**Division of Watershed Management**

**Department of Environmental Protection**

**627 Main Street, Second Floor**

## Worcester, MA

# Standard Operating Procedure

**Title: SOP for Phycocyanin Cyclops Probe and DataBank Datalogger**

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1.0 SCOPE AND APPLICATION

**Background**

The phycocyanin probe (Cyclops 7, Turner Designs, Inc) and DataBank DataLogger (Turner Designs, Inc) can provide real benefit to emergency responders or others monitoring recreational water bodies or drinking water reservoirs for possible cyanobacterial blooms. Phycocyanin is a pigment found almost exclusively in cyanobacteria ensuring specificity in the results. The probe provides instantaneous results and can provide measurements over a large spatial area quickly. The quickness of the results could help alert public health or drinking water officials to the potential for cyanotoxins to be present in the water. Cyanotoxins are not measured directly by the phycocyanin probe, but there is a relationship between the amount of cyanobacteria present as indicated by the phycocyanin readings and the amount of cyanotoxins that biomass could represent. The phycocyanin measurements are more informative if identifications of the cyanobacteria are made to determine if they are known to potentially produce toxins. The difficulty remains however in that not all cyanobacteria produce toxins and blooms of cyanobacteria look the same whether they are toxic or not.

**What phycocyanin values are of concern?**

Phycocyanin (PC) can be used as a screening tool to determine the presence or absence of cyanobacteria. The phycocyanin readings can also be correlated to another known parameter e.g. cell counts, chlorophyll a or microcystin values, to evaluate the level of risk that may be present for the user of the water body. Literature values for phycocyanin concentrations will be used to help determine if a cyanobacterial bloom is present. Readings of around 100 µg /L phycocyanin indicates a bloom of cyanobacteria is occurring and could indicate a cell count of *Microcystis* sp. of approximately 100,000 cells/ml based upon the calibration concentration. Brient et. al. (2008), for example, examined the relationship between PC µg/L and cell counts of the cyanobacteria *Planktothrix agardhii* . At 98 µg/L of phycocyanin the cell count ranged from 86,691-117,116 cells/ml with a 95% confidence interval . Brient et. al. (2008) found a significant correlation between phycocyanin and cyanobacterial cell numbers (R2=0.73), which indicates that phycocyanin concentrations are a good substitute for cyanobacterial cell counts.

Regulators of drinking water and recreational water quality make use of this relationship. Table 1 includes examples of cell counts limits and likely phycocyanin concentrations that would be found if our Cyclops phycocyanin probe was used. The phycocyanin probe (Cyclops 7), has a detection level of 2 µg/L, so any value above this confirms the presence of phycocyanin. However, differences in cell volumes between different species of cyanobacteria and other environmental factors have made determining a ‘safe’ low threshold difficult. Until more data is collected, readings of 50 µg /L phycocyanin should be considered bloom condition, but whether the 70,000 cells/mL (MA DPH recreational protocol) or the 14 ppb microcystin level have been reached would still need to be confirmed. There are other phycocyanin concentrations that have been used by other agencies or countries. They are included in table 1 as a means of evaluating risk levels.

Table 1: Phycocyanin numbers to aide in interpreting single visit data collection

| Approximate phycocyanin reading µg /L | Approximate Cell Count/ml | Alert |
| --- | --- | --- |
| >2 | >2000 | Alert level 1 NZ drinking water is ~2000cells/ml (New Zealand Ministry of Health 2013) |
| 20 | 20,000 | Guidance Level 1 WHO (Bartram and Chorus 1999) |
| 70 | 70,000 | MADPH advisory level-recreational waters (MADPH 2009) |
| 100 | 100,000 | WHO Alert Level 2-drinking water (moderate risk), Guidance Level 2 recreational water (Bartram and Chorus 1999) |

2.0 SUMMARY

MassDEP personnel receive reports of cyanobacteria blooms from several sources including state agency personnel, watershed associations and lake shore property owners. In most cases, there is little or no warning given prior to the need for immediate sampling and use of the phycocyanin probe to determine cyanobacteria densities. However, some lakes and rivers that have experienced cyanobacteria blooms in the past are re-sampled on a scheduled basis which provides more lead time for response. Operators of drinking water facilities are encouraged to develop monitoring programs for their reservoirs so that developing blooms and other changes in their reservoirs can be observed early and remediation efforts can be initiated.

