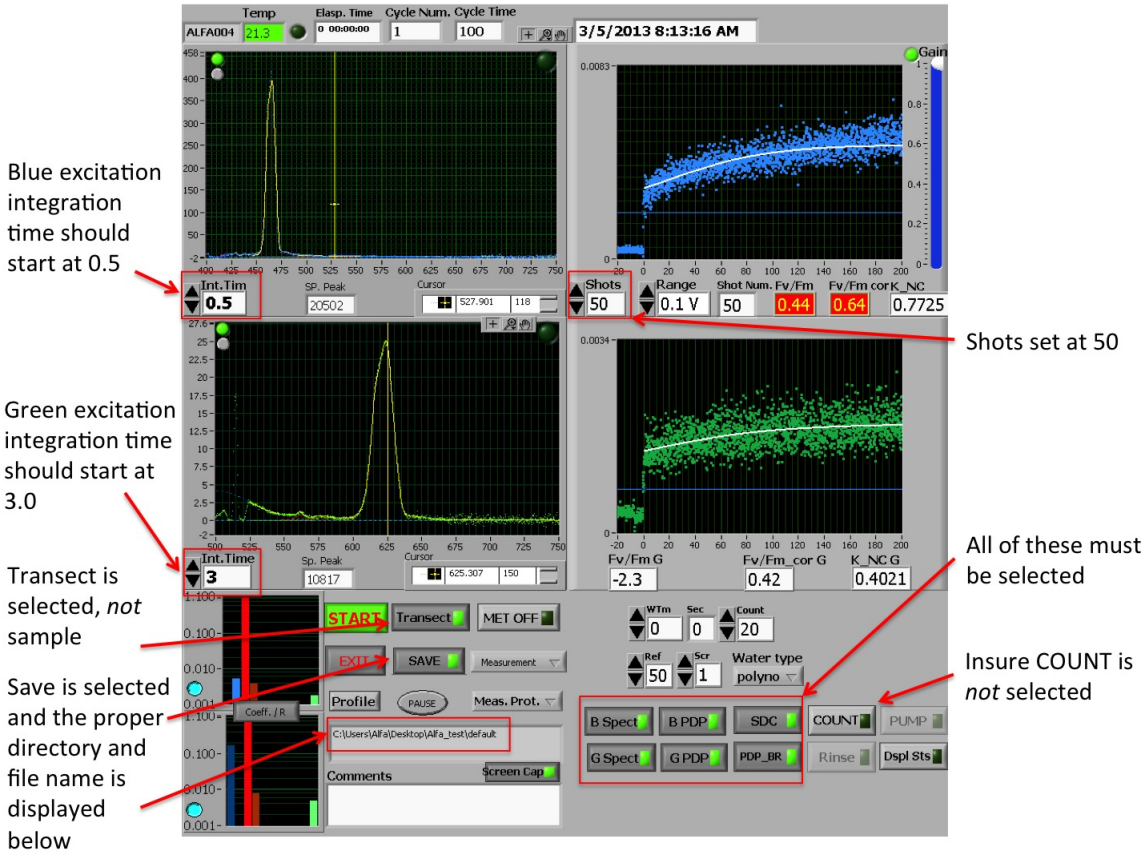
Aquatic Laser Fluorescence Analyzer (ALFA) Daily Maintenance

Thomas Leeuw and Alison Chase

1. Stop sampling using the 'STOP' button.
2. Clean ALFA following the steps in the Cleaning section of the manual
3. Reconnect ALFA to flow through system
4. Integration time for blue excitation set at 0.5, Integration time for green excitation set at 3.0, and Fv/Fm shots set at 50 (integration time may auto range during the day, this is simply a starting point)
5. B Spect, G Spect, B PDP, G PDP, SDC, and PDP BR buttons are all selected
6. Insure the 'COUNT' button is not selected
7. Software should appear like the screen shot in the figure below (same as figure 3 in the manual)
8. Select 'SAVE' and use the naming convention: ALFA_YYYYMMDD_hhmmss. Where the YYYYMMDD is the date and hhmmss is the current time in UTC (e.g. ALFA_ 20130403_142530 is April 3, 2013 at 14:25:30).
9. Select the 'START' button
10. Ensure that fluorescence emission spectra are being collected as well as Fv/Fm for both blue and green excitation (plots should start updating quickly)



