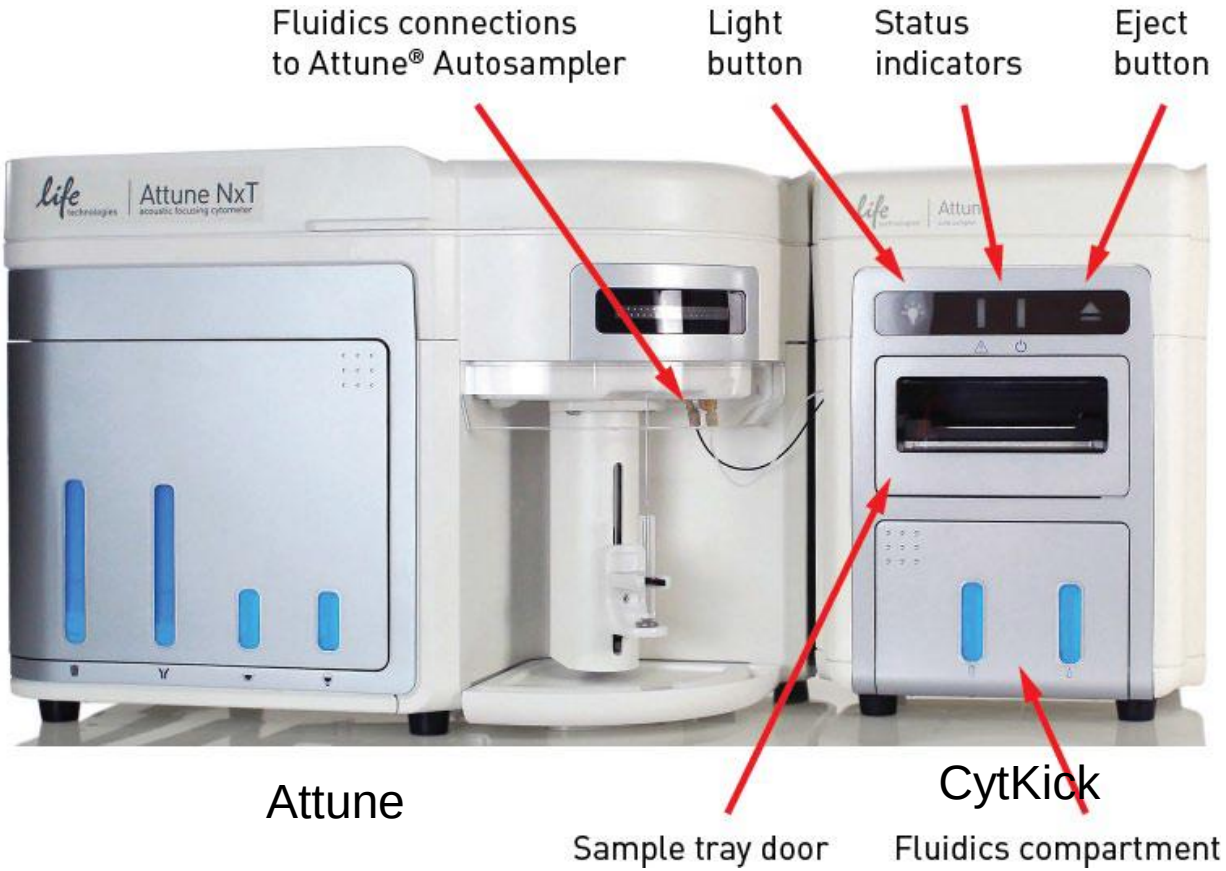
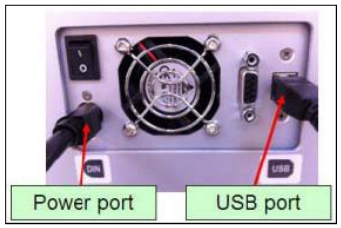
Components



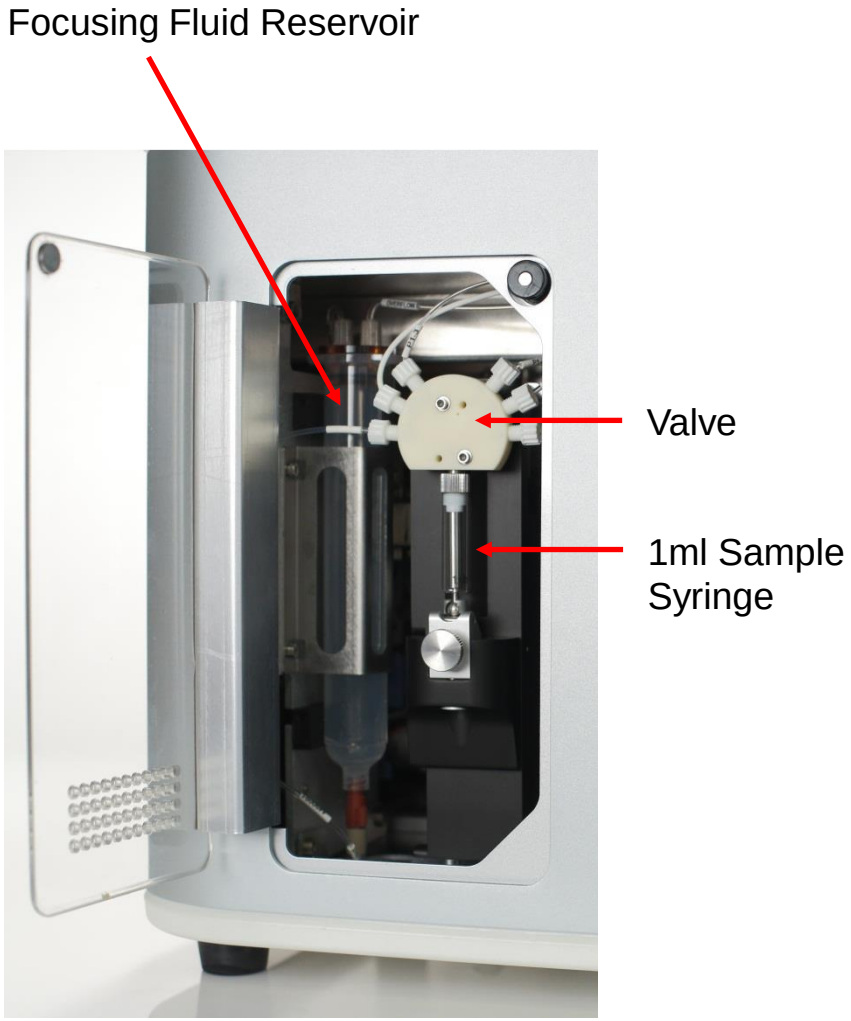
- Compatible with many different standard plate formats, including 96-well, flat, round and V-bottom.
- Option to pierce sealed plates
- Round Bottom plates are recommended for optimal mixing
- Performs automated cleaning between wells (from 1 to 10 rinses) and when the instrument is shutting down

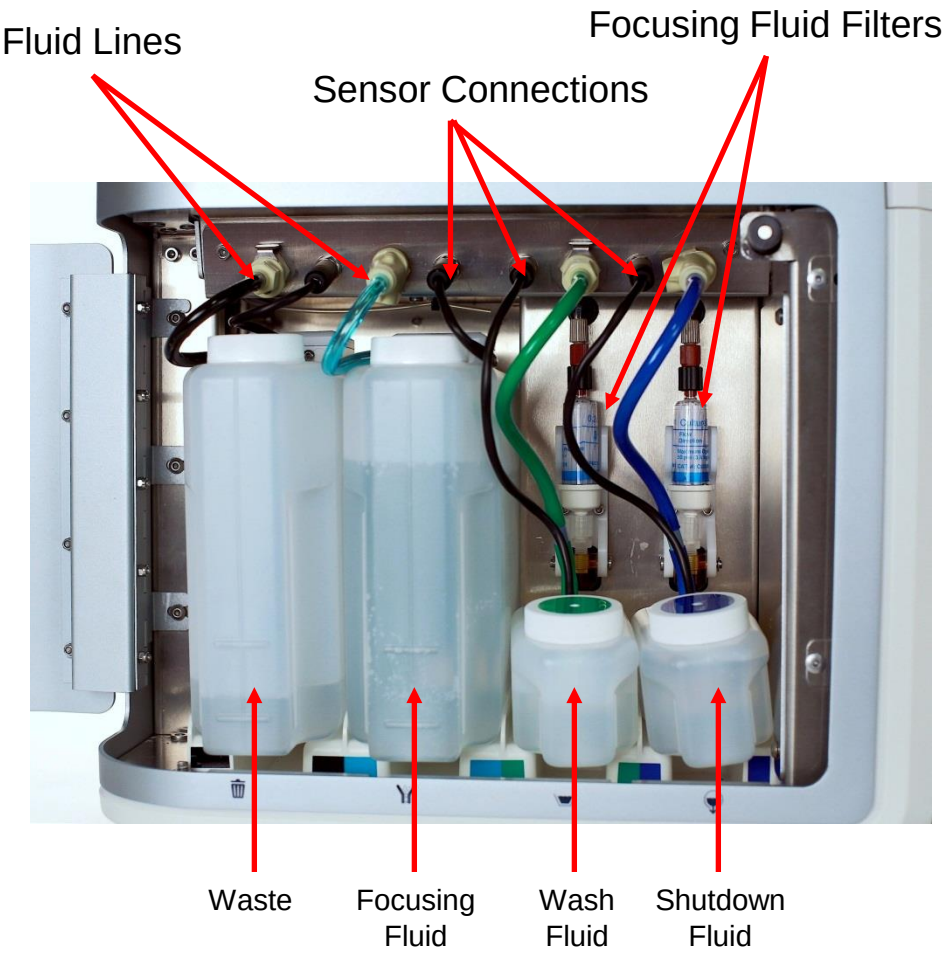
Always turn the CytKick on first!

