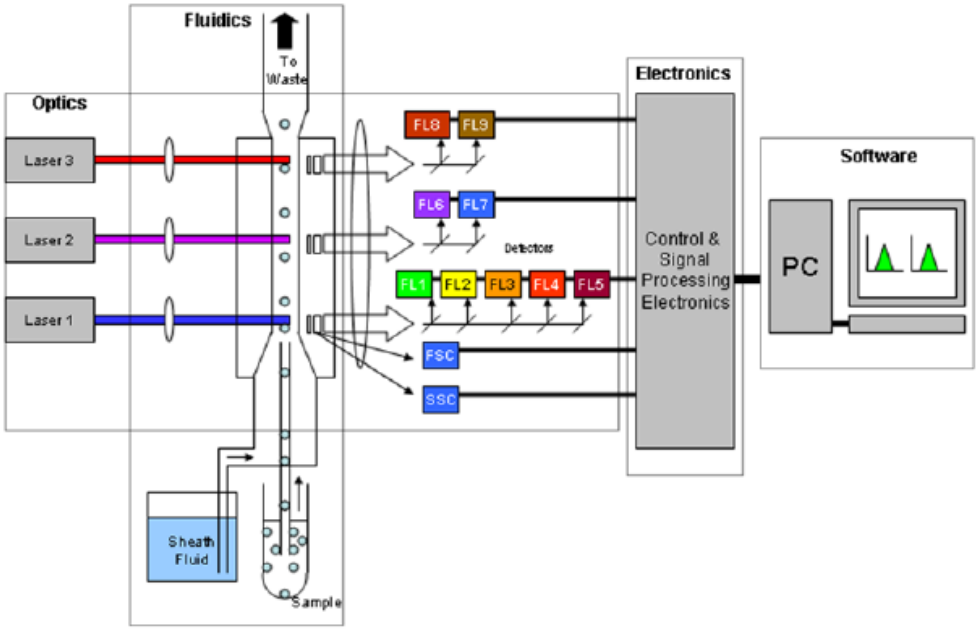
CyAn ADP Guide

Figure 2.2 CyAn ADP Functional Block Diagram



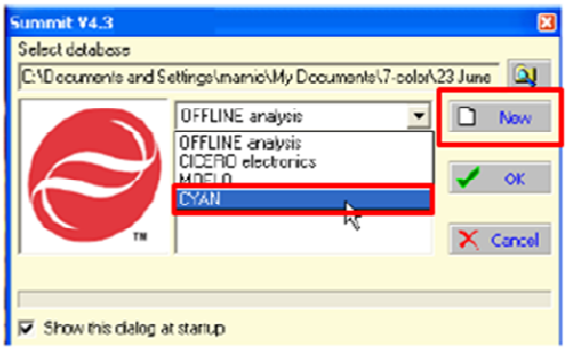
Starting Up

1. Check the sheath and waste fluids, if needed, fill and empty:
    1. To fill the Sheath Tank:
        a. Unscrew the top of the tank labeled “Sheath Tank”, and set it on top of the tank.
        b. Using the Funnel provided on the table just inside the door (or good aim!), add Sheath Fluid (labeled containers on table) up to the line marked on the tank.
        c. Replace the top.
    2. To empty the Waste Tank:
        a. Unhook the waste tank from the Sheath Management System- there are two quick releases for the lines.
        b. Unhook the filter from the waste tank (quick release)
        c. Empty the Waste into the Sink
        d. Pour approx. 15ml of bleach into the Waste Tank
        e. Replace the Waste tank and reattach the lines and filter

2. Log into the computer using your Pawprint ID and Password (the CyAn is turned on in the software)
3. Open Summit by double clicking on this icon on your desktop:

