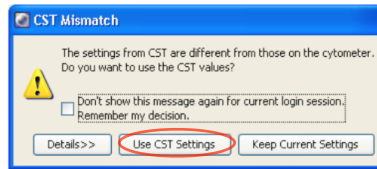


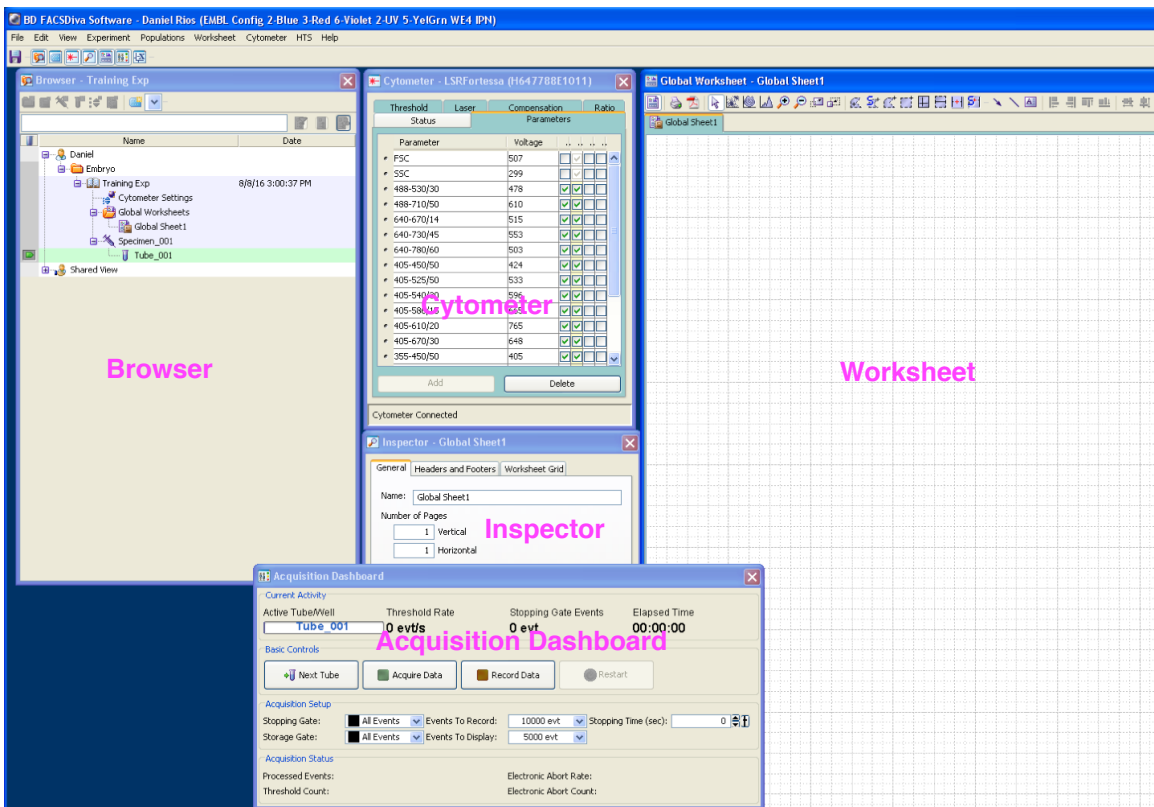


Acquiring samples in the BD LSRFortessa

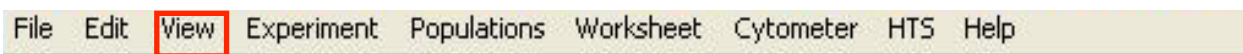
Launch DIVA program and log into you session. When then CST mismatch window pops up click in: "Use CST Settings"



Identify the windows of Diva workspace:




If one of the windows is missing, go to the top menu bar, click on view and select the missing window.