Phycocyanin will be measured using a Cyclops-7 probe (Turner Designs, Inc). The data will be stored using a DataBanks Datalogger (Turner Design, Inc). Readings will be collected by either wading into the water and immersing the probe to the depth desired or from a dock where the probe can be lowered to a maximum of 5 meters. Phycocyanin measurements will be made and the readings used to determine risk. A phycocyanin reading greater than 2 µg/L –the detection limit- is considered to be positive for cyanobacteria. Readings between 50 and 100 µg/L PC would indicate that the waterbody is experiencing a bloom of cyanobacteria which may contain > 70,000 cells/mL the MADPH criteria when it’s likely that a recreational use advisory might be issued (MDPH 2009). To know more about the potential risk present, a cyanobacteria sample should be collected for identification and counts. Phycocyanin readings at this time can only confirm presence absence, help find hot spots and provide indications as to the severity and spatial parameters of a bloom.

3.0 SAFETY CONSIDERATIONS

As with other field sampling, sun screen should be used and precautions should be made to avoid contact with poison ivy or placing equipment down in areas with poison ivy present. Efforts should also be made to protect yourself from mosquito bites with the use of repellants. Wearing light colored long sleeve shirt and pants will aid in searching for ticks after you leave the field. The ‘buddy system’ should be used when conducting sampling.

4.0 SAMPLE COLLECTION, PRESERVATION AND HANDLING

A glass or plastic container can be used if a sample is collected for cyanobacteria identifications. It should be stored in the dark on ice for identification within 24 hours. Samples to be held longer than that should be preserved with Lugol’s solution-2 mls/100 mls of sample.

If a sample is collected for microcystin analysis amber, glass bottles should be used. Clear glass jars or bottles can be wrapped in foil. Samples should be kept cool and in the dark until processing.

5.0 APPARATUS, EQUIPMENT, MATERIALS

**Calibration**

* Carrier case containing DataBank, cables and Cyclops-7 probe
* c-phycocyanin (*Spirulina platensis*) standard (keep refrigerated) from Prozyme, Inc.
* Laptop, kept in room 117
* Two 0.5 liter and three 1 Liter volumetric flask, 400 mL flask, two 500 mL +/- glass measuring jar , four 2 L beakers
* A board or table top with a non reflective black surface to place under volumetric flasks and beakers –not necessary if the shade cap is used.
* Eppendorf Reference micropipette and pipette tips
* Magnetic stirrer
* Thermometer
* Soft, clean cloths to wipe outside of the Cyclops probe and DataBank
* 3-5 liters of Deionized (DI) water
* Squeeze bottle containing DI water
* Lens paper

**Field**

* Carrier case containing DataBank, cables and Cyclops-7 probe
* Field Sheets
* Clipboard
* Small cooler with ice
* Wide mouth, .5 L plastic jars with screw caps
* Wide mouth, 1 L plastic jars with screw caps
* Boots
* Gloves
* GPS unit with extra batteries
* 3-5 liters of Deionized (DI) water
* Squeeze bottle containing DI water
* Lens paper
* Soft, clean cloths to wipe outside of the Cyclops probe and DataBank
* Thermometer
* Camera

6.0 REAGENTS

Lugol’s solution should be used to preserve a sample if it cannot be processed within 24 hours, The dose rate is 2 mls/100 mls of sample.

7.0 CALIBRATION-2-POINT

Following purchase of c-phycocyanin (*Spirulina platensis*) standard (Prozyme, Inc.). Calibration of the Cyclops phycocyanin probe will be conducted in late spring before the likely start of cyanobacteria blooms. The c-phycocyanin standard is compromised by light and increasing temperatures, so its’ exposure to light should be minimized. A solid secondary standard for the Cyclops phycocyanin probe (Turner Design, Inc.) is calibrated concurrently with the pure phycocyanin. It should be also be used at the start of the sampling day to check if the calibration is holding. The value obtained from the secondary standard should be recorded at the top of the field sheet. If the readings of the solid secondary standard are off by 20% the instrument should be re-calibrated.

The following steps should be followed when calibrating the Cyclops probe (Appendix A) Clark and Fitzgerald 2013).