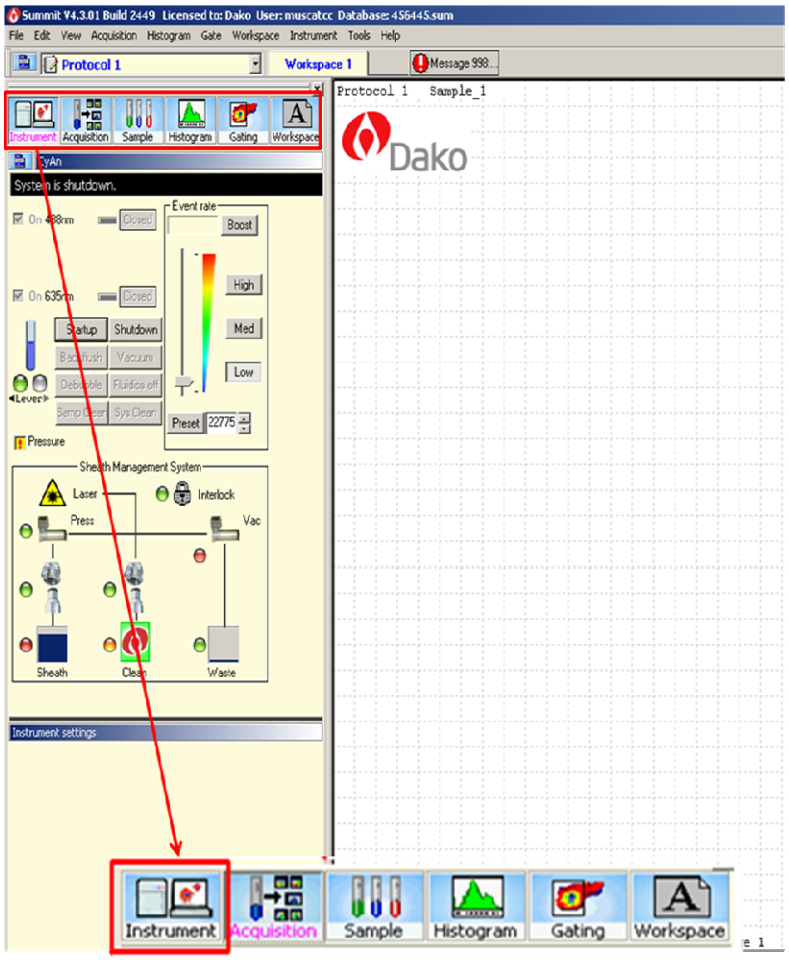
4. This window will appear:

Figure 3.1 Select a New Database

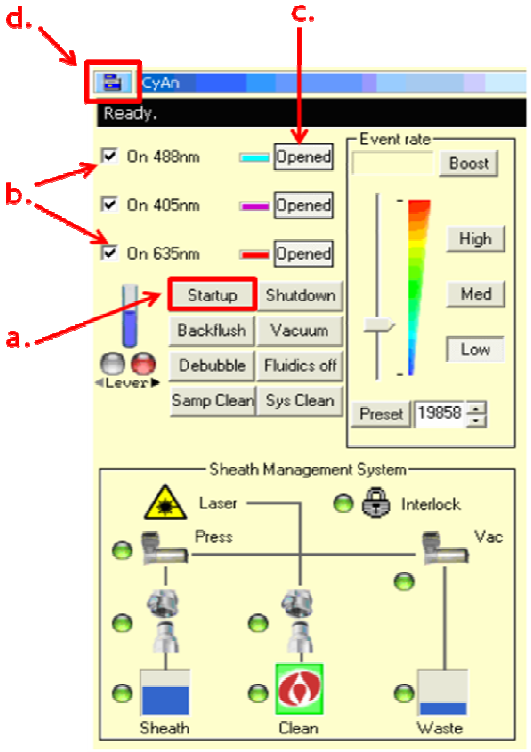


5. Select CyAn
6. Click “new” to create a new database (or select a previous database).
   - A database is a collection of protocols and data. If you would like to pick up where you left off with your protocol and data, open a previous database. This approach is more likely to have ‘bugs’ and crash, so the CIC recommends running in a new database each time and opening your protocol (which will have your previously made histograms, just not your previously recorded data).
   - If you are making a new database, save it on the Data Drive in your Lab’s folder. If you would like to keep the database, label it with a significant name and store it in your lab’s Protocol folder. Unless it is saved in the Protocol folder, it will be erased on a monthly basis.