Power Switch located on the back of both the Attune and CytKick



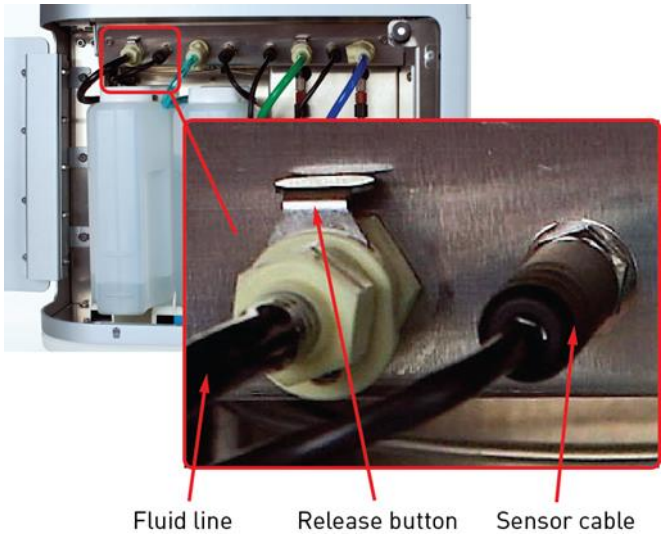
Attune® NxT Fluidics System





Always reconnect fluid lines before sensor!

Filling (or emptying) Fluid Tanks



1. Remove the sensor cable from the instrument
2. Press the metal release buttons to free the tubing
3. Fill or empty as needed with RT solutions
   Large tanks – 1.8 L
   Small tanks – 175 mL
4. Reconnect the fluid line then the sensor line

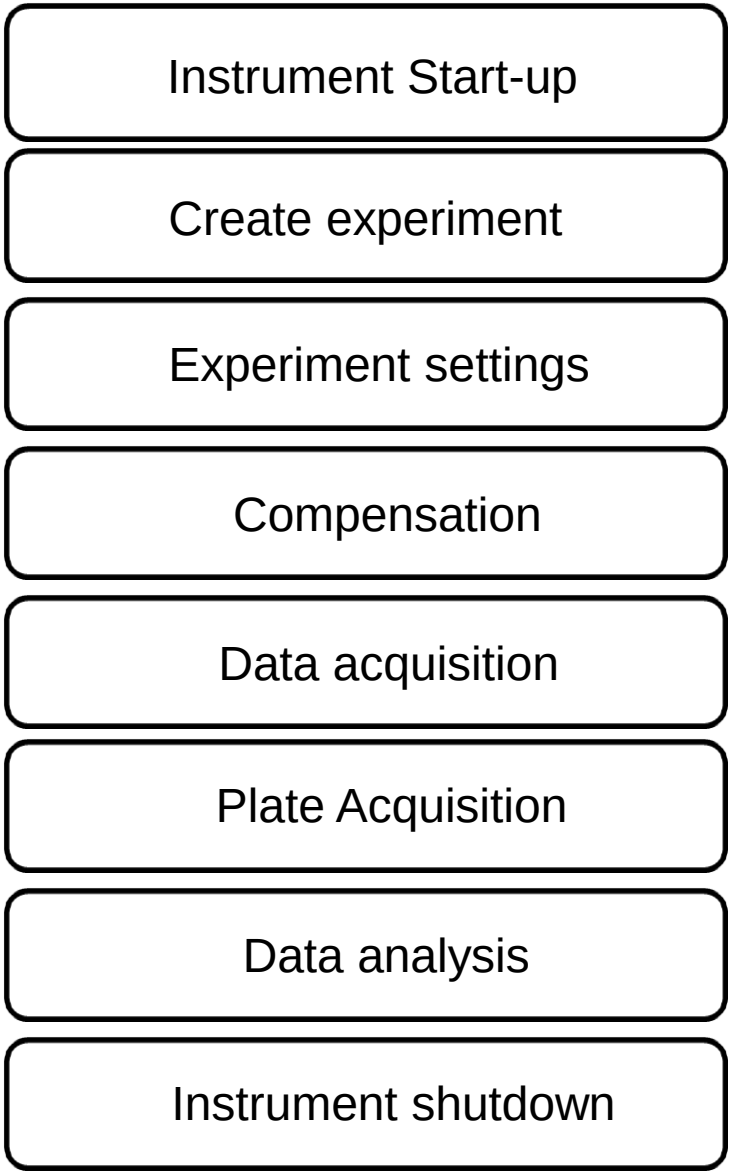
IMPORTANT

1) Connecting the sensor cable while leaving the fluid line disconnected may result in increased back pressure and introduction of air into the system.

2) The Attune® NxT Acoustic Focusing Cytometer must be idle before refilling the fluidics containers.

3) Do not pull on the lines.

Attune NXT Workflow



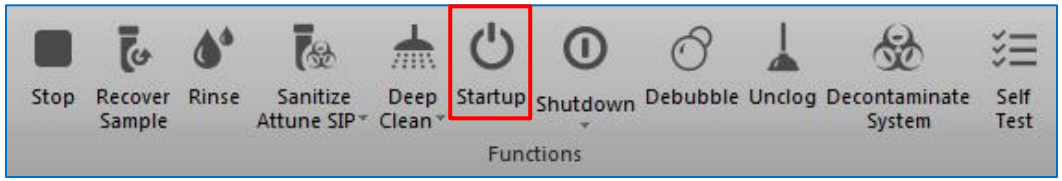
Instrument Startup

From power off

1. Turn on CytKick **first**, remove cleaning plate and close tray door
2. Turn on Attune, wait until status light is solid blue
3. Network login
4. Login Attune Software
5. Launch start-up from instrument tab

From Sleep mode (follows shutdown)

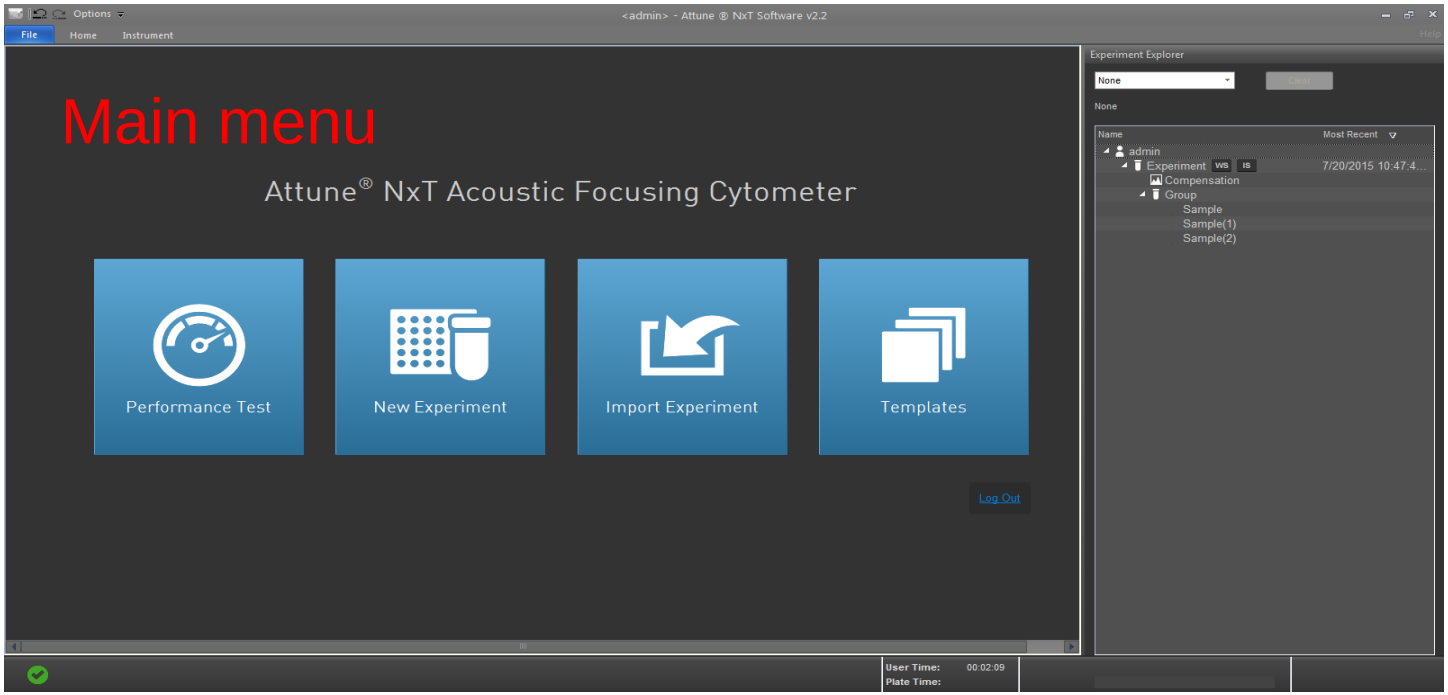
1. Switch user – Network Login
2. Close/restart Attune software - Login
3. Remove plate from CytKick and close tray door if necessary
4. Move Attune sample holder down
5. Launch start-up from instrument tab



