Version K

ogibio.co.uk

Mark II Microbioreactor User Manual

2025

Contents

 This symbol indicates an important point to consider during device use.

Device Manufacturer

Device is manufactured by:

OGI Bio Ltd

Glencorse Building

Pentlands Science Park

Bush Loan

Penicuik

EH26 0PZ

Safety Instructions

If the device is not used as indicated in this manual, its functioning and safety may be impaired.

- **Please turn the device off before replacing and installing optional modules.**
- **Power Supply must be connected to supply with a protective earth connection.**
- **Do not unscrew the base of the bioreactor and open the device. If you have any issues with the device, please contact our team at info@ogibio.co.uk**
- **Do not position the bioreactor so that it is difficult to operate or disconnect the device. The mains supply socket should be easily accessible in order that it can be disconnected quickly in case of emergency.**

  The optional Fluorescence Measurement module (OGI-A-1009) contains a UV LED. This has been tested and assessed in operating mode as Risk Group 0. Please ensure module is firmly secured to the Bioreactor with the fixing screw provided BEFORE turning on the Bioreactor power.

1. Operating the device

This manual is compatible with bioreactor software version BR3.7. If your device is running an earlier version, please contact info@ogibio.co.uk for an update package. If your device is running a later version, be aware that a more up-to-date version of the manual can be found on our website at www.ogibio.co.uk/resources.

Technical specifications can be found in Appendix A. Using the device out with these specifications may result in suboptimal performance and void any warranty in place.

If you are using turbid media, it is recommended that it be filtered. Using turbid media can reduce the range of OD readings or render them inadequate for biomass estimation (depending on the turbidity).

The supplied USB stick is in FAT32 format. Please note that any storage device may be used so long as it is formatted in the same way.

2. Device Description

The ŌGI Bio Microbioreactor is a small-scale bioreactor that can be used as a benchtop device to automate the culturing of microbes. The device comprises four individual reactor flask holders, each holding a single culture flask. The accumulation of biomass of each microbial culture is measured at regular intervals throughout the experiment. Additional optional accessory modules can be installed to add capabilities to the device:

Liquid Control P/N OGI-A-1007

The module is used to maintain the cultures in flask at the desired optical densities, or in chemostat mode to provide a constant flow of fresh media. It contains 8 liquid handling pumps (of which 4 input and 4 output, one pair of each for each flask). Bungs containing needles and pre-cut tubing are provided for use with the Liquid Control Module.

Dissolved Oxygen Monitoring P/N OGI-A-1008

The module can be used to measure the amount of dissolved oxygen in the culture media at regular intervals during an experiment and requires culture flasks with oxygen sensor spots (Part number A200017).

Fluorescence Measurement P/N OGI-A-1009

The module can be used to measure up to 3 different fluorescent signals simultaneously per culture flask. The wavelengths available as standard are:

Excitation: 365nm, 470nm, 590nm.
Emission: 400-450nm, 450-500nm, 500-550nm, 550-600nm, 600-650nm, 650-700nm, 700-750nm, 750-800nm.

All experiment data is saved to a USB drive and can be analysed after the experiment(s).

N.B. Liquid control, oxygen measurements or fluorescence measurements can only be utilised if these modules are connected to the device (refer to the relevant sections of the user manual).

3. Bioreactor Interface

3.1. The Front View

Figure 1A: The front view of the bioreactor with the key interface points labelled.

3.2. The Back View

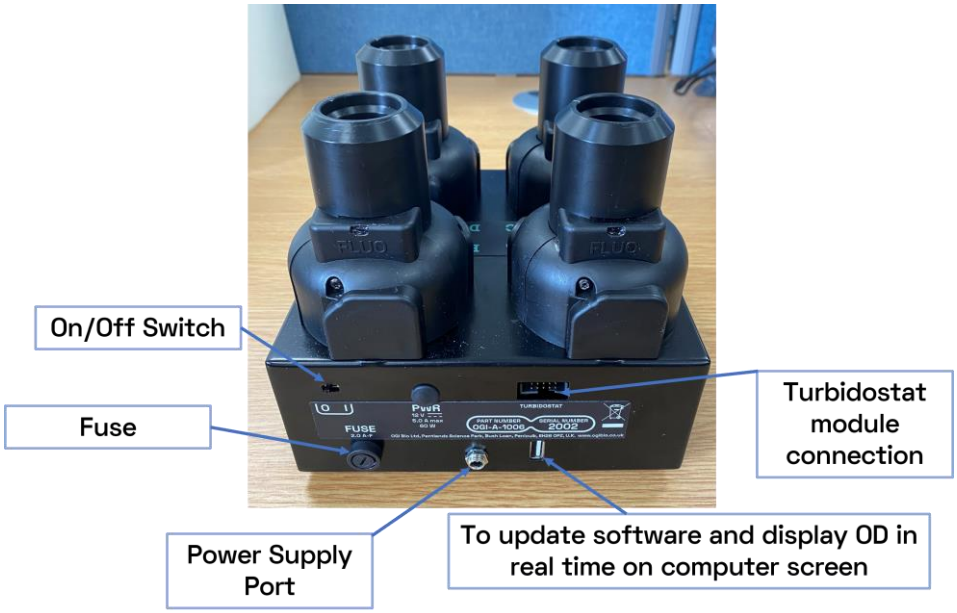

Figure 1B: The rear view of the bioreactor

4. Handling the device

4.1. Connections on the device and turning it on/off

On the rear panel:

- On/off switch.
- Fuse.
- Power supply port
- Connection port for Liquid Control module.
- Connection port to update software and to display OD readings on computer screen

4.2. Supplied Culture Flasks

The flasks are clean but not sterile and can be autoclaved. Please see Section 4.6. for recommendations on cleaning/sterilizing the flasks and other parts of the bioreactor.

4.3. USB drive

Your data will be saved to the USB drive and can be analysed when removed from the device.

The experiment can be carried out without the USB drive in place, but then the data will not be stored. To run the device with no USB drive, select 'Yes' when prompted using the twist/push button (an error message will show up on the screen when there is no USB drive in place, and you will be asked if you want to carry on).

4.4. Warming up

It is required to place the bioreactor at the operating temperature 2-3 hours prior to starting the experiment to heat up or cool down the electronics and ensure accurate measurements.