7. After making the Database, this screen will pop up:



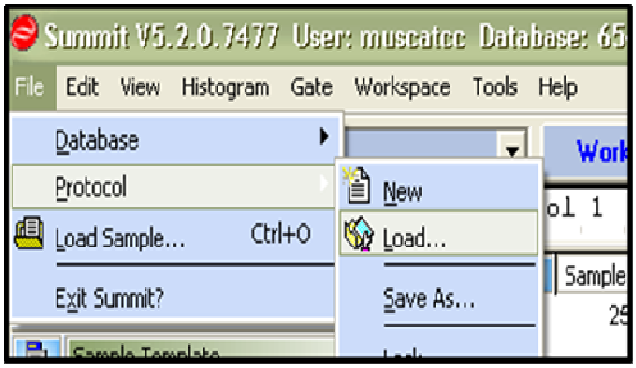
8. In the Summit Control Panel, Click the Instrument tab. The following window will show below the instrument control panel:



a. Click Startup and follow the directions
b. Make sure the required lasers are checked (lasers take 30 min to stabilize)
c. Make sure the laser shutters are closed (click on the 'opened' button to close)
d. If you would like to have a separate window for instrument control (comes in handy when changing event rate while running), click on the blue box in the upper left corner of the window and choose "Detach Floating".

Instrument Setup

- Open up a protocol
  - *If you have a protocol made already:*
    - Choose **File**
      1. Protocol
      2. Load



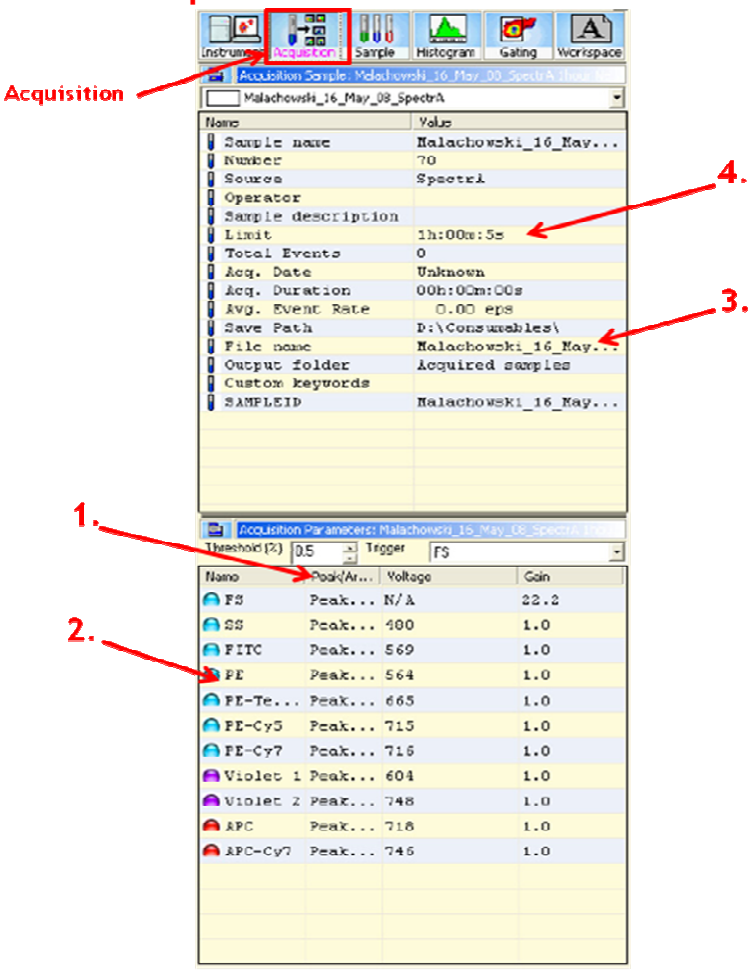
3. Find your protocol:
    a. My Computer
    b. Data (D:)
    c. Customer
    d. Your folder

4. Be sure to Reset your Save Path to a new experimental Folder:

    a. Double-click the save path row (see #3 below)
    b. Set the folder (My computer: Data: Customer: Your Folder)