1. Rinse the optical window in the probe with Dionized (DI) water and clean/wipe dry with lens paper or a soft, clean cloth. Reattach the slotted black plastic shade cap to the probe.
2. Turn on the laptop, but *do not open the DataBank GUI program yet*.
3. Connect the USB interface cable to the DataBank unit using the pin connector, but *do not turn on the DataBank yet*. Be sure to properly align the pins in the adapter to the sockets on the data logger. Screw the connector ring onto the data logger to hold it in place. Plug the USB end of the USB interface cable into the **top** USB port on the **right side, back corner** of the laptop assigned for chlorophyll/phycocyanin analysis. It is currently stored in room 117. A “device connected” message may appear on the laptop; ignore this message.
4. Press and release the **Power/Off** button on the DataBank. Warm-up time is about five seconds.
5. Open the DataBank GUI program by double clicking on the desktop icon. The DataBank GUI program should automatically initiate a connection with the data logger in approximately 5 seconds. When connected, you should see the words “Data Bank: S/N 2900270 Ver. 3.06” under the **Setup Options** tab in the DataBank GUI window. Click on the **Calibration** tab in the DataBank GUI window.
6. Select the parameter group to be calibrated using the **Group #** drop-down menu. You must click on the drop-down menu and then select a number in order to proceed to the next step.
7. Enter µg/L in the **Title** box. The **Gain Values** box should now read “x1, x10, x100”.

*Laboratory use of the Cyclops-7 requires that certain practices are followed (based on Turner-Designs Cyclops-7 Submersible Sensors User’s Manual version 1.6, 2007)*

* *Use a glass beaker as plastic beakers may fluoresce*
* *Place the beaker on a non-reflective surface (black is recommended. This is not needed if the shade cap is used.*
* *The sensor should be held ~3 inches above the bottom of the beaker and at the same height for each sample. This could be done using a lab stand or for most readings the shade cap can be affixed and this will keep everything at the correct height. There should also be at least 2 inches of clearance between the sensor and the inside of the beaker. This clearance is reduced if the shade cap is installed.*
* *The lens should be checked for air bubbles*

1. BLANK CALIBRATION:
   1. Pour DI water into a 500 mL +/- glass jar. Place the probe (with shade cap attached) into the jar, all the way to the bottom. Ensure that there are no air bubbles present on the optical window.
2. Click on the **OK – PROCEED TO STEP 2** button. Wait while the DataBank GUI program collects data from the sensor at each gain setting. This process usually takes around 20 seconds.
3. When the DataBank GUI program is finished with step 2, “The gathering of Blank data has been completed.” will be displayed. Click the **OK – PROCEED TO STEP 3**. Prepare the calibration solution as described below
4. Pour exactly 200 mL of DI water into a 400 mL widemouth flask. Place a magnetic stirrer bar into the flask, and set the flask on a stirrer plate or swirl the flask gently to mix the sample.
5. Obtain the c-phycocyanin “vial” standard from the refrigerator. Check the vial label to learn its concentration (the c-phycocyanin concentration from lot #241014 in 2015 was 24.2 mg/ml (or µg/µL).
6. Use the 10 µL Eppendorf Reference micropipette (found in the cabinet below the Trilogy fluorometer in room 117, pipette tips are found there as well) to obtain a 7 µL volume of the c-phycocyanin standard. Set the control dial on the top of the micropipette to 7 µL.
7. *The pipette must be pre-wetted in order to obtain an accurate volume. To do this push the pipette tip onto the micropipette, then put the pipette tip below the surface of the standard. Press the control button to the first stop release it and repeat. Next press the control button to the first stop. Hold down the control button and pull the tip up the inner wall of the flask (Eppendorf 2001). Totally empty the pipette by pressing the control button down to the second stop (blow-out).To eject the tip press the control button down to the last stop.*
8. PRIMARY STANDARD: Inject the 7 µL of the c-phycocyanin standard into the 200 mL of DI water in the 400 mL flask, placing the pipette just below the water surface. Operate the stirrer on medium speed for 2 minutes. Calculate the concentration of phycocyanin in the 400 mL flask in units of µg/mL:

**[7 *µL of standard*  x *µg/µL conc of standard] / 200 mL*** (*about 0.87 µg/mL*)-solution A

1. SECONDARY STANDARD for CALIBRATION: Transfer 100 mL of the Primary dilution standard from the 400 mL flask to a 1 Liter volumetric flask. Add DI water to bring the total volume to exactly 1 liter. Cover and invert the 1 L flask several times to mix. Calculate the concentration of phycocyanin in the 1 liter flask:

***[µg/mL in 400 mL flask (A) x 100 mL] / 1 L*** (*about 87 µg/L*)-solution B

1. Pour all of the 1 liter of the (about 87 µg/L) Secondary Standard (B) (1 L volumetric) into a 2 L +/- glass beaker. Place the probe (with shade cap attached) into the beaker, all the way to the bottom. Ensure that there are no air bubbles present on the lens surface, and that the lens is two inches above the bottom of the beaker. Record the temperature of the solution in the beaker.
2. In the DataBank GUI program, in the **Enter Standard Value** box, enter the concentration of the calibration solution you prepared in step 17 (e.g., about 87 µg/L for standard used in 2014). Using the **Units** drop-down menu, select µg/L. Make sure that the correct **Group #** and **Title** are displayed.
3. Click the **PROCEED TO STEP 4** button. Wait while the DataBank GUI program collects data from the sensor at each gain setting (there will be 3 consecutive countdowns; takes 20 sec +/-)
4. When the DataBank GUI program is finished, the message “Ready to Calculate and Transmit Results.” will be displayed. Click the **FINALIZE CALCULATIONS** button.
5. After a few seconds, the message **“This Calibration is Complete. The Data Bank is programmed.”** will be displayed in a small box. Click the **OK** button. The calibration procedure is now complete. Close the DataBank GUI program, and remove the USB interface cable.
6. CHECK STANDARDS: Immediately make up additional solutions of the phycocyanin standard, by dilutions of the Primary and Secondary Dilution standards. Begin by pouring the contents of the 500 mL +/- glass jar back into the 0.5 L flask (the Secondary Dilution Standard).
7. Dispense 50 mL of **the Secondary Dilution Standard** (B)(2 L beaker) into a 1 L flask (20X dilution). Fill to volume with DI water. Cover and invert the flask several times to mix. Calculate the concentration of phycocyanin (should be about 4 µg/L). Pour all of the 4 µg/L solution into a 2 L beaker. Analyze on DataBank and record reading.
8. Use a second 1 Liter Flask. Pour 500 mLs of the remaining contents of the **Secondary Dilution Standard** (2L beaker) into the 1 Liter Flask, and adjust to volume with DI. Cover and invert the flask several times to mix. Calculate concentration of phycocyanin (should be about 44 µg/L). Pour all of this solution into a 2 L +/- glass beaker. Analyze and record.
9. Empty and rinse the 0.5 L volumetric flask. Pour 100 mLs of the remaining contents of the **Primary Dilution Standard** (A) into the 0.5 L Volumetric Flask, and adjust to volume with DI (5X dilution). Cover and invert the flask several times to mix. Calculate concentration of phycocyanin (should be about 175 µg/L). Pour all .5 L of this solution into a 500 mL +/- glass glass jar. Analyze and record.
10. All DataBank readings for check solutions should compare within +/- 10% of the calculated solution concentration.
11. At this point the secondary solid standard can be calibrated.

Secondary Solid Standard

1. The optical surface of the Cyclops-7 must be clean and dry before the solid standard is attached.
2. To install press the solid standard on to the optical end of the Cyclops-7 and then rotate it until you can feel the solid standard ‘indexing ball’ click into the ‘indexing mark’ on the Cyclops.
3. To start, a flat-head screwdriver is used to unscrew the locking nut as far as it will go
4. The included green screwdriver is used to change the signal level by inserting the screwdriver blade through the hole in the locking nut (found in the center of the solid standard).
5. The adjustment screw is located beneath the locking nut. It is turned to obtain a signal level similar to the phycocyanin standard (clockwise produces a lower signal).

8.0 PROCEDURE

**Office Preparations**

* **Check or Charge battery**
* Turn on DataBank by pressing power toggle.
* Attach the cable to the top of the DataBank Datalogger. Align the notch on the plug to the one on the top of the DataBank. The rest of the cable has two ends, one has a round hole plug for attaching to the external power source and the other is a USB end. To charge the battery attach the external power source and turn on the DataBank . The battery when charged should flash done and **pv.** If it starts to flash **ot** (over temperature) you need to unplug it let it cool down and then plug in again. The battery should be charged before each sample run.
* If you’re taking readings and it says ‘ low’ in the upper right hand corner it means that the low level of fluorescence gain is being used. It is not an indicator for the battery charger.

**Setup and Operation DataBank**

* After charging the battery, remove the power cable and return the cap.
* Familiarize yourself with the DataBank (figure 1) functions before you go into the field. There are 4 toggles switches, each with two functions. **Press down** for the functions listed across the top-power, store, recall, select and **hold** the toggle down to get the functions listed below-off, log, erase, #01. When the power is on, pressing the power button will turn on and off the backlight too.