Startup Complete

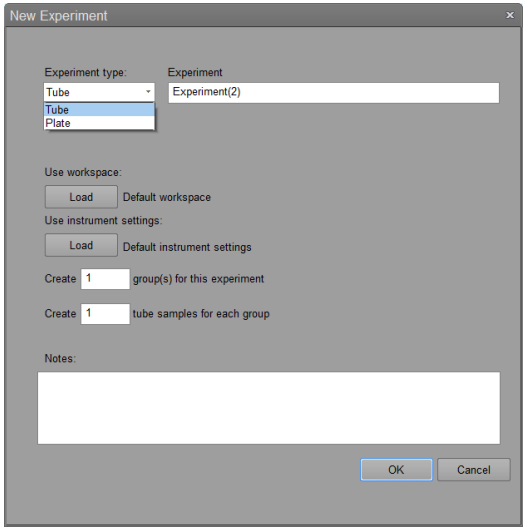
Create Experiment

Create new experiment or import previously saved experiment or template



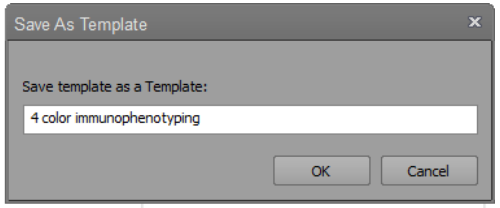
Select default workspace and instrument settings or load saved workspaces and Instrument settings

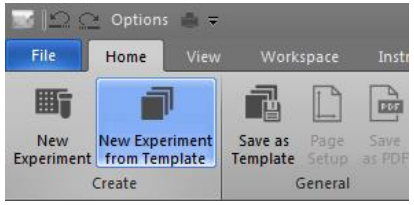
Select tube or plate experiment
Create groups and tubes



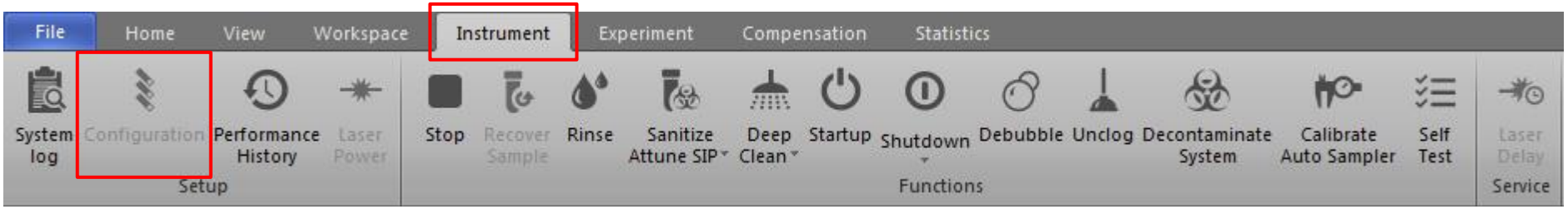
Templates

- Experiment Templates are directions for a new experiment that include workspace plots, run protocol, and instrument settings as defined by the user
- Templates can be accessed from a single user profile
- To create a template from a recorded experiment, select “Save as Template” from Home Tab or by right-clicking on user name in Experiment Explorer
- To create a new experiment from template, select the “Templates” button located on the Main Menu desktop or from the Home Tab
- Once a Template is created, it cannot be deleted

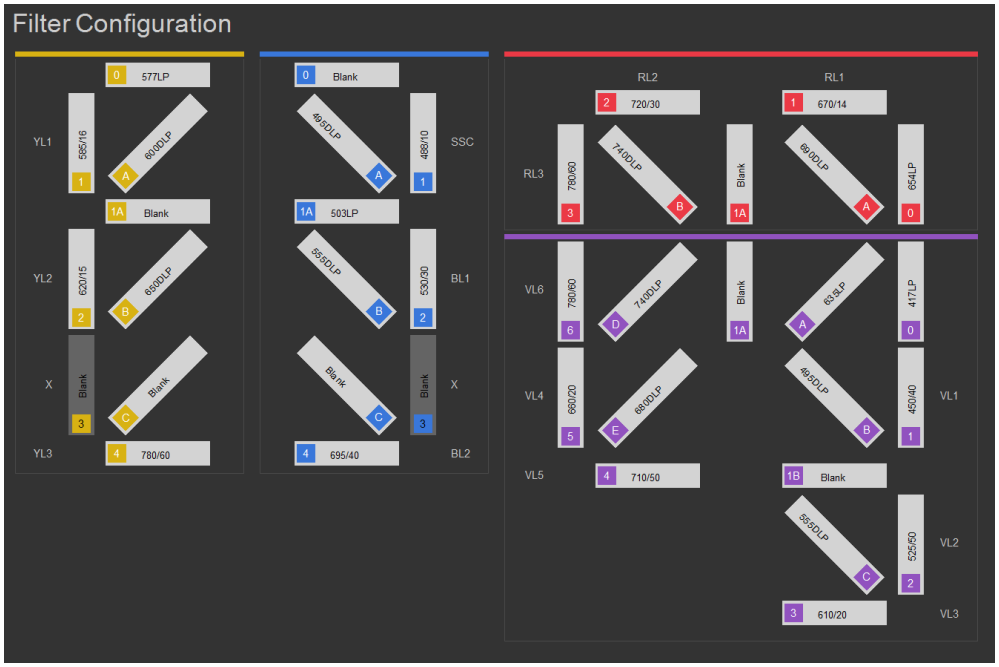




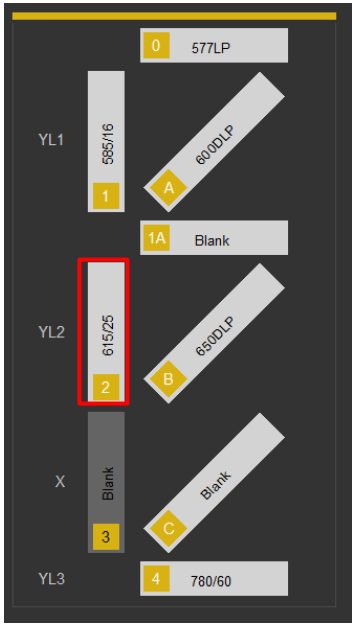
Confirm Optical Configuration



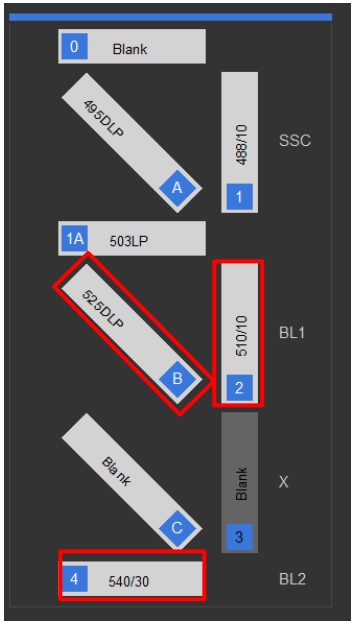
Default Configuration



mCherry Configuration



GFP/YFP Configuration

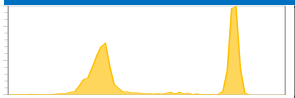
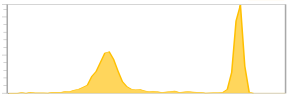
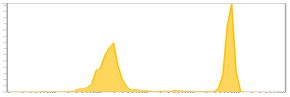
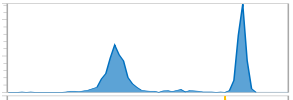
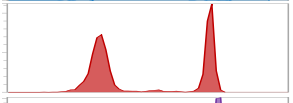
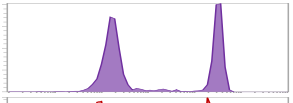
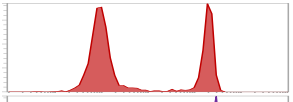
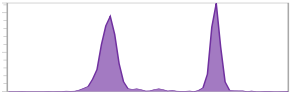


