

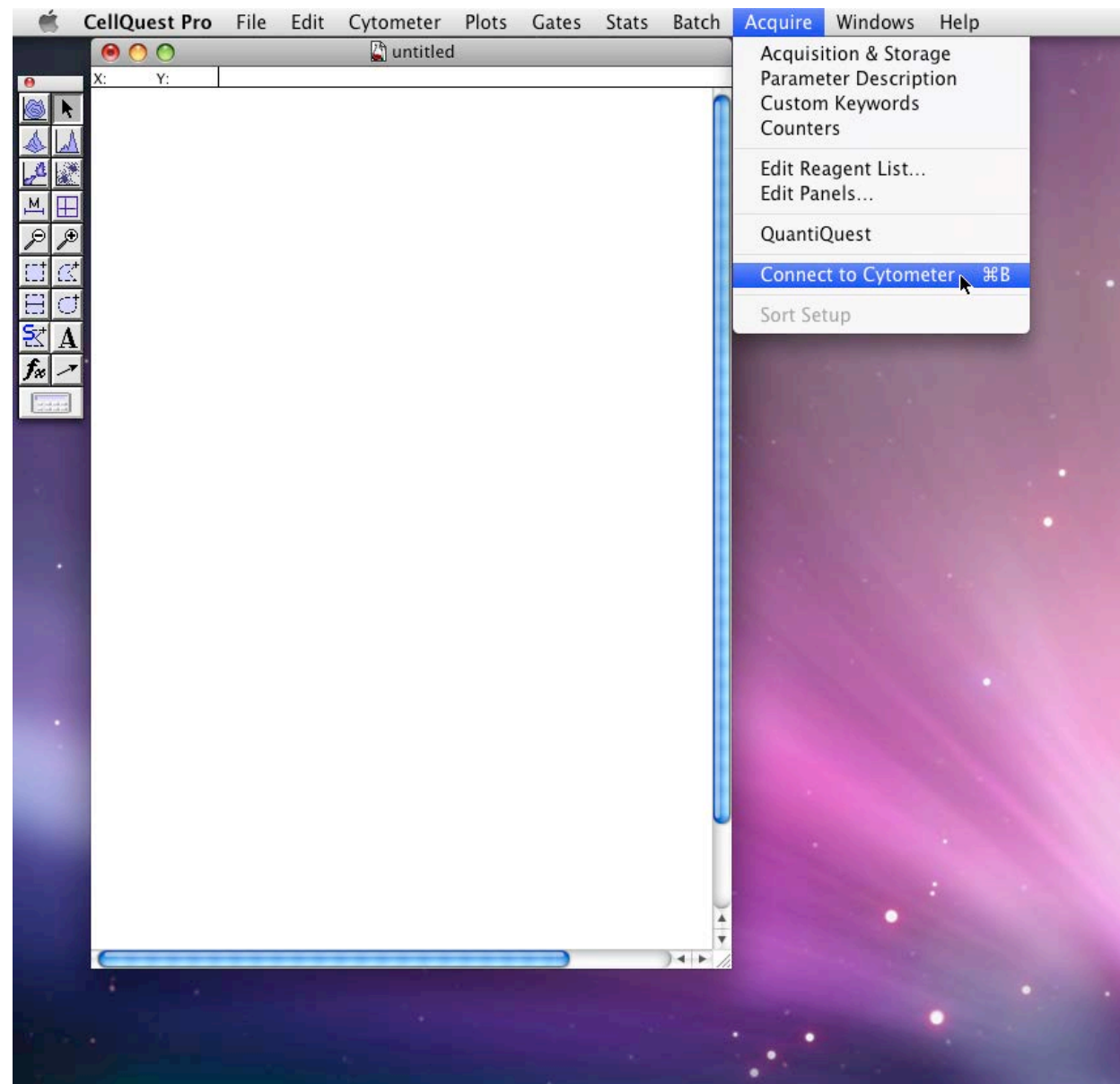
FACS Operation Protocol

V 1.0 – Sept 2013

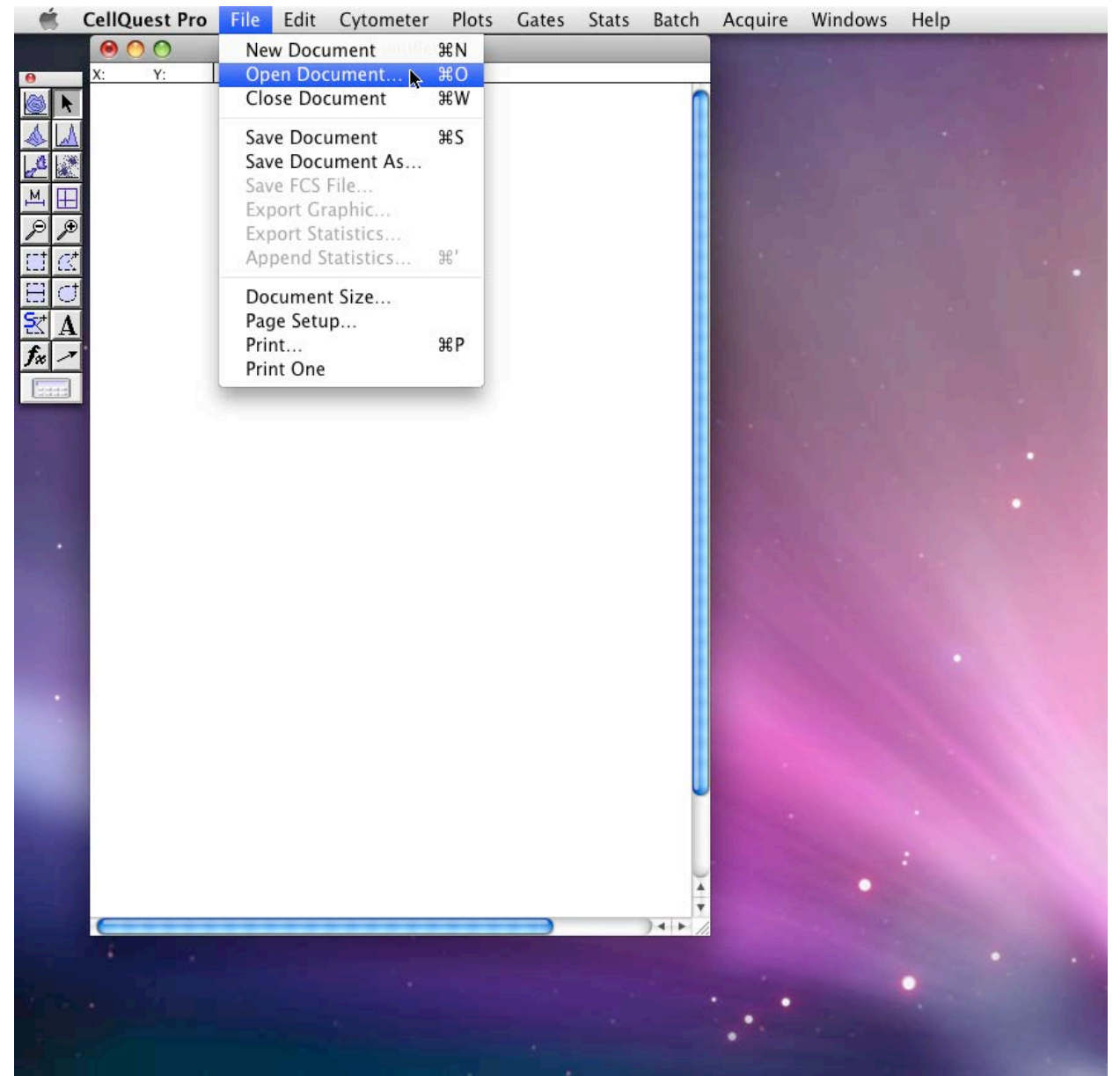
1. Sign in log book
2. Turn on the FACS machine and restart the computer
3. Choose FACS user (no password required)
4. Open the *CellQuest Pro* Program



