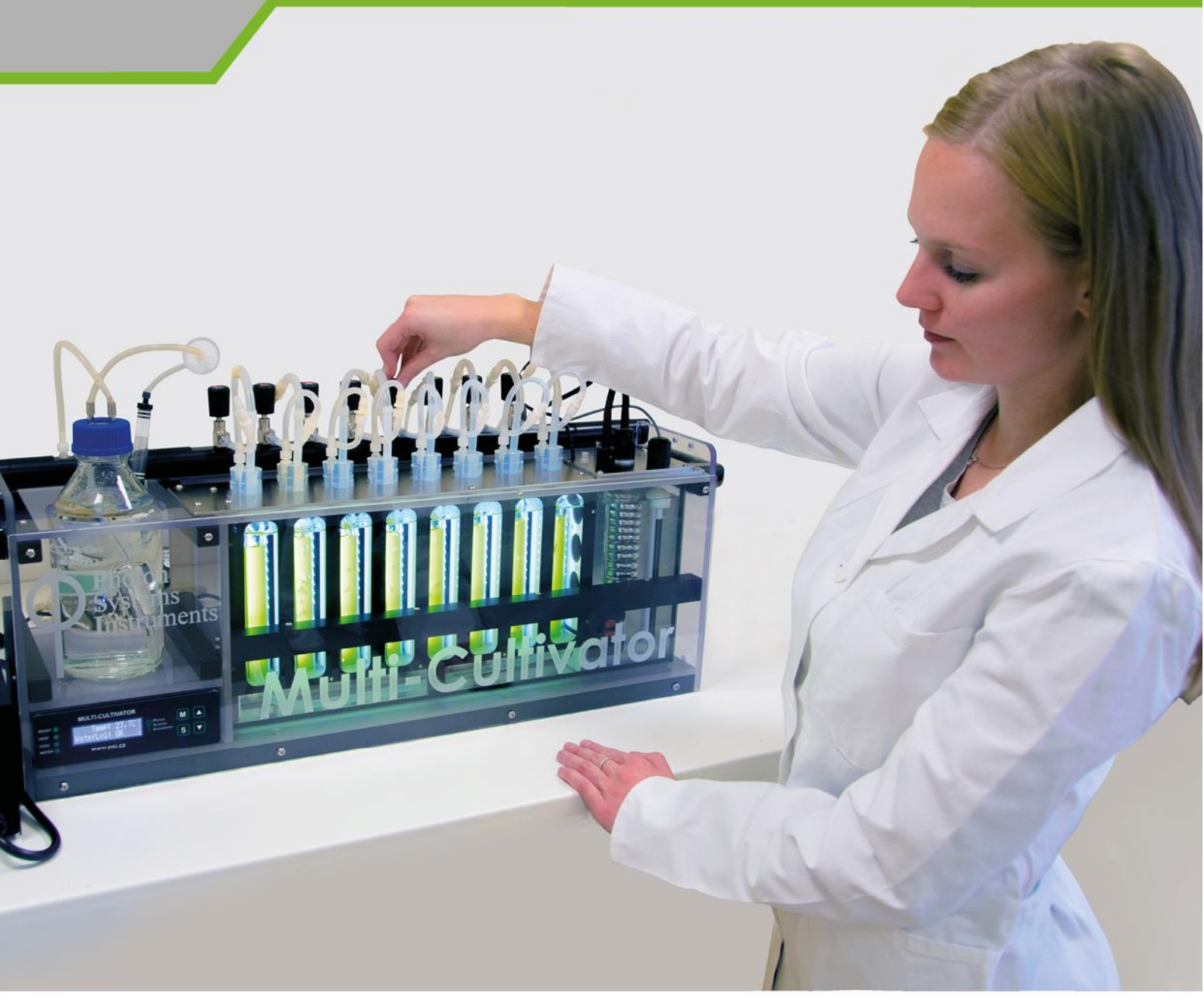
Instruction Guide

PHOTON SYSTEMS INSTRUMENTS

Multi-Cultivator MC 1000-OD

Please read the Guide before operating this product



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1 WARNINGS AND SAFETY PRECAUTIONS

PLEASE READ THE FOLLOWING INSTRUCTIONS CAREFULLY BEFORE TURNING THE MULTI-CULTIVATOR ON:

- Remove all packaging and transport protectors before connecting the Multi-Cultivator to the power supply.
- Use only cables supplied by the manufacturer.
- Keep the device dry outside and avoid working in high humidity environment!
- The manufacturer is not responsible for any damage due to improper operation!
- Water and other liquids should only be placed in vessels designed for the purpose and according to instructions included in this manual.

GENERAL ELECTRICAL SAFETY GUIDELINES:

- Perform a routine check of the devices and their wiring.
- Replace worn or damaged cords immediately.
- Use appropriate electrical extension cords/power bars and do not overload them.
- Place the device on a flat and firm surface. Keep away from wet floors and counters.
- Avoid touching the device, socket outlets or switches if your hands are wet.
- Do not perform any alterations to the electrical parts of the device or its components.

The following table presents basic highlight symbols used in this manual:

Symbol	Description
	Important information, read carefully.
	Complementary and additional information.

Tab. 1 Used symbols.

⚠	The Multi-Cultivator MC 1000-OD is considered Class 1M* LED Product. LED radiation may be harmful to eye. Avoid direct and strongly reflected exposure. Use protective glasses.

**Class 1M: Laser and LED equipment that is safe for the naked eye under foreseeable conditions of operation. Looking directly into the source of radiation by employing optics within the beam, such as magnifying glass, telescope or microscope, can be potentially hazardous.*

2 GENERAL DESCRIPTION

Multi-Cultivator MC 1000-OD is a cost-effective small-scale cultivation device developed for cultivation of multiple samples. The instrument is primarily intended for synchronous growth of algae, bacteria or cyanobacteria under defined conditions with a wide range of applications (i.e. toxicological and eco-toxicological testing, optimization of cultivation conditions, phenotypization of various strains). Multi-Cultivator MC 1000-OD is suitable for cultivations of various microorganisms due to wide range of used LED colors from 405 nm to 730 nm. The device is available in unicolor and multicolor versions.

Multi-Cultivator MC 1000-OD is designed to monitor growth of cultivated organisms by measuring optical density at two wavelengths of 680 nm and 720 nm under controlled environmental conditions (optional). Optical density is periodically measured at selected time intervals and the data are automatically stored in the Multi-Cultivator internal memory for transfer to PC at a later time.

Multi-Cultivator MC 1000-OD consists of 8 cultivation tubes where up to 85 ml of suspension can be maintained under controlled temperature, light and aeration conditions. The cultivation tubes are immersed in temperature controlled water bath. Each tube is illuminated by an array of LEDs (different colors available) that generate incident irradiance up to 1,000 $\mu mol.m^{-2}.s^{-1}$ (optionally up to 2,500 $\mu mol.m^{-2}.s^{-1}$) which is independently adjustable for each cultivation tube in intensity, timing and modulation. Each tube can be bubbled with air or selected gas (optional) of different flow rate through a manually adjustable valve manifold. Multi-Cultivator MC 1000-OD is supplied with a light controlling unit that supports user-defined illumination protocols, such as, flashing light or diurnal regime.

The instrument function can by enhanced by optional accessories:

- Cooling Unit AC-710 for precise thermoregulation of the water bath below ambient temperature from 15 °C.
- PWM Pump for automatic water bath refilling as water evaporates over time.
- Gas Mixing System GMS 150 for control of composition of aeration gas.
- Three-Ways Sampling Valve for sterile culture sampling during cultivation.
- New Monitoring Software designed for user friendly control of several multi-cultivators, clear data visualization and easy online processing, plus remote control.
- Turbidostat module for continuous cultivation at constant optical density.

The multi-well set-up of MC 1000-OD with controlled and adjustable light, temperature and aeration conditions is primarily suitable for small scale, multi-sample or multi-variant experiments. Multi-Cultivator MC 1000-OD can be used in various biotests or optimization studies when different light treatments of the same or different organisms need to be assessed under reproducible conditions.

This manual contains technical information about the Multi-Cultivator device, description of instrumentation delivered with the device and a step by step instructions for successful cultivation of a widely used test alga *Chlorella vulgaris*. The short instructional video about the set-up of MC 1000-OD is provided for demonstration purposes as part of the MC 1000-OD Cultivation kit, illustrates how to set up the MC 1000-OD and initiate the culture under standard conditions.

2.1 TECHNICAL SPECIFICATION

Number of Cultivation Tube Slots:
8
Volume of each Cultivation Tube:
100 ml (maximum recommended cultivation volume of each cultivation tube is 85 ml)
Precision Controlled Temperature:
5 to 10 °C above the ambient temperature - 60 °C (depending to light intensity)
15 °C – 60 °C (optional with cooling unit AC-710 and under standard laboratory conditions)
Heating System:
One 150 W cartridge heater
LED Lighting:
Cool white, warm white, red or blue LED lighting (more about the light spectra of unicolor MC 1000-OD in the Appendix)
In multi-color version is each cultivation slot furnished with different LED color (more in the Appendix)
Light intensity is calibrated and linearized. It is adjustable from 1 $\mu mol.m^{-2}.s^{-1}$ – maximum intensity
Maximum light intensity up to 1,000 $\mu mol.m^{-2}.s^{-1}$ (Light upgrade version has intensity up to 2,500 $\mu mol.m^{-2}.s^{-1}$)
Light Regime:
Static or dynamic (triangle, sinus, daylight, pulse form)
Display:
Two-line display serves to offline system control and visualization of actual readings
Controlled Flow of Bubbled Air:
Manual via manifold valves
Controlled Composition of Bubbled air:
Optional with purchase of GMS 150
Volume of Water Bath:
5 l
Measured Parameters:
Optical density (OD) and temperature
Optical Density Measurement:
Real time measurement of OD by two LEDs (720 nm, 680 nm) per cultivation tube. Measurement made at specified time intervals. Optical path – 27 mm.
Detector Wavelength Range:
PIN photo-diode with 630 nm - 750 nm bandpass filters
Bios:
Upgradeable firmware
Communication Port:
USB A-B
Material:
Glass, stainless steel, silicone gasket, polycarbonate
Dimension:
80.5 x 35 x 21 cm
Weight:
13 kg
Electrical:
110-240 V AC

3 DEVICE DESCRIPTION

Standard version of the MC 1000-OD package consists of the main body comprising control unit, integrated air pump, holder for humidifier bottle, temperature and water control unit, water pump, water bath, main gas dispenser tube, power supply, plus OD measuring sensor (Fig. 1). In addition, the complete packet contains also MC 1000-OD Cultivation Kit (Fig. 2). The main part of the MC 1000-OD is a thermally controlled water bath with 8 slots for the cultivation tubes and the LED panel on the back. Cultivation water bath is a flat, rectangular, glass container with the maximal capacity of 5 liters with inserted cultivation tubes. The homogeneous temperature distribution within the water bath is ensured by continuous circulation of the tempered medium (distilled water) through a water pump. Illumination is designed and controlled to be independent for each cultivation tube. The potential crosstalk with the neighboring tubes is minimized by inserted plastic dividers. The control unit of the MC 1000-OD can be used to individually set of varied light protocols and intensities for each cultivation tube. The dimension of cultivation tubes is 3 cm in diameter and 20 cm height. The maximum volume held by one cultivation tube is 100 ml, the recommended working volume is 85 ml. The array of light emitting diodes (LEDs) is located behind the cultivation tubes. See User's guide chapter on page 25 for cultivation instruction and detailed description of the MC 1000-OD Cultivation Kit.

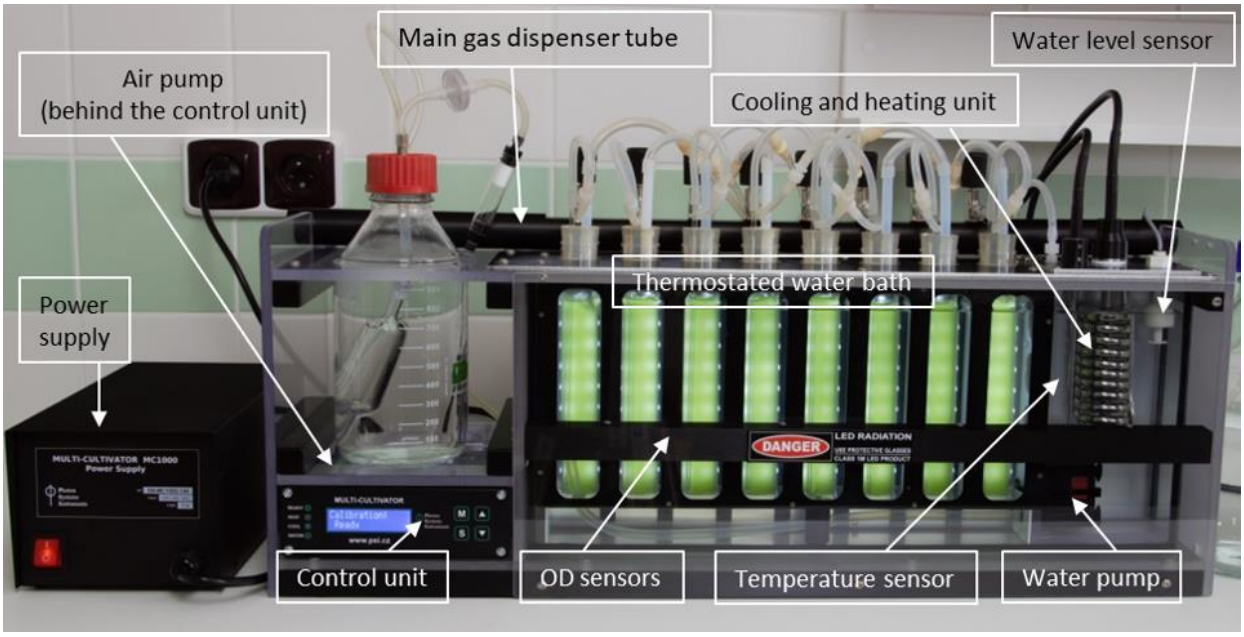


Fig. 1 Front view of the MC 1000-OD.

	Clean the Multi-Cultivator MC 1000-OD and its components only with a mild detergent if not stated otherwise. Don't use alcohol-based cleaners to the plastics.
	If cultivation tube is filled over 85 ml excessive fluid may overflow during bubbling.
	Please note that some light penetration (ca. 2 % when highly illuminated slot is next to dimly illuminated one) occurs between neighboring cultivation slots because the plastic dividers between the slots are not fully light proof.

3.1 COMPONENTS OF MC 1000-OD

Standard MC 1000-OD components delivered to the customer and the view of the final MC 1000-OD set-up are shown in Fig. 1.

	Check the contents of the package and compare it with enclosed standard package list (see below).

3.1.1 LIST OF STANDARD MC 1000-OD COMPONENTS

1. Power supply with the power cord.
2. Main body of the MC 1000-OD consisting of:
    - Water bath,
    - water pump with voltage changer,
    - cooling and heating unit,
    - water level sensor,
    - temperature sensor,
    - integrated air pump,
    - control unit,
    - main gas dispenser tube with manifolds,
    - optical density sensors for each cultivation tube.
3. Cultivation Kit for set up of 8 cultivation tubes (For the complete list of cultivation kit components see Tab. 2 on page 25) and protective glasses.

Fig. 2 Components of the MC 1000-OD Cultivation Kit.

4. Gas Connection Module for establishing the connection between the Multi-Cultivator and an external Gas Mixing System such as GMS 150 or an external air pump. This module is required if the gas supply to the MC 1000-OD is not via the integrated air pump which is provide with the Multi-Cultivator.
5. USB Flash Disc with ODView Software for downloading stored OD and temperature measurements and with ControlDeviceCenter Software for light calibration or firmware upgrade and with demonstration video User´s Guide for Cultivation of Algae and Cyanobacteria in MC 1000-OD.
6. USB cable with for device monitoring by control SW, data transfer or firmware upgrade.
7. Instruction Manual and User's Guide for cultivation.

3.1.2 OPTIONAL ACCESSORIES/COMPONENTS

1. Cooling Unit AC-710 including plastic tubes, power cord and control cable for cooling the water bath below ambient temperature (down to 15 °C). MC 1000-OD built-in temperature regulator allows only to warm up the temperature inside the water bath.

	MC 1000-OD device itself does not have temperature regulator built-in that would allow regulate the temperature in water-bath below the ambient temperature. If high light intensities are used, please be aware that the water bath temperature will increase even above the ambient room temperature.

2. PWM Pump including plastic tubes and plastic connectors for automatic control of water level in the water bath.
3. Gas Mixing System GMS 150 or an external air pump including parker tubing and connectors for control gas concentrations in the MC 1000-OD.
4. Spare Part Kit.
5. Set of 10 Three-Ways Sampling Valves for sterile culture sampling during cultivation.
6. New Monitoring Software.
7. Turbidostat module and USB Flash disc with Turbidostat manual and with PumpCalibrator software.

3.2 DESCRIPTION OF THE MULTI-CULTIVATOR CONTROL UNIT FRONT PANEL

The device is controlled via front panel of the control unit with 4 LED lights on the left and 4 control keys on the right (Fig. 3).

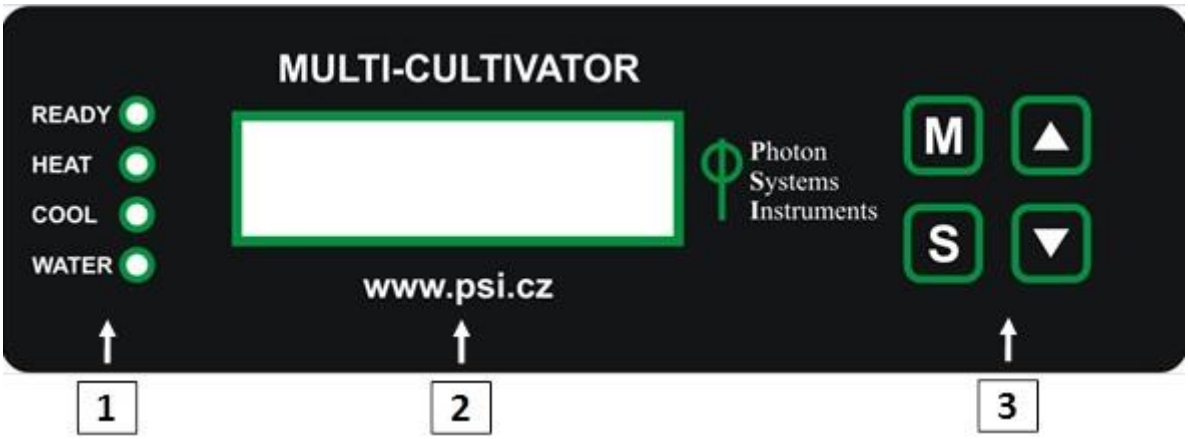


Fig. 3 Multi-Cultivator front panel. 1) Four LED indicators. 2) Two line display. 3) Four control keys.

3.2.1 1. LED INDICATORS

Green LED indicator **READY** is lighting when the current temperature is equal to target temperature.

Orange LED indicator **HEAT** is lighting when the heater turns on in the water bath.

Blue LED indicator **COOL** is lighting when the cooling spiral is cooling water in the water bath when the Cooling Unit AC-710 is operating.

	Without Cooling Unit AC-710 the minimal temperature in the MC 1000-OD will correspond to surrounding room temperature.

Red LED indicator **WATER** is lighting when the water level in the water bath drops under required level.

3.2.2 2. MAIN DISPLAY

The functions as shown in the main display are controlled via the main keys as described below.

3.2.3 3. MAIN KEYS

[M]: Used to move back in the menu tree or to exit the menu.

[S]: Used to move forward in the menu tree, to save the selection, or to turn ON/OFF.

[↑]: Used to move up in the menu or to add value.

[↓]: Used to move down in the menu or to subtract value.

See page 39 of this Manual for more information on Multi-Cultivator control.

3.3 DESCRIPTION OF THE MULTI-CULTIVATOR REAR PANEL

The rear panel houses connectors for connecting cables (Fig. 4). The rear panel differs depending on the model: Model A is inset with bayonet pin connectors whereas model B with circular M12 connectors. Moreover, the model B allows to connect also water level sensor, heater and temperature sensor through the external connectors which facilitates their replacement.