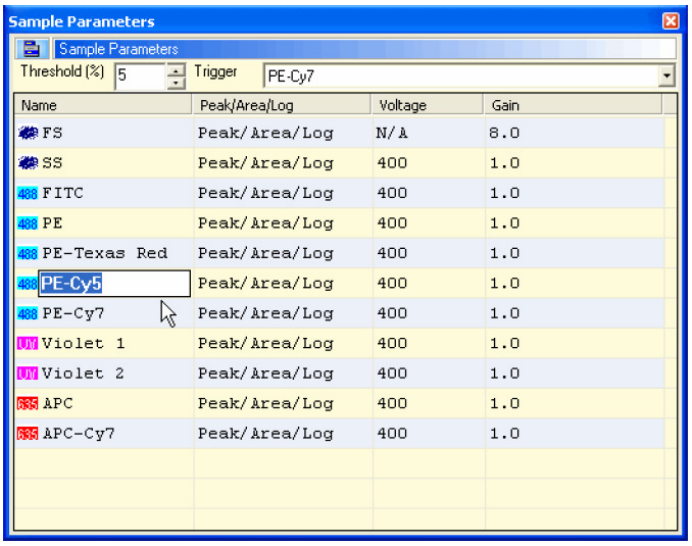
- *If you do not have a protocol*, use the following guide to create one:

    - Click the **Acquisition** tab



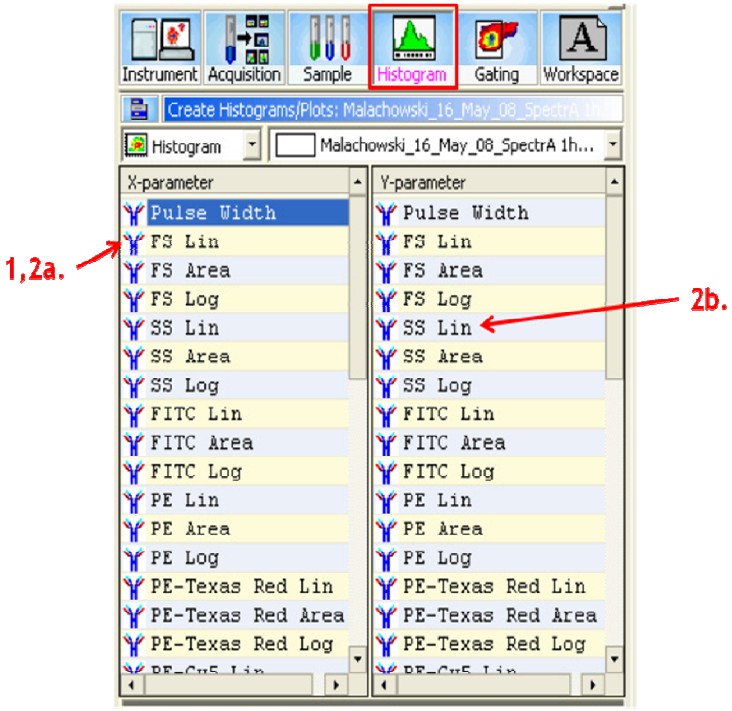
1. Choose the parameters you want to collect information on in your experiment.
    a. In the Peak/Area/Log Column, double click the box next to the channels you would like to use. For FSc/SSc we generally choose peak and area. For fluorescence channels we generally choose Log. **If you are looking at Cell Cycle, choose Peak**

2. Change the parameter names to customize your experiment (not required)



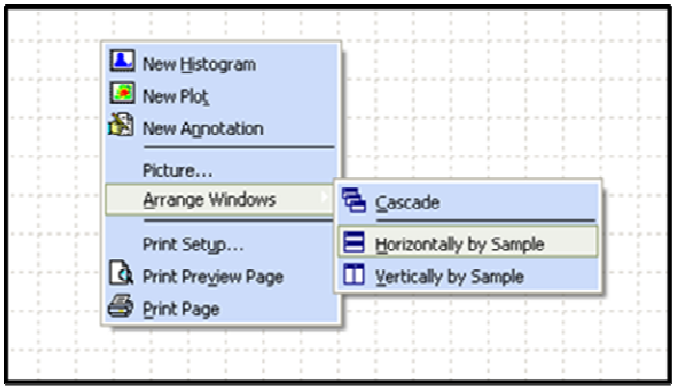
   a. If you would like to change the name of the channels to match your experiment, double click on the name, and write in your label
3. Set your save path
   a. Double-click the save path row
   b. Set the folder (My computer: Data: Customer: Your Folder)

