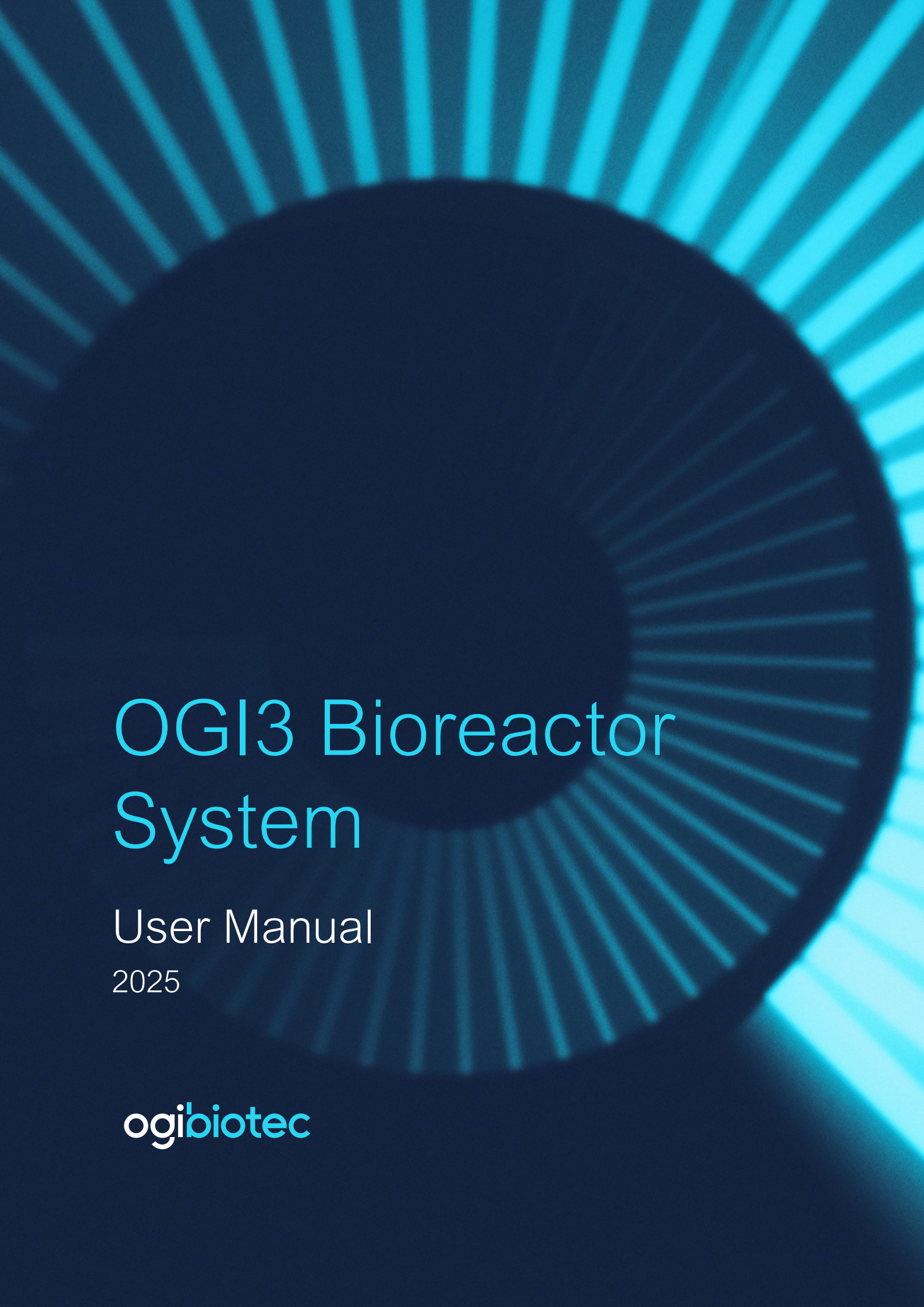


OGI3 Bioreactor System

User Manual

2025

ogibiotec

Contents

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

Device Manufacture

Device is manufactured by:
ŌGI 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.**
- **The Power Supply must be connected to a supply with a protective earth connection.**
- **The optional Fluorescence Measurement module (OGI3-1014) contains a UV LED. This has been tested and assessed in operating mode as Risk Group 0. Please ensure the module is firmly secured to the Bioreactor with the fixing screw provided BEFORE turning on the Bioreactor's power.**
- **Do not unscrew the metal base of the Bioreactor, pH, or sparging modules to open them. If you have any issues with the device, please contact our team at help@ogibiotec.com**
- **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 heating elements have a built in protection for overheating and will pause any in progress experiment if overheating is detected.**

1 Operating the Device

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

Technical specifications can be found in Appendix A.

Using the device outwith the following operating parameters may result in suboptimal performance:

- Environmental operating temperature: 15°C to 60°C (maximum reduced to 40°C if using the Liquid Control Module (P/N OGI3-1011)).
  Note that the maximum culture temperature control setting is 50°C.
- Relative Humidity: 30%-70%.
- Maximum operating altitude: 2000 metres.
- Keep out of direct sunlight or else temperature and optical readings will be unstable.

We do not accept liability for damage to the device incurred by using it outside of the stated certified ranges.

2 Device Description

The ogibiotec OGI3 BioReactor (**P/N OGI3-1010**) is a small-scale bench-top bioreactor primarily designed to automate the culturing of microbes while providing accurate and on-demand culture analytics hands-free. The device comprises four individual and independent reactors. Single culture flasks are held in the central flask holders of each reactor and are equipped with a magnetic stir bar for mixing and oxygenation. The optical density (OD) of each culture is measured at regular intervals throughout the experiment as default. The device has integrated heating elements and can maintain each of the culture flasks at desired temperatures. Silicon bungs are used to close the flask, with plugs provided so that the flask may be closed to the lab environment no matter which modules are installed. Miniature air filters (**KIT-0306**) can be inserted into vent holes on the bung to provide sterile air exchange with the headspace of the flask.

Additional optional accessory modules can be installed to enhance the capabilities to the device:

Wi-Fi Connectivity

OGI3 comes with a Wi-Fi antenna and the ability to connect to a Wi-Fi network in order to retrieve date information for experimental setup. In 2025 this functionality will be extended to setting up, monitoring, and controlling experiments via our online Web App.

Liquid Control Module: OGI3-1011

The Liquid Control module can be used to maintain the cultures at the desired optical densities, or in chemostat mode to provide a constant flow of fresh media into the culture flasks. This module contains 8 pumps for liquid handling: 4 input pumps to add fresh media into the culture flasks and 4 output pumps to remove the excess volume from the culture flask. Bungs containing needles are provided for use with the Liquid Control Module. See Section 8 for more information.

Dissolved Oxygen Module: OGI3-1008

After calibration with reference samples, the Dissolved Oxygen module can be used to measure the amount of dissolved oxygen in the culture media at regular intervals during an experiment. This module requires culture flasks with oxygen sensor spots (**KIT-0301**). See Section 9 for more information.

pH Module: OGI3-1012

The pH module can be used to measure the pH of the culture. The module has 4 pH probes (**KIT-0308**) included, one for each reactor. Used in conjunction with the Liquid Control Module (**OGI-A-1011**) the pH of a culture can be maintained at a set point. See Section 11 for more information.

Sparging Module: OGI3-1013

The Sparging module can be used to bubble air directly into the culture through a sparger. The module contains 4 pumps connected to sparging needles for insertion into each culture. See Section 12 for more information.

Fluorescence Module: OGI3-1014

With our new fluorescence module the fluorescence spectrum of a variety of fluorophores in each of the culture flasks. It is possible to measure the emission spectra from up to 3 different excitations. The excitation wavelengths available as standard are:

Excitation: 365 nm, 470 nm, 590 nm.
Emission: 340 – 780 nm with a sampling resolution of ~ 2 nm.

For the specifications of fluorescence modules bought prior to 2025 please contact us at help@ogibiotec.com.

All experiment data for the OGI3 and any attached modules is saved to a USB drive and can be analysed after the experiment. See Section 10 for more information.

N.B. Any information relating to liquid control, pH, oxygen measurements, fluores-

cence measurements, or sparging can only be utilised if these modules are connected to the device. Refer to the relevant sections of the user manual for further information.

3 Base Unit Overview

3.1 Front Panel

Figure 1: Front view of the OGI3 BioReactor

3.2 Rear Panel

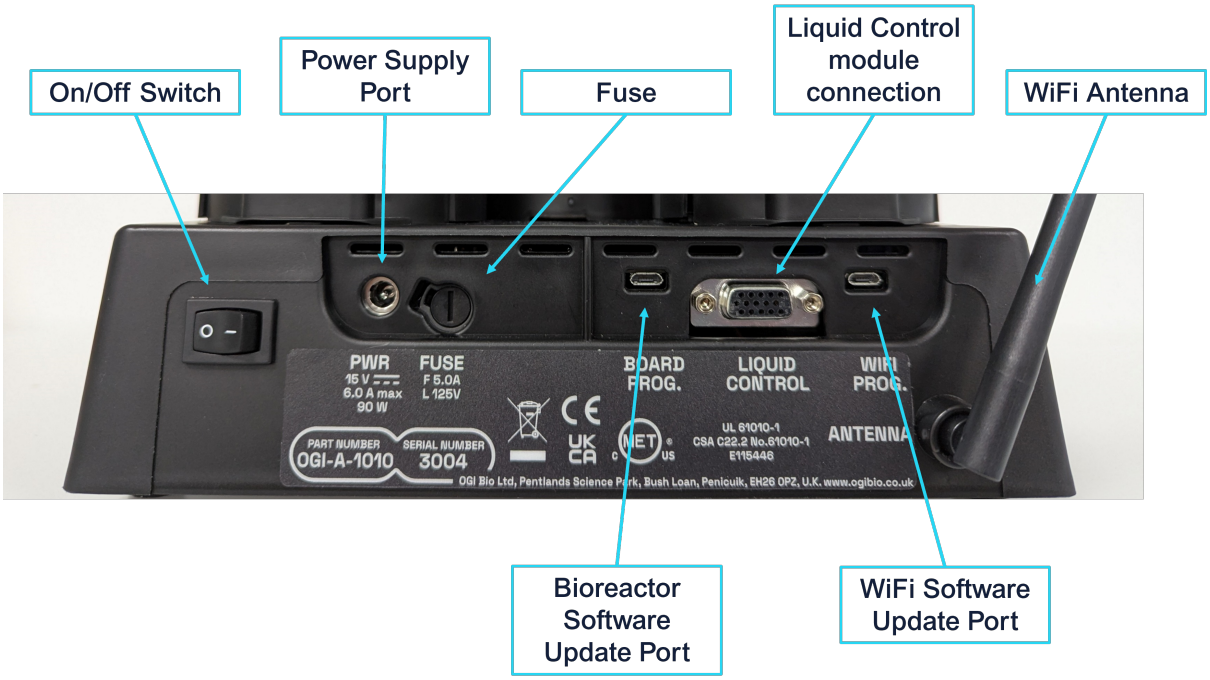


Figure 2: Rear view of the OGI3 BioReactor

On the rear panel of the device:

- On/Off switch
- Fuse
- Power supply port where the power supply is to be connected
- Liquid Control module connector
- Micro USB ports for software updates for both BioReactor and WiFi software packages

⚠ **N.B. Please do not attempt to connect to Wi-Fi Prog. unless to update the device software using one of our update packages. Board Prog. may be used in conjunction with provided script to view ODs live. Incorrect use of these ports could lead to damage to the device.**