5. Under Acquire Menu, *Connect to Cytometer*
6. Make sure FACS is on Vent before opening any reservoir. Fill the PBS tank with PBS
7. If the waste is full, empty it and line the bottom with 10% bleach.
8. Switch to “Pressurize”.
9. **Prime** the machine 3 times (after each priming, the Standby button will be lit – repeat twice).

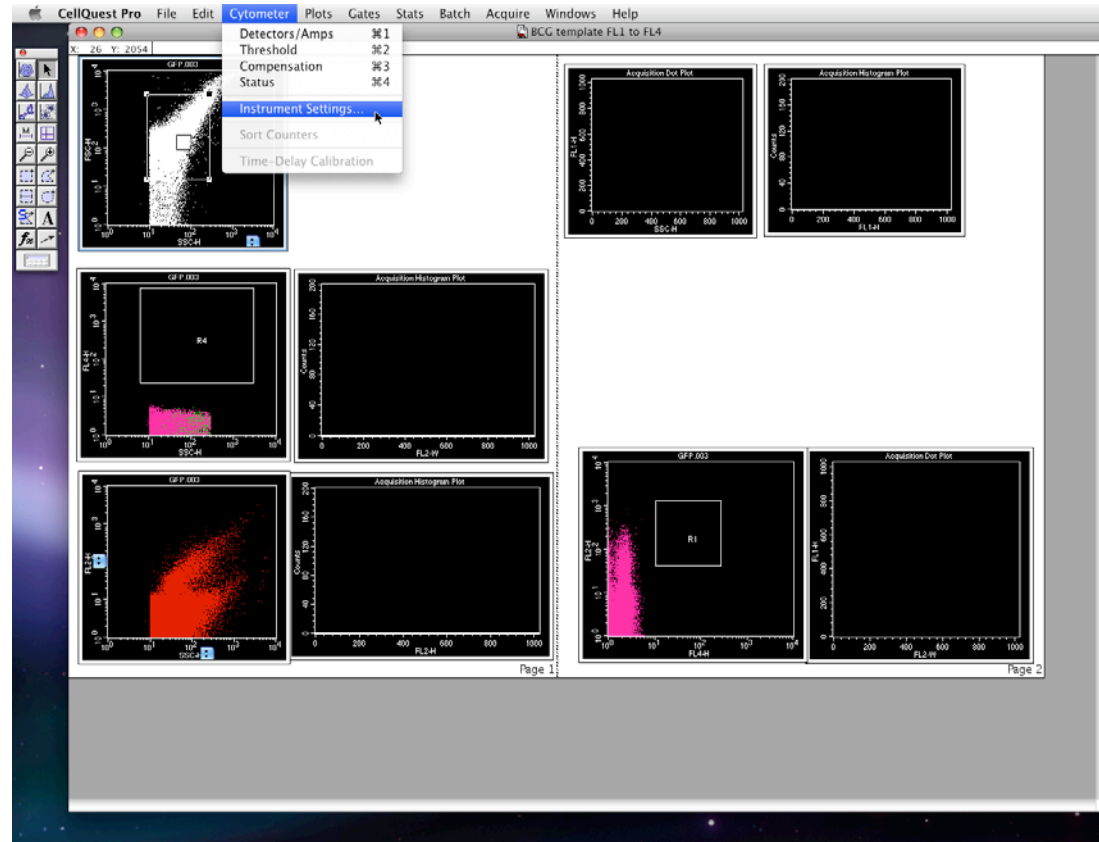


8. Open document template file.
*Your lab may already have a template drawn up; otherwise, you will have to draw your own template.

