FLOW CYTOMETER SETUP: ATTUNE NxT

List of Flow Cytometers & Possible Configurations

Flow Cytometer	Reporter Channel	Channel Emission	Classification Channel	Channel Emission	Compensation Needed
Attune NxT	YL1	585/16	RL1	670/14	No*
iQue Plus Screener	YL1	586/20	YL3	660/20	Yes
*Compensation is not required for the specific flow cytometer when setup properly, but is recommended for consistent results. Using the compensation setup on the instrument (for the Attune NxT) is not recommended as there is no APC compensation particle.					

1. Start up the instrument

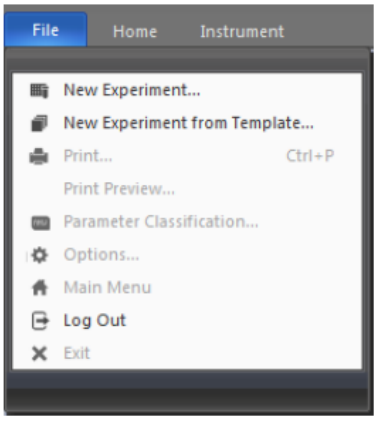
Perform instrument startup and verification checks following the manufacturer's recommendations.

2. Create an Experiment:

To run your samples and collect cytometry data, you need to create an Experiment in the Workspace. This section explains how to create an Experiment using the New Experiment dialog.

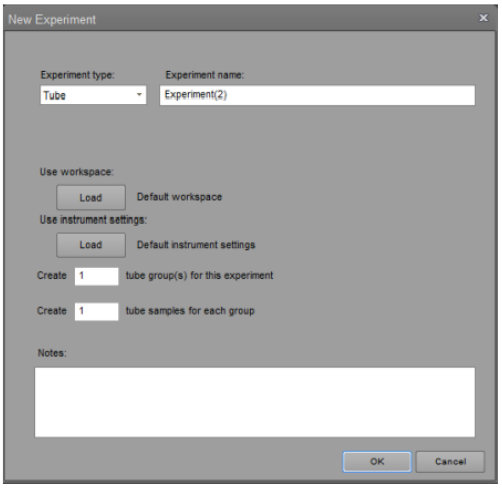
1. Open the New Experiment dialog:
   There are several different ways to access the New Experiment dialog. To open the dialog, you can perform one of the following:

   • click the **New Experiment** icon on Main Menu or the Home tab
   • select **File → New Experiment** on the Ribbon bar
   • right-click on the **User Folder heading** in Experiment Explorer, and then select **New Experiment** from the context menu.

   The New Experiment dialog allows you to create the following experiment types:
   • Tube-only Experiment
   • Plate Experiment
   • Analysis Experiment using imported FCS files

The contents of the New Experiment dialog changes, depending on the experiment type you select. For more information on the New Experiment dialog, refer to the Attune™ NxT Software User Guide.

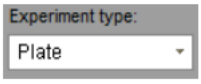


Note: The New Experiment option is enabled only when the instrument is not acquiring.

Create a **Plate Experiment**:

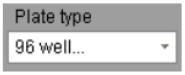
A Plate Experiment includes one plate, and can also include tube groups and tube samples.

1. From the Experiment type dropdown menu on the New Experiment dialog, select **Plate**.



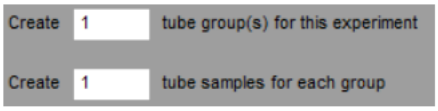
2. Accept the default plate name, or enter a new name.

3. From the Plate type dropdown menu, select the type of plate that you are using for the experiment and enter the plate ID.



4. Accept the default workspace and default instrument settings.

5. If you are including tubes, enter the number of tube groups to create for the experiment.



6. If you are including tubes, enter the number of tube samples to create for each tube group.

7. Click **OK** to create the new experiment and close the **New Experiment** dialog.

Note: Plate Experiment function is not available in early access instruments; it will be available with the next software update.

3. Setup the PMT voltages

NOTE: These instructions have been optimized for the plate reader versus single tubes.
NOTE: Previously, the optimal voltages for each detection channel were determined using a voltage walk using capture beads stained with antibodies detected in each channel. These voltages may differ slightly between instruments.