- Click the **Histogram** tab



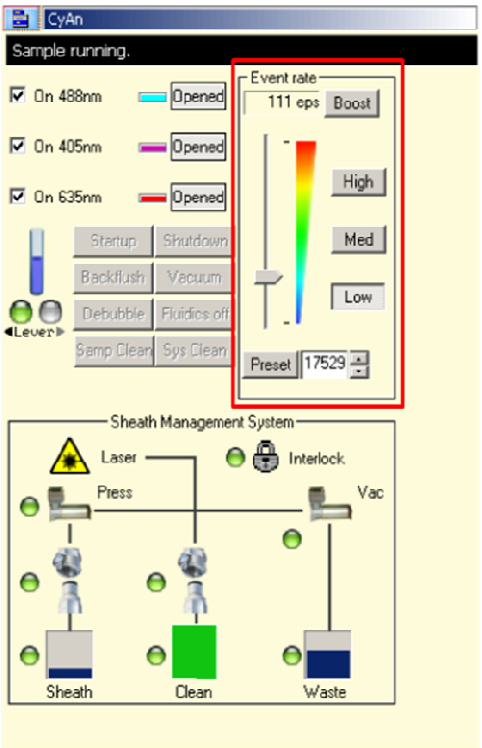
1. To create a histogram
   a. Double-click the x-parameter you want
   b. The plot will show up in the blank workspace to the right of the menu
2. To create a dot plot
   a. Single-click the x-parameter you want
   b. Double-click the y-parameter you want
   c. The plot will show up in the blank workspace to the right of the menu

3. Once you've created all your dot plots and histograms, you can quickly organize them by right clicking in the workspace area and choosing: Arrange Windows, etc:



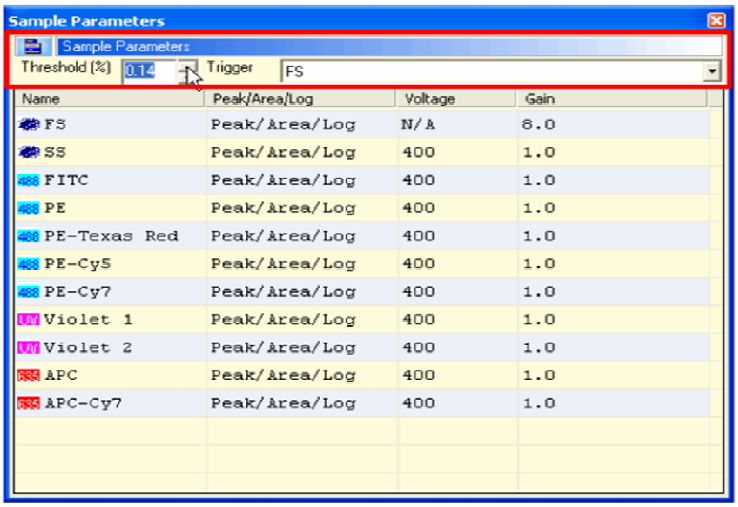
Acquiring Samples for setup

1. Put your negative control sample on the SIP (your samples must be in polypropylene tubes, not the polystyrene that the BD instruments require)
2. Press F2 to start acquiring (adjust the event rate with the speed buttons and scroll bar on the instrument tab).



**If this is a new protocol, and you don't see any events (maybe a small bundle of events on the axis), then THRESHOLD needs to be adjusted. Do the following:

- Click the Acquisition tab
- Make sure Threshold is triggering off of FSC. Raise Threshold until you see cells appear on your plot (make sure you are running at a medium to high rate) this value is usually between 2-3%.



- Once cells appear, adjust event rate so cells are running at about 200eps,
- You may have to lower Threshold to ensure you aren't cutting off your population of interest.