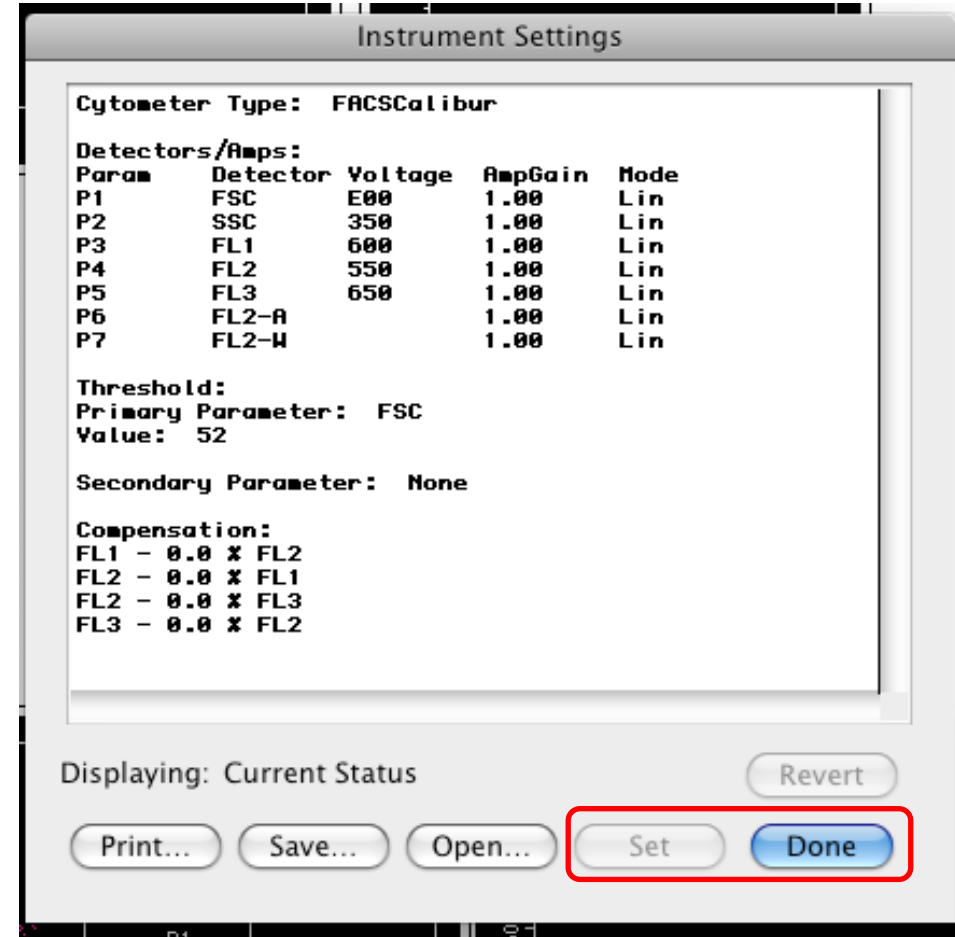


1. Open *Instrument Settings*

*Like Documents, your lab may already have an Instrument Settings file.



1. Click “Set” and let it load
(sometimes the machine appears to have frozen – do not panic, it is simply loading)
2. Then click “Done”



The image shows a software window titled "Instrument Settings" for a FACSCalibur cytometer. It contains several sections: "Detectors/Amps" with a table of parameters, "Threshold" settings, and "Compensation" settings. At the bottom, there are buttons for "Print...", "Save...", "Open...", "Set", and "Done". The "Set" and "Done" buttons are highlighted with a red rectangle.

Instrument Settings

Cytometer Type: FACSCalibur

Detectors/Amps:

Param	Detector	Voltage	AmpGain	Mode
P1	FSC	E00	1.00	Lin
P2	SSC	350	1.00	Lin
P3	FL1	600	1.00	Lin
P4	FL2	550	1.00	Lin
P5	FL3	650	1.00	Lin
P6	FL2-A		1.00	Lin
P7	FL2-W		1.00	Lin