Common Flurochromes for Attune

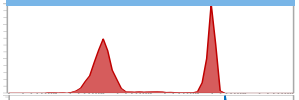
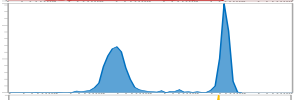
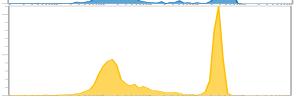
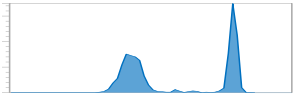
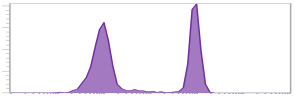
Excitation laser	Emission filter (nm)	Channel	Antibody labels	Compatible fluorescent proteins
Violet–405 nm	440/50	VL1	Super Bright 645 Super Bright 436 eFluor 450 VioBlue Brilliant Violet 421 Pacific Blue BD Horizon V450	Azurite Cerulean eBFP eCFP mTurquoise Sirius **DAPI**
	512/25	VL2	eFluor 506 VioGreen Brilliant Violet 510 Pacific Green BD Horizon V500	T-Sapphire CFP vGFP
	603/48	VL3	Super Bright 600 Qdot 605 Pacific Orange Brilliant Violet 605	
	660/20	VL4	Super Bright 645 Brilliant Violet 650	
	710/50	VL5	Super Bright 702 Brilliant Violet 711 Qdot 705	
	780/60	VL6	Super Bright 780 Brilliant Violet 786	
Blue–488 nm	530/30	BL1	Alexa Fluor 488 FITC	eGFP Emerald eYFP
	695/40	BL2	PE-Alexa Fluor 700 PE-Cy5.5 PerCP-Cy5.5 PerCP-eFluor 710 Qdot 705	
Yellow–561 nm	585/16	YL1	Alexa Fluor 555 Qdot 605 R-phycoerythrin (R-PE, PE)	tdTomato mOrange YFP RFP
	620/15	YL2	Alexa Fluor 568 Alexa Fluor 594 Qdot 605 PE-Texas Red PE-Alexa Fluor 610 PE-eFluor 610	mCherry DsRed mKate mStrawberry
	780/60	YL3	Qdot 800 PE-Cy7 PE-Alexa Fluor 750	
Red–637 nm	670/14	RL1	APC Alexa Fluor 647 Qdot 655 eFluor 660	
	720/30	RL2	Alexa Fluor 680 Alexa Fluor 700 APC-Alexa Fluor 700 Qdot 705	
	780/60	RL3	APC-Alexa Fluor 750 APC-Cy7 Qdot 800 APC-eFluor 780	

Stain Index - Attune™ NxT Flow Cytometer

Brightest

	Reagent	Laser	Filter
	PE	561	585/16
	PE CY7	561	780/60
	PE CY5.5	488	695/40
	PE CF594	561	620/15
	PE e610	561	620/15
	PE CY5	488	695/40
	PE/DAZZLE 594	561	620/15
	BB515	488	530/30
	APC	637	670/14
	BV421	405	450/40
	AF647	637	670/14
	SB436	405	440/40

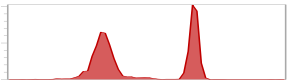
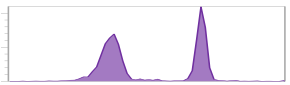
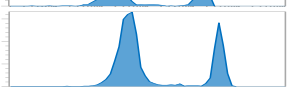
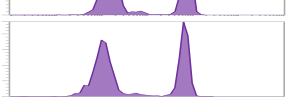
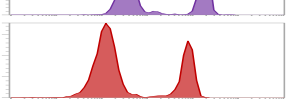
Bright

	Reagent	Laser	Filter
	e660	637	670/14
	PERCP e710	488	695/40
	PE TX RED	561	620/15
	AF488	488	530/30
	PE AF700	488	695/40
	BV786	405	780/60
	APC e780	637	780/60
	BV711	405	710/50

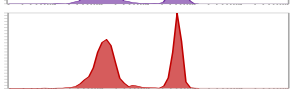
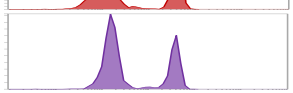
Freshly isolated PBMCs were stained with anti-human CD4 antibody conjugated to various fluorochromes then run on the Attune™ NxT flow cytometer. Ranking of the fluorochromes was based on calculated stain index (SI) of the mean. Brightness was based on the following: Brightest SI 200+, Bright SI 100 – 199, Moderate SI 50 – 99, Dim SI 0 – 49. It is recommended that antibodies be titrated for optimal performance in the assay of interest.

Stain Index Continued

Moderate

	Reagent	Laser	Filter
	SB702	405	710/50
	APC CY7	637	780/60
	SB780	405	780/60
	PERCP CY5.5	488	695/40
	FITC	488	530/30
	SB600	405	610/20
	V450	405	440/40
	BV785	405	780/60
	APC AF750	637	780/60
	e450	405	440/40
	AF405	405	440/40
	BV650	405	660/20
	BV510	405	512/50
	AF700	637	670/14

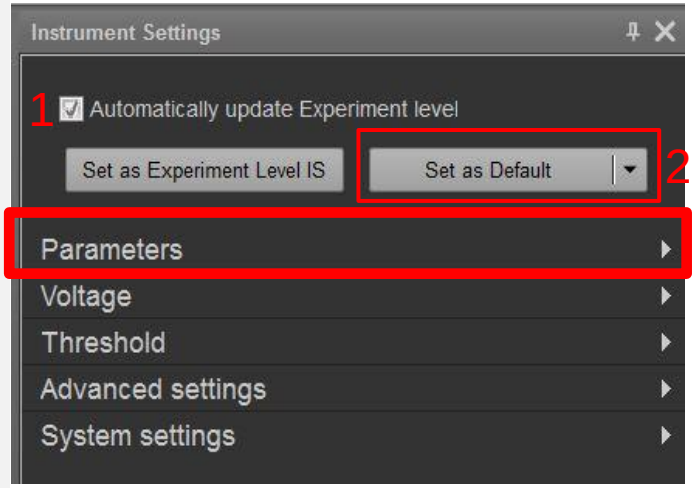
Dim

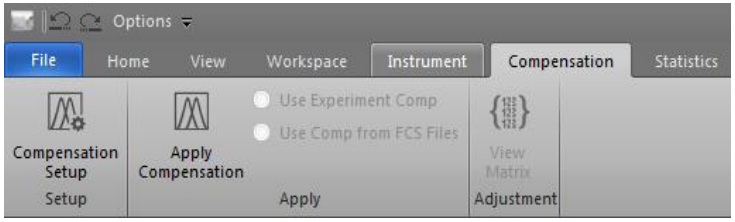
	Reagent	Laser	Filter
	SB645	405	660/20
	BV605	405	610/20
	APC H7	637	780/60
	PAC BLUE	405	450/40
	V500	405	525/50
	PERCP	488	695/40
	e506	405	525/50
	BV570	405	610/20
	PAC ORANGE	405	610/20

Freshly isolated PBMCs were stained with anti-human CD4 antibody conjugated to various fluorochromes then run on the Attune™ NxT flow cytometer. Ranking of the fluorochromes was based on calculated stain index (SI) of the mean. Brightness was based on the following: Brightest SI 200+, Bright SI 100 – 199, Moderate SI 50 – 99, Dim SI 0 – 49. It is recommended that antibodies be titrated for optimal performance in the assay of interest.

