



Operation Manual V1.2

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Overview

This document provides instructions for operating Chi.Bio and running standard Turbidostat-type experiments. Many more complex protocols can be run on the platform, and custom programs can be implemented as required. For notes on writing new software functions see the readme on the project Github <https://github.com/HarrisonSteel/ChiBio>. Commands that must be directly input by the user into the computer terminal are highlighted in [blue](#).

Contents

1	Important Warnings	2
2	Platform Setup	3
2.1	Connecting Hardware	3
2.2	Starting Software	4
3	Experiment Setup	4
3.1	Sterilisation	4
3.2	OD Calibration	5
3.3	Culture Setup	5
3.4	Set Experimental Options	5
4	Running an Experiment	6
5	Finishing an Experiment	6
6	Retrieving Data Files	6
7	Other Protocols & Features	8
7.1	Adding a Chemical Inducer	8
7.2	Sampling During an Experiment	8
7.3	UV	8
7.4	Optogenetics	8
7.5	Custom Programs	8
8	User Interface Reference	9
8.1	Upper	9
8.2	Lower	10
9	Troubleshooting	12

1 Important Warnings

This section is a summary of the most common mistakes people make which can cause the device to not operate as expected, or even break the device. It is presented first to ensure it is read and understood as early as possible. Many of these instructions may not make sense fully until you have read the entire guide, so make sure to refer back to this and read it again once you are finished.

1. Read this entire manual from start-to-finish including the troubleshooting section.
2. When setting the system up for any experiment **always** ensure that the top liquid level in the reactor is higher than the liquid level in the fresh media jar and also the waste media jar *at all times during the experiment*. Therefore, the fresh media liquid level should be below the reactor liquid level at the start of an experiment, and the waste container should be set up such that when all liquid is in the waste container its liquid level is still below that in the reactor. The reason for this is to ensure that if any pumps fail then liquid will not siphon *into* the reactor, potentially breaking it. To achieve this you will generally need to ensure the reactors are raised up on a small box/ledge during experiments.
3. If your reactor software is failing to start (i.e. showing errors when you attempt to start the operating system) the most likely reason is that you have got some liquid (which might well have dried and just left a residue) on the moisture sensing tracks on the top of the reactor (1mm parallel silver lines around both the central hole and outside of the top square face). To fix this use alcohol to wipe down these lines carefully with a tissue.
4. The exterior of the reactor can get hot if you are running the LEDs for long periods at high power, for example if using them for optogenetic/photosynthetic applications. The approximately 1cm*1cm silver square on the outside of the reactor is where most heat is dumped, so do not touch this if you are running LEDs at high power. In general, avoid running the LEDs for prolonged periods at or near maximum power, and particularly avoid running multiple LEDs continuously for long periods. For example, it is fine to run multiple LEDs continuously at power ~ 0.1 , and it is also fine to run the LEDs at any power for standard fluorescence measurements (since they are only switched on for <1 second out of every minute in that case). But, you may damage the device in the long term if you have LEDs running continuously at power ~ 1 (i.e. full power) throughout an experiment. We engineered a *very* powerful light source since it is often necessary to apply short, powerful bursts of light in some applications - but this means it gets very hot if left on for long periods!
5. Do not run the heating/thermostat functionality without a test tube *with liquid* inserted in the reactor as it may be possible to over-heat the heating element. Similarly, always make sure the test tube is pushed down fully within the reactor until you feel it touch the heat plate at the base.
6. Test your pumps before running any experiments to ensure they are all moving liquid as anticipated. If not add some tape to the pump head as described in the “Mechanical Assembly Guide (for purchased devices)” on the Hardware page of the Chi.Bio website.
7. Do not cover the reactor top/sides with aluminium or metal foil since it could accidentally touch and short-circuit connections, damaging the device.
8. If you are having issues with contamination of your samples use the 3D printed lid designs from the Chi.Bio website (both for the reactor, and fresh media jars) which, if printed with suitable autoclavable plastic and additionally connected to input air filters (e.g. disposable syringe filters or similar) can make a fully sterile system.
9. Read this entire manual from start-to-finish including the troubleshooting section (seriously!).