Threshold:
Primary Parameter: FSC
Value: 52

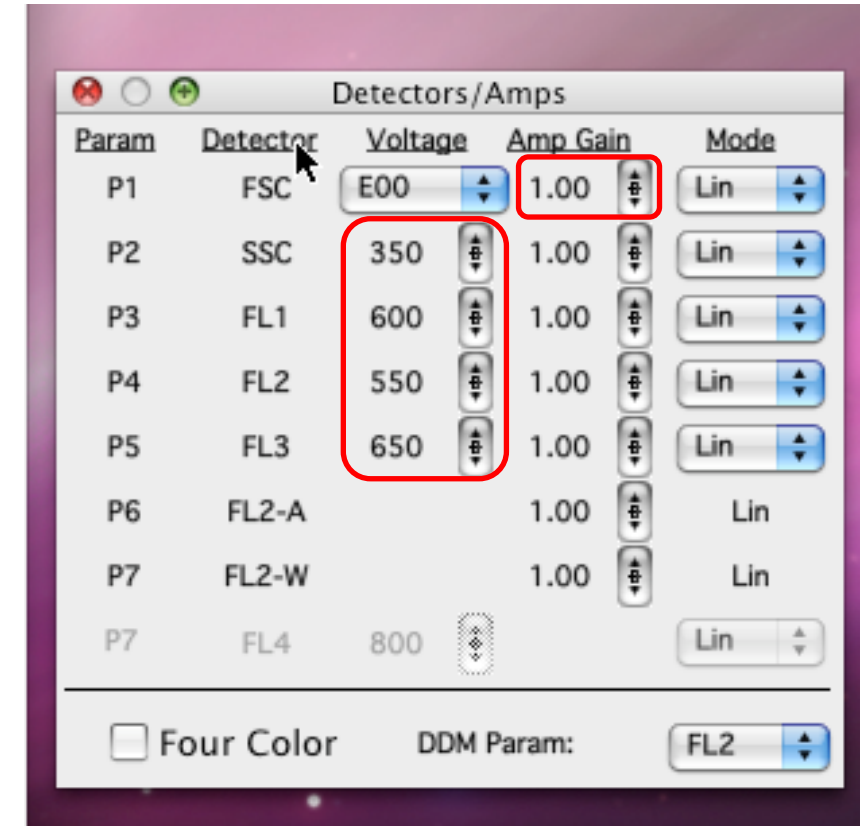
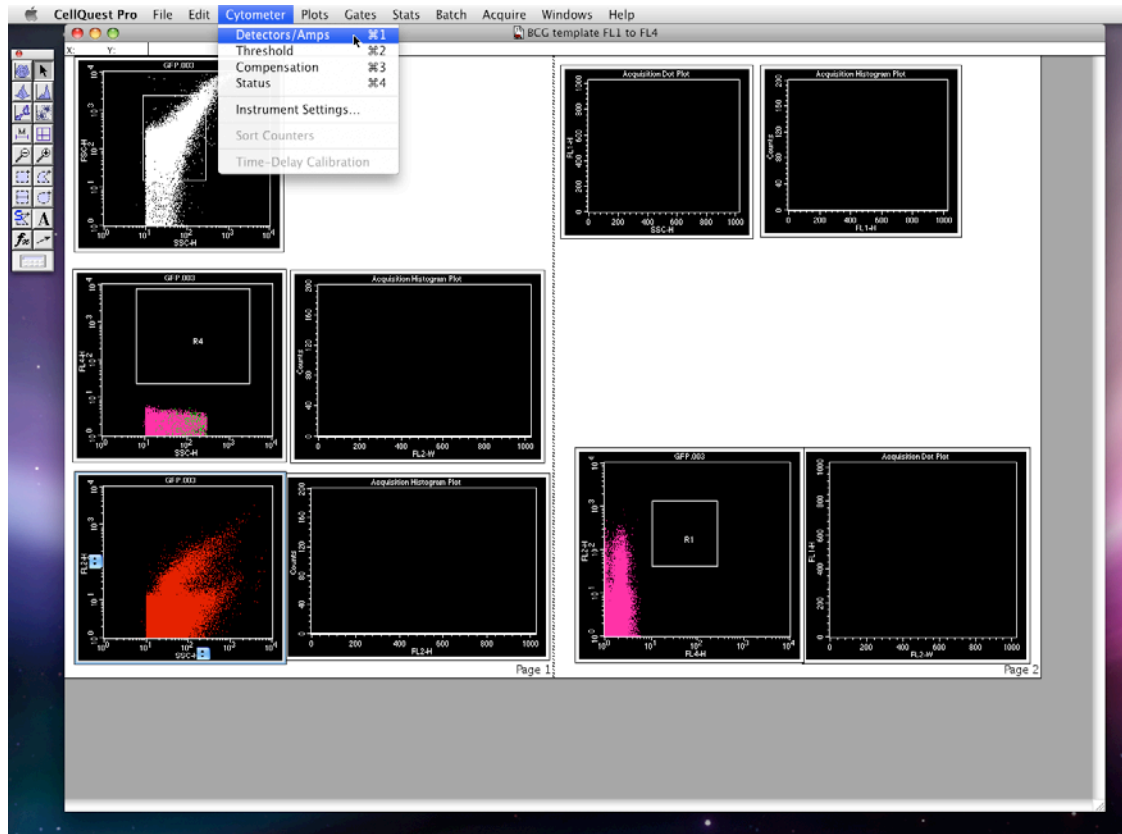
Secondary Parameter: None

Compensation:
FL1 - 0.0 % FL2
FL2 - 0.0 % FL1
FL2 - 0.0 % FL3
FL3 - 0.0 % FL2

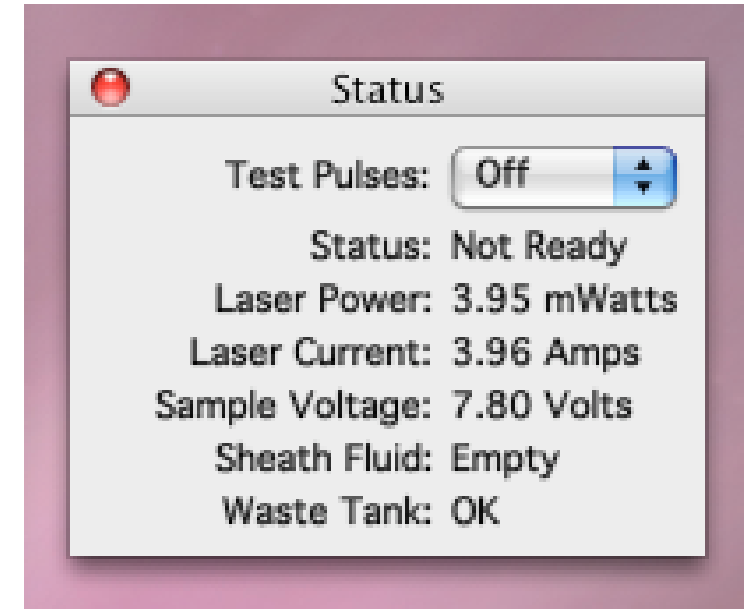
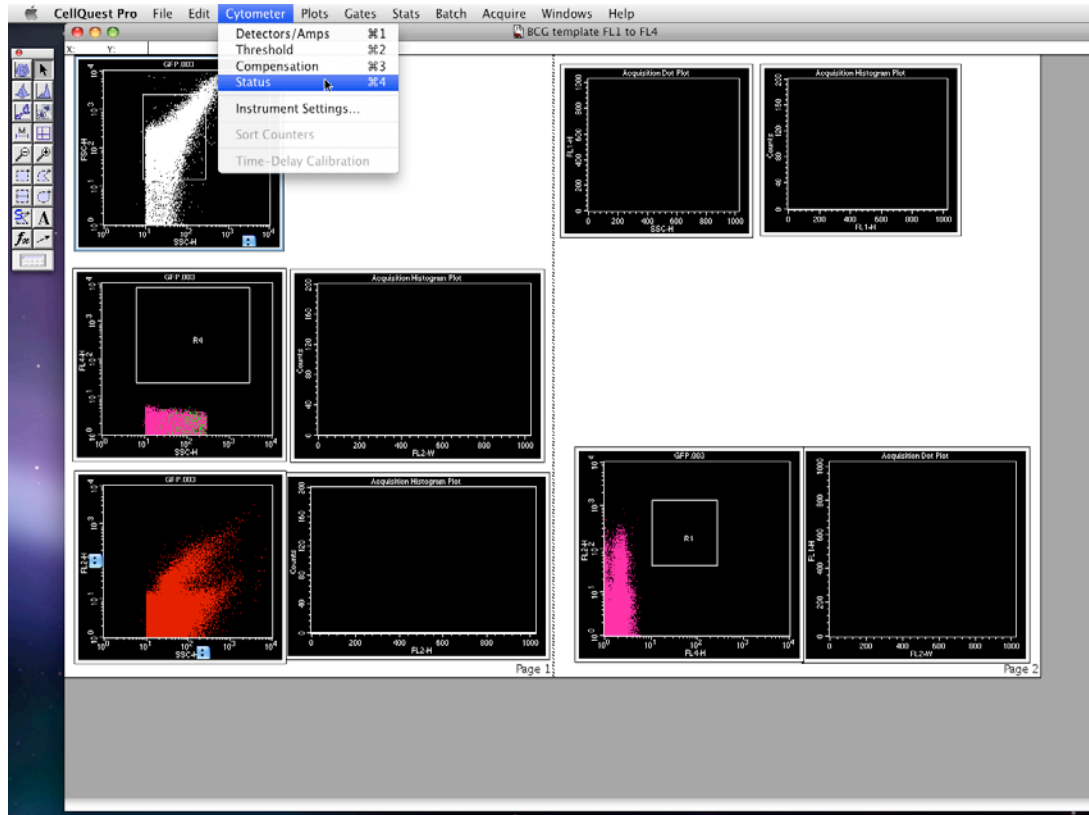
Displaying: Current Status

