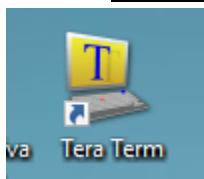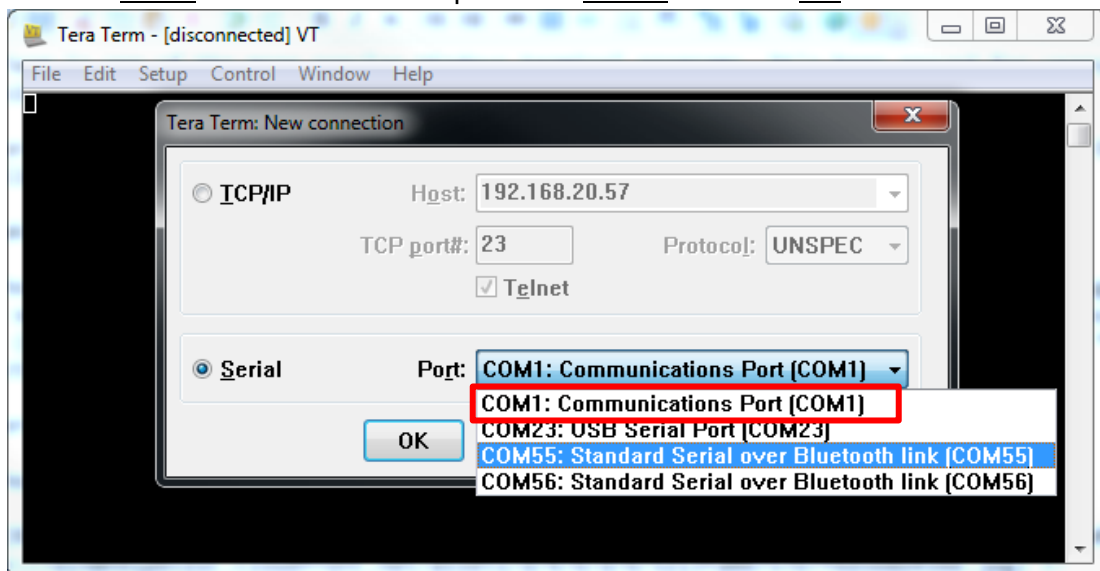# FACS LSRFortessa SORP - QUICK REFERENCE GUIDE

IF THE MACHINE IS OFF OR YOU ARE THE FIRST USER OF THE DAY

1. The computer should be on.
   - To log into the PC: **Username:** BDAdmin **Password:** BDIS

2. Unlock the screen with your PPMS Account.

3. Launch Tera Term.

4. Click on Serial and make sure the port is on COM1 and click OK.

5. Turn on the cytometer by pressing the green button on the side of the instrument.

6. Turn on DIVA Software and log in using the credentials you received after training.


7. Move the SIP arm to the left, remove the DI water tube, and click the prime button twice.

8. Check the sheath and waste tank.
   a. If you are emptying the waste, add a layer of bleach to the bottom of the container before placing it back in its designated space.

9. Clean the fluidics:
   a. For each of the following solutions, fill up half a tube, load the tube on the SIP, and run each for 5 mins on high: 50% Contrad, 10% Bleach, and DI water.

10. Press Standby.

11. After cleaning, go to <u>Cytometer</u> → <u>CST.</u>
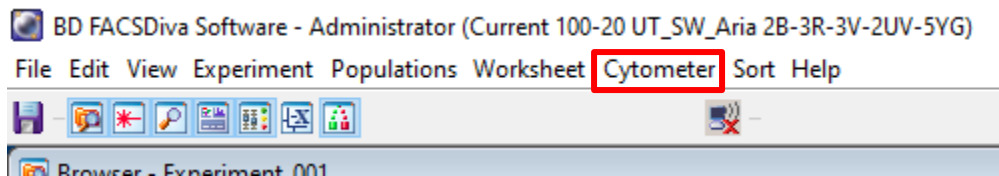
12. Take a clean FACS tube and add two drops of CST beads with ~ 300uL of FACS Flow.

13. Verify that the lot number on the side of the bead's container matches the one on the screen.
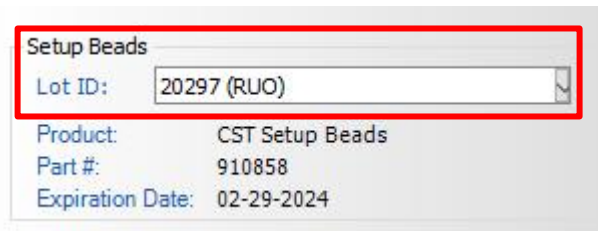
14. Place the CST tube on the SIP, close the arm, and click the buttons <u>Run</u> and <u>Low</u> to control the flow rate.

15. Press <u>Run</u> on setup control.
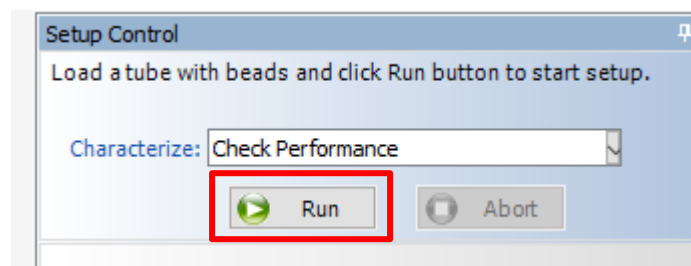
16. Wait for CST to pass.

        a. If it fails, try the following:

                i. Re-run CST.

                ii. Make new CST beads and re-run CST.

                iii. Run another cleaning cycle, then re-run CST.

                iv. Shut down the cytometer, restart the computer, and CST.

