ATTUNE NXT BASIC TRAINING

PRACTICAL MODULE 2: INSTRUMENT SETTINGS

In this module, you will acquire samples of Performance Tracking Beads at multiple flow rates on the Attune NxT Flow Cytometer. You will learn how to set up a tube experiment and adjust the acquisition and instrument settings manually.

Learning Objectives:

1. Learn how to create a new Tube experiment
2. Learn how to set up the Workspace for acquisition and analysis of your sample
3. Learn how to use the Collection Panel to run a sample
4. Learn how to adjust Instrument Settings including PMT voltages and thresholds

For this Lab you will need:

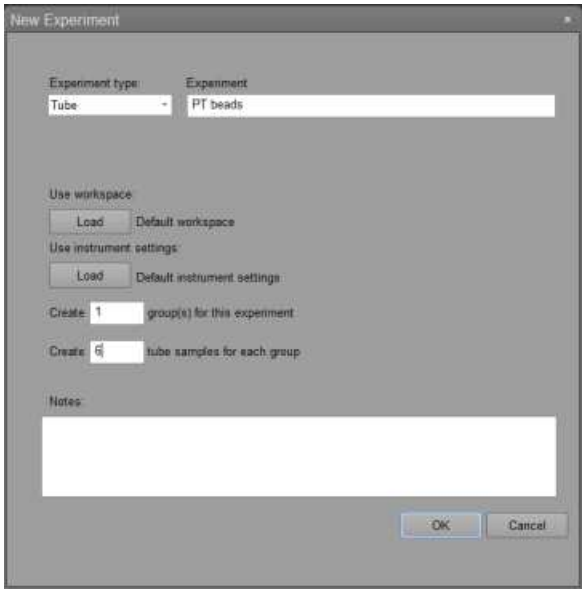
- Attune® Performance Tracking Beads
- 1x Focusing Fluid or 1x PBS
- Flow tubes (12x75mm tubes)

Lab Activity

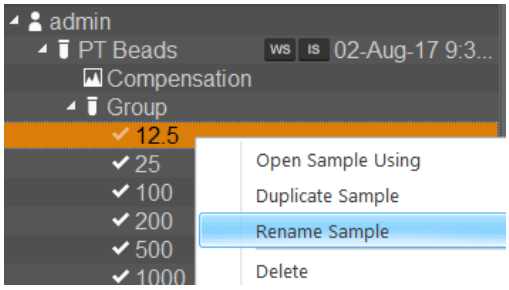
1. Select New Experiment on the Main menu or Right click on the user name in the experiment explorer and select "New experiment" from the drop down menu.

2. Choose the experiment type (tube) and name the experiment "PT beads". Choose to have 1 group and 6 samples (1 for each flow rate).

