Calibur Operational Notes

Initial Set-up:

- Turn the cytometer (green button) on first, followed by the computer, allowing for a 15 minute warm-up time.
- Open the fluidics drawer at the front of the cytometer and check the sheath fluid and waste tank and replenish or empty as needed.
- Flip the black toggle switch at the rear of the fluidics drawer forward in to the "Pressurize-Run" position.
- Using the thumbwheel that comes off of the filter inside the fluidics drawer evacuate any air bubbles in the sheath fluid line.
- With water on the SIP "PRIME" the cytometer several times and then put the cytometer in "RUN" on "HI".
- At the computer there is a two step log-in.

    - Log in to the Calibur with your chosen login name and password.
    - Log in to the TRINCH network with your standard network log-in.

Quality Control: (Make sure lasers and detectors are working properly.)

If you are the first user of the day you will need to run the beads with the FACSComp program.

- Check the FACSComp Results folder on the desktop to see if someone has already run FACSComp for that day in which case you don't need to run it again.
- To check, open the FACSComp results folder and look at the top of the file listing to see if FACSComp has been run. You can check the results of any FACSComp run by clicking on the file that has a "PICT" suffix on it.

Prepare Beads (Calbrite 3 Beads)

- If fresh beads need to be prepared do as follows after checking the fridge for beads that are already made. (Prepared beads should be good for a couple of weeks).
- To make fresh beads get the box of Calbrite 3 beads which contains five vials of different types of beads out of the refrigerator.
- Prepare a dated tube labeled tube #1 with 1 drop unlabeled and 1 drop APC beads in 1mL sheath fluid after thorough vortexing of the beads.
- Prepare a second dated tube (#2) with 1drop unlabeled, 1 drop FITC, 1 drop PE, 1 drop Per-CP and 1 drop APC in 2 mL sheath fluid after thorough vortexing of the beads.

Note: If you need to open a new box of beads you need to let me know so that we can record the lot number in the FACS Comp program.

Run FACSComp

- Open the FACS Comp program via the shortcut on the desktop.
- Type your name in the "Operator" field > Accept > Run. (You will get a warning message about printing here. Ignore it and > "Continue".)
- Remove the water tube from the SIP and place tube #1 (unlabeled and APC) on the SIP.
- Set the cytometer to "RUN" the beads on "HI".
- Click on "START" to begin the FACSComp procedure.
- FACSComp is an automated program so follow the prompts in the "Message" box at the top of the screen. Once tube #1 has finished running replace it with the all color #2 tube > "START".

- Once tube #2 has finished running a report sheet will be generated. You need to look at two areas on the report to see if FACSComp has passed, those being the "Result" column in the first "Parameter" table at the top of the page as well as the "Time Delay Calibration" result at the bottom of the page under "Comments".

  Note: If any of these parameters does not pass you need to remove the beads from the SIP, replace with water and "Prime" the cytometer at least several times. Run FACSComp again and repeat if necessary until all of the FACSComp parameters "Pass". If FACSComp continues to fail you need to let me know.

- Once FACSComp has passed, "Quit" to send the results to the FACSComp results file.
- Cap and place the remaining bead mixtures in the refrigerator for subsequent use.
- Put a tube of distilled water on the SIP, close the arm and "RUN" on "HI".

Experiment Set-up:

To set up an experiment you can either create a new experiment from scratch or open an existing experiment and use its settings as a template for a new experiment.

Set Up an Experiment from Scratch

- Open Cell Quest Pro on the desktop and expand the experiment window > File >Document Size.
- Set the document size to 2 Squares for a new document by clicking on the square to the right of the highlighted square >"OK".
- Create a dot plot from the tool bar which will open its "Inspector" once the plot is drawn on the worksheet.
- Go to the dot plot Inspector> Plot Type > Acquisition. (You have to do this for every plot that is created)
- Go to "Acquire" on the main menu bar > Connect to Cytometer (this will open the acquisition control Browser).
- Set the destination for your data in the Browser by going to Directory: > Change> User Files > Choose destination folder > Select.
- Go to File: in the Browser > Change and enter any file names you want to create.
- Go to "Acquire" on the main menu bar > "Acquisition and Storage" and set the collection criteria (# of events to record etc.) for your experiment.
- Go to "Untitled Parameter Settings" in the Browser and assign names for the various parameter labels on your graphs by using either the drop-down menu or typing in the names you wish.
- Go to "Cytometer" on the main menu bar and open the "Detectors/Amps", "Threshold" and "Compensation" windows.
- Proceed to **Set Voltages** in these instructions.

