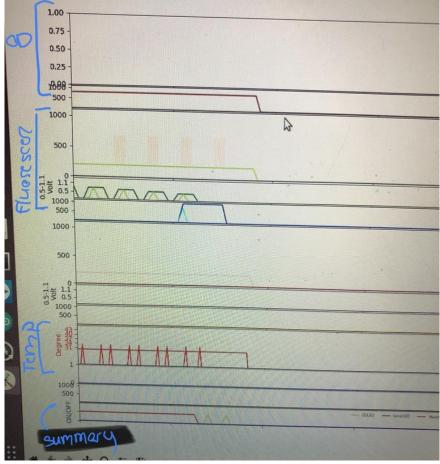
## **Checking on a Fluorostat Run**

- 1) Check that there is still media left in the media bottle (metal capped bottle in the plastic tub)
- 2) Make sure the fluorostat isn't currently taking a measurement
  - a. If you can see the lights are on, the device isn't taking a measurement
  - b. Also, the matplotlib graphic shows you the current state of the device. The top plot shows OD measurements, the second shows fluorescent measurements. The summary graphic at the bottom also shows what functions the device is currently doing. The top two plots should have no peaks (the temperature is always adjusting so it's fine if that is changing), and the summary chart should have the stir state on (green peaks)
  - c. It isn't a big deal if the box is off when measurements are taken, that measurement will be off but it won't hurt the run or device. If the lights switch off while the box is off, try to put it back on but it's ok if you don't.

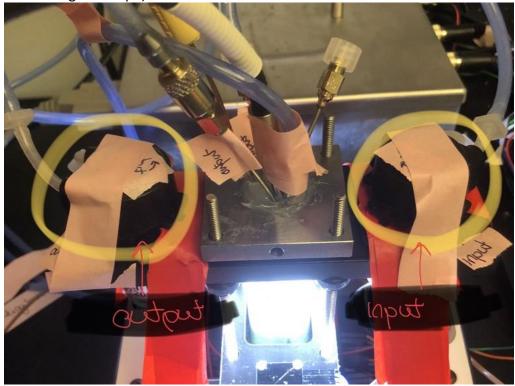


- 3) Take the box off the fluorostat
- 4) Check that nothing is leaking or overflowing (this isn't likely, never happened before but I still like to check)
- 5) Make sure the pumps are still attached
- 6) Check that liquid level is where is should be
  - a. It's totally fine to pick up the vial to get a closer look. Because of all the tubing you can't pull it too far out of the sample holder, but the device and measurements are pretty robust to bumps and readjustments.
- 7) Check that it is still bubbling (see video for target bubbling)
- 8) Check that there isn't substantial wall growth, especially blocking the OD LED path.

# Common things that go wrong and how to fix them:

## Pumps fail/fall off

Pumps consist of a motor, then a plastic housing for the tubing that sits on the motor. Occasionally the housing will pop off of the motor and the pumps fail. This can be pretty easily fixed by replacing the housing on the motor (and securing with tape).



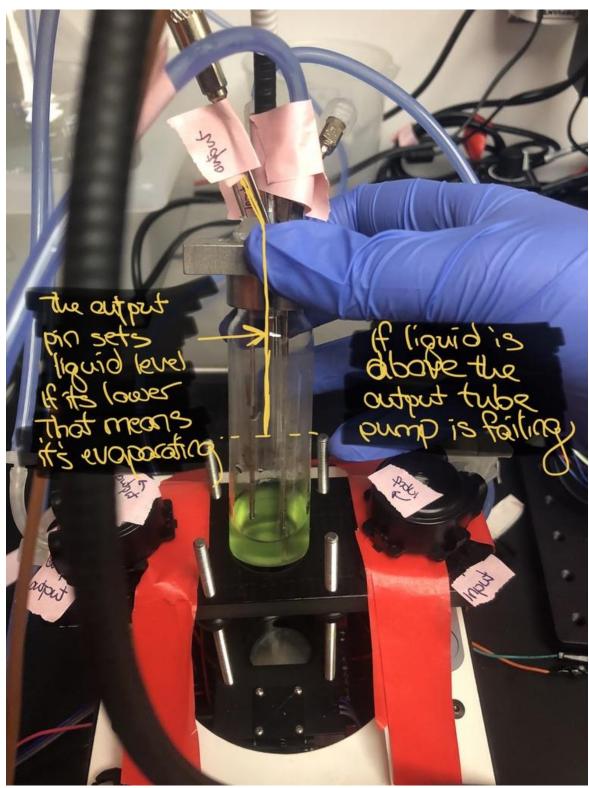
If a pump failed but didn't pop off (which you would see either by the code switching to batch mode or by the liquid level rising/falling) the code likely needs to restart. I will usually try just running the pump code to see if they are individually working. If that doesn't work you can give me a call.

