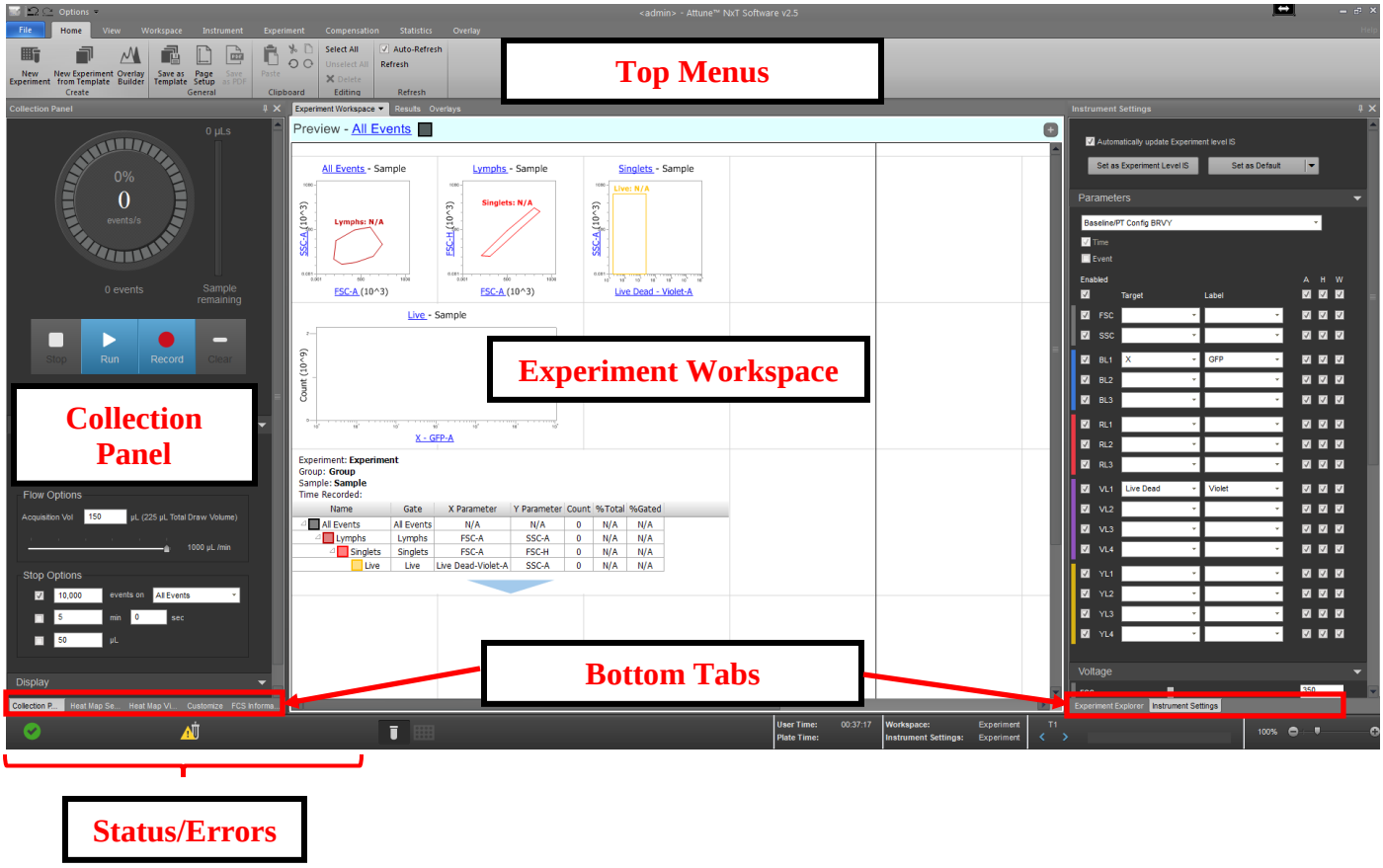
Table of Contents

1. Startup

1. Check **Sheath, Waste, and Cleaning** containers:
   - Empty Waste container in the sink – Add bleach to the first mark (~10% final concentration).
   - Fill Sheath container with Attune 1X Focusing Fluid.
   - Fill Wash container with Attune Wash Solution.
   - Fill Shutdown container with Attune 1X Shutdown Solution.
2. Turn on **PC**, **Autosampler**, and then **Attune NxT** (power switch in the back – follow orange label).

