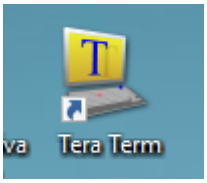
CANTO REFERENCE GUIDE

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

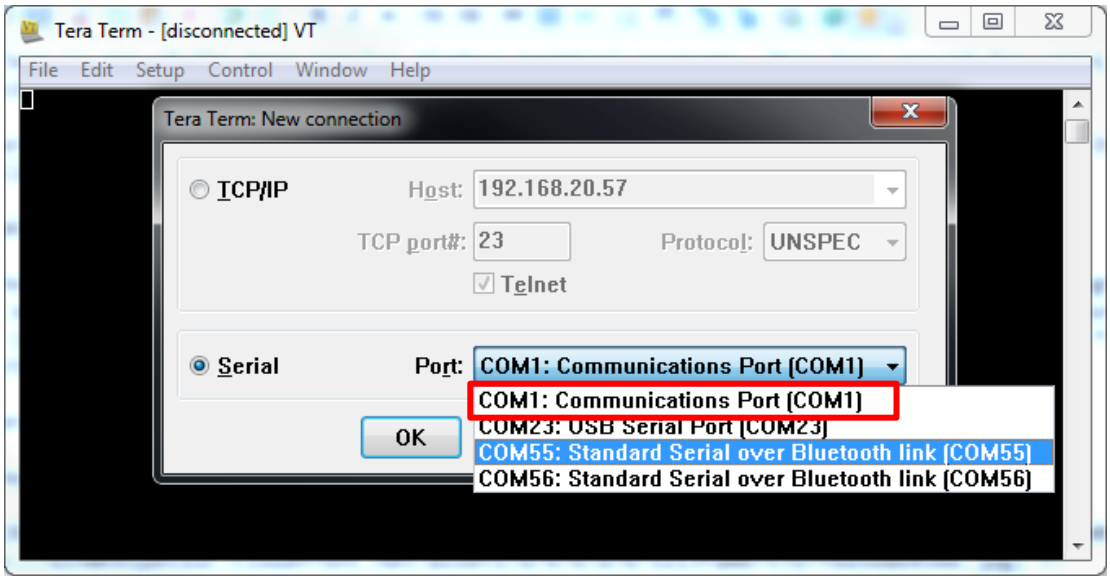
1. The computer should be on.
   a. To log into the PC: **Username:** BDAdmin **Password:** BDIS

2. Unlock the screen with your PPMS Account.

3. Launch Tera Term.



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

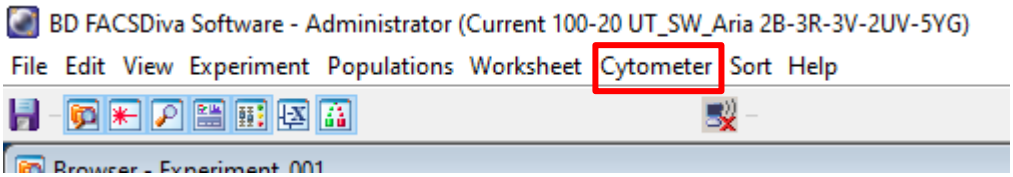


5. Turn on the cytometer by pressing the green button on the side of the instrument.

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

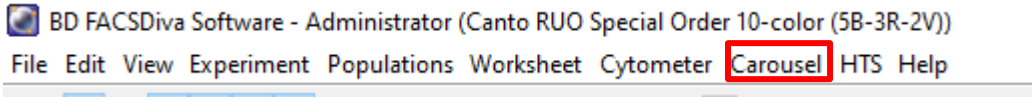
7. Check the sheath and waste tank.
    a. If you are emptying waste, add a layer of bleach to the bottom of the container before placing it back in its designated space.

8. Perform Fluidics Startup:
    a. Cytometer → Fluidics Startup

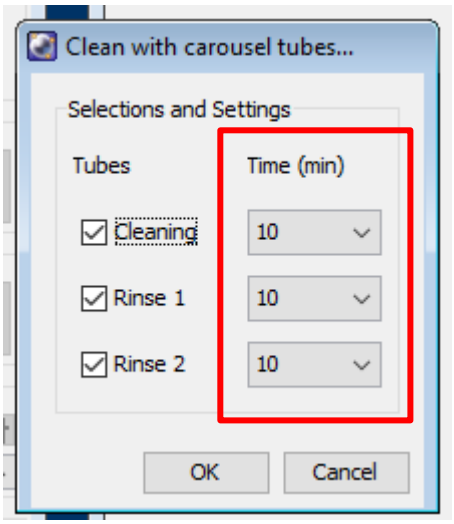


9. Prepare the cleaning carousel:
    a. Place a tube of bleach in the first position.
    b. Place a tube of Contrad in the second position.
    c. Place a tube of shut down solution in the third position.

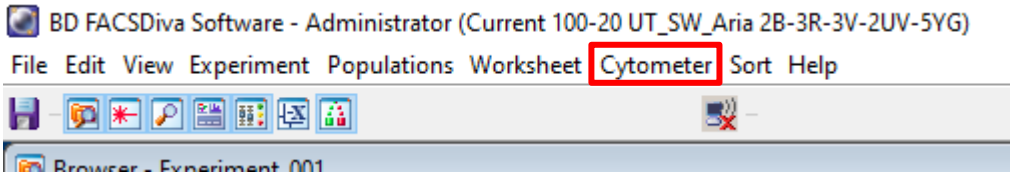
10. Once fluidics startup has finished, insert the carousel, and go to Carousel → Clean.



    a. Select 10 minutes for each tube.