- Vortex the vial of 'Raw Beads' for 30 second to resuspend the beads.
  - To ensure minimal aggregates were present, we prepared beads by water bath sonication for 5 minute, followed by a 1 minute vortex.
  - As you can visualize in Figure 1 & 2, we were able to decrease the debris and aggregates to their lowest levels (~5%) using this increased water bath sonication time, denoting its importance.
- Transfer 300 uL of the 'Raw Beads' to an empty plate well.
- Set the flow cytometer flow rate to '25 ul/min'. The sample volume can be set to 200 uL (240uL draw) to take up most of the sample well.

- If you are confirming instrument setup, as in later runs, you can also use '100 uL/min' without a change. This simply allows for more time change the instrument settings if needed before running through your sample.

- Run the 'Raw Beads'. Adjust the FSC and SSC voltage settings so that both beads populations are visible (**Figure 1 & 2**).
  - The best separation of the bead populations, even at higher flow rates, was found to be at least roughly 200K apart without affecting the spread of the bead population(s).
  - The beads presented were run at a flow rate of 100 uL/min.
  - In **Figure 1**, the median FSC-A is 385k and 640k for Beads A and B, respectively.
  - In **Figure 2**, the median FSC-H is 370k and 573k for Beads A and B, respectively.

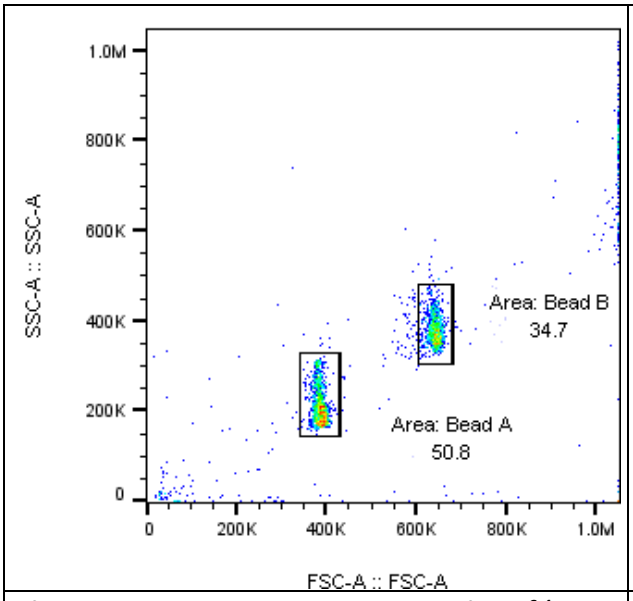


Figure 1. FSC-A vs. SSC-A representation of 'Raw Beads'. The instrument in this figure were set to a FSC of 300v and SSC of 280v.

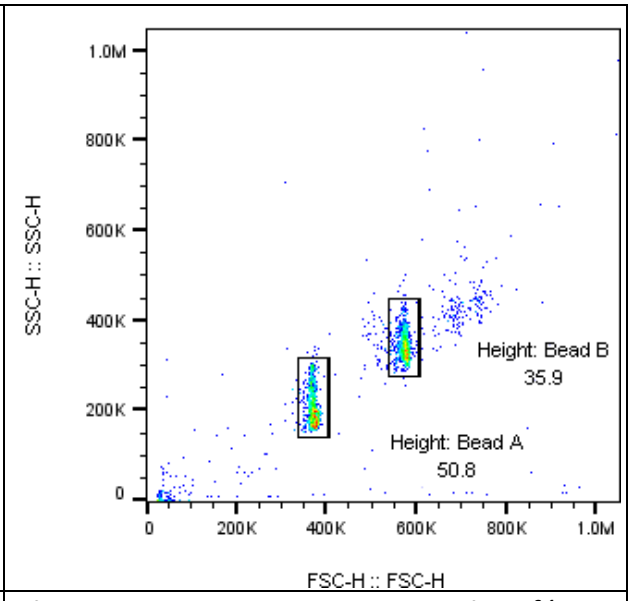
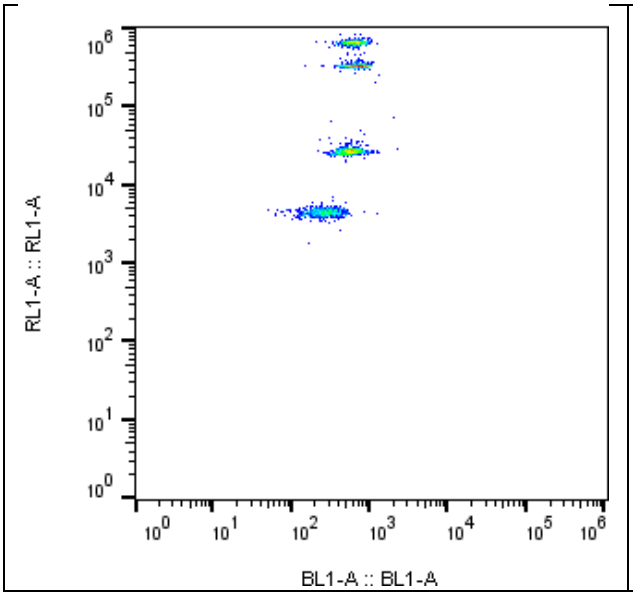