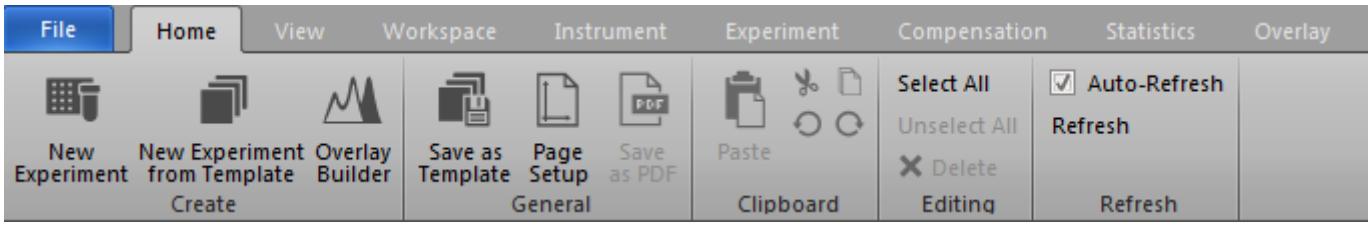
3. Log in to Windows (Onyen + password).
4. Launch **Attune NxT Software** (Username + password).
5. Check that the Performance Test has been performed for the day.

2. Attune NxT Software

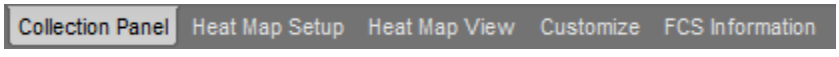


Menus Overview

Top Menus



Bottom Tabs



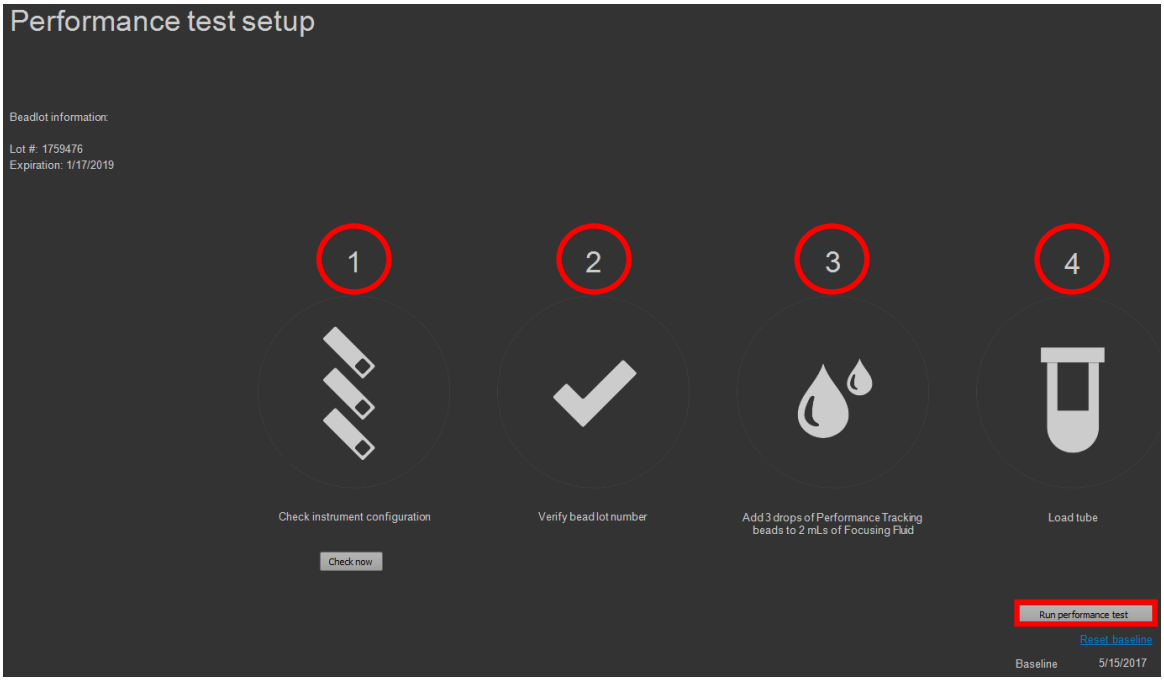
3. Quality Control – Performance Test

After the baseline values are defined, the Attune Performance Tracking Beads are used to run daily performance measurements to track the daily performance of the cytometer. The intensity level 4 bead is placed in the target channel and the PMT voltage is