3.3 Birds-Eye-View

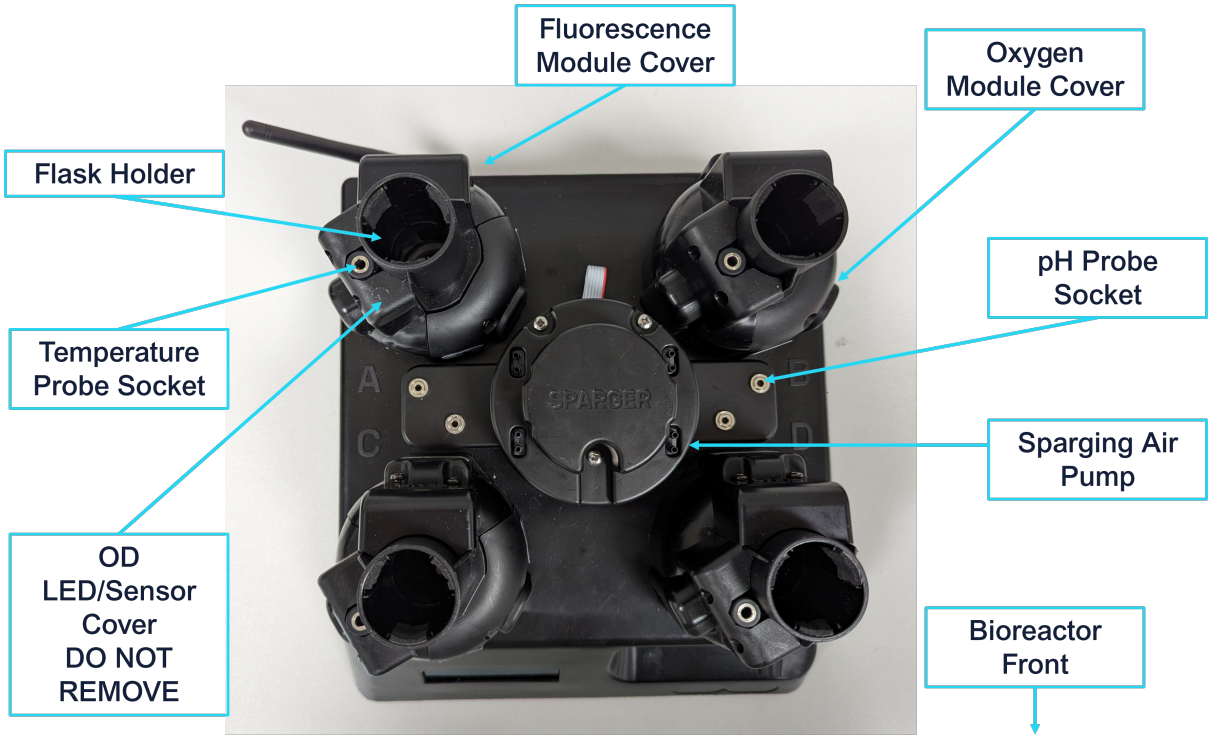


Figure 3: Overview of the complete OGI3 BioReactor system

From above, the locations of key points of interface with the device are highlighted. Note that the modules (Liquid Control, Oxygen, Fluorescence, pH, and Sparging) are optional and may be purchased and added to the OGI3 system as needed, they do not come as standard with the Base Unit. Temperature control is included as standard.

4 Handling the Device

4.1 Powering On

The socket for the power supply cable sits at the back of the OGI3 as shown in Figure 2. Ensure that the USB stick, temperature probes, and any optional modules/probes are connected to the device prior to powering on.

⚠ **N.B Do not attach a module to the OGI3 base unit while it is powered on. Doing so can severely damage the device and module.**

The device is switched on via the On/Off Switch on the rear of the Base Unit (Figure 2).

Upon turning on the device it will automatically detect which modules are installed, and on which reactors they are present. It will display a short message for each module it finds.

The device will then attempt to connect to a network, unless it is your first time powering on, in which case it will tell you it does not have any credentials. In this case, please contact us at help@ogibiotec.com.

When the device has finished booting, you will land on the Main Menu.

4.2 USB Drive

Data is saved to the USB drive as standard and can be analysed when removed from the device. The supplied USB drives are in FAT32 format, if using an alternative USB drive, you must ensure that it is formatted in the same way.

The device can be operated without the USB drive in place. If the USB drive is not in place on device start-up or at the start of an experiment, an error message will show up on the screen and you will be asked if you want to carry out the experiment without the USB. Select 'Yes' or 'No' using the twist/push button. Experiments **will not** save data, and Dissolved Oxygen calibrations **cannot** be started without a USB drive plugged in.

4.3 Culture Volume

The recommended volume to use in each flask is 15 mL. This can be increased to 20 mL if necessary.

⚠ **N.B. Using less than 15 mL will have serious negative effects on the reliability of all device measurements.**

The Liquid Control module will set the volume to 15 mL regardless of starting volume by nature of the height of the drain needle.

4.4 Cleaning the Device and it's Modules

It is recommended to clean contaminated bungs and flasks without oxygen spots via autoclaving. Below are a set of cleaning protocols for the care of your OGI3 BioReactor system:

Bungs

Bungs should be removed from flasks soon after an experiment. Their needles should be flushed and the whole bung autoclaved **after removing the temperature probe from its sleeve**. Plugs and needle covers should be removed from the bung prior to autoclaving, but can be autoclaved **with the exception of the opaque blue vent plugs, these must be sterilised at room temperature**. The filters should be disposed of in lab waste. See below for full detail on Bung cleaning:

dx.doi.org/10.17504/protocols.io.yxmvmeq29g3p/v1

Temperature Probes

The temperature probes should be removed from the bungs post experiment and pre-bung cleaning. **Do not autoclave the bungs with temperature probes still in place**. The sleeves in the bung will prevent them from coming into contact with contaminants, and so they do not require sterilisation. If you have older stoppers without the sleeves, you can sterilise the probes with your preferred stainless-steel-safe method.

N.B, When removing the temperature probes from the bung or OGI3 BioReactor,

please only use the metal grip or plastic jack respectively. Avoid pulling on the wire itself.

Liquid Control Tubing

Liquid control tubing should be disconnected from the bung needles soon after an experiment and cleaned with the Pump Cleaning protocol. They may be autoclaved a maximum of 5 times if necessary. See below for full detail on Liquid Control Tubing cleaning:

dx.doi.org/10.17504/protocols.io.dm6gpz2jdlzp/v1

Oxygen-Spot Flasks

The oxygen spot flasks should be washed with de-ionised water and rinsed with ethanol. They should be left to dry after rinsing with ethanol, and stored in a dark place for at least 24 hours after cleaning. These flasks may be autoclaved a limited number of times if necessary, and should be stored in a dark place for at least 1 week after autoclaving.

If you find ethanol is not strong enough, the spots can tolerate a 1:9 solution of household bleach. Let soak for one hour and rinse heavily with pure water to ensure no bleach particles are left behind.

See below for full detail on cleaning Oxygen-Spot flasks:

dx.doi.org/10.17504/protocols.io.261ged277v47/v2

pH Probes

The pH probes should be removed from the bungs soon after an experiment. Take care to ensure the probes are gently handled, as the glass is delicate. They should be rinsed with de-ionised water and ethanol, and soaked in storage solution (3M potassium chloride) in their rubber storage sleeve. Wrap parafilm around the edge of the sleeve to prevent drying. See below for full detail on pH probe cleaning:

dx.doi.org/10.17504/protocols.io.j8nlkoz31v5r/v2

Routine Cleaning

The device should be wiped with a damp cloth for regular cleaning.

If contaminants come into contact with the flask holders, other parts of the reactors, or the base of the device these should be wiped down with a cloth damped with disinfectant. Do not spray directly onto the system. If you suspect liquid ingress into the unit, power off the device immediately and contact help@ogibiotec.com.

In choosing a method of disinfection be aware that the enclosure is composed of both polypropylene and metal components and a suitable disinfectant should be chosen.

4.5 Device Main Menu

The Main Menu will change slightly depending on which modules you have installed, however, you will always have a default set of options.

- Batch Culture: Every device has a basic batch culture function. This experiment mode allows you to use the device to automate analysis of a growing cell culture. See Section 6 for information on setting up one of these experiments.
- Additional Experiments (Optional): This refers to a selection of menu items that become available when different modules are installed. These experiments are, in order of appearance:
    - Turbidostat (Section 8.5).
    - Chemostat (Section 8.9).
    - pH Control (Sections 8.10 and 11.8).
    - Note that these options will not appear if you do not have the relevant modules installed.
- Quick Measure: This menu item allows you to take individual measurements of Temperature, OD, Dissolved Oxygen, pH, and fluorescence if the relevant modules are installed (Section 4.6).
- Settings: Here is where you can create and view settings presets for convenient device setup.

- Calibration: All calibration functions are found here. By default, only the OD calibration is visible. See Section 5.1 for more information regarding OD calibration. Each module that requires calibration, Dissolved Oxygen and pH modules, has its own menu item within Calibration that appears when that module is connected. Please see the individual module section in this manual for information on calibrating the respective modules.
- Pump Cleaning: This menu item allows you to run the pumps in the Liquid Control module to flush cleaning liquid through the tubing as described in Section 8.3.
- Wi-Fi: This menu contains the functions to connect the device to a Wi-Fi network and to the OGI Web App. The Web App will launch in 2025, but in the meantime you can use the menu option Wi-Fi -> Network connect to connect the device to an available Wi-Fi network to automatically retrieve date information for experimental set up. The device will look for available networks and display the network names on the LCD. After selecting the desired network, you will be prompted to enter the credentials to connect to the network. Depending on your network security protocol, these can be either a simple password or a username, an optional anonymous identity, and a password. Before entering each required credential, you will be prompted to enter its length (i.e. the number of characters you will need to enter).
- Tests: A sub-menu of the standard tests run on the device to ensure it is operating correctly. They are provided to support remote diagnostics by our support team and to ensure that your device is still running as you need. We may ask for the results of some of these tests should troubleshooting be necessary. Also contained in this menu is the Factory Reset function, which will restore your device to factory defaults.

  ⚠ **WARNING: Factory Reset cannot be undone and will replace all user calibrations on the device with factory calibrations. Use with caution.**

4.6 Quick Measure

Quick Measure allows you to take a single measurement from installed analysis modules. As default, each device can measure Temperature and OD. Note that these measurements are not saved to the USB.

- Temperature: Displays a real-time measurement from each temperature probe. If a probe isn't connected, the device will display a high-magnitude negative number.
- OD: You will be asked which OD calibration you would like to use, then to tell the device which reactors you would like to measure in. The device will then warm up the LEDs for 2 minutes and take a measurement, displaying the results on the LCD screen.
- Dissolved Oxygen: You will be asked which O2 calibration you would like to use and then the device will display a real-time measurement from each flask with an O2 sensor spot.
- pH: You will be asked to select a pH calibration and then the device will display a real-time measurement from each pH probe.

  N.B. A temperature probe must also be attached in order to display an accurate pH reading.
- Fluorescence: You will be asked to select which excitation LED you would like to use (all of them and none of them are also available options) and then which wavelength you would like to view the intensity of. The device will display the real-time intensity at the nearest available wavelength band to your selection (based on each sensor's fixed resolution and calibration).

4.7 Keeping Your OGI3 Up To Date

New versions of the OGI3 software may be downloaded from our website. The updater requires a Windows PC or laptop and needs PowerShell 7 to be installed. After extracting the downloaded archive, clicking runme.exe will open the updater interface. Instructions for running the updater can be found in this program, and are also provided in the readme.

Please ensure that adequate time is allowed for the update and verification processes to complete. Partial updates will fail. Ensure that your laptop is connected to the Board Prog. port for updating the main board and the Wi-Fi Prog. port for updating the Wi-Fi card.

The main board and the Wi-Fi board must be kept at versions that are compatible with each other. If the OGI3 detects any incompatibility, it will report this on the LCD screen.

To resolve, simply run the updater and update both boards.

⚠ **N.B. Do not attempt to update via the front USB port. This port is for storage devices only.**

5 Calibrating the device

5.1 Optical Density and Calibration

N.B. The OGI3 and it's calibrate-able modules come with factory calibrations, and so for qualitative results you don't need to calibrate before running for the first time.

Optical density is not often a well-explained method of determining cell density, and it can be difficult to know whether one can trust a given method of obtaining it.