Figure 2. FSC-H vs. SSC-H representation of 'Raw Beads'. The instrument in this figure were set to a FSC of 300v and SSC of 280v.

- Move the gates for beads A and Beads B so that the smaller beads fall into Beads A gate and the larger beads fall into Beads B gate.
- Adjust the FITC setting so that the FITC signal for the majority of beads in between 1 x 10^2 and 1 x 10^3 (**Figure 3 & 4**).



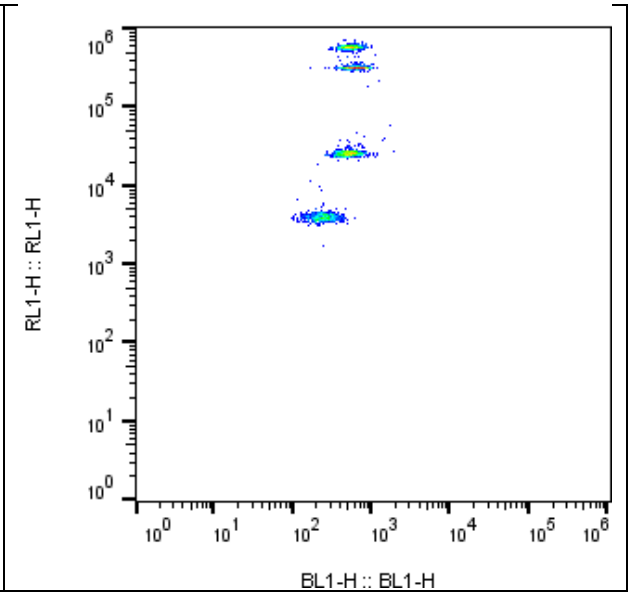
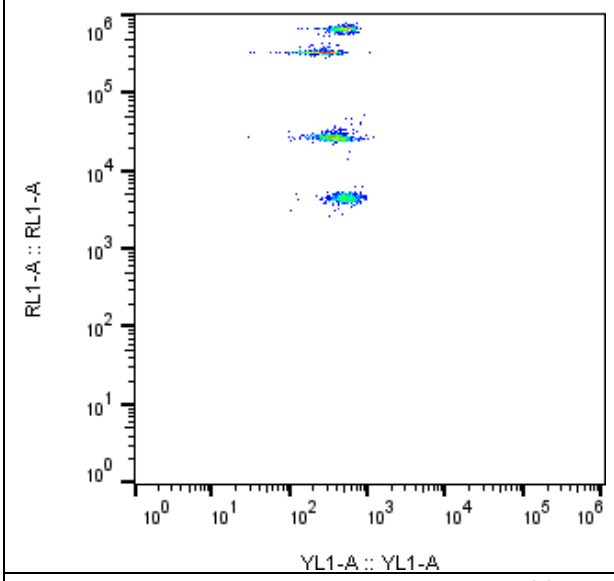


Figure 3. BL1-A vs. RL1-A representation of 'Raw Beads'. The instrument in this figure were set to a BL1 of 375v and RL1 of 325v. The gate incudes both Beads A and Beads B.	**Figure 4. BL1-H vs. RL1-H representation of 'Raw Beads'.** The instrument in this figure were set to a BL1 of 375v and RL1 of 325v. The gate incudes both Beads A and Beads B.

