# Portable Gas Exchange Fluorescence System GFS-3000

Handbook of Operation

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# 1 Safety Instructions

## 1.1 General Safety Instructions

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

## 1.2 Special Safety Instructions

- 1. The 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 16.4
- 4. Before shipment of the instrument release the pressure of the CO<sub>2</sub>-unit (See chapter 13.5) and follow battery transport instructions (see chapter 16.4).

## 2 Introduction

Gas exchange is defined as the interchange of gases between plants and their environment. A number of 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 since forty 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<sub>2</sub>- and H<sub>2</sub>O 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.

## 2.1 Combination of Gas Exchange and 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 parameters and PAR absorptivity (PAR: photosynthetic 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 3100-C
- 2. Standard Measuring Head 3010-S
- 3. LED Light Source 3040-L (if GFS-3000) LED-Array/PAM-fluorometer 3055-FL (if GFS-3000FL)
- 4. AC Power Supply 3020-N
- 5. Li-ion Battery 3025-A
- 6. Li-ion Battery Charger LC-03
- 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 3100-C,

containing CO<sub>2</sub>/H<sub>2</sub>O infrared gas analyzer (IRGA), pump, flow meter, solenoid valves, CO<sub>2</sub> control, H<sub>2</sub>O 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. The components and connectors are described in chapter 3.3.





## GFS3000: LED Light Source 3040-L,

consisting of 24 red and 2 blue LEDs, fan for cooling the LEDs, electronics for managing the portion of the blue light, 2 pins for attachment to the upper or lower part of the cuvette, cable for connection to the electronic box of the

Standard Measuring Head.

# $\label{eq:GFS-3000FL} \textbf{GFS-3000FL} \ \textbf{or optional in addition:}$

## LED-Array/PAM-Fluorometer 3055-FL,

consisting of 24 red and 2 blue LEDs, fan for cooling the LEDs, fluorescence module, 2 pins for attachment to the upper or lower part of the cuvette, two cables for connection to the electronic box of the measuring head.



### AC Power Supply 3020-N,

with cable for connecting the AC Power Supply 3020-N to the mains voltage (100-240 V AC, 50/60 Hz), cable for connecting the AC Power Supply 3020-N to the connector "Battery/DC IN" (1 or 2) of the Control Unit, On/Off switch





## Li-Ion Battery 3025-A,

with cable for connecting the Li-ion battery to the connector Battery/DC IN (1 or 2) of Control Unit 3000-C or 3100-C or to the Li-Ion Battery Charger LC-03. (Battery Charger LC-03, see chapter 18.7)

## LiFePO4 Battery 3035-A

with cable for connecting the LiFePO4 battery to the connector Battery/DC IN (1 or 2) of Control Unit 3100-C or to the Battery Charger LC-03.





## Battery Charger LC-03,

with cable for connecting the indicated battery-type (Li-Ion or LiFePO4) to the Charger LC-03 to the mains voltage (100-240 V AC, 50/60 Hz), two displays, two charging outputs On/Off switch at the rear side

## 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 in order to carry the system in the field.





## Protection cover,

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

## Entrance Filter 5 ml 3000-C/EF,

which is 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 Volume 40 ml 3000-C/MV

which replaces 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.

## Connecting tubes,

tubes replacing drier, humidifier or measuring head connected to the front side of the control unit.



Air-Cycling tube,

for calibration of absolute  $CO_2$ -zero or absolute  $H_2O$  zero.





# USB Communication Cable (only for Control Unit Model 3000-C),

which is used to connect the Control Unit 3000-C with a PC in order to transfer programs or data, to update the software of the Panel PC inside the GFS-3000 or to control the GFS-3000 using an external PC via GFS-Win.

# USB adapter (only for Control Unit Model 3100-C),

connects to the splash proof USB-port at the front side of the Control Unit 3100-C. The



USB-A female connector of the adapter is not splash proof.



# USB Null Modem Cable (only for Control Unit Model 3100-C),

which is used to connect the Control Unit 3100-C with a PC in order to control the GFS-3000 using an external PC. Requires USB adapter (previous item)

## 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 (see chapter 16.3).



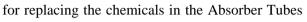


## CO<sub>2</sub> cartridge 000160103430,

fills for CO<sub>2</sub>-supply with pure CO<sub>2</sub>. Not generally included, only deliverable with over-land transport. See chapter 13.5.

### Chemicals

Soda lime ( $CO_2$  absorber) 000160103401, Silica Gel ( $H_2O$  absorber) 000160103402, and Humidifying Granules 000160103403



CO2 ABSORBER, DRIER and HUMIDIFIER. See chapter 16.4 for safety instructions.



## Dust Cap for CO<sub>2</sub> Absorber Tube,

serves to close CO<sub>2</sub> absorber tube, when not in use, part#: 000140701732

## Spare Kit 3000-C/SK,

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





## 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.

## Darkening Plate 3010-DP,

which can be clicked onto the upper or lower part of the cuvette via the 2 pins. If the LED Light Source 3040-L or LED-Array/PAM Fluorometer 3055-FL is attached, the Darkening Plate can be used to exclude ambient light from the other side.

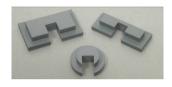


## Optional: Leaf Area Adapter,

for adjusting the leaf area of the Standard Measuring Head 3010-S. Following leaf area adapters are available (some sizes are optional):



Leaf Area Adapter 3010-2x4: 2 by 4 cm, for larger leaves, Leaf Area Adapter 3010-1x4: 1 by 4 cm, for narrow leaves, Leaf Area Adapter 3010-2.19x1.14, area: 2.5 cm<sup>2</sup> (2.19 cm x 1.14 cm) Leaf Area Adapter 3010-2.19x1.14c, area: 2.5 cm<sup>2</sup> excentric Leaf Area Adapter 3010-R3. round, 3 cm<sup>2</sup>, for smaller leaves or for leaves with high transpiration rates.



### Mounting Plates,

for mounting the foam gaskets onto the leaf area adapters. Included with leaf area adapters.

### Foam gaskets,

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



Foam Gaskets 8 cm<sup>2</sup> (2 cm x 4 cm, 10 pcs, part #: 000244009314) Silicone Gaskets 8 cm<sup>2</sup> (2 cm x 4 cm, 10 pcs, part # 000244009324) Foam Gaskets 4 cm<sup>2</sup> (1 cm x 4 cm, 10 pcs, part #: 000244013214) Foam Gaskets 3 cm<sup>2</sup> (round, 10 pcs, part #: 000244012914) Foam Gaskets 2.5 cm<sup>2</sup> (2.19 cm x 1.14 cm, 10 pcs, part #000236406014)



## 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. (Foam Gaskets for 3010-V80, 10 pcs, part#:000244014714)

## **Optional:**

### Cuvette for Lichens/Mosses 3010-V32,

consisting of two adapter plates, two distance holders, two hose clamps, angle for thermocouple, an extension rod and a cylindrical cuvette



(polycarbonate) with a perforated plate made out of steel 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, Ø:3.5 cm, 10 pcs, part#: 000244015514).



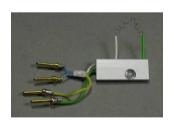
## Optional: Cuvette for Arabidopsis 3010-A,

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 ( $\varnothing$ :8.2 cm, 10 pcs, part#: 000244020214)

# Optional: Spare Thermocouple 3010-CA/TCL,

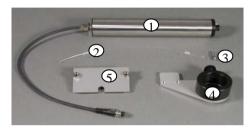
consisting of a thermocouple, cuvette temperature sensor, mounting plate and 4 pins; the unit is usually built into the lower part of the cuvette.



## Optional: Spare Absorber Tube 3000-C/ABS,

for Transpiration rate (labeled "CO2 ABSORBER"), Silica Gel (labeled "DRIER") or Humidifying Granules (labeled "HUMIDIFIER"). A filter pad should be used at he 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: Imaging-PAM**

The Imaging-PAM (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. (See chapter 4.6)





## Optional: Darkening Leaf-Clamp 3010-DLC

Darkening Leaf-Clamp 3010-DLC (weight:11 g, water-proof, with stainless steel-clips) for dark adaptation of leaf samples in the field before fluorescence measurements.

## Optional: Tripod ST-1010,

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





# Optional: Outdoor-Set 3000-C/OS,

consisting of 101 mixing vessel, 2.5 m fiber-glass antenna, tubing with entrance filter, clips, elastic net and antenna holder for running the GFS-3000 in the field, using ambient air

# Optional: DC-DC Converter 3020-DC (Control Unit 3000-C only)

For supplying power to the GFS-3000 via an external 12 or 24 V battery (e.g. car battery). Input voltage is 9 - 32 V, output current is max. 6 A. An under-voltage and over-temperature protection is comprised. See technical data fopr Central Unit 3000-C chapter 18.2.





# Optional: Battery Cable 3100-C/BC (Control Unit 3100-C only)

For supplying power to the GFS-3000 with Central Unit Model 3100-C from an external 12 or 24 V battery (e.g. car battery), which have no special connectors. Input voltage for the Control Unit 3100-C is 12 - 24 V. See technical data for Central

Unit 3100- C chapter 18.1.



### Optional: Oxygen sensor 3080-O2,

which can be attached to the front side of the control unit at the position filter PUMP (see special manual).

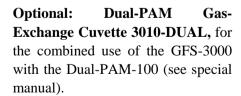
# Optional: Gas Exchange Chamber 3010-GWK,

for large sample sizes or the combined use of the GFS-3000 with or without the Imaging PAM *M-Series* MAXI-Version (see special manual).



## **Optional: LED-Panel RGBW**

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 3100-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<sub>2</sub> control, this absorber tube must be replaced by the Mixing Volume 40 ml "3000-C/MV".
- 4. Outlet "ANALYZER"/"REF", which is the outlet of the reference cells of the analyzer.
- 5. Outlet "ANALYZER"/"SAMPLE", which is the outlet of the sample cells of the analyzer.

- 6. Outlet "SAMPLE", which is the outlet, if the valves 3 or 5 are partly opened.
- Outlet "VENT", which is the outlet of the measuring gas after passing the cuvette in valve position ZP. Also any over-pressure in the CO<sub>2</sub> container is vented here
- 8. Two pneumatic connectors labeled "FILTERS"/"PUMP" for a Filter 000140301225 mounted via two pneumatic adapters; the filter is integrated in the pneumatic pathway behind the gas supply consisting of pump, absorbers and CO<sub>2</sub> injection.
- 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 directly in front of the reference cells of the analyzer.
- 12. Flow indicator labeled "ANALYZER"/"SAMPLE" measuring the flow directly in front of the sample cells of the analyzer.
- 13.  $CO_2$  cartridge holder, which contains a small  $CO_2$  cartridge supplying the  $CO_2$  control with pure  $CO_2$ .
- 14. Inlet, which must be closed by a screw; if CO<sub>2</sub> cartridges are used. If a CO<sub>2</sub> cylinder should be connected, the screw must be replaced by the hose adapter contained in the Spare Kit 3000-C/SK (see chapter 3.4)
- 15. Slot for one Li-Ion Battery 3025-A, which can be connected to one of the two connectors labeled "BATTERY/DC IN"/"1" or "BATTERY/DC IN"/"2". A second battery fits underneath this slot.

16. Short feet, which are suitable, if the control unit 3000-C 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.

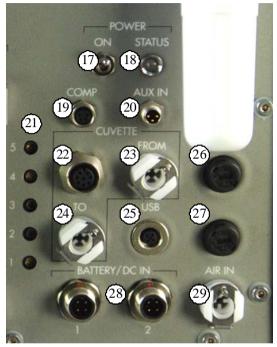


Fig. 3: Components and connectors at the front side (continued)

- 17. Switch "POWER"/"ON": the control unit is switched on, if the STATUS LED is lit up or blinking.
- 18. LED "POWER"/"STATUS" indicates 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 it is working well (for LED code see chapter 15.4)
- 19. Connector "COMP", to which additional components developed by Walz can be connected. The connected component must be implemented in the software; otherwise it cannot be recognized and controlled by the software (for pin assignment see chapter 16.1).

- 20. Connector "AUX IN" (for pin assignment see chapter 16.1), to which two sensors with a voltage output between 0 ... 4095 mV can be connected using the Cable for 2x Auxiliaries 000130606205 (see chapter 3.1 and 16.3).
- 21. Valves labeled from "1" to "5" for adjustment of through the different air pathways (see chapter 13.10).
- 22. 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.
- 23. Pneumatic connector "CUVETTE"/"FROM", to which the tube "FROM" of the measuring head is connected.
- 24. Pneumatic connector "CUVETTE"/"TO", to which the tube "TO" of the Measuring Head is connected.
- 25. Electronic connector "USB" is the USB-port of the internal panel PC. Note the GFS-3000 with Control Unit 3100-C is not a USB-device, but can operate USB-devices like a mouse or memory stick. To connect the Control Unit 3100-C with an external PC use the provided USB adapter and the provided "USB Null-Modem Cable" marked with NMC at its connectors (see chapter 3.1). Do not use a standard USB-cable to make a connection between the GFS-3000 and an external PC. It would create an electrical shortage between the USB ports causing damage. For the Control Unit 3000-C, the USB-port is an interface for the connection of an external PC directly with the provided USB cable.
- 26. Fuse holder "F1: 10.0 AT" (for Control Unit 3100-C, do not use another type of fuses!); (8.0 AT for Control Unit 3000-C).
- 27. Fuse holder "F2: 10.0 AT" (for Control Unit 3100-C, do not use another type of fuses!); (8.0 AT for Control Unit 3000-C).
- 28. Connectors "BATTERY/DC IN"/"1" and "BATTERY/DC IN"/"2", to which batteries of the type Li-ion Battery 3025-A or the AC Power Supply 3020-N can be connected. At the same time two batteries or one

battery and the AC POWER Supply can be connected. Note that the range of input voltage is different between Central Unit Model 3100-C and Central Unit Model 3000-C.

29. 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.

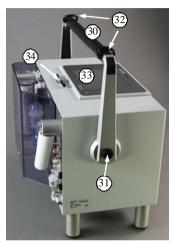


Fig. 4: Components attached to the top and to the sides

- 30. Carrying handle, which can be locked in several positions. Lock horizontal in transport box for transport.
- 31. Push buttons on each side, which must be pushed to disable the lock mechanism and move the handle
- 32 Two rings for mounting the carrying belt (see chapter 3.1)
- 33. Integrated panel PC with touch panel
- 34. Clip holding the PDA pen

# 3.3 Components and Connectors of the Standard Measuring Head 3010-S

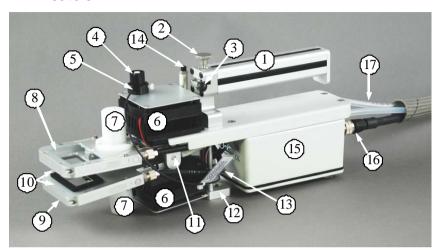


Fig. 5: Components of the Standard Measuring Head 3010-S

- 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 (see chapter 4.4.1.)
- 3. Opening mechanisms which must be pushed forward to open the cuvette (see chapter 4.4.1.)
- 4. Miniature Quantum Sensor MQS-B/GFS for measuring ambient photosynthetic active radiation (PARamb)
- 5. Holder for the external light sensor
- 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. Distance Holder at each side fixing the lower cuvette half
- 12. Tripod adapter
- 13. Connector for the Miniature Quantum Sensor MQS-B/GFS
- 14. Remote push button to initialize the storage of a data record
- 15. Electronic box controlling the Standard Measuring Head 3010-S
- 16. Connection cable between Standard Measuring Head 3010-S and Control Unit 3100-C or 3000-C (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.

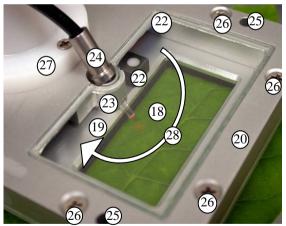


Fig. 6: View from the top onto the upper half of the cuvette

- 18. Enclosed leaf area
- 19. Leaf Area Adapter 8 cm2 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 3040-L or the LED-Array/PAM-Fluorometer 3055-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

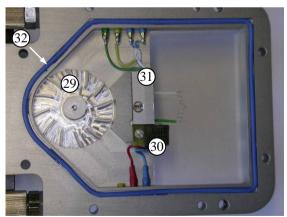


Fig. 7: View into the lower half of the cuvette (leaf area adapter is removed)

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 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. 8: Connectors at the rear side of the electronic box

- 33. Connector for the connecting cable between Standard Measuring Head 3010-S and Control Unit 3100-C or 3000-C. The cable usually remains connected to the measuring head.
- 34. Connector for an additional component; which has the same pin assignment as the connector "COMP" at the front side of the Control Unit 3100-C or 3000-C. If e.g. the LED-Array/PAM-Fluorometer 3055-FL is used, one of the two cables must be connected here.
- 35. Connector for cable of the LED Light Source 3040-L or for one of the two cables of the LED-Array/PAM-Fluorometer



Fig. 9: Connectors at the front side of the electronic box

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

## 3.4 Content of the Spare Kit 3000-C/SK



Fig. 10: Content of the box Spare Kit 3000-C/SK

- 1. Injection 5 ml for humidifier, for adding water to the humidifying granules inside the absorber tube HUMIDIFIER
- 2. 8 spare screws for mounting the leaf area adapters
- 3. 2 spare fuses 2AT (part #: 000130401268) for Li-ion Battery Charger LC-03 (see chapter 18.7)
- 4. 4 spare fuses 8AT (part #: 000130401274) for Control Unit 3000-C spare fuses 10AT (part #: 000130401275) for Control Unit 3100-C
- 5. Hose Connector Straight 6.4 mm (Part #: 000140701956) for connecting a tube with an inner diameter of 6 mm to the pneumatic connector "AIR IN"; if a long tube is used to draw in air.
- 6. Adapter for CO<sub>2</sub> cylinder including sealing, which replaces the screw at the CO<sub>2</sub> unit, if CO<sub>2</sub> should be supplied via a cylinder instead of using little cartridges.
- 7. 4 tube fitting nuts belonging to the pneumatic connectors 4, 5, 6, 7 in Fig. 2.
- 8. Spare PDA pen (part #: 000160201311) for operating the panel PC software via the touch panel.

- 9. 2 Hose Connectors Straight 4 mm (part #: 000140701955) for tubes with an inner diameter of 4 mm.
- 10. Spare filter (part #: 000140301225) for PUMP, CUVETTE and AIR IN (see 8, 9, 10 in)

## 3.5 Battery Charger LC-03

The provided Battery Charger LC-03 is a mains operated, fully automated battery charger with two independent charging connectors and illuminated LCD-displays. The LC-03 is specially adjusted for the Li-ion Battery 3025-A or LiFePO4 Battery 3035-A as indicated on the Battery Charger housing and will **not** work with other types of batteries.

## 3.5.1 Battery Charger LC-03 adjusted to Li-ion Battery 3025-A

After activating the main control switch on the rear panel of the device, the following indication will appear on the display.

Lilon ready for charge 16.4V 3.0A 31.0Ah

The instrument is now ready for operation.

One or two Batteries 3025-A can be connected for charging. After the connection, the battery will be tested by the charger. After 5 s the charging starts automatically and the display shows the following message:

Lilon charge xx.xV x.xA xx.xAh

The charging voltage (xx.xV), the charging current (xx.xA) and the capacity (xx.xAh) are displayed. The charging current is automatically controlled in dependence of battery voltage.

After successful loading the display shows:

Lilon full xx.xV x.xA xx.xAh

After completion of the charge the battery can remain connected to the battery charger LC-03 and will be trickle charged. The length of the

charging process is about 6 h. If necessary the battery can be disconnected before it is completely loaded

In case of instrument errors or electrical power outage the display shows the message:

Lilon error xx.xV x.xA xx.xAh

The accumulators have to be disconnected and the Charging Unit LC-03 has to be switched off and on again. If this error persists please contact the Heinz Walz GmbH for support. Also read the next chapter on the protection circuit of the batteries.

For technical data proceed to chapter 18

#### **Important Note:**

Please take care that the <u>ventilation slots</u> in the housing in particular the fan are not barred.

## 3.5.2 Battery Charger LC-03 adjusted to LiFePO4 Battery 3035-A

After activating the main control switch on the rear panel of the device, the following indication will appear on the display.

LiFe ready for charge 14.8V 3.0A 31.0Ah

The instrument is now ready for operation.

One or two LiFePO4 Batteries 3035-A can be connected for charging. After the connection, the battery will be tested by the charger. After 5 s the charging starts automatically and the display shows the following message:

LiFe charge xx.xV x.xA xx.xAh

The charging voltage (xx.xV), the charging current (xx.xA) and the capacity (xx.xAh) are displayed. The charging current is automatically controlled in dependence of battery voltage.

After successful loading the display shows:

LiFe full xx.xV x.xA xx.xAh

After completion of the charge the battery can remain connected to the battery charger LC-03 and will be trickle charged. The length of the charging process is about 6 h. If necessary the battery can be disconnected before it is completely loaded

In case of instrument errors or electrical power outage the display shows the message:

LiFe error xx.xV x.xA xx.xAh

The accumulators have to be disconnected and the Charging Unit LC-03 has to be switched off and on again. If this error persists please contact the Heinz Walz GmbH for support.

For technical data proceed to chapter 18

# **Important Note:**

Please take care that the <u>ventilation slots</u> in the housing in particular the fan are not barred.

#### 3.6 Li-ion Battery 3025-A

The Li-ion Battery 3025-A is a modern maintenance-free battery with a nominal voltage of 14.4 V and a nominal capacity of 15 Ah. This kind of Battery comprises a very high energy density, only a small amount of self discharge and in contrast to other types of accumulators no noteworthy memory effect.

The Li-ion batteries are packed in a watertight housing with also watertight connector plugs.

The batteries have a protection circuit to prevent extremely low discharging. If this protection circuit is activated, the batteries need a voltage of 16 V to become conductive again. The charger will give this voltage directly after connection of the battery for a short moment. If the charger will not start charging, but continues to displays the message *LiIon ready for charge*, disconnect the battery and reconnect it again, so that the battery will receive 16 V again to become conductive. It may be necessary to repeat this process three to five times until the protective block is released.

#### **Important Note:**

Never store the batteries in their discharged state. For longer storage recharge every 6 month – they keep best in halfway charged condition.

Please read the safety and transport information chapter 16.4.

An upgrade for GFS-3000 using LiFePO4 Battery 3035-A is available

For technical data proceed to chapter 18.

#### 3.7 LiFePO4 Battery 3035-A

The LiFePO4 Battery 3035-A exhibit plenty advantages in comparison to other battery types running GFS-3000. LiFePO4 are commercially available, lightweight and environmental-friendly as they disclaim cobalt and lead. In standard configuration LiFePO4 Batteries 3035-A are easy to transport, they do not require a shippers declaration for dangerous goods (see chapter 16.4). The standard configuration features a battery set containing two parallelly connected, but electronically separated LiFePO4 batteries on a mounting plate with a plug connector for the GFS-3000 or the Battery charger LC-03. The LiFePO4 Battery 3035-A exhibits a nominal voltage of 12.8 V and a nominal capacity of 15 Ah (2 x 7.5 Ah). The operating duration of the GFS-3000 can be raised easily by exchanging the standard LiFePO4 Batteries of 7.5 Ah with commercial available LiFePO4 Batteries with 10 Ah (standard charger can still be used). LiFePO4 Batteries mounted in LiFePO4 Battery 3035-A are of cause maintenance free with a built-in automatic protection for over-charge. Furthermore they are also protected against over-discharge and over-temperature.

# 4 Principle of Operation

## 4.1 Pneumatic Diagram of the GFS-3000

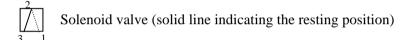
The pneumatic diagram of the GFS-3000 is shown in Fig. 11 and described in chapter 4.2. The pneumatic pathway is the same in Control Unit Model 3000-C and Model 3100-C.

Legend for the pneumatic diagram shown in Fig. 11:

Grey or with pattern: components attached to the front side of the control unit, component is specified in the box

Component

White background: components inside the control unit



- Pneumatic connectors at the front side of the Control Unit 3000-C or 3100-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"
- Pneumatic connector labeled "CUVETTE" / "FROM"
- ② 2 pneumatic connectors labeled "FILTERS" / "CUVETTE"
- (i) Pneumatic connector labeled "ANALYZER" / "REF"
- ① Pneumatic connector labeled "ANALYZER" / "SAMPLE"
- Pneumatic connector labeled "SAMPLE"
- 13 Pneumatic connector labeled "VENT"

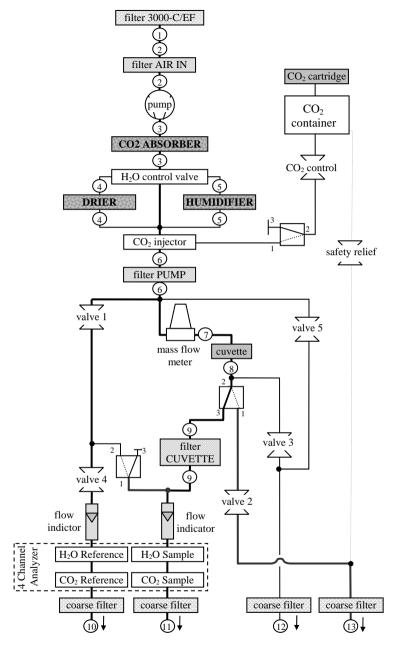


Fig. 11: Pneumatic diagram of GFS-3000

#### 4.2 Description of Pneumatic Pathway and Its Components

The pneumatic pathway of the GFS-3000 shown in Fig. 11 works in the following way:

- 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, which 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 the flow set value.
- The pump is followed by the CO<sub>2</sub> absorber, which should remove all CO<sub>2</sub> of the incoming air. If experiments should be made using ambient air, the CO<sub>2</sub> control must be switched off and the absorber tube "CO<sub>2</sub> ABSORBER" must be replaced by the MIXING Volume 40 ml "3000-C/MV" for ambient air also a 10 l volume needs to be connected (see chapter 13.7).
- The CO<sub>2</sub> free or ambient air is entering the H<sub>2</sub>O control valve. If experiments should be made using ambient air, the H<sub>2</sub>O control must be switched off. In this case the air is going straight through the H<sub>2</sub>O control valve without passing the drier or humidifier. Note that the air has only ambient humidity, if there is no CO<sub>2</sub> absorber connected. If the H<sub>2</sub>O control is switched on, the air will partly be forced through the drier or humidifier, so that the H<sub>2</sub>O value measured by the H<sub>2</sub>O reference cell of the analyzer (H2Oabs) matches the H<sub>2</sub>O set value.
- The CO<sub>2</sub> injector follows. If the CO<sub>2</sub> control is switched off, the CO<sub>2</sub> supply will be shut by a solenoid valve. If the CO<sub>2</sub> control is switched on, pure CO<sub>2</sub> will be added via a thermal CO<sub>2</sub> control valve, so that the CO<sub>2</sub> value measured by the CO<sub>2</sub> reference cell of the analyzer

(CO2abs) matches the  $CO_2$  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 of the pneumatic pathway.
   The following gas pathways belong to the analyzing part.
- After the filter the flow is divided into reference gas and the measuring gas.
- Two solenoid valves are integrated in the following pneumatic pathway. The pneumatic diagram shows the resting position of these two solenoid valves. The pneumatic pathway is now described for this case.
- The analyzer has four cells and can measure 2x CO<sub>2</sub> absolute mole fraction and 2x H<sub>2</sub>O absolute mole fraction. The cells are called "CO<sub>2</sub> Reference", "CO<sub>2</sub> Sample", "H<sub>2</sub>O Reference" and "H<sub>2</sub>O 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<sub>2</sub>O reference" and "CO<sub>2</sub> reference". The measured values (CO2abs and H2Oabs) are used for the H<sub>2</sub>O and CO<sub>2</sub> control. A coarse filter follows before the reference gas is leaving the system through the outlet labeled "ANALYZER" / "REF".
- The measuring gas passes the electronic mass flow meter. The measured value is used for the flow control loop. Then the measuring gas is going through the cuvette connected to the pneumatic connectors labeled "CUVETTE" / "TO" and "CUVETTE" / "FROM". A solenoid valve follows, which in the resting position forces the measuring gas through the filter labeled "FILTERS" / "CUVETTE". The filter removes e.g. spores released by the sample or dust, which might have entered the cuvette. A mechanical flow indicator follows. If the cuvette is not air tight, the flow in this flow indicator will be reduced. The measuring gas flows through the analyzer cells "H<sub>2</sub>O sample" and "CO<sub>2</sub> sample". The absolute values measured (H2Osam and CO2sam) are not displayed in the software except during calibration. Instead the differ-

- ence between sample cell and reference cell is calculated (dH2O, dCO2) and stored, see chapter 4.2.1 and chapter 10.1 to 10.5.
- Valve 5 should be opened with low flow rates to increase the speed in the mixing part. Valve 3 determines the flow rate from the cuvette through the analyzer. It is open with high flow rates. The flow leaves at exit "SAMPLE" (see chapter 13.10).

#### 4.2.1 Definition of CO2abs, H2Oabs, dCO2, dH2O

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". In the software the value measured by the cell " $CO_2$  reference" is displayed and stored under the name  $CO_2$  sample (see chapter 11 and 12). The value measured by the cell " $H_2O$  reference" is displayed and stored under the name  $H_2O_2$  absolute measured by the cells " $H_2O_2$  sample" and " $H_2O_2$  sample" are neither displayed and 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 exact equations see chapter 10.3 and 10.5. In the software the values dH2O and dCO2 are displayed only in the *chart* (*see* chapter 8.5.2). In the *values table* (*see* chapter 8.5.3) and in the *report* (*see* chapter 8.5.4) the values dH2O and dCO2 are displayed and stored under different names (*see* chapters 4.2.3 and 4.2.4) depending on the solenoid valve position.

#### 4.2.2 Mode MP and Mode ZP

The system has two solenoid valves which can switch between the modes MP (Measuring Point) and ZP (Zero Point).

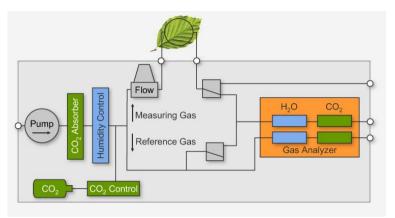


Fig. 12: Simplified pneumatic diagram with solenoid valves being switched to mode MP

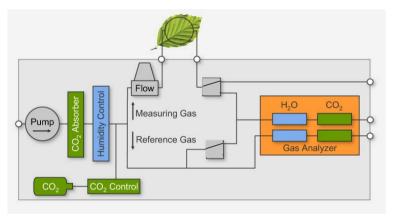


Fig. 13: Simplified pneumatic diagram with Solenoid valves being switched to mode ZP

Fig. 12 shows the pneumatic diagram with the solenoid valves being switched to Mode MP. In Mode MP the reference gas is flowing through the reference cells and the measuring gas is flowing through the sample cells of the absolute analyzer.