4.5. Culture volume

The operating volume in each flask is 15 mL, this must be ensured as below this volume the OD readings will not be reliable. Each flask can support up to 20 ml if necessary. **Note that if used, the Liquid Control module will maintain the culture volume at 15 ml.**

4.6. Cleaning the device

The device can be wiped with a damp cloth for regular cleaning.
If small amount of liquid culture spills into flask holders, other parts of the reactors, or the base of the device these should be wiped down with a disinfectant, while keeping in mind that the bioreactor is made of metal and hence may be sensitive to corrosive chemicals. Contact us at help@ogibio.co.uk if severe spillage occurs as this may affect functioning of the device.

To clean and sterilise contaminated caps, bungs and flasks (apart from flasks with oxygen spots) wash them and autoclave using your standard cleaning/sterilisation protocols. OGI Bio's protocols are available on request.

The tubing for the Liquid Control module can be cleaned with 70% ethanol. The tubing can be autoclaved if necessary, however should be replaced after no more than 5 autoclave cycles.

Flasks with oxygen spots can be sterilised with 70% ethanol. To preserve the performance and lifetime of the sensor spots, do not leave ethanol in the flask for more than 5 minutes. After cleaning with ethanol, let the flask dry completely and wait 24h before using it again.
If necessary, flasks with oxygen spots may be autoclaved a limited number of times. However, the exact safe number of autoclave cycles is unknown and therefore we do not recommend that these flasks are cleaned this way. If the flasks are autoclaved they should be left for at least one week and the system should be recalibrated after the 7-day resting period prior to use.

5. First use

5.1. Using the menu

Rotate the twist/push button to move between options. Press the twist/push button to select the option you require. When in a particular menu, the “Return” option will bring you up one level.

6. Calibration and Pre-sets

6.1. OD Calibration

It is important to calibrate the device at the expected experiment temperature, otherwise OD readings may not be accurate. See also Section 4.4. Warming up

The bioreactor measures scattering of microbial cells in a similar manner as a spectrophotometer, but at a different angle (designed to extend the linear range). This is why calibration is required.
The calibration will be influences by the same variables that influence OD readings: (1) scatterer size and shape, (2) index of refraction of the scatterer, (3) index of refraction of the media, (4) the exact optical path. If these parameters used during the calibration are the same as during the experiment, the OD reading in the bioreactor will be the same as in the spectrophotometer against which it is calibrated. If this is not the case, one of these 4 have changed between the calibration and the experiment.

Given that the exact optical path influences calibration, to get the most accurate calibration, one should calibrate individual flaks, in each bioreactor and for the given media and microbe (the scatterer). Changes between flask and bioreactors will be small (see Figure 2), thus, we recommend that you start with calibrating a full set of 4 flasks, one flask per reactor.
Changing the scatter, for example using a different microbe, or using the same microbe but grown in different conditions, will influence the calibration. The extent of change will depend on the extend of change in size, shape and index of refraction of the microbe (see Figure 2). Ideally, the culture you use for the calibration should

reflect the conditions that you will be interested in for your experiments. For example, if you are especially interested in measuring ODs of cells in the exponential phase of growth, then the culture for the OD calibration should also be grown to exponential phase and immediately used to calibrate the device.

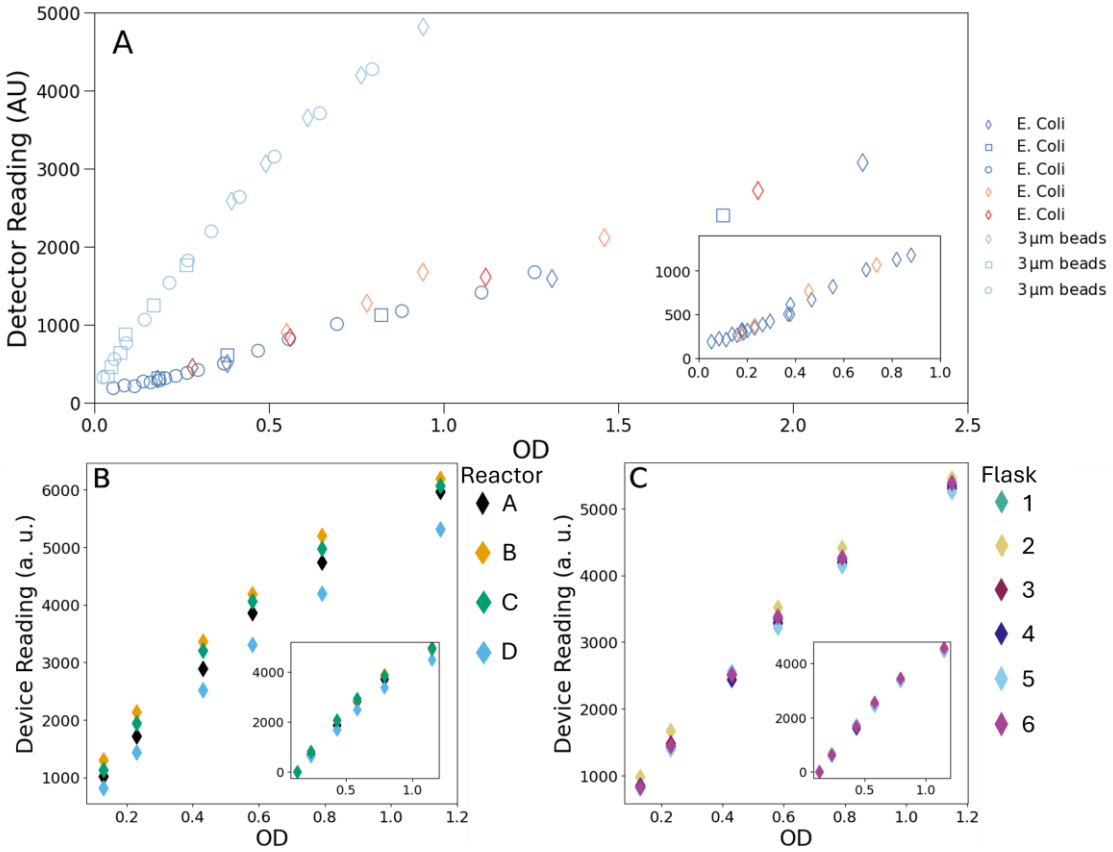

Figure 2: (A) One bioreactor holder was calibrated using the same flask (oriented in the same way) with different scatterers: in dark blue - K12 MG1655 E. coli strain grown to OD=0.3 on three different days, in orange and red – same strain grown in LB to early stationary phase (two different days). Using the same scatterer gives the same calibration. Cells grown to OD=0.3 and early stationary phase are slightly different in size, so there is a small change in the calibration. Using 3 µm in diameter plastic beads (index of refraction ~1.45 as opposed to estimated E. coli index of refraction of 1.38) gives different calibration curves. (B) and (C) show differences in calibration between different bioreactors calibrated with the same flask (B) and same bioreactor calibrated with different flasks (C). These differences are small and mostly due to changes in the offset. Inset shows the calibration curves normalised by the initial offset, highlighting differences in the slope only, which is the only parameter that is used for converting the bioreactor scattering reading to OD.

