FluoroQuik Fluorometer

User's Manual

Standard Operating procedure

Version 1.0

Edited for use with dual channel fluorometers and in conjunction with the Cyanobacteria Monitoring Collaborative (CMC)

Table of Contents:

1. FluoroQuik Fluorometer

1.1 Overview

This Standard Operating Procedure has been written to specifically address the operation of the AMISCIENCE two channel fluorometer designed and built by AmiScience and sold and distributed by Beagle BioProducts, as this is the principal fluorometer currently being used by the Cyanobacteria Monitoring Collaborative (CMC). Other fluorometers are acceptable for use, such as the Turner AquFluor unit. Key procedures that must be followed regardless of instrument type are noted throughout this document in order to ensure the highest level of data consistency and quality throughout the program. This SOP has been adapted from the original AmiScience user manual to specifically address the calibration, operation, and maintenance of the dual channel FluorQuik fluorometer and the protocols of the Cyanobacteria Monitoring Collaborative.

The FluoroQuik fluorometer utilized by the CMC is a portable instrument designed for multipurpose fluorescence measurements with two optical channels built into one unit. The instrument is simple to use, light in weight, and can be powered by either DC power adaptor or AA batteries.

1.2 Key Features

a. Using either 200-µL PCR tubes (model-A), 500-µL micro-centrifuge tubes (model-B), or 1-cm square cuvette (model-C).
b. LCD touch-screen display.
c. User-friendly software with “touch and test” operation.
d. USB interface for data management.
e. Larger than 5 logs of dynamic range (after proper calibration procedure).

1.3 Included Parts

a. The FluoroQuik fluorometer, carry case, sample tubes & pipettes.
b. 5VDC/2A power adapter.
c. Standard-USB-to-mini-USB cable.

d. Operation manual and USB driver/data management software disk.

2. System Operation

2.1 Principle of Operation

The FluoroQuik Fluorometer uses a single-wavelength light source to excite the sample which, as a result, emits a fluorescent signal of a specific wavelength detected by an internal photo sensor. The reading by the photo sensor, represented by RFU (Relative Fluorescence Unit) is used to calculate the nominal concentration of the sample when the fluorometer is properly calibrated.

As shown in Figure 1, during the calibration process, a "Blank" tube (zero concentration) and a "Standard" tube (known concentration) are separately measured by the fluorometer to obtain the RFU readings. The RFU readings and the concentration values are then used to generate a "linear calibration curve" which is stored in the non-volatile memory of the fluorometer. During the measurement operation, the sample's RFU is used to internally calculate the unknown concentration using interpolation or extrapolation based on the stored linear calibration curve.

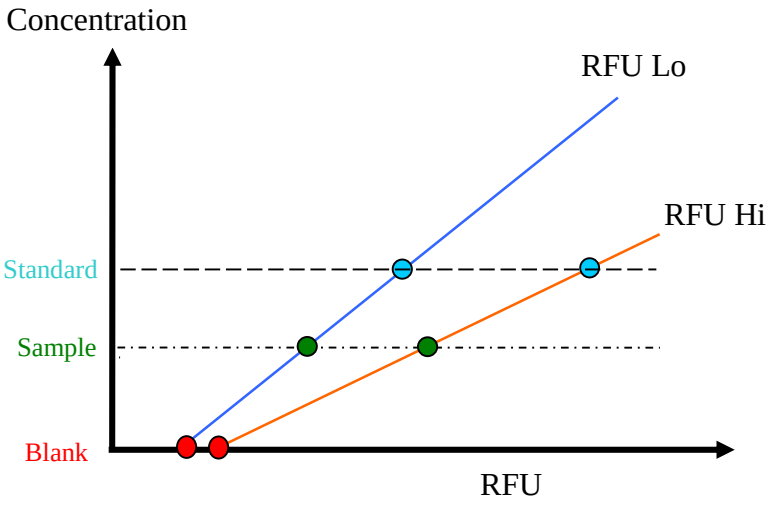

Figure 1

Note that in order to extend the measurable concentration range, two levels of excitation power are automatically used during the calibration and measurement steps, and two different RFU readings ("RFU Hi" and "RFU Lo") are obtained. When the sample's concentration is too high and the fluorescent signal saturates the photo sensor, the fluorometer automatically uses the

"RFU Lo" reading (and the associated linear calibration curve) to calculate the sample concentration, hence extending the upper measurement range. This does not change the output measurements.

