Portable Gas Exchange Fluorescence System GFS-3000

Handbook of Operation

9th Edition: 1-Jul-19
Mai_2019gfs-3000_Manual_9index.docx

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Printed in Germany

1 Safety Instructions

1.1 General Safety Instructions

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

1.2 Special Safety Instructions

1. The GFS-3000 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 GFS-3000 employs high intensity light sources which may cause damage to the eye. Avoid looking directly into these light sources during continuous illumination or saturation pulses.
3. Before handling of chemicals and batteries read chapter 16.6 and 0.
4. Before charging batteries read chapter 13.1.
5. Before shipment of instrument release pressure of CO_2-unit (chapter 13.4) and follow battery transport instructions (chapter 16.6).

2 Introduction

Gas exchange is defined as the interchange of gases between plants and their environment. Several gases take part in this process. However, carbon dioxide, oxygen and water vapor are of basic importance. The exchange of carbon dioxide and oxygen is linked to primary processes of photosynthesis, whereas the water vapor loss concerns the hydrologic balance of plants. The term gas exchange is often restricted to the exchange of these three gases. As the exchange of oxygen and carbon dioxide is equivalent, it is sufficient to measure the gases water vapor and carbon dioxide in order to detect the gas exchange of plants.

Commercial gas exchange measuring devices are available for about fifty years. The first gas exchange measuring devices have been very large, and a detailed knowledge of the measuring principle was required. For this reason, they were in most cases only operated by specialists. Thanks to technological progress, these devices have become much smaller, and operation has been made easier, without any loss in measuring accuracy. The portable gas exchange system GFS-3000 is a very precise and flexible system appropriate for measuring photosynthesis and respiration in many fields of application.

It is small in size and can be battery-powered. Therefore, the GFS-3000 is not only appropriate for use in laboratories but is also an excellent device for use in the field. It offers the possibility to make measurements simulating ambient conditions inside the cuvette or to make measurements, controlling the most important parameters as light, temperature, CO_2- and H_2O concentration over the whole range relevant for plant photosynthesis. In this way, the GFS-3000 allows an extensive functional analysis of the gas exchange process of plants.

2.1 Combination of Gas Exchange and chlorophyll fluorescence

PAM (pulse amplitude modulated) fluorometry and the saturation pulse method provide detailed information on the flux of excitation energy into photochemical pathways. This information complements and extends the gas exchange information. Images of chlorophyll fluorescence parameters and PAR absorptivity (PAR: photosynthetically active radiation) reveal heterogeneities (patchiness and local damage) which cannot be distinguished by gas exchange measurements alone.

3 System Components

3.1 System Components Overview

Fig. 1: Overview on most important components of the GFS-3000 or GFS-3000FL

1. Control Unit 3200-C
2. Standard Measuring Head 3010-S
3. LED Light Source 3041-L (if GFS-3000)
   LED-Array/PAM-fluorometer (if GFS-3000FL)
4. Power Supply 3200-N
5. Li-ion eSMART Battery 98 Wh (000160101434)
6. Li-ion eSMART Battery Charger -03 (000190101115)
7. Leaf Area Adapters
8. Cuvettes for Arabidopsis plants, Conifers or Lichens/Mosses (optional)

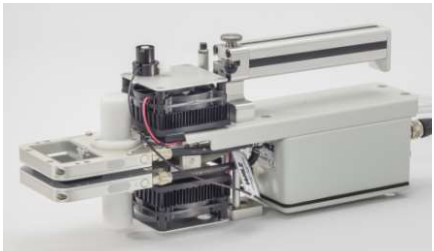
In the following, all components are shown and described:

Control Unit 3200-C,

containing CO_2/H_2O infrared gas analyzer (IRGA), pump, flow meter, solenoid valves, CO_2 control, H_2O control, integrated panel PC, electronics for the battery control and the control unit. Components and connectors attached to the front and other sides are described in chapter 3.2.

Standard Measuring Head 3010-S,

consisting of cuvette for leaves with closing mechanism, temperature control, light control, ventilation system (impeller), 3 light, and 4 temperature sensors, tripod adapter, electronics. Further information see chapter 3.2.1.



GFS/3000: LED Light Source 3041-L,

LED array with warm white LEDs providing homogeneous light on up to 10 cm^2 sample area. Light control can be set to a constant PAR level from 0 up to ca. 3000 µmol m^{-2} s^{-1} or to track the ambient PAR.

GFS-3000FL or optional in addition:

LED-Array/PAM-Fluorometer 3056-FL,

Red/blue LED array for measuring light, actinic light (0…ca. 2500 µmol m^{-2} s^{-1} PAR), saturation pulses (in steps up to > 8000 µmol m^{-2} s^{-1} PAR) and far-red light. Photodiode detectors for parallel measurements of gas exchange and chlorophyll fluorescence on leaf areas up to 8 cm^2

AC Power Supply 3200-N,

with adapter for a battery-slot of the Control Unit 3200-C and cable to the mains voltage (100-240 V AC, 50/60 Hz).

eSMART-Battery 98 Wh 000160101434,

3 pcs eSMART Batteries that can be inserted into the Control Unit 3200-C, 4 pcs are included within the delivery package.

sSMART Battery Charger 000190101115,

for simultaneous charging of four batteries, with display, on/off switch and cable to mains voltage (100-240 V AC, 50/60 Hz).

Legs,

which are mounted to the control unit. The legs are necessary if the Outdoor-Set 3000-C/OS is used. In the field the legs are recommended to keep distance to a moist underground.

Carrying Belt

which can be clicked onto the handle of the control unit for carrying the system in the field.

Protection cover,

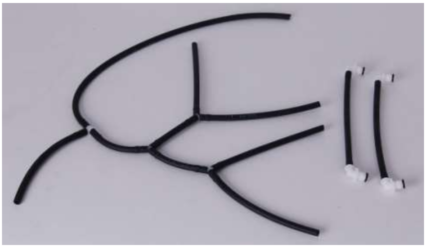
which can be mounted to the front side of the control unit to protect the pneumatic components, especially in the field or greenhouse.

Entrance Filter 5 ml 3000-C/EF,

connected to the inlet AIR IN at the front side of the control unit in order to reduce noise and remove coarse particles of the incoming air.

Mixing Volume40 ml 3000-C/MV

replacing the CO_2 absorber tube connected to the front side of the control unit, if ambient air is used instead of the CO_2 control.



Tube Set 3000-C/TS

with branched air-cycling tube used during H2O absolute zero calibration and connecting tubes replacing drier, humidifier or measuring head connected to the front side.

Dust Cap for CO_2 Absorber Tube,

serves to close CO_2 absorber tube, when not in use, part#: 000140701732

Interface 3010-I/BOX,

USB-RS485 converter with galvanic isolation (1kV) and connecting cables. For connecting the socket COMP of the Control Unit to the USB-port of an external PC.

Cable for 2x Auxiliaries 000130606205,

which can be connected to the socket "AUX IN" at the front side of the control unit. The cable has three bare ended wires, which can be connected to two additional sensors (chapter 16.5).

Chemicals

Silica Gel (H_2O absorber) 000160103402, Humidifying Granules 000160103403, Soda lime (CO_2 absorber) 000160103401 and Molecular sieve 3 Å (strong H_2O absorber for calibration) 000160103414 for replacing the chemicals in the Absorber Tubes CO2 ABSORBER, DRIER, HUMIDIFIER and MOLECULAR SIEVE. See chapter 16.6 for safety instructions.

Resting angle,

for resting the light source or LED-array/ PAM fluorometer when not attached to the cuvette of the Standard Measuring Head 3010-S.

Spare Kit 3000-C/SK,

box with spare parts, which are listed and described in chapter 3.4.

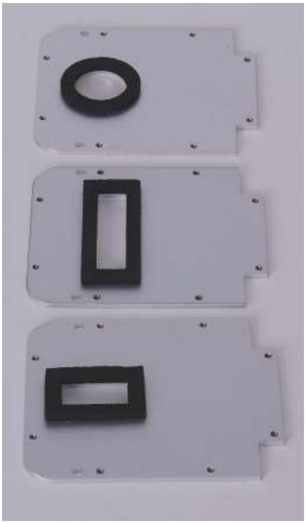
Not included:

CO_2 cartridges 000160103430,

for charging the internal CO_2-container with pure CO_2. Since it is only deliverable with overland transport, it is generally not included. See chapter 13.4.

Optional: Leaf Area Adapter,

for adjusting the leaf area of the Standard Measuring Head 3010-S. Following leaf area adapters are available:



- **3010-2x4:** 2 by 4 cm (included) standard
- **3010-R3:** round, 3 cm^2, for smaller leaves or for leaves with high transpiration rates (see picture, first adapter plate)
- **3010-1x4:**1 by 4 cm, for narrow leaves (see picture, middle adapter plate)
- **3010-2.19x1.14**, area: 2.5 cm^2
- **3010-2.19X1.14L**, area: 2.5 cm^2 lengthwise (see picture, last adapter plate)
- **3010-2.19x1.14C**, area: 2.5 cm^2 excentric in the corner
- **3010-2.58x1.93**, area 5 cm^2 for Imaging-PAM MiniHead with 2/3" camera
- **3010-4.5x2.22** for 10 cm^2 for use with LED Light Source 3041-L.

Mounting Plates,

for mounting the foam gaskets to the leaf area adapters. A matching mounting plate is included with leaf area adapters.

Foam gaskets,

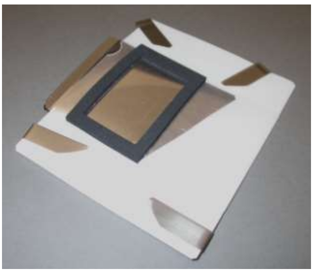
with adhesive tape at one side, fitting onto the leaf area adapters shown above. 10 foam gaskets are included with leaf area adapters. Following sets of foam gaskets are available:

Foam Gaskets 10 cm^2 (2.2 cm x 4.5 cm, 10 pcs, part #: 000244040414)

Foam Gaskets 8 cm^2 (2 cm x 4 cm, 10 pcs, part #: 000244009314)
Silicone Gaskets 8 cm^2 (2 cm x 4 cm, 10 pcs, part # 000244009324)
Foam Gaskets 5 cm^2 (1.93 cm x 2.58 cm, 10 pcs, part #: 000244035814)
Foam Gaskets 4 cm^2 (1 cm x 4 cm, 10 pcs, part #: 000244013214)
Foam Gaskets 3 cm^2 (round, 10 pcs, part #: 000244012914)
Foam Gaskets 2.5 cm^2 (1.14 cm x 2.19 cm, 10 pcs, part #000236406014)

Darkening Plate3010-DP,

which can be clicked onto the upper or lower part of the cuvette via the 2 pins. If the LED Light Source 3041-L or LED-Array/PAM Fluorometer 3056-FL is attached, the Darkening Plate can be used to exclude ambient light from the other side.



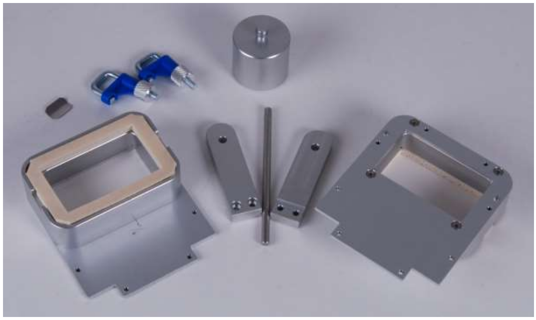
Optional: Darkening Leaf Clamp 3010-DLC,

with sliding windows for dark adaptation of plant leaves and positioning help for insertion into the Standard Measuring Head 3010-S.

Optional:
Spare Thermocouple 3010-CA/TCL,

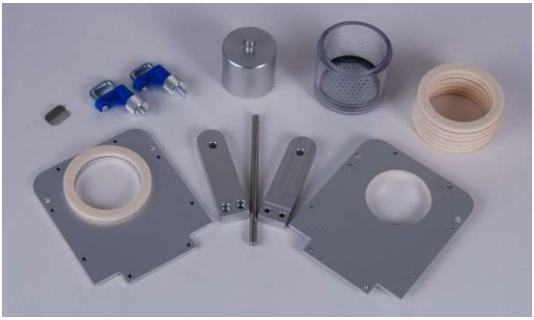
consisting of a thermocouple, cuvette temperature sensor, mounting plate and 4 pins; the unit is usually built into the lower part of the cuvette.

Optional: Cuvette for Conifers 3010-V80,



consisting of two cuvette halves, two distance holders, an extension rod, two hose clamps, and angle for thermocouple. The cuvette for conifers can be used to measure conifers like spruce or twigs with small leaves. (Silicone Gaskets for 3010-V80, 10 pcs, part#:000244014714)

Optional: Cuvette for Lichens/Mosses 3010-V32,



consisting of two adapter plates, two distance holders, an extension rod, two hose clamps, adapter for the tripod, angle for thermocouple and a cylindrical cuvette (polycarbonate) with a perforated steel-plate at the bottom. This cuvette is not only suitable for measuring photosynthesis of detached samples like lichens or mosses but also for measuring respiration of small animals (Silicone Gaskets D35, ∅:3.5 cm, 10 pcs, part#: 000244015514).

Optional: Cuvette for small Petri-Dish 3010-P,

consisting of two adapter plates, one of them closed, two distance holders, an extension rod, two hose clamps, adaptor for mounting the measuring head upside-down on a tripod. (Silicone Gaskets D35, ∅:3.5 cm, 10 pcs, part#: 000244015514)

Optional: Cuvette for Arabidopsis 3010-A,

special cuvette for e.g. small, potted Arabidopsis plants, which can be attached to the Standard Measuring Head 3010-S. The set consists of a foam stand, a cuvette adapter angle, a height-adjustable base plate, 2 pot holders for flowerpots (55-70 mm diameter), a hollow punch, angle for thermocouple, two hose clamps, and silicone gaskets (∅:8.2 cm, 10 pcs, part#: 000244020214)

Optional: Oxygen sensor 3085-O2,

optical oxygen sensor for monitoring O_2 concentrations, can directly be connected to the GFS-3000 gas path. Data logging via USB communication.

Optional: Tripod ST-1010,

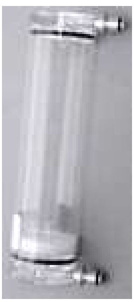
onto which the Standard Measuring Head 3010-S can be mounted. The tripod is chosen so that it has a reasonable height, but still fits into the carrying box of the system.

Optional: Outdoor-Set 3000-C/OS,

consisting of 10 l mixing vessel, 2.5 m fiberglass antenna, tubing with entrance filter, clips, elastic net and antenna holder for running the GFS-3000 in the field, using ambient air.

Optional: Battery Adapter 3200-C/BC

Can be inserted into a battery-slot of the Control Unit 3200-C and has a cable and clamps for external batteries supplying 12 or 24 V battery (e.g. car battery), which have no special connectors. Input voltage for the Control Unit 3200-C via this adapter is 12 - 24 V. Note that there are different battery adapters required for the different Control Unit types.



Optional: Spare Absorber Tube 3000-C/ABS,

for Soda Lime (labeled "CO2 ABSORBER"), Silica Gel (labeled "DRIER"), Humidifying Granules (labeled "HUMIDIFIER") or Molecular sieve 3Å (labeled "MOLECULAR SIEVE"). A filter pad should be used at the top and the bottom. Replacement tube without connectors: part#: 000244004414.

Optional: Fiberoptics/PAM-Fluorometer 3050-F

consisting of a metal tube (1) that encloses the PAM-fluorometer, a fiber (2), an air-tight adapter (3) a holder (4) and a darkening plate (5).

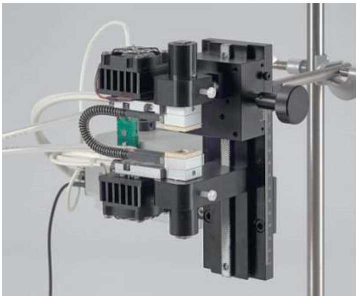
Optional: Adapter Box 3050-F/A,

for splitting the COMP-port of the Standard Measuring Head 3010-S, required to operate the Fiberoptic PAM-Fluorometer 3050-F together with the LED Light Source 3041-L.

Optional: Imaging-PAM

The Imaging-PAM *M-Series* MINI-Head can be connected to the Standard Measuring Head 3010-S using the optional Adapter for GFS-3000 IMAG-MIN/GFS. A complete imaging system is required. The operation will then be controlled via one computer running GFS-Win and Imaging-Win concurrently. Both software will exchange relevant data. (chapter 4.7.8)

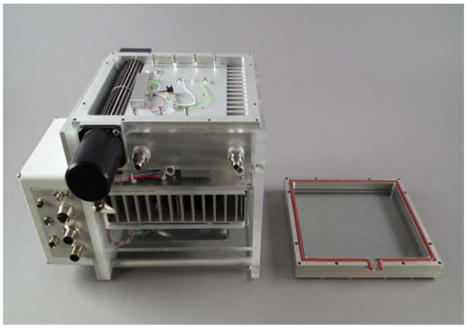
Optional: Dual-PAM Gas-Exchange Cuvette 3010-DUAL,



for the combined use of the GFS-3000 with the Dual-PAM-100 or DUAL-KLAS-NIR (see special manual).

Optional: Gas Exchange Chamber 3010-GWK1,

for large sample sizes or the combined use of the GFS-3000 with the Imaging PAM *M-Series* MAXI-Version (see special manual).



Optional: LED-Panel RGBW-L084

Illumination device containing LED colors red, green, blue and white which can be set individually. Mountable on top of Gas Exchange Chamber 3010-GWK.

3.2 Components and Connectors attached to the Front and other Sides of the Control Unit 3200-C

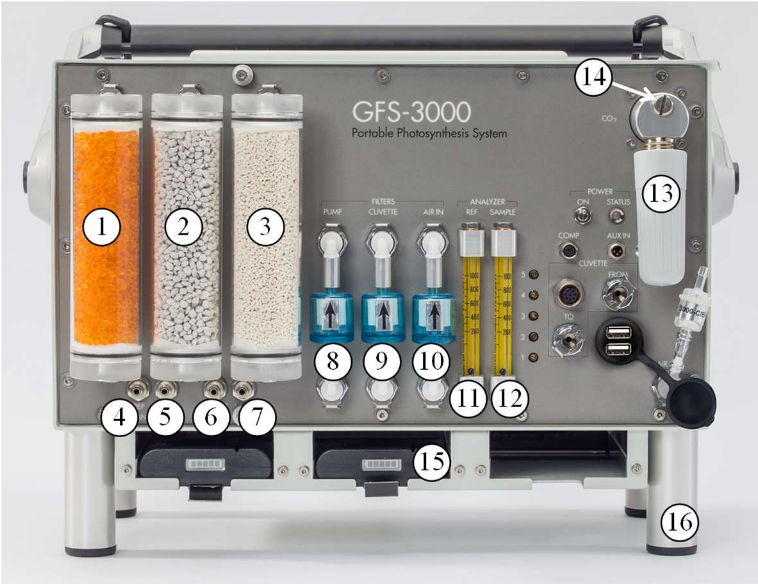


Fig. 2: Components and connectors at the front side

1. Two pneumatic connectors labeled "DRIER" for the absorber tube "DRIER" filled with silica gel.
2. Two pneumatic connectors labeled "HUMIDIFIER" for the absorber tube "HUMIDIFIER" filled with humidifying granules.
3. Two pneumatic connectors labeled "CO2 ABSORBER" for the absorber tube "CO2 ABSORBER" filled with soda lime. If ambient air is used instead of the CO_2 control, this absorber tube must be replaced by the Mixing Volume 40 ml "3000-C/MV".
4. Outlet "ANALYZER"/"REF": outlet of the analyzer reference cells.
5. Outlet "ANALYZER"/"SAMPLE": outlet of the analyzer sample cells.

6. Outlet "SAMPLE": outlet for the measuring gas that is bypassing the analyzer in valve position ZP and through valve 3.
7. Outlet "VENT": outlet for excess air, which is controlled with valve 5.
8. Two pneumatic connectors labeled "FILTERS"/"PUMP" for a Filter 000140301225 mounted via two pneumatic adapters; the filter is integrated in the pneumatic pathway downstream from the gas supply consisting of pump, H_2O control and CO_2 control.
9. Two pneumatic connectors labeled "FILTERS"/"CUVETTE" for a Filter 000140301225 mounted via two pneumatic adapters; the filter is integrated in the pneumatic path between the cuvette and the sample cells of the analyzer.
10. Two pneumatic connectors labeled "FILTERS"/"AIR IN" for a Filter 000140301225 mounted via two pneumatic adapters; the filter is integrated in the pneumatic path directly behind the Entrance Filter 5 ml "3000-C/EF".
11. Flow indicator labeled "ANALYZER"/"REF" measuring the flow upstream of the reference cells of the analyzer.
12. Flow indicator labeled "ANALYZER"/"SAMPLE" measuring the flow upstream of the sample cells of the analyzer.
13. CO_2 cartridge holder for refilling pure CO_2 to the CO_2 container inside the control unit with a small CO_2 cartridge.
14. Inlet, which must be closed by a screw; if CO_2 cartridges are used. If a CO_2 cylinder should be connected, the screw must be replaced by the hose adapter contained in the Spare Kit 3000-C/SK (chapter 3.4)
15. Three equivalent battery slots for up to three Li-ion eSMART batteries, the 3200-N power supply or the 3200-C/BC battery adapter.
16. Short feet, which are suitable, if the control unit is placed on a table. If the control unit is placed on the ground, it is recommended to mount the legs to the short feet.

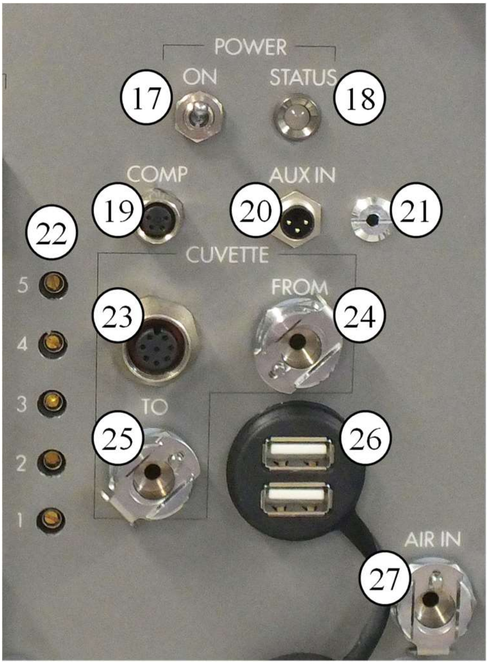


Fig. 3: Components and connectors at the front side (continued)

17. Switch "POWER"/"ON": the control unit is switched on, if the STATUS LED is lit up or blinking.

18. LED "POWER"/"STATUS" indicating the status of the battery control of the GFS-3000. The battery control manages the power input and distribution within the GFS-3000. If the LED is flashing green with a frequency of 1 Hz or 2 Hz, it is working well (for LED code see chapter 16.1)

19. Connector "COMP" to which the 3010-I/BOX for operation with an external computer can be connected (for pin assignment see chapter 16).

20. Connector "AUX IN" (for pin assignment see chapter 16), to which two sensors with a voltage output between 0 ... 4095 mV can be connected using the Cable for 2x Auxiliaries 000130606205 (chapter 3.1 and 16.5).

21. Outlet of safety valve CO_2 container.

22. Valves labeled from "1" to "5" for adjustment of flow through the different air pathways (chapter 12.2).
23. Electronic connector "CUVETTE",to which the electronic cable of the Measuring Head is connected. Different configurations of the measuring head exist; the configuration must be selected within the software.
24. Pneumatic connector "CUVETTE"/"FROM", to which the tube "FROM" of the measuring head is connected.
25. Pneumatic connector "CUVETTE"/"TO", to which the tube "TO" of the Measuring Head is connected.
26. Two electronic USB-connectors of the internal panel PC. Here a USB memory stick can be connected to directly collect data or download collected data. A USB keyboard or mouse can be connected here. To connect the internal PC with an external PC do not use a Standard USB-cable. It would create an electrical shortage between both USB ports causing damage. An optional "USB Null-Modem Cable" marked with NMC at its connectors may be used (chapter 3.1).
27. Pneumatic connector "AIR IN", where the air is drawn into the system and to which the Entrance Filter 5 ml 3000-C/EF or the Outdoor-Set 3000-C/OS should be connected.
28. Carrying handle, which can be locked in several positions? For transport, lock horizontal in transport box.

Fig. 4: Components attached to the top and to the sides

29. Push buttons on each side, which must be pushed to disable the lock mechanism and move the handle
30. Two rings for mounting the carrying belt (chapter 3.1)
31. Integrated panel PC with touch panel (see next chapter 3.2.1)
32. Clip holding the PDA pen

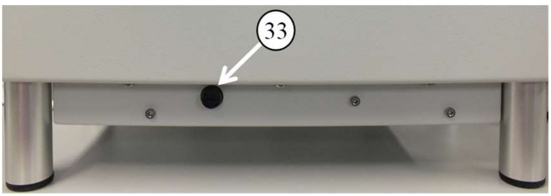


Fig. 5: Components attached to the back

33. Fuse holder for fuse 10.0 AT (Control Unit 3200-C and 3100-C, do not use another type of fuse, there are replacement fuses in the spare kit); (use 8.0 AT for Control Unit 3000-C sold until 2012).

3.2.1 Integrated Panel PC

The integrated panel PC operates the GFS-3000 via the GFS-Win software. It has a color display with touchscreen and a Windows operating system for embedded systems. When the GFS-3000 is switched on, it boots, and GFS-Win starts automatically. Do not install any software that is not recommended by the Heinz Walz GmbH. The panel PC has no virus protection, check any memory stick before insertion. The following software is preinstalled:

C:\GFS3000\GFS-Win\GFS-Win.exe
C:\GFS3000\GFS-Win\OxygenSensor.exe (for optional O_2-sensor)
C:\GFS\Backlight\BL.exe (for backlight of screen)
C: \GFS3000\GFS-Win\Keyboard.exe (onscreen keyboard)
C:\Program Files\Touchside\XTouchMon.exe (touchscreen adjust)
Drivers for USB-Port, Touch Screen, WIFI Adapter.

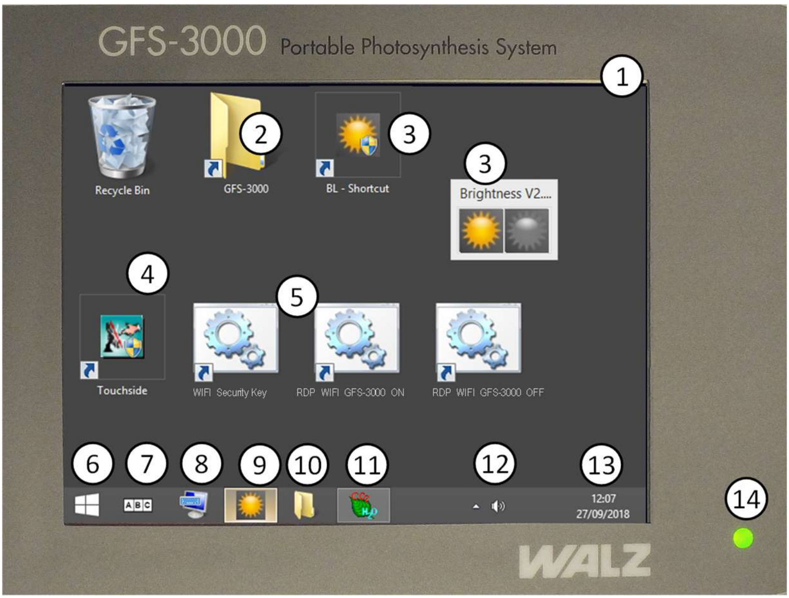


Fig. 6: Integrated panel PC, view on Desktop, GFS-Win is minimized

1. Screen with touch panel. Only operate the touch panel with the provided soft touch pen or a finger. A short tick is equivalent to a left mouse-button click. A long tick is equivalent to a right mouse-button click.
2. Shortcut to the folder "MyDocuments/GFS-3000". This is the standard folder for the stored data and all user programs.
3. Shortcut to the program BL for regulating the brightness of the display and Program window for the adjustment of display brightness.
4. Shortcut to the program Touchside for adjustment of the touchscreen. If the touchscreen is maladjusted it may be necessary to connect a keyboard to the USB-port to access Touchside. If GFS-Win is running the key combination Crtl + T can be used to start Touchside.
5. Three program scripts for WIFI operation of the GFS-3000 via Remote Desktop Connection. (chapter 16.9).
6. Windows Start Button on taskbar
7. Taskbar-Shortcut to Walz-Onscreen-Keyboard
8. Taskbar-Shortcut to Windows-Onscreen-Keyboard
9. Taskbar-Shortcut to Display Brightness Adjustment (program BL)
10. Taskbar-Shortcut to Windows Explorer
11. Taskbar-Shortcut to minimized GFS-Win. For minimizing GFS-Win see Fig. 29. The GFS-Win software for operating the instrument is explained in chapter 7.
12. Adjustment of speaker volume
13. Date and Time display and adjustment
14. Panel PC LED, green: on, orange: accessing hard drive (chapter 16.1)

3.3 Components and Connectors of the Standard Measuring Head

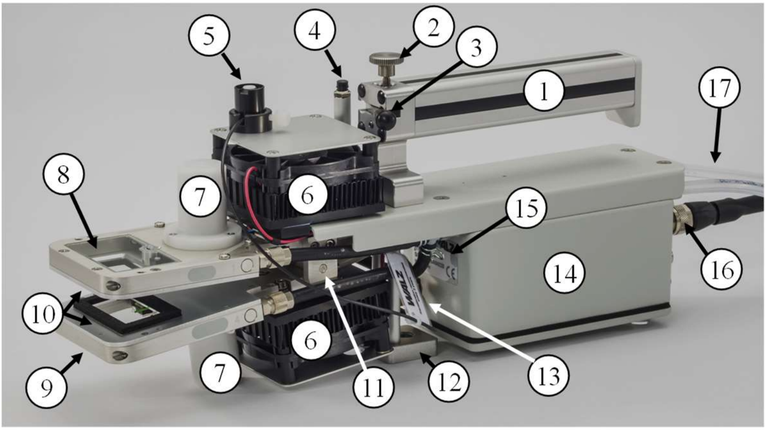


Fig. 7: Components of the Standard Measuring Head 3010-S

1. Handle
2. Closing mechanism: To close the cuvette, the knurled screw (2) needs to be pushed down until the lever (3) engages. The knurled screw can be used to increase or decrease the pressure on the foam gaskets (chapter 4.5.1.)
3. Opening mechanisms which must be pushed forward to open the cuvette (chapter 4.5.1.)
4. Remote push button to initialize a user program or storage of a data record
5. Miniature Quantum Sensor MQS-B/GFS for measuring ambient photosynthetically active radiation (PARamb)
6. External heat exchanger attached to the Peltier elements with fan and cover
7. Motor driving an internal impeller (radial fan) for each cuvette half
8. Upper cuvette frame with large sized window made of glass

9. Lower cuvette frame with large sized window made of glass
10. Leaf Area Adapters with foam gaskets
11. Spacer at each side fixing the lower cuvette half
12. Tripod adapter
13. Connector for the Miniature Quantum Sensor MQS-B/GFS
14. Electronics box controlling the Standard Measuring Head 3010-S
15. Label attached to the electronics box with serial number of Standard Measuring Head 3010-S
16. Connection cable (part#: 000130606210) between Standard Measuring Head 3010-S and Control Unit 3200-C or older control units (the cable should remain connected to the electronics box of the measuring head)
17. Tubes guiding the air from the control unit to the measuring head and backwards.

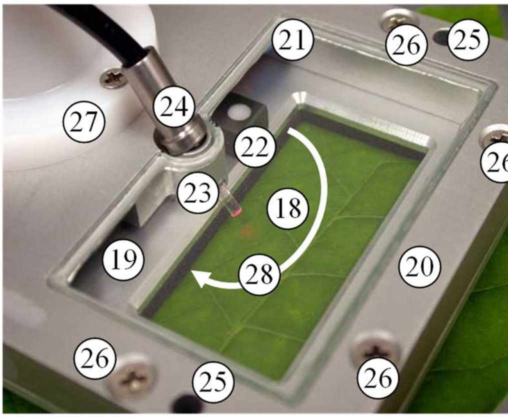


Fig. 8: View from the top onto the upper half of the cuvette

18. Enclosed leaf area
19. Leaf Area Adapter 8 cm^2 attached to the upper frame
20. Upper frame of the cuvette

21. Large sized window made of glass
22. Light sensor (PARtop) inside the upper cuvette half measuring the light intensity incident on the upper leaf area (a light sensor is also available in the lower cuvette half).
23. Adapter for mounting a fiber optics for simultaneous measurement of gas exchange and fluorescence under ambient light conditions (if no fiber optics is inserted the port must be closed by a Perspex screw)
24. Fiber optics 1.5 mm in diameter of a PAM fluorometer (optional)
25. Two holes with click mechanism inside for attaching the LED Light Source 3041-L or the LED-Array/PAM-Fluorometer 3056-FL (the click mechanisms are adjusted from the front side of the frames)
26. 8 screws with which the leaf area adapter is mounted to the upper frame of the cuvette (4 of 8 screws can be seen)
27. Hood for the motor driving the impeller (radial fan) inside the upper half of the cuvette
28. Arrow showing the air flow direction inside the upper cuvette half

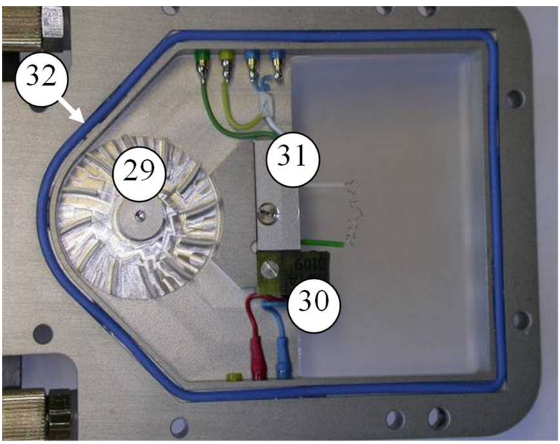


Fig. 9: View into the lower half of the cuvette (leaf area adapter is removed)

29. Impeller (radial fan) inside the cuvette driven by an external motor

30. Internal light sensor sitting on a small board, which is mounted to the frame via a small screw. The internal light sensor is connected via two pins.

31. Thermocouple 3010-CA/TCL consisting of a thermocouple and a cuvette temperature sensor. The measuring side of the thermocouple is directed towards the opening for the leaf, the reference side is glued onto the cuvette temperature sensor.

32. O-ring for sealing between leaf area adapter and cuvette

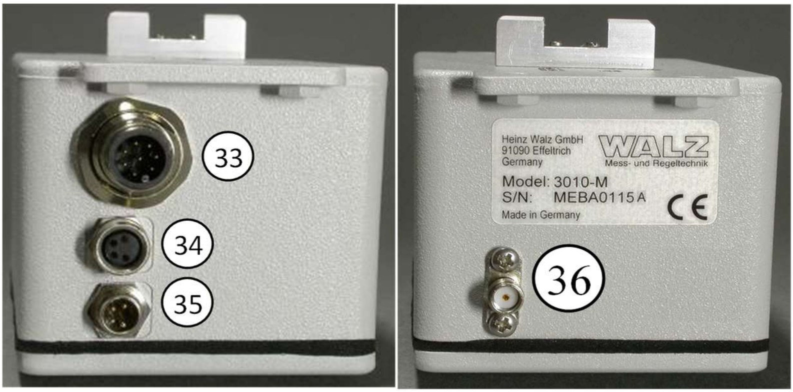


Fig. 10: Connectors at the rear side and front side of the electronics box

33. Connector for the connecting cable between Standard Measuring Head 3010-S and Control Unit. The cable usually remains connected to the measuring head.

34. Connector for a cable of the LED light source or LED-Array/PAM-Fluorometer. Also, an additional component which has the same pin assignment as the connector "COMP" at the front side of the Control Unit can be connected here e.g. 3010-I/BOX.

35. Connector for a cable of the LED light source or LED-Array/PAM-Fluorometer, provides power for the actinic light and fans.

36. Connector for the External Miniature Quantum Sensor MQS-B/GFS

3.4 Content of the Spare Kit 3000-C/SK

Fig. 11: Content of the box Spare Kit 3000-C/SK

1. Syringe 5 ml for adding water to the humidifying granules inside the absorber tube HUMIDIFIER
2. 8 spare screws for mounting the leaf area adapters
3. 1 spare fuse 5x20 mm 10AT (part #: 000130401275) for Control Unit 3200-C
4. Hose connector straight 6.4 mm (Part #: 000140701956) for the use at the upper connector "FILTER PUMP" during H2Oabs span calibration (chapter 12.3.6).
5. Adapter for CO_2 cylinder including sealing. It replaces the screw at the CO_2 unit, if CO_2 is to be supplied from a cylinder instead of small cartridges.
6. 4 tube fitting nuts belonging to the pneumatic connectors 4, 5, 6, 7 in Fig. 2.
7. Two hose connectors straight 4 mm (part #: 000140701955) for tubes with an inner diameter of 4 mm.
8. Spare filter (part #: 000140301225) for PUMP, CUVETTE and AIR IN

9. Spare PDA pen (part #: 000160201311) for operating the touch panel of the panel PC.

3.5 Li-Ion eSMART Battery Charger and Li-Ion eSMART Batteries

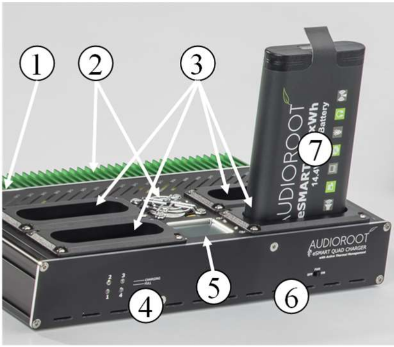


Fig. 12: Li-Ion eSMART Battery Charger (part #: 000190101115) with Li-Ion eSMART Battery (part #: 000160101434)

1. Mains input socket and On/Off switch
2. Heat exchanger and fan
3. Four independent battery charging bays
4. Status LEDs
5. OLED display with battery information
6. Switch for noise and thermal management (fan on/off)
7. Li-Ion eSMART Battery 98 Wh (part #: 000160101434),
   the standard system comes with four eSMART Batteries 98 Wh

   For operation and safety, see chapter 13.1 and 16.6.5

4 Principle of Operation

4.1 General Principle

The GFS-3000 consists of an open gas pathway (Fig. 13), which means that the air is constantly pumped through the unit. Its composition, in particular the CO_2 and H_2O content, can be precisely regulated. After mixing the air, it is divided into two pathways, the measuring and reference gas pathways. The measuring gas flows to the measuring head containing the sample, while the reference gas flows directly to the reference side of the analyzer. The flow rate to the measuring head is precisely regulated. Inside the measuring head the climate is controlled, especially temperature, light and ventilation. During photosynthesis, the sample takes up CO_2. For this purpose, it opens the stomata, which leads to a release of H_2O. These actions generate a concentration difference in CO_2 and H_2O between the measuring gas and the reference gas. Together with the flow rate, these concentration differences are used to calculate photosynthetic CO_2 assimilation and H_2O evaporation of the sample – usually a plant leaf or lichen. Further parameters, like the leaf temperature or chlorophyll fluorescence allow the deduction of further photosynthetic parameters. The system will be explained in more detail in the next chapters.

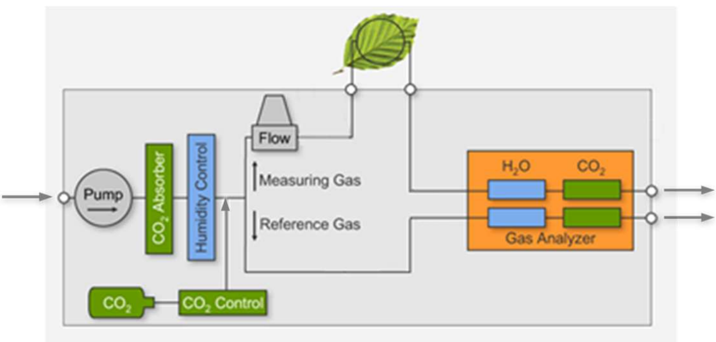


Fig. 13 Simplified diagram of GFS-3000 with the basic principle of operation

4.2 Pneumatic Diagram of the GFS-3000

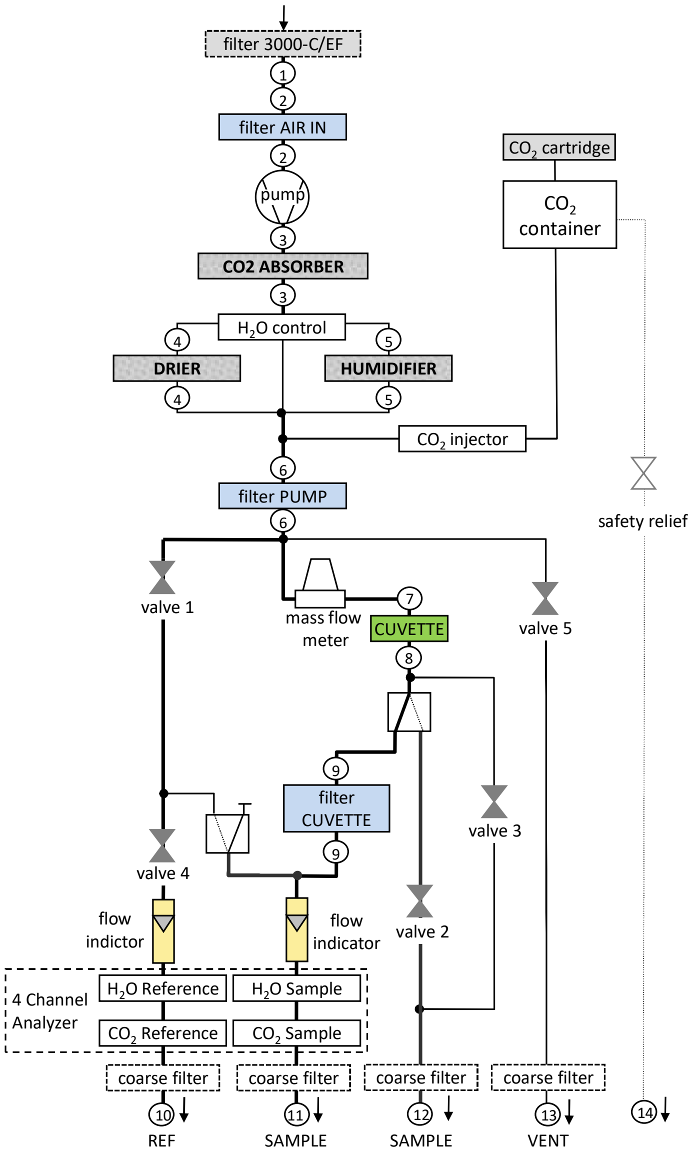
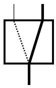


Fig. 14 Pneumatic diagram of GFS-3000 with Control Unit 3200-C

Component	White background: components inside the control unit
Component (filled)	Filled background: components attached to the front side of the control unit


Solenoid valve (solid line indicating the resting position)

(#) Pneumatic connectors at the front side of the Control Unit 3200-C with numbering as follows:

(1) Pneumatic connector labeled "AIR IN"
(2) 2 pneumatic connectors labeled "FILTERS" / "AIR IN"
(3) 2 pneumatic connectors labeled "CO2 ABSORBER"
(4) 2 pneumatic connectors labeled "DRIER"
(5) 2 pneumatic connectors labeled "HUMIDIFIER"
(6) 2 pneumatic connectors labeled "FILTERS" / "PUMP"
(7) Pneumatic connector labeled "CUVETTE" / "TO"
(8) Pneumatic connector labeled "CUVETTE" / "FROM"
(9) 2 pneumatic connectors labeled "FILTERS" / "CUVETTE"
(10) Pneumatic connector labeled "ANALYZER" / "REF"
(11) Pneumatic connector labeled "ANALYZER" / "SAMPLE"
(12) Pneumatic connector labeled "SAMPLE"
(13) Pneumatic connector labeled "VENT"
(14) Pneumatic outlet for safety relief valve of CO_2 container

4.3 Description of Pneumatic Path and its Components

The pneumatic path of the GFS-3000 shown in Fig. 14 has the following parts and functions:

- Air is drawn in passing a coarse filter "labeled "3000-C-/EF", which keeps away particles and small animals and reduces the noise of the pump.
- The filter is connected to the pneumatic connector "AIR IN"
- A fine filter connected to the pneumatic connectors labeled "FILTERS" / "AIR IN" follows. "It keeps away fine particles in the incoming air".
- The pump generates the flow through the system. The pump is controlled, so that the flow measured by the mass flow meter "matches its set value.
- The pump is followed by the CO_2 absorber, which removes all CO_2 of the incoming air. For experiments with ambient air, the CO_2 control must be switched off and the absorber tube "CO2 ABSORBER" must be replaced by the MIXING Volume 40 ml "3000-C/MV" for ambient air also a 10-liter volume needs to be connected (chapter 13.11).
- The CO_2 free or ambient air is entering the H_2O control valve". For experiments with ambient air, the H_2O control must be switched off. In this case the air is going straight through the H_2O control valve without passing the drier or humidifier. Note that the air has only ambient humidity, if there is no CO_2 absorber connected. If the H_2O control is switched on, the air will partly be forced through the drier or humidifier, so that the H_2O value measured by the H_2O reference cell of the analyzer (H2Oabs) matches its set value.
- The CO_2 injector follows. If the CO_2 control is switched off, the CO_2 control valve will be closed. If the CO_2 control is switched on, pure CO_2 will be added via the CO_2 control valve, so that the CO_2 value measured by the CO_2 reference cell of the analyzer (CO2abs) matches its set value. The CO_2 is usually supplied by small CO_2 cartridges.
- A fine filter " connected to the pneumatic connectors labeled "FILTERS" / "PUMP" removes the dust released by the pump and the absorbers. The

filter sits in the end of the mixing part and the pneumatic pathway. The following gas pathways belong to the analyzing part.