You will need 15ml of culture per flask to begin the calibration and have on hand some plain media for the subsequent dilutions. The amount of media required will depend on the OD values you want to use for the calibration.

How do I choose what culture density to use for the calibration? The OD of the initial 15ml of culture should be on the high end of the range that you are interested in for your experiments. For example, if you are especially interested in accurate OD readings in the range 0 to 1, then you should start your calibration with 15ml of culture at OD 1 in each flask, and then dilute it down for subsequent measurements. The maximum linear range of the bioreactor is OD~4.5 for K12 *Escherichia coli* cells.

N.B. The linearity range of the measurements may differ for different types of cultures. If, after checking the results of the calibration (.e.g by plotting them), you experience loss of linearity at the high OD end of the calibration curve, please redo the calibration with a smaller range of ODs to improve the quality of the calibration.

1. Insert the flasks with your initial 15ml of culture in the device. You will be calibrating each flask in a particular reactor. The position of the flasks should not be swapped during the calibration. If you take the flasks out of the reactors to extract samples, you should put them back in the same reactor and with the same orientation.
2. Go to the 'Calibration' menu and select 'Calibrate OD'.
3. You will be instructed to input the date and memory location - where you want to save the calibration data. If you select a memory slot that already contains data, you will be asked if you want to overwrite this data. Selecting 'Yes' also means previous data will be lost.
4. You will be asked to input a 16-character name to identify the calibration dataset. Use the twist button to highlight the first character of the name and then press the button to select. Repeat the process to input the other characters. The process will stop when you have selected the last (16^{th}) character. (Underscores ("_") can be used to fill the 16-character quota). We recommend the name contains the information on the flask, reactor and the scatterer used for the calibration.
5. After the LEDs warm up you will be presented with options: Finish/A/B/C/D.

6. Select the flask (A, B, C or D) you would like to take a reading from.
7. When the measurement is finished, extract a sample, measure the OD in a spectrophotometer and record this value.
8. Dilute the culture by adding the appropriate amount of media to it and repeat steps 7 and 8. Keep in mind that there must be at least 15ml of culture in the flasks when the device takes a measurement.
9. You may take up to 6 measurements per flask, but you don't have to take any if you don't intend to use a particular flask.
10. Once you press 'Finish', you'll be asked to enter the ODs corresponding to all the measurements made. For each measurement, the LCD will display the photodiode reading in parentheses.
11. The device will perform a linear fit to the obtained data set (ODs measured in the spectrophotometer versus values obtained by the bioreactor) and save the resulting fit parameters to memory. The measured values and fit parameters will be saved in a calibration file in the USB drive. The relevant parameter is the slope of the fit, which you can view in Calibration > View Calib. Vals. Use the twist button to scroll through saved calibrations and push the button to select the calibration you wish to view.

6.2. Oxygen Calibration (if oxygen module connected)

1. **Pre-warm the bioreactor.** Section 4.4. Warming up.
2. **Prepare your calibration solutions:**
   - **100% oxygen sample.** You will need approximately 10 ml of liquid per flask. For best results, prepare your calibration media in advance and pre-equilibrate it as follows: put the media in a clean bottle or flask, loosen the cap to allow for gas exchange with the environment and let the media, pre-warmed to the experimental temperature, equilibrate with air for 1-2h, by shaking at moderate speed. If you pre-warmed the media in a bottle or conical flask rather than in the bioreactor flask that you will use for the calibration, it is also advisable to pre-warm the bioreactor tubes before transferring the solution into those.
   - **Prepare your 0% oxygen solution.** You will need at least 10 ml of calibration solution per flask. You can either use commercially available 0% oxygen

solutions/tablets to manufactures instructions or deplete the oxygen in the media by growing cells in sealed flasks. The 0% oxygen solution should be pre-warmed to the working temperature before starting.

3. **Transfer solution in bioreactor flasks.** Once the 100% solution is warmed up and equilibrated, it can be transferred into flasks with oxygen sensor spots, which should then be inserted into the reactors. Using the notch in the flask holder to align the oxygen spots with the oxygen detector as shown in Figure 3. The caps should be loose for the 100% oxygen calibration.

Figure 3: Correct alignment of oxygen sensor spot – Spot to notch in flask holder

4. **Start calibration.** In the 'Calibration' menu, select 'Calibrate Oxygen' and enter the date. You will be prompted to choose a slot. If the slot is empty (i.e. the LCD displays 'Factory' or 'No Data' next to the slot number) or you do not want to keep any existing data, then select 'Overwrite' and continue. If the slot has existing calibration data you would like to keep then select 'Update' and continue. You can use this option if you want to recalibrate only one step (100% or 0%) and keep the previous data for the other step, or if you would like to recalibrate only one reactor. The pre-existing data for the flasks or steps that you don't want to recalibrate will be kept. The device will scan for sensors and

indicate which reactors have the sensing electronics for dissolved oxygen. **N.B. The calibration does not need to be performed in a single step, but you need both a 100% and a 0% calibration point before running accurate experiments.**

5. **Start calibration step.** Select 'Calibrate 100%' > 'Yes'. If the USB drive is not already in the device, you will be prompted to insert one now. Then, you will be asked to make sure that the flasks with the calibration solution are in place and to press the button to start the calibration. At this point, the device will check the alignment of the sensor spots and you will be prompted to either adjust the alignment of misaligned sensors or disable individual oxygen modules. If you want to skip the 100% calibration step and proceed to the 0% calibration, you can select 'Calibrate 100% ' > 'No'.
6. **Wait until calibration is done.** The bioreactor will now stir the solution and take oxygen readings every 15s until the measurements become stable. This can take up to 1h but will complete more quickly if your media is pre-equilibrated. The LCD will display letters ('A', 'B', 'C', 'D') for the flasks that have already been completed. Once the calibration is complete, you will be asked if you want to save this calibration in the memory of the device. At this point, you can take the USB drive out and check the data saved in the file with suffix 'OH'. If the values settle (usually at a value around 20 for the 100% solution and around 54 for the 0%) calibration is successful, and you re-insert the USB and select 'Yes'.
7. **Proceed to 0% calibration.** Discard the 100% solution and transfer the 0% solution in the same set of flasks with oxygen spots used before. Make sure that the caps are fully sealed.
8. Repeat steps 5 and 6 for the 0% calibration step.

