

# Attune® Auto Sampler

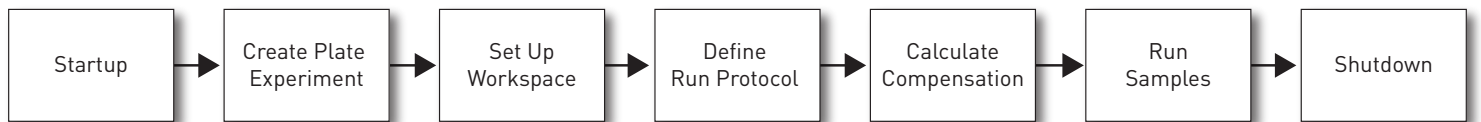
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The functions of the Attune® Auto Sampler are controlled by the Attune® Cytometric Software and the Attune® Acoustic Focusing Cytometer. Instructions for using the Attune® Auto Sampler are described below.

**Note:** For safety and biohazard guidelines, refer to the “Safety” sections in the *Attune® Acoustic Focusing Cytometer User Guide* and the *Attune® Auto Sampler User Guide*. For every chemical, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

## Workflow



For detailed instructions, refer to the *Attune® Acoustic Focusing Cytometer User Guide* and the *Attune® Auto Sampler User Guide* supplied with the product or download the user guides from [www.lifetechnologies.com](http://www.lifetechnologies.com).

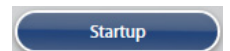
## Startup

### Before You Begin

1. Check the levels in the fluid containers of the Attune® Acoustic Focusing Cytometer and the Attune® Auto Sampler.
2. Power ON the Attune® Acoustic Focusing Cytometer, the Attune® Auto Sampler, and the computer.
3. Log in to Windows and launch the Attune® Cytometric Software.

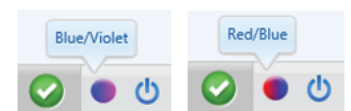
### Run Startup Function

1. Click **Startup** on the upper-right-corner of the *Login* screen.
2. Follow the instructions provided by the software.
3. Login to the Attune® Cytometric Software.



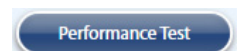
### Verify Optical Configuration

1. Check the optical layout of the cytometer to ensure that the filters are appropriate for the sample type and fluorophores you are using.
2. Place your cursor over the icon in the lower-left corner to verify that the optical configuration is correct. If you need to change the optical configuration, refer to the instructions provided in the *Attune® Acoustic Focusing Cytometer User Guide*.

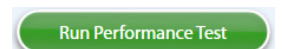


### Perform Performance Test

1. Click **Performance Test** on the *Main Menu*.
2. Verify that the lot number of the Attune® Performance Tracking Beads you are using is identical to the bead lot number used in the current baseline.  
**IMPORTANT!** If you are using a new lot of beads, download the appropriate lot information at [www.lifetechnologies.com](http://www.lifetechnologies.com) and perform new baseline calculations.
3. Prepare the Attune® Performance Tracking Bead suspension.
4. Click **Run Performance Test** and follow the instructions provided by the software.



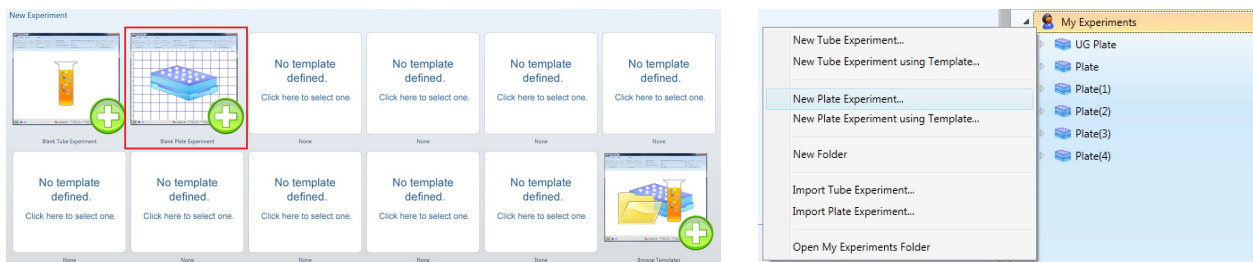
Current Baseline: 6/9/2010 - 756080  
 Lot No.: 756080  
 Expiration Date: 02/11/2013



# Create Plate Experiment

## Create a Plate Experiment

1. On the *Main Menu*, double **Blank Plate Experiment** or a pre-populated template (if available).  
Alternatively, use the *Experiment Explorer* to create a new experiment (right-click **My Experiments** and select **New Plate Experiment**) or duplicate a saved experiment (right-click on an existing experiment and select **Duplicate Plate**).