- After this filter, the flow is split into reference and measuring gas.
- Two solenoid valves are integrated in the analyzing pathway. The pneumatic diagram shows their resting position, which is called MP mode (mode for recording measuring points). The pneumatic pathway is now described in MP mode.
- The analyzer has four cells and can measure 2x CO_2 absolute mole fraction and 2x H_2O absolute mole fraction. The cells are called "CO_2 Reference", "CO_2 Sample", "H_2O Reference" and "H_2O Sample".
- The reference gas is passing valve 1 and valve 4 (restrictor valves), a flow indicator follows. The reference gas flows through the analyzer cells "H_2O reference" and "CO_2 reference". The measured values (CO2abs and H2Oabs) are used for the H_2O and CO_2 control. A coarse filter follows before the reference gas leaves the system through the outlet "ANALYZER" / "REF".
- The measuring gas passes the electronic mass flow meter, which is also used to control the pump speed. The controlled flow then passes through the cuvette of the measuring head connected to the pneumatic connectors labeled "CUVETTE" / "TO" and "CUVETTE" / "FROM". A solenoid valve follows, which in the resting position (MP mode) guides the measuring gas through the filter labeled "FILTERS" / "CUVETTE". The filter removes any spores released by the sample or any dust particles, which might have entered the cuvette. A mechanical flow indicator follows. If the cuvette is not air tight, it will show a decreased flow rate. The measuring gas flows through the analyzer cells "H_2O sample" and "CO_2 sample". The difference between sample cell and reference cell is calculated (dH2O, dCO2) displayed and stored, see chapter 4.3.1 and chapter 9.1 to 9.5. The measuring gas is leaving through the outlet "ANALYZER" / "SAMPLE".
- Valve 5 controls a bypass after the filter "PUMP". It shall be opened with low flow rates to increase the speed in the mixing part. Valve 3 opens a bypass after the cuvette and therefore controls the flow rate of measuring

gas through the analyzer. It shall be open with high flow rates (chapter 12.2). The measuring gas leaves the system at the outlet "SAMPLE"

4.3.1 Definition of CO2abs, H2Oabs, dCO2, dH2O

The analyzer has four cells and can measure two different CO_2 absolute mole fractions and two different H_2O absolute mole fractions simultaneously. The cells are called "CO_2 reference", "CO_2 sample", "H_2O reference" and "H_2O sample". In the software, the values measured in the cells "CO_2 reference" or "H_2O reference" are referred to as CO2abs or H2Oabs (chapter 10 and 11). The values measured in the cells "CO_2 sample" and "H_2O sample" are neither displayed nor stored. Instead the difference between sample cell and reference cell is calculated, displayed and stored:

$dH_2O = H_2O$ sample – H_2O reference
$dCO_2 = CO_2$ sample – CO_2 reference

For more detailed equations see chapter 9.3 and 9.5. In the software the values dH2O and dCO2 are only displayed in the *chart* (chapter 7.4.2). In the *values table* (chapter 7.4.3) and in the *report* (chapter 7.4.4) H2O and dCO2 have the additional identifiers MP or ZP, which indicate the situation of the gas pathway during the measurement. MP is a measuring point where the measuring gas flows to the sample side of the analyzer after it has passed the sample, while ZP is a differential zero point at which both sides of the analyzer are supplied with the same gas. The differential ZP is dependent on the absolute gas concentration and is inherent in the analyzer (*see* next chapters for more detailed information 4.3.2 to 4.3.4). It drifts a bit with changing temperature.

4.3.2 MP mode and ZP mode

The system has two solenoid valves which can be switched between the modes MP (measuring point) and ZP (zero point).

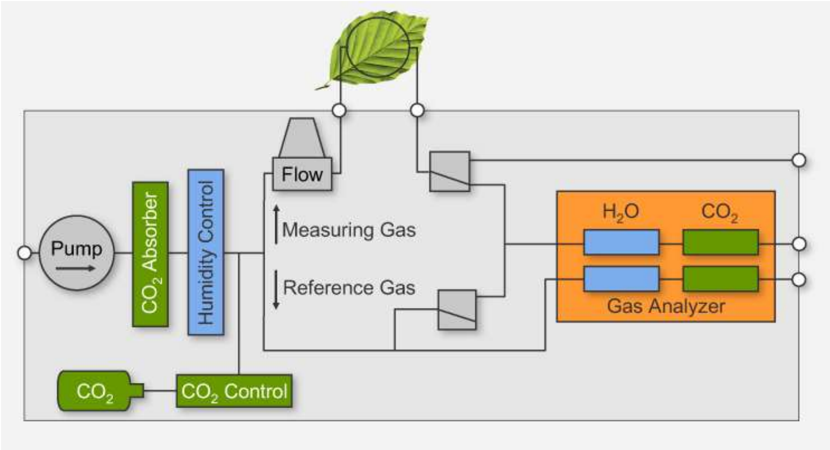


Fig. 15: Simplified pneumatic diagram with solenoid valves in MP mode

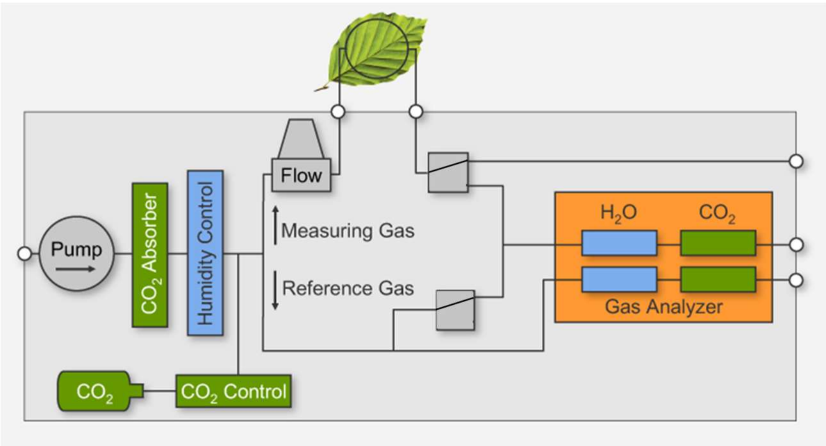


Fig. 16: Simplified pneumatic diagram with solenoid valves in ZP mode

Fig. 15 shows the pneumatic diagram with the solenoid valves in MP mode. In MP mode the reference gas is flowing through the reference cells and the measuring gas is flowing through the sample cells of the analyzer.

Fig. 16 shows the pneumatic diagram with the solenoid valves in ZP mode. In ZP mode, the reference gas is split so that it supplies both sides of the analyzer equally. Both flow indicators (Fig. 14) indicate a lower flow than in MP mode. The measuring gas continues to flow through the cuvette, exposing the sample to the same gas conditions as in MP mode but bypassing the analyzer. ZP-values are close to zero, they are concentration differences inherently indicated by the analyzer.

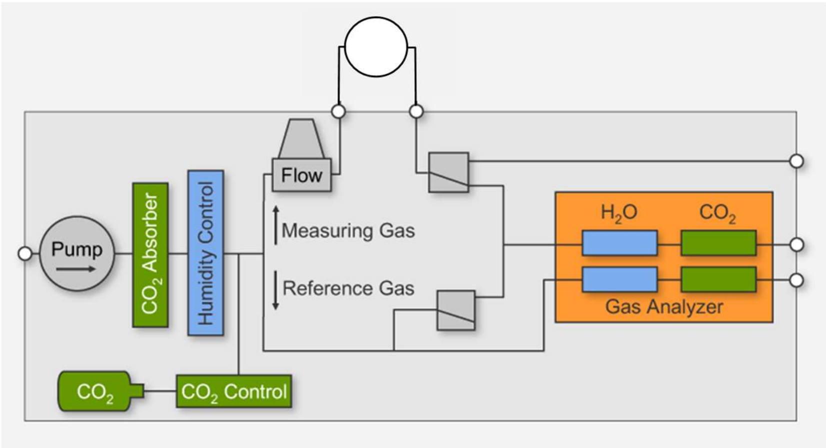


Fig. 17: Simplified pneumatic diagram illustrating ZPcuv. Solenoids are in MP mode, but the sample cuvette is empty.

Fig. 17 illustrates ZPcuv. ZPcuv is a differential zero point, measured in MP mode, but without sample. Again, the same gas is present in both sides of the analyzer.

Before measuring MP-values, a ZP-value must be measured and stored so that it can be taken into account for the calculation of the photosynthesis parameters (see next chapters and chapter 9.7. and 9.10).

4.3.3 Values dCO2ZP, dH2OZP, dCO2MP and dH2OMP displayed in the Values window

In the *Values window* (chapter 7.4.3) the actual values are shown. If the system is in MP mode, the measured values dCO2 and dH2O are displayed as dCO2MP and dH2OMP. The field dCO2ZP and dH2OZP display the last values stored for dCO2ZP and dH2OZP (chapter 4.3.4).

If the system is switched to the ZP mode, the measured values dCO2 and dH2O are displayed under dCO2ZP and dH2OZP, while the field dCO2MP and dH2OMP have no value.

4.3.4 Values dCO2ZP, dCO2MP, dH2OZP and dH2OMP stored in a Data Record

Each stored data record includes the values dCO2ZP, dCO2MP, dH2OZP and dH2OMP and a code. The codeindicates MP or ZP and the averaging time in seconds of the stored data record. It further specifies which method has been used to measure any ZP points with the code ZPi or ZPc.

4.3.4.1 Storing a Zero point "ZPi" or "ZPirga"

To measure a ZPi, the flow and the concentrations must be set-up, the cuvette must be closed (with or without sample). The system must be switched to ZP mode and a steady state for the CO2abs, H2Oabs dCO2 and dH2O concentrations must be reached. Then a data record, which is called zero point (ZP), can be stored. The values dCO2 and dH2O will be stored in the data record as dCO2ZP and dH2OZP; it will be annotated ZPi in the report-column *code* (chapter 11). The letter i stands for infra-red gas analyzer. The stored values CO2abs and H2Oabs are the absolute CO_2 and H_2O mole fraction of the reference gas. Since the reference gas is split, the stored values dCO2ZP and dH2OZP represent the differential offset inherent in the analyzer for the current concentration. In mode ZP, there are no values for dCO2MP or dH2OMP.

4.3.4.2 Storing a Measuring point "MP

To record a measuring point (MP), the system must be set-up, a ZP must have been measured, a sample must be inserted in the measuring head and the system must be in MP mode. After a steady state has been reached, an MP can be stored. The stored values CO2abs and H2Oabs give the absolute CO_2 and H_2O mole fraction of the reference gas. The stored values dCO2MPand dH2OMP indicate the CO_2 uptake and H_2O release by the enclosed sample plus any offset inherent in the analyzer. The values for dCO2ZP and dH2OZP will be carried forward from the last ZP measurement. From such an MP data record the true dCO2 and true dH2O can be calculated as follows:

true dCO2 = dCO2MP - dCO2ZP
true dH2O = dH2OMP - dH2OZP

The true dCO2 and true dH2O are never indicated, but always calculated for the calculation of the gas exchange parameters. For more detailed equations see chapter 9.3 and 9.5. The calculations of gas exchange parameters are done automatically (chapter 9).

4.3.4.3 Storing a Zero point "ZPc" or "ZPcuv"

If the system is running in MP mode and no leaf is enclosed in the cuvette, a ZPcuv can be stored by clicking the Store ZPcuv button. In this case, the values dCO_2 and dH_2O (displayed in the *Values window* as dCO2MP and dH2OMP) will be stored in the data record under dCO2ZP and dH2OZP. The data record is labeled with ZPc, where c stands for cuvette. It will be used for the calculation of the true dCO2 and dH2O in the same way as ZPi.

The data record ZPc contains the offset of the analyzer and additionally absorption and desorption effects of the cuvette. Therefore, the accuracy of the measurement can be increased by measuring a ZPc instead of a ZPi.

4.3.5 Recommendation for Zero points (ZP)

In the beginning always a ZP must be measured, because the calculations of the gas exchange parameters depend on both, the MP and the ZP. After one ZP a lot of MPs can follow. It is recommended to measure one ZP every hour or whenever the absolute CO_2 or H_2O concentration has been changed. ZPs may be measured less often, if the temperature surrounding the instrument is rather constant. If the leaves are enclosed for a short time only, the cuvette is empty in the meantime and it is recommended to measure a ZPc instead of a ZPi to increase accuracy (chapter 4.3.4: remark on Storing a Zero point "ZPc"). If the leaf is enclosed for long-term experiments, or if the CO_2 or H_2O concentration is changed during the experiment, it is recommended to measure a ZPi. A ZPc always requires manual operation because the cuvette cannot be emptied automatically. The measurement of ZPi can be carried out fully automatically with user programs or in automatic measurement sequences that measure a ZPi followed by a defined number of MPs (this cycle will be repeated). Switching between MP and ZP may disturb the CO_2 control. This can be minimized by good adjustment of valve 2 (chapter 12.2). ZPs and MPs should only be measured with stable CO_2 and H_2O concentrations.

4.4 Recommendations for Humidity Control

Always avoid condensation in the cuvette or tubes. Fig. 18 shows the relationship between temperature and humidity.

The cuvette temperature needs to be chosen so that the humidity remains below 100% at the coldest location within the cuvette, which may be up to 2°C cooler than the cuvette temperature, if the system is cooling with full power. Condensation within the tubes may occur, if the cuvette temperature is warmer than the environment of the instrument, or if the tubes touch a cold ground. Use the Tamb sensor to measure the temperature dependence on height.

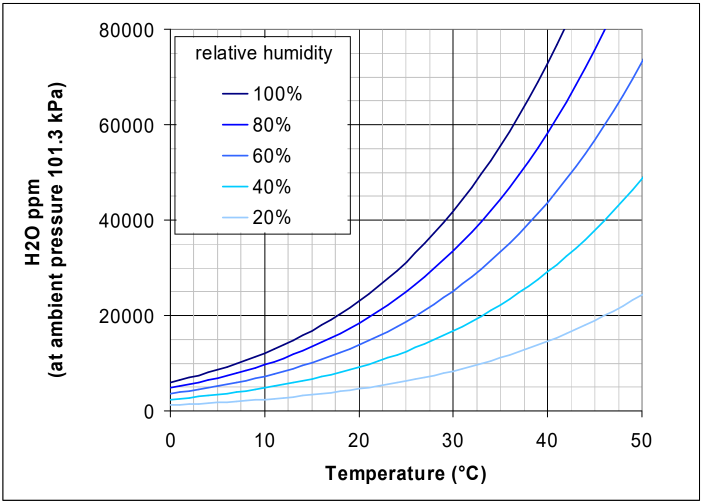


Fig. 18: Relationship between mole fraction H_2O (ppm), cuvette-temperature and relative humidity. See chapter 19 for precise values.

When selecting the H_2O set value, note that it is the humidity entering the chamber that is controlled. Therefore, consider the additional H_2O caused by evaporation of the sample. Also take into consideration, that evaporation is very low, when the surrounding humidity is high. The calculation of some photosynthetic parameters is only possible with sufficient evaporation. Without evaporation the stomatal conductance and the intercellular CO_2 mole fraction cannot be calculated (division by zero or a value close to zero), see chapter 9.11 for equations.

4.5 Measuring Head

4.5.1 Closing Mechanism

The closing mechanism of the Standard Measuring Head 3010-S is shown in Fig. 19. To close the measuring head, the knurled screw needs to be pushed down until the leaver snaps into place. The knurled screw can be used to increase or decrease the pressure on the foam gaskets. If the leaver does not snap in, loosen the knurled screw (counter-clockwise), until the lever locks. Then fasten the knurled screw to increase the pressure on the foam gaskets. To open the measuring head, push the leaver forward. It might be necessary to firstly loosen the knurled screw. When the measuring head is not in use, loosen the knurled screw or keep the measuring head open to allow the foam gaskets to expand.

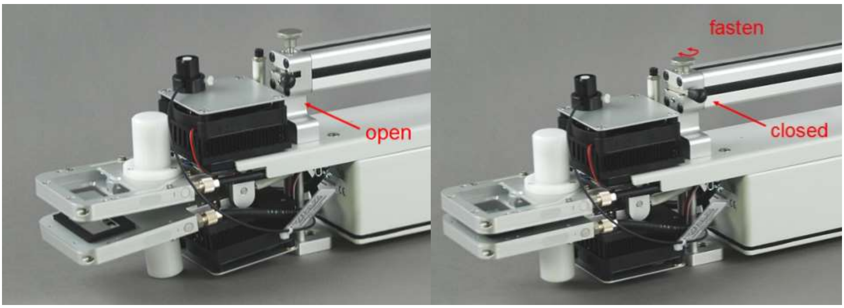


Fig. 19: Closing mechanism of Standard Measuring Head 3010-S.

While closing the measuring head, watch the flow indicators of ANLAYZER SAMPLE in MP mode. The small beads should move upwards so that both flow indicators have the same height (also see valve adjustment chapter 12.2). Do not apply to much pressure on the gaskets, rather use Terostat-IX® (Teroson, Henkel) near a protruding leaf vein to achieve a good sealing.

4.5.2 Ventilation

The Standard Measuring Head 3010-S has one impeller in each cuvette half. Their motors are located under the white covers. For gas exchange measurements or temperature control, the impellers always need to be switched on. The speed can be adjusted in 9 steps, standard setting is 7.

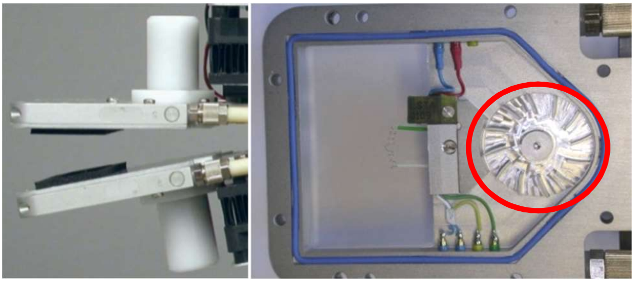


Fig. 20: Impellers: left: white covers; right: inside view, impeller marked with a red circle.

4.5.3 Temperature Control

The Standard Measuring Head 3010-S has four temperature sensors (see Fig. 21): Three Pt100-sensors, *Tcuv*, *Ttop* and *Tamb*, and one thermocouple for *Tleaf*.

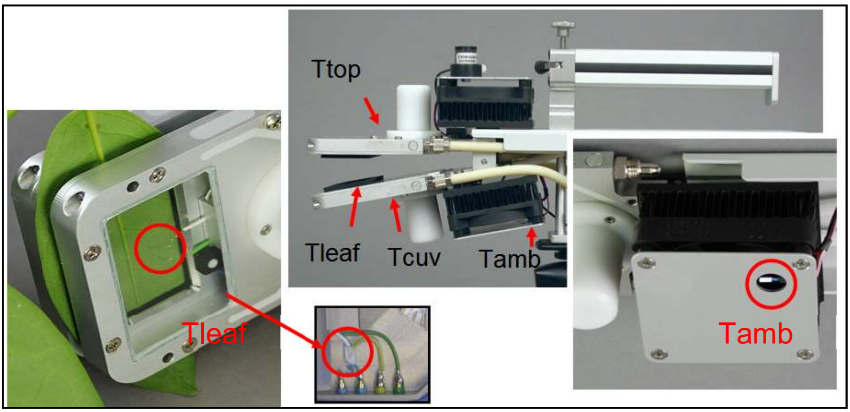


Fig. 21: Standard Measuring Head 3010-S, location of temperature sensors.

Tcuv and *Ttop* are located at the exits of the cuvette halves. *Tamb* is located outside and measures the ambient temperature. Pt100-sensors measure absolute temperature, while thermocouples measure a differential temperature signal. The leaf temperature thermocouple measures the difference between the temperatures at its tip on the leaf and the reference at *Tcuv* (see red circles on the left in Fig. 21). The leaf temperature is the sum of these two signals and is indicated as *Tleaf*. The temperature control of the Standard Measuring Head 3010-S operates with Peltier elements. They are located under the black heat exchangers outside the measuring head. Avoid any force or mechanical stress on these heat exchangers. Three control modes are available: *Tcuv* constant, *Tleaf* constant or *Follow Tamb* with constant temperature offset (see software description, chapter 7.4.1.4).

4.5.4 Light Control

The Standard Measuring Head 3010-S has three PAR sensors: two small sensors, which are located inside the cuvette in the upper (*PARtop*) or lower (*PARbot*) cuvette half; and a cosine corrected Mini Quantum Sensor MQS-B/GFS which is located outside (*PARamb*).

Fig. 22: Standard Measuring Head 3010-S, location of PAR sensors.

The light intensity of the LED Light Source 3041-L (next chapter) or the LED-Array/PAM-Fluorometer 3056-FL (chapter after next) is regulated with these light sensors. There are several modes available. The most commonly used mode is *PARtop*, where the power for the light source is adjusted until

PARtop, measures the given value (see Fig. 22). Since the sensor is located next to the leaf, where the light is lower than at the leaf surface, a so-called light-source factor is applied. The value for *PARtop* is internally multiplied with this light-source factor, whenever the light is controlled with *PARtop* (chapter 7.4.1.4). The light-source factor is stored in the LED Light Source 3041-L or in the LED-Array/PAM-Fluorometer 3056-FL. For compatibility reasons, it is also stored in the measuring head. See chapter 12.5 for the adjustment of the light-source factor and chapter 4.6 about the LED-Light Source 3041-L for further details.

If the light source is connected to the lower cuvette frame, for example, when using the *Arabidopsis* chamber, the light must be controlled with *PARbot* in the same way as with *PARtop*. In *PARbot* mode the measured *PARbot* value is multiplied with the light-source factor and then indicated.

Fig. 23: Standard Measuring Head 3010-S with LED Light Source 3041-L and darkening plate 3010 DP attached.

The other two options for light regulation are *PARamb* and *Follow PARamb*. The regulation mode *PARamb* is only sensible, if the external sensor (*PARamb*) is placed underneath the light source. In the regulation mode *Follow PARamb* the light value measured externally with the *PARamb* sensor is emulated within the cuvette (chapter 7.4.1.4).

4.5.5 Choice when enabling the Measuring Head

No Measuring Head >>

Standard Measuring Head 3010-S >>

Head only with bottom chamber >>

Custom built Head >>

When switching the system on, there is a choice on, which measuring head is connected and shall be operated. To cycle through the choices, click on the field. Usually the choice *Standard Measuring Head 3010-S* is the correct choice. But also, the very similar choice, *Custom built Head* is correct. There is only a slight difference between these two options: With the custom-built head it is not checked whether all impellers, ventilators and Peltier elements are connected. In addition, the volume of the measuring head is taken into account when calculating the gas exchange data.

There are further options for the measuring head which are not shown here: Ring Chamber, Gas-exchange Chamber 3010-GWK1 and Dual-PAM cuvette 3010-DUAL.

4.6 LED-Light Source 3041-L (provided with GFS-3000)

The LED Light Source 3041-L has an LED array with warm-white LEDs. Its spectral intensity is shown in Fig. 24. It results in 16% blue (400-500 nm), 40% green (500-600 nm) and 44 % red (600-700 nm).

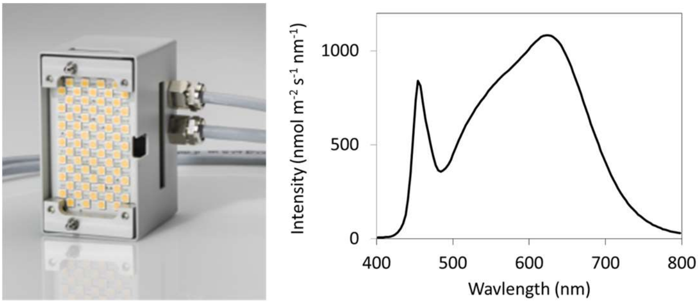


Fig. 24 Left: LED Light Source 3041-L; Right: Spectral intensity

Nothing or Light Source 3040-L >>
Standard Distance Light Source 3041-L >>
Conifer.. Distance Light Source 3041-L >>
Even Voltage Light Source 3041-L >>

The LED Light Source 3041-L has three settings to ensure uniform illumination with different configurations of the measuring head: "Standard Distance", "Conifer Distance" or "Even Voltage". The choice must be made, when switching the system on.

Each setting requires, its own light-source factors. They need to be adjusted, if the light-source or the geometry between the light-source and the sample was changed. See chapter on light-source factor 12.5.

4.7 LED-Array/PAM-Fluorometer 3056-FL (provided with GFS-3000FL)

Chlorophyll fluorometers measure chlorophyll *a* fluorescence mainly emitted from the inner antennae of PSII. They serve to access the photochemistry of PSII.

Fig. 25 Left: LED-Array/PAM Fluorometer 3056-FL

Like all PAM fluorometers, the LED-Array/PAM-Fluorometer 3056-FL uses pulse-modulated measuring light to selectively detect the chlorophyll fluorescence yield (F). The actual measurement of the photochemical yield is performed by first recording F and then applying a short saturation light pulse to completely suppress the photochemical yield and induce maximum fluorescence yield (Fm'). The Y(II)-parameter 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. From the Y(II) data and the estimated light absorbed by PS II, the electron transport (ETR) can be obtained. If the saturation light pulse is applied in the dark, the fluorescence parameters are named 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 widespread, whereby Fv is the variable fluorescence with Fv = Fm – Fo. In formulating this definition, Fo was assumed to be constant – not variable. But soon it turned out that in the light acclimated state, directly after the light was switched off and far-red light was used to only excite PSI and thus open PSII, a quenched Fo level could be observed. It was called Fo' analogous to Fm', the quenched Fm level. The LED-Array/PAM-Fluorometer 3056-FL allows the application of far red light to record Fo'. In addition, it 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 9.12. For further reading on chlorophyll fluorescence see Schreiber U (2004).

4.7.1 Actinic Light

In chlorophyll-fluorescence terminology, actinic light is the photosynthetically active radiation given to drive photosynthesis. The fluorescence module provides actinic light via blue (peak wavelength: 470 nm) and red LEDs (peak wavelength 630 nm). The proportion of the blue light is 10% (mol/mol). Actinic illumination of the LED-Array/PAM-Fluorometer 3056-FL ranges from 0 to 2500 µmol m^{-2} s^{-1}. The light intensity is controlled and measured with the sensor of the measuring head. The light intensity is controlled and measured by the PARtop or PARbot sensors of the measuring head. There is a light-source factor that corrects the PAR measured by one of these sensors to match the PAR of the sample. See chapter on light-source factor 12.5.

4.7.2 Measuring Light

The measuring light (ML) of the LED-Array/PAM-Fluorometer 3056-FL is blue pulse amplitude modulated light with a peak wavelength of 470 nm. The measuring light of cause is also absorbed by the photosystems like the actinic light and it also causes photochemical charge separation, but to measure in the "dark", it is kept so low that any electron is moved away before the next photon hits; consequently, the fluorescence measured in the dark acclimated state with only measuring light shows no transient change.

4.7.3 Frequency of Measuring Light:

The frequency of the measuring light is automatically controlled by the fluorescence module. If the actinic light is off or low (below 50 µmol m^{-2} s^{-1}) the frequency of the measuring light is 10 Hz resulting in a PAR around 0.02 µmol m^{-2} s^{-1}. If the actinic light is above 50 µmol m^{-2} s^{-1} and during the saturating light pulses, the frequency of the measuring light is 500 Hz (PAR about 1 µmol m^{-2} s^{-1}).

4.7.4 Excitation of Chlorophyll

In most photosynthetic organisms, blue light excites chlorophyll fluorescence. However, in organisms with phycobilisomes (cyanobacteria and red algae) the yield of blue light excited fluorescence is rather low. This is because most of the chlorophyll in these organisms is associated with photosystem I and in a low-fluorescent state. Therefore, the use of the blue excitation light cannot be recommended for the study of such organisms (e.g. lichen with cyanobacteria as photobionts) .

4.7.5 Detector Filters

The PIN-photodiode detectors are protected by long-pass filters with $\lambda > 660$ nm.

4.7.6 Saturation Light Pulse

The saturating light pulse is provided via the red LEDs also used for the actinic light. It reaches more than 8000 µmol m^{-2} s^{-1} at setting 12.

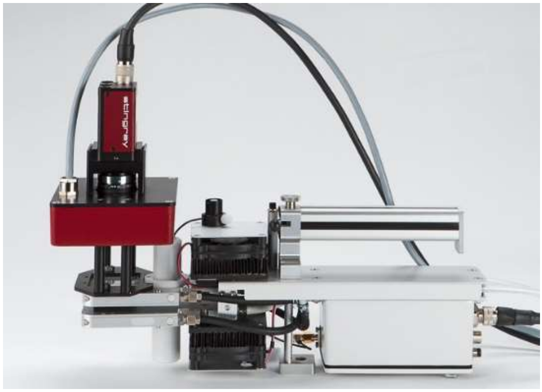
4.7.7 Far Red Light

The far red light LEDs have an emission peak around 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 immediately after strong light is turned off.

4.7.8 Zero-Offset with Non-Fluorescent Foam

The non-fluorescent foam serves to adjust the zero offset of the fluorescence module. If the sample is replaced with this foam the fluorescence value Ft must be zero. See chapter 12.6.

4.8 Imaging-PAM – Optional



The Imaging-PAM can be used together with the GFS-3000. The Imaging PAM *M-series* MAXI-Head version may be used together with the Gas Exchange Chamber 3010-GWK1 , while the Imaging-PAM *M-series* MINI-Head version (IMAG-MIN/B, /R and /GFP) can be clicked onto the Standard Measuring Head 3010-S of the GFS-3000 with the adapter IMAG-MIN/GFS. The Imaging-PAM will be driven by two programs, GFS-Win and ImagingWin, collaborating synchronously on one computer. It is not possible to operate the Imaging-PAM via the internal panel PC of the GFS-3000. For the combined use of the Imaging-PAM with the GFS-3000 see special manual.

4.9 Darkening Leaf-Clamp 3010-DLC - Optional

The darkening leaf-clamp serves to dark-adapt leaf samples in the field before Fv/Fm measurements. Insert the shutters into the darkening leaf clamp with the black sides showing towards the leaf. Use the stainless-steel clips to fix the clamp at the leaf.

Fig. 26: Darkening Leaf Clamp 3010-DLC attached to tobacco

For the measurement, insert the darkening leaf-clamp together with the fixed leaf into the Standard Measuring Head 3010-S, so that the clamp and the measuring head open to the same side. The black foam-frame at the darkening leaf clamp serves to position the sample; it surrounds the foam gasket of the Standard Measuring Head. After positioning the sample, close the Measuring Head infirmly, pull the shutters out and then close the Measuring Head firmly. The pressure of the Measuring Head can be adjusted with the knurled screw at the handle.

5 Switching the System ON and OFF

5.1 Switching the System On, Operation with internal panel PC

First insert two fully charged eSMART-batteries or the Power Supply 3200-N (for charging batteries see chapter 13.1.3 and 13.1.4). Shortly press the POWER ON switch for switching the system and the internal panel PC on. The Power Status LED will shortly light orange and then blink green with 1s intervals. During initialization the solenoid valves can be heard clicking.

If the power status-LED blinks once red and then switches off and stays off, the provided voltage is too low or too high. If it blinks orange/green alternating, a battery is empty. For more code of the power status-LED, see Appendix.

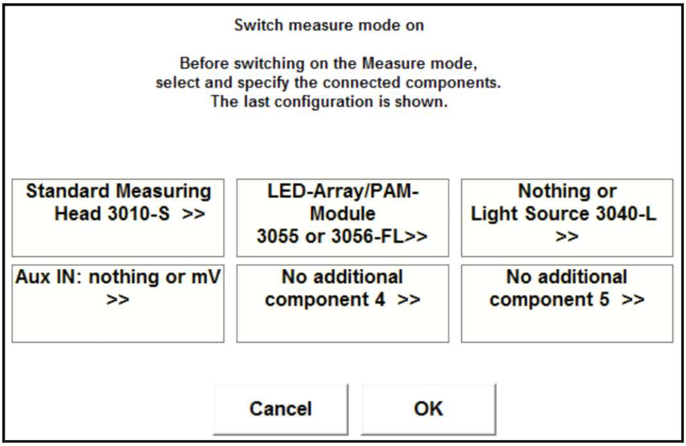


Fig. 27: Measure mode on – window

After the power has been switched on, the GFS-Win software starts and the *measure mode on* window appears (Fig. 27). Here the components attached to the GFS-3000 can be selected and activated. Click on the fields to cycle through possible attachments. Afterwards press OK. The control unit, the gas analyzer and the selected components will be initialized. This takes a short while and afterwards the GFS-3000 is ready to receive settings. The attached components can also be enabled and initialized later.

If the system is in standby mode (panel-PC LED is on, power status-LED is blinking green) touch the screen and wait 6 s, GFS-Win will resume operation. To start the measure mode, select *Menu →On/Off →Measure Mode ON* and continue with selecting the attached components as described above.

If only the screen is turned off, touch it. GFS-Win has not stopped operation and will appear immediately.

5.2 Switching the System On, Operation with external PC

If the system shall be operated with an external PC via the provided Interface 3010-I/Box, the POWER ON switch must be pressed for 5s then the panel PC will remain off. The power-status LED will shortly light red, change to orange, and afterwards blink green with 2 second intervals, indicating that the internal PC remains off. Alternatively, if the internal PC is already on, it can be paused see chapter 7.9.1.3.

Connect the Interface 3010-I/Box and start GFS-Win on the external PC. To start the measure mode, select *Menu →On/Off →Measure Mode ON* and continue with selecting the attached components as described above (*see* Fig. 27).

5.3 Switching the System Off or into Standby Mode

To switch the system off, launch the shutdown dialog via the menu of the GFS-Win software (*Menu →On/Off →Exi*t, see chapter 7.9.1.5). It can be selected how deep the system should be shut down. Most importantly, the gas analyzer remains on when the system is put into standby mode, otherwise it is turned off.

Fig. 28: Shutdown dialog

During shutdown, the system must be flushed with dry air to avoid condensation, which is crucial when the analyzer is switched off and a cool night is expected or during transport. Condensation within the analyzer can lead to destruction. For the drying to be effective, the measuring head must be closed, and the drier must be functional. Depending of the selected option, the system switches off, only keeps the panel PC on (Measure Mode off) or goes into standby mode. We recommend keeping the system in *standby* mode instead of switching it off overnight, if power is available. In standby mode the panel PC LED flashes orange (3200-C only). Touch the screen to resume panel PC from standby mode. The *Measure mode off* function is recommended for updating the GFS-Win software (chapter 13.12.2).

Do not use the Windows operating system shutdown function directly, because it does not shut-down the entire system properly, but forces any software to close immediately, regardless of the current hardware state. Especially, it will not run the drying cycle for the analyzer and measuring head. Sometimes drying is not required, for example, if it just has been done manually. Then the check mark for *flushing the system with dry air* can unchecked before pressing OK.

The steps performed when shutting down the system are explained in the next chapters.

5.3.1 Power Off

The *Power off* procedure goes through the following steps:

Controls are switched off.

If the analyzer was on, it will be flushed with dry air. For this to be effective, the silica gel in the drier needs to be dry enough and the measuring head needs to be closed.

GFS-Win may ask for a filename (replacing null.csv).

The analyzer will be switched off.

The internal PC will be shut down and switched off

The battery control will switch the system and itself off.

5.3.2 Measure Mode off / Exit GFS-Win

The purpose of this function is to enable data-downloads, software-updates or other services. A low battery will not be indicated, if GFS-Win is not running. Watch out for an orange/green blinking of the Status LED. The *Measure mode off →Exit GFS-Win* procedure goes through the following steps:

Controls are switched off.

If the analyzer was on, it will be flushed with dry air.

GFS-Win may ask for a filename (replacing null.csv).

The analyzer will be switched off.

The internal PC stays on.

The battery control remains on.

GFS-Win exits. (GFS-Win may be restarted via the Start-Menu of Windows.)

5.3.3 Standby Mode

The *Standby mode* sets the system into standby mode, which means that the analyzer and battery control stay on. When sent into standby mode, the system goes through the following steps:

Controls are switched off.

The analyzer stays on.

If the option *flush system with dry air* is ticked, the system will be flushed with dry air. Note that if the battery is drained in standby mode, the system switches off without flushing. This situation must be avoided.

GFS-Win may ask for a filename (replacing null.csv).

The internal PC goes into standby mode (LED orange blinking).

The battery control remains on (status LED green blinking).

To resume from standby mode, touch the touch panel and wait 6s.

5.3.4 Low Battery (Capacity <6%)

If the battery status is lower than 6% and GFS-Win is running, the switch-off procedure will go through the following steps:

Controls are switched off.

The analyzer will be switched off immediately afterwards, in order to save power.

If the analyzer was on, it will be flushed with dry air.

Before the internal PC will be shut-down, GFS-Win may copy data of the file null.csv to a file with an automatically generated name: date time.sav. Otherwise immediately after restarting, secure any data that may have been stored in the file "null.csv". Always enter a filename other than "null" before taking measurements to avoid data loss.

5.3.5 Very Low Battery (Voltage <10.8V)

If the input voltage is lower than 10.8V (lower than 6% capacity) usually GFS-Win would have shut down the system already. But if it was in standby mode:

The panel PC is forced to shut down via the battery control and the Windows operating system.

The system is forced to switch off.

Avoid this situation.

6 Checklist for the Operation of the GFS-3000

6.1 Gas Exchange

6.1.1 Checks

- Check humidifier, drier and CO_2 absorber (chapter 13.2).
- Have a new CO_2 cartridge ready (only insert on command, chapter 13.4).
- Check gaskets of Measuring Head and orientation of Tleaf sensor.

6.1.2 Power on

- Switch Power on at GFS-3000 (chapter 5.1). If possible, use standby mode overnight. GFS-Win software (chapter 7) starts automatically.
- Select *Menu →On/Off →Measure Mode ON*, select measuring head and light module (with 3041-L select standard or conifer distance), press OK.

6.1.3 Calibrations before Measurement

- Set Flow-Meter Zero Offset with *Calibration/Maintenance→ Central unit→ Set Flow Meter Zero Offset*.
- Consider zero calibration of CO_2 analyzer (check weekly or after changing absorber, or before and after long-term measurements, chapter 12.3.2).
- Consider zero calibration of H_2O analyzer (once a week, chapter 12.3.4).
- Consider total calibration of analyzer, offset and span (once in three months, chapter 12.1).
- If a different flow rate than before is used, readjust valves (chapter 12.2).
- Consider light source factor, if light source has been changed or cuvette was modified, or system was used by another user (chapter 12.5).
- Consider setting the offset of Tleaf (chapter 13.8.10).

6.1.4 Settings

- Decide on and adjust the measuring parameters (Table 1). Programming functions can be used to set parameters, but if you are using the system for the first time, enter everything step by step.
- The fluorescence measurement should be adjusted first (chapter 6.2).

Table 1: Settings for gas exchange, with example values

parameter	**mode (unit)**	**value**
filename		myplant
area/weight	☑ area (cm^{2}) ☐ weight (mg)	8
impeller speed	(steps)	7
light	☑ PARtop ☐ PARbot (µmol m^{-2} s^{-1}) ☐ PARamb	1000
temperature control	☐ follow Tamb ☑ Tcuv (°C) ☐ Tleaf	25
flow rate	(µmol/min)	750
CO_2	☑ off (remove absorber) ☐ value (requires cartridge and absorber)	
H_2O (drier and humidifier may need purging with CO_2 free or ambient air)	☐ no control (amb) ☑ ppm ☐ rh (%, requires Tcuv to be set)	20000
Interval→ purge time	(s)	60
Interval: Averaging and measuring interval	(s)	005/060

6.1.5 Inserting Leaf

- Before inserting the leaf, consider, whether a ZPcuv shall be measured with an empty measuring head. If yes, in *MP mode* with closed empty measuring head, wait until CO2abs, H2Oabs, dCO2 and dH2O have stabilized, press *Store ZPcuv*. Afterwards insert the leaf.

- The leaf must have a continuous water supply for gas exchange measurements. Either keep it attached to a well-watered plant or keep the branch/petioles under water while cutting.
- The light source can be removed and placed on the resting angle before inserting the leaf, but then the light needs to be switched off beforehand (*see 7.4.1.4*).
- Press "*Regulation standard*" in the *Settings window* to put the regulation into freezing mode "*Gas regulation frozen*". To avoid loud noise, when opening the measuring head, the impeller and temperature control can be switched off.
- While inserting the leaf watch out for the thermocouple. Its tip should touch the leaf.
- Avoid big veins when inserting the leaf, because they may cause a leak. If a big vein cannot be avoided, it may be necessary to use Terostat® beside it for better sealing.
- While closing the measuring head (chapter 4.5.1) watch the mechanical flow indicator ANALYZOR SAMPLE rise. A drop of the flow indicator is an indication of a leak.
- Place the light source back on the measuring head.
- Switch the temperature control, impeller and light back on.
- Wait for some seconds before pressing the button "*Gas regulation frozen*" to switch the flow, CO_2 and H_2O regulation back to "*Regulation standard*".
- Adjust the settings for the *Leaf area*".
- Enter the *Object number*
- Enter a comment about the leaf and intended experiment

6.1.6 Measurements

- If *ZPcuv* has not been measured with an empty measuring head, switch to *ZP mode ZPcuv* or use *AutoZP* to measure *Zpirga*. For *ZPirga* the measuring head does not need to be empty.

- Switch back to *MP mode*, observe *dCO2* and *dH2O* on the chart with fine scale. Directly after switching there is a switching artefact.
- Once the values have stabilized, store measuring points as desired (chapter 4.3.2) either single points by pressing *Store MP* (chapter 7.3) or several by adjusting the timing with *Interval* and pressing *Start storing* (chapter 7.4.1.1).
- Change parameters as desired.
- If the CO_2- or H_2O-concentration is changed, a new ZP has to be recorded before taking any MPs (chapter 4.3.2 for a general explanation on MP and ZP; *see* chapter 7.3 for operation of MP/ZP; and 7.4.1.1 for automatic storage including MP/ZP).

Note! After switching on the measure mode, the instrument needs to reach a constant temperature (preheating of the gas analyzer). Before this temperature is reached, measuring points can be recorded but the absolute and delta values for CO_2 and H_2O drift. Typically, values are constant after 15 minutes, but the absolute H_2O values may need up to 1 hour of preheating, if very high accuracy is required. (in standby the analyzer is kept in operating temperature)

6.2 Fluorescence Module (optional)

6.2.1 Enable Components

After power on, select *Menu →On/Off →Measure Mode ON*, select measuring head, select fluorescence module (chapter 5.1).

6.2.2 Checks

Measure Z-offset with provided black non-fluorescent foam (chapter 7.4.1.5). With superfluous leaf sample try settings for Fluorescence Module (chapter 7.4.1.5). If the measuring light is to high the fluorescence value will oversaturate during Fm determination, if it is to low not the full scale will be used.

6.2.3 Settings

Decide on and adjust parameters for fluorescence measurements

Table 2: Settings for fluorescence

parameter	recommended value
Gain	low, unless the area is very small
ML-Ampl	10 or change to obtain Ft between 100 and 600
ML	always on
Sat Int	recommended 12
Sat Width	0.6 – 0.8 or check whether plateau is reached
ETR Fac	enter leaf absorption
FR Int	12
Far Red	off
Fo' Mode	on or off

- After *Gain*, *ML-Ampl* or the optical set-up have been changed, the *Z-Offset* (zero offset) of the fluorometers needs to be readjusted.
- Continue with setting up the gas exchange parameters now.

- For the determination of Fo and Fm, the sample must be dark acclimated, 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 by definition the maximal photochemical quantum yield, the conditions under which the maximum Fv/Fm can be obtained are the best conditions to obtain Fv/Fm.
- Give flashes as intended, either single flashes by pressing Store Yield + MP or several flashes by adjusting the timing with *Interval* and *1Yield/y*MP* (included in *Interval* in *GFS-Win)* and then pressing *Start storing*.
-

6.2.4 Start of Measurement

- Measure ZPcuv before inserting the leaf or ZPirga after inserting the leaf (chapter 4.3.4).
- Insert a dark acclimated sample, measure Fo/Fm.
- Start gas exchange measurements and fluorescence measurements as desired. Consider that after dark acclimation it will take a while for the leaf to reach a steady state in photosynthesis (12 min to 1 h). To avoid a closure of stomates during dark acclimation, it may help to use low CO_2 concentrations like 200 ppm. Often, photosynthetic rates are best in the morning. The user-program example from the provided CD may be used to perform measurements.

