow-through WATER-PAM.
- 2) The Flow-through WATER-PAM can be either operated in the stand-alone mode in conjunction with the PAM-Control unit or under PC-control using the WinControl software. For getting used to the numerous functions of the instrument, it is recommended to use WinControl.
- 3) WinControl first has to be installed on the PC. A CD for set-up of this program is included in the shipment. Please consult the WinControl manual for the features of this program.
- 4) The Flow-through WATER-PAM is connected to the PAM-Control unit via two cables (PM and AUX sockets at the right hand side of the unit).
- 5) For operation under WinControl, the PAM-Control is connected to the serial interface port of the PC via the RS-232 cable or to a USB port via. Connection to USB shall only be done with the provided USB to RS232 adapter to avoid communication errors
- 6) For optimal operation of the instrument, the proper instrument settings have to be installed. This is done with the help of the *Reset Settings* command under *Main Settings* (corresponding icon in the upper left corner of the screen) by selecting "Water" as current device.
- 7) At its maximal setting the Actinic Light Intensity of the Flow-through WATER-PAM exceeds $2000 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$. In some applications this intensity may be too high. In this case, under WinControl/Main Settings/LED Amplitudes the actinic intensity range can be lowered via the Actinic LED Amplitude setting (default setting 12).

- 8) Under WinControl / Settings 2 the *Internal PAR-List* for the 12 Actinic Light Intensity settings can be defined. As the function of PAR with intensity setting is known, for definition of the PAR-list a 2-point calibration is sufficient. Total intensity is composed of the Measuring Light intensity (high frequency at AL-Int. >3) and the Actinic Light intensity. Under *2-point/Read* a ML-value and an AL-value can be entered manually and confirmed by o.k., if no suitable microquantum sensor is available. You may enter the following values for the Flow-Through unit shipped to you on Dec. 22, 2004:

ML-value, 3

AL-value, 440

In principle, you may determine these values yourself by measuring the PAR in front of the detector window at ML-F. High (ML-value) and at Act. Light Int. 8 (Measuring Light off).

- 9) While the instrument was designed for flow-through of the water samples continuously pumped from some larger reservoir, it may as well be used for the study of smaller samples. The normal position is depicted in Fig. 1 of the preceding description of the device. As shown in Fig. 2, the excitation and detector windows are oriented vertically, thus minimizing any background signal caused by deposited phytoplankton or debris. In this position, pumped water should enter at the bottom and exit at the top of the device

The device can be also positioned with both water in/outlets pointing upwards. In this case, the chamber volume can be filled with a discrete water sample (using a small funnel), the properties of which can be studied in detail under stationary conditions.

In principle, for certain tests it is even possible to study small water samples contained in standard glass cuvettes, when these are placed on top of the excitation window. For this purpose, the cover of the measuring head must be removed and due to disturbance of the photomultiplier by ambient light, the measurements have to be carried out in a darkened room.

- 10) The cover of the measuring head is held by 4 metal screws. After these are removed the cover can be pushed off by screwing in the two nylon screws. These have to be screwed out before the cover can be fixed fastened again.
- 11) The Flow-through WATER-PAM was conceived for low pressures close to atmospheric pressure. It is recommended not to exceed 0.2 bar. Problems can be avoided when the water is sucked instead of being pressed through the device.
- 12) The Flow-through WATER-PAM is optimized for low background signal (measured with pure water) and, hence, high sensitivity. Please note that this background signal may increase significantly when fluorescent material is deposited at the cuvette side opposite to the emitter-window and on the windows of the emitter-detector head. Hence, it is important to wash the cuvette with clean water after use.
- 13) For calibration of Chl concentration, please consult the WATER-PAM manual, p. 17-20. Please note that a calibration is valid only for the particular Gain-setting at which it was made.

- 14) When working with natural surface waters the fluorescence signal is not only determined by phytoplankton, but by gilvin (humics, yellow substances) as well. The contribution of the latter is particularly large with blue excitation. In principle, this "background signal" can be determined by measuring the fluorescence of a filtrate.
- 15) The volume of the flow-through cuvette can be changed. The unit is shipped by default with the volume reducer mounted. The overall volume of the flow-through chamber amounts to about 100 ml, which on request can be reduced to 30 ml which, however, leads to some increase (approx. 50%) of the background signal. The **illuminated volume** in front of the detection window amounts to about **2 ml** only.

Working with cuvettes in the WATER-FT.

Recently the **WATER-FT/SK** has been released. It is an accessory, that enables the WATER-FT to be used as an upright standing cuvette instrument.

The following images show the mounting of the parts shipped in the WATER-FT/SK.



Fig. 4 Firstly the head of the WATER-FT unit has to be opened like shown in Fig. 2. Afterwards the cables can be pulled through the base stand of the WATER-FT/SK.



Fig. 5 shows the WATER-FT head mounted on the base of WATER-FT/S. The cables just have to be pulled through the hole in the base tube so that they can be connected with the PAM Control unit. The head itself can just be stuck into the tube from the top and if the white plastic screw (dashed arrow) is tightened, it can not fall out accidentally.



Fig. 6 here the cuvette holder frame is already mounted on the WATER-FT head. The small plastic part holds the sample cuvette WATER-K in place during the measurement. The part is fixed with two long screws that replace the shorter ones used on this position in the default head.



Fig. 7 the housing is closed with the second tube and a separate lid to prevent ambient stray light to illuminate the sample. Now the WATER-FT head can be used upright with a round cuvette.