

Attune™ CytPix™ Flow Cytometer

USER GUIDE

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Product description

Attune™ CytPix™ Flow Cytometer

The Invitrogen™ Attune™ CytPix™ Flow Cytometer is a benchtop flow cytometer with high-resolution bright field imaging capabilities that uses acoustic pressure to confine the injected particles to a tight central line as the sample passes through the optical cell for interrogation.

Acoustic focusing places the interrogated particles within a narrower depth-of-field compared to conventional imaging cytometers that use hydrodynamic focusing alone, which allows the production of in-focus images at standard flow cytometry rates (up to 6,000 images/second).

Product contents

Attune™ CytPix™ Flow Cytometer is shipped with the system components listed below. All components are shipped at ambient temperature.

Component	Quantity
Attune™ CytPix™ Flow Cytometer, in one of the following configurations: Blue/Red/Violet/Yellow (BRVY) configuration Blue/Red/Violet 6/Yellow (BRV6Y) configuration Blue/Red/Violet (BRV) configuration Blue/Violet/Yellow (BVY) configuration Blue/Red/Yellow (BRY) configuration Blue/Red/Violet 6 (BRV6) configuration Blue/Red (BR) configuration Blue/Violet (BV) configuration Blue/Yellow (BY) configuration Blue/Violet 6 (BV6) configuration	1

(continued)

Component	Quantity
Power cord kit, universal voltage C13 2.5 m RC	3
Cable, USB 3.0 A-B M/M, 2 m RC	1
Cable, network RJ45 M/M CAT6 STP, 7 ft BLUE RC	1
27-inch flat panel monitor	1
Computer, minitower desktop (includes mouse and keyboard)	1
Attune™ CytPix™ Starter Kit (includes the Software Kit and reagents)	1

Attune™ CytPix™ Starter Kit is boxed separately from the instrument components and contains the following items. Store the reagents at the temperatures specified on the product packaging or labels.

- Software Kit (includes a valid DESkey device, which is required for the operation of the Attune™ Cytometric Software)
- Reagents:

Reagent	Quantity	Storage
Attune™ Focusing Fluid, 6 x 1 L	2	15°C to 30°C
Attune™ Wash Solution, 250 mL	1	15°C to 30°C
Attune™ Shutdown Solution, 250 mL	1	15°C to 30°C
Attune™ Debubble Solution, 50 mL	1	15°C to 30°C
Attune™ Performance Tracking Beads, 3 mL	1	2°C to 8°C

IMPORTANT! Reagents can be stored at colder temperatures, but ensure that all reagents are at 15°C to 30°C before running the instrument.



WARNING! CHEMICAL HAZARD Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, work with, or dispose of any chemicals or hazardous materials.

Upon receiving the instrument

Examine the instrument carefully for damage incurred during transit. Ensure that all parts of the instrument and accessories listed are included with the product. Damage claims must be filed with the carrier; the warranty does not cover in-transit damage.

IMPORTANT! See the *Attune™ CytPix™ Flow Cytometer Site Preparation Guide* (Pub. No. MAN0019443) for instrument installation instructions.

Register your instrument

Visit www.thermofisher.com to register your instrument. You will be asked to supply the serial number, your name, and your contact details. Registering your instrument ensures that you will receive notifications of software upgrades and information on new assays for use with the instrument.

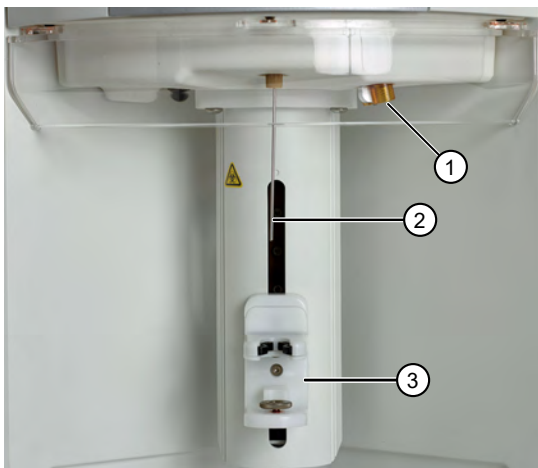
Parts of the instrument

Front view of instrument



- ① Fluidics compartment
- ② Sample injection port (SIP)
- ③ Status indicator lights

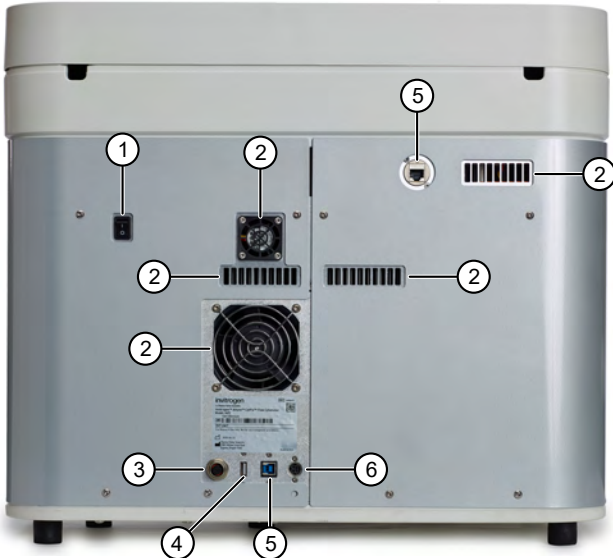
Sample injection port (SIP)



- ① Fluid connection ports for autosampler
- ② Sample injection tube
- ③ Sample tube lifter

Note: The fluid lines that connect the Attune™ CytPix™ Flow Cytometer to the optional autosampler can be attached to either fluid connection port.

Rear view of instrument



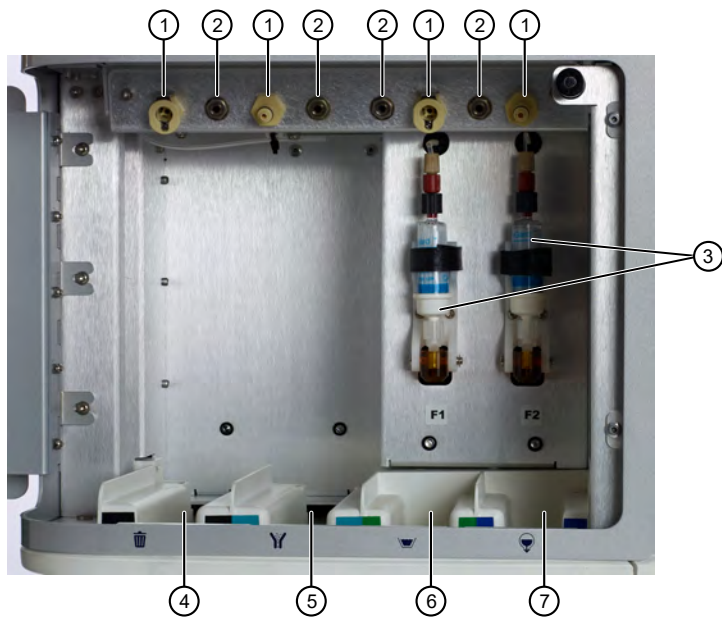
- ① On/Off switch
- ② Air exhausts
- ③ Port for Attune™ NxT External Fluid Supply connection
- ④ Port for autosampler connection
- ⑤ Port for PC connection
- ⑥ Power input jack

Side view of instrument

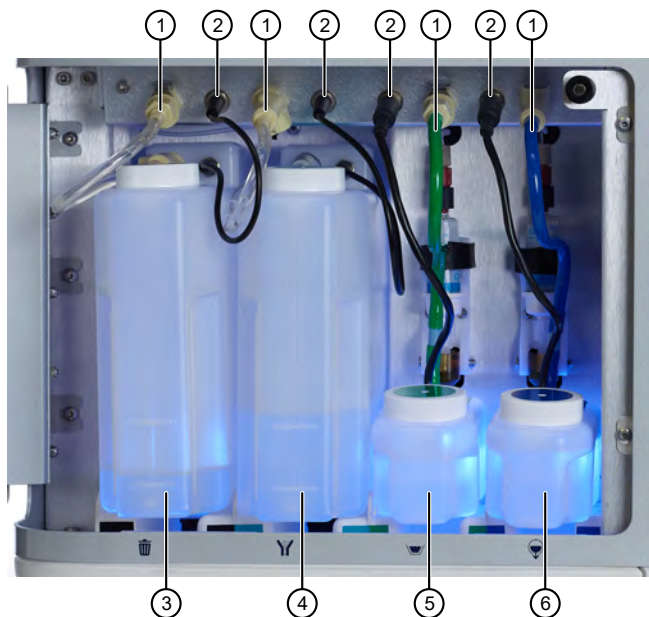


- ① Syringe pump compartment
- ② Fluidics compartment

Fluidics compartment

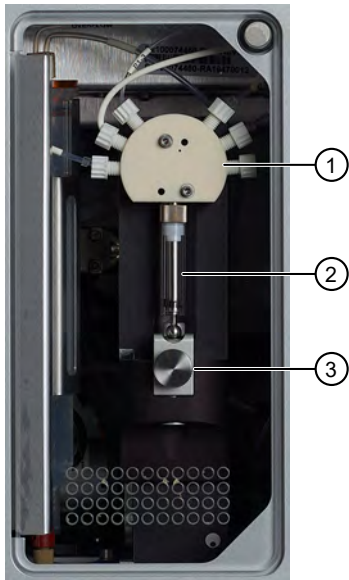


- ① Fluid connection ports
- ② Fill sensor connection ports
- ③ Focusing fluid filters
- ④ Waste container compartment
- ⑤ Focusing fluid compartment
- ⑥ Wash fluid compartment
- ⑦ Shutdown fluid compartment



- ① Fluid lines
- ② Fill sensor connections
- ③ Waste container
- ④ Focusing fluid container
- ⑤ Wash fluid container
- ⑥ Shutdown fluid container

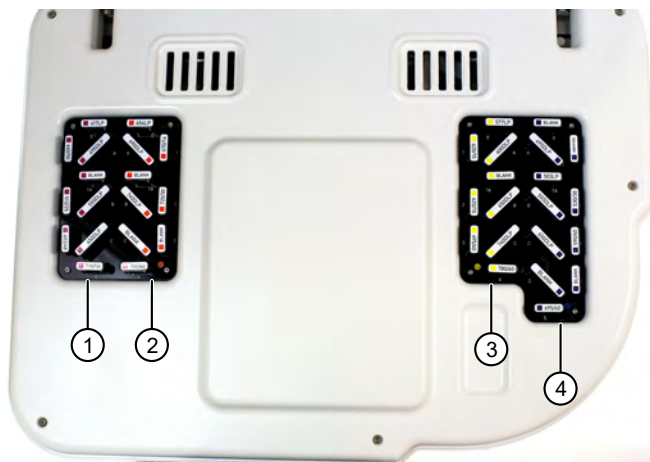
Syringe pump compartment



- ① Valve
- ② Syringe piston
- ③ Syringe capture mechanism

Optics compartment

The optics compartment houses the collection optics (i.e., optical filters and mirrors) for the violet and red lasers on the left, and for the yellow or green and blue lasers on the right.



- ① Collection optics for violet laser
- ② Collection optics for red laser
- ③ Collection optics for yellow laser
- ④ Collection optics for blue laser

Status indicator lights

The status indicator lights above the sample injection port identify the status of the cytometer.

Instrument cycle	Status indicator lights
Startup and all other instrument functions (except acquisition)	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	Flashing blue
Rinse	Flashing blue
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	Flashing blue
Shutdown complete	Multicolor fade
Error	Amber blink

Attune™ Cytometric Software

The functions of the instrument are controlled by the Attune™ Cytometric Software. The software is pre-installed to the computer workstation supplied with the instrument, and the Attune™ Cytometric Software icon (i.e., shortcut) is placed on the computer desktop and under **Start ▶ All Programs ▶ ThermoFisher ▶ Attune™ ▶ Attune™ Cytometric Software**.

About the software

The Attune™ Cytometric Software is a flexible data acquisition and analysis tool that uses a browser view to:

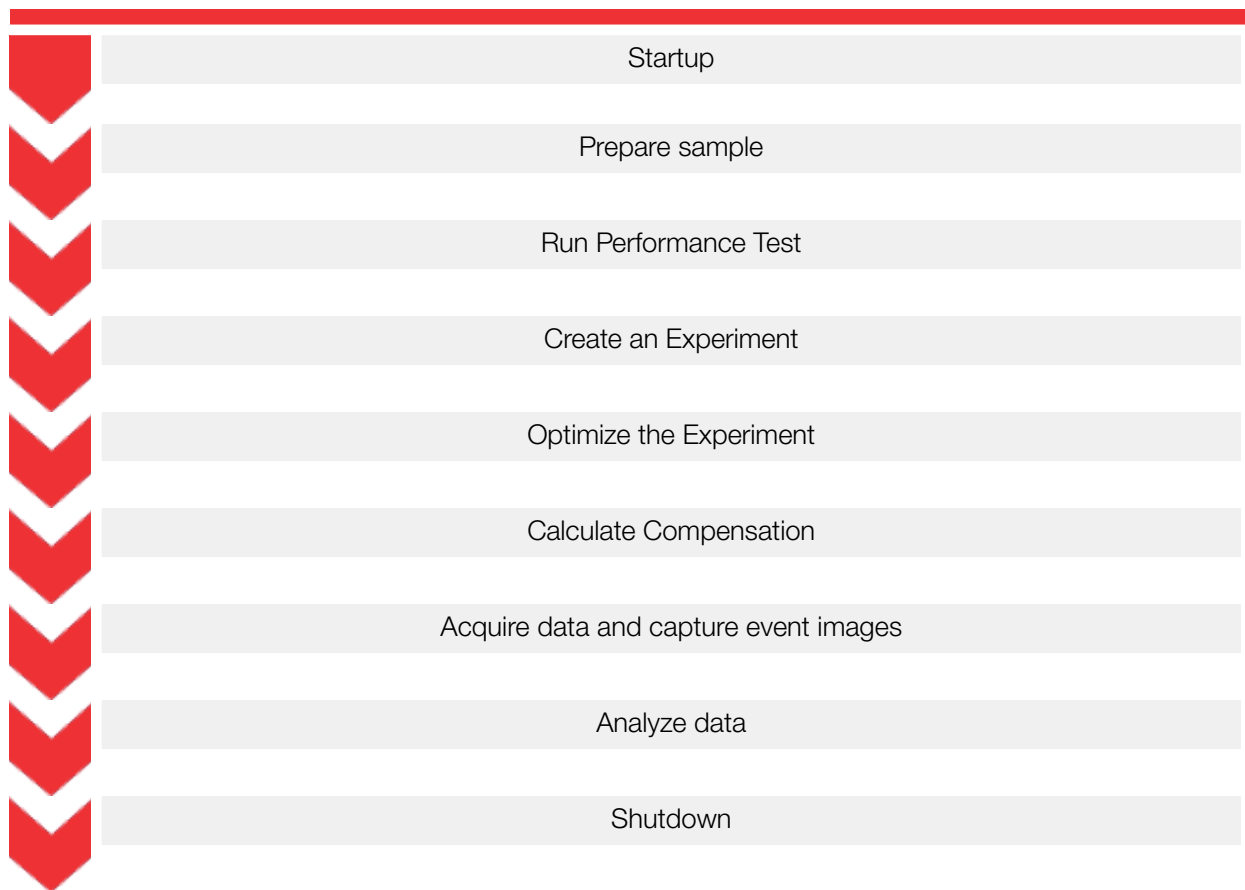
- Design and perform experiments
- Define independent instrument settings and optimize data collection
- Carry out instrument performance checks and track instrument performance
- Acquire and record data
- Capture images of specific events from gated populations
- Manage and process recorded data

Note: For detailed information on the Attune™ Cytometric Software, see the *Attune™ Cytometric Software User Guide* (Pub. No. 100024236), which is provided with the product. You can also download the software user guide from www.thermofisher.com/attune.

Instrument workflow

The following diagram summarizes the workflow of the Attune™ CytPix™ Flow Cytometer. For detailed instructions on each step of the workflow, see the appropriate chapter in this user guide.

Note: For detailed instructions on using the Attune™ Cytometric Software, see the *Attune™ Cytometric Software User Guide* (Pub. No. 100024236). The software user guide is provided with the product and is also available for download at www.thermofisher.com/attune.



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Before you begin

Required solutions

- **Attune™ Focusing Fluid** – is a buffered, azide-free support/carrier reagent for transporting particles through the optical cell. It contains a preservative and detergent designed to minimize bubble formation.
- **Attune™ Wash Solution** – is a ready-to-use solution for removing cellular debris and dyes from the fluidics system of the instrument.
- **Attune™ Shutdown Solution** – is a ready-to-use solution added to the Shutdown container to prevent bubble formation in the fluidics system of the instrument.
- **10% bleach solution in deionized water** – decontaminates the fluidics lines. Prepare this solution fresh daily and use during the shutdown procedure.
- **Deionized water** – used for diluting bleach, as well as for long-term storage of the instrument.
- **Attune™ Debubble Solution** – a solution optimized for removing bubbles from the system.

IMPORTANT! 10% bleach is defined as a 1 in 10 dilution (1 part bleach to 9 parts water) of 5.25% sodium hypochlorite in water. This gives a final concentration of 0.5% sodium hypochlorite equivalent to 5000 ppm of available chlorine. We recommend using laboratory-grade bleach. Avoid bleach with additives (such as perfumes).

IMPORTANT! Recommended storage temperature for reagents and solutions is room temperature (15–30°C), but they can also be stored at colder temperatures. However, running the instrument with cold reagents (<15°C) will affect the data quality. Before you run the instrument, ensure that all fluid temperatures are at least 15°C.

Startup procedure

During Startup, the instrument:

- Warms the lasers to operating temperature
- Initializes the pumps
- Primes the instrument fluidics
- Informs the user of System Status (Ready, Attention, Clog, etc.)

Check fluid and waste levels

1. Check the levels in the fluid containers.
2. Fill the focusing fluid, wash solution, and shutdown solution containers.
3. Empty the waste container. Fill the emptied waste container with full strength bleach solution up to the bleach fill mark on the bottle.



- ① Sensor cable jack
- ② Fluid line connection
- ③ Bleach fill mark

IMPORTANT! The bleach fill mark is the bottom line on the waste container. Be sure to fill the waste container to the middle of the bottom line.

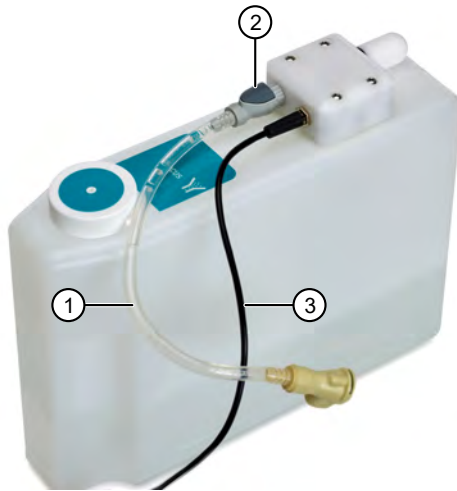
IMPORTANT! Ensure that the instrument is in the idle state (i.e., not acquiring or performing instrument functions) before refilling the fluidics containers.

Fill the empty fluid containers

1. Unplug the sensor cable, then pinch the release button on the fluidic connector to disconnect the fluid line from the container. Repeat for each container.

Note: Do not apply excess pressure to the fluidic connector release button to avoid undue stress on the connector and threads.

Note: To avoid any backpressure issues, we recommend that you unplug the sensor cables before disconnecting the fluid lines.



- ① Fluid line
- ② Release button
- ③ Sensor cable



2. Remove the container from the instrument, unscrew the lid, and fill the container with the appropriate solution.

Note: The focusing fluid container has 1.9 L capacity, and the shutdown and wash solution containers each have 175 mL capacity. Do not overfill the fluid containers.

3. Screw the lid back on without over-tightening it.

4. Replace the container by sliding it into the appropriate slot. First plug the fluid line, then the sensor cable into the instrument.

IMPORTANT! For all fluid containers, always connect the fluid line first. Connecting the sensor cable while leaving the fluid line disconnected can result in increased backpressure and introduce air into the system.

IMPORTANT! Ensure that all fluid containers are placed in the correct orientation with the lids of the containers away from the back of the instrument. If the containers are placed in the wrong orientation, the fluid lines can become kinked and obstruct the flow.

Note: Fluid levels are monitored via floating sensors in all fluid containers. When the fluid level is low, the waste container is full, or the bottle level sensor is unplugged, the software displays the appropriate warning message and the blue LED on the affected fluid container will pulse. To resume the run, follow the displayed instructions, then click **OK**.

Power on the cytometer and computer

1. Flip the rocker switch located on the back of the instrument to the **ON** (up) position to power on the instrument.
2. Power on the computer and monitor, and wait for the computer to boot.
3. Log in to Windows™. The default credentials are:
 - User name: INSTR-ADMIN
 - Password: INSTR-ADMIN

IMPORTANT! You can power on the instrument and the computer in any order. If your system includes the optional Attune™ NxT Auto Sampler or the CytKick™ / CytKick™ Max Autosampler, the auto sampler should be powered on before the cytometer.