For the Imaging-PAM, see special manual.

7 GFS-Win Software

7.1 Introduction

The GFS-Win software is preinstalled on the GFS-3000 Portable Photosynthesis System. It serves to operate the GFS-3000 or GFS-3000FL, for the collection, display and analysis of measured data and for calibration of the sensors. Thus, the GFS-Win software together with the GFS-3000 or GFS-3000FL enables the assessment of the photosynthetic performance of plants. How to start the instrument and the GFS-Win software has been described in chapter 5.1 and 5.2, also see chapter 7.9.1.

7.2 General Information on the User Interface

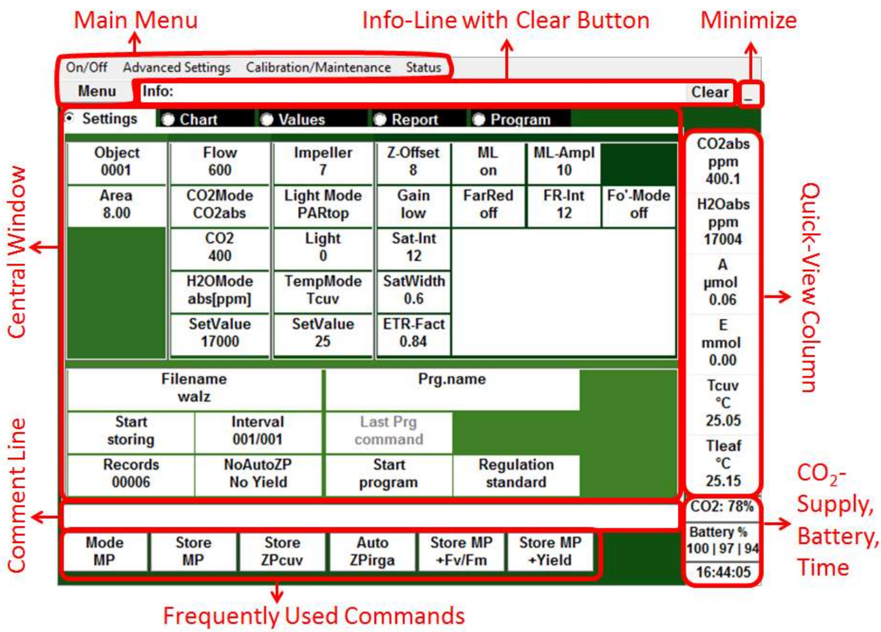


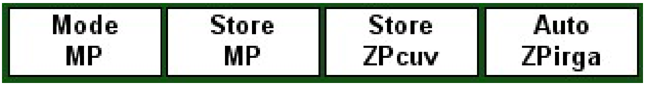
Fig. 29: User interface with outlined zones.

The graphical user interface is divided into zones. They are listed in the order of importance:

- *Frequently used commands* are at the bottom of the main window, so that they can be reached easily.
- A central window that may display one of five index cards (*Settings, Chart, Values, Report, Program*).
- A *comment line*; comments are stored together with the next record set.
- *Quick view column* at the right side, which displays 6 user defined values.
- Fill level of CO_2 supply, charging status for all three battery slots, and PC-time in the lower right corner.
- The main menu at the top of the screen is exposed by clicking on the button *Menu*.
- The *info line* with clear button serves to display information, warnings and errors. Warnings or errors should be confirmed with the clear button, otherwise the info line may be blocked and not display new information.
- The *minimize button*, minimizes the user interface and reveals the taskbar of the Windows operating system.

The functions of these zones are explained in the following chapters.

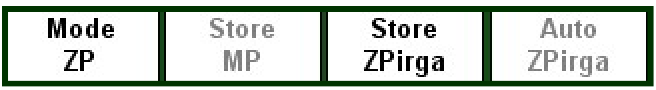
7.3 Frequently Used Commands Buttons



A bar with *frequently used commands* is located at the bottom of the main window. The *MP mode* button on the left indicates the state of the solenoid valves that control the gas flow. The mode can be MP or ZP (chapter: 4.3.2 about MP and ZP). Depending on the mode, the next three keys change their appearance and function.

If *MP mode* is selected, the reference gas flows through the reference cell and the measuring gas flows through the sample cell of the analyzer. If a leaf is enclosed, the *Store MP* button can be used to store a measuring point (MP). If the cuvette is empty, the *Store ZPcuv* button serves to store a zero point (ZPcuv). The buttons are disabled when a user program is running or when continuous data storage is activated.

A zero point must be measured once per hour or after changing the CO_2 or H_2O concentration. If samples are enclosed for a short time, *ZPcuv* with empty cuvette should be preferred. For long term measurements it is not possible to measure *ZPcuv*, but only *ZPirga* (zero point of the infrared gas analyzer alone), where the solenoids are switched to *ZP mode*.



In *ZP mode,* the sample remains in the measuring head and is continuously exposed to the measuring gas, which is vented afterwards. The reference gas is split so that it flows equally through the analyzer reference and sample cell. The *Store ZPirga* button serves to store the zero point of the infrared gas analyzer.

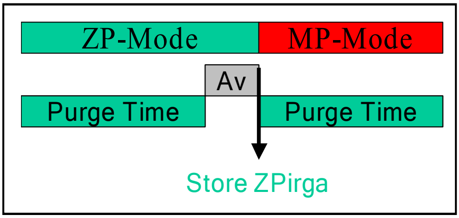


Fig. 30: Time course of an Auto *ZPirga* measurement

Auto ZPirga performs the complete procedure for measuring a *ZPirga* (Fig. 30): The solenoids of the gas paths are switched to *ZP mode*, the gas analyzer is purged for a defined period of time, the so-called *Purge time*. After purging, the dCO2 and dH2O values are averaged and stored as *ZPirga*. The solenoids then switch back to *MP mode* and the gas analyzer is purged again before measuring points can be taken.

The *Purge time* can be set with the button *Interval* in the *Settings window* or in the menu item *Advanced settings*. The averaging time for data storage can be set in the *Settings window* with the button *Interval*.

The additional two buttons on the right *Store MP+Fv/Fm* and *Store MP+Yield* are for fluorescence measurements. They are only visible, if a fluorescence module is connected and enabled (chapter 7.4.1.5).

7.4 Central Window

The central window of the GFS-Win software features five index cards leading to five different windows:

- *Settings*; to change measuring parameters and settings.
- *Chart*; to display a full-size chart of any measured magnitude.
- *Values*; to obtain an overview over all measured values.
- *Report*; to see stored values.
- *Program*; to write, read or change user programs.

7.4.1 Settings window

The *Settings window* opens automatically after starting the GFS-3000 or starting the external GFS-Win software (*see* Fig. 31).

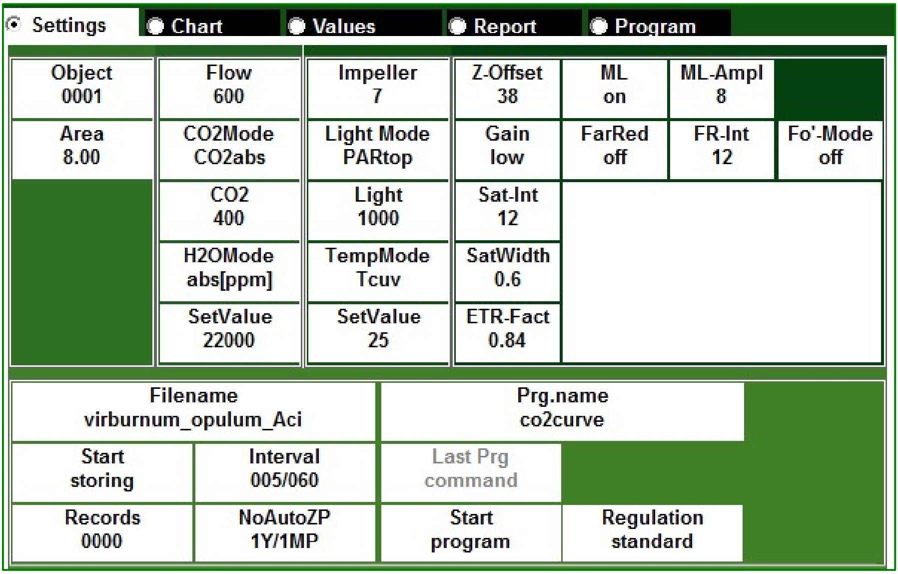


Fig. 31: *Settings window*.

The control elements in the *Settings window* are arranged in five different groups outlined with a thin line. The first column contains parameters that are required for data organization and calculation; in particularly the leaf area respectively weight needs to be entered for exact data calculation. The second column contains control elements for the control unit of the GFS-3000. The third column concerns the controls of the measuring head. The last four columns are for the operation of the fluorescence module, if connected. The group located horizontally at the lower half of the central window determines the data storage, sampling routines and user programs. After switching the measure mode on, the control buttons for connected and enabled components are active. Clicking on a control button displays an input-dialog.

7.4.1.1 General

Filename

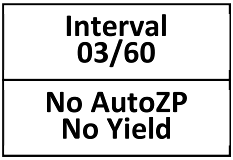
The *Filename* button allows to name an upcoming measurement or to open an existing file. Newly collected data, also called record sets, are appended to the present records file. If a new file is created, the reference type for the calculation (*Area* or *Weight*) of photosynthetic parameter must be entered. The area is suitable as a reference for flat leaves while the weight should be used for conifers, lichens or mosses. The reference type (*Area* or *Weight*) is fixed for the entire file, while the value can be different for each object. Both can be changed after the measurement in the *Report window*; the data will then be recalculated based on the new reference. If the filename "null.csv" is selected, or automatically assigned, data may be deleted without warning. To avoid this, assign a filename before start measuring. If not immediately after restarting the system there may be a last chance to secure any valuable data accidentally stored in "null.csv" also have a look under "null.bak" and the automatically given name "date_time.sav", which may contain a copy of "null.csv".

Start Storing

The four buttons, *Start Storing, Records, Interval* and *NoAutoZP*, control the automatic sampling sequence. This should not be confused with a user program, since automatic sampling only controls the storage of data records and the *MP* or *ZP mode* (chapter 4.3.2), while user programs allow the automatic control of all settings. The *Start Storing* button starts the sampling sequence and record sets are saved under the specified filename (*.csv). It's button title changes to *Stop Storing* and allows stopping the automatic sampling sequence.

Records

The *Records* Button indicates the amount of record sets that have been taken. A record set can be a measuring point (MP) or a zero point (ZP, see chapter 4.3.2). Pressing *Records* is equivalent to pressing *Filename*.



The other two buttons control the sampling sequence settings. A Click on one of them opens the dialog displayed in Fig. 32.

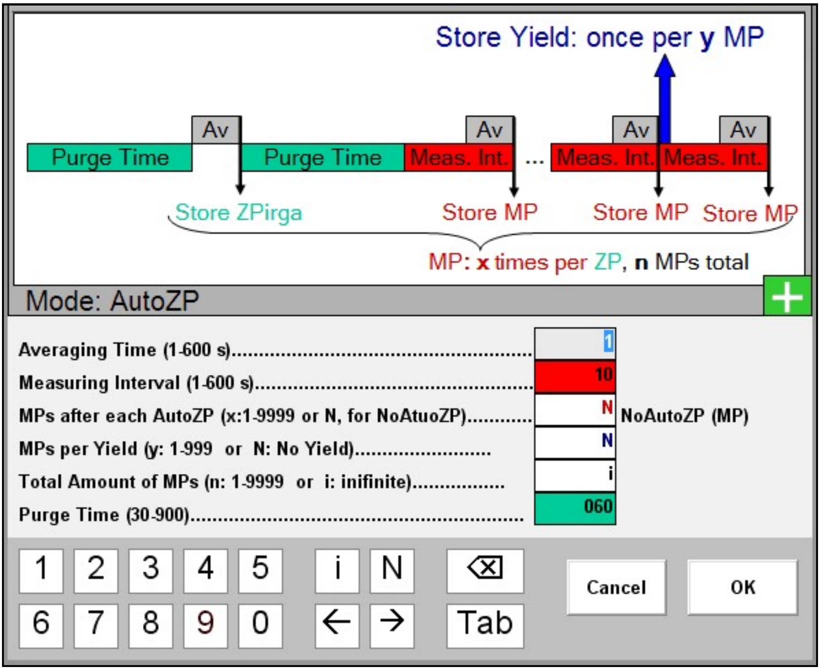


Fig. 32: Dialog with settings for the automatic sampling sequence.

There are several optional sequences. In the simplest sequence only measuring points (MP) are taken, but this can be extended by additionally taking zero points (ZP) and also yield measurements if a fluorescence module is connected. It is also possible to use the automatic sampling sequence to only store zero points ZPs. The different sampling sequences are illustrated in Fig. 32 and Fig. 33. Fig. 32 shows the standard view with fluorescence module, whereas Fig. 33 shows the extended view without fluorescence module.

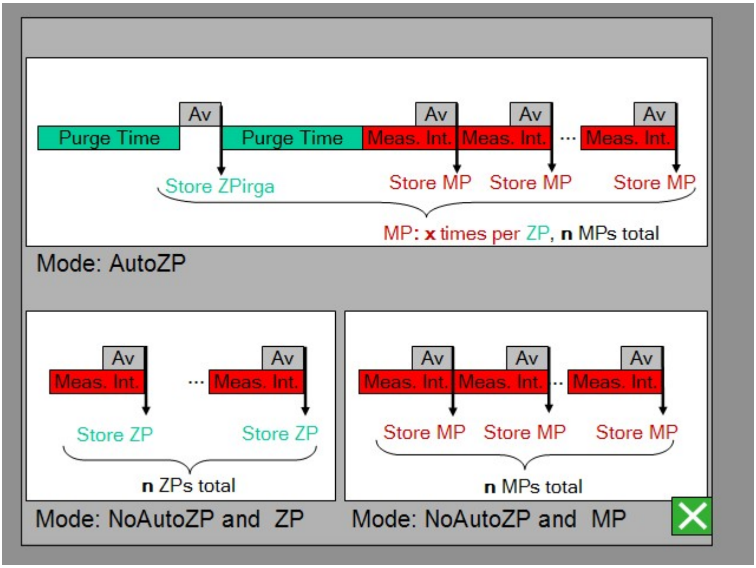


Fig. 33: Illustration of sampling sequences (fluorescence module not enabled).

All settings for the sampling sequences are displayed together in the sampling sequence dialog; some of them are also indicated on the *Interval* and *No AutoZP* buttons in the *Settings* window, and in the *Menu →Advanced Settings*. They are now explained:

- *Averaging time* determines how many values are averaged for each stored record set. Although this value is set within the sampling dialog, it applies to any record set no matter how it is stored, manually by pressing *Store MP/ZP*, automatically in a sequence, or with commands in a user program. The *Averaging time* is displayed in the *Interval* button in front of the slash and in the menu item *Advanced settings*.

- *Measuring interval* determines how often data are stored when the sampling sequence is activated. The *Measuring interval* must be equal to or longer than the averaging interval. The *Measuring interval* is displayed on the *interval* button behind the slash.

- The *value x (MPs after each AutoZP)* defines how often the system should switch to ZP mode and measure a zero point (ZPi) (also *see AutoZPirga* chapter 7.3). The MPs are recorded after each *AutoZPirga*. If *No AutoZP* is selected, there is no automatic mode-change and depending on the current mode only measuring points (MPs) or zero points (ZPs) are stored. This sequence of pure MPs is only recommended for short-term measurements, if a *ZPirga* or *ZPcuv* has been previously measured. If *AutoZP + x*MP* is selected, the cycle (AutoZP + x*MP) will be repeated until *n* MPs have been recorded (= *n/x* cycles). This option is recommended for long-term experiments such as diurnal measurements on single leaves, so that zero points are automatically recorded between series of MPs, resulting in higher accuracy. The *value x* is displayed in the *No AutoZP* or *ZP+xMP* button.

- The *value y (MPs per Yield)*is only active if a fluorescence module is connected and enabled. It determines how often a Yield (fluorescence measurement with saturating light pulse) is measured (chapter 7.4.1.5). Each yield measurement is performed directly after the last MP of a series of y MPs. If N is entered, no yield measurements are performed during the sampling sequence. Depending on the value entered, the button displays either *No Yield* or *1Y/yMP* in the second line.

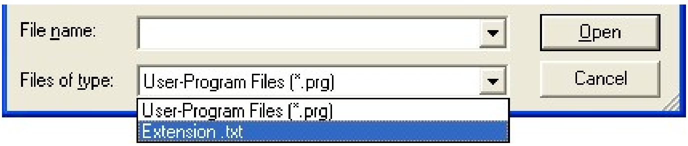
- The *variable n (total number of MPs)* determines the total number of MPs after which the automatic sampling sequence is stopped. If the letter *i* (like infinite) is entered, automatic sampling will not stop until the *Stop storing* key is pressed. This variable can be changed or confirmed in the input dialog that appears when starting the sampling sequence.

- The *Purge time* is required for the *AutoZPirga* sequence. It determines how long the system is flushed in ZP and MP mode before and after a ZP measurement. The *purge time* is also displayed in the Menu *Advanced Settings*.

Prg.name

The three buttons, *Prg.name, Start program*, and *Last prg command* control the execution of user programs (also known as script-files or batch-files). A user program is a user programmable experiment such as a light curve that can be edited in the *Program window* (chapter 7.4.5 and 8 for more information). Before a program can be started, the measure mode must be switched on and a file name must be entered. The *Prg.name* button opens a user program which is a text-file with the extension .prg containing a listing of commands known to the GFS-Win software. Since files with the extension .prg have been classified as extremely risky by Microsoft, files with the extension .txt can now also be opened and executed like a .prg-file. To find them, the extension .txt must be selected for *Files of type* (Fig. 34).

Fig. 34: Selecting file extension .txt when opening a user program.



Start program

When the selected user program is started with the *Start program* button, it becomes a *Stop* button and displays the estimated runtime. If the user program was interrupted with the *Stop* button, it can be continued by pressing the *Start* button again. A dialog appears and the proposed line number for continuing the user program can be confirmed or changed. Similarly, if a line of the user program is marked in the *Program window*. The marked line can be confirmed or changed as the starting point.

Last Prg command

The button entitled *Last Prg command* displays the last command that was executed. GFS-Win executes the commands of the user program as quickly as possible. Therefore, not every command is visible. The button not only displays the last command, but can also display the word *Skip,* such as *Skip Interval*, *Skip AutoZP* or *Skip Wait→Steady*. If the button is pressed, the program continues with the next command. This allows a quick test of user programs or a semi-automatic use of the GFS-3000 so that an automatic user program can be continued based on user-decisions. Generous time intervals can be specified for this type of operation.

The *Last Prg Command* button displays not only commands from a user program, but also commands given internally when settings are changed or during certain procedures such as shutdown. In addition, it shows commands invoked via a COM (Component Object Model) interaction from another program such as a script file of the DualWin software (see special manual).

Regulation Standard **Gas Regulation frozen**

The possibility to hold or "freeze" the gas regulations helps to speed up measurements with frequent sample changes, as opening the measuring head interferes with the flow and gas regulation. It usually takes a while before they reach the set value again. When "freezing", however, the pump, the CO_2 control and H_2O control valve remain in their current position until the regulation is switched back to standard, which should be done a short time (5s) after closing the measuring head.

7.4.1.2 Parameters

Object

The *Object* number is assigned to each measuring point (MP) stored in the record file. It is important to change the object number if objects have a different size or weight and the data are to be recalculated (chapter 7.4.4).

Area

The reference value, either the *Area* in cm² or *Weight* in mg, must be entered by the user. It is required for the accurate calculation of photosynthetic parameters such as assimilation or transpiration rate. The reference type (*Area* or *Weight)* must be specified when creating the data file (*Filename*). It can be changed when recalculating the data (chapter 7.4.4). The value for *Area* or *Weight* can be different for each object number.

7.4.1.3 Central Unit

Flow
0

Enter a *Flow* rate between 300 and 1500 µmol/s (equivalent to ≈400 to 2000 ml/min). The default and recommended value for the Standard Measuring Head 3010-S is 750 µmol/s, but 600 µmol/s is also a reasonable value. If the flow rate setting is changed by more than 100 µmol/s, the valve settings must be adjusted (chapter 12.2). If the *Flow* is switched off, the H_2O and CO_2 control is also stopped, in reverse the H_2O control or CO_2 control can only be switched on if the *Flow* is switched on. This order should also be followed in user programs.

CO2
off

The user can choose between two different options:

CO_2 control off; to use ambient air. The CO_2-supply valve is closed. The CO_2 absorber tube must be replaced by the provided mixing volume (3000-C/MV). A 10 L volume with a long tube must also be placed before the AIR IN inlet, so that the CO_2-concentration of the provided air is very constant and not influenced by the user's breathing.

Absolute CO_2 concentration in ppm: The set value can be selected between 0 and 2000 ppm. The CO_2 control requires the connection of the CO_2 absorber tube filled with soda lime. Note that the soda lime indicator is a pH indicator. Its color change to violet indicates that the soda lime is used and needs to be replaced. However, the color change depends on humidity and is only visible for a short time period. However, the soda lime is not functional if the indicator was once purple. In addition, the indicator does not work in dry air. If 0 ppm CO_2 is selected, do not set the CO_2 control to 0, but switch it off and connect the CO_2 absorber.

The CO_2 in the supply vessel is filled with a small CO_2 cartridge. If the pressure in the supply vessel drops below 250 kPa (see *Menu →Status 7.9.4*), a warning appears that it must be refilled with a new CO_2 cartridge (chapter

13.4). The information field about the filling status of the CO_2 supply in the lower right corner near the battery field also turns yellow.

H2O Mode
off

SetValue
xxx

The user can choose between *off* and three additional modes:

Off; all air is directed through the H_2O valve without passing through the *Drier* or *Humidifier*.

Absolute H_2O* concentration in *ppm; the set value can be selected between 0 and 60000 ppm. Since some users are more familiar with other humidity units, the corresponding relative humidity and dew point are displayed during input of the ppm value.

Relative humidity* in *%; the measuring head must be connected and the *TempMode* set to *Tcuv.* Note that not the relative humidity of the cuvette is controlled, but the relative humidity entering the cuvette. The required humidity is calculated from the set value for *Tcuv* and adjusted if this set value is changed.

Drier; in this function, all air is directed through the drier. This mode can be used to flush the system with dry air. It is then advisable to first switch the flow off and then the H_2O control, so that the air in the gas pathways remains dry.

Once the control mode has been selected, the *Set Value* button is activated. The unit is *ppm* or relative humidity (rH), depending on the H_2O control mode.

7.4.1.4 Measuring Head

Impeller
0

Impeller speed for effective ventilation of the cuvette volume. The values can be set between 0 and 9, setting 7 is recommended.

Light Mode PARtop
Light 0

The *Light Mode* determines which light sensor is used to control the light intensity.

PARtop Mode: The sensor in the upper half of the cuvette is used to control the light to the set value.

PARbot Mode: The sensor in the lower half of the cuvette is used to control the light to the set value (used with the *Arabidopsis* chamber)

PARamb Mode: For this function, the MQS-B/GFS sensor for ambient light must be placed under the light source (e.g. in self-made cuvettes).

PARtop follows PARamb*:* The sensor in the upper half of the cuvette is used to control the light, while the set value is the value measured by the ambient sensor MQS-B/GFS.

PARbot follows PARamb*:* The sensor in the lower half of the cuvette is used to control the light to the value measured by the ambient sensor.

Whenever the light is controlled with the *PARtop* or *PARbot* sensor, the light-source factor is effective (chapter 12.5) for the light value of the controlling sensor. The light-source factor is not effective, if the light mode is PARamb or if the light is off (no regulation). It is then assumed that the measured light comes from the sun. To set a light value, a light source must be connected and mounted on the cuvette. A set value between 0 and 3000 µmol m^{-2} s^{-1} depending on the light source can be selected.

TempMode off
SetValue xxx

The temperature control offers the choice between *off* and three further functions:

Off*:* No temperature control.

Follow ambient temperature: The ambient temperature (*Tamb*) is measured underneath the external fan of the lower cuvette half. In this mode the cuvette temperature is regulated, so that it follows *Tamb*. A temperature offset can be entered to reach a value some defined degrees above or below *Tamb*.

Set cuvette temperature: The cuvette temperature (*Tcuv* and *Ttop*) is kept constant at the set value.

Set leaf temperature: The leaf temperature (*Tleaf*) is kept constant at the set value.

The set value or temperature offset must be entered when starting the temperature control. It can be changed via the *SetValue* button. The minimum temperature that the standard measuring head can reach is about 10 K lower than the ambient temperature; the maximum is indicated by the software and around 55°C for Tcuv.

7.4.1.5 LED-Array/PAM-Fluorometer 3056-FL - Optional

The PAM-fluorometer is enabled in *Menu →On/Off, →Enable/disable components*. When the fluorescence module is enabled, the control elements in the *Settings window* become active. Also, the two additional buttons on the lower bar with *frequently used commands* become active.

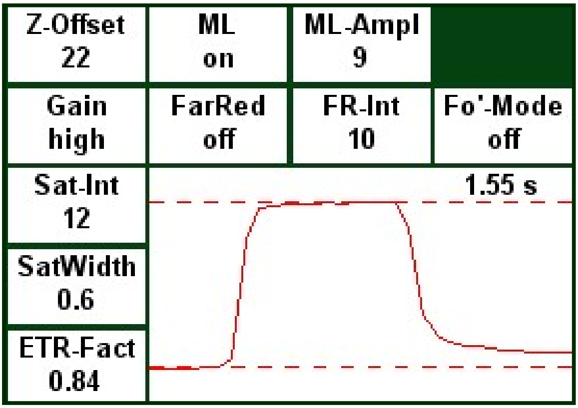


Fig. 35: Settings for the Fluorescence Module 3056-FL with display of fluorescence kinetics during the last saturating flash

Fluorescence is measured in the unit mV. The continuously displayed value is called Ft, where t stands for time, while F is the last stored fluorescence value, usually measured directly before a saturating flash. Ft can be observed in the *chart* and in the *Values window*

Z-Offset is used to determine the zero value of the fluorometer, which consists of background fluorescence and a small preset electronic offset. Background fluorescence can be caused by fluorescent materials or a small interference of reflected measuring light. The purpose of the electronically preset offset is to bring noisy values to zero on average. With the *Z-Offset* button the zero value is determined, stored in the fluorescence module and displayed. Its value is subtracted from each fluorescence value directly in the fluorescence module. Before pressing *Z-Offset*, the fluorescence module needs to be placed in its final measuring position, the sample in the measuring head must be replaced by black non-fluorescent foam and the measuring light must be on for at least 5 s.

The *Z-Offset* must be adjusted each time the *Gain* or *ML-Ampl* settings (*see* below) or the optical configuration, e.g. new measuring head, different leaf area adapter or cuvette, are changed. It is recommended to determine the *Z-Offset* after starting the system.

Gain low

By pressing the *Gain* button, the sensitivity of the sensor is changed by a factor of 3.7 between low and high. High gain is only recommended for small samples. It improves the resolution, but not the signal-to-noise ratio. The *Gain* should be set to high, if the signal remains below 200 mV. But first check whether the measuring light can still be increased to get a higher signal. If the Fiberoptics/PAM-Fluorometer 3050-F is used and the signal is low, make sure the fiber is inserted correctly. After changing *Gain* or *ML-Ampl* the *Z-Offset* must be readjusted.

ML on

Switches the measuring light (*ML*) *on* or *off*. The button indicates the current status.

ML-Ampl 10

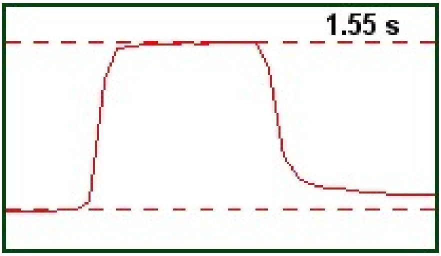
ML-Ampl changes the intensity of the measuring light. The higher the *ML-Ampl* level the better the signal-to-noise ratio. However, if it is too high, it can cause an actinic effect or an overload of the fluorescence signal during Fm determination, which must be avoided. To test whether the measuring 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. To avoid overload, the fluorescence value of the dark acclimated sample should remain below 700 mV. The recommended value for *ML-Ampl* is 10. After changing the *Gain* or *ML-Ampl* the *Z-Offset* must be readjusted.

Sat-Int 12

Sat-Int changes the intensity of the saturation light pulse in steps from 1 to 12. The recommended setting is 12.

SatWidth
0.6

SatWidth changes the duration of the saturation pulse in steps of 0.2 s within the range from 0.2 to 1.2 s. The recommended setting is 0.6 s.



The saturating light pulse serves to completely reduce (also called close) photosystem II. 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. Fm and Fm' are determined between the point at which the plateau is reached and the point at which the light pulse ends. The height of the stored fluorescence values is indicated by dashed lines. The intensity and duration of the saturation light pulse should be adjusted, so that the plateau lasts for 200 to 300 ms. If no plateau is reached, the intensity of the saturation pulse is too low. If the fluorescence is quenched during the pulse, the saturation pulse is too high or too long. For most applications maximal pulse intensity can be recommended.

FR-Int
10

Changes the intensity of the *far red* light (*FR-Int*) in steps from 1 to 12.

FarRed
off

Turns the *far red* light on or off.

Fo'-Mode
off

Activates or deactivates the *Fo'-Mode* in conjunction with *Yield* measurements. If the *Fo'-Mode* is activated, the actinic light is automatically switched off after each saturating light pulse, then far-red light is applied for 5 s before the actinic light is switched on again. During the period of far-red illumination, the *Fo'* is determined. If *Fo'* is determined, it's used instead of the calculated *Fo'* to obtain *qP* an *qN* (chapter 9.12).

ETR-Fact 0.84

The *ETR-Factor* refers to the fraction of the incident light absorbed by the leaf. It is used to calculate *ETR*. It is not measured by the LED-Array/PAM-Fluorometer 3056-FL or Fiberoptics/PAM-Fluorometer 3050-F 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. When using the Imaging-PAM, the *ETR-Factor* is taken from the absorptivity measurements.

When the fluorescence module is enabled, the two additional buttons on the lower bar with *frequently used command*s are active.

Mode MP	Store MP	Store ZPcuv	Auto ZPirga	Store MP +Fv/Fm	Store MP +Yield

Store MP +Fv/Fm

Pressing *Store MP + Fv/Fm* saves a record set (MP) with gas exchange and fluorescence data. 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 or after changing the fluorescence settings (*ML-Ampl, Gain* or *Z-Offset*) the specific Fo and Fm values are required. Especially before 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 (chapter 7.4.4). 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* saves a record set (MP) with gas exchange and fluorescence data. The saturation light pulse is only triggered after the gas exchange data have been measured. 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 *Fo' Mode* is inactivated.

Store MP +Yield,Fo'

If the *Fo' Mode* is activated the title of the button is changed 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 for 5 s to perform the *Fo'* determination (see above under *Fo' Mode*).

For all the three buttons explained above, the saturation light pulse is only triggered after the gas exchange data have been stored. This also applies to automatic sampling sequences (see Fig. 32) and the commands *Fv/Fm* and *Yield* in a user program. The time stamp assigned to the stored record-set is the last second of the averaged gas-exchange measurement taken directly before the saturating flash. After a saturation light pulse it is not possible to give the next flash earlier than after 10 s to avoid damage to the sample or the LEDs.

7.4.1.6 Fiberoptics/PAM-Fluorometer 3050-F - Optional

The Fiberoptics/PAM-Fluorometer 3050-F works very similar to the Fluorescence module 3056-FL. Enable the Fiberoptics-version in the GFS-Win software. Here only additional information required for the fiberoptics-version is given.

ML-Frq low

In difference to the Fluorescence Module 3056-FL the frequency of the measuring light can be set manually. It is recommended to use the low frequency in low light and darkness, where the measuring light would otherwise have an actinic effect on the sample. The high frequency results in a signal with less noise. It can be used, when the environmental light intensity is so high that the measuring light is weak in comparison. During a saturating light flash, the frequency is changed automatically.

ML-Ampl 10

The intensity of the measuring light should be set to 10. Note that the intensity depends on the distance. It must be reduced if a short distance to the sample is used.

Gain high

Set the *Gain* to high, when the fluorescence signal of a dark acclimated sample is lower than 200 mV.

7.4.2 Chart window

The *Chart* window shows the measured values as chart. Recording starts when the *Measure mode* is switched *on*. The chart memory holds all values of the last hour, so that scrolling through the last hour with the highest time resolution is possible. Two readouts can be displayed in parallel. One is shown in red, the other in blue.

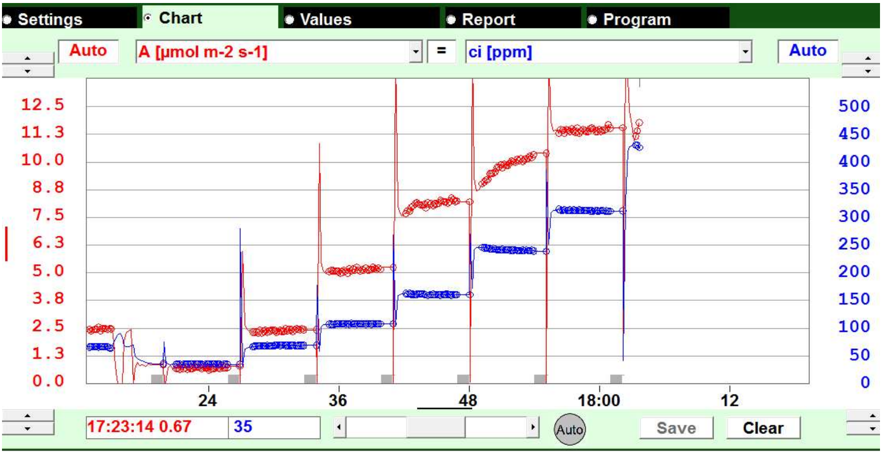
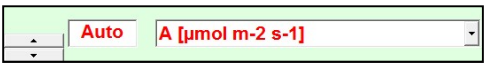


Fig. 36: Chart window displaying CO_2-Assimilation (A, red line and scale) and Intercellular CO_2-Mole Fraction (ci, blue line and scale).



The *Chart* window has control buttons on each side. The control buttons on the left are red, on the right they are mirrored in blue. The variable selected on the left is plotted in red and refers to the red scale on the left, while the variable selected on the right is plotted in blue and refers to the blue scale on the right.

- The *selection box* allows the selection of the data to be plotted. Directly measured (e.g. CO2abs) or derived (e.g. A or gH2O) values can be displayed in the chart.

- *Auto;* if this key is pressed, the range is automatically shifted so that the current value lies within the displayed range.
- The *vertical arrow keys* in the corners of the chart help to change the scaling of the Y-axis. The upper *arrow keys* control the maximum value and the lower *arrow keys* the minimum value.

Fig. 37: Chart window, scaling options

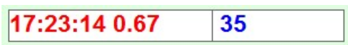
- A click on the *y-axis values* changes the Y-axis scaling in three steps (fine, middle and full scale). The short red and blue lines at the side of the scales (Fig. 37 black arrows) have the length of 5% of total scale.
- Another way to zoom in is to use the touch pen or left mouse button to select a specific area directly in the *Chart* window. The zoom range is rounded up to 5% of the total scale and 5 min. If the area is drawn from left to right, the zoom applies to the red curve. If it is drawn from right to left, it applies to the blue curve. Fig. 37 illustrates both directions together.

- Clicking on the *time axis* with the left or right mouse button changes the scaling in 6 steps. The minimum time range is five minutes, maximum 60 minutes. The black line in the time scale is five min long.

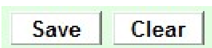
- The *scroll bar* underneath the time scale serves to scroll through the data. When the chart is scrolled, it stops updating. To return to the current measurement click on the round *Auto* button. The current value is marked with a *short gray vertical line* at the top of the chart area.

- The *mode (ZP or MP) is indicated* by a grey bar at the bottom of the chart area (*see* minute 25 and 38 in Fig. 37). If this bar is present, the mode was ZP otherwise MP.

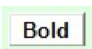
- *Comments* are indicated with a gray vertical line, at the time when they have been stored (*see* Fig. 37 minute 28). Comments can be entered in the Comment line (chapter 7.5) or with a user-program



The value box is active when the mouse is moved over the chart or when the chart is briefly touched with the touch pen. The values at the mouse-position are displayed. For fluorescence, F values are shown in normal font, Fm and Fm' values are shown in italic font, and Fo and Fo' values in pale colors.



Clear, clears the chart and *Save*, saves the content of the chart memory in a .csv-file. This .csv file is a dump of the chart-memory. It gives the possibility to store data when something unexpected was detected. The csv-file of the chart has a different structure than a record file and cannot be read into the record memory. The *Save* button is only active if no automatic storage sequences or user programs are running.



Bold/Thin, , alters the graphic presentation of the chart with a bold or thin line.

If the data reception for the GFS-3000 is stopped, a straight line is drawn over missing data. If the 5 min scale is chosen, this line is pale. Data, which cannot be obtained in ZP mode leave a gap in the chart.

7.4.3 Values window

The *Values* window (Fig. 38) shows all the measured and calculated parameters. Some of the shown units are abbreviated. If the data are saved, they also appear in the *Report window* and *file*. For more information on the underlying calculations proceed to chapter 9 For a detailed description of each value and its full unit, please proceed to chapter 11 (Data Record Structure).

Menu | Info:

Settings | Chart | Values | Report | Program

Date	Time	Code	Object	Area	Status	Aux1	Aux2
yy-mm-dd	hh:mm:ss	string	No	cm2	string	mV	mV
2050-06-21	13:50:33	MP	0001	8.00	FFFFFFF---	0	0
CO2abs	dCO2ZP	dCO2MP	H2Oabs	dH2OZP	dH2OMP	Flow	Pamb
ppm	ppm	ppm	ppM	ppm	ppm	µmol/s	kPa
742	0.32	-13.57	16984	-13	1403	750.1	99.0
Imp	Tcuv	Ttop	Tleaf	Tamb	PARamb	PARtop	PARbot
steps	°C	°C	°C	°C	µmol	µmol	µmol
7	24.05	24.02	23.40	23.47	412	500	2
rH	E	VPD	GH2O	A	ci	ca	wa
%	mmol	PA/kPa	mmol	µmol	ppm	ppm	ppm
60.09	1.35	10.92	123.83	12.04	565	728	18400
Ft	Fo	Fm	Fv/Fm	F	Fm'	Fo'	Fo' calc
mV	mV	mV		mV	mV	mV	mV
446	361	1889	0.809	448	681	355	---
Yield	ETR	qP	qL	qN	NPQ	Y(NPQ)	ETR-Fac
0.342	71.9	0.62	0.49	0.76	1.77	0.421	0.84

Fig. 38: Values window after values tab has been pressed once only

Depending on the number of connected modules, the *Values window* may contain more rows than fit on the screen. Rows that are not of interest can be hidden. *Advanced Settings →Hide rows in Values window* opens a dialog, where row numbers can be entered for hiding (see Fig. 39).

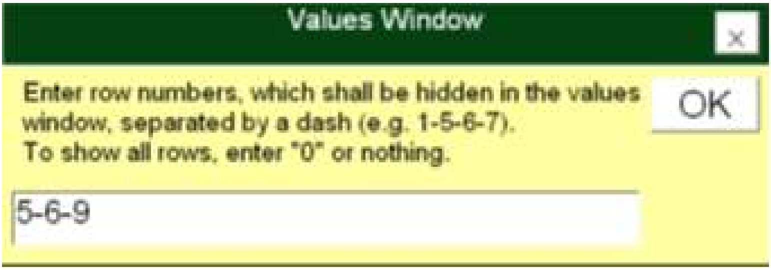


Fig. 39: Dialog for changing the rows displayed in the Values window

7.4.3.1 Stability of Values

For advanced users, who like to automate measurements the automatic stability determination is very useful. But first-time-users may also be interested in this functionality. If the *values tab* is pressed a second time while the values are already being displayed, the *Values window* changes into the *Val slope window*. In addition to the values, the slope is now displayed below each value. The displayed numbers have format xxx +yyy, whereby xxx is the slope in the unit: "unit of the value/min" and yyy is the error of the slope determination in the same unit. The slope values are used to classify the values as steady or not steady. This classification is visible, if the *values tab* is pressed again. The window then changes into the *Val steady window* and the stability classification is displayed below the values. The symbols are:

- =xxx= classified as stable, xxx is slope
- <xxx> slope is zero, but error of slope determination leads to classification as instable, xxx is error.
- /xxx/ classified as rising signal, xxx is slope
- \xxx\ classified as dropping signal, xxx is slope
- |xxx| classified as strongly rising or dropping, xxx is slope
- [xxx] value is distant from set value, xxx is set value.
- (/xxx/) no classification but ignored. xxx is slope

If all values are stable the *values tab* turns red. If in a user program the command: "*Wait until steady*" or "*Wait until steady, max =*" is executed, the user-program will then continue with the next command. The criteria for classifying the stability are in the responsibility of the user. They can be found and changed with *Menu →Advanced Settings →Settings for stability determination*.

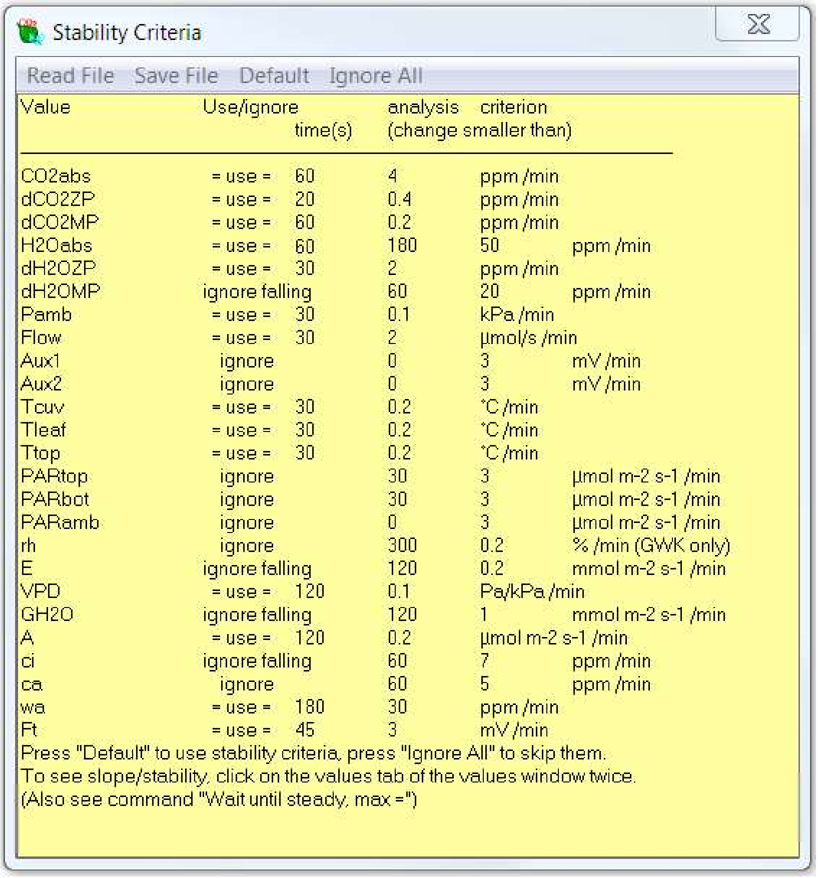
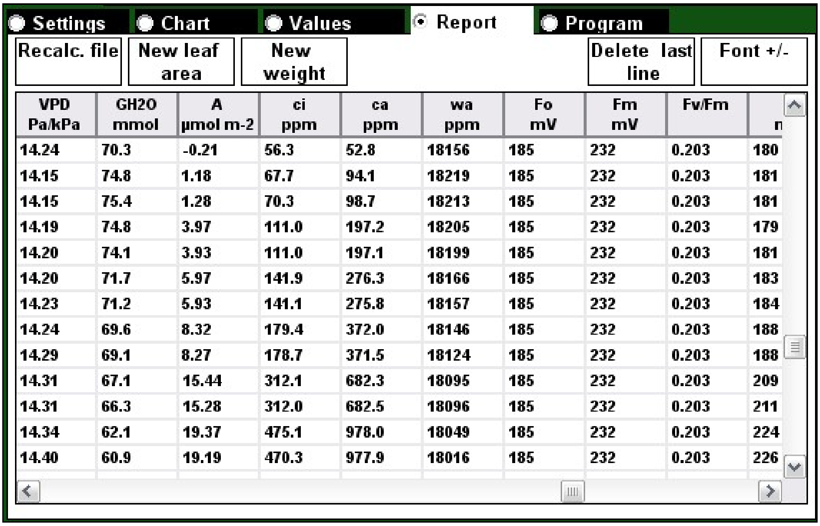


Fig. 40: List of Stability Criteria

Fig. 40 shows a list of the values and their stability criteria. Each value can be used or ignored for the stability determination or it can be only ignored if the value is rising or falling. The time over which the slope shall be calculated can be adjusted and the limit for classifying the slope as stable can be set. Files with different stability criteria can be stored and used. The criteria can also be changed by selecting the values in the *Values window*. If only one value shall be classified, use the menu item *Ignore all* and then change the criteria for this value only. The stability criteria can also be accessed and changed directly from the *Val steady window*. We recommend looking on the chart and deciding whether a value is stable or not. Then if the classification in the *Val steady window* does not match the classification of the user, for example it shows /xxx/ for rising, while it should show =xxx= for stable, then adjust the criteria by clicking on the value in the *Val steady window* and go through the displayed dialogs for changing the criteria.