Figure 1 DataBank

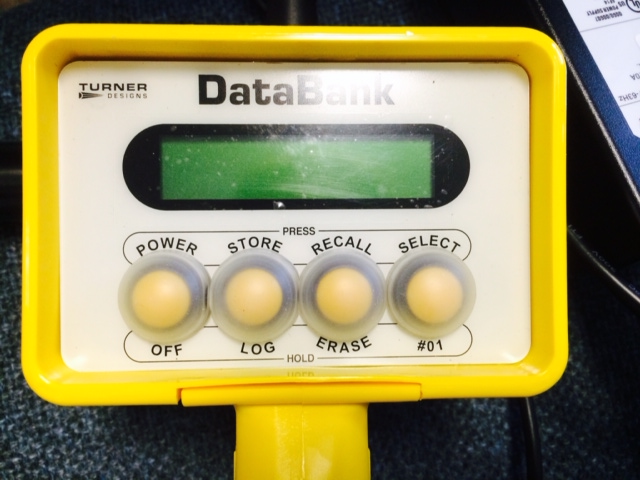


Table 1 Description of functions of DataBank buttons

|  |  |  |
| --- | --- | --- |
| Button | Press- <1 second | Hold> 1 second |
| Power | Turn DataBank on  also  If unit is in logging mode pressing power will stop logging | Turn DataBank off |
| Store | Stores current reading displayed | Starts ***logging mode*** when logging mode has begun the screen will display-**Logging Mode Entered** |
| Recall | Displays the last record stored  Pressing this button again will ‘decrement’ to the next record stored until the first record stored is reached then it will increment to the last record stored | Erases the last record stored  Display will show-***Caution about to clear***-which is followed by-***Cleared top record*** |
| Select | Selects a parameter group | Reverts to first parameter group |
|  |  |  |
|  |  |  |

**Field Measurements**

* Before entering the water, attach the Cylops 7 cable to the DataBank. Make sure that the pins align with the proper holes
* Attach Cyclops 7 (the actual probe for making the phycocyanin measurements) to the other end of the cable and then attach the slotted plastic shade cap.
* Press power button -warm up is 5 sec
* Get a reading of your location by the shore and record on field sheet



Field Procedure for Phycocyanin Measurements

1. Take temperature of water using hand held thermometer, record on field sheet
2. From the shore take a GPS reading of your location and record it.
3. The probe can be deployed by either walking into the water with the probe or by lowering the probe from dock to obtain measurements
4. Wade into water to knee depth. Wait for the sediments to settle before beginning readings
5. Submerge the probe end to allow it to acclimate at least one minute to the water temperature before starting to record readings
6. Lower probe to depth desired for first reading (0.25 m matches MADPH protocol). A yellow tape marks this depth. (If there is a visible ‘algal scum’ the probe end could be lowered just below the surface- Position the probe so that the entire shade cap is immersed and an inch or more of the probe is in the water. Begin by pressing store and then press recall to obtain the last reading and record on field sheet. Write down the time of the reading and the phycocyanin value.
7. Either this value can be used for the depth or to check for variability 4 readings can be obtained and this same depth by hitting store a series of four times. If it is desired to obtain more data at this depth hold down the Store toggle and the DataBank will start logging mode. Once the probe is in logging mode it will take a reading every 15 seconds until you wish to stop. To stop logging mode press the Power button quickly. To review any data, press the Recall button. Stop logging with each change in depth and make sure that you have written down the time and the value of the firs t and last reading before continuing to another depth.
8. Repeating this sequence with every change in depth or location within a waterbody will make it easier to group the readings after the data is downloaded.
9. At least one stored value should be recorded on the field sheet (Appendix B) for each location or depth that is evaluated.
10. To determine if a possible cyanobacteria bloom is present, follow the procedure above. Yellow tape marks 0.25 meters, 0.5 and subsequent meters down to 5. Start by taking readings just below the surface if a bloom is present. Lower the probe.25 meter (~9 inches) repeat the readings at 0.5 m and 1 meter. Depending upon the needs of the study, a profile down to 5 m can be made using this probe and available cable.
11. To power off the DataBank hold down the power button for >1 sec.
12. Take off the shade cap and wipe it, probe and cable with clean rags.
13. Rinse the probe lens with DI water, **gently** blot with a clean cloth, if it’s really dirty bring it back to the lab and put it in soapy water for an hour or so and then rinse well with DI.
14. After cleaning, return the probe and shade cover to the carrier case.
15. Wipe off the cable and coil it in loose coils and place to the carrier case.
16. Back at the lab, the stored data can be accessed and downloaded into Excel.

This sampling routine will help you determine if a blue-green coloration of the water is cyanobacteria or not, how dense a cyanobacteria bloom is, what depth it is found at as well as its spatial extent. Obtaining measurements within this upper part of the water column will quickly help you determine if you have a cyanobacteria bloom present.

