

## Rainbow Software

When the Rainbow software application is launched, the following window appears (the labels on the controls will vary depending on the laser configuration chosen in the preferences window):

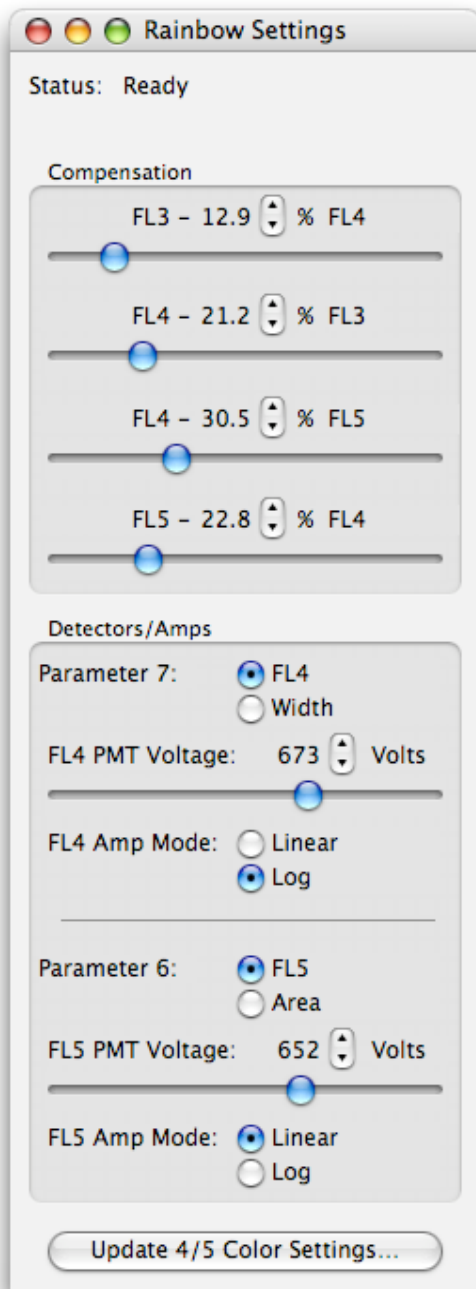


Figure 1

From this Rainbow Settings window, the compensation, detectors and amplifiers settings can be adjusted.

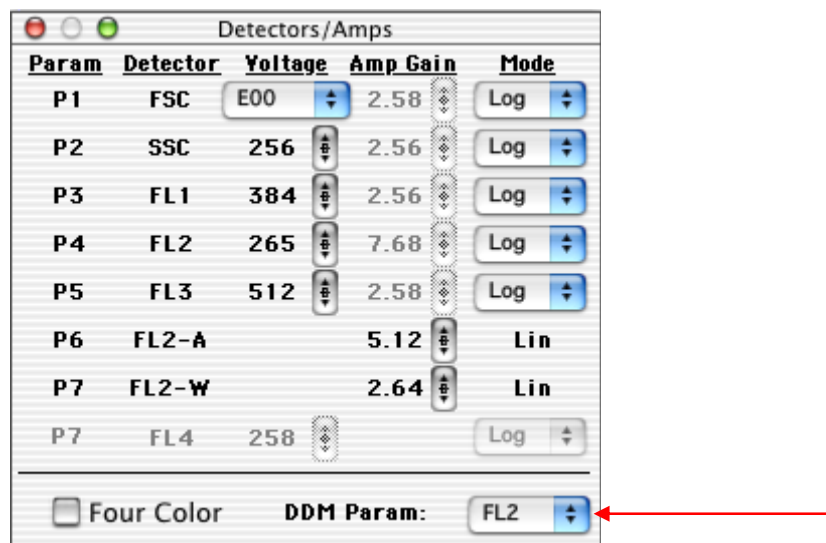
## Setting up the 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> Color Parameters

These parameters are set up as usual in CellQuest (set the PMT voltages to place the unstained cells in the lower left quadrant on the FL1/2 and FL2/3 dot plots).

## Setting up the 4<sup>th</sup> and 5<sup>th</sup> Color Parameters

### CellQuest Settings

**Please Note:** Be sure to specify that DDM is present in the Detectors/Amps window, and be sure to save all 8 parameters in the acquisition and storage menu.



1. Open up a CellQuest document as usual. Under *Acquire*, in the *Edit Reagent List* window, add "FL4-H" for P7 and "FL5-H" for P6. Under *Acquire* in the *Parameter Description* window, enter FL4-H for P7 and FL5-H for P6. Under *Plots* in the *Log Data Units* window, select 0-1023, so the FL4 and FL5 plots look the same as the other plots.
2. Set up a FSC/SSC, FL3/FL4, and FL4/FL5 dot plots. Under *Detectors/Amps*, be sure to click DDM.