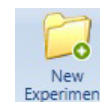


2. In the virtual plate layout, select the wells you want to include in your *Experiment* by dragging the cursor over them.

To select whole columns or rows, click on the number or letter of the column or row you would like to select.

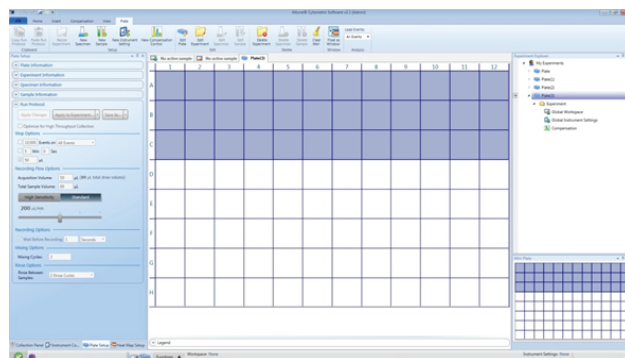
To multi-select wells, columns, or rows hold down the **CTRL** key while selecting.

To select the entire plate, click on the upper left hand corner of the virtual plate.



3. Click **New Experiment** on the *Plate tab* of the *Ribbon bar*.

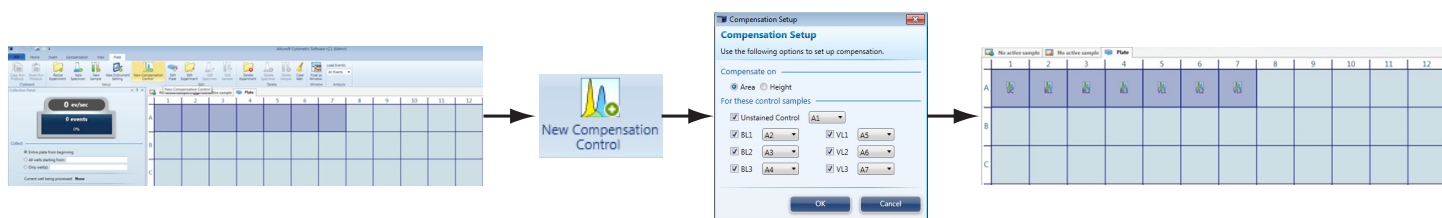
The new *Experiment* is displayed in the virtual plate layout on the Attune® Desktop and the Collection Panel, and the samples associated with the Experiment have the same background color.



## Define Compensation Control Wells

1. Select the desired wells, and then click **New Compensation Control** on the *Plate tab* of the *Ribbon bar*.
2. Choose between height and area for the compensation calculation, map the compensation controls to the desired wells using the appropriate check boxes and drop-down menus, and then click **OK**.

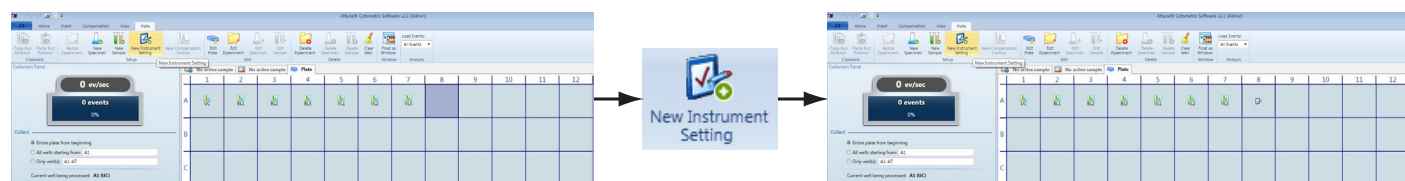
**Note:** You must select a minimum of 2 fluorescent channels or the OK button will be disabled.



## Define Instrument Setting Well

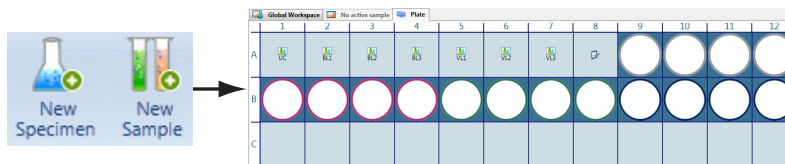
1. Select the desired well, and then click **New Instrument Setting** on the *Plate tab* of the *Ribbon bar*.