2 Platform Setup

2.1 Connecting Hardware

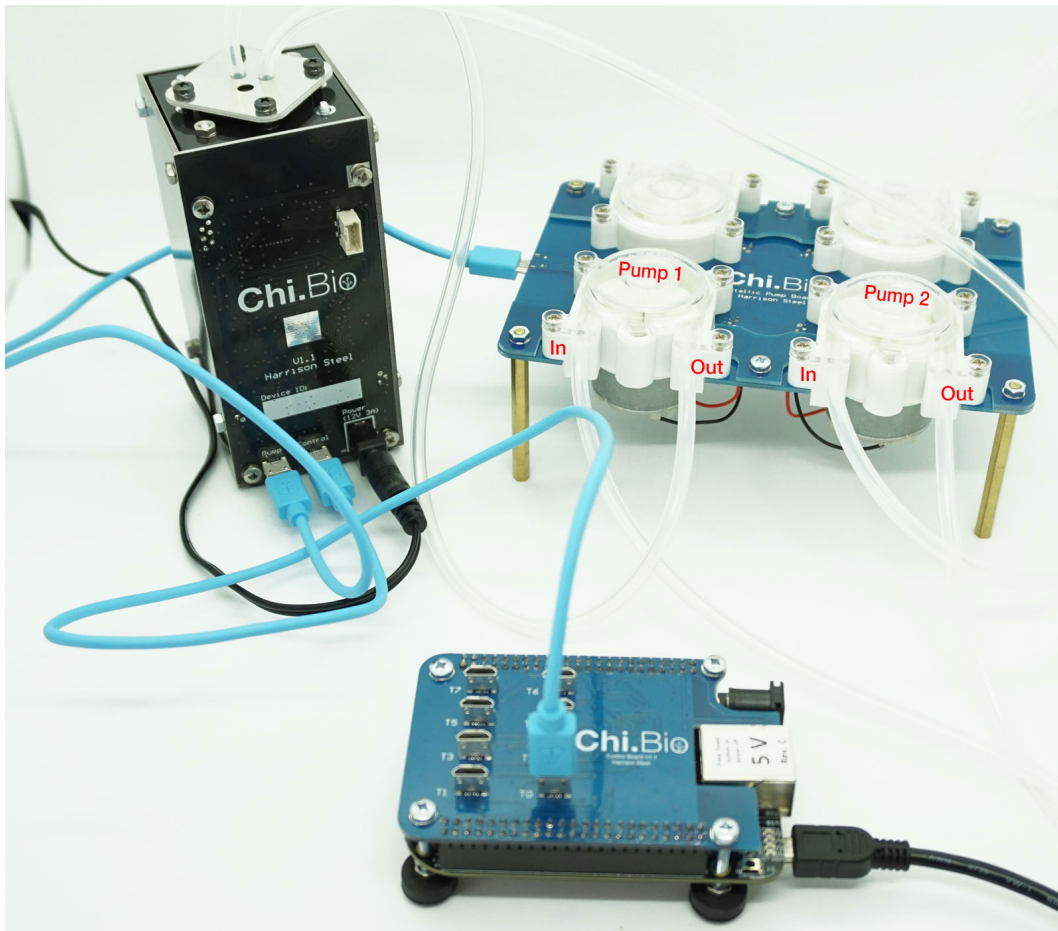


Figure 1: Chi.Bio with all electronic/pump connections. Pump default input/output directions are labelled.

1. Plug control computer into PC using Mini-USB cable.
2. Plug each Reactor into the control computer with Micro-USB cable, and each reactor into its pump board.
3. Plug each reactor into 12V wall power supply and turn on. **Important:** This should always happen *after* steps 1+2 above (that is, don't mess with USB cables while it is powered on) to protect all electronics as best as possible.
4. Position the reactors on your bench such that the liquid level in the reactor will be above the liquid level in either the fresh media and waste media containers at *all* points in an experiment. It must therefore be above the fresh media level at the start of an experiment, and also above the height of liquid in the waste media container assuming all the fresh media ends up there. An easy way to do this is to put the reactors on a small box/ledge. The purpose of this step is to ensure that in the rare case that a pump makes a poor seal or fails during operation, liquid will drain into media reservoirs *rather than in to the reactor itself*.