3. Adjust the voltage and amp gain values to properly set your negative control:

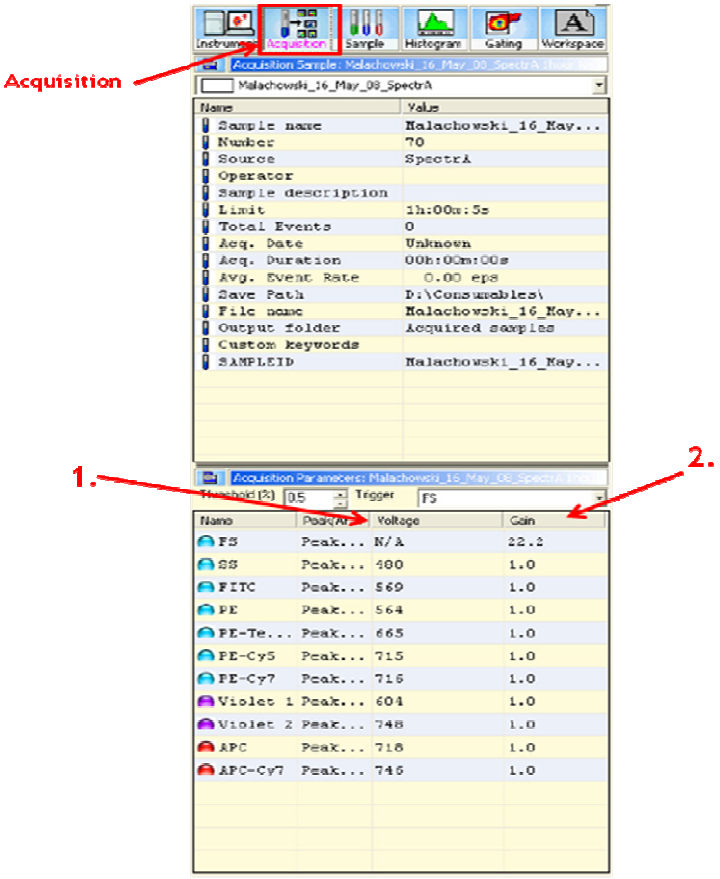


Figure 3.14 Set Voltage for a Parameter

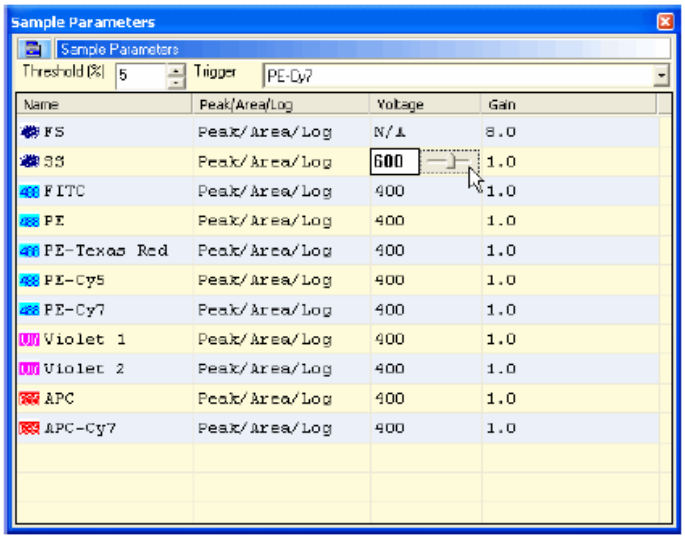
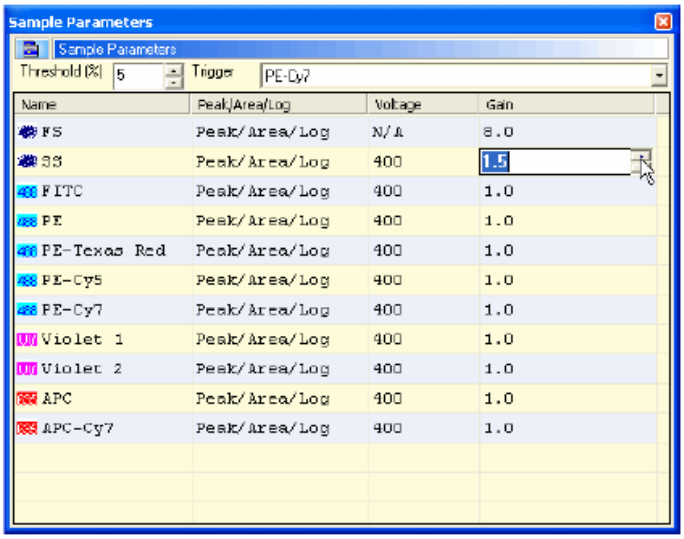
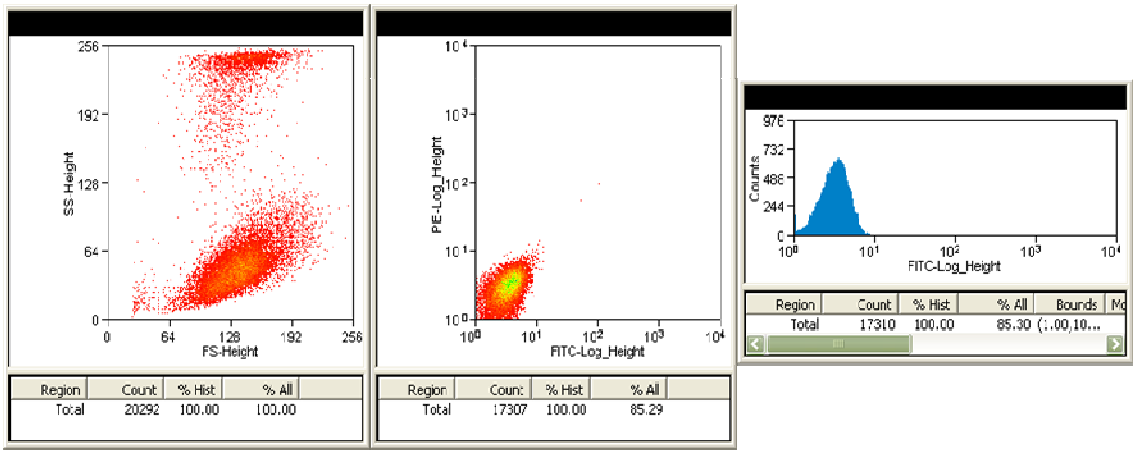


