Standard Operating Procedure for the Quantech Fixed Wavelength Fluorometer

The Quantech Life Sciences Fluorometer has the ability to measure fluorescence at two emission wavelengths (340 and 450nm) during excitation by UV light at 280 nm.

The unit should be equipped with 2 sets of filters (one for use; one spare). There should be one narrow band excitation wavelength filter at 280nm (280NB), one narrow band emission filter at 340nm (340NB), and one narrow band emission filter at 450nm (450NB) in each set; there should also be one blocking filter.

Filter Placement

The positioning of the filters are as follows:

- When facing the instrument for operation with the cover open, the 280nm excitation filter fits into the filter slot at the 6 o'clock position.
- The emission filters (either 340nm or 450nm) fit into the filter slot at the 9 o'clock position.
- The blocking filter fits into the filter slot at the 12 o'clock position; it and the 280nm filters should remain in place (not be moved).

Startup

- At least 1 hour before the first sample is to be analyzed, the instrument is turned on (switch at the back of the unit near the power cord). The instrument will go through a start up sequence and display a 15 minute countdown.
- After 15 minutes has elapsed, the display will show ← Menu →. Push the <Right Arrow> once until the display reads ← Advanced Functions →. Push the <Enter> key. <Date and Time> will appear. Press the <Right Arrow> key until <UV Lamp Options> is displayed. Press <Enter>. The display will read <UV Lamp Installed?> with <No> underneath. Press the <Up/Yes> key to turn the UV lamp on and press <Enter>. You now must wait **20 minutes** for the UV bulb to warm up. ← Menu → should once again be displayed.
- After at least 20 minutes, press the <Right Arrow> to select ← Advanced functions →(Press <Enter>). Press the <Right Arrow> until <Manually Set Gain and PMT Voltages>. Press <Enter>. The display will show <Turn off Auto Gain?> Press the <UP/Yes Arrow>and press <Enter>. Press the <Up/Yes Arrow> until Gain= 100 is displayed and press <Enter>. .

- ← Menu → will be displayed. Press the <Right Arrow> to select ←Advanced Functions→ (Press <Enter>) and <Right Arrow>until <View Diagnostic Information> is on the display. When ←Raw PMT…. Hardware→ id displayed, press the <Left Arrow> key to select <Raw fluorescence>, and press the <Right Arrow> to select <UV Lamp>. Press the <UP/Yes> key to select the gain at 100. The raw fluorescence value should start fluctuating.

Drawing Samples for Analysis

Note: SAMPLES FOR THE FLUORESCENCE INTENSITY RATIO SHOULD ONLY BE TAKEN FROM BOTTLES FIRED IN AREAS OF ELEVATED FLUORESCENCE IN THE CTD TRACE.

- A 20 ml sample is acidified with 20μL of 10% HCl in a 20 mL scintillation vial equipped with a foil or Teflon lined cap and stored at 4^{o}C.
- Samples are drawn from the same cups as used for the LISST with the following exception – pour the contents of sample cup #2 into sample cup #1 (after the LISST sample from cup #2 has been successfully analyzed). This creates an integrated sample from the one niskin bottle/sample depth.
- Draw duplicates (labeled “sampleID”-1 and “sampleID”-2) from the integrated cup. Acidify the sample. (NB – It is easier to add the acid to the vial first and then the sample to ensure adequate mixing).
- Place approximately 3.5 mL of sample into a methacrylate disposable cuvette and allow the samples to approach room temperature; a cold sample will effect the fluorescence measurement. Refrigerate the remaining sample for storage, or discard if not to be archived.

Sample Analysis

- Place the cuvette into the holder of the Quantech fluorometer. Make sure the 340nm emission filter is in the proper filter holder position (9 o’clock). Close the lid.
- Wait a moment (count to five) and glance at the display. As the reading is not stable, glance at the reading and record the first number you see in the back of the fluorometry notebook under a column labelled “340”. Repeat this procedure twice more for a total of three (3) times (for example, sample ID BM550107-2 had 340 nm readings of 55, 63, 44).
- Open the cover of the fluorometer and replace the 340nm filter with the 450nm filter. Close the cover, count to five, and read the display three times in the same way as for the 340nm wavelength (for example: sample ID BM550107-2 had 450 nm readings 27, 23, 33).

- Average the three 340nm readings (eg, 55+63+44=162 / 3= 54) and the three 450 readings (eg, 27+23+33=83 / 3= 27.7)
- Obtain the fluorescence intensity ratio (FIR) by dividing the 340 nm by the 450 nm average (eg, 54 / 27.7 = 1.95)
- Repeat this procedure for the next samples.

Reporting Data

The FIR data are to be included with the LISST particle data in the daily report. FIR should be recorded as a number on the LISST particle graph above the corresponding LISST sample in the histogram.