Launch the software and sign in

1. Double-click the Attune™ Software shortcut icon on the desktop to launch the software.

Alternatively, select **Start ▶ ThermoFisher ▶ Attune™ Software**.

The *Login* screen is the first screen that is displayed after the splash screen when you start the software.



The image shows a login window with a dark background. It contains two white input fields: the top one is labeled 'Username' and the bottom one is labeled 'Password'. Below the password field, there is a blue link that says 'Forgot Password?'. In the bottom right corner, there is a grey button with the text 'Sign in'.

2. To sign in the Attune™ Cytometric Software, type a valid username and password in the Username and Password fields, then click **Sign in**.

Note: When signing into the Attune™ Cytometric Software for the first time after installation, you must sign in as an *Administrator*.

The default username and password for the Administrator are both admin.

After you log successfully in to the software, the *Main Menu* is displayed. For more information on the Main Menu screen, see “Main Menu” in the *Attune™ Cytometric Software User Guide*.



Note: After signing in for the first time, you will be required to change the password. For more information on user management, see the *Attune™ Cytometric Software User Guide*.

IMPORTANT! A license control mechanism in the form of a DESkey device is required for the operation of the Attune™ Cytometric Software. If a valid DESkey device is not present, the software will display a warning message. For more information, see “Main Application Startup” in the *Attune™ Cytometric Software User Guide*.

IMPORTANT! To sign in to the Attune™ Cytometric Software in the SAE (“Security, Auditing, and Electronic Signature”) mode, you must have an SAE-specific DESkey device installed and the SAE module enabled and configured to allow sign in using SAE credentials. For more information, see “Login screen” in the *Attune™ Cytometric Software User Guide*.

Run the Startup function

The Startup function primes the system fluidics. The software guides you through the Startup function. Follow all the instructions provided by the software during the procedure. For more information, refer to the *Attune™ Cytometric Software User Guide*.

1. To initiate the Startup function, click the **Startup** button  on the *Instrument ribbon tab* or the *Collection panel*.

The *Startup dialog* opens and provides instructions to perform the Startup operation.

2. If the tube lifter is raised, lower the tube lifter.

If your system includes the optional auto sampler and a plate is loaded in the auto sampler, remove the plate.

3. Click **Next** to run the Startup function.

During Startup, the software automatically turns on the lasers and instrument systems, initializes the pumps, and primes the fluidics lines. The status window displays the Startup operation being performed.



After the Startup function is completed and no system errors are encountered, the Status bar displays the *Idle* icon.



If any system errors are encountered during the Startup, the status bar displays the *Alarm* icon.

Note: A fading blue status indicator light above the sample injection port indicates that Startup is under way, and a continuous green light indicates that the instrument is ready.



Performance tracking

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Overview

Performance tracking

Performance tracking is a comprehensive set of procedures to monitor the daily performance of the instrument. During the performance tracking procedure, you will:

- Establish the cytometer's initial *Baseline status* by running the Attune™ Performance Tracking Beads
- Run the same performance tracking bead particle set to perform the *Performance test*
- Monitor the changes in the coefficient of variation and the changes in PMT voltages
- Track the linearity of the cytometer
- Evaluate the detector quantum efficiency (Qr) and optical background (Br)

Performance tracking is critical to ensure the accuracy and sensitivity of the cytometer and it provides information about the lasers and detection channels available on the instrument.


Note: Results of instrument performance tracking tests are available for all users, but only users authorized by the administrator can perform Baseline calculations and Performance tests.

Note: For optimal cytometer performance, follow the periodic and scheduled maintenance procedures as described in the *Attune™ Flow Cytometry Maintenance and Troubleshooting Guide* (Pub. No. 100024234).

Baseline and Performance Test (BL/PT) module

The Attune™ Cytometric Software provides automated *Baseline Calculations* and *Performance test* functions that require minimal user interaction and facilitates performance tracking through its *Reports* feature. These functions are controlled by the *Baseline and Performance Test (BL/PT) module* of the software.

To access the BL/PT module, click **Performance test**  on the Main Menu.

Alternatively, click **Performance History**  on the Instrument ribbon tab, when the instrument is connected.

For more information on the BL/PT module, refer to the *Attune™ Cytometric Software User Guide*.

Attune™ Performance Tracking Beads

The Attune™ Performance Tracking Beads (Cat. No. 4449754) are used to define a baseline for cytometer performance and conduct daily performance measurements of the cytometer. They are stained with a combination of fluorophores that can be excited by the lasers used in the cytometer and emit fluorescence signals at designed levels to all the channels in the instrument.

Each vial of performance tracking beads contains a mixture of four beads at an equal concentration that differ in relative fluorescence emission intensity: blank, dim, medium, and bright. The blank beads in the Attune™ Performance Tracking Beads have a nominal diameter of 2.4 µm. The dim, medium, and bright intensity beads have a nominal diameter of 3.2 µm.

IMPORTANT! Prepare the Attune™ Performance Tracking Beads suspension immediately before use. The performance tracking beads are non-hazardous and can be disposed according to local regulations.

Baseline setup

The *Baseline setup* workflow uses Attune™ Performance Tracking Beads to define the cytometer's initial baseline status. During this process, the median fluorescence intensity of each bead and the r%CV (robust percent coefficient of variation) are automatically measured in all fluorescence detectors. Software algorithms use this information to determine cytometer settings and provide target values for subsequent application specific settings.

How Baseline setup works

The software guides you through this process to measure the following values for each fluorescent bead using assigned MESF (molecule of equivalent soluble fluorophore) values:

- PMTV (photomultiplier tube voltage)
- Delta PMTV
- Target MFI (target median fluorescence intensity)
- Measured MFI
- r%CV (robust percent coefficient of variation)
- Quantum Efficiency (Qr)
- Background (Br)
- Linearity
- Area Scaling Factor
- Laser Delay

The software automatically adjusts the PMT voltages to maximize population resolution in each detector, then creates the *Baseline Calculations Report*. For more information on the Baseline Calculations Report, refer to the *Attune™ Cytometric Software User Guide*.

IMPORTANT! Perform baseline calculation after any major troubleshooting or cytometer service.

Set up Baseline

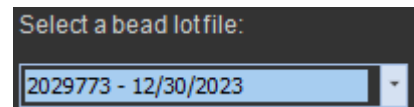
1. Open the **BL/PT module** to view the *Baseline setup* screen, which provides general instructions to set up a Baseline.



Note: If a Baseline already exists, the *Performance test setup* screen is displayed instead of the Baseline setup screen. On the Performance test setup screen, you can run a Performance test or reset the Baseline.

2. Select the appropriate bead lot file from the **Select bead lot file** dropdown menu.

If needed, select **Import** from the menu to import a new bead lot file.

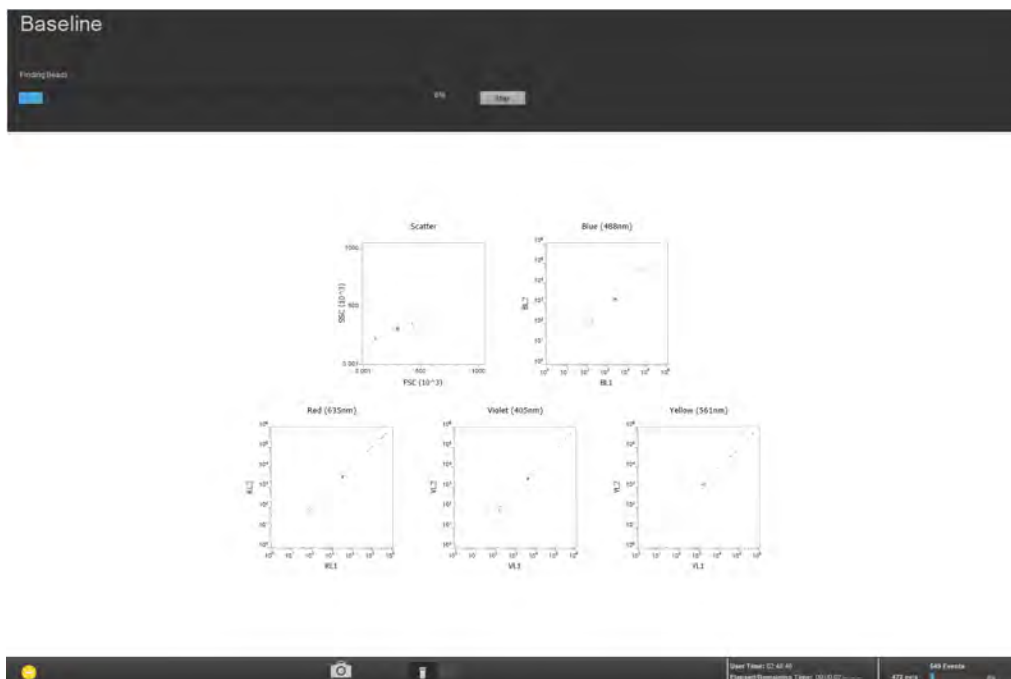


3. Click **Check now** under the filter block image to verify the instrument configuration. The software displays the default instrument configuration that must be used for the Baseline.
4. Verify that the bead lot number you have selected matches the bead lot number of the Attune™ Performance Tracking Beads you are using.
The bead lot number is the **first seven digits** printed on the label (disregard any alpha numeric characters).
5. Vortex the Attune™ Performance Tracking Beads bottle to resuspend the beads, and then add 3 drops of the bead suspension to 2 mL of focusing fluid in a 12 × 75-mm tube. Mix the bead suspension in focusing fluid by gentle inversion or vortexing.
6. Load the tube by placing it in the sample tube lifter.

- Click **Run Baseline** to initiate the automated baseline calculations.

Note: If the *Startup* procedure has not been performed, the button displays **Run Startup** instead of **Run Baseline**. You must run the Startup procedure before proceeding.

- The *Baseline* screen provides progress information for the Baseline procedure.



Baseline completion

If the baseline passes, the software:

- Displays the *Baseline Results* screen. For details on how to interpret the Baseline results, see the *Attune™ Cytometric Software User Guide*.

Baseline Test Results

Baseline test successful

Baseline: 2029773 - 4/7/2021

4/7/2021 1:42:09 PM

Channel	PMTV	Target MFI	MFI	Robust %CV	Qr	Background	Linearity	ASF	Laser Delay	Result
FSC	386	300,000	300,756	1.54 %	0.000	0	0.000	1.12	840	✓
SSC	363	300,000	308,669	4.09 %	0.000	0	0.000	1.12	840	✓
BL1	382	300,000	313,133	1.57 %	0.055	74	1.000	1.12	840	✓
BL2	405	300,000	310,687	2.31 %	0.061	20	1.000	1.12	840	✓
RL1	374	300,000	310,584	3.38 %	0.069	63	0.997	1.16	1095	✓
RL2	392	300,000	313,176	3.21 %	0.012	281	0.995	1.16	1095	✓
RL3	430	300,000	309,816	3.33 %	0.048	83	1.000	1.16	1095	✓
VL1	296	300,000	301,609	0.99 %	0.077	533	1.000	1.18	583	✓
VL2	328	300,000	301,633	1.00 %	0.105	165	1.000	1.18	583	✓
VL3	422	300,000	301,920	1.93 %	0.395	3	1.000	1.18	583	✓
VL4	513	300,000	305,774	3.00 %	0.088	6	0.999	1.18	583	✓
VL5	495	300,000	307,069	3.00 %	0.085	2	0.999	1.18	583	✓
VL6	561	300,000	303,698	3.39 %	0.017	23	0.999	1.18	583	✓
YL1	424	300,000	309,031	1.64 %	0.111	37	1.000	1.05	333	✓
YL2	362	300,000	303,811	2.17 %	0.055	16	1.000	1.05	333	✓
YL3	461	300,000	305,408	3.17 %	0.004	73	1.000	1.05	333	✓

Flow Rate: 500 µl/min

- Calculates the system area scaling factor constant based on the results for each configured laser.
- Updates the default system values for the area scaling factor in the Instrument Settings panel.
- Applies the measured laser delays to the system settings in the Instrument Settings panel.

If the Baseline fails, the software displays a dialog, which provides a list of the channel statistics that failed and the recommended actions to remedy the errors.

Note: For detailed information on the Baseline setup function, see the *Attune™ Cytometric Software User Guide*.

IMPORTANT! At the end of the Baseline procedure, run the **Sanitize SIP** function before running any samples.

Performance test

After the Baseline has been run and the baseline values are defined, the same lot of Attune™ Performance Tracking Beads is used to run the *Performance tests* to measure the variation from those baseline measurements and track the daily performance of the cytometer.

During this process, the software measures and records the following observables in all fluorescence detectors:

- Voltage required to place the bright bead in the target channel
- Change in PMT voltage (Δ PMT)
- r%CV (robust percent coefficient of variation) of the bright bead

Using assigned *MESF* (molecule of equivalent soluble fluorophore) values for each fluorescent bead, the software calculates the following for each channel:

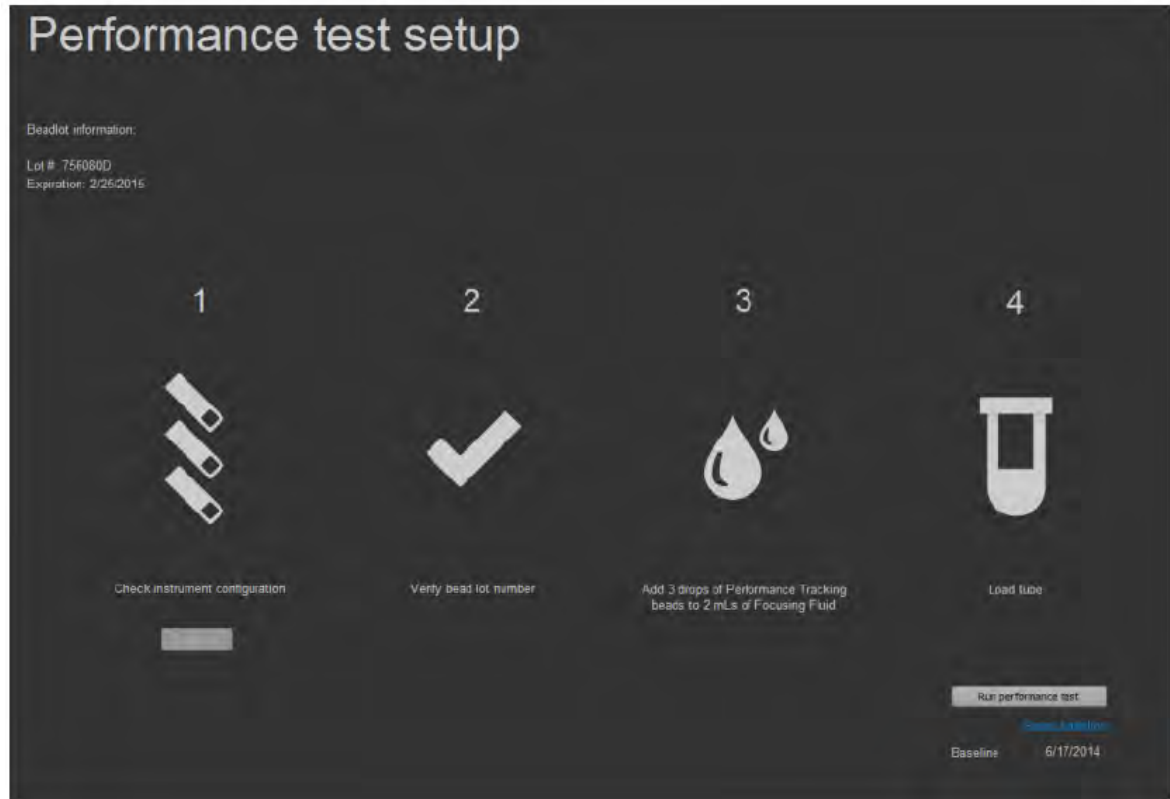
- Detector quantum efficiency (Qr)
- Background level (Br)
- Linear regression
- Laser delay setting

The results of the Performance test can also be viewed as Levey-Jennings charts, which provide a visual to track the r%CV and changes in PMT voltage to check for shifts and trends in cytometer performance.

IMPORTANT! We recommend that you run the Performance test at least once per day when the cytometer is in use.

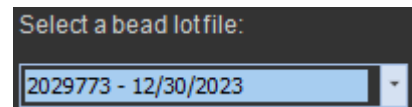
Run Performance test

1. Open the **BL/PT module** to view the *Performance test setup* screen, which provides general instructions to set up and run a Performance test.



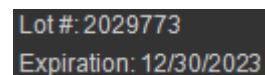
Note: If no Baseline exists, the *Baseline setup* screen is displayed instead of the Performance test setup screen. You must run the Baseline before running the Performance test.

2. Select the appropriate bead lot file from the **Select bead lot file** dropdown. If needed, import a new bead lot file by selecting **Import** from the menu.

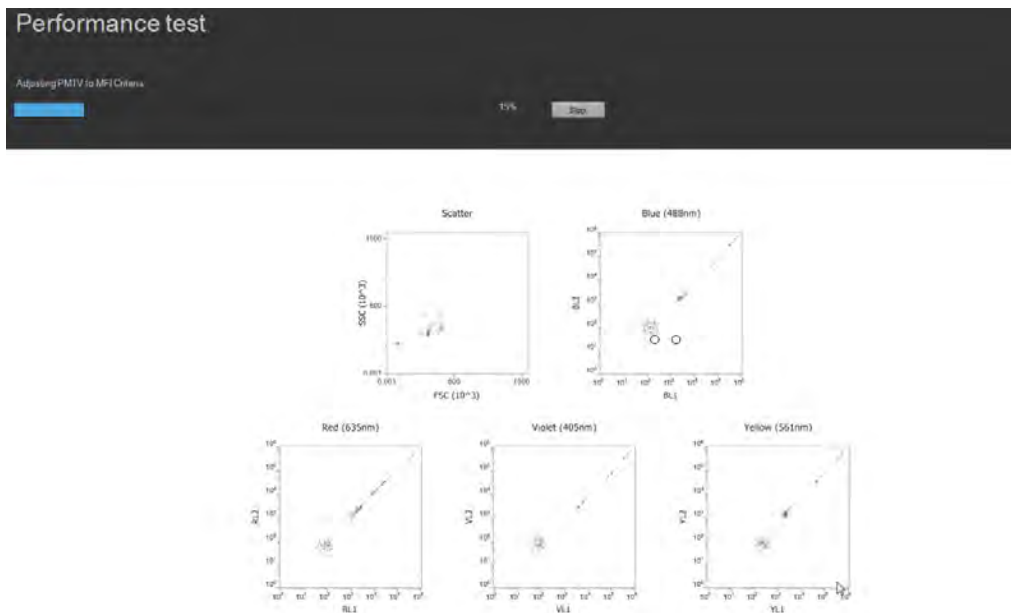


Note: If needed, select a different Baseline against which to run the Performance test, then click **Reset Baseline** to go to the Baseline setup screen.

3. Click **Check now** under the filter block image to verify the instrument configuration. The software displays the current instrument configuration.
4. Verify that the bead lot number printed on the label of the Attune™ Performance Tracking Beads you are using match the bead lot number used in the current baseline.



5. Vortex the Attune™ Performance Tracking Beads bottle to resuspend the beads, then add 3 drops of the bead suspension to 2 mL of focusing fluid in a 12 × 75-mm tube. Mix the bead suspension in the focusing fluid by gentle inversion or vortexing.
6. Load the tube by placing it in the sample tube lifter.
7. Click **Run Performance test** to initiate the automated Performance test.
The *Performance test screen* provides progress information for the Performance test procedure, which takes about 5 minutes to complete.