**Note:** Fluorescence PMT voltages are set during compensation and they cannot be adjusted once compensation has been applied. The *Instrument Settings Well* allows you to update threshold, scatter voltages, and depending on your access level advanced options, custom parameters, necessary in cases where the compensation controls have different scatter properties than experimental samples. The Instrument Settings Well is not recorded during an experiment.



## Define Specimen and Sample Wells

1. Select the desired wells from the virtual plate layout to highlight and click **New Specimen** or **New Sample** on the *Plate tab* of the *Ribbon bar*.
2. To add additional *Specimen* or *Sample Wells*, repeat the process by selecting the desired wells and clicking **New Specimen** or **New Sample** on the *Plate tab* of the *Ribbon bar*. Each new *Specimen* added to the *Experiment* is displayed as uniquely colored circles in the virtual plate layout.



3. To add a name, description, or notes to an *Experiment*, *Specimen*, or *Sample*, select the desired **Experiment**, **Specimen**, or **Sample** in the virtual plate layout, and then use the *Information tabs* in the *Plate Setup tab* to type in the appropriate information. Alternatively, right click an *Experiment*, *Specimen*, or *Sample* in the *Experiment Explorer*, select **Rename** from the drop-down menu, and then enter the desired name.

## Set Up Workspace

1. To access the *Global Workspace*, double-click **Global Workspace** within the *Experiment* in the *Experiment Explorer*. Alternatively, click the **Global Workspace tab** within a *Sample*.  
To access a *Local Workspace*, click **Local Workspace tab** on the Attune® Desktop.  
To access the *Compensation Workspace* double-click the specific compensation control once they have been set up in the *Plate Layout view* or in the *Experiment Explorer*.
2. Add analysis objects (*Plots*, *Gates*, and *Statistics*) to your *Workspace* using the **Insert tab**. For more information on working with *Workspace* objects and customizing the *Workspace*, refer to the Attune® Acoustic Cytometer User Guide.

## Define Run Protocols

### Define Run Protocol for Compensation Controls

1. Select the **Unstained (UC) control well** on the virtual plate display.
2. Define the *Run Protocol* criteria for the unstained compensation control in the *Plate Setup tab* and click **Apply Changes**.  
**Note:** The *Setup Flow Options* and *Recording Flow Options* are set up separately to conserve the sample during setup. The *Setup Flow* options allow you minimize sample flow during instrument configuration.
3. To apply the same *Run Protocol* to the remaining compensation control wells, click **Copy Run Protocol** on the *Plate tab* of the *Ribbon bar*, select the appropriate wells on the virtual plate layout, and then click **Paste Run Protocol**.

**Note:** We recommend analyzing the compensation controls with the same *Run Protocol* as for the samples.

### Define Run Protocol for Instrument Settings Well

1. Select the **Instrument Settings well** on the virtual plate display.
2. Define the *Run Protocol* criteria for the unstained compensation control in the *Plate Setup tab* and click **Apply Changes**.

**Note:** *Run Protocol* for the *Instrument Settings Wells* does not contain the *Recording Flow Options*, which is only available for the *Compensation Control* and the *Sample Wells*. Note that the *Instrument Settings Well* is not recorded.

### Define Run Protocol for Sample Wells

1. Click the first **Sample Well** (previously defined) on the virtual plate display to select.
2. Define the *Run Protocol* criteria for the selected sample well in the *Plate Setup tab* and click **Apply Changes**.
3. To apply the same *Run Protocol* to the remaining sample wells, click **Copy Run Protocol** on the *Plate tab* of the *Ribbon bar*, select the appropriate wells on the virtual plate layout, and then click **Paste Run Protocol**.

**Note:** *Run Protocol* for the *Sample Wells* does not contain the *Setup Flow Options*, which is only available for the *Compensation Control* and the *Instrument Settings Wells*.

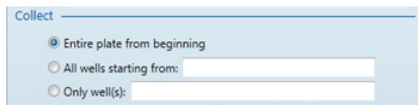
# Calculate Compensation

## Before You Begin

Ensure that the *Sample plate* is loaded into the Attune® Auto Sampler and contains the *unstained* and *single-stained compensation controls* in the appropriate wells.