2.2 Starting Software

1. Start Putty software on connected lab PC. Connect to IP 192.168.7.2 and port 22. Log in with username:password root:root. If the Putty connection does not work you may have not given the control computer sufficient time to boot up (takes ~ 30 seconds from the time it is plugged into the PC).
2. Set the date and time by running command `date -s "Jan 1 2019 13:00:00"`, adjusted for the current date and time. This needs to be done whenever the control computer is started up (it has no battery to keep time when disconnected).
3. Execute command `cd chibio` to enter the Chi.Bio folder in the device's operating system.
4. We now start the Chi.Bio operating system by executing the command `bash cb.sh`. This will take a few seconds, and should put a message in the Putty terminal for each connected reactor as it boots. Leave the Putty window open whilst the device is running. You can stop the Chi.Bio operating system by selecting the Putty terminal and pressing `ctrl+c`.

3 Experiment Setup

1. Open a web browser on the attached PC and navigate to `http://192.168.7.2:5000`, you should see the platform's user interface. In the top left are buttons for each connected device (greyed out for those that are not present).
2. For an explanation of each button in the UI see Section 8. Subsequent sections will assume you have read this section of the guide.

3.1 Sterilisation

1. Sterilise test tubes and stir bars with any method of your fancy.
2. Sterilise reactor lid using alcohol + swabbing.
3. Sterilise pump tubing by pumping alcohol through each tube for ~ 10 seconds, followed by fresh media to flush the alcohol out. Leave the fresh media pump (Pump 1) full of media, but pump all media out of the waste pump (Pump 2). Note that pump heads by default rotate clockwise, and as such have input/output directions as in Fig. 1.
4. Sterilise the exterior of each pump tube's end (5-10 cm or so) with alcohol + swabbing.
5. Push the output end of the fresh media tube approximately 1cm through the reactor lid. Ensure that the end which is going into the lid is the end from which new media will come out.
6. Push the waste pump's input tube 4-5cm through the reactor lid. The distance this is pushed through will determine the steady state level of media in the test tube.
7. Put the waste media tube's output end in a waste container. Put the fresh media tube's input end into the fresh media container.
8. For the first few experiments it may be wise to tape the fresh media tube at the rim/top of the fresh media vessel (to prevent it from moving), since the pumps may tug the tube through the pump head slightly while settling.

3.2 OD Calibration

OD calibration needs to be performed on each device when a new growth media is used in order to zero the density measurement. Calibration values can then be carried over to future experiments on that reactor.

1. Fill a test tube with solution of known OD; either your plain media with “zero” OD (no cells), or media+cells that has been measured in a spectrophotometer or similar. Shake solution to ensure it is well mixed.
2. Clean the sides of test tube (to ensure transparency) using alcohol + swabbing (and dry it!), and put it into reactor.
3. Wait \sim 60 seconds or so for the solution to settle.
4. Click Measure in the OD section of the UI. A new number will appear as the “Raw” measurement below “OD Zero” in the UI. Type this number into the “Measured” box, type the actual OD of the solution into the “Actual” box (which may be zero if its just media), then press Set.
5. The number below “Current” in the OD Zero section will change; this is the new OD calibration value (note it in your laboratory log for convenient reference, though it is also stored in the experiment data files).
6. If you find this OD calibration approach is not sufficiently accurate for your application, use the more accurate (but less convenient) method described in the Troubleshooting section at the end of this document.

3.3 Culture Setup

1. Put your inoculated media into a 25ml glass test tube, add stir bar.
2. Swab the tube’s exterior with alcohol (particularly the mid-section where measurements are made) and dry it. Any marking on the outside of the tube can affect measurements.
3. Put inoculated test tube into the reactor, secure lid on two opposite corners with screws.

3.4 Set Experimental Options

1. If you havent just done OD calibration, put previous calibration value into UI set it as 0 OD measurement.
2. Set desired control parameters such as stirring rate, OD set-point, thermostat set-point.
3. The thermostat and stirring will be automatically enabled once an experiment starts, so you don’t have to turn them on (with “Switch”) now. **Make sure** you don’t turn on the thermostat when no test-tube is in place as it may burn out the heating element.
4. Set up fluorescent protein (FP) measurements: Select excitation LED, select measurement wavelengths (generally leave Baseband as CLEAR), select gain (x512 works in most cases), and then click “Active” to activate the fluorescent protein measurement. For example, to measure GFP optimal settings would be excitation at 457nm, Baseband Clear, Emit1 at 550nm (or 520nm), Gain x512. Emit 2 can be set OFF (or to another wavelength if you want to record at an additional wavelength).
5. You can test the fluorescent protein measurements by clicking “Measure Fluorescent Proteins” at the top of the UI.