ANY USER OF THE DAY

1. Log into PPMS and your DIVA account.

2. For each of the following solutions, fill up half a tube, load the tube on the SIP, and run each for 5 mins on high: 50% Contrad, 10% Bleach, and DI water.

     • Note: Pay attention during the cleaning and do not allow the tube to empty.

FOR A NEW EXPERIMENT

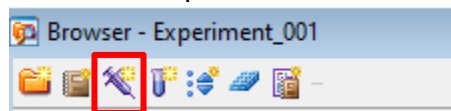**1.** Select Experiment → New Experiment → Blank Experiment
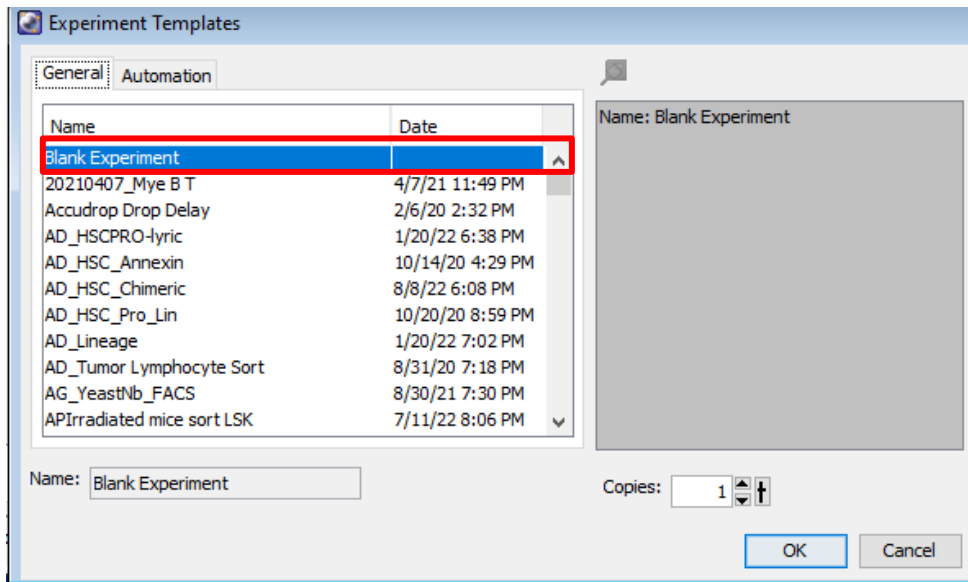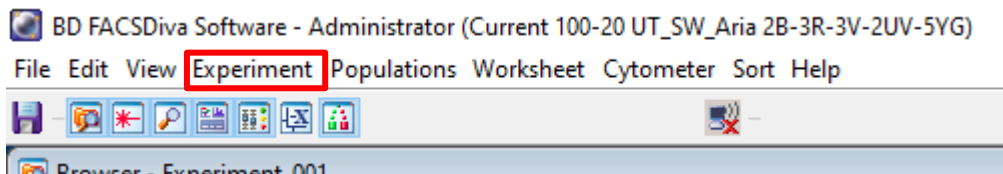
2. Click on the <u>Parameters</u> tab in the cytometer window.
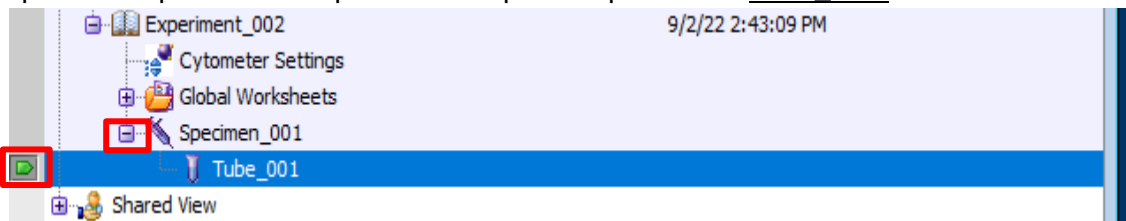

3. Delete all fluorochromes and add the fluorochromes you will be working with.

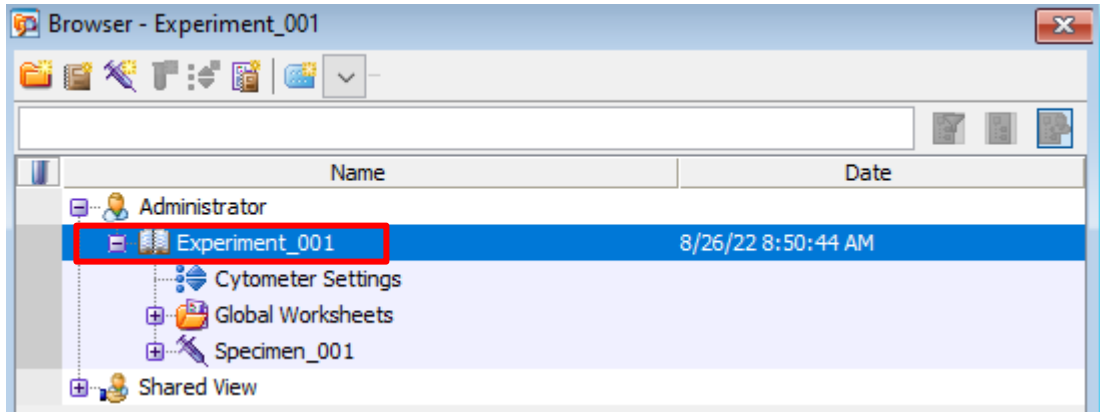**4.** Create a new specimen.