Figure 3.15 Set Gain for the Voltage



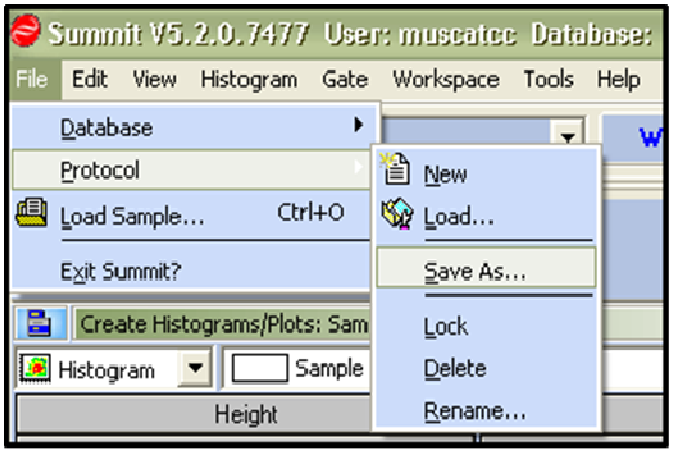
- Your negative control cells should be centered on our FSC/SSC plot and within the first decade of your Fluorescence Log Plots:



a.FSC/SSC in viewing range b/c. FITC/PE dot plot anc FITC histogram within first decade

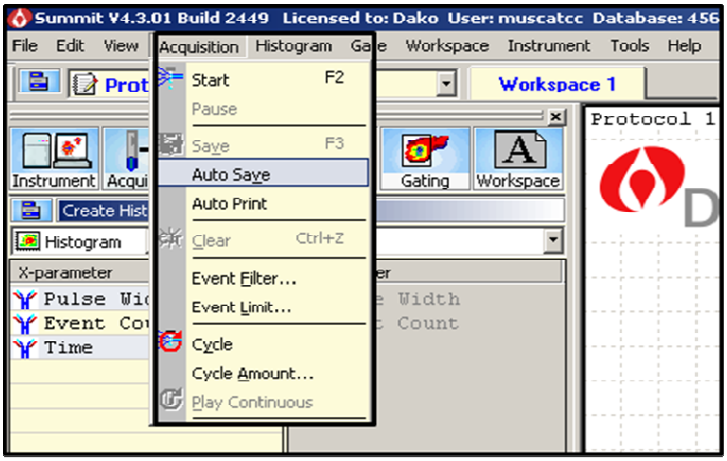
4. Run your other controls to verify the voltage and compensation settings are correct (for compensation guide see below)

5. If you would like to save this protocol for future use, go to File:Protocol: Save As…
   a. My Computer: Data (D:): Customer: YourPI: PROTOCOL FOLDER.
   b. Be sure to save your protocol in your lab's specified Protocol Folder. This is the only folder that will not be erased on a monthly basis.