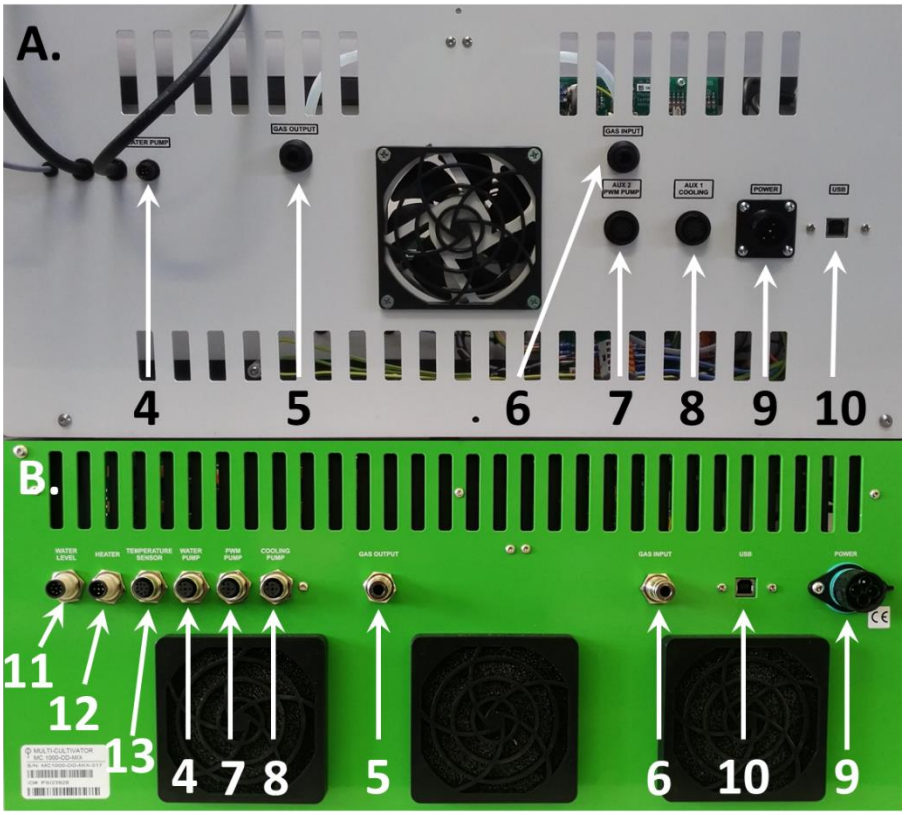


Fig. 4 Multi-Cultivator rear panel. Model A: 4) Water pump connector. 5) Gas Output for the Gas Mixing System. 6) Gas Input for the Gas Mixing System. 7) AUX2 connector for PWM Pump. 8) AUX1 connector for Cooling unit. 9) Power supply connector. 10) USB communication connector. Model B: 11) Water level sensor. 12) Heater. 13) Temperature sensor.

3.3.1 CONNECTION FOR THE WATER PUMP

Power cable for water pump should be plugged into the water pump connector (Fig. 4-4) prior to switching MC 1000-OD device ON.

3.3.2 IDENTIFICATION LABEL WITH SERIAL NUMBER

Each MC device is assigned by the serial number after the final testing. The serial number is marked on the label on the main body. Please note, the serial number of Multi-Cultivator and the power supply must fit (Fig. 5).

Fig. 5 A) Label from the MC 1000-OD. B) Label from the Power Supply of the MC 1000-OD. Note the same serial number.

	Damage can occur when the power supply is incompatible with the MC 1000-OD device.

3.3.3 GAS OUTPUT AND GAS INPUT FOR THE GAS MIXING SYSTEM

Please note that the Gas Mixing System GMS 150 is not included with the standard MC 1000-OD device. See page 23 for detail instructions for use of the Gas Mixing System.

3.3.4 AUX2 CONNECTOR FOR THE PWM PUMP CABLE

Please note that the PWM Pump is not part of standard MC 1000-OD device. See page 22 for detail instructions for use of the PWM Pump.

3.3.5 AUX1 CONNECTOR FOR THE COOLING UNIT AC-710

Please note that the Cooling Unit AC-710 is not included with the standard MC 1000-OD device. See page 18 for detail instructions for use of the device.

3.3.6 USB COMMUNICATION CABLE

USB communication cable is provided as part of the MC 1000-OD package for connecting the device with the computer. This connection has to be made for OD and temperature data download to a PC by ODView program, for SW control of the device or for any firmware updates.

4 INSTALLATION

4.1 DEVICE INSTALLATION

- Multi-Cultivator should be placed on a flat, firm and dry surface.
- Make sure that the power supply is switched OFF.
- Using the provided power cord, interconnect the MC 1000-OD with the power supply. Plug the power cord of the power supply (the thickest cable) into the round connector on the rear panel of the Multi-Cultivator labeled POWER (Fig. 6A).

Fig. 6 Rear panel of the MC 1000-OD with the POWER connector and Power supply device. The upper picture is related to the model A, the lower picture is related to the model B.

- Connect the power supply (Fig. 6B) to a 110/230 V outlet.
- Plug the voltage changer of the water pump into the water pump connector (Fig. 4-4) in the rear panel of the MC 1000-OD.
- Switch ON the power supply.

4.2 LIGHT CALIBRATION

Lights of each MC 1000-OD device are factory calibrated. Lights are calibrated for different light intensities equivalent to 1, 2, 3, 5, 10, 15, 20, 25, 50, 70, 90 and 100 % output of the LED lights. The results of the light calibration with the calibration coefficients are included in a calibration list and provided to the customer together with the purchased device unit.

In case the customer requires re-calibration or the firmware version was changed (please note that in this case light calibration values are lost) the following steps should be followed:

- Fill the water bath with distilled water up to 2/3 of the volume.
- Place cultivation tube filled with distilled water in slot 1 (first slot from the left side of the MC 1000-OD = Light 1).
- Turn on the Multi-Cultivator and connect it to the computer via serial cable. Open ControlDeviceCenter from delivered USB flash disc and find the device via button "**Detect**".
- Place the light measuring sensor inside the cultivation tube at a fixed depth in the center of the tube. **Please note, we use spherical light sensor for the calibration.**
- Write a desired light output (for example "100 %") down in the first window in **Calibration > Displayed Light > Light 1 > New Calibration**.
- Activate the Light 1 with click to the second window. Write a relevant acquired light intensity down in the second window (without using the units).
- Use button "**Add**" to continue with next light intensity of Light 1.
- After finishing of Light 1 calibration use button "**Save to Device**" to save the calibration coefficients and curve for Light 1. Please note, after calibration saving only calibration coefficients and calibration curve without raw calibration data will be displayed in sheet "**Calibration**".
- Follow the same protocol to calibrate all the other lights ensuring that the light measuring sensor is placed at the same depth and in the center of every cultivation tube.

	For optimal calibration performance, proceed with the calibration in dark room where no external light sources can influence the measurement. Please note that when all lights are operating, light intensity measured in individual slots will be higher due to the penetration of some light from the neighboring slots.

Calibration coefficients (either provided by manufacturer or calculated by customer) can be changed and replaced (sheet "**Calibration**"). It is recommended to save the customized calibration coefficients for different calibration curves in separate file because once the coefficients are overwritten and saved in ControlDeviceCenter they aren't accessible anymore.

5 ACCESSORIES/OPTIONAL COMPONENTS

The standard functions of the Multi-Cultivator device can be enhanced about six optional accessory modules – (1) Cooling Unit AC-710 (Fig. 7A), (2) Gas Mixing System GMS 150 (Fig. 7B), (3) Turbidostat module TS 1100 (Fig. 7C), (4) Sampling valve (Fig. 7D), (5) PWM Pump (Fig. 7E) and (6) Monitoring Software.

Fig. 7 A) Cooling Unit AC-710. B) Gas Mixing System GMS 150. C) Turbidostat module TS 1100. D) Three ways sampling valve. E) PWM Pump.

5.1 COOLING UNIT AC-710

Additional Cooling Unit AC-710 is designed to regulate temperature of water bath in the extended range, down to 15 °C with the resolution of ± 1 °C at standard laboratory conditions. This accessory device is also recommended for applications requiring high light intensities as some heating of the water bath by the LEDs always occurs.

	The AC-710 cooling unit is supplied in two versions – for 210-240V AC and 110V AC power line.

5.1.1 COOLING UNIT AC-710 EQUIPMENT

Cooling Unit AC-710 package consists of:

- AC-710 water pump (Fig. 8A),
- Hailea HC-130A water chiller,
- One piece of power cable,
- One piece of AUX cable,
- One piece of elastic silicone tube 8/6 mm – 5 m length.

5.1.2 INSTALLATION

1. Place the Cooling Unit AC-710 on a flat, firm and dry surface! Let it stand in upright position for at least **12 hours before plugging it into power supply!**
2. First, connect the water pump with the water chiller by placing two circular rubber seals (Fig. 8A) around the outlets on the top of the Hailea water chiller (Fig. 8B). Then, put the water pump on the top of the water chiller (Fig. 8C) and place the other two seals around the outlets of Hailea water chiller (Fig. 8D). Finally, fix the water pump to the water chiller with screws (Fig. 8E-F).
3. Plug the AC-710 water pump connector into the **AUX1** output on the rear panel of the MC 1000-OD (Fig. 8G). This connection provides the powering of the pump as well as controls its function in remote mode (MC 1000-OD controls the circulation of the water in water cooling circuit).

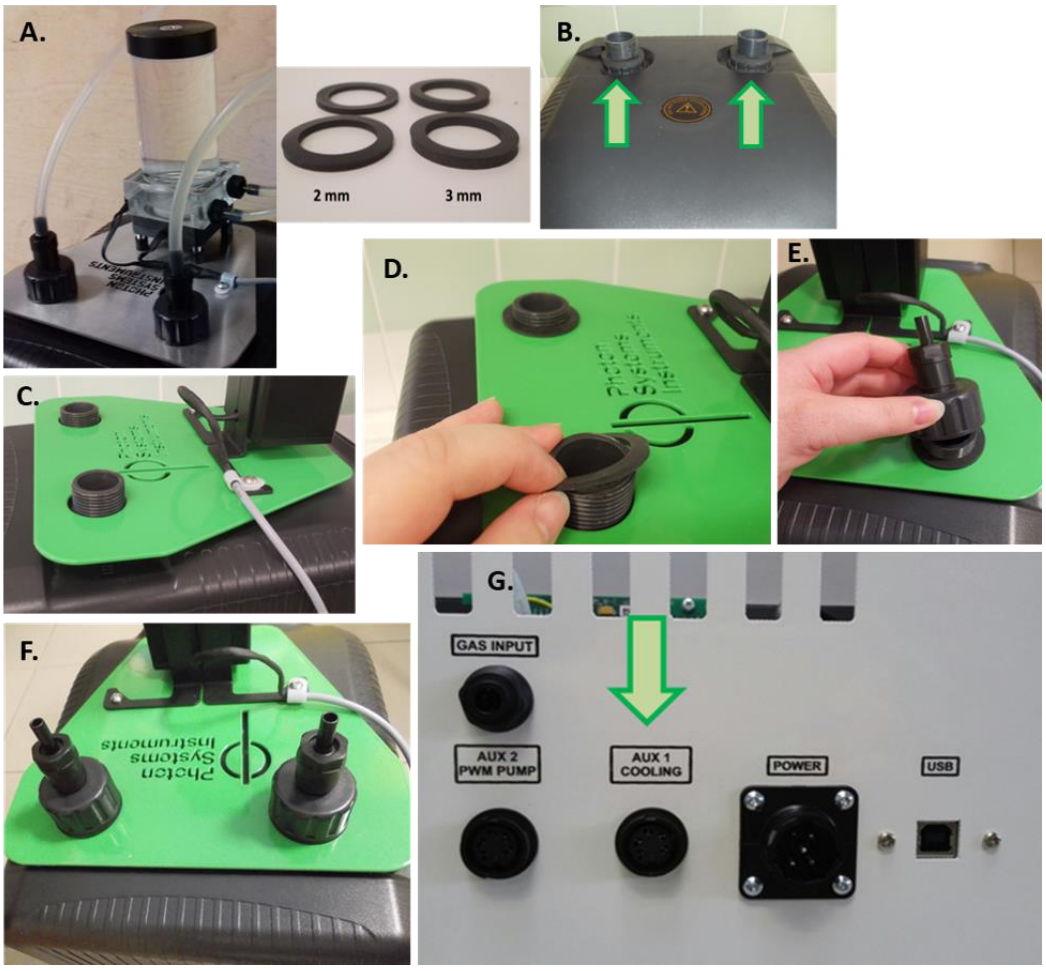


Fig. 8 A) AC-710 water pump and four circular rubber seals. B) Water outlets on the top of the Hailea water chiller. C)-F) Step-by-step montage of the water pump to the water chiller. G) Inner-connection of the cooling unit with the Multi-Cultivator using the AUX cable.

4. Inter-connect the AC-710 water pump, water chiller and MC 1000-OD cooling spiral using the water circulation hose (Fig. 9A-B). First, attach the short, 20 cm silicone hose to the **lower port** (Fig. 9A-1) on the right side of the water pump. Then connect the second end of this tubing to the right top input of the water chiller (Fig. 9A-2).
5. Second, connect the 50 cm long silicone hose to the **upper port** (Fig. 9A-3) on the right side of the water pump and inter-connect it with the right top input of the cooling spiral (Fig. 9B-4).
6. Finally, use the 1 m long silicone hose to connect left output of the MC 1000-OD cooling spiral (Fig. 9B-5) with the HC-130A water chiller (Fig. 9A-6).
7. Plug the Hailea HC-130A water chiller in AC electricity.

	The Hailea HC-130A water chiller is supplied with specific power cable for 220V or 110V AC plug.

8. Switch **ON** the HC-130A water chiller. Front display shows the actual temperature in the small water reservoir positioned inside of the HC-130A. Please read the attached HC-130A manual for more information.
9. Unscrew the top cover of the AC-710 water pump. This way you access the filling tank of the water circuit (Fig. 9C).
10. Switch **ON** the MC 1000-OD device. Fill the MC 1000-OD water bath with distilled water while the water level in water bath is optimal (Fig. 9D).
11. Set the low temperature via display: **Sensors > Temperature > 15°C**. Then set the temperature control ON: **Sensor > TControl > ON** (Fig. 10).
12. Pour carefully approximately 1 liter of distilled water in the water pump reservoir (Fig. 9C). Wait while the water is pumped into the cooling system. Fill the water into the system the water returns from the **upper port** on the right side of the water pump (Fig. 9A-3).
13. Let the bubbles leave out and add the water into the filling tank. It must stay filled up to the **upper port** on the right side of the water pump.
14. Screw back the top cover of the AC-710 water pump (Fig. 9C).
15. Set the required temperature of the water in the water chiller always to 5 °C. It is easily done by long push of the **SET** button on the front panel. Afterwards (set value is blinking) change the temperature to 5 °C and confirm by the short **SET** push.
16. Set the desired temperature via display or via control software.
17. MC 1000-OD is now set to control automatically the temperature in the water bath by circulating the water from the water chiller. Regulation is provided by the MC 1000-OD.
18. For the proper function of the AC-710 cooling device with the MC 1000-OD it is **IMPORTANT** to regularly check the water level in the cooling circuit. Water should be re-filled as described in Fig. 9C, E when the water level in water pump reservoir drops to 50 %. It is recommended not to let the water amount drop below this level as the cooling unit will not operate properly and the required temperature in MC 1000-OD may not be stable and increase.

	It is recommended not to leave the tank without the water. However, the **pump operation without the water will not damage the AC-710 Unit.** The water pump is prevented of overheating as it is automatically switched off in the case when pump temperature rises up too high.

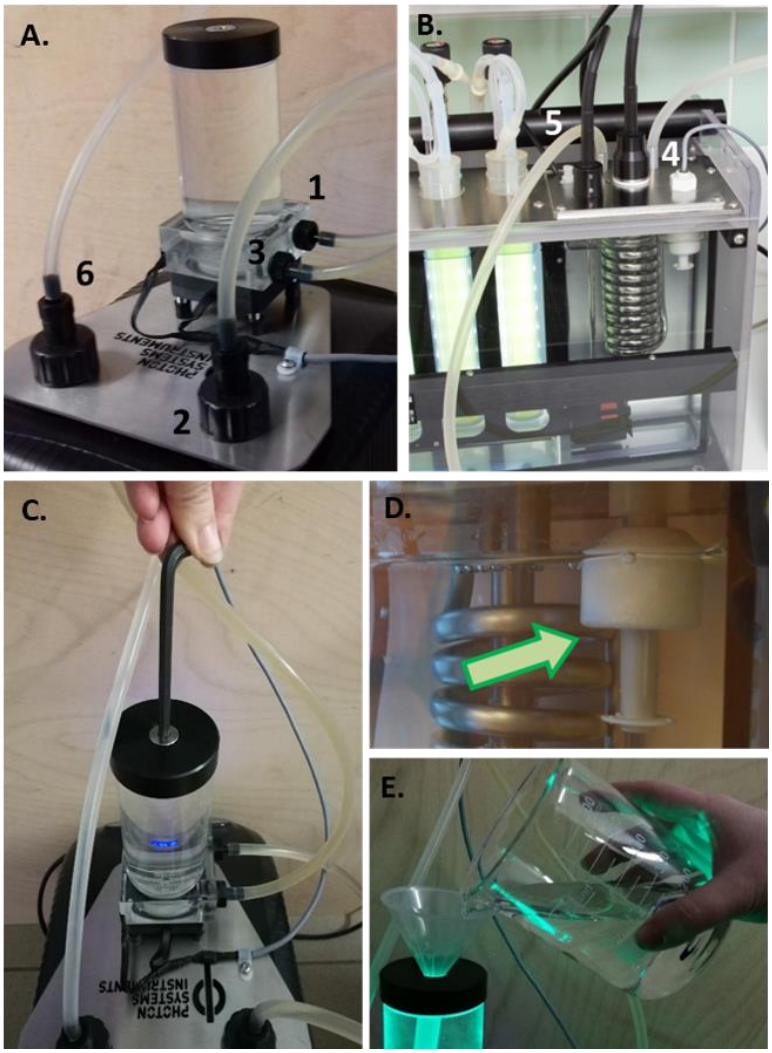


Fig. 9 A), B) Connection of the HC-130A water chiller with water pump and the MC 1000-OD. C) Unscrew/screw back the top cover of the AC-710 water pump. D) Optimal water level in the MC 1000-OD water bath. E) Filling of the water pump with distilled water.

18. When Cooling Unit device is operating and the water bath is cooled down blue LED light indicator **COOL** in the Multi-Cultivator control panel is lit (Fig. 10).

Fig. 10 Display of the MC 1000-OD control unit when Cooling Unit AC-710 is operating.

5.2 PWM PUMP REFILLING MODULE

PWM Pump is used to automatically refill water to the water bath. The water level in the bath is indicated by a water level sensor. The refilling function is useful especially at high cultivation temperatures a risk of higher evaporation. Or it can be a practical tool for long-term automated experiments.

5.2.1 PWM PUMP COMPONENTS

As shown in Fig. 11A:

- PWM Pump with remote control cable,
- silicone tubing with Luer Lock Fittings.

	Check the contents of the package and compare it with the described package content (see above). Component specifications can be found in Tab. 2 on page 25.

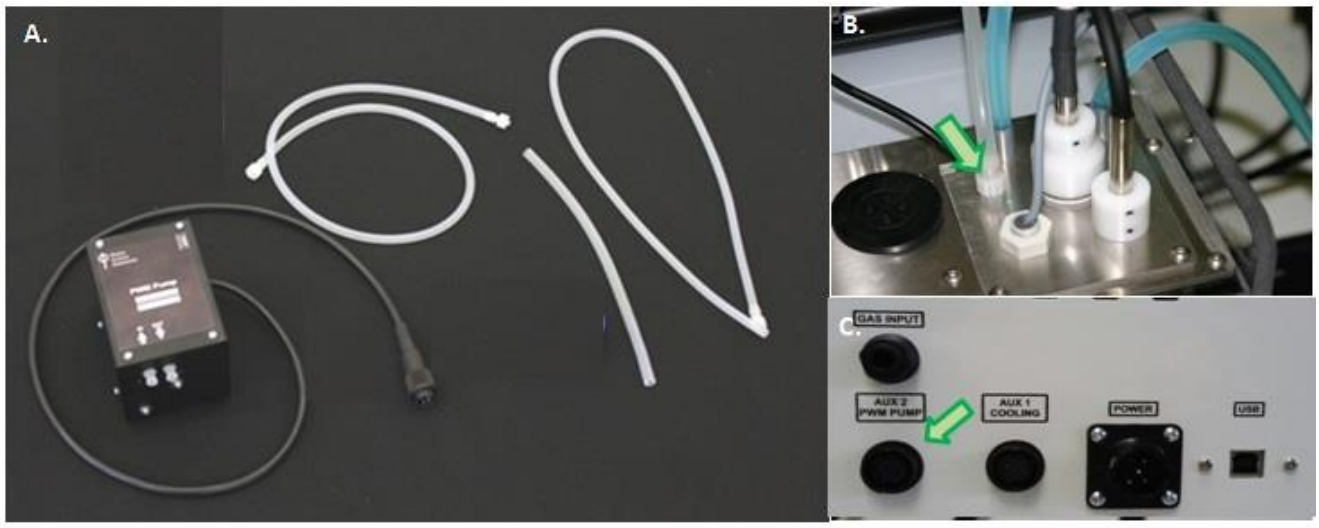


Fig. 11 Setting up the PWM Pump. A) Complete set of components. B) Connection of the "OUT" outlet of the pump with the inlet placed in the lid of water level checking system. C) PWM pump cable is connected to AUX2 plug on the rear panel of MC 1000-OD.