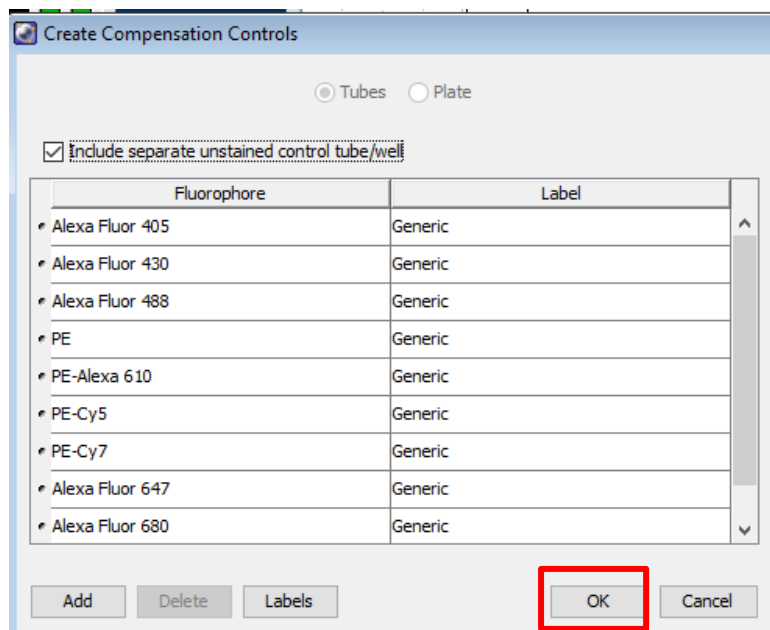**5.** Expand the specimen and place the acquisition pointer on <u>Tube_001.</u>
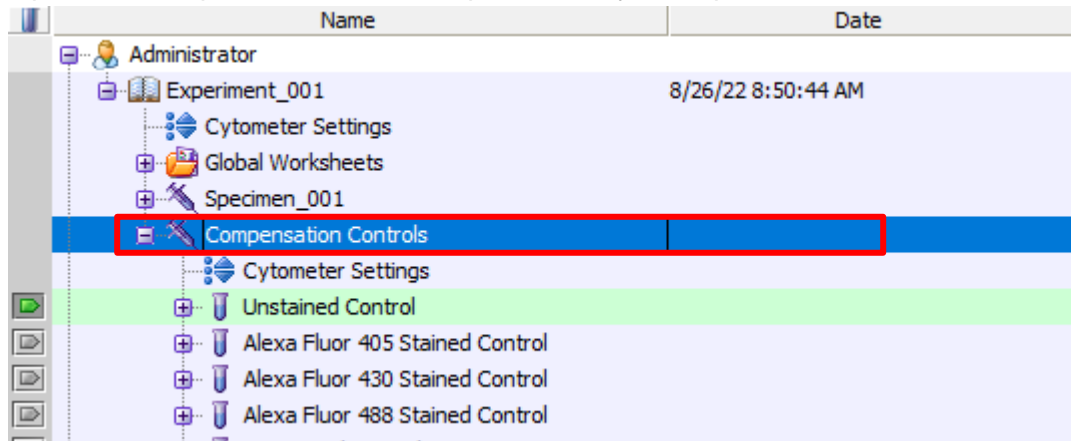
CALCULATING COMPENSATION

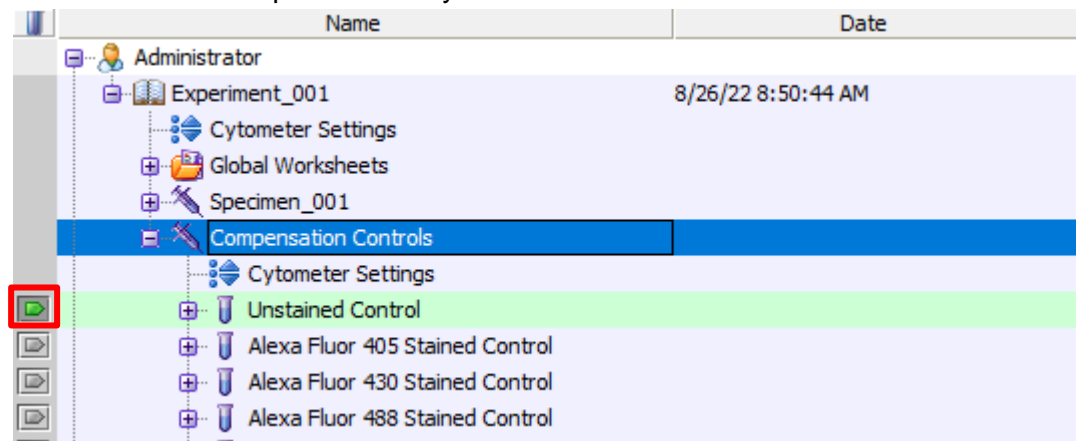1. Select Experiment → Compensation Setup → Create.

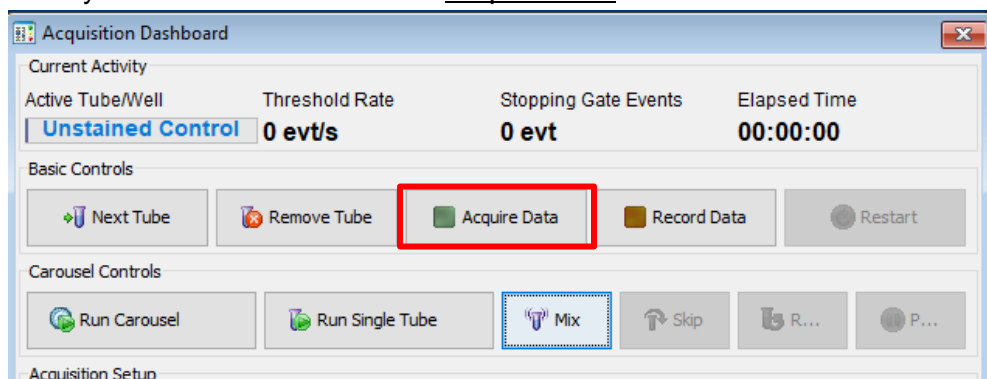   a. Verify compensation controls coincide with your chosen fluorochromes, then press OK.

