Oroboros NextGen O2k-Manual

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PhotoBiology-Module

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1. Introduction

PhotoBiology is the science of the effect of light on biological processes including photosynthesis. The PhotoBiology- (PB-) Module is an add-on module of the NextGen-O2k. It enables the controlled induction of photosynthesis by exchangeable PB light sources (PBLS) emitting light of different wavelengths and intensities.

The optical PBLS (one per experimental O2k-chamber) are inserted into the front window of the O2k, providing light to the experimental chamber. The light spectral range that photosynthetic organisms can use to drive photosynthesis covers 400 to 700 nm and is called Photosynthetically Active Radiation (PAR). Most algae and all terrestrial plants are most efficient in capturing the energy associated to the blue (400-500 nm) and red (600-700 nm) light wavelengths [1]. Therefore, three different light sources are provided with the PB-Module in form of LEDs that emit PAR within the blue, red, and white (full spectrum) range and cover physiologically sub-saturating to super-saturating light intensities for different types of algae and plant cells. The light intensity can be set and regulated (light on, off, and selection of different light intensities) with the DatLab 8 software. In addition, to calibrate the light intensity provided and guarantee its stability, an integrated photodiode quantifies in real-time and continuously corrects the light intensity throughout the experiment.

Net photosynthesis, dark respiration and light enhanced dark respiration are measured by high-resolution respirometry.

2. Setup

2.1. Components

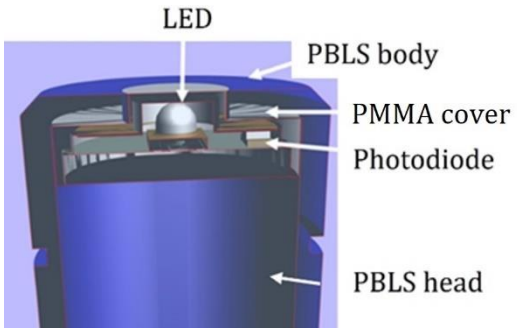
The PB-Module consists of electronic components which are an integral part of the NextGen-O2k and three pairs of PB Light Sources (PBLS) that emit different wavelengths of light. The PBLS-Storage box contains:

- 2x white PBLS (WS)
- 2x blue PBLS (BU)
- 2x red PBLS (RD)

Table 1: Specifications of the PBLS

	LED	Wavelength range [nm]	Wavelength peak [nm]	Max. light intensity [µmol*s^{-1}*m^{-2}]
2x white PBLS (WS)	Duris S5	425-750	440 and 630	3500
2x blue PBLS (BU)	Duris S5	439-457	451	3000
2x red PBLS (RD)	Duris S5	620-632	634	2750

The light is emitted by an LED mounted on the PBLS head behind a transparent protective cover made of PMMA. All LEDs have a viewing angle of 80°. The light intensity can be regulated (table 1) with the DatLab 8 software (see section 3). An integrated photodiode provides real-time correction of the emitted light intensity to ensure a constant value throughout the experiment. The PBLS head is implemented in the PBLS body which can be inserted into the NextGen-O2k front window and connected to the NextGen-O2k main unit via a fixed cable (see section 2.2).

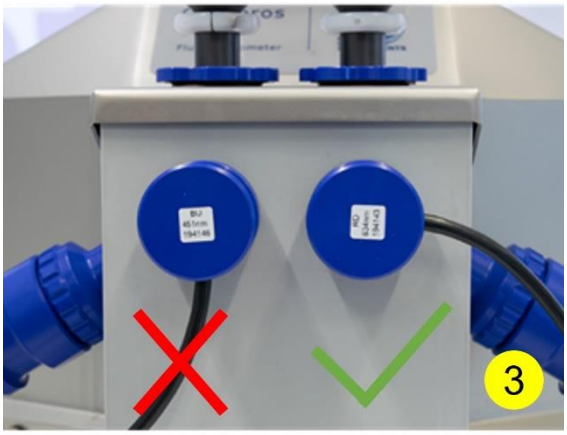


2.2. Assembly

For general operating procedures not related to the PB-Module of the NextGen-O2k please see the O2k-FluoRespirometer manual.

1. Connect the PBLS cable to NextGen-O2k main unit by inserting the male plug of the cable into the female Fluo/PB socket (1). The red dot on the male plug must be pointing straight up when inserting into the socket. Each PBLS can be used on O2k-chamber A or B.