4 Running an Experiment

1. Begin an experiment by clicking “Start”. This starts a standard automated experiment as described on the Chi.Bio software page (<https://chi.bio/software/>). Note that a standard experiment does not automatically enable OD regulation (turbidostat functionality).
2. If you want OD to be maintained at a given set-point click “Switch” in the OD regulation section. Note that doing so will prevent manual control of the input/output pumps. When in Turbidostat mode Pump 2 (removing waste media) will always run at a faster rate than Pump 1 to ensure the media level is maintained at the appropriate height.
3. You can click Stop at any point to stop or pause the experiment. When stopped you can remove/change the test tube if required. You can resume a stopped experiment (but allow it to remain in the stopped state for > 30 seconds) by clicking Start again.
4. A stopped experiment can also be reset, which will clear all setup parameters and data for the current reactor only (i.e. other reactors will continue running). Note, resetting will only clear the data show in the user interface; it won’t clear/delete the experimental data which is stored automatically on the control computer.

5 Finishing an Experiment

1. Once a given reactor’s experiment has finished click stop in its UI to pause its experiment.
2. Remove lid and test tube.
3. Pump alcohol through all pumps used to clean any left over cells (and prevent them growing in the tubes when they are not in use!). Generally it is good practice to leave all pump tubes full of alcohol between experiments to minimise chances of cross-contamination.
4. If you are done with all devices then shut down the Chi.Bio software (press ctrl+c in the Putty terminal) and unplug each reactor’s power supply. The control computer can be happily left plugged in/on indefinitely.

6 Retrieving Data Files

To retrieve data files from an experiment use Filezilla or similar FTP software to log into the control computer (IP: 192.168.7.2, Port: 22, username:password root:root) and navigate to /root/chibio. All experimental data is stored here and named according to the time of experiment start and device it was run on.

Each experiment generates two data files:

Setup file: This .txt file includes all setup parameters, the experiment’s start time, and the reactor’s hardware ID. It is formatted as a JSON, which can be easily imported in many programming languages (e.g. in MATLAB run `file = uigetfile('*.txt');` `fileID = fopen(file,'r');` `A=fscanf(fileID,'%s');` `B=jsondecode(A);`).

Data file: This .csv file stores all measured data and control parameters. The default configuration of its columns is as follows. Note that thanks to Zoltan Tuza the column labels should now *also* appear at the top of the output file:

Column	Parameter
1	Experiment time (s)
2	Measured OD
3	Turbidostat OD setpoint
4	OD zero setpoint
5	Thermostat setpoint
6	Heating rate
7	Internal air temperature (C)
8	External air temperature (C)
9	Media temperature (C)
10	Optogenetic actuation intensity
11	Pump 1 rate
12	Pump 2 rate
13	Pump 3 rate
14	Pump 4 rate
15	Volume
16	Stirring rate
17	395nm LED set-point
18	457nm LED set-point
19	500nm LED set-point
20	523nm LED set-point
21	595nm LED set-point
22	623nm LED set-point
23	6500K LED set-point
24	Laser set-point
25	UV LED intensity
26	Fluorescent protein 1 baseband
27	Fluorescent protein 1 emission band 1
28	Fluorescent protein 1 emission band 2
29	Fluorescent protein 2 baseband
30	Fluorescent protein 2 emission band 1
31	Fluorescent protein 2 emission band 2
32	Fluorescent protein 3 baseband
33	Fluorescent protein 3 emission band 1
34	Fluorescent protein 3 emission band 2
35	Custom program parameter 1
36	Custom program parameter 2
37	Custom program integrator

7 Other Protocols & Features

7.1 Adding a Chemical Inducer

An inducer can gradually be added using Pump 3 or 4 by mixing it in with a second fresh media (which includes the inducer). However, to get a step-change in inducer concentration it should be added manually to the reactor chamber and also to the input fresh media. To do so accurately it is wise to use a fresh media flask that has marked volume measurements, or swap the input tube into a different media flask with known inducer concentration.

7.2 Sampling During an Experiment

Samples can be extracted through the reactor lid using a syringe or long-tip pipette. Alternatively, the experiment can be paused, lid removed, and samples taken if required.