-OR-

Set Up an Experiment from an Existing Experiment

- Open Cell Quest Pro on the desktop > File >Open Document > User Files and open the file you wish to use.
- Go to "Acquire" on the main menu bar > Connect to Cytometer (this will open the acquisition control Browser).
- Set the destination for your data in the Browser by going to Directory: > Change>Choose destination folder > Select.
- Go to "Cytometer" on the main menu bar > Instrument Settings > You can either use the settings associated with this experiment or apply a different group of settings from the Instrument Settings folder > Open> Find the settings file you want > Set > Done.
- Go to "Cytometer" on the main menu bar and open the "Detectors/Amps", "Threshold" and "Compensation" windows.
- Proceed to **Set Voltages** in these instructions.

Set Voltages:

Adjust FSC, SSC and Threshold

Whether or not you are setting up a new experiment or running one with previous settings you should always check and adjust your voltages prior to running your samples.

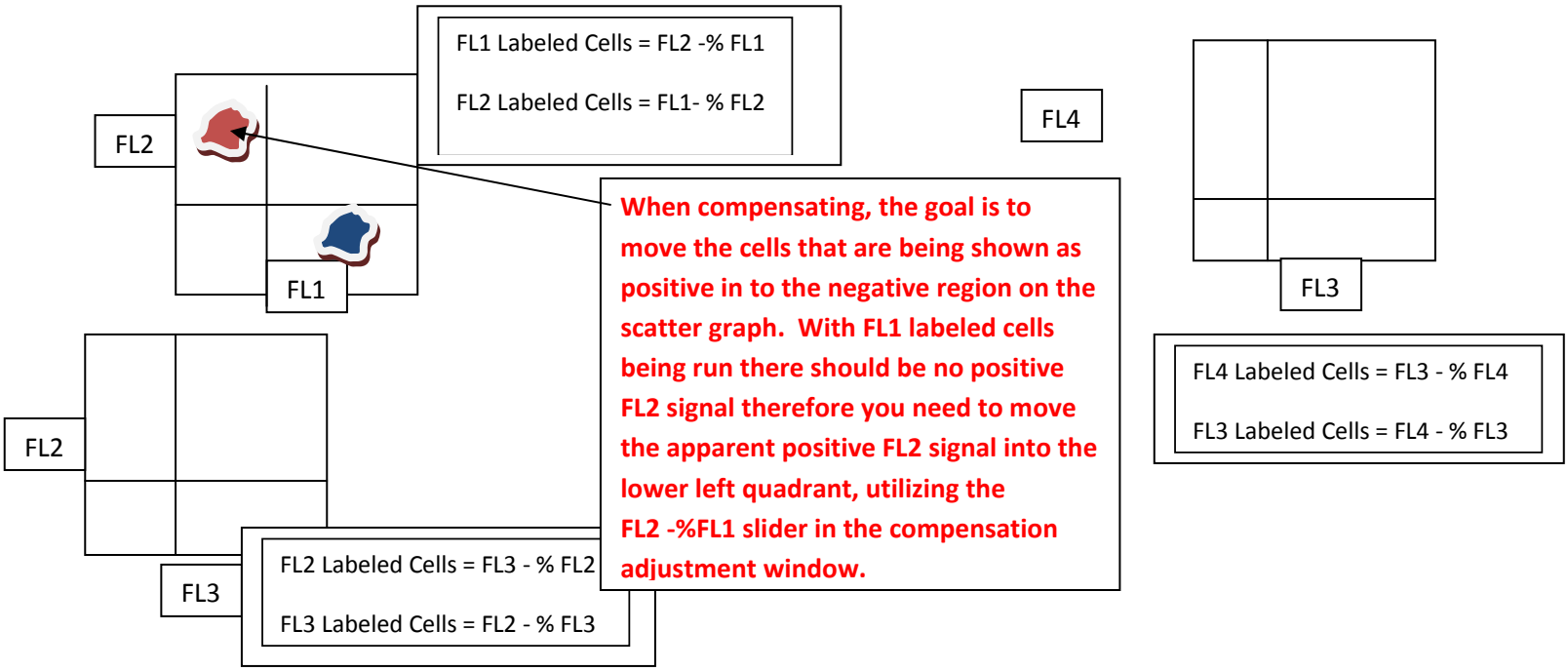
- Place unstained cells on the SIP and "RUN" on "LOW".
- At the Browser click on "Acquire" (the set-up check-box should be checked until done with all settings and then unchecked when ready to proceed with the experiment).
- Adjust the FSC Amp gain and the SSC voltage in the Detectors/Amps window to place the cells in the proper location on the scatter graph.
- Adjust the FSC threshold to remove debris w/out cutting off the cells of interest.
- Once you have the population of interest place a polygon gate around it using the gating tool from the tool palette.
- Once gated, "Pause" the acquisition.