Conventional spectrophotometers present OD via measurement of light transmitted through a sample of culture in a cuvette. For cells, most light that is not transmitted is scattered (rather than absorbed). You will probably be familiar with a so-called "linear regime" or "region", when discussing results taken in your spectrophotometer. The linear relationship between cell number and OD within this region arises from the fact that at low cell densities each photon is either scattered once or transmitted. This 'single-scattering' leads to rays exiting the sample at wide angles, which then do not enter the detector of the spectrophotometer. As the cell number increases, it becomes more likely that rays are scattered multiple times ('multiple-scattering'). The more times a ray is scattered, the more likely it ends up in the detector, and so the detector will see an increasing contribution from scattered light in addition to transmitted light, meaning that the amount of light the detector sees is no longer directly proportional to the number of cells in the sample, and the relationship between OD and cell density is no longer linear.

In the OGI3, we do not measure transmitted light. Rather, we directly measure the light that has been scattered by cells in the sample. The consequence of this is that the relationship between cell number and OD may not be linear at any point in our device, and so it requires calibration against true (i.e. within the linear region) readings from a spectrophotometer. In fact, the relationship between the amount of light we collect (expressed as a 'Device Reading') and OD nicely follows the power law shown below.

Once readings have been gathered using the method in Section 5.2, the device will fit according to a power law:

$$y = p_0 + p_1 x^{p_2}$$

where in our case y is the sensor reading, x is the OD, p_0 is the y-intercept, p_2 is the power, and p_1 is a scaling factor that would be analogous to the gradient when p_1 is close to 1. We find this relationship fits well to our device readings and ODs. If you wish to perform the manual calibration as in Section 5.3, you may do so with that formula as the fitting function. You will find in the Scripts folder in the software update package a python script that will plot the data collected and corresponding fit from an OD calibration file.

5.2 Calibrating your Device

Calibration of the device's OD measurements is performed in experimental conditions, (i.e. each calibration has the same cells, media, and temperature as in respective experiments), beginning with a concentrated culture and diluting down throughout the process. The cells should not be growing throughout the calibration, consider using bacteriostatic antibiotics to halt their growth during your calibration. Additionally, when measuring alongside the reactor with a spectrophotometer, please ensure that you are comfortably within the linear region of your spectrophotometer, otherwise the calibration will not be trustworthy. Before you begin, you should have a good idea of the ODs you want to calibrate over.

N.B. You must have at least one point at or near 0, otherwise the low-OD readings will be affected. There is a MAX OD of 5 in the calibration process. For the most accurate readings, we recommend calibrating the OD in each reactor with the flask you intend to use in that reactor for experiments.

⚠ **N.B. If you are using the Sparging Module, see Section 12, you must calibrate the optical density with a sparging needle in place.**

⚠ **N.B. For all OD calibrations temperature probes are required to be inserted in all the flasks being calibrated - including calibrations performed without heating.**

1. Prior to calibration, you should prepare one flask of at least 15 mL of cell culture at a known OD no higher than an OD of 5 per reactor to calibrate.
2. Start the calibration from Calibration -> Calibrate ODs.
3. You will be prompted for the date, the name and save location for the calibration.

4. You will be asked to select heating for individual reactors, we recommend calibrating at the temperature you intend to experiment at.
5. The motors will briefly turn on and the LEDs will warm up and you will be presented with options: Finish/A/B/C/D, with a number indicating how many readings are left for each reactor. Note that you do not have to use all 6 readings, a minimum of three readings are required per reactor for calibration. However, if it is your first time using the device or in a given condition, we strongly recommend taking all six measurements.
6. Select the reactor you wish to take a measurement point in and you will see Stirring… and then Measuring. After the measurement has been taken, take a measurement of the culture in that reactor in your benchtop spec. Once all the measurements for a given OD have been taken you may dilute the culture and re-measure in the spectrophotometer for all flasks. Continue until you have used all 6 measurements or you are satisfied with the breadth of your calibration.
7. When ready, press Finish. You'll be asked to enter the ODs you measured in your benchtop spec corresponding to all of the measurements made in the device.
8. The calibration values will be saved to the device's memory and to a calibration file on the USB drive.

5.3 Manual OD Calibration

Sometimes you may need to make adjustments after the calibration is complete, e.g. if you entered an incorrect OD value or if you want to discard or add points afterwards. In such cases one may retrieve the readings and ODs from the saved file in the USB and manually fit a curve to it. Before carrying out this operation, please ensure you have read and understood Section 5.1. If anything is not clear, please contact help@ogibiotec.com.

In OD Calibration -> Input Cal one may insert the parameters found by an external fitting, rather than relying on the device to perform the operation.

6 Setting up an Experiment

6.1 Starting an Experiment from the Device

On booting up Batch Culture will be the option on the screen, selecting this will begin an experiment setup. Liquid Control Module experiments (e.g. Turbidostat) will be visible on this menu to the right of Batch Culture if that module is installed.

Select Start Experiment to enter the setup menu. This menu is presented in a flat structure, with options being navigable via scrolling and settable via clicking into them:

- Date yyyy-mm-dd: Clicking into this option allows you to set the date in the format shown. This will be used as the experiment name for the folder and files saved on the USB.
- Load settings: We allow the user to save presets of settings, they can be loaded here to save time on experimental setup. Refer to Section 6.4 to explore presets.
- Ambient Temp: Here you should select the temperature of the environment the device is in. This will be saved to the settings file, and will help the device determine whether it can control temperature in the reactors.
- Temp. Control: Clicking into this setting will enable you to set temperature targets for each reactor. The maximum temperature you can set is 50°C, and the minimum temperature is 6°C above the ambient temperature. This is due to the heat that is inherently built up as the device runs.
- OD Calibration: Clicking into this setting allows you to select an OD calibration for your experiment. At this time, you may not start an experiment without an OD calibration. See Section 5 for notes on calibrating OD.
- Exp Length: Set the length of your experiment. Note that the experiment can be stopped at any time during an experiment and so unless media usage is a concern, this setting is not critical.
- Sampling Rate: Set the sampling rate in minutes for your experiment. Please note that the device enforces a 3 minute minimum rate to ensure all measurement processes have adequate time to complete.

- Stirring Speed: Set the stirring speed in RPM of your experiment. The device can stir at speeds between 500 and 5800 RPM.
- Start: If no OD calibration has been selected there will be a prompt here to return to that menu item and do so. If one has been selected you may start the experiment. At this point, the device will check the alignment of oxygen the oxygen flasks if DO measurements have been enabled (see Section 9 for further information).

  Once the experiment is running, you will have access to a new menu with options to pause or stop the experiment, update the settings, and view the last measurement.

6.2 During an Experiment

When an experiment is running the most recent measurement of each reactor is displayed on the LCD screen. By clicking the Twist/push knob you may cycle through the measurement type (e.g. OD -> O2).

In addition to viewing current measurement readings you have a few options for interacting with the device. Note that while the screen displays Measuring… interactions are suspended to ensure data integrity.

- Stop Experiment: If you wish to stop the experiment, select this option. It will ask if you are sure with a Yes/No response. Upon stopping, the device will exit the experiment mode and the USB may be taken out to review data.
- Pause Experiment: If you wish to pause the experiment, for example if you wish to check data without stopping it, you may use this option. The screen will then update to display Resume Experiment for when you are ready for the experiment to continue.

  a) The time that the experiment was paused will be saved to the settings file, as will the time the experiment was restarted.

  b) While the experiment is paused you will not be able to change any other settings until you resume the experiment.

  c) While paused, you may remove the USB to access the data. If the USB drive is not replaced when Resume Experiment is clicked, you will be warned that

it is not present and asked if you wish to continue. You may replace the USB at this time, or you may continue the experiment without it if you so desire.

⚠ **N.B. If you remove the files from the USB stick and do not replace them, the device will generate a new file when it begins saving measurements again. This new file will not contain headers.**

d) Update Settings: During an experiment you may update the settings in the device. This option opens a reduced version of the settings menu from in Section 6.1 where you may update the desired settings.

6.3 Data Format and Handling

- The data recorded during the experiment will be saved to the USB drive. Each experiment is contained in a folder with the day's date. Multiple experiments on the same day will generate new folders with the experiment number appended. E.g. 240131_01 would be the second experiment performed on 2024/01/31.
- Files with suffix 'A'. These are the analysis files. These contain the time each measurement was taken at and the corresponding OD.
- Files with suffix 'S'. These record the settings and 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.
- Files with suffix 'TM'. These are records of the flask temperature during the experiment.

6.4 Saving 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.

To select settings, use the twist button to select the desired value and push to confirm selection. The settings that can be saved are:

1. Experiment Length
    - Total length of the experiment in hours.
    - The default experiment length is 96 hours.
    - Minimum experiment length is 1 hour but an experiment can be stopped at any time by selecting Stop Experiment.
2. Sampling Rate
    - The period of time between measurements after which the motors stop spinning and an OD measurement is taken.
    - Default sample rate is 5 minutes.
    - Minimum sample rate is 3 minutes to ensure all measurements execute correctly.
3. Stirring Speed
    - The speed at which the motors spin, mixing the culture.
    - Stirring speed is in rpm and can be changed in increments of 100 RPM.
    - The minimum speed is 0 RPM and the maximum is 5800 RPM. Note that there is a step between 0 RPM and 500 RPM as the motors cannot drive anywhere in between those values.
    - 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, immediately prior to a measurement cycle they will turn on for 10 seconds at 500 RPM to gently resuspend the cells and avoid sedimentation.

Saved settings can be used in an experiment by selecting them during the experiment setup. 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 User Scripting

Users may control the device from a computer (set experiment parameters, retrieve data etc.) with simple commands sent via the Serial port (BOARD PROG. on the rear).

7.1 Communication Protocol

Commands are plain text in a UTF-8 encoding. The settings are: 115200 Baud Rate, 8 Data Bits, 1 Stop Bit, no Parity. The reactor will always return a response to a command which consists of the following:

```
<original message><payload | ERR#><\r>
```

The return response always begins by repeating the original message. This can help debug issues with transmission integrity, for example, if characters go missing. Depending on the command, for example ones asking for data, there may be a "payload" which is a comma separated list of numbers in human-readable form. Alternatively, if there was an error in parsing the command the message, this section of the response will be characters "ERR" followed by a negative number indicating the issue (see Section 7.4). Lastly, all return messages are terminated by a carriage return character (0x0D).

All numbers sent to/from the bioreactor are in human readable form. For example, the number 128 is sent as the characters "1", "2" and "8"; not the bits "10000000".

7.2 How to use

Any application that can open a serial connection can be used. We provide some example python scripts in the updater package at https://ogibiotec.com/resources/ and more at https://github.com/OGIBio/user-scripting, where you are welcome to contribute your own examples. Other options for simple serial monitors include the Arduino IDE, the Serial Monitor extension in VSCode, or CoolTerm.

Switch your bioreactor on then connect via the USB cable supplied with the bioreactor. Establish the serial connection. Note that the bioreactor will restart unless you set the DTR and RTS flags off. This has been done in the example scripts provided, i.e., the

bioreactor will not restart when running these scripts.

Initial experiment settings (Section 7.3.1) should be applied *before* starting the experiment (Section 7.3.2), but can also be updated during.

The bioreactor will only start listening for commands when in the main menu. Some menu items, such as updating experiment settings, will block the commands until you exit that menu item. Similarly, parts of the experiment control flow are blocking, such as measurements.

7.3 Commands

Most experiment commands take two forms: one that applies to all flasks, and one that applies to a single specified flask.

`<f>` indicates a number that can be interpreted as a floating point number. `<i>` indicates a number that can be interpreted as an integer. `<A-D>` are flask labels.

7.3.1 Experiment settings

Command	Range	Units
`set exp length <f>`	0+	hrs
`set sample interval <i>`	3+	mins
`choose OD cal <i>`	0–11 [1]	
`choose O2 cal <i>`[2]	0–3	
`choose pH cal <i>`[3]	0–5	
`choose sparging cal <i>`[4]	0–1	
`set motors rpm <f>`	0, 500–5800	rpm
`set motor rpm <A-D> <f>`	0, 500–5800	rpm
`set ambient temp <f>`	0–50	°C
`set temp controls <0,1>`[5]		
`set temp control <A-D> <0,1>`		
`set temp control targets <f>`	ambient+6 – 50	°C

Continued on next page...