7.3 UV

The UV light can be enabled in the user-interface by the user, and won't be automatically de-activated during an experiment's automated running. Due to its low wavelength it doesn't interfere with OD/fluorescence measurements.

7.4 Optogenetics

There is a rudimentary optogenetics function implemented in the UI, which will activate a user-selected LED for part of each 60 second experiment iteration (when measurements are not being made). More complex optogenetic control can be implemented via custom programs (below).

7.5 Custom Programs

Custom programs can be written in Python in the operating system's back-end. These can then be selected and enabled in the UI.

8 User Interface Reference

8.1 Upper

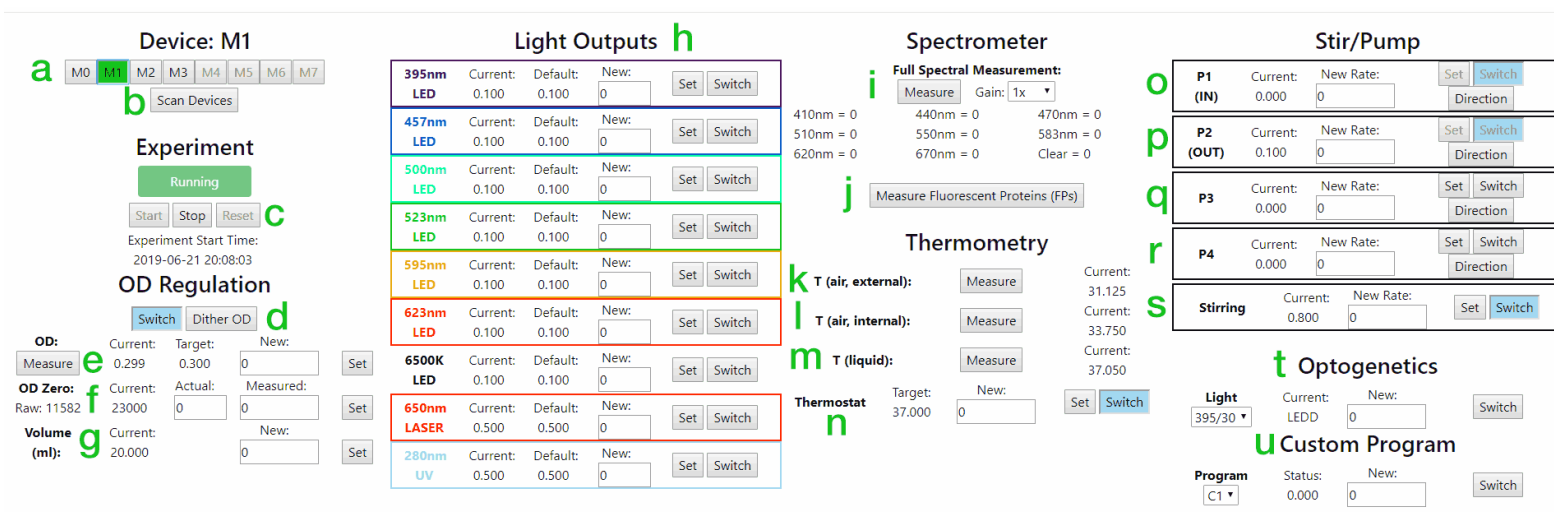


Figure 2: Operating system upper control panel.

- Turbidostat Selection:** Clicking an active button will switch to view of that turbidostat's controls/data. Note that all values for parameters should update when you do this, but values in the user-input boxes will not.
- Scan:** The Scan Devices queries all eight possible connected devices, and can be pressed to recognise new devices if they are plugged in during an experiment.
- Experiment Start-Stop-Reset:** When an experiment is running (i.e. started) this box is green. You can stop the experiment as required, and reset the setup/data whilst it is stopped. You will never delete data by resetting an experiment; all past data is recorded in external data files.
- OD Regulation Switch:** Enables turbidostat functionality. The "Dither" button makes growth follow a zig-zag pattern near to the OD set-point for growth rate estimation.
- OD Regulation Controls:** Used to manually measure OD and set a new target for the turbidostat algorithm. To set a new value type it into the New box and click Set.
- OD Zeroing Controls:** Operated as described in Section 3.2. When calibrating the Raw value displayed at the left of this control should be typed into the Measured box, and the actual value that this corresponds to in the Actual box, then click Set.
- Volume:** Approximate volume of experimental media in the test tube. This is used as a tuning parameter on the OD regulation and can generally be left as 20ml. Note that this does NOT set the physical volume of media in the test tube, which is determined solely by the depth of the waste media removal tube.
- LED/Laser Controls:** Each works essentially the same; an intensity between 0 and 1 can be input to the New box and then Set as this output's power. Switch turns that item on/off.
- Manual Spectrometer Measurement:** By selecting a gain and clicking Measure the current spectrum is measured and values at each wavelength are presented below.
- Measure Fluorescent Proteins:** Thus button performs all fluorescent protein measurements that are currently activated and outputs their values in the lower part of the UI.