For displaying the stability classification criteria, the Values window can also be changed into the *Val crit window* by pressing the *values tab* a third time. In the *Val crit window* the stability classification criteria are displayed underneath the values in the format xx’ >yy<. The first value xx’ indicates the time in seconds over which the slope is determined. The second value, yy, is the classification limit for the slope.

7.4.4 Report window



VPD Pa/kPa	GH2O mmol	A µmol m-2	ci ppm	ca ppm	wa ppm	Fo mV	Fm mV	Fv/Fm	n
14.24	70.3	-0.21	56.3	52.8	18156	185	232	0.203	180
14.15	74.8	1.18	67.7	94.1	18219	185	232	0.203	181
14.15	75.4	1.28	70.3	98.7	18213	185	232	0.203	181
14.19	74.8	3.97	111.0	197.2	18205	185	232	0.203	179
14.20	74.1	3.93	111.0	197.1	18199	185	232	0.203	181
14.20	71.7	5.97	141.9	276.3	18166	185	232	0.203	183
14.23	71.2	5.93	141.1	275.8	18157	185	232	0.203	184
14.24	69.6	8.32	179.4	372.0	18146	185	232	0.203	188
14.29	69.1	8.27	178.7	371.5	18124	185	232	0.203	188
14.31	67.1	15.44	312.1	682.3	18095	185	232	0.203	209
14.31	66.3	15.28	312.0	682.5	18096	185	232	0.203	211
14.34	62.1	19.37	475.1	978.0	18049	185	232	0.203	224
14.40	60.9	19.19	470.3	977.9	18016	185	232	0.203	226

Fig. 41: Report window

The *Report* window contains the saved record sets. Each of them is a line. There are three types of record sets, MP, ZPi or ZPc, that are specified in the *Code* column (not shown). MP stands for measuring point, ZPi for zero point measured in ZP mode, and ZPc for zero point measured measured with an empty cuvette in MP mode. For more details on the values, please read the special chapter on data record structure (chapter 11). See also chapter 9 on data calculation.

The order of the columns can be rearranged by firstly clicking on a column to select it and then clicking on another column to insert the selected column to the left of it. The rearrangement requires that there are only a few lines.

These buttons can be used to recalculate stored data with new reference values. *New leaf area* or *New weight* allows the specification of a new leaf area for particular objects and carries out the recalculation only for the specified object. *Recalc.*

file recalculates the entire file. The values are calculated either in relation to *Weight* or to *Area* as specified in the header. It is also possible, to change the reference type by using the *New Weigh*t button on a file that has been measured area-related or *vice versa*. The headers of the data change to the new reference type (*Area in* cm^2 or *Weight in mg, but also* $\mu mol\ m^{-2}\ s^{-1}$ *or* $\mu mol\ g^{-1}\ s^{-1}$). However, only the data for the selected object will be recalculated, resulting in a discrepancy between values and displayed units for the other objects. The reference type is indicated for each record set under *Status* beginning with A or W (for the interpretation of the status string see chapter 7.9.4) it needs to be the same for all objects. This can be achieved by either entering the new reference type and value for each object or by recalculating the entire file with the new reference type using the *Recalc. file* button.

For deleting the last record set, press *Delete last line*. Note that this button only deletes the line in the report file. But if the last line was a ZP, the ZP value will still be used for the calculation of subsequent measurements, the same with the Fv/Fm values.

This button changes the font size. With the left mouse button (short touch on the touch screen), the font size increases until the maximum is reached and then jumps to the smallest font size. With the right mouse button (long touch), the font size decreases.

7.4.5 Program window

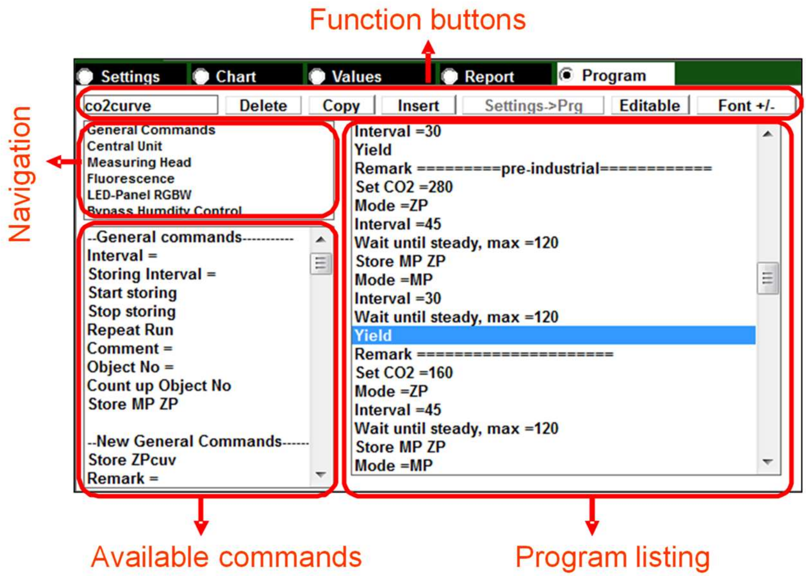


Fig. 42: Program window

The *Program window* serves to write user programs. It is only active if automatic data storage is inactive and no program is running. First open a program file by clicking on the left box in the upper line. The program can be a new file or an existing file. Originally, user program files have the extension .prg, but since those files are considered risky by Microsoft, files of the type .txt can now be used in the same way. In Fig. 42 the user program has the name co2curve. The program listing is shown in the text field on the right. Commands can be selected and changed by double clicking on them. If the command requires a value, an input dialog appears.

The available commands are listed on the left. Double-click on a command to insert it into the program after the marked position. The commands are organized in groups. Navigate to them by clicking on a group name in the navigation field (Fig. 42). Also see chapter 8 for a detailed description on programming.

The control fields in the top line of the Program window serve to organize the programming.

- *co2curve;* is the name of the current user program. Another program can be opened by clicking here.
- *Delete;* deletes the selected lines in the program listing.
- *Copy* takes the selected lines to an internal memory and the clipboard.
- *Insert;* inserts the copied lines from the internal memory after the marked command.
- *Settings->Prg;* inserts all actual settings from the *Settings window* into the program after the selected line. This function is useful if settings have been made and are to be used again another day. In addition, it is useful at the beginning of a user program. Superfluous lines can then be deleted. The two commands "*Purge Time* =" and "*ZP + xMP* =" only serve to store the settings for manual operation and have no influence on a program run. They should be deleted, if the program is used for program runs, to avoid confusion. The order of the commands written with *Settings→Prg* depends on the respective settings.

  Note, that if the *Gain* or *ML* settings of the fluorescence module are changed, its *Zero Offset* must be readjusted. Do not change them unintentionally with a user-program. For more information on user programs read chapter 8.
- *Editable / Read only; ;*any change of the program listing is immediately saved in the currently opened file. To avoid unintended changes, the file property can be changed to "read only".
- *Font +/-;* changes the font sizes for the program listing, increasing (left mouse-button or short touch) or decreasing (right mouse-button or long touch). If the font size has reached the maximum, it jumps to the smallest font size.

7.5 Comment Line

Underneath the central window is a comment line.

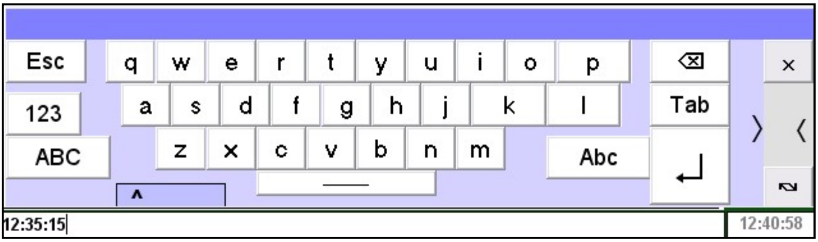
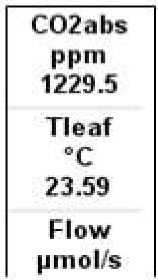


Fig. 43 Comment line with on-screen keyboard above after a double click.

The text entered in this line is stored with the next record set (MP or ZP). On the panel PC, the on-screen keyboard appears when the comment line is activated. A double-click on the comment line inserts the current time. The comment is saved in the comment column of the report. A maximum of 32 characters are displayed in the Report window. It may be necessary to make the column wider to see them. A maximum of 200 characters per comment are stored in the csv-file (commas and semicolons are automatically removed from comments before saving). Commands can also be entered during a program run. They will be stored with the next record-set.

7.6 Quick View Column



The *Quick View* column at the right side of the main window shows six user-selectable values. To select a new value for a specific field, click on that field. A table appears with all available values. If a value is selected, it is now displayed in the *Quick View* column. If this function is accidentally activated, normal operation can be resumed by pressing the *Escape* key displayed above the *Quick View* column.

7.7 CO2 supply

Underneath the *Quick View* column is a field indicating the filling status of the CO_2 reservoir. 100% corresponds to 670 kPa, which is obtained with one cartridge. When CO_2 can be added (chapter 13.4), the field turns yellow (< 37%). A new cartridge adds another 670 kPa, so that a filling of well over 100% is indicated. At 1000 kPa (10 bar) a safety valve opens to release the overpressure, which should be avoided. To see the exact pressure use the menu item *Status* (chapter 7.9.4).

7.8 Battery %

The charging status for all three slots are indicated. The system switches off, if the battery power is too low (chapter and 5.3.4 to 5.3.5). A warning is displayed if the level has reached 16% (adjustable in *Menu →Advanced settings →Battery Warning* 7.9.1.6). If the battery level is down to 6% the GFS-Win software automatically shuts down the system. Once the shut-down procedure has started, the complete shutdown can only be stopped by inserting a battery with a capacity of more than 70%. If the battery voltage is lower than 10.8 V, which is usually less than 6% and may occur, if the instrument is in sleep mode, the internal battery control will force the system to shut down immediately.

7.9 Main Menu

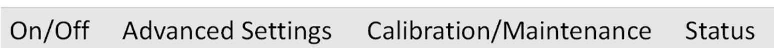


Fig. 44 Main Menu

The main menu can be revealed by pressing the *Menu* button in the upper left corner of the screen. It contains the items *On/Off*, *Advanced Settings, Calibration/Maintenance* and *Status*. *On/Off* is for switching the instrument on or off or down loading data. *Advanced settings* contain various settings concerning the touch-screen as well as additional advanced settings for the measurement. *Calibration/Maintenance* is for calibrating the components of the GFS-3000. With *Status* the current system status, system components with version and serial numbers and system values are displayed.

7.9.1 Menu On/Off

The items in the *On/Off* menu point depend on whether GFS-Win is used on the internal panel PC or on an external PC.

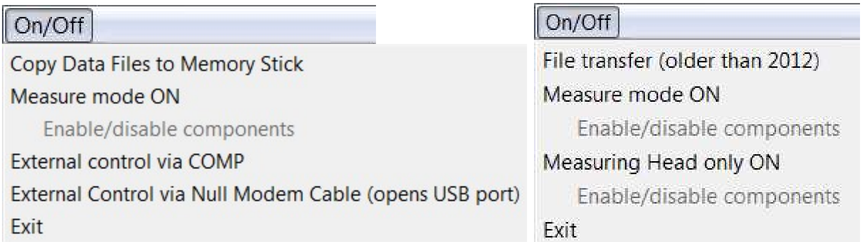


Fig. 45: Menu On/Off: GFS-Win running on internal Panel PC (left) or external PC (right).

7.9.1.1 Copy Data Files to Memory Stick

To copy data, only insert virus-free memory sticks. When the item *Copy Data Files to Memory Stick* is chosen, the Windows Explorer will open in the directory, where the current data are stored. Alternatively, the minimize button  in the upper right corner of the GFS-Win software can be used to access the *Desktop-link* to the standard subdirectory of the GFS-3000 data.

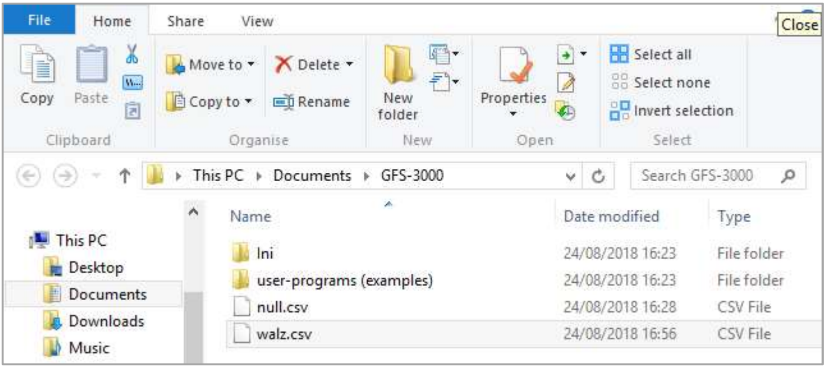


Fig. 46: Windows Explorer for File Transfer to Memory Stick

Fig. 47: Menu item: *Copy Data Files to Memory Stick*

In the Windows Explorer select the files and click on *Copy to* and select the memory stick (*Removable Disk*).

7.9.1.2 Measure Mode ON and Enable/disable components

The menu item *Measure mode ON* opens a dialog for system configuration (Fig. 27). In this dialog the connected components should be enabled by selecting them with the associated button, see chapter 5.

After the measure mode has been switched on, a check mark appears to the left of the *Measure mode ON* menu item, and the menu item *Enable/Disable components* becomes accessible. With this menu item components can also be activated later, after the system and analyzer have been switched on (*see* Fig. 48).

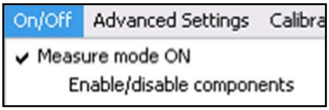


Fig. 48: Dialog with *Measure mode ON* check mark and *Enable/disable components* accessible.

7.9.1.3 External Control via COMP

If the system shall be operated with an external PC, it needs to be connected to the GFS-3000 via the provided interface box 3010-I/Box. Since both computers cannot operate the system simultaneously, GFS-Win on the internal PC needs to be stopped before the external PC can take control. To do this, follow chapter 5.2 or for systems older than 3200-C chose *Menu →On/Off →External Control via COMP*. A window appears in which GFS-Win can be paused or the panel PC can be switched off (Fig. 49).

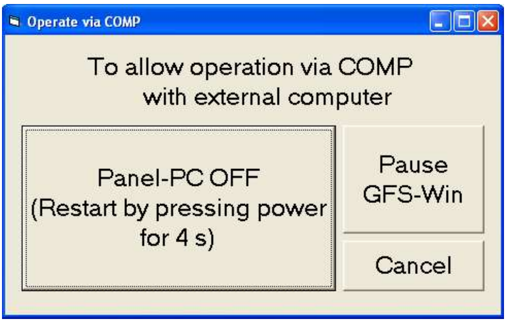


Fig. 49: Choice to allow operation via COMP with an external PC

If the button *Panel PC OFF* is clicked, GFS-Win sends a command to the battery control to switch the panel PC off. This is only successful, if no program prevents the shutdown of the Windows operating system. The status-LED blinks slowly (once per 2 s green) indicating that the system expects external control. To restart the panel PC, press the power button for about 4 s or until it blinks shortly red.

If *GFS-Win* has only been paused, control can be regained by selecting *Menu→External Control via COMP* again.

Once the internal PC has been paused or switched off, the *Measure mode ON* item can be selected in the external GFS-Win software to take control and switch the system on.

7.9.1.4 External Control via Null Modem Cable

Since 2018 the 3010-I/Box is available and shall be used for operation with an external PC. The menu-item *External Control via Null Modem Cable* is only there for downward-compatibility. It is only accessible, if the measure mode is off or in standby mode. Only then the internal panel PC searches for a null-modem cable and opens it. Note that the null modem cable is marked with NMC. Any other USB cables create a short connection and very likely destroy the USB port.

In the external control mode via null modem cable, the internal GFS-Win software only passes commands forwards and backwards between the external software and the GFS-3000 without monitoring them. Any data-files, configuration settings, calibration protocols or error messages are directly stored on the external PC. After selecting *External Control via Null Modem Cable* an input dialog will appear, which allows setting the value for timeout. If the info line displays the sentence: "Expecting external Control", the panel-PC has found the Null Modem Cable and the control from an external computer can be started.

Fig. 50: Info line after enabling external control mode.

On the external PC now start GFS-Win and select: *Menu →On/Off →Measure Mode ON* as explained in chapter 5.

If the USB null-modem cable is disconnected, at the side of the GFS-3000, the external mode will be disrupted, while the external PC will still try to reestablish the connection. Either reconnect the cable and press *OK* on the internal PC and *Retry* on the external PC, or keep the cable disconnected and press *OK* on the internal PC and *Abort* on the external PC to give the control back to the internal PC.

7.9.1.5 Exit

The *Exit* item leads to the shutdown dialog. See chapter 5.3.

7.9.1.6 Measuring Head only ON

This menu item can be used, if the GFS-Win software is used without a control unit, e.g. to operate the Gas Exchange Chamber 3010-GWK or the LED-Panel RGBW-L084 stand alone, or the Gas-Exchange Cuvette 3010-DUAL with the Dual-PAM-100 only. If the *Measuring Head only ON* item is selected, the GFS-Win software first searches the connected measuring head and then opens the dialog for system configuration (Fig. 27). The connected components must be selected, so that they will be enabled, see chapter 5.

7.9.1.7 File Transfer (older than 2012)

The item *File transfer* on an external GFS-Win serves to download files from instruments with the control unit type 3000-C with a black and white screen, which have been built before 2012.

7.9.2 Menu Advanced Settings

The menu *Advanced Settings* gives access to additional settings that are rarely required. All settings given here are stored on the hard disk of the PC, which is running the GFS-Win software (panel PC or external PC) and not within the instrument. The item *Touchscreen Adjustment* is only available on the internal panel PC and can also be reached via a link on the Desktop.

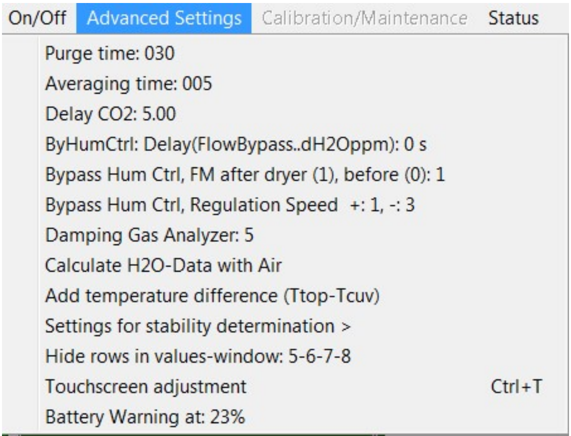


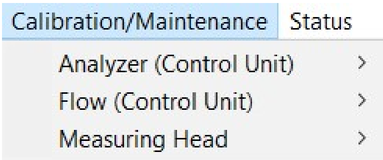
Fig. 51: Advanced Settings

- *Purge time;* applies to the *AutoZP* function. It determines how long the system is purged in ZP or MP mode before and after an automatic *Zero point* is recorded. Times between 30 and 600 sec can be entered.
- *Averaging time;* this item leads to the same setting as the button *Interval* in the *Settings window*. The averaging time will be used for the recording of gas exchange data.
- *Delay CO2;* is only relevant, if there is a self-built measuring head used or no measuring head connected. Otherwise the value is stored in the electronics of the measuring head and can be found under *Menu →Calibration/Maintenance →Measuring Head →Time lag at Standard Flow*. The gas flowing through the measuring head arrives later in the analyzer than the gas flowing through the reference pathway (see pneumatic diagram). When dCO2 is calculated, this time delay is taken into account (chapter 9.3).
- *ByHumCrtl or Bypass Hum Ctrl;* is only relevant, if the system is used with a bypass humidity control. These items are explained in the manual

for the bypass humidity control. When the system is switched on, these menu items are hidden.

- *Damping Gas Analyzer;* Damping setting of the gas analyzer, default value is 5.
- *Calculate H2O-Data with Air;* Low oxygen changes the IR-sensitivity for H_2O. Therefore, the used oxygen concentration (%) can be entered, so that this effect is taken into account for data calculation. The values for H2Oabs, dH2MP and dH2OZP will remain as originally measured. Only the calculated values as rH, wa etc. will be corrected. If the oxygen sensor is used, enter "on", so that the currently measured oxygen concentration will be used for the calculation of data. Also see manual for the Optical Oxygen Sensor 3085-O2.
- *Add temperature differences (Ttop-Tcuv);* allows the setting of a temperature difference (-10 to +10 K) between *Ttop* and *Tcuv* for the setvalue during temperature regulation in mode *Tcuv*.
- *Settings for Stability Determination;* opens a window for adjusting the stability classification criteria (chapter 7.4.3.1).
- *Hide rows in values-window;* opens a dialog for entering row numbers to be hidden (chapter 7.4.3).
- *Touch Screen Adjust;* opens the program *Touchside.exe*, which serves to adjust the touch screen. After adjustment, close this program by clicking on the cross in the upper right corner. *Touchside.exe* can also be accessed via a link on the Windows Desktop (chapter 3.2.1).
- *Battery (eSmart) Warning;* sets the capacity level (standard: 16%) at which a battery warning shall be displayed. See also chapter 0 about charging and safety advices and chapter 13.1.1 for information on Li-ion batteries and chapter 5.3.4 to 5.3.5 about the automatic switching off procedures at low battery levels.

7.9.3 Menu Calibration/Maintenance



The menu item *Calibration/Maintenance* contains calibrations and adjustments of the gas exchange system. All the settings adjusted here are not stored in the panel PC, but directly in the hardware components. The menu point is only accessible, when the system is switched on. Maintenance and Calibration is explained in chapter 12.

- *Analyzer (Control Unit);* calibration procedures for the gas analyzer (chapter 12.1).
- *Flow (Control Unit);* zero offset of mass flow meter and valve adjustment (*see* 12.2 and chapter 15.2.7).
- *Measuring Head*; calibration and adjustment of values concerning the sensors and properties of the attached measuring head (chapter 12.4).

7.9.4 Menu Status

The menu item Status opens a window (see

Fig. 52) that contains the current system values, the status string, the components info and the system constants and calibration values stored in the components.

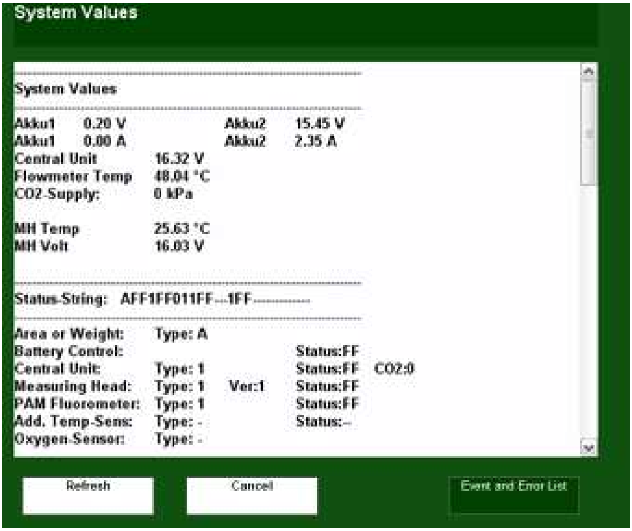


Fig. 52: Status Window displaying system values.

Of importance is the CO_2-supply, which indicates the remaining CO_2 pressure in the CO_2-container. Further information on the CO_2-supply can be found in chapter 7.7. The status string is displayed below the system values. It is decoded in the paragraph beneath. Scrolling down with the scroll bar leads to further information about the connected components. The button *Event and Error List* opens Notepad.exe displaying the file *error.rpt* or *error2.rpt.* These files can be useful for troubleshooting, but also contain information only intended for system development.

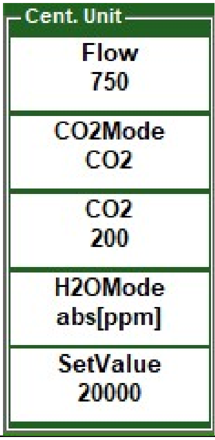
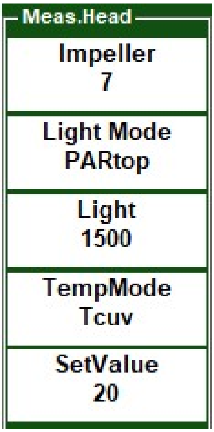
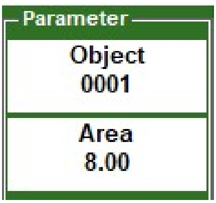
8 Programming

8.1.1 General Programming Information

User programs serve to automate and standardize measurements. A user program is a text file with a list of commands that are executed one after the other. Any setting that can be set in the Settings window can also be set with a command in a user-program. They can be easily written or modified within the Program window. It is not required to really write anything for writing a program, just double click on a command in the to insert it or change it (explained in chapter 7.4.5). Any change is stored immediately in the file. User programs can be very simple, like only storing several values, or they can be very complex, for example doing several CO2 curves at different light intensities. Just realize each setting you set, when operating the instrument step by step. A list of these settings is a user program, which can be used to automate your actions. Table 3 shows the elements of the *Settings window* with their matching commands to give an idea of the simplicity.

Before using automatization, we recommend doing some measurements, CO_2 curves and light curves by hand and evaluate the obtained data to become familiar with the system. Nevertheless, even for first-time-users the button "*Settings* $\rightarrow$ *Prg.*" is very useful to store the current settings in form of a user program for protocol purposes. This button becomes enabled as soon as a *program name* has been entered and the measure mode is on. And once a user program has been created in this way, it can be tested immediately by pressing the *Start* button in the *Settings window* and watching its progress. The execution of the user program terminates, after the last command. Any settings made during a program-run, remain active. For changing or entering commands, double click on them (chapter 7.4.5). The user program may be useful the next day to quickly set the same settings again.

Table 3: Elements of the *Settings window* with matching commands

Settings window	***Corresponding Commands***
	***Control Unit***
	Set Flow =
	Set CO2 =
	CO2 Control off
	Set H2O(ppm) =
	Set H2O(rH) =
	H2O Control off
	***Measuring Head***
	Impeller =
	Set PARtop =
	Set PARbot =
	Set Tcuv =
	Set Tleaf =
	Follow Tamb plus =
	TempControl off=
	***General Commands***
	Object No = =
	Count up object No
	Area/Weight =
	Fluorescence Module

Fluor.Module: Z-Offset 105	*Set Z-Offset FL=*
Gain high	*Gain FL =*
Sat-Int 9	*Sat-Int FL =*
SatWidth 0.6	*SatWidth FL =*
ETR-Fact 0.84	*ETR-Fact FL =*
ML on	*M-Light FL=*
ML-Ampl 10	*ML-Ampl FL=*
FarRed off	*FarRed FL=*
FR-Int 12	*Far-Int FL=*
Fo'-Mode off	*Fo'-Mode FL =*
General	***General Commands***
Filename	*New_Record_File =*
Start storing	*Start storing*
	Stop storing
Interval 001/001	*Storing Interval =*
NoAutoZP	*ZP +xMP =* (only affects settings)
	PurgeTime =060 (only affects settings)
No Yield	*1Yield/y*MP =* (in Program window under Fluorescence)
Records	

Regulation standard	***For opening Measuring Head***
	Freeze Gas Regulations
	Continue Gas Regulations=
Mode MP	***Frequently used commands***
	Mode =
Store MP / Store ZPcuv	*Store MP ZP=*
	Store ZPcuv
Mode ZP / Store ZPirga	*Mode =*
	Store MP ZP Mode =
Auto ZPirga	*Auto ZP =*
Store MP +Fv/Fm / Store MP +Yield	***Frequently used commands (fluorescence)***
	Fv/Fm=
	Yield Fv/Fm=
	Comment line
	Comment =

The next step in becoming familiar with user programs is to use an example for a light curve and watch the system while it executes one command after the other. Almost all commands give a set value, e.g. set flow, CO_2, H_2O, light, cuvette temperature or impeller. This kind of commands takes less than a second.

Other commands request the system to wait until a certain time period has passed. The most prevalent is "*Interval* =". It defines a time that must elapse before the next command is executed. The commands "*Wait until steady*" and "*Wait until steady, max* =" also pause the execution of the user program until all stability criteria are fulfilled or the maximum waiting time has elapsed (only with "*Wait until steady, max* ="). See chapter 7.4.3.1 for more details about stability determination and criteria. In addition, the command "*Messagebox* =" will stop the execution of the user-program until the user has actively pressed ok.

After starting a user program, only two buttons are active; the *Stop program* button and the button above it, which displays the currently or last executed command. If the current command specifies a wait time, the button turns into a *Skip*-button and displays *Skip Interval*, *Skip Wait→Steady*. It can be pressed to jump directly to the next command. This allows quick testing of a user program or semi-automatic use of the GFS-3000 so that planned settings can be executed automatically but timing is based on user decisions. For the semi-automatic operation it is recommended to select the time intervals longer than necessary and to interrupt them as required. We recommend semi-automatic operation to familiarize oneself with the system and user programs, and for optimizing the stability criteria and intervals. Note that a user program can be stopped and resumed at any step. Comments can still be entered during execution. They will be stored together with the next record set.

Table 4 explains every command in detail. It also includes some additional commands, some of them are easy, like "*Beep*", some are useful, like "*Repeat Run*" some are advanced like "*Load_Stability_File* =".

8.1.2 Command List

Table 4: Command List

<table>
<tr><th></th><th>General Commands</th></tr>
<tr><td>Interval =</td><td>Defines an interval (between 1 and 3600 s) that must elapse before the next command is executed. For longer intervals, use the command several times in a row.</td></tr>
<tr><td>Storing Interval =</td><td>Defines the averaging time and interval for automatic sampling sequences:
The command corresponds to the button Interval in the Settings window. If the "Storing Interval" command is not given in the user program, the actual settings will be used.
Use this command before storing any data.</td></tr>
<tr><td>Start storing</td><td>Starts an automatic sampling sequence.
To specify, which data (MP or ZP) with which averaging time and how often shall be stored and how often a saturating flash shall be given, use the commands for these settings beforehand. The following list shows an example of a program section that contains the "Start storing" command.
<table>
<tr><td>Mode = MP</td><td>Sets mode to MP</td></tr>
<tr><td>Storing Interval = 005/020</td><td>Sets the averaging time (5s) and storing interval (every 20 s)</td></tr>
<tr><td>1Yield/y*MP= 3</td><td>Setting for yield measurements (only with fluorescence module). A saturating flash will be given after every third MP, i.e. every 60 s (Fig. 32)</td></tr>
<tr><td>Start storing</td><td>Starts sequential data storage</td></tr>
</table>
</td></tr>
</table>

	Interval = 300 — The data is sampled for 15 min (300 s) every 20 s gas exchange date, every 60 s a Yield measurement. *Stop storing* — Stops the sequential data storage Also see chapter 8.1.5 for further explanations.
Stop storing	Stops the sequential storage of data points.
Repeat Run	Repeats the execution from the beginning or from the line with the command "*--Repeat from here-----*".
Comment =	To enter a predefined comment that appears in the comment line during execution and is saved together with the next record set. The comment line can also be used directly during execution.
Object No =	Each record set is marked with an *Object number*. Use this number if the reference value (area or weight) is different for different objects.
Count up object No	Increases the value for Object by 1..
Store MP ZP	Stores a record set. This is the same as pressing the button *Store MP* or *Store ZP*. The type of data depends on the actual mode (*MP* or *Z*P).
	New General Commands This category contains commands, which can only be used with GFS-Win, but not with the old control unit (3000-C sold before 2012).
Store ZPcuv	Stores a ZPcuv. The cuvette must be empty, and the mode must be MP during execution of this command.
Remark =	Enter a remark into the program listing for orientation, e.g.: *"Remark = -- Start of Light Curve --"* A remark has no effect during execution.

Messagebox =	Shows a message and pauses the execution until OK has been pressed.
New_Record_File =	Opens a new record file with the given name.. If the code word *"date_time"* is entered as the name, the record file is automatically named with the current date and time during execution. (Imaging-PAM only: If the name ends on the letter "q", Fo and Fm images are used from the previous record file).
Area/Weight =	Sets the reference value respectively to area or weight.
Load_Stability_File =	Loads the specified stability file. It is first searched in the subdirectory of the configuration file (GFS3000.cfg) and, only if not found, in the subdirectory of the user program itself. If the code word *default* is entered instead a *file name*, the default criteria are loaded (default values may change with GFS-Win versions) .
Wait until steady	Pauses execution until the criteria specified in the stability file are fully met and all values used for the stability determination are classified as stable.
Wait until steady, max =	Equivalent to *"Wait until steady"* but continues after a maximum waiting time (s). The command "*Interval =*" can be used beforehand for a minimum waiting time.
Wait until remote button (MH) pressed	Pauses the execution until the remote button at the Measuring Head has been pressed by the user.
Repeat from here	Sets the position, where "Repeat Run" shall start the repeat.
Beep	Triggers a beep, with the speakers of the computer.

	Central Unit
Set Flow =	Set the gas flow rate (µmol/s) through the measuring head. Give this command always before switching the CO_2 control or H_2O control on, but after switching them off.
Set CO2 =	Sets the CO_2 control to the indicated value (0 to 2000 ppm) . Set the flow before giving this command.
CO2 Control off	Switches the CO_2 control off. If the CO_2 absorber is used, the CO_2 concentration becomes zero otherwise ambient. Give this command while the flow is still on.
Set H2O (ppm) =	Sets the H_2O control to the indicated value (0 to 60000 ppm). Set the flow before giving this command.
Set H2O (rh) =	Sets the H_2O control to the indicated value (relative humidity % entering cuvette). Note the interdependence of the commands: The relative humidity control requires that the Temperature control is set to *Tcuv*, because the set value of *Tcuv* is used to calculate the control value for the H_2O concentration. Use the commands *"Set Tcuv ="* and "*Set Flow ="* before this command.
H2O Control off	Switches the H_2O control off. The H_2O valve moves to a position, where the gas will not pass through the drier or humidifier.
Mode =	Sets the gas pathway to *MP* or *ZP mode*
Auto ZP=	Performs an automatic ZP measurement with the given purge time and averaging time. Firstly, the solenoids switch to *ZP mode*, the gas pathways are purged for the indicated time, then *dCO2ZP* and *dH2OZP* are averaged and stored. Afterwards, the solenoids switch back to *MP*

	mode, the pathways are purged again for the given time interval before the user program continues. "*Auto ZP = 005/040*" replaces the following series of commands: <table><tr><td>*Stop Storing*</td><td>Only if an automatic sampling sequence was active</td></tr><tr><td>*Mode = ZP*</td><td>Sets mode to *ZP*</td></tr><tr><td>*Interval = 40*</td><td>40 s purge time</td></tr><tr><td>*Store_MP_ZP*</td><td>Stores a ZP, with the given averaging time.</td></tr><tr><td>*Mode = MP*</td><td>Sets mode to *MP*</td></tr><tr><td>*Interval = 40*</td><td>40 s purge time</td></tr><tr><td>*Start Storing*</td><td>Only if it was active before.</td></tr></table>
	New Commands Central Unit
Set ca =	Sets *CO2abs*, so that *ca* (CO_2 concentration leaving the he Gas Exchange Chamber) has the given value. Use this command only with the Gas exchange Chamber 3010-GWK1.
Set wa =	Sets *H2Oabs*, so that *wa* (H_2O concentration leaving the he Gas Exchange Chamber) has the given value. Use this command only with the Gas exchange Chamber 3010-GWK1.
Set VPD =	Sets *H2Oabs*, so that the *VPD* in the Gas Exchange Chamber has the given value (Pa/kPa). Use this command only with the Gas exchange Chamber 3010-GWK1.
CO2 push valve =	Moves the CO_2 control valve to the given position to accelerate the CO_2 regulation. Especially useful for low CO_2 values.
Freeze Gas Regulations	The pump, CO_2 control and H_2O control valve remain in their current position and are not adjusted when deviating from the set value.

	This command can be used before opening the Measuring Head.
Continue Gas Regulations	The regulation of the pump, CO_2 control and H_2O control are resumed after pausing due to the command "*Freeze Gas Regulations*". This command shall be used about 5s after closing the Measuring Head.
Calculate H_2O with low Oxygen =	Use this command for experiments in low oxygen to give the current oxygen concentration (%). It will influence the data calculation only. It is not required, if the oxygen sensor 3085-O2 is used.
Aux1 =	to scale Aux1-Values
Aux2 =	to scale Aux2-Values
	Settings Measuring Head
Impeller =	Sets the impeller speed in steps (0 to 9). Set the impeller speed before switching the temperature control or light on.
TempControl off	Switches the temperature control off. Don't use this command while the H2O mode is set to relative humidity.
Follow Tamb plus=	Sets the temperature control for *Tcuv* to follow the ambient temperature measured with *Tamb*. A positive or negative offset value can be entered, if the Measuring Head Version is higher than 1.20.
Set Tcuv =	Sets the value for cuvette temperature; the temperature control mode is set to *Tcuv* mode. Set impeller speed beforehand ("*Impeller =*").
Set Tleaf =	Sets the value for the leaf temperature; the temperature control mode is set to *Tleaf* mode. Set impeller speed beforehand ("*Impeller =*").
Light Control off	Switches the light off

PARtop= *PARbot =* *PARamb =*	Controls the light (PAR in µmol m^{-2} s^{-1}). The command determines which sensor is used to control the light source. *PARtop/PARbot*: The light source will be regulated with the sensor in the upper/lower cuvette half. For *PARtop* and *PARbot*, the *light-source factor* is taken into account. *PARamb*: The ambient sensor must be placed under the light source.
PARtop follows PARamb *PARbot follows PARamb*	Imitates the light measured with the external sensor *PARamb* for example with the Fluorescence Module 3056-FL. The light is controlled with *PARtop* or *PARbot*. The *light-source factor* is taken into account. Requires Measuring Head Version 1.20 and higher.
	New Commands Meas. Head-
Add Temperature Difference (Ttop-Tcuv) =	Sets a temperature difference (-10 K…+10 K) between upper and lower cuvette half. This command becomes effective as soon as *"Set Tcuv ="* is executed sometime afterwards.
	Settings Meas. Chamber 3010-GWK1 see also commands for Measuring Head.
Inside Fan =	Sets the speed of the inside fan of the Gas Exchange Chamber 3010-GWK1 in steps from 0 to 5. Set the inside fan speed before switching the temperature control or light on.

	Fluorescence Module 3055-FL, 3056-FL or 3050-F
*1Yield/y*MP =*	Determines the repetition rate of saturation pulses for fluorescence measurements. A yield is measured every certain number 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 gas exchange data are measured, averaged and stored before the saturating flash. The sample should be dark acclimated for this measurement.
Yield	Trigger a Yield measurement: In a yield measurement a saturation light pulse is applied to determine F and Fm'. If the *Fo'-Mode* is switched *on*, Fo' is determined immediately afterwards. Gas exchange data are averaged and stored before the yield measurement.
Default F	Sets all settings of the fluorescence module to default values. Note, readjust the zero-offset of the fluorescence module after this command.
Fo'-Mode FL =	Sets the Fo'-Mode: On/Off: Fo' is determined/not determined with every Yield measurement.
Gain FL =	Sets the gain (high/low) . Note, after changing this setting, the zero-offset of the fluorescence module needs to be set again.
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
	Fiberoptics/PAM-Fluorometer 3050-F
ML Frequency F =	The frequency of the measuring light can be set to high or low with the Fiberoptics PAM-Fluorometer 3050-F.
	Imaging-PAM
Set PARtop = *Set PARbot =*	Sets the actinic light of the Imaging-PAM in steps (0-20). This command is the same as above except that when the Imaging-PAM is connected, the values scaled in steps, not µmol m^{-2} s^{-1}. If the Imaging PAM is shining on *PARtop* or *PARbot* use *PARtop* or *PARbot* respectively. The *lightsource factor* is applied accordingly.
*1Yield/y*MP*	*see* Fluorescence Module 3056 above
Fv/Fm	*see* Fluorescence Module 3056 above
Abs	Triggers the measurement of an Absorptivity-Image
Yield	*see* Fluorescence Module 3056 above

	LED-Panel RGBW L084 for operation, see separate manual
Red =	Sets the intensity value for red LEDs (steps)
Green =	Sets the intensity value for green LEDs (steps)
Blue =	Sets the intensity value for blue LEDs (steps)
White =	Sets the intensity value for white LEDs (steps)
Red/Green/Blue/White =	Sets intensity values for all colors together in one command (steps)
LED-Panel RGBW total =	Sets the total brightness (0-100%). Use this command to switch the LEDs on or off.
Red/Green/Blue/White/Tot =	Sets the intensity values for all colors and the total brightness 0-100% together in one command.
	Bypass Humidity Control for operation and commands, see separate manual

8.1.3 Order of commands

When the GFS-3000 is controlled manually via the *Settings window*, some settings are blocked if another setting is not made before. For example: the CO_2 control cannot be switched on, if the flow was not set beforehand. Also, during a program-run, the flow should be switched on first and off last. Simply use the commands in the following order to avoid any kind of non-sense:

Switching the system or controls on:
First measuring head: impeller followed by temperature and light.
Then central unit: flow followed by CO_2 and H_2O control.
Switching the system or controls off, use reverse order:
First central unit: CO_2 and H_2O control off, followed by flow = 0.
Then measuring head: temperature and light followed by impeller.

The command "*Storing Interval =*" defines both the averaging time and the storing interval. Because averaging should be set before saving data, use this command before using commands that save data, such as "*Store MP ZP*", "*Start Storing*", "*Fv/Fm*" or "*Yield*".

8.1.4 File organization

To keep the original version of a user program, a backup copy must be made before opening the file. It is also possible to copy all lines from a template program, open a new file and insert it. Files can be set to *read-only* to prevent unintentional changes. The programming function becomes only enabled when a *program name* is entered.

The user programs are text files with the extension *.prg*. However, files with the extension *.txt* can be created and handled in the same way as files with the extension *.prg*. The contents of a user program can be inserted into a word file for documentation. With the *copy* button copy the selected lines to the windows clipboard and then to a *Word* file. It is also possible to change user programs with a text editor. However, this is difficult because the exact syntax must be kept. The text-editor *WordPad* will destroy the syntax, while the text-editor *NotePad* keeps the required syntax. To open a user program with *NotePad*, right-click on the file name in the *open file dialog* and chose *open with* from the context menu to open the file with *NotePad*.

8.1.5 Timing during Programming

If you use the command sequence "*Start storing*", "*Interval = 120*" and "*Stop storing*" for recording an automatic sampling sequence please pay attention to the storing intervals, set with "*Storing Interval =*". To obtain more than one measurement “Interval” needs to be longer than “Storing Interval =”.

Fluorescence measurements require some additional time, especially if they include Fo'-measurements, since the saturation light pulse is only given after averaging the gas exchange data. The maximal time required for a Yield measurement in *Fo'-mode* is 8s. In a data sampling sequence, where the number of Yield measurements is given with the command "*1Yield/y*MP=*" command, the idle time of the next measuring interval (idle time = measuring interval - averaging time) is used for this. But if the idle time of the specified measuring interval is too short, the measuring interval of the following MP is prolonged. As a result, the total Interval set with "Interval =" may contain fewer MPs than intended. If a yield measurement is started at the end of an interval, the yield measurement is finished before proceeding with the next command in the user program.

A simple way avoiding data sampling sequences during a program run is using the simple commands "*Store MP ZP*", "*Interval = 20*", "*Store MP ZP*", "*Interval = 20*", "*Yield*"… instead. The program performs one command after the other no matter how long it takes.