[1] see `list OD cals`
[2] enables O2 measurements
[3] enables pH measurements
[4] enables sparging
[5] may return error if target is incompatible with ambient temperature

Command	Range	Units
set temp control target <A-D> <f>	ambient+6 – 50	°C
set sparging flowrates <f>	0+[6]	ml per min
set sparging flowrate <A-D> <f>	0+	ml per min
set turbidostat controls <0,1>		
set turbidostat control <A-D> <0,1>		
set turbidostat targets <f>		
set turbidostat target <A-D> <f>		
set chemostat flowrates <f>	0–100	ml per hr
set chemostat flowrate <A-D> <f>	0–100	ml per hr
set pH controls <0,1>		
set pH control <A-D> <0,1>		
set pH control targets <f>	0–14	
set pH control target <A-D> <f>	0–14	
set pH control types <acid,base>		
set pH control type <A-D> <acid,base>		
set pH control durations <f>	0.3–15	s
set pH control duration <A-D> <f>	0.3–15	s
set pH2x controls <0,1>		
set pH2x control <A,B> <0,1>		
set pH2x control targets <f>	0–14	
set pH2x control target <A,B> <f>	0–14	
set pH2x control durations <f>	0.3–15	s
set pH2x control duration <A,B> <f>	0.3–15	s
set pHchemo controls <0,1>		
set pHchemo control <A,B> <0,1>		
set pHchemo control targets <f>	0–14	
set pHchemo control target <A,B> <f>	0–14	
set pHchemo control types <acid,base>		
set pHchemo control type <A,B> <acid,base>		
set pHchemo control durations <f>	0.3–15	s
set pHchemo control duration <A,B> <f>	0.3–15	s
set pHchemo flowrates <f>	0–100	ml per hr

Continued on next page...

[6] Max sparging flowrate is module dependent

Command	Range	Units
`set pHchemo flowrate <A,B> <f>`	0–100	ml per hr

7.3.2 Starting an experiment

Command
`start batch culture`
`start turbidostat`
`start chemostat`
`start pH control`
`start pH2x control`
`start pHchemo control`

7.3.3 Retrieve data

Command	Payload format	Payload Description
`get OD <A-D>`	`<f>,<f>`	time (hrs), OD
`get O2 <A-D>`	`<f>,<f>`	time (hrs), O_2 (%)
`get temperature <A-D>`	`<f>,<f>`	time (hrs), °C
`get pH <A-D>`	`<f>,<f>`	time (hrs), pH
`list OD cals`	<string>	prints OD calibrations in a table

7.4 Error codes

Error code	Description
ERR-1	Could not parse command - check that returned message matches original command; check spelling of original command.
ERR-2	Command was valid but flask was not (e.g. `get OD F`)
ERR-3	Command was valid but value was not (e.g. `set exp length -10`)
ERR-4	No calibration data found in given slot
ERR-5	Temperature fuse blown - could not enable control
ERR-6	Temperature sensor error - could not enable control
ERR-7	Could not start experiment - there is already one running
ERR-8	Module not installed

8 Liquid Control Module (P/N OGI3-1011)

The Liquid Control module is a separate unit designed to sit underneath the OGI3 Bioreactor. 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 (Section 8.5), to run an experiment in chemostat conditions by specifying a continual flow rate of media (Section 8.9), or to keep cultures at a specified pH (Section 8.10).

8.1 Connecting the Liquid Control module

Ensure that the Liquid Control module is connected to the OGI3 BioReactor before switching on the device. The LCD screen on the OGI3 will display Liquid ctrl ok if the module is properly connected.

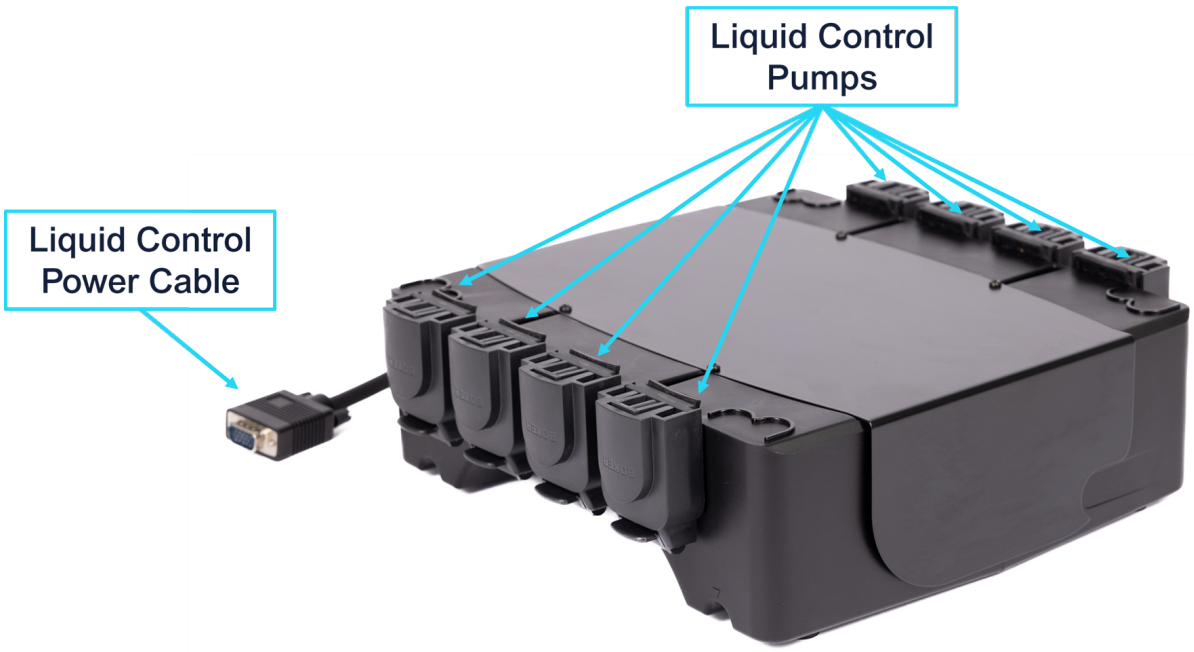


Figure 4: Liquid Control Module

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 2).

Please ensure that the screws on the connector are tightened to avoid accidental

disconnection of the Liquid Control module from the main device.

Figure 5: Liquid Control Module in position

8.2 Fitting the Liquid Control Tubing

To operate the Liquid Control system tubing must be fitted to each pump. 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 in line with the side of the Liquid Control module (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 has 2 tubing clips (highlighted in Figure 9, left). The clips are slotted into place in the pump cover with the opening facing out. The tubing should be pressed into the right-hand clip (Figure 9, middle) 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. Note that as the tubing stiffens with use, you may wish to mark the position of the tube-clamps on the tubing with indelible marker.

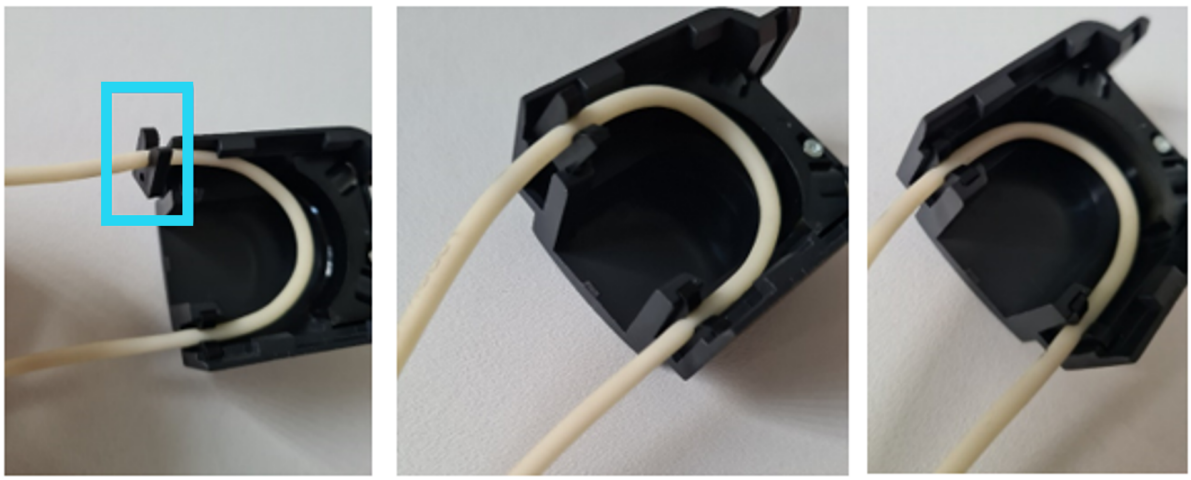


Figure 9: The tubing fits into the pump tube clips which then attach to the pump cover

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. The tubing will naturally stiffen over the first 10 hours of use or so. If you notice that the flow rate drops significantly or becomes unreliable check the tubing. If it is noticeably stiff or crumbly it is time to replace that tubing.

8.3 Cleaning the Pump Tubing

- The recommended method for cleaning the tubing is with 70% ethanol and 18 MΩcm water for rinsing. The tubing can be autoclaved if necessary, however should be replaced after no more than 5 autoclave cycles. The cleaning process utilises the function of the pumps themselves to propagate the cleaning fluid through the length of the tubing. Tubing can also be removed from the pumps and cleaning media flushed through 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 tube obstruction is increased. This is dependent on the type of organism being used. Obstructions can affect the function of the pumps and the

ability of the device to maintain desired conditions. If obstructions are noticed it is recommended to use a fresh set of tubing for subsequent tests.

In order to clean your Liquid Control tubing, please follow the following instructions carefully.

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 of 85 cm 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 tubing from any needles. Place all the inputs (right side of the pumps) in the ethanol, and all the outputs (left side of the pumps) in the empty waste flask. Note that the handedness is the same for all pumps and so one side of the device will be oppositely orientated to the other,
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. Press the button and the pumps will be turn on and propagate the liquid through the pumps,
7. The screen will show Press button to stop pumping,
8. 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 optimise for your organism), press the button again to turn off and the pumps will turn off,
9. The screen will display Press button to flush pumps,
10. Swap your cleaning fluid for flushing fluid (distilled water),
11. Press the button and the pumps will restart, flushing distilled water through the pumps,
12. The screen will display Remove tubing + press button,
13. 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,

14. 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,
15. 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. You will then be notified that the cleaning process is complete, and the system will return to the main menu.

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.