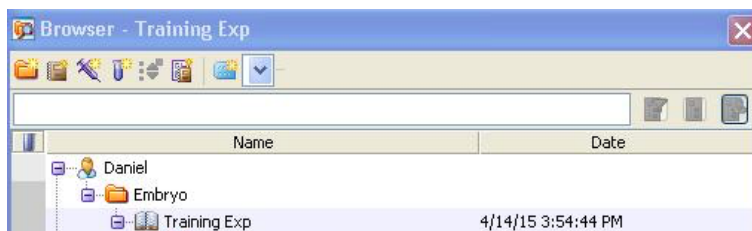


Use the Browser window to create experiments:

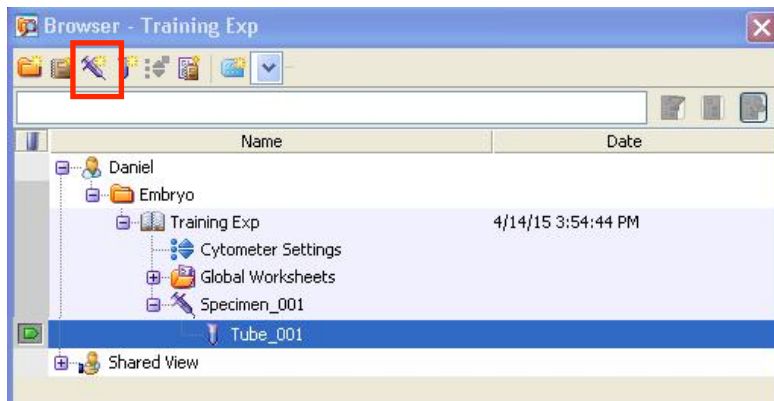
Click in the folder icon to create a folder, right click to rename.



Now you could create a new experiment in the folder. Click in the icon:  Double click to open the experiment and right click to rename it.



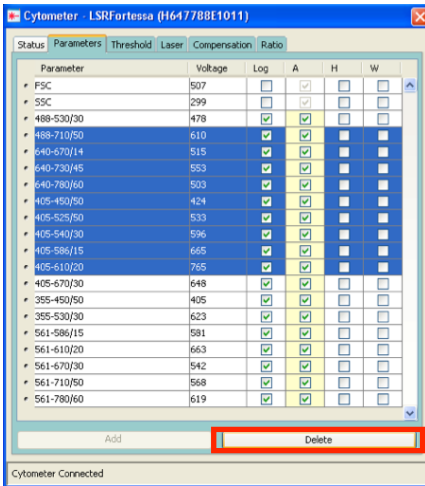
Create a Specimen by clicking the Specimen icon:



The specimen contains a tube, when clicking in the tube pointer you will visualise the list of available parameters in the cytometer window/parameter tab:

The screenshot shows the 'Cytometer - LSRFortessa (11647788E1011)' window. It displays a list of parameters with columns for 'Parameter', 'Threshold', 'Laser', 'Compensation', and 'Ratio'. The 'Ratio' column has sub-columns for 'Log', 'A', 'H', and 'W'. The 'Status' column has a dropdown arrow. At the bottom, there are 'Add' and 'Delete' buttons. The status bar at the bottom indicates 'Cytometer Connected'.

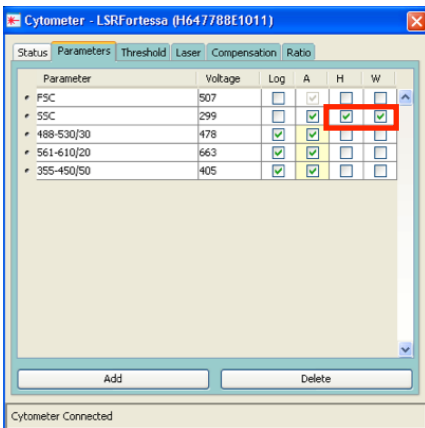
Parameter	Threshold	Laser	Compensation	Ratio			
				Log A H W			
FSC	507	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
SSC	299	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
488-530/30	478	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
488-710/50	610	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
640-670/14	515	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
640-730/45	553	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
640-780/60	503	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
405-450/50	424	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
405-625/50	533	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
405-540/30	596	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
405-586/15	665	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
405-610/20	765	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
405-670/30	648	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
355-450/50	405	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
355-530/30	623	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
561-586/15	581	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
561-610/20	663	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
561-670/30	542	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
561-710/50	568	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
561-780/60	619	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>



Erase the parameters that you do not need to measure in your experiment.

