
ATTUNE NXT BASIC TRAINING

PRACTICAL MODULE 1: STARTUP

In this module, we will go through the best practices for inspecting the Attune NxT Cytometer before it is used. Next, you will learn the procedure for starting up the instrument and execute a performance test as part of the daily maintenance of the Attune NxT Cytometer. Conducted daily, the performance test monitors the accuracy and sensitivity of the instrument and allows easy identification of potential shifts or trends in instrument performance.

PRE-USE INSTRUMENT CHECK

Learning Objectives:

1. Learn how to what the indicator lights should like after shutdown is completed
2. Learn best practices for inspecting an instrument before it is used daily

Lab Activity

Ensure that your instrument is in good working performance. Before the instrument is turned on check the following:

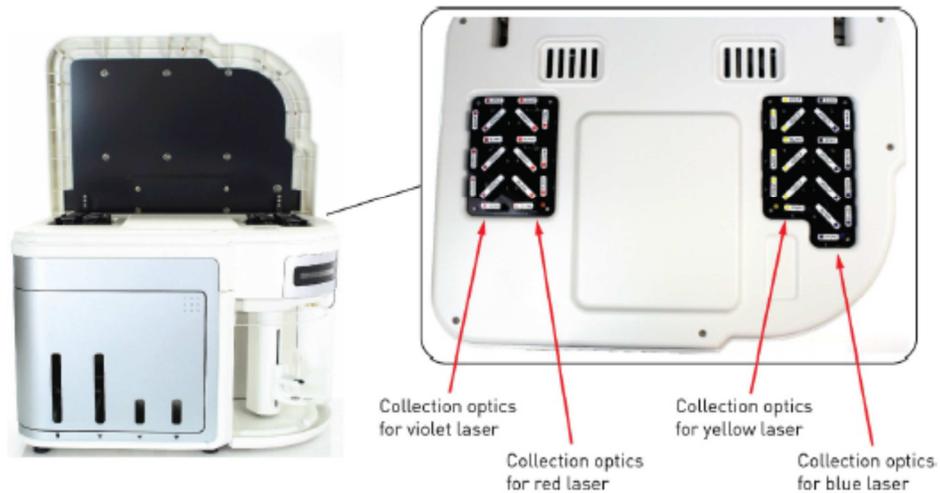
1. **Status Indicator Lights:** What color are they? At the end of a shutdown the status indicator lights should be colored multicolor and fade-in/fade-out.