5.2.2 INSTALLATION

1. Use any glass or plastic bottle as an additional water re-filling bottle and fill it with minimum 1 liter of distilled water. Please note that additional water re-filling bottle is not part of PWM Pump set.
2. Cut two 35 – 40 cm long pieces of silicone tubing. One of them is placed into re-filling bottle. End of this tubing is connected with the MTLL Luer Lock Fitting to the "**IN**" outlet of the PWM Pump.
3. Second silicone tubing has MTLL Luer Lock Fitting on both ends. Connect one of the two MTLL Luer Lock Fittings to the "**OUT**" outlet of the PWM pump. The second end with the FTLL Luer Lock Fitting is attached to inlet placed in the lid of water level checking system as shown in Fig. 11B.
4. Insert the PWM Pump cable into the plug labeled AUX2 on the rear panel of the Multi-Cultivator as shown in Fig. 11C.
5. Switch the PWM pump **ON** via setting in the control unit of the MC 1000-OD: **Settings > PWM Pump > ON**.

⚠	Place the water-refilling bottle to the same elevation or below the PWM Pump to avoid water overflow from the water bath. Make sure that there is always water in the water re-filling bottle and the end of the silicone tubing inside the re-filling bottle is always submerged in the water.

5.3 GAS MIXING SYSTEM GMS 150

Gas mixing system GMS 150 serves to produce precise defined mixtures of up to 4 different gasses (e. g. Air, CO2, N2). The gas supplies should be pressurized for the proper operation of the GMS. Recommended inlet pressure is in the range of 3 - 5 bars. The GMS is a stand-alone instrument which is controlled locally from its front panel or alternatively via Multi-Cultivator Software.

The new versions of MC 1000-OD are manufactured with Parker connectors for the Gas Mixing System. It is possible to connect external air pump instead of GMS.

ℹ	MC 1000-OD version with connectors for the Gas Mixing System contains additional bubble interruption valve inside of the MC 1000-OD that is operating gas flow from the external gas mixing system. The control of the gas flow is done solely by the gas mixing device. No specific operation and set up is required to be done in the MC 1000-OD control unit.

5.3.1 INSTALLATION

- Use parker tubing to connect gas mixing unit with the gas inlet connectors of the MC unit and gas outlet connectors with the humidifier bottle. As shown in Fig. 12 Gas Mixing Unit outlet tubing (in yellow) is connected with the MC gas inlet connector.
- Gas outlet tubing from the MC (in blue) is directly connected with the humidifier bottle. It is recommended to connect Parker tubing with silicone tubing (optionally via Luer Lock fittings) which is then connected to the humidifier bottle as shown in detail Fig. 20F.

For a more detailed description please follow the manual for Gas mixing system GMS 150.

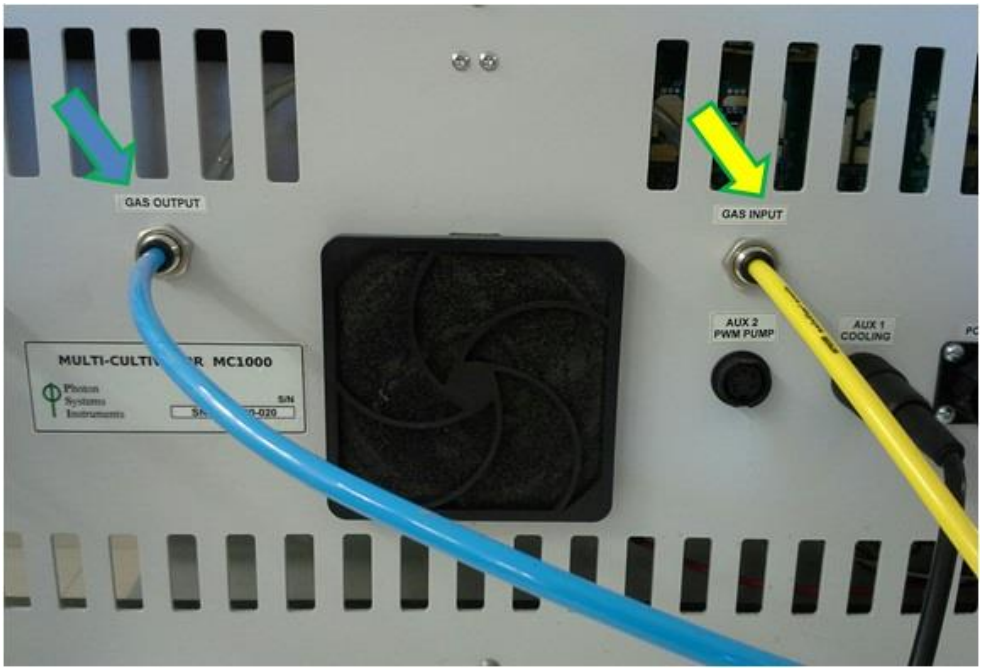


Fig. 12 Rear panel of MC 1000-OD version with gas/air mixing module. Note the gas inlet from GMS 150 in yellow and gas outlet connector bringing the desired air composition to the humidifier bottle.

5.4 THREE-WAYS SAMPLING VALVE

The valve serves for sterile culture sampling during cultivation (Fig. 13). The sampling valve is provided with the thread for quick and secure connection. Connected sampling valve has no influence on the flow rate and it is suitable for long-term application. Pressure resistance is guaranteed up to 4.5 bars.

5.4.1 INSTALLATION

- Connect the sampling valve between female luer lock (number 3 in Tab. 2) and male luer lock (4) as shown in Fig. 13A.
- Sterile syringe is used for sampling. Connect the syringe to the third connection of the sampling valve (Fig. 13B), turn the blue valve as shown in Fig. 13C and performed sampling.

	Sterile syringe is not included of the spare part kit (Tab. 2).

- After the sampling is finished, close the valve to initial position and remove the syringe.

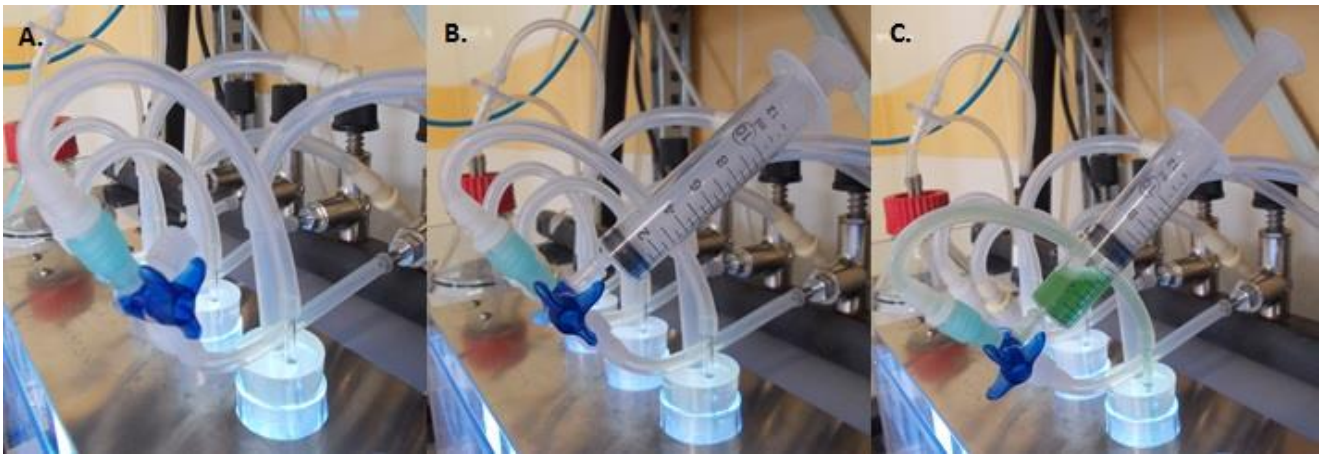


Fig. 13 A) Valve between Luer Locks. B) Valve with the attached syringe. C) Sampling of cyanobacteria.

5.5 MONITORING SOFTWARE

MC SW provides intuitive and dynamic interface for online monitoring and visualization of all measured data as well as creation of user-defined protocols. All recorded experiments are saved in SW database. Advanced data analysis functions are integrated for complex regression analysis and dynamic real-time calculation of growth rates for defined periods are possible. The SW is available in two versions: Basic and Advanced. The advanced version is optimized for simultaneous control of different and multiple cultivation devices such as Multi-Cultivators as well as Photobioreactors from one PC. Software controlled communication with additional devices such as Gas Mixing System GMS-150 is also supported.

5.6 TURBIDOSTAT

Turbidostatic version of Multi-Cultivator MC 1000-OD allows to control the biomass growth via OD680 or OD720 independently in each vessel. The selected cultivation optical density can be set to a constant level or automatically modified in dependence on the conditions during the experiment.

For more information see the Turbidostat manual.

6 USER'S GUIDE FOR CULTIVATION OF ALGAE AND CYANOBACTERIA

6.1 MC 1000-OD CULTIVATION KIT COMPONENTS

In the next section individual components are described that are delivered as part of the MC 1000-OD kit and are required for the initiation of 8-multi-well culture cultivation in the MC 1000-OD.

Tab. 2 lists standard Kit Components of MC 1000-OD and their specifications. The number corresponds with the numbering in the Fig. 14.

Component Number (Fig. 14)	Component Description	Specification, Lenght (number)
1	Outlet silicone tubing	Ø 8/5 mm cca 120 mm (8x) cca 120 mm (1x)* cca 40 mm (1x)*
2	Aeration silicone tubing	Ø 6/3 mm cca 100 mm (8x) cca 140 mm (8x) cca 300 mm (8x) cca 100 mm (2x)** cca 200 mm (1x)** cca 300 mm (1x)**
3	Fitting Luer Lock FTLL 240-1***	5/32" (4.0 mm)
4	Fitting Luer Lock MTTL 240-J1A***	5/32" (4.0 mm)
5	Reduction Tube Fitting 5060-1***	1/4" (6.4 mm) and 3/16 (4.8 mm)
6	Silicone Plug	Ø 29x23, 30 mm
7	Effluent teflon tubing	Ø 8/1 mm, 80 mm
8	Aeration glass tubing	Ø 4/0.8 mm, 230 mm
9	Cultivation tubes (2 sets)	Ø 30/1.4 mm, 200 mm
10	USB communication cable	
11	Humidifier	1 liter
12	Screw cap GL 45 with hole and sealing ring	
13	Plastic (or metal) lid with ports	
14	Air filter	0.2 µm
15	Lock ring plug LP 240-J1A***	
16	Teflon runner	
17	Specimen tube	
18	USB Flash Disk	
19	Hex Key	2.5 mm

Tab. 2 Standard Component Kit

* silicone tubing for specimen tube

** silicone tubing for humidifier

*** provided by: www.valueplastics.com

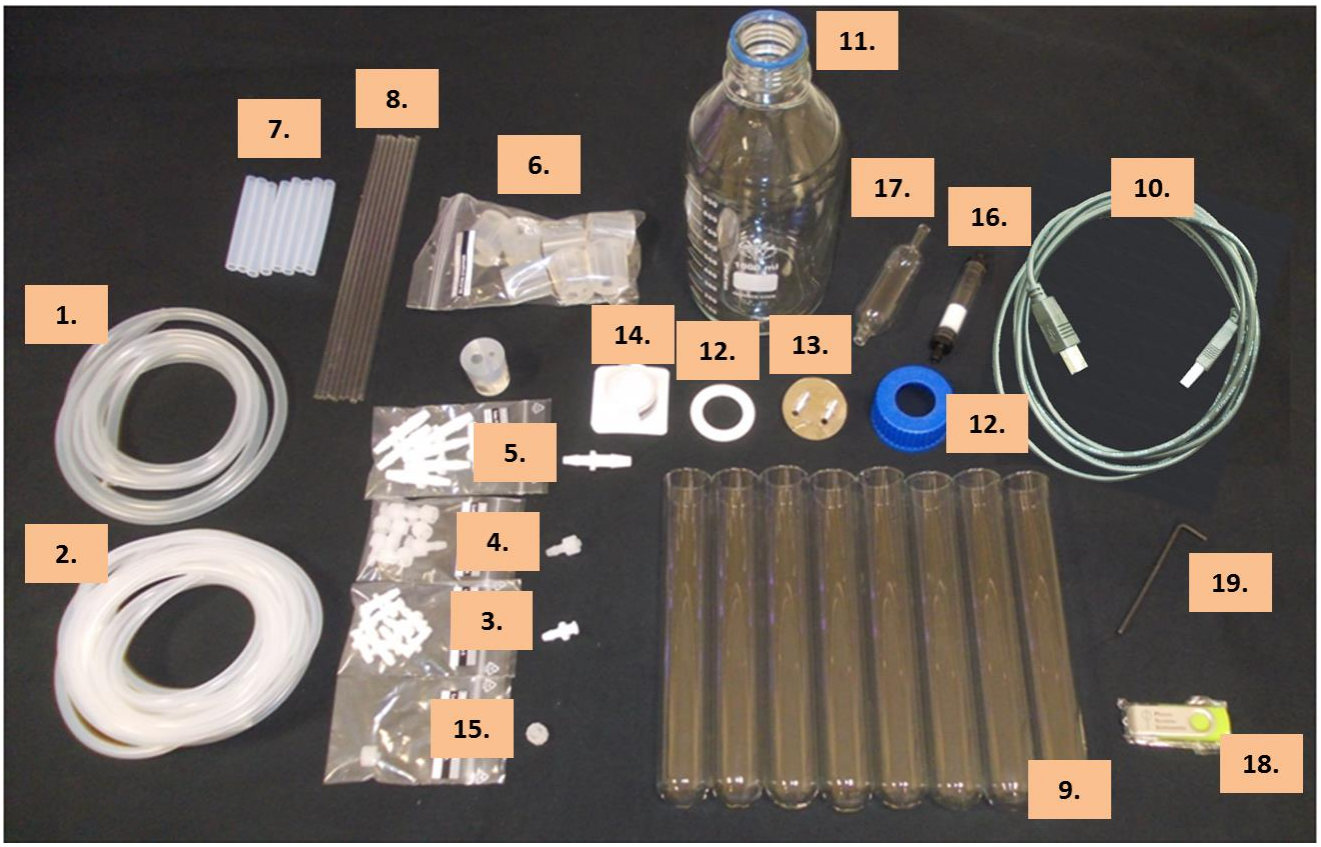


Fig. 14 MC 1000-OD kit components delivered with the standard package. The labelling corresponds to the number in Tab. 2

6.2 PREPARATION AND SET UP OF THE COMPONENTS FOR MC 1000-OD

1. First prepare the glass cultivation tubes. All components required for setting up the tubes are shown in the **Error! Reference source not found.**A.

   First silicone tubs are cut in pieces of different length according to the parameters described in Tab. 2. Aeration glass tubing (8; number corresponds to the number in Tab. 2) and effluent teflon tubing (7) is carefully inserted into the silicone plug (6). The end of the wider effluent teflon tube should be aligned with the narrower bottom side of the silicone plug as shown in Fig. 15B. Silicone tubing (2) is joined together using FTLL a MTLL Fitting Luer Locks (as shown in Fig. 15C) and are connected to the aeration glass tubing. Silicone tubing (1 and 2) is joined together with by Tube-to-Tube connector (5) and connected to the effluent teflon tubing according to the Fig. 15D. This way assembled silicone plug is inserted into the glass cultivation tube (9). Prepare all remaining cultivation tubes following these instructions.

	It is recommended to use protective gloves when inserting glass tubes into the silicone plug.

2. To prepare the cultivation tubes for autoclaving wrap end parts of the silicone tubing and the silicone plugs of assembled cultivation tube with aluminum foil (not included).

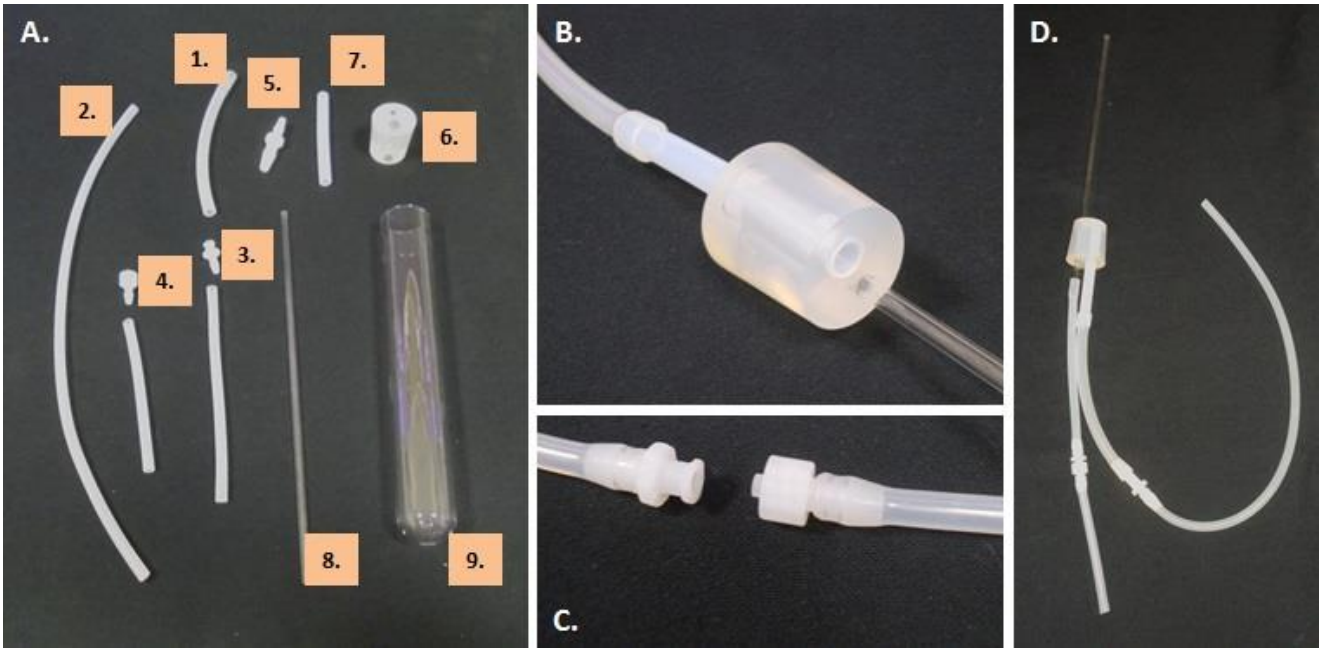


Fig. 15 A) Components required for assembly of one cultivation tube. The labelling corresponds to the number in Tab. 2. B) Optimal positioning of the effluent teflon tubing in the plug. C) Schematic illustration of FTLL (left) and MTLL (right) Luer Lock assembly. D) Final set up of the cultivation tube plug.

3. Components required for the assembly of the humidifier bottle with the metal lid with ports are shown in the Fig. 16A. First assemble the lid (12). Connect silicone tubing (2) to the metal portion of the lid with the ports (13) and insert into the screw cap. One end of the silicone tubing has FTLL Luer Lock and Lock Ring Plug, the second with MTLL Luer Lock (as shown in Fig. 16B). Other silicone tubing of about 12 cm is connected to the other side of the metal sleeve on one side and to the specimen tube (17) on the other side place short silicone tubing of about 4 cm to the bottom end of the specimen tube as shown in Fig. 16B. Fill the bottle up to 1 liter with distilled water, insert the sealing ring (12) into the screw cap and close the bottle with the assembled lid. End parts of the silicone tubs should be covered with aluminium foil. The assembled aeration bottle (as shown in Fig. 16C) is now prepared for autoclaving.

⚠	Specimen tube is recommended to be used with MC 1000-OD as it assures proper stopping of aeration during the process of measuring OD.