Aquatic Laser Fluorescence Analyzer (ALFA)

Thomas Leeuw and Alison Chase

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1 Introduction

The Aquatic Laser Fluorescence Analyzer, ALFA, is a flow-through instrument capable of fast, broadband, laser-stimulated fluorescence emission spectral measurements and phytoplankton photophysiological assessments. The ALFA will be run in flow through mode on board the Tara and will sample continuously.

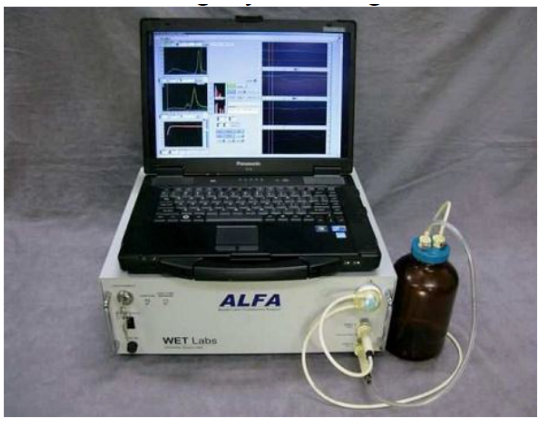

Figure 1: ALFA with computer and sample bottle (bottles only used for cleaning)

2 Generalized Setup Procedure

1. Ensure input and output ports are connected to flow through system
2. Turn ALFA on (rocker switch located on the front of the ALFA) then turn lasers on (key switch on the front of the ALFA) (figure 2)
3. Open software (always done after the ALFA has been powered on)
4. Integration time for blue excitation set at 0.5, Integration time for green excitation set at 3.0, and Fv/Fm shots set at 50
5. B Spect, G Spect, B PDP, G PDP, SDC, and PDP BR buttons are all selected
6. Insure the 'COUNT' button is not selected
7. Software should appear like the screen shot in figure 3
8. Select 'SAVE' and use the naming convention: ALFA_YYYYMMDD_hhmmss. Where the YYYYMMDD is the date and hhmmss is the current time in UTC (e.g. ALFA_ 20130403_142530 is April 3, 2013 at 14:25:30).

9. Select the 'START' button
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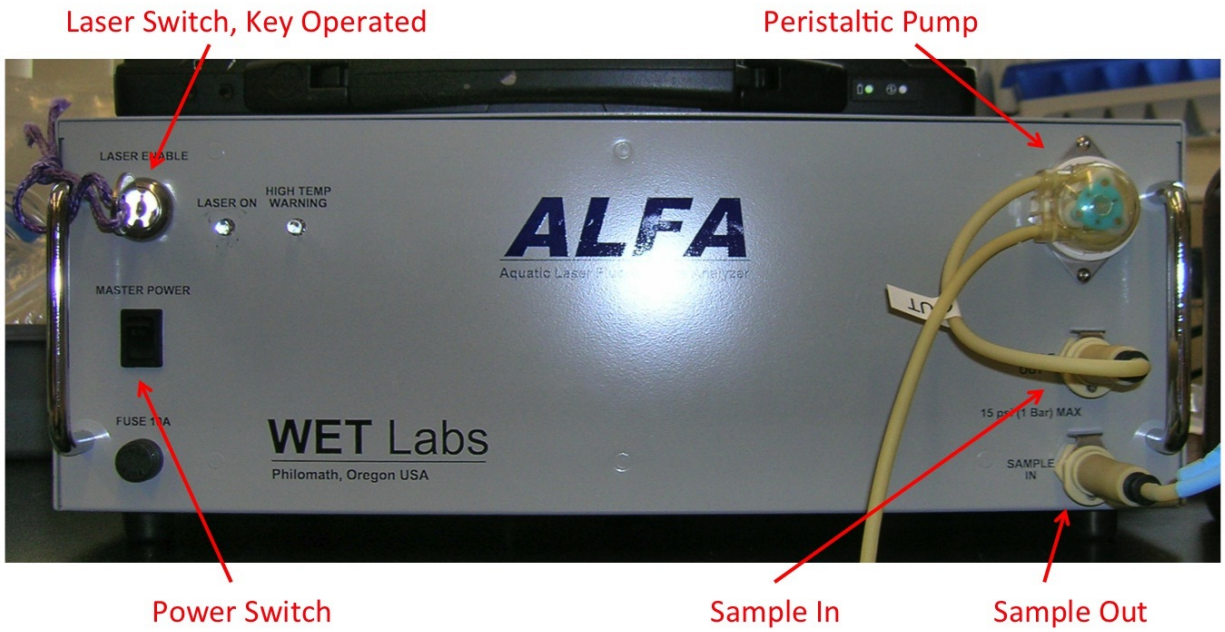


