Fluorometry Protocol

1. Select the correct Aquafluor fluorometer to measure your parameter; each fluorometer is only set up to measure 2 of 4 parameters. One Aquafluor measures Phycocyanin (channel A) and CDOM (channel B), while the other measures optical brighteners (channel A) and Chlorophyll (channel B). Check this by turning on the device and pressing the <**A/B**> button, which will show you the parameters it measures.
2. Turn on the fluorometers by pressing the **<ON/OFF>** button, and wait at least 5 seconds for the instruments to boot up.
3. Using gloves, obtain a plastic cuvette (**NO GLASS CUVETTES)**, making sure that the outer surfaces are clean and free of noticeable scratches or marks. **Check for the sharpie mark on the rim of the cuvette; make sure to insert the cuvette with this side facing you for every reading.**
4. Run one blank sample **FIRST** (using autoclaved DI water) for all 4 channels before measuring the other samples. Follow the instructions below and repeat steps 5 -14 for each sample.
5. Rinse the cuvette with DI water **3 times**.
6. Gently agitate your water sample to resuspend any particles that have settled to the bottom.
7. Rinse the cuvette with your sample **3 times**, then fill the cuvette until it is ¾ full (do not fill the cuvette to its maximum volume).
8. Gently clean off any smudges or liquid droplets from the outside of the cuvette with a KIMTECH wipe.
9. After opening the small hatch to the sample bay, place your sample into the fluorometer, **making sure not to spill any of the contents of the sample into the interior of the device. If a spill occurs, quickly invert the device and immediately let a member of the BWL know.**
10. Select your desired parameter to measure using the <**A/B**> button.

11. Press either of the two <**READ**> buttons, and record the measured parameter in RFU. Make sure measure all 4 parameters (Chla, PC, OB, and CDOM) for each sample.
12. Empty the cuvette and start at step 5 to measure the next sample.
13. **Finally, read one more blank sample after all of the other samples have been measured.**

IMPORTANT NOTES:

- Positive control: Measure the Site 2 sample twice (denoted on the data sheet as 2A and 2B) for each parameter to provide an indication of instrument variation.
- If your cuvette becomes too dirty or scratched, you may discard it and take a new one. However, you MUST run another blank for all 4 parameters before proceeding to the next sample. This allows us to account for variability between cuvettes.