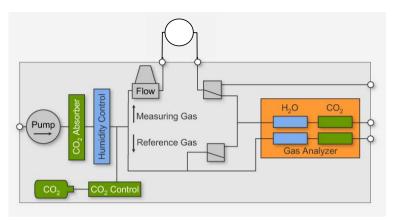


Fig. 14: Simplified pneumatic diagram illustrating ZPcuv. Solenoids are in mode MP, but cuvette is empty.

Fig. 13 shows the pneumatic diagram with the solenoid valves being switched to mode ZP. In mode ZP the measuring gas is vented after passing the cuvette of the measuring head (an enclosed leaf will be exposed to the same conditions as in mode MP). The reference gas is divided so that the same gas is in both sides of the analyzer with one half flowing through the reference cells and the other half the sample cells. The flow indicators will show a lower flow than in mode MP.

Fig. 15 illustrates ZPcuv. For ZPcuv the system is in mode MP, but the cuvette is empty. Again the same gas is in both sides of the analyzer. ZPcuv is a Zero Point measured in mode MP, but with an empty cuvette.

ZP-values are differential gas concentrations indicated inherently by the analyzer. A ZP-value needs to be measured and stored before measuring MP-values, so that they can be taken into account for the calculation of plant action (see chapter 10.7. and 10.10).

# 4.2.3 Values dCO2ZP, dH2OZP, dCO2MP and dH2OMP displayed in the Values Window

In the *values window* (*see* chapter 8.5.3) the actual values are shown. If the system is switched to the mode MP, the measured values dCO2 and dH2O will be displayed in the *values window* as dCO2MP and dH2OMP. The field dCO2ZP and dH2OZP show the values dCO2ZP and dH2OZP of the data record ZP, which was stored last (*see* chapter 4.2.4).

If the system is switched to the mode ZP, the measured values dCO2 and dH2O will be displayed in the *values window* as dCO2ZP and dH2OZP. The values in the field dCO2MP and dH2OMP show "----".

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

Each stored data record includes the values dCO2ZP, dCO2MP, dH2OZP and dH2OMP.

#### 4.2.4.1 Storing a Zero Point "ZPi" or "ZPirga"

If the system is switched to mode ZP, a data record, which is called zero point, can be stored. The values dCO2 and dH2O will be stored in the data record as dCO2ZP and dH2OZP (*see report* chapter 8.5.4). The stored values CO2abs and H2Oabs give absolute CO<sub>2</sub> and H<sub>2</sub>O mole fraction of the reference gas, which is equal to the gas at the entrance of the cuvette. The reference and sample cells can have a small offset between each other. Since the reference gas is split in mode ZP, the stored values dCO2ZP and dH2OZP correspond to this offset. The stored data record is labeled as ZPi, where it stands for infra red gas analyzer. The values dCO2MP and dH2OMP of the stored data record ZPi are filled with "----".

## 4.2.4.2 Storing a Measuring Point "MP"

If the system is switched to Mode MP, a data record, which is called measuring point, can be stored. The values dCO2 and dH2O will be stored in the data record as dCO2MP and dH2OMP (see report chapter 8.5.4). The

stored values CO2abs and H2Oabs give absolute CO<sub>2</sub> and H<sub>2</sub>O mole fraction of the reference gas, which it is equal to the gas at the entrance of the cuvette. Since in mode MP the reference gas is going through the reference cells and the measuring gas is going through the sample cells (after passing the cuvette with an enclosed leaf), the stored values dCO2MP and dH2OMP indicate the CO<sub>2</sub> uptake and H<sub>2</sub>O release by the enclosed leaf area plus any offset inherent to the analyzer. The stored data record is labeled as MP. The values for dCO2ZP and dH2OZP will be carried forward from the last ZP measurement and indicated in the MP data records.

Beside the  $CO_2$  uptake and  $H_2O$  release by the enclosed sample, the values dCO2MP and dH2OMP also include the offset between the two cells of the analyzer. From an MP data record the true dCO2 and true dH2O can be calculated as follows:

true dCO2 = dCO2MP - dCO2ZPtrue dH2O = dH2OMP - dH2OZP

The calculation of gas exchange parameters is done automatically (see chapter 10).

#### 4.2.4.3 Storing a Zero Point "ZPc"or"ZPcuv"

If the system is switched to mode MP and if no leaf is enclosed in the cuvette, the user can store a ZP by clicking on a special button. In this case the values dCO<sub>2</sub> and dH<sub>2</sub>O (displayed in the *values window* as dCO2MP and dH2OMP) will be stored in the data record as dCO2ZP and dH2OZP, the values dCO2MP and dH2OMP of the stored data record are filled with "----". The data record is labeled as ZPc, where c stands for cuvette.

If a measuring point is stored, the values dCO2ZP and dH2OZP of the MP data record will be filled with the values dCO2ZP and dH2OZP of the last stored data record ZP, no matter whether it was a ZPi or a ZPc.

The data record ZPc includes the offset between the two cells 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.

#### 4.2.5 Recommendation for Measuring Zero Points

In the beginning always a zero point should be measured, because the calculations of the gas exchange parameters for a measuring point MP depends on a zero point ZP. If a zero point was measured, a lot of measuring points can follow. It is recommended to measure a zero point at least every hour or whenever the CO<sub>2</sub> or H<sub>2</sub>O concentration has been changed. If leaves are enclosed only for a short time, the cuvette will be empty inbetween and it is recommended to measure a ZPc instead of a ZPi in order to increase accuracy (see chapter 4.2.4: remark on Storing a Zero Point "ZPc"). If a leaf is enclosed for long-term experiments, or if the CO<sub>2</sub> or H<sub>2</sub>O concentration is changed during the experiment, it is recommended to measure a zero point ZPi. A zero point ZPc can only be measured manually by clicking on a button since the cuvette can not be empty automatically. The zero point ZPi can be measured manually by clicking on a button. Zero points ZPi can also be integrated in automatic programs or in automatic measuring sequences which measure a zero point ZPi followed by a defined number of measuring points (this cycle will be repeated). The switching between MP and ZP may disturb the CO<sub>2</sub> regulation. This disturbance can be minimized by good adjustment of valve 2 (see chapter 13.10). MPs should only be measured with stable CO<sub>2</sub> concentration.

# 4.3 Recommendations for Humidity Control

When setting up the system avoid condensation in the cuvette or tubes. Fig. 15 shows the relationship between temperature and humidity.

The cuvette temperature needs to be chosen so that the 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 system is set-up in a cool environment.

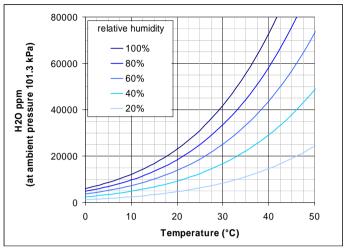


Fig. 15: Relationship between mole fraction H2O (ppm), cuvette-temperature and relative humidity. See chapter 19 for precise values.

For controlling humidity, consider that the humidity entering the chamber is regulated; please take the transpiration of the sample into account, when choosing the value. Also take into consideration, that transpiration is very low, when the external humidity is high. The calculation of some photosynthetic parameters is only possible, if there is some transpiration. Without any transpiration the stomatal conductance and the internal  $CO_2$  mole fraction can not be calculated (division by zero or a value close to zero), see chapter 10.11 for equations.

# 4.4 Measuring Head

#### 4.4.1 Closing Mechanism

Fig. 16 demonstrates how the Standard Measuring Head 3010-S is opened or closed. 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, so that the foam gaskets can expand.



Fig. 16: Closing mechanism of Standard Measuring Head 3010-S.

When closing the measuring head, watch the flow indicators of ANLAYZER SAMPLE in mode MP. It should come up, so that both flow indicators have equal height (also see calve adjustment chapter 13.10). Do not apply to much pressure on the gaskets, rather use Terostat-IX® (Teroson, Henkel) near a protruding vein of the leaf to obtain a good sealing.

#### 4.4.2 Ventilation

The Standard Measuring Head 3010-S has an impeller in each cuvette half. The motors of the impellers are located under the white hoods. The

impellers always need to be switched on during gas exchange measurements or during temperature regulation. The speed can be adjusted in 9 steps. Standard speed is 7.

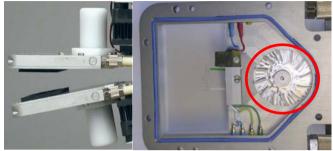


Fig. 17: Impeller of Standard Measuring Head 3010-S, left: white hoods; right: view from inside, impeller marked with a red circle.

## 4.4.3 Temperature Regulation

The Standard Measuring Head 3010-S has four temperature sensors (see Fig. 18). Three Pt100-sensors, *Tcuv*, *Ttop* and *Tamb*. and one thermocouple for *Tleaf*. Pt100-sensors measure absolute temperature, thermocouples measure a differential temperature signal.

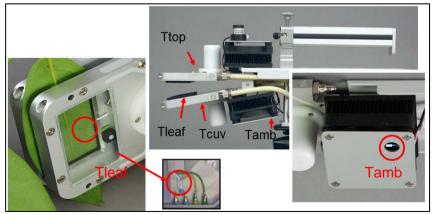


Fig. 18: Standard Measuring Head 3010-S, location of temperature sensors.

Tcuv and Ttop are located at the exits of the cuvette halves. Tamb is located outside to measure ambient temperature. The thermocouple for the leaf temperature measures the difference between the temperature at its tip positioned at the leaf and the reference positioned at Tcuv (see red circles left side in Fig. 18) the leaf temperature is the sum of these two signals and is indicated as Tleaf. The temperature regulation of the Standard Measuring Head 3010-S operates with Peltier elements. They are located under the black heat exchangers outside the measuring head. Ovoid any force or mechanical stress on these heat exchangers. There are three regulation modes available, Tcuv constant, Tleaf constant or Follow Tamb with a constant temperature offset (see software description, chapter 8.5.1.4).

#### 4.4.4 Light

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

The light intensity of the LED Light Source 3040-L or the LED-Array/PAM-Fluorometer 3055-FL is regulated with these light sensors. There are several modes available. The most commonly used mode is *PAR-top*, where the power for the light source is adjusted until *PARtop*, measures the given value (see Fig. 20). Given that the sensor is located beside the leaf, where the light is lower than over the leaf area, there is a so-called

light-source factor applied. The value indicated for *PARtop* is internally multiplied with this light-source factor, whenever the light is regulated with *PARtop*. Also *see* chapter 8.5.1.4 and 13.11.3.2). The light-source factor is stored in the measuring head.



Fig. 20: Standard Measuring Head 3010-S with LED Light Source 3040-L and darkening plate 3010 DP attached.

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

The other options for light regulation are *PARamb* and *Follow PA-Ramb*. The regulation mode *PARamb* is only sensible, if the external sensor is placed under the light source. With *Follow PARamb* the light value measured outside with *PARamb* are mimicked inside the cuvette (*see* chapter 8.5.1.4 and 13.11.3.2).

## 4.5 LED-Array/PAM-Fluorometer 3055-FL (Optional)

The fluorescence module, like all PAM fluorometers, applies pulse-modulated measuring light for selective detection of chlorophyll fluorescence yield. The actual measurement of the photosynthetic yield is carried out by applying a saturating light pulse which briefly suppresses photochemical yield to zero and induces maximal fluorescence yield. The given photochemical yield is calculated, displayed and stored. Numerous studies with the previously introduced PAM fluorometers have proven a close correlation between the thus determined YIELD-parameter (Fm'-F/Fm') and the effective quantum yield of PSII in leaves, algae and isolated chloroplasts. The yield data are used to calculate the apparent electron transport (ETR). In addition to this information, the fluorescence module also provides the possibility of measuring fluorescence quenching coefficients (qP, qN, NPQ). The Fo'-mode allows the application of far red light and the recording of Fo'.

#### 4.5.1 Measuring Light

The measuring light is a blue pulse amplitude modulated light with a peak wavelength of 470 nm.

## 4.5.2 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  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>) the frequency of the measuring light is automatically set to 10 Hz resulting in a PAR around 0.02  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>. If the actinic light is above 50  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> and during the saturating light pulses, the frequency of the measuring light is automatically set to 500 Hz (PAR about 1  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>).

#### 4.5.3 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 due to the fact that 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. also lichen with cyanobacteria as photobionts).

#### 4.5.4 Detector Filters

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

#### 4.5.5 Actinic Light

In chlorophyll-fluorescence terminology, the actinic light is the continuous photosynthetic active radiation which drives photosynthesis. The fluorescence module provides actinic light via 24 red (peak wavelength: 640 nm) and 2 blue LEDs (peak wavelength (470 nm). The proportion of the blue light is 10% (mol/mol), if the intensity is below 1500  $\mu$ mol/ (m² s). Above 1500  $\mu$ mol/ (m² s) the blue light remains at 150  $\mu$ mol/ (m² s).

#### 4.5.6 Saturating Light Pulse

The saturating light pulse is provided via the red LEDs of the actinic light. It reaches about  $4,500 \, \mu \text{mol/} \, (\text{m}^2 \, \text{s})$ .

#### 4.5.7 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.

#### 4.6 Imaging-PAM – Optional

The Imaging-PAM can be used together with the GFS-3000. The Imaging PAM version MAXI-Head may be used together with the Gas Exchange Chamber 3010-GWK1, while the Imaging-PAM version MINI-Head (IMAG.MIN/B, /R and /GFP) can be clicked onto the Standard Measuring Head 3010-S of the GFS-3000 after the adapter IMAG MIN/GFS has been attached to the MINI-Head. 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 panel PC of the GFS-3000.



Fig. 21: Imaging-PAM MINI-Head attached to Standard Measuring Head 3010-S with the Adapter IMAG-MIN/GFS.

Note: For proper communication between GFS-Win and ImagingWin Software. Choose "Run ImagingPam now" during setup of ImagingWin Software (see Fig. 22)

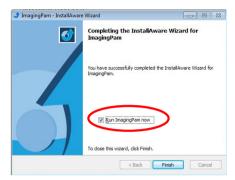


Fig. 22: Choose "Run Imaging Pam now" during setup of Imaging Win Software

If the Imaging-PAM is used with the GFS-3000 the absorptivity settings need to be readjusted by the user, since the transmission of the cuvette-glass differs for NIR and red light. Also it is advisable to cover the outer NIR and red LEDs (*see* yellow arrows in Fig. 23), because they cast a shadow at the cuvette frame. This can be done with some lengthwise-cut black tube.



Fig. 23: Imaging PAM Mini-Head with outer NIR and red LEDs covered

The absorptivity determination of the IMAGING-PAM relays on the principle that plants reflect near infrared radiation (NIR), but absorb red radiation. A red reflectance and NIR reflectance image are measured. The IMAGING-PAM has red and NIR LEDs for that purpose. In the MINI-Head, they are located between the blue LED-units.

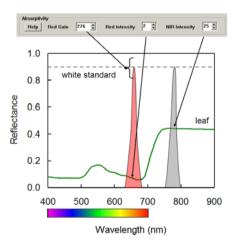


Fig. 24: Settings for absorptivity determination demonstrated together with reflectance spectrum of a leaf and a white standard and sketched spectrum of red and NIR-LEDs.

The parameters for adjustment are *Red Gain*, *Red Intensity* and *NIR Intensity*, which are located in the Group *Absorptivity* in the windows *Settings* of ImagingWin.

With a white standard (e.g. a white paper) the parameters for absorptivity determination shall be changed, so that the absorptivity measured for white paper is zero. This is the case if the reflectance-images, obtained with NIR or red light, have the same intensity.

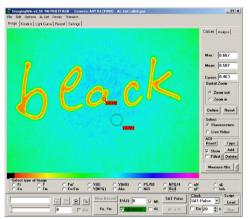


Fig. 25: Image of the red reflectance of a white standard with black writing before adjustment; value is 563 and shall be adjusted to match the NIR value.

Before starting the adjustment put the value for red gain to 100. Place a white standard (several layers of white paper) instead of the sample into the cuvette and adjust the value for the NIR intensity until the NIR image has a value between 700 and 750, which is equivalent to dark blue on the color scale. Then adjust the red intensity until the red image reaches a similar value (take the value of area1). With red gain the result for the red image can be fine adjusted until its values are as close as possible to the NIR image. After adjustment write the values down for future measurements.

## 4.7 Usage of the Darkening Leaf-Clamp 3010-DLC

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.

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 Software and Driver, Installation and Update

#### 5.1 Installation or Updating of the GFS-Win Software

The GFS-Win software has two setup types: updating the internal PC or installation of GFS-Win on an external PC. During the setup-procedure the correct type needs to be selected. 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, even though updates are free of charge, they are valuable). For updating the software on the GFS-3000, start the instrument and exit GFS-Win by choosing  $Menu \rightarrow On/Off \rightarrow Exit$ . In the shut-down dialog select Measure  $Mode\ off \rightarrow Exit\ GFS-Win$  and click OK (see chapter 7.7.2). Insert a USB memory stick with the setup-file into the USB-port. After GFS-Win has been stopped and the Taskbar has appeared chose  $Start \rightarrow Run \rightarrow Browse$ , navigate to the setup file and press Open and OK. The installation will start (see Fig. 26). Press Next to read the installation information and Next again.



Fig. 26: Installation of GFS-Win.

For updating the software on the GFS-3000 use the default directory by pressing *Next*. If the software is installed on an external PC, the installation directory may be changed. Please note that Windows versions higher than Windows XP have special restrictions in the subdirectory "Program Files".

Operation may be more convenient, if GFS-Win is not installed within this subdirectory.

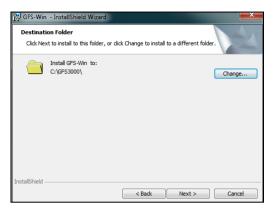


Fig. 27: On the internal panel PC use default destination folder.

After the directory has been confirmed, the installation type needs to be selected. For updating the internal PC of the GFS-3000 use the preselected option *GFS-3000* (*update for internal PC*) and press *Next*. For installation on an external PC chose the option *GFS-Win* (*external Computer*), indicated by the red arrow in Fig. 28, and click *Next*.

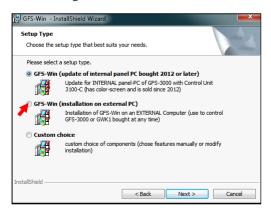


Fig. 28: For installation on external PC, change selection.

After installation press *Finish*. When updating the internal PC of the GFS-3000 another setup for the updating the on-screen keyboard may be started automatically. Again *Next* needs to be pressed until the installation has finished. Also if no modification needs to be done, *Next* needs to be pressed until the process is finished. After installing the software on an external PC, the USB-Port driver needs to be installed, see next chapter.

If the wrong software type has been installed, it may be necessary to remove the software. Also old software sometimes need to be removed before installation. To remove a GFS-Win version press  $Start \rightarrow Control$   $Panel \rightarrow Add$  or  $Remove\ Programs \rightarrow GFS-Win\ (Read\ support\ info,\ which\ contains\ version\ number) \rightarrow Remove$ . To remove the Keyboard.exe file press  $Start \rightarrow Control\ Panel\ \rightarrow Add\ or\ Remove\ Programs \rightarrow Keyboard\ (Read\ support\ info,\ which\ contains\ version\ number) \rightarrow Remove$ .

The external installation, will provide an uninstall shortcut at  $Start \rightarrow Programs \rightarrow GFS 3000 \rightarrow Uninstall GFS-Win for software removal.$ 

After installation please start the GFS-Win software by clicking on the link in the *Start-Menu* of Windows.

After installation on an external PC make sure that the regional settings of the external PC are set to point for the decimal character and space for the thousand-separator, also use a semi colon as list separator. The 24 h time-format should be used ( $Start \rightarrow Settings \rightarrow Control\ Panel \rightarrow Regional\ Settings \rightarrow customize$ ) or ( $Start \rightarrow Run \rightarrow "intl.cpl" \rightarrow customize$ ).

#### 5.2 USB-Driver Installation

#### 5.2.1 Windows 95, 98, ME

The GFS-3000 can be connected to an external computer using the supplied USB null-modem cable (NMC). When the USB-null modem cable is connected to an external computer for the first time, the PC automatically recognizes the new hardware and the "New Hardware Wizard" window appears.

Select the option *Install from a list or specific location (Advanced)* and click *next* and browse for the location on the provided CD with the appropriate USB-Port driver. After clicking *Next* the Hardware Wizard gives a warning. Click on *Continue anyway* and *Finish*. If the procedure starts once more, please repeat the procedure as described before for the next hardware port.

#### 5.2.2 Windows 2000

Start the program CDM\_Setup.exe from the subdirectory C:\GFS-WIN\USBPORT. It will take some time until the installation will be confirmed.

#### 5.2.3 Windows XP and Windows 7 (32 or 64 bit)

The program CDM(version number).exe needs to be started to install the USB-driver. To do this chose  $Start \rightarrow Programs \rightarrow GFS\ 3000 \rightarrow Install\ USB-Port.$ 

#### 5.2.4 Changing the Port number

The internal PC uses the COM port number 3 for communication with the external PC. This number is preset, but it may be required to correct this setting, if this number has been changed for the use of other USB-devices. Start the *Device Manager* with ( $Start \rightarrow Run \rightarrow "devmgmt.msc" \rightarrow OK$ ) or ( $Start \rightarrow Control\ Panel \rightarrow System \rightarrow Hardware \rightarrow Device\ Manager$ ), select *View devices by type*, then *Ports* ( $COM\ \&\ LPT$ ). Select the USB serial port and click *USB Serial Port*  $\rightarrow Port\ Settings \rightarrow Advanced$ . Set the COM Port Number to 3. To find the correct entry you may connect and disconnect the USB null-modem cable (NMC). The number can be set for both sides of the USB null-modem cable separately.

#### 5.2.5 Changing the Latency Time (for Imaging-PAM only)

Start the *Device Manager (Start*  $\rightarrow Run$   $\rightarrow$  "devmgmt.msc"  $\rightarrow OK$ ); select *View devices by type*, then *Ports (COM & LPT)*. Select the USB serial

port and click *USB Serial Port*  $\rightarrow$  *Port Settings*  $\rightarrow$  *Advanced*. Change Latency Time: to 8 ms. The Latency time is the time commands are buffered before they are sent to the control unit.

# 6 Checklist for Running the GFS-3000

## 6.1 Gas Exchange

#### 6.1.1 Checks

- Check humidifier, drier and CO<sub>2</sub> absorber (*see* chapter 13.1)
- Have a new CO<sub>2</sub> cartridge ready, (only insert on command, chapter 13.5)

#### 6.1.2 Power on

- Switch Power on at GFS-3000 (*see* chapter 7.6). If possible, use standby mode over night (touch screen to reawake).
- The GFS-Win software (*see* chapter 8) starts automatically.
- Select *Menu* → *On/Off* → *Measure Mode ON*, chose measuring head and light module, press OK.

#### 6.1.3 Calibrations before Measurement

- Set Flow-Meter Zero Offset with Calibration → Central unit → Set Flow Meter Zero Offset.
- Consider zero calibration of CO<sub>2</sub> analyzer (check weekly or after changing absorber, or before and after long-term measurements, chapter 13.11.1.1).
- Consider zero calibration of H2O analyzer (once a week, chapter 13.11.1.3).
- Consider total calibration of analyzer, offset and span (once in three months, chapter 13.11.1).
- If a different flow rate than before is used, consider readjustments of valves (*see* chapter 13.10).
- Consider light source factor, if system has been used by another user, if light source has been changed if cuvette was modified (see chapter 13.11.3.2).
- Consider setting the offset of Tleaf (see chapter 13.6.9)

# 6.1.4 Settings

- Decide on and adjust the measuring parameters (*see* Table 1). You might use programming functions to set parameters.
- You might want to set-up the fluorescence measurement first (*see* chapter 6.2 or 6.3)

Table 1: Settings for gas exchange, with example values

parameter	mode (unit)	value
filename (8 letters only)		test
area/weight	☑ area (cm <sup>2)</sup>	8
-	☐ weight (mg)	
impeller speed	(steps)	7
light	☑ PARtop	
	$\square$ PARbot ( $\mu$ mol m <sup>-2</sup> s <sup>-1</sup> )	1000
	☐ PARamb	
temperature control	☐ follow Tamb	25
	☑ Tcuv (°C)	
	☐ Tleaf	
flow rate	(µmol/min)	750
$CO_2$	✓ off (remove absorber)	
	☐ value (requires cartridge	
	and absorber)	
$H_2O$	☐ no control (amb)	20000
(drier and humidifier	☑ ppm	
may need purging with	☐ rh (%, requires Tcuv to	
CO <sub>2</sub> free or ambient air)	be set)	
Interval→ purgetime	(s)	60
interval: averaging and	(s)	005/060
measuring interval		

#### 6.1.5 Measurements

- You might use programming functions for measurements (see chapter 8.5.5 and 9). If new to the system start with manual measurements.
- Measure *ZPcuv*: In *MP mode*, close empty measuring head, wait until CO2abs, H2Oabs, dCO2 and dH2O have stabilized, press Store *ZPcuv*. Insert leaf and adjust setting for area, (*see 8.5.1.4*).
- Alternatively switch into *modeZP* or use *AutoZP* to measure *Zpirga*. For *ZPirga* the measuring head needs not to be empty.
- Start measuring in *mode MP* with leaf, observe *dCO2* and *dH2O* on the chart with fine scale.
- Store measuring points as desired (*see* chapter 4.2.2) either single points by pressing *Store MP* (*see* chapter 8.4) or several by adjusting the timing with *Interval* and pressing *Start storing* (*see* chapter 8.5.1.1).
- Change parameters as desired.
- If the CO<sub>2</sub>- or H<sub>2</sub>O-concentration is changed, a new ZP has to be recorded before taking any MPs (*see* chapter 4.2.2 for a general explanation on MP and ZP; *see* chapter 8.4 for operation of MP/ZP; and 8.5.1.1 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<sub>2</sub> and H<sub>2</sub>O drift. Typically values are constant after 15 minutes, but the absolute H<sub>2</sub>O values may need up to 1 hour of preheating, if very high accuracy is required.

#### 6.2 Fluorescence Module (optional)

#### 6.2.1 Power on

After power on, chose  $Menu \rightarrow On/Off \rightarrow Measure\ Mode\ ON$ ,-chose measuring head, chose fluorescence module (see chapter 7.6).

#### 6.2.2 Checks

Measure offset with provided black non-fluorescent foam (*see* chapter 8.5.1.5). With superfluous leaf sample try settings of Fluorescence Module (*see* chapter 8.5.1.5).

#### 6.2.3 Settings

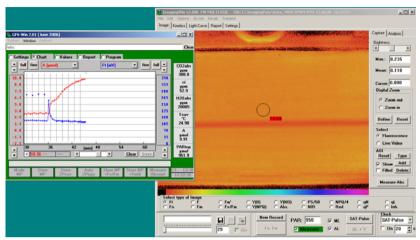
Decide on and adjust parameters for fluorescence measurements

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
FarRed Mode	on or off

- After gain, ML-Ampl or the optical set-up have been changed, the 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, dark adapt sample.
- Give flashes as intended, either single flashes by pressing Store Yield + MP or several flashes by adjusting the timing with *Interval* and *IYield/y\*MP* (included in *Interval* in *GFS-Win*) and then pressing *Start storing*.

# 6.3 Imaging-PAM (optional)



To use the Imaging-PAM together with the GFS-3000, the software ImagingWin and GFS-Win can be run in parallel on one computer. They both communicate with each other.

#### 6.3.1 Setup

- The Imaging-PAM *M-Series* MINI-Head can be mounted on the Standard Measuring Head of the GF-3000 with a special adapter plate (IMAG-MIN/GFS). The Imaging-PAM M-Series Maxi Head can be used together with the Gas Exchange Chamber 3010-GWK1 and the GFS-3000.
- Connect everything.
- Start Imaging-Win (the AL-List can be any, because it will not be used). In case of problems starting Imaging-Win, *see* chapter 15.1.
- Adjust camera settings (Window: Settings of Imaging-Win)
  - Measure a correction image with uniform fluorescence standard and camera out of focus.
  - o Adjust Absorptivity, see chapter 4.6.

- Settings: Press Reset Default Settings to obtain standard settings
- o The intensity of the saturating light pulse can be measured with a user-program command (*see* MeasureSatPuls in chapter 9)
- Try settings with a superfluous leaf and adjust, (see Table
   for recommended values

Table 3: Recommended Settings for Imaging-PAM

Setting (I-PAM)	Standard value
Meas Light	2-3
Frequency	1 changes automatically, when
	actinic light is on
Gain	2
Damping	2
Act Light	0 (change with GFS-Win)
Sat Pulse	8-10

- o Adjust focus
- The Imaging-PAM can be used to roughly determine the leaf areas within its imaged area. To check the settings, place a fluorescent sample of known size into the cuvette. Chose Ft and Analysis (upper right-hand corner). Adjust the shifters for Low and High until only the fluorescent sample is displayed in red. Under Red Pixel Assessment read the area. If the indicated area is wrong, click on it and enter the correct area. The factor to calculate areas from amount of red pixels will be adjusted.
- Start *GFS-Win* (In Imaging-Win under Options, GFS-3000 shall be ticked now)
- In GFS-Win start Measure mode with:
  - $Menu \rightarrow On/Off \rightarrow Measure\ Mode\ on \rightarrow select\ Standard$  $Head \rightarrow select\ Imaging-PAM \rightarrow OK.$

- In GFS-Win set light-source factor:
  - o In Settings-Window, set Light Mode
  - Select Calibration → Measuring Head → Light Source Factor → No
- In GFS-Win consider further calibrations (see chapter 6.1.3)
- Adjust the settings of the GFS-3000 (*see* chapter 6.1.4), but do not switch the light on.

#### 6.3.2 Start of Measurement

- Measure ZPcuv with an empty cuvette or ZPirga.
- Insert a dark adapted sample, measure Fo/Fm, then Abs.
- Start gas exchange measurements and fluorescence measurements as desired. Consider that after dark adaptation it will take a while for the leaf to reach a steady state in photosynthesis (12 min to 1 h). Often, photosynthetic rates are best in the morning. The program example may be used to perform measurements.

Images (pim-file) will be stored directly to the hard drive without special indication. Do not open or close files with the Imaging-Win software, but use GFS-Win software only to avoid loss of imaging data.