- Adjust the PE setting so that the PE signal for the majority of beads in between 1 x 10^2 and 1 x 10^3 (**Figure 5 & 6**).



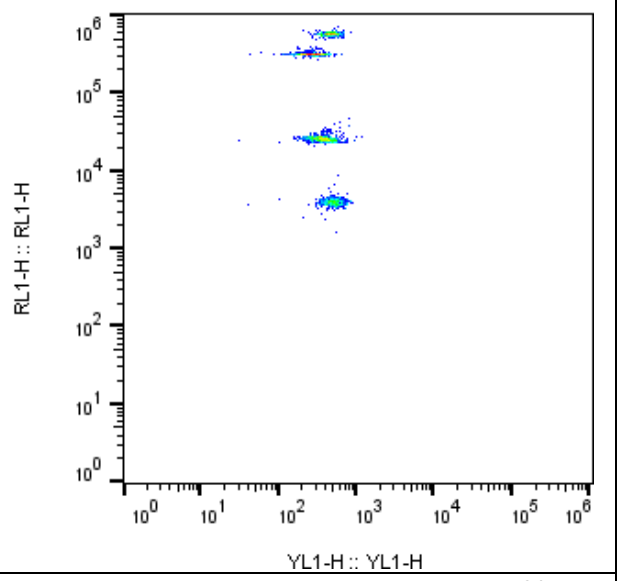
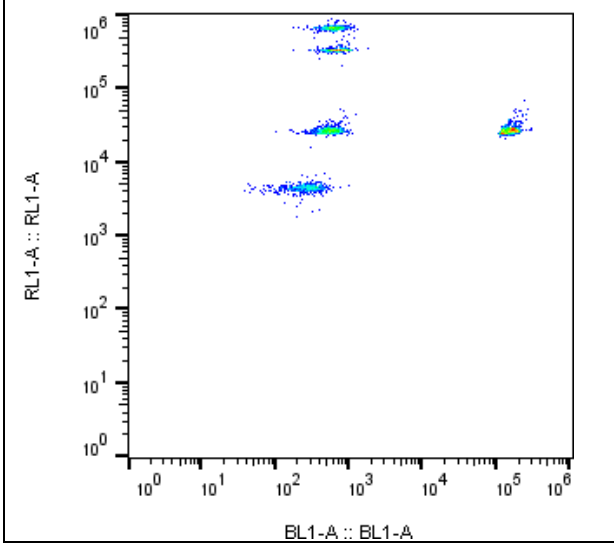


Figure 5. YL1-A vs. RL1-A representation of 'Raw Beads'. The instrument in this figure were set to a YL1 of 350v and RL1 of 325v. The gate incudes both Beads A and Beads B.	**Figure 6. YL1-H vs. RL1-H representation of 'Raw Beads'.** The instrument in this figure were set to a YL1 of 350v and RL1 of 325v. The gate incudes both Beads A and Beads B.

- Adjust the APC setting so that the APC signal for the majority of beads in between 4 x 10^3 and 7 x 10^5 (**Figure 5 & 6**).
- This setup can be saved as a template for future runs.
- While the FITC and PE Beads are not required for uncompensated runs, additional images are included below to show the bead populations for FITC + Raw Beads (**Figure 7 & 8**) and PE + Raw Beads (**Figure 9 & 10**). Recall that only Beads A are utilized in the FITC and PE samples, thus, only a single population of the four populations will show increase fluorescence.



