**FLUOROPROBE CALIBRATION CHECK SOP**

**Last updated:** 02JUN20 by MEL

**Important note on purpose of this SOP:** If you have never used the FluoroProbe before or are not familiar with basic operation in the field and lab, this document is NOT the place to start. You should read the “Flora\_Cheatsheet” housed under LabDocuments on the CareyLabVT GitHub first. You should also read the FluoroProbe manual (hopefully kept in the same room where the FluoroProbe is housed in Derring). This SOP is not intended to either provide a beginner’s introduction to how to operate the sensor or to describe its principles of operation and software interface in great detail. Rather, once a user has become familiarized with the instrument, principles of operation, and basic functionality of the software, this SOP details current calibration procedures specific to the Carey Lab at Virginia Tech. As such, this SOP is under continuous development and nothing written here should be considered a substitute for thinking critically about the problem yourself or deter you from coming up with your own procedures if they are a practical improvement over what’s described here! :-) Make it your own!

**Overview:** When the FluoroProbe (FP) returns from bbe-moldaenke after maintenance, it’s best practice to check all functions of the instrument EVEN IF maintenance was only performed on one part of the instrument. We have had issues in the past in which instrument settings during FP’s time with the manufacturer resulted in altered temperature and algal calibrations, so it is important to go through this complete process every time she returns from Germany. This document describes how to check the algal fingerprint calibrations, the temperature calibration, and the depth sensor.

1. Check temperature calibration first; complete both lab and field checks
   1. In the lab (if doing this at home, you could do this in the bathtub!)
      1. Prepare two water baths (you can just use a bucket) at two different temperatures. I usually do one at room temperature and one with ice in it.
      2. Put the FP in benchtop mode (instructions below in **Other notes**).
      3. Open the bbe++ software and click “Start measurement” and “Stop measurement” to start and stop measurement. The temperature reading will show up in the software interface data table. You may have to scroll to the right to see it.
      4. Using a YSI or similar temperature sensor, take a reading with the FP in each of the water baths and compare the value to the temperature sensor immersed in the same bath.
      5. If temperature readings do NOT match, you can perform a temperature re-calibration; the instructions for that are in the FP manual. However, they really should match if the instrument has just come back from the shop, so if they don’t I would recommend sending an email to Cayelan and also to PP Systems to get in touch with the appropriate technician at bbe-moldaenke. The email would go to: Bob Fowler ([bf@ppsystems.com](mailto:bf@ppsystems.com)). You can also email bbe directly if you already have a contact there, but I’ve found the PP Systems has less turnover than bbe so Bob at PP is probably the best place to start.
      6. **Save your FP files from the temperature calibration process in both proprietary (.bdb or .flp) and non-proprietary (.txt) formats for future reference as needed. If you end up needing to send them to bbe for troubleshooting, you’ll need proprietary files. If you want to manipulate them later in R, non-proprietary is easier. If you’ve never saved files from the FP before, see the FluoroProbe cheatsheet or field day instructions for further information. We don’t currently have a specified lab repository for calibration files like this but if you want to create one, check in with CCC about her preference for where these should be stored and then have at it! :-)**
   2. In the field (Duck Pond or WVWA reservoir)
      1. Take a cast with the FP in conjunction with a CTD cast at the same site and roughly the same time (within one hour).
      2. Download and compare the two temperature profiles. You can both visually compare the profiles and do a little data manipulation in R to come up with a correlation plot by depth. Obviously the correlation plot by depth strategy is more rigorous. You can also calculate RMSE. Check with CCC about her preference for how this should be done; our standards here are evolving.
      3. **Save your FP files in both proprietary (.bdb or .flp) and non-proprietary (.txt) formats.**
   3. Notes from previous calibration years:
      1. In 2020, when compared to the SCC sensors, two separate FP casts had a combined RMSE of 1.3 degrees C, and this was deemed acceptable
2. Check depth sensor
   1. In the lab
      1. There is not a great way to do this in the lab in my opinion.
      2. However if you really want to do a lab test, you could:
         1. Fill a bucket or trash can with water
         2. Put the FP in field mode
         3. Slowly lower the FP in and out of the water several times
         4. Download the file and see if the FP records being submerged (positive depth value) vs. out of the water (negative depth value)
         5. **Save your FP files in both proprietary (.bdb or .flp) and non-proprietary (.txt) formats.**
   2. In the field (Duck Pond or WVWA reservoir)
      1. Take a cast with the FP in conjunction with a CTD cast at the same site. By “in conjunction” I mean at the same time and lowered together best as possible.
      2. Download and compare the two depth profiles. If you’ve lowered the two sensors at the same time, you should be able to use the timestamp to help you verify that the depths are lining up. I do not currently have a standardized method for this beyond visual comparison so this would be a great area for further SOP development if you are so motivated.
      3. **Save your FP files in both proprietary (.bdb or .flp) and non-proprietary (.txt) formats.**
3. Check algal fingerprint calibration: Perform both A and B.
   1. In the lab
      1. Note: You can use the lab Scenedesmus culture to test the green algae fingerprint as an alternative to buying cultures from Carolina Biological for that group.
      2. Coordinate with Cayelan/Bobbie to order cultures from Carolina Biological to cover the “green”, “bluegreen”, and “brown” spectral groups, for example:
         1. Greens: Chlorella, Item # 152069, <https://www.carolina.com/algae/chlorella-living/152069.pr>
         2. Browns: Gymnodinium, Item # 153260 <https://www.carolina.com/algae/gymnodinium-living/153260.pr?question=>