For more complete information, grab samples of the scum or within the water column could be collected for identification and counts. If the information may be used by Mass. Dept of Public Health (MADPH) to determine if a waterbody is safe for recreational use, collection of the sample should follow their protocols and be collected by inverting a clean glass or plastic jar and submerging it down to the 0.25 m depth (approximately 9 inches). Then cap it underwater and bring it back up to the surface. The sample should be kept in the dark and on ice for analysis within 24 hours. It could alternatively be preserved with Lugol’s solution.

**Samples for lab analysis (in place of field samples)**

If the probe is not available to take out in the field, samples (of at least a liter of sample water) can be brought back to the lab for analysis. Samples are collected by wading into the water to ~ knee depth and then inverting the sample jar and plunging it into the water to a depth of approximately 9 inches. The sample jar is brought back up, capped, labeled and put into a cooler containing ice for transport to the DEP-DWM lab in the Central Regional Office building in Worcester.

At the lab, set up the probe as described in section 8 on the non-reflective black surface. If the samples were held in the refrigerator take them out ~2 hours before you expect to do your analysis to allow them to come to room temperature, but still keep them in the dark. Samples should be at room temperature. Other equipment needed are two 2 L beakers (stored in the cabinet above chlorophyll analysis area )(room 117), and the shade cap to keep the probe at the correct height in the beaker.

1. First fill one 2 L beaker with DI water, submerge the probe, allow it to acclimate to room temperature and then take a reading. The reading should be <2 µg/L.
2. Shake the sample container well to mix the sample and fill the second beaker with the liter of water.
3. Gently lower the probe down into the water. Make sure that air bubbles do not form on the lens surface.
4. Let the probe acclimate for 1 minute and then either record one reading or hold down the store button to record for one minute.
5. If more than one reading is made per minute the results should be averaged.

**To access the data**

1. Data can either be transferred from the field sheet or accessed by plugging the Datalogger into the laptop using the cable with the 8 pin end which is connected to the top of the DataBank Datalogger.
2. The other USB end is plugged into the laptop computer used solely for chlorophyll and phycocyanin.
3. The Datalogger program is started up and the tab SAVE DATABANK RECORDS is selected. Currently, the files should be saved to laptop C: drive (local) AND N:\cyanobacteria phycocyanin probe data
4. Name the file for the date the readings were recorded e.g. 11Jul14.txt. If a single waterbody was sampled include the name e.g. CharlesR11Jul14.txt. The file extension .txt should be included in the filename. The file is comma delimited.
5. Choose to replace the file.
6. Choose to **record all records as stored**.
7. Confirm that the records were transferred over.
8. Next go to Excel-file import, locate the file and then download it to Excel. Save it with same name, but with extension .xls.
9. Insert a line and add the names of the columns; e.g Record #, Range used, Date and Time (24 hr clock), Sensor mV, Concentration µg/L.
10. At this time the DataBank records can safely be deleted

9.0 CLEANING PROCEDURE

Wipe Cyclops probe and cable with clean, absorbent cloths.

Rinse the probe lens with DI water, **gently** blot with a clean cloth, if it’s really dirty bring it back to the lab and put it in soapy water for an hour or so and then rinse well with DI. Any cleaning of the lens should be done with microscope/optical lens paper.

10.0 QUALITY CONTROL

There is a solid secondary standard for use with the DataBank. To use it attach it to the probe end. The value read should be within 20% of the value that was initially set (e.g., 100 ug/l for secondary calibration standard used in 2015. Use the solid secondary standard prior to going out in the field to check the probe calibration. Record result on the field sheet (Appendix B) and on the Calibration Record Sheet (Appendix C) kept in the Phycocyanin drawer in the room 117.

11.0 INTERFERENCES

Humic acid can cause interference with phycocyanin readings, but according to *Turner Designs Fluorometer Application Notes* ‘humic interference while detectable is statistically insignificant’.

12.0 PREVENTIVE MAINTENANCE

The phycocyanin probe lens should be rinsed with DI water. If very dirty, it can be soaked in a mild detergent and then rinsed in DI. Never clean the lens with anything other than microscope lens paper. **Do not** use Kimwipes, paper towels or tissue paper. All contain abrasives that can permanently damage the lens.

The battery does best if it is charged regularly before a lot of the storage capacity is used up.

13.0 CORRECTIVE ACTIONS

If when charging the battery an **‘ot’** signal appears, this means that the battery is overheated and should be unplugged, allowed to cool and then plugged in again so that the charging can resume.

14.0 WASTE AND POLLUTION PREVENTION

Since phycocyanin is water soluble and non-toxic, waste solvents are not a problem. All containers should be washed with a mild detergent and rinsed three times-twice with tap water and the last with DI water, with wastewater disposal down the drain.