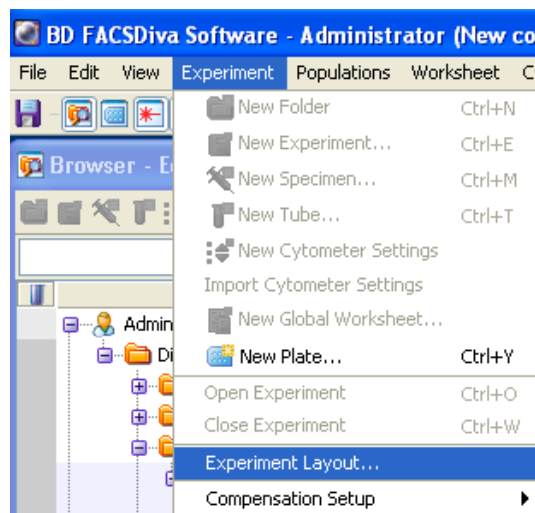
To delete parameters, click the selection button next to each unneeded parameter. Hold down the Ctrl key to select more than one. When you are finished selecting, click Delete.

Verify that Log mode is selected for all fluorescence detectors but not for FSC and SSC (Lin mode). Tick H and W for SSC parameter in order to discriminate doublets.

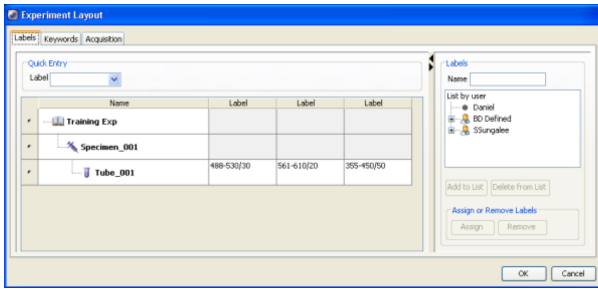


*For cell cycle, don't forget to measure the parameter of your DNA dye in Lin mode and to tick H and W for doublet exclusion.

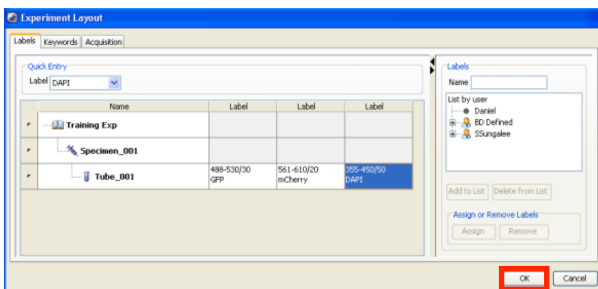
Add your specific labels to the parameters names. Choose Experiment (top menu bar) > Experiment Layout, and define labels for each parameter.



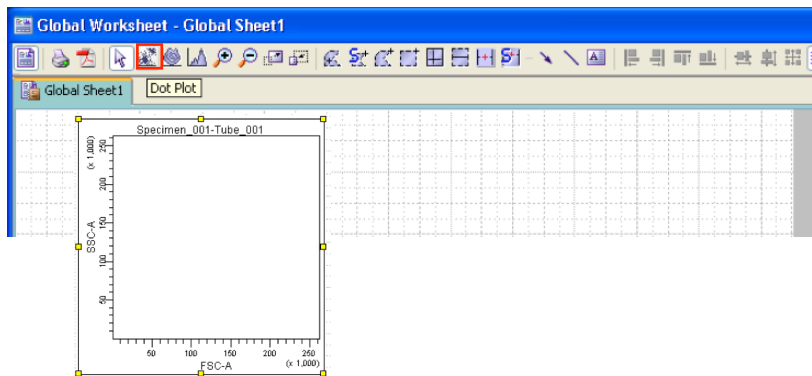
The experiment layout window opens up:



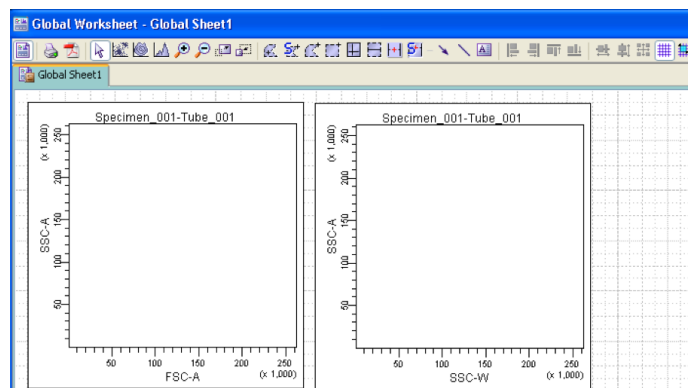
Click on the parameter box and type in the label for each parameter.