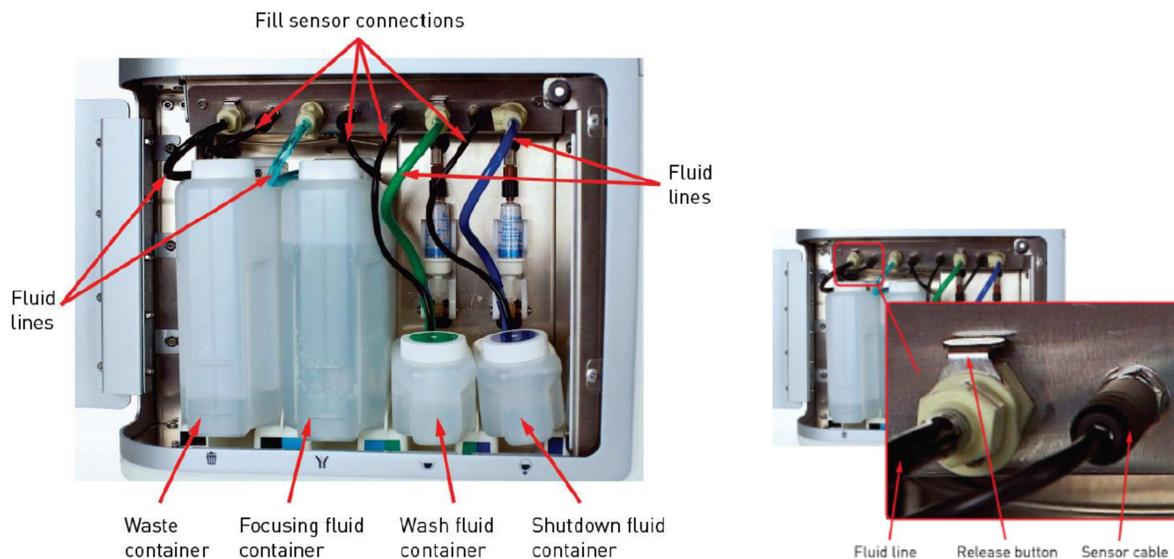
Status indicator lights

| Instrument cycle | Status indicator lights |
|---|-------------------------|
| Startup and all other instrument functions (except Rinse) | Flashing blue |
| Startup complete | Green solid |
| Idle | Green solid |
| Warm up | Blue fade |
| Warm up complete | Blue solid |
| Acquiring data/Run | Flashing green |
| Run complete | Green solid |
| Wash/Unclog/De-bubble | Green solid |
| Rinse | Green solid |
| Clog detected | Amber blink |
| Focusing fluid container empty | Amber blink |
| Waste container full | Amber blink |
| Wash container empty | Amber blink |
| Shutdown fluid container empty | Amber blink |
| Shutdown | Green solid |
| Shutdown complete | Blue fade |
| Error | Amber blink |

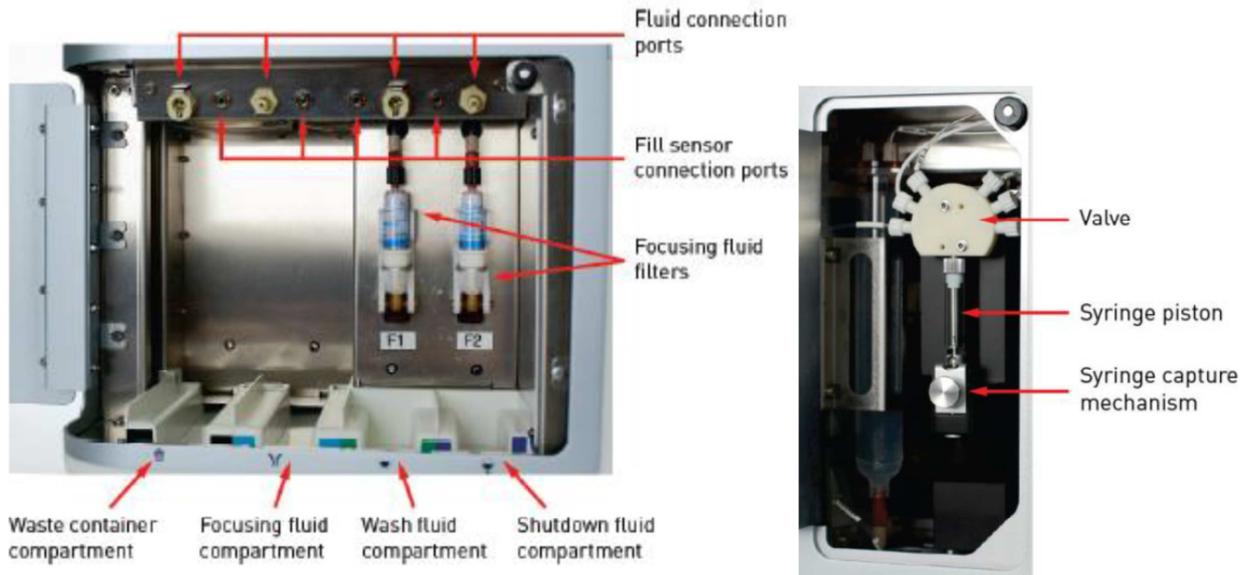
2. **Filters:** are the optical filters in the correct configuration? Open the lid of the cytometer and check. Compare it to the filter configuration in the software



3. **Fluids:** Are there enough fluids in the instrument? Is the waste full? Are the connections in the ports firmly plugged in? **Always remove the sensor cable first, followed by the fluid cable. Then plug in the fluid cable first before ending with the sensor cable**



4. **Leaks:** Are there any leaks? Open the fluidics door on the Attune NxT and Autosampler (if applicable) to check for leaks under the bottles and the focusing fluid filters. Are any of the bottles cracked? Open the syringe pump compartment and check for leaks under the sample syringe. Look for any salt buildup on the syringe.