8.1.6 Programming Example for making Settings

Remark ========Settings Start =========	
Impeller =7	Impeller switched on to step 7
Add Temperature Differenz (Ttop-Tcuv) =0	Set to no temperature difference between upper and lower cuvette half (standard)
Set Tcuv =23.0	*Tcuv* set to 23 °C
Set PARtop =0	mode set to *PARtop*, no light
Set Flow =600	Flow set to 600 µmol/s
Set CO2 =400	CO_2 entering the measuring head set to 400 ppm
Set H2O(ppm) =20000	At a cuvette temperature of 23°C this corresponds to 70% RH and 17°C dew point.
Mode =MP	Mode set to MP
Remark ===== Settings Fluorescence ====	Settings for Fluorescence Module, here standard values
SatWidth FL =0.6	
Sat-Int FL =12	

FR-Int FL =12	
Fo'-Mode FL =off	
FarRed FL =off	
Gain FL =low	Note, *Zero Offset* of the PAM-Fluorometer must be adjusted, if the *Gain* or the *ML-Ampl* have been changed
ML-Amp FL =10	
M-Light FL =on	
ETR-Fact FL =0.84	
Remark ===== Settings Data Storage ====	
Storing Interval =005/005	Storing interval for data sequences and averaging time are both set to 5s here
*1Yield/y*MP =0*	No fluorescence measurements with automatic data sequences
Load_Stability_File =Stability_Criteria_test	The file with stability criteria must exist, otherwise default criteria are used

8.1.7 Programming Example for a Light Curve

	Settings can be made here, manually, or in a separate user program
Remark ======= Light Curve ========	
Remark ===whatever light is preferred==	Remarks only serve to structure the user program
Set PARtop =400	Activate the leaf at its usual environmental condition, here 400 µmol m^{-2} s^{-1}.
Interval =180	Give the system some time to stabilize and store a ZP afterwards, while the leaf is already experiencing light.
Mode = ZP	
Interval = 60	
Store MP ZP	Stores a ZP
Mode = MP	
Wait until steady, max =1800	Depending on whether the leaf opens its stomata or not it

	can take longer or shorter until all values are steady.
Remark ==== First Storage of MP ======	
Yield	If only a Light Module is connected, always use the command "*Store MP ZP*" instead of "*Yield*". "*Store MP ZP*" can also be used several times before the "*Yield*" command .
Remark ==========================	
Set PARtop =220	Next light step (lower)
Interval =60	
Yield	
Remark ==========================	
Set PARtop =100	Next light step (lower)
Interval =60	
Yield	
Remark ==========================	
…	More lower light steps
Remark ==same light as start conditions==	
Set PARtop =400	Same light as start condition
Interval =120	
Wait until steady, max =1800	Since the light was low, it may take again some time until the leaf reaches the same value as before
Yield	
Remark ==========================	
Set PARtop =600	Next light step (higher)
Interval =120	
Wait until steady, max =300	Since the light was increased, stomatal opening may take a while.
Yield	
Remark ==========================	
…	More higher light steps
Remark ==========================	
Set PARtop =0	

Interval = 600	10 min dark acclimation for rough FvFm estimation
Fv/Fm	Measurement of dark respiration and rough Fv/Fm
Remark ============End==========	
Beep	
Interval =1	
Beep	
Freeze Gas Regulations	Prepare for opening chamber
Impeller = 3	
Count up Object No	
Messagebox = change leaf, then press ok	User can change sample
Set PARtop =400	
Interval = 5	
Continue Gas Regulations	Continue regulations
Impeller = 7	
Repeat Run	Starts from the beginning

8.1.8 Programming Example for a CO_2 Curve

Flow = 600 *…..*	Settings can be made here, manually or in a separate user program.
Set PARtop =1000	
Remark ======== CO2 Curve ==========	
Set CO2 =340	CO_2 entering the measuring head set to 400 ppm
Remark ===whatever light is preferred==	Remarks only serve to structure the user program
Set PARtop =600	Activate the leaf at its usual environmental condition, here 600 µmol m^{-2} s^{-1} and 340 ppm CO_2.
Mode = ZP	
Interval = 45	
Wait until steady, max = 240	
Store MP ZP	

Mode MP	
Interval =30	
Wait until steady, max =1800	
Yield	If only a Light Module is connected use the command "*Store MP ZP*" always instead of "*Yield*". "*Store MP ZP*" can be used several times before the "*Yield*" command to store several data sets per CO_2 level.
Remark -----------------------------------	
Set CO2 =35	very low CO_2 level
Mode = ZP	
Interval = 30	
Wait until steady, max = 300	adjustment of very low CO_2 may take some time
Store MP ZP	
Mode MP	
Interval =30	
Wait until steady, max =120	
Yield	
Remark -----------------------------------	
.....	Repeat the same code with different CO_2 levels going up step by step
Remark --------------End-----------------	
Set PARtop =0	10 min dark adaptation for rough FvFm estimation. In addition, dark respiration is recorded
Set CO2 =340	
Mode = ZP	
Interval = 120	
Store MP ZP	
Mode MP	
Interval =480	
Fv/Fm	Measurement of dark respiration and rough Fv/Fm
Beep	

Interval =1	
Beep	
Freeze Gas Regulations	Prepare for opening chamber
Impeller = 3	
Count up Object No	
Messagebox = change leaf, then press ok	User can change sample
Set PARtop =1000	
Interval = 5	
Continue Gas Regulations	Continue regulations
Impeller = 7	
Repeat Run	Starts from the beginning or from the line: "--Repeat from here-------\" if present

8.1.9 Discussion of Light or CO2 curves

In the previous chapters, an example of a light curve and a CO_2 curve are given. In science, there are several opinions on how to measure a light curve depending on their research interests and experiences. Scientists who are most interested in the light reaction may use a dark acclimated leaf that is cut under water before sunrise. Then they first measure Fv/Fm and only afterwards expose the leaf to light and wait until the stomata have opened, they may even use a higher CO_2 concentration than ambient and low oxygen to avoid photorespiration and measure maximum electron transport rate. These scientists usually want to measure the low light values before the leaf has experienced any high light in order to obtain maximum quantum efficiency without any photoinhibition. Other scientists may be interested in stomates and the conductance reached under every light level and how much CO_2 can be assimilated then. They need to wait much longer in low light than programmed in the given examples. In addition, they may want to save several data to save the dynamics of the stomates and then give one saturating flash at the end of each light level. Other scientists may be interested in measuring light curves during the day as fast as possible. They may want to avoid any dark adaptation period before measuring the light curve, because it leads to a closure of stomates. It may even be sensible to measure from ambient down and from ambient up with different samples or to measure form the highest value downwards.

The dark acclimated Fv/Fm measurement is required for calculating the fluorescence parameters. Only the Y(II) can be calculated without this reference. In the examples a compromise is suggested by measuring Fv/Fm last not first. There are other compromises in the literature on how the Fo and Fm value can be estimated.

A CO_2 curve is usually also started at ambient conditions or with a CO_2 concentration a bit below ambient concentration until the stomata are well opened. Subsequently, the CO_2 concentration is completely decreased and then increased step by step. Avoid long term exposure to very low CO_2 concentrations. Low CO_2 concentrations may theoretically affect the activity of the RubisCO. Very long exposure to low CO_2 concentrations can lead to wilting especially with dry air. The more open the stomata are the more accurate is the calculation of the intercellular CO_2 concentration. Therefore, do not spend more time than necessary at each CO_2 level. However, it should be noted that not only the stomata, but also the amount of metabolites in the Benson-Calvin cycle and enzyme activity adapt with each change. Also note that the diurnal rhythm influences the measured curves. Traditionally, a light curve or CO_2 curve published in textbooks has a steady state level reached at each step. But since there are environments in nature, where there is no steady state, scientists may be interested in other measurement approaches. GFS-Win allows the scientists to adapt the user programs freely to their needs.

9 Calculations

9.1 Parameters for Calculations

The following table (Table 5) lists the parameters on which the calculations in GFS-Win are based. A brief description of each parameter and its units is given.

Table 5: Parameters used for calculation

Measured value	Description	Unit
Area or Weight	Reference value of the sample (area or weight), provided by the user	cm^2 or mg
CO2abs	CO_2 mole fraction in reference cell of analyzer, equals CO_2 concentration at measuring head inlet.	ppm*
CO2sam	CO_2 mole fraction in sample cell of analyzer.	ppm
CO2delay	Time difference between the gas arriving in the sample or reference side of the gas analyzer	s
dCO2ZP	= $CO2sam_{(t)}$ - $CO2abs_{(t)}$ (in ZP mode)	ppm
dCO2MP	= $CO2sam_{(t)}$ - $CO2abs_{(t\text{-}CO2delay)}$ (in MP mode)	ppm
H2Oabs	H_2O mole fraction in reference cell of analyzer, equals H_2O concentration at measuring head inlet.	ppm
H2Osam	H_2O mole fraction in sample cell of analyzer.	ppm
dH2OZP	= H2Osam - H2Oabs (in ZP mode)	ppm
dH2OMP	= H2Osam - H2Oabs (in MP mode)	ppm
Pamb	Ambient barometric pressure	kPa
Flow	Gas flow into cuvette	µmol/s
Tcuv	Air temperature within measuring head	°C
Tleaf	Leaf temperature	°C

* ppm = parts per million = $\mu mol\ mol^{-1} = 10^{-6}$

9.2 CO_2 Mole Fraction (ca)

The CO_2 mole fraction around the sample (ca) approximately corresponds to the CO_2 mole fraction at the outlet of the measuring head. It is measured in the sample side of the analyzer (CO2sam). The value (CO2sam) must be corrected for the differential zero-value (dCO2ZP) to receive ca:

(1)

$$\text{ca} = \text{CO2sam} - \text{dCO2ZP}$$

Since the relationship between CO2sam and ca is very simple, CO2sam is not indicated.

9.3 Differential CO_2 Mole Fraction in MP Mode (dCO2MP)

The differential CO_2 mole fraction (dCO2MP) is the difference between the CO_2 mole fraction in the sample side (CO2sam) and in the reference side (CO2abs) of the analyzer. The gas flowing through the measuring head enters the analyzer later than the gas flowing through the reference paths. For the differential CO_2 determination, this time difference (delay CO_2;) is taken into account.

(2)

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

The time difference (CO2delay) is a value stored in the measuring head and can be adjusted for a changed tube length. The subscript t denotes the current time.

The next equation is another form of equations (1) and (2):

(3)

$$\text{dCO2MP}_{(\text{t})} - \text{dCO2ZP} = \text{ca}_{(\text{t})} - \text{CO2abs}_{(\text{t-CO2delay})}$$

9.4 H_2O Mole Fraction (wa)

The H_2O mole fraction around the sample (wa) is calculated in the same way as the CO_2 mole fraction (ca). The gas leaving the measuring head is measured in the sample side of the analyzer.

(4)

$$\mathrm{wa = H2Osam - dH2OZP}$$

9.5 Differential H_2O Mole Fraction in MP mode (dH2OMP)

The differential H_2O mole fraction (dH2OMP) is the difference between the H_2O mole fraction in the sample side (H2Osam) and in the reference side (H2Oabs) of the analyzer. Since the H_2O concentration changes only slowly in comparison to CO_2, the time delay is not taken into account for the calculation of dH2OMP as for dCO2MP.

(5)

$$\mathrm{dH2OMP_{(t)} = H2Osam_{(t)} - H2Oabs_{(t)}}$$

The next equation is another form of equation (4) and (5).

(6)

$$\mathrm{dH2OMP_{(t)} - dH2OZP = wa_{(t)} - H2Oabs_{(t)}}$$

9.6 Relative Humidity (rh %)

The relative humidity is the ratio of the actual water vapor pressure of the air to the saturation water vapor pressure. Relative humidity is usually expressed as a percentage.

(7)

$$\mathrm{rh} = \frac{\text{Actual Vapor Pressure}}{\text{Saturation Vapor Pressure}}$$

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

(8)

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

Whereby:

SVP(Tcuv) = saturation vapor pressure at Tcuv calculated according to Goff-Gratch [kPa] (chapter 19).

9.7 Transpiration Rate (E)

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

(9)

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

where

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

The terms in equation (9) relate to the values provided by the GFS-3000 as follows:

(10) $u_e = Flow$

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

(12) $w_o = wa$

(13) $LA = Area$

Using the values provided by the GFS-3000 and equations (9) - (13) the transpiration rate E can be calculated as follows:

(14)

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

9.8 Vapor Pressure Deficit (VPD)

According to Caemmerer and Farquhar (1981) the ALVPD (or VPD) is calculated as follows:

(15)

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

Whereby:

VPD = (Air-to-Leaf-) Vapor-Pressure-Deficit [Pa/kPa],
w_i = Intercellular H_2O mole fraction within the leaf [ppm],
w_a = H_2O mole fraction in the leaf chamber [ppm].

The intercellular H_2O concentration (w_i) is determined from the temperature of the leaf assuming 100% humidity in the intercellular air spaces:

(16)

$$w_i = \frac{SVP(Tleaf)}{Pcuv}$$

Whereby:

SVP (Tleaf) = saturation vapor pressure at Tleaf calculated according to Goff-Gratch [kPa] (chapter 19),
Pcuv = total pressure in the cuvette [kPa].

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

(17)

$$\mathrm{Pcuv} = \mathrm{Pamb}$$

Using the values provided by the GFS-3000 and equations (15) - (17) the VPD can be calculated as follows:

(18)

$$\mathrm{VPD} = \frac{\left(\frac{\mathrm{SVP(Tleaf)}}{\mathrm{Pamb}} - \mathrm{wa}\right)}{1 - \frac{\left(\frac{\mathrm{SVP(Tleaf)}}{\mathrm{Pamb}} + \mathrm{wa}\right)}{2}}$$

9.9 Water Vapor Conductance (GH2O)

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

(19)

$$\mathrm{GH2O} = \frac{\mathrm{E}}{\mathrm{VPD}}$$

where

GH2O = total water vapor conductance [mmol m^{-2} s^{-1}],
E = transpiration rate [mmol m^{-2} s^{-1}],
VPD = (Air-to-Leaf-) Vapor-Pressure-Deficit [Pa/kPa].

VPD can also be expressed in kPa. To obtain this unit, multiply the VPD value by the ambient pressure and divide by 1000 .

GH2O can be calculated using the results of equations (14) and (18).

9.10 Assimilation Rate (A)

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

(20)

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

Whereby

A = assimilation rate [µmol m^{-2} s^{-1}],

u_e = molar flow rate at the inlet of the cuvette [µmol s^{-1}],

c_o = CO_2 mole fraction at the outlet of the cuvette [ppm],

c_e = CO_2 mole fraction at the inlet of the cuvette [ppm].

LA = leaf area [cm^2],

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

The terms in equation (20) relate to the values provided by the GFS-3000 as follows:

(21) $u_e = \text{Flow}$

(22) $c_e - c_o = \text{dCO2ZP} - \text{dCO2MP}$

(23) $c_o = \text{ca}$

(24) $\text{LA} = \text{Area}$

Using the values provided by the GFS-3000, equations (20) - (24) and the result of equation (14), the assimilation rate A can be calculated as follows:

(25)

$$A = \frac{\text{Flow} \cdot (\text{dCO2ZP} - \text{dCO2MP})}{\text{Area}} - E \cdot \text{ca}$$

9.11 Intercellular CO_2 Mole Fraction (ci)

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

(26)

$$c_i = \frac{\left(g_{CO2} - \frac{E}{2}\right) \cdot c_a - A}{g_{CO2} + \frac{E}{2}}$$

Whereby

c_i = intercellular CO_2 mole fraction [ppm],
g_{CO2} = conductance for CO_2 [mmol m^{-2} s^{-1}],
E = transpiration rate [mmol m^{-2} s^{-1}],
c_a = CO_2 mole fraction in the cuvette [ppm],
A = assimilation rate [µmol m^{-2} s^{-1}].

The conductance for CO_2 (GCO2) relates to the conductance for H_2O (GH2O) (simplified equation):

(27)

$$G_{CO2} = \frac{G_{H2O}}{1.56}$$

Using the values provided by the GFS-3000, equations (1) - (27) and the results of equations (14) and (25), the intercellular CO_2 mole fraction ci can be calculated as follows:

(28)

$$ci = \frac{\left(\frac{G_{H2O}}{1.56} - \frac{E}{2}\right) \cdot ca - A}{\frac{G_{H2O}}{1.56} + \frac{E}{2}}$$

Note that the value given for the intercellular CO_2 concentration (ci) generally makes no sense with closed stomates or only slightly open stomates or low evaporation, since the equation then contains a division by zero or close to zero.

9.12 Equations for Fluorescence Parameters - Optional

The following parameters are calculated from the fluorescence values Fo, Fm, F, Fo' and Fm' and the photosynthetically active radiation PARtop, PARbot or PARamb (depending on the mode). For the derivation of the equations see Klughammer and Schreiber 2008:

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

(29)

$$Y(II) = \frac{Fm' - F}{Fm'} = 1 - \frac{F}{Fm'}$$

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

(30)

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

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.

ETR-Fac: PAR absorbed by the sample. The value needs to be given by the user.

Fo' is either measured, if Fo' mode is on, or calculated following the relationship published by Oxborough and Baker (1997).

(31)

$$Fo' = \frac{Fo}{1 - \frac{Fo}{Fm} + \frac{Fo}{Fm'}}$$

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 among each other (Schreiber et al. 1986 as formulated by van Kooten and Snel, 1990):

(32)

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

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

(33)

$$qL = qP \cdot \frac{Fo'}{F}$$

The non-photochemical quenching is defined as the fluorescence quenched by processes other than photochemistry. There are three different approaches in the literature. qN, 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 has the advantage, that it does not require the determination of F, Fo or Fo', but only fluorescence values measured during a saturating light pulse. It is the only quenching coefficient, which can be greater than 1, it ranges to around 4.

(34)

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

(35)

$$NPQ = \frac{Fm}{Fm'} - 1$$

(36)

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

(37)

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

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 Values and Ranges

Table 6: Values and ranges

Value	Definition	Range, options	Unit, format
Date	Date (international standard date notation)		yyyy-mm-dd
Time	Time (international standard time notation)	00:00:00 -23:59:59	hh:mm:ss
Code	Position of solenoid valves	MP, ZP	String
Object	Object number, for differentiation of several objects within one report file	0001-9999	#
Area or Weight	Reference value of the sample used for calculations	Area: .01...999.99, Weight: 1...999999	cm^2 or mg
Status	Operating status of components		String
CO2abs	CO_2 mole fraction in reference cell of analyzer, equal to CO_2 concentration at the inlet of cuvette.	0 ... 5000	ppm
CO2sam	CO_2 mole fraction in sample cell of analyzer (usually not shown in the software)	0 ... 5000	ppm
dCO2ZP	= CO2sam - CO2abs (in ZP mode)	-99.99 ... +99.99	ppm
dCO2MP	= $CO2sam_{(t)}$ - $CO2abs_{(t-CO2delay)}$ (in MP mode)	-99.99 ... +99.99	ppm
H2Oabs	H_2O mole fraction in reference cell of analyzer, equal to H2O concentration at the inlet of cuvette.	0 ... 75000	ppm
H2Osam	H_2O mole fraction in sample cell of analyzer (usually not shown in the software)	0 ... 75000	ppm
dH2OZP	= H2Osam - H2Oabs (in ZP mode)	-60000 ... +60000	ppm
dH2OMP	= H2Osam - H2Oabs (in MP mode)	-60000 ... +60000	ppm
Flow	Gas flow through the cuvette	-75 ... +1500	µmol/s
Pamb	Ambient barometric pressure	60 ... 110	kPa
Aux1	Input signal of an additional sensor connected to AUX IN	0 ... 4095	mV
Aux2	Input signal of an additional sensor connected to AUX IN	0 ... 4095	mV
Tcuv	Cuvette temperature measured in lower half	-10 ... +55	°C

Value	Definition	Range, options	Unit, format
Ttop	Cuvette temperature measured in upper half	-10 ... +55	°C
Tleaf	Leaf temperature	Tcuv-30 ... Tcuv+30	°C
Tamb	Ambient temperature	-10 ... +55	°C
PARtop	Photosynthetically active radiation measured with sensor in upper cuvette half	0 ... 3200	µmol/(m²*s)
PARbot	Photosynthetically active radiation measured with sensor in lower cuvette half	0 ... 3200	µmol/(m²*s)
PARamb	Ambient photosynthetically active radiation measured with external sensor MQS-B/GFS	0 ... 3200	µmol/(m²*s)
Imp	ImpSet value for impeller	0 ... 9	steps
Tmin	Minimum or maximum temperature (measured near Peltier element instead of Ttop in Standard Measuring Head 3010-S Version 1 or in Gas Exchange Chamber 3010-GWK1)	-10 ... +55	°C
rH	Relative humidity in the cuvette	calculated	%
E	Transpiration rate	calculated	mmol/(m²*s)
VPD	Vapor pressure deficit between object (leaf) and air	calculated	Pa/kPa
GH2O	Water vapor conductance	calculated	mmol/(m²*s)
A	Assimilation rate	calculated	µmol/(m²*s)
ci	Intercellular CO_2 mole fraction	calculated	ppm
ca	CO_2 mole fraction in the cuvette (=CO2sam-dCO2ZP)	calculated	ppm
wa	H2O mole fraction in the cuvette (=H2Osam-dH2OZP)	calculated	ppm
Optional			
Fo	Fluorescence of the dark-adapted leaf with only the measuring light on	0…4000, should range between 100 and 600	mV
Fm	Fluorescence of the dark-adapted leaf during a saturating light pulse	0…4000	mV
Fv/Fm	Maximal quantum efficiency of photosystem II (Kitajima and Butler 1975 and Butler and Kitajima 1975)	0…0.84	
F	Fluorescence	0… 4000	mV

Value	Definition	Range, options	Unit, format
Ft	transient Fluorescence in Chart	0…4000	mV
Fm'	Fluorescence of the illuminated leaf during a saturating light pulse	0…4000	mV
Fo'	Fluorescence of reoxidized photosystem II illuminated with only far red illumination and measuring light after a saturating light pulse.	0…4000	mV
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)
qN	Non-photochemical quenching (Schreiber et al. 1986)	0..1	
qP	photochemical quenching with no connectivity (Schreiber et al. 1986)	0..1	
qL	photochemical quenching with infinite connectivity	0..1	
NPQ	Non-photochemical quenching (Bilger and Björkman, 1990)	0..10	
Y(NPQ)	Quantum yield of NPQ related energy loss	0..1	
Y(NO)	Quantum yield of 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 photosystems. The value needs to be entered by the user	0..1	

11 Data Record Structure

Each file starts with two lines of headers. The first line contains the description for each column; the second line contains the units. The two header lines are followed by data record sets consisting of measured and calculated values and additional information. Each column is explained below. The text fields contain the headers and one or more example values.

Date
yyyy-mm-dd
2005-04-08

The date is stored in the international standard notation yyyy-mm-dd, where yyyy is the year in the usual Gregorian calendar, mm is the month of the year between 01 (January) and 12 (December), and dd is the day of the month between 01 and 31. When the file is imported into a spreadsheet program, e.g. Excel, the appearance of the date depends on the format selected by the spreadsheet program.

Time
hh:mm:dd
23:14:07

The time is stored in the international standard notation for the time of day hh:mm:dd, where hh is the number of complete hours that have passed since midnight (00-23), mm is the number of complete minutes that have passed since the beginning of the hour (00-59), and ss is the number of complete seconds since the beginning of the minute (00-59). When the file is imported into a spreadsheet program, e.g. Excel, the appearance of the time depends on the format selected by the spreadsheet program.

Code
String
ZPc001
ZPi005
MP_010

The first two characters of Code indicate whether the stored record set is a zero point "ZP" or a measuring point "MP". For ZP, the third letter indicates the switching position of the two solenoid valves. "c" means that ZP was measured with an empty cuvette and the solenoid valves being switched to MP mode, "i" means that ZP was measured in ZP mode. The last three digits indicate the number of data points averaged for this record set. If the averaging has been disturbed there is "err" instead of a number. The value should still be correct but has fewer averaged values.

Object
#
107

Object number between 1 ... 9999 for distinguish between samples or treatments.

Area
cm^2
8

Weight
mg
1000

Reference type *Area* in cm^2 or *Weight* in mg selected when creating a new file. The reference type cannot be mixed within a file, because the header is valid for the entire file. The units of the calculated parameters A, E and GH2O depend on the selected reference type.

Status
String
AFF1FF612FF---1FF--…

In the *report* the complete status string is saved, while in the *Values window* only the status characters of the components are displayed "FF" = component is functioning, "--" = component is not connected, neither "FF" nor "--" = error code of the component (chapter15.2.7).

Character 1:	A: Reference is Area, W: Reference is Weight
Character 2&3:	Status of battery control (FF = ok)
Character 4:	Type of central control unit (here type 1)
Character 5&6:	Status of central control unit (FF = ok)
Character 7:	CO_2 supply (here 6 bar)
Character 8:	Type of measuring head (here type 1)
Character 9:	Version measuring head (here version 2)
Character 10&11:	Status of measuring head (FF = ok)
Character 12:	Type additional temperature sensor
Character 13, 14:	Status additional temperature sensor (FF = ok)
Character 15:	Type of PAM fluorometer (here type 1)
Character 16&17:	Status of PAM fluorometer (FF = ok)
Character 18:	Type of oxygen sensor
Character 19:	Type of cold trap
Character 20&21:	Status of cold trap (FF = ok)

In the *Menu Status*, the resolved status string is shown (chapter 7.9.4).

CO2abs
ppm
350.6

CO_2 mole fraction measured in the CO_2 reference cell of the analyzer, which corresponds to the CO_2 mole fraction at the inlet of the cuvette.

dCO2ZP
ppm
0.491816

Difference between CO_2 mole fraction in the sample cell and reference cell of the analyzer measured as zero point ZP. If the record set is a zero point (ZP), the value is newly measured. If the record set is a measuring point (MP) the value from the last ZP measurement was carried over.

dCO2MP
ppm

-2.499774

Difference between CO_2 mole fraction in the sample cell and reference cell of the analyzer measured in MP mode. If the record set is a zero point (ZP), the value of dCO2MP is filled with "----".

H2Oabs
ppm
15826

H_2O mole fraction measured in the H_2O reference cell of the analyzer, which corresponds to the H_2O mole fraction at the entrance of the cuvette.

dH2OZP
ppm
-168.7

Difference between H_2O mole fraction in the sample cell and reference cell of the analyzer measured as zero point ZP. If the record set is a zero point ZP, the value is newly measured. If the record set is a measuring point (MP) the value from the last measured dH2OZP-value was carried over.

dH2OMP
ppm
819.1

Difference between H_2O sample and reference cell of the analyzer measured in MP mode. If the record set is a zero- point (ZP), the field dH2OMP contains "----".

Flow
µmol/s
749.6

Air flow to the measuring head, measured with the electronic mass flow meter.

Pamb
kPa
99.34

Ambient barometric pressure.

Aux1
mV
2019

Input signal in mV of a sensor connected to Aux1 of AUX IN (chapter 16). The input voltage for Aux1 is 0 ... 4095 mV. A function can be applied with a command in a user program.

Aux2
mV
137

Input signal in mV of a sensor connected to Aux2 of AUX IN (chapter 16). Characteristics re the same as for Aux1.

Tcuv
°C
25.01567

Cuvette temperature measured with a Pt100 sensor in the lower half of the cuvette of the Standard Measuring Head 3010-S.

Ttop
°C
25.01567

Cuvette temperature measured with a Pt100 sensor in the upper half of the cuvette of the Standard Measuring Head 3010-S.

Tleaf
°C
24.92682

Leaf temperature measured with a thermocouple in the lower half of the cuvette of the Standard Measuring Head 3010-S. The reference for the thermocouple is Tcuv.

Tamb
°C
22.18981

Ambient temperature measured by a Pt100 sensor, which is located at the external fan of the lower side of the Standard Measuring Head 3010-S.

PARtop
µmol/(m²*s)
20.99068

Photosynthetically active radiation (PAR) measured in the upper half of the cuvette of the Standard Measuring Head 3010-S with a light sensor type LS-A (chapter 16.8 for spectral sensitivity).

PARbot
µmol/(m²*s)
3.274362

Photosynthetically active radiation (PAR) measured in the lower half of the cuvette of the Standard Measuring Head 3010-S with a light sensor type LS-A (chapter 16.8 for spectral sensitivity).

PARamb
µmol/(m²*s)
5.625673

Photosynthetically active radiation (PAR, since the MQS is cosine corrected the term "photosynthetic photon flux density" (PPFD) is also correct) measured on top of the upper part of the cuvette of the Standard Measuring Head 3010-S by a light sensor type MQS-B/GFS (chapter 16.7 for spectral sensitivity).

Imp
step
5

Set value in steps from 0 to 9 for the impellers inside the upper and lower half of the cuvette of the Standard Measuring Head 3010-S.

rH
%

52.87663

Relative humidity inside the cuvette of the Standard Measuring Head 3010-S. If a measuring point MP is stored and a *Tcuv* sensor is present, rh is calculated as described in chapter 9.6, otherwise the field rh contains "----".

E
mmol/(m²*s)

0.941

Transpiration rate of the enclosed sample calculated as described in chapter 9.7. If the record set is a ZP, the field contains "----".

VPD
Pa/kPa

15.3

Vapor pressure deficit between leaf and air of the enclosed sample. If a measuring point MP is stored and the sensors for *Tcuv* and *Tleaf* are present, VPD is calculated as described in chapter 9.8, otherwise the field contains "----".

GH2O
mmol/(m²*s)

61.5

Water vapor conductance of the enclosed sample. If a measuring point MP is stored, GH2O is calculated as described in chapter 9.9, otherwise the field GH2O contains "----".

A
µmol/(m²*s)

1.57

Assimilation rate of the enclosed sample. If a measuring point MP is stored, A is calculated as described in chapter 9.10, otherwise the field contains "----".

ci
ppm

300.3

Intercellular CO_2 mole fraction in the enclosed sample. If a measuring point MP is stored and sensors for Tcuv and Tleaf are present, ci is calculated as described in chapter 9.11, otherwise the field contains "----".

ca
ppm

348.1

CO_2 mole fraction in the cuvette of the measuring head. If a measuring point MP is stored, ca is calculated as described in chapter 0, otherwise the field contains "----".

wa
ppm

17000

H_2O mole fraction in the cuvette of the measuring head. If a measuring point MP is stored, wa is calculated as described in chapter 9.4, otherwise the field contains "----".

comment
whatsoever

comments that have been entered in the comment line before saving data. If the Imaging-PAM is used, the filename and the image number are stored in this comment column.

Fo
mV
311

Fo is the fluorescence of a dark acclimated sample, measured with no additional light except measuring light. It is measured with the button *Store Fv/Fm +MP* or the command "*Fv/Fm*".

Fm
mV
1604

Fm is the maximal fluorescence of a dark acclimated sample during a saturating light flash. It is measured together with Fo with the button *Store Fv/Fm +MP* or the command "*Fv/Fm*".

Fv/Fm
0.806

Fv/Fm is calculated from Fo and Fm and corresponds to the maximum yield obtained in the dark acclimated state as described in chapter 9.12.

F
mV
294

F is fluorescence as it is recorded. If it is recorded during a yield measurement, it is the fluorescence measured before the saturating light flash was given.

Fm'
mV
361

Fm' is the fluorescence during a saturating light flash of a sample which is exposed to actinic light. It is measured with the button *Store Yield +MP* or the command *Yield* or automatically with *y MP/1 Yield*.

Fo'
mV
0

Fo' is a measured value, measured after each Yield measurement in the dark with far red light switched on (Fo'-mode).

Fo' calc
mV
0

Fo'calc is a calculated value replacing Fo' see chapter 9.12.

Yield
0.186

Yield (PSII) is a value calculated from F and Fm' only (chapter 9.12).

ETR
46.77

ETR electron transport rate calculated from the Yield value, ETR-Fac and PAR as described in chapter 9.12.

qP
1.3400

qP photochemical quenching calculated as described in chapter 9.12.

qL
1.3400

qL photochemical quenching (lake model) calculated as described in chapter 9.12.

qN
0.9613

qN non-photochemical quenching (chapter 9.12.).

NPQ
mV
3.443

NPQ non-photochemical quenching (chapter 9.12). When comparing NPQ values from the record file of the GFS-Win software and the report-file of the ImagingWin software, note that the NPQ value in ImagingWin is divided by 4.

Y(NPQ)
mV
0

Yield(NPQ) is the fraction of absorbed quanta lost in the processes of non-photochemical quenching (chapter 9.12).

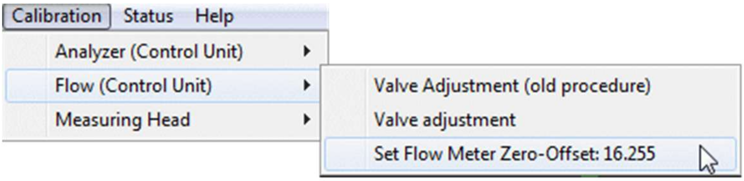
ETR-Fac
0.84

The ETR-factor needs to be measured for every sample and entered manually. It is measured best with an Ulbricht sphere (not produced by the Heinz Walz GmbH). If an Imaging-PAM is used, the value indicated as ETR-Fac is taken from the absorptivity measurement.

12 Calibrations and Adjustments

All calibration values are stored in the parts themselves, so that the Standard Measuring Head e.g. can freely be interchanged between instruments. But note that if the light source is exchanged between measuring heads, the light-source factor needs to be adjusted.

12.1 Offset of Flow meter



Switch the flow and impeller off, then chose the item *Menu→ Calibration/Maintenance →Central Unit →Flow Meter Zero-Offset*. The Offset will immediately be adjusted to a new value and stored in the flow meter.

12.2 Valve Adjustment

The adjustment of the internal restrictor valves (*see* Fig. 3) can be necessary after changing the flow rate or the measuring head of the GFS-3000. The valve adjustment tunes the system, so that the pressure in the reference and sample cell of the analyzer is equal. Also the total flow through the system or analyzer in MP mode and ZP is adjusted with the valves. The flow through the analyzer is shown by the mechanical flow indicators attached to the front panel (Fig. 2).

The flow adjustment requires a small screw driver. The valves close clockwise (screw moves into the instrument) and open counter-clockwise (screw moves in the outward direction). Do not turn the valves all the way to the end. They are closed or open before they feel tight. Forceful closing/opening or an inappropriate screw-driver may destroy the soft metal of the valves.

The valve adjustment needs to be done with a closed measuring head. It is easier to close it airtight, if something (e.g. 1 mm neoprene, a plastic-covered card-board or plastic-covered foam) is put in place of the leaf. For the valve adjustment, the system should be set up in the configuration, which is used for the experiments and the flow that is usually used for the experiments should be entered. We recommend a flow rate of 750 µmol/s with the Standard Measuring Head 3010-S. If the impeller speed is quite often changed, the standard value 7 should be entered. It has hardly any effect on the valve adjustment.

The flow indicated on the computer screen is measured with the electronic flow meter located inside the system with the unit µmol/s. The flow indicated by the mechanical flow indicators at the front panel has the unit ml/min.

Fig. 53 shows a simplified version of the pneumatics with emphasis on the purpose of the valves. Table 1 gives an overview on the valves, and its adjustment criteria. You may directly adjust the valves by following these short instructions or by using a guided protocol available in GFS-Win (*Menu →Calibration/Maintenance →Central Unit →Valve adjustment*). It is suitable for flow rates between 350 and 1500 µmol/s.

The guided protocol will firstly ask for the flow rate and impeller speed. The given values need to be confirmed or set. To start with defined conditions and a steady running pump, valve 3 and 5 need to be roughly adjusted before the fine adjustment is possible. With low flow (below 700 µmol/s) valve 3 needs to be closed, so that all air coming from the measuring head travels through the analyzer.

Also, with a low flow (below 800 µmol/s) valve 5 needs to be open to assure a proper operation of the pump and good air mixing in the first part of the gas pathway. Valve 5 should be opened until the *DAC Flow,* which is the pump voltage in digital numbers (DAC: digital analog conversion), indicates a value around 1500 (±200). The *DAC Flow* value is the last choice in the chart. It will also be indicated on the computer screen in the quick view column during the guided protocol. For high flow rates valve 5 needs to be closed and the *DAC Flow* value may reach higher values than 1500.

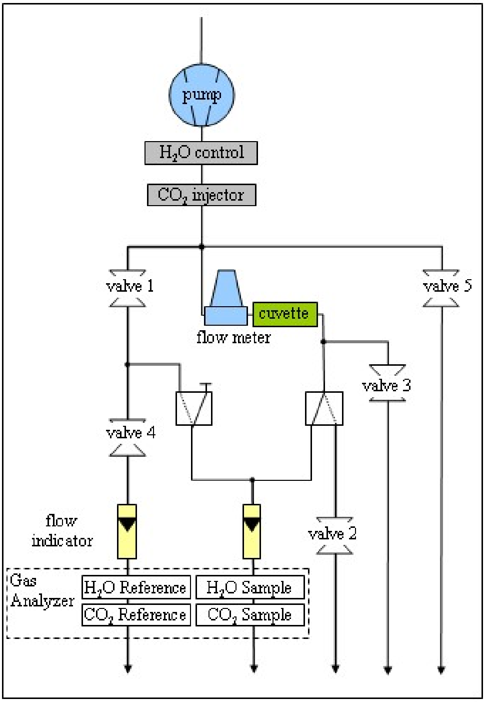
Valve 4 balances the flow between sample and reference of the analyzer in ZP mode. Therefore, the guided protocol switches to ZP mode and valve 4 needs to be adjusted until the mechanical flow indicators have equal height.

Valve 1 and 3 determine the flow through the analyzer in MP mode (the measuring head needs to be connected and closed properly). With valve 3 adjust the flow of the sample side; and with valve 1 balance the flow at the reference side. Valve 5 may need to be readjusted afterwards.

Valve 2 determines the total flow in ZP mode, since the pump is regulated by the flow measured with the electronic mass flow meter. The regulated value is called *DAC Flow*, which is the pump voltage in digital numbers. It needs to be adjusted so, that *DAC Flow* is equal in ZP and MP mode. The better this adjustment the shorter the purge time for *AutoZP* can be chosen.

In the guided protocol for the adjustment of valve 2, the regulation of the pump is switched to a constant voltage (*DAC Flow* value constant) and valve 2 is asked to be adjusted until the flow measured with the electronic flow meter (see number on computer screen) in ZP-Mode is the same as before in MP-mode. This can be checked by switching during this step from ZP mode to MP and back. The flow should not change – or change only very little during switching. If adjustment is done directly without the guided protocol, you may switch between MP and ZP and watch the value for *DAC Flow* (last option in chart). The DAC value should change as little as possible, when switching between MP and ZP.

Most probably the best position of valve 2 is completely open or nearly completely open.



Valve 1 balances flow between analyzer sample and reference in MP mode.

Valve 2 determines total flow (pump speed) in ZP mode only.

Valve 3 determines flow through analyzer sample in MP mode - open with high flow rates.

Valve 4 balances flow between analyzer sample and reference in ZP mode.

Valve 5 determines total flow (pump speed) independent of mode, ZP or MP – open with low flow rates.

Fig. 53: Pneumatics of GFS-3000 with emphasis on valves. Solenoids are in position of MP mode. In ZP mode both solenoids will be switched to the position indicated by the dotted line.

Table 7: Direct valve adjustment, valves are listed in order of adjustment. It may be necessary to roughly adjust valve 3 and 5 before starting.

Valve	Mode	Adjustment
4	ZP	Equal flow at flow indicators ANALYZER REF and SAMPLE.
3	MP	Flow through Analyzer 800 ml/min at flow indicator ANALYZER SAMPLE or until closed (with low flow rate). It might be necessary to close valve 5 first.
1	MP	Equal flow at ANALYZER REF and SAMPLE.
5	MP	Pump Voltage Adjust so that *DAC Flow* is 1500 +- 200 or until closed (with high flow rate) DAC-Flow can be found: Control Unit Model 3200-C, GFS-Win: Chose in chart (last item) Control Unit Model 3000-C: *Option2 →System Values*
2	MP ↔ ZP	Total flow in ZP Mode: Watch DAC-Flow while switching between ZP and MP. Adjust valve 2 in ZP mode, so that the DAC-Flow value is the same in MP and ZP mode. You may write and start a user program for the adjustment. MP mode Interval = 20 ZP mode Interval = 40 Repeat Run The beads will be lower in ZP than in MP mode.

12.3 Calibration of Gas Analyzer

The calibration procedure has been changed in GFS-Win version 3.71. We recommend doing the calibration with the set-up described here (e.g. air-cycling) and the latest version of GFS-Win for all types of instruments, having Control Unit Model 3000C, 3100C or 3200-C.

The gas analyzer used in the GFS-3000 is a non-dispersive infrared photometer (NDIR). It consists of two infra-red radiators, two pairs of gold-surfaced gas cells, a chopper wheel and detectors. A pyroelectric (solid-state) detector serves for H_2O-detection and an opto-pneumatic sensor, for CO_2-detection. The rotating chopper wheel produces periodically changing signals within the detectors. For the GFS-3000 an analyzer with a dual-frequency chopper has been developed. It allows a very sensitive and exact determination of the difference value between sample and reference and simultaneously the determination of the absolute concentration of H_2O and CO_2.

The full calibration includes the zero point and afterwards a span calibration of the absolute signals. In order to carry out a calibration, the analyzer should be purged with calibration gas until the measured values no longer display a drift. There are two causes for drift, the warm-up drift and the flushing drift. The warm-up drift is a consistent drift after the gas analyzers have been switched on. Take your time for calibrations.

The warm-up drift is typically 15 min for CO_2 and 30 min for H_2O but may take up to 1 h for CO_2 and up to 2 h for H_2O. The warm-up drift can be avoided by keeping the gas analyzer on (standby mode) when not in use e.g. overnight. The calibration is best performed after this warm-up period. For calibration purposes, we recommend a longer waiting time than for measurements. It may be advisable to calibrate the analyzer in the evening after measurements for the next day (or week) rather than in a hurry in the morning. The gas analyzer is switched on, when the *measure mode* is switched on. It remains on, if the GFS-3000 is switched into *standby mode*. It is possible to feel by hand whether the analyzer is/was on, because the instrument is getting warm. The flushing drift is short for CO_2 less than 5 min, but long for H_2O 1-2 hours.

To carry out the CO_2 zero or H_2O zero calibration a tube-set (3000-C/TS) is requires. In addition, for the CO_2 zero calibration the CO2 absorber needs to be fresh and for the H_2O zero calibration the silica gel needs to be freshly dried. The CO_2 span calibration requires a tank equipped with a pressure reducer and a fine adjustment valve containing calibration gas. For the H_2O span calibration, a cold trap including a humidifying bottle is required.

The calibration process will be initiated by running the protocol that can be found in the GFS-Win software under the *Menu* → *Calibration/Maintenance*→ *Analyzer*.

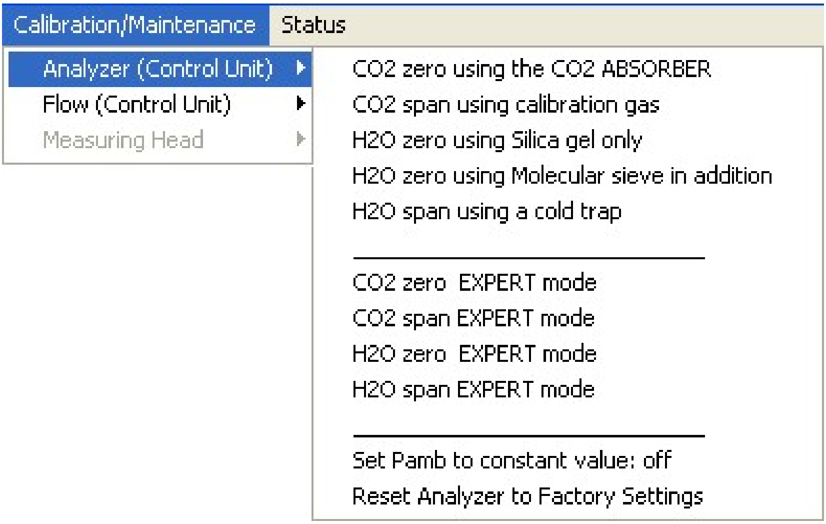


Fig. 54: Menu Calibration Analyzer

The upper four options are guided calibration procedures. The lower four additional options are expert calibration procedures. In the guided modes, the user is recommended how to set up the system step by step. In the expert-modes it is expected that calibration gas is passing the analyzer and only the actual calibration needs to be executed and stored. The last item *Reset Analyzer to Factory Settings* resets both, the CO_2 and the H_2O calibration of the analyzer to company values that were valid before delivery of the GFS-3000.

Every analyzer-calibration is automatically documented in the file "calibrat.rpt" in the subdirectory *My Documents/GFS-300/ini* or *<Install Dir>/GFS-Win*. Calibrat.rpt is a common text file. The calibration values themselves are stored directly in the analyzer.

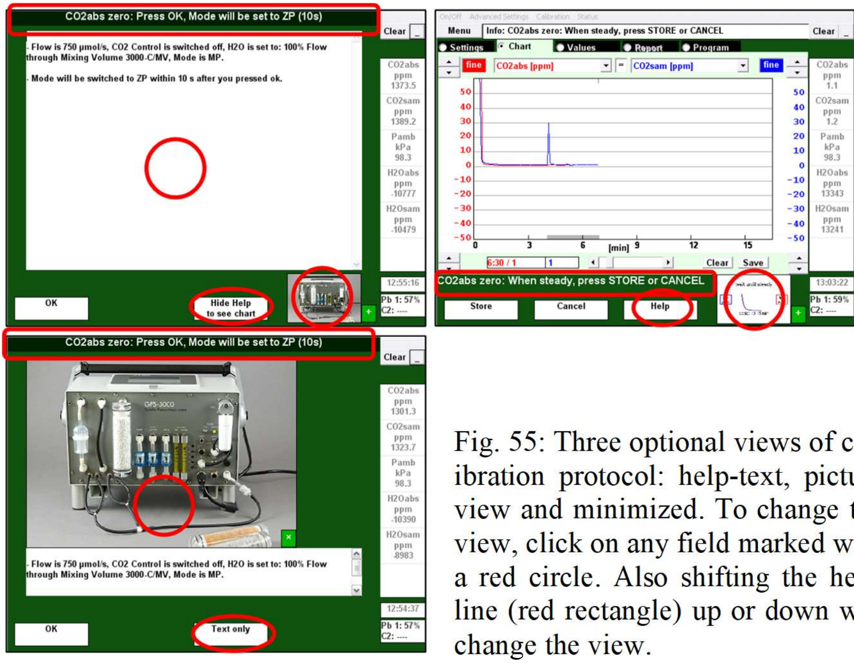


Fig. 55: Three optional views of calibration protocol: help-text, picture view and minimized. To change the view, click on any field marked with a red circle. Also shifting the head line (red rectangle) up or down will change the view.