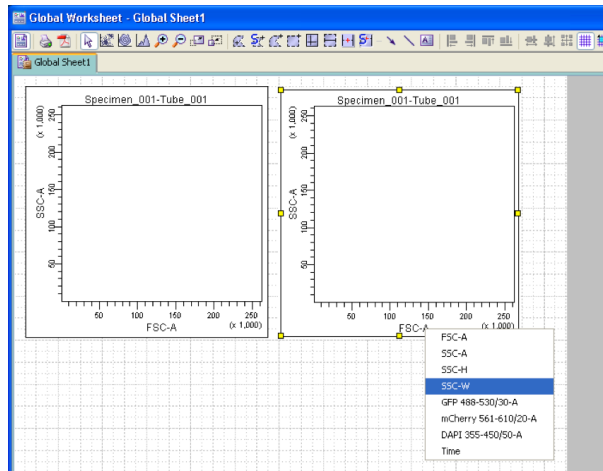
Create dot plots and histograms on the global worksheet to visualise the data:
Click the Dot Plot button on the Worksheet toolbar and click in the worksheet, a plot of a default size is drawn.



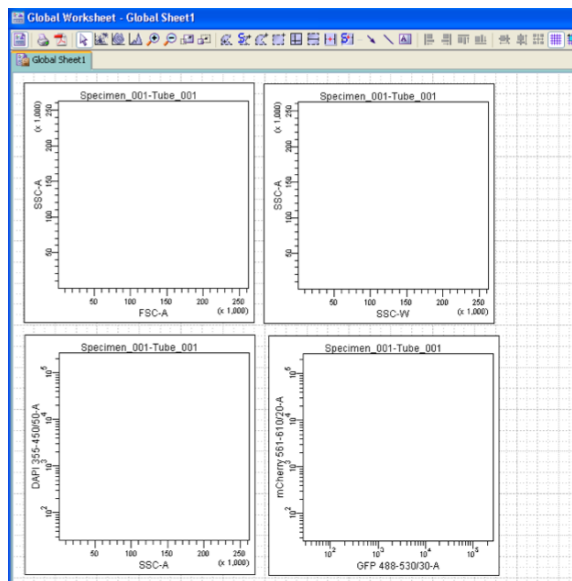
Start with a FSC vs SSC plot, create a second plot SSC-A vs SSC-W to exclude doublets.



To change the plot parameter, click the x-axis label and choose the parameter from the menu that appears. Notice that the parameter names include the labels you defined previously.



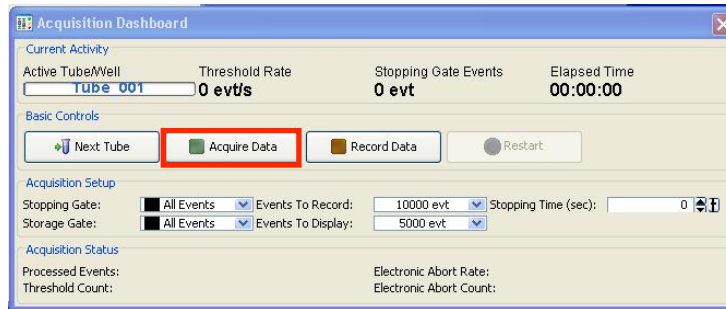
Create additional plots and/or histograms with the parameters that you need to measure in your samples. Do not forget to include a plot/histogram to exclude dead cells.



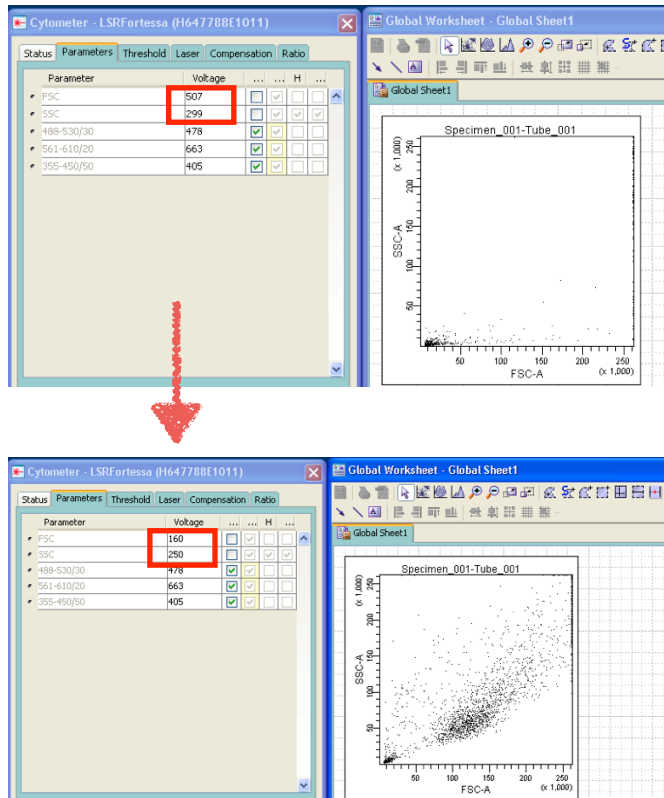
Place your sample tube in the SIP, close the arm and press RUN-Low in the cytometer control panel.