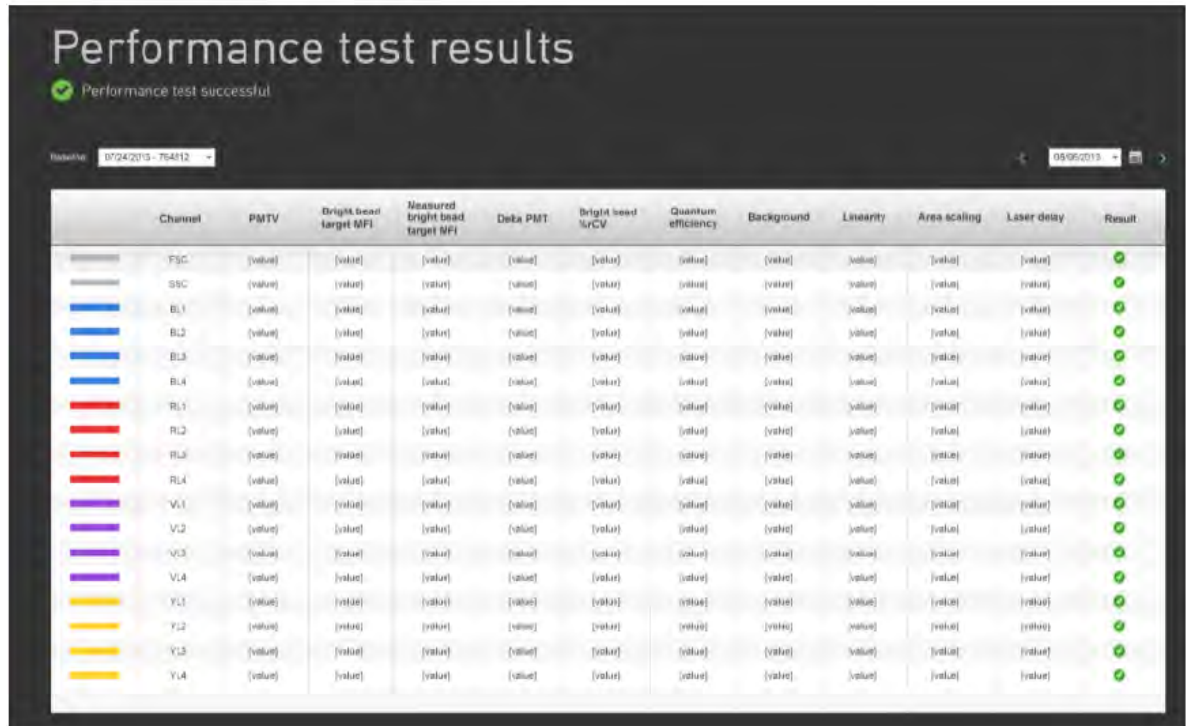


Note: If the *Startup* procedure has not been performed, the button displays **Run Startup** instead of **Run Performance test**. You must run the Startup function before proceeding.

Performance test completion

If the Performance test completes without errors, the software:

- Displays the *Performance test results* screen. For details on how to interpret the Performance test results, see the *Attune™ Cytometric Software User Guide*.




The screenshot shows the 'Performance test results' screen with a green checkmark and the text 'Performance test successful'. Below this is a table with the following columns: Channel, PMTV, Bright bead target MFI, Measured bright bead target MFI, Data PMT, Bright bead %CV, Quantum efficiency, Background, Linearity, Area scaling, Laser delay, and Result. The table contains 20 rows of data, each representing a different channel (FSC, SSC, BL1-4, RL1-4, VL1-4, YL1-4) and showing various performance metrics. All 'Result' columns contain a green checkmark, indicating that all tests passed successfully.

Channel	PMTV	Bright bead target MFI	Measured bright bead target MFI	Data PMT	Bright bead %CV	Quantum efficiency	Background	Linearity	Area scaling	Laser delay	Result
FSC	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	✓
SSC	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	✓
BL1	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	✓
BL2	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	✓
BL3	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	✓
BL4	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	✓
RL1	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	✓
RL2	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	✓
RL3	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	✓
RL4	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	✓
VL1	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	✓
VL2	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	✓
VL3	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	✓
VL4	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	✓
YL1	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	✓
YL2	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	✓
YL3	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	✓
YL4	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	[value]	✓

- Calculates the system area scaling factor constant based on the Performance test results for each configured laser.
- Updates the default system values for the area scaling factor in the Instrument Settings panel.
- Applies the measured laser delays to the system settings in the Instrument Settings panel.

If the Performance test completes with errors, the software displays the *Errors Detected* dialog, which provides a list of the channel statistics that failed and the recommended actions to remedy the errors.

Note: To access the most recent Performance test report, click **Current PT result**  on the Performance Test ribbon tab of the BL/PT module. For more information, see the *Attune™ Cytometric Software User Guide*.

IMPORTANT! At the end of Baseline setup and Performance test procedures, run the **Sanitize SIP** function before running any samples.

Performance test reports

After running the Performance tests, you can generate the following reports in the Attune™ Cytometric Software:

- Performance history
- Current PT results
- Levey-Jennings report
- Baseline results

Note: For more information on viewing and interpreting the Performance Test reports, see “Performance test reports” in the *Attune™ Cytometric Software User Guide*.

4

Run samples

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Before you begin

Recommended flow rates

The Attune™ CytPix™ Flow Cytometer has a range of flow rates from 12.5 µL/minute to 1000 µL/minute.

- At low sample rates (e.g., 12.5 µL/minute and 25 µL/minute), the instrument operates predominantly as a hydrodynamic focusing instrument. These rates are recommended for small particles (diameter <2 µm) and for dim expressing assays relative to an unbound, fluorescent background contributor.
- Sample input rates 100 µL/minute, 200 µL/minute, and 500 µL/minute are not recommended for particles <2 µm in size.
- The highest rate is not recommended for particles <4 µm in size.
- At higher flow rates some loss of sensitivity may occur.
- The instrument can collect high-quality images at all flow rates from 12.5 µL/minute to 1000 µL/minute.

For more information, see “Flow rate” on page 76.

Guidelines for imaging

- The Attune™ CytPix™ Flow Cytometer can collect high-quality images at all flow rates from 12.5 µL/minute to 1000 µL/minute.
- The visual detection limit of the instrument is 800-nm particles and the pixel resolution is 0.3 µm/pixel.
- The image capture rate of the instrument is 3,000–6,000 images/second, which is dependent on image size and event rate.
- The maximum and minimum size of the image window is 248 × 248 pixels and 96 × 96 pixels (width × height), which correspond to an image size of 74 × 74 µm and 29 × 29 µm, respectively. Because larger images require more storage, we recommend capturing smaller images for small cells and increasing the image size as needed for larger cells or cell complexes when cell-cell interactions are of interest.

- The maximum event rate of the instrument is 35,000 events/second. While most events can be imaged at typical flow cytometry event rates, event and image rates diverge at higher rates. We recommend image gating to capture images only from populations or cells of interest.
- Addition of imaging to the flow cytometry workflow greatly increases data storage requirements. To reduce the volume of data generated, we recommend using the Image Frequency tool to capture only a subset of images in the gated population and choosing the smallest image size that satisfies your experimental needs.

Table 1 Data requirement examples

Experiment	No images (FCS file only)	100 reference images from a gated population	All images from a gated population (25% of total events)	All events imaged
1 tube (20,000 events)	4 MB	8 MB	232 MB	916 MB
96-well plate	384 MB	768 MB	14,000 MB	89,000 MB

Sample requirements

- The Attune™ CytPix™ Flow Cytometer is designed to handle samples in tubes that range from 17 × 100-mm to 8.8 × 45-mm in size.
- The method used to prepare a sample depends on the sample type and the assay.

Startup

If not yet performed, execute the **Startup** procedure.

Performance test

If not yet performed, execute the **Performance test** procedure and verify that the instrument is in good working order.

Wash procedure between users

We recommend that you sanitize the system after completing Baseline and Performance tests and between users:

1. Run the **SIP sanitize** function with 3 mL of 10% bleach solution (1 in 10 dilution of 5.25% sodium hypochlorite in water).
2. Run the **Rinse** function after the SIP sanitize function is complete.

Note: For more information on SIP sanitize and Rinse functions of the instrument, see the *Attune™ Flow Cytometry Maintenance and Troubleshooting Guide* (Pub. No. 100024234), available for download at www.thermofisher.com/attune.

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.


The New Experiment dialog allows you to create the following experiment types:

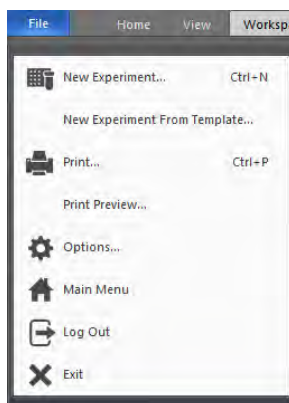
- Tube-only Experiment
- Plate Experiment


The contents of the *New Experiment* dialog depend on the Experiment type you select. For more information on the New Experiment dialog, see the *Attune™ Cytometric Software User Guide*.

Open the New Experiment dialog

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

- Click the **New Experiment** icon  on the Main Menu or the Home tab.
- Select **File** ▶ **New Experiment** on the Ribbon bar.

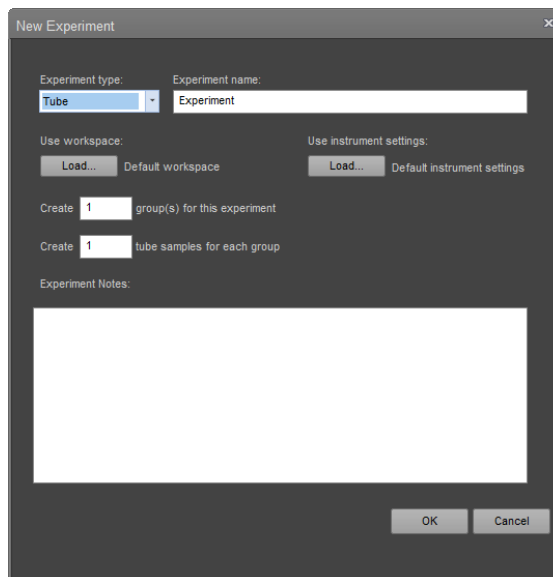
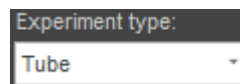


- Right-click on the **User** icon  in Experiment Explorer, then select **New Experiment** from the context menu.

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

Create a Tube-only Experiment

1. From the **Experiment type** dropdown menu on the New Experiment dialog, select **Tube** (default selection).

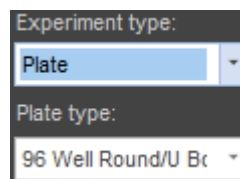


2. Accept the default experiment name or enter a new name.
3. Accept the default workspace and default instrument settings or load saved files.
4. Enter the number of tube groups to create for the Experiment.
5. Enter the number of tube samples to create for each tube group.
6. Click **OK** to create the new experiment and close the *New Experiment* dialog.
The software opens the first sample in the Experiment and the Experiment Workspace.

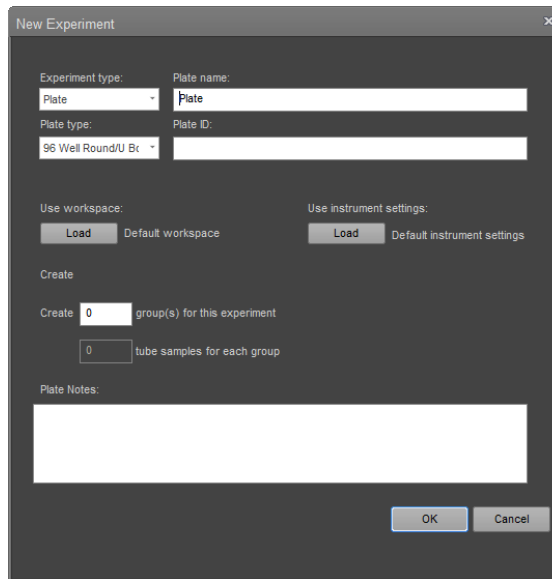
Create a Plate Experiment

A *Plate Experiment* includes one plate, but it 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. *Optional* If you are using a foil cover to protect the sample plate from condensation or evaporation, select **Use Foil Cover**.
5. *Optional* If you are using a cooling block, select **Use Cooling Block**.

When **Use Cooling Block** option is selected, the autosampler accounts for the extra height that the cooling block adds to the plate specification. This option is enabled only for standard 96-well round bottom plates.



Note: Use Foil Cover and Use Cooling Block options are available only when a CytKick™ Max Autosampler is connected to the cytometer.

6. Accept the default workspace and default instrument settings, or load saved files.
7. If you are including tubes, enter the number of tube groups to create for the experiment.
8. If you are including tubes, enter the number of tube samples to create for each tube group.
9. Click **OK** to create the new Experiment and close the *New Experiment* dialog.

Optimize instrument settings

Before you can record data for a sample, you need optimize instrument settings. Optimizing instrument settings involves fine-tuning the PMT voltages, compensation, and threshold settings for each dye and sample used in the experiment, which allows you to adjust the positions of populations of interest on scale for the scatter and fluorescence parameters.

Note: We recommend that you optimize each Experiment before collecting data. If compensation is to be applied, ensure that all Voltage settings (except FSC and SSC) have been finalized for all samples before recording any data. After compensation is recorded, it is not possible to adjust voltages for samples.

Before you begin

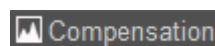
- If the experiment requires compensation, prepare the necessary compensation controls. Each fluorophore used for compensation requires single-stained controls (i.e., compensation beads or cells). Unless you select to use a negative gate or none, an unstained or isotype-labeled control is also required.
- We recommend that you optimize the instrument settings for compensation controls in the *Compensation Workspace*.
To optimize instrument settings for compensation control samples, open the *Compensation Setup dialog* as described in “Define control samples” on page 38 and select the necessary parameters for compensation.
- The Compensation Setup dialog provides different options for setup based on your selection of the compensation source. See page 40 – page 42 for details about optimizing compensation controls.
- If no compensation is necessary, you can optimize the instrument settings within the *Sample Workspace* in the Experiment Explorer. The procedure for optimizing samples is similar to that described for compensation control samples.
- Adjust all voltages to put the population of interest on scale in all necessary channels. Set voltages to maximize the signal-to-noise ratio.
- You can adjust the threshold and voltages using the *Instrument Configuration* tab on the Collection Panel. Alternatively, you can use *Instrument Settings* in the View tab, which gives you the additional ability to rename parameters.
- For more information on the Instrument Settings tab, see the *Attune™ Cytometric Software User Guide*, available for download at www.thermofisher.com.

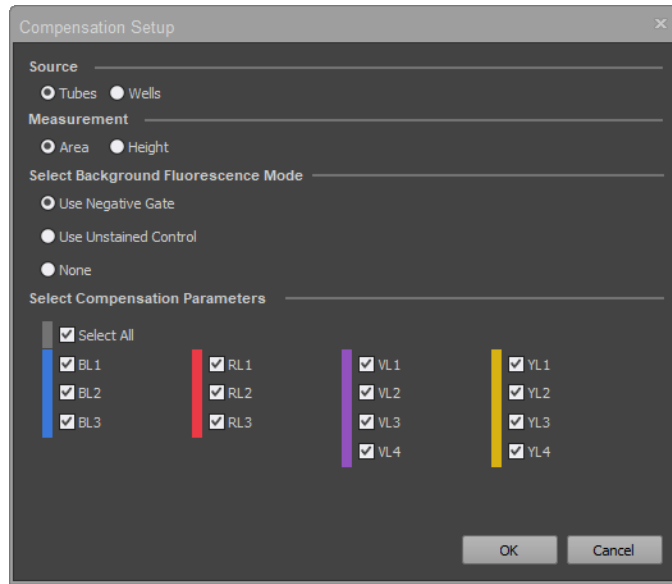
Define control samples

1. To define the control samples, click the **Compensation Setup** button on the *Compensation ribbon tab* to open the Compensation Setup dialog.

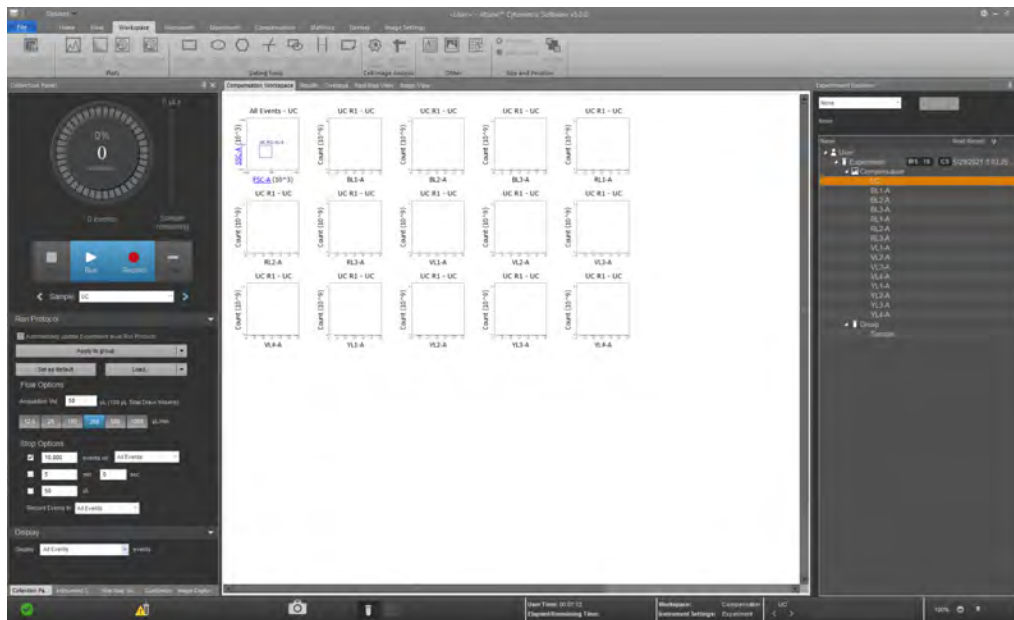
Alternatively, double-click on the **Compensation node** within the Experiment on the *Experiment Explorer* when there are no compensation controls present.

Each of these methods launches the *Compensation Setup dialog*.



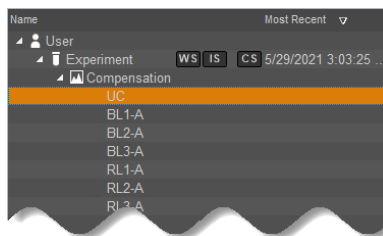


2. On the Compensation Setup dialog, select **Tubes** or **Wells** as the source of compensation controls. The Compensation Setup dialog provides different controls based on your selection of the compensation source.
3. Next, select the **Measurement** parameter, **Background Fluorescence Mode**, and the **Compensation Parameters**. For more information on each option, refer to the “Compensation” chapter in the *Attune™ Cytometric Software User Guide*.
4. Click **OK**. The dialog box closes and the software creates or updates the compensation control for each selected parameter in the Experiment Explorer. The *Compensation Workspace* for the first control or the unstained control (if **Use Unstained Control** was selected) opens automatically. The following example shows the Compensation Workspace for the unstained control.



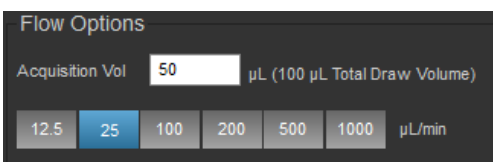
Optimize instrument settings for unstained control

1. Double-click **UC** (i.e., unstained control) under Compensation in the Experiment Explorer.



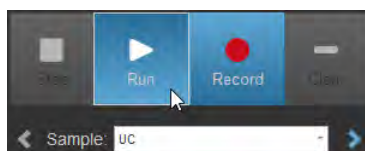
The Compensation Workspace for the unstained control contains one SSC vs. FSC plot with a polygon gate, and histogram plots for each of the fluorescent parameters selected during the compensation setup.

2. Install the unstained control on the tube lifter.
3. In the *Collection Panel*, enter the **Acquisition Volume**, and then select the **Flow Rate**.



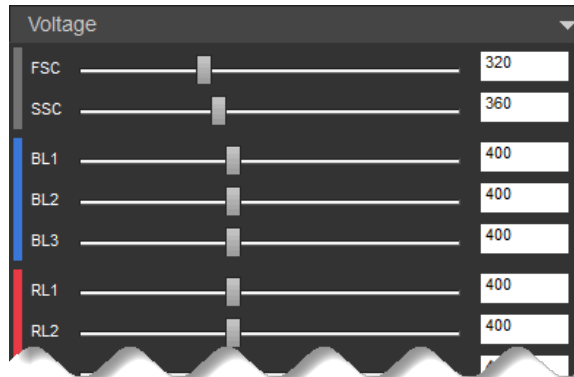
Note: For setup, you can conserve your sample by running the cytometer at a 25 µL/minute collection rate.

4. Click **Run**. Events will appear in the FSC vs. SSC plot. You can obtain data in real-time without saving to a file.

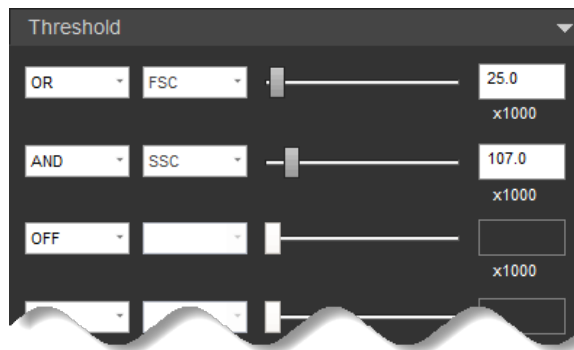


IMPORTANT! DO NOT click **Record** at this point. Once you click **Record** on any of the compensation controls within the Compensation Setup, the instrument settings for fluorescent channels cannot be changed. It is critical that you optimize voltages before recording any sample or any compensation controls.

5. Select *Instrument Settings*, then adjust the **FSC voltage** to place the population on scale. Alternatively, type a specific numerical value in the settings window above each channel.



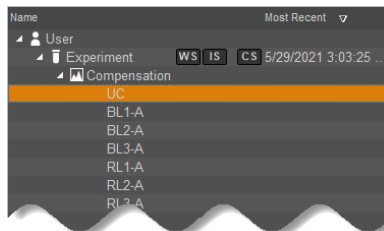
6. Adjust the **SSC voltage** to place the population on scale.
7. Adjust the **Threshold** to remove unwanted events and background.