2. Open the Compensation Controls specimen in your experiment.

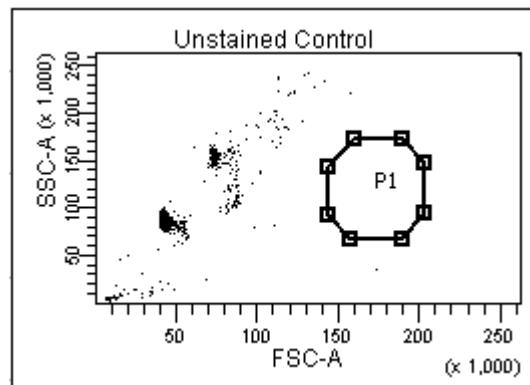3. Make sure the tube pointer is on your unstained control.

4. Load your unstained tube and hit Acquire Data.

5. Adjust the FSC and SSC voltage until your cells are on-scale in the plot.

6. Stop acquiring.

7. Move the P1 gate until it encompasses the population of interest.
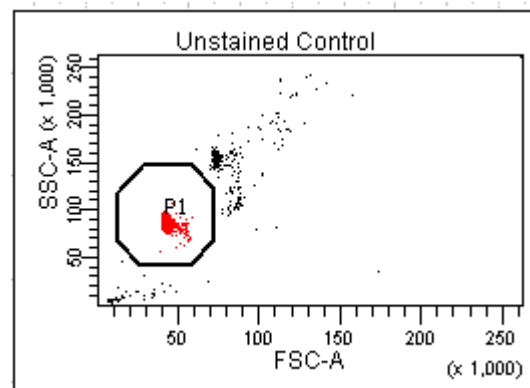
8. Right-click the P1 gate → apply to all compensation controls.

9. Acquire data again and <u>Record</u>.

10. Unload your unstained tube.

11. Load your first compensation tube.

12. Make sure that your tube pointer is on the correct tube.
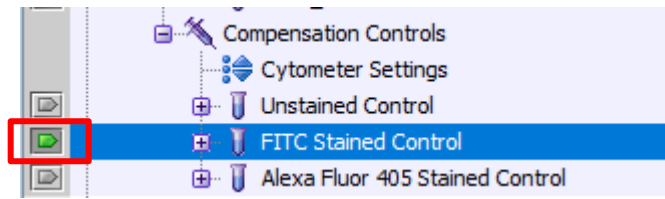
13. Click on the next worksheet panel.
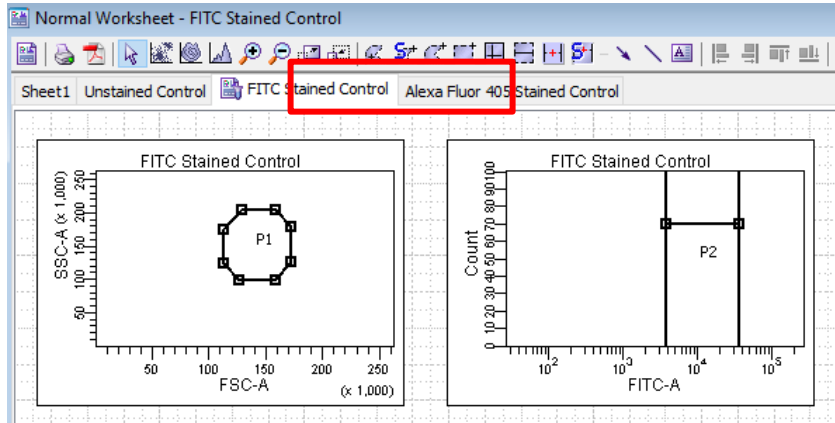
14. Click on your histogram plot.

15. Click the X-axis <u>Biexponential Display</u> in the inspector window.
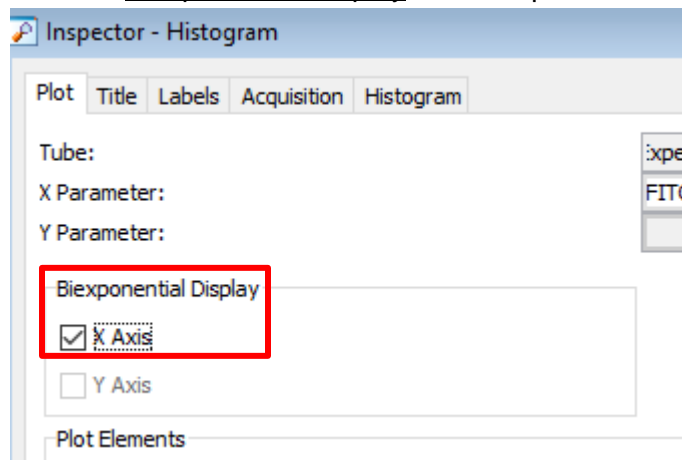
16. Click <u>Acquire Data</u>.
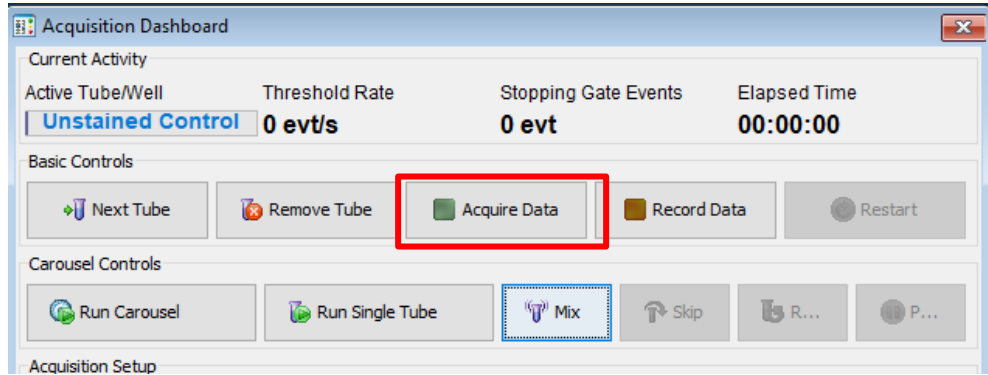
17. Adjust the voltage of the fluorochrome until the negative population is close to zero.