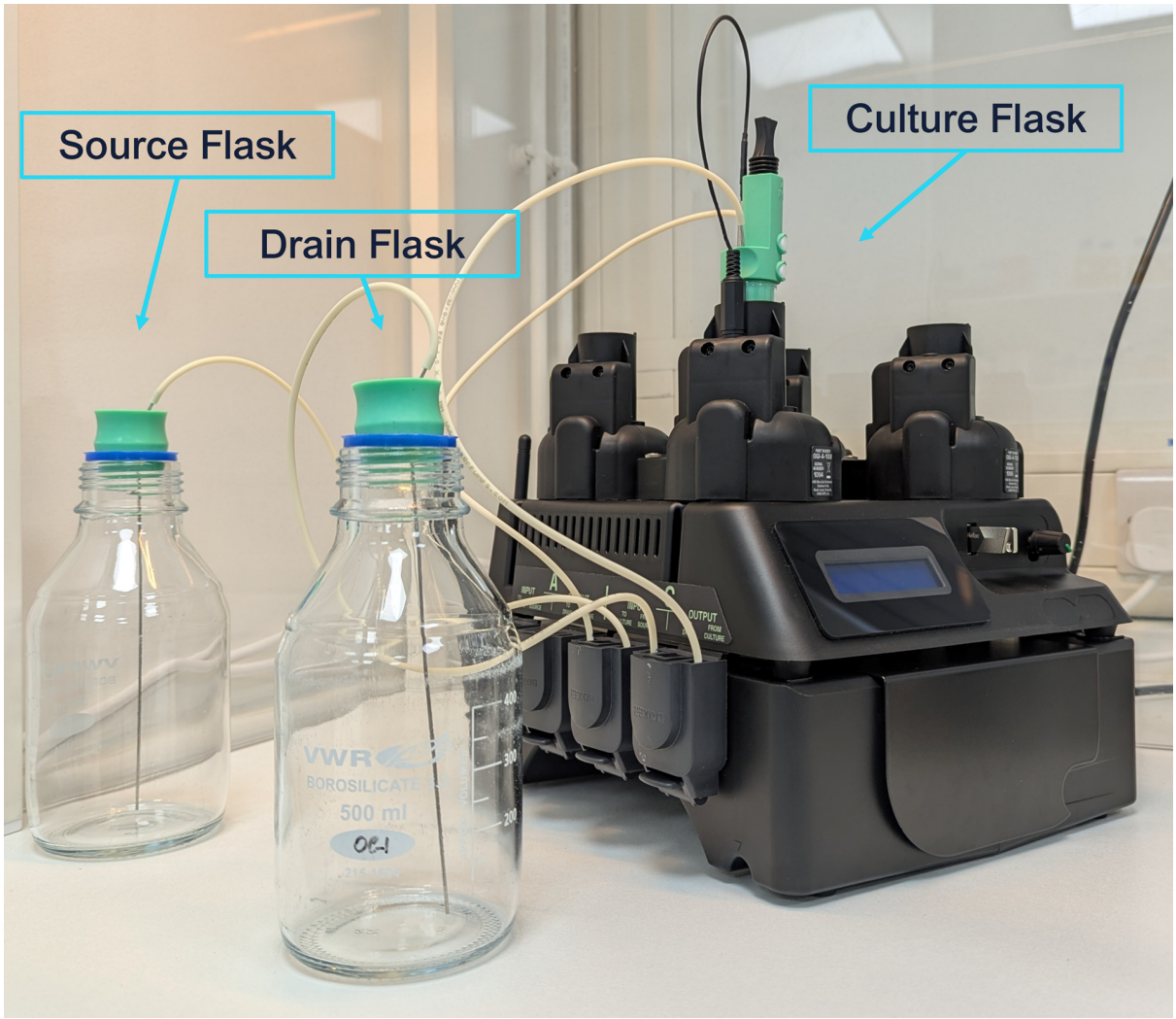


Figure 10: OGI3 Base Unit with a Liquid Control Module fully connected to Reactor C

8.4 Preparing for a Liquid-Controlled Experiment

Before conducting a turbidostat experiment it is recommended to clean the pump tubing. See Section 8.3. 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

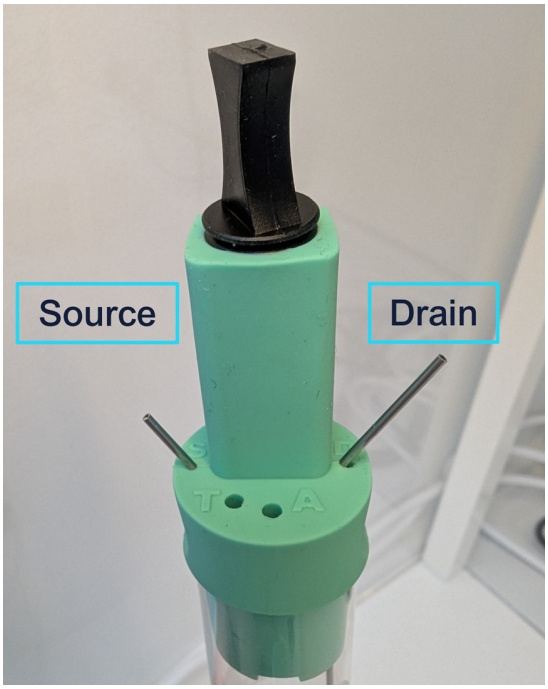


Figure 12: Ensure the input flask is connected to S (Source) and the output or waste flask is connected to D (Drain).

2. Use volumes of media that are suitable based on cell growth rates and desired experiment length – refer to Section 8.6 for information on estimating the media usage of your Turbidostat experiment.
3. Put your 15 ml cell cultures into the reactors you wish to use during the experiment.
   The fixed needles in the culture flask bungs should give a maintained culture volume of 15 ml (slight variation may occur due to the manufacturing process).

4. Connect your pump tubing to the appropriate part of the system.
    a) Refer to Section 8.2 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) The source flask bungs each have a long needle (Figure 11) and the drain flask bungs each have a short needle (Figure 12).
    d) The culture flasks each have a long needle (Drain) and a short needle (Source).
    e) 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. Refer to Bioreactor labelling for input and output pumps.
    f) Do this for all reactors that you are using.

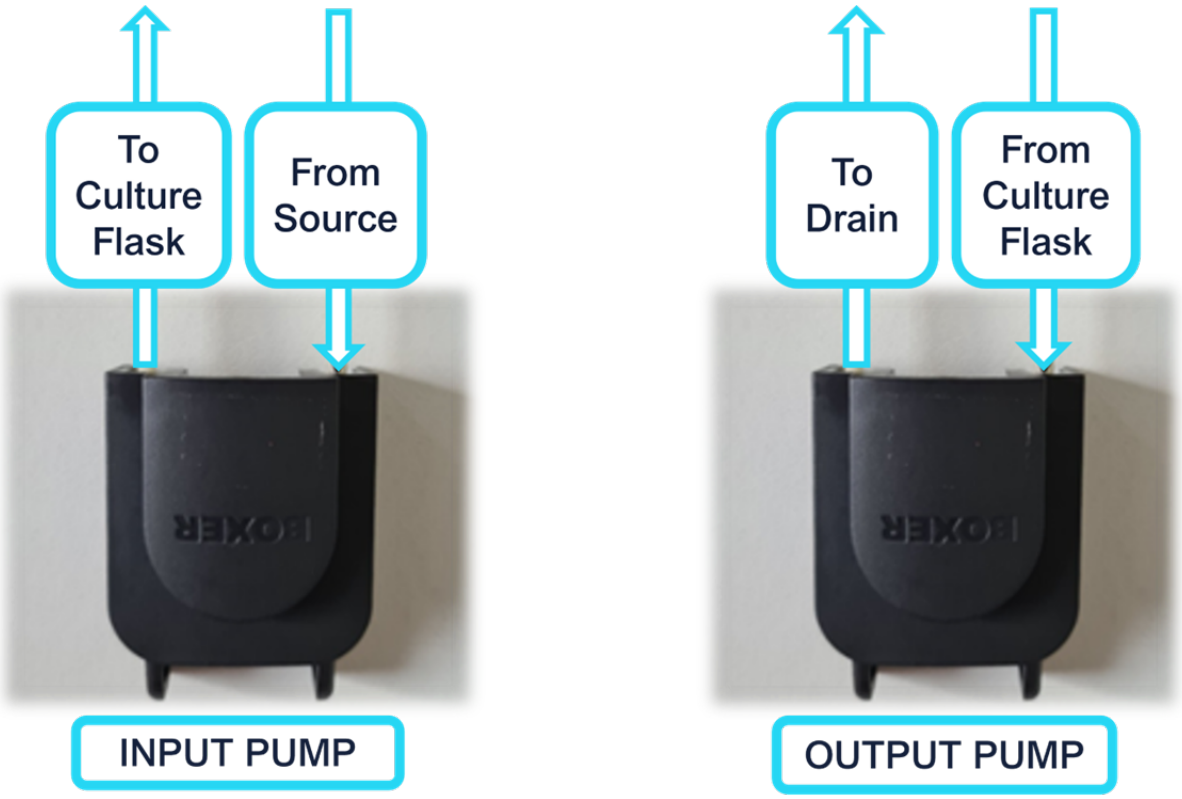


Figure 13: Input and output pump configuration showing tubing connections

8.5 Turbidostat Experiment Settings

Section 6 covers the basic Batch Culture settings. By connecting a Liquid Control

module and starting a Turbidostat experiment you unlock two new sets of settings under special menu items. For Chemostat, skip ahead to Section 8.9.

- Turbidostat:
  a) Maintain ODs: Enabling this setting will switch all reactors to 'Maintain' mode, and so they will work to maintain a cell culture at the OD it was at when it was placed in the reactor. While this affects all reactors, you can still turn individual reactors on and off by scrolling to the right.
  Note that the OD of your culture will be rounded to the nearest 0.05.
  b) If maintain ODs is not selected each reactors target OD can be set individually. To do this select the reactor in this sub-menu, choose whether it is ON or OFF, and set the target OD you desire. You can change this setting later, while the device is running.
  c) Tube Length: This is the length of your tubing, and is required to ensure correct priming function of the Liquid Control module. The default value is set at 85 cm - the length of tubing supplied by us. Please note that Factory Reset will restore this value to this default value.

⚠ **N.B. Please ensure all tubing used is the same length.**

8.6 Turbidostat Media Usage Prediction

When setting up a Turbidostat 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.

- 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.
- This will then display an approximate time period in which it is expected that your media will run out for this specific experiment.
- This will display on the screen for 5 seconds before going back to the Turbidostat menu.

- This value can then be used as a guide as to when the experiment must be stopped or fresh media 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.

8.7 During a Liquid Control experiment

After pressing Start during the experimental setup, you will be asked if you wish to prime the pumps, i.e. fill the tubing with input liquid up to the input needle. Currently, the slight variation in pump rates means that they will over-prime (and a few drops of fluid will exit the input tube during the process). If you wish to prime accurately, you may at this point disconnect the input tube and prime into a sterile tissue or bottle before replacing the tube. Once priming is complete, you will be able to Press to Continue and start the experiment.

N.B. The experiment cannot be stopped during a dilution period, measurement or priming period. The same rules apply to Turbidostat experiments as apply to Batch Culture experiments with regards to USB removal and experiment pausing and stoppage. 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 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. Reactors'.

8.8 Data Format and Handling

If performing a turbidostat test, then an additional file with suffix 'T' will be generated. This contains the time, OD, the status of each pump, and the difference between measured and target OD.

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 drive.

8.9 Chemostat Functionality

The Liquid Control module may also be used as a Chemostat, where experimental conditions are maintained through continuous pumping of fresh media at a given rate. The special settings menu item for this is called Chemostat.

- Chemostat: Each reactor (or all at once) may be given its own unique flow rate. The minimum flow rate you can specify is 0.1 mL/hour, and the maximum is 100 mL/hour. Please ensure that you have enough media for a given flow rate.

8.10 pH Control

New with the OGI3 is the ability to control the pH of your culture. This is achieved through a combination of the Liquid Control module and the pH module, and instructions on carrying out this experiment mode are found in Section 11.

9 Oxygen Module (P/N OGI3-1008)

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.
3. 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.
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 flask holder with the previously removed screw.

⚠ **N.B. Screws should be touch-tight. Do not overtighten as this will distort the plastic cover.**

9.2 Calibrating the Oxygen Modules

1. Prepare your 100% oxygen solution. You will need 15 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 equilibrate with air for 1-2 h, by shaking at moderate speed and pre-warming the media to the temperature that you are planning to use for your experiments.
2. Transfer solution into the Bioreactor flasks. Once the media is warmed up, it can be transferred into flasks with oxygen sensor spots. Insert the flasks into the reactors using the notch in the flask holder to align the oxygen spots with the oxygen detector as shown in Figure 14.
3. Prepare your 0% oxygen solution. You will need at least 15 ml of calibration solution per flask. You can either use commercially available 0% oxygen solutions/tablets (please follow manufacturer's instructions for correct use of these), 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 in a sealed container before starting the calibration.

Figure 14: 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. all flasks show Factory) 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. 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.

5. Calibrate 100%. If you want to calibrate 100%, select Yes when the device reads Calibrate 100%. If there isn't already a USB drive 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. N.B. It is highly recommended to have both a 100% and a 0% calibration point before running experiments.