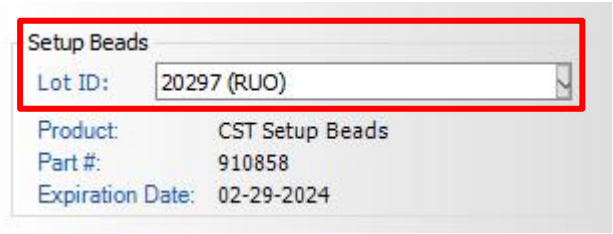


11. After cleaning, go to Cytometer → CST.



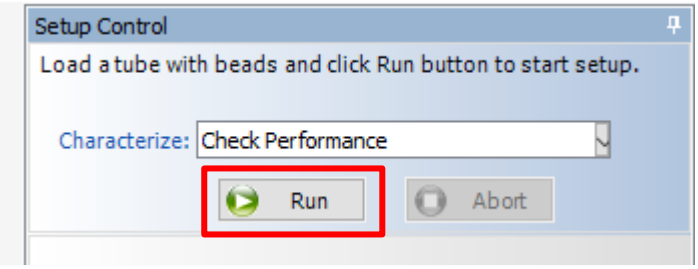
12. Take a clean FACS tube and add two drops of CST beads with ~ 300uL of FACS flow.

13. Verify that the lot number on the side of the bead's container matches the one on the screen.



14. Take out the cleaning carousel and replace the tube in position 1 with the CST tube.

15. Press Run 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 run CST.

ANY USER OF THE DAY

1. Log into PPMS and your DIVA account.

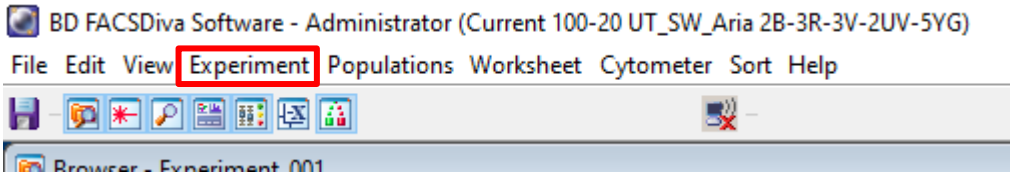
2. Prepare the cleaning carousel:
    a. Place a tube of bleach in the first position.
    b. Place a tube of Contrad in the second position.
    c. Place a tube of shutdown solution in the third position.

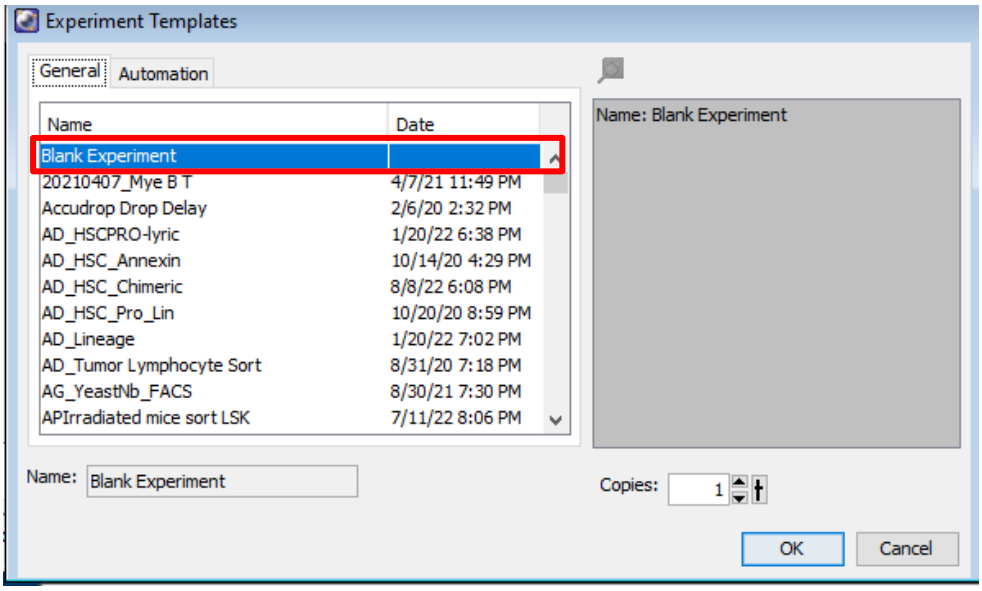
3. Insert the carousel and go to Carousel → Clean.

    a. Select two minutes for each tube, click OK.

FOR A NEW EXPERIMENT

1. Select Experiment → New Experiment → Blank Experiment.

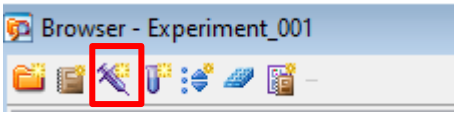




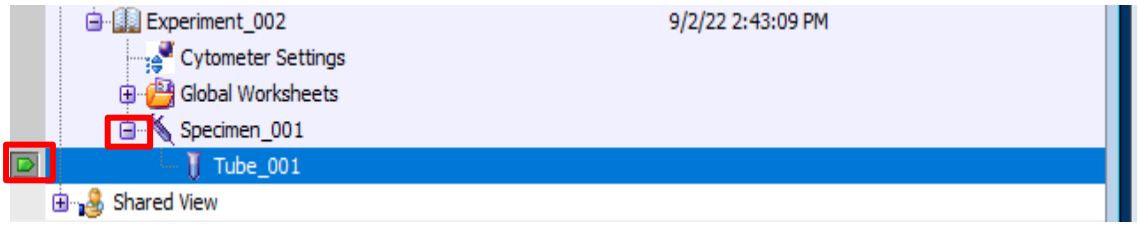
2. Click on the Parameters tab in the cytometer window.