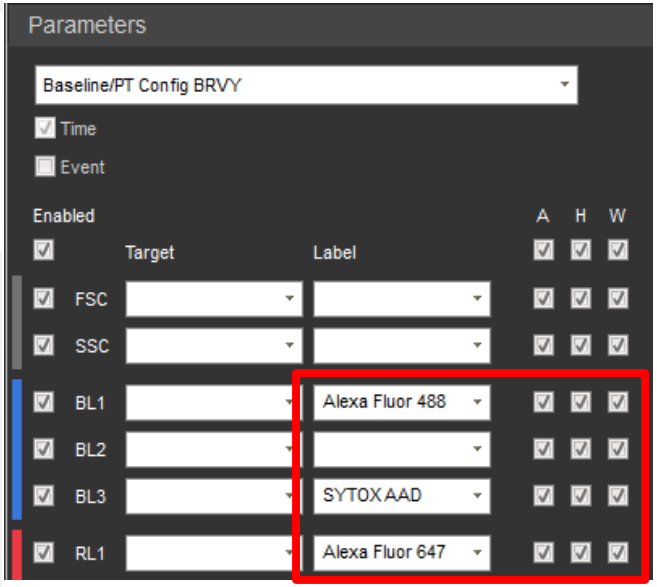
Choosing Parameters

- Set to Automatically update Experiment level 1
- Optional: Load/export customized configuration 2
- Parameters tab - select detectors (A-H-W) as required
- Select fluorchrome ***label*** from drop down menu and enter target/marker name if desired



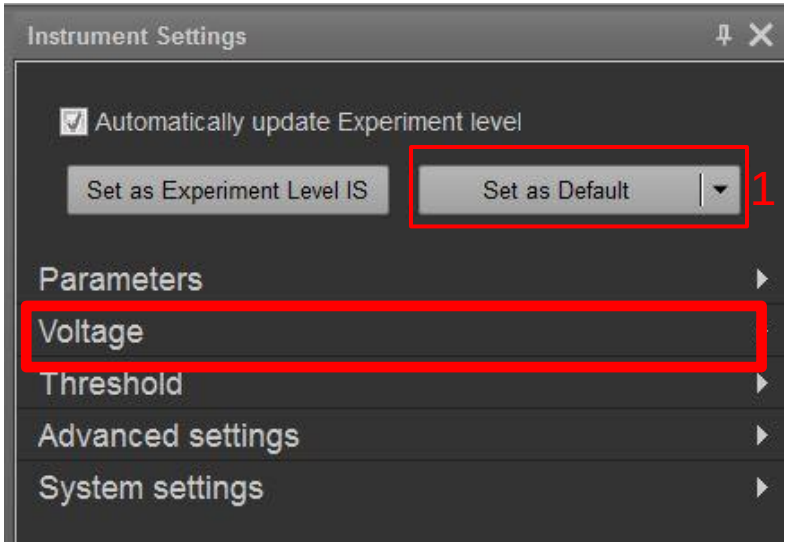


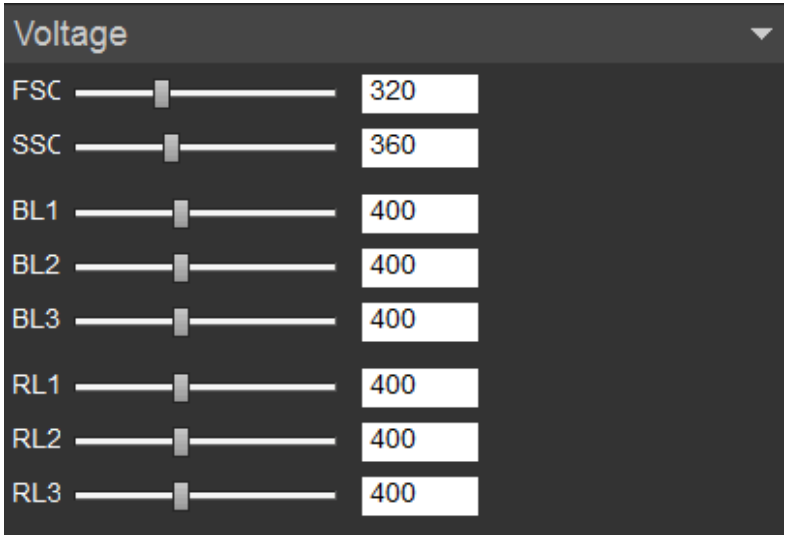
- If compensation is required detectors can be selected through compensation setup
- *Once compensation is recorded, all fluorescence channel voltages will be disabled



2. Adjust PMT Voltages

- Adjust FSC & SSC voltages to position cell population on the scatter plot
- Load default PMT Voltages (voltage walk values) from desktop folder and set as default 1
- Reduce PMT voltages as necessary to keep fluorescence on scale

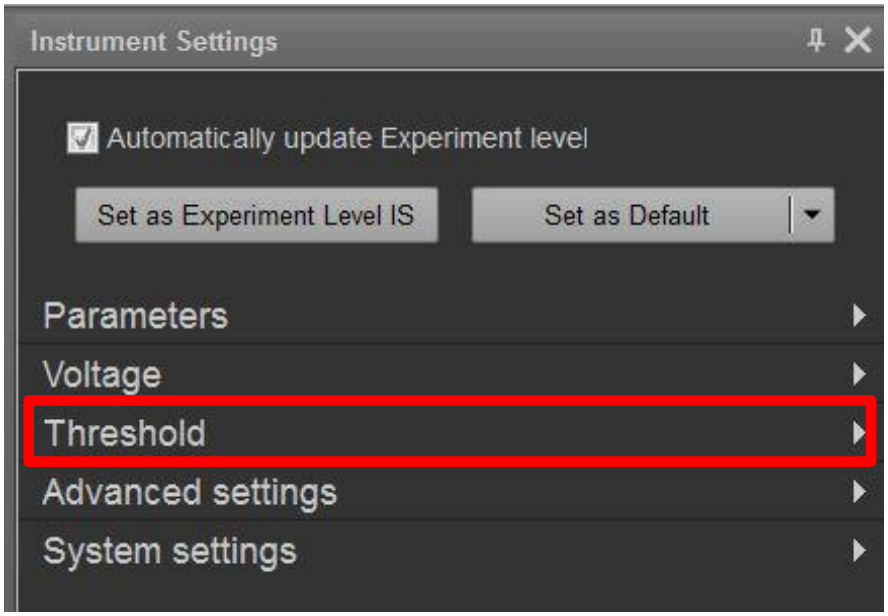


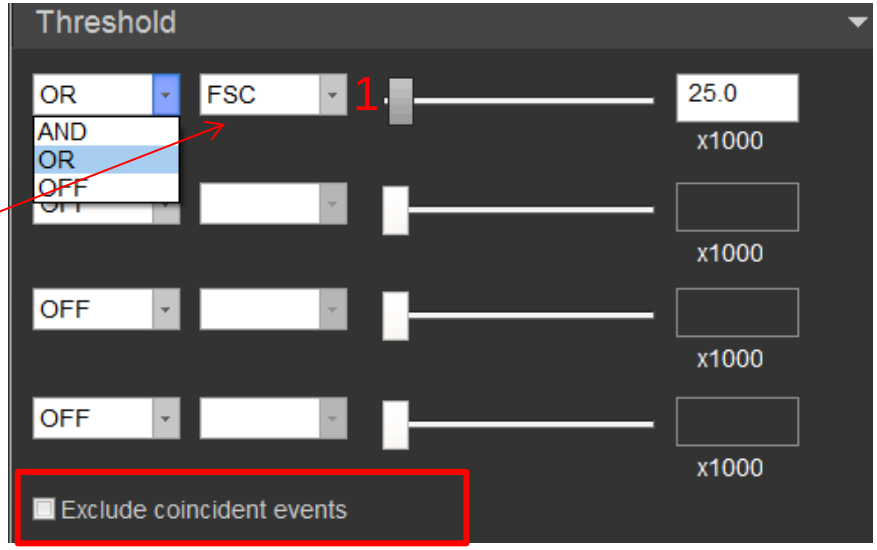


3. Adjust Threshold

- Default setting: OR FSC - Between 50-100 X1000 is a good starting point 1
- Can be set on a single or up to 4 scatter and/or fluorescence channels
- Data not meeting threshold criteria is <u>permanently lost</u>

- Exclude coincident events option:
  - checkbox



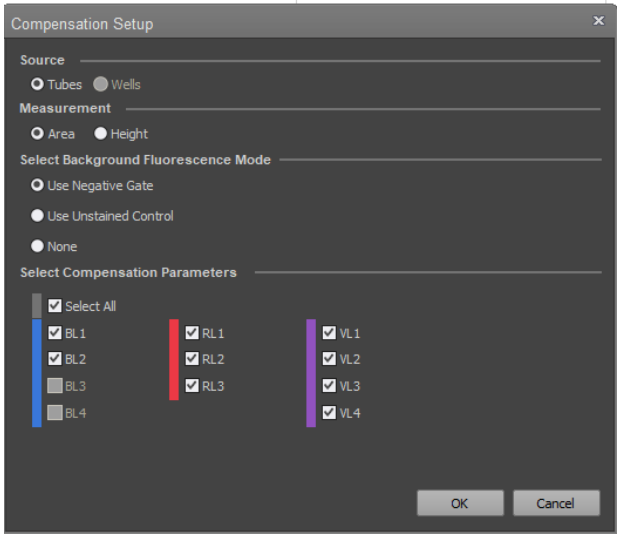


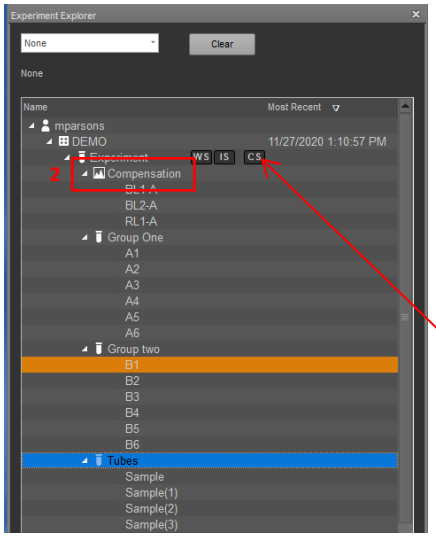
At least 1 threshold must be set – typically the FSC . If all are set to OFF, no data is displayed on the workspace!