Figure 2: Power switches and input and output ports. Peristaltic pump will only be connect to sample in and out when cleaning the instrument

3 Cleaning

3.1 Preparation of Cleaning Solutions

1. Prepare three bottles as follows:
   (a) Water (1.0 L) - Deionized (or distilled) water.
   (b) Detergent - Dilute to 50:1. 250 ml of water and 5 ml of mild detergent (dishsoap, Triton-XTM, LiquiNox) will make enough solution for one cleaning.
   (c) Bleach - Dilute household bleach to 50:1. 250 ml of water and 5 ml of bleach will make enough solution for one cleaning.
2. *Cautions*:
   (a) Do not use stronger solutions or solutions other than those recommended.
   (b) Soak time is not required. Always flush promptly with water.

3.2 Software Settings

1. Use Sample Mode (Transect button is deselected),
2. 'COUNT' not selected.

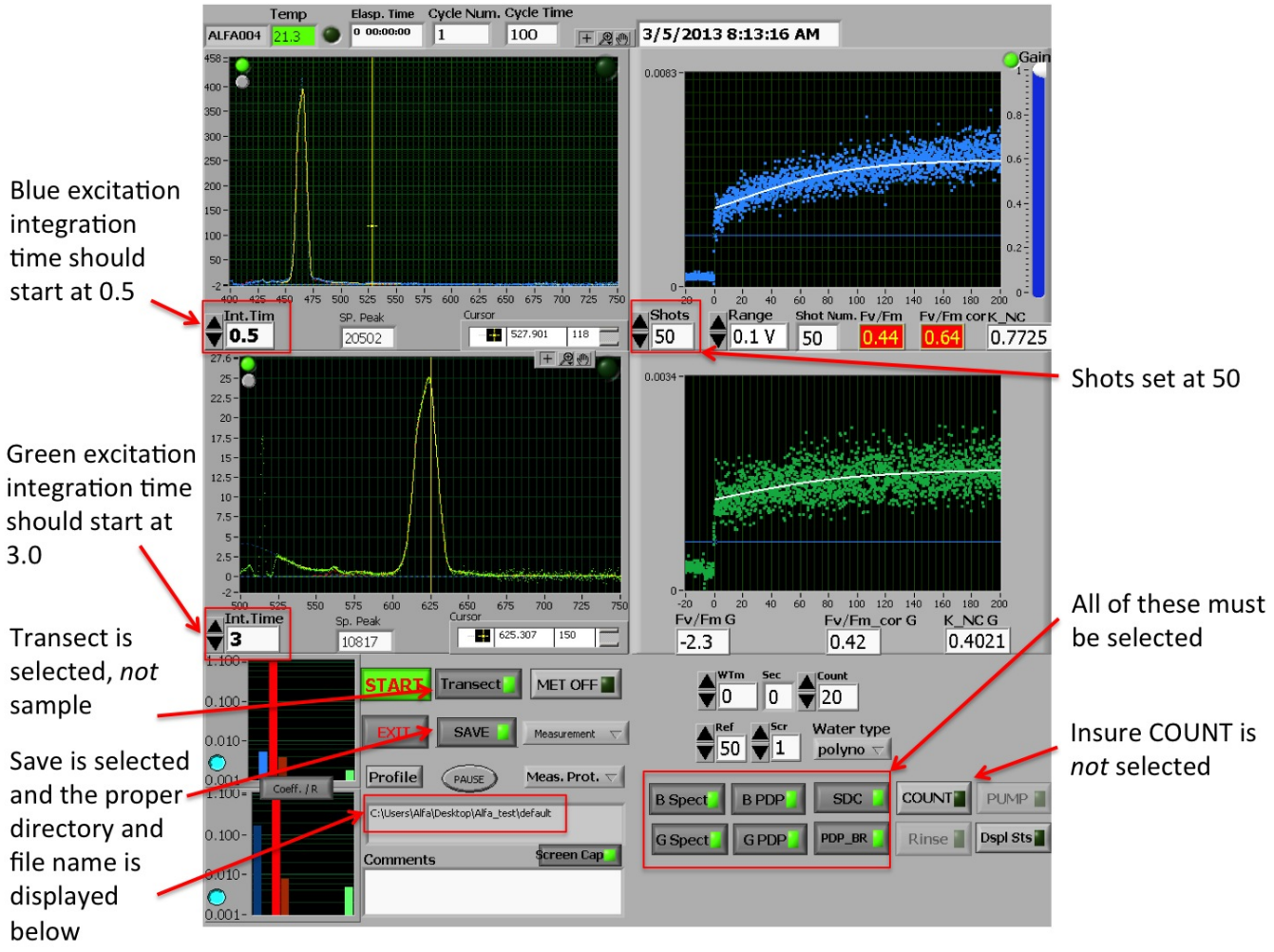