Buttons: Print... Save... Open... Set Done

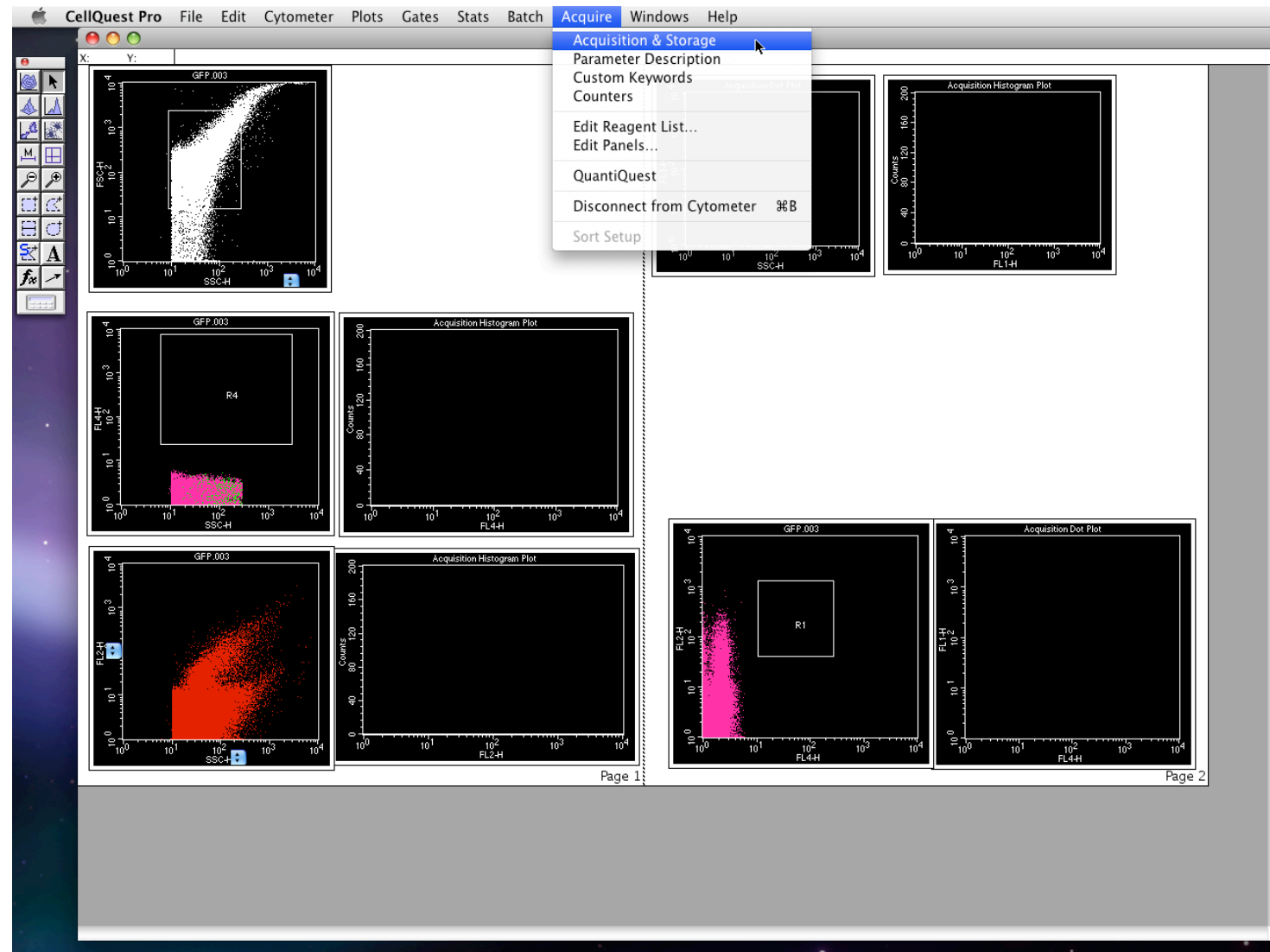
1. Open the *Detectors/Amps*
2. To adjust your cell population positions on the histograms, use “Amp Gain” for FSC, and “Voltage” for the remaining