# 7 Integrated Panel PC (Control Unit 3100-C)

#### 7.1 Introduction

The integrated panel PC features a color display with touch-screen functionality as well as a Windows installation specifically designed for embedded systems. This allows for a direct control of the GFS-3000 via the GFS-Win software running on the panel PC. Upon startup of the GFS-3000 the integrated PC boots and the GFS-Win software starts. The panel PC should only be operated in conjunction with the GFS-Win software. Do not install additional software other than recommended by the Heinz Walz GmbH for operation of the GFS-3000.

# 7.2 Display Brightness



The color-display of the GFS-3000 can be comfortably read in direct sun light. Its backlight can be adjusted in 7 levels with the *Backlight* Window, which is located on the desktop and can be accessed from

within GFS-Win via the menu function Advanced  $Settings \rightarrow Display$  Brightness or by touching the field displaying the battery capacity in the lower right corner of the GFS-Win software.

### 7.3 Touch Screen

The touch-panel should only be operated 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. The software to adjust the touch panel settings (Touchside) can be accessed via the desktop or from within GFS-Win via the menu function: *Advanced Settings/Touch Screen Adjust*. If a keyboard input is required during the operation of the GFS-Win software, an on-screen keyboard is shown. The on-screen keyboard can also be started independently of the GFS-Win software from the desktop.

#### 7.4 USB-Port

The USB connector at the front side of the Control Unit 3100-C connects directly to the USB-port of the internal panel-PC. The connector is splash-proof. Additional devices can be plugged into the USB port via a short connector with a standard USB-A female connector. Keep this connector pointing downwards to avoid water (rain) causing an electrical shortage. The use of an USB-hub is also possible to enable the simultaneous use of several devices, but humidity needs to be avoided. A USB storage device (only attach virus-free storage devices) can be used to transfer measured data. The provided WLAN adapter enables wireless access to the GFS-3000. A USB-mouse or keyboard can be connected for comfortable operation. Please do NOT(!) connect a standard USB-cable for devices to the USB connector. It will cause a shortage and may cause damage. Only use the provided USB null-modem cable that is marked with USB-NMC for the connection of the GFS-3000 to an external PC.

#### 7.5 Pre-Installed Software

In addition to the operating system, there is preinstalled software on the panel-PC, which is required for the correct function of the GFS-3000:

C:\GFS3000\GFS-Win\GFS-Win.exe

C:\FanBL\Fan\_BL.exe (never stop this software)

C:\Program Files\Keyboard\Keyboard.exe

C:\Program Files\CopyData\CopyDataToStick.exe

C:\Program Files\Touchside\XTouchMon.exe

Drivers for USB-Port, Touch Screen, W-Lan Adapter.

Do not change, uninstall or delete or change the names or location of any preinstalled software.

# 7.6 Switching the System On

If the system is off, the panel PC LED and status LED of the battery control at the front side of the GFS-3000 are off. Connect the provided AC

Power Supply 3020-N or a fully charged battery. The Control Unit Model 3100-C may be run with input voltage between 12 and 24 V. The battery control will automatically determine, whether a 16V Li-ion Battery 3025-A or a 12V standard Pb battery is connected, when the system is switched on or every time a new battery is inserted. Only use the Battery Cable 3100-C/BC available form the Heinz Walz GmbH to connect 12 V batteries. Control Unit Model 3100-C upgraded with 3100-C/U-LIFE and control units with the serial number KETCxxxx can be used additionally with LiFePO4 Batteries (LiFePO4 Battery 3035-A). The recognized battery type (Li-ion, LiFePO4 or Pb) will be indicated together with the battery status in the software.

Note: An upgrade 3000C/U-LIFE for Control Unit Models 3000-C is available, too.

Shortly press the power on switch at the front side of the GFS-3000 to switch the system on. The status LED will be orange/yellow blinking during initialization and power-on voltage check. The initialization of the pump can be heard. If after pressing the power-on switch the status LED only blinks once red and then switches back off, the provided voltage is to low. If the provided voltage is high enough, also the internal PC will be switched on and its LED will glow green or orange indicating hard drive access, the operating system will boot and the GFS-Win software will start. Once the GFS-Win software has taken control, the status LED at the front side blinks green. If the indicated battery capacity is lower than 6%, GFS-Win will shut-down and the GFS-3000 will be shut off, while the status LED at the front side blinks yellow/orange.

After the power of the GFS-3000 has been switched on, the display shows the *measure mode on* window of the GFS-Win software (*see* Fig. 29). In this window the components that are attached to the GFS-3000 can be selected and activated. Clicking on one of the five panels cycles through the possible attachments for that slot. By clicking OK the system initialize the control unit, the gas analyzer and the selected components. This takes a short while and after the process is done, the GFS-3000 can be used for

measurements. Any component can also be activated later. Cancel will leave the system in its actual state. To be able to do measurements it is required to switch the system on by clicking OK.

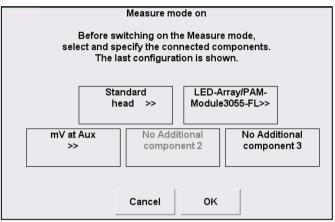


Fig. 29: Measure mode on – window

If the system is in standby mode (panel-PC LED is on, status LED at the front panel of the GFS-3000 is green blinking) touch the screen and wait 6 s. GFS-Win will resume operation. To start the measure mode select  $Menu \rightarrow On/Off \rightarrow Measure\ Mode\ ON$  and continue as described in the previous chapter.

If the screen is turned off to save power (panel-PC LED is on, status LED at the front panel of the GFS-3000 is green blinking), touch the screen to turn it back on. GFS-Win has not stopped operation and will appear immediately.

The GFS-3000 can also be operated with an external PC via the provided null-modem cable (see chapter 8.6.1.4 and 8.6.1.5).

# 7.7 Switching the System Off or into Standby Mode

When shutting the system down there are three options. The system can be switched off completely, just the measure mode can be switched off, or it can be set into standby mode. We recommend keeping the system in standby mode instead of switching it off over night, if power is available.



Fig. 30: shutdown dialog for switching the system off.

When shutting the system off, always do this via the shutdown dialog of the GFS-Win software. Do not use the shut down function of the Windows operating system, because it will not orderly shut down the complete system, but force any software to close independently of its current hardware operation. The shutdown dialog can be launched by pressing the power-on switch at the front panel of the GFS-3000 for about 5 s or via the menu of the GFS-Win software ( $Menu \rightarrow On/Off \rightarrow Exit$ , see chapter 8.6.1.6).

#### 7.7.1 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 flushing 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.

### 7.7.2 Measure Mode off / Exit GFS-Win

The purpose of this function is to enable software-updates or other services. A low battery will not be indicated, if GFS-Win is not running, but if the battery is low, the system will stop operation without warning. The *Measure mode off*  $\rightarrow Exit$  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.)

### 7.7.3 Standby Mode

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

Controls are switched off.

The analyzer stays on.

Depending on user request (option flush system with dry air ticked or not) the system will be flushed with dry air or not.

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

The internal PC goes into standby mode (LED remains on).

The battery control remains on (status LED green blinking). If the capacity is lower than 1%. The system will be switched off forcefully without flushing.

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

# 7.7.4 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.

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

GFS-Win may copy data of the file null.csv to a file with an automatically generated name: date\_time.sav.

The internal PC will be shut-down unless a battery capacity of 70% or higher is detected. Afterwards the battery control will switch the system completely off unless a battery capacity above 70% is de-

tected before the internal PC has started to shut down. Also see chapter 8.8.

# 7.7.5 Low Battery (Capacity <1%)

If the battery status is lower than 1% or if the power-on switch is pressed for more than 12s:

The panel-PC is forced to shut down via the Windows operating software.

The system is forcefully switched off.

Avoid this situation. Immediately after restarting the system secure any data which may have been stored in the file "null.csv". Always enter another filename before taking any measurements to avoid loss of data. Also see chapter 8.8.

# 8 GFS-Win Software

#### 8.1 Introduction

The GFS-Win Software is software preinstalled on the GFS-3000 Portable Photosynthesis System. It serves for the operation of the GFS-3000 or GFS-3000FL, in particular for the collection, display and analysis of measured data and for calibration of the sensors of the GFS-3000. Thus the GFS-Win software together with the GFS-3000 or GFS-3000FL enables the assessment of the photosynthetic performance of plants. The start of the software has been described in chapter 7.6 also see chapter 8.6.1.3 to 8.6.1.5

#### 8.2 General Information on the User Interface

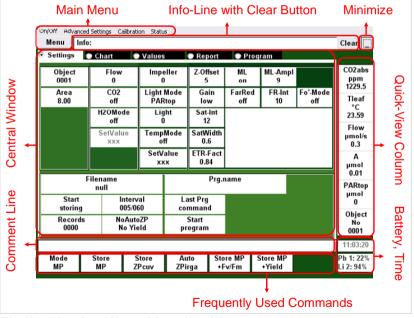


Fig. 31: User interface with outlined zones.

The graphical user interface is divided into zones:

- The main menu at the top of the screen has to be revealed by clicking on the button *Menu*.
- The info line with clear button serves as a display for info, warnings and errors, which shall be cleared or reset with the clear button.
- A central window that may display one of five index cards (*Settings, Chart, Values, Report, Program*).
- Comments entered into the comment line will be stored with the next record set.
- Frequently used commands at the bottom of the main window.
- Quick view column at the right side, which displays 6 user defined values.
- Battery capacities and PC-time in the lower right corner.
- The minimize button, minimizes the user interface and reveals the taskbar of the Windows operating system.

The functions of these zones will be explained in the following chapters.

#### 8.3 Quick View Column

The *quick view column* at the right side of the main window shows six values, which are user-selectable. To allocate a new value to a certain field of the *quick view column* select this field. A table with all available values will appear. Select your value of choice. It will now be shown in the *quick view column*. If this function has been activated accidentally, the *Esc*-button, which appeared above the quick view column, can be used to resume normal operation.



# 8.4 Frequently Used Commands Buttons

Mode	Store	Store	Auto	
MP	MP	ZPcuv	ZPirga	

A bar with *frequently used commands* is located at the bottom of the main window. The *Mode MP*-button on the left shows the state of the solenoid valves controlling the gas flow. The mode can be MP or ZP (*see* chapter: 4.2.2 about MP and ZP). Depending on the mode the next three buttons change their appearance and function.

If mode MP (Measuring Point) is chosen, the reference gas is flowing through the reference cell and the measuring gas is flowing through the sample cell of the analyzer. If a leaf is enclosed, a measuring point (MP) can be stored, using the button Store MP. If the cuvette is empty, a zero point ZPcuv can be stored using the button Store ZPcuv. This button is disabled if Start Storing is activated or if a program is running.

Once an hour or after changing the  $CO_2$  concentration or  $H_2O$  absolute concentration a zero point must be measured. If samples are enclosed for a short time, ZPcuv should be preferred. For long term measurements it is not possible to measure ZPcuv, but only Zero Points of the infrared gas analyzer alone (ZPirga).

Mode	Store	Store	Auto
ZP	MP	ZPirga	ZPirga

If *Mode ZP* is chosen the measuring gas is vented after passing the measuring head. The reference gas is split and flows through the reference and sample cell of the analyzer. A zero point of the infrared gas analyzer can be stored using the button *Store ZPi* (short for *Store ZPirga*). It will be marked as *ZPi* in the record file. A leaf can be enclosed in the cuvette during this procedure.

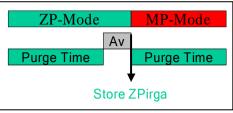


Fig. 32: Time course of an Auto ZPirga measurement

Auto ZPirga does the following procedure, which is illustrated in Fig. 32, to measure a ZPirga: The solenoids of the gas ways are switched into ZP-Mode, the gas analyzers are purged for a defined period of time, called purge time. After purging, data are averaged and stored as ZPirga. The time stamp of the last averaged data value will be assigned to the stored ZPirga record set. The solenoids will be switched back to mode MP and again the gas analyzers are purged.

The *Purge time* can be set with the button *Interval* in the *settings* window or in the menu *advanced settings*. The averaging time for zero points is the same as the averaging time chosen for measuring points. It can be set in the *settings* window with the button *Interval*.

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

### 8.5 Central Window

and enabled (see chapter 8.5.1.5).

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.

## 8.5.1 Window Settings

The *Settings* window opens automatically after starting the GFS-3000 or starting the external GFS-Win software (*see* Fig. 33).

Settings	Chart •	Values	;	Report	Prog	ram	
Object 0001	Flow 750	Impeller 7		Z-Offset 22	ML on	ML-Ampl 9	
Area 8.00	CO2 off	Light Mode PARtop		Gain high	FarRed off	FR-Int 10	Fo'-Mode on
	H2OMode abs[ppm]	Light 0		Sat-Int 12			
	SetValue 17000	TempMode Tcuv		SatWidth 0.6			
		SetValue 24		ETR-Fact 0.84			
III	Filename Viburnum_Opulum_CO2				name DULE		
Start storing	Interval 005/060		Last Prg command				
Records 00055	NoAuto No Yie			Start ogram			

Fig. 33: Settings window.

The control elements in the *settings* window are arranged in five different groups, which are outlined with a small line. The first column contains gas-exchange parameters that are not measured by the instrument, but need to be entered by the user; in particularly the leaf area respectively weight is required 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 elements of connected and enabled components are activated. Clicking on a control element starts an input-dialog.

#### 8.5.1.1 General

The Filename button allows to entitle an upcoming Filename measurement or to open an existing file. Newly collected data, also called record sets, will be 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. Area is suitable as reference for flat leaves while weight should be used for conifers, lichens or mosses. The reference type (Area or Weight) is fixed for the complete file, while the value can be different for each object. Both can be changed after the measurement in the Report window; data will then be recalculated based on the new reference. If the filename "null.csv" is chosen, which may have been given automatically, when no filename has been entered, data may be deleted without warning. To avoid this situation only use this name for data that shall be discarded. Immediately after restarting the system secure any valuable data accidentally stored in "null.csv" also have a look under "null.bak" and the automatically given name

Start Storing The four buttons, Start Storing, Records, Interval and NoAutoZP, serve to control the automatic sampling sequence. This should not be confused with a user program, since automatic sampling does only control the storage of data records and the measure mode MP or ZP (see chapter 4.2.2), while user programs allow the control of all settings during the time-course of a measurement protocol. The Start Storing button starts the sampling sequence and record sets will be stored into the given filename (\*.csv). The button will be titled Stop Storing and allows stopping the automatic sampling sequence.

"date time.sav", which may contain a copy of "null.csv".

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 4.2.2). Pressing *Records* is equivalent to pressing *Filename*.

Interval 03/60 No AutoZP No Yield

The other two buttons control the settings of the sampling sequence. Clicking on either of them will open the dialog that is displayed in Fig. 34.

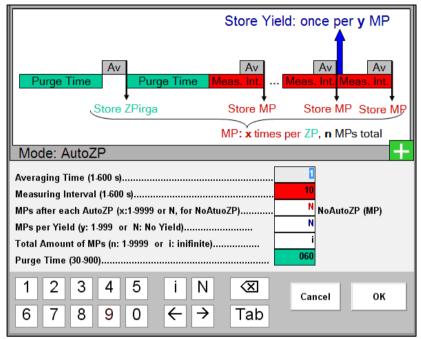


Fig. 34: Dialog with settings for the automatic sampling sequence.

There are several optional sequences. The simplest one is to just take measuring points (MP), however this can expanded upon by taking additional zero points (ZP) and yield measurements if a fluorescence module has been attached. Also the instrument can be switched to ZP mode to only take ZPs instead of MPs. These different modes are illustrated in Fig. 34 and Fig. 35. Fig. 34 shows the standard view with fluorescence module, whereas Fig. 35 shows the expanded illustration without fluorescence module.

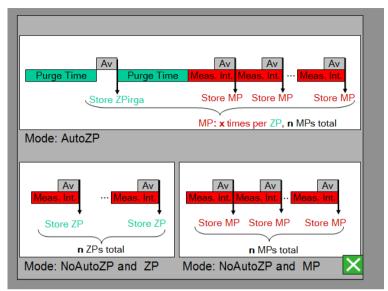


Fig. 35: Illustration of sampling sequences (fluorescence module not enabled).

The settings for the sampling sequences are displayed all together in the sampling sequence dialog; some of them are also indicated on buttons Interval and  $No\ AutoZP$  in the settings window, and in the  $Menu \rightarrow Advanced$  Settings.

Settings for the automatic sampling sequence:

- Averaging time determines how many values are averaged for each stored record set. Although this value is adjusted within the sampling dialog, it is valid for 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 indicated in the button Interval in front of the slash and in the Menu Advanced settings.
- Measuring interval determines how often data are stored, if the sampling sequence is activated. The measuring interval needs to be the same or bigger than the averaging interval. The Measuring interval is indicated on the button interval behind the slash.

- Value x (MPs after each AutoZP) defines how often the system shall switch into the mode ZP and measure the differential zero point of the gas analyzer (see AutoZPirga chapter 8.4). The MPs are taken after each AutoZPirga. If No AutoZP is chosen, there is no automatic mode-change and only measuring points (MPs) or zero points (ZPs) will be stored depending on the actual mode. This sequence of purely MPs is only recommended, if a ZPirga or ZPcuv has been measured beforehand, for short-term measurements. If AutoZP + x\*MP is selected, the cycle (AutoZP + x\*MP) will be repeated until n MPs have been recorded (= n/x cycles). This choice is recommended for long-term experiments like complete day courses of single leaves. Zero points will automatically be recorded between series of MPs, giving a higher accuracy. The number for the value x is indicated in the button named No AutoZP or ZP+xMP.
- 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 (see chapter 8.5.1.5). Each yield measurement is directly taken after the last MP of each series of y MPs. If N is entered, no yield measurements are performed during the sampling sequence. Depending on the entered value, the button will either display No Yield or 1Y/yMP in the second line.
- Variable n (total amount of MPs) determines the total amount of MPs, which shall be taken before the automatic sampling sequence stops. When the letter i (like infinite) is entered the automatic sampling will not stop until Stop Storing has been pressed. This variable may be changed or confirmed in the input dialog, which appears before the sampling sequence is started.
- The *Purge time* is required for the *AutoZPirga* sequence. It determines how long the system is purged in mode ZP and MP before

and after a ZP measurement respectively. The purge time is also indicated 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 like e.g. a light curve, which can be edited in the *program* window (for further information, proceed to chapter 8.5.5 and 9). Before a program can be started, the measure mode needs to be switched on and a filename and program name has to be entered. With button *Prg.name*, a user-program, which is a text-file with the extension .prg containing a listing of commands known to the GFS-Win software, can be selected.

Start program When the selected user-program is started with the *Start* button, it turns into a *Stop* button and indicates the estimated running time. If the user-program

did not finish normally, but was interrupted with the *Stop* button, it can be continued by pressing the *Start* button again. A dialog will appear and the suggested line can be confirmed or changed. Similar when in the *program* window a line of the user-program has been marked. The marked line can be confirmed as starting point or changed.

Last Prg

In the field with the title *Last Prg command*,, the last command, which has been executed, is indicated. With GFS-Win single commands of the user-program

are executed as fast as possible. Therefore not every command becomes visible. The button does not only show the last command, but may also indicate the word *Skip*, like *Skip Interval*, *Skip AutoZP* or *Skip Wait→Steady*. If the button is pressed while showing *Skip*, the program proceeds with the next command. This allows a fast check of user-programs or a half automated use of the GFS-3000, so that an automatic user-

program can continue based on user-decisions. For this kind of operation time intervals may be given generously.

The *Last Prg Command* field does not only indicate commands from a user-program, but also commands given internally, when settings are changed or during certain procedures like the shutdown procedure. Additionally it shows commands invoked via a COM connection from another program like a script-file of the DualWin software (see special manual).

#### 8.5.1.2 Parameters

The *Object* number will be 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 data shall be recalculated after recording in the *record* window.

The reference value (for *Area* or *Weight*) needs to be entered by the user. It is required for the exact calculation of photosynthetic parameters like the assimilation or transpiration rate. The reference type (*Area* or *Weight*) can be changed, when a new data-file is named (*Filename*) or in the Report window when data shall be recalculated. The value for *Area* or *Weight* can be different for each object number.

#### 8.5.1.3 Central Unit

Input of the *Flow* rate between 300 and  $1500 \mu \text{mol/s}$  (equivalent to  $\approx 400 \text{ to } 2000 \text{ ml/min}$ ).

The default and recommended value for the Standard Measuring Head 3010-S is 750  $\mu$ mol/s. If the setting for the flow rate is changed by more than 100  $\mu$ mol/s, consider readjusting the valve settings (see chapter 13.10). The *Flow* can only be switched off, when neither H<sub>2</sub>O nor CO<sub>2</sub> are controlled by the instrument ( $H_2O$ - and  $CO_2$  Mode are off). Vice versa the H<sub>2</sub>O control or CO<sub>2</sub> control can only be switched on, when *Flow* is on. Also within user-programs keep this order.

CO2 off The user can choose between two different options:

- **1.**  $CO_2$  control off; to use ambient air. In this case the  $CO_2$  absorber tube has to be exchanged against the provided mixing volume (3000-C/MV). In this mode the  $CO_2$ -supply is closed by a solenoid valve, so that no  $CO_2$  can diffuse to the system.
- **2.** Set an absolute  $CO_2$  concentration in *ppm*: The set value can be chosen between 0 and 2000 ppm. The  $CO_2$  regulation can only be started, when the flow is switched on and also requires the connection of the  $CO_2$  absorber tube filled with functional soda lime. The soda lime is not functional and has to be exchanged when turned violet once. Note, that the indicator may turn its color back over night. Nevertheless the soda lime is not functional the next morning. Also with very dry air the indicator may not work. If 0ppm  $CO_2$  is desired, do not regulate to 0, but switch the regulation off with  $CO_2$  absorber connected.

A small cartridge is used to fill  $CO_2$  into the supply vessel. If the  $CO_2$  control is on and the pressure in the supply vessel drops below 250 kPa (see  $Menu \rightarrow Status~8.6.4$ ), a warning appears, that the  $CO_2$  cartridge has to be exchanged (see chapter 13.5).



The user can choose between *off* and two further modes:

**1.**  $H_2O$  Mode off; The air is directed straight through the  $H_2O$  valve, without passing the *Drier* or *Hu*-

midifier.

- **2.**  $H_2O$  *Mode absolute* concentration in ppm; the resulting relative humidity inside the cuvette depends on the cuvette temperature.
- **3.**  $H_2O$  Mode relative humidity in %; the Measuring Head has to be connected and the *TempMode* must be set to Tcuv. Note that not the relative humidity of the cuvette is controlled, but the relative humidity entering the cuvette. The humidity required is calculated from the set value for Tcuv, it is adapted when Tcuv is changed.

**4.** *Completely dry*; with this function the H2O control is not regulated, but set,so that all air streams through the drier. This mode can be used to flush the system with dry air. Then it is advisable to turn the flow off before the H2O control mode is switched back off.

For option 2 or 3 a set value must be entered. After the control mode has been defined the button underneath for the set value is active. The unit of the set-value is ppm or relative humidity (rH), depending on the  $H_2O$  control mode.

### 8.5.1.4 Measuring Head

Impeller 0

*Impeller* speed to ventilate the cuvette volume effectively. The values can be set between 0 and 9,

setting 7 is recommended. At lower speed the response time is slower and the temperature control gets less effective.

Light Mode PARtop

> Light 0

The *Light Mode* determines which light sensor is used to control the light intensity, *Mode PARtop*: sensor in the upper chamber side is used to control the light to the given set-value.

Mode PARbot: sensor in the lower chamber side is used to control the light to the given set-value (used with the Arabisopsis chamber)

*Mode PARamb*: MQS-B/GFS sensor for ambient light needs to be placed under the light source for this function (e.g. in self-built cuvettes).

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

*PARtop* or *PARbot*: the sensor in the lower chamber side is used to control the light to the value measured by the ambient sensor.

Whenever the light is controller with the PARtop or PARbot sensor, the light-source factor is effective (see chapter 13.11.3.2) 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). Because then any measured light is not coming from the connected light source, but assumed to come from the sun. To set a light value a light source must be connected and mounted onto the cuvette. A set value between 0 and 2000  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> can be chosen.

# TempMode off SetValue xxx

The temperature control gives a choice between *off* and three further functions:

Off: No temperature control.

Follow ambient temperature: The ambient temperature is measured at the external heat exchanger fan of the lower cuvette half and used for the control of the cuvette temperature. In this mode the cuvette temperature is regulated, so that it follows the measured value of this sensor. A temperature offset can be entered to reach a value some degrees above or below ambient temperature.

Set cuvette temperature: the cuvette temperature is kept constant at the given set value.

*Set leaf temperature*: the leaf temperature is kept constant at the given set value.

The set value or temperature offset must be entered when starting the temperature control. It can be changed, using the *Set Value* button. The minimal temperature, that the standard measuring head can reach, is about  $10~^{\circ}\text{C}$  below ambient temperature and can be increased up to  $50~^{\circ}\text{C}$ .

### 8.5.1.5 LED-Array/PAM-Fluorometer 3055-FL - Optional

The control elements for the fluorescence module are located in the four last columns of the *settings* window. They become active, when the LED-Array/PAM-Fluorometer 3055-FL or the Fiberoptics/PAM-Fluorometer 3050-F is connected (see next chapter).

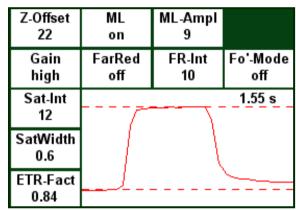


Fig. 36: Settings for the Fluorescence Module 3055-FL with display of fluorescence kinetics during the last saturating flash

The fluorometer is enabled in  $Menu \rightarrow On/Off$ ,  $\rightarrow Enable/disable\ components$ . Create a file for data storage (with Filename), before performing any fluorescence measurements. The fluorescence will be measured in the unit mV. It can be continuously observed (as Ft) in the *chart* or *values* window or selected to be displayed in the *quick view column*.

Z-Offset serves to determine the zero-value of the

fluorometer, which consists of background fluorescence and a small preset offset. The background fluorescence may be caused by fluorescing materials or a small interference of reflected measuring light. The purpose of the small electronically preset offset is to allow noisy values average to zero. With the *Z-Offset* button the zero-value will be determined, stored in the fluorescence module and indicated. Its value will be subtracted from every fluorescence value directly within the fluo-

Z-Offset

rescence module. Before pressing *Z-Offset*, the fluorescence module needs to be placed in its final measuring position, the sample in the measuring

head needs to be replaced with black non-fluorescing foam, and the measuring light needs to be on for at least 5 s. The *Z-Offset* needs to be adjusted, with any change in the *Gain* or *ML-Ampl* (*see* below) settings or any change in the optical setup (e.g. new measuring head different cuvette or leaf area adapter). It is good practice to determine the *Z-Offset* after the system has been started.

Gain low Pressing the *Gain*-button changes the sensitivity of the sensor between low and high by a factor of 3.7.

A high gain is only recommended for small samples. It improves the resolution, but not the signal to noise ratio. It can be set to high, if the signal remains below 200 mV, even after adjusting the measuring light. After the adjustment of *Gain* or *ML-Ampl* the *Z-Offset* needs to be adjusted.

ML on Switches the measuring light (*ML*) on or off. The button indicates the actual state of the light.

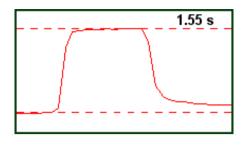
ML-Ampl 10 *ML-Ampl* changes the intensity of the measuring light. The signal to noise ratio is the better the higher the measuring light. Nevertheless, if it is chosen to

high it may cause an actinic effect or overloading of the fluorescence signal during Fm determination, which should be avoided. To test, whether the measuring light causes an actinic effect, observe the fluorescence in a dark-adapted sample after switching the measuring light on, or after inserting a dark adapted sample. If the measuring light causes a slow fluorescence increase by itself, it is too high. To avoid overloading, the fluorescence value for the dark adapted sample should range between 150 and 700 mV. The recommended value for *ML-Ampl* is 10. After the adjustment of *Gain* or *ML-Ampl* the *Z-Offset* needs to be readjusted.

Sat-Int 12 *Sat-Int* changes the intensity of the saturating light pulse in steps from 1 to 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 saturating light pulse serves to fully reduce (also called close) photosystem II. It is activated with the button  $Store\ MP + Fv/Fm$  or the button  $Store\ MP + Yield$  at the bottom of the screen. After every saturating light pulse, the kinetic of the

fluorescence signal is displayed in a small graph. The fluorescence value before and during the flash is stored. *Fv/Fm* serves to measure the reference fluorescence values Fo and Fm with the dark adapted sample. With a *yield* measurement the saturating light pulse is given and measured in the same way, but the fluorescence values are assigned as F and Fm'. The Fm or Fm' fluorescence values are determined between the point, where the plateau is reached and the point, where the light pulse is switched off. The level of the stored fluorescence values are indicated by broken lines. The intensity and duration of the saturating 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 to low. If the fluorescence is quenched during the pulse, the saturation pulse is to high or 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

Switches the far red light on or off.

Fo'-Mode off

Enables or disables the Fo'-Mode in conjunction with Yield measurements. Usually Fv/Fm of a dark

adapted sample is measured before a series of *Yield* measurements. If the Fo'-Mode is enabled, the actinic light is automatically switched off after every saturating light pulse, then far-red light is applied for 2 s before the actinic light is switched back on. During the far-red illumination the Fo' is measured and used instead of the calculated Fo' for the determination of qP an qN (see chapter 10.12).

ETR-Fact 0.84 The *ETR-Factor*, relates to the fraction of the incident light, which is absorbed by the leaf. It is used

for the calculation of *ETR*. It is not measured by the LED-Array/PAM-Fluorometer 3055-FL or Fiberoptics/PAM-Fluorometer 3050-F, but needs to be entered by the user. A mean value for green leaves in moderate climate is 0.84, which should be used, if the *ETR-Factor* can not be determined. When using the Imaging-PAM, the *ETR-Factor* is automatically determined during the absorption measurement.

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

When the fluorescence module is enabled, the two buttons of the bottom bar on the right, which serve to trigger saturating light pulses, become active. They are inactive for 10 s after each saturating light pulse to allow cooling time for the LEDs.

Store MP +Fv/Fm Store MP + Fv/Fm Serves to store a measuring point including gas exchange and fluorescence data.