6.3. Pre-set Settings

It is possible to save settings to memory for convenient loading in an experiment. When you enter the 'Settings' menu, pre-sets will show on the screen with details of the settings scrolling on the screen. If you select a saved setting, you will be asked if you want to overwrite the settings in this location. If you want to save a new group of settings in a new location, use the twist/push button to highlight a space labelled 'Empty' and push the twist/push button to input values for 'New Settings' in this data space.

1. Experiment Length
    - Use the twist/push button to change the experiment length then push the button to select the experiment length you require.
    - The minimum period of testing is 1 hour however the experiment can be stopped during a test by selecting 'Stop Experiment'.
    - Default experiment length is 96 hours.
2. Sampling Rate
    - Use the twist button to change the sampling rate then push the button to select the sampling rate you require.
    - This is the period after which the motors stop spinning and an OD measurement is taken.
    - The minimum sampling rate to ensure correct fluorescence measurements is 3 minutes.
    - Default sample rate is 5 minutes.
3. Stirring speed
    - Use the twist button to change the stirring speed and push the button to select the speed you require.
    - This is the speed at which the motors spin.
    - Stirring speed is in rpm and can be changed by 100 RPM.
    - The minimum speed is 0 RPM and the maximum is 6520 RPM.
    - Default stirring speed is 4000 RPM.
    - If you select 0 RPM, the motors will be off for most of the time during the experiment. However, they will still turn on for a short time to gently resuspend the cells before each measurement to avoid sedimentation.

Each group of saved settings can then be used in an experiment. Otherwise, new settings can be selected in an experiment setup. Experiment length, stirring speed and sampling rate can all be changed during an experiment in the experiment menu by choosing 'Update Settings'.

7. Setting up an experiment

7.1. Starting an experiment

See Section 4.4. Warming up for instructions for setting up the device.

1. In the main menu, select from 'Batch Culture', 'Turbidostat' or 'Chemostat' test when they are displayed on the screen by pressing the twist/push button.
2. Select 'Start Experiment'.
3. After attached modules are detected, you'll be faced with the 'startup' menu where you can select the various experimental settings (such as stir speed, sample rate etc) plus relevant metadata (date, ambient temperature etc.)
    a. The date will be used as the title of the documents saved on the USB drive for that experiment.
4. You must select the OD calibration that you wish to use for the experiment.
5. You can load basic settings by selecting Load Settings > Basic Settings. If you wish to use a preset then press the twist/push button to select it. If you wish to choose new settings then select the 'New settings' option.
    a. Refer to Section 6.5. Pre-set Settings to explore setting options.
6. If starting a liquid control experiment, you will be asked for specific settings pertaining to the type of control, e.g., target OD. Refer to Section 8. Liquid Control Module for further information on the available settings.
7. If oxygen modules are connected, the screen will display which reactors have oxygen modules and you will be asked if you wish to measure oxygen in the experiment. Use the twist/push button to select. If you select 'Yes', you will be prompted to select a calibration and to input the salinity of the culture media. The salinity values will be used to calculate the oxygen content in µmol/l. The oxygen percentage and mbar are not affected by the salinity value. Before the experiment starts, the spot alignment will be checked. If the alignment is poor, you'll be asked to realign them. If you wish to deactivate oxygen measurements for a specific reactor, use the twist/push button to select 'Disable'.
8. If fluorescence modules are connected, you will be asked if you wish to measure fluorescence in the experiment. Use the twist/push button to select.

a. Additional fluorescence settings can be found in the 'Fluo. Settings' section of the startup menu. These are described in Section 10.3 Using the fluorescence module.

9. The 'Triggers' option allows you to automatically change the stirring speed when a given length of time has passed and/or when the OD in a flask reaches the desired value. You can set up to 10 triggers for an experiment. The Settings file for the experiment will contain timestamps indicating the activation of the triggers.
10. If you want to normalise your measurements against plain media, select Yes under 'Blanking needed?' (If you use a very high dilution for your culture then blanking will not be necessary – the first measurement taken of your cell culture will be used to normalise subsequent measurements. Otherwise, you will need to blank your test.)
    a. When the experiment starts, you will be instructed to insert a flask with your blank sample into reactor A (15-20 ml). Then a measurement will be taken. Then you will be asked to repeat the process for the remaining reactors. When the blanking process is complete, place your culture flasks into the reactor and press the button to start the experiment.
11. When you are ready to proceed, select 'Start'. You'll be prompted for any further steps such as blanking or oxygen spot alignment. When finished, the screen will say 'Setup complete, press to continue'. Press the button and you are now finished setting up the experiment.
12. During the experiment a new menu will be present. Here you can scroll through 'Stop Experiment', 'Pause Experiment', the remaining experiment time, a display of the most recent measurements from each reactor, and an option to update experiment settings.
13. The 'Stop Experiment' option simply allows you to stop the experiment, and will bring you to the main menu.
14. It is recommended not to remove flasks from the flask holders during an experiment unless it is paused. You may pause the experiment by scrolling to and selecting 'Pause Experiment' in the menu. If removing the flask, be sure to replace it with the same orientation. Press 'Resume' to continue.

15. Twist the twist/push button to display the remaining experiment time and measurements for each flask. The measurement type, e.g. O2, can be changed by pushing the twist/push button on each flask.

7.2. Pause Experiment

If you wish to pause your experiment, twist the twist/push button until it displays 'Pause Experiment' and then press the button. The screen will then display 'Resume Experiment'; press the button to resume.

- The time that the experiment was paused will be saved to the setting file, as will the time the experiment was restarted.
- While the experiment is paused you will not be able to change any other settings until you resume the experiment.
- You can remove the USB drive whilst the experiment is paused if you wish to access the data. If the USB drive is not put back in before the experiment is restarted, you will be asked if you want to continue without the USB drive or to put the USB drive back in.

7.3. Stopping an experiment

The device will run until the experiment length has elapsed or the experiment is stopped by the user.

- During an experiment, the screen will display 'Stop Experiment' unless the device is undergoing a measurement.
- Press the twist/push button when the screen shows this display to stop the experiment.
- If you select this option in error then select 'No' when the screen displays 'Are you sure?'. Otherwise, select 'Yes' and the experiment will stop.

7.4. Changing settings during an experiment

During an experiment, settings can be updated while in the experiment menu.

- Use the twist/push button to scroll until the screen displays 'Update Settings'.
- Then use the twist/push button to make changes to the appropriate settings.