3. Right click on the group name and sample names in the experiment explorer to rename them. Label the

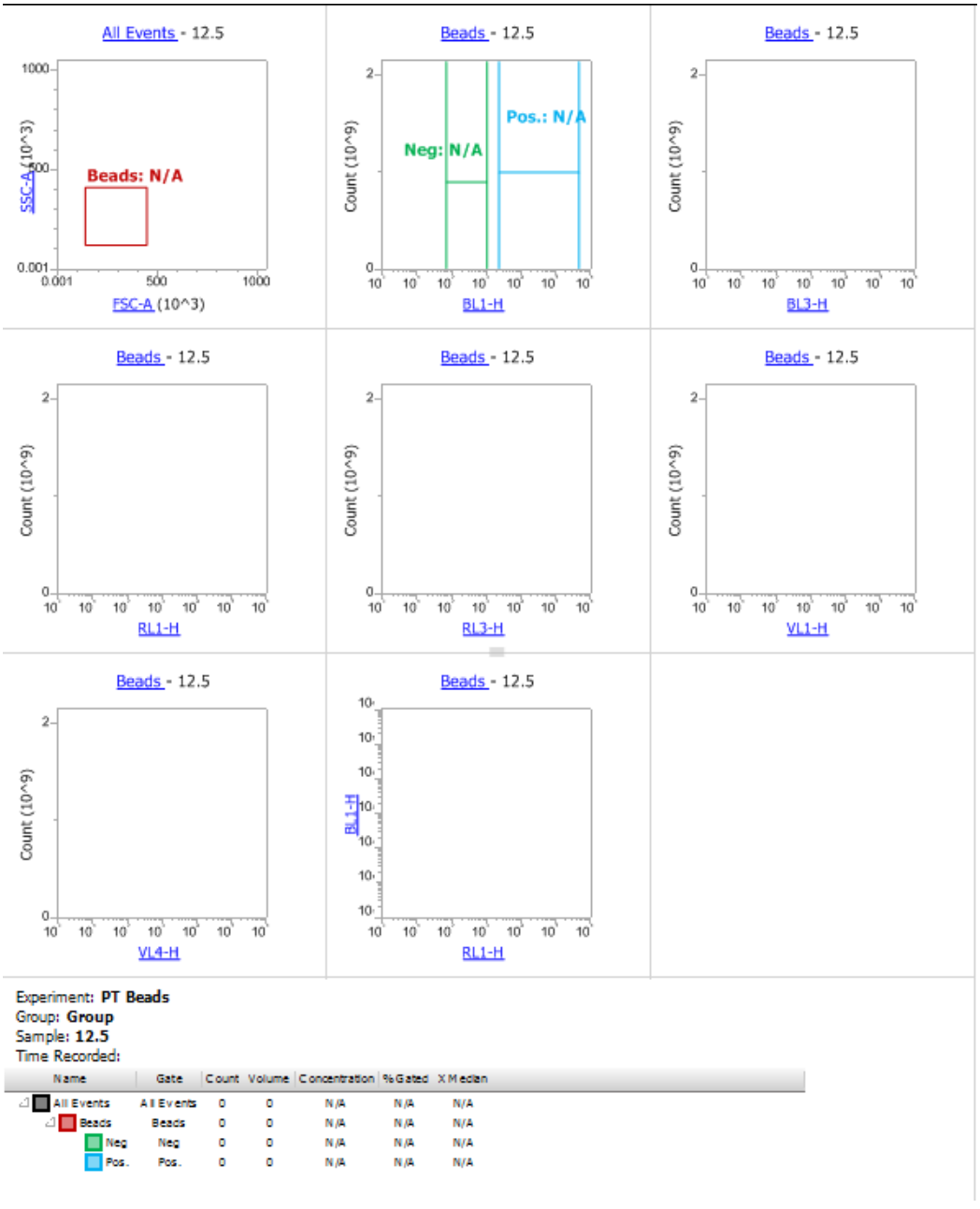


group “PT beads” and label the samples by each flow rate



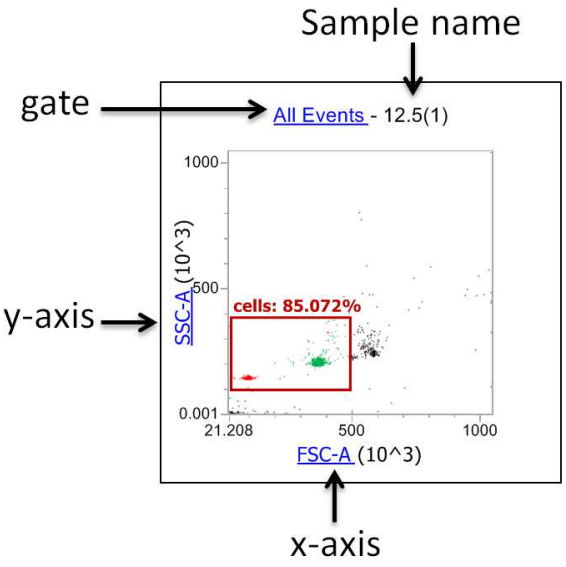
4. Under the “Instrument Settings” tab, open the “parameters” and check that all channels are turned on.

5. In the Workspace area in the middle, use the tools in the “Workspace” ribbon to generate a workspace like the one below.