## Compensation Setup

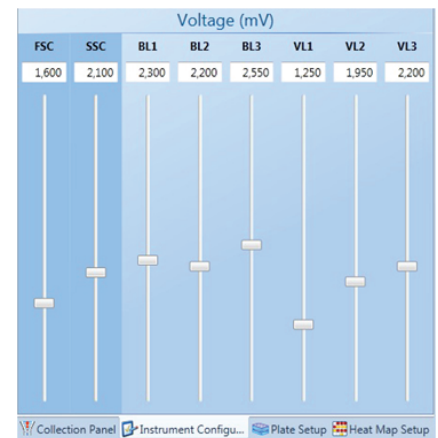
1. On the *Collection Panel tab*, set the plate to collect **Entire plate from beginning**.
2. Click **Set Up Comp** (i.e., the Set Up/Collect button) to begin compensation setup procedure, starting with the unstained control.



3. While unstained control is running, use the voltage slider bars on the *Instrument Configuration tab* to optimize the voltages for the scatter parameters for FSC and SSC.

**Note:** The parameter appropriate to the compensation control is highlighted during setup.

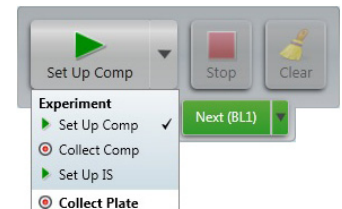
4. Set the **R1 gate** over the population of interest on the scatter plot, and then right-click on the gate and select **Apply gate shape to all controls**.
5. Click **Stop** when you are satisfied with the data and then click **Next** to move onto the next compensation control.  
Alternatively, allow the instrument to dispense the entire acquisition volume of the compensation control before clicking **Next** and then **Set Up Comp** to begin the set up of the next compensation well.



6. Using the voltage slider bars on the *Instrument Configuration tab*, optimize the voltage for the selected compensation control, and set the **R2 gate** on the peak of interest on the histogram plot.
7. Repeat this procedure for the remaining compensation controls, ensuring that the gates are set on the appropriate populations for all controls.

**Note:** You can skip the compensation setup procedure entirely and select one of the following commands using the **Set Up/Collect** drop-down menu.

- **Collect Compensation** to record of all compensation controls automatically
- **Set Up IS** to optimize instrument settings before sample collection
- **Collect Plate** to start the collection procedure to record all samples on the plate

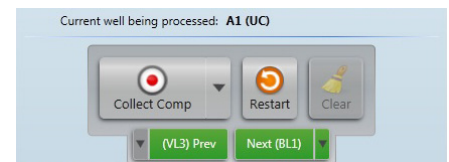


## Collect Compensation

1. After compensation setup is complete, click **Next** on the *Collection Panel tab*. The workspace for the first defined control compensation control (e.g., unstained control or UC) opens.
2. Click **Collect Comp** to start the automated compensation collection procedure. The Attune® Cytometric Software acquires and records compensation controls starting with the first defined control using the Run Protocol applied to each individual well.

**Note:** While the compensation controls are being recorded, ensure that the gates created during compensation setup are still set on the populations of interest.

3. When the last compensation control is recorded, *Compensation Matrix* automatically opens for your review. Click **OK** to accept the compensation values.  
If not satisfied, you can also manually edit or reset the compensation values in the matrix (not recommended).



Area	BL1	BL2	BL3	VL1	VL2	VL3
BL1	100.53	-0.48	-0.1	5.77	-41.88	-0.05
BL2	-39.03	106.76	-27.2	0.97	-6.58	-0.07
BL3	3.48	-25.49	106.53	0.19	3.25	-8.14
VL1	0.08	0	-0.01	101.44	-12.89	-0.04
VL2	-0.83	0	0	-14.17	100.23	-0.01
VL3	1.5	-3.53	0.15	6.89	-78.88	100.1



## Optimize Instrument Settings

1. After accepting the compensation matrix, click **Next** or select **Set Up IS** (if an Instrument Settings well has been defined) from the drop-down menu on the **Set Up/Collect** button. Global Workspace opens to allow the optimization of instrument settings.