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

4. Create a new specimen.

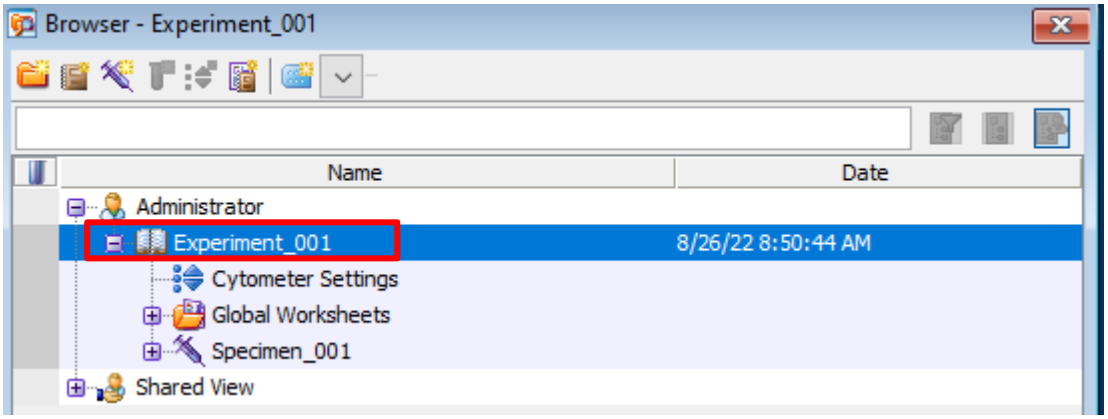


5. Expand the specimen and place the acquisition pointer on Tube_001.

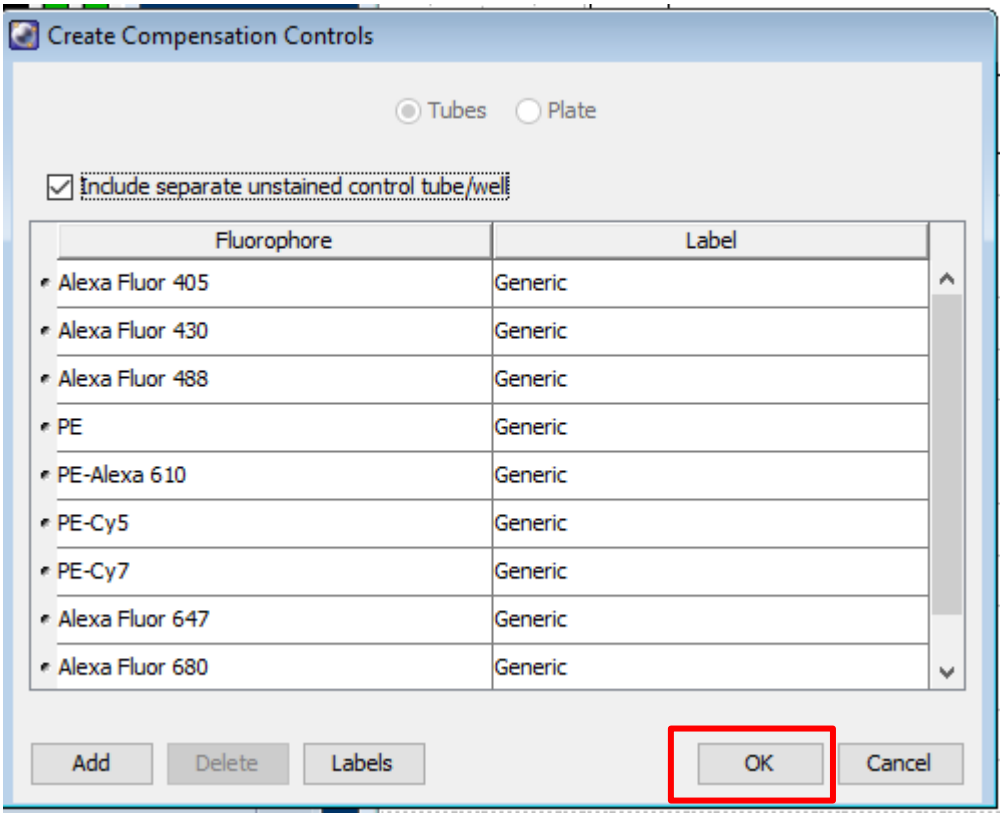


CALCULATING COMPENSATION:

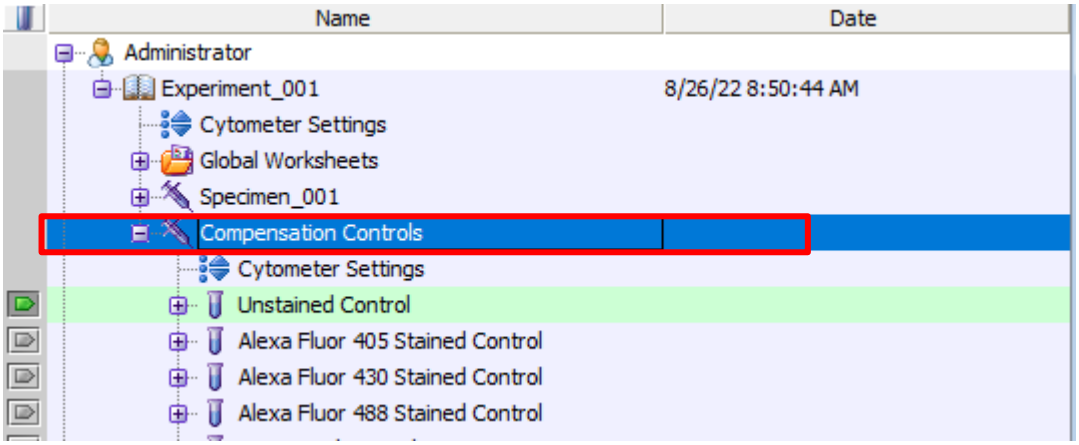
1. Select Experiment → Compensation Setup → Create.