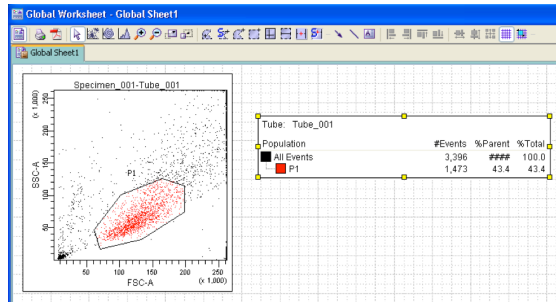
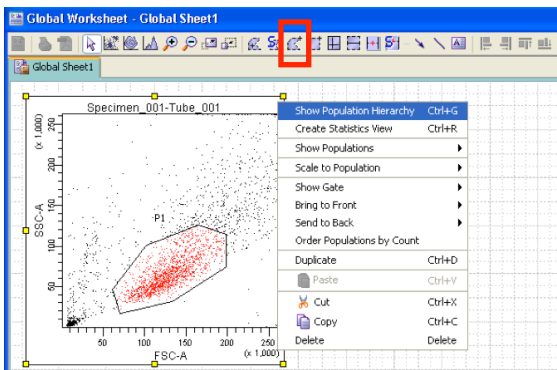
Press acquire data in the acquisition dashboard window as well.



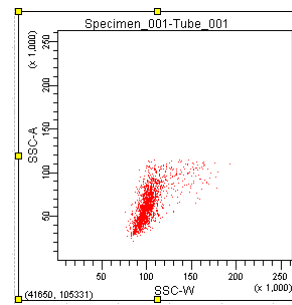
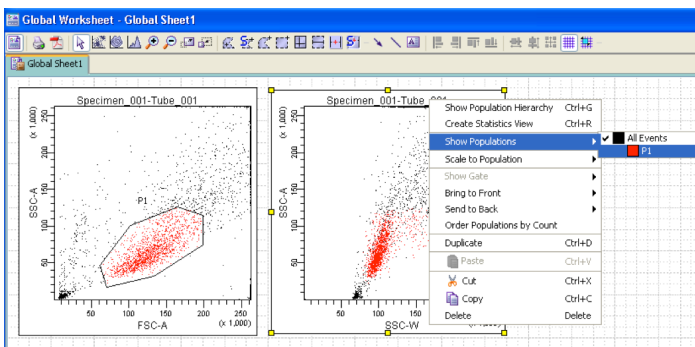
Adjust FSC and SSC voltages in the parameter tab, to bring the population of interest onto scale. Press restart (acquisition dashboard window) after modifying the voltages to refresh the image and quickly visualise the effect of the changes.



Draw a gate to select the cell population and exclude debris. Click on the polygon gate tool at the worksheet menu and draw a polygon around the population, the gate will be automatically named P1. Right click inside the plot and choose Show Population Hierarchy. The population hierarchy is used to view defined populations and display the relationships between different gated populations.

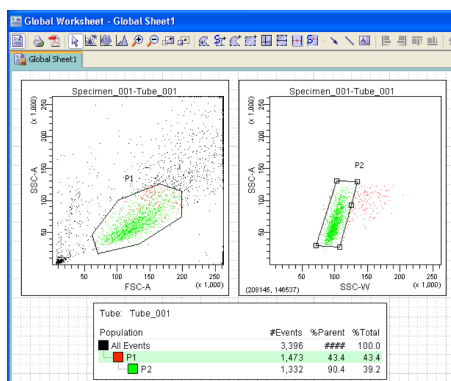


Right click inside the SSC-A vs SSC-W plot and choose Show Populations > P1.