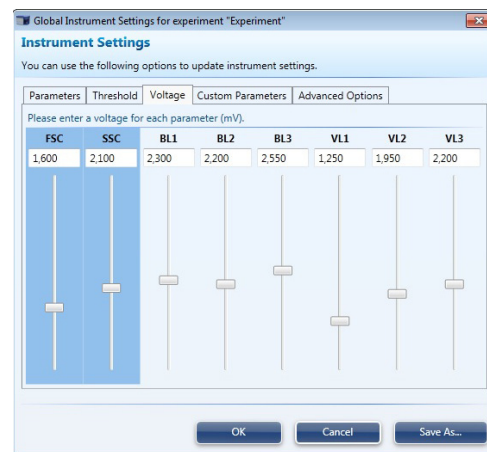
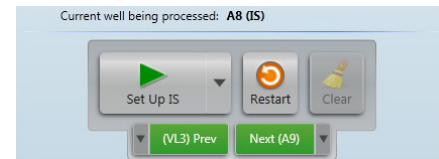
**Note:** The data in Instrument Settings well(s) are not recorded.

2. Click **Instrument Settings** in the *View tab* to open the *Instrument Settings window* and adjust the voltages and threshold settings for the scatter channels (FSC and SSC) and the fluorescent channels (if compensation is not applied) while the sample is acquiring.

Alternatively, you can use the *Instrument Configuration tab* to adjust the instrument settings.

**Note:** Once compensation has been collected, only the scatter channels will be available for adjustment, and the channels for fluorescence voltages will be grayed out. However, if the experiment contains no compensation wells, then the fluorescence voltages will also be available for adjustment.

3. Adjust the gates in the workspace as necessary.
4. Click **Stop** when the voltages have been optimized for all available channels. Alternatively, allow the instrument to dispense the entire acquisition volume for the Instrument Settings sample.



## Run Samples

### Collect Samples (Plate Collection Mode)

1. After you have optimized instrument settings, click **Next** to move to sample wells or select **Collect Plate** from the drop-down menu on the **Set Up/Collect** button.

If you have set up multiple experiments on the same plate and wish to collect only from specific wells, you can enter your selection in the **All wells starting from** or the **Only well(s)** fields.

2. Click **Collect Plate** to start the automated sample collection procedure. The Attune® Cytometric Software acquires and records samples starting with the first designated sample well using the appropriate *Run Protocol* applied to that well.

**Note:** While collecting samples, you can create plots, daughter plots, and statistics, and insert and adjust gates. However, once compensation has been recorded and sample collection has started, you cannot adjust the PMT voltages and Threshold values.

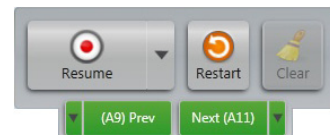
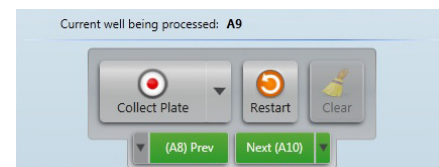
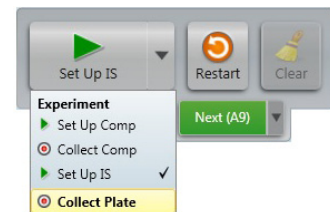
3. Click **Pause** to temporarily halt sample collection, if needed. The instrument completes the collection of the current well and then pauses the collection procedure. When the instrument is paused between wells, you can make changes to the Run Protocols, Heat Map settings, and Workspace objects for the remaining wells.

Click **Clear** to erase all collected data from that well without pausing the collection procedure.

Click **Resume** to resume the data collection from the point where it was paused.

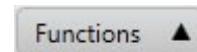
Click **Restart** to restart collection from the first well and overwrite all collected data including compensation controls and Instrument Settings wells.

4. Sample collection automatically stops after all the selected samples in the plate are collected and the Attune® Cytometric Software automatically saves the data in a unique FCS file.



### Sample Recovery (Plate Collection Mode)

1. Select **Sample Recovery** from the **Functions menu** of the lower taskbar. The *Sample Recovery prompt screen* appears.
2. Follow the instructions on the screen to recover your sample.



## Data Analysis and Processing

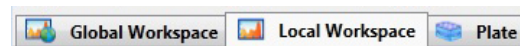
1. To analyze and process data globally, open the *Global Workspace* for any sample well by double-clicking the well in the *Plate tab* or the *Mini Plate*, or double-clicking the appropriate file in *Experiment Explorer*.

Adjust gates and add or remove plots as desired.

Right-click the border area of a plot and choose **Customize** to adjust the axes, display information, and other plot-specific settings, as needed. Multiple plots can be customized at the same time by selecting multiple plots then right-clicking and selecting **Customize**.