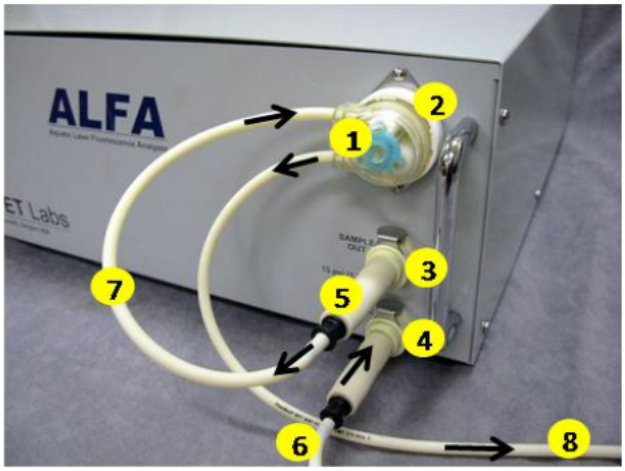


Figure 3: Screen shot of correct ALFA software settings to collect data continuously.

3.3 Circulating Cleaning Solution

1. Install the peristaltic pump (ignore if already installed)
   (a) Push the pump onto the pump seat located above the sample out port
   (b) Pump should be oriented so the tubing points to the left
   (c) Turn pump slightly clockwise to lock the pump into the pump seat
   (d) Attach tubing according to figure 4



ALFA setup for Bottle Samples

(1) Pump
(2) Pump Seat
(3) ALFA sample out
(4) ALFA sample in
(5) Hose coupling adapter
(6) Tubing from sample bottle
(7) Tubing to pump (pump pulls fluid through ALFA)
(8) Tubing to waste receptacle

Arrows show flow direction

Figure 4: Proper pump setup for cleaning of the ALFA. Pump should draw fluid through the system (i.e. pump is connected to the sample out port)