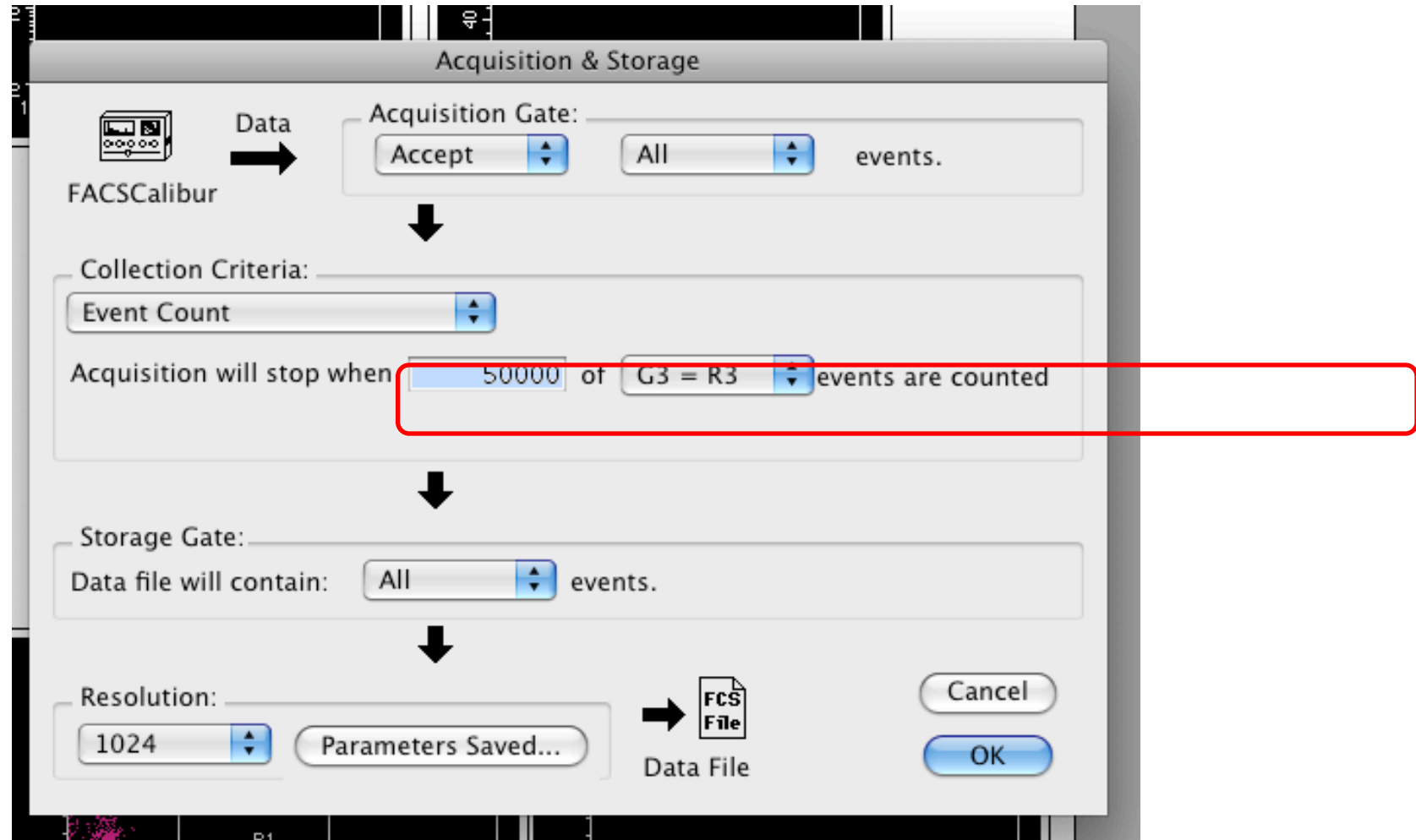
1. Open the *Status*.
2. Check the status: the machine should say “Ready”.
3. If it says “Not Ready”, it will tell you why. (eg. The sheath fluid tank is empty).



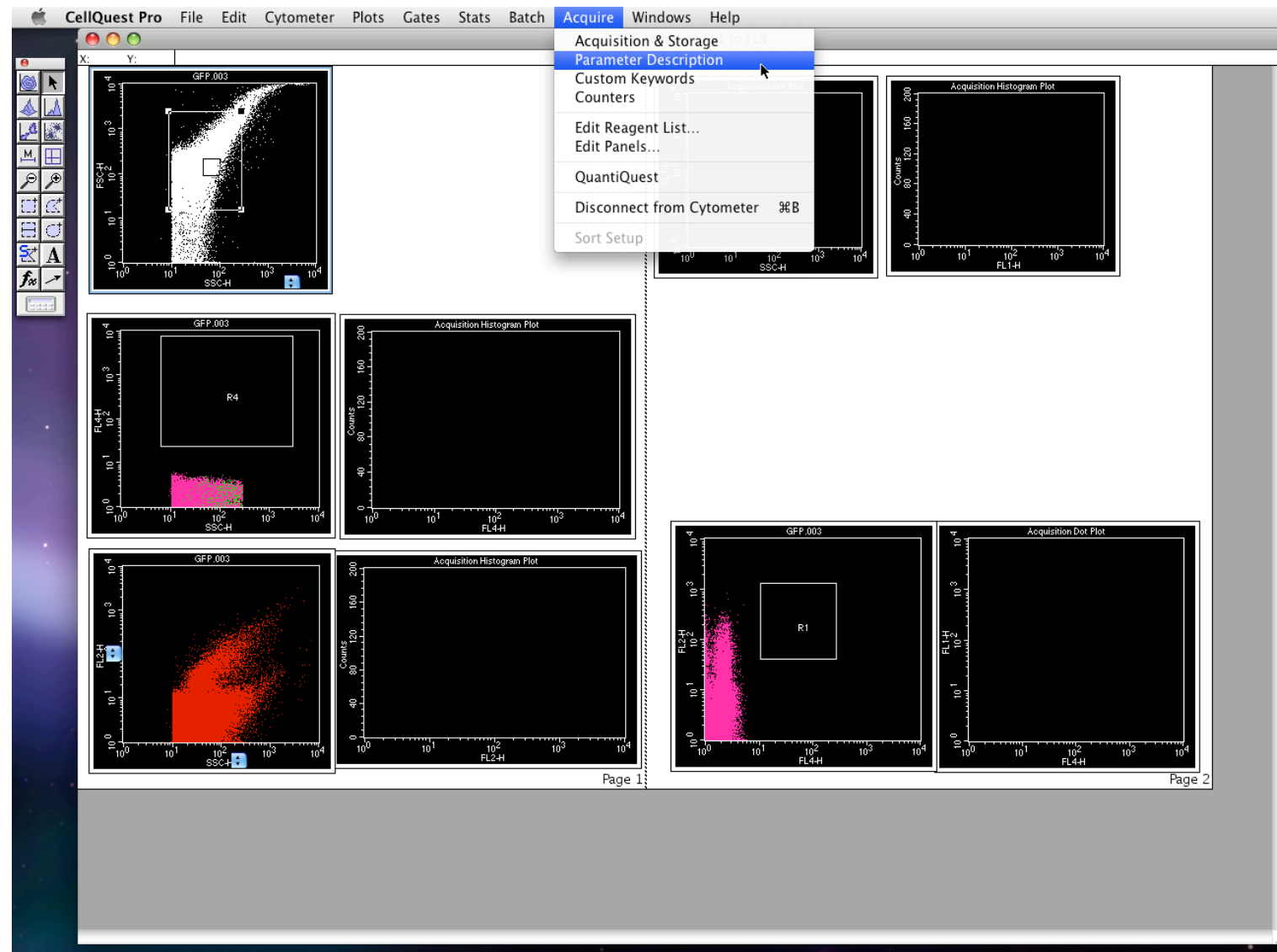
1. To select the area of your cell population and number of events to acquire, select *Acquisition & Storage*