All plots containing modified parameters will be automatically updated after each change and for all subsequent samples in the global workspace.

2. To analyze and process data locally (i.e., only for the selected well), click the **Local Workspace tab**. Any changes made within the local workspace will not be propagated to other samples' workspaces.



3. Run Protocol is displayed at bottom of Collection Panel, however this protocol cannot be changed via the Collection Panel; it will have to be modified on the Plate Setup tab.

## Shutdown

**IMPORTANT!** Perform the Shutdown procedure at least once a day, even if the instrument is intended for continuous use. Proper cleaning of the instrument ensures its consistent and accurate operation.

**CAUTION! BIOHAZARD.** Cytometer hardware may be contaminated by biohazardous material. Using fresh 10% bleach solution in deionized water is the only procedure we recommend for decontaminating the cytometer.

## Check Fluid and Waste Levels

1. Check the levels in the fluid tanks.
2. Ensure that the wash and shutdown solution tanks are at least half-full. If empty, fill the appropriate tanks with Attune® Wash or Attune® Shutdown Solution.
3. Empty the waste tank on both the Attune® Acoustic Focusing Cytometer and the Attune® Auto Sampler.

## Run Shut Down Function

1. **Log Out** of the Attune® Cytometric Software, and click **Shut Down**. The *Shut Down prompt screen* appears.

2. Select **Standard** for 10 wash cycles or **Custom** to enter the desired number of wash cycles. Click **Next**.

3. When prompted, place 3 mL of **10% bleach solution** in a tube on the sample injection port (SIP) and raise the tube lifter.

**Note:** 10% bleach is defined as a 1 in 10 dilution (1 part bleach to 9 parts deionized water) of 5.25% sodium hypochlorite in deionized water. This gives a final concentration of 0.5% sodium hypochlorite equivalent to 5,000 ppm of available chlorine.

4. Ensure that the manual valve is set to **tube mode** and click **Next**.

5. When prompted, place 3 mL of **deionized water** in a tube on the SIP, lift the tube filter, and click **Next**.

6. When prompted, place an empty 96-well round bottom plate in the Auto Sampler and close the door.

7. Move the manual valve to **plate mode** and click **Next**.

8. At the end of the shutdown operation, the Attune® Cytometric Software automatically powers down the Attune® Acoustic Focusing Cytometer and the Attune® Auto Sampler.

The Attune® Auto Sampler is in a low power standby mode and will automatically power on if the software is started and then the Attune® Acoustic Focusing Cytometer is powered on.

The eject button of the Attune® Auto Sampler will blink in the standby mode until the instrument is turned off using power switch located at the rear of the Attune® Auto Sampler.



**IMPORTANT!** If you intend to leave the Attune® Acoustic Focusing Cytometer in the shutdown state for longer than two weeks, perform **System Decontamination** instead of running the Shut Down function.

## Sanitize Between Uses

Follow the procedure below to sanitize the Attune® Auto Sampler between uses. Note that this procedure is intended for a quick cleaning of the instrument to minimize cross-contamination. For a more thorough decontamination, perform the System Decontamination procedure (see below).

1. Run a well of Milli-Q® water.
2. Run a well of freshly prepared 10% bleach (1 part 5.25% sodium hypochlorite to 9 parts of deionized water).
3. Repeat a well of Milli-Q® water.

## System Decontamination

**IMPORTANT!** Perform the System Decontamination operation:

- as a monthly maintenance routine to prevent and reduce microbial growth within the instrument
- if the system is likely to be idle for more than two weeks (run it in place of the Shut Down function)
- if the instrument has been idle for more than two months
- if the instrument has been idle for more than two weeks without decontamination run prior to it becoming idle

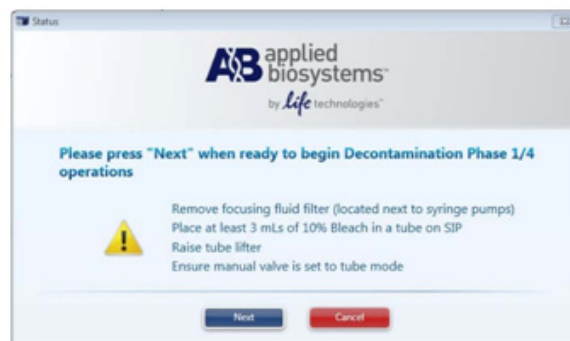
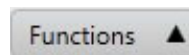
**CAUTION! BIOHAZARD.** Cytometer hardware may be contaminated by biohazardous material. Using fresh 10% bleach solution in deionized water is the only procedure we recommend for decontaminating the cytometer.