The saturating light pulse is triggered directly after gas exchange data have been taken, averaged and stored. The fluorescence data are assigned Fo, Fm and Fv/Fm. The leaf needs to be dark adapted for an Fv/Fm measurement. A healthy dark adapted leaf reaches values around 0.8 for Fv/Fm. For the calculation of qP, qN and NPQ, an Fv/Fm measurement must be recorded prior to a sequence of Yield measurements, since the calculation of qP, qN and NPQ require Fo' (Fo) and Fm. With every new sample or change of sample position or change of fluorescence settings (ML-Ampl, Gain or Z-Offset) a new Fv/Fm measurement is required

Store MP +Yield Serves to store a *measuring point* including gasexchange data and fluorescence data, The saturating

light pulse is triggered directly after gas exchange data have been taken, averaged and stored. The fluorescence data are assigned F, Fm', Yield and ETR. If an Fv/Fm measurement has been performed, also qP, qN and NPQ is calculated. Note that Fo' is calculated if 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 saturating flash 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).

The time stamp assigned to the record-set is always the last second of the averaged gas-exchange measurement taken directly before the saturating flash.

If the automatic sampling sequence shall be used for storing data yield measurements can be triggered automatically. The value for *y* in the setting *MPs per Yield* will cause a yield determination directly after the last MP of a sequence of y MPs (see chapter 8.5.1.1). If the Fo'-Mode is on also Fo' will be determined automatically.

In a user-program the commands Fv/Fm and Yield will cause the measurement of gas exchange together with fluorescence data.

### 8.5.1.6 Fiberoptics/PAM-Fluorometer 3050-F - Optional

The Fiberoptics/PAM-Fluorometer 3050-F works very similar to the Fluorescence module 3055-FL. In the GFS-Win software enable the Fiberoptics-version.

ML-Frq low In difference to the Fluorescence Module 3055-FL, the frequency of the measuring light can be set

manually. It is recommended to use the low frequency in low light and in the dark, where the measuring light would otherwise have an actinic effect on the sample. The high frequency results in a smoother signal. 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 unless it needs to be decreased, when the distance to the sample is very low.

Gain high The Gain should be set to high, when the fluorescence signal of a dark adapted sample is lower than

200 mV.

#### 8.5.2 Window Chart

right side.

The *chart* window simulates the function of a chart recorder. The record starts instantly when the *Measure mode* is switched *on*. The chart memory of GFS-Win holds all values of the last hour, so that it is possible to scroll through the entire last hour with the highest time resolution. Two magnitudes can be displayed in parallel.

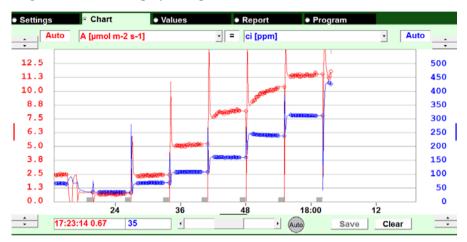
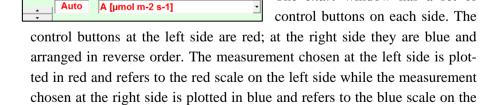


Fig. 37: Chart window displaying Fluorescence (red line and scale) and Intercellular CO<sub>2</sub>-Mole Fraction (blue line and scale).

The chart window has a set of



• The *selection box* allows choosing the measurement to be plotted. Directly assessed (e.g. CO<sub>2</sub>abs) or calculated (e.g. A or gH<sub>2</sub>O) values can be displayed in the chart.

- *Auto* When this button is pressed, the range is automatically adjusted, so that the actually measured value is displayed.
- The *vertical arrow keys* change the scaling of the Y-axis. The range is not limited. The upper *arrow keys* control the maximum value and the lower control the minimum value.

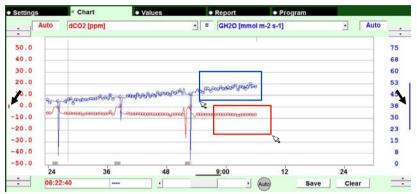


Fig. 38: Chart window, scaling options

- The *Y-axis bars* (Fig. 38 black arrows) change the y-axis scaling in three increments (fine, middle and full).
- Another way of changing the scale is to select a certain area with the touch pen directly in the chart window or with the mouse, when the left button is pressed. The chosen range will be rounded to 5% of the full range and 5 min. If the area is drawn from left to right, it concerns the red curve. If it is drawn from right to left, it concerns the blue curve. Fig. 38 illustrates both directions together.



• The *time axis* is active. Clicking on it either with the left or right mousebutton or clicking onto the black time axis bar scrolls through the scaling by 6 increments. The minimum time range is five minutes, maximum is 60 minutes.

- 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 actual measurement, click somewhere inside the chart-area. The actual value is marked with a *short gray vertical line* at the upper border of the chart area.
- The *mode* (*ZP or MP*) *is indicated* at the lower border of the chart area by a grey bar (*see* minute 25 and 38 in Fig. 38). 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. 38 minute 28).

The value box is active, if the mouse is moved over the chart or if the chart is touched shortly with the touch pen. It shows the measured values corresponding to the horizontal mouse-position. For fluorescence also the vertical mouse position is evaluated, 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 to a .csv-file. This .csv file is a dump of the chart-memory. It gives the chance to store data, if something unexpected has been seen, but storing was not active. The csv-file of the chart has a different structure than a record file and can not be read into the record memory. The Save button is only active, when automatic storage and program run are inactive.

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.

### 8.5.3 Window Values

Menu Info:							
<ul><li>Settings</li></ul>	tings ● 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	FFFFFF	1000	1000
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
lmp	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	Α	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 mV 446	Fo mV 361	Fm mV 1889	Fv/Fm 0.809	F mV 448	Fm' mV 681	Fo' mV 355	Yield 0.342
ETR 71.9	qP 0.62	qN 0.76	NPQ 1.77	ETR-Fac			

Fig. 39: Values window

The *Values window* (Fig. 39) shows all the measured and calculated parameters, which also appear in the *report file* after saving a *measuring point* or *zero point*. For further information about the underlying calculations proceed to chapter 10 (Calculations). For more details please proceed to the chapter 12 (Data Record Structure).

After pressing the values tab a second time the *Values window* changes into the *Val slope window*. In addition to the values, the slope will be indicated underneath each value. The indicated number is xxx +yyy, whereby xxx is the slope in the unit: "indicated unit/min" and yyy is the error of slope determination.

Pressing the values tab again will change the window into the *Val steady window* indicating stability. 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 away from set value xxx is set value.

Pressing the values tab again will lead to  $Val\ crit\ window\ displaying$  the criteria for stability determination indicated as xx' >yy<. The first value xx' is the time in seconds over which the slope shall be determined. The second value, yy, is the limit, which determines the classification limit for the slope. The criteria for stability determination can also be set under  $Menu\ o Advanced\ Settings$ . The slope determination and the criteria for stability determination is preliminary. This function is still under test and may be changed in future.



Rows which are not of interest can be hidden in the value window. *Advanced Settings/Hide rows in values window:* opens up a dialog window to enter the rows favored for hiding (see Fig. 40).

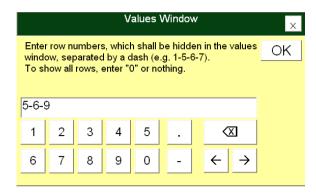


Fig. 40: Dialog window to alter the number of rows displayed in the values window

Setting Recalc.	file Nev	hart v leaf rea	New weight		Report		Program Delete line		nt +/-
VPD Pa/kPa	GH20 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

### 8.5.4 Window Report

Fig. 41: Report window

The *report* contains the record-sets that have been stored. One record set is one line. There are three kind of record sets, MP, ZPi or ZPc, which is indicated in the column assigned code string (not shown). MP stands for measuring point, ZPi for zero point measured in mode ZP, and ZPc for a zero point measured with an empty cuvette in mode MP. For more details on the values please proceed to the chapter on data record structure (chapter 12). Also read chapter 10 about the calculation of data.

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 in front of it.

Recalc. file	New leaf	New
	агеа	weight

These switches can be used for the recalculation of saved data with new reference values.

New leaf area or New weight allows the specification of a new leaf area for single objects, and performs the recalculation only for the specified object. Recalc. file recalculates the entire file. Thereby, the values are either calcu-

lated in respect to *Weight* or *Area* as indicated in the header of the file. It is also possible, to change the reference type by use the *New Weight* button on a file, which has been measured based on the area or *vice versa*. The file-header will change to the new reference type (*Area* or *Weight*). Nevertheless, only the data for the chosen object will be recalculated, leading to a mismatch between values and indicated units. This can be avoided by either entering a new reference value for each object or by using *Recalc. file* to recalculate the entire file with the new reference type. The reference type used for the calculation is indicated for each record set in the status line starting with A or W (for the interpretation of the status string see chapter 8.6.4).

**Delete last line**If the last record set shall be deleted, press *Delete last line*. Note that, this button will only delete the line in the report file. Nevertheless, if the last line was a ZP, the ZP value will still be used for the calculation of subsequent measurements, the same with Fv/Fm.

Font +/This buttons cycles through font sizes for the display of the report. With the left mouse button (short touch), the font-size cycles in the direction of increasing fonts until the maximum size has been reached. With the right mouse button (long touch), the font size cycles in the reversed direction.

### 8.5.5 Window Program

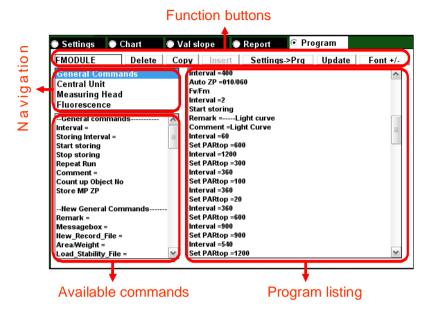


Fig. 42: Program window

The *program* window serves to write user-programs. It is only active, if the automatic data storage is inactive and if no program is running. First a program file needs to be opened by clicking on the left box with the program name in the upper line. The program can be new file or an existing file. In Fig. 42 it has the name FMODULE. The content of the program file, the program listing, is shown in the text box at the right half of the screen. Commands can be chosen and changed by a double click. If the command requires an additional value, an input dialog will appear as known already from the handling of the instrument with the *settings* window.

The left half of the screen shows the available commands. The commands are organized in groups similar to the groups in the *settings* window. Clicking on a line in the upper left box will navigate to the commands belonging to that group. To insert a new command into the program listing, double click on a command in this list. The command will be inserted after

the marked position in the program listing. If the command requires a set value, an input dialog will appear beforehand. Also see chapter 9 on programming.



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

- *FMODULE* is the name of the actual user-program. Another program can be opened with this control box.
- Delete deletes the selected lines in the program listing.
- Copy takes the selected lines into an internal memory and clipboard.
- *Insert* inserts the copied lines after the marked line.
- Settings->Prg writes the actual settings, set of the settings window, into the program listing behind the selected line. This function is useful, if settings have been established and shall be used another day again. But this function is also useful to write the start 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 measurements and have no effect on a program run. They should be deleted, if the program is used for program runs to avoid confusion.

Note, that if the settings for Gain or ML of the fluorometer are changed, the Zero-Offset needs to be readjusted. The sequential order of the commands written with  $Settings \rightarrow Prg$  depends on the actual settings. It complies with the programming rules (chapter 9).

- *Update* has been introduced with GFS-Win version 2.01. It serves to automatically update the programming commands to the latest syntax.
- *Font* +/- cycles through different font sizes for the program listing, increasing (left mouse-button) or decreasing (right mouse-button).

#### 8.6 Main Menu

On/Off Advanced Settings Calibration Status

The main menu can be accessed by pressing the *Menu* button in the upper left corner of the screen. It reveals the items *On/Off*, *Advanced Settings*, *Calibration* and *Status*. *On/Off* concerns switching the instrument on or off or to down-load data. *Advanced settings* exposes settings concerning the touch-screen and display brightness as well as additional advanced settings for the measurement. *Calibration* 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.

#### 8.6.1 Menu On/Off

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

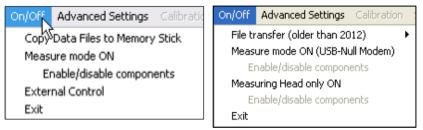


Fig. 43: Menu On/Off: GFS-Win running on internal Panel-PC (left) or external PC (right).

### 8.6.1.1 Copy Data Files to Memory Stick

The menu option *Copy Data Files to Memory Stick* serves to down load measured data to an USB storage device. Only insert virus-free memory sticks. The program CopyDataToStick.exe will be launched. It consists of two explorer-windows and an operation bar. The left explorer window opens the folder *MyDocuments\GFS-3000*.

Here all the data should be stored. It is possible to create and access a special sub-directory for each user. Also it is advisable to store and archive user-programs together with the measured data in the same subdirectory. The other explorer window displays the content of the USB storage device. The operation bar offers special buttons for the operation of the explorer windows. The buttons are equivalent to pressing or releasing the keys Crtl, Shift, Crtl+c, Crtl+v and Del. Also remember that a long touch is equivalent to a right-button mouse click giving access to context-menus in the explorer windows.

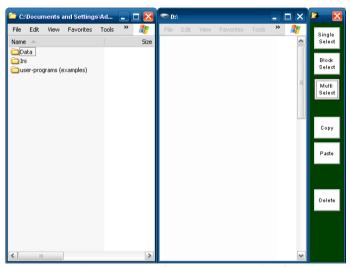


Fig. 44: Menu item: Copy Data Files to Memory Stick

### 8.6.1.2 File transfer (older than 2012)

This function exists for downward compatibility. It serves to transfer data from the Control Unit Model 3000-C, which has been sold until the end of 2011. Collected data files (.csv) can be downloaded from the GFS-3000 and user-programs (.prg) can be uploaded to the GFS-3000. Downloaded files will be stored in the actual subdirectory chosen with filename in the *settings* window. Also the GFS3000.ini and GFS3000.err file

can be downloaded. These files will be stored in the subdirectory of the GFS-Win software or in My Documents\GFS-3000\ini.

Fluorescence parameters can be calculated in various ways. If checkbox is marked fluorescence parameters will be recalculated during transfer with equations given in chapter 10.12. Further information on the equations used by the panel pc of GFS3000 and GFS-Win software are given in chapter 10.12.

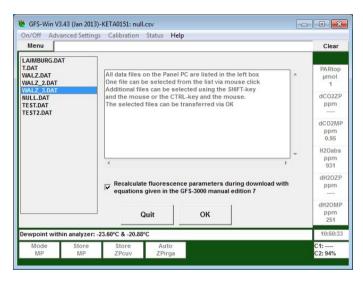


Fig. 45: File transfer (older than 2012)

### 8.6.1.3 Measure Mode ON and Enable/disable components

The menu item *measure mode ON* opens a dialog with the system configuration (see Fig. 29 and similar Fig. 46). In this dialog the attached components should be chosen by clicking on the corresponding button. A fluorescence module can only be selected, if an appropriate measuring head has been selected before. The system will be switched on, and the selected components will be initialized, after pressing OK. Also the analyzer within the central unit will be switched on and initialized with this step.

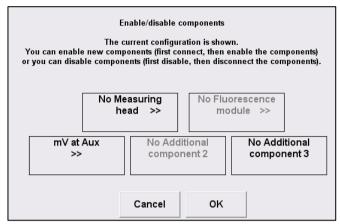
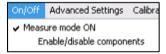


Fig. 46: Dialog for system configuration: Enable/disable components.

Components can also be activated later, after the system and analyzer has been switched on (menu function: *Enable/disable components see* Fig. 46).



After the Measure Mode has been switched on, there is a check symbol right besides the *Measure mode ON* menu point indicating that the

system is on.

### 8.6.1.4 External Control, Control Unit 3100-C

If the system shall be operated with an external PC, it needs to be connected to the GFS-3000 via the provided USB null-modem cable. To allow external operation chose  $Menu \rightarrow On/Off \rightarrow External\ Control$ . This item is only available, if the measure mode is off or in standby mode. After pressing external mode an input dialog will appear, which allows setting the value for timeout. After pressing ok, the control from an external computer can be started.



Fig. 47: Info line after enabling external control mode.

In the external control mode, 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 or error messages are directly stored on the external PC. To start the external control on the external PC chose in GFS-Win:  $Menu \rightarrow On/Off \rightarrow Measure\ Mode\ ON\ (USB\ Null\ Modem)$  as explained in the next chapter.

### 8.6.1.5 Measure Mode ON (USB Null Modem)

This item establishes the control of the GFS-3000 with an external PC. As described in the previous chapter the external control needs to be enabled on the internal PC before the external access can be established. The external GFS-Win software contacts the internal GFS-Win software and takes control. The measure mode can now be switched on with the external GFS-Win software. To give the control back to the internal PC, use the Exit function in the external PC (see next chapter).

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 try to reestablish 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. Also now the control is back at the internal PC.

#### 8.6.1.6 Exit

The *Exit* item serves to launch the shutdown dialog, which allows to switch the system off. The shutdown dialog will also be launched if the *Measure mode ON* item is chosen or if the power on switch at the front panel of the GFS-3000 is pressed for about 5s. The shutdown dialog gives a choice on how deep the system shall be switched off. Most important the gas analyzer stays on, if the system is sent into standby mode otherwise it is switched off.



Fig. 48: Shutdown dialog (internal PC).

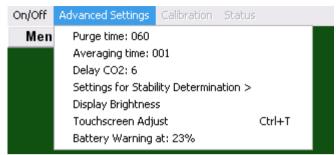


Fig. 49: Shutdown dialog (external PC).

During the shutdown procedure, the system may be flushed with dry air to avoid condensation, which is most important, if the analyzer is off and a cool night is expected or during transport. Condensation within the analyzer may lead to destruction. For the flushing to be effective, the measuring head needs to be closed and the drier needs to be functional. After flushing, the H<sub>2</sub>O control is turned off. Depending of the choice the system switches itself off, keeps only the panel PC on or goes into standby mode. In standby mode the panel-PC LED is glowing. Touch the screen and wait for about 6s to resume from standby. For a detailed description of the different shutdown procedures see chapter 7.7. Since GFS-Win also obtains the information on the battery status, it should always be kept running on

the internal PC. The function *Measure mode off / Exit GFS-Win* is only recommended to use when updating the GFS-Win software. The option *Show Desktop and Taskbar* has the same function as the button [-] in the upper right corner of GFS-Win. It allows minimizing the GFS-Win software window and has now influence on the measurement. It should only be required for service functions on the Windows operating system, which should only be done with extreme care. The shutdown dialog on the external PC gives an additional choice for the GFS-Win software. It can be stopped (exit) or continued (keep).

### 8.6.2 Menu Advanced Settings

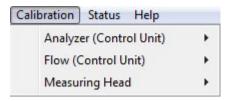


The menu *Advanced Settings* gives access to additional settings, which are rarely required. All settings here concern the operation of the GFS-Win software or the operation of the panel-PC. These values are all stored on the hard drive of the operating PC, which is running the GFS-Win software (panel-PC or external PC) and not within the instrument. The item advanced setting is also available, when the instrument is switched off. The items *Display Brightness* and *Touch Screen Adjust* are only available when GFS-Win is running on the internal panel-PC.

- *Purge time* concerns the *AutoZP* function. It determines how long the system is purged in ZP-mode respectively MP-mode before and after an automatic *Zero Point* is recorded. Times between 30 and 600 sec can be chosen.
- Averaging time only indicates the averaging time. To change the averaging time, use the command *Interval* in the *settings* window. The averaging time will always be used for the recording of gas exchange data, no matter whether it is via automatic storage or a manual storage triggered with a button on the frequently used commands bar.
- 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 → 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 Fig. 11). When dCO2 is calculated, this time delay is taken into account (see chapter 10.3).
- Add temperature differences (Ttop-Tcuv) enables to enter a temperature difference (-10 to +10 °C) between Ttop and Tcuv for set-value of temperature regulation in Mode Tcuv.
- Settings for Stability Determination opens a new window for the adjustment of stability criteria. This function is still preliminary and may be changed in future (see chapter 8.5.3).
- *Hide rows in values-window:* opens up a dialog window to enter the values' rows favored for hiding (Chapter8.5.3)
- Display Brightness puts the window of the program Fan\_BL.exe into the foreground see chapter 7.2. Clicking on the battery-status field (lower right corner in GFS-Win) has the same effect. To hide the window for display brightness, click somewhere in GFS-Win. Never stop the program Fan\_BL.exe since it also controls the temperature of the CPU.
- *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.
- *Battery Warning* sets the capacity level at which a battery warning shall be displayed. See also chapter 8.8 about battery status, chapter 3.5 about charging, chapter 3.6 about Li-ion batteries and chapter 7.7.4 to 7.7.5 about the automatic switching off procedures at low battery levels.

#### 8.6.3 Menu Calibration



The menu item *Calibration* contains not only calibrations, but also 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. Adjustments concerning the internal panel PC are in the *Menu Advanced Settings*, so that they are available as soon as the panel PC is switched on.

- Analyzer (Control Unit) leads to the calibration procedures for the gas analyzer (see chapter 13.11.1).
- Flow (Control Unit) contains menu points for the valve adjustment and adjustment of mass flow meter zero (see 13.10 and chapter 13.11.2).
- Measuring Head contains menu points for the calibration and adjustment of values concerning the sensors and properties of the attached measuring head (see chapter 13.11.3).

#### 8.6.4 Menu Status

The menu item *status* opens a window (see Fig. 50) that contains the current system values, the status string, the components info and the system constants and calibration values stored in the components.

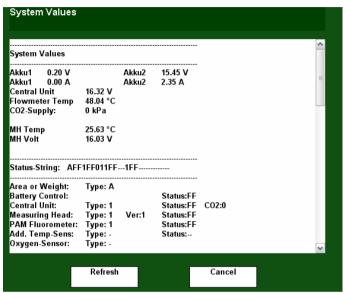


Fig. 50: Status Window displaying system values.

Of major importance for planning experiments is the  $CO_2$ -supply, which indicates the remaining  $CO_2$  pressure in the container. The maximum value is 950 kPa. If the pressure is below 250 kPa, a message will notify that a new  $CO_2$  cartridge should be inserted. One cartridge adds 600 kPa of  $CO_2$  into the  $CO_2$  container.

The status string is indicated underneath the system values. It is decoded in the paragraph below.

Scrolling down with the scroll bar at the right side leads to further information on the components.

#### 8.7 Comment Line

Underneath the central window is a comment line.

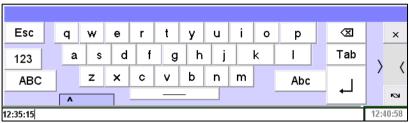


Fig. 51 Comment line with on-screen keyboard above after a double click.

Comments entered in this line will be stored with the next record set (MP or ZP) in the comment column. When the comment line is activated by a click, the on-screen keyboard will appear. Maximal 32 characters are shown in the *report* window. It may be necessary to pull the comment-column of the *report* window wider, to see the comment. Maximal 200 characters per comment are stored in the csv-file (commas and semicolons are automatically removed from comments before storing). A double-click on the comment line will insert the actual system-time into the comment line.

### 8.8 Battery Control, Time

The Control Unit Model 3100-C can be operated with a wider voltage range (12-24 V) than the Control Unit Model 3000-C (14-16 V). Thus the LiFePO4 Battery 3035-A (Control Unit Model 3100-C upgraded with 3100-C/U-LIFE or serial number KETCxxxx) and the Li-ion Battery 3025-A provided by us as well as 12V or 24V batteries may be used for operations in the field. Car batteries should only be connected via a 4 m cable provided by us. The battery type (Pb,Li or LiFe) and status will be indicated in the lower right-hand corner.

Note: An upgrade 3000C/U-LIFE is available for Control Unit Models 3000-C.

The system switches off, if the battery power is too low (see chapter and 7.7.4 to 7.7.5). A warning is displayed if the level has reached 23% (adjustable in  $Menu \rightarrow Advanced\ settings \rightarrow Battery\ Warning\ 8.6.2$ ). Note that the battery level is indicated in units of percentage, nevertheless this indication is not linear. If the battery level is down to 6% the GFS-Win software will automatically shut down the system. Once the shut-down procedure has started, the only way to stop the complete power off is by inserting a battery with a capacity of more than 70%. If the battery is down to 1% the internal battery control of the control unit will forcefully and immediately shut off the system.

The field indicating the battery status has a second function: clicking on it will launch the window for display brightness adjustment, also see chapter 8.6.2:  $Menu \rightarrow Advanced Settings \rightarrow Display Brightness$ .

### 8.9 Changes to GFS-Win Software, with Imaging-PAM enabled

When ImagingWin and *GFS-Win* (external control only) run in parallel, the ImagingWin software is not restricted in its functionality. Nevertheless, everything which can now be controlled with *GFS-Win* should be controlled with it, otherwise loss of imaging data may occur:

- o opening and closing files
- o taking images and triggering a saturating light flash
- o changing the light intensity
- o user-program runs for light curves

### 8.9.1 Settings

Light Mode is used to indicate which light sensor is used to measure the light from the Imaging-PAM. Since the light sensor is located at the side of the sample, where it does not obtain the full actinic light from the Imaging-PAM, a light-source factor is used. If the chosen sensor is PARbot or PARtop the light will be corrected with this light-source factor and sent to the ImagingWin software. The value will appear in the PAR-box of the ImagingWin software as well as in GFS-Win. If the Imaging-PAM is enabled, the light-source factor is also active, if the light is set to 0 (different to usual).

PAR: 353

The PAR-box from Imaging-Win software indicates the light value obtained from GFS-Win.

Light 16 If the Imaging-PAM is enabled, the given number is referring to the steps of the Imaging-PAM, not μmol m<sup>-2</sup> s<sup>-1</sup>. It is recommended to use this button in the GFS-Win software for changing the light of the Imaging-PAM. The Imaging-Win software will notice the change, but not *vice versa*.

Store MP +Fv/Fm

Store MP + Fv/Fm Storserves to store a measuring point including gas exchange data and fluorescence images. Gas

exchange data are averaged and stored first. Directly afterwards the Imaging-Win software stores an Fo-image, gives a saturating light pulse, and stores an Fm-image. The images are stored in a pim-file in the subdirectory of the Imaging-Win software: \ImagingPAM\Data\_MINI as usual. The averaged data from the current AOI 1 (Area Of Interest 1) are sent to the GFS-Win software and stored in the record-file together with the gas exchange data. Only one Fo/Fm-image pair can be stored per image file. The image number will appear in the comment-column of the record-File in the GFS-Win software. Only one Fv/Fm-image and one Abs-image can be stored per pim-file. Always the last absorptivity-image taken is stored.

The Store MP + Yield button is similar to the button Store MP + Fv/Fm, but instead of Fo and Fm, F and Fm' images are stored. In difference to Fv/Fm, this yield-button is used, when the actinic light is on.

Measure Absorptivity serves to measure the absorptivity of the leaf. Only one absorptivity-image can be stored per pim-file. Always the last absorptivity-image taken is stored. No gas exchange data are recorded with an absorptivity measurement. In the GFS-Win software the result of the absorptivity measurement of the current AOI1 is stored in the results-file with the next saturating flash under ETR-Fac. It is used to calculate the electron transport rate (ETR).

#### 8.9.2 Chart

The measured and calculated fluorescence values of AOI1 can be plotted with the chart function of the *GFS-Win* as well as with the chart function of the Imaging-Win software. Other areas are controlled via Imaging-Win only.

### 8.9.3 Report

Date		Tin	ne	No.	PAR	Fm'1	F1		Y(II)1	NPQ1	qN1		qP1	ETR1
27.07.0	06	12:	36:43	1	0	0.2123	0.05	583	0.725	0.000	0.00	0	1.000	0.0
27.07.0	06	12:	39:59	2	148	0.1799	0.08	328	0.540	0.043	0.20	8	0.780	35.6
27.07.0	06	12:	42:58	3	148	0.1338	0.11	47	0.143	0.145	0.48	2	0.227	9.4
27.07.0	06	12:	45:59	4	147	0.1284	0.10	083	0.156	0.165	0.51	4	0.255	10.3
PARtop µmol	Comm Strin		Fo mV	Fm mV	Fv/Fm	F mV	Fm' mV	Fo m\		ETR	qΡ	ql	N NPQ	ETR-Fac
147.5	IPAM4	_04	58	212	0.725	108	128	49	0.157	10.26	0.255	0.5	15 0.653	3 0.891
147.5						110								
148.5						112								
147.5	IPAM4	_03	58	212	0.725	115	134	50	0.143	9.44	0.228	0.4	84 0.58	7 0.891
149.4						120								
145.6						132								
147.5	IPAM4	_02	58	212	0.725	83	180	56	0.540	35.57	0.781	0.2	07 0.180	0.891
148.5						102								
0.0						62								
0.9	IPAM4	_01	58	212	0.725									

Values in the report-file of GFS-Win correspond to values in the report-file of Imaging-Win. But in GFS-Win they are stored permanently and will not adapt as in Imaging-Win, if AOI1 is changed. The units of fluorescence values are arbitrary and they are displayed at different scales, depending on the software used: in Imaging-Win, they range from 0 to 1, in GFS-Win from 0 to 1000. The fluorescence values are calculated from fluorescence values averaged over the area AOI1. In GFS-Win the equations are the same as for the fluorescence module (3055 FL) except for Fo', which can not be measured with the Imaging-PAM, but calculated (see chapter 10.12). This calculated value for Fo' is also used for the calculation of qP and qN.

In difference to the Fluorescence Module (3055FL) ETR-Fac can be measured with the Imaging-PAM ( $\rightarrow$  absorptivity). Therefore it can not be set manually.

The image-number is stored in the comment file of the report in GFS-Win. If images are taken with the buttons of the ImagingWin software the GFS-Win software will not notice and the information belonging to this image will not be stored by GFS-Win.

# 9 Programming

### 9.1.1 General Programming Information

User-programs can be used to only store the actual settings for simplifying the start-up of further measurements once the settings have been established or they can be used to run complete measurements.

User programs are listed in the *program* window and started with the button *Start program* in the *settings* window. A program consists of a list of commands, which will be executed line by line. Almost all commands in Table 4 (below) are commands for entering a set value (e.g. for flow, light, cuvette temperature, storing interval). If during a program-run such a command line is reached, the command will be carried out immediately as fast as possible. Other commands require some time to elapse. The most prevalent is "Interval =". It is used to define a time that has to elapse before the next command is carried out. If it is preceded by "Start storing =", values are recorded during this Interval. Another command requiring time is "Auto ZP =". It is equivalent to pressing the button "Auto ZPirga" in the Settings Window. Also the fluorescence commands: "Fv/Fm" and "Yield" require some time.

The set-value of a parameter remains active until it is overwritten by a command with a new set-value.

The program will stop after the last command is carried out. If the storage of data-records is enabled at the end of the program, it will be stopped. All other settings made during the program-run, remain active. Of course, they can now be changed manually.