8. Set the scatter gate on the population of interest so that the fluorescence histograms are reflective of the population for which you are optimizing your voltages.
9. Adjust the voltage for **Fluorescence Channels** to get the best separation of signal-to-noise ratio.
10. Remove the unstained control from the sample injection port.

Optimize instrument settings for single-stained controls

After you have optimized the instrument settings for the unstained control (if applicable), optimize the instrument controls for each of the single-stained controls.



IMPORTANT! DO NOT click **Record** during this procedure. Once you click **Record** on any of the compensation controls within the Compensation Setup, the instrument settings for fluorescent channels cannot be changed. It is critical that you optimize voltages before recording any sample or any compensation controls.

1. Stay on the **UC** (i.e., unstained) compensation control.
2. Install the first single-stained control on the tube lifter.
3. Using the same optimization procedure, adjust the instrument settings and set the scatter gate on the population of interest.
4. For each compensation control sample (i.e., fluorophore), observe the corresponding histogram to optimize the voltages.
5. Perform the optimization procedure for all single-stained controls.
6. After you have optimized instrument settings for each single-stained control, proceed to “Calculate compensation” on page 43.

Note: To apply the Scatter Gate defined in the **UC** (i.e., unstained) control to all other compensation controls, right-click the gate (R1) and select **Apply gate shape to all controls...**

Note: The Attune™ Cytometric Software automatically executes the **Rinse** function each time the tube lifter is pushed down to remove the sample from the sample injection port. This ensures that the fluidics system of the instrument is flushed and any remaining sample is removed to minimize carryover.

Calculate compensation

Fluorophores emit light over a range of wavelengths. Although optical filters limit the range of frequencies measured by a given detector, when two or more fluorophores are used in an experiment, there is often an overlap between the wavelength ranges. Compensation is the mathematical method used to correct the overlap of one fluorophore's emission into another fluorophore's emission channel.

The software calculates the compensation settings automatically as it guides you through the process.

Before you begin

Before you begin the compensation acquisition procedure to record compensation controls and calculate the compensation matrix, ensure that you have:

- Defined compensation controls and selected compensation parameters
- Optimized instrument settings

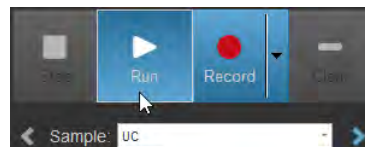
See for “Optimize instrument settings” on page 38 more information.

IMPORTANT! Once you have recorded compensation controls and calculated and applied the compensation matrix, you cannot change Instrument Settings for fluorescent channels and adjust the PMT voltages for experiment data. It is critical that you optimize voltages before you record any compensation controls or run samples.

Compensation acquisition from tubes

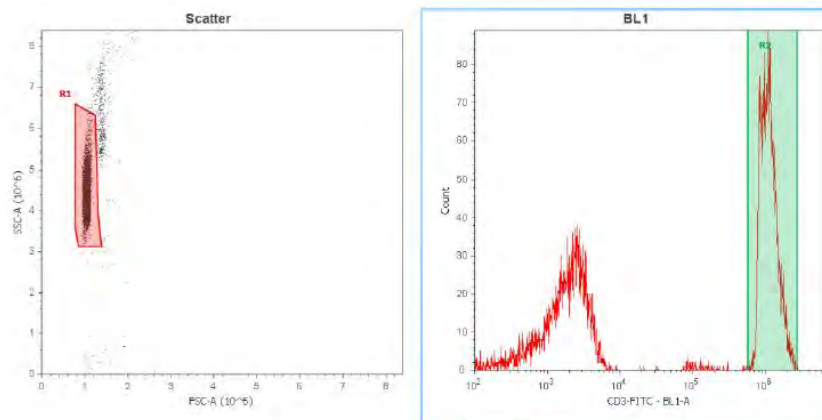
Compensation setup and acquisition from tubes is performed manually.

1. Open the Compensation Setup dialog, then select **Tubes** as the compensation source.
2. Select the type of compensation to perform and the desired channels, then click **OK**.
Compensation Workspace opens and is automatically populated with the plots necessary to calculate compensation.
3. Install the tube containing the unstained control beads/cells on the sample injection port as prompted by the software.
4. Push up the tube loader to the active position in the sample injection port, then click **Run** on the Collection Panel.



5. Wait until the sample equilibrates, then click **Record**.

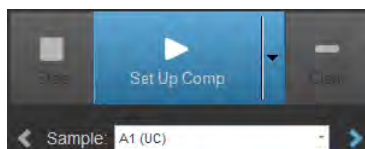
- Repeat the process for each of the single-stained controls, making sure that the positive signal for all samples is on scale.




Note: The Compensation matrix is calculated as new compensation controls are recorded. The calculation will only include the controls that have been recorded.

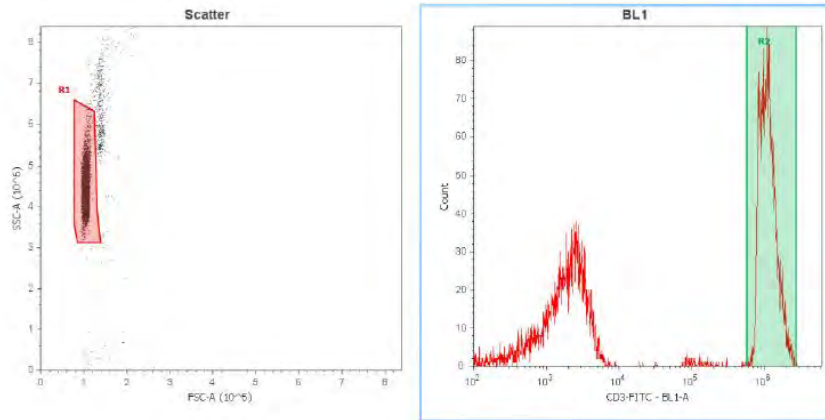
Compensation acquisition from wells


Compensation setup and acquisition from wells is initiated using the **Set Up Comp** button on the *Collection panel*. This button is displayed by default if the plate contains compensation wells and the system is ready for acquisition setup.

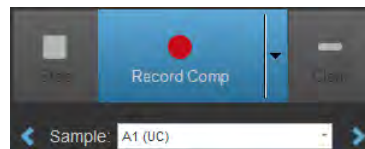


- Open the Compensation Setup dialog, then select **Wells** as the compensation source.
- Select the type of compensation you want to perform and the desired channels, then click **OK**. Compensation Workspace opens and is automatically populated with the plots necessary to calculate compensation.
- Ensure that the sample plate containing the compensation control beads is loaded into the cytometer or the auto sampler as prompted by the software.
- Click **Set Up Comp** to initiate the optimization of compensation control samples in the *Set Up mode*, starting with the unstained control (UC).
- While unstained control is running, use the **Voltage** controls on the Instrument Settings tab to optimize the voltages for the scatter parameters for FSC and SSC.
- Set the scatter gate on the population of interest so that the fluorescence histograms are reflective of the population for which you are optimizing your voltages.
- Right-click on the gate, then select **Apply gate shape to all controls....**

8. Click **Stop** on the Collection Panel when you are satisfied with the data, then click **Next**  to move onto the next compensation control.
9. Again, using the **Voltage** controls on the Instrument Settings tab, optimize the voltage for the selected compensation control.
10. Set the **R2 gate** on the peak of interest on the histogram plot.



11. Repeat the setup steps for the remaining compensation controls and ensure that the gates are set appropriately on the population of interest.
12. After you have completed the optimization of instrument settings, click the **Next**  navigation button on the last compensation control sample to advance the task button to **Record Comp**.
13. Click the **Record Comp** button on the Collection panel to initiate the recording of the compensation control wells.



The software acquires and records compensation controls starting with the first defined control using the Run Protocol applied to each well.

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

Note: The parameter order for compensation controls in plates is unstained control, blue laser controls, red laser controls, violet laser controls, and yellow laser controls, and it is independent of the well location on the plate.

Compensation acquisition from files

- Files can also be imported into each compensation control created using the **Tube** option. Imported files behave like recorded compensation and force the matrix to recalculate.
- The Compensation matrix is calculated as each control workspace is loaded and the gates are adjusted.

IMPORTANT! Once you have recorded all compensation controls and calculated and applied the compensation matrix, you cannot adjust the PMT voltages for experiment data.

If you need to adjust voltages after you have recorded any of the compensation controls, you must first remove the data from compensation controls that contain recorded data. To remove recorded data from a compensation control, do one of the following:

- Right-click on the control sample, then select **Remove FCS file**.
- Right-click on the **Compensation** node in the Experiment Explorer, then select **Remove Compensation**.

Note: If you choose the **Remove Compensation** option, you must perform the Compensation setup procedure again to recreate all compensation control samples.

Review the Spillover matrix

When the last compensation control is recorded, the *View Matrix dialog* automatically opens for your review. The View Matrix dialog displays the *Spillover matrix*.

View Matrix

Matrix - 20210531_JurkatEtOH_P14

Spillover values are read across rows

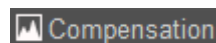
Spillover

	BL1-A	BL2-A	BL3-A	RL1-A	RL2-A	RL3-A	VL1-A	VL2-A	VL3-A	VL4-A	YL1-A	YL2-A	YL3-A	YL4-A
BL1-A	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
BL2-A	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
BL3-A	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
RL1-A	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
RL2-A	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
RL3-A	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
VL1-A	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
VL2-A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00
VL3-A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00
VL4-A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00
YL1-A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00
YL2-A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00
YL3-A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00
YL4-A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00

OK Cancel Reset

To launch the View Matrix dialog at other times, you can also do one of the following:

- Click the **View Matrix** button on the *Compensation ribbon tab*.
- Double-click the **Compensation node** on the Experiment Explorer when compensation controls are present.



Click **OK** to accept any changes made to the matrix and apply the updated compensation values to the dataset. You can also manually edit or reset the compensation matrix values (not recommended).

Note: The *Spillover matrix* shows the amount of spillover from each fluorophore into each of the other fluorescent channels. Data compensation is calculated using the *Compensation matrix*, which is the inverse of the Spillover matrix.

Run samples and collect data

After you have calculated the compensation settings, you are ready to run your samples to acquire and record data.

Run and Record modes

The Attune™ Cytometric Software has two modes for data collection, the *Run* mode and the *Record* mode.

Run mode

The *Run* mode is initiated when you select **Run** on the Collection Panel.

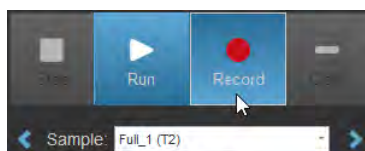


- In the Run mode, the software starts data acquisition but no FCS file containing the data is created.
- Run mode is primarily used for adjusting the various parameters for cytometer and experiment optimization and customizing the charts for recording.
- During the run, the events are displayed on the plots as the graphs are being populated.

Note: If compensation is available and turned ON, data that is displayed will be compensated during acquisition.

Record mode

The *Record* mode is initiated when you select **Record** on the Collection Panel.



- During recording, the events are displayed on the plots as the graphs are being populated.
- In the Record mode, the software automatically creates an FCS file when you click **Stop**.
- The software creates a unique name for each FCS file so that an existing file is not overwritten.
- If the Sample already has an FCS file, the software displays a warning that the FCS file is going to be overwritten. You can choose to append the existing FCS file or overwrite it (see “Optional: Append data” on page 55).

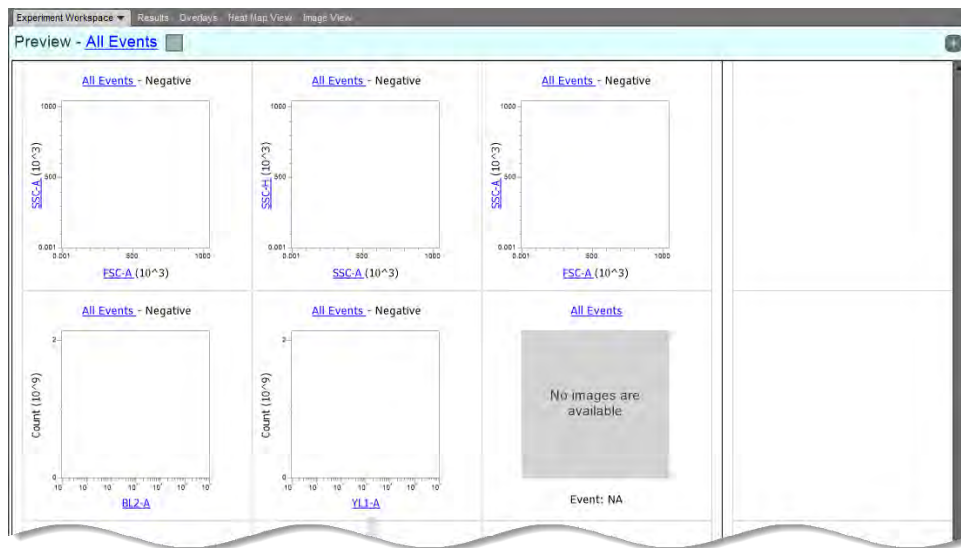
Prepare the Experiment Workspace

The *Experiment Workspace* can display plots, gates, statistics, text boxes, and cell image containers, all of which can be created using the options available on the *Workspace ribbon tab*. Prepare your Experiment Workspace and customize your plots by selecting each plot one at a time. Change the axis labels and other properties appropriate to your experiment.

Note: For detailed information about the Experiment Workspace and the Workspace ribbon tab, see the *Attune™ Cytometric Software User Guide*.

1. From the Workspace ribbon tab, select the desired **Plot**, then click on the Experiment Workspace to add the plot to the workspace. Repeat until you have added all the plots you want to use for your experiment.
2. To display cell images on the Workspace, select **Cell Image** from the Cell Image Analysis tools on the Workspace ribbon tab, then click on the Experiment Workspace to add a **Cell Image Container**.

Note: *Cell Image Containers* allow you to add cell images specific to the sample to the current Workspace. They act like plots and can be batch printed with the Experiment Workspace (cell images cannot be printed from the *Image view* tab).

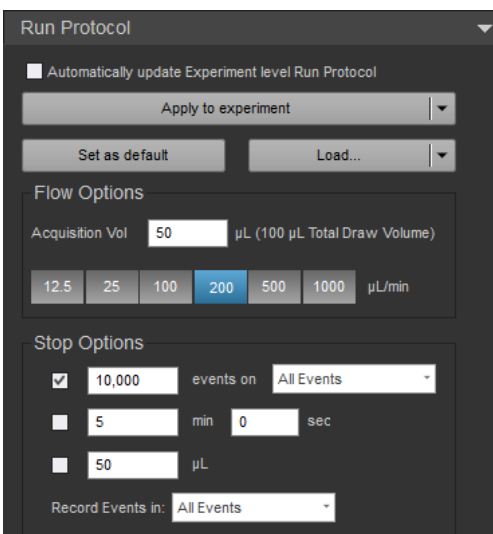


3. To change the parameter displayed on a plot axis, click the **plot parameter hyperlink**, then select the desired parameter.

Alternatively, right-click on the axis label, then select the desired parameter from the **Parameter** sub-menu.

Acquire data

1. On the Experiment Explorer panel, double-click the **Sample** you wish to run to activate it. Experiment Workspace displays the default sample plots set up for the Experiment when the Workspace was prepared. If desired, you can delete or modify these plots.
2. Enter the collection criteria in the **Run Protocol** on the Collection Panel. Set limits to collection by the number of events for specified gates, total sample volume analyzed, or by elapsed time.



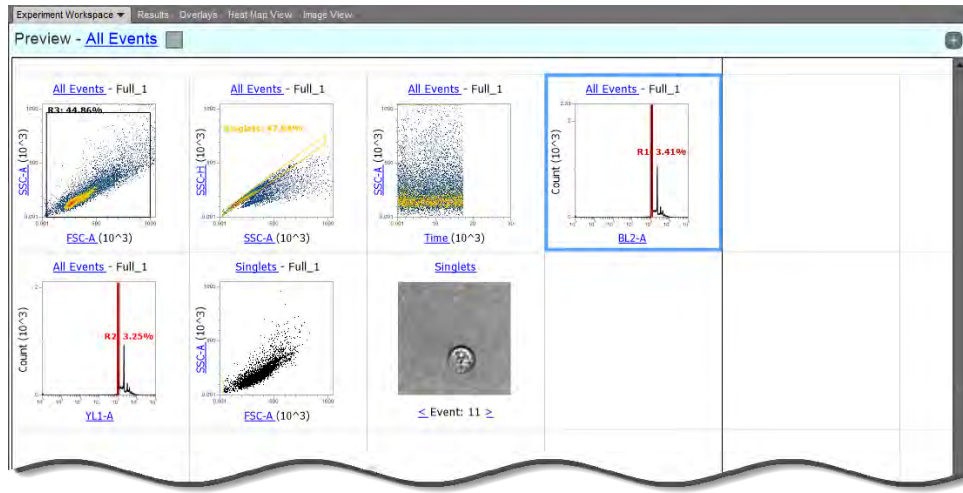
3. Install the tube containing the sample on the sample injection port, then lift up the tube loader to the active position.
4. On the Collection Panel, click **Run**.



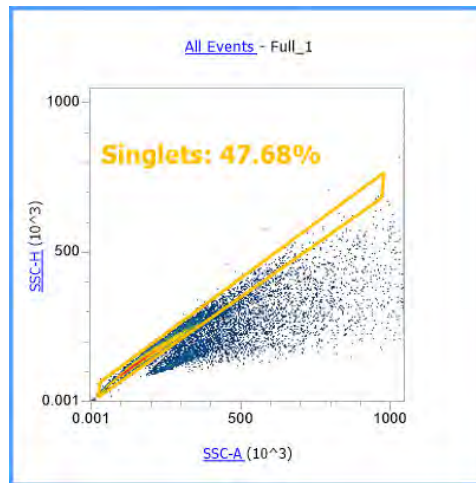
The events are displayed on the plots as the graphs are being populated. Wait a short time for the sample to equilibrate.

5. Using the **Voltage** and **Threshold** controls on the Instrument Settings panel, adjust the PMT voltages and threshold values for the appropriate channels. Ensure that the events are on scale.

- From the **Gating Tools** on the Workspace ribbon tab, select the desired gates to add to the plots on the Experiment Workspace to isolate and analyze selected populations.



Note: You can use gates to create subsets within defined populations, generate statistics, and limit the number of events collected or stored.



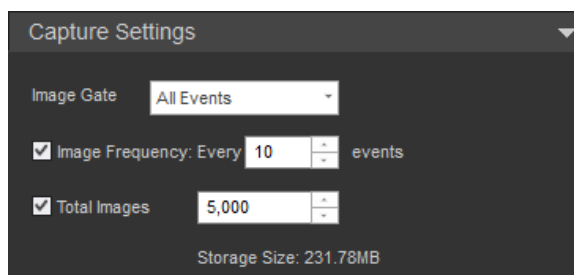
Determine Image Capture Settings

The *Image Capture Settings* panel allows you to adjust settings that affect the capture of images, which are grouped under Capture Settings, Camera Settings, and Image Size and Position Settings.

- You can adjust the Image Capture Settings at any time before or during Run mode to optimize image capture and store the settings at Sample- or Experiment-level.
- During Record mode or after data recording is completed, the Image Capture Settings are disabled to reflect the state in which the sample was recorded.
- For imaging guidelines and strategies to reduce the amount of space required for image data storage, see “Guidelines for imaging” on page 33.

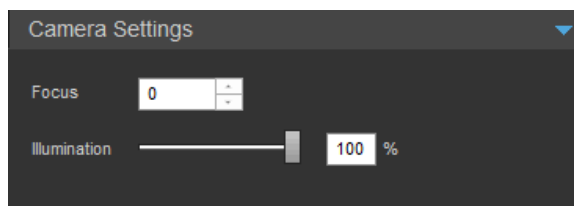
Note: For detailed information about the Image Capture Settings panel, see the *Attune™ Cytometric Software User Guide*.

1. Use the **Capture Settings** controls to choose the **Image Gate**, then select the **Image Frequency** and **Total Images** options.



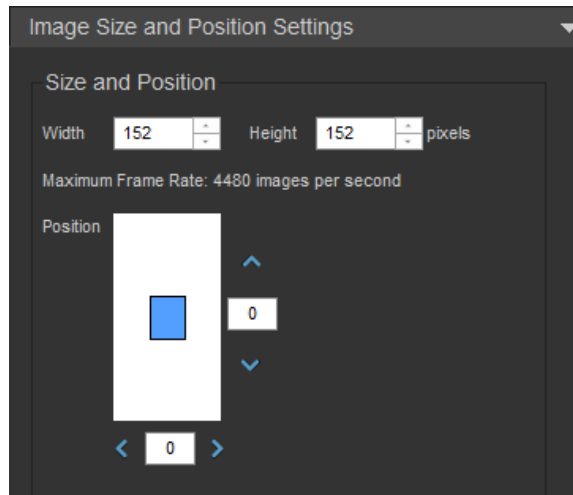
- Image Gate allows you to capture images only from a specific subset of events on a Workspace plot and exclude all events outside that gate.
- Image Frequency allows you to capture only a subset of reference images rather imaging all events.
- Total Images sets the maximum number of images to be captured during a run.