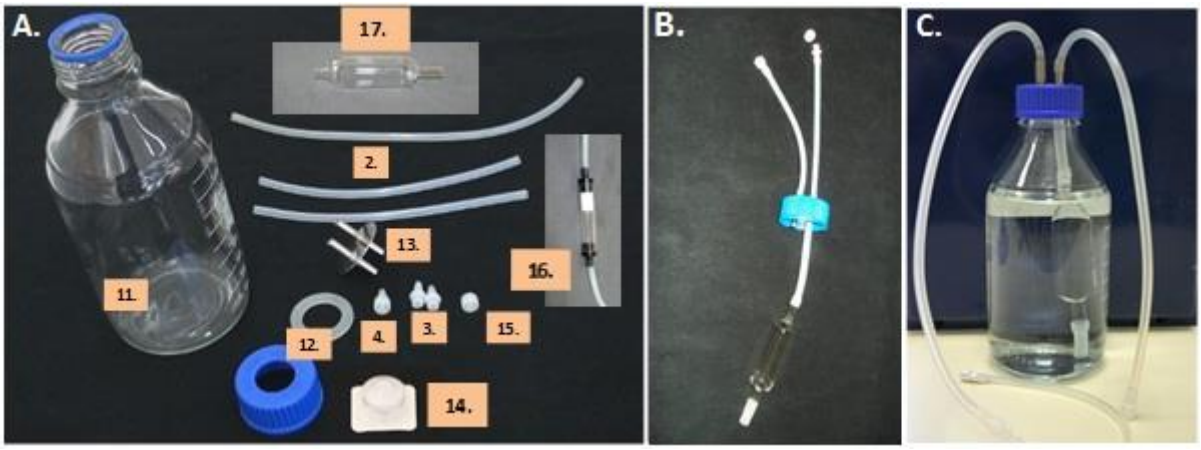


Fig. 16 A) Components required for assembling of the humidifier bottle. The labelling corresponds to the number in Tab. 2. B) Correct assembly of the silicone tubing to the lid is shown. C) Final set up of the humidifier bottle.

4. Should the MC 1000-OD delivery include the plastic lid with ports, assemble the humidifier bottle according the following schemes in Fig. 17. Please note, the silicon tubing connected to the Specimen tube (outlet silicone tubing Ø 8/5 mm) is inserted into the glass sleeves. The humidifier bottle with cap, plastic ports and Specimen tube is autoclavable whereas the Air filter and Teflon runner are not autoclavable.

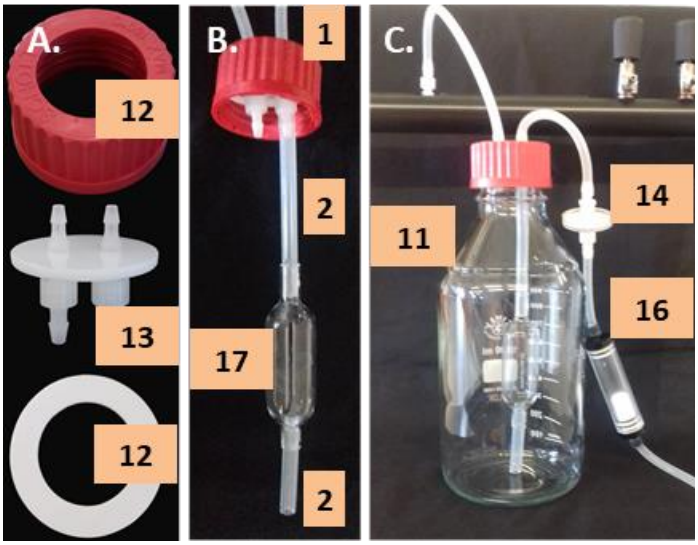


Fig. 17 A) Lid with plastic ports and silicon sealing. B) Specimen tube connection with the plastic port. C) Final set up of the humidifier bottle with Air filter and Teflon runner.

5. Prepare suitable medium for the cultivation and autoclave. For standard experimental set up with 8 cultivation tubes of 80 ml volume per tube (up to 85 ml of media per cultivation tube can be used), total of 650 ml of media are required. But twice as much should be prepared as half of it will be required for OD calibration (should be done with the cultivation tubes filled with medium and no inoculum). The other half will be used for cultivation.

	The volume of 80 ml is recommended as optimal volume for the setting up of inoculation.

6. All the cultivation kit components as listed below are now prepared for the sterilization by autoclaving:

   - 8 assembled cultivation tubes,
   - assembled humidifier bottle

   Other equipment required for the initiation of the cultivation that should be sterilized by autoclaving:

- 2 x 100ml measuring cylinder,
- 2 x 250ml beaker,
- 2l Erlenmeyer flask or other glass flask for the pre-cultivation of the inoculums,
- cultivation media.

7. Sterilize medium and all the instruments as described in step 5 by autoclaving at 121 °C for 30 min.
8. Dry the glass in a drier and let it cool down to room temperature.

6.3 PREPARATION OF INOCULUM

	Please note that not all 8 cultivation tubes have to be used. In such case scale down accordingly, the volume of inoculums and required media and close the unused cultivation tube slots by a lid to avoid water evaporation (Fig. 18). The lids are not a part of the delivery.

All described steps should be done in sterile conditions in a flow-hood.

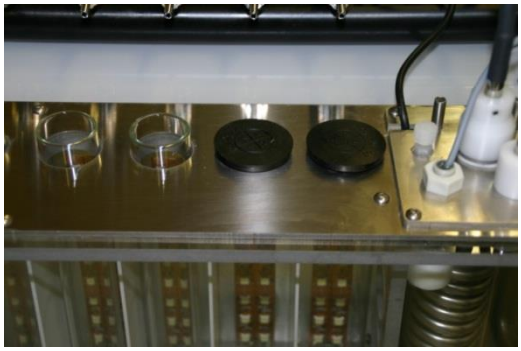


Fig. 18 Closed slots of the water bath (an illustrative picture).

9. In case of organisms such as *Chlorella vulgaris*, *Cyanothece* or *Synechocystis* the optimal growth rate was obtained when initial inoculum with concentration of 3 million cells per ml was used to establish culture for growth measurements in the MC 1000-OD cultivation tubes.
10. Concentration of *Chlorella vulgaris* culture in a stationary phase when grown and kept under 60 µmol.m^{-2}.s^{-1} illumination, standard aeration and 24 °C is about 55 - 60 million of cells. This corresponds to OD680 of about 1.0. The measurement of OD can be quickly and easily obtained with an AquaPen (not included; http://handheld.psi.cz/products/aquapen-c-and-aquapen-p/).
11. To obtain the recommended concentration of the initial culture dilute the inoculum down to OD680 of 0.1 - 0.15 (3 million cells/ml). Prepare appropriate volume of the inoculum according to the number of the cultivation tubes used in the experiment. Optimal volume of the inoculum needed for setting up 8 tubes of 80 ml is 650 ml of initial culture.
12. It is recommended to pre-cultivate the diluted inoculum for few hours prior to the initiation of the cultivation in MC 1000-OD. This ensures synchronization of the culture before start of an experiment. For diluted culture of *Chlorella vulgaris* (OD680 of 0.1) pre-cultivation of 12 hours under 150 µmol.m^{-2}.s^{-1} illumination, standard aeration and 25 °C is used to prepare optimized culture (OD680 of 0.15) that is sufficient used for the initiation of growth in MC 1000-OD.

6.4 SETTING UP AND PREPARATION OF MC 1000-OD DEVICE

13. In the flow hood sterilize the main gas dispenser tube with 70% ethanol by squirting ethanol into the tube and rinsing it. Allow the tube to dry and place it back in its position.

14. Fill the MC 1000-OD water bath with distilled water up to 2/3 of the volume.
15. Switch ON the MC main power switch. Warning “water level low” will be displayed as shown in Fig. 19A.

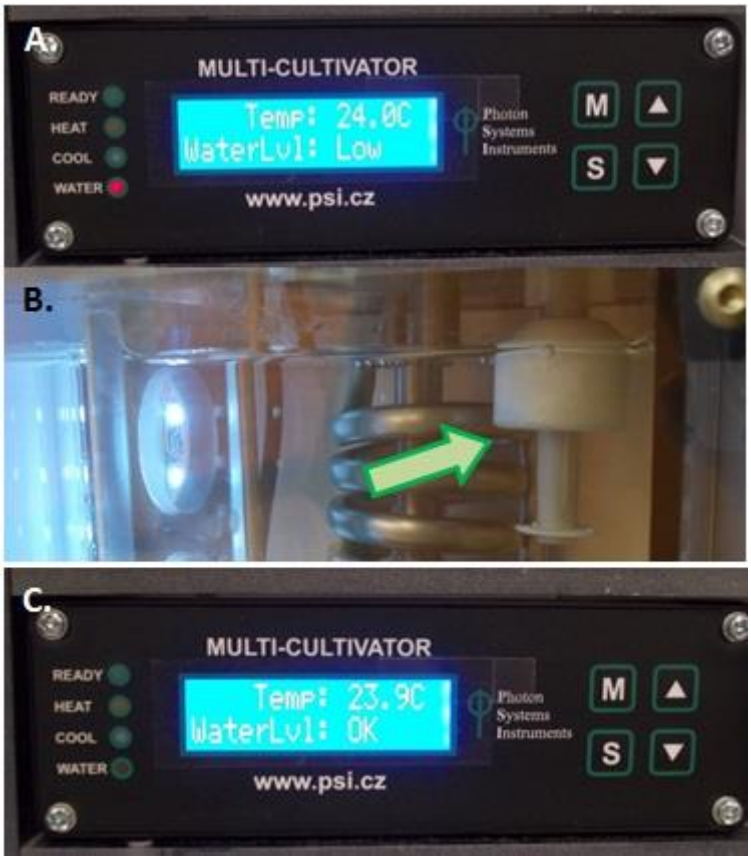


Fig. 19 A) Display of the control unit when the water level in water bath is low. B) Position of the water level controller with optimal amount of water in water bath. C) Display of the control unit when the water level in water bath is optimal.

16. Place the sterilized humidifier bottle in MC 1000-OD above the control unit and connect the tubing as described in the following steps. Remove the Lock ring plug (15; number corresponds to the number in Tab. 2) from the silicone tubing and connect the MTLL Luer Lock (4) with FTLL Luer Lock (3) placed in the main gas dispenser tube (Fig. 20A). Prepare silicone tubing of approximately 30-35 cm length and connect it with the integrated air pump as shown in Fig. 20B. The second end of the tubing should be pulled through the hole in the plastic lid where the humidifier bottle is inserted (Fig. 20C) and connected with teflon runner (Fig. 20D). The side of the teflon runner with the small hole in the transparent plastic tube should be connected with the silicone tubing from the humidifier bottle. The side without the hole is connected with the silicone tubing from the integrated air pump. Connect supplied 0.2 µm air filter with the MTLL Luer Lock end (3) of the silicone tubing from the humidifier bottle and via other FTLL Luer Lock connect with the silicone tubing coming from the teflon runner (Fig. 20E). Final set up of the humidifier bottle is shown in Fig. 20F.

⚠	Keep teflon runner in a vertical position so that the side with small hole in the transparent plastic tube is always on top. This assures proper stopping of aeration during the OD measurement. Do not clean teflon runner with an ethanol.

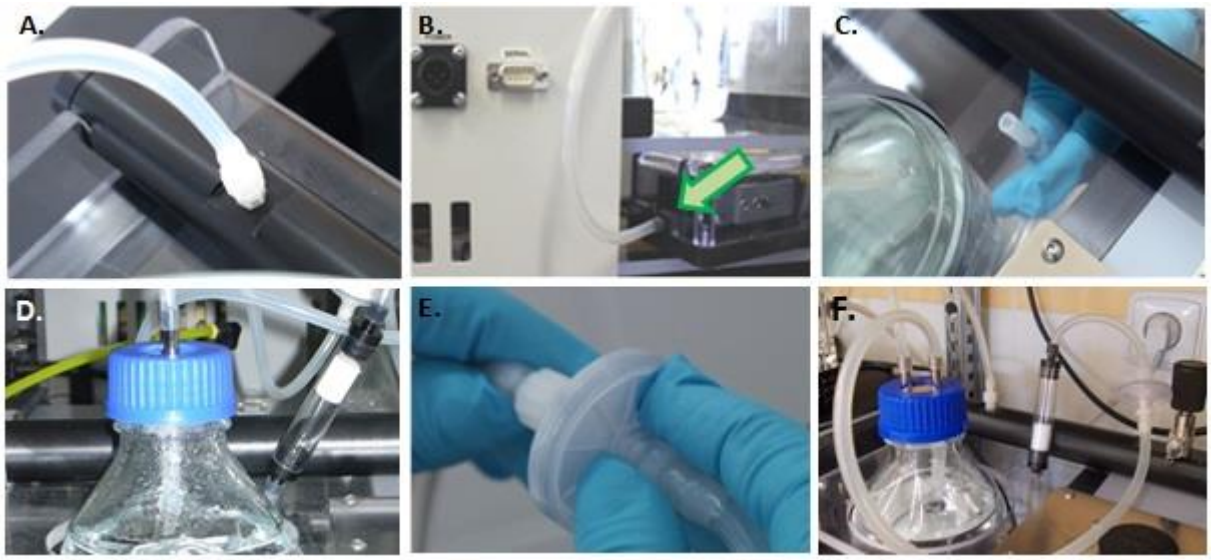


Fig. 20 Steps for set up of the humidifier bottle. A) Connection of the silicone tubing from the bottle to the main gas dispenser tube. B) Connection of silicone tubing with the integrated air pump. C) Set up for the connection of the integrated air pump with the humidifier bottle. D) Silicone tubing from the air pump is connected with the teflon runner. E) Connection of the humidifier bottle with teflon runner via 0.2 µm filter. F) Final set up of the humidifier bottle.

17. To switch the aeration pump ON go to **Settings > Air pump > ON**.
18. Prepare the MC 1000-OD for the OD calibration step.

    Place all 8 glass cultivation tubes, cylinder and glass beakers in the flow-hood and fill the tubes with required volume of cultivation medium. The volume corresponds to the volume of inoculum to be cultivated in individual tubes. Prior placing cultivation tubes for OD calibration into MC 1000-OD, clean the outer side of the tubes with 70% ethanol to remove any dust or finger prints on the surface.

	When performing this step under sterile conditions, medium used for the OD calibration can be further re-used. As a result, the same set of cultivation tubes can be used for OD calibration for culture cultivation. Alternatively, a second set of cultivation tubes can be used for calibration that are provided as part of the standard set up.

19. Switch the lights ON: **Lights > All lights > ON**. For *Chlorella vulgaris* light intensity in the range 100 – 250 µmol.m^{-2}.s^{-1} is used.

	MC 1000-OD control unit allows set static and dynamic light regimes (pre-designed sinus, daylight and pulse form regimes. For dynamic light regimes please refer to Menu Tree Control description on page 40.

20. In case the warning “water level low” is still displayed fill the water bath with distilled water up to the level shown in Fig. 19B until the message “water level ok” is displayed on the control (Fig. 19C).
21. **OPTIONAL:** for the users of MC 1000-OD instruments, which are supplied with PWM Pump follow the installation instructions as described on page 22.
22. Set the temperature control ON: **Sensors > TControl > ON**. Set the cultivation temperature: **Sensors > Temperature > 25°C.** For *Chlorella vulgaris* temperature of 25 °C is used. It is important to calibrate the OD sensors at the temperature used in the experiment.

⚠	MC 1000-OD device itself does not have temperature regulator built-in that would allow regulate the temperature in water-bath below the ambient temperature. If high light intensities are used, please be aware that the water bath temperature will increase even above the ambient room temperature. MC 1000-OD built-in temperature regulator allows only to warm up the temperature inside the water bath.

23. **OPTIONAL:** for the users of MC 1000-OD instruments, which are supplied with Cooling Unit AC-710 follow the installation instructions as described on page 18.

24. The MC 1000-OD device is now prepared for the OD calibration step. Prior to start of each experiment OD calibration protocol should be performed with the medium used in that experiment. Blank cultivation medium should be used for calibration in all 8 cultivation tubes. Run the OD calibration protocol: **Settings > OD Calibration > Run**.

    Now the MC device is ready for initiation of the culture growth.

6.5 ESTABLISHMENT AND GROWTH OF SELECTED CULTURE IN THE MC 1000-OD

25. Place autoclaved glass cultivation tubes, cylinder, beakers and pre-cultivated inoculum into the flow-hood. Under sterile conditions measure 80 ml of the inoculum and pour it into the cultivation tube. Sterilize the end of the tube in the flame and close the tube with the assembled silicone plug. Follow the procedure for all the cultivation tubes.

26. Place the cultivation tubes with the pre-cultured inoculums in the MC 1000-OD slots. Prior placing the cultivation tubes into MC 1000-OD, clean the surface with 70% ethanol to remove any dust or finger prints on the surface. Repeat this step for each cultivation tube. If same set of cultivation tubes is used as in the OD calibration remove carefully the medium under sterile conditions and replace with 80 ml of pre-cultivated inoculum.

⚠	If number of cultivation tubes is removed from the water bath at the same time, water level will drop and warning “water level low” on the control unit display will appear and the alarm will sound. Press the **S** button on the MC 1000-OD control unit to stop the alarm system.

27. Remove the aluminum foil at the end of the aeration silicone tubing and connect to the valve of the main dispenser tube (Fig. 21). Repeat the step for all the cultivation tubes.

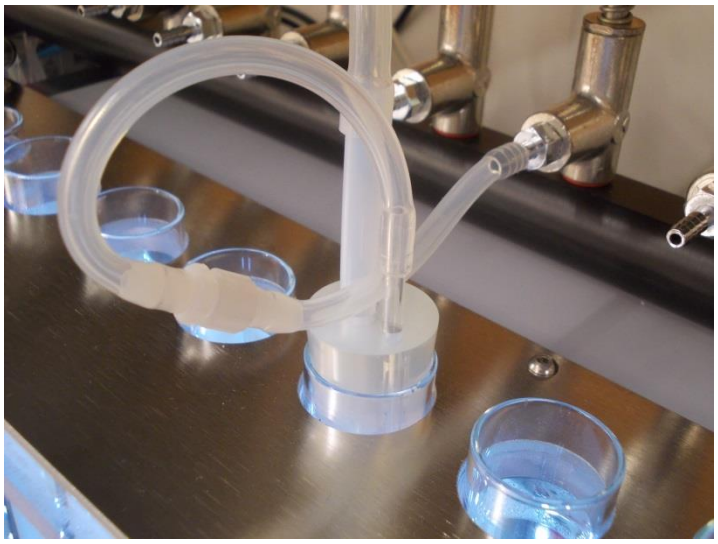


Fig. 21 Connection of the cultivation tube to the main gas dispenser tube.

⚠	The OD sensor is aligned with the center of each cultivation tube so to avoid interference of aeration glass tubing with OD measurements it is important to place the aeration glass tubing to either side of the tube as shown in Fig. 22A.

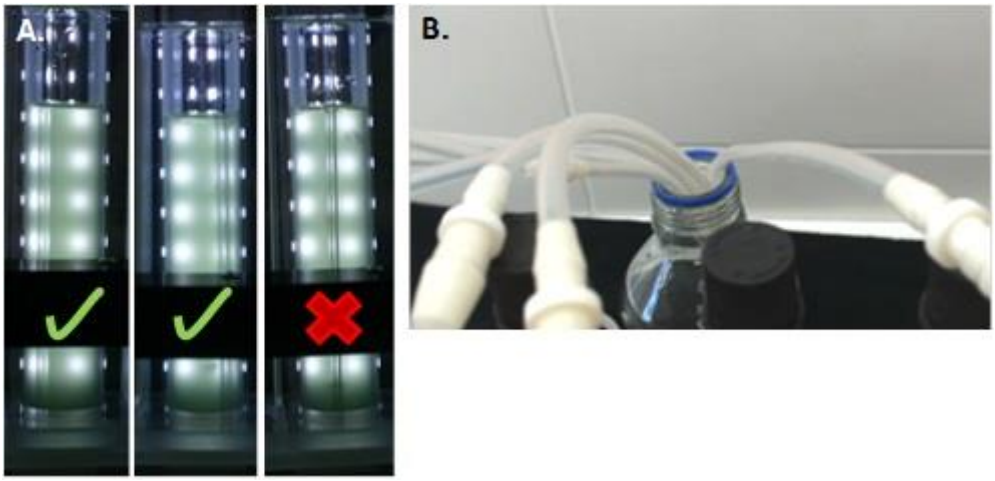


Fig. 22 A) Left and middle image show correct placement of the glass aeration tubing in the cultivation tube. B) End portion of the effluent tubing from the cultivation tubes are placed into the waste bottle.

28. Effluent tubing from the cultivation tube should be placed into a beaker or glass bottle in case any waste water comes out of the tubing (Fig. 22B). Place the bottle behind the MC 1000-OD device.
29. Adjust the air flow rate for each cultivation tube by using the individual valves on the main gas dispenser tube. It is important to check that all cultivation tubes, silicone plugs and aeration glass tubing are at the same position. All aeration glass tubing should be the same distance from the bottom of the cultivation tube. Note that the position of the aeration tubing end affects the size of the bubbles. Optimal position of the end should be few millimeters from the bottom of the tube. Ensure that there are no kinks in the silicone tubing that may impede the flow of gas.
30. Completion of all the steps outlined above will ensure optimal inoculation and homogenous and reproducible growth rate of the culture.
31. OD measurements are not continuous. They are done at specific time interval. To set up OD measuring protocol go to: **Sensors > OD Protocol > ON**. Set the time interval for the OD measurement between 5 minutes up to 1 hour.
32. Measured OD values can be read during the growth of the culture and without stopping of the experiment by going through the menu system as follows: **Sensors > OD measure > Light1 - Light 8**

    To visualize all of the stored OD values these have to be downloaded onto a PC using the ODView software supplied with the MC 1000-OD and the USB cable. Follow the instructions described in the next chapter on page 35.