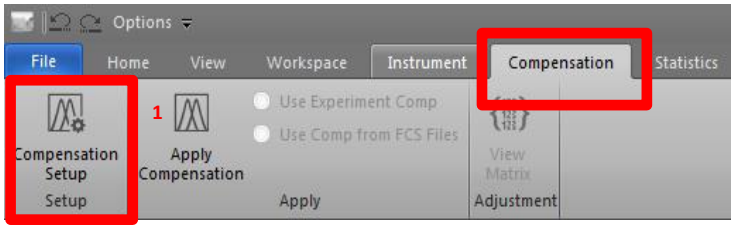
The "exclude coincident events" option can deal with the 2nd and 3rd types of coincidence where events showing a 'valley' (defined percentage of the pulse height/amplitude) are abort or thrown away

Compensation Set up

Under the *Compensation* tab, click on **Compensation Setup**[1]. Or double-click on Compensation in Experiment Explorer[2]







Compensation settings can also be dragged into a new experiment from a previous experiment

Source - tubes or wells

Measurement – Area or Height

Background fluorescence – negative gate vs. unstained control

Compensation Parameters

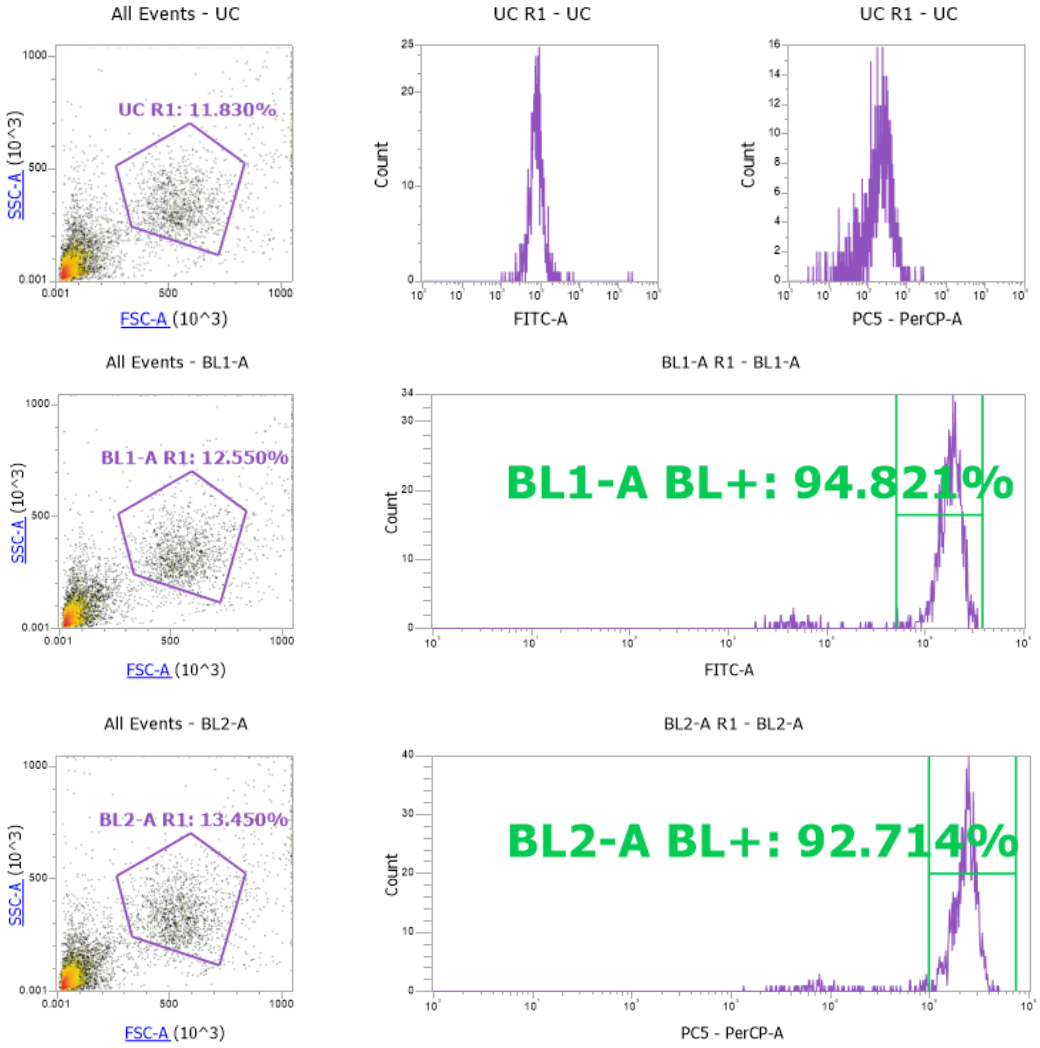
Compensation: Background Fluorescence Modes

Auto Fluorescence Correction Choices:

- Negative Gate
- Unstained Control
- None

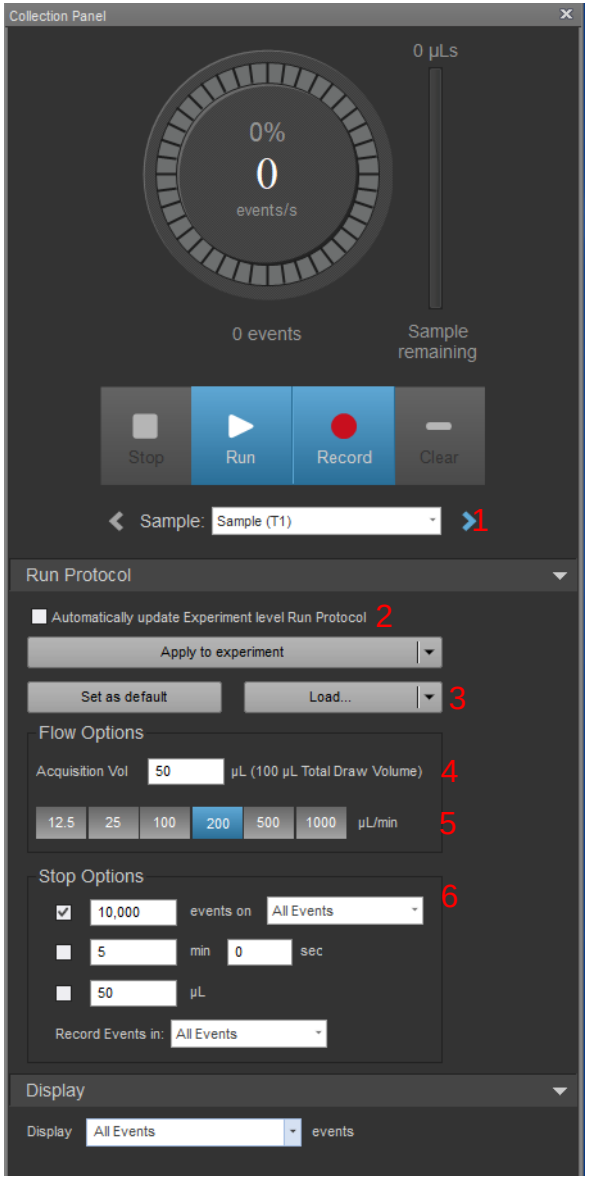
Background Mode	When to Use?
Negative Gate	With different controls such as cells and beads; or using different cell populations (lymphs and monos).
Unstained Control	When all controls are of the same type (beads, all lymphs)
None	Rarely used but in cases where background is negligible or cannot be ascertained.