2. If needed, adjust **Focus** and **Illumination**.



Note: Although the camera focus is pre-set to the optimal position by service engineers, depending on cell size, cell type, or flow rate, you might choose to modify the focus value to suit the needs of your experiment. Usually, a change of ± 10 units is sufficient to find the ideal focus position for most applications.

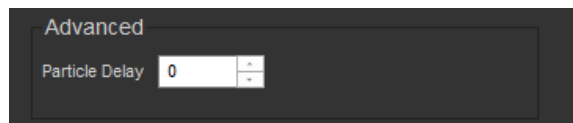
- Use the **Size and Position** controls to adjust the size and position of the image window.



- Width** and **Height** allow you to set the dimensions of the captured image. The maximum and minimum size of the image window is 248 × 248 pixels and 96 × 96 pixels (width × height), which correspond to an image size of 74 × 74 μm and 29 × 29 μm, respectively.
- Position** controls allow you to adjust the position the image window within the scanning region. By default, the image window (blue rectangle) is centered around the coordinates 0 and 0.

IMPORTANT! Changing image capture settings is not instant; it takes 1–2 seconds for the camera to be cleared and streaming to restart. We do **not** recommend changing the image size and position incrementally with mouse clicks.

- If needed, adjust **Particle Delay** (not recommended), which allows you to change the image delay relative to the image trigger event.

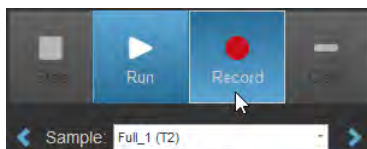


Note: Particle Delay changes the position of the particle to keep it within the image capture region. Size and Position settings move the image capture region within the scanning area. If the Particle Delay and Size and Position settings are mismatched, you will not be able to see and image the particle.

To reset the Position and Particle Delay settings, enter 0/0 for Position to center the image field in the scanning region and enter 0 for Particle Delay.

Record data

1. While still in the *Run mode*, adjust the **Voltage** and **Threshold** values for the appropriate channels using the Instrument Settings tab. Make sure that the events are on scale.
2. Click **Record** to start data collection.

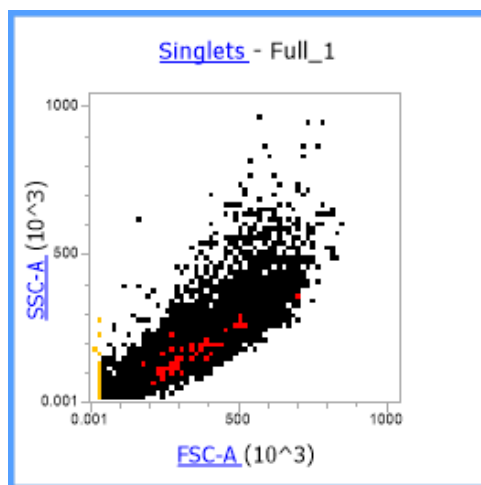


Data is recorded until any of the conditions set in **Run Protocol** is satisfied.


3. Click **Stop** to stop the data collection. The software automatically saves the data in a unique FCS file.

Optional: Backgate images

Image backgating allows you to identify the event that is associated with the captured cell image (displayed in *Image View*) on a Dot plot or a Precedence Density plot in Workspace view. When image backgating is applied, the events that have a corresponding image are automatically identified in red.



Note: We recommend that you lower the resolution of the plot associated with the captured cell images to 64 × 64 pixels so that the events are more easily identified.

Backgated images display the "eye" icon  in Image View.



1. Right-click on the cell image you want to backgate in Image View, then select **Show Image(s) on Plots**. You can select the Active image or any image from the Image Gallery.
2. To backgate multiple images, multi-select the images you want to backgate in the Image Gallery, then right-click and select **Show Image(s) on Plots**.
3. To backgate all imaged events on the corresponding Workspace plots, press Ctrl+A on the keyboard to select all images in the Image Gallery, then right-click and select **Show Image(s) on Plots**. Alternatively, click **Backgate All Images** in the Image Settings ribbon tab.

Optional: Append data

After recording data, you can append the data file with additional data. If you wish to append a data file for a Sample, you cannot modify the Instrument Settings or collection rate, or change the Sample (e.g., go to the next Sample). However, you can execute a **Rinse** function prior to appending data.

To append data:

1. Click **Run** button, and then click **Record**.
A dialog box opens and prompts you to select **Append**, **Overwrite**, or **Cancel**.
2. Click **Append** if you wish to add the new data to the existing FCS.

Note: When appending data, the stop criteria is based on the individual record cycle and not the total resulting FCS file.

For example, a file containing 10,000 events appended with a run that has stop criteria of 20,000 events will result in a file with 30,000 total events.



Shutdown

- Workflow 56
- Shutdown procedure 56

Workflow

IMPORTANT! Although the daily **Shutdown** procedure is automated and requires minimal user input, we recommend that you familiarize yourself with the instrument and its operating principles by reading “Appendix A: System Overview” (page 59) and “Appendix B: Technical Overview” (page 75) before starting your experiments.

For a detailed description of the software user interface, see the *Attune™ Cytometric Software User Guide* (Pub No. 100024236).

Shutdown procedure

The **Shutdown** function of the Attune™ Cytometric Software facilitates the automated shutdown of the instrument. The function removes all sample fluid and dyes from the system, decontaminates the fluidics lines and sample pumps, and fills them with the Attune™ Shutdown Solution to prevent the formation of salt crystals.

Depending on the Shutdown option selected, the procedure takes 25 to 70 minutes to complete; however, most steps are automated and do not require monitoring. At the end of the Shutdown procedure, the cytometer is automatically powered down.

IMPORTANT! Perform the following shutdown procedures 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.

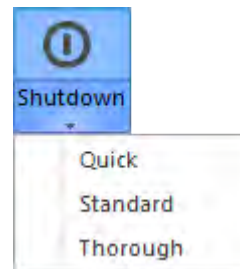
Check fluid and waste levels

1. Check the levels in the fluid tanks.
2. Ensure that the Wash and Shutdown solution tanks are at least half-full.

Shutdown options

There are three options available for the **Shutdown** function:

- **Quick** – Quick option uses 15 bleach scrubs and 10 wash scrubs through sample pathway lines.
- **Standard** – Standard option uses 25 bleach scrubs and 20 wash scrubs through sample pathway lines.
- **Thorough** –Thorough option uses 35 bleach scrubs and 30 wash scrubs through sample pathway lines.



For daily use, we recommend the **Standard Shutdown** function.

Run shutdown function

1. Before running the Shutdown function, empty the Waste containers and refill the fluid tanks with the appropriate solutions.
2. On the Instrument ribbon tab, click the **Shutdown** button to run the Standard shutdown option. To choose from the **Quick**, **Standard**, or **Thorough** options, click the arrow on the **Shutdown** button, then select from the dropdown menu.

The *Shutdown dialog* opens and provides step-by-step instructions to perform the Shutdown operation. The steps of the Shutdown operation differ depending on whether an auto sampler is connected to the cytometer.

3. If the tube lifter is raised, lower the tube lifter.
4. Click **Next** to perform an automatic Rinse procedure.

Note: Steps 3 and 4 are required only if the tube lifter was raised at the start of the Shutdown procedure.

5. When the rinse is complete, empty the instrument and auto sampler waste containers.
6. Refill the fluid bottles with the appropriate solutions.
7. Fill a clean tube with 3 mL of 10% bleach, load the tube into the instrument, then raise the tube lifter.

8. Click **Next** to start the Shutdown procedure.
9. At the end of the Shutdown operation, the software automatically puts the instrument in a low power state.

IMPORTANT! If you cancel the shutdown, allow at least 3 minutes for the lasers to reach operating temperature before running any samples. You will also need to re-run Startup.

IMPORTANT! If you intend to leave the instrument in the shutdown state for longer than two weeks, perform system flush and leave the instrument in deionized water to prevent salt crystals from clogging the fluidics system.

Optional: Perform system flush

Perform system flush if you intend to leave the cytometer shut down for longer than two weeks.

1. Replace all fluidic containers (focusing fluid, waste, wash, and shutdown tanks) with deionized water.
2. Run the **Shutdown** function using deionized water on the SIP instead of bleach.
3. Empty all fluidic tanks and decontaminate with 10% bleach.
4. Allow empty fluidics tanks to dry.



System overview

Technical specifications

Optics

Fluorescence detection

Excitation lasers:

Laser	Wavelength (nm)	Divergence (degrees) ^[1]	Power output (mW)	Pulse description
Violet	405	<7.6	50	CW ^[2]
Blue	488	<7.6	50	CW
Yellow	561	<7.6	50	CW
Red	637	<7.6	100	CW

^[1] Measured at the output of Beam Shaping Optic (BSO).

^[2] Continuous wave output.

Laser excitation: Optimized excitation for minimized stray laser-line noise and losses to reflection

Laser profile: 10 × 50 μm flat-top laser providing robust alignment

Emission filters: Up to 14-color channels with wavelength-tuned photomultiplier tubes (PMTs); user-changeable, keyed filters

Laser separation: 100 μm

Optical alignment: Fixed alignment with pre-aligned welded fiber; no user maintenance required

Onboard thermoelectric cooler: No warm-up delay; fiber is not affected by “on/off”

Simmer mode: Instant “on/off” reduces usage and/or aging by 10X; only turns on when acquiring samples; reports hours of usage

Flat-top laser specified at the flow cell: Instant “on/off” reduces usage and/or aging by 10X; only turns on when acquiring samples; reports hours of usage

Upgradable: Convenient field changes

Imaging

Excitation laser:

Laser	Wavelength (nm)	Divergence (degrees) ^[1]	Power output (mW)	Pulse description
Violet	405	<30	1500	≤ 100 ns at 7 kHz

^[1] Measured at the output of fiber optic.

Laser profile: No warm-up delay; fiber is not affected by “on/off”

Emission filters: <50 nanoseconds

Fluidics

Flow cell: Quartz™ cuvette gel coupled to 1.2 numerical aperture (NA) collection lens, 200 x 200 μm

Sample analysis volume: 20 μL–4 mL

Custom sample flow rates: 12.5–1,000 μL/min

Sample delivery: Positive-displacement syringe pump for volumetric analysis

Sample tubes: Accommodates tubes from 17 x 100 mm to 8.5 x 45 mm

Fluid-level sensing: Active

Standard fluid reservoirs: 1.8 L focusing fluid tank, 1.8 L waste tank, 175 mL shutdown solution tank, and 175 mL wash solution tank

Fluid storage: All fluids stored within instrument

Extended fluidics option: Configuration for 10 L fluid

Nominal fluid consumption: 1.8 L/day

Automated maintenance cycles: ≤15 min start-up and shutdown—deep clean, sanitize, and debubble modes

Performance

Fluorescence detection

Fluorescence sensitivity: ≤80 molecules of equivalent soluble fluorochrome (MESF) for FITC, ≤30 MESF for PE, ≤70 MESF for APC

Fluorescence resolution: CV <3% for the singlet peak of propidium iodide–stained chicken erythrocyte nuclei (CEN)

Data acquisition rate: Up to 35,000 events/sec, 34 parameters, based on a 10% coincidence rate per Poisson statistics

Maximum electronic speed: 65,000 events/sec with all parameters

Carryover: Single-tube format: <1%

Forward and side scatter sensitivity: Able to discriminate platelets from noise

Forward and side scatter resolution: Optimized to resolve lymphocytes, monocytes, and granulocytes in lysed whole blood

Forward scatter: Photodiode detector with 488/10-nm bandpass filter

Side scatter: PMT with default 488/10-nm bandpass filter; optional 405/10-nm bandpass filter

Fluorescence detectors: 14 individual detectors

Electronic pulse: Measured area; height and width pulse for all detectors

Violet side scatter resolution: Can be configured for violet side scatter to better resolve particles from noise

Minimum particle size: 0.2 μm on side scatter using submicron bead calibration kit from Bangs Laboratories—0.1 μm on side scatter under following conditions:

- using an Attune™ CytPix™ Flow Cytometer with standard 0.5-mm blocking configuration
- using an Invitrogen™ Attune™ 488/10 Filter (Cat. No. 100083194)
- using Attune™ Focusing Fluid (Cat. No. 4488621, 4449791, or A24904) that has been passed through a 0.025- μm filter

Imaging

Pixel resolution: 0.3 μm /pixel

Objective magnification: 20X

Objective numerical aperture (NA): 0.45

Theoretical resolution: 0.6 μm

Detection limit: Visually detect 800 nanometer particles

Image capture rate: 3,000–6,000 images/second (image is size dependent)

Image size: 96 x 96 pixels–248 x 248 pixels

Field of view: 29 x 29 μm –74 x 74 μm

Attune™ Cytometric Software

Compensation: Full matrix—automated and manual modes, on-plot compensation tools for fine adjustment; use of tubes and wells

Flow rate: Precise flow rate control via software; no hardware adjustments

Live streaming: Live update of statistics during acquisition of events up to 35,000 events/sec

Overlays: Comparative analysis between samples; 3D view

Sample recovery: System able to return unused samples

Concentration: Direct concentration measurement without use of counting beads

Software layout: Fully customizable for each user account

Bubble detection technology: Stops automated run to preserve sample integrity

Maximum single-event file: 20 million with option to append

Heat map: Set up for definition of plate layout; screening view for analysis for tubes and plates



Threshold: Up to 4 individual thresholds with user option to apply Boolean logic

Gating: Hierarchical gating with the ability to derive gates

Voltage: User adjustable

Windows extensions: User adjustable

Area scaling factor (ASF): User adjustable

Acquisition settings: Documented in FCS files and maintained upon import

Templates: Create from existing experiments—instrument settings, workspaces, run protocols, heat map settings, and compensation settings optimized and defined previously

Tube-to-plate conversion: One-click transition from tubes to plates and vice versa; no disassembly, no additional QC, no reboot required for conversion between plates and tubes

Graphics resolution: Publication-quality images; support for TIF, PNG, BMP, JPG, GIF, and EMF; quickly copy and paste plots to any external application (e.g., Microsoft™ PowerPoint™ software)

User account administration: Administrative creation of individual user accounts with designated roles, advanced setting permissions, management of individual accounts, user time tracking, and sample count

Imaging software

Image capture settings: Set total number of recorded images, image frequency, image capture gate, image size and position, focus and illumination for control over experiment design and data footprint

View images: Image view option allows overview of the gallery of images combined with cell image option to view individual images on the workspace for any cell population

Image backgating: Correlate images to flow cytometry data by backgating either all or selected images onto supported workspace plots

Image measurement tool: Elliptical tool to measure the area of events in images in μm^2

Image export options: Export images as 8 bit TIF, PNG, GIF, BMP, JPG or EMF

Computer

Software requirements: Invitrogen™ Attune™ Cytometric Software

Monitor: 27-in. flat panel (1,920 x 1,080 resolution); dual-monitor capability

Computer: Minitower desktop

Operating system: Microsoft™ Windows™ 10 software, 64-bit

FCS format: FCS 3.1, 3.0

Processor: Intel™ Core™ i7 processor

RAM: 64 GB

Hard drives: 2 x 8 TB SSD, 560 MB/sec; controller RAID1, integrated

GPU: NVIDIA™ Quadro™ P2200

Installation requirements

Electrical requirements:

- 100–240 VAC, 50/60 Hz, <150 W
- Thermo Fisher Scientific certifies that the Attune flow cytometers conform to relevant directives to bear the CE mark
- The instrument conforms to the UL and CAN/CSA general requirements (61010.1)
- The Attune flow cytometers are class I laser products per Center for Devices and Radiological Health (CDRH) regulations and EN/IEC 60825

Heat dissipation: <150 W**Temperature operating ranges:** 15–30°C (59–86°F)**Operating humidity:** 10–80%, noncondensing**Audible noise:** <65 dBA at 1.0 m**Instrument size (H x W x D):** ~49 x 58 x 43 cm (19 x 23 x 17 in.), including fluid bottles**Weight:** ~33 kg (73 lb)

Operation principles

The Invitrogen™ Attune™ CytPix™ Flow Cytometer is a benchtop high-resolution imaging cytometer that uses acoustic pressure to confine the injected particles to a tight central line as the sample passes through the optical cell for interrogation.

Acoustic focusing places the interrogated particles within a narrower depth-of-field compared to conventional imaging cytometers that use hydrodynamic focusing alone, which allows the production of in-focus images at standard flow cytometry rates (up to 5,000 images/second at 500 µL/min flow rate).

This section explains how the Attune™ CytPix™ Flow Cytometer measures scattered light and fluorescence as cells pass through the laser beam and how acoustic-assisted hydrodynamic focusing allows the production of in-focus images at standard flow cytometry rates.

Sample loading

The sample is loaded into the cytometer via the sample injection tube, which automatically delivers the sample to the flow cell after the tube lifter is engaged and the user defines collection criteria.

The sample is pushed through a capillary assembly and wrapped in a sheath of focusing fluid before it is intercepted by the laser beam for interrogation. The capillary assembly is an acoustic resonant device that focuses cells or particles into a single, tight line using a capillary coupled to a piezoelectric transducer.

Acoustic focusing

Acoustic focusing exploits the size, density, and compressibility differences between cells or particles relative to the background carrier medium to position the particles or cells into a single, focused line along the central axis of a flow channel. The Attune™ CytPix™ Flow Cytometer allows a tight control on particle positioning, which enables high sensitivity and precision at high sample input rates.

Sample interrogation

As the sample traverses the interrogation point, the cytometer uses multiple lasers to illuminate the particles or cells in the sample, which scatter the laser light and emit fluorescent light from fluorescent dyes attached to them. The optical filters and mirrors route specified wavelengths of the resulting light scatter and fluorescence signals to the designated optical detectors.

Signal processing

The cytometer uses PMT detectors and a diode detector (FSC) for converting the fluorescence signals and collected light scatter into electrical signals (i.e., voltage pulses), which are proportional to the intensity of the light received by the detectors. The PMTs are dedicated to fluorescence and SSC detection, and the FSC is obtained from the diode detector. The analog and digital electronics of the cytometer amplify and analyze these pulses, and transfer them to the workstation computer for further processing by the Attune™ Cytometric Software.

Results

Results are saved as FCS 3.0 or 3.1 files in the appropriate user folders.

Fluidics

The fluidics system of the Attune™ CytPix™ Flow Cytometer handles the flow of fluids required for acoustic focusing cytometer operation. This includes the fluid functions during the main data collection operation as well as during the Startup, Deep Clean, Unclog, Sanitize SIP, System Decontamination, Rinse, Run, De-bubble, Stop, and Shutdown operations.

The sample to be analyzed is driven by a syringe displacement pump and passes through a bubble sensor along the path of the sample loop before arriving at the capillary assembly. A separate continuous flow pressure pump controls the focusing fluid through the focusing fluid filter and combines it with the sample fluid to allow for particle hydrodynamic focusing.

The capillary assembly is an acoustic resonant device that focuses cells or particles in the sample fluid into a single tight line (i.e., the sample core) using a capillary coupled to a single piezoelectric transducer. The capillary carries the sample core upward through the center of the optical cell, where the particles to be analyzed are intercepted by a tightly-focused laser beam for interrogation.

After passing through the optical cell, the stream arrives at the waste container.

Fluidics functions

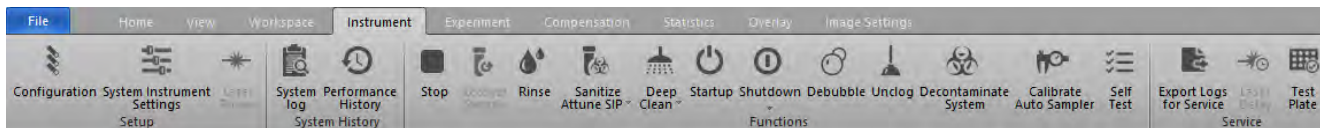
The fluidics functions of the Attune™ CytPix™ Flow Cytometer are controlled by the Attune™ Cytometric Software. These functions are:

- **Sanitize SIP** – Quickly washes and sanitizes the SIP and sample lines. It is especially important to perform the Sanitize SIP function when running sticky samples, DNA stains, or beads. This function requires user-supplied bleach or detergent.
- **System Decontamination** – Sanitizes the system and fluidics bottles with bleach and Wash solutions for the proscribed time. Ensures full system cleanliness at regular maintenance intervals to prevent buildup of contaminants in the system or fluidics bottles.
- **De-bubble** is a user-initiated function for clearing bubbles in the fluidics lines of the cytometer.
- **Deep Clean** is a user-initiated system cleaning between sticky samples. This function requires user-supplied 10% bleach solution.

IMPORTANT! We recommend that you sanitize the system between users. See “Wash procedure between users” on page 34.