⚠	Note that the MC 1000-OD control unit can collect limited number of data points before it runs out of memory. Maximum number of data points for each OD sensor is 3533. This number corresponds to 147 days of measurement if 1 h measuring interval is used. To check the memory status of the MC 1000-OD wait few seconds without manipulating with the control unit display. An idle screen will appear with actual measured temperature of the water bath displayed. On the bottom section of the display shows % memory occupied. By pushing the button **S** memory space in hours will be displayed. Optionally the memory status can be found in: **Sensors > OD Protocol.** Remaining memory space is displayed as shown in Fig. 23.

Fig. 23 Control unit display with remaining memory space in % and in hours is shown.

7 TERMINATION OF CULTIVATION AND MC 1000-OD MAINTENANCE AND STORAGE

Below recommendations are given that help the user to successfully terminate the growth of selected culture in MC, evaluate the measured data and maintain the MC device itself.

7.1 DATA TRANSFER AND VISUALISATION

1. Prior initiation of new experiment and starting new OD protocol it is **essential** to download the measured OD values. To proceed with data transfer, connect the USB adaptor and the serial cable between the PC and the serial plug in the back of the MC 1000-OD.

	Data saved to the MC 1000-OD memory will be stored **ONLY** until new OD protocol is started, since initiation of new OD protocol will automatically reset the memory space and measured OD values will overwrite stored data set.

2. To visualize all of the measured and stored OD values for each cultivation tube the data has to be first downloaded from the MC 1000-OD control unit to the PC. This is done using the ODView software that is included with the MC 1000-OD device.

3. Copy the ODView software from the Flash Disc onto the PC first. Run the software. The initial screen will appear as shown in Fig. 24.

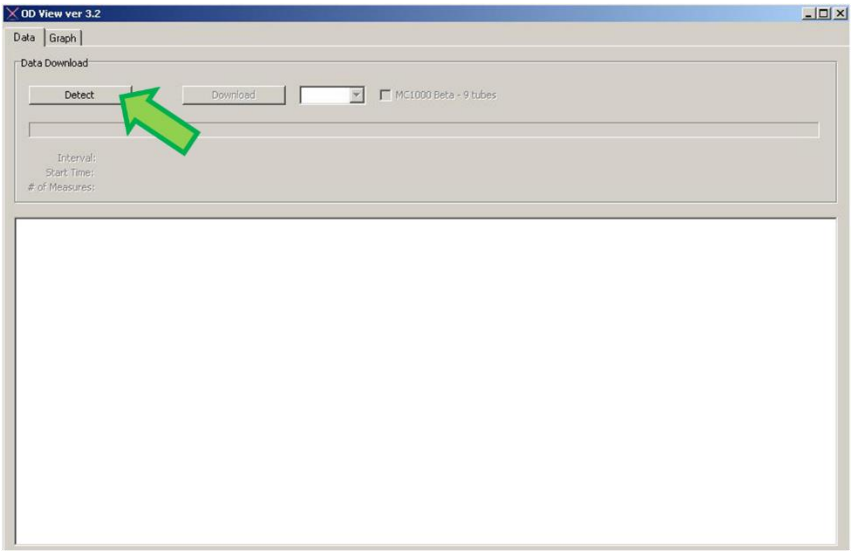
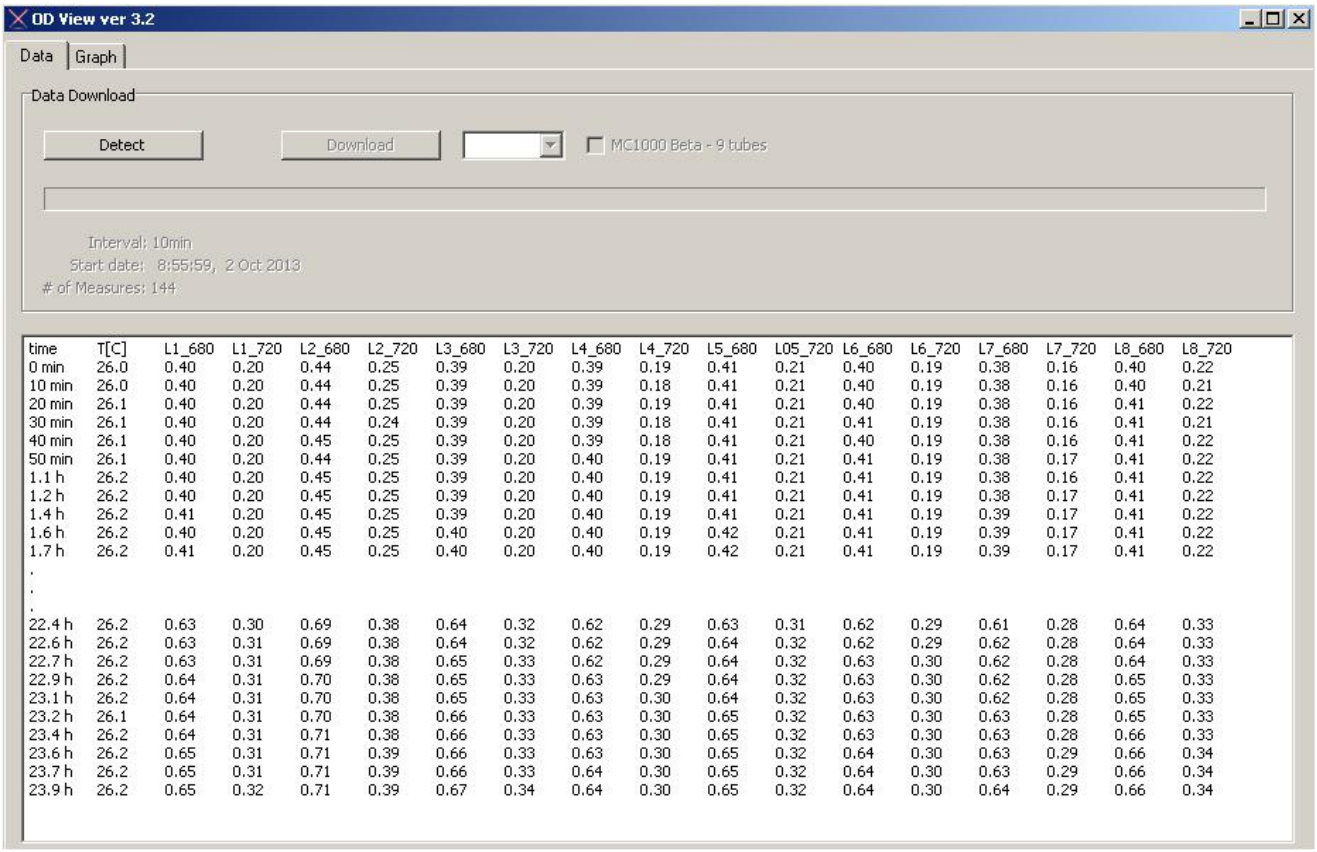


Fig. 24 The initial window of the ODView program

4. Click "**Detect**" button for the software to connect with the Multi-Cultivator control unit. Once the connection has been established click **"Download"** button that appears. The stored data from the MC 1000-OD control unit will be downloaded and the following screen will appear as shown in Fig. 25.

	In the table format (data) only the first 10 and the last 10 values will be shown. To visualize all of the data collected for each cultivation tube click on "**Graph**" and select the cultivation tubes with appropriate OD wavelength as shown in Fig. 26. In this window description of the experiment and notes associated with the experiment can be made.



time	T[C]	L1_680	L1_720	L2_680	L2_720	L3_680	L3_720	L4_680	L4_720	L5_680	L05_720	L6_680	L6_720	L7_680	L7_720	L8_680	L8_720
0 min	26.0	0.40	0.20	0.44	0.25	0.39	0.20	0.39	0.19	0.41	0.21	0.40	0.19	0.38	0.16	0.40	0.22
10 min	26.0	0.40	0.20	0.44	0.25	0.39	0.20	0.39	0.18	0.41	0.21	0.40	0.19	0.38	0.16	0.40	0.21
20 min	26.1	0.40	0.20	0.44	0.25	0.39	0.20	0.39	0.19	0.41	0.21	0.40	0.19	0.38	0.16	0.41	0.22
30 min	26.1	0.40	0.20	0.44	0.24	0.39	0.20	0.39	0.18	0.41	0.21	0.41	0.19	0.38	0.16	0.41	0.21
40 min	26.1	0.40	0.20	0.45	0.25	0.39	0.20	0.39	0.18	0.41	0.21	0.40	0.19	0.38	0.16	0.41	0.22
50 min	26.1	0.40	0.20	0.44	0.25	0.39	0.20	0.40	0.19	0.41	0.21	0.41	0.19	0.38	0.17	0.41	0.22
1.1 h	26.2	0.40	0.20	0.45	0.25	0.39	0.20	0.40	0.19	0.41	0.21	0.41	0.19	0.38	0.16	0.41	0.22
1.2 h	26.2	0.40	0.20	0.45	0.25	0.39	0.20	0.40	0.19	0.41	0.21	0.41	0.19	0.38	0.17	0.41	0.22
1.4 h	26.2	0.41	0.20	0.45	0.25	0.39	0.20	0.40	0.19	0.41	0.21	0.41	0.19	0.39	0.17	0.41	0.22
1.6 h	26.2	0.40	0.20	0.45	0.25	0.40	0.20	0.40	0.19	0.42	0.21	0.41	0.19	0.39	0.17	0.41	0.22
1.7 h	26.2	0.41	0.20	0.45	0.25	0.40	0.20	0.40	0.19	0.42	0.21	0.41	0.19	0.39	0.17	0.41	0.22
.																	
.																	
.																	
22.4 h	26.2	0.63	0.30	0.69	0.38	0.64	0.32	0.62	0.29	0.63	0.31	0.62	0.29	0.61	0.28	0.64	0.33
22.6 h	26.2	0.63	0.31	0.69	0.38	0.64	0.32	0.62	0.29	0.64	0.32	0.62	0.29	0.62	0.28	0.64	0.33
22.7 h	26.2	0.63	0.31	0.69	0.38	0.65	0.33	0.62	0.29	0.64	0.32	0.63	0.30	0.62	0.28	0.64	0.33
22.9 h	26.2	0.64	0.31	0.70	0.38	0.65	0.33	0.63	0.29	0.64	0.32	0.63	0.30	0.62	0.28	0.65	0.33
23.1 h	26.2	0.64	0.31	0.70	0.38	0.65	0.33	0.63	0.30	0.64	0.32	0.63	0.30	0.62	0.28	0.65	0.33
23.2 h	26.1	0.64	0.31	0.70	0.38	0.66	0.33	0.63	0.30	0.65	0.32	0.63	0.30	0.63	0.28	0.65	0.33
23.4 h	26.2	0.64	0.31	0.71	0.38	0.66	0.33	0.63	0.30	0.65	0.32	0.63	0.30	0.63	0.28	0.66	0.33
23.6 h	26.2	0.65	0.31	0.71	0.39	0.66	0.33	0.63	0.30	0.65	0.32	0.64	0.30	0.63	0.29	0.66	0.34
23.7 h	26.2	0.65	0.31	0.71	0.39	0.66	0.33	0.64	0.30	0.65	0.32	0.64	0.30	0.63	0.29	0.66	0.34
23.9 h	26.2	0.65	0.32	0.71	0.39	0.67	0.34	0.64	0.30	0.65	0.32	0.64	0.30	0.64	0.29	0.66	0.34

Fig. 25 OD and Temperature downloaded data window

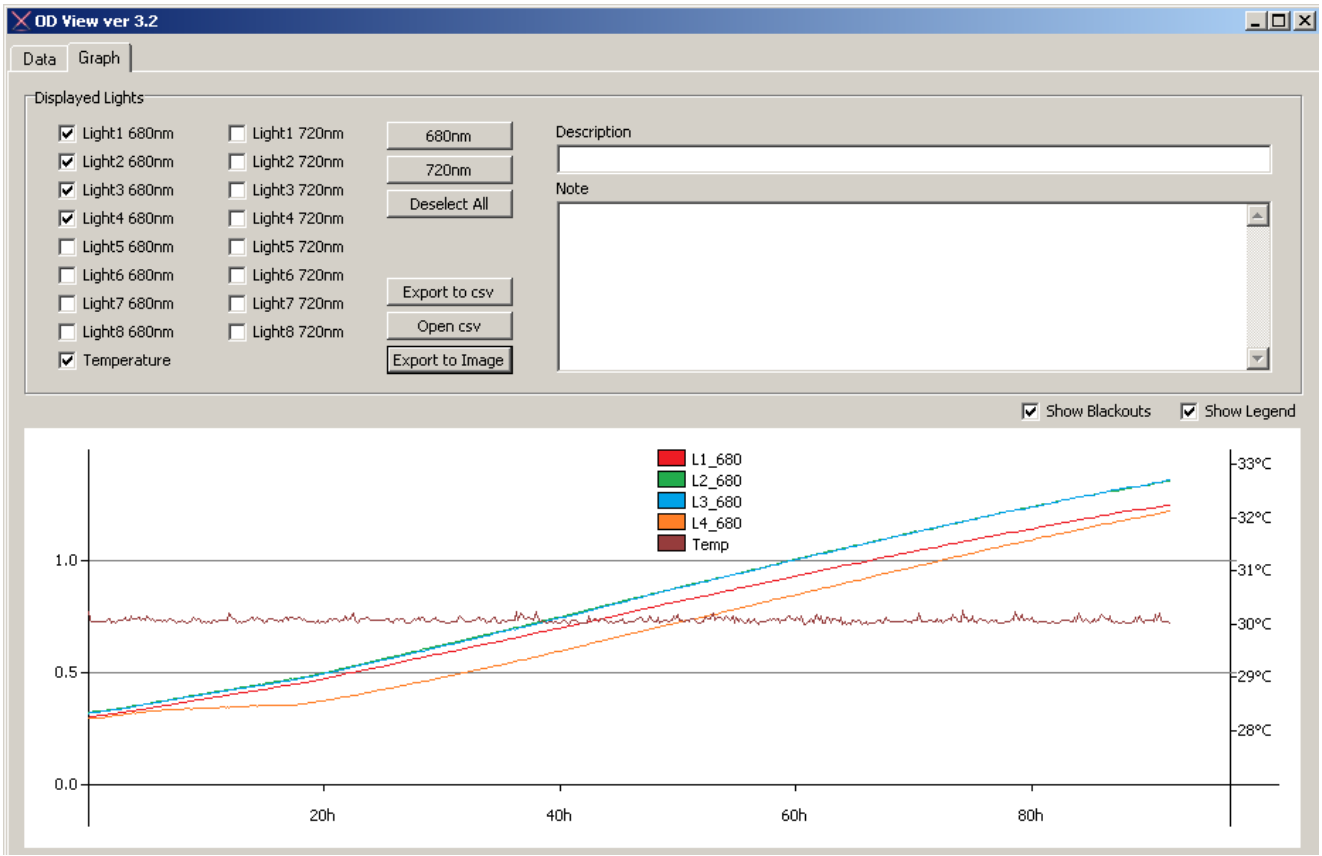


Fig. 26 The graph window of the data. OD680 was measured for Chlorella vulgaris culture in 5 min intervals for 40 hours. Temperature values can be displayed together with OD values.

5. The logged data can then be exported to .csv file using the buttons shown in Fig. 27 and further analyzed as required using a spreadsheet program such as Excel.

	The ODView software can only be used to download and visualize the data stored in the MC 1000-OD control unit while the computer and the unit are connected with the USB cable. This software will not save downloaded data and allow its visualization later while the MC 1000-OD is disconnected. Downloaded data has to be exported as a .csv file so it can be saved and manipulated later.

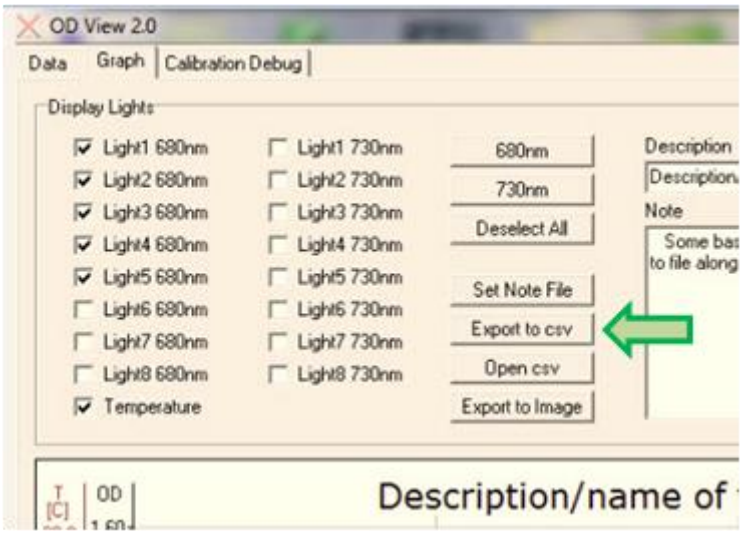


Fig. 27 Data export and data selection functions window

7.2 TERMINATION OF CULTIVATION EXPERIMENT

6. After downloading the logged data proceed with the termination of the cultivation experiment.
7. First stop the OD protocol: **Sensors > OD Protocol > OFF**
8. Switch off the lights: **Lights > All lights > OFF**
9. Stop the temperature controller: **Sensors > TControl > OFF**
10. Stop the aeration: **Settings > Air Pump > OFF**
11. Unscrew the FTLL and MTLL Luer Lock fittings on the aeration tubing and remove the cultivation tubes.
12. Remove and empty the humidifier bottle.

7.3 WATER BATH MAINTENANCE

13. Clean the water bath approximately once every two months.
14. Use only distilled water in the water bath to avoid boiler incrustation.
15. Use a tube to pump water out of the water bath. Do not place the water bath on an angle to empty it out as damage may occur.
16. To clean the water bath, unscrew the 4 screws on the top of the small square metal cover with inlets for heater, cooler, water level sensor and remove it (Fig. 28A). Then unscrew 8 screws in the lid of the cultivation water bath with slots for cultivation tubes and remove it (Fig. 28B). Pull out the stand for cultivation tubes and water pump.

    Wash inside of the water bath and its content with a mild detergent or diluted vinegar (provide 50% dilution).

17. To remove rough pollution on the walls of the water bath, use a soft brush or plastic scouring pad.
18. When assembling the water bath, please, do not overtighten the screws to avoid stripping the threads.
19. Plastic tubing, plastic connectors and glass components of the Multi-Cultivator can be autoclaved at temperatures not exceeding 121 °C.

	Avoid spilling water on any parts of the Multi-Cultivator except inside of the water bath.

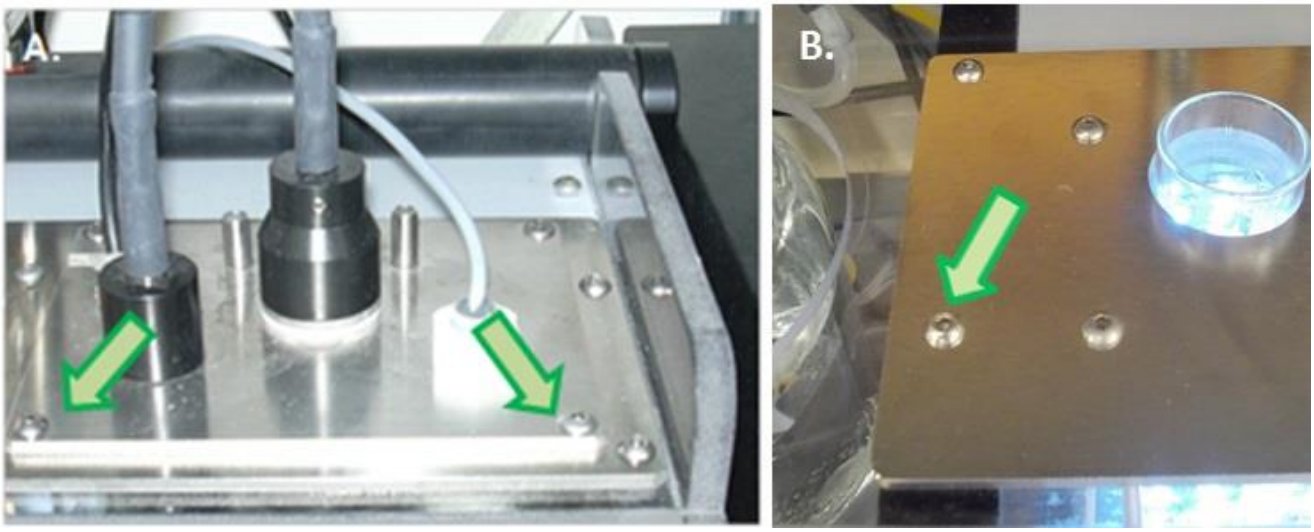


Fig. 28 A) Unscrew 4 screws in the metal cover of the cooling and heating unit. B) Unscrew 8 screws in the lid of the water bath.