Fig. 55 shows the software during a calibration procedure. After starting a guided calibration protocol, a window appears with a header line, a help text and an image for the instrument setup. This window can be minimized so that it only covers the lower part of the screen below the central window.

Fig. 56: Info line during actual analyzer calibration

In each calibration protocol there is a step where a button is named *Store*. The actual calibration is performed with the *Store* key. Alternatively, the procedure can be aborted with *Cancel*. After pressing S*tore,* the analyzer receives a signal that the actual calibration is to be performed. The analyzer averages the measured values while a countdown is displayed in the info line.

Perform the calibration only if the values shown in the chart are stable and sensible. Use a fine scale for the y-aches and a long time period for the x-aches to judge stability. Check the displayed values after each calibration. Never attach a hose to the outlets of the gas analyzer as it changes the pressure inside the analyzer, except during absolute zero calibrations. It may be necessary to adjust the valves after calibration (chapter 12.2).

12.3.1 Pneumatic Path during Calibration

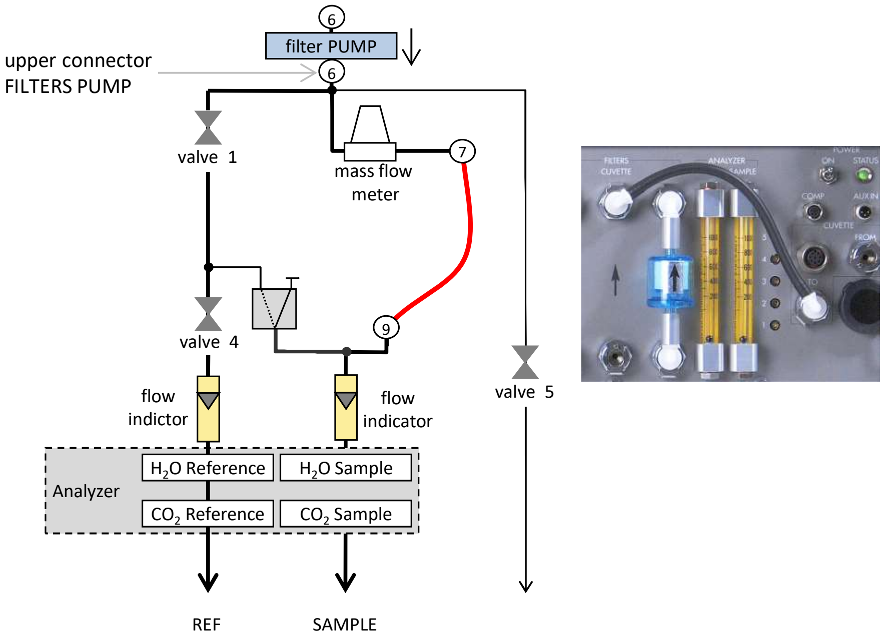


Fig. 57: Gas pathway in the down-stream section during calibration

A short pneumatic path is advantageous for all calibrations of the gas analyzer. This can be achieved by connecting the CUVETTE TO connector to the upper FILTERS CUVETTE connector (see red line 7 to 9 in Fig. 57). It may also be advantageous to close valve 5. But with the exception of the H_2O span calibration, the advantage is minimal. Nevertheless, all connected paths should be purged briefly with the current calibration gas. This can be achieved by briefly opening valve 5 and briefly switching to ZP mode. The calibration itself must be carried out in MP mode when using the setup shown in Fig. 57.

When calibrating H_2O or CO_2 span the flow through the flow indicators should both be at 800 ml/min and nothing should be connected to the outlets of the analyzer. This is not necessary when calibrating H_2O or CO_2 zero, since zero concentrations are not influenced by pressure.

12.3.2 CO_2 Zero using the CO_2 Absorber

Fig. 58: Set-up of the system for CO_2 absolute zero calibration.

- Chose *Menu→ Calibration/Maintenance → Analyzers→ CO2 zero using CO2 absorber*. Or on the Panel PC chose *Option1→ Cal. Gas Analyzers→ CO2 zero*

- The CO_2 zero calibration requires fresh soda lime (CO_2 absorber). Note that the pH-indicator of the soda lime only turns purple immediately during and after usage. The purple color may have disappeared the next day. Nevertheless, the soda lime is used up. If the inlet gas is very dry, the pH-indicator will not work.

- The drier tube and humidifier must be replaced by the Mixing Volume 3000C/MV and a tube.

- Disconnect the tubes to the Measuring Head

- Connect the CUVETTE TO connector to the upper FILTERS CUVETTE connector (see red line 7 to 9 and picture in Fig. 57).

- For the CO_2 zero, we do not recommend air-cycling, since the CO_2 absorber is usually wet. Continuous cycling may lead to water condensation in the tubes.

- Valve 5 may be closed to increase the flow through the analyzer; also valve 1 may be changed to balance the flow between analyzer reference and sample and therefore increase the speed of flushing the reference. Nevertheless, for the CO_2 zero calibration in contrast to any span calibration a balanced flow is not mandatory. Note that the valves need to be readjusted after the calibration, if they have been changed.

- Any connected paths should be purged briefly with CO_2 free gas. Open valve 5 briefly, if it has been closed. Also, the mode will briefly be switched to ZP mode (see Fig. 57).

- The two upper fields in the quick view column and the chart show the CO_2 absolute value of the reference cell (CO2abs) and the sample cell (CO2sam). Adjust the chart and observe the values. If both values have stabilized, click *Store*. The info line will display a countdown while the analyzer is performing the calibration, please wait.

- Check the calibration: Are CO2abs and CO2sam close to 0?

- Either set up the system for the next calibration (CO_2 span or H_2O zero) or for measurements:

  If necessary, enable the measuring head with *On/Off →Enable Components*.

  If valve 1 has been changed, it must be readjusted for measurements. The measuring head needs to be connected and closed. Switch the flow on. In MP mode, set valve 1 so that both flow indicators have the same height (chapter 12.2).

  If valve 5 has been changed, select the *Flow DAC* in the chart. In MP mode, adjust valve 5 so that Flow DAC is 1500 ± 100. For high flow rates that require a higher DAC value close valve 5. Afterwards check the adjustment of all valves (see Table 7 and chapter 12.2)

12.3.3 CO_2 Span using Calibration Gas

The zero point must always be calibrated before span-calibration. CO_2 span calibration requires a tank of CO_2 calibration gas equipped with a pressure reducer and a fine adjustment valve. Since the pump remains off, it is irrelevant whether absorber tubes are connected or not.

- In GFS-Win chose *Menu→ Calibration/Maintenance → Analyzers→ CO2 span* using calibration gas.
- No tube shall be connected at the outlet of the analyzer, ref and sample. Therefore, remove any cycling-tubes connected during H_2O zero calibration.
- Connect the CUVETTE TO connector to the upper FILTERS CUVETTE connector (see red line 7 to 9 and picture in Fig. 57).

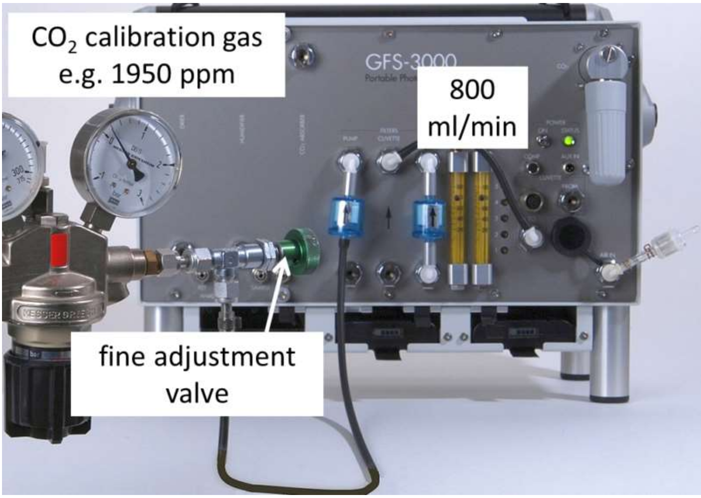


Fig. 59: System during absolute CO_2 span calibration.

- After pressing OK, the flow, CO_2 control and H_2O control will be switched off automatically.
- Practice the handling of the pressure reducer and fine adjustment valve of the calibration tank without connection to the GFS-3000 before proceeding. A fine stream may be adjusted before connection.
- Valve 5 may be closed in order to save calibration gas.
- After the pump is off, disconnect the filter labeled PUMP. The CO_2 calibration gas must be connected to the upper connector labeled FILTERS PUMP. The filter from the spare kit should be used in the feed tube of the calibration gas. Carefully adjust the pressure reducer and fine adjustment valve of the tank so that the flow indicators are at 800 ml/min. Valve 5 can be used for additional fine adjustment of the total flow. Use valve 1 to equalize the flow between analyzer reference and sample until both indicators are at 800 ml/min (see Fig. 59).
- Flush all connected gas pass ways: If valve 5 is closed, open it briefly. The mode will briefly be switched to ZP mode.

- In the next step, enter the set value of the calibration gas (between 200 ... 2200 ppm) into the GFS-Win software.
- The two upper fields in the quick view column and the chart show the CO_2 absolute value of the reference cell (CO2abs) and the CO_2 absolute value of the sample cell (CO2sam). When both values have stabilized, a CO_2 span setting can be performed using the *Store* key.
- The info-line shows a count-down during calibration, please wait.
- Check the calibration: Are CO2abs and CO2sam at the set value?
- After calibration, set up the system for measurements or another analyzer calibration. See chapter 12.2 for valve adjustment or continue with H_2O calibration.

  If necessary, enable measuring head with *On/Off →Enable Components*.

12.3.4 H_2O Zero using Drier

H_2O is a small molecule that is well absorbed by surfaces. Therefore, it takes a while until the system is completely flushed dry. We therefore recommend regular checking of the H_2O zero value, but only a complete calibration, if there is enough time for good drying. In order to be able to provide dry air as long as required even in humid climate, we recommend air-cycling (*see* Fig. 60). Note that not all silica gel on the market is suitable. Only use high quality silica gel from BASF or Walz; do not use burnt silica gel. Warm silica gel may not sufficiently dry the air. Therefore, we provide Molecular sieve 3 Å in addition. It has a higher drying efficiency than Silica gel, especially when warm, but it should only be used with dry air behind Silica gel.

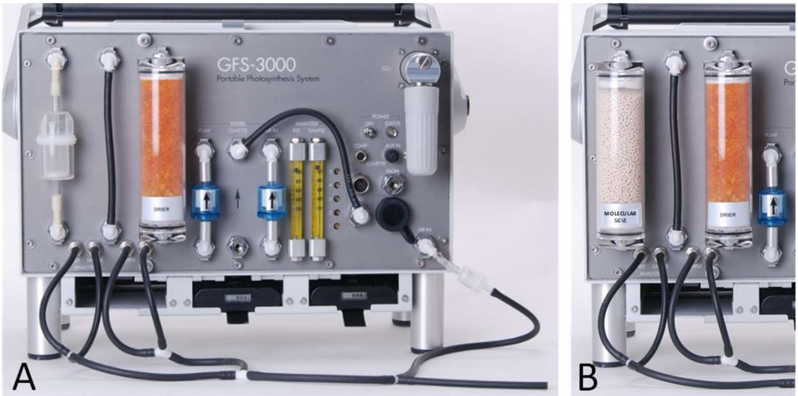


Fig. 60: Set-up for H_2O zero calibration with air-cycling, A: First dry with Silica gel, B: Additionally, use MOLECULAR SIEVE for drying.

- In GFS-Win chose *Menu→ Calibration/Maintenance→ Analyzers→ H2O zero using Molecular sieve in addition*. If no additional column for Molecular sieve 3 Å is at hand, use the menu option *H2O zero using Silica gel only.* With Molecular sieve, set up the system as shown in Fig. 60A, otherwise follow the pictures in the software.
- Use freshly dried silica gel in the drier tube and insert it in the place for the CO2 absorber.
- Insert the Mixing Volume 3000-C/MV in the place of the DRIER.
- Remove the humidifier and replace it with a tube.
- Use the air-cycling tube to connect all outlets with the filter at AIR IN. An open branch serves to avoid pressure problems.
- Connect the CUVETTE TO connector to the upper FILTERS CUVETTE connector (see red line and picture in Fig. 57).
- The parameters for flow, CO_2 control and H_2O control will be adjusted automatically. To speed up the purging process, valve 5 can be closed, but it should be opened briefly to also flush the connected paths. The mode is automatically switched briefly to ZP to purge all gas paths with dry air.

- In MP mode, opening valve 1 will equalize the air flow through the analyzer reference and sample and therefore speed-up the drying procedure. Watch the mechanical flow indicators rise as the flow through the analyzer reference increases while opening valve 1.
- The two upper fields in the quick view column show the H_2O absolute value of the reference cell (H2Oabs) and the sample cell (H2Osam). Also, the chart shows H2Oabs and H2Osam. If both values have decreased (after 10 to 20 min), replace the Mixing Volume 3000-C/MV with the MOLECULAR SIEVE (Fig. 60B).
- Wait until H2Oabs and H2Osam have stabilized (after 10 to 20 min), a H_2O zero-point calibration can be carried out via STORE. Both H_2O absolute values will be set to zero.
- Check the calibration: Is H2Oabs and H2Osam close to 0?
- After calibration, the system can be set up either for the next calibration or for measurements:

  Disconnect the air-cycling tubes. Connect absorber tubes, filters and the measuring head. If necessary, enable the measuring head with *On/Off →Enable Components*.

  If only valve 1 has been changed, it can be readjusted in the following way. Connect the closed measuring head. Switch flow and impeller on. Adjust valve 1 until both flow indicators show equal flow through both sides of the gas analyzer (also see Table 7 and chapter 12.2).

  If valve 5 has been changed, select the *Flow DAC* (value indicating the regulated voltage to the pump) in the chart. In MP mode, adjust valve 5 so that Flow DAC is 1500 ± 100, or close valve 5 for high flow rates that require a higher DAC value. Afterwards check the adjustment of all valves (see Table 7 and chapter 12.2).

12.3.5 H_2O Zero using a nitrogen cylinder

The system can be setup as shown in Fig. 59 for the CO_2-span calibration, but with a nitrogen cylinder or technical air or even CO_2 calibration gas. Switch the system into MP mode and wait until H2Oabs has stabilized on a low value. Then use the *Calibration/Maintenance→Analyzer(Control Unit) →H2O zero EXPERT MODE* to calibrate the zero value of the H2O analyzer. For saving pressurized gas, use silica gel to dry the system before using gas from the cylinder.

12.3.6 H_2O Span using a Measuring Gas Cooler

The zero point always needs to be calibrated before the span-calibration. For the H_2O span calibration, a cold-trap is required. Use the 6.4 mm connector from the spare-kit at the outlet of the cold trap. Follow the instructions slowly and very carefully, step by step, to avoid water being forced into the system, which would be a fatal mistake. Switch on the cold trap as early as possible before calibration. The set temperature of the cold trap needs to be at least 2°C below room temperature so that the gas in the water bottle will be correctly saturated. Only when the set temperature has been reached and condensation occurs, does the cold trap reach the set humidity value. To check for condensation, tilt the cold trap forward and watch for water running into the condensate hose. Fill only a small amount of water into the humidifier bottle to keep the back pressure low (see Fig. 62).

Fig. 61: Set-up of the GFS-3000 for H_2O span calibration with GFS-Win version 3.31 or higher.

- Chose *Menu → Calibration/Maintenance → Analyzer→ H2O span using cold trap*. And set up the system as shown in Fig. 61.
- Close the upper connector labeled DRIER, connect the CUVETTE TO connector to the upper FILTERS CUVETTE connector (see red line and picture in Fig. 57).
- The outlets from the analyzer must be open and no tube shall be connected.
- Close valve 5 (do not use force, stop one turn before the end).
- Replace the CO_2 absorber with the Mixing Volume 3000-C/MV, insert the humidifier tube (humidity is irrelevant) and disconnect the filter labeled PUMP.
- The flow, CO_2 control, H_2O control and mode will be adjusted automatically.
- After the system has been set up, continue with OK.

Follow the following steps very accurately:

- While the pump is off, connect the lower connector of the filter PUMP to the inlet of the humidifier bottle. Use the 4 mm hose connector and the filter from the spare kit for this connection. Connect the outlet of the humidifier bottle to the inlet of the cold trap (see Fig. 62).

Attention: Keep the outlet of the cold trap disconnected. This is to first check the correct orientation of the humidifier bottle, so that no water will be forced into the system causing serious damage.

Fig. 62: Connection of the humidifier bottle. Water bubbles, when the pump will be switched on. Outlet of cold trap must be disconnected.

- After clicking OK, the pump will be switched on (see Fig. 62) and water will bubble through the humidifying bottle.
- If everything is fine, the outlet of the cold trap can now be connected to the upper connector labeled FILTERS PUMP. Use a short and wide tube for this connection (6.4 mm hose connector = widest hose connector from spare-kit) to keep the back pressure low.

Fig. 63: Final set-up for H_2O span calibration .

Fig. 64: Set-up for H_2O span and software sliding bar.

- In the next step the calibration protocol displays a sliding bar (see Fig. 64). Use this sliding bar to adjust the total flow, so that the flow indicators are at 800 ml/min. If the flow is unequal between sample and reference adjust valve 1 with a small screw driver.
- To flush all connected pathways, briefly open valve 5 also the mode will briefly be set to ZP mode.

- In the next step the temperature of the cold trap needs to be entered into the GFS-Win software. The ppm-value will be calculated taking the ambient pressure into account. This value can be changed, in case different calibration equipment is used. For a very exact calculation of the ppm-value, the pressure difference between the cold trap and the analyzer can be entered in the next step; the ppm-value will then be corrected.
- The two upper fields in the quick view column show the absolute H_2O value for the reference cell (H2Oabs) and the sample cell (H2Osam). If both values have stabilized and the also the cold trap is stabilized, press *Store*. H_2Oabs and H2Osam will be set to the set values.
- Check calibration: are H_2Oabs and H2Osam close to the set value?
- Remove the tube between CUVETTE TO and FILTERS CUVETTE, open upper connector DRIER. Insert absorber tubes and filters. Adjust valve 1 and 5. Check all valves (chapter 12.2)

Remark: If measurements with other oxygen concentrations than ambient (21%) are planned, the "band broadening" effect of oxygen on the infra-red detection of H_2O needs to be taken into account. The "band broadening" effect causes the H_2O signal in nitrogen to be about 8% smaller than in air (also see manual for Optical Oxygen Sensor 3085-O2). The effect of oxygen on the CO_2 signal is much smaller and can be neglected.

12.4 Measuring Head

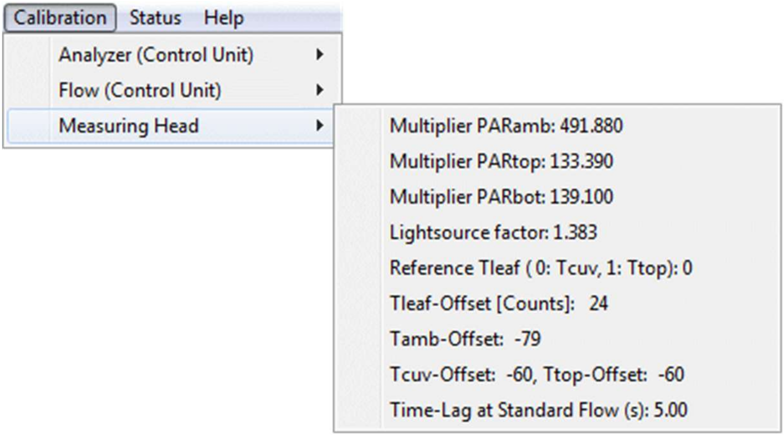


Fig. 65: Menu with Settings concerning the Measuring Head.

12.4.1 Multiplier PARamb, PARtop, PARbot

The multipliers for *PARamb*, *PARtop* and *PARbot* are given on the calibration certificate. The multiplier for *PARamb* is in addition given on the label attached to the sensor. The values are stored in the electronics box of the measuring head and loaded by the GFS-Win software, when the measuring head is enabled.

12.4.2 Light-Source Factor

The light-source factor depends on the measuring head as well as the light source and is therefore explained in a special chapter on its own, see chapter 12.5.

12.4.3 Offset of Thermocouple for Leaf Temperature (Tleaf)

The thermocouple measures the temperature difference between *Tcuv* and its tip touching the leaf. During the offset adjustment, this difference will be set to zero. It is important, that the temperature control is off and has not been used for a while before this function is used. Insert a piece of paper, close the cuvette. The impeller speed will be set to 5 after choosing the menu point *Tleaf-Offset*. After the temperature of *Tcuv* and *Tleaf* have stabilized and show no drift (about 10 min) press OK. The offset will be set to the new value and stored in the measuring head.

After the offset has been set, you may check the function of the thermocouple. To do this, switch the impeller of, have the temperature control off. Switch the flow off. Insert a black (or dark) piece of paper. Switch the light on (e.g. 2000 µmol m-2 s-1). Tleaf should rise by several degrees (> 5°C). Switch the light off, Tleaf should decrease back.

12.4.4 Offset of Tamb, Tcuv and Ttop

The offset values for the temperature sensors for *Tamb*, *Tcuv* and *Ttop* do not require recalibration. If the values are out, the measuring head should be checked by Walz. Nevertheless, offset values can be adjusted here. An independent correctly calibrated air thermometer is required as reference. It needs to be inserted into the closed measuring head for *Tcuv* and *Ttop* or placed outside near *Tamb* for the adjustment of the particular sensor settings. The temperature regulation shall be switched off. The impeller speed will be set to 5. After the temperature is steady, the temperature value of the reference thermometer can be entered. Over the next 10 s the system will average the measured temperature values and suggest a new offset. When pressing OK the new offset will be stored in the measuring head.

12.4.5 Time Lag at Standard Flow

With GFS-Win version 3.15 and higher the CO2delay is stored in the measuring head under the name *Time Lag at Standard Flow (s)*, because it is dependent on the tube length of the measuring head. The stored value of the Standard Measuring Head 3010-S is the value for the flow rate at 750 μmol s^{-1}. It will be recalculated for the chosen flow rate. Nevertheless, the value can be adjusted with other flow rates.

12.5 Determination of the Light-Source Factor

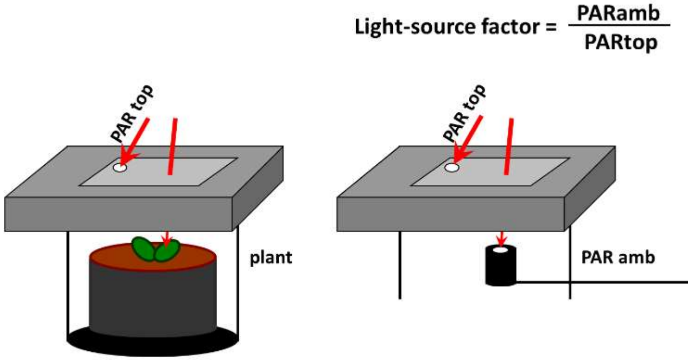


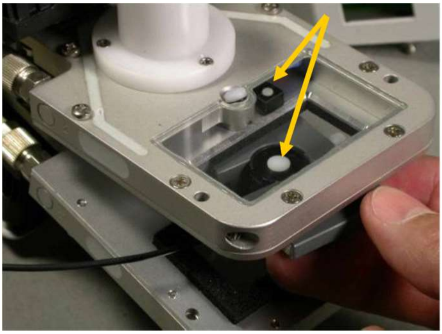
Fig. 66: Illustration of the Light-source factor. For the lower cuvette half replace PARtop with PARbot.

The intensity of the light source is measured and regulated with the *PARtop* or *PARbot* sensor inside the cuvette in dependence of the side of attachment. These light sensors are located at the rim of the cuvette frame, respectively. In contrast to sunlight, which is uniform over large areas, the light coming from an artificial light source may decrease towards the rim, where the light sensor is located. Also, the distance between the light source and the sample depends on the geometry of the setup.

The *light-source factor* is specific for every light source. If the light source (LED Light Source 3040-L, 3041-L, LED-Array/PAM-Fluorometer 3055-FL, 3056-FL, or the Imaging-PAM) is changed, a new adjustment is required. Also, if the cuvettes of the measuring head are changed, so that the distance to the sample changes, the lights-source factor needs to be read-justed.

The *light-source factor* is used, when *PARbot* or *PARtop* are chosen as sensor for light regulation, meaning that the indicated value has been multiplied with it. The *light-source factor* is only used for the one sensor regulating the light. When the *light mode* is *PARamb* or the light is switched off, the *light-source factor* is not used.

With the IMAGING-PAM the *light-source factor* is used whenever the *light mode* is *PARbot* or *PARtop*, independent of the set-value.



For carrying out the determination of the *light-source factor,* the external Miniature Quantum Sensor MQS-B/GFS and the provided adapter plate (chapter 3.1 and picture) will be necessary. Chose *Menu→ Calibration/Maintenance→ Measuring Head→ Light-source factor*. Open the cuvette and hold the external light sensor mounted in the adapter plate at leaf-level in the center of the cuvette.

Attach the light-source and click OK or press the button at the measuring. It has the same function as the OK or STORE button during the light-source factor detemination. The actual values for *PARtop*, *PARbot* and *PARamb* are displayed in the *quick view column* of the GFS-Win software. After OK has been clicked, the light will be regulated to 1000 µmol m^{-2} s^{-1} *PARamb* (or in the case of IMAG-MIN/B set to step 16). In the next step, the *light-source factor* will be determined. The averaging will take some time. Afterwards the value is automatically stored in the measuring head or the new LED Light Source 3041-L and LED-Array/PAM Fluorometer 3056-FL. These LED modules provide are several light-source factors, which are stored within the LED modules and adjusted in the same way, see next chapters.

12.5.1 Light-Source Factor in the LED Light Source

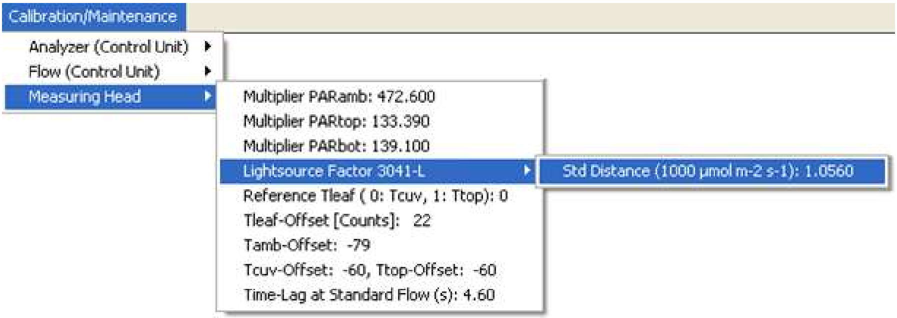
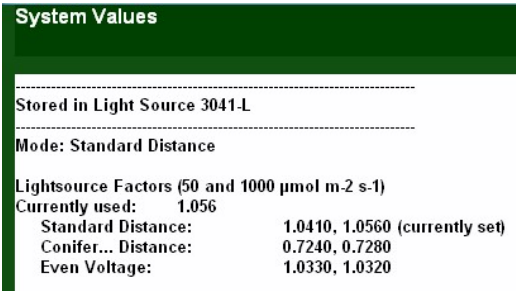


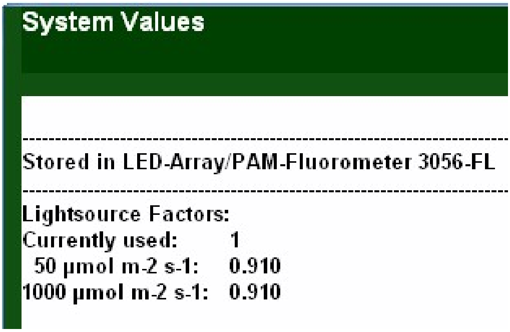
Fig. 67: Menu with settings concerning the light-source factor



When enabling the LED-Light Source 3041-L, there is a choice concerning the light distribution: "Standard distance", "conifer distance" or "even voltage". For each setting two light-source factors are stored in the LED Light Source 3041-L, one measured at 50 µmol m^{-2} s^{-1}, the other measured at 1000 µmol m^{-2} s^{-1}. The first one is used for values below 100 µmol m^{-2} s^{-1} the other one above 200 µmol m^{-2} s^{-1}. Between 100 and 200 µmol m^{-2} s^{-1} the light- source factor is linearly extrapolated. The light-source factors and the currently used factor can be seen with the menu item *Status* under *System Values*.

For reasons of downward compatibility, the light-source factor measured at 1000 µmol m^{-2} s^{-1} is also stored in the measuring head. If this light-source factor differs considerably from the respective value stored in the LED Light Source 3041-L, a message appears that the light-source factor must be adjusted. The light-source factor always needs to be readjusted if the light source is changed or the geometry between the light and the sample is changed, independent of whether there is a warning or not.

12.5.2 Light-Source Factor of LED-Array/PAM-Fluorometer



For the LED-Array/PAM-Fluorometer 3056-FL, the light- source factor is stored in the PAM-fluorometer . It can be seen with the menu item *status* in the system values. The LED-Array/PAM-Fluorometer 3056 contains two light-source factors one determined at 1000 µmol m-2 s-1 as before with older versions and another one determined at 50 µmol m-2 s-1, for higher accuracy at low light levels. As in the LED-Light Source 3041-L, the light-source factor determined at 50 µmol m^{-2} s^{-1} is used for lower light levels in the same way as explained for the LED-Light Source 3041-L. Also, as explained before, the light-source factor needs to be adjusted if the geometry between the light and the sample is changed, or if the light-source itself is changed.

12.6 Zero-Offset PAM-Fluorometer

The PAM-Fluorometer should show zero fluorescence for Ft, when there is no fluorescence. Insert the non-fluorescent foam, switch the measuring light on and press Z-Offset in the Settings window to set the zero offset (chapter 7.4.1.5 and 7.4.1.6. The *Z-Offset* must be adjusted each time the *Gain* or *ML-Ampl* settings (*see* below) or the optical configuration, e.g. new measuring head, different leaf area adapter or cuvette, are changed.

13 Maintenance and Modification

13.1 Handling Batteries

13.1.1 Li-Ion eSMART Battery 98 Wh

The Li-ion eSMART Battery (article no: 000160101434) is a modern maintenance-free battery with a nominal voltage of 14.4 V and a nominal capacity of 6.8 Ah. It features a state of charge display and embedded micro controller for monitoring key parameters such as cycle count, serial number, power, temperature etc. It communicates with the Li-Ion eSMART charger for optimal charging, resulting in longer battery life.

Important Note:

Never store the batteries in their discharged state. For longer storage check status every 6 month – they keep best if charged to 30% every 6 month and stored below 21°C in a dry well-ventilated area without corrosive gases.

Please read the safety and transport information chapter 16.6.5.
For technical data proceed to chapter 18.

13.1.2 Exchanging Batteries

The instrument is powered by two batteries, but has three equivalent battery-slots, so that batteries can be exchanged without interrupting the power supply. When the batteries are discharged, insert a fully charged battery with the yellow label facing up into the empty slot, before replacing one of the empty batteries and removing the second one.

13.1.3 Li-Ion eSMART Battery Charger

The provided Li-Ion eSMART Battery Charger is a mains-operated, fully automated battery charger with four independent charging connectors and illuminated OLED-display. It is compatible with our Li-98Wh batteries (article number 000160101434). Do not use similar batteries from third party vendors. Place the charger on a flat level surface away from sources of moisture or heat. Plug the AC cord to the charger and a compatible wall socket (90-240 V AC, 150 W).

The main switch is located on the rear panel of the device. Switch it on and insert a battery to one of the four battery slots to start the charging process.

The front panel provides four status LEDs to monitor the charging process. Flashing: in process, constant light: fully charged. A full charge starting from zero takes about 3.5 hours.

The display shows detailed information for each battery: battery capacity, charging current, charging/battery voltage, battery temperature (°C) and battery cycle count. The LEDs next to the display have the same function as the status LEDs of the front panel.

The charger provides an active thermal management system. For fast charging keep the fan switched on.

For safety warnings read the next chapter (13.1.4), for technical data proceed to chapter 18.

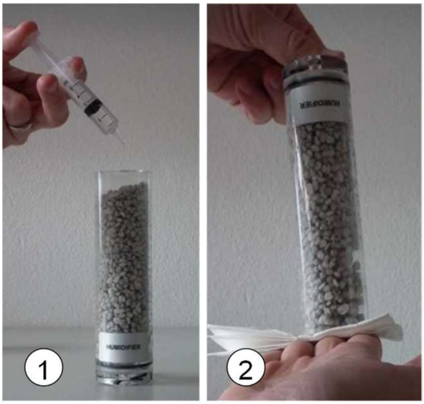
13.1.4 Safety instructions for Li-Ion eSMART Battery Charger

- Do not expose the charger to liquids. It is not water resistant.
- Do not open it – high AC voltage is present inside the charger.
- Do not cover the air vents, fan or heatsink, do not obstruct air flow, this will cause overheating.
- Place the charger in a cool spot away from direct sunlight or any external heat source. Only charge batteries in an environment warmer than 0°C.

13.2 Replacing Drier, Humidifier or CO_2 Absorber Material

To remove the absorber tubes from the front panel, press the clamp button at the connectors and pull the absorber tube forward. The lower lid is fixed with adhesive tape. To open the absorber tube, use the upper lid. It can be removed by laterally rotating it. Do not use the connector as lever for opening the lid as it may cause damage. Before closing the absorber tube, make sure the tube and the seals are clean. Turn the lid until the connector fits properly into the notch of the tube. The filter pad at the top and bottom of the absorber material should be inserted with the smooth side facing the material. Watch out for cracks, leaks cause malfunction of flow and CO_2 control.

The drier contains silica gel (Sorbead Orange Chameleon, BASF), a H_2O absorber that is orange when dry and becomes colorless at a water content of approx. 6 % (w/v). The material can be reused after drying in the oven (130-140°C 8h, pay attention to chapter 16.6.2). Note that if the material is burnt, it may look orange despite being wet. Also, if the material is old, it becomes grayish instead of white when wet, and should be disposed of. Silica gel of different quality is available on the market. During normal operation the quality is less important, but when recalibrating the absolute zero of the H_2O analyzer (chapter12.3.4), it is very important to obtain completely dry air. Therefore, we also provide Molecular sieve 3 Å (Roth). It can be regenerated at 350°C and shall only be used during H_2O zero calibration behind Silica gel. Always keep it dry and closed, use orange dust caps.



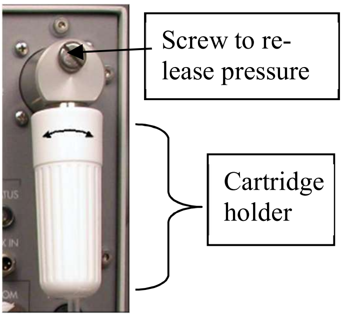
Ceramic granulate (Stuttgarter Masse) is used for humidifying the air. It must be wetted with distilled water. Please ensure that no water accumulates at the bottom of the tube. To prevent this, add the water slowly (1) so that it can be absorbed by the granulate. Carefully shake out excessive water before connection (2). The ceramic granulate can be autoclaved to prevent algae growth. The humidifier column has a capacity of approx. 20 ml water (chapter 16.6.4).

Soda lime (Sofnolime®, molecular) is used as CO_2 absorber. It has a pH-indicator that changes color from colorless to violet, when used. Note that the color change may not work when the inlet gas is dry. In addition, CO_2 diffuses into the core of the granules overnight which causes the surface to turn white again. However, the absorbing capacity is exhausted with the first color change and the soda lime needs to be replaced. As the material is corrosive, please observe the safety and disposal instructions in the safety data sheet (chapter 16.6.1).

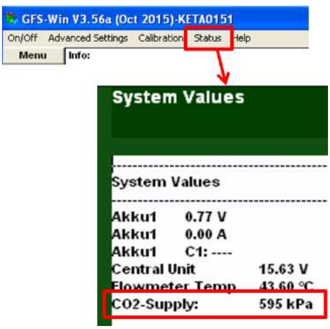
13.3 Replacing Filters

The filters for PUMP, CUVETTE and AIR IN on the front panel can be removed by pressing the clamp buttons at the connectors. Turn the filter slightly when pulling it out of the pneumatic adapters. A spare filter (article number 000140301225) is supplied with the Spare kit 3000-C/SK. Please regard the arrow marked on the filter and on the front panel behind the filter. The direction of flow enables the detection of dirt.

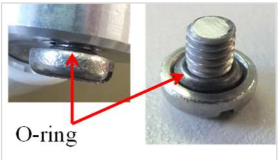
13.4 Adding CO_2 with Cartridges or Releasing CO_2 Pressure



Inside the control unit there is a CO_2 supply tank for the CO_2 control. It is filled with a soda charger (CO_2 cartridge). One filling is usually enough for one week. If a warning appears that the CO_2 pressure of the CO_2 supply is low, switch off the CO_2 control, unscrew the white cartridge holder by turning it counterclockwise. Insert a new cartridge and retighten the holder. A gentle hiss can be heard when CO_2 flows into the supply tank. Switch the CO_2 control on again.



The pressure within the CO_2 supply is indicated in GFS-Win under *Menu →Status*. The same information is given in the lower right corner of GFS-Win as a percentage value, the content of one cartridge corresponds to 100%. The warning appears when the pressure in the CO_2 tank drops below 250 kPa while the CO_2 control is in use. After the first warning, the CO_2 control can still run for a few hours. Do not overfill the CO_2 supply but wait for the described warning before additional charging. Each cartridge adds 600 kPa. The maximum pressure that the CO_2 supply can hold is 950 kPa. At 1000 kPa (10 bar) a safety valve will open.



The pressure within the CO_2 supply tank can be released by carefully opening the screw at the front of it. Protect your eyes as the screw may be blown out if turned too far. The seal ring could also burst. Before closing the screw, open it so far that the seal-ring can pull back into the groove of the screw otherwise it may become damaged. Instead of the screw a connector can be attached, so that a CO_2 tank can be used for filling the CO_2 supply.

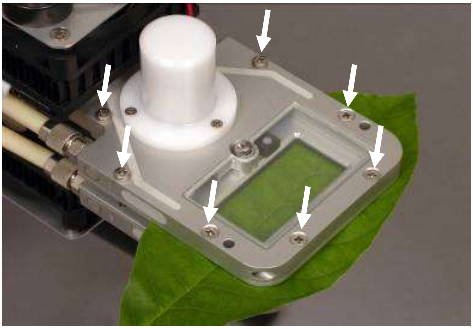
Please note that CO_2 cartridges (article number: 000160103430) have been classified as dangerous goods; and with some exceptions only land transport is allowed. We therefore recommend buying compatible CO_2 cartridges directly from the super market, if possible. Note that golden soda chargers contain CO_2, while similar pink chargers for cream contain N_2O.

It is important that the size of the cartridges matches the connector of the instrument (height: 65 mm, diameter: 18 mm, neck diameter: 8.7 mm, puncture depth: 0.25 mm, Filling: 8 g CO_2). Do not use cartridges other than those for food supply. We recommend soda chargers from ISI GmbH, Vienna (http://www.isi.at/consumer/products/chargers/en/). In some countries these cartridges are available at Amazon for example. The webpage https://www.isi.com/en/culinary/meta/about-isi/contact-isi-sales-partners-worldwide/ provides a list of suppliers of ISI cartridges world-wide.

13.5 Adjusting the Clip Mechanism for the Light Source

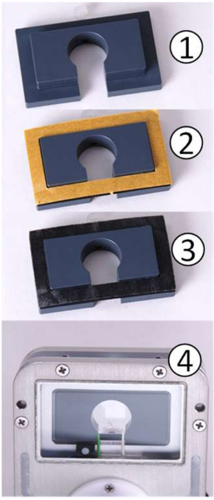
The light source, imaging-PAM or darkening plate can be mounted on either side of the Standard Measuring Head 3010-S by clicking its pins into the coupling of the respective cuvette frame. To tighten or loosen this connection, the screws on the front of the cuvette frames can be adjusted (*see* picture).

13.6 Replacing Leaf Area Adapters



The measured leaf area can be easily adapted with different leaf area adapter plates. There are 8 screws per cuvette frame which fix the area adapter plate. Unscrew them to remove the plate. Make sure that the blue O-rings (see picture in chapter 13.8.10) under each leaf area adapter plate remains in the correct position to ensure a good seal, before mounting the new plate. This can be achieved by facing the screws downwards and the O-ring upwards when opening the screws. If the O-ring has moved out and cannot be inserted easily, it may help to place it into a fridge, so that it becomes shorter. Tighten the screws evenly in several rounds. Note that the metal of the screws is harder than the metal of the adapter plates. Therefore, tighten the screws gently to avoid destruction of the threads. The O-ring underneath the adapter plates provides an airtight seal. If a thread of a leaf area adapter plate is worn, the plate needs to be replaced with a new one.

13.7 Replacing Foam Gaskets



The gaskets can be mounted exactly with the provided mounting plate (1). Usually, the leaf area adapters can remain attached to the cuvette frames when replacing the gaskets. Remove the old gaskets. The remaining adhesive can be carefully removed with the adhesive itself or Eucalypt oil or a razor blade. Place the new gasket on the mounting plate (2), remove the protective film (3), position the gasket with the mounting plate and press it to the leaf area adapter plate (4).

When attaching the gaskets to dismounted leaf area adapter plates, make sure that the gaskets are always mounted on the non-chamfered side of the leaf area adapter plate.

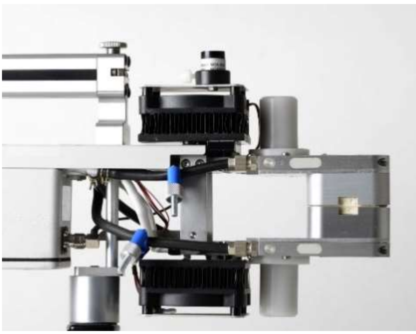
13.8 Exchanging Cuvettes

Whenever the setup of the cuvette is change it may be necessary to adjust the valve settings and in most of the cases the light-source factor needs to be adjusted, since the distance between the sample and the light source changes.

13.8.1 Mounting the Cuvette for Conifers 3010-V80

To replace the standard leaf area adapters with the conifer cuvette, it is necessary to increase the distance between the two cuvette frames. The hinge of the lower frame is mounted to the upper frame with two spacers.

Each spacer is fixed with two Allen screws. They must be unscrewed. Now the extension rod of the closing mechanism can be replaced with the longer rod. The extension rod sits on the arm of the lower cuvette frame and extends to the closing mechanism in the handle. To release the extension rod, pull the lower unscrewed cuvette frame forward. This may require some sleight of hand as the white sheathed electronic cables, which lay in a loop in the space above the electronics box, must be pulled out with some force. When the extension rod of the measuring head becomes free, it falls out through the hole of the tripod adapter. No tool is required. Insert the long extension-rod of the conifer cuvette through the drill hole of the tripod adapter, so that the **blunt end points upwards to the handle** and the rounded end outwards. If the rod is not easy to insert, loosen the Allen screws of the tripod adapter beside the drill hole slightly, so that it slides in.

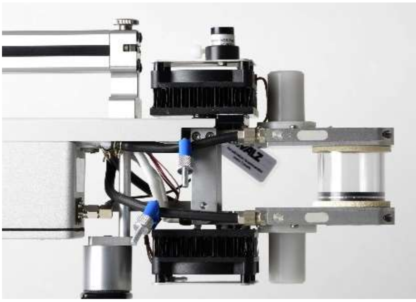


First remove the lower leaf area adapter plate (chapter 13.5). For some applications, the thermocouple shall be positioned higher. Now that the adapter plate has been removed, it can be bent upwards with the metal angle provided (chapter 13.8.10). Fix the conifer cuvette halves with 8 screws to the cuvette frames as explained for the leaf area adapter plates (chapter 13.5). Keep the blue O-rings underneath the adapter plates in place. The easiest way is, to first connect the lower conifer cuvette half, then fix the lower frame to the upper frame with the long spacers and afterwards replace the upper leaf area adapter plate with the other conifer cuvette half. Screw the metal cylinder under the tripod adapter for extension.

Modify hoses: Use the two supplied hose clamps to close the outgoing hose #2 and the feed hose #3 (the numbers can be found on each side of the cuvette frames).

Never unscrew the three screws underneath the heat exchanger holding the Peltier elements in place, it will cause severe damage to the temperature control.

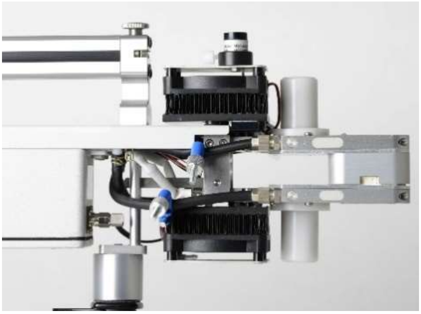
13.8.2 Mounting the Cuvette for Lichens/Mosses 3010-V32



The assembly of the cuvette for lichens/mosses is very similar to that of the cuvette for conifers. Please see there (chapter 13.8.1) and proceed in the analogous way.