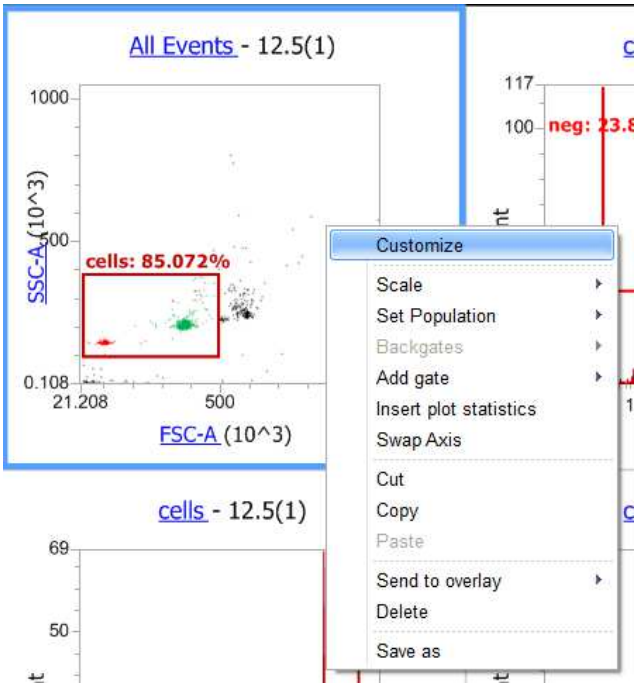


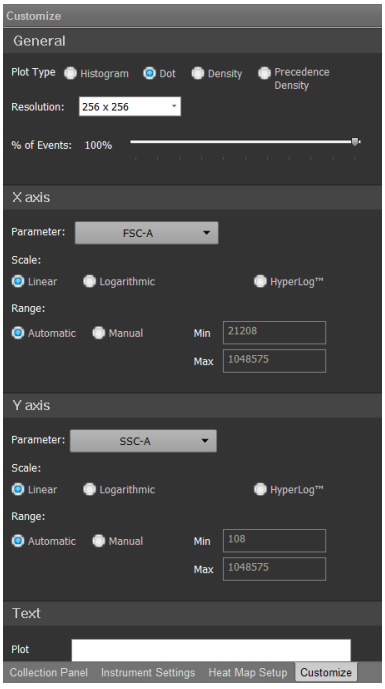
TIP: You can either use density plots to reflect the concentration of events using heat map colours or dot plots to reflect the gates. Precedence density plots show both the density as well as backgating. Use the density plot for the FSC/SSC plot

TIP: Click on axis names (eg. FSC-A) to change parameters.
Click on gate names (eg. “All Events”) to change the gates



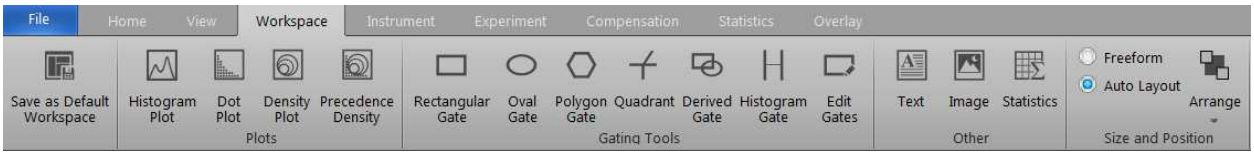
TIP: Select a plot or gate to customize their axis, names or colours in the customize tab.