Example - compensation using unstained autofluorescence control



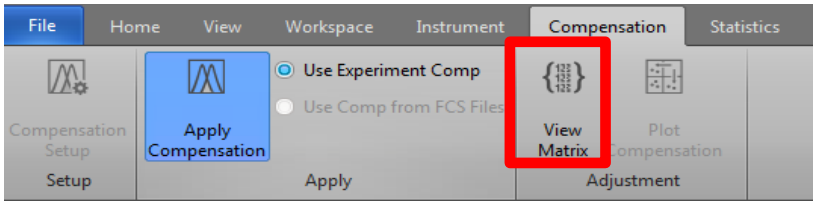
Data Acquisition- Tube (Collection Panel)

1. Select sample to acquire
2. Select automatically update experiment level run protocol
3. Possible to load a saved run protocol
4. Choose volume to acquire (**minimum volume required is indicated as total draw volume which takes into account the dead volume**)
5. Select acquisition speed – normally between 12.5 and 200
6. Choose between “stop options”
7. After data is recorded the remaining sample can be returned to the tube



Spillover Matrix

- At the end of Auto-compensation:
  - Spillover Matrix is automatically calculated
  - Compensation is applied to all samples



Spillover BL1-A BL2-A BL3-A RL1-A

BL1-A
BL2-A
BL3-A
RL1-A

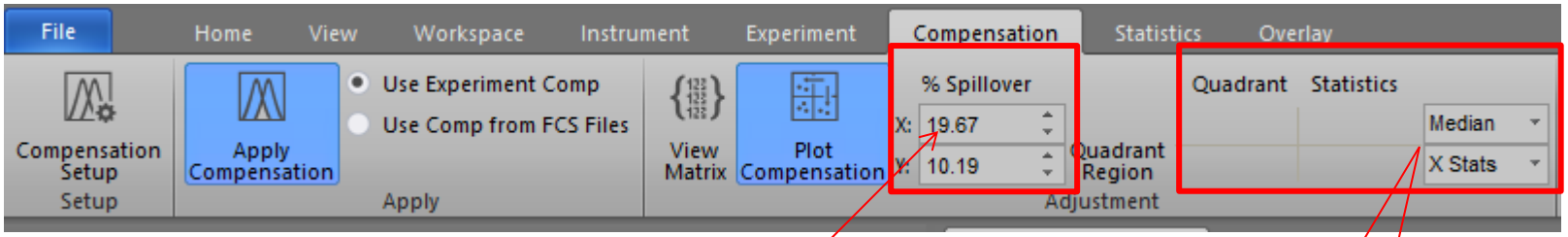
	BL1-A	BL2-A	BL3-A	RL1-A
BL1-A	100.00	38.10	5.86	0.01
BL2-A	0.51	100.00	24.18	0.00
BL3-A	0.22	25.40	100.00	13.92
RL1-A	0.06	0.04	0.01	100.00

Matrix assigns numeric value (percent spillover) of a fluorophore into other detectors. To read the matrix – fluorochromes in the column on the left spill into the detectors indicated on the top. Eg BL1A (FITC) spills into detector BL3-A (5.86%)

On Plot Compensation Adjustment Tools

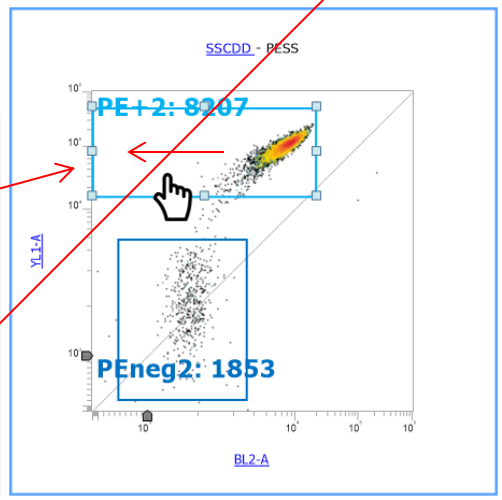
Tool allows manual adjustment of compensation

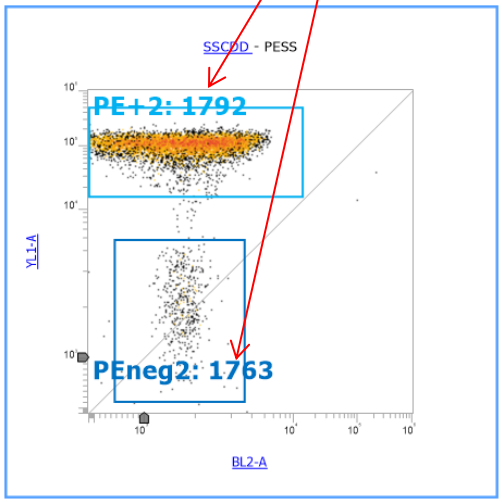
*manual compensation must be used carefully to avoid adversely affecting results



Drag populations to make coarse adjustments to compensation.

Fine adjustments can be made using the *%spillover* dialogue box

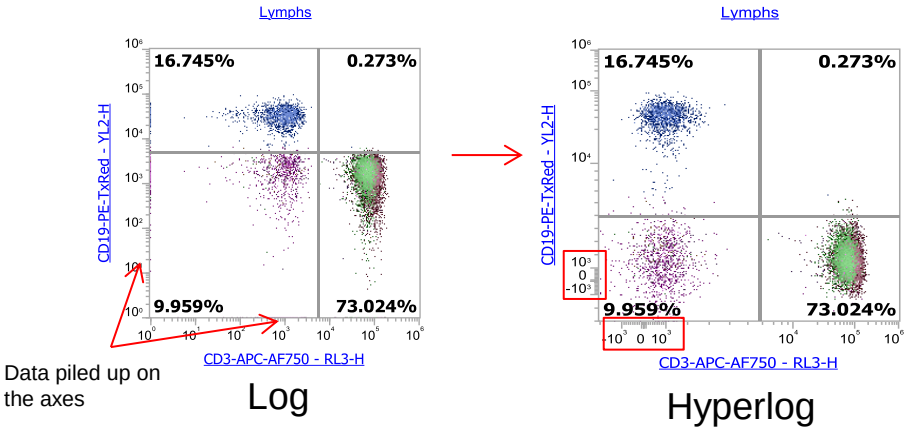




Use statistics to match relevant median populations

How to view compensated data - Hyperlog

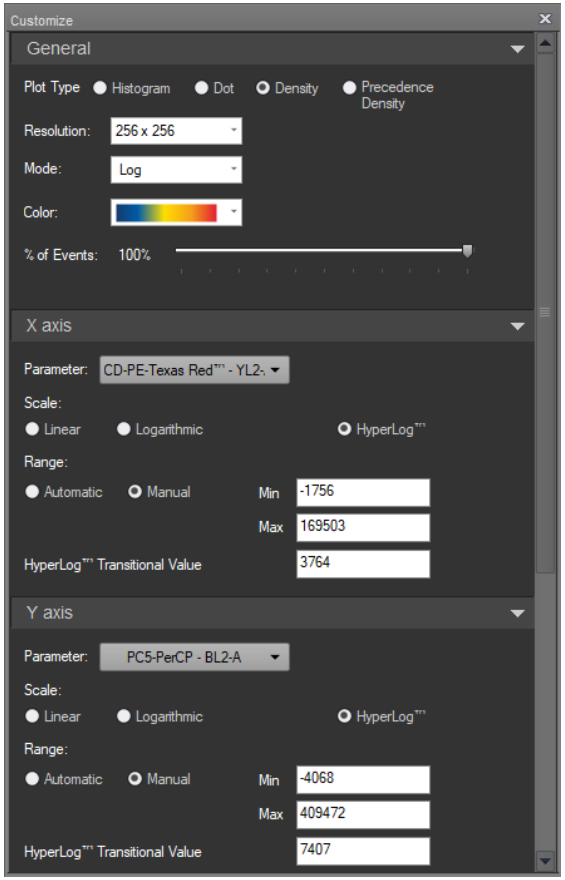
Compensated data can be viewed in Hyperlog in order to see data that enters negative log space or is close to zero AFTER compensation is applied.



Data piled up on the axes

Log

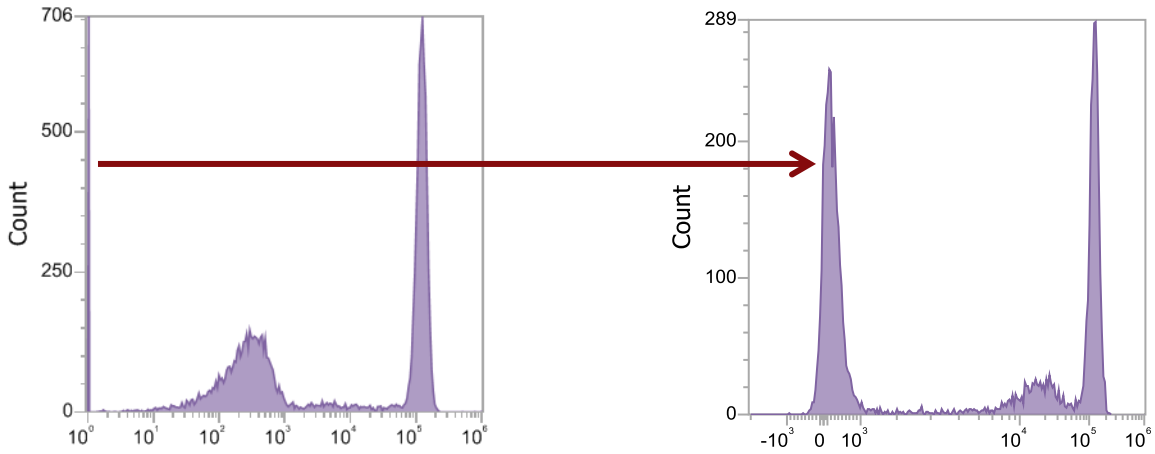
Hyperlog



Once plot is selected the customize panel may be opened to change plot type and scaling