6. Wait until the 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 completed the calibration as an indication of progress. 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 somewhere around 20 and are consistent they are good, and you can replace the USB drive in the device and select 'Yes'. Note that the settle point may be different between devices.

7. Proceed to 0% calibration. If you want to proceed with the 0% oxygen calibration now, you can now select Calibrate 0% -> Yes. If there is not already a USB drive in the device, you will be prompted to insert one now. If you want to calibrate the 0% oxygen level at another time, you can repeat the above Bioreactor's setup, skip the 100% calibration, and proceed to the 0% calibration.

8. Start 0% calibration. Once your calibration solution is warmed-up, insert the flasks in the reactor flask holders, aligning the oxygen spots with the oxygen detector. Make sure that the caps are fully sealed. When prompted, press the button to start the 0% calibration process. 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.

9. Wait until the calibration is done. The Bioreactor will now take oxygen readings until the measurements become stable. This can take up to 1h, but complete more quickly if the calibration solution is prepared correctly (e.g. ensure that the

flasks are fully sealed, do not leave a large empty headspace above the liquid in the flask to prevent diffusion of oxygen into the calibration solution). 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 '**OL**'. If the values settle somewhere around 50 and are consistent they are good, and you can replace the USB drive in the device and select 'Yes'. Note that the settle point may be different between devices.

9.3 Measuring Dissolved Oxygen in Experiments

Having an Oxygen module installed in any reactor will unlock a special menu item for it in experimental setups:

- Measure O2? If you select Yes, you will be prompted to select an oxygen calibration and then set the salinity of each reactor.

If oxygen measurement is enabled, the device will check alignment after Start Experiment is selected. If you have no oxygen flask in a given reactor, simply scroll once and click Disable when you are warned that that flask is not aligned. 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 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.

9.4 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, module temperature in °C, ambient pressure in mbar).
- File with suffix '**O**'. This file contains the results of the oxygen measurements taken during your experiment. For each reactor, you will find a status code to indicate

errors, the dissolved oxygen in µmol/l, the dissolved oxygen in mbar, the dissolved oxygen in % of air saturation, the temperature in °C within the module, the signal intensity in mV, the ambient light in mV, the ambient air pressure in mbar and the % relative humidity within the module.

- 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.

9.5 Notes

- Flasks with oxygen sensor spots must be stored such that the spot is kept 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 24 h 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 must be left for at least one week and the system should be recalibrated prior to use.

10 Fluorescence Module (P/N OGI3-1014)

10.1 Connecting the Fluorescence Module

1. Loosen the screws on the blank cover for the fluorescence sector (facing the back of the device).
2. Remove the blank cover, keep the screw.
3. Connect the fluorescence module to the device using the cable, and secure the cover ensuring that the tab at the bottom of the cover fits into the slot in the device's shell.
4. Move the top part of the cover towards the reactor in a rotary motion, hinging on the tab/box connection.
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 reactor with the fixing screw provided BEFORE turning on the device.**

10.2 Description of the Fluorescence Module

The standard configuration of the fluorescence module is the following:

- Excitation LEDs (peak wavelength):
  - LED 1: 365 nm
  - LED 2: 590 nm
  - LED 3: 470 nm
- Detection range:

- 340 - 780 nm

- Spectral resolution (FWHM):
  - 12 nm typical, 15 nm max (sampling resolution ~ 2 nm)

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 will have an additional Measure Fluo? option in the experimental startup menus. It is a simple Yes/No toggle to activate the modules for an experiment. At each measurement cycle, a spectrum will be taken from each flask with each of the fitted LEDs in turn.

Fluorescence settings can be changed via the Fluorescence menu item in the main device menu and experiment set-up menu. In this menu, you will find options to change the intensity of the excitation LEDs, spectrometer settings, alongside selecting which fluorophores, if any, you wish to measure.

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 menu. If you would like all or any of the LEDs to remain off during an experiment then you can set the intensity of the desired LED(s) to '0' in the Fluorescence menu.

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.

The spectrometers will default to an integration time of 100 ms and a gain setting of 'LOW', these can be changed to optimise the detection of your fluorophore's emission.

The pre-set fluorophore list is as follows:

Fluorophore	Excitation peak (nm)	Detection range (nm)
CFP	375	455 - 540
DAPI	375	425 - 550
DsRed	470	545 - 630
EGFP	470	510 - 550
mCherry	590	625 - 680
YFP	470	520 - 560

10.4 Data Format and Handling

The results of fluorescence measurements are saved in the files with suffixes '**F**' and '**FS**'.

File with suffix '**FS**'. This file contains the spectral intensity measurements from each reactor and with each excitation LED. The column headers in the file contain the relevant information to interpret the data. The headers in the file are 'Time (hrs), Reactor, LED, <wavelength>nm', where the wavelengths range from 340 nm to 780 nm in 2 nm steps.

File with suffix '**F**'. This file contains the results of the reduced fluorescence measurements depending upon which, if any, fluorophores were selected. For each reactor, you will find measurements for each selected fluorophore, with up to four fluorophore channels available for selection per measurement. The headers in the file are 'Time (hrs), Reactor, Channel, Reading (arb)' where the reading is the dark corrected, average intensity given by the fluorescence spectrometer over the chosen fluorophore's detection range.

N.B. if no fluorophores are selected for a measurement then the spectra will still be saved in the '**FS**' file but the '**F**' file will remain empty.

10.5 Updating the Spectrometer Calibrations

If your system arrives with fluorescence modules pre-installed then they will already be calibrated and ready to use. However, if you have purchased fluorescence modules as additional sensors to your system, they will come with a calibration sheet and executable to run. Download and open the executable on a PC next to your OGI3,

then follow the instructions provided in the 'help' section of the executable. This will parse and upload the calibration data to your device making it ready to use.

Please do not hesitate to contact help@ogibiotec.com if you have any questions regarding your device or modules.

N.B. Each fluorescence module is labelled with its corresponding flask and requires individual calibration. Do not move the modules to different flasks/devices as the calibration will no longer apply.

11 pH Module (P/N OGI3-1012)

The pH module is a new addition with the OGI3 BioReactor. It uses four pH probes that fit into the large hole in the bung to accurately measure the pH of your liquid sample. It can be used in tandem with the Liquid Control module to keep a growing culture at a stable pH.

11.1 Connecting the pH Module

1. If you have purchased a pH module alongside your OGI3 BioReactor unit, it will already be connected.
2. If you purchase a base unit without the pH module it will have a plastic square covering the sparging and pH connectors. To connect the pH module, first remove this square and keep safe.
3. Find the connector on the underside of the pH module, and connect to the 8-pin connector as shown in Figure 15:

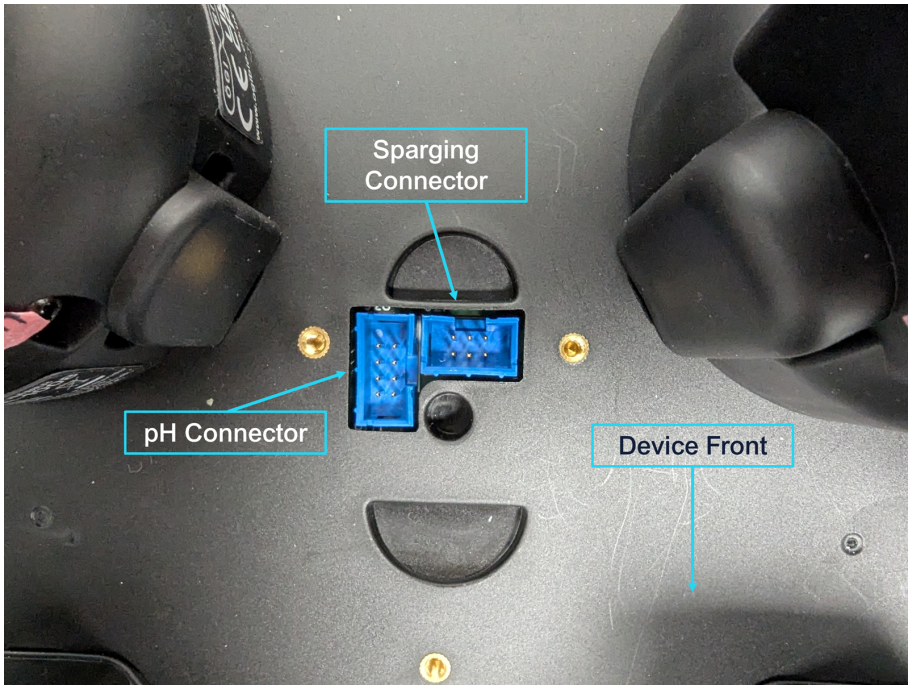


Figure 15: Top Connectors and screw holes

4. Sit the module flat on the top of the BioReactor unit, this may take some small rearrangement of the connector wire underneath the module.

5. Use the three butterfly screws provided to fix the module to the base unit. Do not overtighten these screws as this may cause deformation in the top of the base unit and the module casing.

11.2 Constructing and Using the Optional pH Probe Holder

If you have purchased the pH probe holder, this section will instruct you on its construction and use. If you have not, you may continue to Section 11.3. for information on handling and connecting the pH probes to the device.

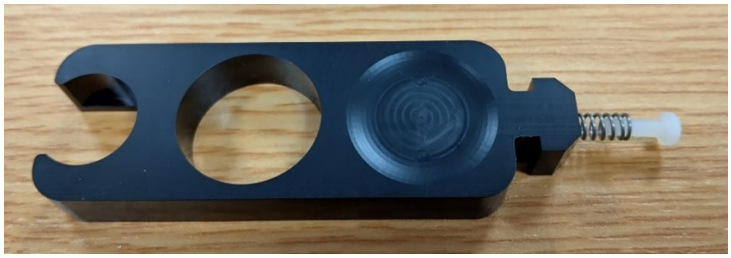
11.2.1 Parts

From left to right, top to bottom.

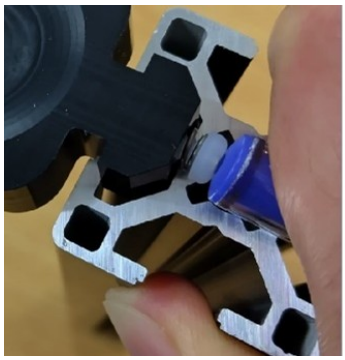
- 4x Plastic holders (black)
- 4x Nylon screws (white)
- 4x Compression springs
- 1x End cap (black)
- 1x Base with aluminium post

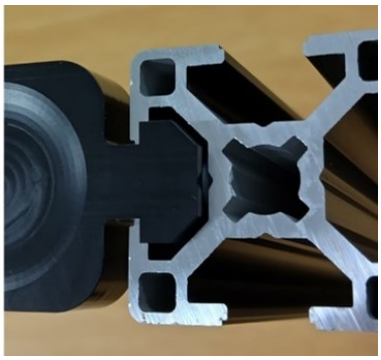
11.2.2 Assembly

1. Insert one nylon screw and one compression spring into the side of each plastic holder.

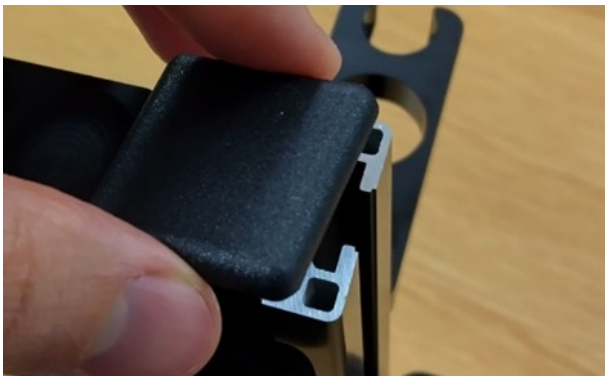


2. Using a pen, to protect the hand, press the nylon screw (1) so that it lies flush with the side of the plastic holder (2) and insert into the top of the aluminium post. Repeat this process for the other three holders. Be careful when pressing the screw down as it will spring back if let go.





3. Once all holders are inserted, place the end cap to cover the sharp edges of the aluminium profile and press it until fully inserted. The holder is now ready to use.