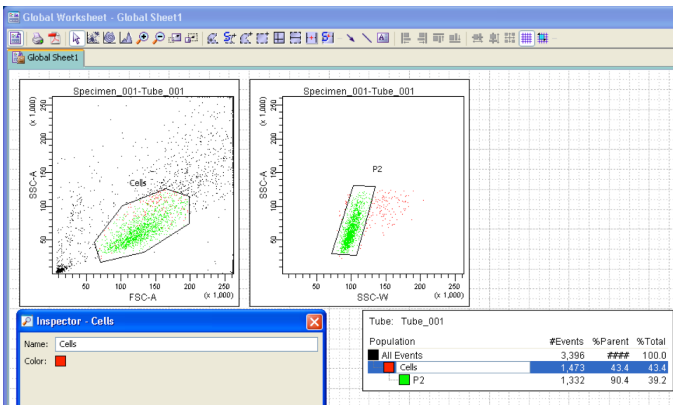
Only events within the P1 gate are shown in the plot. Notice that all events in the plot are now coloured red, the color of the P1 population.

Make a new gate to select the singlet population, named P2.

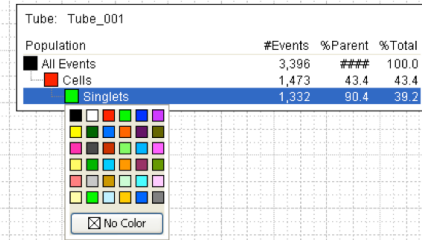


To make a new population from P1, make sure P1 population is highlight in the population hierarchy. When a population is highlighted in the population hierarchy, any new populations will be a subset of the population that is highlighted.

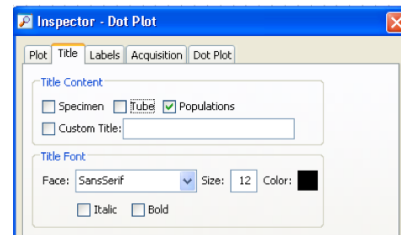
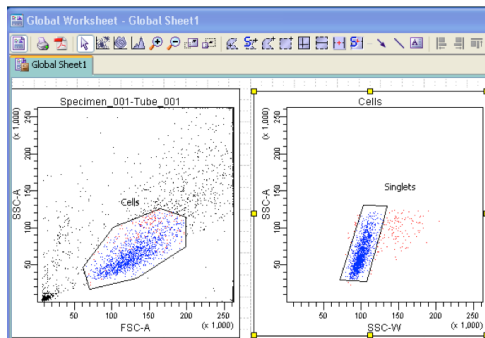
The name of the populations could be modified in the population hierarchy list: double click in the name to enter a new one, or in the inspector window.



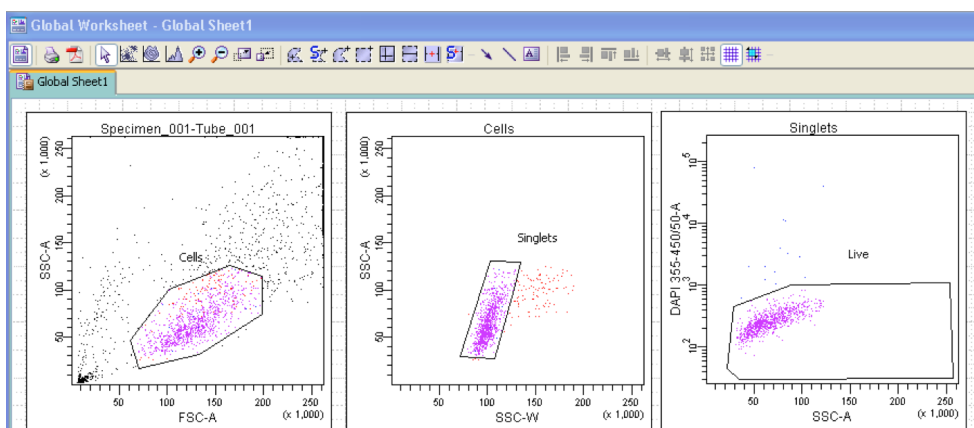
Click on the coloured square to choose a new colour for the population.