8 MULTI-CULTIVATOR 1000-OD CONTROL

Parameters such as light intensity and light regime, temperature, bubbling and online optical density measurement can be controlled via the Control Unit of MC 1000-OD. Use four keys located at the right side of the front panel to control the settings of the instrument (Fig. 3, label 3):

[M] key: Used to move back in the menu tree or to exit the menu.

[S] key: Used to move forward in the menu tree or to save your selection.

[↑] key: Used to move up in the menu or to add value.

[↓] key: Used to move down in the menu or to subtract value.

The pages 40 – 47 show the graphical re-presentation of the operation scheme for the Multi-Cultivator. This scheme is structured in five levels:

Main menu (blue)

First-level nested sub-menu (yellow)

Second-level nested sub-menu (green)

Third-level nested sub-menu (orange)

Fourth-level nested sub-menu (gray)

Explanation of symbols and color differentiation* used in the graphical presentation:

Full-line arrows are used for the **[S]** key.

Dashed-line arrows are used for the **[M]** key.

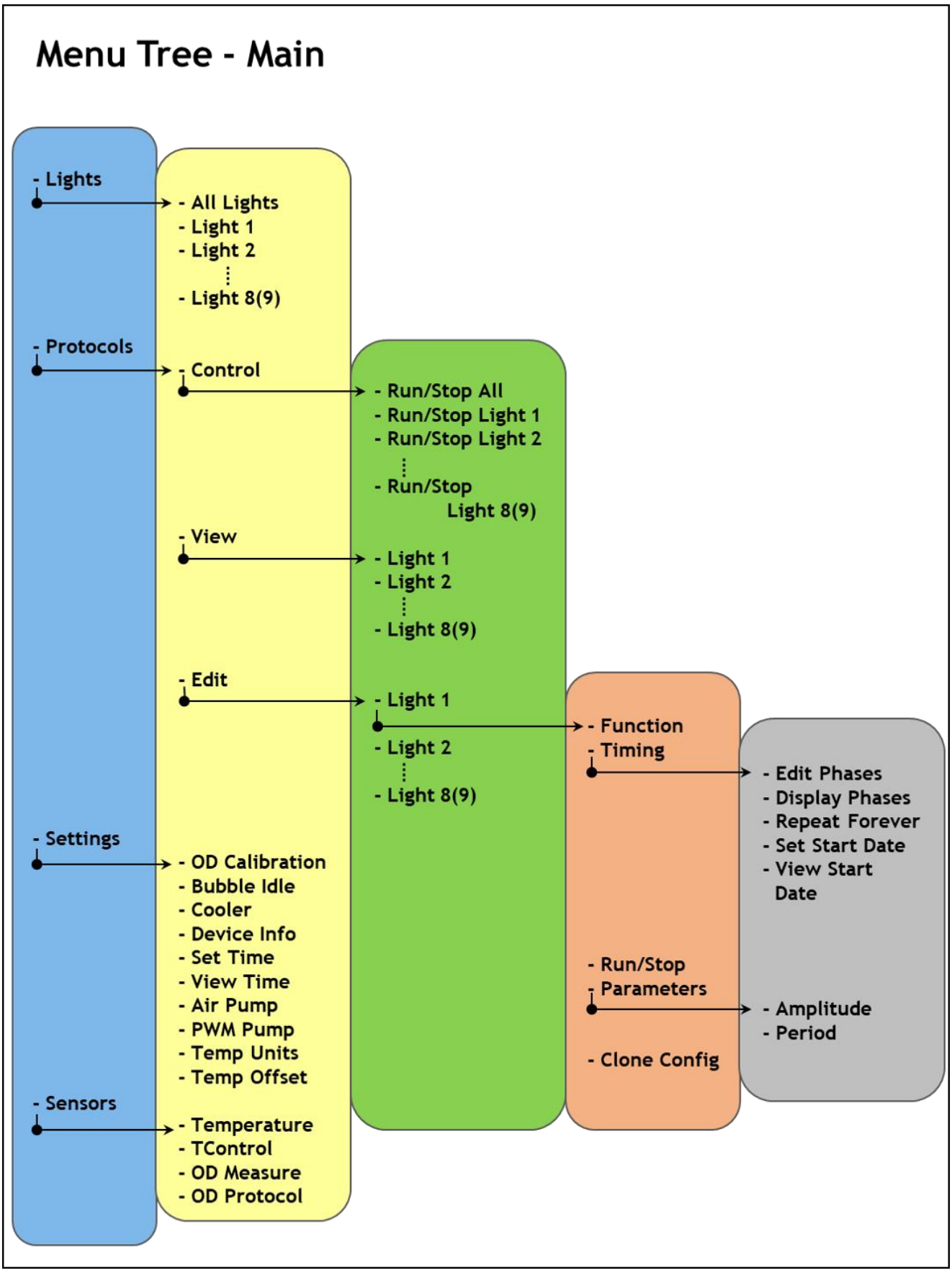
Dotted-line arrows are used for the **[UP/DOWN]** keys.

* The Multi-Cultivator screen does not reflect this color differentiation.

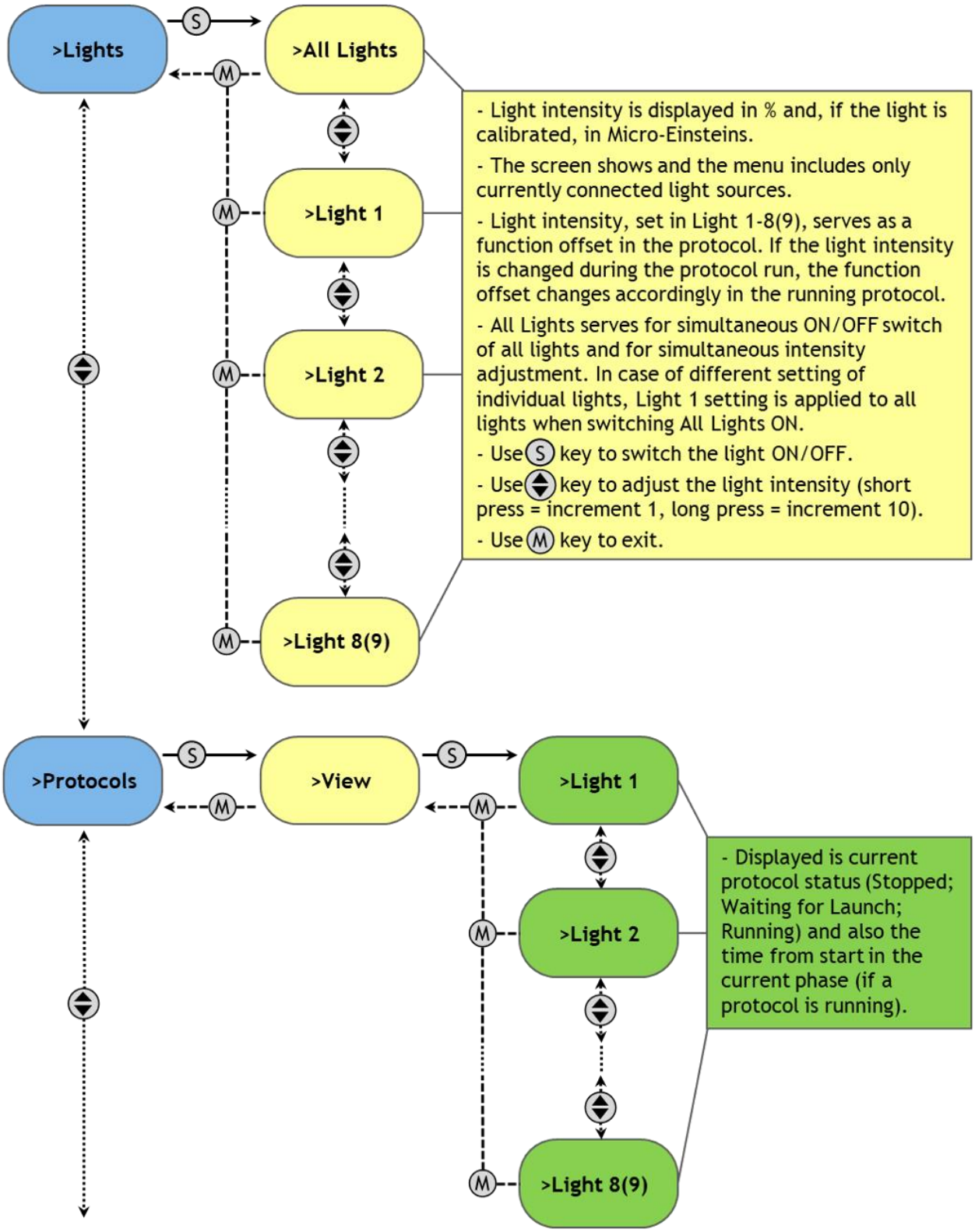
	After 10 seconds of no action, an idle screen appears on the control unit which displays the actual measured temperature inside the Multi-Cultivator water bath (Fig. 19C).
	When error message “BOInfo: ODProt. resumed!” is shown on the display (Fig. 29), MC 1000-OD was turned off while the protocol was running (for example in case of electric power outage). In this case OD Protocol and OD measurement was automatically re-started after the MC 1000-OD was again switched ON.

Fig. 29 Control unit display after OD protocol was resumed following MC 1000-OD power outage.

8.1 CONTROL MENU TREE



Menu Lights. Menu Protocols → View



Menu Protocols→Control. Menu Protocols→Edit→LightN

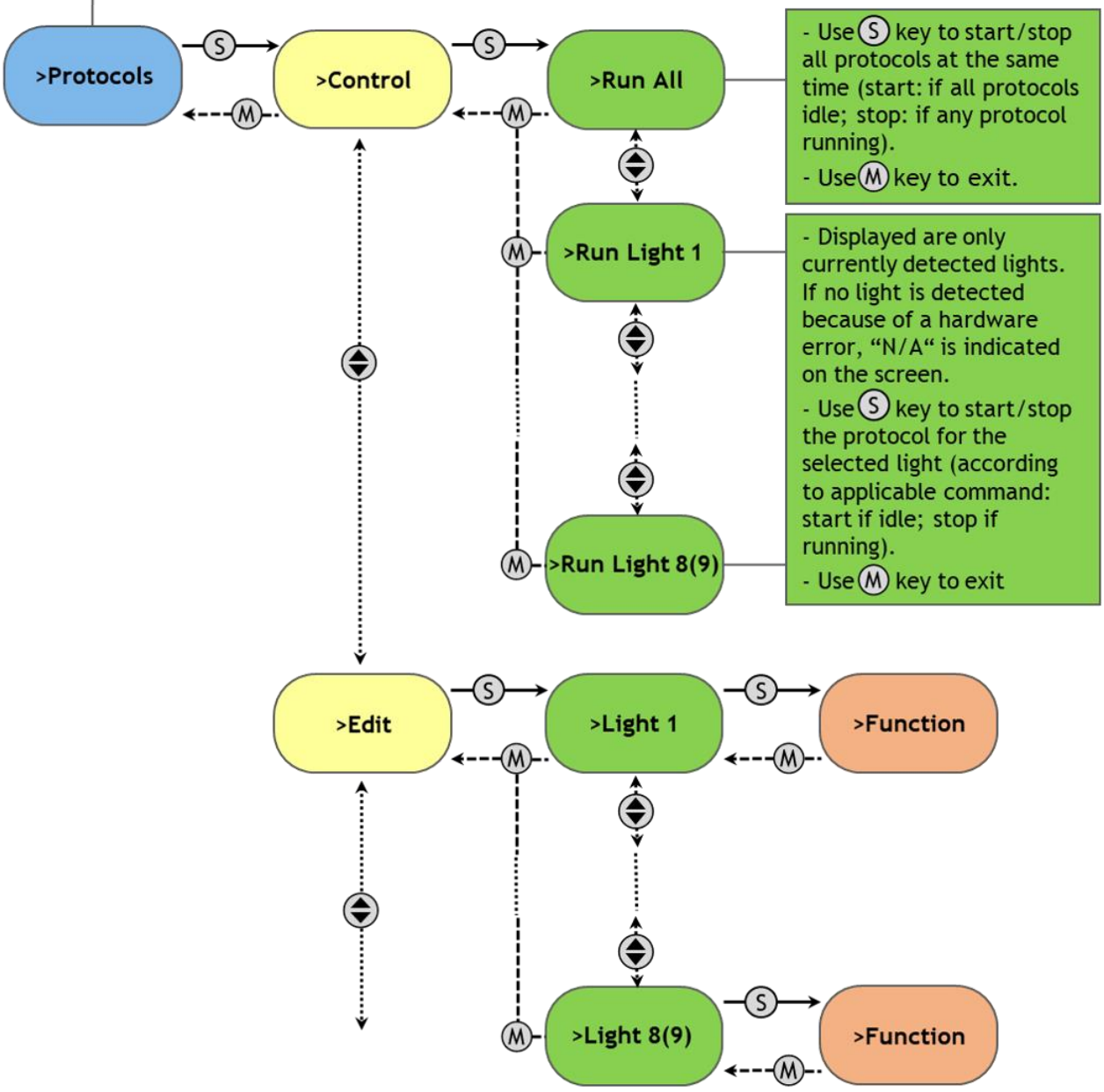
EACH PROTOCOL CONSISTS OF THREE INDEPENDENTLY CONFIGURABLE PHASES:

1) **Light Period (LP)** = Time period during which the defined function is performed.
2) **Dark Period (DP)** = Time period during which the light is off.
3) **Repeats** = Number of repeats for the phase.

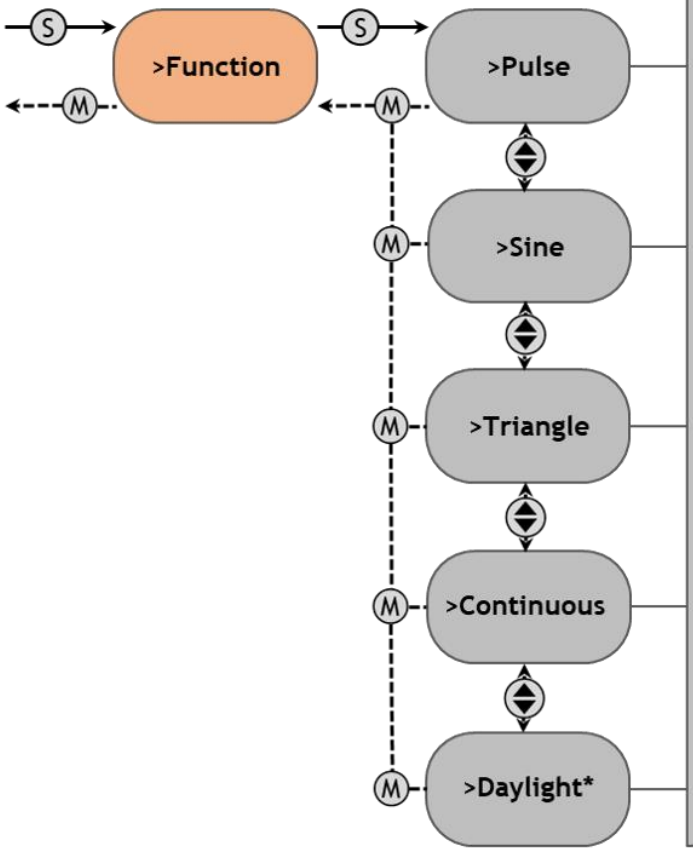
OTHER EDITABLE PROTOCOL FUNCTIONS:

Repeat forever = The whole protocol runs in infinite loop.

Zero phase = LP + DP = 0; or Repeats = 0. Editing of phases is finished when the Zero phase is confirmed.



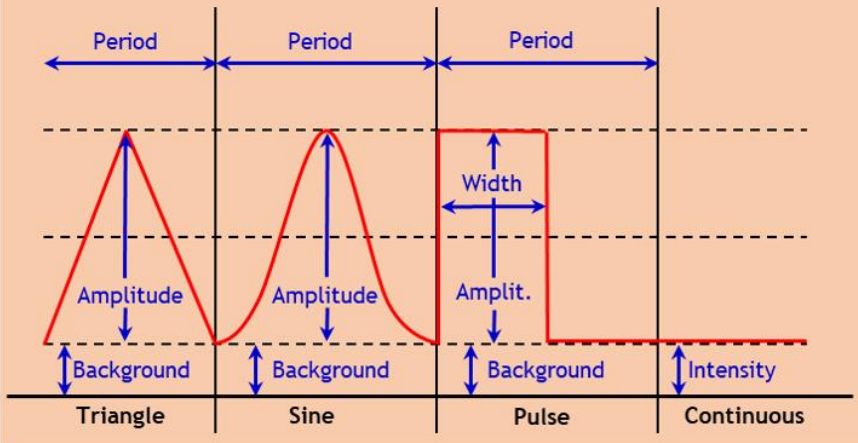
Menu Protocols→Edit→LightN→Function



- AVAILABLE ARE FOUR LIGHT FUNCTIONS: PULSE, SINE, TRIANGLE, AND CONTINUOUS.
- The choice of function influences active or inactive items in the menu (see Fig. 1 below).
Amplitude is displayed in the Sine, Triangle, Pulse, and Continuous function.
Period is displayed in the Sine, Triangle, and Pulse function.
Width is displayed in the Pulse function.
- Use ⇕ key to scroll.
- Use (S) key to save your selection and to exit.
- Use (M) key to exit (no save).
- Daylight function serves for "cloudy sky" simulation. Programmable parameters are: offset, amplitude, period (standard or random seed), and drop coefficient.
- Drop coefficient serves for "setting more or less clouds (the smaller number, the more clouds)."
- Ideal period setup (for daylight function) equals one light phase, i.e., one day coincides with one light phase of the protocol.