Adjust Detectors for Your Fluorophores

- Create histograms for each of your single colors and set-up each graph thru its Inspector by choosing "Acquisition" as the plot type and choosing the proper fluorophore designation as the x-axis parameter for each graph.
- In the Inspector for each graph choose "gate" G1=R1.
- Go to the browser and "Resume" the acquisition and adjust voltages for autofluorescence levels for each of your colors in order to set background.

Compensation: (If you are using more than one color)

- Whether you have set up an experiment from scratch or are using previous settings you must compensate your data with new compensation values for each experiment.
- In order to compensate set up scatter graphs for each dual color combination.
- Run each of your single stained controls and make appropriate compensation adjustments in the compensation window for each of the detectors picking up bleed over from the single color control.



Running Samples:

- Once you have made all of your voltage and compensation adjustments you may proceed with running your samples.
- Prior to running the first sample make sure you "Uncheck" the "Set-up" check box.
- Enter a "Sample ID" in the Browser.
- Place sample on SIP and acquire until you hear the tone indicating that collection criteria have been met (i.e. number of events to record). Your data has been recorded at this point.
- Proceed to your next sample by entering a new sample ID > placing the sample on the SIP on the cytometer > "Acquire", and so on.

Save Settings

- Instrument settings can be saved to a file and retrieved later for subsequent analyses.
- Go to "Cytometer" on the main menu bar > Inst. Settings > Save.
- Find the destination folder desired in the Save File dialog box.
- Enter a name for the settings file in the "Save As" text box > Save > Done.

Save an Experiment Document

- Your experiment document, including plots, gates etc. can be saved.
- Go to "File" on the main menu bar > Save Document.
- Choose a destination folder.
- Enter a name for the experiment document > Save.

Export Data

- Close Cell Quest > "User Files" on desktop > Find your files.
- Go to the Apple Icon in the upper left corner of the desktop> Recent Servers > Research > Choose data destination > Drag and Drop from User Files.

Shut Down

- Put tube with 10% bleach on SIP leaving the support arm to the side for 1 minute.
- Close the support arm and RUN the cytometer on HIGH for 5 minutes.
- Repeat above steps using distilled water.
- Leave tube of distilled water on the SIP with support arm in the closed position.
- Set cytometer to STANDBY.
- Shut down the computer first followed by the cytometer.
- Open the sheath fluid drawer and "DEPRESSURIZE" the system by flipping the black toggle switch to the vent position.
- Refill the sheath fluid container.
- Empty the waste container into the sink and place a small amount of bleach in the container.
- Replace the waste container and its lid but do not tighten the lid.