   a. It is important to make sure you can see both ends of your population. If it bleeds into the side of the graph, increase the voltage until you can see the bottom left of the peak.

18. Click <u>Record Data.</u>

19. Move the histogram gate until it surrounds your positive population.

20. Repeat steps 10-17 until all your compensation tubes have been completed.

21. Select Experiment → Compensation setup → Calculate compensation → Apply only.
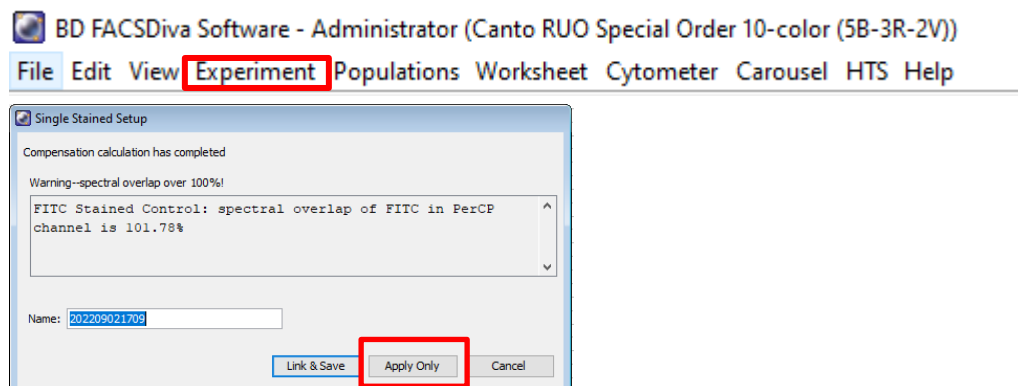
IF USING HTS (Plate Loader):

1. Click Experiment → New plate (V, U, or flat bottom).

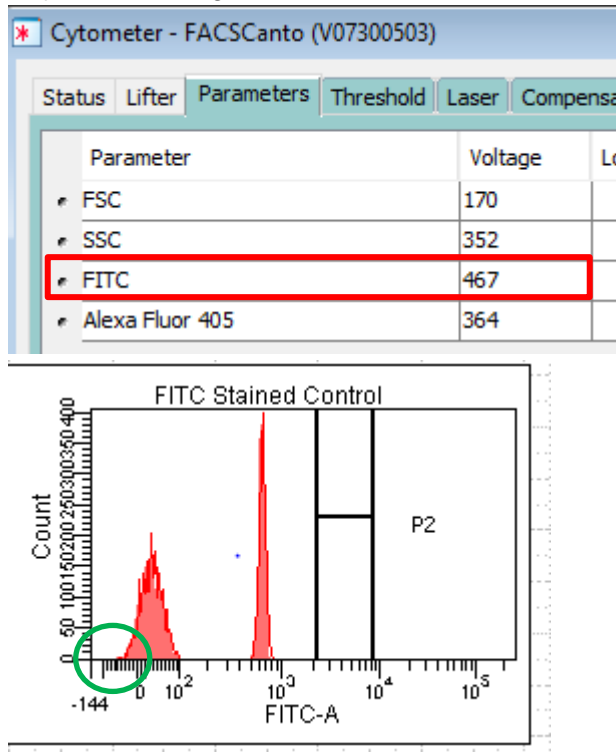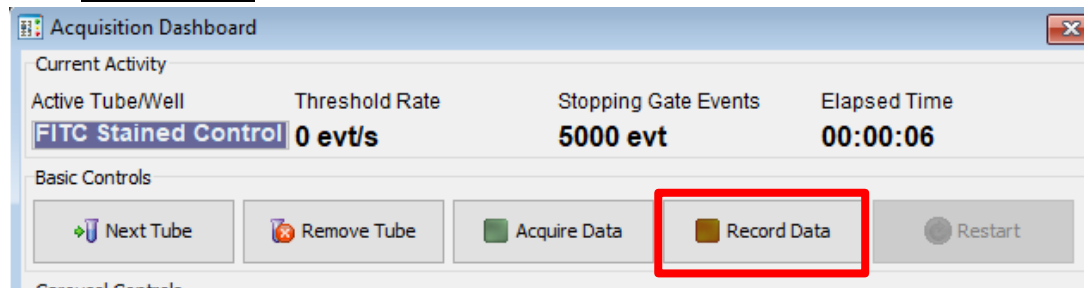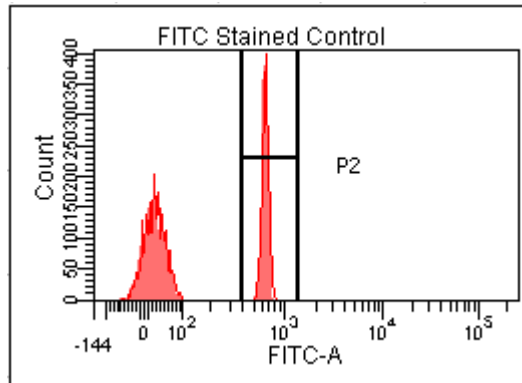2. Right-click on the first well of your compensation → Setup → Create Compensation Controls. **Note:** Compensation wells should be arranged horizontally.