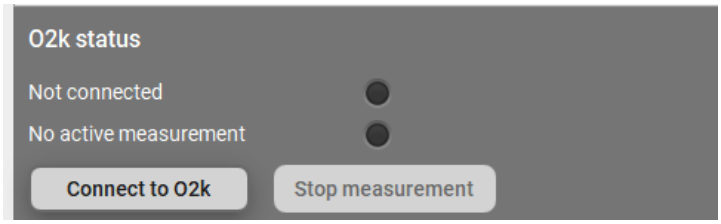
2. The blue frame of the chamber window and the PBLS are specially designed to only connect at a specific orientation, when the flat edges align (2). In this optimal position, the PBLS is carefully inserted in the window until the sensor covers the chamber window without any gap. In this position the cable routing must be horizontal (3). The correct position needs to be ensured to guarantee proper illumination of the chamber. To remove the PBLS, carefully pull out the PBLS body with slight back and forth rotations. Do not pull on the cable.



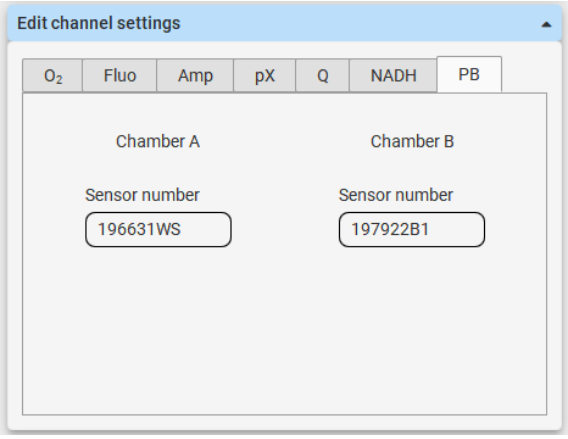
3. Operating instructions using DatLab 8

3.1. Launch DatLab 8 and NextGen-O2k configuration

1. Switch on the O2k and launch “Oroboros DatLab 8” software. Enter or select a username. Click on Connect to O2k.



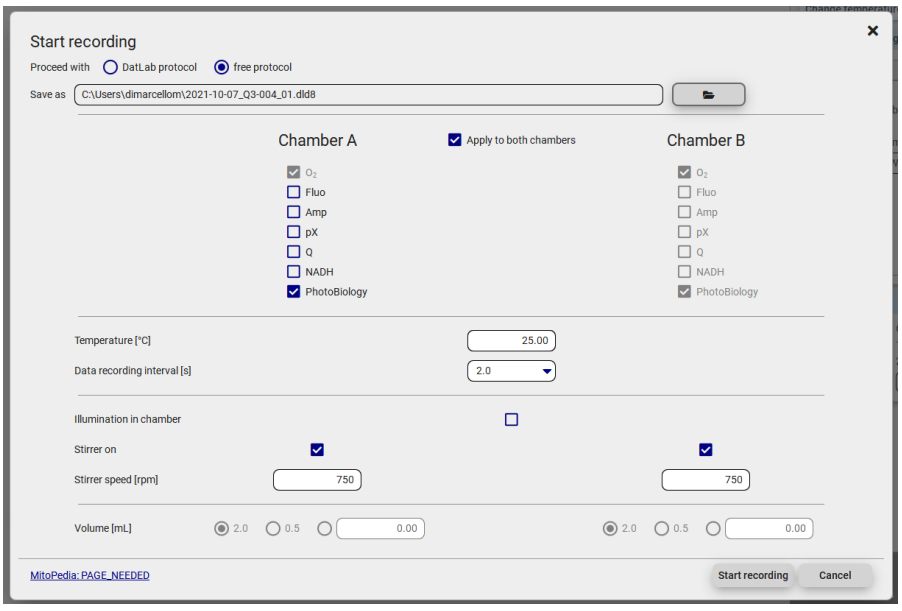
2. Plug in the PBLS and click on Edit channel settings. Click on the “PB” tab and the sensor number will be shown.



The PBLS serial number and color of the emitted light can be found on the inside of the PBLS body. The color of the light emitted by the PBLS is engraved on the outside of the PBLS body. Only when a PBLS is connected, the serial number is automatically detected and set in the PB tab “sensor number #”.