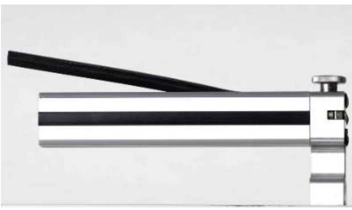
The cylindrical sample tube can also be used for attaching new gaskets to the adapter plates of the lichens/mosses cuvette. Simply place the gasket on the cylindrical body, remove the protective film and press it against the cuvette plate. This can be done before mounting the plates, but also afterwards.

13.8.3 Rectangular Cuvette for Lichens/Mosses 3010-V40



As an alternative to the cylindrical chamber, a rectangular chamber can be used for lichens or mosses. It consists of half a conifer chamber at the upper side and a flat adapter plate on the lower side. This set-up includes shorter spacers for the hinges and a shorter rod for the closing mechanism than the conifer chamber.

The conifer chamber and the flat adapter plate can also be mounted the other way around. Then the complete measuring head must be turned upside down. As a result, the thermocouple reaches the sample from the top.

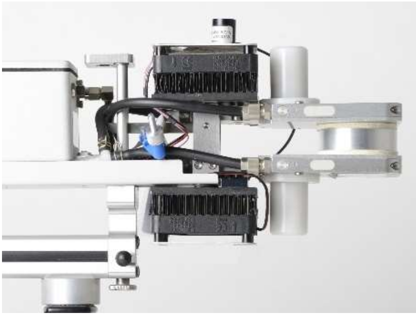


For mounting the measuring head at its handle upside down a handle-tripod adapter is required. Remove the metal plate at the end of the handle and pull out the upper plastic stripe. Now the handle-tripod adapter can be slide in.

(article numbers for handle-tripod adapter: 000244033414, 000150113500, 2 times 000150402429, 2 times 000150105520).

When the measuring head is turned upside down, everything that is called "top" in the software, is now at the bottom. In particular, when switching the light on use: Set PARbot = xxx, not PARtop as usual.

13.8.4 Cuvette for Petri-Dish 3010-P



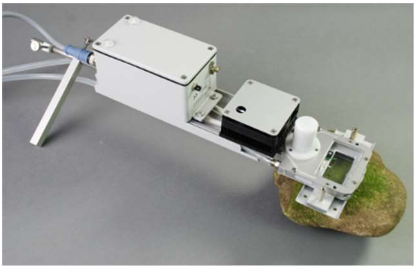
The cuvette for Petri dishes fits the Schott Petri dish (article number at Schott: 237553903, outer diameter of lid: 40 mm height: 12 mm). In this setup the measuring head is turned upside down and fixed with the handle-tripod adapter to the tripod as described in the chapter above. The setup has a closed plate underneath the aluminum block and a round adapter plate with opening at the upper side.

Connect the closed plate and the open to the correct sides of the measuring head. Exchange the rod of the closing mechanism and the spacers for the hinges. Insert the aluminum block for the Petri dish and close the measuring head.

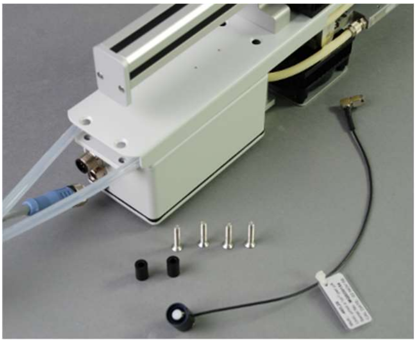
Now connect the measuring head to the control unit, switch the flow on and the H_2O control to “drier”, so that the measuring head is flushed with dry air. Once wa is low (below 2000 ppm) and while still flushing, use the two hose clamps to disconnect the side with the closed adapter plate from the gas pathways. The air inside the closed space should now be dry, so that no condensation can occur.

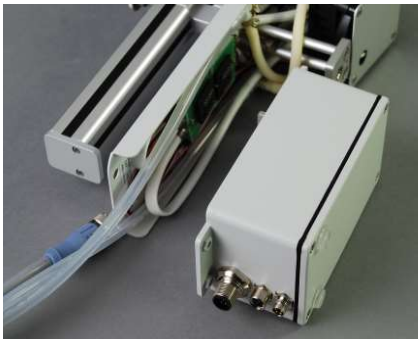
In the software it is possible to set a temperature offset between both cuvette halves (Advanced Settings). This option makes it possible to keep the Petri dish the coldest point so that no water evaporates from it. Caution, if liquid water enters the gas pathways, it will destroy the analyzer.

13.8.5 Mounting the Cuvette for Flat Surfaces

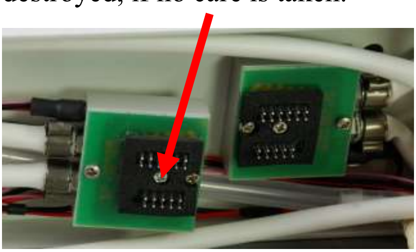


For measurements, on flat surfaces the Standard Measuring Head 3010-S can be assembled without upper cuvette frame. For this purpose, it must be disassembled into three parts: electronics box, lower cuvette frame, and upper cuvette frame including handle. Additional mechanical parts are required, which are not included in the Standard Measuring Head 3010-S.

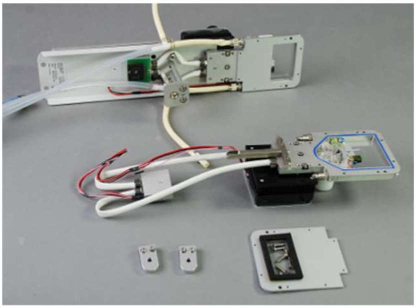


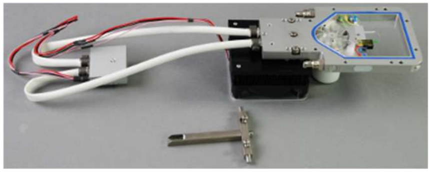


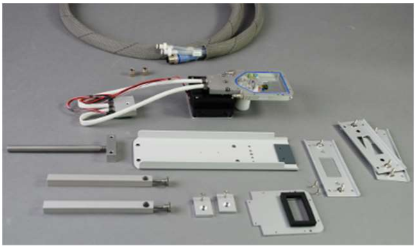
Disconnect the cable and remove the external light sensor. Remove the four screws holding the electronics box in place. Remove the electronics box from the measuring head as described in chapter 13.8.6. To not proceed without reading this chapter, because the electronics and the pins may become destroyed, if no care is taken.



Remove the leaf area adapters, the hinges, and the axis with lever. Do not(!) unscrew any screw of the heat exchanger.

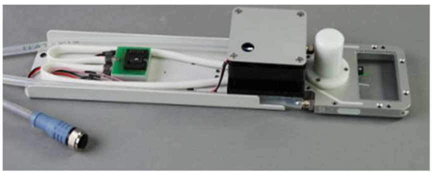






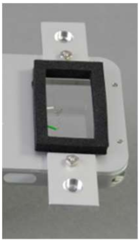
The Standard Measuring Head 3010-S is now completely disassembled and can be reassembled in the new configuration.

Screw the flat adapter plate to the lower cuvette frame and connect the tubing without any branch connections. Connect the tube labeled "To" to inlet #3 and the tube labeled "From" to outlet #4.



When connecting the electronics box, take care not to bend any of the tiny electronic pins.

Place the connecting block for the back-legs into the gap between the box and the upper metal plate and secure the box with all four screws.

Attach the leaf area adapter plate to the cuvette frame, keep the blue O-ring in place. Use two long screws to attach the protruding plates. Connect the rear legs as shown in the first picture of this chapter.

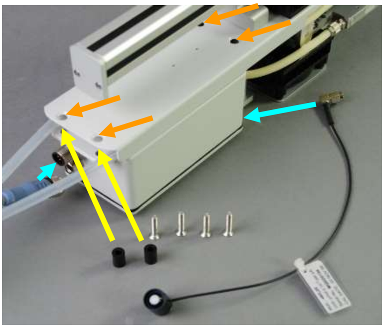
Use a surface-sealing plate for the air-tight connection to the sample. Make sure to model a defined area with Terostat IX® (Teroson, Henkel) with no dark gaps.

The cuvette frame can now be connected to the surface-sealing plate. Reconnect the cable and connect the *PARamb* sensor.

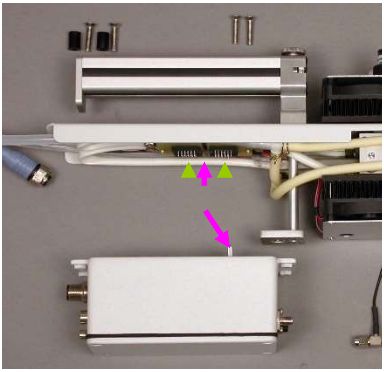
The system is now ready for measurements. When starting the GFS-3000 do not chose "*Standard Measuring Head*", but "*Head only with bottom chamber*". Use light mode *PARbot* for controlling the light, since now *PARbot* points upwards.

13.8.6 Disconnecting the Electronics Box

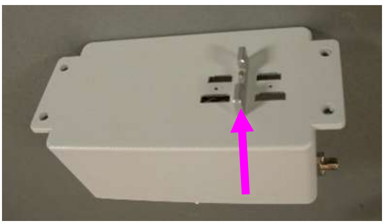
Avoid disconnecting the electronics box. Usually the white sheathed electronic cables can be pushed back under the space above the electronics box from the front. Additionally, they can be pulled from the back by using a long alien key as hook.



To disconnect, switch off the GFS-3000 and disconnect the measuring head. Eliminate any electrostatic charge by touching a central heating system or water tap together with the measuring head. Remove screws (orange arrows), spacers (yellow arrows), cable to GFS-3000 and *PARamb* sensor (blue arrows).

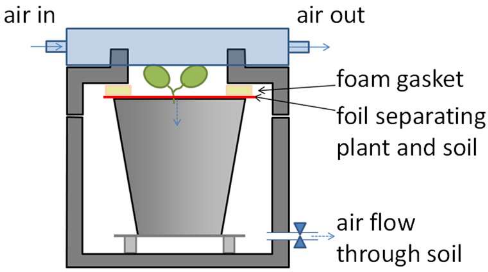


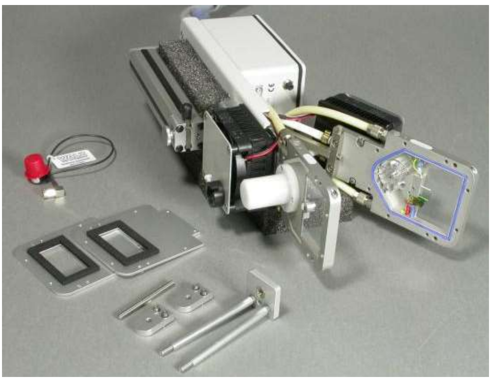
Place the measuring head with its side on a flat smooth surface and pull the electronic box carefully straight (!) off. Do not pull to one side as the small pins (green arrows) must not be bent. The pink arrows indicate a distance between the connectors and a metal part (positioning aid) that fits exactly into this space. Use the flat surface and the positioning aid to pull in a straight direction.



To reconnect, place the head and the electronics box back on their sides and carefully slide them together. When tightening the screws, be careful not to overtighten the front screws. There are no spacers, but the distance should be the same as in the back, otherwise the upper metal plate will be bent, and the cuvette will not close properly.

13.8.7 Mounting the *Arabidopsis* Chamber 3010-A



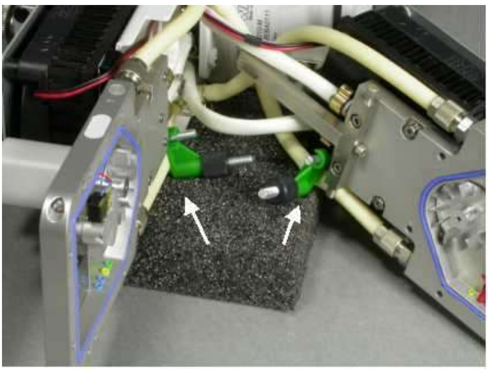


In the setup for the *Arabidopsis* chamber, the measuring head is laid on its side. The bottom side is detached, so that it can be turned upwards and connected to an angled adapter with opening for a pot holder.

The plant is placed in a pot holder. A sealing film is placed on the soil to separate it from the upper parts of the plant. Some air is pushed through the soil, to avoid any influence of the soil transpiration and respiration on the measurement.

For assembling the *Arabidopsis* chamber, first detach the Mini Quantum Sensor MQS-B/GFS using an 8 mm flat spanner. Remove the complete tripod attachment, including the extension rod for the closing mechanism. To detach the lower cuvette frame, remove the spacers holding its hinge. Each spacer is attached with two hexagon socket screws, which must be unscrewed. Now lay the measuring head on its side in the provided foam stand, so that the upright part of the foam-stand fits into the handle. Detach the leaf area adapter plates by removing 8 screws on each side as shown in chapter 13.6. After removing the lower adapter plate, the position of the Tleaf sensor should be modified as described in chapter 13.8.10, so that comes closer to the leaves of the plantlets.

Now the angled adapter can be mounted to the cuvette frames instead of the leaf area adapter plates using the same screws. The easiest way is to plug the angled adapter onto the black pot holder and mount the cuvette frames to it. The thin plate of the angled adapter is mounted on the top cuvette frame (now in the upright position) and the lower cuvette frame is rotated 90 degrees and mounted on top of the angled adapter. Pay attention that the blue O-rings in the cuvette frames stay in place and that the hoses are not buckled. The white sheathed cables form a loop inside the housing, so that they can be pulled out for some additional centimeters.

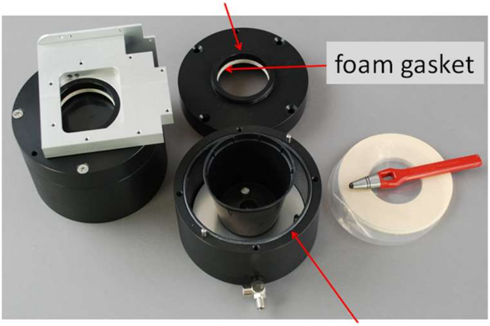


The tubing must be modified. Use the two provided hose clamps to close the outgoing hose #2 and the feed hose #3 (the numbers can be found on each side of the cuvette frames). After these preparations, the external light sensor can be reconnected.

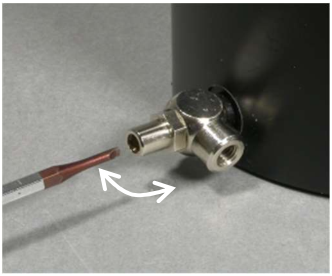
Different sizes of flowerpots can be used (55 to 70 mm in diameter). The pot holder can be adapted to all heights between 45 and 58 mm with the provided height adjustable base plate. The easiest way to adjust is to use an empty pot of the same size.

Lay the base plate into the lower part of the pot holder and place the pot in the middle of it. The adapter plate has the right elevation when the applied but not fixed top of the pot holder shows a gap of about 1 mm to the bottom part. Only in this configuration the upper brim of the flower pot is sealing completely with the foam in the upper part of the pot holder. Now a plant can be inserted into the chamber:

Preparation of *Arabidopsis* plants: For best sealing, the plants should be potted as high as the upper brim. To arrange the film, lift the rosette leaves carefully and lay the sealing film around the plant like a collar between the rosette leaves and potting soil. The film should lie flat on the brim of the pot and the plant should protrude through the hole in the middle of the film. Please pay attention that the leaves do not overlap the brim of the flowerpot. The cutting in the film can additionally be sealed with an adhesive tape or a second film with the opening at the opposite side. Additional sealing foils can be produced with the supplied hollow stamp, whereby the supplied foils serve as a template.



Keep all O-rings of the pot holder, especially the O-ring with 8.6 cm diameter in place.



To avoid measuring artifacts, resulting from H_2O and CO_2 released from the soil that can pass through gaps of the sealing film, a restrictor valve has been built in the pot holder. It can be adjusted with a screwdriver, so that air flows constantly outwards through the soil.

Do the valve adjustment at the GFS-3000 with the closed measuring head and *Arabidopsis* chamber including a test plant or soil-filled pot sealed with a punched film. Proceed with the valve adjustment as described chapter 12.2, but keep valve 3 closed and instead of valve 3 use the restrictor valve of the *Arabidopsis* chamber. There should be at least 100 ml/min flow out through the restrictor valve. The flow through the restrictor valve can be checked in MP mode by closing the restrictor valve, the bead of the flow indicator ANALYZER SAMPLE should rise by at least 100 ml/min, afterwards open the restrictor valve the bead should drop back. Still in MP mode, adjust valve 1, so that the flow indicators have the same height.

Note that with the *Arabidopsis* chamber the light mode *PARbot* must be used, since the light sensor of the lower cuvette frame points upwards and measures the light. Also, the light-source factor must be adjusted because of the changed sample distance. To do that, switch the light mode to *PARbot*, plug the upper part of the pot holder into the opening of the angled adapter and use the positioning help to place the Miniature Quantum Sensor MQS-B/GFS in the position of the sample. Proceed as described in chapter 12.5 with the light-source factor adjustment.

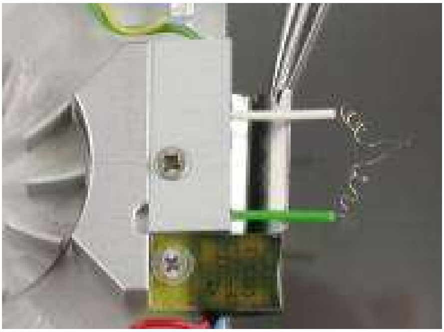
13.8.8 Mounting the Fiberoptics/PAM-Fluorometer 3050-F (optional) to the Standard Measuring Head

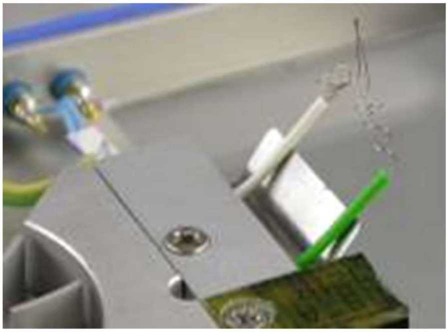
Before mounting the Fiberoptics/PAM-Fluorometer 3050-F remove the plastic screw in the upper cuvette frame in front of the impeller and replace it with the air-tight adapter provided with the-Fiberoptics/PAM-Fluorometer 3050-F. Keep the sealing around the thread in place. Remove the back plate at the handle of the Measuring Head and mount the holder instead. Now the fluorometer can be inserted into the holder and fixed. Insert the fiber carefully; the long end fits into the fluorometer, the short end into the adapter at the measuring head. Do not push the fiber in forcefully to not destroy the optics inside the fluorometer. If the fiber is pushed in 30 mm a resistance may be felt. The fiber may need to be turned a bit for fitting in fully (35 mm). If the fiber is fully inserted, the signal from the fluorescence standard (at position of leaf) is around 400 mV (Gain: high, ML: 10).

13.8.9 Adapter 3050-F/A (optional)

The adapter 3050-F/A extends the number of connectors of the measuring head. It is required, when using the Fiberoptics /PAM-Fluorometer 3050-F together with the Light Source 3041-L. Insert the adapter under the handle and connect its cable to the measuring head. Use the two sockets for the Fiberoptics/PAM-Fluorometer 3050-F and the Light Source 3041-L.

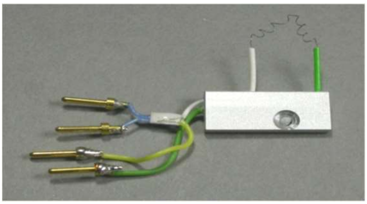
13.8.10 Adjusting theThermocouple (Angle optional)



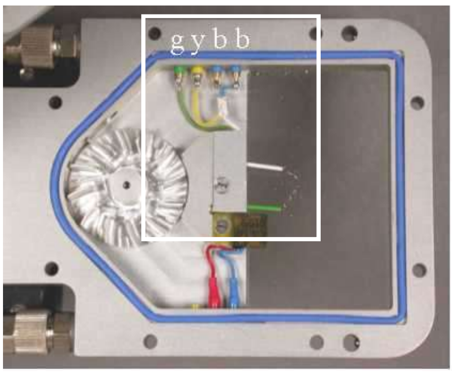


For some applications, it is required that the thermocouple protrudes upwards. An additional angle can be inserted using tweezers after loosening the aluminum plate of the thermocouple (about three turns). Afterwards refasten the screw.

13.9 Exchanging the Spare Thermocouple 3010-CA/TCL



The Thermocouple 3010-CA/TCL for Tleaf measurements consists of thermocouple (type K), an aluminum plate for mounting, four pins, and a Pt100 sensor. It is mounted with the aluminum plate in the lower cuvette frame.



To access it, the lower cuvette frame needs to be detached and the leaf area adapter needs to be taken off. See chapter 13.14 for an explanation on this procedure. The four pins (g,y,b,b) realize the electrical connection. In the graphic the colors of the thermocouple pins are from left to right: green (g), yellow (y), blue (b) and blue (b) (the two blue cables can be interchanged). To remove the thermocouple, unscrew the aluminum plate and disconnect the pins. The pins sit very tight and a fine flat nosed-pliers or tweezers may be required. Insert the pins of the new sensor and mount the aluminum plate. Make sure that the Pt100 sensor attached to the blue wires is surrounded by air and does not touch the cuvette frame or leaf area adapter. The thermocouple tip should have contact to the leaf and can be slightly bent with a tooth stick if necessary.

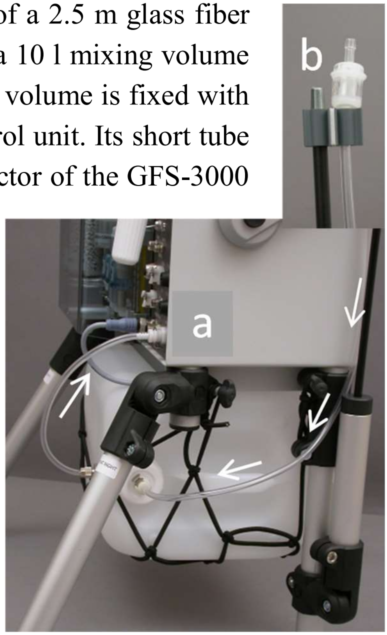
After the temperature sensor has been exchanged, it is important to determine the new Tleaf offset of the thermocouple. This can be done with the help of the GFS-Win software *Menu → Calibration/Maintenance → Measuring Head → Tleaf Offset [Counts]*. For instructions see chapter 15.2.7.

13.10 Installation of Sun Screen (optional)

A sun screen can be installed underneath the handle of the control unit in the indicated way.

13.11 Mounting the Outdoor-Set 3000-C/OS (optional)

The Outdoor-Set 3000-C/OS consists of a 2.5 m glass fiber pole to which the air inlet is attached and a 10 l mixing volume mounted under the GFS-3000. The mixing volume is fixed with a luggage net between the legs of the control unit. Its short tube is connected to the pneumatic air in connector of the GFS-3000 instead of the 3000-C/EF filter, while its long tube is mounted on the glass fiber pole to drawn in air 2.5 m above ground.



The pole-holder must be mounted on the rear right leg of the GFS-3000 (a). The glass fiber pole is inserted there. Make sure that the upper end of the tube is equipped with a coarse filter to avoid contamination and insects entering the instrument (b).

13.12 Software Update and Installation on External PC

13.12.1 Installation or Updating of the GFS-Win Software

The GFS-Win software is preinstalled on the GFS-3000 and delivered on a CD (updates can be downloaded at: www.walz.com, please make extensive use of this option, updates are free of charge). The currently installed version number can be seen under *Menu→Status→Components Info→GFS-Win.*

13.12.2 Updating of the GFS-Win Software

For updating the internal PC, a memory stick with the GFS-Win setup file must be used. First exit GFS-Win by selecting *Menu →On/Off →Exit*. In the shut-down dialog select *Measure Mode off → Exit GFS-Win* and click *OK* (chapter 5.3.2). Insert the USB memory stick with the setup file into the Control Unit.

After the taskbar appears, select the *Windows Start button* to access *File Explorer* from the *Start Window* (Fig. 68). If it is not visible, touch the green background to obtain more options.

Fig. 68: Start-Window with access to File Explorer.

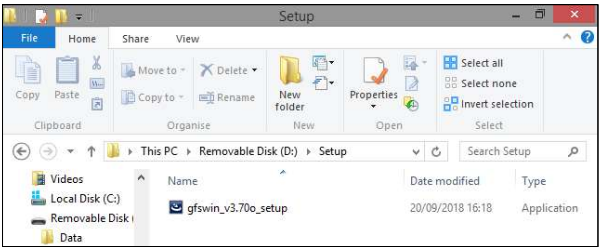


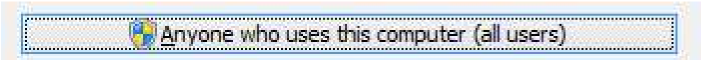
Fig. 69: *File Explorer* with gfswin-setup file.

In the *File Explorer* navigate to the removable disk and double-click on the setup-file to start it.

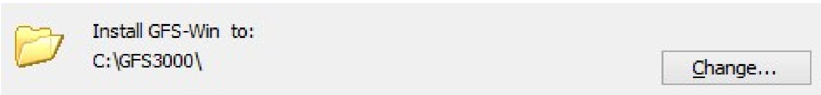
The Installation Wizard will do the following:

- extract GFS-Win.msi
- show welcome and copyright information; click *Next*

- show the user choice; select:



- show readme information; press *Next*
- show the destination folder; use *C:\GFS3000* and press *Next*



- give a choice of installation types (Fig. 70):
  When updating keep the first option and press *Next.*
- After *Next* has been pressed, the installation wizard copies GFS-Win.exe and other files to the installation-directory and makes changes to the Windows registry. GFS-Win itself creates the *Documents/GFS-3000* subdirectory for data-storage the first time it is started.

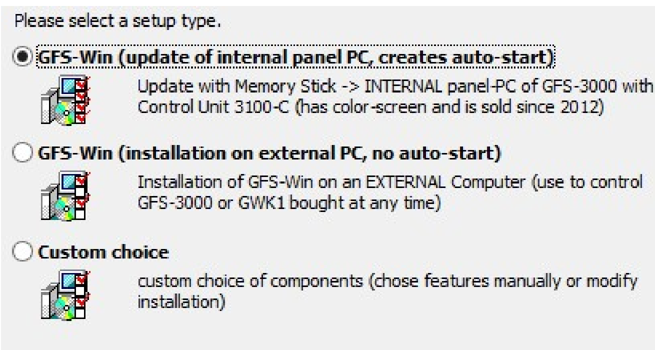


Fig. 70: Setup types: When updating the internal PC, select first type; for installation on an external PC, select second type.

If the wrong software type has been installed, it has to be removed. Also old software sometimes has to be removed before installation. To remove a GFS-Win version press *Windows Start button* in the taskbar. To navigate to the App-Groups, touch the green background in the *Start Window* (see Fig. 68) and navigate sideways to the *GFS 3000* group with the *Uninstall GFS-Win* option (see Fig. 71).

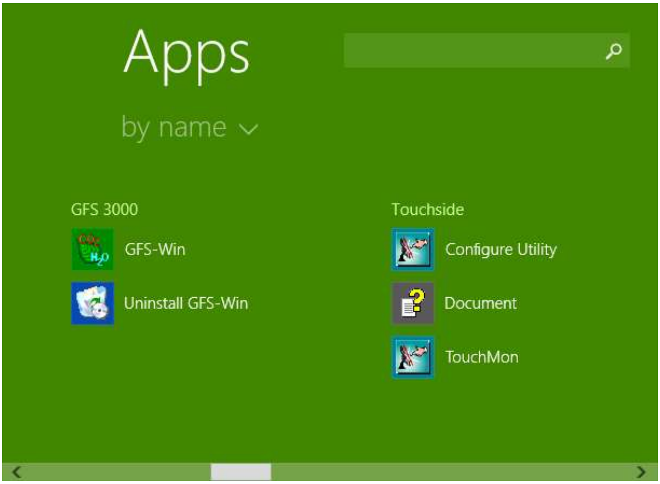


Fig. 71: GFS 3000 Group with Uninstall option.

Alternatively, in the *Windows File Explorer*, navigate to *This PC*. The *Uninstall or change a program* option will appear in the menu bar, if the size of the window is increased. Use this option to uninstall GFS-Win. If there are multiple GFS-Win installations, uninstall them all. There is also a batch file on the provided CD, which can be used to remove old software versions.

13.12.3 Installation of an external PC for Operation

If the GFS-3000 shall be operated with an external PC, install GFS-Win with gfswin_setup.exe. It can be started directly from the Walz website or from the supplied CD. The installation is very similar to the update described above, except that, when the window with the choice of setup-types appears, the second option must be selected (see Fig. 70). Also the installation directory can be changed, but note that Windows Vista and later versions have special restrictions in the subdirectory "Program Files". GFS-Win works better, if it is not installed under this subdirectory.

For the Interface 3010-I/Box a driver from FTDI is required. The program "CDM(version number).exe" installs it. Navigate to the *GFS 3000 group* and click on the shortcut *USB-driver* or in the subdirectory *C:\GFS3000\USB driver* in the *Windows File Explorer* double-click on the file "CDM(version number).exe" to start the driver installation.

13.13 Cleaning the GFS-3000

Wipe off any dirt with a damp cloth. Be careful not to scratch the display or other surfaces. Make sure the instrument is flushed with dry air when switching off to avoid condensation. Regard chapter 13.15.1 before storing the instrument.

13.14 Cleaning the Standard Measuring Head 3010-S

For thorough cleaning of the measuring head, the adapter plates should be removed, and the cuvette halves should be detached from each other for better access. (Do not! unscrew any screw of the external heat exchangers, also avoid any stress on the external heat exchangers).

Remove the leaf area adapters as described in chapter 13.6. To detach the connection between the *lower and upper cuvette frames*, unscrew the four hexagon socket screws that fix the *distance holders* of the hinge and take them off. The lower cuvette frame can be pulled a bit forward since the electronic wiring forms a loop above the electronics box. Now the inside of the measuring head can be accessed. Remove dust, sand and plant parts by blowing inside the cuvette and underneath the impeller wheels. The impeller wheels may be moved carefully without stress during cleaning. Carefully wipe the glass clean with SIDOLIN window cleaner or a cleaner for glasses. Do not spill the cleaner on the O-ring or any other part. After cleaning, the measuring head should be reassembled (make sure the O-rings are well placed). Regard the checklist in chapter 13.15.1 before storing.

13.15 Checklists

13.15.1 Checklist before Storing the Instrument

- Are the filters still clean?
- Flush the instrument with dry air to avoid condensation with coldness
- Charge batteries, they keep best 30% charged
- Depending on storage time also the CO_2 in the supply vessel may be released (chapter 13.4).
- Discard used soda lime (read chapter16.6.1)
- Is the measuring head inside still clean of sand, dust and plant parts? If not, remove the adapter plates and clean it
- Clean the windows of the measuring head with cleaner for optical glasses
- Is the measuring head dry inside? Otherwise flush with dry air overnight before storing (the setup shown in Fig. 60 may be used for drying, connect the measuring head, switch the flow on and switch the H_2O control mode to drier), dry from outside.
- Is the *Tleaf* sensor still intact?
- Replace the gaskets if necessary
- Store the measuring head open, so that the gaskets are not compressed
- Download all data and relevant user programs
- Pack everything (chapter 3.1 for all components).

13.15.2 Checklist for Transport

- Flush the system with dry air to avoid condensation during cold transport.

- Remove CO_2 cartridge. Note that the CO_2 cartridges may not be transported via air-mail.
- Release the pressure in the CO_2 supply vessel of the central unit (chapter 13.4).
- When packing the central unit, make sure that the handle is in the horizontal position and locked in this position, so that the instrument cannot move forwards or backwards during transport.
- Avoid bending the cables, especially the cable and tubes of the measuring head. Lay them in big loops.
- Observe laws on the transport of batteries (chapters 16.6).
- The MSDS-sheets are on the CD provided with the instrument.

- If for some reason the pump cannot be used to flush the instrument with dry air (no power, repair needed), the instrument can be dried with the setup shown below. Push air through the drier. Watch at the mechanical flow indicators, that the dried air flows through the analyzer. Avoid using your breath, because it will cause the CO_2 concentrations as high as 50000 ppm, disturbing the next measurement considerably. Nevertheless, if there is no other way, 10 deep breaths through dried silica gel can dry the pathways.

14 Practical Hints

- If a constant power supply is available, the GFS-3000 should be kept switched on or in standby mode, even over weeks-ends.
- The gas analyzer of the GFS-3000 is sensitive to severe shocks and strong vibrations and may need recalibration afterwards.
- Protecting the control unit from fast changing temperatures during measurements increases accuracy.
- Resting the control unit horizontally increases accuracy.
- When measuring with ambient air a 10 L buffer-volume (or bigger) in front of the gas inlet is required to obtain a constant CO_2 and H_2O concentration.
- Calibration of gas analyzer and other devices need to be checked regularly (chapter 12).
- As control of the measurement, the measuring routine should be repeated with an empty measuring head.
- Control the fluorescence measurement with a fluorescence standard routinely.
- Don’t put any stress or force on the fans or heat exchangers of the measuring head and never unscrew them.
- Avoid condensation inside the cuvette or tubes. Do not place tubes on the ground. There may be strong temperature gradient in the room, use the *Tamb*-Sensor to find out.

15 Trouble Shooting

15.1 Symptoms and Solutions

Table 8: Symptoms and Solutions

Symptoms	Solutions
GFS-Win Software	
System shows error 72 during starting GFS-Win	Write permission in the sub-directory of GFS-Win is necessary. Also, files like *GFS-3000*.cfg, *GFS-3000*.err and *GFS-3000*.ini should not be write-protected.
No communication can be established between PC and *GFS-3000* with 3010-I/Box	On the Panel PC chose the *On/Off→External Control via COMP*. Then pause GFS-Win or switch the panel-PC off, before using the external PC to control the instrument.
Pneumatic Pathway	
Pump runs high, but the flow reached is to low	Check whether there is a leak at one of the columns or a filter is not properly inserted.
Pump is not giving a constant flow	The Measuring Head needs to be closed for the pump to work steadily. The chosen flow might be too low for the operation range of the pump. Open valve 5 to increase the pump through-put (see valve adjustment chapter 12.2).
CO_2 concentration varies and is not stable	Is the CO_2 absorber fresh? Switch the CO_2 control off, does the CO2abs concentration go to zero. Under some circumstances CO_2 changes may also be caused by the water control, directly after the humidifier or drier has been changed or after changing from working without CO_2 absorber to working with CO_2 absorber, or vice versa. The humidifier and the drier should be flushed with air of the appropriate CO_2 concentration (either free or ambient). Switch water control for about 2 min to maximal H_2O

	(60 000 ppm) and for 2 min on minimal H_2O (0 ppm).
ci is not stable	There may be condensation in the system (chapter 4.4). But also note, ci is a calculated magnitude and not necessarily stable. With no or low evaporation, the calculation of ci includes a division by a value close to 0. ci will then vary a lot being meaningless.
Air humidity indicated is higher than the set value.	The regulated value is the inlet humidity not the actual humidity within the cuvette.
Measuring Head	
Temperature of cuvette cannot be controlled; the leaf does not transpire properly	The impellers always need to be switched on.
Temperature and/or PAR-values are generally strange	Check the calibration values for the sensors. The original calibration values for the specified measuring head may be obtained from the Heinz Walz. GmbH.
Temperature of *Tleaf* varies by more than 0.5°C per s and is close to *Tcuv*.	Thermocouple is broken, or a saturation light pulse has caused a fast temperature change.

15.2 Simple function checks

15.2.1 Pneumatic pathway

- The function of the pneumatic pathway can be checked by watching the flow indicators while changing the pneumatic settings.
- Both indicators should show the same or a similar value. If this is not the case, the cuvette is not closed properly, or the valves are not adjusted well.
- If the flow rate is changed, the indicators should change.
- If the analyzer reference or sample outlets are hindered, the respective indicators should descend. Careful, do not block the outlets, the analyzers might get damaged, if too much pressure builds up within its analyzer cells.
- If the cuvette is bridged with a tube the flow shown in the Values window of GFS-Win should readjust to the previous value by changing the flow through the pump.
- If the flow is chosen low (600 µmol/s) and the cuvette is open, it is normal, that the pump cannot maintain the flow and switches off and on.
- If the mode is switched from MP to ZP, the flow in both indicators should decrease to the same level. Otherwise adjust valve 4.
-

15.2.2 H_2O Mixing Valve

- The CO_2 absorber column can be used to check, whether the H_2O valve can move into the correct position to direct the air flow through the drying or humidifying side.

  In the Settings window, switch the flow on and switch the H_2O mode to 3 (completely dry) to move the H_2O valve into the drying position. Place the CO_2 absorber first in the position of the dryer and afterwards in its correct position. Use the Mixing Volume 3000-C/MV for the other position. In both positions, the CO2abs value should decrease to zero after some time. Return the dryer to its correct positions to dry the gas paths before turning off the unit.

- If the CO_2 absorber is used to check the valve on the humidifying side, the CO2abs value does not necessarily decrease to zero, typically up to 10% of the incoming CO_2 remain in the gas stream.

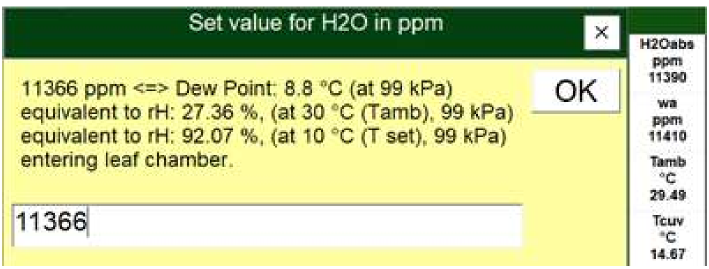
15.2.3 Reasonable CO_2 Detection

- Use fresh CO_2 absorber (note that the indicator only works with humid air and turns pale after a while). Switch to ZP mode. Switch the CO_2 control off. The CO2abs should descent to zero (±8 ppm, if the analyzer is not warmed up), also the dCO2 should reach 0 (±1 ppm).
- Replace the CO_2 absorber with the Mixing Volume 3000-C/MV. Use air from outside, don't breathe near the air-inlet. Ambient CO_2 concentration varies between 350 and 600 ppm in dependence on the location, day time and time of the year.

15.2.4 Reasonable H_2O Detection

- Replace the CO_2 absorber with the Mixing Volume 3000-C/MV. Switch the flow on and switch the H_2O mode to 3 (completely dry) and watch the H2Oabs value decrease slowly to zero.

  If the current relative humidity together with its measurement temperature are known, the ppm-value for H2Oabs can be compared. Replace the CO_2 absorber with the Mixing Volume 3000-C/MV and the drier and humidifier with tubs. Switch the flow on and the H_2O control off. Watch the H2Oabs value until it is stable.



  To calculate the relative humidity from the indicated H2Oabs value, use the humidity calculator of the H2O control dialog. Simply enter the currently measured ppm value for H2Oabs into the set-value dialog without pressing *OK*, the corresponding relative humidity value will be indicated for Tamb and Tcuv. If the relative humidity is not known for Tamb, but for a different temperature value, Tcuv can be set to this value, so that the humidity calculator also calculates the relative humidity for this temperature. For the calculation it is not necessary to really reach the set Tcuv value.

15.2.5 Testing Impellers separately

- With Standard Measuring Head Version 3.xx and GFS-Win Version 3.72 and higher, it is possible to run the impellers separately. There are to user- programs “Impeller1.prg” and “Impeller2.prg” on the provided CD, which contain the lines.

Impeller1.prg	**Impeller2.prg**
"Imp1 =","7"	"Imp2 =","7"
"Interval =","30"	"Interval =","30"
"Imp1 =","0"	"Imp2 =","0"

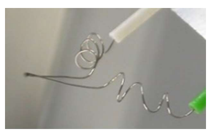
For different times and speeds, simply change the user-program (chapter 7.4.5). Switch the impeller off, before running them together as usual.

15.2.6 Temperature Control of Measuring Head

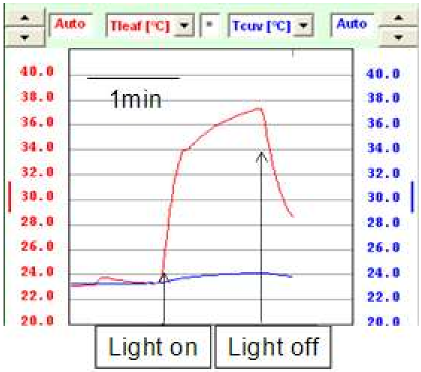
- If the Temperature control has been off for a while, Ttop, Tcuv and Tleaf should show very similar values.
- Set Tcuv to a value lower than ambient and touch both sides to check whether they are cooling.

15.2.7 Testing temperature sensor Tleaf

- Visually inspect the thermocouple. The temperature is measured at its tip, which should touch the leaf.



- For testing the thermocouple attach a light source to the measuring head. Insert a black piece of paper with the thermocouple touching it. Keep the flow, impeller and temperature control off. Switch the light to 2000 µmol m^{-2} s^{-1}. An intact thermocouple shows a temperature rise of more than 5°C, but a broken thermocouple shows no temperature difference to Tcuv.



15.3 Error Messages

15.3.1 Errors notified by GFS-Win Software

Errors are indicated as "Warnings" in the info-line as text-message. The info line will turn yellow. Erase the error message by pressing *Clear* in *GFS-Win*. The error will be reset or if necessary the complete module will be reset. If the problem still exists, the error message will come up again. All error messages are stored in the error lists error.rpt and error2.rpt in the subdirectory (My Documents/GFS-3000/ini) or the *GFS-Win*-subdirectory (depending on the program setup). Check this file to find out, whether there have been more errors before the last displayed error.

Table 9: Errors notified by *GFS-Win*

Type	**Explanation**
GFS Error	*See* Table 10 GFS-3000 errors.
No contact with…	*GFS-Win* tried to contact a module of the GFS-3200 but did not get a reply. Modules are: central unit, battery control, gas analyzer, H_2O control, flow meter, measuring head, fluorescence module, light module. Usually some cable (e.g. USB) is disconnected. The battery control is the first module contacted, when receiving data, therefore this error usually indicates that the total GFS-3000 is disconnected from the operating computer.
Communication error	The relevance of communication errors depends on their circumstances. It could be that a module was disconnected without disabling it beforehand. It could be that a data string was not received and is missing, that a new setting was not successfully sent and changed or that it happened during calibration. Depending on the circumstances, the last action should be repeated. Sometimes there are harmless echoes detected in the line and reported as communication errors. (junk in line)
File error	These errors occur during writing or reading a file. Some of these files might be temporary files, not seen by the user. The most common reasons is that a file has been opened or stored by another program in a new format. For example, program-files opened with Notepad, or report-file opened and stored with Excel. Or *GFS-Win* is running from a CD.
Unexpected file error	These are file errors, where we have no suggestion for a cause.
Calibration failed	If this error occurs, please repeat calibration.

Table 10: Errors detected and notified by modules of the GFS-3000. Some voltage errors might have serious causes like a short but could also be generated during switch-on/off processes. Voltage or current errors, initialization errors, most memory errors and some other errors lead to a halt of the GFS-3000 instrument.