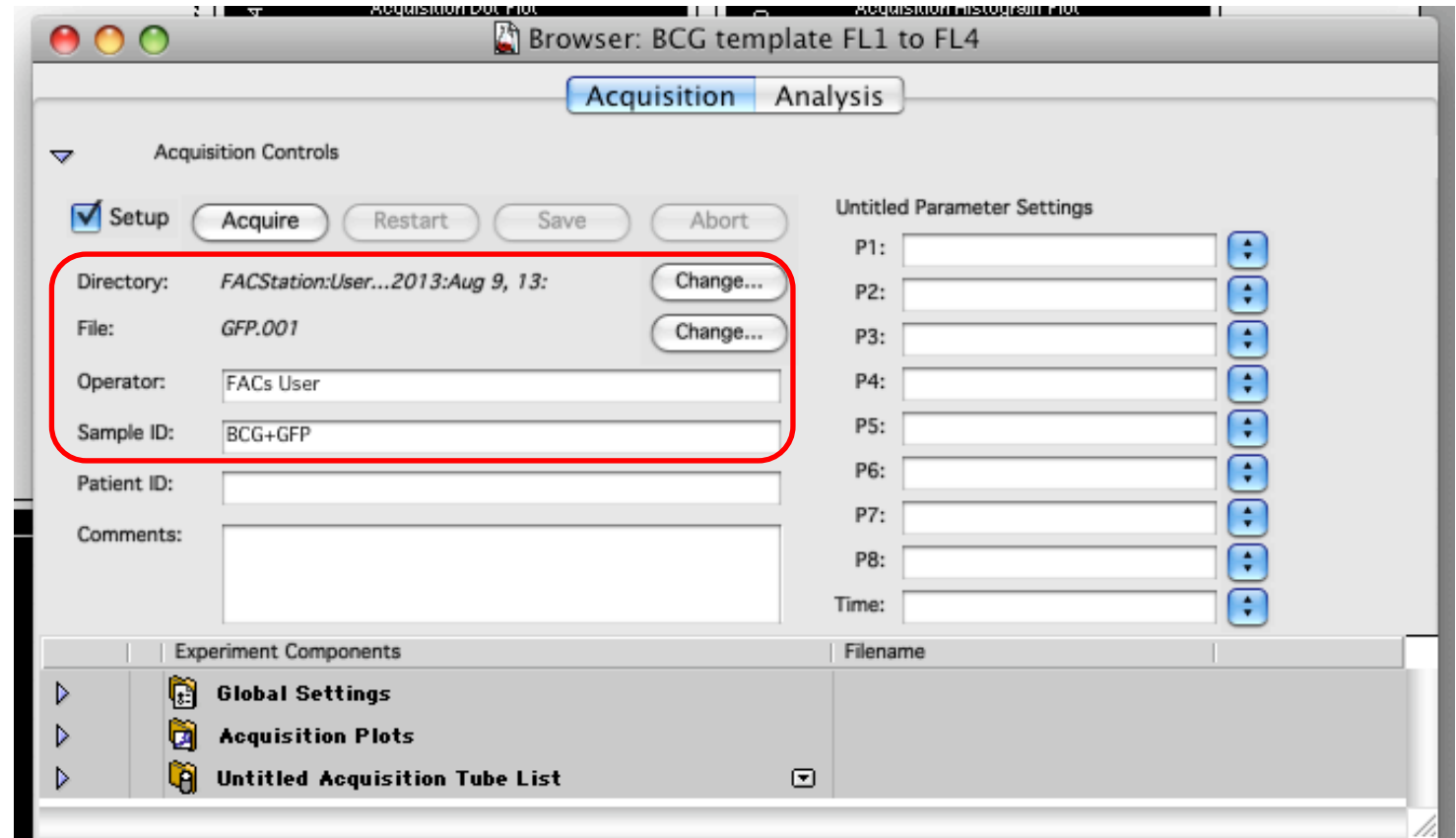
1. Set the number of events you would like to record and check it is recording the events in the box you have drawn.