If a program is started, the manual input of a file name, set-values or other parameters is blocked. Only the button *Stop program* for stopping the program-run will be enabled. The button underneath displays the last executed command. Beside that it has another function. If it indicates the word *Skip*, like *Skip Interval*, *Skip AutoZP* or *Skip Wait* → *Steady*, the button can be pressed to proceeds directly with the next command. This allows a fast

check of user-programs or a half automated use of the GFS-3000, so that an automatic user-program can continue based on user-decisions. For this kind of operation time intervals may be given generously.

Comments can be entered during a program run. They will be stored with the next data-record.

### 9.1.2 Command List

Table 4: Command List

	General Commands
Interval =	to define an interval (s), which has to elapse before the next command of the program is car- ried out:
	If you use "Start storing" before the "Interval"-command, data points will be stored during the interval. The nature of data points will depend on the settings ( <i>see</i> "Mode", "Storing Interval" and "1Yield/y*MP"). The maximum length for an interval is 3600s. If you need longer intervals you can use the command several times in a row.
Storing Interval =	to set the Measuring Interval- and Averaging Time for the storage of data points:
	This command corresponds to the button <i>Interval</i> in the Settings Window. If the Storing interval is not defined in the program, the actual settings will be used.
	Avoid this command between "Start Storing" and "Stop Storing" to avoid ambiguous timing situations.
Start storing	to initialize the storage of data records:
	This command is only performed, if it is followed by the command "Interval =". Storing will continue until it is stopped by the command "Stop storing" or until the run has finished. Depending on the mode (MP or ZP), measuring

	points or zero points will be stored. The mode can be set with the command "Mode =". The storing interval can be set with the command "Storing Interval =".
Stop storing	to stop the storage of data points.
Repeat Run	to repeat the run from start.
	With <i>GFS-Win</i> the command: "Repeat from here\" can be used, to repeat from a later position.
Comment =	to enter a comment for the report-file:
	after clicking on "Comment =" an input window appears and a comment can be entered. The comment will be stored in the report-file with the next storage of gas exchange data, like usual comments entered manually.
Count up Object No	Increases the value for Object by 1.
Store MP ZP	performs the same action as pressing the button <i>Store MP</i> or <i>Store ZP</i> depending on the actual mode. Easier to handle than the command sequence: Start Storing, Interval=, Stop Storing.
	New General Commands
	This category contains commands, which are not implemented on the panel PC of the Control Unit 3000-C and can only be used with GFS-Win (external control or Control Unit 3100-C).
Remark =	to enter a remark into the program-code:
	after clicking on "Remark =" an input window appears and a remark can be entered; the remark has no effect on the program; it can only be seen in the "Program Listing".
Messagebox =	to show a message during a program:
	after clicking on "Messagebox =" an input window appears and a message can be entered; when the program reaches the line with the messagebox command, it will show the message and stop

	41.4 1 1.1 CEC.W. 1
	until the user has pressed ok. In <i>GFS-Win</i> , also the receiving of data will be interrupted.
New Record_File =	To open a new record file.
	Useful, when using the Imaging-PAM, where the number of images is limited to 100 per file. If the name ends on the letter "q" Fo and Fm images are used from the previous record file.
	If the letters <i>date_time</i> are entered as name, the record file will be named automatically during the program run with the actual date and time.
Area/Weight	To change reference value.
Load_Stability_File	To load a given stability file (test version). It is searched in the subdirectory, where the configuration file GFS3000.cfg is stored; and if not found, in the subdirectory where the user-program is stored.
	If the letters <i>default</i> are entered, the default criteria are loaded (these default values will be changed and adjusted with future GFS-Win versions).
Wait until steady	Waits with proceeding the user-program until the criteria given in the stability file are full filled (test version)
Repeat from here	to set the position, where "Repeat Run" shall start the repeat.
	Central Unit
Set Flow =	to set the gas flow rate (µmol/s) through the measuring cuvette.
	Note the order of commands: This command needs to be given before switching the CO <sub>2</sub> control or H <sub>2</sub> O control on, but after switching them off.
Set CO2 =	to set the CO <sub>2</sub> control to the indicated value (ppm).
	Note the order of commands: This command

	needs to be given after switching the flow on, otherwise it will not be performed.
CO2 Control off	to switch the CO <sub>2</sub> control off:
	This command switches the CO <sub>2</sub> control off, by closing a solenoid valve. If the CO <sub>2</sub> absorber is used, the CO <sub>2</sub> concentration becomes zero otherwise ambient.
Set H2O(ppm) =	to set the H <sub>2</sub> O control to the indicated value (ppm).
	Note the order of commands: This command needs to be given after switching the flow on, otherwise it will not be performed.
Set H2O(rh) =	to set the H <sub>2</sub> O control to the indicated value (relative humidity % entering cuvette).
	Note the interdependence of commands: The relative humidity control requires the Temperature control to be set to Tcuv, because the set value of Tcuv is used to calculate the control value for the H2O concentration. Give the commands "Set Tcuv" and "Set Flow" before this command.
H2O Control off	to switch the H <sub>2</sub> O control off:
	This command switches the H <sub>2</sub> O control off, gas is neither guided through the drier nor through the humidifier.
Mode =	to chose between MP and ZP-Mode:
	Solenoids switch to MP-Mode (outlet from cuvette enters infrared gas analyzer) or ZP-Mode (reference gas is distributed between both infrared gas analyzers, outlet from cuvette is vented).
Auto ZP =	to set the purge time and averaging time (temporarily), and perform an automatic ZPirga determination:
	Firstly, the solenoids switch to ZP-Mode, then the system is purged for the indicated time,

	77D' 1 1 1 1 1 1 1 1 1
	ZPirga is averaged over the indicated time and stored. Afterwards, the solenoids switch back to MP-Mode, and the system is purged for the indicated time before the run continues. Auto ZP replaces the following series of commands:  Stop Storing Mode = ZP Interval = Purge Time
	Storing Interval = Av Time / Interval equal Store_MP_ZP
	Storing Interval = back to what it was Mode = MP
	Interval = Purge Time
	Start Storing, if it was active before
	New Commands Central Unit
CO2 push valve =	to enter a valve position for acceleration of CO2 regulation
Aux1 =	to scale Aux1-Values
Aux2 =	to scale Aux2-Values
	Settings Meas. Head
Impeller =	to set the impeller speed.
	If the impeller speed is set to 0, the light and the Temperature Control needs to be switched off first.
TempControl off	to switches the temperature control off.
	Don't use this command, if the H2O mode is set to relative humidity.
Follow Tamb plus	to set the temperature control for <i>Tcuv</i> to follow the ambient temperature measured with <i>Tamb</i> . A positive or negative offset value can be entered, if Measuring Head Version is higher than 1.20.
Set Tcuv =	to enter a set value for cuvette temperature; the temperature regulation will be set to the mode <i>Tcuv</i> .
	Set impeller speed beforehand ("Impeller =").

Set Tleaf =	to enter a set value for leaf temperature; the temperature regulation will be set to the mode <i>Tleaf</i> .
	Set impeller speed beforehand ("Impeller =").
Light Control off	to switch the light off
PARtop = PARbot =	to control the light. The command determines which sensor is used to control the light source.
PARamb =	PARtop/PARbot: The light source will be regulated with the sensor in the upper/lower cuvette half. With PARtop and PARbot, the light-source factor will be taken into account.
	<i>PARamb</i> : The ambient sensor needs to be placed under the light source.
	The value determines the light value in $\mu$ mol m <sup>-2</sup> s <sup>-1</sup> .
PARtop follows PARamb PARbot follows PARamb	Only for Measuring Head Version 1.20 and higher. These commands can be used to imitate the light measured with the external sensor PARamb with the Fluorescence Module 3055-FL. The light is controlled with PARtop or PARbot according to the value measured by PARamb. The Light-Source Factor is taken into account.
	New Commands Meas. Head-
Add Temperature Differenz (Ttop- Tcuv) =	to enter a temperature differenz value (-10°C - +10°C) between upper and lower cuvette half
	LED-Panel RGBW
Red =	to set the intensity step value for red colored LEDs
Green =	to set the intensity step value for green colored LEDs
Blue =	to set the intensity step value for blue colored LEDs
White =	to set the intensity step value for white colored

	LEDs
Red/Green/Blue/W hite =	to set intensity step values for each color at the same time.
LED-Panel RGBW total =	to enter the total brightness 0-100% or switch the LEDs off.
Red/Green/Blue/W hite/Tot =	to enter the intensity step values for each color and the total brightness 0-100%
	Fluorescence Module 3055-FL or 3050-F
1Yield/y*MP	to set the clock for giving saturating pulses during fluorescence measurements. A yield will be measured every yth MP.
Fv/Fm	to trigger a single Fv/Fm measurement:
	An Fv/Fm measurement includes a saturating light pulse with Fo and Fm determination. Gas exchange data will be determined and stored beforehand. They will be averaged according to the actual Averaging Time, which can be set with the command "Storing Interval". The Measuring Interval, that is also set with this command, is ignored here.
Yield	to trigger a single Yield measurement:
	A yield measurement includes a saturating light pulse with F and Fm' determination. In dependence on the Fo-Mode an Fo' determination will be performed after the light pulse. Gas exchange data are averaged and stored before the yield measurement. They will be averaged according to the actual averaging time, which can be set with the command "Storing Interval". The measuring interval, that is also set with this command, is ignored here.
Default F	to set fluorescence module to default settings
Fo'-Mode FL =	to set the Fo-Mode:
	On/Off: Fo' is determined/not determined with every Yield determination.

Gain FL =	to set the gain (high/low).
	Note, after changing this setting, the zero-offset of the fluorescence module needs to be set again.
M-Light FL =	to switch the modulated measuring light on or off.
ML-Amp FL =	to set the amplitude (intensity) of the modulated measuring light.
	Note, after changing this setting, the zero-offset of the fluorescence module needs to be set again.
SatWidth FL =	to set the duration (s) of the saturating light pulse.
Sat-Int FL =	to set the light intensity (steps) of the saturating light pulse.
Set Z-Offset FL	to set the zero-offset for the fluorescence module: This command will interrupt the user-program and ask the user to insert a black non-fluorescent foam into the cuvette. The user can circumvent this measurement by choosing "Cancel". The user-program will be interrupted until the user has chosen ok or cancel.
ETR-Fact FL =	to set the ETR-Factor, which is the factor used to calculate the electron transport rate (ETR) from yield measurements and PAR. The factor is equal to the proportion of light absorbed by the leaf.
FR-Int FL =	to set the intensity of the far red light without switching it on (use command "FarRed FL =").
FarRed FL =	Switches far red light on or off
	Module 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 (external control only)
PARtop =	to set the light of the Imaging-PAM in steps.

PARbot =	This command is the same as usual, but if the Imaging-PAM is connected the values means steps, not µmol m <sup>-2</sup> s <sup>-1</sup> . Range: 0-20.
	If the ImagingPAM is shining on PARtop or PARbot use PARtop or PARbot respectively. The Light-Source Factor is used accordingly.
1Yield/y*MP	see Fluorescence Module 3055
Fv/Fm	see Fluorescence Module 3055
Abs	to trigger the measurement of an Absorptivity- Image

### 9.1.3 Programming Rules

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. The same is true for most settings during the program-run. When programming, it is important to pay attention to the following rules for the sequential order of commands:

**Switching the system or controls on:** First measuring head: impeller followed by temperature and light.

Then central unit: flow followed by gas concentrations.

**Switching the system or controls off:** Use reverse order: first central unit: gas controls off, followed by flow. Then measuring head: temperature and light followed by impeller.

The command "Storing Interval =" shall be called before any measurements, also before fluorescence measurements.

It is recommended to define all settings in the program run so that they are always the same with the same program-run.

To simplify programming the button "Settings->Prg" can be used (*GFS-Win* software only), *see* chapter 9.1.4.

### 9.1.4 How to Program

Programs can be written or modified within the *program* window. With the button *Program name* an existing program can be loaded, or a new program can be created. Any changes to the program file will be stored immediately without warning. To keep the original version, a safety-copy should be done before opening the file. It is also possible to copy all lines from a template program, open a new file and insert all lines. The programming function is disabled, as long as no program name is entered.

In the commands-box a subset of available commands is listed. First select the line in the program listing, behind which the new command shall be inserted. Then navigate to a command-subset with the upper control-box. Now a specific command can be chosen. Double-click on it. The selected command will be inserted into the program listing behind the selected line. If the command requires a value, an input box will appear.

For further descriptions on how to enter, delete, copy, insert, change or update program code *see* software description chapter 8.5.5.

### 9.1.5 Timing during Programming

The timing in user-programs is sometimes ambiguous. In general, ambiguous situations can be avoided by generous timing of the storing interval. In the following the proceeding of the program in ambiguous situations is described.

Fluorescence measurements performed with the command "1Yield/y\*MP=" take additional time, especially if they include Fo'-measurements, because the saturating light pulse is given after the gas exchange measurements has been taken. Usually the idle time of the next measuring interval (idle time = measuring interval -averaging time), which has been set with "Storing Interval =" is used. The maximal time required

for a Yield measurement in *Fo'-mode* is 8s. If the idle time of the given measuring interval is to short, the measuring interval of the following MP is prolonged. As a result, the total Interval set with "Interval =" might contain less MPs then intended. If a yield measurement has been started at the end of an Interval, the count-down of the interval is paused the yield measurement is finished before proceeding with the program.

If averaging of gas exchange data, has not been finished at the end of an Interval, the program proceeds without storing the data-set.

### 9.1.6 Format of Program Files

In GFS-Win, the user-programs are text files with the extension prg. Therefore it is possible to also insert the content into a word file, protocol or email for documentation. The button copy may be used for this purpose, since with this command the marked lines may be copied into the clipboard. It is also possible to change user-programs with a text-editor. Nevertheless this is difficult since the exact syntax needs to be kept. The text-editor WordPad will destroy the syntax, while the text-editor NotePad keeps the required syntax. For testing the syntax, the Update button can be used.

# 9.1.7 Programming Example for a Light Curve

<b>Program Listing</b>	Comments		
Remark =Light curve	(only GFS-Win) Internal comment		
Messagebox =Insert a leaf	(GFS-Win only) Message for experimenter,		
	the program will not continue until the user		
	clicks on OK		
	The following settings can also be made		
	manually before starting the program, but it is		
	recommended to include them in the program		
Set Tcuv =25.0	Set cuvette temperature to the desired value		
Set Flow $=750$	Set flow to desired value (the flow must be set		
	before the two following commands)		
Set CO2 =350	Set CO <sub>2</sub> to desired value		
Set $H2O(ppm) = 14000$	Set H <sub>2</sub> O to desired value		
Impeller =5	Set Impeller to desired value (the impeller		
	must be set before the two following com-		
	mands)		
Storing Interval =005/010	Define the storing interval with storing inter-		
	val = 10 s and averaging interval = 5 s		
Interval=300	Allows the system and plant to settle		
	Start of light curve		
PARtop =1500	Set the light intensity (first step of the light		
1	curve)		
Interval =600	An interval of 600 s will elapse before the next		
	command is carried out in order to give the		
	CO <sub>2</sub> and H <sub>2</sub> O control some time to stabilize		
	and to give the leaf time to adapt to the first		
	light intensity.		
	-		

First measure	a Zero	Point ZPi	as follows
---------------	--------	-----------	------------

Auto ZP =060/010 Switches solenoid valves to mode ZP, purges

60 s, measures ZP with averaging time of 10s,

switches mode back to MP

Measuring 3 Measuring Points for the first

light step:

Interval =5 Interval of 5 s will elapse

Store MP ZP Data point with 5 s averaging (first number in

storing interval).

Interval =5

Store MP ZP

Interval =5

Store MP ZP

Next light step

PARtop = 1200 Set the light intensity to the next step of the

light curve

Interval =600 An interval of 600 s will elapse without stor-

ing, during which the leaf can adapt to the new

light intensity

Measuring 3 Measuring Points for the next

light step

Interval =5 Interval of 5 s will elapse

Store MP ZP Data point with 5 s averaging (first number in

storing interval) will be taken.

Interval =5

Store MP ZP

Interval =5

Store MP ZP

. . .

A CO<sub>2</sub> curve can be written very similar to the light curve. Instead of the light intensity the CO<sub>2</sub> concentration is changed step by step. As long as the

light curve does not take to long, it is sufficient to measure one Zero Point in the beginning of the light curve. In the case of the  $CO_2$  curve a ZP must be measured after each change of the  $CO_2$  concentration.

#### Programming Example for Usage with Imaging-PAM 9.1.8

### **Program Listing**

## Remark =----Settings Timing----

Storing Interval =005/060

1Yield/y\*MP = 3

Remark =----Settings Meas. Head-

Impeller =5Set Tcuv = 25.0

PARtop = 0

Remark =----Settings Central Unit-

Set Flow =750Set CO2 = 380

Set H2O(ppm) = 20000

Mode = MP

Remark =----Settings End----

Interval =300

Auto ZP = 010/060

#### Comments

Internal Comment

Dfine the storing interval storing interval 60s and aging interval 5s (only active Command start storing) Defines how often a Saturating light pulse shall be given. Here every 3<sup>rd</sup> Meas-

uring Point.

Settings for Measuring

Head

Indicates that PAR of Imaging-PAM will be measured with upper light sensor. Light is still off. If the Imaging-PAM is connected the unit for the Light control is steps and ranges from 0-20. Settings for Central Unit,

Interval, for system and plant to stabilize, dark

adapt....

note order

The system will be switch to ZP mode and a zero point will be taken. Alternatively the Program run could be finished here and a zero

point with empty cuvette

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d be performed manu
Then the next steps
ld have to be in a dif-
nt Program-File.
ger an Fo and Fm-
surement
ger an Absorptivity

Abs trigger an Absorptivit

Measurement

Start storing From now on values will be stored with the storing and averaging interval set in the beginning of the program

Remark =----Light curve---

Interval =60 PARtop =10

first measure respiration measure with the light intensity the leaf has been grown in

Return to normal light envi-

Interval =1200

A long interval to give the dark adapted leaf time to accumulate RuBP, activate Calvin Cycle enzymes and

open stomates.

PARtop =8 decrease light values to continue with light curve.

Interval =360

. . .

**FvFm** 

PARtop =2 Interval =360 PARtop =8

ronment

Interval =900 Leave again a long interval after dark values

PARtop =10 Continue with light curve Interval =420

...

PARtop =20 Interval =420 Stop storing Remark =----CO2 curve

PARtop = 10

CO<sub>2</sub>-Curve

Return to normal light envi-

ronment

Interval =600

Start storing

Start storing

Measure CO<sub>2</sub> curve

The long purge times for Auto ZP are to make sure that new CO<sub>2</sub> concentration

is stable before a ZP is

measured.

Set CO2 = 50

Auto ZP =005/180

Interval =360

Set CO2 =1500

Auto ZP =005/180

Interval =360

Remark =----System off---

H2O Control off

CO2 Control off

Set Flow =0

TempControl off

Light Control off

Impeller =0

Remark =----Settings End----

End of CO<sub>2</sub> curve Switch everything off

# 10 Calculations

# 10.1 Parameters for Calculations

The following table (Table 5) lists the parameters integrated in the formulas and directly assessed by the GFS-3000, a short description of the parameters and their unit.

Table 5: Parameters used for calculation

Measured	Description	Unit
value		
Area/Weight	Reference value of the sample used for calculations	cm <sup>2</sup> /mg
CO2abs	CO <sub>2</sub> mole fraction in reference cell of analyzer, equal to CO <sub>2</sub> concentration at inlet of cuvette.	ppm
CO2sam	CO <sub>2</sub> 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 Mode ZP)	ppm
dCO2MP	= CO2sam <sub>(t)</sub> - CO2abs <sub>(t-CO2delay)</sub> (in Mode MP)	ppm
H2Oabs	H <sub>2</sub> O mole fraction in reference cell of analyzer, equal to H <sub>2</sub> O concentration at inlet of cuvette.	ppm
H2Osam	H <sub>2</sub> O mole fraction in sample cell of analyzer.	ppm
dH2OZP	= H2Osam - H2Oabs (in Mode ZP)	ppm
dH2OMP	= H2Osam - H2Oabs (in Mode MP)	ppm
Pamb	Ambient barometric pressure	kPa
Flow	Gas flow entering cuvette	μmol/s
Teuv	Air temperature within cuvette	°C
Tleaf	Leaf temperature	°C

#### 10.2 Calculation of CO<sub>2</sub> Mole Fraction in the Cuvette – (ca)

For the Standard Measuring Head 3010-S, the  $CO_2$  mole fraction in the cuvette and around the sample (ca) corresponds to the  $CO_2$  mole fraction at the outlet of the cuvette. It is measured in the sample side of the analyzer (CO2sam). The value (CO2sam) needs to be corrected for the differential zero-value (dCO2ZP). The  $CO_2$  mole fraction in the cuvette (ca) is calculated as follows:

$$(1) ca = CO2sam - dCO2ZP$$

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

# 10.3 Calculation of the Differntial CO<sub>2</sub> Mole Fraction in Measure Mode MP – (dCO2MP)

With the Standard Measuring Head 3010-S, the differential CO2 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 travelling through the measuring head arrives later in the analyzer than the gas travelling through the reference. For the differential  $CO_2$  measurement this time difference (CO2delay) is taken into account.

(2) 
$$dCO2MP_{(t)} = CO2sam_{(t)} - CO2abs_{(t-CO2delay)}$$

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

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

(3) 
$$dCO2MP_{(t)} - dCO2ZP = ca_{(t)} - CO2abs_{(t-CO2delay)}$$

#### 10.4 Calculation of H<sub>2</sub>O Mole Fraction in the Cuvette – (wa)

The  $H_2O$  mole fraction in the chamber (wa) is calculated in the same way as the  $CO_2$  mole fraction (ca). It is the actual  $H_2O$  concentration within the chamber and around the sample (wa) and is measured at the outlet of the chamber in the sample side of the analyzer.

$$(4) wa = H2Osam - dH2OZP$$

# 10.5 Calculation of the Differntial H<sub>2</sub>O Mole Fraction in Measure Mode MP − (dH2OMP)

With the Standard Measuring Head 3010-S, the differential H<sub>2</sub>O mole fraction (dH2OMP) is the difference between the H<sub>2</sub>O mole fraction in the sample side (CO2sam) and in the reference side (CO2abs) of the analyzer. Since the H<sub>2</sub>O concentration changes only slowly in comparison to CO<sub>2</sub>, the time delay is not taken into account for the calculation of dH2OMP as for dCO2MP.

(5) 
$$dH2OMP_{(t)} = H2Osam_{(t)} - H2Oabs_{(t)}$$

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

(6) 
$$dH 2OMP_{(t)} - dH 2OZP = wa_{(t)} - H 2Oabs_{(t)}$$

# 10.6 Calculation of Relative Humidity - (rh %)

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

(7) 
$$rh = \frac{Actual\ Vapor\ Pr\ essure}{Saturation\ Vapor\ Pr\ essure}$$

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

(8) 
$$rh = \frac{wa * Pamb}{SVP(Tcuv)}$$

Whereby:

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

# 10.7 Calculation of 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<sup>-2</sup> s<sup>-1</sup>],

 $u_e = \text{molar flow rate at the inlet of the cuvette } [\mu \text{mol s}^{-1}],$ 

 $w_0 = H_2O$  mole fraction at the outlet of the cuvette [ppm],

 $w_e = H_2O$  mole fraction at the inlet of the cuvette [ppm],

 $LA = leaf area [m^2].$ 

Note that ppm means ( $\times$  0.000001)

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

(10) 
$$u_e = Flow$$

$$(11) w_o - w_e = dH 2OMP - dH 2OZP$$

$$(12) w_o = wa$$

$$(13) LA = Area$$

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

(14) 
$$E = \frac{Flow*(dH2OMP - dH2OZP)}{Area*(1-wa)}$$

# 10.8 Calculation of 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 cuvette [ppm].

The intercellular  $H_2O$  concentration ( $w_i$ ) is calculated from the temperature of the leaf (assuming 100% humidity in the intercellular space):

(16) 
$$w_i = \frac{SVP(Tleaf)}{P_{CUV}}$$

Whereby:

SVP (Tleaf) = saturation vapor pressure at Tleaf calculated according to Goff-Gratch [kPa] (*see* 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:

$$(17) Pcuv = Pamb$$

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

(18) 
$$VPD = \frac{\left(\frac{SVP(Tleaf)}{Pamb} - wa\right)}{1 - \frac{\left(\frac{SVP(Tleaf)}{Pamb} + wa\right)}{2}}$$

#### 10.9 Calculation of Water Vapor Conductance - (GH2O)

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

$$(19) GH2O = \frac{E}{VPD}$$

where

GH2O = total water vapor conductance [mmol m<sup>-2</sup> s<sup>-1</sup>],

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

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

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

#### 10.10 Calculation of Assimilation Rate - (A)

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

(20) 
$$A = \frac{u_e * (c_e - c_o)}{I \Lambda} - E * c_o$$

Whereby

A = assimilation rate  $[\mu \text{mol m}^{-2} \text{ s}^{-1}]$ ,

 $u_e = \text{molar flow rate at the inlet of the cuvette } [\mu \text{mol s}^{-1}],$ 

 $c_0 = CO_2$  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<sup>2</sup>],

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

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

$$(21) u_{e} = Flow$$

$$(22) c_{e} - c_{o} = dCO2ZP - dCO2MP$$

$$(23) c_o = ca$$

$$(24) LA = Area$$

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

#### 10.11 Calculation of Intercellular CO<sub>2</sub> Mole Fraction – (ci)

According to Caemmerer and Farquhar (1981) the internal CO<sub>2</sub> mole fraction ci is calculated as follows - ci:

(26) 
$$c_{i} = \frac{(g_{CO2} - \frac{E}{2}) * c_{a} - A}{g_{CO2} + \frac{E}{2}}$$

#### Whereby

 $c_i$  = intercellular  $CO_2$  mole fraction [ppm],

 $g_{CO2}$  = conductance for  $CO_2$  [mmol m<sup>-2</sup> s<sup>-1</sup>],

E = transpiration rate [mmol m<sup>-2</sup> s<sup>-1</sup>],

 $c_a = CO_2$  mole fraction in the cuvette [ppm],

A = assimilation rate [ $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>].

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

$$GCO2 = \frac{GH2O}{1.56}$$

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

Note that the value given for the intercellular CO<sub>2</sub> concentration (ci) generally has no meaning with closed stomates or only slightly open stomates, since a division by zero or close to zero takes place.

#### 10.12 Fluorescence Calculations - Optional

The following parameters are calculated from the fluorescence values Fo, Fm, F, Fo' and Fm'and the photosynthetic active radiation PARtop, PARbot or PARamb (depending on the mode):

The photosynthetic yield indicates the quantum efficiency of photosystem II (Genty et al. 1989, for a mathematical derivation see Schreiber et al. 1995 or Schreiber 2004).

$$(29) YIELD = (Fm'-F) / Fm'$$

The electron transport rate through PSII is calculated with the following equation:

(30) ETR = YIELD \* 
$$PAR/2 * ETR$$
-Factor

whereby:

PAR: photosynthetic active radiation is divided by two, because it is reasonable to assume, that the absorbed light is equally distributed be-

tween photosystem I and II.

ETR-Factor is the total light absorption of the leaf.

Fo' is either measured (Fo' Mode on) or calculated following the relationship published by Oxhorough and Baker (1997).

(31) 
$$Fo' = Fo / (1 - Fo/Fm + Fo/Fm')$$

The photochemical quench is an empirical indication for the fraction of open photosystems and calculated using Fo' (Schreiber et al. 1986 as formulated by van Kooten and Snel, 1990):

(32) 
$$qP = (Fm'-F) / (Fm'-Fo')$$

The coefficient of photochemical fluorescence quenching assuming interconnected PSII antennae is calculated according to Kramer et al, 2004:

(33) 
$$qL = qP (Fo'/F)$$

The non-photochemical quench is defined as the fluorescence quenched by other processes than photochemistry. There are two different approaches in the literature. qN, where the non-photochemical fluorescence quench is determined in relation to the maximal variable fluorescence (Schreiber 1986; van Kooten and Snel 1990), or NPQ, where the non-photochemical fluorescence quench is determined in relation to the remaining maximal fluorescence (Bilger and Björkman 1990). qN has the advantage, that it ranges between 0 and 1. NPQ has the advantage, that it does neither require the determination of Fo nor Fo'.

(34) 
$$qN = 1 - (Fm'-Fo') / (Fm-Fo)$$

(35) 
$$NPQ = (Fm/Fm') - 1$$

Note: GFS-win Versions older than Jan 2013 do not calculate Fo'. If Fo' Mode is switched off qP and qN are calculated by equations 36 and 38. Panel PCs of the GFS-3000 Model GFS-3000-C calculate qP and qN by equations 32 and 37 if Fo' is determined (Fo' Mode on) otherwise Fo is used for the calculations (equations 36 and 38). Fluorescence parameters can be recalculated during file transfer with equation 31- 35 by activating the checkbox "Recalculate fluorescence parameters ..." (see chapter 8.6.1.2).