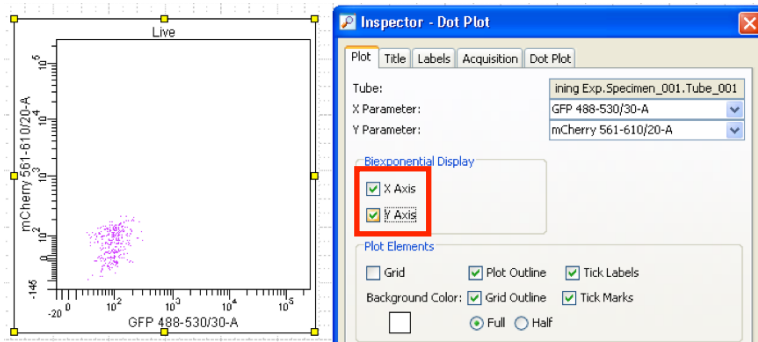
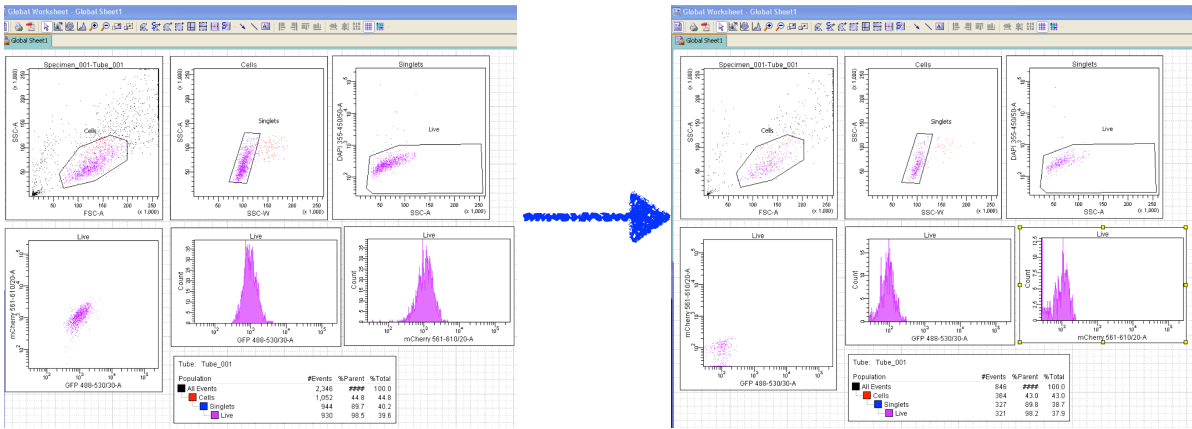
Modify the title of the dot plots to display a selected population: Select the plot, go to the inspector >Title tab: Tick populations



Select the viable cells from your Singlets population (DAPI negative) and restrict your further analysis to events contained in Live gate.

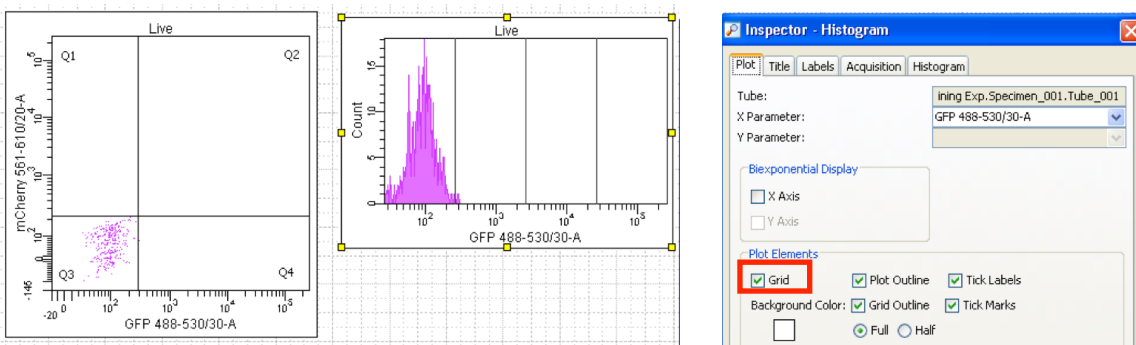


Once the FSC and SSC voltages are set-up, do the same for the fluorescent detectors. Load your negative/unstained sample and adjust the voltages while the same is running. Negative population should be placed in the lower left quadrant of a plot. When using an histogram place the negative population on the left.



If the population appears below the axes (in the dot plot), enable Biexponential display to view populations with negative events. Click on the plot and check the boxes in the Inspector window.