8.2 Lower

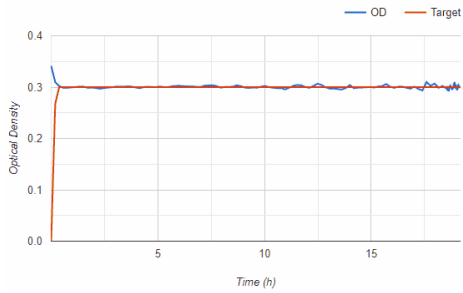
- k. External Air Temperature: Measured on the control computer board.
- l. Internal Air Temperature: Measured on the horizontal PCB within the reactor.
- m. Media Temperature: Measured by the infrared thermometer in the reactor.
- n. Thermostat: Change the set-point with Set, and switch on/off with Switch. The Thermostat will enable/disable automatically when an experiment starts/stops. DO NOT run the thermostat when no test-tube is in place as it may burn out the heating resistors.
- o. P1 (Input) Pump Rate: To achieve slow pumping rates pumps are run for a short period during each 60 second cycle. Pumps can be set to a rate between -1 and 1, clicking the Direction is equivalent to multiplying the current rate by -1. Manual control of P1 and P2 is disabled when OD regulation (turbidostat functionality) is enabled.
- p. P2 (Output) Pump Rate: Similar to P1.
- q. P3: Additional pump rate which can be manually controlled / programmed.
- r. P4: Additional pump rate which can be manually controlled / programmed.
- s. Optogenetics: Rudimentary optogenetics function which allows a given LED to be periodically enabled during an experiment at the set intensity.
- t. Custom Program: Selectable custom functionality can be programmed into the operating system, see examples in the Python code. For more information on how to implement one see the project Github.

8.2 Lower

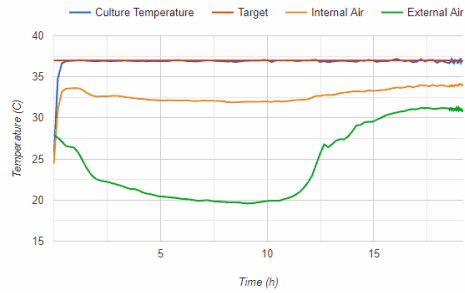
- a. OD: Real-time plot of OD and target OD (which will be zero if turbidostat functionality is disabled). The data plot in this (and all other) graphs is interpolated to lower resolution each 3 hours (to prevent the UI from becoming too laggy). Consequently, for long experiments it will look like recent data is being collected at a higher frequency. Note that only displayed data is interpolated; that stored in the experiment record is not altered.
- b. Thermometry: Plot of each thermometer's reading as well as the Thermostat set-point.
- c. Pump rate for Pump 1 (Input).
- d. FP Activation: This button must be enabled for a fluorescent protein measurement (set up below) to happen automatically during an experiment.
- e. Excitation LED: The LED used for excitation can be selected from the drop-down box.
- f. Baseband Filter: Used to normalise by the total excitation intensity, can generally be left as Clear. The most recently measured Baseband value is displayed below the selection box.
- g. Emit 1: First emission measurement filter. The value displayed below this box is the intensity measured in the emission band divided by that measured in the baseband.
- h. Emit 2: Similar to Emit 1.
- i. Gain: Measurement gain can be selected from this drop-down box, which can generally be left as x512 (which allows us to avoid running the excitation LED at very high power).