STARTUP AND PERFORMANCE TEST

Learning Objectives:

1. Learn how to turn on and log into the Attune NxT system
2. Learn how to perform Performance test
3. Learn how to read results from performance test

Performance Test

The Attune® Performance Tracking Beads are designed for use with the Attune® NxT Software to automatically characterize, track, and report performance measurements of the Attune® NxT Acoustic Focusing Cytometer. The beads are used to define a baseline and conduct daily measurements of the cytometer. Each vial of PT Beads contains a mixture of equal concentrations of beads of four fluorescence emission intensities (intensity levels 1, 2, 3, and 4). Vials contain enough beads for 25 measurements, and specific information for each lot is downloaded to the Attune® NxT Software prior to use. The performance tracking process involves:

- Running performance-tracking beads
- Monitoring changes in the coefficient of variation and in the associated PMT voltage
- Tracking linearity of instrument performance
- Evaluating the detector sensitivity and background over time
- Automatic setting of laser delay

For this Lab you will need:

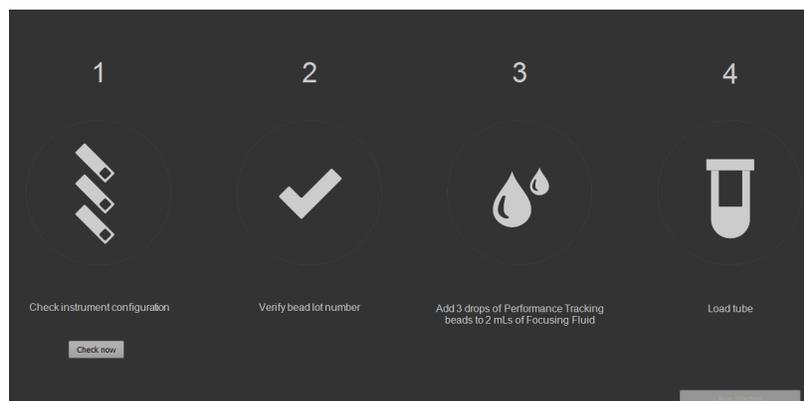
- Attune® Performance Tracking Beads
- 1x Focusing Fluid or 1x PBS
- 10% bleach (freshly diluted from 10x stock)
- Flow tubes (12x75mm tubes)

Lab Activity

1. Turn on the Autosampler. Wait until startup actions are complete (few seconds)
2. Turn on the Attune NxT cytometer
3. Turn on computer. Log into INSTR-ADMIN, password: INSTR-ADMIN
4. Double-click to launch Attune NxT software. Log into Username: admin, Password: admin
5. In the main menu, select the left most Performance test button



6. At the bottom right of the page, select Run Startup



7. While waiting, follow steps 1-3 (do not raise tube lifter yet). Remember to vortex or vigorously shake performance tracking beads to mix.
8. When Startup is done, load tube and click “Run Performance Test”. Performance test should take about 2 minutes if the beads were made at the correct dilution.
9. Once the test is completed the PT Results Table will be displayed
10. Drop the tube lifter and listen for the rinse cycle to initiate; ensure the rinse is completed.
11. Interpret PT Results
12. **If PT has passed, perform a SIP sanitize:** place 3mL 10% bleach in a flow tube. Place on tube lifter and execute instructions after pressing “SIP sanitize” from the Instrument ribbon. SIP sanitize takes less than 3min to complete. Ensure the tube lifter is lowered following SIP sanitize to rinse.

If the performance test fails, a dialogue box may be viewed that provides instructions on next best steps. In general, follow these steps to troubleshoot performance test:

- **Wrong filter configuration:** Check filter configuration inside instrument and in software
- **Bubbles in instrument:** Perform the debubble script (repeat if necessary) followed by 1-2 rinse.
- **Clog:** Is there liquid being drawn into the SIP? If not, execute an unclog script to backflush.
- **Problem with beads.** Prepare fresh well-mixed beads (ensure 1x focusing fluid is used and not water). Ensure that no less than 3 drops are applied.
- **If all else fails:** exit software, turn instrument(s) off, restart instruments in the correct sequence, re-launch software. Make sure that performance test is performed immediately following startup. Do not view any experiment file prior to this.
- If continues to fail: notify us for help