3. On the pop-up window, make sure the parameters order matches the compensation well order on your plate; unstained sample will be created by checking the box on the top of the table.

4. The "Normal Worksheets" have been created for each compensation well.

5. If compensation beads are used, optimize FSC and SSC voltages to the beads values by using small amounts (e.g. 10uL for sample volume, 10uL for mixing volume, and flow rate on 0.5uL/sec).

6. Select all compensation wells and click Run Wells to acquire/record selected

samples.

7. Check the gates under "Normal Worksheets" (if unstained sample was not created, drop a new gate for negative population for each sample).

8. Right-click on experiment tab on the top → Compensation Setup → Calculate Compensation. Choose Apply Only.

9. Optimize the FSC and SSC voltages to noted values for cells when switching from compensation beads to cells.

ACQUISITION

IF USING SINGLE TUBES:

1. Place the Acquisition Pointer on Tube_001.
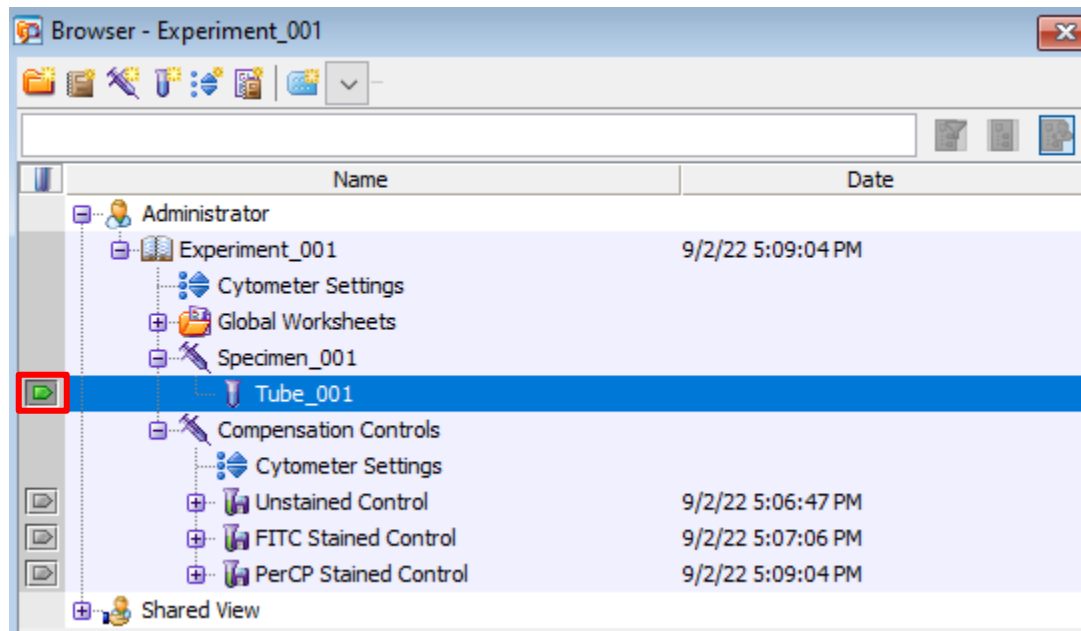
2. Create a new global worksheet.
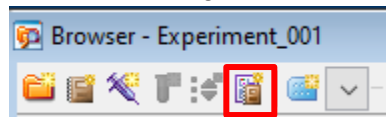
3. Create your graphs, dot plots, and gates to run your experiment.

   a. Hover over each item to view more information.
   b. Once you have your graphs set up, right-click one, and press Show Population

<u>Hierarchy.</u>

4. Add additional tubes to the experiment if needed.
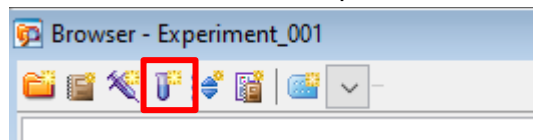
5. Select <u>Experiment</u> → <u>Experiment Layout.</u>
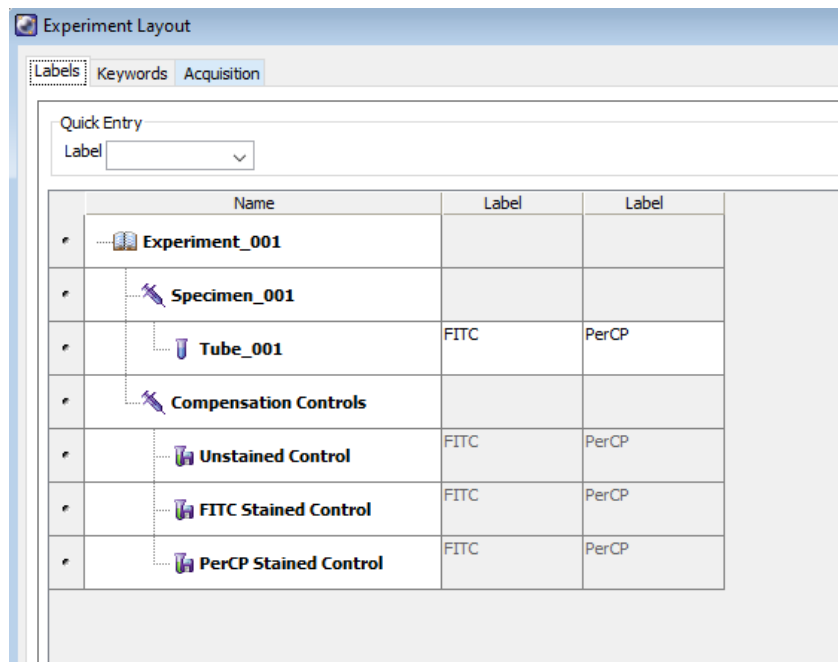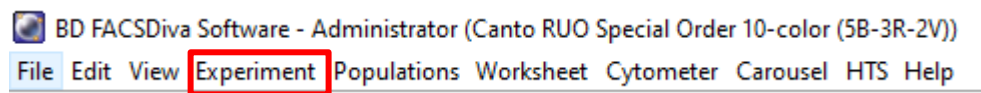
6. In the Labels tab:
   a. Change tubes names and the axis labels.
   b. Highlight the name you want changed and press Shift button.