7.5. Data format and handling

The first OD measurement taken of the sample in each experiment will be used to normalise subsequent measurements, i.e. the initial measurement will be subtracted from the subsequent measurements for that reactor. If you have chosen to normalise your samples against plain media, the plain media measurement will be subtracted from subsequent measurements. All times in these files are in hours.

- The data recorded during the experiment will be saved to the USB drive.
- Documents with suffix 'A' is the analysis file. This contains the time each measurement was taken at and the corresponding OD.
- Documents with suffix 'S' record the settings & calibration used for the experiment. They will also record times of pauses, any settings that were changed and other relevant information about any other modules activated in the experiment.
- Documents with suffix 'C' are generated when an OD calibration is performed. These documents contain:
  - The name of the calibration set.
  - The OD values of your samples and the measurements taken during the calibration process.
  - The slope, offset and R^2 value of the calibration curves.

Accessory Modules

8. Liquid Control Module (P/N OGI-A-1007)

The liquid control module is a separate box upon which the base bioreactor sits during function. It can be used to hold continuous cultures at a set target OD from either a high dilution or a pre-grown culture as a turbidostat, or to run an experiment in chemostat conditions by specifying a continual flow rate of media. **Note that although a batch culture experiment volume may range from 15-20 ml, a Liquid Control experiment is by default limited to 15 ml. Contact us at info@ogibio.co.uk if alternate volumes are required.**

8.1. Connecting the liquid control module

Ensure that the liquid control module is connected to the main device before switching on the device.

The liquid control module comes as a separate enclosure to the main device with a connection cable on the back.

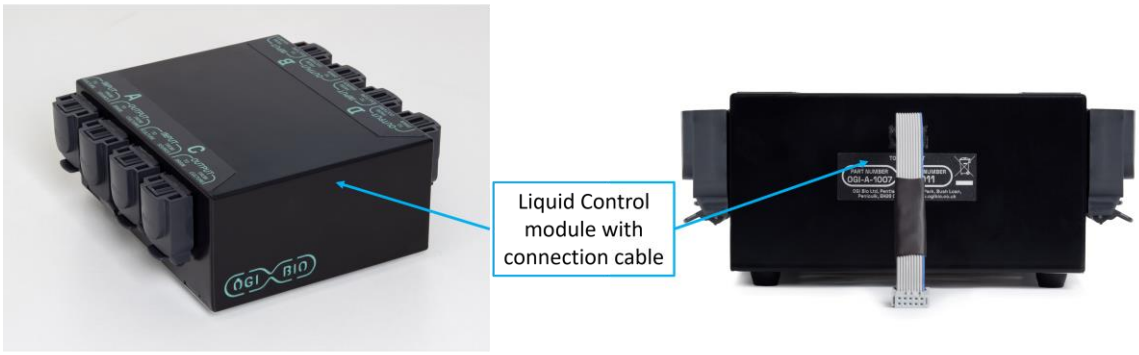

Figure 4: Liquid control Module

The bioreactor should be placed on top of the liquid control module, and the connection cable inserted into the connection port at the rear of the device.

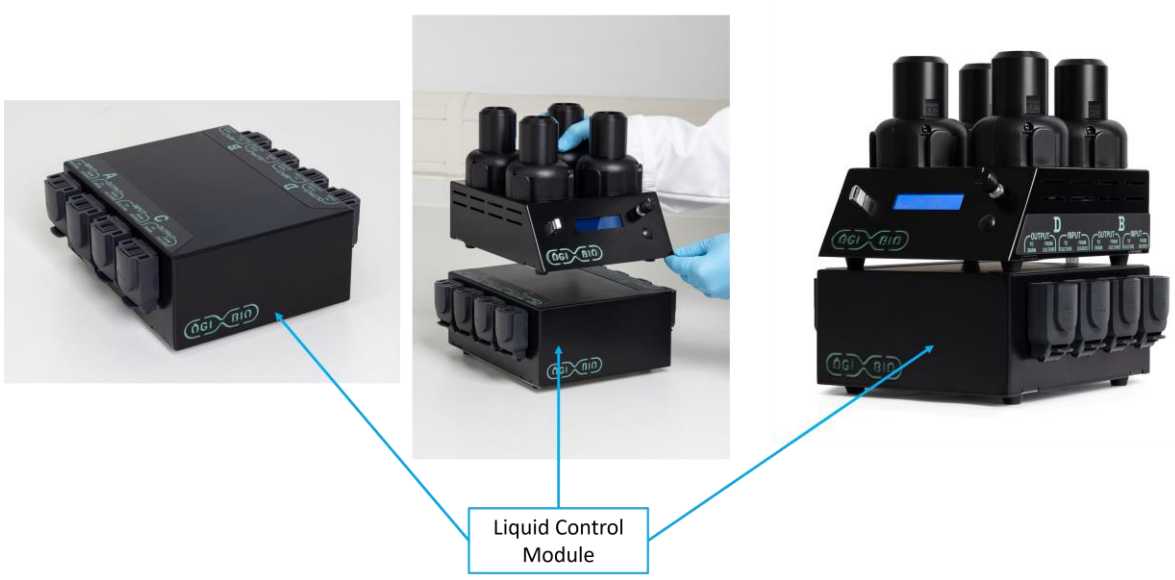

Figure 5: Liquid control module in position and connected to bioreactor

8.2. Fitting the tubing

The device will be delivered without tubing in the pumps. Follow the guide below to put tubing into the pumps.

1. The flap on the pump cover should be sitting parallel to the bench in the locked position (figure 6). The flaps **must** be in the locked position during a liquid-controlled experiment.

Figure 6: Pump cover flap in the locked position

Figure 7: Pump cover flap in the open position

Figure 8: Line up latch and groove to reconnect pump cover

2. To remove the pump cover, press down on the flap until it is at 90 degrees to the bench so it is in the open position (figure 7). The pump cover can then be removed from the pump so that the tubing can be put in place in the pump cover.

3. Each pump should have 2 tubing clips (highlighted in figure 9, left). The clips are slotted into place in the pump cover with the opening facing up. The tubing should be pressed into the right-hand clip (figure 9, left) and wrapped around the tubing guide (figure 9, middle) and then the second clip slotted in place (figure 9, right). These clips are necessary for correct pump functioning. Position the tubing so that it can connect without stretching to the appropriate flasks.