- Kill the current run
- turn on the pumps by uncommenting the following lines of Arduino code and upload (no need to run the python script)
- o Comment out the pump commands and uncomment the run mode of choice and restart

```
// the loop routine runs over and over again forever:
void loop() {
    //-------
    //Choose one function to set turbidostat mode:
    //------
    //turbidostat(target_OD);
    //turbidostat_cyano(target_OD);
    //fluorostat(Fluorostat_target_channel, Target_fluoro[Fluorostat_target_channel]);//(channel (0 or 1), gain, target re
    //Pump_for_Exp_Start();
    //rolling_measure();
    //ODbatch();
    //Thorlab();
    //batch();
    //batch();
    //pump_out(5);
    //pump_in(5);
    //wait(10);
```

# **Evaporation**

Especially when the air is set too high or the cells are growing too slowly, the vial volume can evaporate away. Fixes for this include checking that the media input pump is working (as described above), and making sure that there is enough aeration. After making these adjustments, you can turn the pumps on as described above to get back the appropriate liquid level, and then restart a run. If neither of these fixes work it's probably a growth limitation that doesn't have an easy fix... This hasn't been a problem in the turbidostatic aeration runs though.



#### **Culture washes out**

When this happens the run needs to be restarted. I will often do a "quick and dirty" restart where I:

- o Kill the current run
- turn on the pumps by uncommenting the following lines of Arduino code and upload (no need to run the python script)
- Let it run for maybe 30s or so to flush out the current media in the sample
- o Pipet in ~5mLs of healthy actively growing culture into the sample vial directly
- o Comment out the pump commands and uncomment the run mode of choice and restart

I typically have entrained cells actively growing while I'm doing a run in case this happens but the Percivals are currently set to constant light so this isn't the case. If there is a washout event/failure we will probably need to start a culture growing in constant light and then entrain in the fluorostat.

```
// the loop routine runs over and over again forever:
void loop() {
 //Choose one function to set turbidostat mode:
 //-----
 //turbidostat(target_OD);
 //turbidostat_cyano(target_OD);
 //fluorostat(Fluorostat_target_channel, Target_fluoro[Fluorostat_target_channel]);//(channel (0 or 1), gain, target re
 //Pump_for_Exp_Start();
 //rolling_measure();
 //ODbatch();
 //Thorlab();
 //batch();
 //OD_calib();
 //pump_out(5);
 //pump_in(5);
  //wait(10);
 //ODbatch_light();
 //ODbatch_light_LD(starttime, transtime, delaytime);
 //ODbatch_light_air();
 //OD_signal_read();
 //ODbatch_light_air_PMT();
 //AirPin_ON();
 //digitalWrite(AirPin, HIGH);
 //turbidostat_cyano_air_LD(target_OD, starttime, delaytime, counter);
 turbidostat_cyano_air(target_OD,counter);
 //chemostat_cyano_air(chem_pump_interval,chem_starttime);
```

### "biofilm" growth on the vial

I sometimes see growth accumulate on the vial, especially after long runs. So far I haven't seen cells accumulating in the tubing (although that could change in longer runs). To fix this one could either clean the whole set up or simply replace the sample vial. I propose we would just replace the vial, at least while I'm away. I have clean (but not yet autoclaved) extra vials on my bench. We can just pipet the culture from the current vial to the new clean vial, but use the same tubing and media.

#### Media runs low

While I don't anticipate media running out, if it does there is more freshly made media sitting on my bench. The media can be replaced by switching out the bottles. The source media bottle is the one with the metal cap.

# Location of Stuff you may need