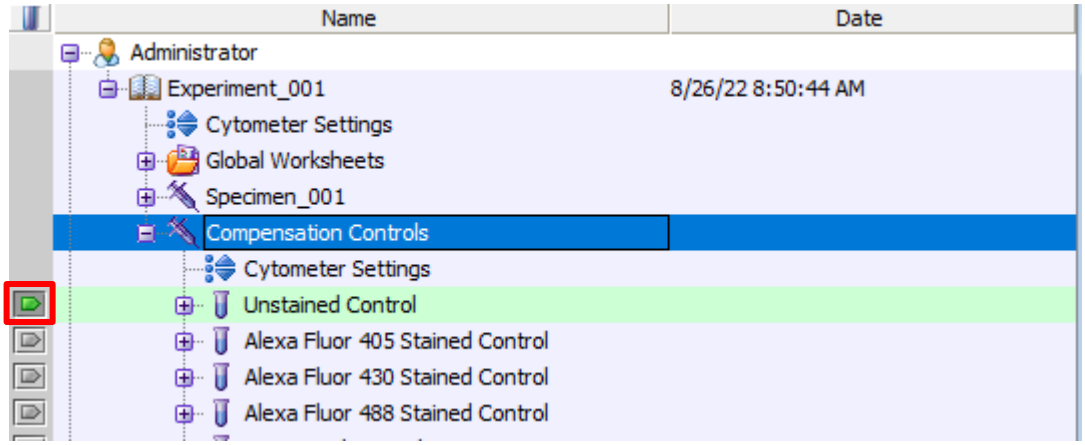
   a. Verify compensation controls coincide with your chosen fluorochromes, then press OK.



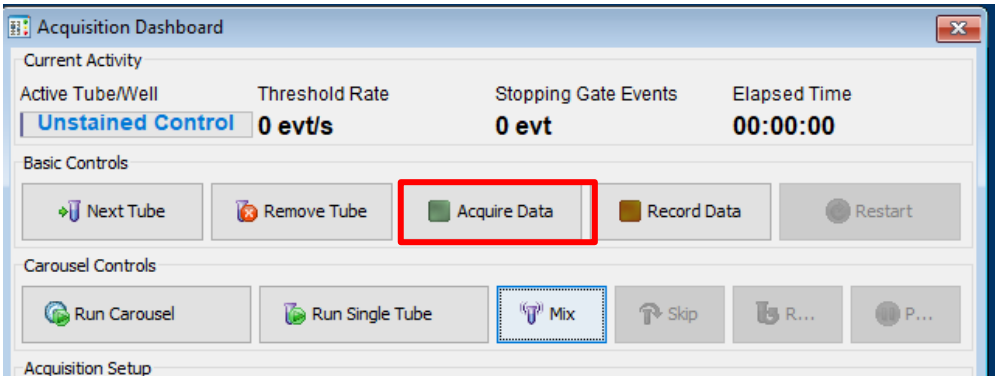
2. Open the Compensation Controls specimen in your experiment.



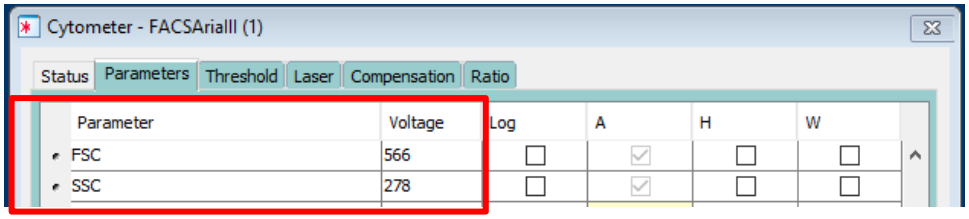
3. Make sure the tube pointer is on your unstained control.

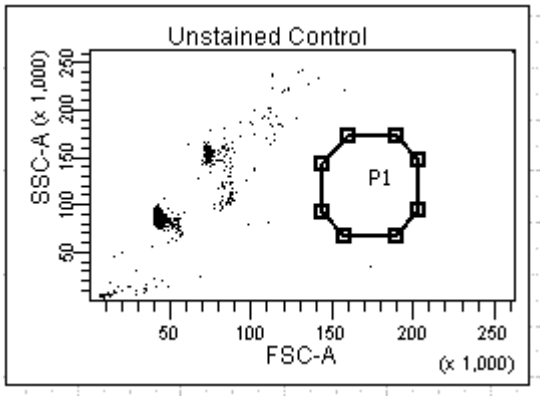


4. Load your unstained tube and hit Acquire Data.



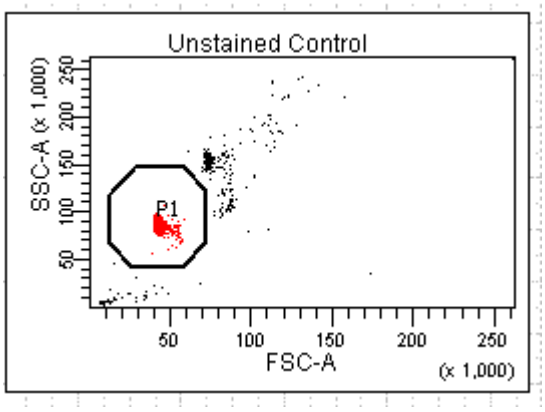
5. Adjust the FSC and SSC voltage until your cells are on-scale in the plot.





6. Stop acquiring.

7. Move the P1 gate until it encompasses the population of interest.



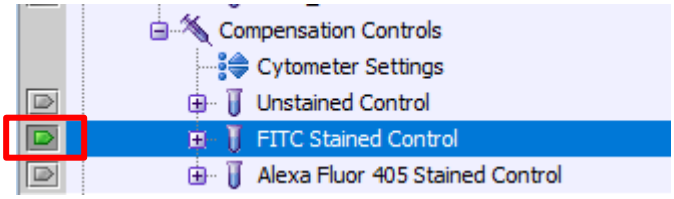
8. Right-click the P1 gate → apply to all compensation controls.