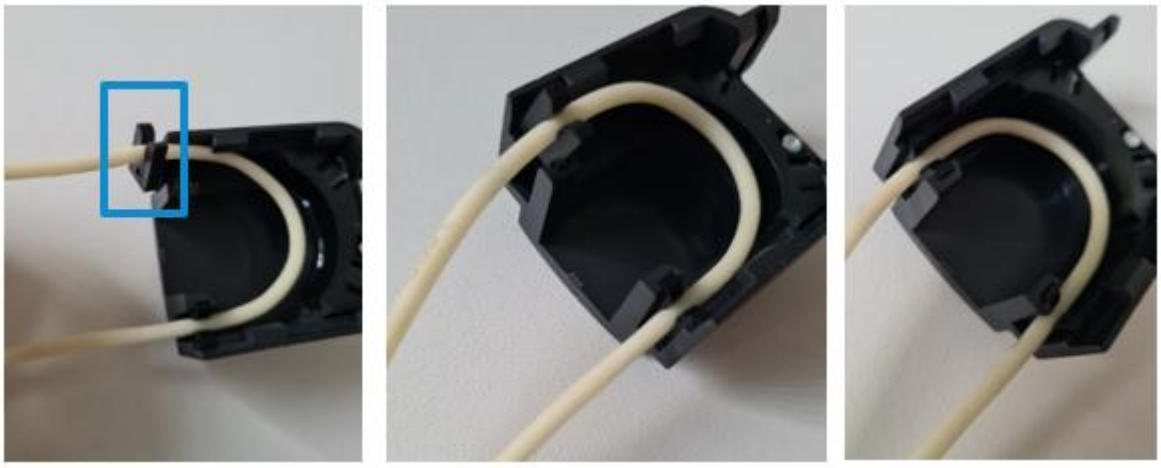

Figure 9: The tubing fits into the pump tube clips which then attach to the pump cover

4. To put the pump cover back on, there are 2 latches that should be slotted into the grooves as shown in figure 8 and then the flap should be returned to the locked position (figure 6). Push the cover towards the pump if the movement is stiff.

5. If your tubing begins to have a notable difference in texture, becomes more stiff or fragile, it is recommended to replace the tubing.

8.3. Cleaning the pump tubing

The default method for cleaning the tubing is with 70% ethanol and 18 MΩ-cm water for rinsing. The tubing can be autoclaved if necessary, however should be replaced after no more than 5 autoclave cycles. The cleaning process utilizes the function of the pumps themselves to propagate the cleaning fluid through the length of the

tubing. An alternative would be to remove the tubing from the pumps and flush through the cleaning media by hand.

- During the cleaning process, you will have control over how long the cleaning fluid is inside the tubing itself.
- It is recommended to clean the pumps before and after a liquid-controlled experiment has been performed.

N.B. If a liquid-controlled experiment is carried out for a period of several days then note that the risk of biofilm formation is increased. This also depends on the type of microbe that is being used for the test. Biofilms can affect the function of the pumps and hence the success of the liquid control process. If biofilms are noticed it is recommended to use a fresh set of tubing for subsequent tests.

8.4. **Liquid Control** pump cleaning process

1. Make up a flask with around 5-10 ml of 70% ethanol. Make up another flask with around 10 ml of distilled water for flushing the pump tubing. (These volumes are recommended for the default tubing length with the minimum period of cleaning recommended – you will require larger volumes for increased cleaning times). Use another empty flask to collect the used fluid.
2. Disconnect the tubing from the needles.
3. Use the main menu to select 'Pump Cleaning'.
4. Then select 'Start Cleaning'.
5. The screen will show 'Press button to clean pumps'.
6. Put appropriate tubing into the cleaning fluid:
7. Refer to figure 14 for the below.
    a. Put tubing that connects 'From culture flask removal needle' and 'From source flask' into a flask of cleaning fluid (i.e. 70% ethanol).
    b. Put tubing that connects 'To culture flask addition needle' and 'To drain flask' into an empty flask.
    c. Once you have finished this setup, press the button and the pumps will be turn on and propagate the liquid through the pumps.
    d. The screen will show 'Press button to stop pumping'.

e. Once you feel that you have pumped the cleaning fluid through the tubing for long enough (at default tubing length we recommend a minimum of 30s but optimize for your microbe type), then press the button and the pumps will turn off.
f. The screen will show 'Press button to flush pumps'.
g. Then you should swap your cleaning fluid for flushing fluid (distilled water)
h. Put tubing connected 'From culture flask removal needle' and 'From source flask' into a flask of flushing fluid.
i. Leave the other tubing in empty flask.
j. Once you have finished this setup, press the button and the pumps will be turn on and propagate the flushing liquid through the pumps.
k. The screen will show 'Remove tubing + press button',
l. Once you feel that you have pumped the flushing fluid through the tubing for long enough (at default tubing length we recommend a minimum of 60s – double the cleaning time), then press the button.
m. The pumps will continue to run while all of the flushing fluid is removed from the tubing and the screen will show 'Press button to stop pumping'
n. Once you can see that there is no more fluid coming out of the output tubing into the collection flask, press the button and the pumps will turn off.
o. You will then be notified that the cleaning process is complete, and the system will return to the main menu.

8. Bungs should be flushed with water soon post-experiment using a small piece of tubing attached to a syringe to avoid blockage. They may be sterilised via autoclave.

N.B. When using brand new tubing, it is recommended that you increase the recommended cleaning times by at least 5 seconds, note that this will require additional cleaning fluid.

8.5. Turbidostat Media Usage Prediction

When setting up a liquid-controlled experiment 500 ml bottles should be setup and your fresh media added. In the Turbidostat menu there is a tool for predicting how long it will take for this media volume to be depleted due to dilution of your cultures.

1. Input the values for volume of media used in the setup, target OD, doubling time of your culture, culture volume, stock OD, dilution of this stock culture and lag time.
2. This will then display an approximate time period in which it is expected that your media will run out for this specific experiment.
3. This will display on the screen for 5 seconds before going back to the Turbidostat menu.
4. This value can then be used as a guide as to when the experiment must be stopped or fresh media must be added to the setup. If any of these variables are unknown it is recommended to monitor your first experiment to check the time for use of media.

N.B. the media usage for a Chemostat experiment is a simple function of flow rate and planned experimental time.

Figure 10: Base bioreactor with a Liquid Control Model fully connected to Reactor A

8.6. Preparing for a liquid-controlled experiment

Before doing a turbidostat test it is recommended to clean the pump tubing. Look at Section 8.3. Cleaning the Pump Tubing for further information.

1. Set out your source flasks and drain flasks (figure 11) and culture flasks (figure 12) for each reactor that you wish to use. 500 ml standard laboratory flasks should be used for the source and drain flasks. The flasks provided should be used as the culture flasks.

