

# Attune NxT Flow Cytometer



A detailed user guide is available on the demo computer desktop. File name: Attune NxT Flow Cytometer User Guide.

## I. Start-up

**Note:** Standard operation—leave the Invitrogen™ Attune™ NxT Flow Cytometer and Autosampler turned on.

1. Turn on the computer if it's not already on.
2. Log in with the user name "INSTR-ADMIN." Password is "INSTR-ADMIN" (all caps).
3. Click the "Attune software" icon. User is "admin." Password is "password1" (all lowercase).
4. Click the "start-up" icon. This is found in the instrument ribbon tab. Follow the prompts.
  - a. Start-up takes about 3 minutes. There is a progress bar.
  - b. The instrument indicator will turn green. This turns on the lasers and primes the system.

**Note:** If a hard reboot is necessary, turn off the software first, then the Attune NxT cytometer, then the Autosampler. Turn on in reverse, allowing 30 seconds between each step (i.e., turn on the Autosampler, wait 30 seconds, turn on the Attune NxT cytometer, wait another 30 seconds, and then turn on the software). Failure to wait an appropriate amount of time will result in a connection error.

## II. Run performance test

1. Click "performance test" on the main menu to view the performance test setup screen.
2. Shake the Invitrogen™ Attune™ Performance Tracking Bead bottle vigorously to resuspend the beads.
3. Add 3 drops of the bead suspension to 2 mL of focusing fluid in a 12 × 75 mm tube. Mix.
4. Load the tube.
5. Click "run performance test." This test takes about 3 minutes.

## III. Create experiment

### 1. Duplicate experiment

- Right-click an “existing experiment” file—choose “duplicate experiment.”
- You will be asked if you’d like to import Compensation and Instrument Settings (IS).  
If you import both, you’ll only need to check the FSC/SSC (forward-scatter and side-scatter) settings and collect data.  
If you import only the Instrument Settings, you can create Compensation and change all instrument settings at will.
- The duplicate experiment file will be identical to the original except it will be free of data.
- Simply click a “tube” file and “run” or “record” data in the data collection tab.

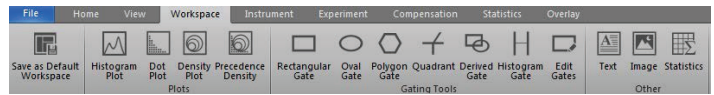
### 2. Template experiment

- Any existing experiment can be saved as a template.
- Templates are like “duplicate experiment” but they cannot be deleted.
- Templates do not include compensation files; settings can be changed if desired.

### 3. Creating an experiment from scratch

- On the main menu, choose “new experiment.”
- Select the experiment type: “tube” or “plate” (you can add a tube to a plate-based experiment).
- Name the new experiment and click “OK.”
- A new experiment is created in the experiment tab window.
- Go to the Instrument Settings Menu (left side of screen).
  - Open Parameters.
  - Select the channels you wish to use, and label them as you like.
  - Close Parameters window.

- Create your workspace.
- Choose the “workspace function” in ribbon bar or drag-and-drop a Workspace (WS) from an existing experiment.



## IV. Calculate compensation

- Copy previous settings using drag-and-drop compensation (CS), or you may create from scratch using the Create Compensation tab. You may do this prior to collecting data, or after data acquisition.

## V. Collect data

- Open the Collection Menu (right side of screen), press Run, and set your Instrument Settings.
- Open the Instrument Settings Tab and expand the Voltage section. Remember, you can always drag-and-drop Instrument Settings (IS) and change them afterward.

## VI. Shutdown

- Click “shutdown” in the instrument ribbon tab. Follow the instructions.
- Place a tube with 3 mL of 10% bleach on the tube holder and an empty plate in the Autosampler. Click “next.”
- Do not turn off the software, and do not interrupt the shutdown process, which will take 35 minutes. The instrument will shut itself down.

**Note:** If for any reason you must abort the shutdown procedure, you’ll need to hard-reboot the entire system and rerun the start-up procedure. Please refer to the start-up note for guidance.