9. Acquire data again and Record.

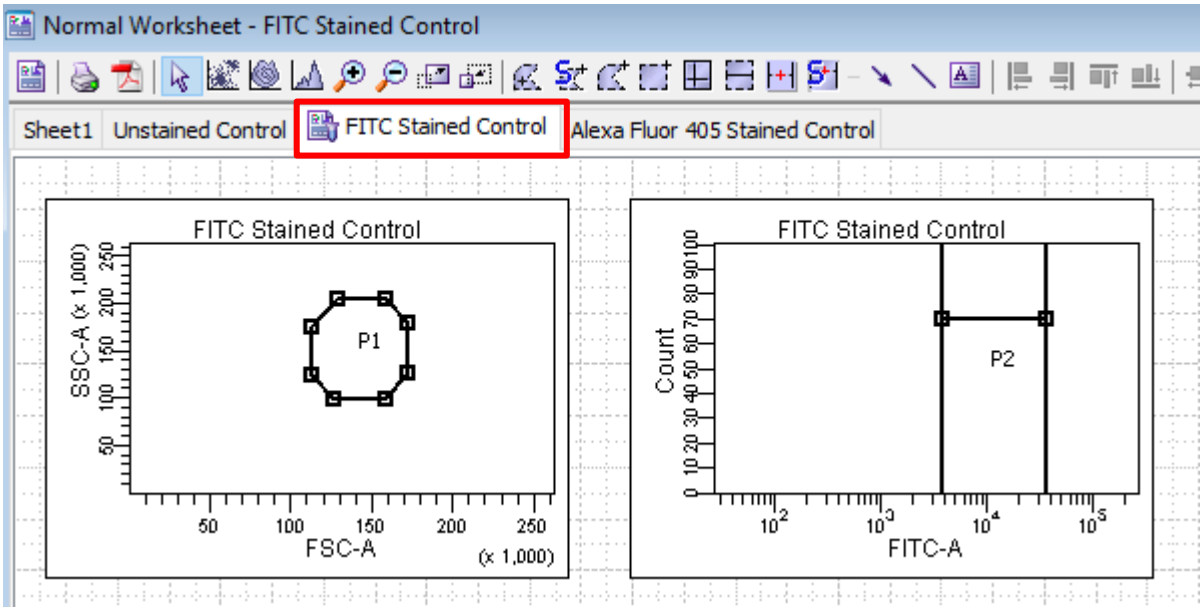
10. Unload your unstained tube.

11. Load your first compensation tube.

12. Make sure that your tube pointer is on the correct tube.

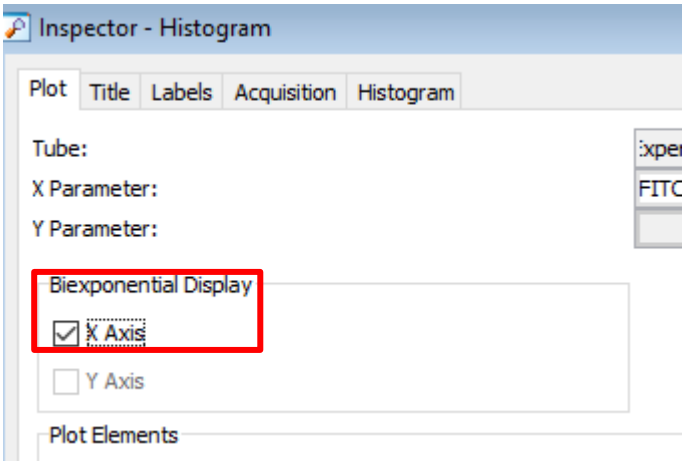


13. Click on the next worksheet panel.

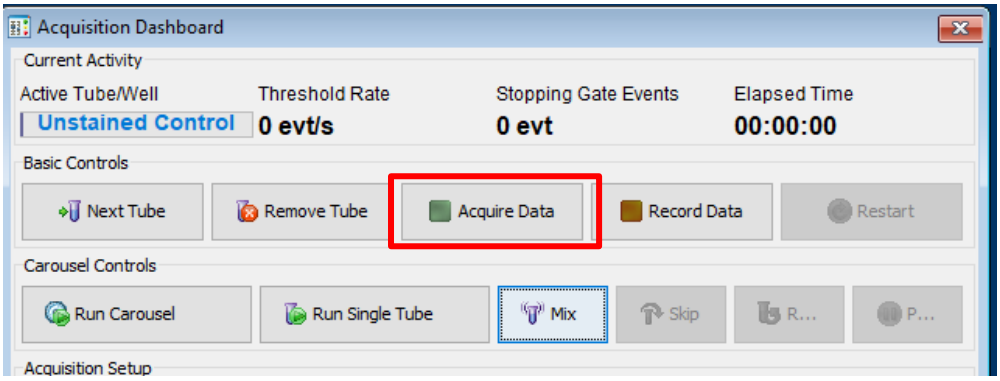


14. Click on your histogram plot.

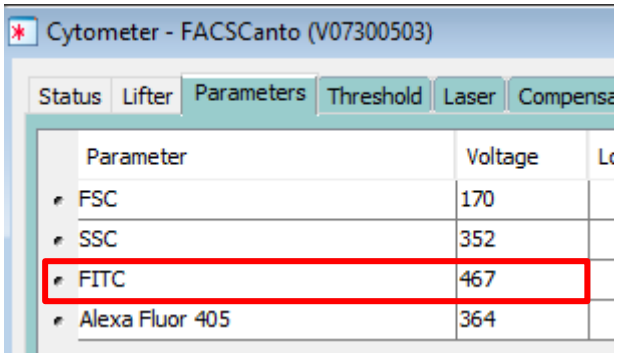
15. Click the X-axis <u>Biexponential Display</u> in the inspector window.

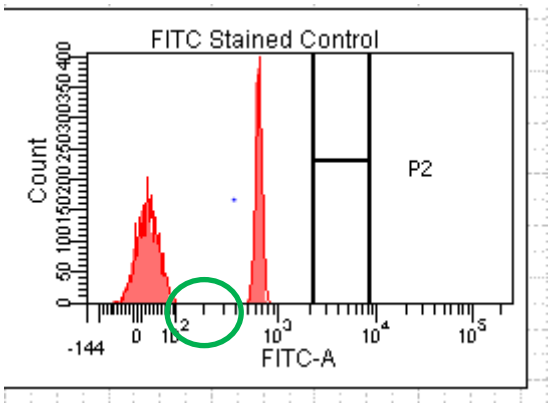


16. Click Acquire Data.



17. Adjust the voltage of the fluorochrome until the negative population is close to zero.

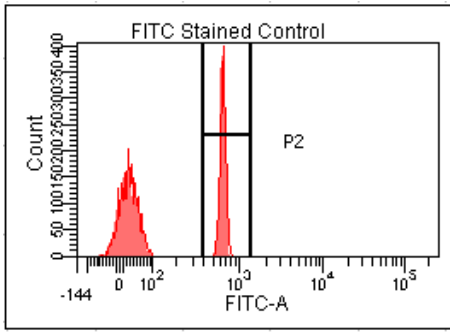




a. It's important to make sure you can see both ends of your population. If it bleeds into the side of the graph, increase the voltage until you can see the bottom left of the peak.