recorded, and then compared to the previous PMT voltage to calculate the delta. The %HPCV (half-peak coefficient of variation) of the bead is recorded. Using assigned MESF values for each fluorescent bead, the relative quantum efficiency (rQ) and relative background (rB) is calculated for each channel, and the linear regression is calculated and recorded. The laser delay setting is also automatically calculated. Levey-Jennings charts provide a visual to track the %HPCV and PMT voltage to check for shifts and trends.

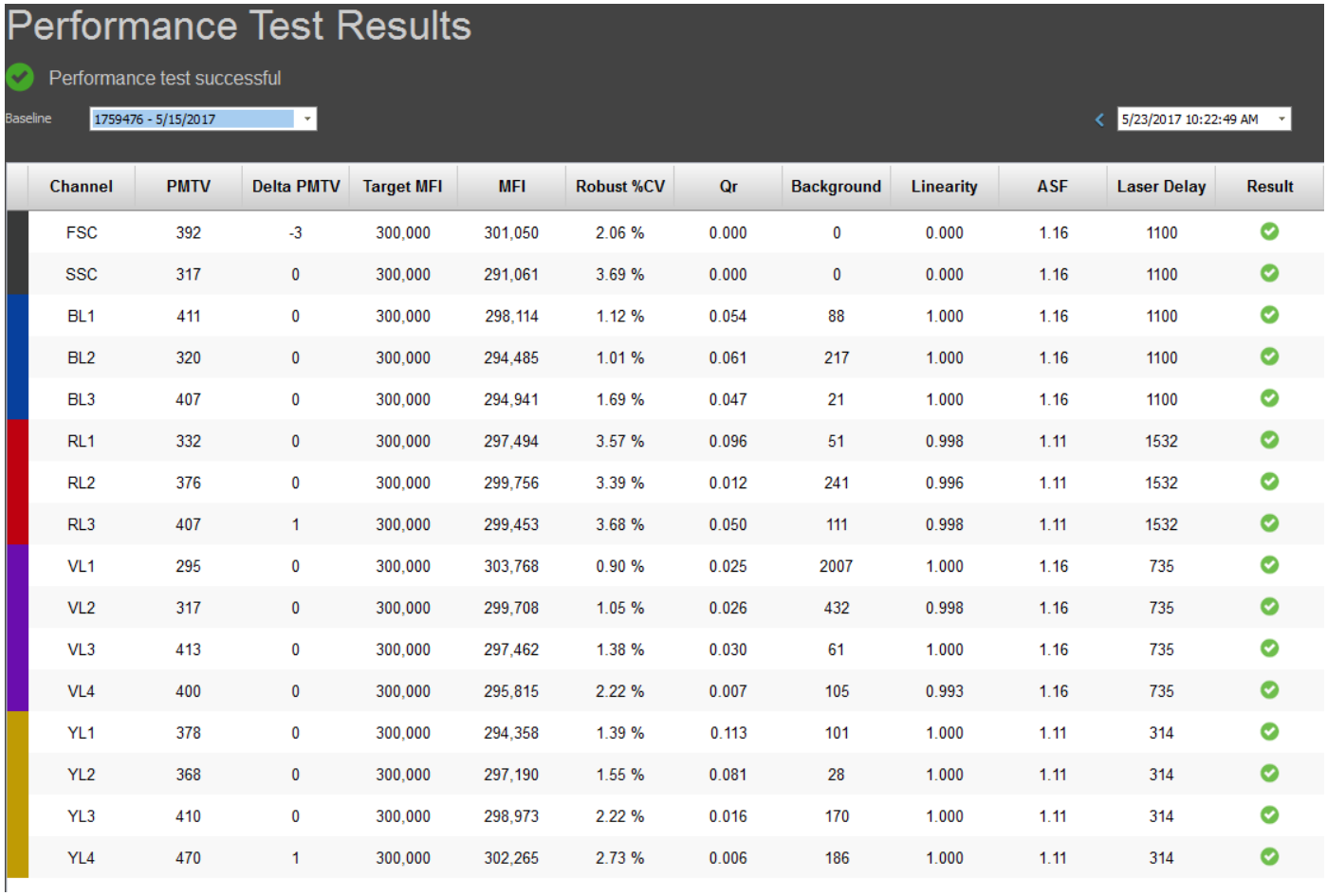
Launching the Performance Test

On the Main page, click on the **Performance Test** button.