### Rainbow Settings

1. Open the Rainbow software. Note that switching between CellQuest and Rainbow is automatic when a window is clicked in either application. However, the first click is required to switch the application to the foreground, while a second click is required to actually change the desired parameter.
2. Set the FL4 and FL5 Amp Modes to "Log".
3. Select FL4 and FL5 parameters. If "Width" is selected instead of "FL4", the width parameter specified in CellQuest will be acquired rather than the FL4 parameter. Similarly, if "Area" is selected instead of "FL5", the area parameter specified in CellQuest will be acquired rather than the FL5 parameter. In the Rainbow Settings window, if "Area" or "Width" is selected, the corresponding Amp Mode is forced to "Linear", and the PMT Voltage controls are disabled.

### Running Samples

Set up gates, data file names and data acquisition termination criteria as usual. Note that it is possible to gate on FL4 and FL5 parameters if desired. Data files will be saved with the same axis labels used to acquire the data (e.g. FL4-H or a width parameter).

It is necessary to use the "Linear" Amp Mode setting in FL4 and FL5 to obtain valid CVs. Other stats, such as means and percent positives, are valid in log or linear modes.

### **Using Rainbow to Save 4/5 Color Information to Data Files**

Since Rainbow and CellQuest are run as separate applications, certain FCS data file header information will not be set correctly unless you set up Rainbow to automatically update the file with the correct 5 color header information as the files are acquired.

#### **Auto Scan**

To allow Rainbow to automatically save your data files with the correct 5-color keywords please follow these steps:

1. Click on the *Update 4/5 Color Settings...* button at the bottom of the Rainbow Settings window. The following window will open:

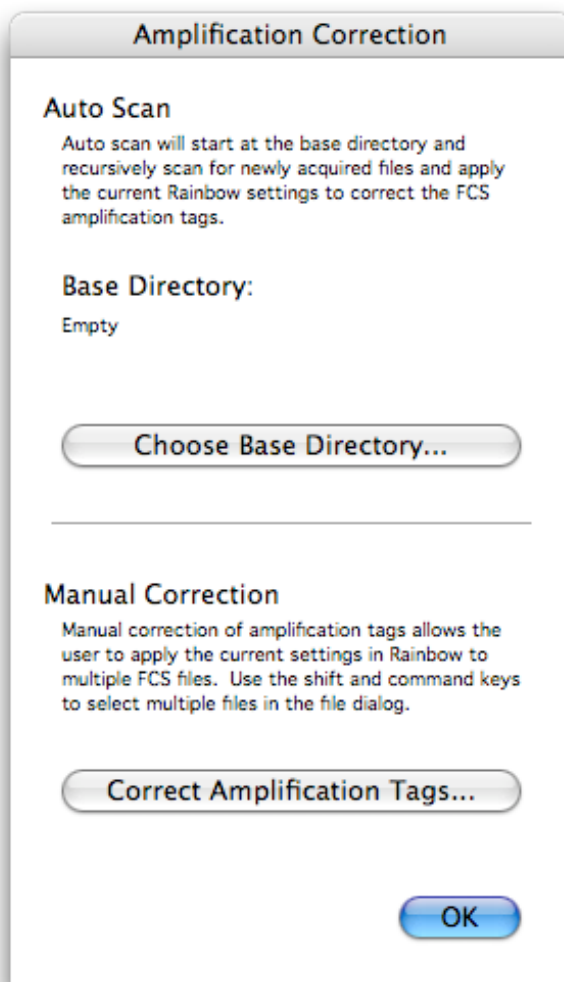


Figure 2

2. Click on the *Choose Base Directory...* button and select the directory where CellQuest will be saving the FCS data files. By choosing a directory to "Auto Scan", Rainbow will automatically update your CellQuest data files with the correct 5-color header information as they are saved by CellQuest into that directory.

### **Importing Rainbow Settings**

The 5-color instrument settings can be imported into the Rainbow software from any CellQuest data file that has previously been modified by Rainbow, either automatically or manually. This can be done by selecting the *Import Settings...* menu item in the File menu, or by pressing the “command (apple)–O” keys to open the data file containing the Rainbow settings you wish to import. After you choose the data file, the Rainbow Settings window will change to reflect the imported instrument settings.

**Warning:** If you import a file that was created on a system with a different laser configuration, the laser configuration in the preferences window will change as well. You will need to open the preferences window and ensure that the laser configuration is correct for your hardware.