8.2 Lower

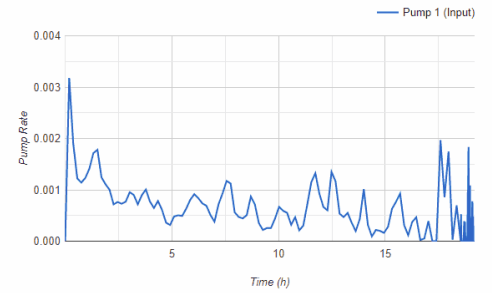
a OD



b Thermometry

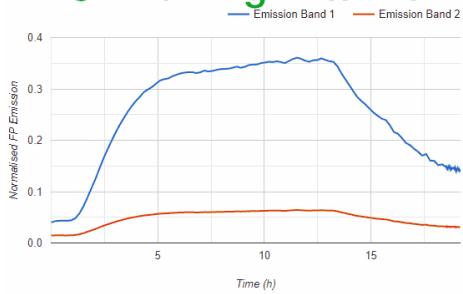


c Pumping



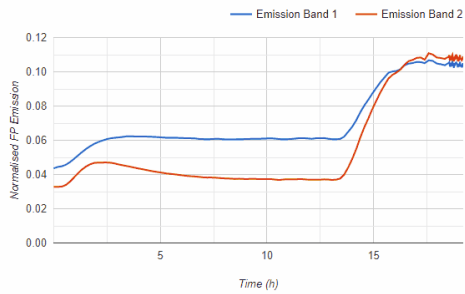
d FP 1
Active

Excite: Baseband: Emit 1: Emit 2: Gain:
 Current: 19702 0.139 0.030



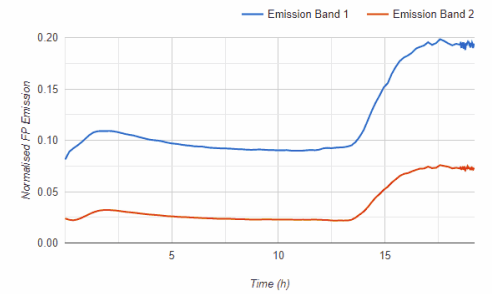
FP 2
Active

Excite: Baseband: Emit 1: Emit 2: Gain:
 Current: 7832 0.105 0.109



FP 3
Active

Excite: Baseband: Emit 1: Emit 2: Gain:
 Current: 5842 0.193 0.073



Status Terminal -

```
2019-06-22 15:19:07 - Cycle 1150 Started
2019-06-22 15:19:07 - Cycle 1149 Complete
2019-06-22 15:18:31 - Terminal Cleared
```

Figure 3: Operating system data plots.

9 Troubleshooting

Operating system wont start: The system has built-in functionality to prevent the software from running if there is an electrical error in *any* of the connected devices. Therefore, you won't be able to start the operating system if (for example) a reactor is plugged into the control computer but is not connected to its power supply, or if (in the process of putting test-tubes into a reactor) you have spilled some liquid onto its top cover. Liquid on the top cover is detected as a short-circuit between the exposed silver tracks - these are quite sensitive, so even *dried* spilled liquids can trigger an error. If the reactor is failing to start and this may be the cause a good first step is to wet a tissue with alcohol and wipe down the moisture sensing tracks on the top of the reactor very thoroughly.

My system starts but sometimes crashes mid experiment: The most likely cause of this (by far) is that some liquid is getting on the moisture sensing tracks as described above. So, first step is to clean these. Failing this, it may be that your lid is not making a particularly good seal with the glass test tube and thus liquid is getting out and around, then dripping down the outside of the test tube and triggering the moisture sensor. If this is a problem the best approach is to add a rubber o-ring to the underside of the 3D printed lid. The optimal specification is something made of autoclavable rubber (e.g. FKM), with approximately 15mm inner diameter and 18mm outer diameter (i.e. o-ring thickness 1.5mm). Here is a suitable item from RS Components: <https://uk.rs-online.com/web/p/gaskets-o-rings/1965666>

My UI is unresponsive: Look at the Putty terminal and check that the Chi.Bio operating system is running.