Follow the four steps and click the **Run Performance test** button.



Upon completion of the Performance Test, the results are presented in a table.



Performance Test Results

Performance test successful

Baseline 1759476 - 5/15/2017 5/23/2017 10:22:49 AM

Channel	PMTV	Delta PMTV	Target MFI	MFI	Robust %CV	Qr	Background	Linearity	ASF	Laser Delay	Result
FSC	392	-3	300,000	301,050	2.06 %	0.000	0	0.000	1.16	1100	
SSC	317	0	300,000	291,061	3.69 %	0.000	0	0.000	1.16	1100	
BL1	411	0	300,000	298,114	1.12 %	0.054	88	1.000	1.16	1100	
BL2	320	0	300,000	294,485	1.01 %	0.061	217	1.000	1.16	1100	
BL3	407	0	300,000	294,941	1.69 %	0.047	21	1.000	1.16	1100	
RL1	332	0	300,000	297,494	3.57 %	0.096	51	0.998	1.11	1532	
RL2	376	0	300,000	299,756	3.39 %	0.012	241	0.996	1.11	1532	
RL3	407	1	300,000	299,453	3.68 %	0.050	111	0.998	1.11	1532	
VL1	295	0	300,000	303,768	0.90 %	0.025	2007	1.000	1.16	735	
VL2	317	0	300,000	299,708	1.05 %	0.026	432	0.998	1.16	735	
VL3	413	0	300,000	297,462	1.38 %	0.030	61	1.000	1.16	735	
VL4	400	0	300,000	295,815	2.22 %	0.007	105	0.993	1.16	735	
YL1	378	0	300,000	294,358	1.39 %	0.113	101	1.000	1.11	314	
YL2	368	0	300,000	297,190	1.55 %	0.081	28	1.000	1.11	314	
YL3	410	0	300,000	298,973	2.22 %	0.016	170	1.000	1.11	314	
YL4	470	1	300,000	302,265	2.73 %	0.006	186	1.000	1.11	314	

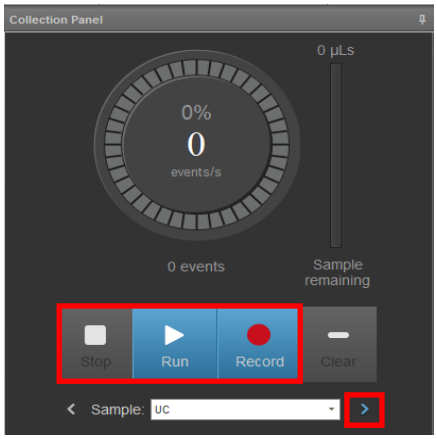
4. Experiment Setup (TUBE)

- Click **New Experiment** or **Templates** (New Experiment from Template) – Experiment type = tube.

- Name the experiment (optional: select the number of groups and the number of tubes). Click OK.
- Click the **Instrument Setting** tab>**Parameters**.
  - ✓ Select the parameters used in the experiment.
  - ✓ Name the Target (i.e. CD3) and the Label (i.e. BV421).
  - ✓ Select the Height and Width parameters for aggregate discrimination.
- Click the **Experiment Explorer** tab and R-click **Compensation**>**Compensation Setup**.
  - ✓ Check the Use Unstained Control (UC) box and click OK.
- On the **Collection Panel** tab, adjust the **Flow Options** (50µL @ 12.5µL/min) and click **Apply to Experiment**.
- Select (double-click) the UC (highlighted in orange).
- Mix and load a fully stained tube and click **Run** (Collection Panel tab).
- Click the **Instrument Setting** tab>Voltage.
  - ✓ Adjust the FSC and SSC voltages.
  - ✓ Adjust the fluorescent parameter voltages to insure all signals are on scale (optimal voltage ranges should have already been defined with a voltage walk).
- Click **Stop** then **Recover** (leave the tube in place).
- Click **Next** to return any unused sample to the fully stained tube.
- Lower the tube lifter (catch the drop on the SIP using the lip of the tube) and click **Next** to initiate the **Rinse** cycle.
- Load and **Run** each single stained control to insure each positive signal is brighter in its intended detector.