7. In the Keywords tab, <u>Add Patient ID</u> and <u>Sample ID.</u>
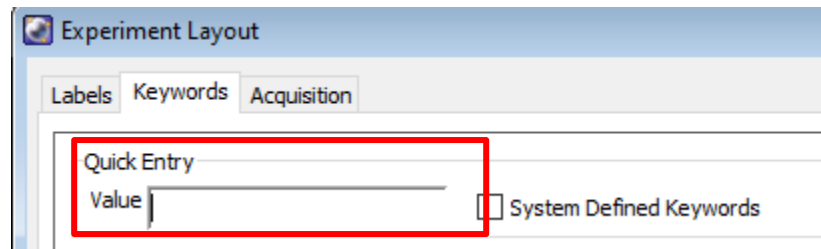
8. In the Acquisition tab:
   a. change the number of events to record/acquire.

9. Run a fully stained sample and set up your gates.
   a. Check to make sure the gates are collecting the desired populations.

IF USING HTS (Plate Loader):

1. Click Global Worksheet to go back to original worksheet.

2. Click New Plate on the browser window.
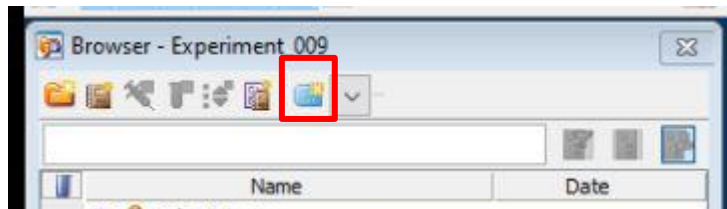
3. Select appropriate wells on the plate and create specimen(s).

4. Enter the appropriate setting (see below for throughput mode, speed, volume, number of mixes, and wash volume).

5. Make sure the "HTS probe" is on and the HTS connector is installed on the probe (short probe + screwing Brown HTS connector).

6. In the Compensation Tab, make sure Enable compensation is selected.

7. To develop the Global Worksheet, Create your plots (dot plots, contour plots, and/or histograms), stats (Median Fluorescence Intensity, CV, etc.), and Population Hierarchy for your analysis.
   a. Create gates (polygonal gates, squares, quadrants, or intervals) to analyze your data.

8. Select Experiment → Experiment Layout.

9. In the Labels tab:
    a. Change the names of the tubes and the axis labels.
    b. Highlight the name you want to change and press the Shift button.

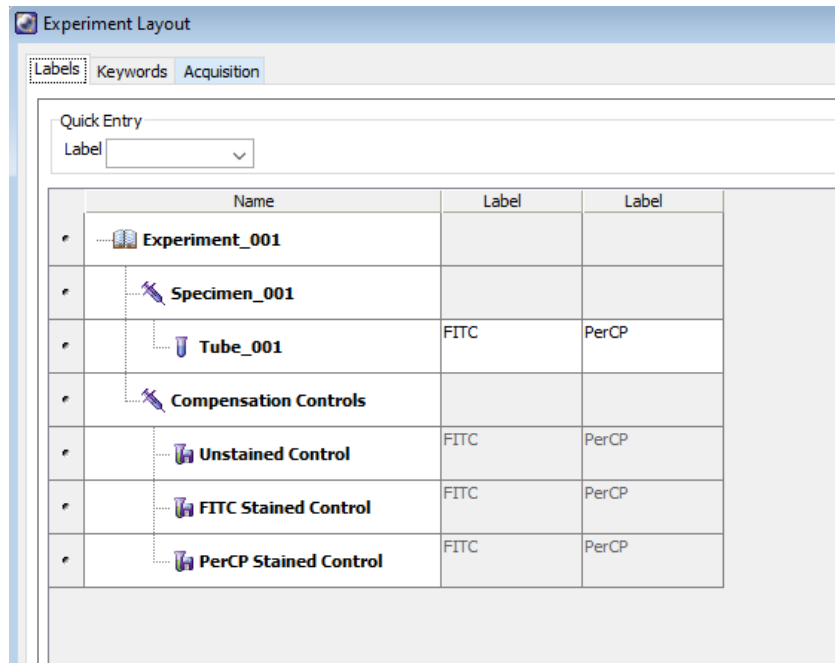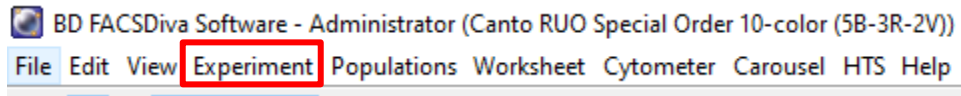10. In the Keyword tab, <u>Add Patient ID</u> and <u>Sample ID.</u>
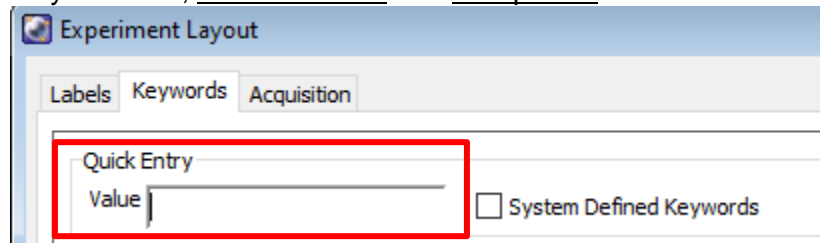
11. In the Acquisition tab:
    a. Click Global Worksheet column header then select the correct Global Sheet on the Global Worksheet pull-down tab.
    b. Select the number of events to acquire as well as the stopping gate by selecting the appropriate column header first.