2. Connect output to waste bottle.
3. Insert the intake tube in a beaker of DI water. Pump (by selecting the pump button) 200 mL of clean water through the system to clear remaining sample. If water does not flow, prime the pump by attaching a syringe to the output and drawing some vacuum.
4. Pump 200 mL of diluted detergent through the system. While pumping this solution, lift the tube out of the solution for about 1 sec to allow a burst of air. Do this several times before exhausting your supply of detergent.
5. Flush the system with at least 400 mL of clean water. While flushing, lift the tube out of the solution for about 1 sec to allow a burst of air. Repeat every 5-10 sec. Continue flushing with water until the effluent is no longer foamy or soapy.
6. Pump 200 mL of diluted bleach solution through the system.
7. Flush the system with at least 400 mL of DI water.
8. Verify your clean water spectra while flowing DI water. Any contaminant spectra should gradually fade and the main feature present on the spectral plots should be Raman scattering (as seen in figure 1).

4 Software Notes

Provides an overview of software features.

4.1 Logging Data

1. Selecting the 'SAVE' button will allow you to select a directory and name for the output files.
2. New data will not be appended to an existing file after sampling has been interrupted using 'STOP.'

3. A new file name will have to specified every time sampling is interrupted using the 'STOP' button.

4. Software outputs 11 text files (.txt) and one folder into the selected directory. The folder contains 7 screen captures (.png) taken at the end of logging session (when the 'STOP' button is selected).

5. Text files:

bm	Fluorescence emission spectra under **blue** excitation
bp	Protocol file for **blue** excitation (contains settings such as integration time)
Bpm	Variable fluorescence using **blue** excitation
gm	Fluorescence emission spectra under **green** excitation
gp	Protocol file for **green** excitation (contains settings such as integration time)
Gpm	Variable fluorescence using **green** excitation
GPS	GPS coordinates (not in use)
m	Output from spectral deconvolution
mavg	Averaged data
pp	PDP measurements
s	Output from spectral deconvolution (continued)

4.2 Sample Mode (used only for cleaning)

1. Sample Mode - Count button selected.

   (a) After selecting start, the pump will begin to run and a single variable fluorescence measurement will be made, then fluorescence emission spectra will begin to be collected.

   (b) The pump will continue to run until the number of fluorescence emission spectra specified in the 'Count' box have been collected (when 'Cycle Num.' in the upper left equal the count value).

   (c) Both variable fluorescence and fluorescence excitation spectra will be displayed while the pump runs. No time series measurements (right three graphs) will be displayed.

   (d) Can be used to check for residual fluorescence after cleaning.

   (e) Changing integration time using this mode will not change SP. Peak height.

2. Sample Mode - Count button deselected

   (a) Pump will not run after hitting start, will only run if 'PUMP' button is selected.

   (b) Instrument will take measurements until 'STOP' button is selected.

   (c) Fluorescence emission spectra, variable fluorescence, and time series measurements will all be displayed.

   (d) Similar to transect mode (see below).

3. Pump Button

   (a) Runs pump, no measurements taken, no data logged.

   (b) Used to empty or clean the instrument.

4. Rinse Button

(a) Runs pump for specified number of seconds specified in 'rinse controls' (found in the drop down menu next to the pause button), before taking any measurements.

(b) Will immediately begin sampling after rinse is completed.

4.3 Transect Mode (used to collect continuous data)

1. Pump will not run, used for flow through only.
2. Fluorescence emission spectra, variable fluorescence, and time series measurements will all be displayed in real time.
3. Pump and rinse buttons are not available in this mode, switch to Sample mode to use these features.
4. Count will limit the number samples taken to the number listed in the 'Count' box (should not be used in this mode).

4.4 Graphs

1. Fluorescence Emission (Top Left)
   (a) Top graph for blue excitation, raman peak at 465nm
   (b) Bottom graph for green excitation, raman peak at 625nm
   (c) Scrolling and zooming can be accomplished by using the toolbar located at the top right of each graph
   (d) Grey button on top left of each graph toggles between linear (default) and log scale
2. Coefficients (Bottom Left)
   (a) Spectral deconvolution coefficients for the blue excitation (top) and green excitation (bottom)
   (b) Teal button to the left toggles between log (default) and linear scale
   (c) Bar graph key:

Number (from left to right)	Color	Coefficient
1	Dark Blue	Elastic Scattering
2	Light Blue	CDOM
3	Light Red	Water Raman
4	Dark Red	Phycoerythrin 565nm (PE1)
5	Brown-Orange	Phycoerythrin 575nm (PE2)
6	Yellow	Phycoerythrin 589nm (PE3)
7	Purple-Pink	Phycoerythrin 613nm (R1)
8	Purple-Pink	Phycoerythrin 625nm (R2)
9	Purple-Pink	Phycoerythrin 642nm (R3)
10	Purple-Pink	Phycoerythrin 662nm (R4)
11	Light Green	Summed Chlorophyll

3. Variable Fluorescence (Middle)

   (a) Fv/Fm magnitude over time (μs) under blue excitation (top) and green excitation (bottom)

   (b) Gain adjusts automatically

   (c) if there are problems with the vertical scale, right click the axis turn off 'autoscale y' and set scale manually by changing the number in the 'Range' box.

   (d) Fv/Fm is the raw value

   (e) Fv/Fm core is the value corrected for background non-photophysiological fluorescence

4. Time Series (Right)

   (a) Measurement number displayed on x-axis (measurement number is equal to 'Cycle Num.' minus one).

   (b) Different y-axis displayed on left and right side of each graph.

   (c) y-axis can be changed by selecting the drop down box below each axis.

   (d) Line color and styles can be adjusted by selecting the icon next the variable name on the right side.

   (e) Description of variables:

FChl (Top Graph)	Chlorophyll fluorescence normalized to raman, **blue** excitation
Fcom	Fluorescence of CDOM
Fv/Fm B	Variable fluorescence under **blue** excitation
Fv/Fm G	Variable fluorescence under **green** excitation
PAR	Photosynthetically active radiation (not in use)
FPE1	Phycoerythrin fluorescence at 565nm normalized to raman
FPE2	Phycoerythrin fluorescence at 575nm normalized to raman
FPE3	Phycoerythrin fluorescence at 589nm normalized to raman
FChl (Second Graph)	Chlorophyll fluorescence normalized to raman, **green** excitation
Chl., ug/L	Chlorophyll in micrograms per liter
AC	Allophycocyanin
SYN	Synechococcous
SYN/AC	Synechococcous to allophycocyanin ratio
Chl. g	Chlorophyll in grams
FR2	Phycoerythrin fluorescence at 625nm normalized to raman
FR3	Phycoerythrin fluorescence at 642nm normalized to raman
FR4	Phycoerythrin fluorescence at 662nm normalized to raman