Pump is making a poor seal on its tube: It may be that the pump's tube was not well-placed within the pump head; see the Hardware section on the Chi.Bio website (<https://chi.bio/hardware/>). If a pump breaks / has a poor seal you may notice that the fresh media is moving slowly backward through its tube toward the fresh media flask; the OD regulation controller is equipped to deal with this to some extent, but to improve performance you can put tape in the pump head as demonstrated at the end of the "Mechanical Assembly Guide (for purchased devices)" on the Hardware page. Another possibility to improve this is to add a one way check valve in the tubing between the pump and reactor, for example we have used part number 7757K41 from <https://www.mcmaster.com/products/one-way-flow-valves/check-valves-/body-material-plastic-1/check-valves-with-barbed-fittings-9/>.

OD regulation algorithm is over/under shooting target cell density: Particularly if this is accompanied by error messages in the Putty window along the lines of "Failed Pump Comms..." then ensure you have updated your Chi.Bio operating system (specifically *app.py*) to the latest version on the Github, which includes fixes for these errors.

I am seeing many errors in the Putty terminal such as "Failed to Disconnect Multiplexer" or "Failed XYZ comms" which either prevent the operating system from starting, or make it crash a few minutes into each experiment: If this is the case it is likely a hardware fault in manufacturing. If you purchased your system from Labmaker let them know about it and they can help you out.

I am seeing many errors in the Putty terminal as above, and the system starts, but pumping is inaccurate and the OD regulation algorithm often over/under shoots: Try updating your Beaglebone with the latest operating system image as described on the Chi.Bio Software page, in which we have made changes to the operating system to help it deal with infrequent errors in digital signalling that can arise in noisy lab environments.

I put a test-tube into the device and the OD reading is not what I expected/calibrated: This may occur if the exterior of the test tube is dirty, even a small amount of dried water that is inopportunistically placed can cause a deviation in the measurement. Make sure the exterior of every tube is swabbed (and dried!) before putting it into the device.

My OD regulation algorithm is enabled, and Pump1 is running every experiment cycle, but OD isn't decreasing: There may be a biofilm on the inside of the test tube which makes the OD appear to be significantly higher than it is. For experiments maintained at low to moderate OD, and running up to a few days, biofilms are unlikely. However, they seem to be exacerbated by allowing cells to get to very high densities, by leaving the culture sit without stirring for extended periods, or by using certain combinations of media/cells. Several users have also had issues with certain media types (e.g. some M9 mixtures) forming precipitates on the sides of the test tube eventually leading to inaccurate measurements. Another potential cause of OD regulation not working (though Pump1 is running) can be that the fresh media supply has become contaminated - meaning no matter how much you pump in the OD inside the reactor will not decrease!

My stir bar is not working very well: Many stir bars work with Chi.Bio, but some may be ineffective due to two failure modes. 1) A large stir bar with a very strong magnet or unfavourable shape may be too difficult for the stirring motor to move and hence cause it to stall; no stirring will occur. 2) Stir bars with very weak magnets may “jump” in the test tube and not make an effective vortex. If one of these issues arises we recommend using a different stir bar, or running it at a different speed (generally somewhere around 0.6-0.7 is ideal in many cases and gives sufficient aeration even for fast growing organisms). If you want to see how well a particular stir bar works disconnect the reactor, take off its left and right side panels, and then run the stirring. Note that most recent versions of Chi.Bio have an updated stirring assembly allowing them to accommodate larger/heavier stir bars without stalling.

My OD “zero” point is not as accurate as I require: The OD calibration method described in Section 2.2. above is the easiest, but not most accurate approach to calibrating OD measurements. If you want to make the calibration as reliable as possible generally the best approach is to 1) Put your test tube of media (and stir bar) into the reactor. 2) Turn on the thermostat and stirring in the reactor and let the media (only) heat up to your target temperature. 3) After 5-10 minutes do the OD calibration (stop stirring first) with your test-tube fixed in place and at the appropriate temperature. 4) Pipette in your sample of cells (i.e. through a hole in the lid) and then “Start” the experiment. This approach minimises errors due to both misalignment of the tube/laser/sensor (since it is calibrated *in situ*), as well as any temperature dependence of the laser/sensor.

Over time my pumps seem to change behaviour: The peristaltic pumps will eventually wear on the tubing, potentially giving a poor seal. It is therefore wise to change out the tubing after several months of heavy operation. You can either completely swap out the tube (i.e. as done during assembly), or gently pull the existing tube through the pump head to put a fresh part inside the pump (this is most easily done by turning the pump on at rate 1 then pulling *with* the direction it is rotating).