12. Set up the Global Worksheet and turn on the bi-exponential display for the fluorescent log plots.

13. Run a fully stained sample to set up gates with small amounts (e.g. 10uL for sample volume, 10uL for mixing volume, and flow rate on 0.5uL/sec).

14. Adjust the settings as shown below and click on <u>Run Plate</u>.

15. Click the <u>HTS tab</u> → Home before starting next plate to avoid pump 1 error.
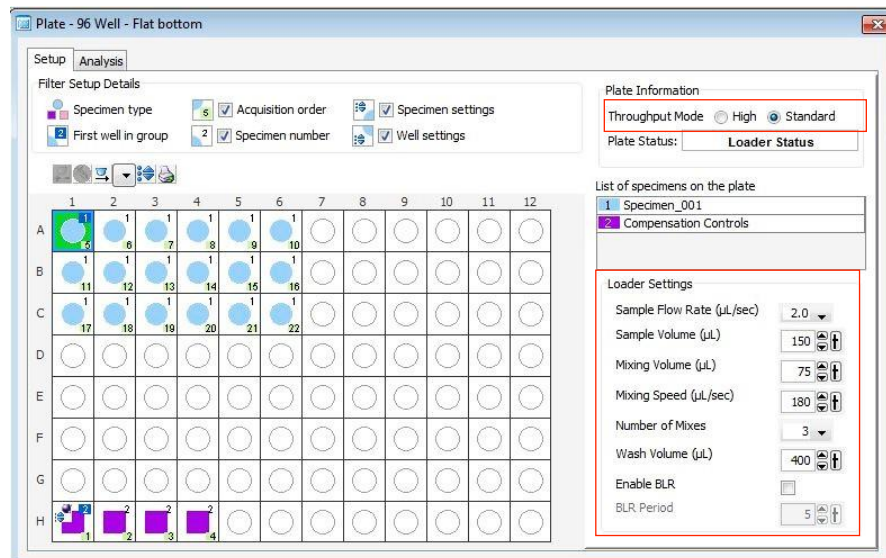
*Note 1: Export the Experiment Template to the hard drive for future use: File →
Export → Experiment Template→ Name, etc.*

*Note 2: Even though the saved experiment template contains Application
Settings, these Application Settings are not automatically synchronized with
current CS&T every time a new document is created from the template. When
you return to perform a similar experiment and you want the settings to be
relevant to previous runs, import the experiment template; right-click Cytometer
Settings → Application Settings → Apply. If the Confirm dialog appears and you
have a new set of compensation tubes, click, "Set all compensation to zero" and
proceed with step 9 above.*

*Alternatively, you can keep compensation values from a previous run or
"Duplicate Experiment without Data," but in the latter two examples the settings
won't be relative to the current CST.*

<u>EXPORTING DATA:</u>

1. **For single tubes**: <u>Export</u> → <u>Experiment</u> to your computer folder.
   For HTS: <u>Export</u> → <u>FCS</u> → <u>3.0</u> to your computer folder.

2. Connect to your online tools (Cytobank.org/cytobank with your personnel
   username and password) to upload the folder on this online server.

3. Upload your files using Cytobank or BioHPC.

*NOTE: The flow core deletes experiments off DIVA on the first of every month. They are

stored in a cloud backup. Please make sure that you are saving your experiments after every session.*

CLEANING BETWEEN USERS

1. Clean the fluidics:
    a. **Tubes***:* For each of the following solutions, fill up half a tube, load the tube on the SIP, and run each for 5 mins on high: 50% Contrad, 10% Bleach, and DI water.
    b. **Plates***:* Run the cleaning plates (4 wells of Contrad, 2 wells of Bleach, and 2 wells of DI water).

2. Wipe down workspace.

3. Check sheath and waste levels.

4. Log out FACS DIVA software.

5. Log out of PPMS.

DAILY SHUTDOWN

1. Clean the fluidics:
    a. **Tubes***:* For each of the following solutions, fill up half a tube, load the tube on the SIP, and run each for five mins on high, 50% Contrad, 10% Bleach and DI water.
    b. **Plates***:* Run the cleaning plates (4 wells of Contrad, 2 wells of Bleach, and 2 wells of DI water).

2. Wipe down workspace.

3. Check sheath and waste levels.

4. Quit FACS DIVA software, Laser software (BD Coherent 3), and Tera Term.

5. Turn off cytometer and fluidics cart by pressing the green buttons.

6. Log out of PPMS.