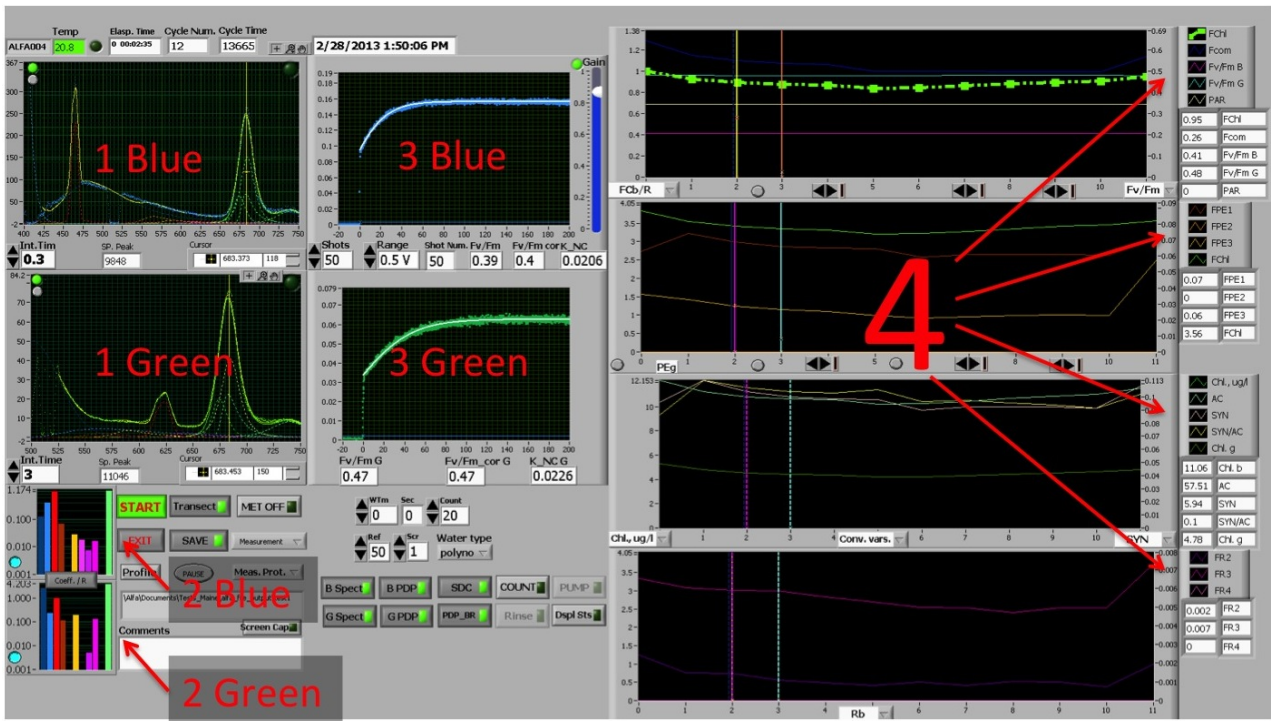


Figure 5: Graphs displayed in ALFA software (explanation in section 4.4)

4.5 Exiting Software

1. Do not press the small round red button in the upper left
2. Press the 'EXIT' button, located next to the 'SAVE' button (it will look deselected after you press it), then the software window can be closed.
3. Failing to the press the 'EXIT' button before closing the software will cause an error. If it does occurs, it should not affect the instrument or the data and the program can be resumed normally.

5 Packing ALFA for storage or shipment

1. Clean the flow system as described above.
2. Shut down ALFA (turn off laser, turn off main power switch), the computer, and disconnect from the power supply and the computer.
3. Disconnect the hose coupling inserts from the input and output.
4. Connect a short piece of tubing between the input and output (figure 6). This will keep the plumbing passages moist, making ALFA easier to prepare for future use. Also, and excess water in the system will be unable to leak during transit.
5. Pack ALFA and accessories in the shipping case in the same arrangement as it was in when unpacked.

6. *Cautions*:

   (a) Protect ALFA from freezing.

   (b) Flush and clean ALFA before subsequent use.

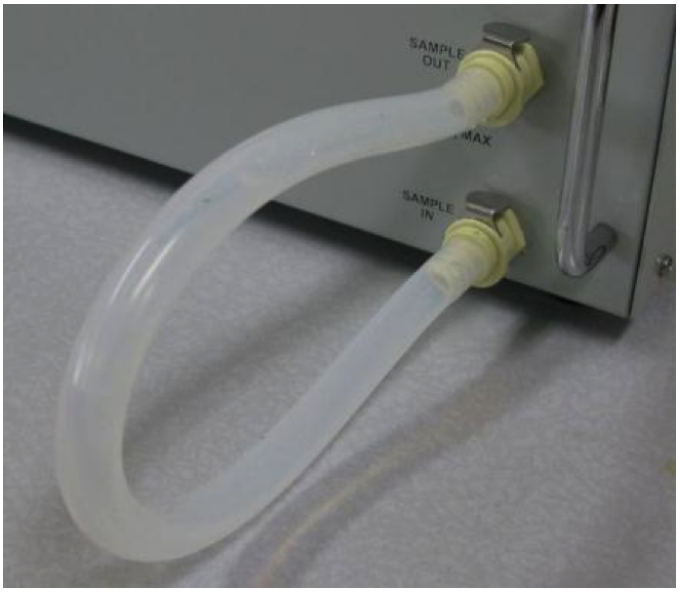


Figure 6: Input and output ports connected with a short piece of tubing prior to shipment