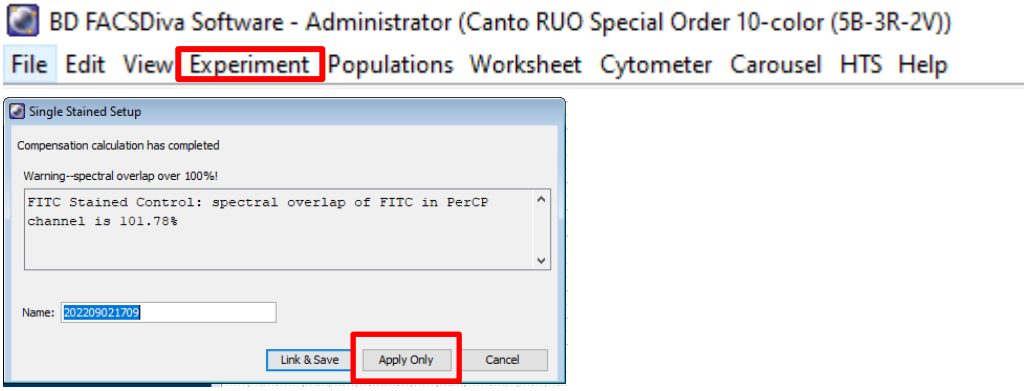
18. Click Record Data

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



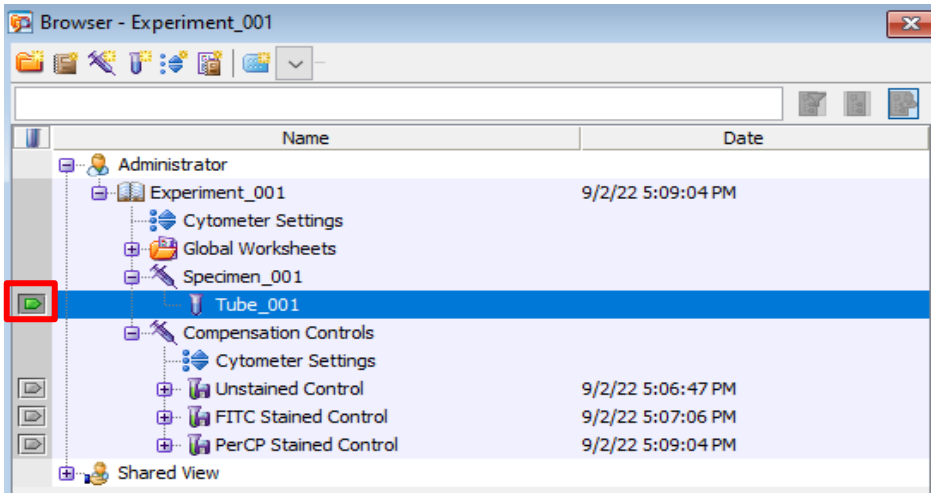
20. Repeat steps 10-17 until all your compensation tubes have been completed.

21. Select Experiment → Compensation Setup → Calculate Compensation → Apply Only.

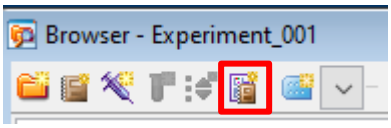


ACQUISITION

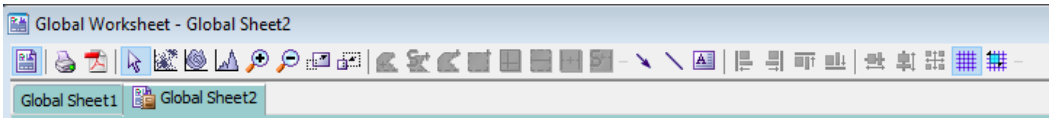
1. Place the Acquisition Pointer on Tube_001.



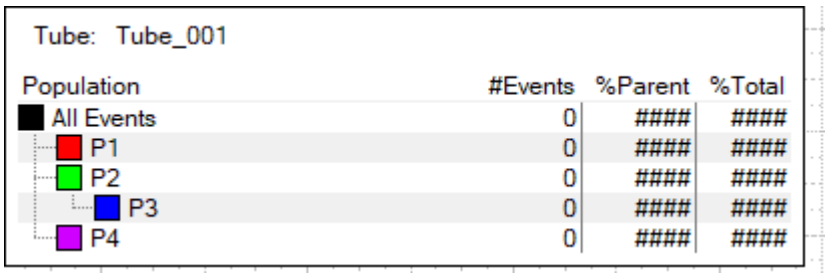
2. Create a new global worksheet.



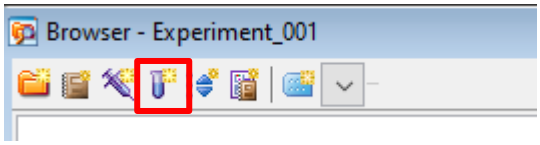
3. Create your graphs, dot plots, and gates to run your experiment.



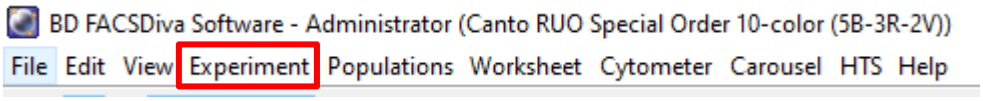
   a. Hover over each item in the toolbar for more information.
   b. Once you have your graphs set up, right-click one and press Show Population Hierarchy.