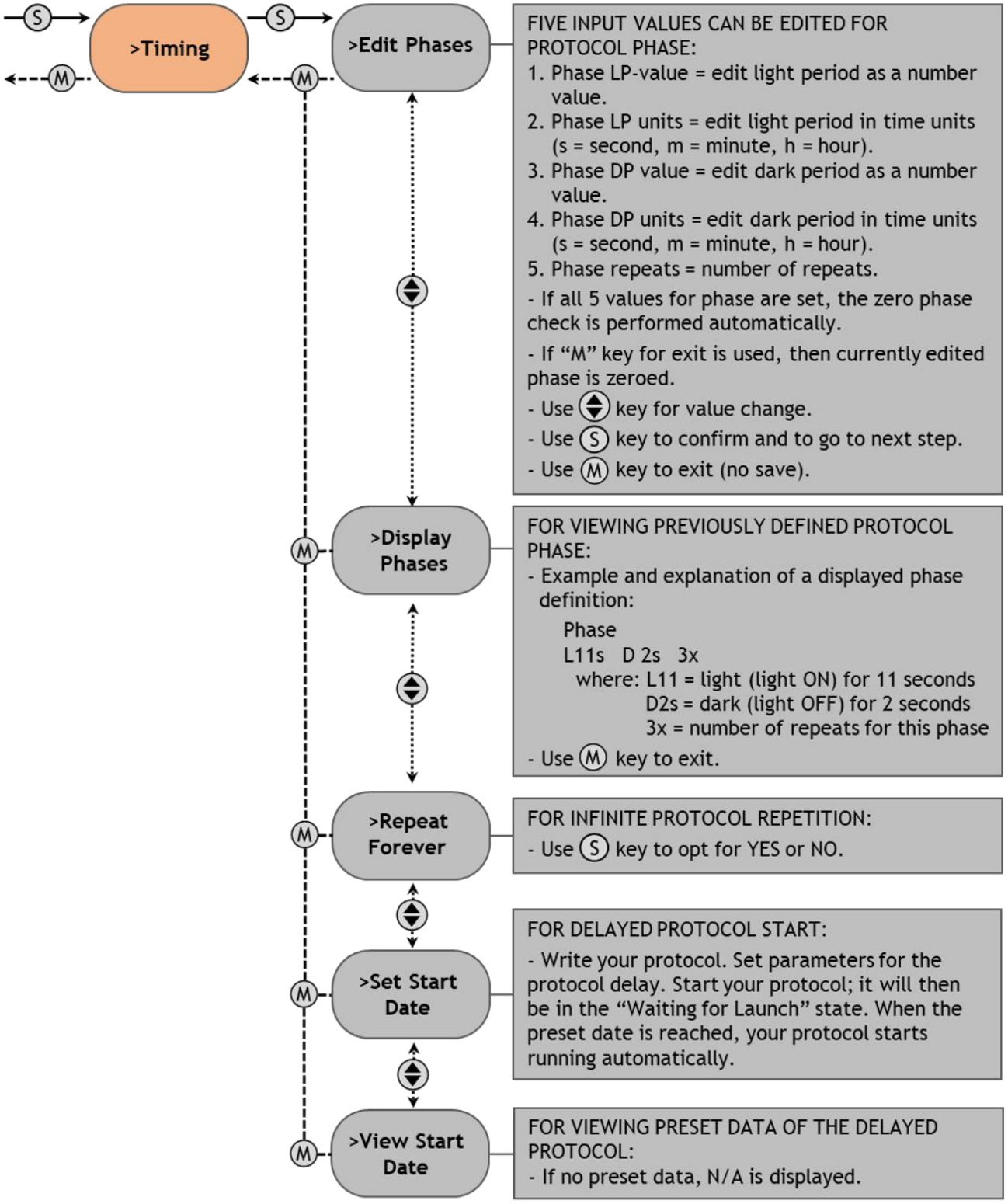
Fig. 1

Light Function Visualization

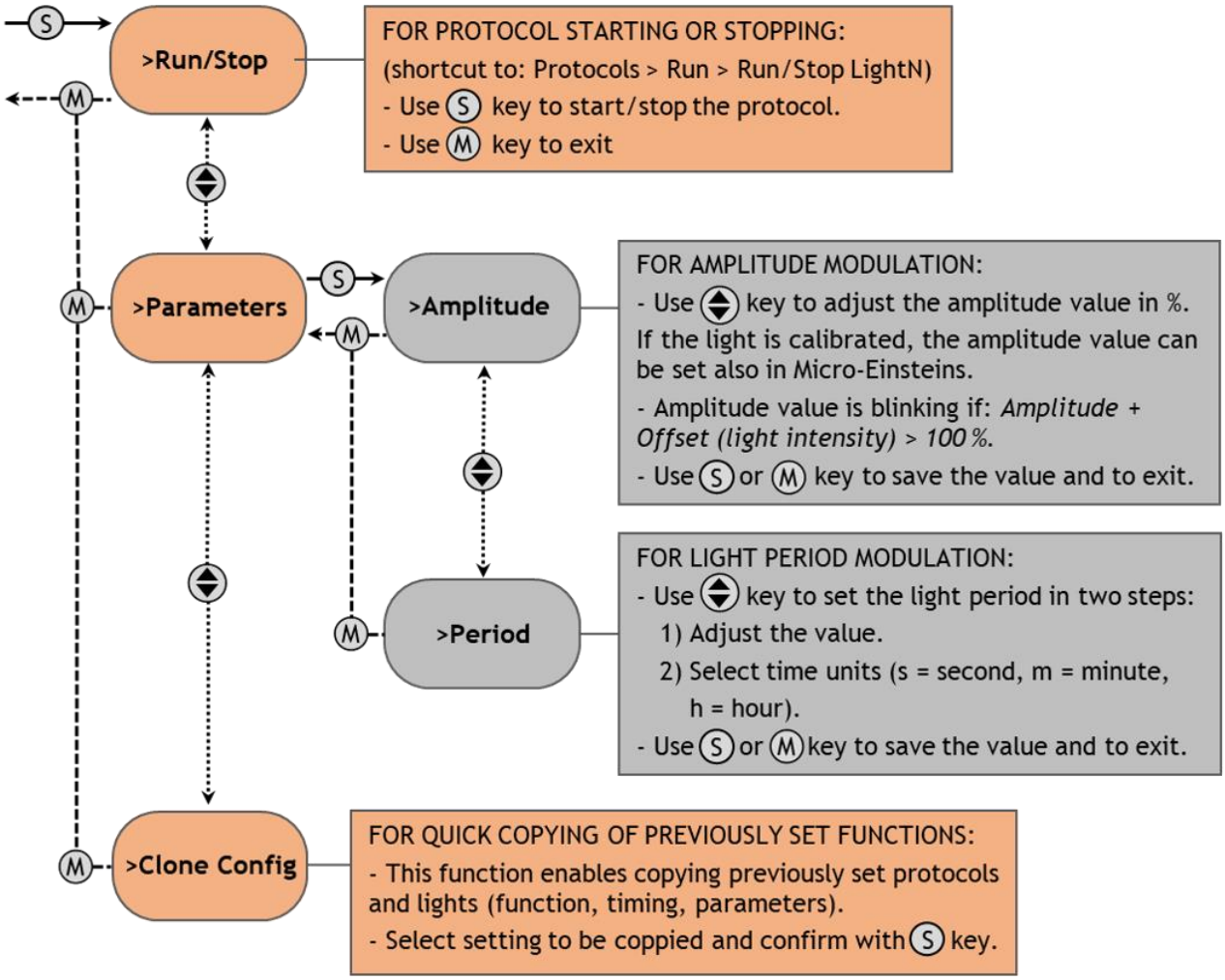


* There is a PC application for daylight protocol light curve visualization available as an upgrade to the standard Multi-cultivator package. Seed parameter in the Daylight protocol is used to synchronize this application and the Multi-cultivator, so with the same protocol settings, it produces identical light curve.

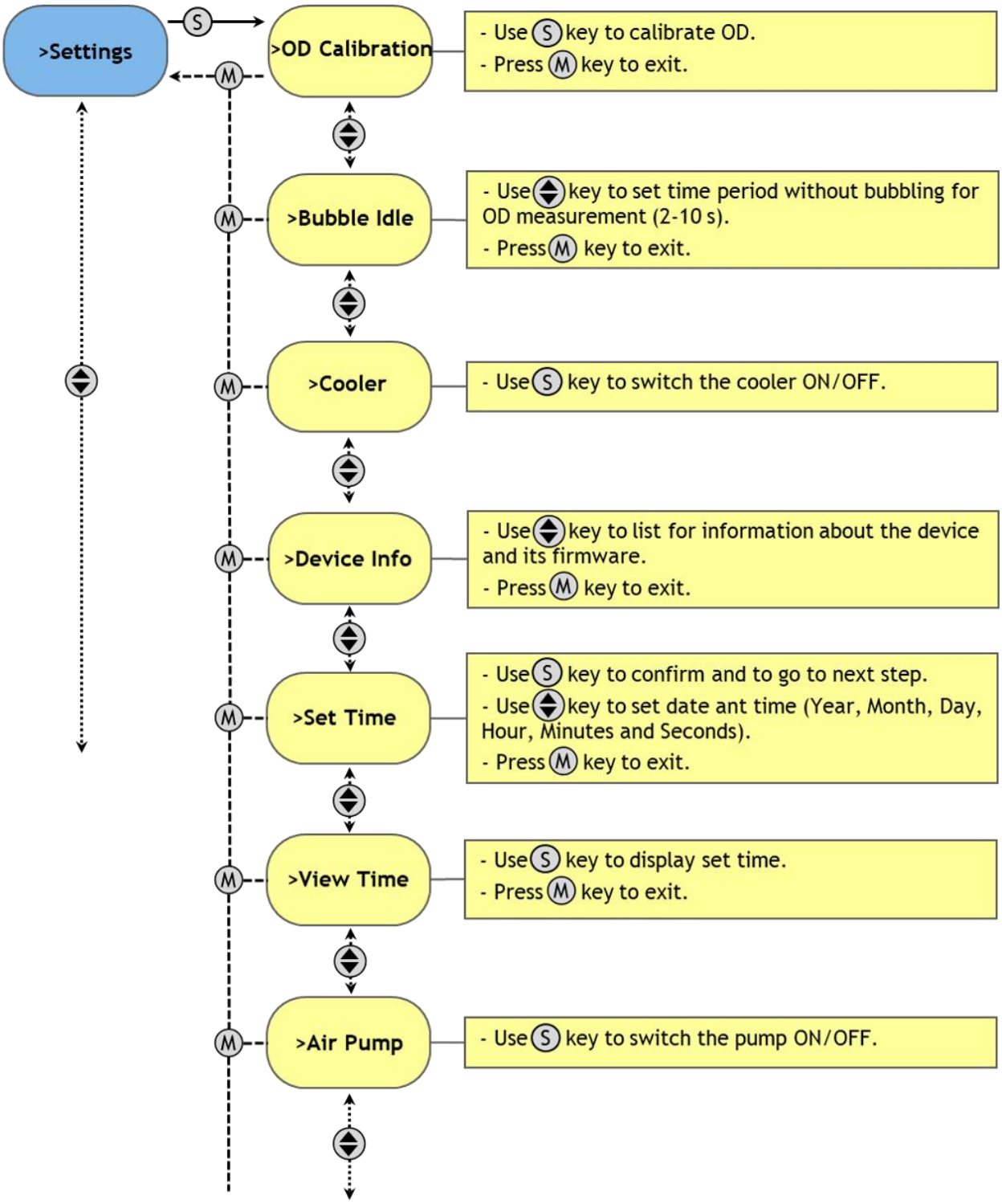
Menu Protocols→Edit→LightN→Timing



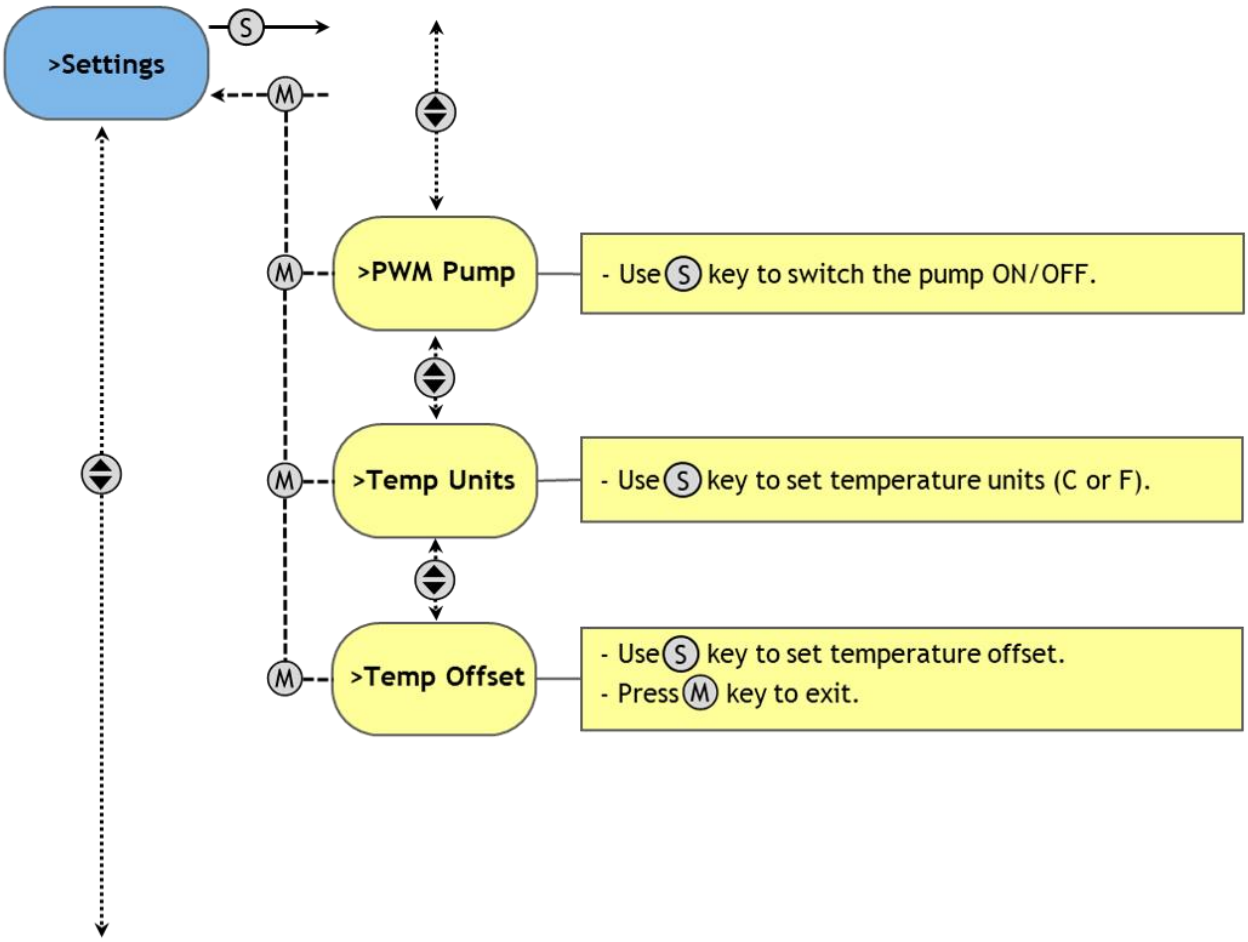
Menu Protocols→Edit→LightN→Run/Stop... Clone Config



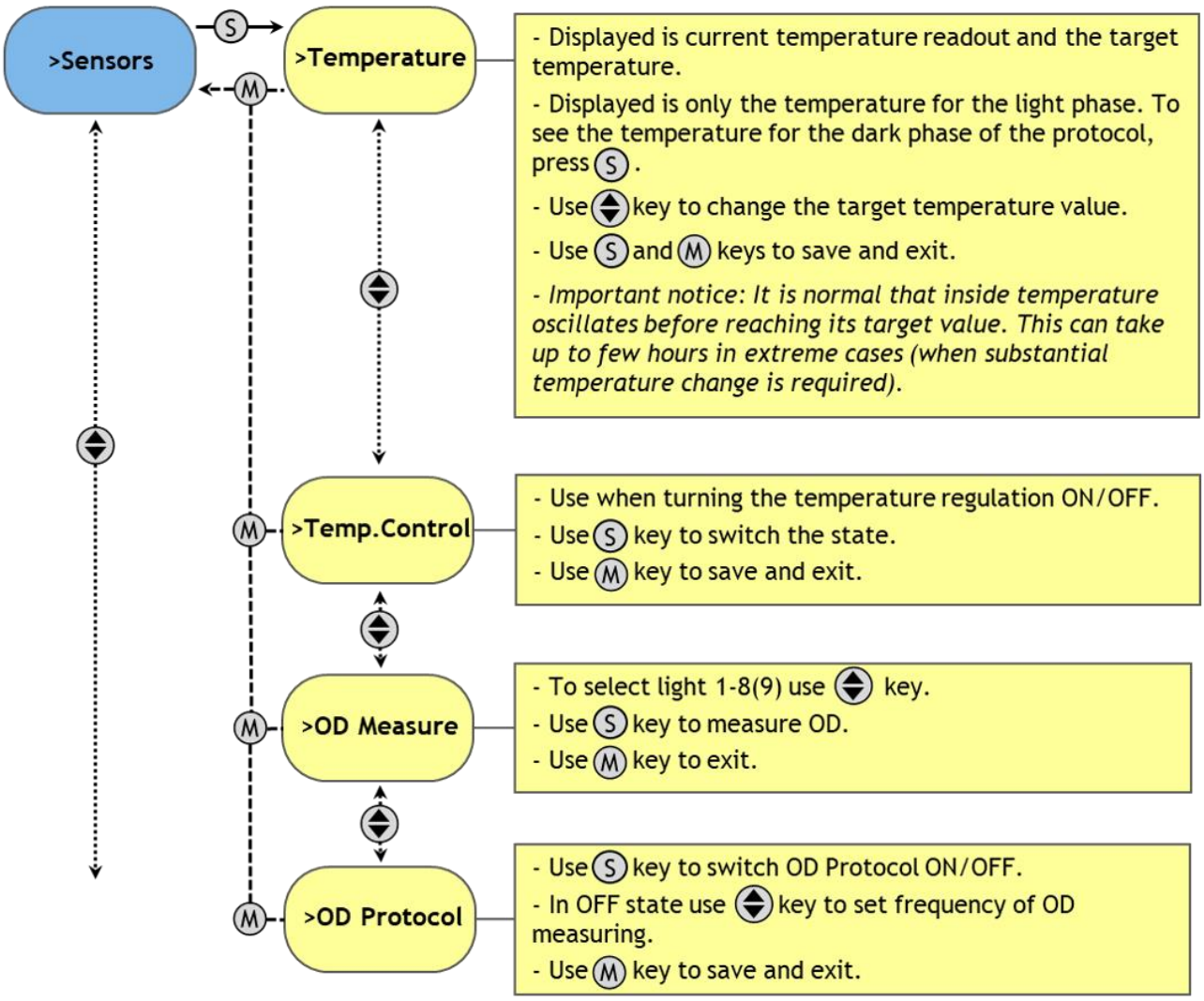
Menu Settings → OD Calibration … Air Pump



Menu Settings → PWM Pump ... Temp Offset



Menu Settings → OD Calibration … Air Pump



8.2 CONTROL MENU TREE – UPDATE VERSIONS

Please note that updated firmware versions are available for the MC 1000 and MC 1000-OD. Firmware versions might differ depending on the production date of the device. The Menu Tree Protocols might differ slightly for different versions of firmware from the Control Menu Tree described on page 40 - 47.

Please find below the description of the modifications for given firmware version based on production date. To find the production date go to **Settings > Device Info**.

Menu Tree – Protocols Versions for:

Firmware version from 9. 5. 2013 and higher

To start the protocol **FUNCTION, TIMING** and **START** must be defined. Follow the next steps to set the protocol.

- **FUNCTION** refers to one period.
- **TIMING** refers to Light Period (**LP**) during which the defined **FUNCTION** is performed, Dark Period (**DP**) refers to dark phase without any light illumination. This is repeated according to predefined number of repetitions or can be repeated forever with **REPEAT FOREVER** function.
- **START DATE** – it is possible to set the start date to start/terminate the protocol. If the date is set to future, the protocol is waiting until desired start time is reached and the protocol is automatically initiated. If the desired start date is in past, the protocol is switched on and set to the phase where the predefined protocol is in the current moment. For example: If 16 h light/8 h dark daylight protocol is defined with start time from 7:00 am and the user starts the protocol at 11:00 am, in the Menu **Protocols > View** the time displayed will be 4 hours, such that the protocol is already 4 h running.
- To **START** the protocol, go to **Run Light x** in **Protocols > Control**.

Firmware version from 22. 4. 2015 and higher

- **BUBBLE IDLE** allows to set a time period without gas sparging for the OD measurement mode. As the bubbles disturb the OD measurement the gas sparging is automatically stopped for this purpose for a while. As a default setting 3.5 seconds are used but the user can adjust this period in range from 2 up to 10 seconds.

Firmware version 1.0.3.4 and higher supports the bioreactor Control Software. The recommended FW version is 1.0.3.8.

9 WARRANTY TERMS AND CONDITIONS

- This Limited Warranty applies only to the Multi-Cultivator device. It is valid for one year from the date of shipment.
- If at any time within this warranty period the instrument does not function as warranted, return it and the manufacturer will repair or replace it at no charge. The customer is responsible for shipping and insurance charges (for the full product value) to PSI. The manufacturer is responsible for shipping and insurance on return of the instrument to the customer.
- No warranty will apply to any instrument that has been (i) modified, altered, or repaired by persons unauthorized by the manufacturer; (ii) subjected to misuse, negligence, or accident; (iii) connected, installed, adjusted, or used otherwise than in accordance with the instructions supplied by the manufacturer.
- The warranty is return-to-base only and does not include on-site repair charges such as labor, travel, or other expenses associated with the repair or installation of replacement parts at the customer's site.
- The manufacturer repairs or replaces faulty instruments as quickly as possible; the maximum time is one month.
- The manufacturer will keep spare parts or their adequate substitutes for a period of at least five years.
- Returned instruments must be packaged sufficiently so as not to assume any transit damage. If damage is caused due to insufficient packaging, the instrument will be treated as an out-of-warranty repair and charged as such.
- PSI also offers out-of-warranty repairs. These are usually returned to the customer on a cash-on-delivery basis.
- *Wear & Tear Items* (such as sealing, tubing, padding, etc.) are excluded from this warranty. The term *Wear & Tear* denotes the damage that naturally and inevitably occurs as a result of normal use or aging even when an item is used competently and with care and proper maintenance.

10 TROUBLESHOOTING AND CUSTOMER SUPPORT

In case of troubles and for customer support, please, visit **FAQ** on our websites, write to **support@psi.cz** or contact your local distributor.

11 APPENDIX

11.1 LED LIGHT SPECTRA USED IN MC 1000-OD

The Multi-Cultivator MC 1000-OD is available in various color versions:

Unicolor MC 1000-OD is a version in which each tube is illuminated by an array of cool white LEDs (optionally warm white, red, or blue LEDs).