In a dual-channel fluorometer, there are two independent excitation/emission wavelength pairs (Channel 1 and Channel 2) whose calibration curves are independently defined by the user, but each channel can define only one linear calibration curve (in other words, only one "Assay" for each channel.)

2.2 Power Up

The FluoroQuik Fluorometer can be powered by four AA batteries or the supplied power adapter (5VDC/2A). After connecting to power, switch the ON/OFF button on the upper-right of the unit to turn on the fluorometer. After a flash of the welcome screen, the screen automatically turns into the "**Main Menu**", as shown in Figure 2.

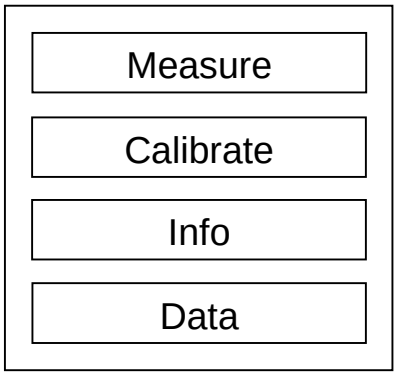

Figure 2. **Main Menu** screen

2.3 Calibration

a. In order to measure the concentration of unknown samples, a calibration procedure needs to be performed. If a calibration has already been completed, you can skip this calibration step and go to Sec. 2.4 to perform sample measurement. Once calibrated, the calibration curve is stored in a non-volatile memory and is not affected by powering the instrument on or off.

b. Touch "**Calibrate**" tab on the "**Main Menu**" screen. A confirmation screen asking "Create new calibration?" will show in order to prevent unintentional calibration steps. Touch

"**Return**" if you don't intend to perform the calibration, otherwise touch "**Continue**" to enter the channel selection screen. Select the Channel on which you want to calibrate your standard (1 Phycocyanin or 2 Chlorophyll).

NOTE: *For the Cyanobacteria Monitoring Collaborative, you will need to use phycocyanin and chlorophyll standards for calibrating your fluorometer. This helps reduce measurement error, improves on quality assurance, and provides standardization across the program.*

c. Now enter the "**Calibrate**" screen similar to Fig. 3. Put in the Blank tube in the sample chamber and close the cap (the blank tube does not need to be filled with de-ionized water). Make certain your tube is clean of fingerprints/debris and is oriented so that you can re-insert it if need be in the exact same position. Touch "**Blank**" to take the blank value.

d. After the Blank is read, the screen will look like the one shown in Fig. 4.

e. To set the nominal value of the Standard tube to calibrate the fluorometer, use the "<" and ">" arrow keys on the second row to move the underline to select the digit you want to change, and use the "+" or "–" keys to increase or decrease the value of the underlined digit. (A zero standard value is not allowed.)

f. Put the Standard tube in the chamber and touch the "**Measure**" tab to take the measurement. After a few seconds, "**Calibration Finished**" will show on the screen. Press "**Return**" to go back to "**Main Menu**".

g. If the Standard measured value is equal or less than the Blank, an error message "**Reading Too Low!**" will show. Prepare the right Blank or Standard and measure again.

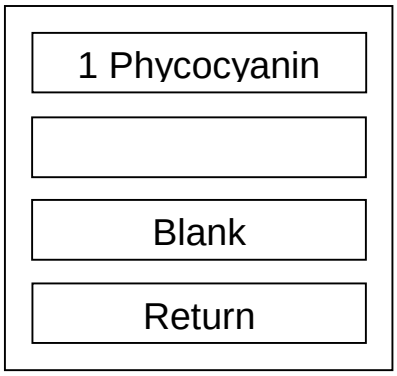

Figure 3. **Calibrate** screen

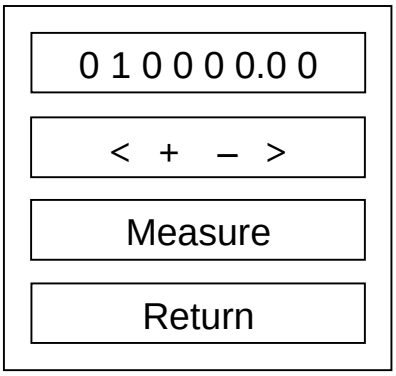

Figure 4. **Standard setting** screen

2.4 Measurement

a. Refer to Section 2.3 for calibration procedures if the fluorometer has not been calibrated.

b. Touch the "**Measure**" tab on the "**Main Menu**" screen to enter the Channel selection screen. In the screen you can select 1 Phycocyanin, or 2 Chlorophyll depending on which you want to measure your sample for. This selection will measure the sample and calculate the nominal concentration value using the linear calibration curve stored in the fluorometer.