Figure 11: Source/Drain flask with bung

Figure 12: Culture flask with Bung. S – Addition from source. D – Removal to drain. Centre hole is a vent.

2. Use volumes of media that are suitable based on cell growth rates and desired experiment length – refer to Section 8.5. Turbidostat Media Usage Prediction for information.
3. Put your 15 ml cell cultures into the reactors you wish to use during the experiment.
    a. The fixed needles in the culture flask bungs should give a maintained culture volume of 15 ml (slight variation may occur due to manufacture).
4. Connect your pump tubing to the appropriate part of the system.

a. Refer to Section 8.2. Fitting the tubing for information on putting the tubing into the pumps.
b. N.B. when connecting your tubing/needles, first wipe with ethanol to remove any contamination on the tubing/needle openings.
c. Connect the correct pumps to the correct reactors according to the labelling on the device.
d. The source flask bungs each have a long needle (figure 11) and the drain flask bungs each have a short needle (figure 12).
e. The culture flasks each have a long needle (removal needle) and a short needle (addition needle) (figure 13).
f. Connect the tubing from the source flask bung needle to the culture flask addition needle and from the culture flask removal needle to drain flasks according to the diagram in figure 14. Refer to bioreactor labelling for input and output pumps.
g. Do this for all reactors that you are using.

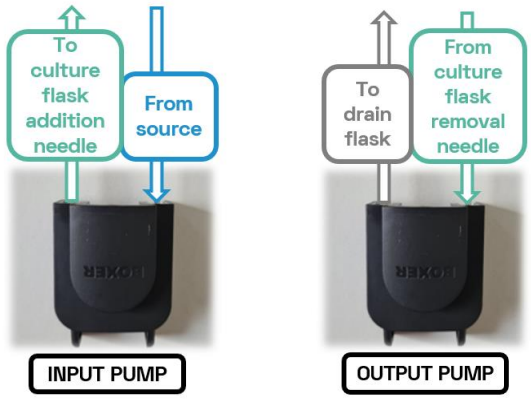

Figure 14: Input and output pump configuration showing tubing connections

8.7. Starting a turbidostat experiment

Having completed the steps in Section 8.6. Preparing for a liquid-controlled experiment above, follow the steps as set out in Section 7.1. Starting an experiment. Specific options for the turbidostat are further explained below.

- 'Maintain ODs' will keep the cultures at the current OD. When you press Start, you'll be asked to 'blank' the reactors with plain media and the current ODs will be measured and displayed on screen. The process is

described further in Section 8.10. Maintain ODs. (Note that this option is incompatible with manually setting a target OD.)

- 'All' lets you set a target OD for all four flasks.
- Individual reactors can be turned on/off or have unique target ODs.

'Pump tube length' is important for drawing the correct amount of liquid into the tubes – if you ever cut the tubes, be sure to adjust this value.

8.8. During a turbidostat experiment

The first time that the pumps are turned on, they will go through the 'Priming process'. This turns the pumps on for an appropriate length of time to prime the tubing. Once this is complete the dilution process will begin. This will be displayed on the LCD screen and no action is required.

N.B. The experiment cannot be stopped during a dilution period, measurement or priming period (all displayed on LCD screen during process).

- Twist the twist/push button to display the remaining experiment time; the most recent OD and O2 values, if available, for each reactor between measurements; to update settings; and turn on and off turbidostat function in reactors.
- If you wish to take culture out of the culture flask during the experiment, it is recommended to do this while the experiment is paused. Do not remove the flask from the holder during a measurement (the LCD screen displays 'Measuring'), or dilution ('OD>Target') as this may affect readings.
- Currently our devices have a minimum working volume of 15 ml, therefore we do not recommend that more than 1 ml is removed from any flask during a single measurement cycle. Note that this may affect the OD as the system will not remove media for the next couple of pumping cycles.

N.B. If one of the reactors runs out of fresh media during a turbidostat run, the system will detect that it is not diluting and will turn off this reactor. An error note will be saved in the settings file. If you then top up the media, the reactor will still remain off (no dilution) so if you require to use more than 500 ml of media in one experiment, ensure you swap in fresh media before the system has run out and switched off dilution. If turbidostat function on a reactor has been turned off

during an experiment, it can be reactivated using the twist/push button and selecting 'Turb control'.

8.9. Setting the pump tubing length

If you are using the default tubing length (85 cm as supplied), you do not need to change the tubing length in the startup menu (this is the tubing length that is sent with the device).

If you want to change the tubing length due to your own setup then this should be input to the system using the startup menu. **(All tubing should be the same length)**.

It is recommended that tubing is left long enough so that there are no restrictive bends in the tubing that would impede fluid flow.

8.10. Maintain ODs

The 'Maintain ODs' option is used to maintain your culture at the OD at the first reading, (i.e. if cells have been grown in another environment and you wish to maintain the culture in this state).

1. Select 'Maintain ODs' in the Turbidostat section of the startup settings.
2. You will be prompted to blank the flasks before the setup is complete. The blanking process will automatically start when the LEDs have initialised. You will be instructed to insert flasks for blanking into all 4 reactors.
3. After blanking, you will be instructed to put your cultures into the reactors. Press the button to determine target ODs for your experiment.
4. Once the system has taken the first measurements, (i.e. those which will be used as the target ODs), they will be displayed on the screen.
5. You will be instructed to press the button to accept each target OD and begin the turbidostat experiment. If the ODs are reading below 0.1 then it is recommended to stop the experiment and use different cultures.
6. Do not use a target OD of 0.

7. The OD read will be rounded to the nearest 0.05 and this will be used for the target OD. (If this has rounded down then the system will dilute because the reading is slightly higher than the target).
8. This will then be saved in the settings file.
9. If you change the target OD in the settings menu during the experiment, it will no longer maintain the OD of the first reading but will apply the target OD of the new selected value.
10. This functionality may only be activated at the start of the experiment.

8.11. Data format and handling

If performing a turbidostat test, then an additional file with suffix 'T' will be generated. This contains the time, OD, whether dilution is initiated or not, error from target OD and dilution time.

N.B. If you want to remove the USB drive during an experiment to view your files, first pause the experiment and then remove the USB.

8.12. Chemostat Functionality

The Liquid Control module may also be used as a Chemostat, wherein the experimental conditions are maintained through continuous pumping of fresh media at a given rate. The setup for Turbidostat and Chemostat functionality is almost identical, with the difference that no target OD is given to the device. Instead, a flow rate is set for each reactor. The flow rate and individual reactor state can be altered mid-experiment just as above with the Turbidostat function.

There is no media consumption estimate for a Chemostat experiment as it is equivalent to the users' desired flow rate multiplied by the duration of the experiment.