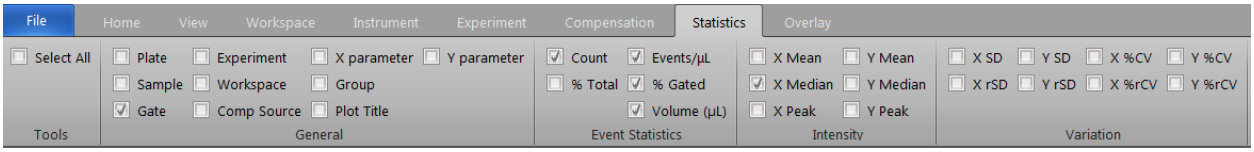




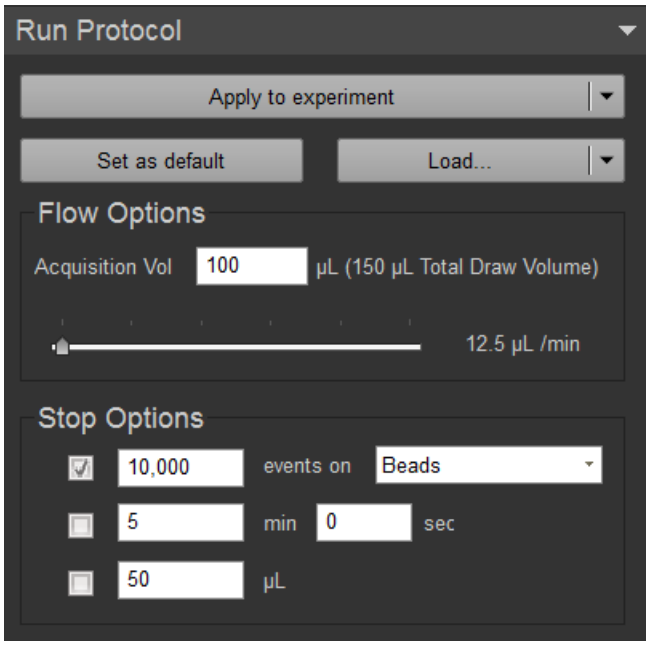
TIP: Copy and paste to duplicate plots

TIP: Select on “Statistics” in the Workspace ribbon to make a statistics box. Choose which statistics you need by first clicking on the statistics box and choosing the statistical categories on the “Statistics” ribbon





6. Select the “Collection Panel” tab to change the acquisition settings. Change it as follows. When you have set everything, remember to select “Apply to experiment”



You are now going to set the voltages for FSC and SSC as well as for each of the channels.

7. Place the tube on the tube lifter. Raise the tube lifter. Press Run.

8. Open the instrument settings tab. Open the Voltage drop down menu.

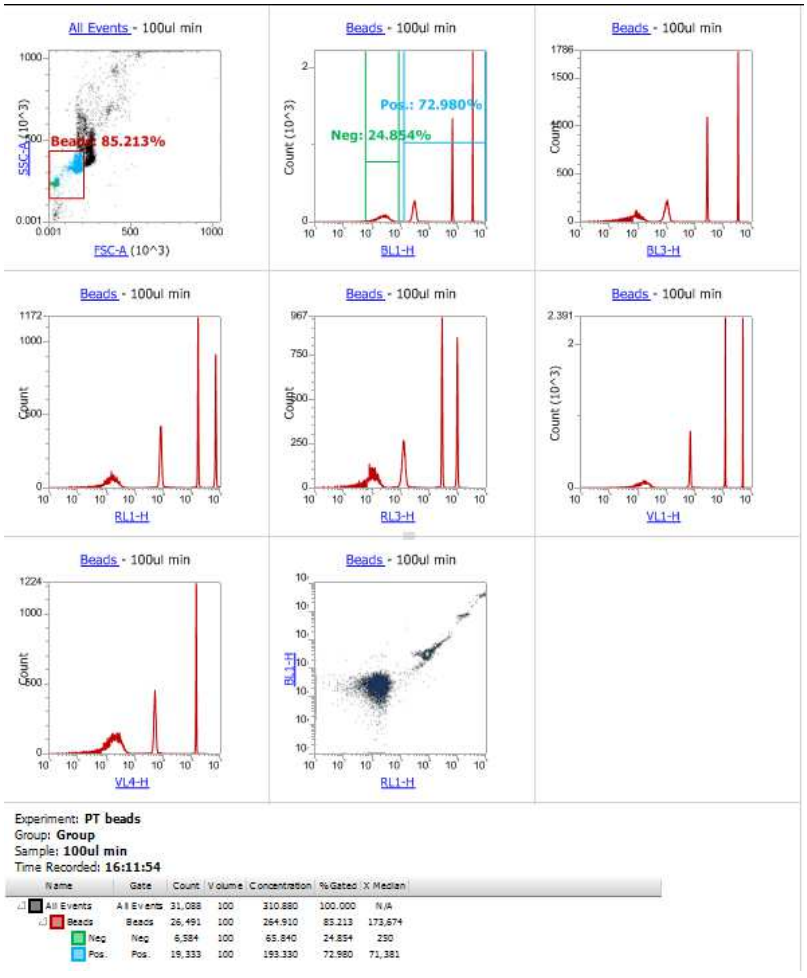
9. Adjust FSC and SSC voltage so the bead population is on scale. IN GENERAL: increasing the voltage will move the population or peak to the right (or top) of the axis.

10. Move the gate to cover both the small and large bead populations. You will also likely see multiple populations corresponding to doublets and other multimer populations.