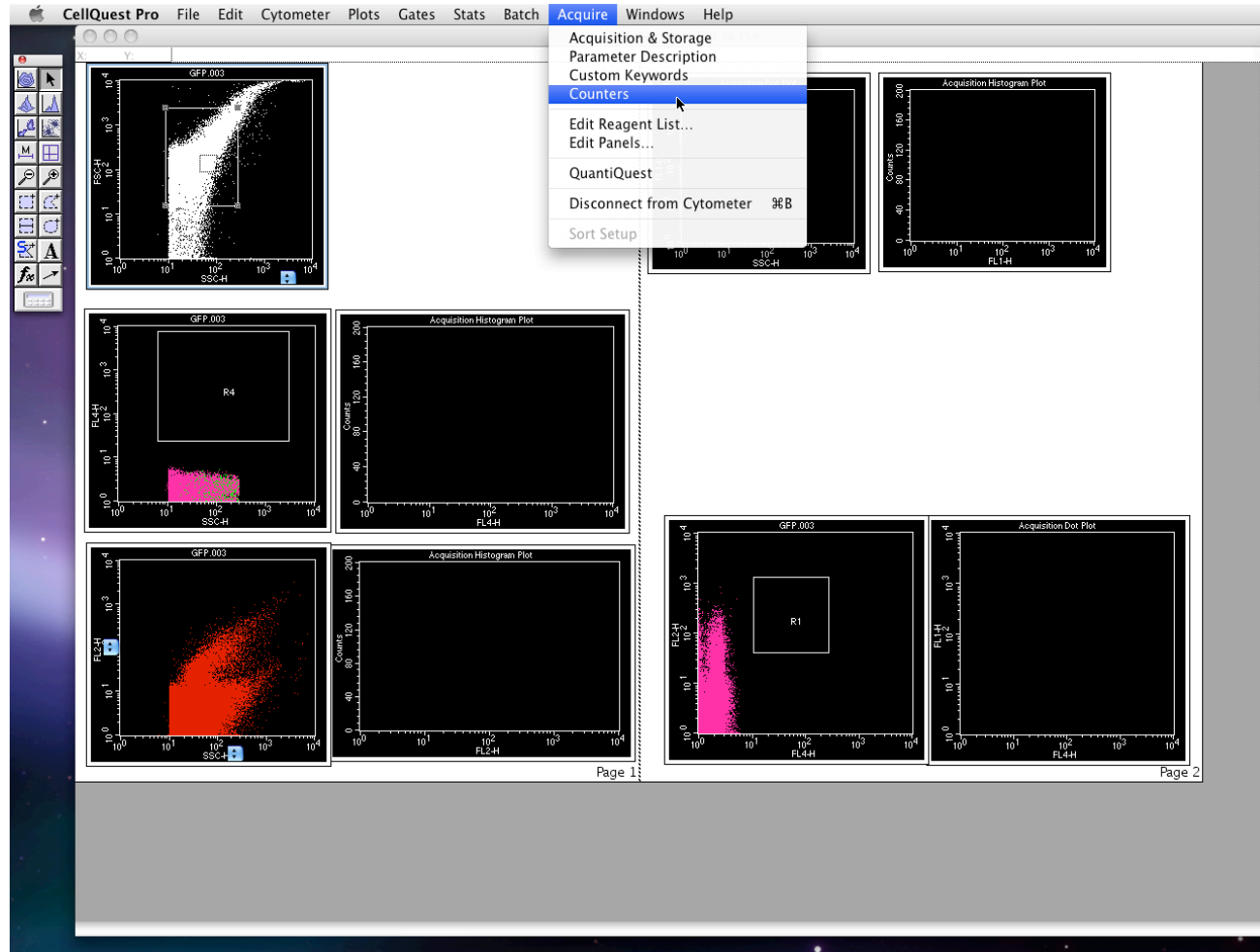
1. Click on *Parameter Description* to select location of file saving.



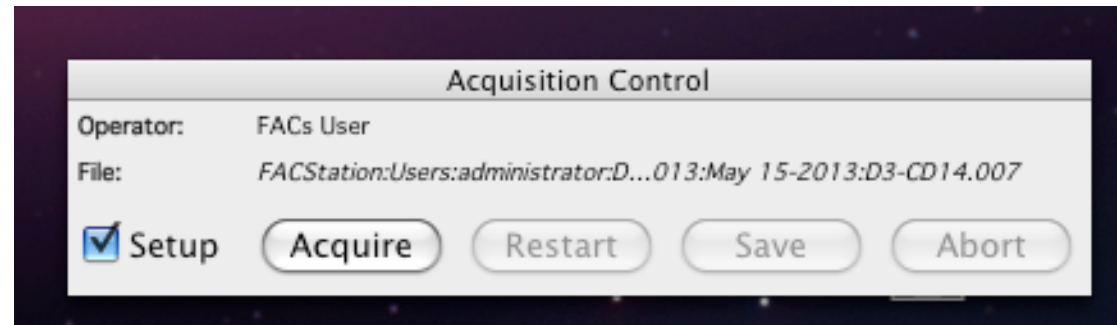
1. Directory: the folder where you want to save your files to
2. File: a prefix that will appear on all your file names, followed by a number (naturally, you will start the numbering with “1”)
3. Sample ID: a meaningful title that you can distinguish your files by (ie. “Control”, “Wild type”). This naming will not appear on the file name, but it is embedded in the meta data.



1. Open *Counters* to monitor the number of events per second.
- *You may use “Lo”, “Med”, and “High” to control the event counting speed. However, anything beyond 800 events/second should be closely monitored. If you see events (the dots) jumping radically all over the plot, it is time to decrease the speed.



1. Now you are ready to starting reading your samples.
2. Firstly, with the “Setup” box checked, start acquiring your unstained sample to gate for the proper box and voltage.
3. Once you are satisfied with the histograms, uncheck “Setup” box and press “Acquire” to begin saving your acquisitions.
4. ***If the “Setup” box is checked, no data will be saved.
5. When the machine finished acquiring the events, it will stop by itself and you do not need to press the “Save” button.



1. Probe must always be submerged in something – water, 10% bleach or sample
2. From STBY mode, turn it on to **RUN** before changing the sample
3. Changing from STBY mode to run with your sample inside may cause it to expel some water in the sample
4. When changing tubes, do not force the wing to move if you feel resistance. Use the O-ring base to adjust the seal height to make a perfect fit.
5. Shutdown procedure: set the machine on Lo.
6. Run with bleach for 1 min with the wing open and then water for 5 mins with the wing closed.
7. Set back to **STBY** mode
8. Turn the Pump back to **Vent** and empty the waste tank.
9. Turn off the machine

Love the FACS machine and it will love you back :D

For technical assistance contact Alice or Alex in the Hmama Lab – Rm 436 – x68494