c. Prepare sample tube (and blank tube if to be used), referring to Section 3.

d. Select the Channel to enter the "**Measure**" screen similar to Fig. 5.

e. If the fluorometer has been calibrated before, the Blank value is already stored in the fluorometer. But for low-concentration sample measurement, it is recommended that a new "Blank" is performed at this step to compensate for any minor inconsistencies in the walls of the sample tube. Insert the Blank tube into the testing chamber and secure the cap, and touch "**Blank**" to take the blank reading. The tube does not need to be filled with de-ionized water for a blank measurement.

f. Insert the sample tube and touch the "**Measure**" tab to start the sample measurement. The measurement result will be displayed on the "**Result**" screen in a few seconds, as shown in Figure 6. The timer clock will start from zero so you can do a follow-up measurement after a certain time. NOTE: *If doing repeat measurements of the same sample, wait a minimum of 10 seconds between measurements.*

g. If the reading is too high and saturates the photo-detector, an "**Over Limit**" message will display. In other cases where the concentration of material is too high, light cannot pass through the material and reach the measurement optics. This is commonly referred to as "quenching" and can be resolved by diluting your sample. This usually results in a lower reading (See section 3, Sample Preparation and Measurement Tips).

h. If you want to save the measurement data in the meter's on-board memory, you can touch the "**Save**" tab. The data will be saved in the memory of the specific assay/channel that you selected earlier, with the stored data sequential number displayed on the upper-right corner.

i. Touch "**Return**" to go back to the previous "**Measure**" screen. Touch "**Measure**" tab again will repeat the measurement. The timer clock will restart from zero.

j. If batteries are used as the power source, and the voltage has dropped too low and the accuracy of the measurement may be affected, a "**Battery Low**" warning message will show on the bottom of the screen during measurement. The batteries should be replaced as soon as possible.

NOTE: *Do not leave your batteries in the unit for any extended periods, as they can leak and damage the instrument. Always check the battery compartment for any corrosion or battery leakage prior to use.*

k. Touch "**Return**" tab will return to "**Measure**" screen, and touch "**Return**" again will go back to the "**Main Menu**".

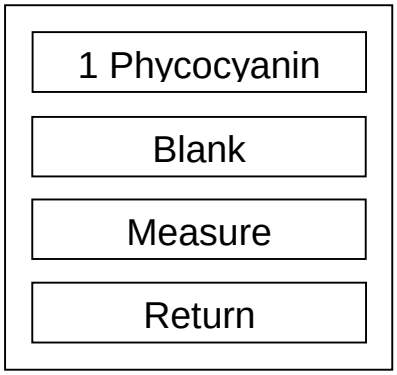

Figure 5. **Measure** screen

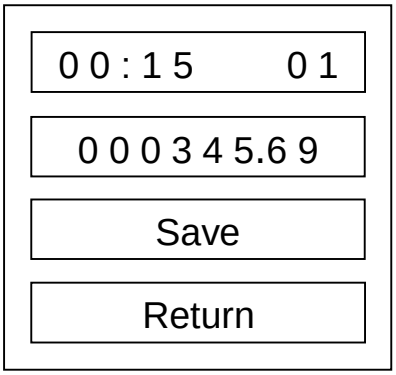

Figure 6. **Result** screen

2.5 Data Retrieval

a. Touch "**Data**" tab on the "**Main Menu**" screen will let you select the assay/channel in which you want to inspect the data. After selection the "**Data**" screen now shows similar to Fig. 7. The first row shows the saved data, and the second row shows the data sequential number. You can touch the left and right arrow key to change the data number to inspect other saved data.

b. If you want to erase the saved data of the assay/channel you are inspecting now, touch "**Erase All**" and confirm the action in the next screen. The data of the other assays/channel will not be affected by this erase.

c. Touch "**Return**" tab will return to "**Main Menu**" screen.

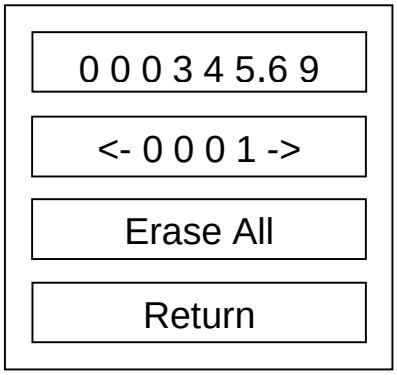

Fig. 7 "**Data**" screen

3. Sample Preparation and Measurement Tips

a. Prepare Standard and Sample solution within the concentration range that can be read by the fluorometer. You can use the "**RFU Hi**" or "**RFU Lo**" mode in the "**Measure**" function to measure the sample if you are not certain. It is also better that the Standard doesn't saturate the RFU Hi reading in order to maximize sensitivity.