(36) 
$$qP = (Fm'-F) / (Fm'-Fo)$$

(37) 
$$qN = (Fm-Fm') / (Fm-Fo')$$

(38) 
$$qN = (Fm-Fm') / (Fm-Fo)$$

# 11 Values and Ranges

Table 6: Values and ranges

Value	Definition	Range, options	Unit, format
Date	Date or date of external PC (inter-		yyyy-mm-dd
	national standard date notation)		
Time	System time or time of external PC	00:00:00 -23:59:59	hh:mm:ss
	(international standard time nota-		
	tion)		
Code	Position of solenoid valves	MP, ZP	String
Object	Object number, for differentiation	0001-9999	#
	of several objects within one report		
	file		
Area or	Reference value of the sample	Area: .01999.99,	cm <sup>2</sup> or mg
Weight	used for calculations	Weight: 1999999	
Status	Operating status of components		String
CO2abs	CO <sub>2</sub> mole fraction in reference cell	0 3000	ppm
	of analyzer, equal to CO <sub>2</sub> concen-		
	tration at the inlet of cuvette.		
CO2sam	CO <sub>2</sub> mole fraction in sample cell	0 3000	ppm
	of analyzer (usually not shown in		
	the software)		
dCO2ZP	= CO2sam - CO2abs	-99.99 +99.99	ppm
	(in Mode ZP)		
dCO2MP	$= CO2sam_{(t)} - CO2abs_{(t-CO2delay)}$	-99.99 +99.99	ppm
	(in Mode MP)		
H2Oabs	H <sub>2</sub> O mole fraction in reference cell	0 75000	ppm
	of analyzer,		
	equal to H2O concentration at the		
	inlet of cuvette.		
H2Osam	H <sub>2</sub> O mole fraction in sample cell	0 75000	ppm
	of analyzer (usually not shown in		
	the software)		
dH2OZP	= H2Osam - H2Oabs	-60000 +60000	ppm
	(in Mode ZP)		
dH2OMP	= H2Osam - H2Oabs	-60000 +60000	ppm
	(in Mode MP)		
Flow	Gas flow through the cuvette	-75 +1500	μmol/s
Pamb	Ambient barometric pressure	60 110	kPa
Aux1	Input signal of an additional sensor	0 4095	mV
	connected to AUX IN		
Aux2	Input signal of an additional sensor	0 4095	mV
	connected to AUX IN		
Tcuv	Cuvette temperature measured in	-10 +50	°C

Value	Definition	Range, options	Unit, format
	lower air exit		
Ttop	Cuvette temperature measured in	-10 +50	°C
	upper air exit		
Tleaf	Leaf temperature	Tcuv-30	°C
		Tcuv+30	
Tamb	Ambient temperature	-10 +50	°C
PARtop	Photosynthetic active radiation	0 2400	$\mu$ mol/(m <sup>2</sup> *s)
	measured with sensor in upper		
	cuvette half		
PARbot	Photosynthetic active radiation	0 2400	$\mu$ mol/(m <sup>2</sup> *s)
	measured with sensor in lower		
	cuvette half		
PARamb	Ambient photosynthetic active	0 2400	μmol/(m <sup>2</sup> *s)
	radiation measured with external		
	sensor MQS-B/GFS		
Imp	Set value for impeller	0 9	steps
Tmin	Minimum or maximum tempera-	-10 +50	°C
	ture (measured near Peltier ele-		
	ment instead of Ttop in Standard		
	Measuring Head 3010-S Version 1		
	or in Gas Exchange Chamber		
	3010-GWK1)		
rH	Relative humidity in the cuvette	calculated	%
Е	Transpiration rate	calculated	mmol/(m <sup>2</sup> *s)
VPD	Vapor pressure deficit between	calculated	Pa/kPa
	object (leaf) and air		
GH2O	Water vapor conductance	calculated	mmol/(m <sup>2</sup> *s)
A	Assimilation rate	calculated	$\mu \text{mol/(m}^2*\text{s})$
ci	Intercellular CO <sub>2</sub> mole fraction	calculated	ppm
ca	CO <sub>2</sub> mole fraction in the cuvette	calculated	ppm
	(=CO2sam-dCO2ZP)		
wa	H2O mole fraction in the cuvette	calculated	ppm
	(=H2Osam-dH2OZP)		
Optional			
Fo	Fluorescence of the dark-adapted	04000, should	mV
	leaf with only the measuring light	range between 100	
	on	and 600	
Fm	Fluorescence of the dark-adapted	04000	mV
	leaf during a saturating light pulse		
Fv/Fm	Maximal quantum efficiency of	00.84	
	photosystem II (Kitajima and		
	Butler 1975 and Butler and Kita-		
	jima 1975)		

Value	Definition	Range, options	Unit, format
F	Fluorescence	0 4000	mV
Ft	transient Fluorescence in Chart	04000	mV
Fm'	Fluorescence of the illuminated	04000	mV
	leaf during a saturating light pulse		
Fo'	Fluorescence of reoxidized photo-	04000	mV
	system II illuminated with only far		
	red illumination and measuring		
	light after a saturating light pulse.		
Yield	Quantum yield of photosynthetic	00.85	
	electron transport (Genty et al.		
	1989, for a mathematical deriva-		
	tion see Schreiber et al. 1995)		
ETR	Electron transport rate (see chapter		μmol/(m² s)
	10)		
qN	non photochemical quenching	01	
	(Schreiber et. al. 1986)		
qP	photochemical quenching (Schrei-	01	
	ber et. al. 1986)		
NPQ	non photochemical quenching	010	
	(Bilger and Björkman, 1990)		
ETR-Fac	Factor used to calculate the elec-		
	tron transport. Coresponds to the		
	proportion of light absorbed by		
	photosystems. The value needs to		
	be entered by the user. Or can be		
	measured with the Imaging-PAM		
	(Absorptivity)		

# 12 Data Record Structure

Each file starts with two header lines. The first line gives the description of each column; the second line shows the unit or the format. After the two header lines, data records follow, which consist of information, measured and calculated values. Each column is explained in the following. The text boxes include the two fields of the header lines and one or more values.

Date
yyyy-mm-dd
2005-04-08

The date is stored using the international standard date 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. If the file is imported into a spread sheet program e.g. Excel, the appearance of the date depends on the format selected by the spread sheet program.

Time
hh:mm:dd
23:14:07

The time is stored using 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 start of the hour (00-59), and ss is the number of complete seconds since the start of the minute (00-59). If the file is imported into a spread sheet program e.g. Excel, the appearance of the time depends on the format selected by the spread sheet program.

Code
String
ZPc001
ZPi005
MP_010

The first two characters of Code define whether the stored data record is a Zero point "ZP" or a measuring point "MP". In case of ZP the third character 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 mode MP, "i" means that ZP was measured with the solenoid valves being switched to mode ZP. The last three digits indicate the number of data points, which have been averaged for this data record. If the

averaging was disturbed there is err instead of a number. The value should still be correct but has less averaged values.

Object
#
107

Object number between 1 ... 9999 for differentiation of several samples.

Area	
cm <sup>2</sup>	
8	

Weight	
mg	
1000	
	Ī

Reference type *Area* in cm<sup>2</sup> or *Weight* in mg, which is selected when a new file is created. Within one file the reference type cannot be

mixed, since the header is valid for the complete file. The units of the calculated parameters A, E and GH2O depend on the selected reference type.

Status
String
AFF1FF612FF1FF

In the *repor*t, the complete status string is stored, in the *values* window only the statuscharacters of the components are displayed

"FF" = component is functioning, "--" = component is not connected, neither "FF" nor "--" = error code of the component (*see* chapter 15.3).

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

Character 5, 6: Status of central control (FF = ok)

Character 7: CO<sub>2</sub> supply (bar)

Character 8: Type of measuring head Character 9: Version measuring head

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

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 (see chapter 8.6.4).

CO2abs
ppm
350.6

CO<sub>2</sub> mole fraction measured in the CO<sub>2</sub> reference cell of the analyzer, which corresponds to the CO<sub>2</sub> mole fraction at the entrance of the cuvette.

dCO2ZP
ppm
0.491816

Difference between CO<sub>2</sub> 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 has been meas-

ured if the record-set is a measuring point (MP) the value is carried over from last ZP measurement.

dCO2MP
ppm
-2.499774

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

filled with the measured dCO2MP value.

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<sub>2</sub>O sample and reference cell of the analyzer: If the record-set is a zero point ZP, the field dH2OZP is filled with the measured dH2OZP value. If the

record-set is a measuring point (MP), the indicated value has been carried over from the last measured dH2OZP-value.

dH2OMP
ppm
819.1

Difference between  $H_2O$  sample and reference cell of the analyzer: If the record-set is a zero point (ZP), the field dH2OMP is filled with "----". If the record-set is a measuring

point (MP), the field dH2OMP is filled with the measured dH2OMP value.

Flow
µmol/s
749.6

Air flow through the cuvette measured by 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 (*see* chapter 16.1). The input voltage for Aux1 is 0 ... 4095 mV. A linear function (offset and gain) can be applied

to the input signal with a command in a user-program.

Aux2
mV
137

Input signal in mV of a sensor connected to Aux2 of AUX IN (see chapter 16.1). The input voltage for Aux2 is 0 ... 4095 mV. A linear function (offset and gain) can be applied

to the input signal with a command in a user-program..

Tcuv
°C
25.01567

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

Tleaf
°C
24.92682

Leaf temperature measured by a thermocouple in the lower part of the cuvette of the Standard Measuring Head 3010-S.

Tamb
ô
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

Photosynthetic active radiation (PAR) measured in the upper part of the cuvette of the Standard Measuring Head 3010-S by a light sensor type LS-A (*see* chapter 16.6 for spectral

sensitivity).

PARbot µmol/(m²\*s) 3.274362

sensitivity).

Photosynthetic active radiation (PAR) measured in the lower part of the cuvette of the Standard Measuring Head 3010-S by a light sensor type LS-A (see chapter 16.6 for spectral

PARamb µmol/(m²\*s) 5.625673 Photosynthetic active radiation (PAR, since the MQS is cosine corrected also PPFD="photosynthetic photon flux density" is a correct denotation) measured on top of the up-

per part of the cuvette of the Standard Measuring Head 3010-S by a light sensor type MQS-B/GFS (*see* chapter 16.5 for spectral sensitivity).

Imp	
step	
5	

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

Ttop
Ŝ
24.74489

Temperature measured by a Pt100 sensor in the exit of the upper part 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 recognized, rh is calculated as described in chapter 10.6, otherwise the field rh is filled with "----".

Е
mmol/(m <sup>2*</sup> s)
0.941

Transpiration rate of the enclosed sample. If a zero point ZP is stored, the field E is filled with "----". If a measuring point MP is stored, E is calculated as described in chapter 10.7.

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 recognized, VPD is calculated as described in chapter 10.8, otherwise the field VPD is filled with

"----"

Water vapor conductance of the enclosed sample. If a measuring point MP is stored and the sensors for Tcuv and Tleaf are recognized, GH2O is calculated as described in chapter 10.9, otherwise the field GH2O is filled with "----".

Α
µmol/(m²*s)
1.57

Assimilation rate of the enclosed sample. If a zero point ZP is stored, the field A is filled with "----". If a measuring point MP is stored, A is calculated as described in chapter 10.10.

ci
ppm
300.3

Intercellular CO<sub>2</sub> mole fraction in the enclosed sample. If a measuring point MP is stored and sensors for Tcuv and Tleaf are recognized, ci is calculated as described in chapter 10.11, otherwise the field ci is filled with "----".

ca
ppm
348.1

CO<sub>2</sub> mole fraction in the cuvette of the measuring head. If a zero point ZP is stored, the field ca is filled with "----". If a measuring point MP is stored, ca is calculated as described in chapter 10.2.

wa
ppm
17000

 $H_2O$  mole fraction in the cuvette of the measuring head. If a zero point ZP is stored, the field wa is filled with "----". If a measuring point MP is stored, wa is calculated as described in chapter 10.4.

comment
whatsoever

comments which have been entered in the comment line before a storing event are stored here. 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 in the dark - or nearly dark since some measuring light is required. It is measured with the button *Store Fv/Fm +MP* or the command Fv/Fm. Fo should only

be measured with a dark adapted sample.

Fm	
mV	
1604	

Fm is the maximal fluorescence. It is measured together with Fo with the button  $Store\ Fv/Fm + MP$  or the command Fv/Fm on a dark adapted sample.

Fv/Fm	
0.806	

Fv/Fm is calculated from Fo and Fm and corresponds to the yield in the dark adapted state as described in chapter 10.12. It is the maximal yield.

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. 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 if the fluorescence module 5033FL is used and Fo'-mode is switched on. With the Imaging-PAM it is a calculated value (chapter 10.12).

Yield	
0.186	

Yield is a value calculated from F and Fm' (chapter 10.12).

ETR
46.77

ETR is calculated from the Yield value, ETR-Fac and PAR. The PAR used for calculating ETR is determined by the light control mode. ETR-Fac depends on the absorptivity of the

leaf.

qΡ	
1.3400	1

calculated value (chapter 10.12.).

qN	calculated value (chapter 10.12.).
0.9613	

NPQ	calculated value (chapter 10.12). When comparing NPQ
mV	values from the record file of the GFS-Win software and the
3.443	report-file of the ImagingWin software, note that the NPQ
	report-the of the imaging will software, note that the IVIQ

value in ImagingWin is divided by 4.

ETR-Fac	The ETR-factor needs to be measured for every sample and				
	entered manually. It is measured best with an Ulbricht sphe				
0.84	(not produced by the Heinz Walz GmbH). If an Imaging-				
	THOU DIOLUCCU DY LIIC HCHIZ WAIZ CHIDHIJ. H AH HHAYHIY-				

PAM is used, the value indicated as ETR-Fac is the result of the absorptivity measurement.

# 13 Maintenance

# 13.1 Replacing Drier, Humidifier or CO<sub>2</sub> Absorber Material



For removing the absorber tubes from the front panel press the clamp button at the connectors and pull the absorber tube forward off the holder. The lower lid is fixed by a tape. To open the absorber tube, use the upper lid. It can be removed with sideward turning movements. Do not use the connector as lever for opening the lid, it will damage the tube. Before closing the absorber tube, please take care that the tube

and the seals are clean. Turn the lid until the connection pipe fits properly into the notch of the tube. The filter pad at the top and bottom of the absorber material should be inserted, so that they are facing with the fine side towards the absorber material.

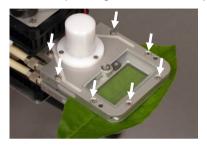
The drier contains silica gel (Sorbead Orange Chameleon, Engelhard), a  $H_2O$  absorber, which has an orange color in the dry state and turns colorless at approx 6 % (w/v) water content. The material can de reused after drying in the oven (130-140 °C 8h, alternatively use a micro wave oven, pay attention to chapter 16.4.2). Note that if the material is burnt, it may look dark despite being wet.

For humidification a ceramic granulate (Stuttgarter Masse) is used. It needs to be wetted with distilled water, if there are no water droplets visible at the tube wall. Please pay attention that no spare water assembles at the bottom of the tube. To prevent this, add the water slowly, so that it can be taken up by the granulate and do not add too much water. Shake any excessive water out before connection. The ceramic granulate can be autoclaved to prevent the growth of algae. The completely dry column has a capacity of circa 20 ml water (pay attention to chapter 16.4.3).

Soda lime (Merck) is used as CO<sub>2</sub> absorber. It changes its color from colorless to violet. Note, that the color change may not work in a dry at-

mosphere. Also over night, CO<sub>2</sub> diffuses into inside of the granules leaving the outside change back to white. Nevertheless, the absorbing capacity is exhausted with the first color change and needs to be replaced. Since the substance is corrosive, please pay attention to the R- and S-Phrases and disposal advice in the safety data sheet (pay attention to chapter 16.4.1).

# 13.2 Replacing Leaf Area Adapters

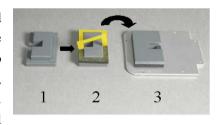


The measured leaf area can easily be adapted with different leaf area adapters. There are 8 screws per cuvette frame holding the area adapter in place. They need to be unscrewed for detachment. When attaching the new one, make sure, that the blue o-rings underneath the leaf

area adapters are properly in their place so that they give a good sealing. Tighten the screws evenly. Note that the metal of the screws is harder than the metal of the adapter plates. Therefore tighten the screws gently to prevent destruction of the thread. The o-ring underneath the adapter plates provides airtight sealing. If the threads of the leaf area adapters are worn out, they need to be replaced.

#### 13.3 Replacing Foam Gaskets

The gaskets can exactly be fitted with the provided mounting plate. The leaf area adapters can remain fixed to the cuvette frames for this procedure, do not unscrew them unnecessarily. Remove the old gaskets. Eucalypt oil



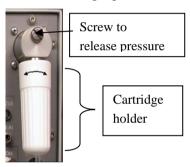
can be used to carefully remove any remaining glue. Take the mounting plate (1) and put the gasket on it, pull off the protective film (2) and press it to the leaf area adapter (3). Consider that the gaskets are always mounted on the non-chamfered side of the leaf area adapter.

#### 13.4 Replacing Filters



The filters for PUMP, CUVETTE and AIR IN on the front panel can be removed by pressing the clamp button at the connectors. Slightly turn and pull the filter out of the pneumatic adapters. One spare filter is supplied with the Spare kit 3000-C/SK. Please pay attention to the flow direction, marked on the filter and on the front panel behind the filter. The orientation of the filter is so that the dirt can be seen.

# 13.5 Exchanging CO<sub>2</sub> Cartridges or Releasing CO<sub>2</sub> Pressure



The CO<sub>2</sub> cartridge serves to fill a CO<sub>2</sub> container of the CO<sub>2</sub> control. When the warning appears that the CO<sub>2</sub> pressure is low switch the CO<sub>2</sub> control off, detach the white CO<sub>2</sub> cartridge holder by screwing it counter clockwise and dispose the empty CO<sub>2</sub> cartridge. Insert a new cartridge into the holder and screw it on again. A gentle

fizzle can be heard. Switch the CO<sub>2</sub> control back on.

The warning for the cartridge change appears, when the CO<sub>2</sub> control is running and the pressure in the vessel drops below 250 kPa. After the first warning the CO<sub>2</sub> control can still run for a few hours. Do not add another CO<sub>2</sub> cartridge before the warning has appeared. Each cartridge adds 600 kPa. The maximum pressure the CO<sub>2</sub> supply can hold is 950 kPa. Above 1000 kPa (10 bar) a safety valve attached to the CO<sub>2</sub> supply vessel within the system will vent the over-pressure through the outlet VENT.

The pressure within the CO<sub>2</sub> supply can be released by carefully opening the screw at the front of the CO<sub>2</sub>-control. Protect your eyes; since the screw may be propelled out, when turned to far. Also the seal-ring may burst. Before closing the screw, open it so far that the seal-ring can pull back into the groove of the screw.

The pressure within the  $CO_2$  supply is indicated in GFS-Win under  $Menu \rightarrow Status$ . On the panel-PC of the Control Unit Model 3000-C version 1.04 or higher it is indicated under *Option2*.

Please note, when planning experiments, that  $CO_2$  cartridges (article number: 000160103430) have been classified as dangerous goods; and only surface transport is allowed. Therefore, we recommend buying compatible  $CO_2$  cartridges directly from the super market, if possible. 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<sub>2</sub>). Do not use other cartridges than cartridges for food supply, since those may contain oil. We recommend soda chargers from ISI GmbH, Wien (http://www.isi.at/consumer/products/chargers/en/). In some countries these cartridges can be obtained from Amazon. Table 7 gives a list of suppliers for ISI cartridges world-wide.

Table 7: List of suppliers for ISI-cartridges world-wide:

Australia	Pearl Int. Pty. Ltd.	+618 / 8271 2616	+618 / 8271 1396	pearlint@tne.net.au
Brazil	Italian Coffee do Brasil Ltda.	+5511 / 3357 8093	+5511 / 3223 1155	janaina@italiancoffee.com.br
Canada	Jascor Housewares	+1 (905) 477 5420	+1 (905) 4777424	cshipton@salton.com
	Jose Rivero Llamazales y Cia			
Chile	Ltda.	+56 (2) 698 9342	+56 (2) 672 8172	frivero@elvolcan.cl
China	Angelhood	+86 (21) 51691855	+86 (21) 51687575	eileen@angelhood.com.cn
France	Matfer (Food Service)	+331 43626040	+331 43625082	fbidoux@matfer.fr
India	Transworld Enterprises	+91 (11) 29232848	+91 (11) 29237693	transwenterprises@hotmail.com
Italy	Trabo S.R.L.	+3902 58014389	+3902 / 5801 5783	traboslr@tin.it
Japan				
Korea	SUFECT CORPORATION	+82-2-400-2038	+82-2-400-2089	sufect@kita.net
Mexico	Import. Y Export. Sifon's SA	+5255 / 5319 8563	+52 (55) 2626 4841	martha@sifons.com
Netherlands	Interhal	+31 (78) 699 2990	+31 (78) 699 2998	vanderborgt@interhal.nl
New Zealand	Safco Ltd.	+649 / 274 9019	+649 / 274 6741	info@safco.co.nz
	Schmidt-Stosberg Lda.	+35 (1) 219 24 22 82	+35 (1) 21923 49 78	info@s-stos.com
Russia	Complex Bar	+7 (495) 980 68 68	+7 (495) 980 68 66	elena.n@complexbar.ru
Singapore	Butler-Mason	+65 / 67521191	+65 / 67521311	sales@butler-mason.com
South Africa	BCE	+2711 4334301	+2711 4334587	apostolos@bce.co.za
Sweden	Creative Design (Household)	+46 (8) 642 18 04		jonas@creativedesignab.eu
Taiwan	Haru International Corp.	+8862 / 2766 6547	+8862 / 2766 5920	haru.cafe@msa.hinet.net
USA	iSi North America, Inc.	+1 973 / 227 2426	+1 973 / 227 9140	customerservice@isinorthamerica.com

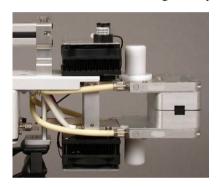
#### 13.6 Exchanging Cuvettes

#### 13.6.1 Mounting the Cuvette for Conifers 3010-V80



For exchanging the standard leaf area adapters against the conifer cuvette, it is necessary to disassemble the hinge that holds the lower cuvette frame. Two allen screws fix each

distance holder of the hinge. They need to be unscrewed.

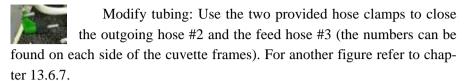


Now the short extension rod of the closing mechanism needs to be exchanged with the longer one. The extension rod sits on an arm of the lower cuvette frame and extends to the closing mechanism in the handle. To release the extension-rod, pull the lower unscrewed cuvette frame forward. This may require some sleight of hand, since

the electronic cords, which lay in a loop in the space above the electronic box, need to be pulled out with some force. When the extension rod of the measuring head becomes free, it falls out through the drill hole of the tripod adapter. No tool is required. (Do not! unscrew any screw of the external heat exchangers, also avoid any stress on the external heat exchangers). Insert the long extension-rod of the conifer cuvette through the drill hole of the tripod adapter, so that the blunt end points towards the handle.

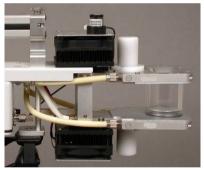
The leaf area adapters have to be removed by loosening the 8 screws on each side of the cuvette frame (see chapter 13.2). After unscrewing the adapter of the lower half, you may insert an additional metal angle underneath the holder of the thermocouple (see chapter 13.6.9). It bends the thermocouple upwards to bring it closer to the sample. The conifer cuvette halves have to be fixed to the cuvette frames similar as described for the

leaf area adapters (chapter 13.2). Afterwards the hinge of the lower cuvette frame can be fixed with the longer distance holders.



#### 13.6.2 Mounting the Cuvette for Lichens/Mosses 3010-V32



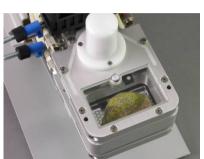


The assembling of the cuvette for lichens/mosses is very similar to the assembling of the cuvette for conifers. Please see there (chapter 13.6.1) and proceed in an analogous way, including the fitting of the hose clamps.

For mounting new gaskets to the adapter plates of the lichens/mosses cuvette, the cylindrical part can be used. Just put the gasket on the cylindrical body, pull off the protective film and press it against the cuvette plate. This can be done, before the plates are mounted, but also afterwards.

# 13.6.3 Rectangular Chamber for Lichens/Mosses (optional)





Alternatively to the cylindrical chamber also a rectangular chamber can be used for lichens or mosses. It can be mounted to one cuvette frame, while a flat adapter plate is mounted to the other frame. This set-up is similar to the conifer chamber, but requires shorter hinges and a shorter rod for the closing mechanism.

13.6.4 Positioning the Measuring Head Upside-Down

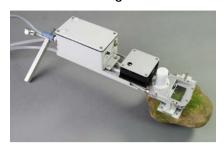


The Standard Measuring Head 3010-S can also be operated in upside-down orientation. In this orientation, it is possible to place the thermo-couple on the upper surface of the measured object.



For the upside-down orientation, screw off the back-plate at the handle; pull out the upper plastic stripe in the handle. Slide the handle over the nut of the screw in the stand (optional) and fasten it.

#### 13.6.5 Mounting the Cuvette for Flat Surfaces



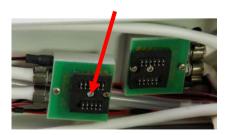
For measurements, on flat surfaces the Standard Measuring Head 3010-S can be assembled without upper cuvette frame. For that, it needs to be disassembled into three parts: electronic box, lower cuvette frame, and upper cuvette frame including the

handle. Additional mechanical parts are required, which are not included in the Standard Measuring Head 3010-S.





Disconnect the cable, and take the external light sensor off. Remove the four screws, which are holding the electronics box in place. Remove the electronics box from the measuring head. Take care that the tiny electronic pins will not bend or break, while pulling the box off. For that, do not push the box to any side, while pulling



Disconnect the plug of the lower cuvette frame by unfastening the indicated screw.



Remove the leaf area adapters, the hinges, and the fulcrum.





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 branching connection. The tube labeled "To" connects to inlet #3 while the tube labeled "From" connects to outlet #4.





When connecting the electronics box, take care not to bend or break any of the tiny electronic pins. Place the connecting block for the back-legs into the gap between the box and the plate and secure the box with all four











Close the cuvette frame with the leaf area adapter plate. Use two long screws for attaching the protruding plates. Connect the back-legs as shown in the first picture of this chapter.



Use a surface-sealing plate to obtain an 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.

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

#### 13.6.6 The Lazy Option for Measurements on Flat Surfaces

To avoid the complete rearrangement of the measuring head, the surface-sealing plate may be used directly at the Standard Measuring Head 3010-S, if the samples are flat and small. The opening of the other cuvette

frame can be covered with a closed adapter plate.





#### 13.6.7 Mounting the *Arabidopsis* Chamber 3010-A

First detach the Mini Quantum Sensor MQS-B/GFS using an 8 mm flat spanner. Remove the complete *tripod adapter*, including the *extension rod* for the closing mechanism (Do not! unscrew any screw of the external heat exchangers, also avoid any stress on the external heat exchangers.).



To detach the connection between the *lower* and *upper* cuvette frame, unscrew the four hexagon socket screws that fix the distance holders of the hinge take the distance holders off. Now lay the measuring head on its side in the provided foam stand so that the upright part of the foam stand fits into

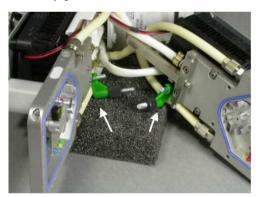
the handle. Detach the *leaf area adapters* by removing 8 screws on each side as shown in chapter 13.2. The position of the Tleaf sensor should be

modified as described in chapter 13.6.10, so that it will touch a leaf of the plantlets.



Now the Arabidopsis cuvette adapter angle can be mounted on the cuvette frames instead of the leaf area adapters using the same screws. The easiest way is to plug the cuvette adapter angle onto the black Arabidopsis pot holder and mount it in this position to

the cuvette frames. The thin plate of the *adapter angle* is mounted on the formerly top cuvette frame (now in the upright position) and the formerly lower cuvette frame is rotated 90 degrees and mounted on top of the *Arabidopsis cuvette adapter angle*, please pay attention that the hoses are not buckled. The cables form a loop inside the housing, so that they can be carefully pulled out for some additional centimeters.



Finally the tubing has to 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 mounted again.

For gas exchange measurements in the *Arabidopsis pot holder*, various sizes of flowerpots can be used (55 to 70 mm in diameter). With the help of the provided *height adjustable base plate*, the pot holder can be adapted to all these sizes. Adapting can be done easiest with an empty pot of the desired size.



Lay the *base plate* into the lower part of the *Arabidopsis pot holder* and place the pot in the middle of it. The adapter plate has the right elevation when the applied (but not screwed) 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. Taking the sample film as a standard the user can easily prepare additional sealing films (a hollow punch for the middle hole is provided in the set). 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.



To avoid measuring artifacts, resulting from the CO<sub>2</sub> produced in the soil that can pass through gaps of the sealing film, a *restrictor valve* has been built in the *Arabidopsis pot holder*. It can be adjusted with a screwdriver, so that air flows constantly outwards through the soil. For starting the

adjustment completely close the *restrictor valve* by right turns. Do the valve adjustment at the GFS-3000 (see chapter 13.10) with the closed measuring head and *Arabidopsis chamber* including a test plant or empty

pot sealed with a punched film. After the valve adjustment set the system into mode MP and open the *restrictor valve*, so that the bead of the flow indicator ANALYZER SAMPLE at the GFS-3000 drops by about 50-100 ml/min. Afterwards readjust valve 1 at the GFS-3000 until the flow indicators show the same height.

Note that now the light mode PARbot has to be chosen, since the light sensor of the lower cuvette frame needs to be used to regulate/measure the light module. Also the light source factor needs to be adjusted, when changing the setup of the cuvette (see chapter 13.11.3.2).

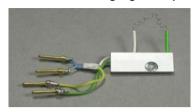
# 13.6.8 Mounting the Fiberoptics/PAM-Fluorometer 3050-F (optional) to the Standard Measuring Head



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 airtight adapter provided with the Fiberoptics/PAM-Fluoro-

meter (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).

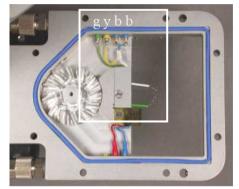
#### 13.6.9 Exchanging the Spare Thermocouple 3010-CA/TCL



lower cuvette frame.

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

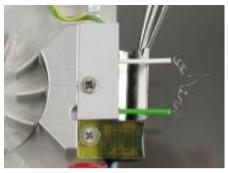
To access it, the lower cuvette frame needs to detached and the leaf area adapter needs to be taken off. See chapter 13.13 for an explanation on this procedure. The four pins (g,y,b,b) realize the electrical connection. In the graphic the colors of the thermocouple pins are from left to right: green (g), yellow

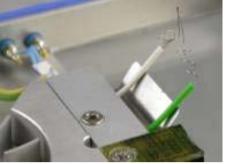


(y), blue (b) and blue (b) (the two blue cables can be interchanged). To remove the thermocouple, unscrew the aluminum plate and disconnect the pins. The pins sit very tight and a fine flat nosed-pliers or tweezers may be required. Insert the pins of the new sensor and mount the aluminum plate. Make 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  $Menu \rightarrow Calibration \rightarrow Measuring$   $Head \rightarrow Tleaf Offset [Counts]$ . For instructions see chapter 13.11.2.

### 13.6.10 Adjusting the Thermocouple (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.

## 13.6.11 Testing the Thermocouple

For testing the thermocouple attach the LED Light Source 3040-L or LED-Array/PAM-Fluorometer 2055-FL to the lower part of the measuring head. Insert a black piece of paper into the cuvette with the leaf thermocouple touching it. After setting the light-mode to PARbot switch on the light to  $100~\mu\text{mol}~\text{m}^{-2}~\text{s}^{-1}$  without turning on the flow and the impeller. An intact thermocouple shows a rise of leaf temperature of more than  $5^{\circ}\text{C}$  after switching the light to  $2000~\mu\text{mol}~\text{m}^{-2}~\text{s}^{-1}$ .