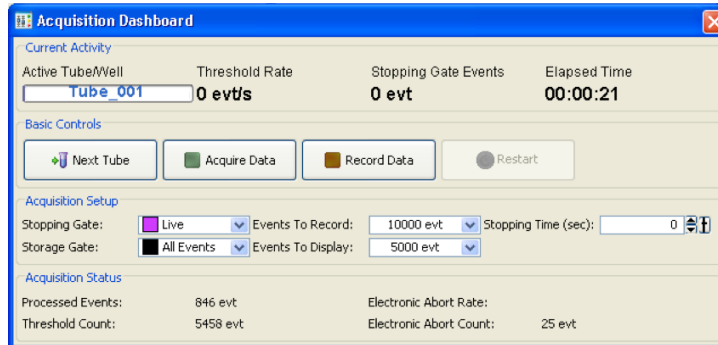
Adding a quadrant (worksheet menu) or grid onto the plot and histogram respectively, will help you to place the negative population correctly.



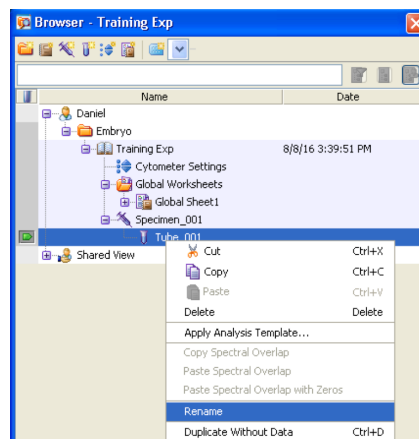
Establish in the acquisition dashboard the amount of events that you want to acquire:

Storage gate: keep all events

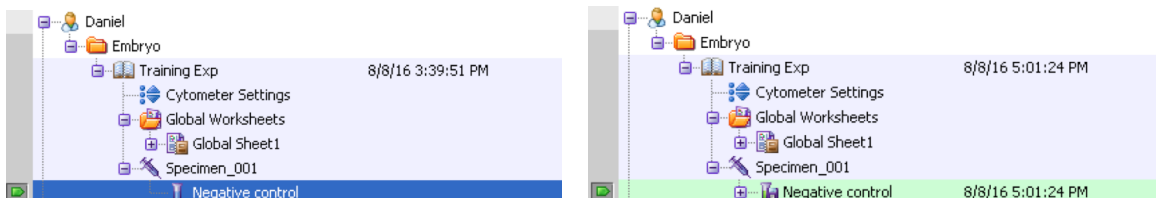
For stopping gate: choose live/P3 and record 10.000 events, enough for most of the applications. If you population of interest is very rare and you need to analyse additional parameter within, choose it as a stopping gate and make sure to collect at least 2000 positive events per population.



Rename tube: Right click on the tube > Rename



Now that all your settings have been established press: Record in the acquisition dashboard window. Note that after acquire the samples the tube icon changed indicating that events have been recorded in the tube.



Add new tubes, rename them and start the acquisition:
 Make sure to select the tube pointer and verify in the dashboard the name of the tube where events will be recorded.



Follow the steps below when loading a new tube:

- Press acquire in the acquisition control
- Visualise the sample in the plots, allow the number events to stabilise (Threshold Rate)
- Press Record Data

The acquisition will be stopped automatically when the sample reach the events stated in the stopping gate. If you do not have enough sample, stop manually first the recording and then the acquisition.

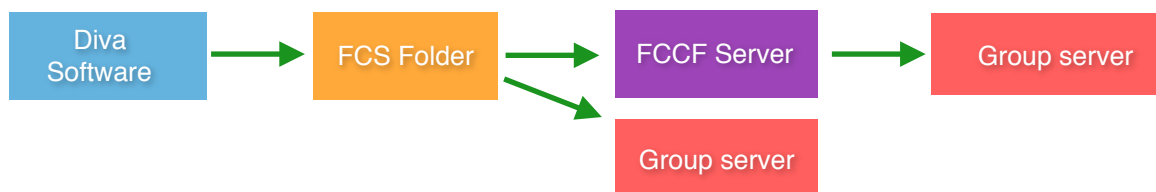


Once you finish, make sure to export your FCS files to the FCS folder and to transfer them to the fccf server or the server of you group. Once your files are safely storage, please remove them from the fccf server.

Data Management:

At the facility

From your PC/Mac



For more info check the [data transfer protocol](#) and the protocol to [create experiment templates](#).