9. Oxygen Module (P/N OGI-A-1008)

9.1. Connecting the oxygen module

1. Loosen the screw on the blank cover for the O2 sector on the side of the flask holder.
2. Remove the blank cover and store the cover and screw for future use.Insert the tab at the bottom of the oxygen module into the hole on the bioreactor box and plug in the connector at the base of the module.
3. Move the top part of the module's cover to bring it into contact with the flask holder with a rotatory motion.
4. Secure the module to the flask holder with the previously removed screw.

N.B. Do not overtighten the screw as this will distort the plastic cover.

9.2. Measuring dissolved oxygen in experiments

When you start an experiment, the device will detect which reactors have the electronics to measure dissolved oxygen and if there are any it will ask you if you want to measure oxygen in your experiment. If you select 'Yes', the device will take oxygen measurements before and after the OD readings and save the results in files with suffixes 'O' and 'OE'. At the beginning of the experiment (or after the blanking, if required), the device will check the alignment of the sensor spots and you will be prompted to either adjust the alignment of misaligned sensors or disable individual oxygen modules.

9.3. Data format and handling

- **File with suffix 'OH'.** The 100% oxygen calibration will produce an output file with suffix 'OH'. This file contains the raw readings of phase shift (dphi) taken during the calibration process and a status code to indicate errors. Status code 0 indicates that no errors occurred during the oxygen measurement. At the end of the file you will find the results of the calibration (dphi: phase shift in degrees).
- **File with suffix 'OL'.** The 0% oxygen calibration will produce an output file with suffix 'OL'. This file contains the raw readings of phase shift (dphi) taken during the calibration process and a status code to indicate errors. Status code 0

indicates that no errors occurred during the oxygen measurement. At the end of the file you will find the results of the calibration (dphi: phase shift in degrees).

- **File with suffix 'O'.** This file contains the results of the oxygen measurements taken during your experiment. For each reactor, you will find the dissolved oxygen measurements in µmol/l, mbar and % of air saturation.
- **File with suffix 'OE'.** This file contains the early oxygen measurement. Each measurement has a corresponding timestamp which is different from those in 'O' allowing the oxygen uptake rate of the culture to be calculated.

9.4. Notes

- Flasks with oxygen sensor spots should be stored in the dark.
- It is advisable to always use the same flask in each reactor and recalibrate the oxygen module when you use a different flask.
- Flasks with oxygen spots can be sterilised with 70% ethanol. To preserve the performance and lifetime of the sensor spots, do not leave ethanol in the flask for more than 5 minutes. After cleaning with ethanol, let the flask dry completely and wait 24h before using it again.
- If necessary, flasks with oxygen spots may be autoclaved a limited number of times. However, the exact safe number of autoclave cycles is unknown and therefore we do not recommend that these flasks are cleaned this way. If the flasks are autoclaved they should be left for at least one week and the system should be recalibrated after the 7-day resting period prior to use.

10. Fluorescence Module (P/N OGI-A-1009)

10.1. Connecting the fluorescence module

1. Loosen the screw on the blank cover for the fluorescence sector on the side of the flask holder.
2. Remove the blank cover and store the cover and screw for future use.
3. Insert the tab at the bottom of the fluorescence module into the hole on the bioreactor box and plug in the connector at the base of the module.
4. Move the top part of the module's cover to bring it into contact with the flask holder with a rotatory motion.
5. Secure the module to the reactor with the previously removed screw.

N.B. Do not overtighten the screw as this will distort the plastic cover.

N.B. This module contains a UV LED. This has been tested and assessed in operating mode as Risk Group 0. Please ensure module is firmly secured to the Bioreactor with the fixing screw provided BEFORE turning on the Bioreactor power.

10.2. Description of the fluorescence module

The standard configuration of the fluorescence module is the following:

- Excitation LEDs (peak wavelength): LED 1 = 365nm, LED 2 = 590nm, LED 3 = 470nm.
- Detection : F1 (400-450nm), F2 (450-500nm), F3 (500-550nm), F4 (550-600nm), F5 (600-650nm), F6 (650-700nm), F7 (700-750nm), F8 (750-800nm).

10.3. Using the fluorescence module

When the device is switched on, it will automatically detect if the fluorescence modules are installed in the reactors. If a fluorescence module is detected in at least one reactor, you can measure fluorescence in a batch culture or turbidostat experiment as described in Section 7 Setting up an experiment. At each measurement cycle, measurements will be taken with all combinations of fitted LEDs and available detection bands. The results of these measurements are saved in a file with suffix 'F'.

In the experiment startup menu, you will also find options to change the intensity of the excitation LEDs.

By default, the LEDs are only turned on for a short time for the fluorescence measurement. If you want to keep an LED on for a prolonged period of time (e.g. for optogenetic activation) you can do so by changing the 'LEDx always on' settings in the Fluorescence section of the startup menu or the main menu after the experiment has started.

LEDs in the fluorescence module can be turned on/off and the intensity can be changed at any time during the experiment. The time and type of change will be recorded in the settings file.

LEDs that are set "always on" will be switched off for short periods of time during measurements to avoid interference with other optical readings.

10.4. Data format and handling

File with suffix 'F'. This file contains the results of the fluorescence measurements. For each reactor, you will find measurements taken with every combination of excitation LEDs and detection bands. The column headers in the file contain the relevant information to interpret the data. For example, LED1_F1_A is the light detected from channel F1 in reactor A when the sample is excited with LED 1.

10.5. Notes

- The minimum sampling rate to ensure correct fluorescence measurements is 3 minutes.

Disposal

This product should be sorted for environmental-friendly recycling

Do not dispose of products into household waste!

Only for EU Countries:

According to the European Directive 2012/19/EU on Waste Electrical and Electronic Equipment and its implementation into national law, products that are no longer useable must be collected separately and disposed of in an environmentally friendly manner. At the end of its useful life, the Microbioreactor may be returned to OGI Bio for disposal if required.

Appendix A - Technical Specification

- This device is rated for the power supply provided
  - Power supply input
    - Input voltage: 100-240V AC (+/- 10%)
    - Input current: 1.6-0.7A
    - Input frequency: 50/60Hz
  - Power supply output
    - 12V DC, 5.0A maximum
  - Bioreactor power input
    - 12.0V DC, 5.0A, 60.0W

- Fuse: 2A Fast Blow

- The device is intended for indoor use only.
- Pollution Degree: 2
- Operating temperature: 15 to 40°C
- Relative humidity: 30% - 70%
- Maximum operating altitude: 2000 meters