11. For each of the fluorescence histograms, increase or decrease the voltage of each channel so that the **least bright peaks are around 10^2-10^3 region**. This is known as Voltage Walking. There should be 4 peaks in total.

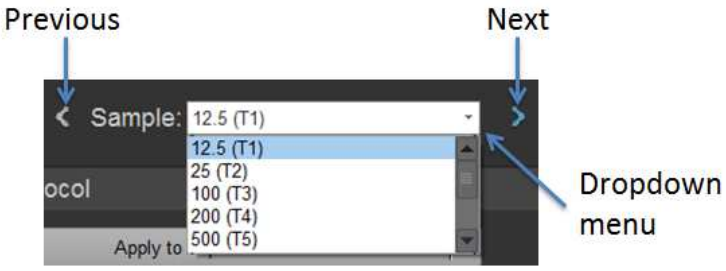
12. Gate the dimmest peak as the "Neg" population and the 3 other peaks as the "Pos" population.

Example data



13. When all the voltages have been adjusted, press STOP and drop the tube lifter to rinse.

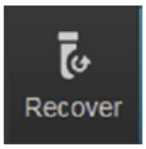
14. Start acquiring each of the samples by varying the speed on the RUN PROTOCOL. Once a file is successfully recorded, there will be a tick to the left of its name. Scroll to next sample by double clicking on its name or using the following options in the collection panel.



NOTE: Ensure the tube lifter is lowered between samples to initiate a rinse cycle. The rinse cycle is important to decrease carryover and will improve the quality of results. It is "OK" to run a sample a couple of times without lowering the tube lifter BUT persistently doing so will result in poor data quality.

15. Simulate re-recording a sample by: right-clicking the sample name, select remove fcs file and repeat the sample acquisition

16. Practice Sample Recovery by clicking on the **Recover Sample** button that has appeared in the place of the STOP button. Follow instructions on screen.



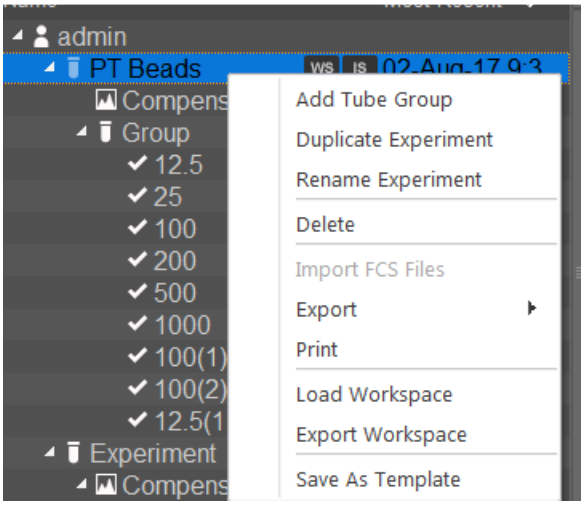
> **Recover Sample:** Allows you to recover unused sample. The button is enabled when the instrument status is idle (i.e., not actively acquiring) and there is enough sample within the sample loop or enough preloaded sample to recover. When the button is pressed, the remaining sample is returned into a tube (from the sample loop) or back into the sample well (preloaded sample in a plate).

17. After all samples have been acquired, perform SHUTDOWN – Quick option

BONUS ACTIVITY: FIND OUT WHETHER THE NEGATIVE BEADS ARE THE SMALL OR LARGE BEAD POPULATION USING BACK-GATING ON THE FSC/SSC PLOT.

Data processing options

18. **EXPORT** the experiment (.atx files) by right clicking on the experiment name and selecting export "Experiment". Save it to the desktop.



19. **EXPORT FCS files** by right clicking on the experiment name and selecting export "FCS files". Save it to a suitable folder.

20. **DELETE** the experiment by right clicking on the experiment name and selecting delete.

21. **IMPORT** the experiment by right clicking the username and selecting import experiment.

22. OPTIONAL: Save this experiment as a template

23. **DUPLICATE** entire experiment to copy every element of it to a new experiment.

24. Create a new experiment and copy the workspace of the "PT beads" experiment by dragging the WS badge. Copy the instrument settings by dragging the IS badge.

25. OPTIONAL: Save instrument settings, workspaces and run protocols