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

The Outdoor-Set 3000-C/OS consists of a 2,5 m fiberglass antenna, which carries the air intake and a 10 l mixing volume that is mounted underneath the GFS-3000. The mixing volume is fixed with a luggage net between the legs of the instrument and the short tube is connected with the pneumatic connector *air in* of the GFS-3000 instead of the 3000-C/EF filter, whereas the long tube is mounted on the antenna to suck in air 2,5 m above the ground.



For mounting the antenna the antennaholder has to be mounted at the rear right leg of the GFS-3000 (a). There the an-

tenna can be inserted. Make sure that the upper end of the tube is provided with a coarse filter to prevent contaminations to the instrument (b).



#### 13.8 Installation of Sun Screen - Optional





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

#### 13.9 Adjusting the Clip Mechanism for the Light Source



The Light Source 3040-L, LED-Array/PAM-Fluorometer 3055-L, Imaging-PAM *M-series* MINI-Head and the Darkening Plate 3010-DP are mounted on the upper or lower cuvette frame of the Standard Measuring Head 3010-S by clicking their pins into the clutches of each cuvette frame. To tighten this connection, the screws on the front of the cuvette frames can be turned (*see* picture).

#### 13.10 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 mode MP and ZP is tuned 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. a plastic-covered card-board or plastic-covered firm 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  $\mu mol/s$  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  $\mu$ mol/s. The flow indicated by the mechanical flow indicators at the front panel has the unit ml/min.

Fig. 52 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 ( $Menu \rightarrow Calibration \rightarrow Central\ Unit \rightarrow Valve\ adjustment$ ). It is suitable for flow rates between 350 and 1500  $\mu$ mol/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  $\mu$ mol/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  $\mu$ mol/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 ( $\pm$ 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.

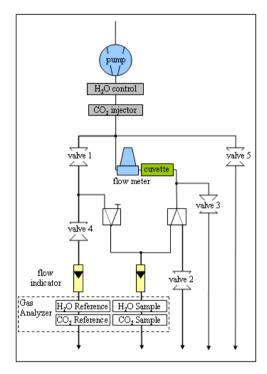
Valve 4 balances the flow between sample and reference of the analyzer in mode ZP. Therefore the guided protocol switches to mode ZP and valve 4 needs to be adjusted until the mechanical flow indicators have equal height.

Valve 1 and 3 determine the flow through the analyzer in mode MP (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 mode ZP, 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 mode ZP 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 mode MP.

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

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

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

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

Fig. 52: Pneumatics of GFS-3000 with emphasis on valves. Solenoids are in position of mode MP. In mode ZP both solenoids will be switched to the position indicated by the dotted line.

**Table 8**: 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 <i>DAC Flow</i> is 1500 +- 200 or until closed (with high flow rate)
		DAC-Flow can be found:
		Control Unit Model 3000-C:
		Option2 →System Values
		Control Unit Model 3100-C, GFS-Win: Chose in chart
2	$MP \leftrightarrow ZP$	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.
		Mode MP Interval = 20 Mode ZP Interval = 40 Repeat Run
		The beads will be lower in ZP than in MP mode.

#### 13.11 Calibration

All calibration values are stored in the parts themselves, so that the Standard Measuring Head e.g. can freely be interchanged between instruments. This is also true for the parts within the control unit.

#### 13.11.1 Calibration of Gas Analyzer

The calibration procedure has been improved since GFS-Win version 3.31. We recommend doing the calibration with the set-up described here (e.g. air-cycling) and with the latest version of GFS-Win for both types of instruments, having Control Unit Model 3000C or 3100C.

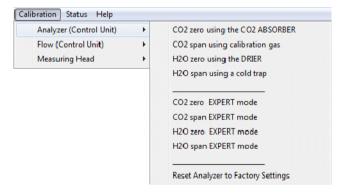
The gas analyzer used in the GFS-3000 is a non-dispersive infrared photometer (NDIR). It consists of two infra-red radiators, two pairs of gold-surfaced gas cells, a chopper wheel and detectors. A pyroelectric (solid-state) detector serves for H<sub>2</sub>O-detection and an opto-pneumatic sensor, for CO<sub>2</sub>-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<sub>2</sub>O and CO<sub>2</sub>.

The full calibration includes the zero point and span calibration of the absolute signals. In order to carry out a zero-calibration, the analyzer should be purged with zero gas until the measured values no longer display a drift. There are two causes for drift, the warm-up drift and the purge-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 calibration is best performed after this warm-up period. For calibration purposes, we recommend a longer waiting time than for measurements. If the gas analyzer can be kept on overnight the warm-up drift would be avoided. 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 is only switched off, if the *measure mode* or *Power* is switched off, but not, 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 would have become warm. The purge-drift is short for  $CO_2$  less than 5 min, but very long for  $H_2O$  1-2 hours.

To carry out the  $CO_2$  span calibration, a tank equipped with a pressure reducer and a fine adjustment valve containing calibration gas is required. 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  $Menu \rightarrow Calibration \rightarrow Analyzer$ .

Fig. 53: 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 advised on how to set-up the system and gas settings are changed automatically. In the expert-modes there is no advise on how to set-up the system, also no gas setting is changed automatically, but the procedure jumps directly to the step before the actual calibration of the

gas analyzer is executed and stored. The last item *Reset Analyzer to Factory Settings* should only be used after completely messing up the calibration. It will reset the  $CO_2$  as well as the  $H_2O$  calibration of the analyzer to company-values, which with the GFS-3000 was delivered. If this option is not visible, please consider to send your instrument to the Heinz Walz GmbH for recalibration and updating.

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.

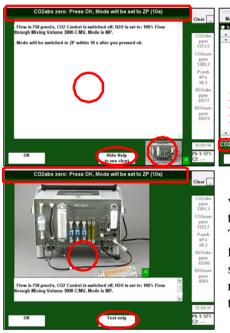




Fig. 54: Three optional views of calibration protocol: help-text, picture view and minimized. To change the view, click on any field marked with a red circle. Also shifting the head line (red rectangle) up or down will change the view.

Fig. 54 shows the software during a calibration procedure. After starting any of the calibration protocols a window appears that shows a head line a help text and a picture for the instrument setup. This window can be minimized, so that it only covers the lower part of the screen underneath

the central window, or expanded, so that the help text or picture can be seen.

Menu CO2 zero calibration, please wait 223 Clear

Fig. 55: Info line during actual analyzer calibration

In each calibration protocol there will be a step where a button is named *Store*. The actual calibration will be performed with pressing *Store*. The process can be cancelled with *Cancel*. After pressing *Store*, the analyzer will obtain a signal that the actual calibration shall be performed. The analyzer will average the measured values while a countdown is indicated in the info line.

Perform the calibration only if the values shown in the chart are stable and sensible. Use fine-scale for the y-aches and a long time period for the x-aches to judge stability. After each calibration check the indicated values. Never attach any tube to the outlets of the gas analyzer, since it changes the pressure within the analyzer, except during absolute zero calibration. It may be necessary to adjust the valves after calibration (see chapter 13.10).

#### 13.11.1.1 CO<sub>2</sub> Zero using the CO<sub>2</sub> Absorber



Fig. 56: Set-up of the system for  $CO_2$  absolute zero calibration with aircycling .

- Chose Menu→ Calibration→ Analyzers→ CO2 zero using CO2 absorber. Or on the Panel-PC chose Option1→ Cal. Gas Analyzers→ CO2 zero
- The CO<sub>2</sub> zero calibration requires fresh soda lime (CO<sub>2</sub> absorber) connected. Note that the indicator of the soda lime only indicates violet directly after usage. The next day the violet color may have disappeared. Nevertheless the soda lime is used. With very dry gas, the indicator may not work.
- The drier tube must be replaced by the Mixing Volume 3000C/MV.
- The humidifier should be replaced by a tube.
- Although it is possible to calibrate the CO<sub>2</sub> zero with an open flow-trough, we recommend air-cycling. This will save CO<sub>2</sub> absorber material and is a more fail-safe way of calibration. In order to establish air-cycling, connect all outlets with AIR IN via tubes as shown in the picture. Note only(!) during zero calibrations the air may be cycled.

- Connect the measuring head and close it. Alternatively the gas connections for the measuring head can be replaced with a short tube.
- The settings for Flow, CO<sub>2</sub> control and H<sub>2</sub>O control will be adjusted automatically. In order to speed up the purging process, valve 5 may be opened for a short period of time. Also the Measure Mode is automatically switched between MP and ZP to flush all gas ways with calibration gas. Click OK to go to the next step and OK again, so that the system will be switched into mode ZP.
- In mode ZP the flow through the analyzers may be increased by opening valve 1. It can be opened while watching the increase of flow in the flow indicators. If valve 1 is not changed, reaching zero will take longer, but its readjustment can be avoided.
- The two upper fields in the quick view column and the chart show the CO<sub>2</sub> 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 count-down 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<sub>2</sub> span or H<sub>2</sub>O zero) or for measurements:

Disconnect air-cycling tube.

If valve 1 has been opened, it should be readjusted for measurements, to do this, the system needs to be in mode MP, the measuring head needs to be connected and closed. Switch the flow on. Adjust valve 1 until both flow indicators show equal height (see chapter 13.10).

If valve 5 has been changed, in the chart chose *Flow DAC*. In mode MP adjust valve 5 until the value for Flow DAC shows  $1500 \pm 100$ , or close valve 5 for high flow rates, where DAC values that low can not be reached.

If necessary, enable the measuring head with  $On/Off \rightarrow Enable$  Components.

#### 13.11.1.2 CO₂ Span using Calibration Gas

The zero-point always needs to be calibrated before a span-calibration can be performed. For the  $CO_2$  span calibration a tank with  $CO_2$  calibration gas, which is equipped with a pressure reducer and a fine adjustment valve, is required. Since the pump is switched off during  $CO_2$  span calibration, it is irrelevant, whether there is any absorber tube attached.

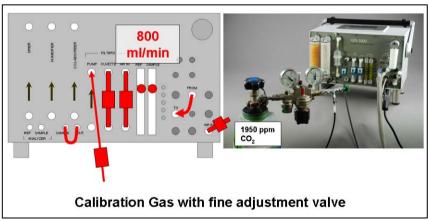


Fig. 57: Set-up of the system for absolute CO<sub>2</sub> span calibration.

- In GFS-Win chose *Menu*→ *Calibration*→ *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 zero calibration.

- The measuring head must be connected and closed. Alternatively, the gas connections for the measuring head can be replaced with a short tube.
- After pressing OK, the flow, CO<sub>2</sub> control and H<sub>2</sub>O control will be switched off automatically. The mode will be switched automatically to ZP.
- If valves 3 or 5 are open, it is recommended to close the outlet SAMPLE in order to save calibration gas. Also VENT should be closed. This can be done by connecting the outlet SAMPLE and VENT, which are located directly beside each other (3<sup>rd</sup> and 4<sup>th</sup> outlet), with a tube.
- 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.
- After the flow is off, disconnect the filter labeled PUMP. The CO<sub>2</sub> calibration gas needs to be connected to the upper connector labeled PUMP. The filter from the spare kit may be used in the feeding tube of the calibration gas. Carefully adjust the pressure reducer and fine adjustment valve, so that the flow indicators show 800 ml/min. Valve 1 may be used for additional fine adjustment. If the flow is unequal between reference and sample, it shall be adjusted with valve 4.
- Flush all gas pass ways by opening the outlet VENT and SAMPLE for a short moment. If valve 5 is closed, also open it for a short moment while the tube is disconnected.
- In the next step, enter the set value of the calibration gas (between 1 ... 2200 ppm) into the GFS-Win software.
- The two upper fields in the quick view column as well as the chart show the CO<sub>2</sub> absolute value of the reference cell (CO2abs) and the

 $CO_2$  absolute value of the sample cell (CO2sam). If both values have stabilized, a  $CO_2$  span adjustment can be carried out via pressing *Store*.

- The info-line will display a count-down while performing the calibration, please wait.
- Check the calibration: Are CO2abs and CO2sam close to the set value?
- After calibration, remove tube between the outlets sample and vent, reconnect filter pump. Set-up system for measurements. If valve 1 has been changed for CO<sub>2</sub> zero calibration see chapter 13.10 for valve adjustment or continue with H<sub>2</sub>O calibration.

If necessary, enable measuring head with  $On/Off \rightarrow Enable\ Components$ .

#### 13.11.1.3 H<sub>2</sub>O Zero using the H<sub>2</sub>O Absorber

H<sub>2</sub>O is a small molecule, which is well absorbed by surfaces. Hence it takes a while until the system is completely dried for the H<sub>2</sub>O zero calibration. We therefore recommend regular checking of the H2O zero value, but only a complete calibration, if there is enough time for good drying. In order to be able to dry as long as desired even in wet climates, without flushing too long, so that the silica gel would become wet during the drying procedure, we recommend air-cycling (*see* Fig. 58).



Fig. 58: Set-up for H<sub>2</sub>O zero calibration with air-cycling.

- Chose Menu→ Calibration→ Analyzers→ H2O zero using H2O absorber. Or on the Panel-PC chose Options1→ Cal. Gas Analyzers→ H2O zero
- Use freshly dried silica gel in the drier tube. Note, if silica gel is burnt, it may stay orange-black although being wet.
- Remove the tube CO<sub>2</sub> absorber and replace it by the Mixing Volume 3000-C/MV.
- Remove the humidifier and replace it with a tube.
- Use the air-cycling tube to connect all outlets with the filter at AIR IN.
- The measuring head must be connected and closed. Alternatively, the gas connections for the measuring head can be shortened with a tube.
- The parameters for flow, CO<sub>2</sub> control and H<sub>2</sub>O control will be adjusted automatically. In order to speed up the purging process,

valve 5 may be opened for a short period of time. Also the Measure Mode is automatically switched between MP and ZP to flush all gas ways with calibration gas.

- Click OK twice. The system will switch into mode ZP.
- In ZP mode, opening valve 1 will increase the air flow through the analyzer and therefore speed-up the drying procedure. Watch the mechanical flow indicators rise as the flow through the analyzers increases while opening valve 1.
- The two upper fields in the quick view column show the H<sub>2</sub>O absolute value of the reference cell (H2Oabs) and the sample cell (H2Osam). Also the chart shows H2Oabs and H2Osam. If both values have stabilized (after 20 to 40 min), a H<sub>2</sub>O zero point calibration can be carried out via STORE. Both H<sub>2</sub>O absolute values will be set to zero.
- Check the calibration: Is H2Oabs and H2Osam close to 0?
- Either set-up the system for the next calibration or for measurements:

Disconnect air-cycling tube.

If valve 1 has been opened, it should be readjusted for measurements, to do this, the system needs to be in MP mode, measuring head needs to be connected and closed. Switch flow on. Adjust valve 1 until both flow indicators show equal flow through both sides of the gas analyzer (see chapter 13.10).

If valve 5 has been changed, chose Option2 oup System Values. In mode MP adjust valve 5 until the value for Flow DAC shows 1500  $\pm$  100, or close valve 5 for high flow rates, where DAC values that low can not be reached. If necessary, enable measuring head with On/Off oup Enable Components.

#### 13.11.1.4 H<sub>2</sub>O Span using a Measuring Gas Cooler

The zero-point always needs to be calibrated before a span-calibration can be performed. For the H2O 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 and step by step to avoid pushing water into the system, which would be a fatal mistake. Switch the controller of the cold trap on as early as possible before calibration. The temperature of the cold trap needs to be at least 2 °C lower than room temperature to allow proper saturation of the gas in the water bottle. Only when the set temperature is reached and condensation occurs, the cold trap has reached the set humidity value. To check whether condensation occurs, tilt the cold trap forward and watch whether water is running into the condenser hose. Fill only a small amount of water into the humidifying bottle to keep the back pressure low (see Fig. 61).



Fig. 59: Set-up of the GFS-3000 for H2O span calibration with GFS-Win version 3.31 or higher.

- Chose Menu → Calibration → Analyzer → H2O span using cold trap.
- Close three openings: The upper connector labeled DRIER, the connector CUVETTE TO and the outlet labeled SAMPLE. Fig. 59 shows one possibility, how this could be done.
- Do not(!) close any outlet from the analyzer and do not(!) connect any tube to the outlets of the analyzer.
- Completely open valve 1 (stop one turn before the end).
- Replace the CO<sub>2</sub> absorber with the Mixing Volume 3000-C/MV, insert the humidifier tube (humidity is irrelevant) and disconnect the filter labeled PUMP.
- The parameters for flow, CO<sub>2</sub> control, H<sub>2</sub>O control and mode will be adjusted automatically.

Proceed with OK. The following steps are described very accurately to avoid that water will be pushed accidentally through the gas analyzer:

• While the pump is off, connect the lower connector of the filter PUMP to the inlet of the humidifying bottle. Use the 4 mm hose connector and the filter from the spare kit for this connection. Connect the outlet of the humidifying bottle to the inlet of the cold trap (see Fig. 60).

Attention: Do not(!) connect the outlet of the cold trap to the upper connector PUMP during this step (If it is connected and the humidifying bottle is inserted the wrong way, water will be pushed out, the analyzer will be damaged.)

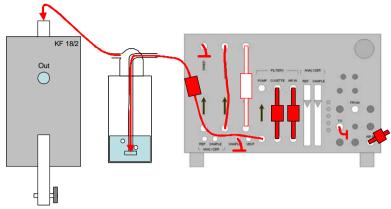


Fig. 60: Drawing with connection of humidifying bottle and cold-trap.

• After clicking OK, the pump will be switched on (Fig. 61) and water will bubble through the humidifying bottle.



Fig. 61: Water bubbling through humidifying bottle after pump has been switched on.

• If everything is fine, the outlet of the cold trap can now be connected to the upper connector labeled 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. 62: Final set-up for H<sub>2</sub>O span calibration.

• In the next step the calibration protocol displays a sliding bar (see Fig. 63). Use this sliding bar to adjust the flow, so that the flow indicators show 800 ml/min. If the flow is unequal between sample and reference adjust valve 4 with a small screw driver.



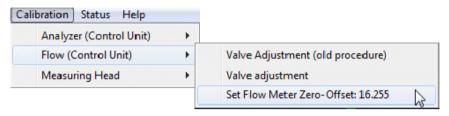
Fig. 63: Calibration window with sliding bar between OK and Help button during H2O span calibration.

- 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 from this temperature value 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<sub>2</sub>O value for the reference cell (H2Oabs) and the sample cell (H2Osam). If both values have stabilized and the cold trap is stabilized, press *Store*. H<sub>2</sub>Oabs and H2Osam will be set to the set values.

- Check calibration: are H<sub>2</sub>Oabs and H2Osam close to the set value?
- Remove the tubes closing the outlet SAMPLE, CUVETTE TO and upper connector DRIER. Insert, drier and the filter labeled PUMP.
   Connect measuring head. Adjust valve 1 (see chapter 13.10)

Remark: If measurements with other oxygen concentrations then ambient (21%) are planned, the "band broadening" effect of oxygen on the infra-red detection of  $H_2O$  needs to be taken into account. In other words, the  $H_2O$  signal in nitrogen is about 8% smaller than in air. The effect of oxygen on the  $CO_2$  signal is much smaller and can be neglected

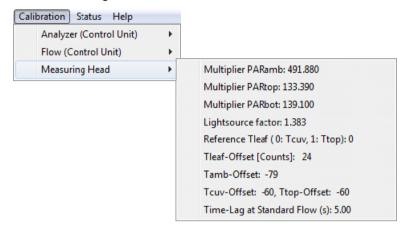
#### 13.11.2 Offset of Flow meter



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

For valve adjustment see chapter 13.10.

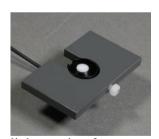
#### 13.11.3 Measuring Head



#### 13.11.3.1 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.

#### 13.11.3.2 Determination of the Light-Source Factor

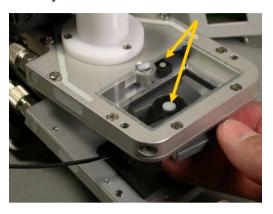


The intensity of the light source is measured with the *PARtop* or *PARbot* sensor inside the cuvette in dependence of the side of attachment. These light sensors are located at the rim of the cuvette frame, respectively. In contrast to sunlight, which is uniform over large areas, the

light coming from an artificial light source may decrease towards the rim, where the light sensor is located. Whenever one of the light sources (LED Light Source 3040-L, LED-Array/PAM-Fluorometer 3055-FL, or the Imaging-PAM) are switched on, the measured intensity of *PARtop* or *PARbot*, depending on the chosen *light mode*, is multiplied with the *light-source factor* stored in the measuring head. The *light-source factor* is specific for

every light source. If the light source is changed, a new adjustment is required. Once the *light-source factor* is determined for a given light source, the known value can be entered manually without a new determination.

For carrying out the determination of the *light-source factor* the external Miniature Quantum Sensor MQS-B/GFS and the provided adapter plate (*see* chapter 3.1 and picture) will be necessary. If a fluorometer is used, it must be enabled within the GFS-Win software. Chose



 $Menu \rightarrow Calibration \rightarrow Measuring \ Head \rightarrow Light-source \ factor$ . 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.

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<sup>-2</sup> s<sup>-1</sup> *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.

When *PARamb* is chosen as *light mode* or the set-value is set to zero, the *light-source factor* is not used. When *PARbot* or *PARtop* are chosen as sensor for light regulation and the set-value is not zero, the *light-source factor* is used for the chosen light sensor, meaning that the indicated value has been multiplied with the light-source factor. With IMAG-MIN/B the *light-source factor* is used whenever the *light mode* is *PARbot* or *PARtop*, independent of the set-value.

#### 13.11.3.3 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  $\mu$ mol m-2 s-1). Tleaf should rise by several degrees. Switch the light off, Tleaf should come back.

#### 13.11.3.4 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.

#### 13.11.3.5 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<sup>-1</sup>. It will be recalculated for the chosen flow rate. Nevertheless, the value can be adjusted with other flow rates. For a determination select this menu-item and enter D. A CO<sub>2</sub> pulse will be given. Observe the CO<sub>2</sub> signal and adjust the value until the influence of the time delay on the dCO<sub>2</sub> signal in mode MP is minimized.

#### 13.12 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 13.14.1 before storing the instrument.

#### 13.13 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 13.2. 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. Regard chapter 13.2 for the reattachment of the adapter plates. Regard the checklist in chapter 13.14.1 before storing.

#### 13.14 Checklists

#### 13.14.1 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 not completely charged
- Depending on storage time also the CO<sub>2</sub> in the supply vessel may be released (see chapter 13.5).
- Discard used soda lime (read chapter 16.4.1)
- 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
- Is the measuring head dry inside? Otherwise flush with dry air overnight before storing (the setup shown in Fig. 58 may be used for drying, connect the measuring head, switch the flow on and switch the H<sub>2</sub>O 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 (do not delete them before checking)
- Pack everything (see chapter 3.1 for all components).

#### 13.14.2 Checklist for Transport

- Flush the system with dry air to avoid condensation during cold transport.
- Remove CO<sub>2</sub> cartridge. Note that the CO<sub>2</sub> cartridges may not be transported via air-mail.
- Release the pressure in the CO<sub>2</sub> supply vessel of the central unit (see chapter 13.5).
- When packing the central unit, make sure that the handle is in the horizontal position and locked in this position, so that the instrument can not move forwards or backwards during transport.
- Avoid bending the cables, in particular the cable and tubes of the measuring head, lay them in big loops.
- Observe laws on the transport of batteries (see chapters 16.4.4 and 16.4.5).

#### 14 Practical Hints

- If a constant power supply is available, the GFS-3000 should be kept switched on or in standby mode, even over weeksends.
- 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<sub>2</sub> and H<sub>2</sub>O concentration.
- Calibration of gas analyzer and other devices need to be checked regularly (*see* chapter 13).
- 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. Use aircycling to dry the system (compare setup of H<sub>2</sub>O zero calibration Fig. 58)
- If the Measuring Head became wet outside, use a warm temperature for Tcuv to dry it.

# 15 Trouble Shooting

# 15.1 Symptoms and Solutions

Table 9: Symptoms and Solutions

Symptoms	Solutions
GFS-Win Software	
Values are very strange within GFS-Win software or in downloaded files, also you ignored a warning during the start of GFS-Win	Check whether the regional settings of your computer are set to number: 123 456 789.00
System shows error 72 during starting GFS-Win	You need to have write permission in the sub-directory of GFS-Win. Also files like <i>GFS-3000</i> .cfg, <i>GFS-3000</i> .err and <i>GFS-3000</i> .ini should not be write-protected.
All values are blank, all constants are zero	The system did not initialize properly. Please switch the measure mode off and afterwards measure mode on again. If this does not help, switch the power off and on again.
No communication can be established between PC and Panel PC of <i>GFS-3000</i>	-Control Unit Model 3000-C: On the Panel PC chose the Window→Settings or Window→Program. The Panel PC is not ready to transfer data, if Option or Option1 is chosen.
Control Unit 3000-C	
The screen of the Panel PC is black	Occurs, when it is hot. Try to cool it. Generally, the screen contrast and brightness can be adjusted under <i>Option2</i> → <i>Display</i> . In the lower right corner above the battery control the brightness can be adjusted. Touching it will cycle through the contrast levels. Also the contrast and brightness can be set to its default setting during the startup of the system by pressing the letter

	"W" of the Walz-logo, which can not be
	seen, if the screen is black, but the loca-
	tion can be guessed.
Pneumatic Pathway	
Pump sounds good, but the	Check whether there is a leak at the hu-
flow reached is to low	midifier or drier column.
Pump is not giving a constant	The Measuring Head needs to be closed
flow	for the pump to work steadily. The cho-
	sen flow might be to low, Open valve 5
	to increase the flow rate (see valve ad-
	justment chapter 13.10).
CO <sub>2</sub> concentration varies to	This may happen directly after the hu-
much and is somehow con-	midifier or drier has been changed or
nected to the water control	after changing from working without
	CO <sub>2</sub> absorber to working with CO <sub>2</sub> ab-
	sorber, or vice versa. The humidifier and
	the drier need to be flushed with air of
	the appropriate CO <sub>2</sub> concentration (either
	free or ambient). Switch water control for
	about 2 min to maximal H <sub>2</sub> O (60 000)
	ppm) and for 2 min on minimal H <sub>2</sub> O (0
	ppm).
CO <sub>2</sub> -control is slow for high	If the CO <sub>2</sub> pressure is low (a warning is
values	displayed every 15 min), change the car-
	tridge.
ci is not stable	ci is a calculated magnitude and not nec-
	essarily stable. With no or low evapora-
	tion, the calculation of ci includes a divi-
	sion by 0 or a value close to 0. ci will
	then vary a lot being meaningless.
	There may be condensation in the system
Ain boundier indicated in	(see chapter 4.3)
Air humidity indicated is	, 2
higher than the set value.	not the actual humidity within the cu-
Measuring Head	vette.
	779
Temperature of cuvette can not	The impellers always need to be switched
be controlled, the leaf does not	on.

transpire properly		
Temperature and/or PAR-	Check the calibration values for the sen-	
values are generally strange	sors. The original calibration values for	
	the specified measuring head may be	
	obtained from the Heinz Walz. GmbH.	
Temperature of Tleaf varies by	Thermocouple is broken.	
more than 0.5°C per s and is	-	
close to Tcuv.		
<b>Battery Charger</b>		
Battery Charger shows "Li-Ion	If battery has been discharged to low,	
error"	disconnect and reconnect battery.	
	Model LC-02: Note that, if the charger is	
	not switched on, the display is powered	
	by the connected battery, which will	
	discharge.	
Imaging-PAM		
GFS-Win reacts slow	Minimize Imaging-Win during GFS-Win	
	operation.	
	Since the Imaging-Win software requires	
	a lot of computer time and memory, a	
	powerful computer is required (CPU at	
	least 1.7 GHz, RAM at least 256 Mb, the	
	more the better). It might be necessary to	
	disable resident programs in particular	
	virus software (Norten-AntiVir is very	
	disruptive even when disabled but still	
Imaging-Win shows black	resident). Disable WLAN-software.	
Imaging-Win shows black pictures instead of Ft or F	Take lens cap off. In Imaging-Win: switch Measure Mode	
pictures instead of Ft of F	on, switch ML on.	
	Reduce latency time of USB Port even	
	lower than the recommended 8 ms: Start	
	$\rightarrow Run \rightarrow "devmgmt.msc" \rightarrow Ports (COM)$	
	& LPT) $\rightarrow$ double-click on the specific	
	USB Serial Port $\rightarrow$ PortSettings $\rightarrow$ Ad-	
	vanced port	
	Update fire-wire driver.	
	Disable any resident programs.	

	TT 1 . C' 11
	Use shorter fire wire cable.
	Connect all instruments (computer, Im-
	aging PAM, light source) to the same
	electrical circuit.
	Keep cables close together so that they
	do not form loops.
The message is displayed: No	Switch the power at the imaging control-
Imaging-PAM found. Check	unit on.
serial connection	If the battery is low, the charge needs to
	be connected as well - even if the power-
	LED is green.
	Check whether the USB cable is con-
	nected.
	Select: $Start \rightarrow Run \rightarrow "devmgmt.msc" \rightarrow$
	Ports (COM & LPT). To find out, where
	the Imaging-PAM is connected, unplug
	its USB-connection for a short while and
	watch, which USB-port disappears. Open
	the identified USB-Port to change the
	settings: $USB \ Serial \ Port \rightarrow Port Settings$
	$\rightarrow Advanced.$
	Set COM Port Number to a number be-
	tween COM 1 and COM 8 (also com-
	ports with the comment "in use" might be
	available).
	Change latency time: to 8 ms. The la-
	tency time is the time commands are
	buffered before they are sent to the con-
	trol unit.
The Power-LED of the imag-	The battery can not be loaded. A new
ing control-unit is first red but	battery in the imaging control-unit might
then switches off	be required.
then switches on	oc required.

#### 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 to much pressure builds up within its cuvettes.
- If the cuvette is bridged with a tube the flow shown in the program 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 can not maintain the flow and switches off and on.
- If the mode is switched from MP to ZP, the flow in both indicators should decrease by the same amount. Otherwise adjust valves.

#### 15.2.2 H<sub>2</sub>O Mixing Valve

- If the H<sub>2</sub>O regulation is switched on or off while the humidifier and drier are bridged with a tube, there should be no change (except for transient changes) in the H2Oabs value, otherwise the mixing valve is damaged.