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| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | | | Appendix A: Modification of NERO MassDEP CheAt Sheet | | | | Phycocyanin Meter Calibration |  |
| Basics | | The Turner Designs Cyclops-7/DataBank Fluorescence unit should be calibrated once per year, or if and when readings for the Solid Secondary Standard (SSS) are off by more than 20%.  This document outlines a **two-point** calibration procedure for µg/L of phycocyanin pigment. | | | | | | |
| Supplies | | CYCLOPS-7 probe connected to DataBank via the supplied cable  Laptop and USB interface cable (part number 021-2903)  Two 0.5 liter and three 1 Liter volumetric flask, one 400 mL Erlenmeyer wide-mouth flask, 4 two liter beakers, two 500 mL glass measuring jars (beakers)  ~3-liters of Deionized (DI) water, gloves  10 µL Micropipette, magnetic stirrer, thermometer, lens paper  c-phycocyanin (*Spirulina platensis*) standard (keep refrigerated) | | | | | | |
| Step By Step Instructions | | | | | | | | |
| 1 | Rinse the optical window in the probe with Dionized (DI) water and clean/wipe dry with lens paper. Reattach the slotted black plastic shade cap to the probe. | | | | | | | |
| 2 | Turn on the laptop, but *do not open the DataBank GUI program yet*. | | | | | | | |
| 3 | Connect the USB interface cable to the DataBank unit using the pin connector, but *do not turn on the DataBank yet*. Be sure to properly align the pins in the adapter to the sockets on the data logger. Screw the connector ring onto the data logger to hold it in place | | | | | | | |
| 4 | Plug the USB end of the USB interface cable into the **top** USB port on the **right side, back corner** of the laptop. A “device connected” message may appear on the laptop; ignore this message. | | | | | | | |
| 5 | Press and release the **Power/Off** button on the DataBank. Warm-up time is about five seconds. | | | | | | | |
| 6 | Open the DataBank GUI program by double clicking on the desktop icon. The DataBank GUI program should automatically initiate a connection with the data logger in approximately 5 seconds. When connected, you should see the words “Data Bank: S/N 2900365 Ver. 3.08” under the **Setup Options** tab in the DataBank GUI window. Click on the **Calibration** tab in the DataBank GUI window. | | | | | | | |
| 7 | Select the parameter group to be calibrated using the **Group #** drop-down menu. You must click on the drop-down menu and then select a number in order to proceed to the next step. | | | | | | | |
| 8 | Enter µg/L in the **Title** box. The **Gain Values** box should now read “x1, x10, x100”. | | | | | | | |
| Blank | 9 | | | Pour DI water into a 500 mL +/- glassbeaker. Place the probe (with shade cap attached) into the beaker, all the way to the bottom. Ensure that there are no air bubbles present on the optical window, and that the window is at least one inch below the water surface. | | | | |
| 10 | | | Click on the **OK – PROCEED TO STEP 2** button. Wait while the DataBank GUI program collects data from the sensor at each gain setting. This process usually takes around 20 seconds. | | | | |
| 11 | When the DataBank GUI program is finished with step 2, “The gathering of Blank data has been completed.” will be displayed. Click the **OK – PROCEED TO STEP 3**. Prepare the calibration solution as described below. | | | | | | | |
| 12 | Pour 200 mL of DI water into a 400 mL flask. Place a magnetic stirrer bar into the flask, and set the flask on a stirrer plate. | | | | | | | |
| 13 | Obtain the c-phycocyanin “vial” standard from the refrigerator. Check the vial label to discern its concentration (should be around 24.2 mg/mL, which is equivalent to 24.2 µg/µL). | | | | | | | |
| Prepare Standards | 14 | | | | Use the 10 µL Eppendorf Reference micropipette (found in the cabinet below the Trilogy fluorometer in room 229, pipette tips are found there as well) to obtain a 7 µL of the c-phycocyanin standard. Set the control dial on the top of the micropipette to 7 µL. *The pipette must be pre-wetted in order to obtain an accurate volume. To do this push the pipette tip onto the micropipette, then put the pipette tip below the surface of the standard. Press the control button to the first stop release it and repeat. Then to totally empty the pipette press the control button down to the second stop (blow-out). Hold down the control button and pull the tip up the inner wall of the flask (Eppendorf 2001). To eject the tip press the control button down to the last stop.* | | | |