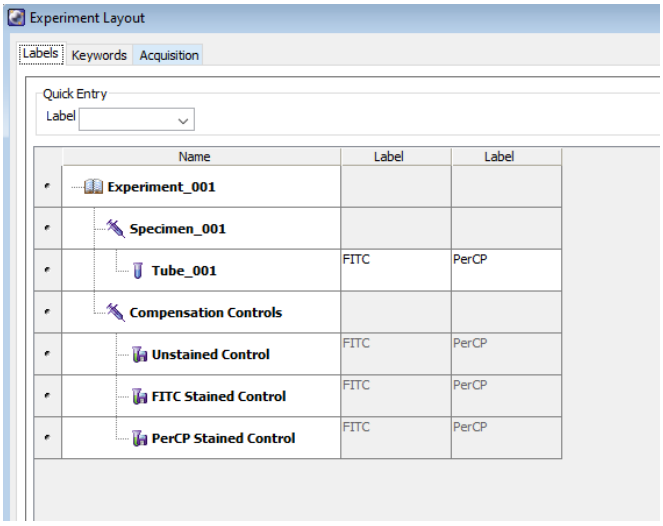


4. Add additional tubes to the experiment, if needed.



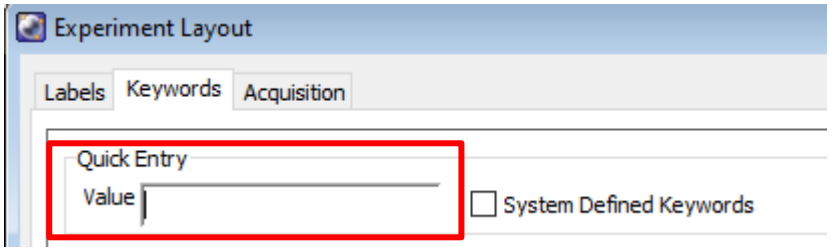
5. Select Experiment → Experiment Layout.





6. 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.

7. In the Keywords tab, Add Patient ID and Sample ID.



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 SINGLE TUBES

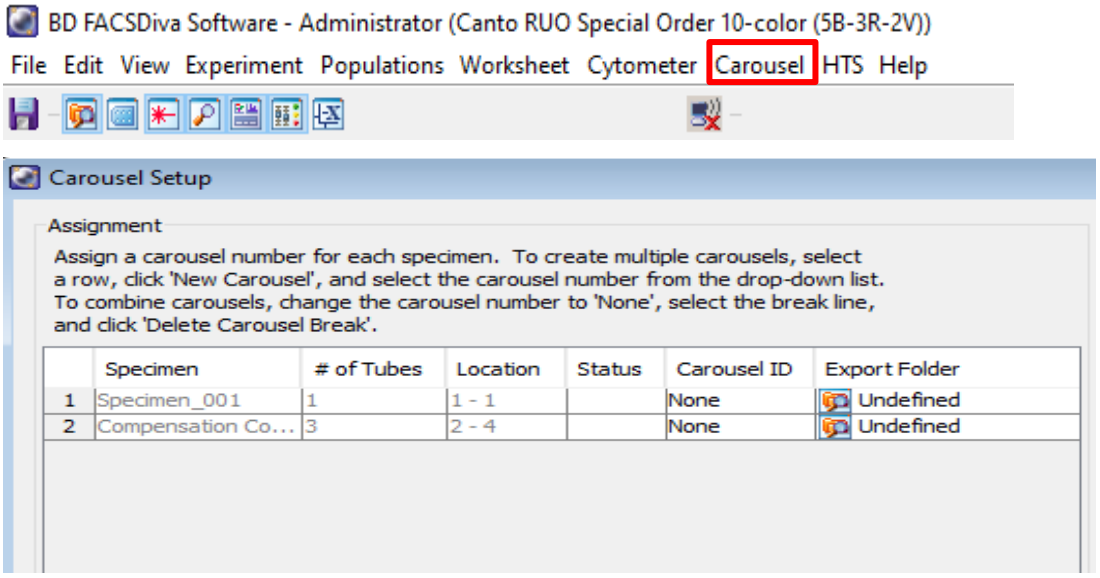
1. Make sure the probe is in "single tube" mode.
    a. Move the tube guide to the left.
    b. Place the probe in the upward position.

2. Run tube.

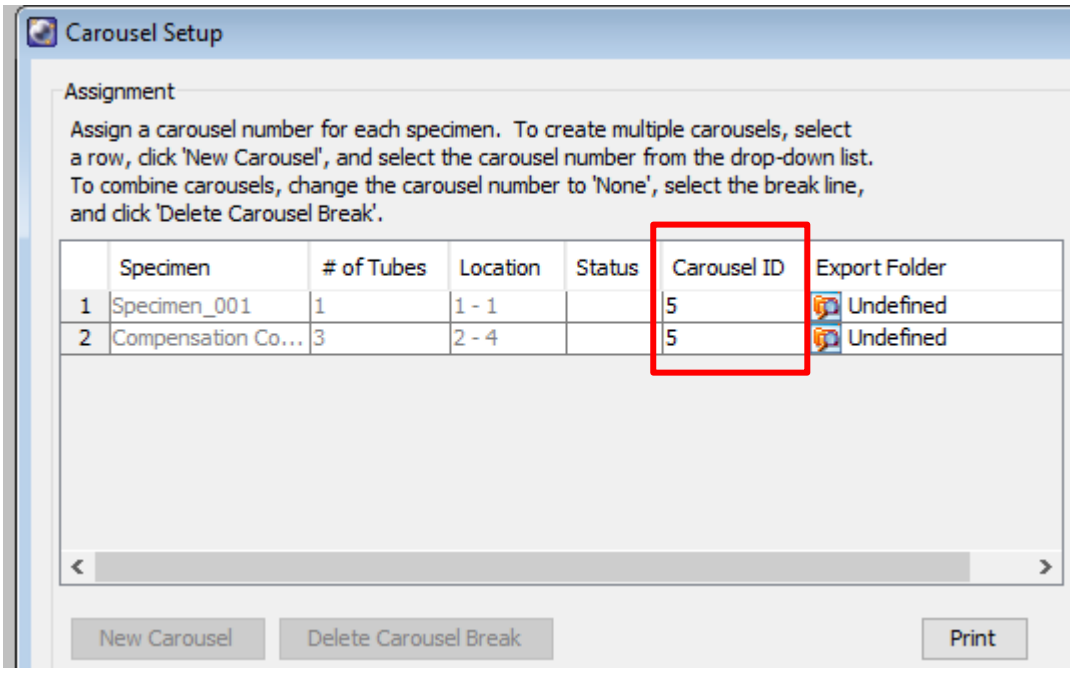
3. Record data.