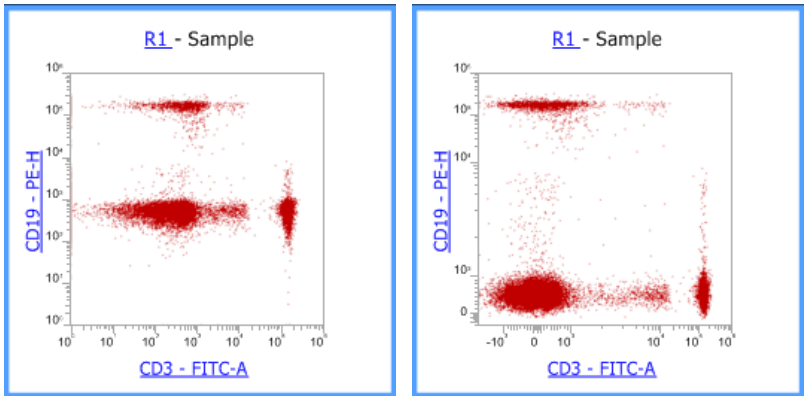
Note: if you are not using an unstained control for compensation, return to the Compensation Setup and check the Use Negative Gate box.

- Modify the **Flow Options** (100µL @ 100µL/min), and **Stop Options** (5,000 events), and click **Apply to Experiment**.
- Load and **Record** the UC and each single stained control, using the right arrow to navigate to the next tube.



- Adjust the gates for each control.
- When all compensation controls have been recorded, the software automatically calculates the compensation matrix. To view the matrix, go to the **Compensation** menu>**View Matrix**.
- Select the experimental sample.
- Use the **Workspace** menu to create plots, gates, and statistics (can be customized in the **Statistics** menu).
- Modify the **Flow Options** and **Stop Options** and click **Apply to Experiment**.
- Mix, load, and **Record** the sample.

Note: if a bottom tab is missing, go to the View menu to select it.
Note: if desired, modify the scaling from log to HyperLog (Biexponential) using the Customize tab.



Exporting Data

- R-click Experiment>Export>FCS Files [3.0 or 3.1].
- Select Location (path).
- When prompted, update FCS keywords for all files.
- R-click Experiment>Save As Template (rename if desired), click OK.
- **R-click Experiment>Delete.**

5. Experiment Setup (PLATE)

- Click **New Experiment** or **Templates** (New Experiment from Template) – Experiment type = plate.

- Name the experiment and select Plate type (optional: select the number of groups and the number of tubes). Click OK.
- Open the Experiment by double-clicking on its name.
- Click the **Instrument Setting** tab>**Parameters**.
  - ✓ Select the parameters used in the experiment.
  - ✓ Name the Target (i.e. CD3) and the Label (i.e. BV421).
  - ✓ Select the Height and Width parameters for aggregate discrimination.
- Click the **Experiment Explorer** tab and R-click **Compensation**>**Compensation Setup**.
  - ✓ Check the Use Unstained Control (UC) box and click OK.
- On the **Collection Panel** tab, adjust the **Flow Options** (50µL @ 12.5µL/min) and click **Apply to Experiment**.
- Select (double-click) the UC (highlighted in orange).
- Mix and load a fully stained tube and click **Run** (Collection Panel tab).
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  - ✓ Adjust the FSC and SSC voltages.
  - ✓ Adjust the fluorescent parameter voltages to insure all signals are on scale (optimal voltage ranges should have already been defined with a voltage walk).
- Click **Stop** then **Recover** (leave the tube in place).
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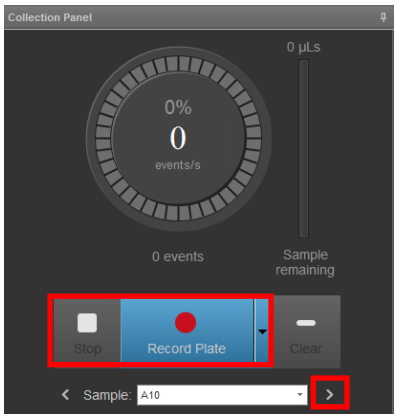
- Load and **Run** each single stained control to insure each positive signal is brighter in its intended detector.