## Prepare for System Decontamination

1. Rinse out all fluid containers.
2. Fill the shutdown solution tank with 200 mL of 10% bleach. Ensure that the wash solution tank is empty.
3. Fill focusing fluid tanks on both the Attune® Acoustic Focusing Cytometer and the Attune® Auto Sampler with 450 mL of 10% bleach.
4. Ensure that all fluid lines and sensor cables are connected.

## Run System Decontamination Function

1. Select **System Decontamination** from the **Functions** menu.  
The *System Decontamination* prompt screen appears.



2. Make sure that the appropriate tanks are filled with 10% bleach and all fluid lines and sensor cables are connected, and then click **Next** to start Decontamination Phase 1.
3. When prompted, remove focusing fluid filter located next to the syringe pump on the Attune® Acoustic Focusing Cytometer, place at least 3 mL of 10% bleach in a tube on SIP, raise the tube filter, and click **Next**.
4. When prompted, move the manual valve to **Plate mode**, and then click **Next** to start Decontamination Phase 2.
5. When prompted, rinse the focusing fluid tanks on both the Attune® Acoustic Focusing Cytometer and the Attune® Auto Sampler and fill them with 450 mL of deionized water.
6. Move the manual valve to **Tube mode**, place at least 3 mL of 10% bleach in a tube on SIP, raise the tube filter, and click **Next** to start Decontamination Phase 3.
7. When prompted, move the manual valve to **Plate mode**, and then click **Next** to start Decontamination Phase 4.
8. After Decontamination Phase 4 is complete, replace the focusing fluid filter with a fresh filter, and perform a full system reset by unplugging the instrument and leaving it unplugged for at least 30 seconds.
9. Replace all fluids in all tanks with the appropriate solutions.

## System Maintenance

The following routine maintenance procedures keep the Attune® Acoustic Focusing Cytometer and all its peripheral systems in good working condition. For more information, refer to the Attune® Acoustic Focusing Cytometer User Guide.

Procedure	Frequency	Notes
Shutdown	Daily	Perform daily shutdown even if the instrument is intended for daily use.
Visual inspection of sample injection port (SIP)	Daily	See "Visual Inspection", below.
Visual inspection of fluidics tanks and connections	Daily	See "Visual Inspection", below.
Visual inspection of syringe pumps	Daily	See "Visual Inspection", below.
Fluidics maintenance	Daily and as needed	Run De-bubble function daily. Run Unclog and Wash functions daily or as needed.
Attune® Auto Sampler calibration	Monthly	Performed automatically every 30 days and takes about 1 minute to complete.
Auto Self test	Monthly	Use the Functions menu to initiate and follow the instructions in the wizard.
Computer maintenance	Monthly	
Optical filter cleaning	Monthly and as needed	
Fluidics container decontamination	Monthly	
System Decontamination	Monthly and as needed	
Changing focusing fluid filter	Monthly	
Replacing syringes	As needed	Depends on the frequency of use. On average, syringes last about 6 months.

## Daily Maintenance

### Daily Shutdown

Daily shutdown involves executing the *Shutdown function*. This function ensures that all sample fluid and dyes have been removed from the fluidics lines and the two pumps have been decontaminated and filled with Attune® Shutdown Solution to prevent the formation salt crystals.

### Visual Inspection

Visually inspect the sample injection port, fluidics tanks and connections, and the syringe pumps for any leakage and for traces of salt residue. If you notice any leaks in the fluidics lines, contact your service representative. Decontaminate any spills by wiping the area with 10% bleach solution.

### Fluidics Maintenance

Daily fluidics cleaning involves executing the *De-bubble*, *Unclog*, and *Wash functions* as needed.

- *De-bubble* is a user-initiated function for clearing bubbles in the fluidics lines of the cytometer.
- *Unclog* is a user-initiated back flush operation to remove clogs from the sample probe and flow cell.
- *Wash* is a user-initiated system cleaning between sticky samples and requires user supplied bleach or detergent.

**Note:** For monthly and periodic maintenance of the system, refer to the Attune® Acoustic Cytometer User Guide.

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