(you could also order chrysophytes; we see plenty of those in the WVWA reservoirs too and they are also in the “brown algae” spectral group)

* + - 1. Cyanobacteria: Anabaena, Item # 151710 <https://www.carolina.com/cyanobacteria/anabaena-living/151710.pr?question=>
    1. Put the FP in benchtop mode (instructions below in **Other notes**). Take the field cover off the FP and put the FP in the benchtop holder. Refer to the FP manual for instructions as to how to set up the FP in the benchtop holder. I don’t have pictures of all this to add right now, but again, if you are so motivated that would be a wonderful addition to this SOP in future.
    2. **Method A: Dilution method - do this every time the FP returns from Germany**
       1. Prepare a “primary standard” for the cell culture you are working with by adding a fixed volume of water (up to the marked line on the cuvette) and a fixed volume of algal culture (it doesn’t take much! I’d start with 1 mL and then adjust as needed) to the benchtop cuvette of the FluoroProbe
       2. Cover the top of the cuvette tightly with Parafilm and invert several times.
       3. Put the cuvette in the benchtop holder slot.
       4. Turn off the lights.
       5. Take several (at least three) repeated benchtop readings with the FP. Invert the cuvette between readings.
       6. **Ensure that the readings are not over 500 ug/L, as the instrument is only calibrated to 500 ug/L. If the readings are too high, start over and inoculate the cuvette with less culture.**
       7. Dilute the algal culture in the cuvette by a factor determined by YOU, depending on the initial concentration of the sample (which is very hard to predict *a priori* as it depends on the initial concentration of the culture). Your ultimate goal is to get one or two measurements at field-relevant concentrations of each fingerprint group. In our reservoirs, this would be the following approximate ranges:
          1. Green algae: 5-50 ugL
          2. Brown algae: 5-100 ugL
          3. Cyanobacteria: 2-200 ugL
          4. Cryptophytes: 2-70 ugL
       8. In general, the process of dilution works like this:
          1. Decant (fancy science word for pour) your “primary standard” (first dilution) into a clean container and temporarily set it aside.
          2. Rinse the FP cuvette and refill with water to the volumetric line.
          3. Add a \*small\* amount of the primary standard to the cuvette (remember, the exact volume is a matter of your judgment based on concentration of the first dilution!!).
          4. Ex: in 2020, when I didn’t have access to proper volumetric equipment because of COVID, I diluted by adding ¼ tsp (approximately equal to 1.2 mL) of my primary standard to the water-filled cuvette. That resulted in approximately a tenfold dilution. But again, this will vary depending on the initial concentration of your culture and the type of phytoplankton!