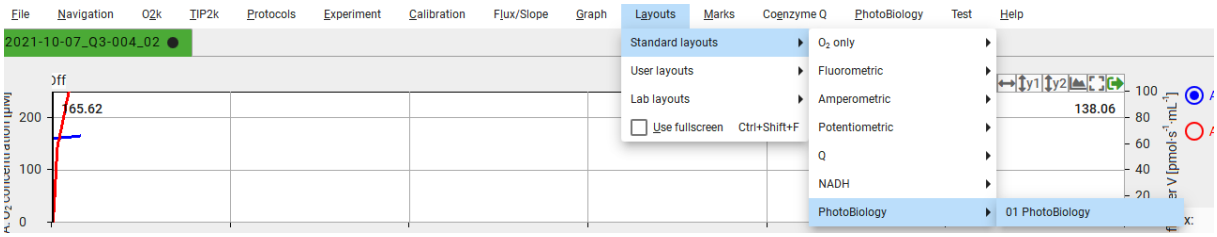
3.2. Start Measurement

1. Click on Start Measurement.
2. In the Start recording window proceed by selecting “DatLab protocol” or “free protocol”. When selecting “free protocol”, the PhotoBiology check box needs to be selected.
3. In the Start recording window, check and modify the measurement parameters (Temperature, Data recording interval, Illumination in chamber, Stirrer) if required. “Illumination in chamber” must be switched off for the use of the PB-Module. The Volume of the chamber cannot be modified as the PB-Module can only be used with the 2.0 mL O2k-chamber.
4. Click on Start recording.



3.3. Select Layout

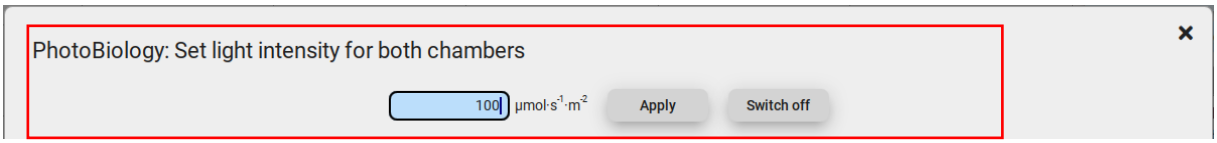
Select the proper Layout to be able to see the PB plot/channel. Click on “Layout” on the Menu bar. In the drop-down menu select “Standard layouts.” In the next drop-down menu select “PhotoBiology” and then “01Photobiology.”



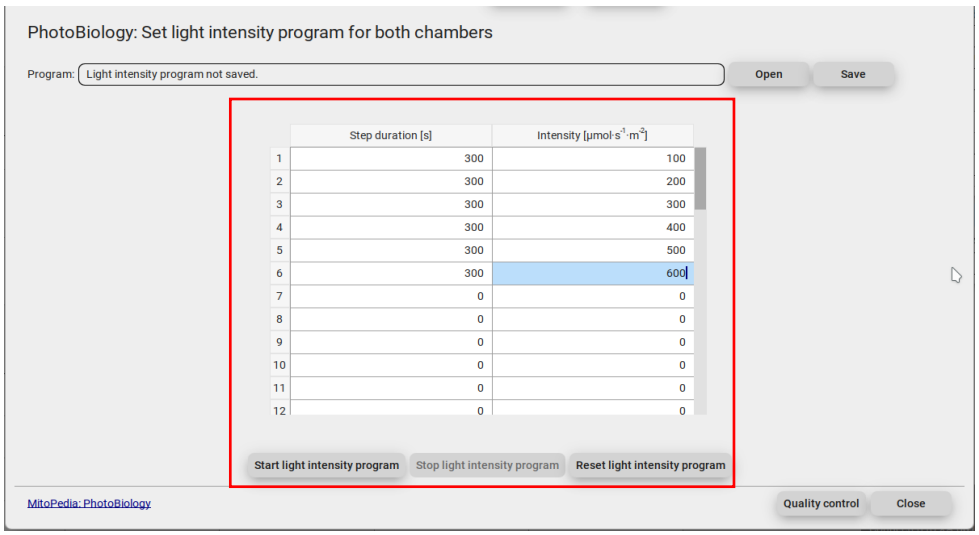
3.4. Set light intensity

1. Click on “PhotoBiology” in the Menu bar and select “Set light intensity” in the drop-down menu. Alternatively, you can press Ctrl+I.