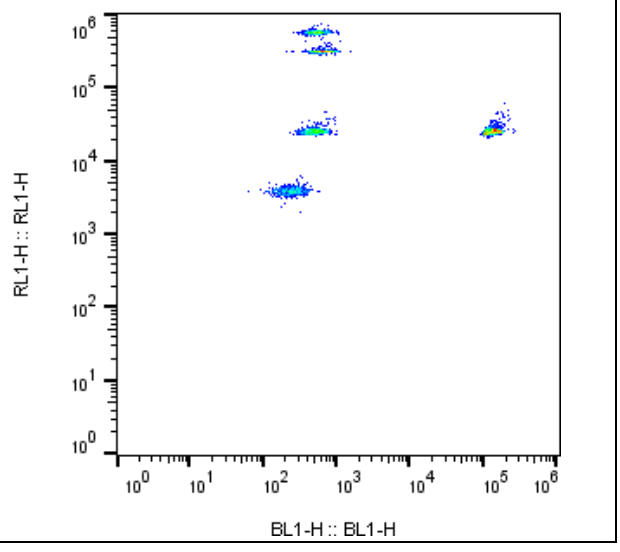


Figure 7. BL1-A vs. RL1-A representation of 'Raw Beads' & 'FITC Beads' mixed 1:1. The instrument in this figure were set to a BL1 of 375v and RL1 of 325v. The gate incudes both Beads A and Beads B.

CV% of (+) population = 16.4%
rCV% of (+) population = 18.3%

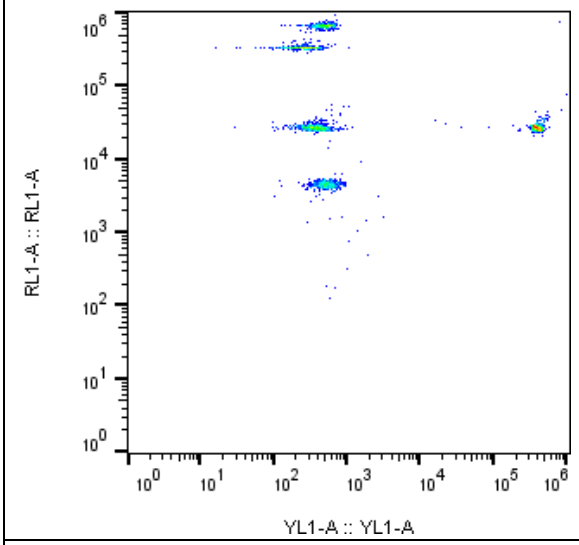


Figure 8. BL1-H vs. RL1-H representation of 'Raw Beads' & 'FITC Beads' mixed 1:1. The instrument in this figure were set to a BL1 of 375v and RL1 of 325v. The gate incudes both Beads A and Beads B.

CV% of (+) population = 16.1%
rCV% of (+) population = 17.9%

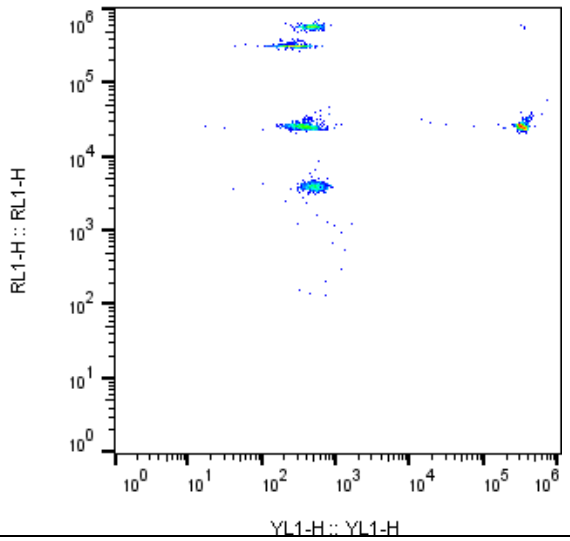


Figure 9. YL1-A vs. RL1-A representation of 'Raw Beads' & 'PE Beads' mixed 1:1. The instrument in this figure were set to a YL1 of 350v and RL1 of 325v. The gate incudes both Beads A and Beads B.

CV% of (+) population = 9.7%
rCV% of (+) population = 9.9%

Figure 10. YL1-H vs. RL1-H representation of 'Raw Beads' & 'PE Beads' mixed 1:1. The instrument in this figure were set to a YL1 of 350v and RL1 of 325v. The gate incudes both Beads A and Beads B.

CV% of (+) population = 9.2%
rCV% of (+) population = 9.5%

4. Notes on sample analysis

- If you are running the sample in the provided kit filter plate with the sample resuspended in 150uL. If you want to run the sample twice, or reserve volume in case it needs to be re-run, set the sample run volume to 40uL. Due to the dead volume of the needle, this will draw 70uL of sample total.