| 15 | | | | **Primary** | Inject the 7 µL of the c-phycocyanin standard into the 200 mL of DI water in the 400 mL flask, placing the pipette just below the water surface. Operate the stirrer on medium speed for 2 minutes. Calculate the concentration of phycocyanin in the 400 mL flask in units of µg/mL:  **[7 *µL of standard*  x *µg/µL conc of standard] / 200 mL*** (*about 0.87 µg/mL*)—solution **A** | | |
| 16 | | | | **Secondary** | Transfer 100 mL of the Primary dilution standard from the 400 mL flask to a 1 Liter volumetric flask. Add DI water to bring the total volume to exactly 1 liter. Cover and invert the 1 L flask several times to mix. Calculate the concentration of phycocyanin in the 1 liter flask:  ***[µg/mL in 400 mL flask (A) x 100 mL] / 1 L*** (*about 87 µg/L*)—solution **B** | | |
| Calibration | 17 | | | | Pour all of the 1 liter of the (about 87 µg/L) Secondary Standard (**B**) (1 L volumetric) into a 2 L +/- glass beaker. Place the probe (with shade cap attached) into the jar, all the way to the bottom. Ensure that there are no air bubbles present on the optical window, and that the window is at least one inch below the water surface. Record the temperature of the solution in the jar. | | | |
| 18 | | | | In the DataBank GUI program, in the **Enter Standard Value** box, enter the concentration of the calibration solution you prepared in step 16 (i.e., about 87 µg/L). Using the **Units** drop-down menu, select the µg/L. Make sure that the correct **Group #** and **Title** are displayed. | | | |
| 19 | | | | Click the **PROCEED TO STEP 4** button. Wait while the DataBank GUI program collects data from the sensor at each gain setting (there will be 3 consecutive countdowns; takes 20 sec +/-) | | | |
| 20 | | | | When the DataBank GUI program is finished, the message “Ready to Calculate and Transmit Results.” will be displayed. Click the **FINALIZE CALCULATIONS** button. | | | |
| 21 | | | | After a few seconds, the message “This Calibration is Complete. The Data Bank is programmed.” will be displayed in a small box. Click the **OK** button. The calibration procedure is now complete. Close the DataBank GUI program, and remove the USB interface cable. | | | |
| Check Calibration Curve | 22 | | | | Immediately make up additional solutions of the phycocyanin standard, by dilutions of the Primary and Secondary Dilution standards. | | | |
| 23 | | | | Dispense 50 mL of **the Secondary Dilution Standard**-(**B**)(2 L beaker) into a 1 L volumetric flask. Fill to volume with DI water. Cover and invert the flask several times to mix. Calculate the concentration of phycocyanin (should be about 4 µg/L). Pour all of the 4 µg/L solution into a 2 L +/- glass beaker. Analyze on DataBank and record reading. Solution **C** | | | |
| 24 | | | | Use a second 1 Liter Flask. Pour 500 mLs of the remaining contents of the **Secondary Dilution Standard** (**B**)(0.5 Liter Flask) into the 1 Liter Flask, and adjust to volume with DI. Cover and invert the flask several times to mix. Calculate concentration of phycocyanin (should be about 44 µg/L). Pour all of this solution into a 2 L +/- glass beaker. Analyze and record.—solution **D** | | | |
| 25 | | | | Empty and rinse the 0.5 L volumetric flask. Pour 100 mLs of the remaining contents of the **Primary Dilution Standard** (**A)** into the 0.5 L Volumetric Flask, and adjust to volume with DI. Cover and invert the flask several times to mix. Calculate concentration of phycocyanin (should be about 175 µg/L). Pour all .5 L of this solution into a 500 mL +/- glass measuring jar. Analyze and record.—solution **E** | | | |
| 26 | | | | All DataBank readings should compare within +/- 20% of the calculated solution concentration. | | | |
| SSS | 27 | | | | Once the linearity of the calibration curve has been adequately demonstrated, attach the Solid Secondary Standard (SSS) to the probe. Turn on the DataBank and obtain a reading for the SSS. Unscrew the locking nut on the back of the SSS as far as it will go. Insert a small screwdriver though the hole in the locking nut, and rotate until the reading you desire based upon the value of the secondary standard is obtained. Then re-secure the locking nut. The SSS is now readjusted. | | | |

 Appendix C: Calibration Record Databank Datalogger with Cyclops 7 Phycocyanin Probe and Secondary Solid Standard

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| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Standard | Source | Batch # | Expiration Date | Date Calibrated | Name Calibrator | Solid Standard set at 100 µg/L, reading should be within +/-20% | | |
|  |  |  |  |  |  | Reading µg/L | Date | Reader |
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