Location and error code	**Explanation**
Battery control	**Version 4 (in Control Unit 3200-C)**
1100-1122	Errors reported by the CPU of the battery control (memory error [0, 3, 18, 20], run-time error [9], initialization failure [1, 2, 3, 4, 5, 7, 16, 17], voltage error [6, 8], or communication errors [21, 22])
1123	Voltage error (short, low power, heat)
1124	Voltage input to high > 27 V
1125	Voltage input low < 10V, battery not recognized
1132	Panel PC not connected
1133	Current too high
1134	Battery protection activated
1137-1140	UART communication errs: Battery ctrl. ↔ GFS-Win
1141	TWI communication errs: no acknowledgment from multiplexer within battery control
1148	LED-power of panel PC is off
1149	Batter control was never contacted by GFS-Win
1150	Switching off not under control from GFS-Win
1151	Switching off while panel PC is off (not controlling)
1153	TWI communication searching address, start
1154	TWI communication searching address, nothing found
1164-1170	TWI communication errs: battery ↔ battery control
Central Unit	**Version 2.10**
2100-2122	Errors reported by the CPU of central unit (memory error [0, 3, 18, 19, 20], run-time error [9], initialization failure [0 … 7, 16, 17], voltage error [8] or communication errors [21, 22])
2108	Voltage, CPU central unit
2123	Voltage, central unit 12V-DC converter: short/heat
2124	Voltage, central unit at input low < 13 V
2125, 2132	Voltage, CO_2 valve < 22V [25]; not 0 V [32]

	for Version < 2.00: voltage pump
2136	Voltage, gas analyzer (short, heat).
2137	Gas analyzer was switched on …
2138	Voltage, solenoids ZP/MP (short/heat)
2148, 2149	Communication err: GFS-Win ↔ CPU central unit
2150 – 2157	Communication err: CPU central unit ↔ gas analyzer
2156	CO_2 regulation: out of range (check CO2 absorber)
2164 – 2165	Communication err: CPU central unit ↔ GFS-Win.
2172	New segment used, when writing to EEPROM
Flow Meter	
2166	No communication: flow meter ↔ CPU of central unit
2167/00-2167/20	Errors reported by the CPU of the flow meter (memory err [0, 3, 18, 20], run-time err [9, 20], initialization failed [0 … 5, 7, 16, 17], voltage err [6, 8]))
2167/23	flow meter, analog digital converter
2167/24-2167/25	Voltage, flow meter (short, low voltage)
2167/33-34	Communication err: flow meter ↔ CPU of central unit
H_2O Control	
2169	No communication: H_2O control ↔ CPU of central unit
2170/00-2170/20	Errors reported by the CPU of the H_2O control (memory error [0, 3, 18, 20], run-time error [9], initialization failure [1, 2, 3, 4, 5, 16, 17], voltage error [6, 8])
2170/24	Voltage, H_2O control, no power at motor
2170/25	Current, heat problem at H_2O control
2170/32-33	Reference detection failed
2170/34-35	Communication err: H_2O control ↔ CPU central unit
Measuring Head	**Ver 3.00**
3040	Peltier error, resistance of Peltier elements is low (mechanical or electrical damage). Temperature control may be not as good as possible. Watch temperature of measuring head during experiments. Contact the Heinz Walz GmbH for repair service.
3100 – 3120	Errors reported by the CPU of the measuring head (memory err [0, 3, 18, 20], run-time err [9, 20], initialization failed [0 … 5, 7, 16, 17], voltage err [6, 8])

3123	Fuse: temperature control and light
3124	Voltage low, measuring head at input
3125	Voltage error, upper Peltier (short/overload) (Ver 1.xx: Voltage error: both Peltier elements)
3132	Voltage error, light circuit (short/overload)
3133	Voltage error, 12 V DC converter (short/ low input)
3134	Voltage error, 5 V DC converter (short/overload)
3135	Measuring Head push button pressed
3136	Voltage error, lower Peltier (short/overload) (Ver 1.21: Impeller 1, problem with motor signal)
3137-3140	(Ver 1.xx, 2.xx: Impeller-control error)
3148	Analog digital converter error
3153, 3154 3172, 3173	Communication error
3155	Impeller 2 stopped, blocked? (Ver 1.xx: Impeller 1)
3156	Impeller 2 rough-running, dirty? (Ver 1.xx: Impeller 1)
3157	(Ver 1.xx: Communication error, impeller 1)
3164	Impeller 1 stopped, blocked? (Ver 1.xx: Impeller 2)
3165	Impeller 1 rough-running, dirty? (Ver 1.xx: Impeller 2)
3166	(Ver 1.xx: Communication error, impeller 2)
3167	Fan, upper Peltier temperature-control, blocked?
3168	Fan, lower Peltier temperature-control, blocked?
3169	Lightening unit not connected or fan in lightening unit stopped, blocked?
3172, 3173	Communication error, measuring head
Impeller Control	**Measuring Head Version 3.00 only**
3180/00- 3180/20	Errors reported by the CPU of the impeller control (memory err [0, 3, 18, 20], run-time err [9, 20], initialization failed [0 … 5, 7, 16, 17], voltage err [6, 8])
3180/21, 22	UART communication error
3180/32, 33	Impeller motor: voltage low [32], high [33]
3180/34, 35	Speed control: high temperature
3180/38, 41	Speed control Impeller 1 / 2: communication error
3180/64, 68	Speed control Impeller 1 / 2: low voltage
3180/65, 69	Speed control Impeller 1 / 2: PWM failed
3180/66, 70	Speed control Impeller 1 / 2: did not start
3180/81-	Communication error: impeller control ↔ impeller

3180/84	
3180/240	Impeller control CPU is resetting
LED Light Source 3041	
Light 3041-L: 0d … 25d	Errors reported by the CPU of the LED light source 3041-L memory err [0, 3, 18, 20], run-time err [9, 20], initialization failed [0 … 5, 7, 16, 17], voltage err [6, 8, 25]) or communication [21, 22]
34d … 36d	Communication error, when receiving settings: light source ↔ GFS-Win
37d … 40d	Communication error: light source ↔ GFS-Win

15.4 Sending for Repair

Before sending the instrument for repair, please contact the Heinz Walz GmbH for advice to exclude simple errors or faults, which can be fixed on-site. There is a service form on the web page of the Heinz Walz GmbH, which contains all items required for smooth administration and shipment:

https://www.walz.com/support/repair_service.html

Please fill in the form and send it together with the instrument. Also provide information on any problems. Only include the parts in the shipment, which are required fort the repair. Please read chapter 13.15.2 on how to prepare the instrument for transport.

16 Appendix

16.1 LED Codes

Table 11: Power LED of Control Unit 3200-C, function of power switch depends on status

LED power-status	Status	Function of power
off	instrument is off	press shortly: switch instrument on press 5s: switch instrument on, but keep panel PC off
orange	battery/power control is booting	
green/red blinking	battery/power control is waiting to be contacted	press 3 s -> switches off
green/orange blinking	at least one of the inserted batteries is empty	
green blinking (1s)	power-input is fine, internal panel PC operation	press 4 s (until red blink)-> emergency off (do not use, it may cause damage)
slowly green blinking (2 s off, 1 s on)	power-input is fine, external panel PC operation	press 4 s (until red blink)-> switches panel PC on
orange blinking	software error (in GFS-Win read error code and press clear)	
red blinking	serious error check power-in voltage (in GFS-Win read error code and press clear)	
one red blink, then switching off	Supplied voltage to low (< 10V)	
green permanent	battery/power control has a program-crash or hardware error	

Table 12: LED-Code for any big LED inside the Control Unit 3200-C, the Standard Measuring Head 3010-S or 3010-GWK1

LED	Status
off	module is off

green blinking (1s)	ok
orange	module is booting, self-test
red/orange blinking	self-test
green, red or off permanent	unknown error: hardware error or software crash
orange/green blinking	-
orange blinking	software error (check file error.rpt for error codes)
red blinking	serious error (check file error.rpt for error codes)

Table 13: LED code for 3010-I/Box

LED	3010-I/Box (USB-RS485 converter)
red continuous	USB provides power, ready for unidirectional data transfer (RS485 to USB, command: ßßßGFS)
green blinking	Ready to send data bidirectional with a set timeout period (Command ßßß012: example for the timeout period 12s)
green continuous	Continuous mode bidirectional data transfer; do not use (Command: ßßß-ON)

Table 14: LED code for panel PC LED of Control Unit Model 3200-C

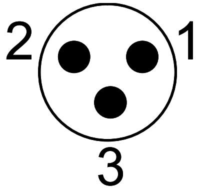
LED	panel PC
green	Power is on
orange	Accessing hard drive
orange blinking	Standby modus

Table 15: LED code of Fluorescence Module 3056-FL and Fiberoptics PAM-Fluorometer 3050-F

LED	Fluorometer
green blinking	Power is on, operation ok
red	Saturation light pulse (may include Fo' measurement) in progress or updating program
red/green blinking	booting

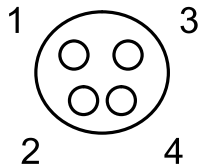
16.2 Pin Assignments of Connectors Control Unit 3200-C

"AUX IN"



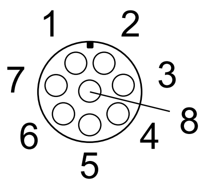
1: input AUX2 (+0...4095 mV),
2: input AUX1 (+0...4095 mV)
3: GND
1: blue, 2: brown, 3: black

"COMP"



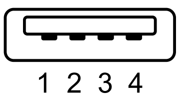
1: RS485/A
2: output (+14 ...16 V)
3: RS485/B
4: GND

"CUV"



1: output (+14 ...16 V)
2: GND
3: GND
4: GND
5: RS485/B
6: output (+14 ...16 V)
7: output (+14 ...16 V)
8: RS485/A

"2 USB 2.0"

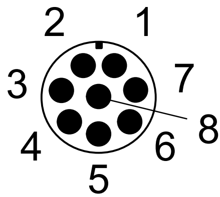


USB 2.0 Ports of panel PC
1: + 5V
2: Data -
3: Data +
4: GND
For connection of USB-devices to the internal PC.
Use a special Null Modem Cable for connecting a PC.

16.3 Pin Assignments of Connectors Standard Measuring Head

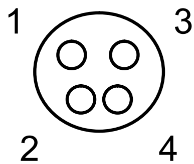
The connectors at the Standard Measuring Head 3010-S are not labeled, in favor a short description of each connector is given!

Connector for the connecting cable between Standard Measuring Head 3010-S and Control Unit 3000-C or 3200-C. The cable usually remains connected to the measuring head.



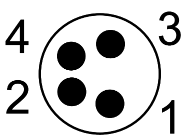
1: input (+14 ...16 V)
2: GND
3: GND
4: GND
5: RS485/B
6: input (+14 ...16 V)
7: input (+14 ...16 V)
8: RS485/A

Connector for an additional component; which has the same pin assignment as the connector "COMP" at the front side of the Control Unit 3000-C. If e.g. the LED-Array/PAM-Fluorometer 3056-FL or the LED Light Source 3041-L is used, one of the two cables must be connected here.



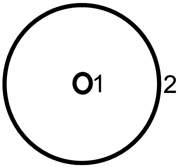
1: RS485/A (brown)
2: output (+14 ...16 V)
3: RS485/B (blue)
4: GND (black)

Connector one of the two cables of the LED Light Source 3041-L or the LED-Array/PAM-Fluorometer 3056-FL:



1: output for LEDs (+0 ... 12 V)
2: GND for LEDs
3: output for fan (+12 V)
4: OC (open collector) for fan

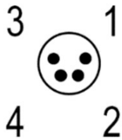
Connector for the External Miniature Quantum Sensor MQS-B/GFS



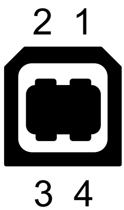
1: cathode of quantum sensor
2: anode of quantum sensor

16.4 Pin Assignment of 3010-I/Box

USB-RS485 converter.



1: RS485/A (brown)
2: not connected
3: RS485/B (blue)
4: not connected



USB 2.0 Connector
1: + 5V
2: Data -
3: Data +
4: GND

16.5 Pin Assignment of AUX Cable

Cable for 2x Auxiliaries (part #: 000130606205), which has three wires with bar ends for connecting two sensors:

Wire color	Assignment	Voltage input
black	Ground	
blue	AUX 1	+0 ... 4095 mV
brown	AUX 2	+0 ... 4095 mV

16.6 Information on Chemicals and Batteries

16.6.1 Soda Lime

Please read all MSDS-sheets for soda lime provided on the CD or our web page. Soda lime contains 2-5% sodium hydroxide and more than 50%

calcium hydroxide. It may be corrosive. Avoid generating dusts, do not breathe dust, wash hands and face thoroughly after working with material. Poison class CH: 2 (very strong toxins). WGK: slightly polluting substances.

Sodium hydroxide:
CAS-Number: 1310-73-2
R-Phrase: C: R35.
H314: Serious skin burns and eye damage

Calcium hydroxide:
CAS-Number: 1305-62-2
R-phrase: Xi: R38, R41.
H318: Serious eye damage
H315: Skin irritation
WEL assigned.

Storage: Recommended storage temperature: 15-25°C. Keep well closed and protected from direct sunlight and moisture.

Disposal: If possible, recycle to approved recycling company. If not, dispose of in accordance with local authority regulations. Rinse out used containers thoroughly.

16.6.2 Molecular sieve 3 Å

Please read all MSDS-sheets provided on the CD or our web page. Avoid dust. Dispose in accordance with local authority regulations.

CAS-Number: 1318-02-1

EC-Number: 215-283-8

REACH: 01-2119429034-49-xxxx

Storage: recommended storage temperature: 20°C. Keep dry.

H2O ads. capacity (10 % R.H. / 25 °C) ≥15,5 %

Melting point/freezing point >400 °C

16.6.3 Silica Gel (Sorbead Orange Chameleon)

Silica Gel	beads, desicant ~ 2 – 5 mm

Formula Hill: O_2Si

Molar mass: 60.09 g/mol

CAS number: 7631-86-9

EC-No.: 231-545-4

HS Code: 2811 22 00

Storage class (VCI): 10-13 (Other liquids and solids)

WGK: nwg (nonpolluting substance)

Poison class CH: F (Not subject to toxicity classification)

16.6.4 Humidifying Granules (Stuttgarter Masse)

Stuttgarter Masse	Grain Size 3 – 5 mm

Technical data:

Material: ceramic bulk

Filtration finesse: micro organism carrier

Temperature res.: up to 600 °C

Chemical res.: pH 0 - 9

Field of application:
Fixed bed filter for water treatment
Carrier material for gas detector devices
Carrier material for catalysators
Carrier material for micro organisms Granules

16.6.5 Li-ion eSMART Battery 98 Wh

Read the MSDS-Sheet provided on the CD for complete information. The Li-ion eSMART Battery 98 Wh (000160101434) with 14.4V and 6.8Ah do not require shipper's declaration for transport. Only a handling label is required since it is below 100 Wh and passed the UN

Manual of Tests and Criteria Part III Subsection 38.3. All current regulations for air shipment can be found on the website of the International Air Transport Association http://www.iata.org.

- Do not transport when broken.
- Avoid shorting the battery with conductive (i.e. metal) goods.
- Only transport <30% charged
- Never use a battery that appears to have suffered abuse.
- Battery must be charged in appropriate charger only.
- Never use a modified or damaged charger.
- Store in a cool, dry and well-ventilated area.
- Do not disassemble, deform, crush or drop the battery from height.
- Do not expose to heat, high humidity, open flame, sunlight, water, seawater, strong acids, strong oxidizers, strong reducing agents, strong alkalis or metal wire.

16.7 Spectral Sensitivity of the Light Sensor

Fig. 72 and Fig. 73 show the relative spectral sensitivity of the quantum sensor type MQS-B/GFS, which is used to measure the ambient light intensity PARamb. Fig. 72 gives the spectral sensitivity regarding the energy flux density, Fig. 73 regarding the photon flux density. The solid lines show the ideal response.

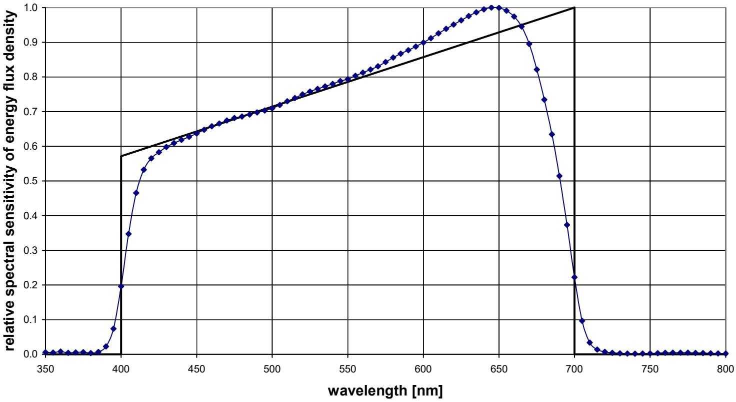


Fig. 72: relative spectral sensitivity of energy flux density

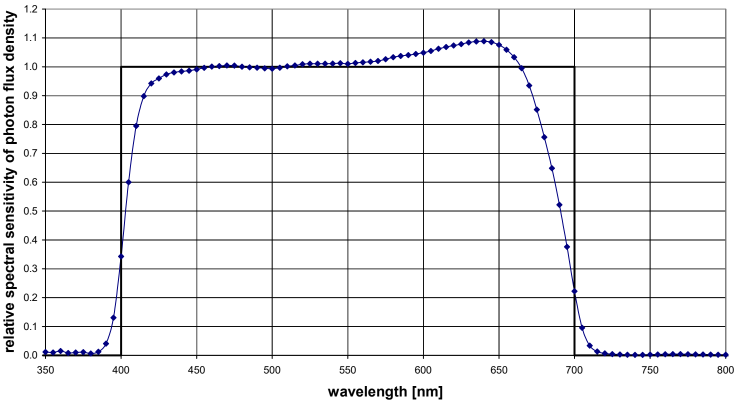


Fig. 73: relative spectral sensitivity of photon flux density

16.8 Spectral Sensitivity of the Light Sensor LS-A

Fig. 74 and Fig. 75 show the relative spectral sensitivity of the quantum sensor type LS-A, which is used to measure the light intensity in the upper (PARtop) or lower part (PARbot) of the cuvette. Fig. 74 gives the sensitivity regarding the energy flux density, Fig. 75 regarding the photon flux density. The solid lines show the ideal response.

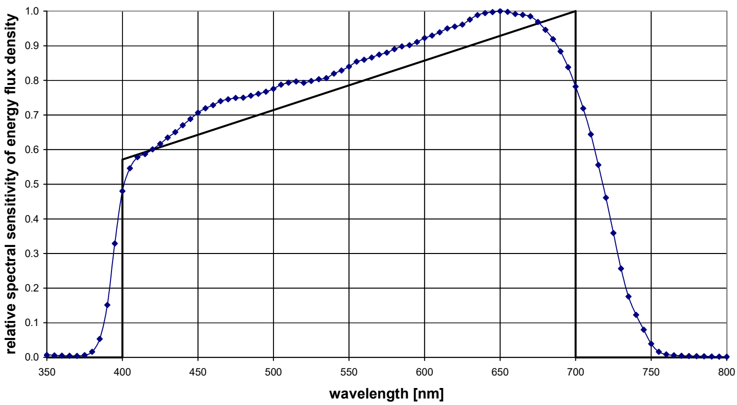


Fig. 74: Relative spectral sensitivity of energy flux density

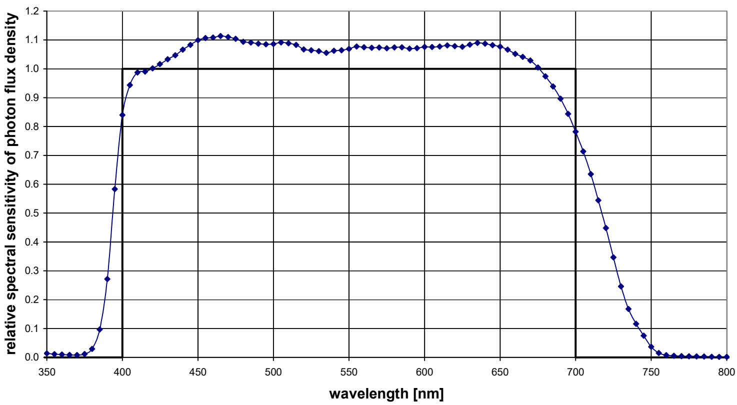


Fig. 75: Relative spectral sensitivity of photon flux density

16.9 Remote Desktop Connection

If the GFS-3000 shall be connected via a Remote Desktop Connection, insert the Wi-Fi adapter (=WLAN-adapter) into one of the USB-ports:

- From the panel PC desktop run the script "WIFI set SecurityKey" (Fig. 6) to show the current Security Key. Close the window or enter a new key.
- Run the script "RDP WIFI GFS3000 ON" to provide a network

On your PC use the network picker  to connect to Wi-Fi, right-hand click on GFS3000_81 to adjust properties:

- Profile Name: GFS3000_81
- Security Type: WPA2-Personal (WPA2-PSK)
- Data encryption: AES
- Network-Key: Security Key as shown or set above

On your PC start Remote Desktop Connection (mstsc.exe):

- Computer: GFS3000 or IP-address of GFS3000 (not GFS3000_81)
- Login: GFS3000\GFS
  (for serial number KETB0xxx and lower: GFS3000\Administrator)
- Password: Please ask Walz. (You may be asked twice for it)

It may be necessary to adjust the address for TCP/IpV4 of the Wi-Fi adapter on your PC. Use the network picker and right-hand click on GFS3000_81, select status (or Network and Internet Settings, Wi-Fi, change adapter options, Wi-Fi) right-hand click→properties, TCP/IpV4, properties:

- IP address: 192.168.0.111
- Subnet mask: 255.255.255.0
- Standard Gateway and DNS Server: empty

For closing the network (more secure):

- On the panel PC run "RDP WIFI GFS3000 OFF"

17 Literature

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18 Technical Data

Subject to change without prior notice.

18.1 Control Unit 3200-C

Design: Aluminum housing featuring integrated PC module, large graphical color-display well readable in sun-light with touch screen, 4-channel CO_2/H_2O gas analyzer, flow control, CO_2 control and H_2O control (for drying and humidifying).

Pneumatic connectors: air inlet, measuring head and four vents.

Sockets for cable connections:

Cuvette: Standard Measuring Head 3010-S,
DUAL-PAM Gas-Exchange Cuvette 3010-DUAL,
Gas-Exchange Chamber 3010-GWK1 or other special design.

Aux in: two Auxiliaries

3 Battery Slots: Li-Ion eSMART Batteries (each 14.4V/6.8Ah),
Mains Power Supply Unit 3200-N
or 3200/BC adapter for 12 V or 24 V Battery

Comp (RS 485): 3010/I-Box for external PC-control or one additional component

2 USB sockets (USB 2.0) to internal PC: USB storage device, other USB device or USB null modem cable (USB-NMC) for control by external PC via internal PC

CO_2/H_2O gas analyzer:

Design: 4-channel CO_2/H_2O absolute NDIR gas analyzer, separate cuvettes for CO_2 and H_2O.

CO_2 measurement: Simultaneous absolute and differential measurements, absolute range 0 to 5000 ppm, cuvette length 20 cm, cuvette volume of one cell 6 cm3, gas-filled detector

H_2O measurement: Simultaneous absolute and differential measurements, absolute range 0 to 75000 ppm, cuvette length 20 cm, cuvette volume of one cell 6 cm^3, pyroelectric detector (solid state)

Max. noise in absolute mode: <0.2 ppm CO_2 and <30 ppm H_2O

Resolution: 0.01 ppm CO_2, 1 ppm H_2O

Linearization: CO_2 absolute: Max error of absolute signal between 0 and 600 ppm: 12 ppm, above 600 ppm: 2% of measured value.
Deviation of differential CO_2 zero in dependence on absolute CO_2: Typically less than 0.5 ppm CO_2 over full range.
H_2O absolute: Max error between 0 and 15000 ppm: 330 ppm, above 15000 ppm: 2% of measured value
Deviation of differential H_2O zero in dependence on absolute H_2O: Typically less than 150 ppm H_2O over full range.

Barometric air pressure measurement: Range 60 to 110 kPa, accuracy ±0.1 %

Mass flow measurement: Thermal mass flow meter, range 0 to 1500 µmol s^{-1}, accuracy ±1 %

Integrated user interface: Panel PC MS-98FG/1.83 GHz with graphical color-display 640 x 480 dots (effective display area 13 cm x 10 cm) with back-light (well readable in direct sun-light), touch screen, 2 USB 2.0 connector and audio speaker.

Data storage capacity: Solid State Drive 32 GB

CO_2 control: Integrated CO_2 control, range 0 to 2000 ppm, CO_2 supply via CO_2 cartridges (8 g CO_2, provide more than 48 hours continuous supply at 380 ppm CO_2, reserve is indicated) or via CO_2 cylinder with pressure reducer.

H_2O control: Integrated H_2O control via step motor for humidifying and drying, range 0 to 100 % rh (non-condensing)

Measured and calculated parameters: CO_2 absolute, CO_2 difference, H_2O absolute, H_2O difference, flow, ambient pressure, 2x auxiliaries, cuvette temperature (upper and lower half), leaf temperature, ambient temperature, PAR in upper part of the cuvette, PAR in lower part of the cuvette, external PAR, impeller frequency, evaporation, VPD, H_2O conductance, net photosynthesis, intercellular CO_2 concentration. Recalculation of stored data is possible,
Fluorescence parameters in combination with LED-Array/PAM Fluorometer 3056-FL (GFS-3000FL): Fo, Fm, Fm', F, Fo', Fv/Fm (max. PS II quantum yield), ΔF/Fm' = Y(II) (effective PS II quantum yield), qP, qL, qN, NPQ, Y(NPQ), ETR (i.e. PAR x ΔF/Fm'), recalculation of stored data with other Fo and Fm is possible.

PC interface: 3010-I/Box

Auxiliaries: Two analog inputs, range 0 to 4095 mV, user programmable

Input voltage: 12-24V

Power Supply: Up to three (standard is two) field replaceable rechargeable Li-ion eSMART batteries 14.4 V/6.8 Ah (000160101434, four batteries supplied), or AC Power Supply 3200-N for laboratory operation. The Control Unit 3200-C may also be operated with a 12 or 24 V battery (only with optional battery cable 3200-C/BC provided by us).

Operating time: 3 to 4.5 typ. with two Li-ion eSMART batteries, 6 to 9 hours typ. with four Li-ion eSMART batteries.

Operating temperature: -5 to +45°C

Dimensions: 43 cm x 29.5 cm x 29.5 cm (L x W x H) with handle in transport position

Weight: 12.3 kg (incl. two Li-ion eSMART batteries)

18.2 Interface 3010-I/Box

Design: USB-RS485 converter with galvanic isolation (1kV) and connection cables. Serves to establish a connection between the COMP socket of the GFS-3000 and the USB port of an external PC. Suitable for all types of Control Units (3000-C, 3100-C or 3200-C). Also suitable for direct operation of the Standard Measuring Head 3010-S, the DUAL-PAM Gas-Exchange Cuvette 3010-DUAL, the Gas-Exchange Chamber 3010-GWK1, the LED-Panel RGBW-L084 with an external PC.

Dimensions: box: 8 cm x 4 cm x 2 cm attached cable 30 cm, USB-device cable: 1.5 m

18.3 Standard Measuring Head 3010-S

Design: Universal measuring head featuring small-sized cuvette volume (40 ml), wide range temperature control and effective ventilation. Electronics box detachable for custom-built cuvettes, upper and lower cuvette halves pneumatically separated with one impeller each for upper and lower parts, interchangeable adapter plates for different leaf areas, cuvette expandable to different volumes and shapes (small cylinder or cuboid) for measuring mosses, lichens or conifers. Sockets for cable connections with Control Unit 3000-C to 3200-C and sockets for LED Light Source 3041-L or LED-Array/PAM-Fluorometer 3056-FL or Fiberoptics/PAM-Fluorometer 3050-F.

Cuvette temperature (in upper and lower cuvette half) ambient temperature: Pt 100 type A, range -10 to +50°C, accuracy ±0.1°C

Temperature control: Three modes of temperature control: tracking ambient temperature (with or without offset), set value for cuvette temperature and set value for leaf temperature; cuvette temperature ranging from 10 degrees below ambient temperature (decreasing with light intensity) to +50°C

Leaf temperature. measurement: Thermocouple, range -10 to +50°C, accuracy ±0.2°C

External miniature quantum sensor: Quantum Sensor MQS-B/GFS sits on top of the Standard Measuring Head 3010-S. Selective PAR measurement, range 0 to 2500 µmol m^{-2} s^{-1}, accuracy ±5 %, cosine corrected (measuring photosynthetic photon flux density, PPFD)

Internal quantum sensor: two sensors, one in the upper and one in the lower part of the cuvette: Selective PAR measurement, range 0 to 3000 µmol $m^{-2}s^{-1}$ PAR, accuracy ±10 %.

Cuvette ventilation system: Two frequency-controlled impellers, one in the upper and one in the lower part of the cuvette, speed adjustable in 9 steps

Leaf area: 8 cm^2 standard, interchangeable adapter plates from 1 to 10 cm^2, flexible shape

Cuvette volume: 40 ml

Operating temperature: -5 to +45°C

Dimensions: 31 cm x 7 cm x 13 cm (L x W x H)

Weight: 1.6 kg (incl. cable and tubes 2 m long)

18.4 AC Power Supply 3200-N

Design: DC power supply unit for laboratory use

Output voltage: 16 V DC

Output power: 135 W Max (depends on version, see label)

Mains power supply: 100 to 240 V AC, 50/60 Hz

Operating temperature: 0 to 60°C (depends on version, see label)

Dimensions: 20 cm x 8 cm x 5.5 cm (L x W x H) with connector inserting into the battery slot 15.3 cm x 7.9 cm x 2.3 cm (L x W x H), cable length 90 cm.

Weight: 1.07 kg

18.5 Li-ion eSMART Battery 98 Wh (000160101434)

Design: High-performance maintenance-free rechargeable Li-ion battery with protection circuit. Use two batteries simultaneously.

Nominal voltage: 14.4 V

Typical capacity: 6.8 Ah

Maximum Current Discharge: 8A continuous, current delivery may be limited below 10°C

Operating temperature: -5 to +60°C (discharge) and 0 to 45°C (charge)

Storage temperature limits: -20 to 60°C, < 80%RH

Recommended storage temperature: < 21°C, in low humidity free from corrosive gas. Temperatures above 45°C could degrade life time.

Dimensions: 15.3 cm x 7.9 cm x 2.3 cm (L x W x H)

Weight: 0.45 kg

18.6 Li-Ion eSMART Battery Charger (000190101115)

Design: Charger for charging four eSmart Li-ion Batteries simultaneously. With active thermal management system, cooling fan and OLED display showing for each battery: capacity (%), temperature, voltage, number of cycles and charging current.

Input voltage: 90-240 AC input /150 W

Maximum charging current per bay: 2 A

Recharging time for each battery: 3-4 hours

Dimensions: 27 cm x 17.6 cm x 5.3 cm

Weight: 1.6 kg

18.7 LED Light Source 3041-L

Design: LED array with 67 warm white LEDs

Light intensity: Range 0 to 3000 µmol $m^{-2}s^{-1}$ PAR max.

Homogeneity of light distribution: ±7 % over 90% of sample area

Leaf area: standard 8 cm^2 up to 10 cm^2

Power consumption: 5.3 W max., power supply via Standard Measuring Head 3010-S

Operating temperature: -5 to +45°C

Dimensions: 7.5 cm x 4.5 cm x 5.5 cm (L x W x H)

Weight: 196 g

18.8 LED-Array/PAM-Fluorometer 3056-FL

Design: Combined PAM chlorophyll fluorometer and LED light source consisting of LED array with 24 red LEDs (for actinic illumination and saturation pulses), 16 blue LEDs (for measuring light and actinic illumination), 20 far-red LEDs and 6 photodiodes (for chlorophyll fluorescence detection)

Measuring light: Blue LEDs (470 nm), modulation frequency 5 to 60 Hz and 1.2 kHz (during saturation pulse)

Actinic light: Blue LEDs (470 nm) and red LEDs (630 nm), range 0 to 2500 µmol m^{-2} s^{-1} PAR, typ. 90 % red and 10 % blue

Saturation light: Red LEDs (630 nm), typically > 8000 µmol m^{-2} s^{-1} PAR

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

Signal detection: PIN-photodiode protected by long-pass filter (> 660 nm), selective window amplifier

Leaf area: 8 cm^2

Power consumption: 15 W max. (during saturating light pulse), power supply via Standard Measuring Head 3010-S

Operating temperature: -5 to +45°C

Dimensions: 7.5 cm x 4.5 cm x 6.5 cm (L x W x H)

Weight: 220 g

18.9 Fiberoptics/PAM-Fluorometer 3050-F

Design: PAM chlorophyll fluorometer enclosed in a metal tube for connection to the Standard Measuring Head 3010-S. Measurement via an optical fiber entering the leaf chamber of the Standard Measuring Head 3010-S through an air-tight connection

Measuring Light: Blue LED (peak: 450 nm), modulation frequency: 10 and 500 Hz. PAR at 2 mm distance and *ML-Ampl*: 10: ca. 0.3 µmol m^{-2} s^{-1} with low frequency, 15 µmol m^{-2} s^{-1} with high frequency

Saturating light: Blue LED (peak: 450 nm). At 1 mm distance: typically, 11000 µmol m^{-2} s^{-1} at 2 mm: typically 6000 µmol m^{-2} s^{-1}

Relationship between light and distance

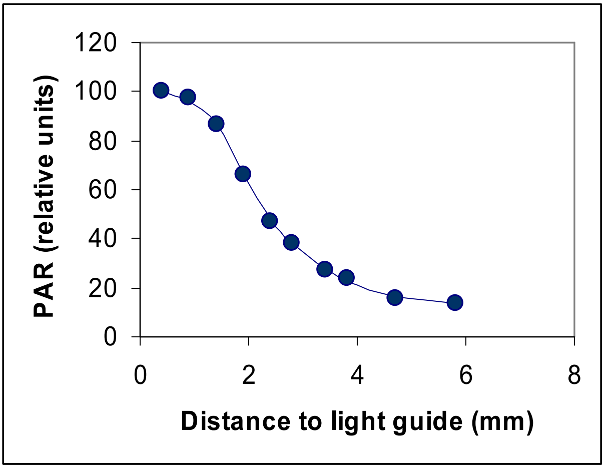

Fig. 76: Relative PAR in dependence of distance. The fiber was placed perpendicular to the sensor (pin-hole: 1 mm diameter).

Far-red light: LED (peak: 730 nm)

Signal detection: PIN-photodiode protected by a long pass filter, selective window amplifier

Power consumption: 0.25 W continuously, 6.5 W (during saturating light pulse), supplied via Standard Measuring Head 3010-S

Operating temperature: -5 to + 45°C

Dimension of light guide: Optical fiber, length: 21 cm, diameter: 1.5 mm

Dimensions of housing: length: 20 cm, diameter: 3 cm

Weight: 150 g

19 Saturation Vapor Pressure above Water

The following three tables show the saturation water vapor pressure above water.

The table values were calculated with the formula of Goff-Gratch (List, Robert J.; Smithsonian Meteorological Tables; Smithsonian Institution Press; Washington, D.C.; Fifth reprint issued 1984):

$$\log_{10}[SVP(T)] = -7.90298 \cdot \left(\frac{Ts}{T} - 1\right) + 5.02808 \cdot \log_{10}\left(\frac{Ts}{T}\right)$$

$$-1.3816 \cdot 10^{-7} \cdot \left(10^{11.344*(1-T/Ts)} - 1\right)$$

$$+8.1328 * 10^{-3} * (10^{-3.49149*(Ts/T-1)} - 1) + \log_{10}\left(SP_{ws}\right)$$

SVP(T) = saturation vapor pressure over a plane surface of pure ordinary liquid water dependent on T [hPa],

T = absolute (thermodynamic) temperature [K],

Ts = steam-point temperature [373.16 K],

SP_{ws} = saturation pressure of pure ordinary liquid water at steam-point temperature [1013.246 hPa] (= 1 standard atmosphere).

The following two tables indicate the saturation vapor pressure in a temperature range between 0 and 100.9°C. The temperature can be read in steps of tenth degrees (add left side with head-line).

Table 16: Saturating Vapor Pressure above water

Temp. °C	.0 hPa	.1 hPa	.2 hPa	.3 hPa	.4 hPa	.5 hPa	.6 hPa	.7 hPa	.8 hPa	.9 hPa
0	6.108	6.152	6.197	6.242	6.288	6.333	6.379	6.426	6.472	6.519
1	6.566	6.614	6.661	6.709	6.758	6.807	6.856	6.905	6.955	7.004
2	7.055	7.105	7.156	7.207	7.259	7.311	7.363	7.416	7.469	7.522
3	7.575	7.629	7.683	7.738	7.793	7.848	7.904	7.960	8.016	8.072
4	8.129	8.187	8.245	8.303	8.361	8.420	8.479	8.538	8.598	8.659
5	8.719	8.780	8.842	8.903	8.966	9.028	9.091	9.154	9.218	9.282
6	9.346	9.411	9.477	9.542	9.608	9.675	9.742	9.809	9.877	9.945
7	10.013	10.082	10.152	10.221	10.291	10.362	10.433	10.505	10.577	10.649
8	10.722	10.795	10.869	10.943	11.017	11.092	11.168	11.243	11.320	11.397
9	11.474	11.552	11.630	11.708	11.788	11.867	11.947	12.028	12.109	12.190
10	12.272	12.355	12.438	12.521	12.605	12.690	12.774	12.860	12.946	13.032
11	13.119	13.207	13.295	13.383	13.472	13.562	13.652	13.742	13.833	13.925
12	14.017	14.110	14.203	14.297	14.391	14.486	14.582	14.677	14.774	14.871
13	14.969	15.067	15.166	15.265	15.365	15.466	15.567	15.668	15.770	15.873
14	15.977	16.081	16.185	16.290	16.396	16.503	16.610	16.717	16.825	16.934
15	17.044	17.154	17.265	17.376	17.488	17.600	17.714	17.827	17.942	18.057
16	18.173	18.289	18.406	18.524	18.643	18.762	18.881	19.002	19.123	19.245
17	19.367	19.490	19.614	19.739	19.864	19.990	20.116	20.244	20.372	20.500
18	20.630	20.760	20.891	21.022	21.155	21.288	21.421	21.556	21.691	21.827
19	21.964	22.101	22.240	22.379	22.518	22.659	22.800	22.942	23.085	23.229
20	23.373	23.518	23.664	23.811	23.958	24.107	24.256	24.406	24.557	24.708
21	24.860	25.014	25.168	25.323	25.478	25.635	25.792	25.950	26.109	26.269
22	26.430	26.592	26.754	26.918	27.082	27.247	27.413	27.580	27.748	27.916
23	28.086	28.256	28.428	28.600	28.773	28.947	29.122	29.298	29.475	29.653
24	29.831	30.011	30.192	30.373	30.556	30.739	30.923	31.109	31.295	31.483
25	31.671	31.860	32.050	32.242	32.434	32.627	32.821	33.017	33.213	33.410
26	33.608	33.808	34.008	34.210	34.412	34.615	34.820	35.026	35.232	35.440
27	35.649	35.858	36.069	36.281	36.494	36.709	36.924	37.140	37.358	37.576
28	37.796	38.017	38.239	38.462	38.686	38.911	39.138	39.365	39.594	39.824
29	40.055	40.287	40.520	40.755	40.991	41.228	41.466	41.705	41.946	42.187
30	42.430	42.674	42.920	43.166	43.414	43.663	43.914	44.165	44.418	44.672
31	44.927	45.184	45.442	45.701	45.961	46.223	46.486	46.750	47.016	47.283
32	47.551	47.821	48.092	48.364	48.637	48.912	49.188	49.466	49.745	50.025
33	50.307	50.590	50.874	51.160	51.447	51.736	52.026	52.317	52.610	52.904
34	53.200	53.497	53.796	54.096	54.397	54.700	55.004	55.310	55.618	55.926
35	56.237	56.548	56.862	57.176	57.492	57.810	58.130	58.450	58.773	59.097
36	59.422	59.749	60.077	60.408	60.739	61.072	61.407	61.744	62.082	62.421
37	62.762	63.105	63.450	63.796	64.143	64.493	64.844	65.196	65.551	65.907
38	66.264	66.624	66.985	67.347	67.712	68.078	68.445	68.815	69.186	69.559
39	69.934	70.310	70.688	71.068	71.450	71.833	72.219	72.606	72.994	73.385
40	73.777	74.172	74.568	74.966	75.365	75.767	76.170	76.575	76.982	77.391
41	77.802	78.215	78.629	79.046	79.464	79.885	80.307	80.731	81.157	81.585
42	82.015	82.447	82.881	83.317	83.754	84.194	84.636	85.080	85.525	85.973
43	86.423	86.875	87.329	87.785	88.243	88.703	89.165	89.629	90.095	90.563
44	91.034	91.506	91.981	92.458	92.937	93.418	93.901	94.386	94.873	95.363
45	95.855	96.349	96.845	97.343	97.844	98.347	98.852	99.359	99.868	100.380
46	100.894	101.410	101.929	102.449	102.972	103.498	104.025	104.555	105.088	105.622
47	106.159	106.698	107.240	107.784	108.330	108.879	109.430	109.984	110.540	111.098
48	111.659	112.222	112.787	113.355	113.926	114.499	115.074	115.652	116.233	116.816
49	117.401	117.989	118.579	119.172	119.768	120.366	120.967	121.570	122.176	122.784
50	123.395	124.009	124.625	125.244	125.865	126.489	127.116	127.745	128.378	129.012

Temp. °C	.0 hPa	.1 hPa	.2 hPa	.3 hPa	.4 hPa	.5 hPa	.6 hPa	.7 hPa	.8 hPa	.9 hPa
50	123.395	124.009	124.625	125.244	125.865	126.489	127.116	127.745	128.378	129.012
51	129.650	130.290	130.933	131.578	132.227	132.878	133.531	134.188	134.847	135.509
52	136.174	136.842	137.512	138.185	138.861	139.540	140.222	140.907	141.594	142.284
53	142.978	143.674	144.373	145.074	145.779	146.487	147.198	147.911	148.628	149.347
54	150.070	150.795	151.524	152.255	152.990	153.727	154.468	155.211	155.958	156.708
55	157.461	158.217	158.976	159.738	160.503	161.271	162.043	162.818	163.595	164.376
56	165.161	165.948	166.738	167.532	168.329	169.130	169.933	170.740	171.550	172.363
57	173.180	173.999	174.823	175.649	176.479	177.312	178.149	178.989	179.832	180.679
58	181.529	182.382	183.239	184.099	184.963	185.830	186.701	187.575	188.453	189.334
59	190.218	191.107	191.998	192.893	193.792	194.695	195.601	196.510	197.423	198.340
60	199.260	200.184	201.112	202.043	202.978	203.917	204.859	205.805	206.755	207.708
61	208.665	209.626	210.591	211.560	212.532	213.508	214.488	215.472	216.459	217.451
62	218.446	219.445	220.448	221.455	222.466	223.480	224.499	225.522	226.548	227.579
63	228.613	229.652	230.694	231.741	232.791	233.846	234.905	235.967	237.034	238.105
64	239.180	240.259	241.343	242.430	243.522	244.617	245.717	246.821	247.930	249.042
65	250.159	251.280	252.405	253.535	254.669	255.807	256.949	258.096	259.247	260.403
66	261.562	262.727	263.895	265.068	266.246	267.428	268.614	269.805	271.000	272.200
67	273.404	274.612	275.826	277.043	278.266	279.493	280.724	281.960	283.201	284.446
68	285.696	286.951	288.210	289.474	290.742	292.016	293.294	294.577	295.864	297.156
69	298.453	299.755	301.062	302.373	303.690	305.011	306.337	307.667	309.003	310.344
70	311.689	313.040	314.395	315.756	317.121	318.491	319.867	321.247	322.633	324.023
71	325.418	326.819	328.225	329.635	331.051	332.472	333.899	335.330	336.766	338.208
72	339.655	341.107	342.565	344.027	345.495	346.968	348.447	349.931	351.420	352.914
73	354.414	355.919	357.430	358.946	360.467	361.994	363.527	365.064	366.608	368.156
74	369.711	371.270	372.836	374.407	375.983	377.565	379.153	380.746	382.345	383.950
75	385.560	387.176	388.798	390.425	392.058	393.697	395.342	396.992	398.649	400.311
76	401.979	403.652	405.332	407.017	408.709	410.406	412.109	413.818	415.533	417.255
77	418.982	420.715	422.454	424.199	425.950	427.708	429.471	431.241	433.016	434.798
78	436.586	438.380	440.180	441.987	443.800	445.619	447.444	449.276	451.114	452.958
79	454.808	456.665	458.528	460.398	462.274	464.156	466.045	467.941	469.842	471.751
80	473.665	475.587	477.515	479.449	481.390	483.337	485.292	487.252	489.220	491.194
81	493.175	495.162	497.156	499.157	501.165	503.180	505.201	507.229	509.264	511.305
82	513.354	515.410	517.472	519.541	521.617	523.701	525.791	527.888	529.992	532.103
83	534.221	536.346	538.479	540.618	542.765	544.918	547.079	549.247	551.423	553.605
84	555.795	557.991	560.196	562.407	564.626	566.852	569.085	571.326	573.574	575.830
85	578.093	580.363	582.641	584.926	587.219	589.520	591.827	594.143	596.466	598.796
86	601.135	603.480	605.834	608.195	610.564	612.941	615.325	617.717	620.117	622.524
87	624.940	627.363	629.794	632.233	634.680	637.134	639.597	642.068	644.546	647.033
88	649.527	652.030	654.541	657.059	659.586	662.121	664.664	667.215	669.775	672.342
89	674.918	677.502	680.094	682.695	685.303	687.920	690.546	693.180	695.822	698.472
90	701.131	703.799	706.475	709.159	711.852	714.553	717.263	719.981	722.708	725.444
91	728.188	730.941	733.703	736.473	739.252	742.040	744.836	747.642	750.456	753.278
92	756.110	758.951	761.800	764.658	767.526	770.402	773.287	776.181	779.084	781.997
93	784.918	787.848	790.788	793.736	796.694	799.661	802.637	805.622	808.616	811.620
94	814.633	817.656	820.687	823.728	826.778	829.838	832.907	835.986	839.074	842.171
95	845.278	848.395	851.521	854.657	857.802	860.957	864.121	867.295	870.479	873.672
96	876.876	880.089	883.311	886.544	889.786	893.038	896.300	899.572	902.854	906.146
97	909.448	912.759	916.081	919.413	922.755	926.107	929.469	932.841	936.223	939.615
98	943.018	946.431	949.854	953.287	956.731	960.184	963.649	967.123	970.608	974.104
99	977.609	981.126	984.652	988.189	991.737	995.296	998.864	1002.44	1006.03	1009.64
100	1013.25	1016.87	1020.50	1024.14	1027.80	1031.46	1035.14	1038.83	1042.52	1046.23

20 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.

20.1 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.

20.2 Instructions

• To obtain guarantee service, please follow the instructions below:

• The Walz Service Information Form available at http://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.

20.3 Applicable law

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

Alphabetical Index