- **Unclog** function is a user-initiated back flush operation to remove clogs from the sample probe and flow cell.
- **Rinse** automatically flushes the system between samples to minimize carryover. This function can also be user-initiated. During the rinse, the Red Status Indicator Light will stop blinking when it is okay to load a new sample onto the SIP.
- **Shutdown** is an automated function that initiates the cleaning cycle and post-cleaning rinse. This mode requires user supplied bleach, Attune™ Wash Solution, and Attune™ Shutdown Solution.
- **Startup** is an automated function that starts the fluidics, optics, and electronics of the system. The Startup functions include priming the instrument fluidics and allowing the laser time to warm.
- **Stop** is used to end all data collection.
- **Clear** is used to delete the data from the screen. It refreshes the Workspace while the instrument is in Run or Record mode.

You can initiate the available fluidics functions by selecting it from the Instrument ribbon tab. For more information, refer to the *Attune™ Cytometric Software User Guide*.



IMPORTANT! The lasers are powered down during the Deep Clean and Shutdown cycles. The lasers must warm up for at least 5 minutes before running additional samples.

Instrument configurations

Default instrument configurations

The following images show the Instrument Configuration panel for each laser configuration.



Figure 1 BRVY (Blue, Red, Violet, Yellow)

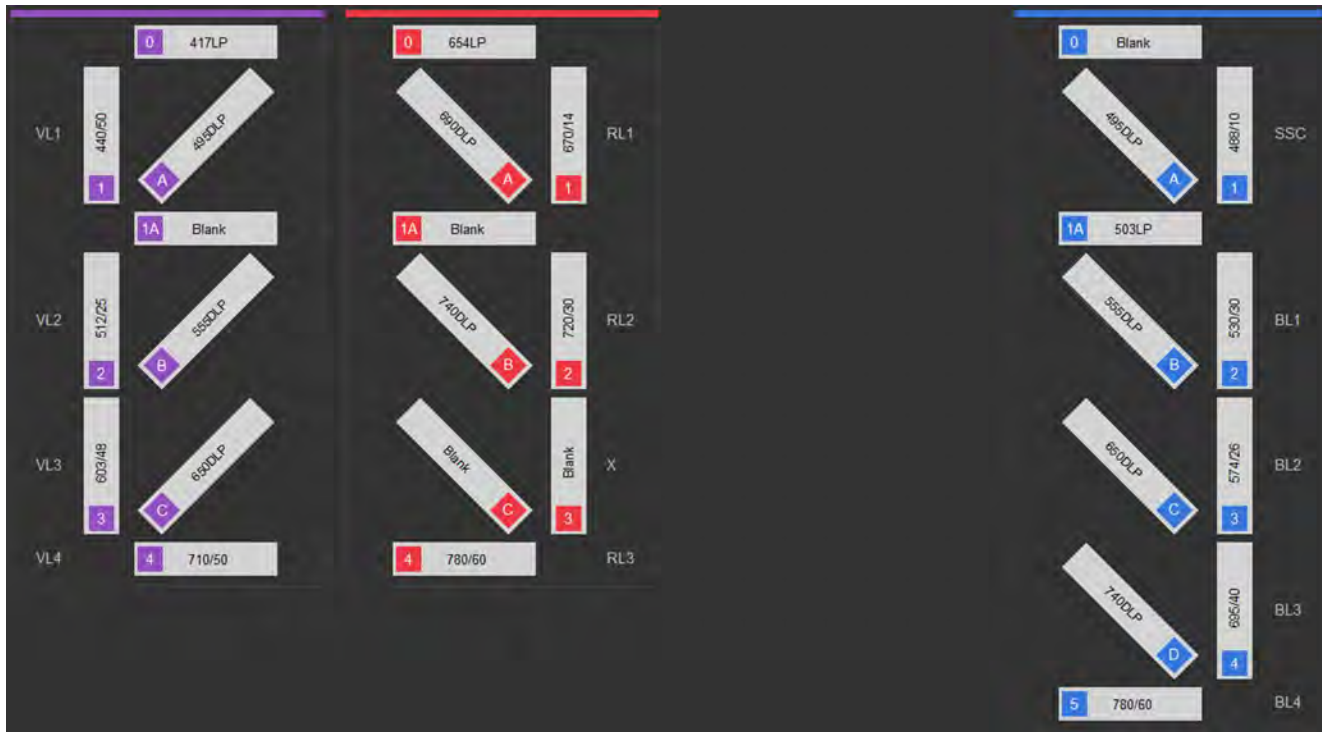


Figure 2 BRV (Blue, Red, Violet)



Figure 3 BVY (Blue, Violet, Yellow)

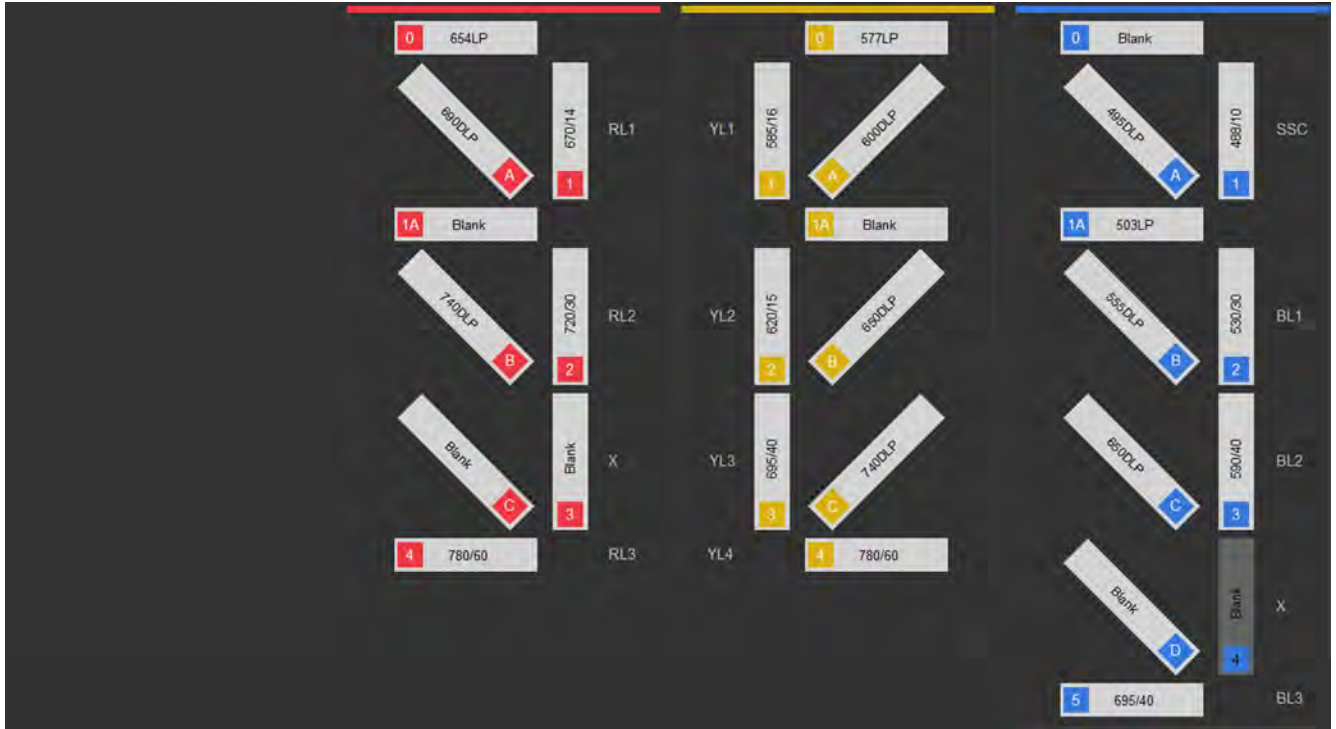


Figure 4 BRY (Blue, Red, Yellow)

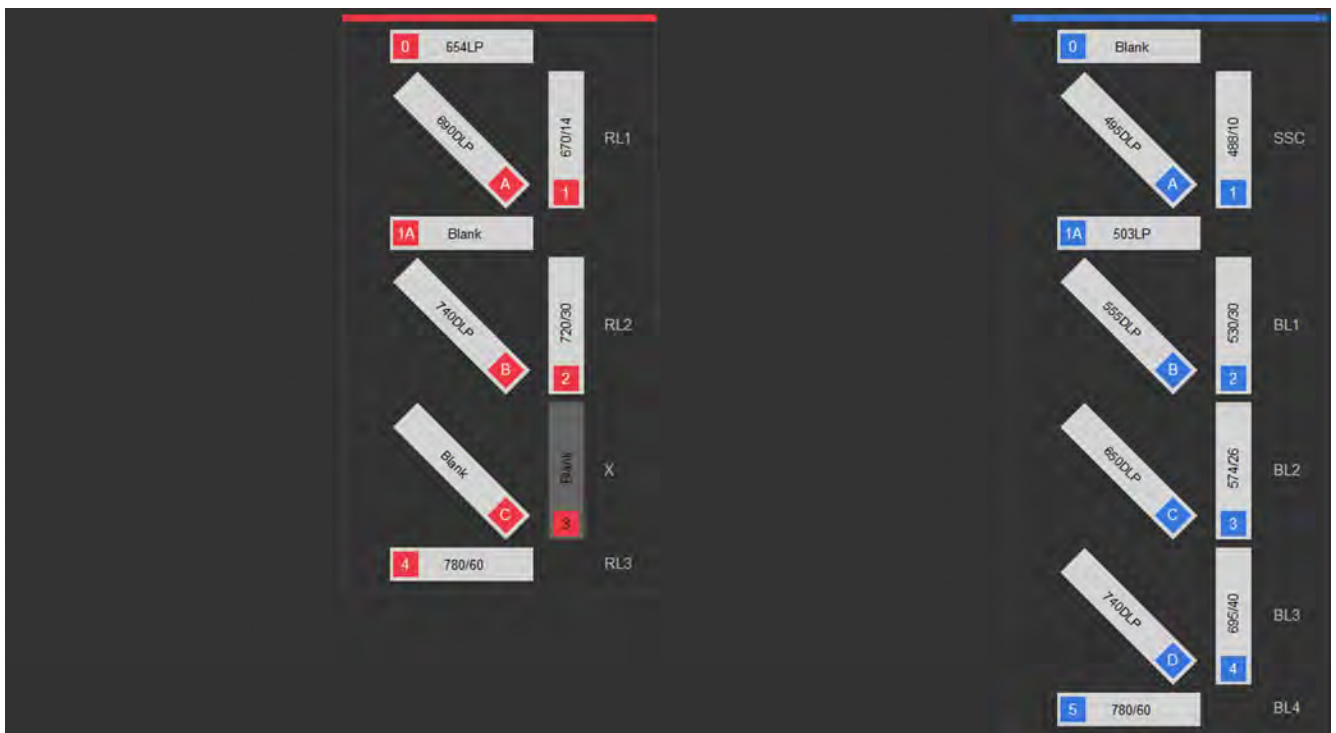


Figure 5 BR (Blue, Red)

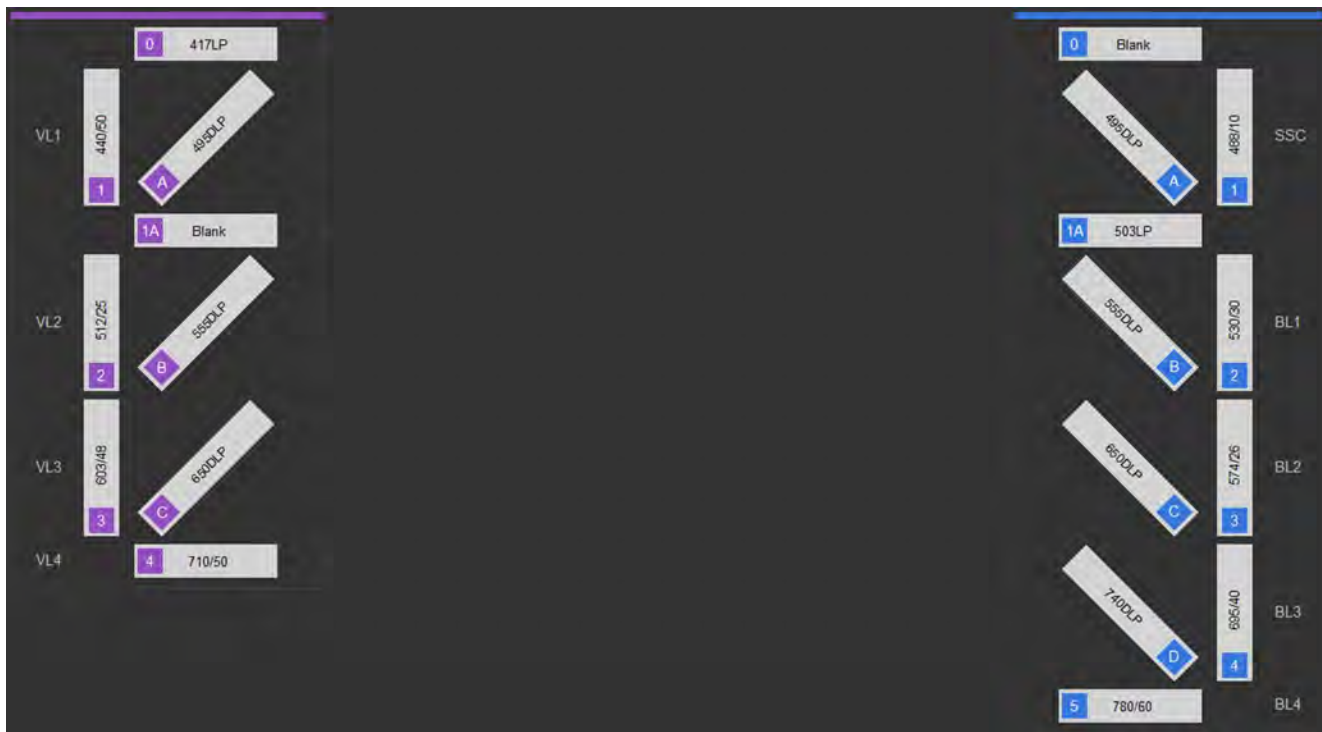


Figure 6 BV (Blue, Violet)

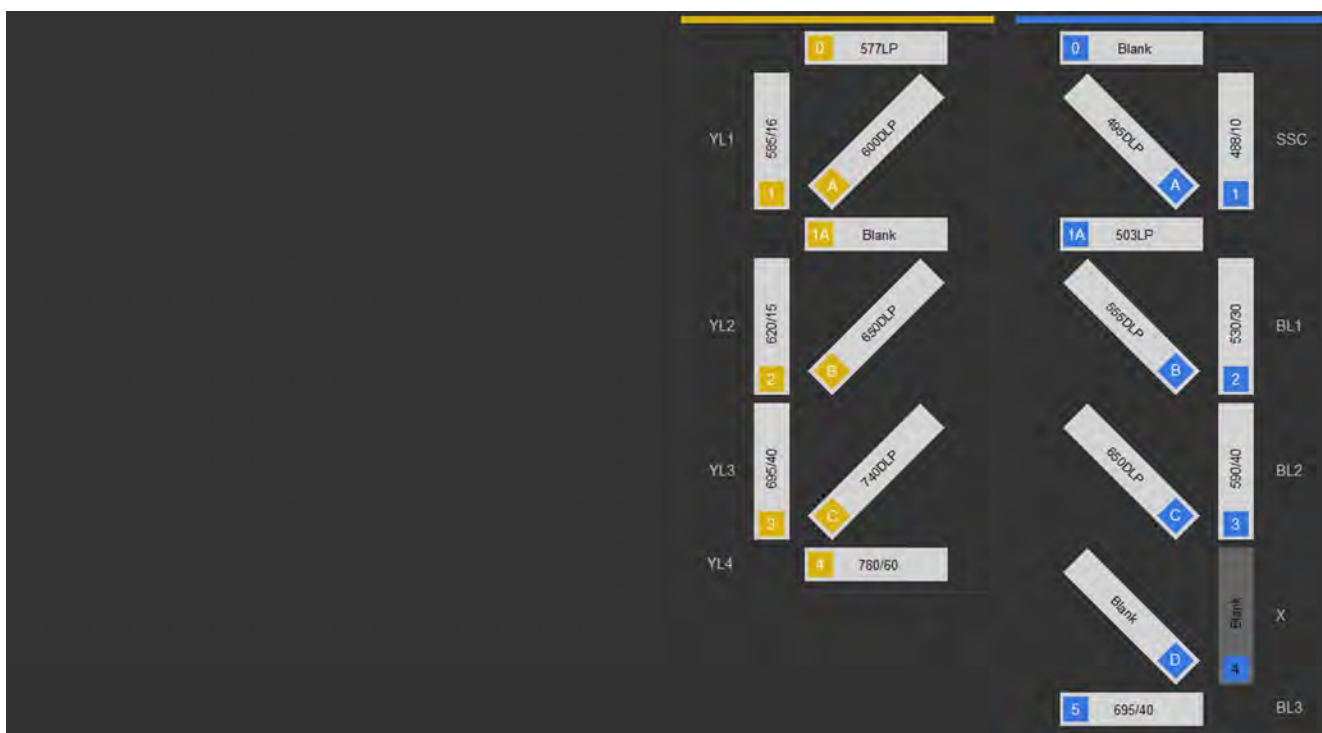


Figure 7 BY (Blue, Yellow)

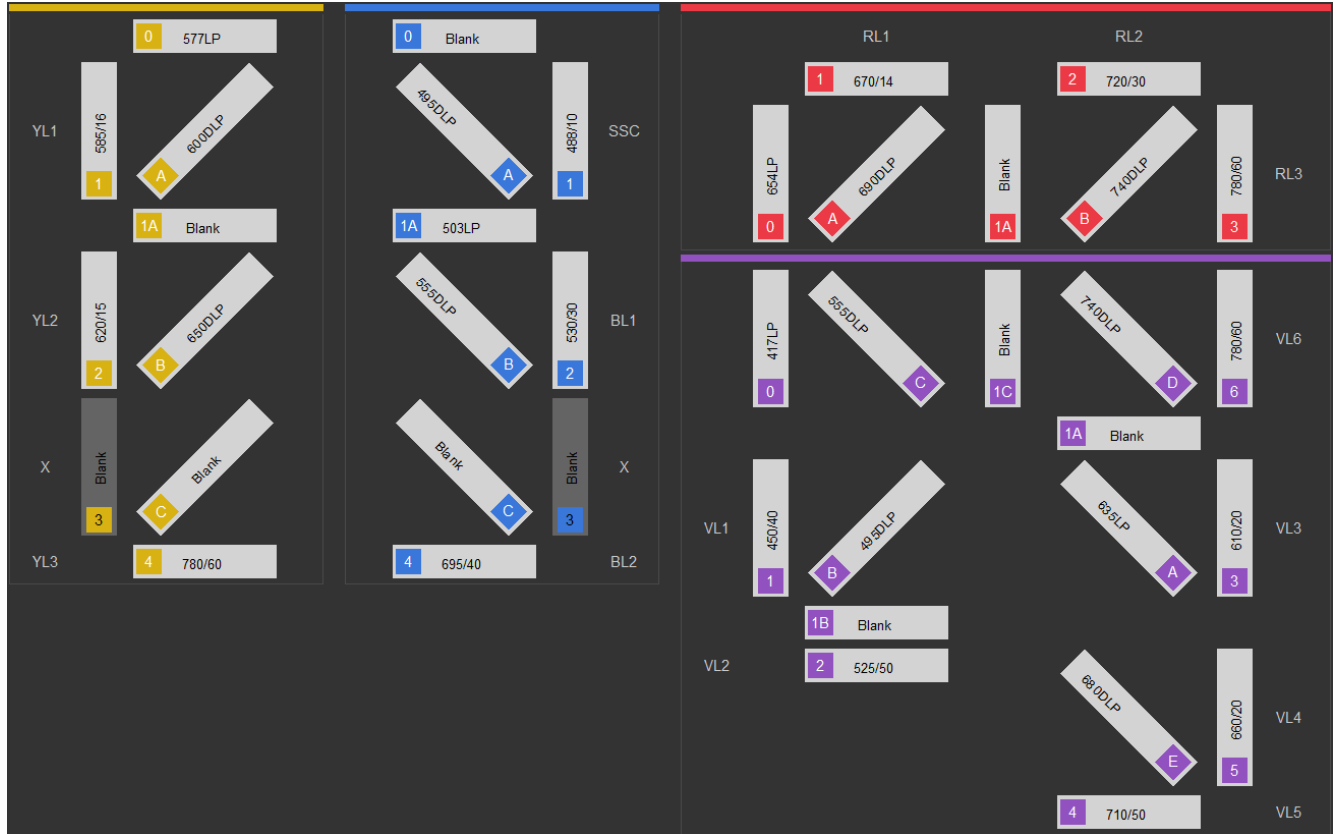


Figure 8 BRV6Y (Blue, Red, Violet 6, Yellow)