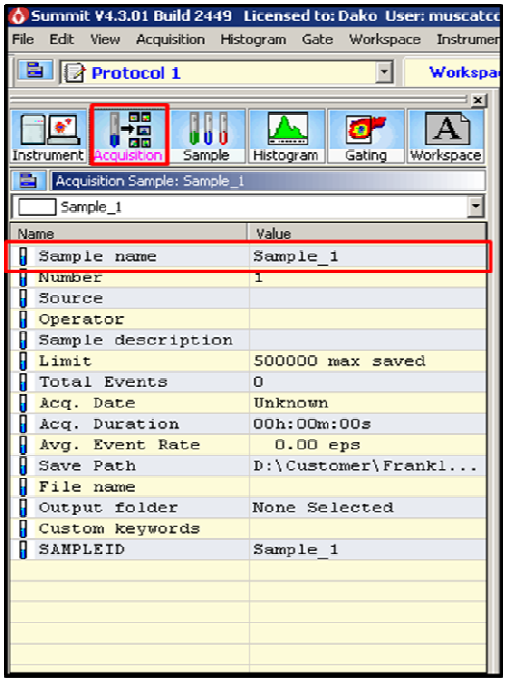


Acquisition for your Experiment:

1. Set up Auto Save to ensure you save every sample:



2. Label your Sample by double clicking on the Sample Name Row:



- A window will pop up, and you can label in the "rules string" area.

3. Acquire the sample (F2 or go to Acquisition: Start)
    - Sometimes, the sample doesn't start properly, and by using the short cut Ctrl + Z, you can refresh the data shown on the histogram so everything is plotted properly.
4. If you've set limits, allow the run to finish
5. If you prefer to run without limits, watch your event count in the window below your histogram plots, and press F2 to stop acquisition (or go to Acquisition: Start… which will stop it)
6. Save the sample (F3 or if you've set up auto save, the save window will pop up automatically

Compensation

Single-color compensation

1. Click the blue button on the plot you would like to compensate
    - Compensate (for manual control)
    - Autocompensate (this will only compensate the x-parameter)

Multi-color compensation

1. Click the Sample tab
    - Collect and save your single color controls (once you've set voltages)
    - Click the blue button on the compensation menu (lower left of screen)
    - Choose "Autocompensate"
    - Set your live gate and enter your control files in the correct parameter
    - Press OK and follow the directions

CyAn Shutdown Procedure

Standby- the following should be done if someone has reserved the instrument within 2 hours of your appointment (i.e. you are done at 1:00 and someone is reserved for 3:00 or earlier). To check the reservation schedule please go to: http://biotech.missouri.edu/cgi-bin/ureserve.pl

1. Click on the "FLUIDICS OFF" button in the Instrument Panel of Summit
2. You will be prompted to put a tube of cleaning detergent (Triton-X 100) on the SIP
3. You will then be prompted to pull the tube of detergent off, and allow the instrument to back flush.
4. After the back flush, the fluidics should be off
5. Close the Shutters on the lasers (in the instrument panel of Summit, click on the box that reads "open".. you will hear a clicking noise and the button will then read "closed"
6. Close the software (click on the X in the upper right hand corner of the Summit window)
7. Log out of your account

Shutdown- the following should be done if someone has reserved the instrument within 2 hours of your appointment (i.e. you are done at 1:00 and no one is signed up until 3:30 or later).

1. Click on the "SHUTDOWN" button in the Instrument Panel of Summit
2. You will be prompted to put a tube of cleaning detergent on the SIP
3. You will then be prompted to put a tube of water on the SIP
4. After the water runs through, the instrument should shut down completely (no more noise)
5. Close the Software (click on the X in the upper right hand corner of the Summit window)
6. Log out of your account