* + - 1. Repeat steps b-e.
      2. Repeat step h, for a second dilution.
      3. This should give you a series of 2 serial dilutions of the original solution made in step a (3 readings total) which you can compare to the values reported by the FP for the relevant spectral group. Again, you are essentially just checking to make sure that you get proportional dilution:concentration differences. Since you’ve diluted using the same volume twice, you shouldn’t see decreases in concentration by a factor of 3 between the first and second dilution followed by decreases by a factor of 7 between the second and third dilutions.
      4. Repeat steps a-j for the other two cultures.
      5. If desired, this method could also be used to test ratios of multiple spectral groups combined together (i.e., create a “primary standard” with 7 drops of “brown algae” culture and 3 drops of “green algae” culture).
      6. **Save your FP files in both proprietary (.bdb or .flp) and non-proprietary (.txt) formats.**
    1. **Method B: Cell count method - check with CCC as to whether this is necessary**
       1. To further verify the results of the dilution method or if there are issues with the Method A above, you may choose to prepare slides of each algal cell dilution to count under the microscope.
       2. Keep in mind that the biomass:biovolume of different taxa are likely to vary.
       3. If desired, this method could also be used to test ratios of multiple spectral groups (i.e., create a “primary standard” with 7 drops of “brown algae” culture and 3 drops of “green algae” culture).
       4. **Save your FP files in both proprietary (.bdb or .flp) and non-proprietary (.txt) formats.**
  1. In the field (Duck Pond or WVWA reservoir): check with CCC about preferences between Method A, Method B, or both. We have done both in the past.
     1. **Method A: Comparison to other sensors**
        1. Take a cast with the FP in conjunction with a CTD cast at the same site and roughly the same time (within one hour).
        2. Download and compare the “total phytoplankton” profile from the FP with the chl-a profile from the CTD.
        3. Note that we have never observed a 1:1 relationship between these two instruments; the CTD tends to report lower biomass. However, there should be a linear relationship between the values for the two instruments and both should show roughly the same dynamics (e.g., similar depths of peaks, even if their magnitude is slightly different)
        4. Ideally, this comparison should be done using data from several different days at different reservoir sites.
        5. Keep in mind that the CTD can’t “see” the same pigments as the FP; so, for example, the CTD does not do a good job of picking up the deep cyanobacterial bloom at BVR because it does not measure phycocyanin or phycoerythrin. This can lead to reporting differences between the two instruments depending on the algal community structure.
        6. **Save your FP files in both proprietary (.bdb or .flp) and non-proprietary (.txt) formats.**
     2. **Method B: Comparison to chl-a measured on spec**
        1. Take a cast with the FP.
        2. Within an hour, collect several 2L depth samples in dark bottles to be analyzed for chl-a. At FCR, I usually do 0.1, 1.6, 3.8, and 5 m. At BVR, I usually do 0.1, 3, and 6 m.
        3. Once chl-a samples are analyzed on the spec, compare the “total phytoplankton” values at each depth to the relevant chl-a samples. Do this comparison by plotting an x-y relationship of the two datasets against one another.
        4. Note that we have never observed a perfect 1:1 relationship between these two instruments; however, there should be a linear relationship between the values for the two instruments.
        5. Ideally, this comparison should be done using data from several different days at different reservoir sites.
        6. **Save your FP files in both proprietary (.bdb or .flp) and non-proprietary (.txt) formats.**

1. Check percent transmission
   1. You can do this in the lab, followed by a sanity check in the field
   2. In tap or distilled water in a bucket, transmission should be close to 100%. Transmission is reported in the same data table as algal concentrations, temperature, etc. in the bbe software.
   3. Brew some strong tea (black is best) to mimic DOC
   4. Add to bucket
   5. Transmission should go down. I don’t have an exact estimate for how much it should decrease, since that obviously depends on the strength of your tea :-) If you want to be more quantitative, you could brew teas of different strengths (1 bag vs. 2 bags) and compare. I have never done this, but encourage you to be a pioneer in further developing this SOP to suit your taste!
   6. Field sanity check: in general, since lake water is not tap water or distilled water, transmission should not be 100%. In general, transmission is a little higher in the epilimnion than in region of the thermocline or the hypolimnion (especially later in the summer when there are a lot of particulate/dissolved constituents around the thermocline and in the hypolimnion). While I have not tracked transmission ranges over time for the FP, I would say transmission values below 50% are worrisome (you are in the sediments or something is wrong), and that transmission values that do not vary across depth are probably also wrong, because they should vary as algal concentrations and dissolved constituents vary over depth. Again, this would be a great area for further SOP development if you are so inclined!

**Other notes:**

* **DateTime and timezones:** It’s good to double-check the timezone of the FP when it arrives. It should automatically prompt you to set to the same time zone as the laptop you use to download it. The current preference is to have all sensors set to EST(whatever the field crew wants to use; coordinate with the CTD crew to make sure these are both set to the same time zone!).
* **Putting the FP in benchtop mode:** This setting allows you to take one, fixed measurement at a time that is immediately transmitted to bbe++, so you can see real-time measurements on the lab laptop. This is particularly useful when doing in-lab testing because you don’t have to download each data file in between readings; also, I \*think\* you can provide notes with each reading that describe what it corresponds to (like the dilution factor of a particular algal culture). However, I always recommend taking handwritten notes about what each sample is for as a back-up. The only absolutely necessary setting change
* **Write things down for future you:** Always record what you did!! The FP has a log (marble composition book) in the same drawer as the manual in the super-secret FP closet off of Middle Earth (room with all the freezers between 2025 and Jeb’s lab).
* **What do I do if something looks wrong?** Chat with Cayelan about it first. Double-check your procedure (this may mean repeating the calibration procedure again) and your R code to make sure it’s not just an error on your part. If something really seems amiss, the next step is to email Tim Doyle at PP Systems ([td@ppsystems.com](mailto:td@ppsystems.com)), cc’ing Cayelan, to describe the problem in detail. He will either get you in touch with the appropriate person at bbe moldaenke or act as a liaison with them. **You will probably be asked to provide either .bdb or .flp files, NOT .txt files, to help bbe moldaenke personnel troubleshoot, so be prepared for that.** You may also find it useful to provide plots or other visualizations to bbe moldaenke to help explain the problem. Final tip: be incredibly appreciative and polite and avoid American colloquialisms when emailing with bbe moldaenke folks; they are mostly not native English speakers so often things are lost in translation!