#### 15.2.3 Function Check of Gas Analyzer for Reasonable H<sub>2</sub>O Detection

- Use freshly dried drier. Switch to ZP-Mode. Set H2Omode to abs and 0 ppm. The H2Oabs should descent to 0 (±200 ppm), also the dH2O should reach 0 (±400 ppm).
- Check the humidifier tube, whether it is wet (often condensation is visible). Place the cuvette beside the humidifier tube. Read the ambient temperature from the display. Switch Mode to ZP. Set Tcuv to off. Set the flow to 600 μmol/s. Set H2Omode to abs and 60000 ppm. The temperature in the humidifier tube will be about 2.0-2.5°C cooler than ambient (due to latent heat of evaporation). When the indicated H2Oabs has stabilized, read Tamb again and subtract 2.2°C. Use the obtained temperature to find the corresponding saturation vapor pressure (Table 15, chapter 19). Multiply the obtained value in hPa with 1000 to roughly obtain the H2Oabs in ppm. The calculated value for H2Oabs should match the measured value for H2Oabs. The dH2O should reach 0 (±400 ppm). Careful, this is just a rough function check not a calibration-method.

#### 15.2.4 Function Check of Gas Analyzer for Reasonable CO<sub>2</sub> Detection

- Use fresh  $CO_2$  absorber, (the indicator only works with humid air and turns pale after a while). Switch to ZP-Mode. Switch  $CO_2$  control off (to close the solenoid valve of the  $CO_2$  control), but keep the absorber in place. The  $CO_2$  abs should descent to 0 ( $\pm 5$  ppm), also the d $CO_2$  should reach 0 ( $\pm 4$  ppm).
- Replace CO<sub>2</sub> absorber with Mixing Volume (3000-C/MV). Use air from outside, don't breath near the air-inlet. Ambient CO<sub>2</sub> concentration varies between 350 and 500 ppm in dependence on the location, day time and time of the year.

#### 15.2.5 Temperature Control of Measuring Head

- If the Temperature control has been off for a while, Tmin, Tcuv and Tleaf should show very similar values.
- Set Tcuv to a temperature lower than ambient and touch both sides to check whether they are cooling.
- Switch the impeller and temperature regulation off. Insert a black piece of paper touching Tleaf. Observe Tleaf, it should be similar to Tcuv and have a low noise (<0.3°C). Switch the light on (PARtop 2000  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>). Observe Tleaf, it should clearly be warmer (more than 5°C) than 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 an error list: error.rpt in the subdirectory (My Documents/GFS-3000/ini) or the *GFS-Win*-subdirectory (depending on the program setup). For diagnosis check this file to find out, which error occurred before the displayed error.

Table 10: Errors notified by GFS-Win

Type	Explanation		
GFS Error	See Table 11 GFS-3000 errors.		
No contact with	GFS-Win tried to contact a module of the GFS-3000,		
	but did not get a reply. Modules are: central unit,		
	battery control, gas analyzer, H <sub>2</sub> O control, flow me-		
	ter, measuring head, fluorescence 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		
g : .:	computer.		
Communication	The relevance of communication errors depend on		
error	their circumstances. 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 a file was not		
	successfully transferred or that it happened during calibration. Depending on the circumstances, the last		
	action should be repeated. Sometimes there are harm-		
	less echoes detected in the line and reported as com-		
	munication 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 <i>GFS-Win</i> is running from a CD.		
Unexpected file	These are file errors, where we have no suggestion for		
error	a user error as cause.		
Calibration failed	If this error occurs, please repeat calibration com-		
	mands.		

Table 11: 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	Explanation
error code	
<b>Battery control</b>	
1100-1122	Errors reported by the CPU of the battery control
	(memory error [0, 3, 18, 20], run-time error [9], Ini-
	tialization failure [1, 2, 3, 4, 5, 7, 16, 17], voltage error
	[6, 8] or communication errors [21, 22])
1123, 1124	Voltage error (short, low power, heat)
1125	Voltage input low <10V
1132	Panel PC not connected
1133, 1134	Current to high, battery 1 or 2, respectively
1135,1136	Voltage at input to high, battery 1 or 2, respectively
1137-1140	Communication errors
<b>Battery control</b>	Control Unit 3100-C only
1148	LED-power of panel PC is off
1149	BC 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)
1157	RESET was called
<b>Central Unit</b>	
2100-2122	Errors reported by the CPU of centrol unit (memory
	error [0, 3, 18, 20], run-time error [9], initialization
	failure [1, 2, 3, 4, 5, 6, 7, 16, 17], voltage error [8] or
	communication errors [21, 22])
2108	Voltage, CPU central unit
2123, 2124	Voltage, central unit to low (short, low input)
2125, 2132	Voltage, pump
2134	Voltage, CO <sub>2</sub> control valve
2136	Voltage, gas analyzer (short, heat).
2137	Gas analyzer was switched on
2138	Voltage, solenoids ZP/MP (short/heat)
2139	Voltage, solenoid CO <sub>2</sub> (short/heat)

Communication error between PC and central unit	
Communication error between CPU central unit and gas analyzer	
Communication error between CPU central unit and	
PC.	
No communication between flow meter and CPU of central unit	
Errors reported by the CPU of the flow meter (mem-	
ory error [0, 3, 18, 20], run-time error [9], initialization failure [1, 2, 3, 4, 5, 6, 7, 16, 17], voltage error [8])	
flow meter, analog digital converter	
Voltage, flow meter (short, low voltage)	
Communication error, between flow meter and CPU of central unit	
No communication between H <sub>2</sub> O control and CPU of central unit	
Errors reported by the CPU of the H <sub>2</sub> O control (mem-	
ory error [0, 3, 18, 20], run-time error [9], initialization	
failure [1, 2, 3, 4, 5, 16, 17], voltage error [6, 8])	
Voltage, H <sub>2</sub> O control, no power at motor	
Current, heat problem at H <sub>2</sub> O control	
Reference detection failed.	
Communication error, between H <sub>2</sub> O control and CPU central unit.	
Peltier error, resistance of peltiers 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.	
Errors reported by the CPU of the measuring head	
(memory error [0, 3, 18, 20], run-time error [9], ini-	
tialization failure [1, 2, 3, 4, 5, 6, 7, 16, 17], voltage	
error [8])	

3123	Fuse: temperature control and light
3124	Voltage low, measuring head in
3125	Voltage error, Peltier temperature control
	(short/overload)
3132	Voltage error, light (short/overload)
3133, 3134	Voltage error, measuring head (short)
3136-3140	Impeller-control error
3148	Analog digital converter error
3153, 3154	Communication error
3172, 3173	
3155	Lower impeller stopped, blocked?
3156	Lower impeller rough-running, dirty?
3157	Communication error, lower impeller
3164	Upper impeller stopped, blocked?
3165	Upper impeller rough-running, dirty?
3166	Communication error, upper impeller
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
3040	Peltier temperature-control, out of order

#### 15.4 LED-Code

Table 12: Power LED of Control Unit

LED	Control Unit 3100-C	Control Unit 3000-C
off	Power is off or hardware error	
green blinking	Power is on	, operation ok
slowly blinking	Power is on, PC is off,	
green	operation ok	
green perma-	Program crash or hardware	Program crash or hardware
nently	error. Switch power off and	error. Switch power off and
	back on	back on
red permanently	Power switch permanently	Program crash or hardware
	pressed or defect	error. Switch power off and
		back on
green/orange	Battery low, change battery	
blinking	(version 2.1 and higher)	
red/green blink-	Waiting for contact from	Supplied voltage to low.
ing	GFS-Win software	
one red blink	Supplied voltage to low	
after power on	( < 10V)	
red blinking	Error detected by software, read error code	
orange blinking	Error detected by software, read error code	

Table 13: LED code for panel-PC LED of Control Unit Model 3100-C

LED	panel-PC
green	Power is on
orange	Accessing hard drive

Table 14: LED Code of Fluorescence Module 3055-FL and Fiberoptics PAM-Fluorometer 3050-F

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

#### 15.5 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:

http://www.walz.com/downloads/global/Service03.pdf

Please fill in the form and send it together with the instrument. Please also provide information on any problems. Only include the parts in the shipment, which are required fort the repair. Please read chapter 13.14.2 on how to prepare the instrument for transport.

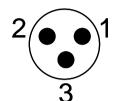
# 16 Appendix

## 16.1 Pin Assignments of Connectors Control Unit 3100-C and 3000-C

Connectors are turned upside down in some units, pay attention to lug for orientation.

"AUX IN"

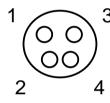




- 1: input AUX2 (+0...4095 mV),
- 2: input AUX1 (+0...4095 mV)
- 3: GND
- 1: blue, 2: brown, 3: black

"COMP"

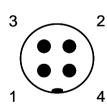




- 1: RS485/A
- 2: output (+14 ...16 V)
- 3: RS485/B
- 4: GND

"USB" Control Unit 3000-C"

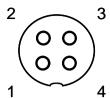




- 1: input
  - (+5 V from ext. PC)
- 2: Data -
- 3: Data +
- 4: GND

"USB": USB 2.0 from internal PC of Control Unit 3100-C





- 1: GND 2: D-
  - 3: D+
  - VCC(+5V)4:

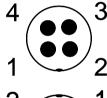
"CUV"

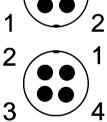


- output (+14 ...16 V) 1:
- 2: GND
- 3: **GND**
- GND 4: 5: RS485/B
- 6: output (+14 ...16 V)
- 7: output (+14 ...16 V)
- RS485/A 8:

"BATTERY/DC-IN"







- input (+15 ... 17 V)
- 2: GND
- 3: GND
- 4: input (+15 ... 17 V)

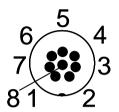
## 16.2 Pin Assignments of Connectors Standard Measuring Head 3010-S

**APPFNDIX** 

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 3100-C. The cable usually remains connected to the measuring head.

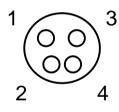




- 1: input (+14 ...16 V)
- 2: GND
- 3: GND
- 4: GND
- 5: RS485/B
- 6: input (+14 ...16 V)
- 7: input (+14 ...16 V)
- 8: RS485/A

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 is used, one of the two cables must be connected here.

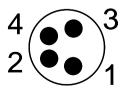




- 1: RS485/A
- 2: output (+14 ...16 V)
- 3: RS485/B
- 4: GND

Connector for the LED Light Source 3040-L or for one of the two cables of the LED-Array/PAM-Fluorometer:



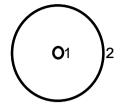


- 1: output for LEDs (+0 ... 12 V)
- 2: GND for LEDs
- 3: output for fan (+12 V)
- 4: OC (open collector) for

fan

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





1: cathode of quantum sensor

2: anode of quantum sensor

## 16.3 Assignment of AUX Cable

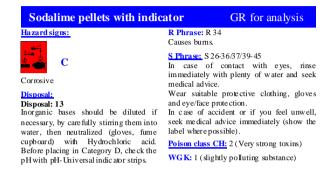
Cable for 2x Auxiliaries (part #: 000130606205), which has three wires with bar ends for connecting two sensors:

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

#### 16.4 Information on Chemicals and Batteries

#### 16.4.1 Soda Lime

contains 2-5% Natrium hydroxide, which can cause severe burns (R35) and > 50% Calcium hydroxid, which can cause serious damage to eyes (R41).



#### 16.4.2 Silica Gel (Sorbead Orange Chameleon)

Silica Gel	beads, desicant $\sim 2-5$ mm
Formula Hill: O <sub>2</sub> Si	WGK: nwg (nonpolluting substance)
Molarmass: 60.09 g/mol	Poison class CH: F (Not subject to toxicity classification)
CAS number: 7631-86-9	
EC-No.: 231-545-4	
HS Code: 2811 22 00	
Storage class (VCI): 10-13 (Other liquids and solids)	

#### 16.4.3 Humidifying Granules (Stuttgarter Masse)

sse	
550	Grain Size 3 – 5 mm
organism carrier	
600 °C	
Fixed bed filter for water Carrier material for gas d Carrier material for cataly	etector devices ysators
	organism carrier 600°C Fixed bed filter for water Carrier material for gas d

#### 16.4.4 Li-lon Battery 3025-A

The Li-Ion Battery 3025-A passed the UN Manual of Tests and Critera Part III Subsection 38.3. The certificate PB\_E6077\_WALZ\_UN\_ T38.3 can be downloaded from our homepage http://www.walz.com/products/gas\_exchange/gfs-3000/downloads.html.

Li-Ion Batteries 3025-A are classified as dangerous good class 9. Shipping needs labelling with UN3480 for the battery alone and UN3481 for the battery attached to a device. A maximum of two Li-Ion Batteries 3025-A are allowed to be transported together with the GFS-3000.

For more transport information see Packing instruction 965 and 966 http://www.iata.org/html\_email/CAR1001654/lithium\_batteries.pdf or http://www.iata.org/whatwedo/cargo/dgr/Documents/Lithium-Battery-Guidance-2013-V1.1.pdf

Do not transport when broken.

Do not dissemble, crush or drop the battery from height.

Do not short (+) and (-) battery terminals with conductive (i.e. metal) goods.

Charge only with specified charger designed for this battery.

Wrong handling can cause fire or explosion.

For more information see provided product safety data sheet.

#### 16.4.5 LiFePO4 Battery 3035-A

LiFePO4 Batteries 3035-A do not need shippers declaration for transport. Only a handling label is required as the two LiFePO4 batteries in LiFePO4 Battery 3035-A are electronically separated, each below 100 Wh and passed the UN Manual of Tests and Criteria Part III Sub-

CHAPTER 16 APPENDIX

section 38.3. The UN-Transport certificate is provided on the delivered CD in the folder MSDS sheets

For more transport information see http://www.iata.org/whatwedo/cargo/dgr/Documents/Lithium-Battery-Guidance-2013-V1.1.pdf

Do not transport when broken.

Do not dissemble, crush or drop the battery from height.

Do not short (+) and (-) battery terminals with conductive (i.e. metal) goods.

Do not mix new and used batteries.

Charge only with specified charger designed for this battery.

For more information see provided material safety data sheet.

#### 16.5 Spectral Sensitivity of the Light Sensor

Fig. 64 and Fig. 65 show the relative spectral sensitivity of the quantum sensor type MQS-B/GFS, which is used to measure the ambient light intensity PARamb. Fig. 64 gives the spectral sensitivity regarding the energy flux density, Fig. 65 regarding the photon flux density. The solid lines show the ideal response.

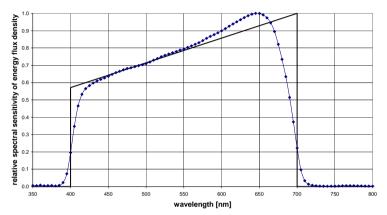


Fig. 64: relative spectral sensitivity of energy flux density

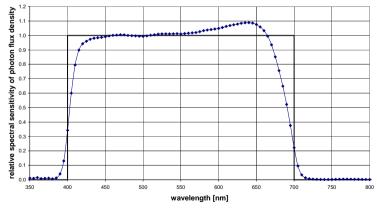


Fig. 65: relative spectral sensitivity of photon flux density

#### 16.6 Spectral Sensitivity of the Light Sensor LS-A

Fig. 66 and Fig. 67 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. 66 gives the sensitivity regarding the energy flux density, Fig. 67 regarding the photon flux density. The solid lines show the ideal response.

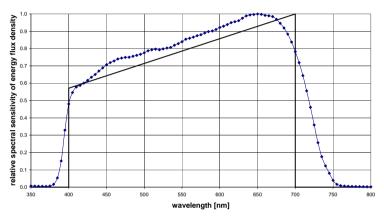


Fig. 66: relative spectral sensitivity of energy flux density

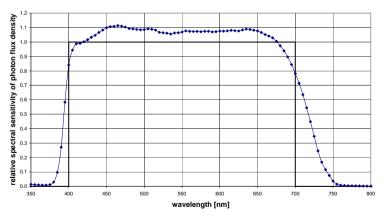


Fig. 67: relative spectral sensitivity of photon flux density

## 17 Literature

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#### 18 Technical Data

Subject to change without prior notice.

#### 18.1 Control Unit 3100-C

**Design:** Aluminum housing featuring integrated PC module, large graphical color-display well readable in sun-light with touch screen, 4-channel CO<sub>2</sub>/H<sub>2</sub>O gas analyzer, flow control, CO<sub>2</sub> control (supplied via small cartridges or cylinders) and H<sub>2</sub>O control (for drying and humidifying).

Pneumatic connectors for air inlet, measuring head and four vents. Sockets for cable connections:

- Cuvette: Standard Measuring Head 3010-S,
   DUAL-PAM Gas-Exchange Cuvette 3010-DUAL
   or Gas-Exchange Chamber 3010-GWK1
- Aux in: two Auxiliaries
- Battery DC/in: two Li-ion Batteries 3025-A,
   Mains Power Supply Unit 3020-N,
   12 V Battery,
   24 V Battery,
   or external DC
- Comp(RS 485): one additional component
- USB (USB 2.0): USB storage device,
   USB null modem cable (USB-NMC)
   or other USB device

#### CO<sub>2</sub>/H<sub>2</sub>O gas analyzer

**Design:** 4-channel CO<sub>2</sub>/H<sub>2</sub>O absolute NDIR gas analyzer, separate cuvettes for CO<sub>2</sub> and H<sub>2</sub>O

• **CO2 measurement:** Simultaneous absolute and differential measurements, absolute range 0 to 3000 ppm, cuvette length 20 cm, cuvette volume of one cell 6 cm<sup>3</sup>, gas-filled detector

- **H2O measurement:** Simultaneous absolute and differential measurements, range 0 to 75000 ppm, cuvette length 20 cm, cuvette volume of one cell 6 cm<sup>3</sup>, pyroelectric detector (solid state)
- Max. noise in absolute mode: <0.2 ppm CO<sub>2</sub> and <30 ppm H<sub>2</sub>O
- **Resolution:** 0.01 ppm CO<sub>2</sub>, 1 ppm H<sub>2</sub>O
- Linearization: CO<sub>2</sub> absolute: Max error between 0 and 600 ppm: 12 ppm, above 600 ppm 2% of measured value

**Deviation of differential CO<sub>2</sub> zero in dependence on absolute CO<sub>2</sub>:** Typically less than 0.5 ppm CO<sub>2</sub> over full range.

**H2O absolute:** Max error between 0 and 15000 ppm: 330 ppm, above 15000 ppm: 2% of measured value

**Deviation of differential H<sub>2</sub>O zero in dependence on absolute H<sub>2</sub>O:** Typically less than 150 ppm H<sub>2</sub>O over full range.

- **Barometric air pressure measurement:** Range 60 to 110 kPa, accuracy  $\pm 0.1$  %
- Mass flow measurement: Thermal mass flow meter, range 0 to 1500  $\mu mol$  s  $^{-1}$  , accuracy  $\pm 1~\%$
- **Integrated user interface:** Panel PC NX800LX/500 MHz with graphical color-display 640 x 480 dots (effective display area 13 cm x 10 cm) with backlight (well readable in direct sun-light) and touch screen and USB 2.0 connector and audio speaker.

Data storage capacity: 4 GB compact flash card

- CO2 control: Integrated CO<sub>2</sub> control via thermal valve, range 0 to 2000 ppm, CO<sub>2</sub> supply via CO<sub>2</sub> cartridges (8 g CO<sub>2</sub>, provide more than 48 hours continuous supply at 350 ppm CO<sub>2</sub>, reserve is indicated) or via CO<sub>2</sub> cylinder with pressure reducer
- **H2O control:** Integrated H<sub>2</sub>O control via step motor for humidifying and drying, range 0 to 100 % rh (non-condensing)
- **Measured and calculated parameters:** CO<sub>2</sub> absolute, CO<sub>2</sub> difference, H<sub>2</sub>O absolute, H<sub>2</sub>O difference, flow, ambient pressure, 2x auxiliaries, cuvette temperature (upper and lower half), leaf temperature, ambi-

ent temperature, PAR in upper part of the cuvette, PAR in lower part of the cuvette, external PAR, impeller frequency, evaporation, VPD, H<sub>2</sub>O conductance, net photosynthesis, internal CO<sub>2</sub> concentration. Recalculation of stored data is possible,

In combination with LED-Array/PAM Fluorometer 3055-FL (GFS-3000FL): Fo, Fm, Fm', F, Fo', Fv/Fm (max. PS II quantum yield),  $\Delta$ F/Fm' = Y(II) (effective PS II quantum yield), qP, qN, NPQ, ETR (i.e. PAR x  $\Delta$ F/Fm')

PC interface: USB 2.0 with USB null modem cable (NMC).

**Auxiliaries:** Two analog inputs, range 0 to 4095 mV, user programmable

**Input voltage:** 12-24V

Power Supply: Field replaceable rechargeable Li-ion Battery

14.4 V/15 Ah 3025-A (two batteries supplied), external 16 V DC, AC Power Supply 3020-N for laboratory operation. The Control Unit 3100-C may also be operated with a 12 or 24 V battery (use only the optional battery cable 3100-C/BC provided by us).

**Operating time:** 2 to 4 hours typ. with one Li-ion battery, 4 to 8 hours typ. with two Li-ion batteries

**Operating temperature:** -5 to +45 °C

**Dimensions:** 43 cm x 28 cm x 26 cm (L x W x H)

Weight: 11.1 kg (incl. Li-ion battery)

#### 18.2 Control Unit 3000-C (sold until 2011)

**Design:** Aluminum housing featuring integrated PC module, large graphical LC-display with touch screen, 4-channel CO<sub>2</sub>/H<sub>2</sub>O gas analyzer, flow control, CO<sub>2</sub> control (supplied via small cartridges or cylinders) and H<sub>2</sub>O control (for drying and humidifying). Pneumatic connectors for air inlet, measuring head and four vents. Sockets for cable connections with Measuring Head 3010-S (or other measuring heads supplied by us), PC (USB interface), two Auxiliaries, two Li-ion Batteries 3025-A (or AC Power Supply 3020-N or external DC) and one additional component

# CO<sub>2</sub>/H<sub>2</sub>O gas analyzer, Mass flow measurement, CO<sub>2</sub> control, H<sub>2</sub>O control, Auxiliaries:

same as Control Unit 3100-C

**Integrated user interface:** Panel PC 486 SX/33 MHz with transflective graphical LC-display 320 x 240 dots (effective display area 11.5 cm x 8.6 cm) with backlight and touch screen

Data storage capacity: 64 MB flash card

Measured and calculated parameters: CO<sub>2</sub> absolute, CO<sub>2</sub> difference, H<sub>2</sub>O absolute, H<sub>2</sub>O 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<sub>2</sub>O conductance, net photosynthesis, internal CO<sub>2</sub> concentration. Recalculation of stored data is possible,

In combination with LED-Array/PAM Fluorometer 3055-FL (GFS-3000FL): Fo, Fm, Fm', F, Fo', Fv/Fm (max. PS II quantum yield),  $\Delta$ F/Fm' = Y(II) (effective PS II quantum yield), qP, qN, NPQ, ETR (i.e. PAR x  $\Delta$ F/Fm')

PC interface: USB 2.0 Input Voltage: 14-16 V

- **Power Supply:** Field replaceable rechargeable Li-ion Battery 14.4 V/15 Ah 3025-A (two batteries supplied), external 16 V DC, AC Power Supply 3020-N for laboratory operation
- **Operating time:** 2 to 4 hours typ. with one Li-ion battery, 4 to 8 hours typ. with two Li-ion batteries
- **Operating temperature, Dimension, Weight:** same as Control Unit 3100-C

#### 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 or 3100-C, LED Light Source 3040-L or one additional component (e.g. LED-Array/PAM-Fluorometer 3055-FL)
- Cuvette temperature, (in each air exit of 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  $^{\circ}$ C, accuracy  $\pm 0.2 \,^{\circ}$ C

**External miniature quantum sensor:** Quantum Sensor MQS-B/GFS sits on top of measuring head. Selective PAR measurement, range 0 to  $2500 \ \mu mol \ m^{-2} \ s^{-1} \ PAR$ , accuracy  $\pm 5 \ \%$ , cosine corrected (measuring photosynthetic photon flux density, PPFD)

**Internal quantum sensor:** Selective PAR measurement, range 0 to  $2500 \ \mu mol \ m^{-2}s^{-1}$  PAR, accuracy  $\pm 10 \ \%$ , two sensors, one in the upper and one in the lower part of the cuvette

**Cuvette ventilation system:** Two frequency controlled impellers, one in the upper and one in the lower part of the cuvette, speed adjustable

**Leaf area:** 8 cm<sup>2</sup> standard, interchangeable adapter plates from 1 to 8 cm<sup>2</sup>, 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 3020-N

Design: DC power supply unit for laboratory use

Output voltage: 16 V DC

Output power: 135 W (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 cm (L x W x H) – depends on version

Weight: 1 kg

#### 18.5 Li-ion Battery 14.4 V/15 Ah 3025-A

**Design:** High-performance maintenance-free rechargeable Li-ion battery with protection circuit, black Perspex-aluminum housing

Nominal voltage: 14.4 V Typical capacity: 15 Ah

Operating temperature: -10 to +60 °C (discharge) and 0 to 50 °C (charge)

**Dimensions:** 28 cm x 15.5 cm x 2.8 cm (L x W x H)

Weight: 1.7 kg

#### 18.6 LiFePO4 Battery 3035-A

**Design:** Mounting plate with GFS-3000 connector plug, containing two electronically separated LiFePO4 batteries placed in parallel, with protection circuit

Nominal voltage: 12.8 V

**Typical capacity:** 15 Ah (2 x 7.5 Ah)

**Operating temperature:** -30 to +60 °C

**Dimensions: 28 cm x 16 cm x 7.5 cm (L x W x H)** 

Weight: 2.78 kg

#### 18.7 Battery Charger LC-03

**Design:** Mains operated automatic Li-ion or LiFePO4 battery charger (as indicated on the instrument housing) with two independent charge outputs, 2-line LC-display with backlight for each charge output

**Charging voltage:** 16.4 V max.

**Charging current:** 0 to 3 A

Recharging time: 6 hours for one or two batteries 3025-A or 3035-A

Mains power supply: 100 to 240 V AC, 50/60 Hz

Operating temperature: -5 to 45 °C

**Dimensions:** 20.5 cm x 19 cm x 9 cm (L x W x H)

Weight: 1.7 kg

#### 18.8 LED Light Source 3040-L

**Design:** LED array with 24 red and 2 blue LEDs

**Light intensity:** Range 0 to 2000  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup> PAR max., type. 90 % red (640 nm) and 10 % blue (470 nm)

**Homogeneity of light distribution:** ±20 % at leaf level

Leaf area: 8 cm<sup>2</sup>

**Power consumption:** 5 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: 160 g

#### 18.9 LED-Array/PAM-Fluorometer 3055-FL

**Design:** Combined PAM chlorophyll fluorometer and LED light source consisting of LED array with 24 red LEDs (for actinic illumination and saturation pulses), 2 blue LEDs (for measuring light and actinic illumination), 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 (640 nm), range 0 to 2000  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> PAR, typ. 90 % red and 10 % blue

**Saturation light:** Red LEDs (640 nm), up to 4500 µmol m<sup>-2</sup> s<sup>-1</sup> 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<sup>2</sup>

**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: 230 g

#### 18.10 Fiberoptics/PAM-Fluorometer 3050-F

**Design:** PAM chlorophyll fluorometer enclosed in a metal tube, which can be connected 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  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> with low frequency, 15  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> with high frequency

**Saturating light:** Blue LED (peak: 450 nm). At 1 mm distance: typically 11000 μmol m<sup>-2</sup> s<sup>-1</sup> at 2 mm: typically 6000 μmol m<sup>-2</sup> s<sup>-1</sup>

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

#### Relationship between light and distance

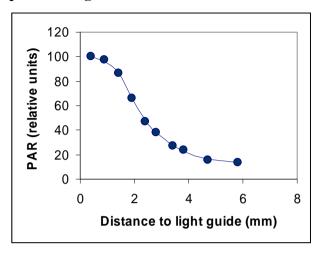


Fig. 68: Relative PAR in dependence of distance. The fiber was placed perpendicular to the sensor (pin-hole: 1 mm diameter).

**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):

$$\log_{10}[SVP(T)] = -7.90298 \cdot \left(\frac{Ts}{T} - 1\right) + 5.02808 \cdot \log_{10}\left(\frac{Ts}{T}\right)$$
$$-1.3816 \cdot 10^{-7} \cdot \left(10^{11.344*(1-T/Ts)} - 1\right)$$
$$+8.1328*10^{-3}*(10^{-3.49149*(Ts/T-1)} - 1) + \log_{10}(SP_{ws})$$

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 steampoint 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.

Table 15: Saturating Vapor Pressure above water

Temp.	.0	.1	.2	.3	.4	.5	.6	.7	.8	.9
°C	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.	.0	.1	.2	.3	.4	.5	.6	.7	.8	.9
<u>°C</u>	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	204.859 214.488	205.805	206.755	207.708
61 62	208.665 218.446	209.626 219.445	210.591 220.448	211.560 221.455	212.532 222.466	213.508 223.480	214.488	215.472 225.522	216.459 226.548	217.451 227.579
63	218.446	219.445 229.652	220.448	221.455	232.791	233.846	234.905	225.522	220.548	238.105
	239.180									238.103
64		240.259	241.343	242.430	243.522	244.617	245.717	246.821	247.930 259.247	260.403
65	250.159	251.280	252.405	253.535	254.669	255.807	256.949	258.096		
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 71	311.689	313.040	314.395	315.756	317.121	318.491	319.867	321.247 335.330	322.633	324.023
	325.418	326.819	328.225	329.635	331.051	332.472	333.899		336.766	338.208
72 73	339.655 354.414	341.107 355.919	342.565 357.430	344.027	345.495	346.968	348.447 363.527	349.931 365.064	351.420	352.914
73 74				358.946	360.467	361.994			366.608	368.156
75	369.711 385.560	371.270 387.176	372.836 388.798	374.407 390.425	375.983 392.058	377.565 393.697	379.153 395.342	380.746 396.992	382.345 398.649	383.950 400.311
75 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
78 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 Warranty Conditions

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

#### The warranty is subject to the following conditions:

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

#### To obtain warranty service, please follow the instructions below:

- 1. The Warranty Registration form must be completed and returned to Heinz Walz GmbH, Germany.
- 2. The product must be returned to Heinz Walz GmbH, Germany, within 30 days after Heinz Walz GmbH, Germany has received written notice of the defect. Postage, insurance, custom duties, and/or shipping costs incurred in returning equipment for warranty service are at customer expense.
- 3. All products being returned for warranty service must be carefully packed and sent freight prepaid.
  - Heinz Walz GmbH, Germany is not responsible or liable, for missing components or damage to the unit caused by handling during shipping. All claims or damage should be directed to the shipping carrier.

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