Each plastic holder can safely hold one pH probe and one stopper and they can be moved up and down individually, by pulling or pushing the filled indented circle. When placing probes into the holders, ensure that the rubber cover on the re-fill port faces away from the post, as shown in Figure 16.

Figure 16: pH Probe held in the constructed pH probe holder

11.3 Handling and Connecting the pH Probes

⚠ **N.B. The glass pH probes are very fragile. When inserting into the silicon bung be very careful to keep pressure along the length of the probe. Any bending motion or force applied perpendicular to the probe can result in its breakage. We do not accept liability for broken pH probes.**

- The pH probes will come with a black storage sleeve wrapped in parafilm. This is how they should be stored when not in use.
- Carefully remove the parafilm and storage sleeve.
- Rinse the tip of the pH probe with pure water (at least 18 $M\Omega$), and insert into the large hole in the silicon bung. The probe should enter without much force required, but if it is a little stiff you may use a light twisting motion to encourage fit.
- Once probe is in place, the bung may be inserted into the culture flask.
- The jack should be connected to the device prior to turning the device on.
- When finished with the pH probes, they should be cleaned and stored in accordance with the following protocol: dx.doi.org/10.17504/protocols.io.j8nlkoz31v5r/v2

11.4 Calibrating the pH Module

Before you start a calibration, please ensure that the pH probes are firmly connected to the pH module and that each probe is labelled as the reactor that it is connected to. This is to avoid mixing them up if you unplug them for cleaning/storage.

1. To calibrate the pH probes, use the twist/push button to select Calibration -> Calib. pH probes.
2. You will be instructed to enter the date and to select an available memory slot to save the pH calibration.
3. Then you will be asked to enter the temperature of the pH buffers that you are going to use for the calibration (e.g. the room temperature if you are doing a

calibration at room temperature). All the pH buffers should be at the same temperature to ensure good linearity of the calibration data points.

N.B. The device will apply temperature compensation to the pH measurements during experiments, therefore it is possible to calibrate the pH module at room temperature and then use it for experiments at different temperatures.

4. Then you will need to select the number of buffers that you want to use for the calibration, and select the desired buffers from the list of available buffers in the device memory.
5. If one or more of the buffers you want to use is not in the pre-loaded list of buffers, you can select the option “Custom” from the list. You can select “Custom” more than once if you want to use more than one custom buffer value.
6. After selecting all the buffers for the calibration, you will be prompted to enter the pH value for the custom buffers, if you have selected any.
7. Then remove the rubber sleeve from the tip of the probes, rinse the probes with deionised water and dab them dry.
8. Dip the tips of the 4 probes in the buffer solution displayed on the LCD and press the button to start the calibration. The device will now take measurements until the readings are stable and then instruct you to repeat the process with the next buffer solutions.
9. Please remember to rinse the probes with deionised water and dab them dry after each step (i.e. before you dip them in the next buffer solution).
10. When the calibration is complete, rinse the probes with deionised water, dab them dry.
11. Fill the rubber sleeves with fresh storage solution (or 3M KCl), slide the sleeves onto the electrodes’ tips and wrap laboratory film around the sleeves to prevent evaporation.

11.5 Measure pH in Experiments

pH can be measured in any experiment mode (e.g. Batch Culture, Turbidostat etc.). When you start an experiment, after setting the main parameters for the culture, you will be asked whether you want to measure pH in the experiment. If you select Yes, you will be prompted to select a pH calibration for the measurements.

11.6 pH Control

By utilising both the Liquid Control module and the pH module, you may control the pH level in a sample or culture in the Bioreactor with a single feed with the pH Control experiment mode. This mode will control the pH with either an acid or a base, but not both. The pH will be measured according to the sampling time and, if necessary, the source media will be pumped in for a user specified time. The device will remeasure the pH and repeat if the target pH has not been reached. The tubing should be cleaned and connected as for the Turbidostat – please see the user manual or quick start guide for instructions. Your source flask should contain the acid or base to be used to correct the pH.

Follow the on screen instructions as usual. The Select reactors prompt allows you to enable or disable the control of individual flasks. You can set the target pHs of each enabled flask. Next, choose whether each source bottle contains acid or base. Finally, you can set the duration of pumping when a target is not met. We recommend short durations, especially for strong pHs.

11.7 2-Way pH Control

You may wish to control pH in both directions, with acid and base. This mode will maintain the pH to within ± 0.05 of a target.

Note that this is a Beta feature and is still being optimised. Additionally, it is not available in the web application. It can be accessed from the main menu under Beta Features -> pH 2xControl.

The connection of tubing to Source, Drain, and Culture flasks in 2xControl is different to other Liquid Control experiments. In order to pump two media types into a single flask, we have to use input pumps A & C to pump into flask A; and input pumps B & D to pump into flask B. Flasks C & D, therefore, are not available for control, though you can still take measurements of OD and other modules.

Connect the tubes as follows:

- From Source side of Inputs A & B to the acidic media
- From Source side of Inputs C & D to the basic media

- To Culture side of Inputs A & C to the short needles of flask A
- To Culture side of Inputs B & D to the short needles of flask B
- From Culture side of Output A to the long needle of flask A
- From Culture side of Output B to the long needle of flask B
- To Drain side of Output A to the waste flask
- To Drain side of Output B to the waste flask

Follow the on screen instructions as usual. The Select reactors prompt allows you to enable or disable the control of individual flasks. You can set the target pHs of each enabled flask.

11.8 pH with Chemostat

This experiment mode combines pH control with a chemostat feed.

Note that this is a Beta feature and is still being optimised. Additionally, it is not available in the web application. It can be accessed from the main menu under Beta Features -> pH + chemostat.

In order to pump two media types into a single flask, we have to use input pumps A & C to pump into flask A; and input pumps B & D to pump into flask B. Flasks C & D, therefore, are not available for control.

Connect the tubes as follows:

- From Source side of Inputs A & B to the pH control media
- From Source side of Inputs C & D to the feed
- To Culture side of Inputs A & C to the short needles of flask A
- To Culture side of Inputs B & D to the short needles of flask B
- From Culture side of Output A to the long needle of flask A
- From Culture side of Output B to the long needle of flask B
- To Drain side of Output A to the waste flask
- To Drain side of Output B to the waste flask

11.9 Data Handling

- File with suffix '**PC**'. The pH calibration will produce an output file with suffix '**PC**'. This file contains pH values for the buffer solutions and the corresponding raw voltage readings taken during the calibration process. At the end of the file you will find the slope % of the calibration curve, the mV offset and the R2 value for each probe. A good quality calibration should have R2 value close to 1, slope between 95-105% and offset between ±30 mV.
- File with suffix '**P**'. This file contains the results of the pH measurements taken during your experiment.

12 Sparging Module (P/N OGI3-1013)

The Sparging Module is a new addition to the OGI3 system that enables air sparging into the culture flasks for the aerobic benefit of your cells during any OGI3 experiment mode. It comprises four diaphragm pumps capable of running up to 400-450 mL/min connected to sparging needles for insertion into each culture flask. This provides improved aeration and a lower-shear mixing environment over stir bars in the OGI3 system.

This module currently ships with two pre-humidification flasks, which are used to minimise losses of culture volume through evaporation by humidifying the air from the pump to the culture. Spill guards are also supplied, which fit over the tops of the flasks and prevent liquid ingress into the OGI3 base unit.

We appreciate that using two of the four OGI3 reactors for pre-humidification is not an ideal solution. We are working on a solution and pre-humidifier flasks will be upgrade to full, dedicated, pre-humidifier systems when they are ready. If you would like to use the system without pre-humidification, please note that the evaporative losses can be quite severe (0.1-0.6 mL/hour between 30-50°C), and care should be taken to maintain the minimum volume of 15 mL within an OGI3 culture flask.

Additionally, please be aware that all fixings in the pre-humidifier flasks are glued in place to maintain a seal, and as such cannot be removed. These flasks should be filled with water via syringe connected to sparging tubing using the sparging needle input, and drained by inverting and drawing out via the shorter output needle. Pre-humidifier flasks may be cleaned by UV sterilisation or by chemicals compatible with silicon.

⚠ **Do not autoclave these flasks, as this will compromise the temperature probes.**

If anything is not clear, please do not hesitate to get in touch at help@ogibiotec.com.

12.1 Connecting the Sparging Module to the OGI3 Base Unit

The sparging module is designed to easily fit on top of your OGI3 device, with, or without, a pH module also installed.

1. If you have purchased a sparging module alongside your OGI3 device, it will arrive pre-installed.
2. If you are upgrading your OGI3 to include sparging, and have not installed a pH module, there will be a square of plastic covering the sparging module connector.
   a) Remove this plastic square and keep safe.
   b) Connect the sparging module connector to the six-pin connector on the OGI3 unit.
   c) Sit the sparging module flush with the top of the device and use the long screws to fasten it in place. Some rearrangement of the sparging module cable may be required.
3. If you are upgrading, and you have a pH module already installed, the sparging module will sit on top of it.
   a) Remove the wing-screws and keep safe.
   b) Lift the pH module enough to connect the sparging module to the six-pin connector on the OGI3 system.
   c) Replace both modules so that the sparging module sits snugly on top of the pH module and use the long screws provided to fasten both modules to the top of the device. Some rearrangement of the module cables may be required.

12.2 Connecting the Sparging Module to the OGI3 Flasks

⚠ **N.B. Please pay close attention to the information sticker on top of the module. If the pumps are connected the wrong way around, liquid culture will enter them potentially damaging the module. If liquid is allowed to enter the pumps, please contact help@ogibiotec.com. We do not accept liability for pumps damaged in this way.**

- Air is drawn in through the pumps through the IN ports and passed out through the OUT ports.
- Syringe filters may be fitted immediately before or after the pump (i.e. on the IN line or immediately after the OUT line). Please note that the inner section of the pump is not sterile and cannot be cleaned.

- If using filters, connection to sparging tubing requires barb fittings on the filter. If connecting a filter directly on the IN line, only one side needs to be barbed.

The order of connection from pump to culture flask in Reactor A, assuming the use of pre-humidifiers, is as follows:

1. If using a filter before the pump, connect it via sparging tubing to the IN port of Pump A. Note that the filters we provide only allow connection to the IN port and may be attached to the tubing as in Figure 19.
2. If using a filter after the pump, connect it via sparging tubing to the OUT port of Pump A.
3. If 2, connect the other end of the syringe filter to the Sparging Needle in a pre-humidifier flask, located in a reactor other than Reactor A as shown in Figure 19.
4. If not using a filter, simply connect the OUT port of Pump A to the Sparging Needle in the pre-humidifier using sparging tubing.
5. Connect the other, shorter, needle in that pre-humidifier to the Sparging Needle in the culture flask bung of a culture flask in Reactor A.

For correct function of the device, ensure Pump A is used with a culture flask in Reactor A, Pump B with Reactor B etc.