> **Note***: If the ambient algal or cyanobacteria concentration is too high, the fluorometer may give an incorrect reading. This is caused when light passing from one side of the instrument and through the sample is blocked so much by the material in the sample that it cannot be appropriately measured by the detector on the receiving side of the instrument. In these cases, samples should be diluted until the proportion that the sample is diluted by reflects an equal reduction in concentration (i.e. diluting the sample by 50% shows a 50% reduction in concentration). This phenomenon is commonly referred to as "quenching."* A rule of thumb is if you cannot see through your sample in your fluorometer tube, then it is likely too concentrated (Fig. 8).

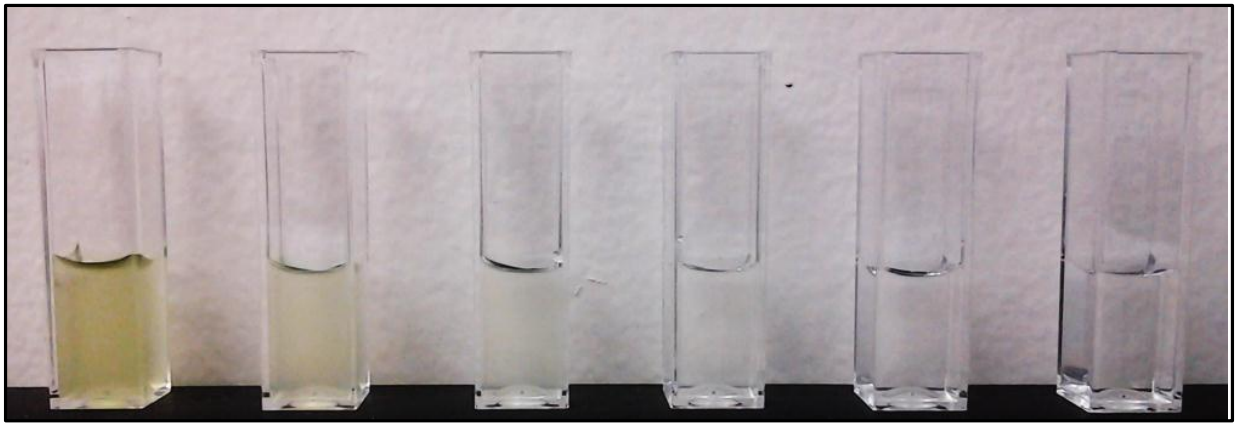

Fig. 8: Dilutions of a *Microcystis aeruginosa* sample. The left-most sample is too turbid to read accurately, but the fluorescence from all other samples can be accurately measured. The right-most sample, though it looks clear, still has a measureable amount of phycocyanin due to the cyanobacteria present, (Beagle BioProducts, April 2013).

b. Make sure the sample tube is clean internally before you put in the solution, and the outside of the tube is clean and dry. Any materials on the outside of the tube may cause measurement error.
c. If glass mini-tube, PCR tube, or micro-centrifuge tube is used, fill the tube with at least 200μL sample solution. For 1-cm cuvette, 1mL sample solution is needed.
d. Make sure no bubbles are in the sample solution.
e. Due to the poor tube-wall consistency of plastic tubes, if PCR tube or micro-centrifuge tube is used, align the cap-lip with the chamber mark so each time the measurement is consistent.
f. Allowing more than 10 seconds between each measurement can minimize the thermal build-up of the light source and maintain the measurement consistency.
g. Due to the possible variation of back-ground level produced by different sample tubes, for very low concentration measurements, you can use the same tube to perform the "Blank" reading, then remove the blank solution and fill with sample solution to perform the "Sample" reading. This technique can ensure the consistent back-ground level to achieve the optimal sensitivity.

4. Maintenance

a. Avoid over-filling the test tube and contaminate the outside wall of the tube. If the contamination is transferred to the inside wall of the test chamber, it may cause increased

signal level and hence reading error. If this happens, use a cotton swab with clean water or alcohol and gently clean the inside wall of the test chamber.

b. The touch screen can be periodically cleaned with alcohol or mild detergent.
c. If the meter will not be used for a while, remove the battery from the battery compartment before put into storage.
d. Always turn off the meter after use if the battery power is used, and remove batteries if the unit will not be in use for a week or longer.