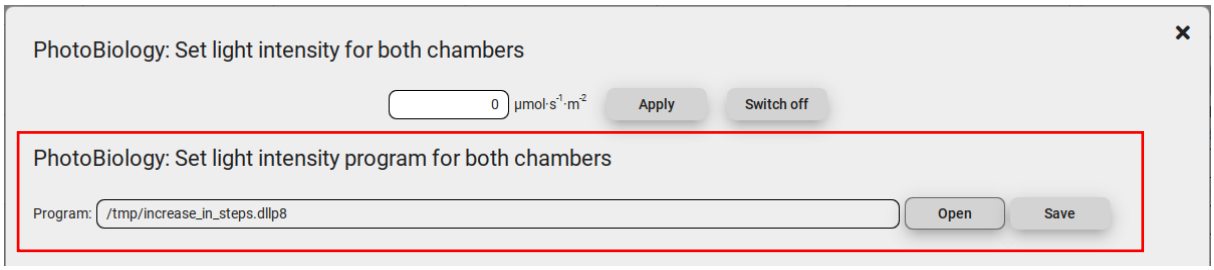
2. The light intensity for the PBLS of both chambers can be set with two different methods.
   a. First section of the PhotoBiology window: “Set light intensity for both chambers” will provide the intensity without factoring in the time. After clicking Apply, both PBLS will emit the given light intensity. This function will work also if the PBLS are different colors.



   b. Second section of the PhotoBiology: With “Set light intensity program for both chambers”, the operator has the ability to set multiple steps for light intensity and intervals over time. Once all steps are set, clicking Start light intensity program will start the whole program automatically. This function will work also if the PBLS are different colors.



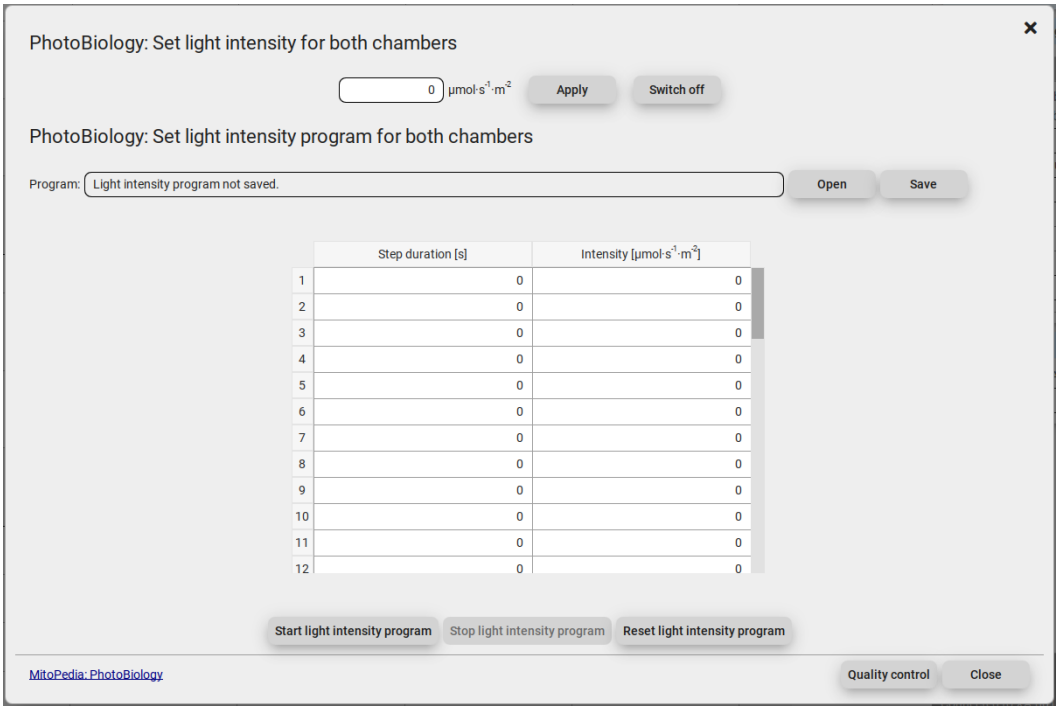
Once the program has started, it will end automatically after the last step, or can be stopped by clicking on the Stop light intensity program in the window.
Clicking on Reset Light intensity program sets all values back to zero.
Opening a preexisting light intensity program is also possible, as well as saving the current one for future use.