Multi-Cultivator MC 1000-OD-MULTI is a multi-color instrument version, in which each cultivation slot is furnished with illumination of different color. Covered is the spectrum from 405 nm to 730 nm.

Multi-Cultivator MC 1000-OD-MIX is a mixed-color instrument version which allows to combine up to 8 different LED colors within each cultivation slot for definition of specific spectra.

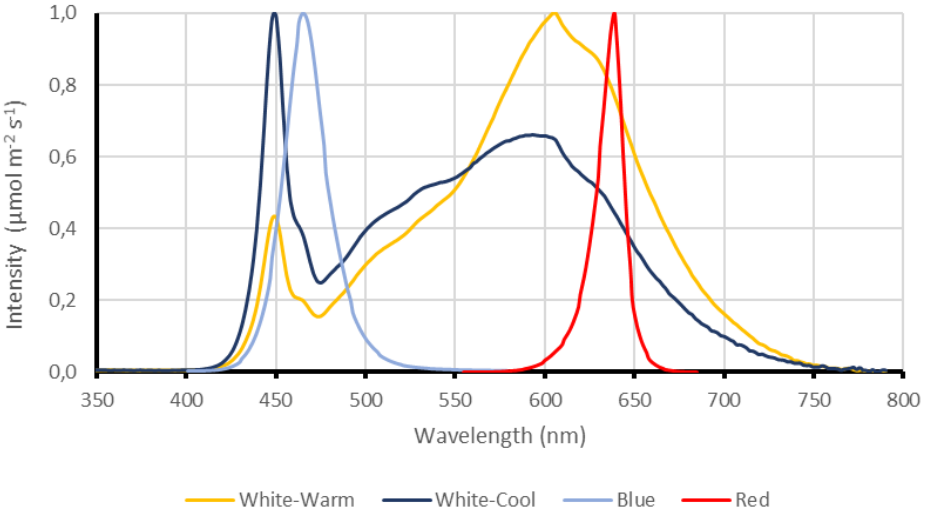


Fig. 30 The representative LED spectra used in unicolor versions of MC 1000-OD.

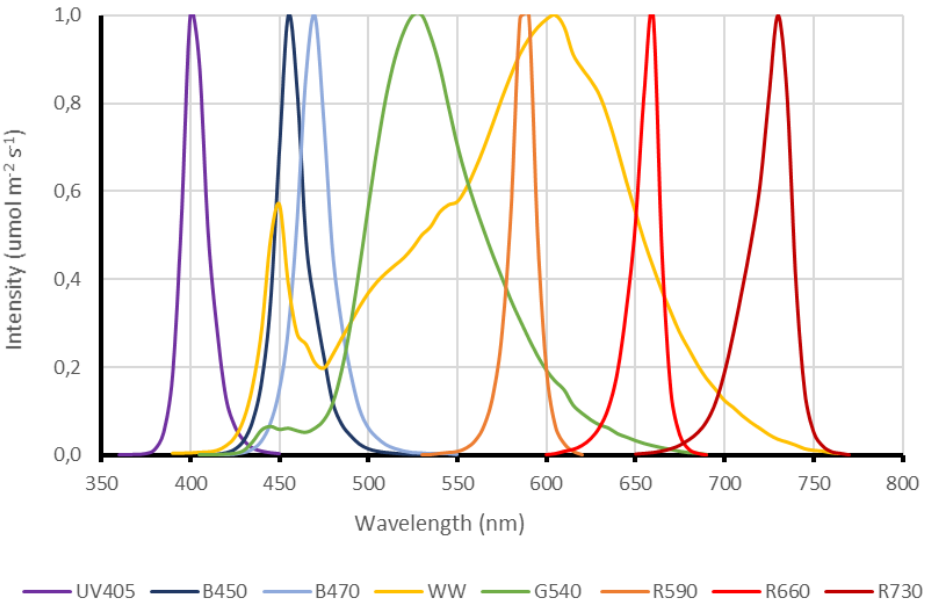


Fig. 31 The representative LED spectra used in multi-color versions of MC 1000-OD.

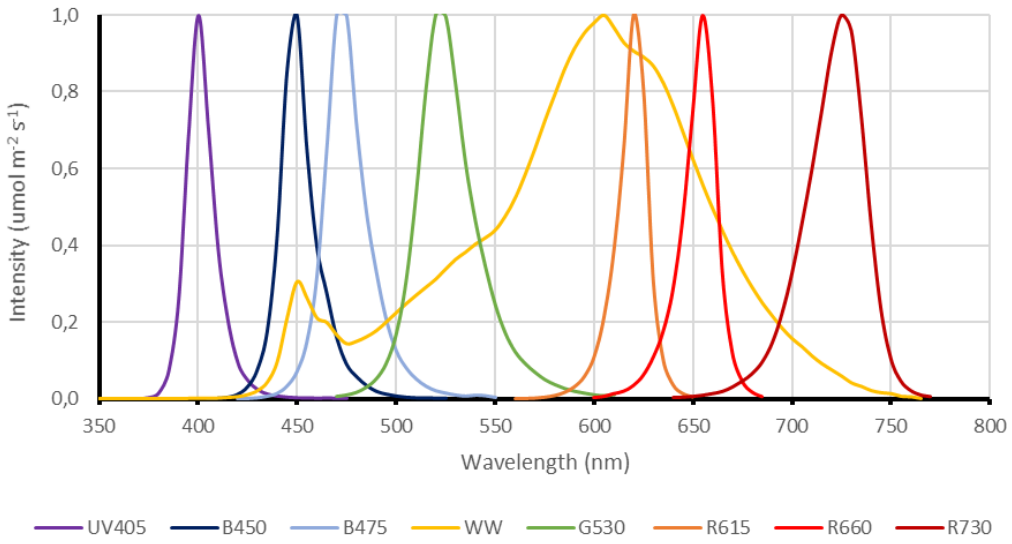


Fig. 32 The representative LED spectra used in mixed-color versions of MC 1000-OD.

11.2 EXAMPLES OF LIGHT PROTOCOLS CONFIGURED VIA THE CONTROL UNIT FRONT PANEL

	Please note that after light protocol termination the lights will be adjusted according to the lights setting in the main menu.

11.2.1 CIRCADIAN CYCLE

The example of five-days long experiment using a diurnal Light/Dark phases is described below. Light phase (LP) takes 16 hours whereas Dark phase only 8 hours (DP; automatically 0 µmol.m^{-2}.s^{-1}). Light intensity during the Light phase is set to a constant level at 200 µmol.m^{-2}.s^{-1}. The Light protocol is identical for all 8 cultivation tubes. The cultivation temperature is different for Light phase (25 °C) and Dark phase (20 °C).

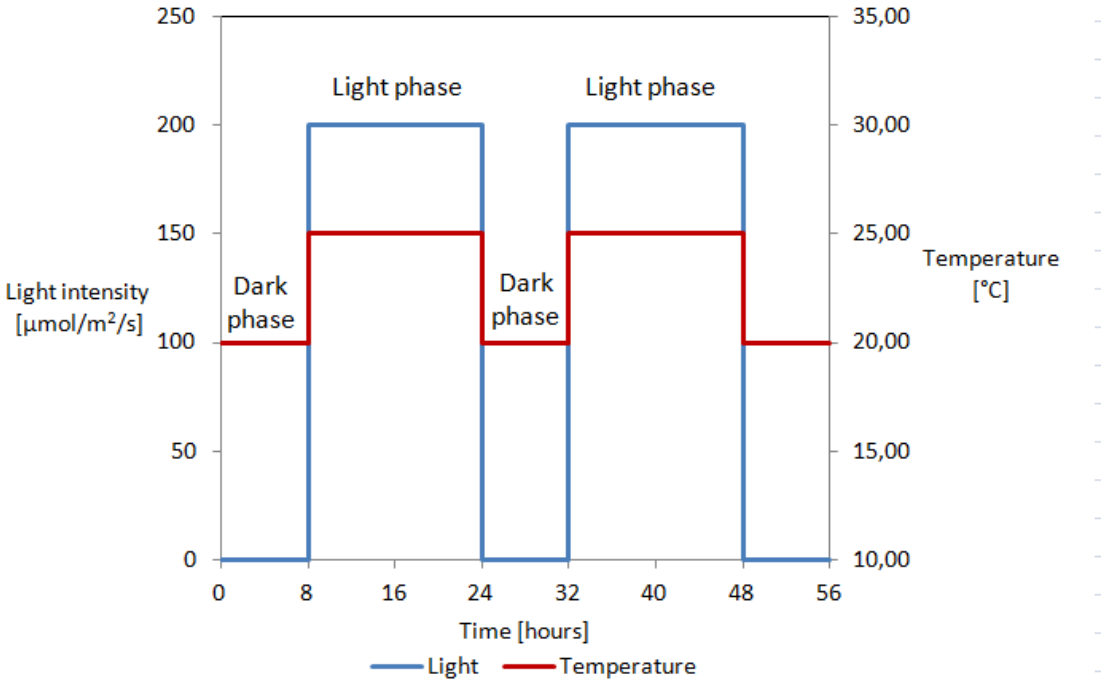


Fig. 33 Graph illustrating circadian cycle protocol.

1. Setting of the light intensity during the Light phase.

   Lights > All Lights > 200 µmol.m^{-2}.s^{-1}

2. Definition of phases timing for Light 1.

   Protocols > Edit > Light 1 > Timing > Edit Phases > Timing: LP value

   LP [16] s

   > Timing: LP units

   LP 16 [h]

   > Timing: DP value

   DP [8] s

   > Timing: DP units

   DP 8 [h]

   > Timing: repeats

   Repeats [5] x

3. Cloning of phases timing to the rest of lights.

   Protocols > Edit > Light 2 > Clone Config > Light 1

   .

   .

   .

   Protocols > Edit > Light 8 > Clone Config > Light 1

4. Simultaneous start of light protocol in all cultivation tubes.

   Protocols > Control > Run

5. Temperature setting.

Sensors > Tcontrol > ON

Sensors > Temperature > Current t X °C

Target L t 25 °C

>Current t X °C

Target D t 20 °C

11.2.2 PULSE CYCLE

An illustration of an alternation of Dark and Light phases with short light Pulses during the Light Phase. The duration of Dark as well as Light phase is 2 minutes (= 120 s). The PULSE amplitude is 300 µmol.m^{-2}.s^{-1}, period 24 s (120/24 = 5 pulses within one Light phase) and width is 50 % (= 12 s PULSE + 12 s background). No background is set during the Light phase in the first example (Fig. 34); 150 µmol.m^{-2}.s^{-1} as the background is set in the following example (Fig. 35). The Light protocol is same for all 8 cultivation tubes.

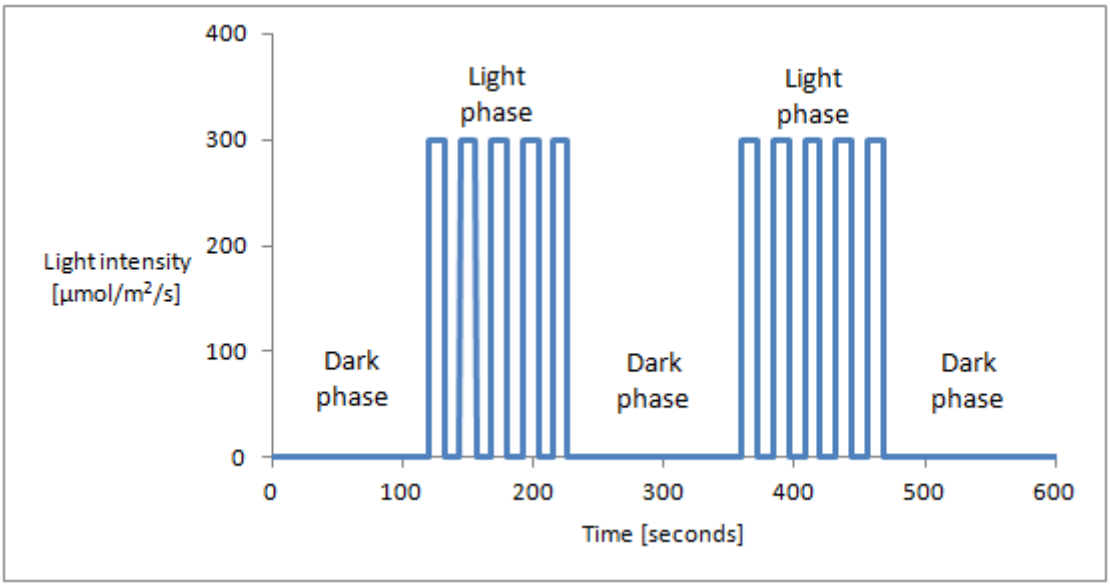


Fig. 34 Graph illustrating the pulse protocol without background.

1. PULSE light function without background – all lights in main menu should be set to 0 µmol.m^{-2}.s^{-1}

   Lights > All Lights > 0 µmol.m^{-2}.s^{-1}

2. Definition of phases timing of Light 1.

   Protocols > Edit > Light 1 > Timing > Edit Phases > Timing: LP value

   LP [2] s

   > Timing: LP units

   LP 2 [m]

   > Timing: DP value

   DP [2] m

   > Timing: DP units

   DP 2 [m]

   > Timing: repeats

   Repeats [1] x

   > RepForever > YES

3. Definition of PULSE light function of Light 1.

   Protocols > Edit > Light 1 > Function > PULSE

   > Params > Amplitude 300 µmol.m^{-2}.s^{-1}

   > Period 24 s

   > Width 50 %

4. Cloning of phases timing to the rest of lights.

   Protocols > Edit > Light 2 > Clone Config > Light 1

   .

   .

   .

   Protocols > Edit > Light 8 > Clone Config > Light 1

5. Simultaneous start of light protocol in all cultivation tubes.

   Protocols > Control > Run

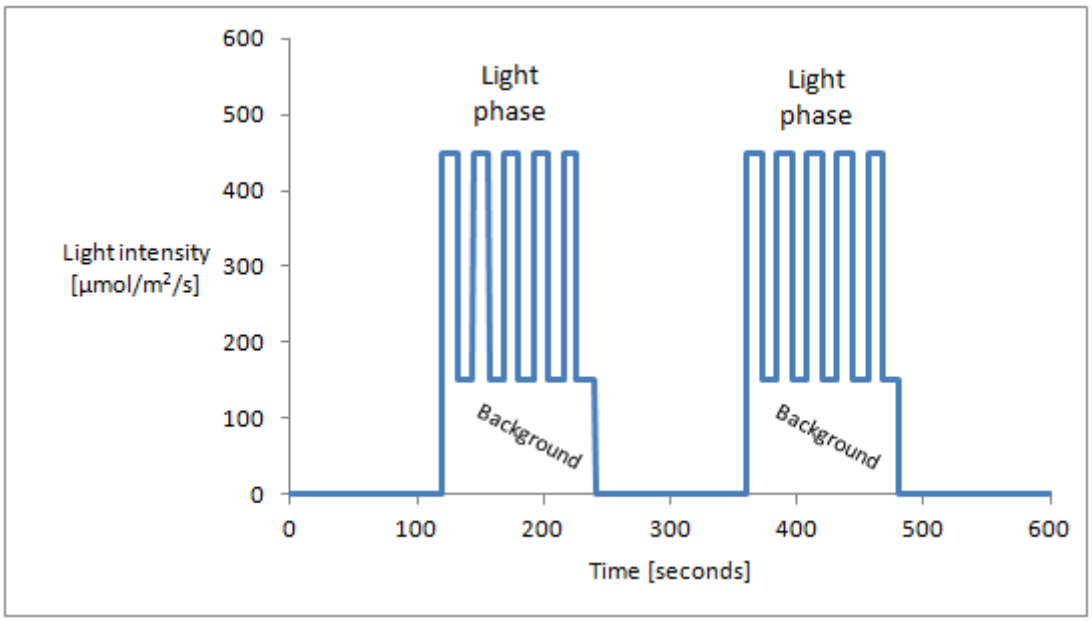


Fig. 35 Graph illustrating the pulse protocol with background.

1. PULSE light function with background – all lights in main menu should be set to 150 µmol.m^{-2}.s^{-1}

   Lights > All Lights > 150 µmol.m^{-2}.s^{-1}

2. Definition of phases timing of Light 1.

   Protocols > Edit > Light 1 > Timing > Edit Phases > Timing: LP value

   LP [2] s

   > Timing: LP units

   LP 2 [m]

   > Timing: DP value

   DP [2] m

   > Timing: DP units

   DP 2 [m]

> Timing: repeats

Repeats [1] x

> RepForever > YES

3. Definition of PULSE light function of Light 1.

   Protocols > Edit > Light 1 > Function > PULSE

   > Params > Amplitude 300 µmol.m^{-2}.s^{-1} (+ 150 µmol.m^{-2}.s^{-1} background)

   > Period 24 s

   > Width 50 %

4. Cloning of phases timing to the rest of lights.

   Protocols > Edit > Light 2 > Clone Config > Light

   .

   .

   .

   Protocols > Edit > Light 8 > Clone Config > Light 1

5. Simultaneous start of light protocol in all cultivation tubes.

   Protocols > Control > Run

11.2.3 SINE CIRCADIAN CYCLE

An example of 24-hours cycle consisting a Light phase with SINE function and a Dark phase. The duration of each phase is 12 hrs. The SINE amplitude is 150 µmol.m^{-2}.s^{-1}, period 12 h (Fig. 36). The Light protocol is same for all 8 cultivation tubes.

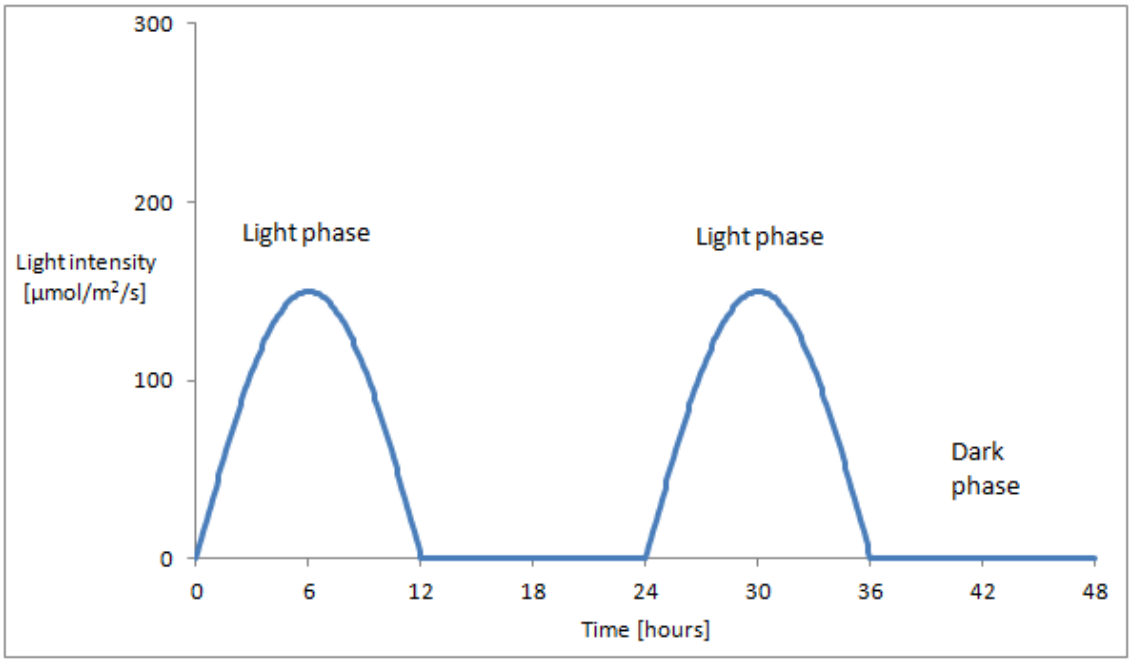


Fig. 36 Graph illustrating sine circadian light protocol.

1. SINE light function without background – all lights in main menu should be set to 0 µmol.m^{-2}.s^{-1}

   Lights > All Lights > 0 µmol.m^{-2}.s^{-1}

2. Definition of phases timing of Light 1.

   Protocols > Edit > Light 1 > Timing > Edit Phases > Timing: LP value

LP [12] s

> Timing: LP units

LP 12 [h]

> Timing: DP value

DP [12] h

> Timing: DP units

DP 12 [h]

> Timing: repeats

Repeats [1] x

> RepForever > YES

3. Definition of SINE light function of Light 1.

   Protocols > Edit > Light 1 > Function > SINE

   > Params > Amplitude 150 µmol.m^{-2}.s^{-1}

   > Period 12 h

4. Cloning of phases timing to the rest of lights.

   Protocols > Edit > Light 2 > Clone Config > Light 1

   .

   .

   .

   Protocols > Edit > Light 8 > Clone Config > Light 1

5. Simultaneous start of light protocol in all cultivation tubes.

   Protocols > Control > Run