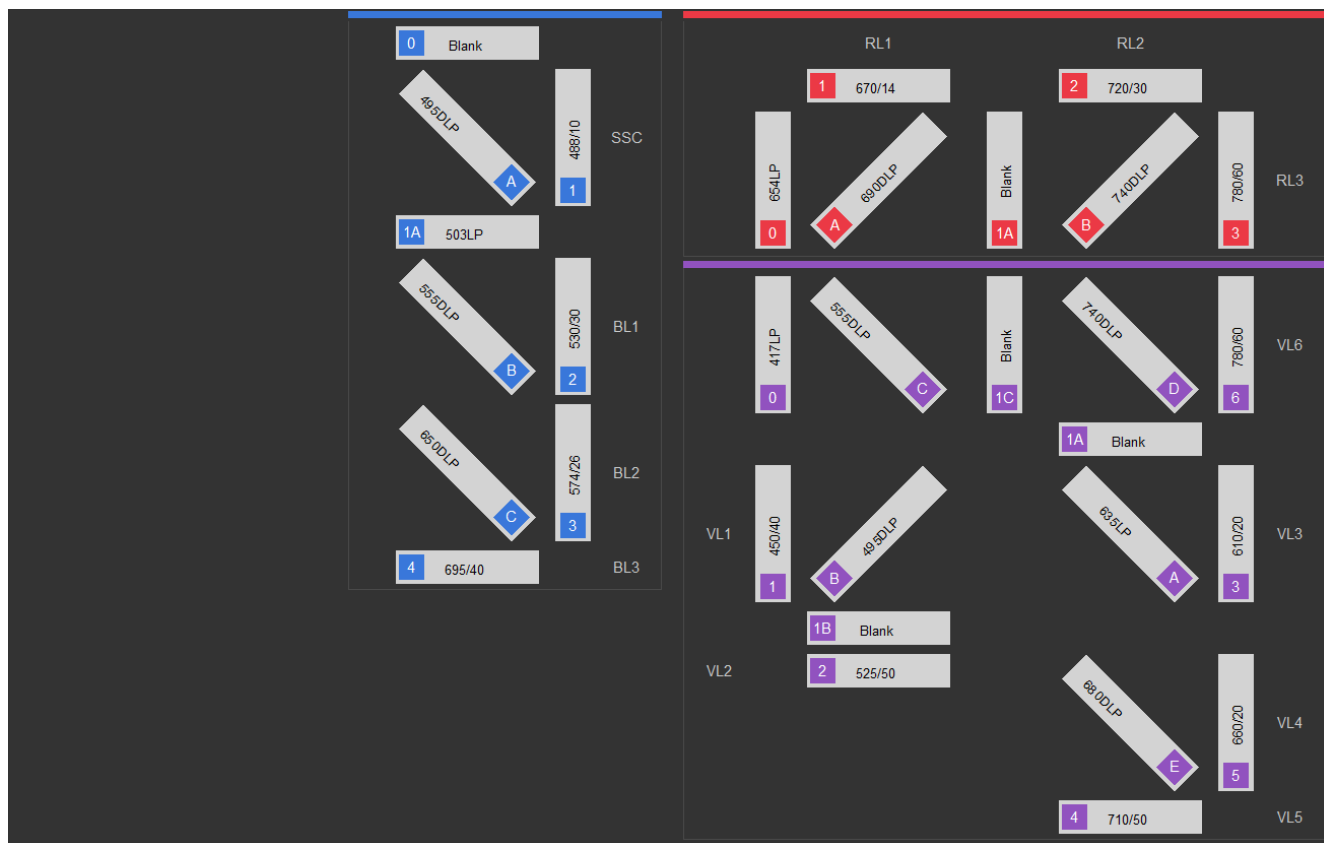


Figure 9 BRV6 (Blue, Red, Violet 6)



Figure 10 BV6 (Blue, Violet 6)

Default filter label list

The table below lists the filter labels that are displayed for each channel for various system default configurations available.

The naming convention for the system default instrument configuration is as follows:

Baseline/PT Config <CCCC>,

where <C> is the first letter of each laser color (**B**lue, **R**ed, **V**iolet, or **Y**ellow) and an **X** in the name indicates that one of the lasers is not present.

An **X** in the Instrument Configuration columns indicates that the filters listed in the table apply to that specific instrument configuration.

Configuration		BYXX	BRXX	BV4XX	BV6XX	BRV4X	BYRX	BYV4X	BRV6X	BYRV6	BYRV4
No. of detectors		7	7	7	9	10	11	11	12	14	14
Laser	Channel	Emission filter (nm)									
Blue	BL1	530/30	530/30	530/30	530/30	530/30	530/30	530/30	530/30	530/30	530/30
	BL2	590/40	574/26	574/26	574/26	574/26	574/26	590/40	574/26	695/40	590/40
	BL3	695/40	695/40	695/40	695/40	695/40	695/40	695/40	695/40		695/40
	BL4		780/60			780/60	780/60				
Yellow	YL1	585/16					585/16	585/16		585/16	585/16
	YL2	620/15					620/15	620/15		620/15	620/15
	YL3	695/40					695/40	695/40		780/60	695/40
	YL4	780/60					780/60	780/60			780/60
Red	RL1		670/14			670/14	670/14		670/14	670/14	670/14
	RL2		720/30			720/30	720/30		720/30	720/30	720/30
	RL3		780/60			780/60	780/60		780/60	780/60	780/60
Violet	VL1			440/50	450/40	440/50		440/50	450/40	450/40	440/50
	VL2			512/25	525/50	512/25		512/25	525/50	525/50	512/25
	VL3			603/48	610/20	603/48		603/48	610/20	610/20	603/48
	VL4			710/50	660/20	710/50		710/50	660/20	660/20	710/50
	VL5				710/50				710/50	710/50	
	VL6				780/60				780/60	780/60	



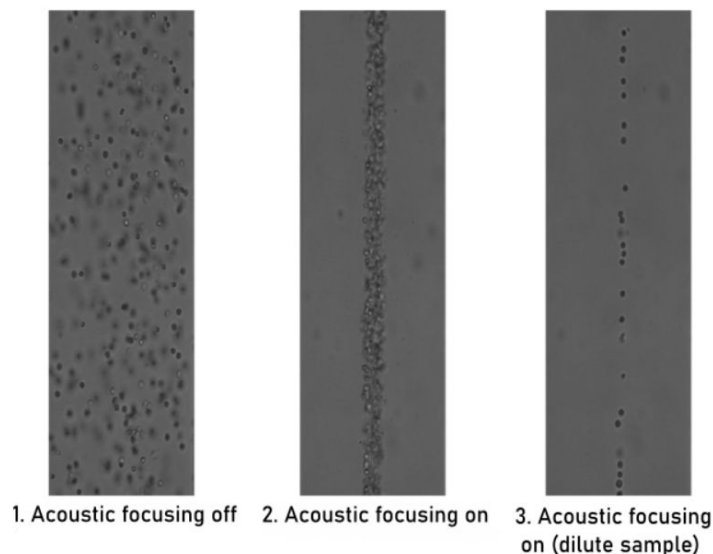
Technical overview

Acoustic focusing

The objective in flow cytometry is to measure the properties of individual particles as they move through the laser beam. When a sample in solution is injected into a flow cytometer, the cells or particles are randomly distributed in three-dimensional space and must be ordered into a stream of single particles.

Acoustic focusing exploits the physical differences between cells or particles relative to the background carrier medium to position the particles or cells into a single, focused line along the central axis of a flow channel independent of sample fluid flow. In contrast to a conventional hydrodynamic sheath-focused cytometer, the Attune™ CytPix™ Flow Cytometer uses acoustic radiation pressure in addition to hydrodynamic focusing to allow for more sample input options, enabling the user to choose higher throughput, shorter run time, higher sensitivity, or a combination of them all depending upon their individual needs.

The images below show the alignment and concentration effects of acoustic focusing on a whole-blood sample.



Effects of acoustic focusing on cell viability

Acoustic focusing differs fundamentally from ultrasonic lysis of cells and is generally gentler on cells than the forces occurring in hydrodynamic focusing.

Ultrasonic lysis of cells relies on cavitation produced at sub-megahertz frequencies where tiny gas bubbles form and collapse with immense local shear and heating in the solution containing the sample. In contrast, the acoustic focusing capillary of the Attune™ CytPix™ Flow Cytometer operates at a frequency well above 1 MHz, where the possibility of cavitation is greatly reduced.

Further, acoustic cytometry is performed with relatively low energy levels at very high sample flow rates and the design of the acoustically-driven capillary spreads this energy over the entire length of the capillary, significantly reducing the probability of cellular damage.

Flow rate

In a conventional cytometer, the diameter of the sample core is varied by the pressure difference between the sample stream and the sheath fluid stream. Increasing the sample flow rate enlarges the core diameter, which allows faster data acquisition but lower resolution because the cells are distributed across the sample core stream and may pass through the laser spot off center.

In contrast, the alignment of cells in the Attune™ CytPix™ Flow Cytometer is independent of the total fluid flow through the cytometer. While large changes in the amount of sample injected or the total fluid flow may alter the diameter of the sample core, acoustic focusing arranges the particles in a very small region within the core, which ensures that the cells remain in the optimal position for interrogation by the lasers. This feature of the cytometer allows even dilute samples to be analyzed at a much higher sample input rate and saves the user valuable time.

The Attune™ CytPix™ Flow Cytometer has a range of flow rates from 12.5 $\mu\text{L}/\text{minute}$ to 1000 $\mu\text{L}/\text{minute}$, representing sample delivery that is predominantly hydrodynamically focused (low flow rate) to sample delivery that is predominantly acoustically focused (high flow rate).

- At low sample flow rates (e.g., 12.5 $\mu\text{L}/\text{minute}$ and 25 $\mu\text{L}/\text{minute}$), the instrument operates predominantly as a hydrodynamic focusing instrument. At these lower rates, the core diameter is the smallest, which yields the best resolution for dim expressing assays relative to an unbound, fluorescent background contributor. These rates are also recommended for small particles (diameter $<2 \mu\text{m}$).
- At higher flow rates, acoustic focusing is implemented at greater degrees to keep the particles in a tight stream as they enter the laser interrogation region. These rates give the user the flexibility to choose a rate to match their sample concentration. As the sample becomes more and more dilute, the user can move to greater sample input rates. Sample input rates 100 $\mu\text{L}/\text{minute}$, 200 $\mu\text{L}/\text{minute}$, and 500 $\mu\text{L}/\text{minute}$ are not recommended for particles $<2 \mu\text{m}$ in size. The highest rate is not recommended for particles $<4 \mu\text{m}$ in size.

Sample concentration

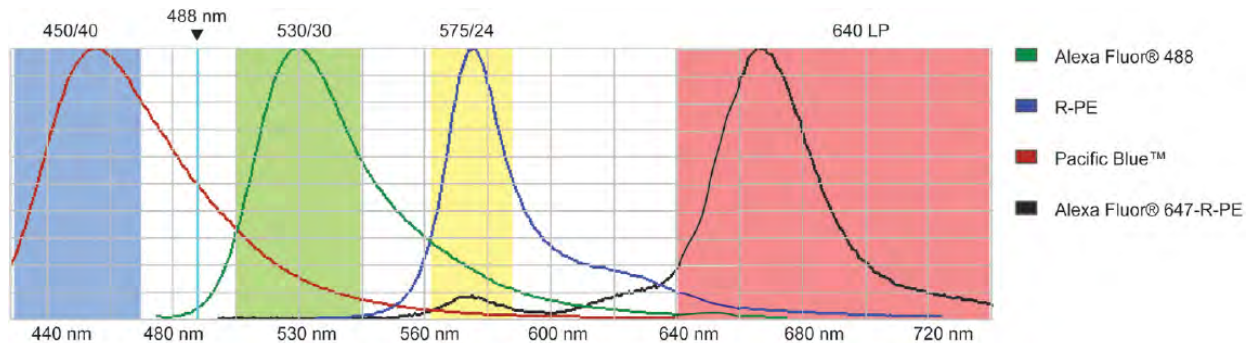
All cytometers are governed by Poisson statistics, which predict the probability of a given number of cells or particles being intercepted by the interrogating laser beam. While increasing the sample concentration results in a higher sample throughput, it also increases the probability of a coincident event, defined as more than one cell present in the interrogating laser beam.

The Attune™ CytPix™ Flow Cytometer can maintain its maximum particle analysis rate over a large range of initial sample concentrations without the need to concentrate using centrifugation or filtration. The ability to analyze dilute samples has the added benefits of reduced background fluorescence from free fluorophores in the sample and capability to analyze very small initial samples sizes.

Compensation

Fluorophores emit light over a range of wavelengths. Although optical filters limit the range of frequencies measured by a given detector, when two or more fluorophores are used in an experiment, there is often an overlap between the wavelength ranges. *Compensation* is the mathematical method used to correct for the overlap of one fluorophore's emission into another fluorophore's emission measurements.

Every fluorescent molecule emits light with a particular spectrum unique to that molecule. These emission spectra overlap, in some cases very significantly. The example below shows the emission spectra of Pacific Blue™, Alexa Fluor™ 488, R-phycoerythrin (R-PE), and Alexa Fluor™ 647-R-PE dyes.



Each dye emits with a characteristic emission spectrum that is specific for the fluorophore: Alexa Fluor™ 488 dye has a maximum around 520 nm, R-PE at about 575 nm, Pacific Blue™ dye at about 454 nm, and Alexa Fluor™ 647-R-PE dye at about 666 nm. The teal line represents the laser excitation wavelength of an argon ion laser (488 nm).

In this example, the emission spectra of each dye is backlit with a shaded area indicating the emission filter where the specific light is captured by the cytometer. In general, filters are chosen which collect the emitted light near the emission maximum. For example, to capture the emission from Alexa Fluor™ 488 dye, a BP 530/30 filter is used (i.e., the filter has a pass-band centered at 530 nm, and the width of the pass-band is 30 nm).

However, it is impossible to choose filters that measure only one dye. For instance, the Alexa Fluor™ 488 dye has a significant emission in the region that R-PE is measured (575 nm). Therefore, the emission from Alexa Fluor™ 488 dye will register in 530 nm and 575 nm bands. If R-PE is also present, it will contribute to the 575 nm band. Compensation is the mathematical process for correcting for the amount of the Alexa Fluor™ 488 dye fluorescence in the 575 nm band so that R-PE fluorescence can be accurately measured. Performing multicolor analyses can complicate this process further, because fluorophores are not usually spectrally separated very well.

Fluorescence spill over can be estimated by running single fluorescence controls specific for a certain dye and then subtracting out the fluorescence in the other detection channels, thus leaving the true signal of the other fluorophores. If a fluorescent dye emission is collected through three different filters, then the amount of fluorescence captured through the first filter can be estimated based on how much spillover or contaminating signal is present in the second or the third filters. In the example above, the Pacific Blue™ conjugate has some fluorescence in the 530/30 filter and very little in the 575/24 filter; therefore, the amount of compensation required in the 530/30 filter will be more than in the 575/24 filter.

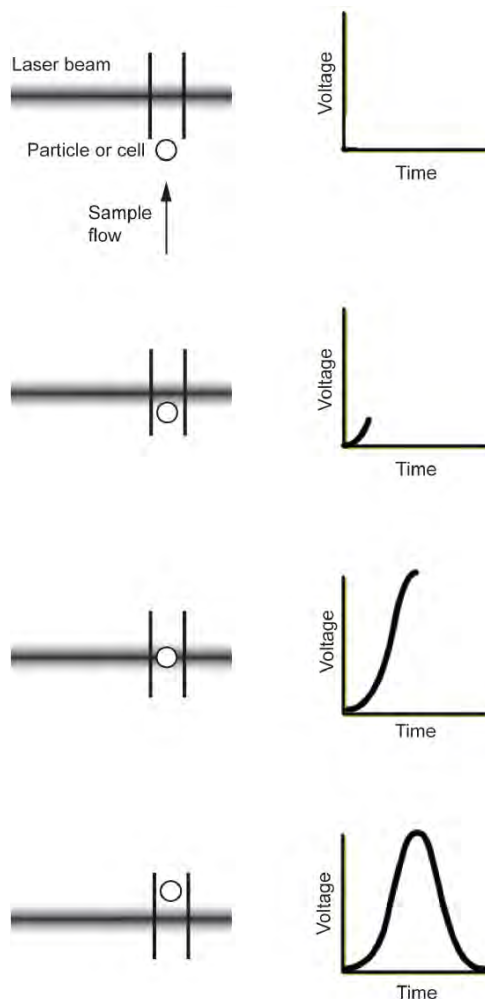
Voltage pulse

When a cell or particle passes through a focused laser beam, it refracts or scatters light in all directions and can emit fluorescence. The scatter and the fluorescence last only a few microseconds, because the cells or particles are moving very rapidly through the focused laser beam. The detectors convert the momentary flash of light into an electrical signal called a *voltage pulse*.

When the cell or particle begins to enter the intercepting laser beam, the signal intensity is low, because only a small portion of the particle scatters the light.

The pulse reaches its maximum when the cell or particle is in the middle of the laser beam, and the whole particle scatters the light. Further, the laser beam is brightest in the middle, thus causing more light to scatter off of the particle.

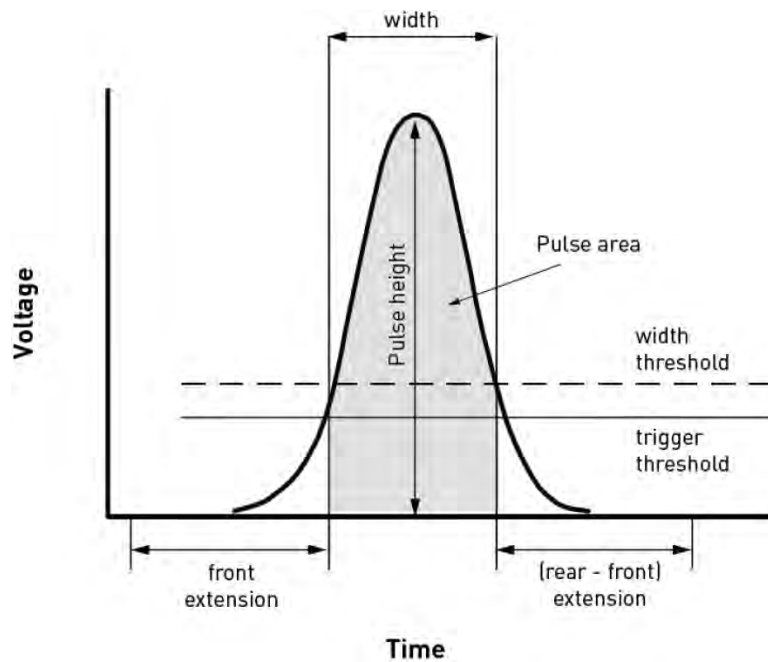
As the cell or particle exits the beam, the signal starts decreasing and eventually trails off below the threshold.



Pulse measurement

The analog signal from the detectors are amplified and relayed to the 16-bit analog-to-digital converter (ADC), which samples the signals at a rate of up to 20 MHz, converting the continuous signal into digital data up to 35,000 events per second.

The data is further processed by the field programmable gate array (FPGA), which simultaneously calculates pulse height, area, and width when the pulse exceeds the user-specified threshold values. The height is defined by the peak voltage of the pulse, the area is the integrated value of the pulse extending into the front and rear extensions, and the width of the pulse (in units of ADC points) is measured at the user-specified width threshold value.





Ordering information

Attune™ CytPix™ Flow Cytometer

The Attune™ CytPix™ Flow Cytometer is available with the following laser configuration options from Thermo Fisher Scientific. For more information, go to www.thermofisher.com or contact Technical Support (page 98).

Item	Amount	Source
Attune™ CytPix™ Flow Cytometer, Blue/Red (BR)	1 each	A48658
Attune™ CytPix™ Flow Cytometer, Blue/Violet (BV)	1 each	A48660
Attune™ CytPix™ Flow Cytometer, Blue/Yellow (BY)	1 each	A48661
Attune™ CytPix™ Flow Cytometer, Blue/Violet 6 (BV6)	1 each	A48662
Attune™ CytPix™ Flow Cytometer, Blue/Red/Violet (BRV)	1 each	A48654
Attune™ CytPix™ Flow Cytometer, Blue/Red/Yellow (BRY)	1 each	A48655
Attune™ CytPix™ Flow Cytometer, Blue/Violet/Yellow (BVY)	1 each	A48656
Attune™ CytPix™ Flow Cytometer, Blue/Red/Violet 6 (BRV6)	1 each	A48657
Attune™ CytPix™ Flow Cytometer, Blue/Red/Violet/Yellow (BRVY)	1 each	A48652
Attune™ CytPix™ Flow Cytometer, Blue/Red/Violet 6/Yellow (BRV6Y)	1 each	A48653



Flow cytometer accessory products

The following products and replacement parts for the instrument are available separately from Thermo Fisher Scientific. For more information, go to www.thermofisher.com or contact Technical Support (page 98).