4. Quality Control: Test of the PBLS LED performance over time

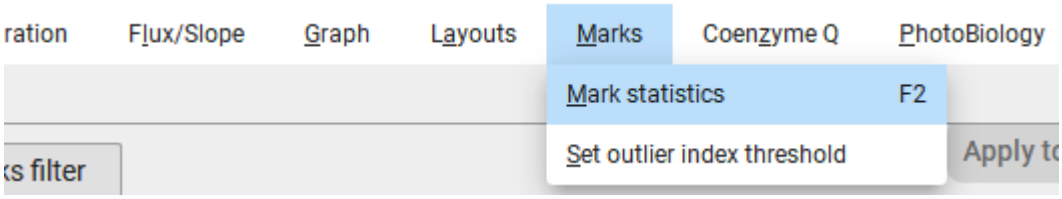
The NextGen-O2k PB-Module has a source of light provided by the LED located in the PBLS head whose intensity can be controlled by the user. To guarantee the selected intensity, the photodiode of the PBLS head continuously measures real-time the light intensity emitted by the LED. The NextGen-O2k automatically corrects for any deviation that may happen, including those that may be caused by any decay or malfunctioning over time in any component of the PBLS head. However, as a quality control it is recommended to assess the performance of the PBLS LEDs over time. This includes determination of changes in the LED's output compared to the selected light intensity and detection of any damage in the PBLS head that may affect light emission. It is recommended to perform the test of the PBLS LED performance every 6 months or any time when there is a suspicion that something may be damaged in the PBLS head.

1. Fill the O2k-chambers with distilled water, close the chambers with the stopper and connect the PBLS as described above in section 2.2.
2. Start a measurement, load the proper layout as described above in section 3.3.
3. Click on PhotoBiology and then Set light intensity program as described above in section 3.4.
4. Select Quality control and then close the window. The software will automatically set a specific light intensity for both PBLS, even if they are of different colour .

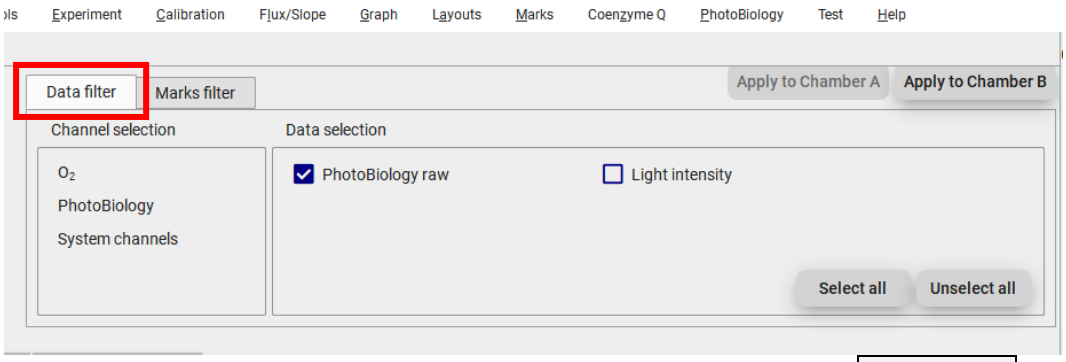


5. Stop recording and go to the menu File\Save and disconnect.
6. Click on the
7. Set a mark in the Light Intensity plot between the event "switch on" and the event "switch off "

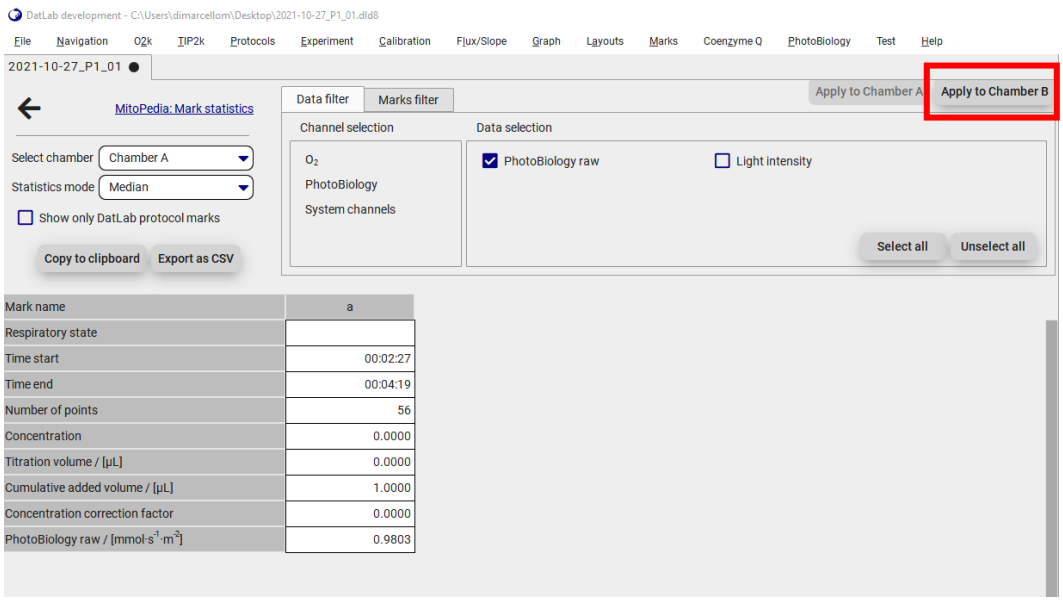
8. Select “Marks”, then “Marks Statistic”