Note: if you are not using an unstained control for compensation, return to the Compensation Setup and check the Use Negative Gate box.

- Modify the **Flow Options** (100µL @ 100µL/min), and **Stop Options** (5,000 events), and click **Apply to Experiment**.
- Load and **Record** the UC and each single stained control, using the right arrow to navigate to the next tube.

- Adjust the gates for each control.
- When all compensation controls have been recorded, the software automatically calculates the compensation matrix. To view the matrix, go to the **Compensation** menu>**View Matrix**.
- Open the **Heat Map View** tab and R-click well(s) to be acquired>New Sample>Add to New Group.
- Select the experimental sample (Experiment Explorer tab or Heat Map View tab).
- Use the **Workspace** menu to create plots, gates, and statistics (can be customized in the **Statistics** menu).
- Modify the **Flow Options** and **Stop Options** and click **Apply to Experiment** (or copy/paste Run Protocol to select wells).
- Load plate and click **Record Plate**.



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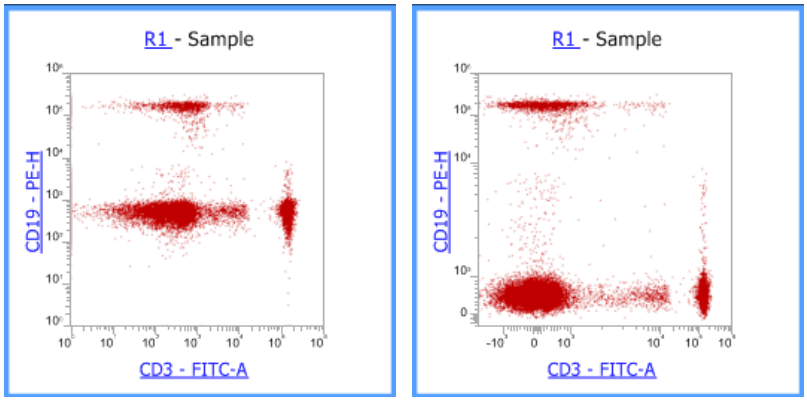


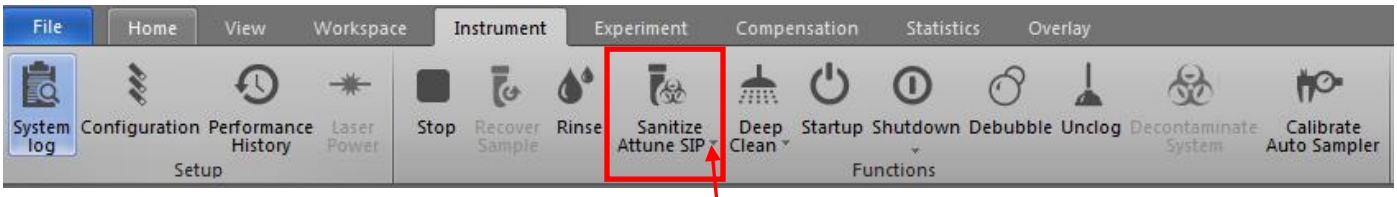
Exporting Data

- R-click Experiment>Export>FCS Files [3.0 or 3.1].
- Select Location (path).
- When prompted, update FCS keywords for all files.
- R-click Experiment>Save As Template (rename if desired), click OK.
- **R-click Experiment>Delete.**

6. Cleaning / Shutdown

Cleaning (~2:30min.)

- Empty the Flow Cell Clean tube and fill with 2 ml of Cleaning solution.
- On the Instrument menu, click **Sanitize Attune SIP** and follow the onscreen instructions (use FACSClean instead of Bleach).



- If the Autosampler was used, click on the small triangle and select **Auto sampler SIP**.

Shutdown

The last user power off the Attune & the Autosampler.

7. References

- Attune™ NxT Acoustic Focusing Cytometer 100024235
- Attune™ NxT Software User Guide 100024236