Item	Amount	Source
Attune™ Focusing Fluid (1X)	1 × 1 L	4488621
	6 × 1 L	4449791
	1 × 10 L	A24904
Attune™ Wash Solution	250 mL	A24974
Attune™ Shutdown Solution (1X)	250 mL	A24975
Attune™ Debubble Solution (1X)	50 mL	A10496
Attune™ NxT Flow Cell Cleaning Solution	30 mL	A43635
Attune™ Performance Tracking Beads	3 mL	4449754
Attune™ NxT Wash Bottle, 175 mL	1 each	100022151
Attune™ NxT Shutdown Bottle, 175 mL	1 each	100022154
Attune™ NxT Waste Bottle, 1.9 L	1 each	100085928CST
Attune™ NxT Focusing Fluid Bottle, 1.9 L	1 each	100085929CST
Attune™ NxT Sample Syringe, 1 mL	1 each	100022591
Attune™ NxT Focusing Fluid Filter	1 each	100022587
Attune™ NxT Custom Filter Holder Kit	1 each	A27784
Attune™ CytPix™ No-Wash No-Lyse Filter Kit	1 kit	A50920
Attune™ NxT Fluorescent Protein Filter Kit	1 kit	100022775



Attune™ optical filters

The following replacement filters used in the optics path of the instrument are available separately from Thermo Fisher Scientific. For more information, go to www.thermofisher.com or contact Technical Support (page 98).

Item	Amount	Source
Long Pass filters		
Attune™ NxT Filter, 413LP	1 filter	100022752
Attune™ NxT Filter, 496LP	1 filter	100022753
Attune™ NxT Filter, 569LP	1 filter	100022754
Attune™ NxT Filter, 646LP	1 filter	100022755
Dichroic Long Pass filters		
Attune™ NxT Dichroic Filter, 495DLP	1 filter	100022769
Attune™ NxT Dichroic Filter, 555DLP	1 filter	100022776
Attune™ NxT Dichroic Filter, 600DLP	1 filter	100022771
Attune™ NxT Dichroic Filter, 650DLP	1 filter	100022772
Attune™ NxT Dichroic Filter, 690DLP	1 filter	100022773
Attune™ NxT Dichroic Filter, 740DLP	1 filter	100022774
Band Pass filters		
Attune™ NxT Emission Filter, 440/50BP	1 filter	100022756
Attune™ NxT Emission Filter, 512/25BP	1 filter	100022757
Attune™ NxT Emission Filter, 530/30BP	1 filter	100022758
Attune™ NxT Emission Filter, 585/16BP	1 filter	100022759
Attune™ NxT Emission Filter, 590/40BP	1 filter	100022760
Attune™ NxT Emission Filter, 603/48BP	1 filter	100022761
Attune™ NxT Emission Filter, 620/15BP	1 filter	100022762
Attune™ NxT Emission Filter, 670/14BP	1 filter	100022763
Attune™ NxT Emission Filter, 695/20BP	1 filter	100022764
Attune™ NxT Emission Filter, 710/50BP	1 filter	100022765
Attune™ NxT Emission Filter, 720/30BP	1 filter	100022767
Attune™ NxT Emission Filter, 780/60BP	1 filter	100022766



CytKick™ / CytKick™ Max Autosampler and accessory products

The following products and replacement parts for the CytKick™ and CytKick™ Max Autosampler are available separately from Thermo Fisher Scientific. For more information, go to www.thermofisher.com or contact Technical Support (page 98).

Item	Amount	Source
CytKick™ Autosampler	1 each	A38975
CytKick™ Max Autosampler	1 each	A38976
CytKick™ Autosampler Focusing Fluid Bottle Assembly	1 each	100054670
CytKick™ Autosampler Waste Bottle Assembly	1 each	100054669
CytKick™ Autosampler syringe, 1 mL	1 each	100054593
CytKick™ Autosampler Tubing Assembly	1 each	100054481

Attune™ NxT External Fluid Supply and accessory products

The following products and replacement parts for the Attune™ NxT External Fluid Supply are available separately from Thermo Fisher Scientific. For more information, go to www.thermofisher.com or contact Technical Support (page 98).

Item	Amount	Source
Attune™ NxT External Fluid Supply	1 each	A28006
Attune™ Focusing Fluid (1X), 10 L Cubetainer	1 × 10 L	A24904
Attune™ NxT External Fluid Supply Waste Container, 20 L	1 each	100027470
Attune™ NxT External Fluid Supply Waste Bottle Interface Assembly	1 each	100028800
Attune™ NxT External Fluid Supply Cubetainer Interface Assembly	1 each	100027471
Attune™ NxT External Fluid Supply Bottle Connections Assembly	1 each	100027251
Attune™ NxT External Fluid Supply Tube/Cable Harness Assembly	1 each	100027250
Attune™ NxT External Fluid Supply Cable, Cytometer to EFS	1 each	100026482



Safety



WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.









- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, see the “Documentation and Support” section in this document.

Symbols on this instrument




Symbols may be found on the instrument to warn against potential hazards or convey important safety information. In this document, the hazard symbol is used along with one of the following user attention words.

- **CAUTION!**—Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.
- **WARNING!**—Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.
- **DANGER!**—Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury.

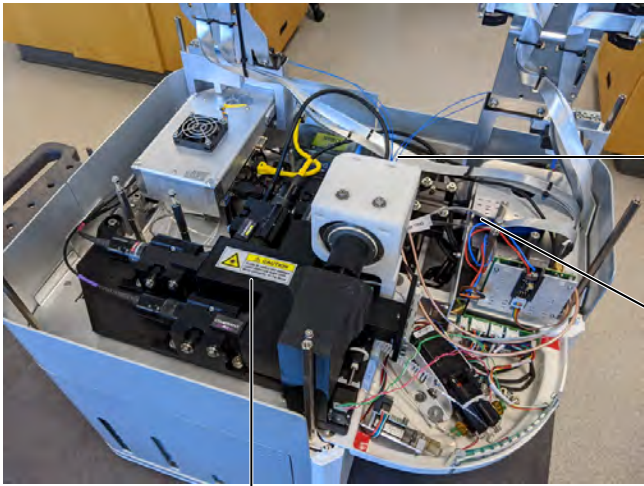



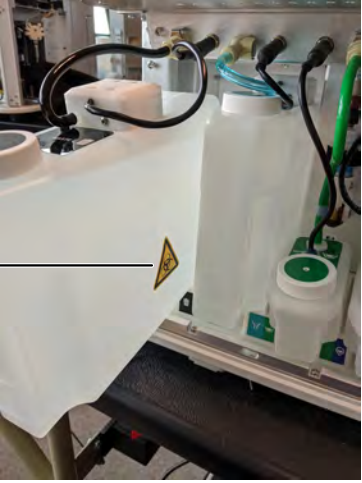
Standard safety symbols

Symbol	English	Français
	CAUTION! risk of danger. Consult the manual for further safety information.	MISE EN GARDE ! Risque de danger. Consulter le manuel pour d'autres renseignements de sécurité.
	CAUTION! Risk of electrical shock.	MISE EN GARDE ! Risque de choc électrique.
	CAUTION! Hot surface.	MISE EN GARDE ! Surface chaude.
	CAUTION! Laser radiation.	MISE EN GARDE ! Rayonnement laser.
	CAUTION! Moving parts.	MISE EN GARDE ! Parties mobiles.
	CAUTION! Piercing hazard.	MISE EN GARDE ! Danger de perforation.
	CAUTION! Potential biohazard.	MISE EN GARDE ! Danger biologique potential.
	CAUTION! Ultraviolet light.	MISE EN GARDE ! Rayonnement ultraviolet.



Safety alerts on this instrument

Symbol	English	Français
	CAUTION! Hazardous chemicals. Read the safety Data Sheets (SDSs) before handling.	MISE EN GARDE ! Produits chimiques dangereux. Lire les fiches signalétiques (FS) avant de manipuler les produits.
	CAUTION! Hazardous waste. Refer to SDS(s) and local regulations for handling and disposal.	MISE EN GARDE ! Déchets dangereux. Lire les fiches signalétiques (FS) et la réglementation locale associées à la manipulation et à l'élimination des déchets.
	DANGER! High voltage.	DANGER ! Haute tension.
	WARNING! To reduce the chance of electrical shock, do not remove covers that require tool access. No user-serviceable parts are inside. Refer servicing to Thermo Fisher Scientific qualified service personnel.	AVERTISSEMENT ! Pour éviter les risques d'électrocution, ne pas retirer les capots dont l'ouverture nécessite l'utilisation d'outils. L'instrument ne contient aucune pièce réparable par l'utilisateur. Toute intervention doit être effectuée par le personnel de service qualifié venant de chez Thermo Fisher Scientific.
	DANGER! Class 4 laser radiation when open. Avoid eye or skin exposure to direct or scattered radiation.	DANGER ! Rayonnement laser de classe 4 A l'ouverte. Éviter l'exposition des yeux ou de la peau rayonnement direct ou éparpille.

Locations of safety labels

Label and location	
	
	
<p>Figure 1 Optics compartment top view (cover removed)</p>	
	
<p>Figure 2 Sample injection port</p>	<p>Figure 3 Waste bottle in the fluidics compartment (compartment door open)</p>

Environmental symbols

Symbol	English	Français
	<p>Do not dispose of this product in unsorted municipal waste.</p> <p>CAUTION! To minimize negative environmental impact from disposal of electronic waste, do not dispose of electronic waste in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provision and contact customer service for information about responsible disposal options.</p>	<p>Ne pas éliminer ce produit avec les déchets usuels non soumis au tri sélectif.</p> <p>MISE EN GARDE ! Pour minimiser les conséquences négatives sur l'environnement à la suite de l'élimination de déchets électroniques, ne pas éliminer ce déchet électronique avec les déchets usuels non soumis au tri sélectif. Se conformer aux ordonnances locales sur les déchets municipaux pour les dispositions d'élimination et communiquer avec le service à la clientèle pour des renseignements sur les options d'élimination responsable.</p>
	<p>Environmental protection symbol of the China RoHS directive. The number in the symbol indicates the "Environment-friendly Use Period" of the product in years. The symbol is used if a substance restricted in China is used in excess of the maximum permitted limit.</p>	<p>Symbole de protection de l'environnement de la directive RoHS de la Chine. Le nombre indiqué dans le symbole indique "période d'utilisation favorable à l'environnement" du produit au cours des années. Le symbole est utilisé si une substance restreinte en Chine est utilisée au-delà de la limite maximale autorisée.</p>

Conformity symbols

Conformity mark	Description
	Indicates conformity with safety requirements for Canada and U.S.A.
	Indicates conformity with European Union requirements for safety and electromagnetic compatibility.
	Indicates conformity with Australian standards for electromagnetic compatibility.
	Indicates conformity with United Kingdom requirements.

Instrument safety

General



CAUTION! Do not remove instrument protective covers. If you remove the protective instrument panels or disable interlock devices, you may be exposed to serious hazards including, but not limited to, severe electrical shock, laser exposure, crushing, or chemical exposure.

Physical injury



CAUTION! Moving and Lifting Injury. The instrument is to be moved and positioned only by the personnel or vendor specified in the applicable site preparation guide. Improper lifting can cause painful and permanent back injury.

Things to consider before lifting or moving the instrument or accessories:

- Depending on the weight, moving or lifting may require two or more persons.
- If you decide to lift or move the instrument after it has been installed, do not attempt to do so without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques.
- Ensure you have a secure, comfortable grip on the instrument or accessory.
- Make sure that the path from where the object is to where it is being moved is clear of obstructions.
- Do not lift an object and twist your torso at the same time. Keep your spine in a good neutral position while lifting with your legs.
- Participants should coordinate lift and move intentions with each other before lifting and carrying.
- For smaller packages, rather than lifting the object from the packing box, carefully tilt the box on its side and hold it stationary while someone else slides the contents out of the box.



CAUTION! Moving Parts. Moving parts can crush, pinch and cut. Keep hands clear of moving parts while operating the instruments. Disconnect power before servicing.

Electrical safety



WARNING! Fuse Installation. Before installing the instrument, verify that the fuses are properly installed and the fuse voltage matches the supply voltage. Replace fuses only with the type and rating specified for the unit. Improper fuses can damage the instrument wiring system and cause a fire.



AVERTISSEMENT ! Installation des fusibles. Avant d'installer l'instrument, vérifier que les fusibles sont correctement insérés et que leur tension correspond à celle fournie par le circuit d'alimentation. Ne remplacer les fusibles que par des modèles du type et de la puissance spécifiés pour l'appareil. L'utilisation de fusibles inadaptés peut endommager le circuit électrique de l'instrument et provoquer un incendie.



WARNING! Ensure appropriate electrical supply. For safe operation of the instrument:

- Plug the system into a properly grounded receptacle with adequate current capacity.
- Ensure the electrical supply is of suitable voltage.
- Never operate the instrument with the ground disconnected. Grounding continuity is required for safe operation of the instrument.



AVERTISSEMENT ! Veiller à utiliser une alimentation électrique appropriée. Pour garantir le fonctionnement de l'instrument en toute sécurité :

- Brancher le système sur une prise électrique correctement mise à la terre et de puissance adéquate.
- S'assurer que la tension électrique est convenable.
- Ne jamais utiliser l'instrument alors que le dispositif de mise à la terre est déconnecté. La continuité de la mise à la terre est impérative pour le fonctionnement de l'instrument en toute sécurité.



WARNING! Power Supply Line Cords. Use properly configured and approved line cords for the power supply in your facility.



AVERTISSEMENT ! Cordons d'alimentation électrique. Utiliser des cordons d'alimentation adaptés et approuvés pour raccorder l'instrument au circuit électrique du site.



WARNING! Disconnecting Power. To fully disconnect power either detach or unplug the power cord, positioning the instrument such that the power cord is accessible.



AVERTISSEMENT ! Déconnecter l'alimentation. Pour déconnecter entièrement l'alimentation, détacher ou débrancher le cordon d'alimentation. Placer l'instrument de manière à ce que le cordon d'alimentation soit accessible.

Cleaning and decontamination



CAUTION! Cleaning and Decontamination. Use only the cleaning and decontamination methods that are specified in the manufacturer user documentation. It is the responsibility of the operator (or other responsible person) to ensure that the following requirements are met:

- No decontamination or cleaning agents are used that can react with parts of the equipment or with material that is contained in the equipment. Use of such agents could cause a HAZARD condition.
- The instrument is properly decontaminated a) if hazardous material is spilled onto or into the equipment, and/or b) before the instrument is serviced at your facility or is sent for repair, maintenance, trade-in, disposal, or termination of a loan. Request decontamination forms from customer service.
- Before using any cleaning or decontamination methods (except methods that are recommended by the manufacturer), confirm with the manufacturer that the proposed method will not damage the equipment.



MISE EN GARDE ! Nettoyage et décontamination. Utiliser uniquement les méthodes de nettoyage et de décontamination indiquées dans la documentation du fabricant destinée aux utilisateurs. L'opérateur (ou toute autre personne responsable) est tenu d'assurer le respect des exigences suivantes:

- Ne pas utiliser d'agents de nettoyage ou de décontamination susceptibles de réagir avec certaines parties de l'appareil ou avec les matières qu'il contient et de constituer, de ce fait, un DANGER.
- L'instrument doit être correctement décontaminé a) si des substances dangereuses sont renversées sur ou à l'intérieur de l'équipement, et/ou b) avant de le faire réviser sur site ou de l'envoyer à des fins de réparation, de maintenance, de revente, d'élimination ou à l'expiration d'une période de prêt (des informations sur les formes de décontamination peuvent être demandées auprès du Service clientèle).
- Avant d'utiliser une méthode de nettoyage ou de décontamination (autre que celles recommandées par le fabricant), les utilisateurs doivent vérifier auprès de celui-ci qu'elle ne risque pas d'endommager l'appareil.

Instrument component and accessory disposal

To minimize negative environmental impact from disposal of electronic waste, do not dispose of electronic waste in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provision and contact customer service for information about responsible disposal options.

Laser safety



WARNING! LASER HAZARD. Under normal operating conditions, the Attune™ CytPix™ Flow Cytometer is categorized as a Class 1 laser product. However, removing the protective covers and (when applicable) defeating the interlock(s) may result in exposure to the internal Class 4 laser. Lasers can burn the retina, causing permanent blind spots. To ensure safe laser operation:

- Never look directly into the laser beam.
- Do not remove safety labels, instrument protective panels, or defeat safety interlocks.
- The system must be installed and maintained by a Thermo Fisher Scientific Technical Representative.
- Remove jewelry and other items that can reflect a laser beam into your eyes or those of others
- Wear proper eye protection and post a laser warning sign at the entrance to the laboratory if the laser protection is defeated for servicing
- DO NOT operate the laser when it cannot be cooled by its cooling fan; an overheated laser can cause severe burns on contact.



CAUTION! Use of controls or adjustments or performance of procedures other than those specified herein may result in hazardous radiation exposure.

The following table lists laser safety symbols and alerts that may be present on the instrument.

Alert	
	DANGER! DANGER! Class 4 laser radiation when open. Avoid eye or skin exposure to direct or scattered radiation.
Alerte	
	DANGER ! DANGER ! Rayonnement laser de classe 4 A l'ouverte. Éviter l' exposition des yeux ou de la peau rayonnement direct ou eparpille.

Safety and electromagnetic compatibility (EMC) standards

The instrument design and manufacture complies with the standards and requirements for safety and electromagnetic compatibility as noted in the following table:

Safety standards

Reference	Description
EU Directive 2014/35/EU	European Union “Low Voltage Directive”
EN 61010-1 UL 61010-1 CAN/CSA C22.2 No. 61010-1	<i>Safety requirements for electrical equipment for measurement, control, and laboratory use—Part 1: General requirements</i>
EN 61010-2-081	<i>Safety requirements for electrical equipment for measurement, control and laboratory use—Part 2-081: Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes</i>
IEC 60825-1 EN 60825-1	<i>Safety of laser products—Part 1: Equipment classification and requirements</i>
21 CFR 1040.10 and 1040.11 as applicable	U.S. FDA Health and Human Services (HHS) “Radiological health performance standards for laser products” and “Radiological health performance standards for specific purpose laser products”

EMC standards

Reference	Description
EU Directive 2014/30/EU	European Union “EMC Directive”
EN 61326-1	<i>Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements – Part 1: General Requirements</i>
FCC Part 18 (47 CFR)	U.S. Standard “Industrial, Scientific, and Medical Equipment”
AS/NZS CISPR 11	<i>Limits and Methods of Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radiofrequency Equipment</i>
ICES-001, Issue 3	<i>Industrial, Scientific and Medical (ISM) Radio Frequency Generators</i>

Environmental design standards

Reference	Description
Directive 2012/19/EU	European Union “WEEE Directive” – Waste electrical and electronic equipment
Directive 2011/65/EU	European Union “RoHS Directive” – Restriction of hazardous substances in electrical and electronic equipment

Chemical safety



WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.



AVERTISSEMENT ! PRÉCAUTIONS GÉNÉRALES EN CAS DE MANIPULATION DE PRODUITS CHIMIQUES. Pour minimiser les risques, veiller à ce que le personnel du laboratoire lise attentivement et mette en œuvre les consignes de sécurité générales relatives à l'utilisation et au stockage des produits chimiques et à la gestion des déchets qui en découlent, décrites ci-dessous. Consulter également la FDS appropriée pour connaître les précautions et instructions particulières à respecter :

- Lire et comprendre les fiches de données de sécurité (FDS) fournies par le fabricant avant de stocker, de manipuler ou d'utiliser les matériaux dangereux ou les produits chimiques. Pour obtenir les FDS, se reporter à la section « Documentation et support » du présent document.
- Limiter les contacts avec les produits chimiques. Porter des équipements de protection appropriés lors de la manipulation des produits chimiques (par exemple : lunettes de sûreté, gants ou vêtements de protection).
- Limiter l'inhalation des produits chimiques. Ne pas laisser les récipients de produits chimiques ouverts. Ils ne doivent être utilisés qu'avec une ventilation adéquate (par exemple, sorbonne).
- Vérifier régulièrement l'absence de fuite ou d'écoulement des produits chimiques. En cas de fuite ou d'écoulement d'un produit, respecter les directives de nettoyage du fabricant recommandées dans la FDS.
- Manipuler les déchets chimiques dans une sorbonne.

- Veiller à utiliser des récipients à déchets primaire et secondaire. (Le récipient primaire contient les déchets immédiats, le récipient secondaire contient les fuites et les écoulements du récipient primaire. Les deux récipients doivent être compatibles avec les matériaux mis au rebut et conformes aux exigences locales, nationales et communautaires en matière de confinement des récipients.)
- Une fois le récipient à déchets vidé, il doit être refermé hermétiquement avec le couvercle fourni.
- Caractériser (par une analyse si nécessaire) les déchets générés par les applications, les réactifs et les substrats particuliers utilisés dans le laboratoire.
- Vérifier que les déchets sont convenablement stockés, transférés, transportés et éliminés en respectant toutes les réglementations locales, nationales et/ou communautaires en vigueur.
- **IMPORTANT !** Les matériaux représentant un danger biologique ou radioactif exigent parfois une manipulation spéciale, et des limitations peuvent s'appliquer à leur élimination.

Biological hazard safety



WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

- U.S. Department of Health and Human Services guidelines published in Biosafety in Microbiological and Biomedical Laboratories found at: <http://www.cdc.gov/biosafety>
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; http://www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html).
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.

Additional information about biohazard guidelines is available at: <http://www.cdc.gov>

In the EU:

- Check your local guidelines and legislation on biohazard and biosafety precaution, and the best practices published in the World Health Organisation (WHO) Laboratory Biosafety Manual, third edition www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/



Documentation and support

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 - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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