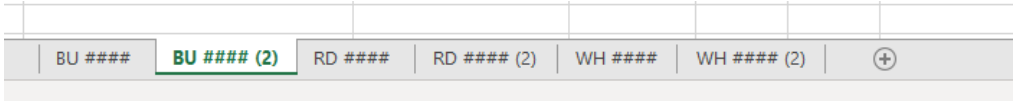
9. Make sure that Data filter tab has been selected



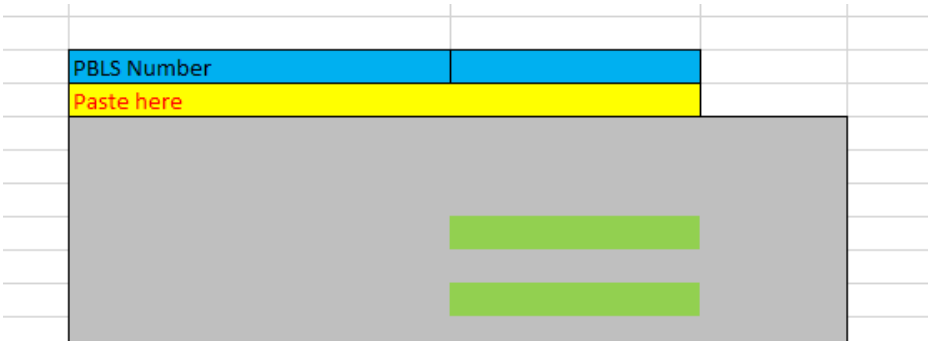
10. Under Channel selection, click on O_2 and on the right, press on Unselect all.
11. Under Channel selection, click on PhotoBiology and unselect Light Intensity, leave only PhotoBiology Raw checked.
12. Under Channel selection, click on System channels and on the right press on Unselect all.
13. Click on Apply to Chamber B (top right). Thereby, all the settings are applied to chamber B as well)



14. Click Copy to clipboard
15. Open the PBLS Quality Control Excel file and select the Excel sheet according to the color of the PBLS



16. Paste and add the PBLS Number



17. On the right side a graph will be automatically generated with all the measurement added
18. If the deviation for the light intensity measured is greater than ±10 % of the target intensity, check the PB-Sensor tip for any signs of dirt or damage. For any sign of dirt clean the sensors following the procedure described in section 5. For any sign of damage please contact O2k Technical Support.

5. Cleaning of the PBLS head

There is no need to wash the PBLS body frequently, providing that it is handled with care and no scratches or dirt appears on the PMMA cover of the LED and the photodiode. If some dirt or smearing appears on the surface of the tip, wash it with a soft paper towel and 70 % ethanol. Afterwards, it is recommended to perform a Quality Control as described in section 4: Assessment of the PBLS LED performance over time.

6. Troubleshooting

6.1. Optimization of cell density for conducting assays.

Before conducting experiments with the PB-Module it is recommended to optimize the cell density, to avoid the production of too much oxygen. High oxygen concentrations could alter the cell physiology and cause noise in the O_2 flux. When using high-resolution respirometry for measurements of O_2 production during photosynthesis, an initial ROUTINE respiration of 10-30 pmol·s^{-1}·mL^{-1} is recommended.

7. References

Bidlack JE, Jansky S, Stern KR (2013) Stern's Introductory Plant Biology; McGraw Hill International Editions.

8. Author contributions

Gnaiger E was responsible for the project development. Gnaiger E, Gradl P, Gollner M, Merth A, Schwaninger H and Walter-Vracevic M were responsible for instrumental development. Gnaiger E, Di Marcello M, Doerrier C, and Schmitt S prepared the manual.

9. Acknowledgements

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