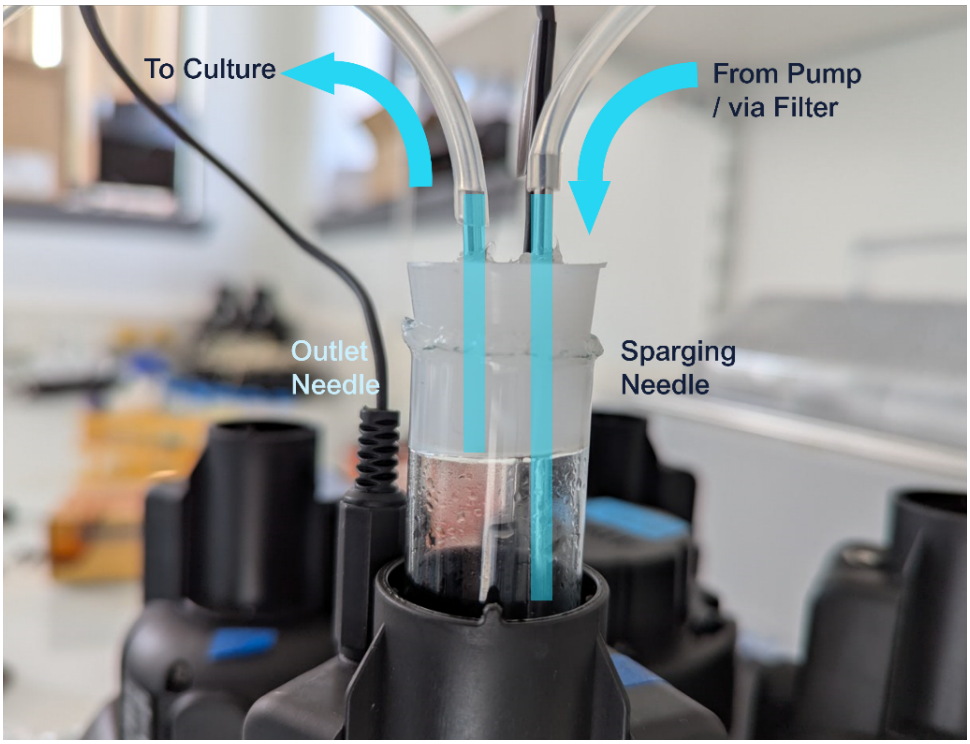


Figure 17: Connection of the pre-humidifier flasks. Ensure that the pump connects ONLY to the long sparging needle, not the short outlet needle. Improper connection will lead to water being pumped into the culture flask, filling it quickly.

Figure 18: Hydrophobic filters are used to ensure safety of the pumps and sterilise the air input. These should be placed in the line prior to pre-humidifier flasks. Note that they will not prevent backflow, but will prevent liquid ingress into the sparging pumps.

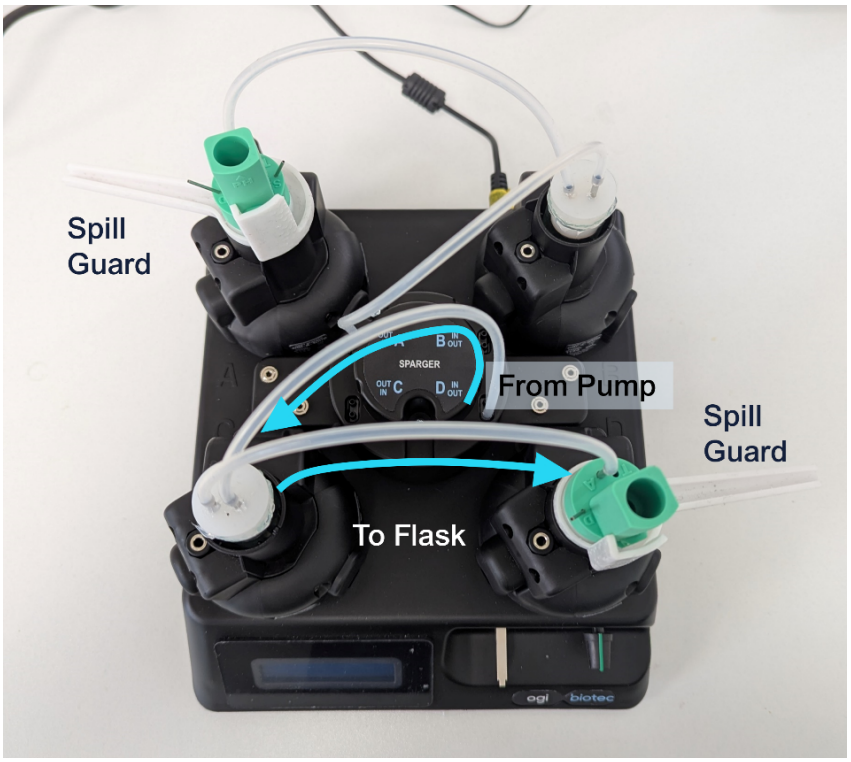


Figure 19: Sparging Module connected to culture flasks (green bungs) through pre-humidifier flasks (white bungs) with the route from Pump D to Flask D highlighted. Spill guards are fitted to the culture flasks.

12.3 Fitting Spill Guards to Culture Flasks

The sparging module comes with a set of spill guards for attachment to the culture flasks. These prevent any overflow of liquid from the flask from damaging the device. We recommend using spill guards for all sparged experiments.

They may be fitted over the top of the culture flasks as shown in Figure 20.

- Ensure that the divot in on the underside of the spill guard is matched to the line on the culture flask as in Figure 20 left.
- When the bung is on the flask and the marker on the bung is lined up with that on the culture flask, the vent holes will be correctly aligned with the vent guard portion of the spill guard as in Figure 20 middle.
- The raised plastic marker on the flask holder will then fit into the spill guard as shown in Figure 20 right.

Please ensure that the spill guards are fitted as in Figure 20, such that the spouts lead off the device.

Figure 20: Correct application of the spill guards to the top of the OGI3 culture flask. Note that the inset portion on the underside of the spill guard should line up with the marker on the culture flask. The bung may then be aligned with the same marker for complete alignment of the now guarded culture flask.

12.4 Antifoam

⚠ **N.B. We strongly recommend using antifoam with the sparging module. Details of our testing processes are available on request. Please ensure that the provided spill guards are always in place while using the sparging module . Please do not assume that your process does not require antifoam and be aware that you may have to add antifoam on a preventative basis. We do not accept liability for damage to the device that arises from liquid ingress resulting from lack of spill guarding or process control.**

12.5 Calibrating the Sparging Module

Before the Sparging Module can be used in experiments, it must be calibrated. This is because each pump provides a slightly different flow rate, and so the device does not know inherently what flow rate a given voltage will provide.

To calibrate the module, you will need a large reservoir of water (a container or bucket)

and a vessel of at least 100 mL in volume that you can comfortably hold upside down. On this vessel, mark a volume ≥ 50 mL in clear marker. A cylindrical measuring tube works well for this. You will also need a sparging needle, and a single length of sparging tubing.

1. The Sparging Module calibration is found in Main Menu -> Calibration -> Calib. Sparger.
2. Choose a slot for the calibration. Currently there are two slots as the pre-humidifier flasks (and in future, unit) have different calibration parameters to free flow.
3. Set the date and name of the calibration.
4. Set the volume at which you have placed your marker. The default is 50 mL, and the maximum is 400 mL. After setting the volume the device will instruct you to press the button to run Pump A at its first voltage step.
5. Fill your chosen vessel with water and invert in the larger container so that the filled part is above the bulk surface.
6. Place the end of the sparging needle at the opening of your inverted vessel and press the button to begin pumping air.
7. When the level of water in the inverted vessel reaches the marker at your chosen volume, press the button to stop the pump. The device will record the time taken to displace that volume of water and ask if you would like to keep or retry that point.
8. If you are happy with that volume displacement point, keep the measurement and repeat for all voltage steps on all pumps. Between pumps it will calculate its calibration parameters. If it detects a problem with the calibration, it will ask you to retry that pump. You may decide to continue anyway - say you wanted to skip a pump in calibration - by selecting 'keep'.
9. Once the calibration is complete the device will display Calib. Complete and return to the calibration menu. The resulting calibration file will be found in the calibrations folder of the USB drive with suffix '**aC**'.

12.6 Using the Sparging Module in an Experiment

Once the sparging module has been calibrated, you may use it during any experiment mode by navigating to the Sparging menu item in the experiment settings. On selecting this item, you will be asked to choose a sparging calibration prior to setting any flow rates.

⚠ **N.B. Please note that during a sparged experiment, all ports on the bung (liquid control needles, pH hole) must be closed if not in use with the provided hoods and plugs. Failure to do so will result in leakage due to condensation within those parts of the bung.**

We recommend running a short, supervised, experiment with your media and antifoam of choice prior to running any longer-term experiments. This is to ensure that the process is stable and safe prior to letting the device run un-supervised. If you are particularly concerned about byproducts increasing the foamability of your culture, it is worth using that condition for this test.

The pre-humidifier flasks should be heated to the same temperature as the culture flasks by default. However, the evaporative loss during your process will depend on various factors such as laboratory temperature, relative humidity, media etc. You may find you need to increase or decrease the temperature in the pre-humidifier flask reactors to maximise the effectiveness of the pre-humidification.

⚠ **N.B. At temperatures above 37°C, the culture flask may begin to gain up to 0.1 mL of volume per hour due to condensation effects. This can be minimized by removing the filters in the bung. Positive air pressure inside the flask will help to keep the flask sterile, but we are working on a suitable solution that maintains strict sterility without need for a hood.**

Currently the device may be operated at a fixed flow rate. We are working on introducing a threshold control which will allow you to set a desired percentage of dissolved oxygen that the device will support by controlling the flow rates itself.

You may set the flow rates of all pumps together, or on an individual basis.

If setting all flow rates at the same time, the maximum and minimum allowed values will be calculated such that all pumps can provide them.

If setting individual flow rates, the above constraint is not needed and as such a slightly wider array of flow rates can be chosen for each pump. This is due to natural tolerances in the flow rates of each pump.

N.B. Please note that, currently, stir bars are not compatible with sparging needles in the culture flasks. The turbulence from the bubbles provides ample mixing of culture at lower shear rates than the stir bars.

13 Disposal

This product should be sorted for environmentally-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 OGI3 BioReactor and any modules 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: 100-240 V AC (± 10%)
    - Input current: 1.2-0.5 A
    - Input frequency: 50/60 Hz
  - Power supply output:
    - 15 V DC, 6.0 A maximum
  - Bioreactor power input:
    - 15 V DC, 6.0 A, 90 W
- Fuse: 5 A Fast Blow
- The device is intended for indoor use only
- Pollution Degree: 2
- Operating (ambient) temperature: 15 to 60°C
  - If you are using the Liquid Control module, maximum operating temperature is 40°C
    - Can be used up to 45°C but this may affect lifetime of pumps.
    - Note that temperature control will still extend to 50°C.
- Ambient operational relative humidity: 30% - 70%
- Maximum operating altitude: 2000 meters

Appendix B: Troubleshooting

As much as possible, we work to improve your experience of the device and ensure there are no hitches or problems. This isn't always possible however, and so this section aims to provide you with the tools to understand various errors the device may communicate to you. If anything is not clear or your problem persists, please don't hesitate to contact us at help@ogibiotec.com.

OD Calibration Warnings

The device will attempt to ascertain whether or not a calibration has gone well. It may, upon saving or viewing a calibration, give the following messages:

- Note: <Reactor> Not Calibrated: This message shows when the reactor detects zero in each of the parameters. If you did not intend to calibrate the reactor it mentions, disregard the message.
- Warning: <Reactor> Poor Fit: This indicates that the fit that the device has for the given reactor has a low R2 value, i.e. that the fit does not accurately represent the OD and reading data. We consider "low" to be below 0.98.
- Error: <Reactor> Contains Zero: This is a different error to above, it indicates that a single parameter in the fit is zero. This warrants inspection of the calibration as it is generally a reason to re-calibrate.
- Error: <Reactor> Intercept -ve: The '-ve' standing for negative, this indicates that the calibration is showing a negative reading for 0 OD. This is a problem as it means the device will not be able to measure low ODs with any accuracy. In this case, it is generally sufficient to recalibrate the device with a lower OD range.
- Error: <Reactor> Bad Power: This indicates that the power parameter, p2, is greater than 1. This warrants inspection, and generally requires recalibration.

Oxygen Spot Errors

The Dissolved Oxygen module will provide some error codes in the Oxygen Calibration files if there are any problems, printed in the 'Status' column. Each error code in

this column is a cumulative sum of warning and error values from the module. We appreciate that this does add a level of complexity to troubleshooting.

- Add 1: Warning – automatic amplification level active
- Add 2: Warning – sensor signal intensity low
- Add 4: ERROR – optical detector saturated
- Add 8: Warning – reference signal intensity low
- Add 16: ERROR – reference signal too high
- Add 64: Warning – 1000xOxygen enabled
- Add 128: Warning – high humidity (>90%RH) within the module
- Add 256: ERROR – failure of case temperature sensor
- Add 512: ERROR – failure of pressure sensor
- Add 1024: ERROR – failure of humidity sensor