IF USING CAROUSEL

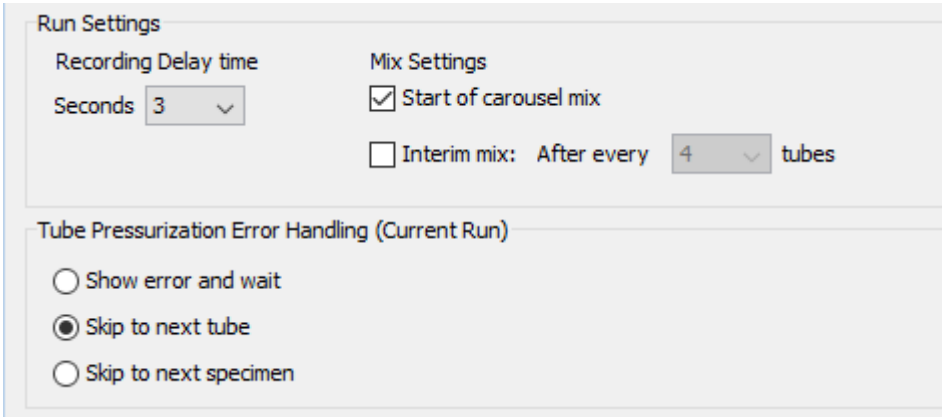
1. Select Carousel → Carousel Setup.



2. Under the Carousel ID tab, select the carousel number.

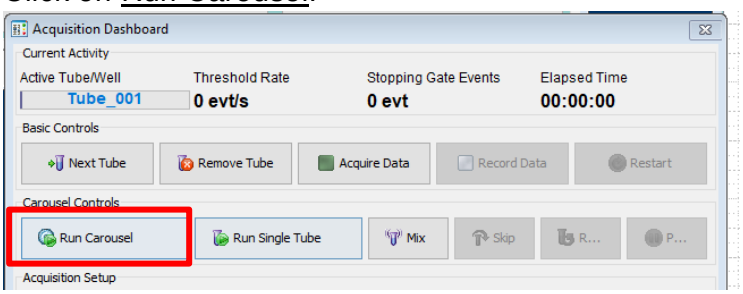


3. Select the appropriate settings and press OK when done.



4. Make sure the probe is in Carousel mode when using the carousel.
   a. Ensure the tube guide is on.
   b. Place the probe in the downward position.

5. Click on Run Carousel.



EXPORTING DATA

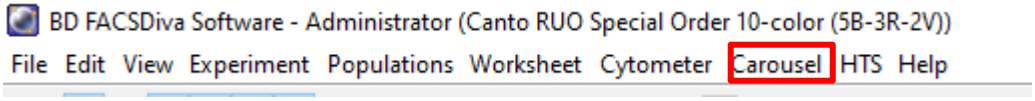
1. BioHPC

2. Cytobank

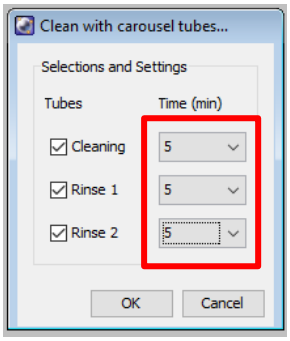
***NOTE:** The flow core deletes experiments off DIVA on the first of every month. They are stored in a cloud backup. Please make sure that you are saving your experiments after every session.*

CLEANING BETWEEN USERS

1. Prepare the cleaning carousel:
    a. Place a tube of bleach in the first position.
    b. Place a tube of Contrad in the second position.
    c. Place a tube of shut down solution in the third position.

2. Insert the carousel and go to Carousel → Clean.
    a. 

    b. Select 5 minutes for each tube.



    1. Wipe down workspace.

    2. Check sheath and waste levels.

    3. Log out of FACS DIVA.

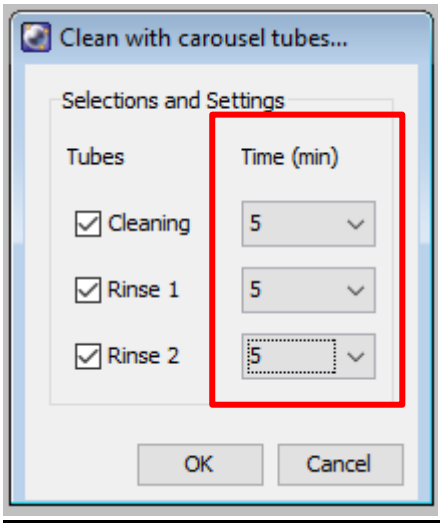
    4. Log out of PPMS.

DAILY SHUTDOWN FOR THE LAST USER OF THE DAY

1. Prepare the cleaning carousel:
    a. Place a tube of bleach in the first position.
    b. Place a tube of Contrad in the second positon.
    c. Place a tube of shutdown solution in the third position.

2. Insert the carousel and go to Carousel → Clean.

a. Select 5 minutes for each tube.



3. Wipe down the workspace.
4. Check sheath and waste levels.
5. Log out of DIVA.
6. Exit out of TeraTerm.

7. Log out of PPMS.
8. Turn off the cytometer.