HyperLog™ Scale

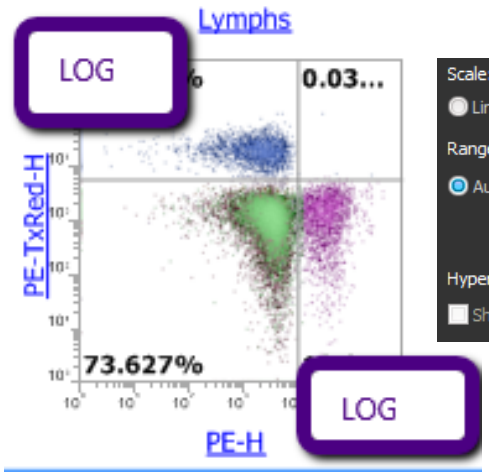
- HyperLog™
  - Logarithmic scale at the high end
  - Transitions to linear scale in the region around zero

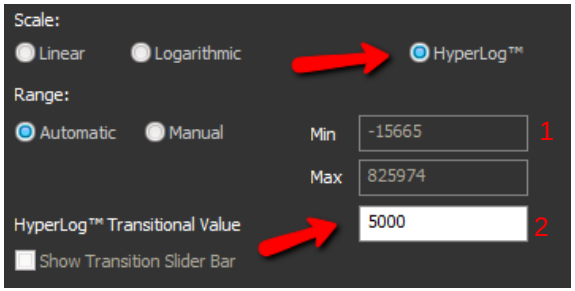


Used to better display fluorescence values on or below “0” that pile up on the axis due to measurement error when using a log scale.

Better separation of dim signals

Adjusting Hyperlog Setttings





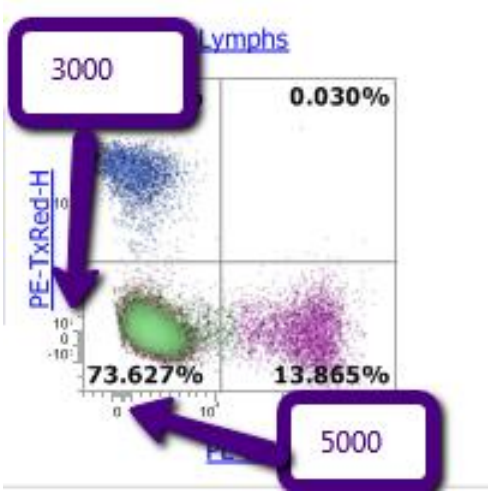
1.To determine the most negative outlier set range to automatic then change the range to manual and set negative outlier as minimum value

2. Use sliders to adjust the transition value

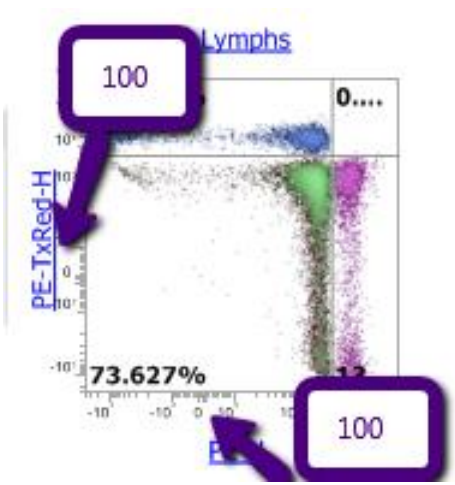
A large transition number will condense a spread population

A small transition value will spread out a population.

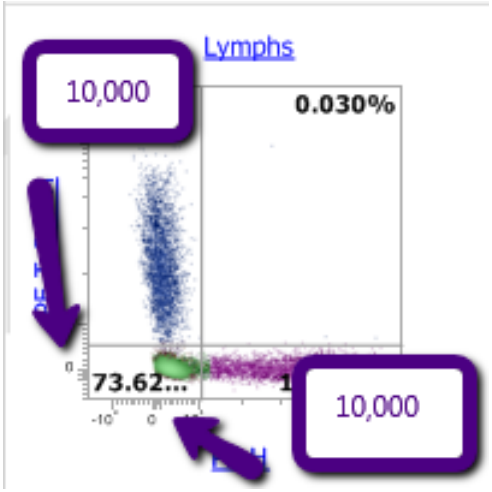
Correct



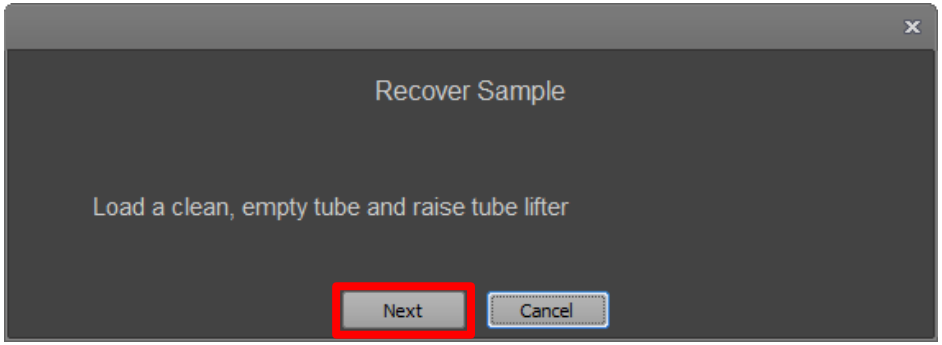
Too small



Too big



Sample Recovery - Tubes



When is ***sample recovery*** available?

Anytime sample remains in the sample loop

- Stop option has been reached
- Operator clicks stop

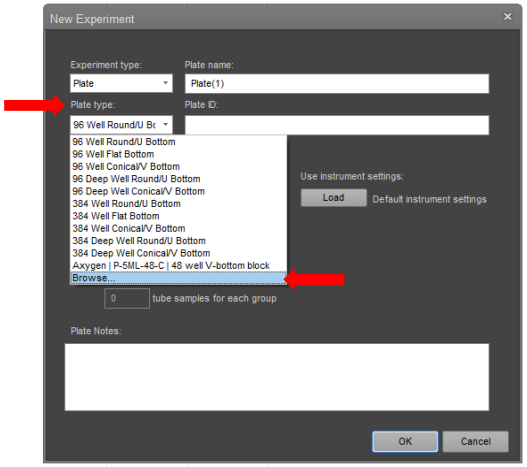
NOTE: Must select **recover sample** before lowering the tube lifter

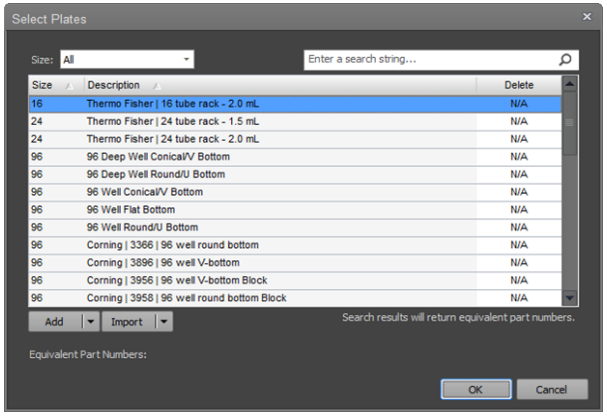
Attune NxT AutoSampler – Cytkick Max

Specification	CytKick Max Autosampler
Plate types	• 96 deep-well (flat, U-bottom, and V-bottom). • 96-well standard depth (flat, U-bottom and V-bottom) • 384-well standard depth (flat, U-bottom and V-bottom) • 384 deep-well (flat, U-bottom and V-bottom) • **1.5 mL and 2 mL microcentrifuge tube rack (up to 16 per rack)** • **Ability to create user defined custom plate definitions using specific plate dimensions**
Cooling	Yes, **passive** using a cooling block for standard 96-well U bottom plate and microcentrifuge tube rack (up to 16 per rack) A standard plate is defined as a plate that is within the height range of 14.35 mm ± 0.76 mm (such as Thermo Scientific™ 96-well Microtiter™ Microplates, Cat. No. 2205).
Plate cover	Foil covered 96-well (U-bottom only) 384-well (U- and V-bottom only) mixing (limited by mixing efficacy)
Mixing method	Combination of aspiration and stirring based on the inputted sample volume, plate type, and well geometry

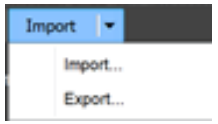
Select Plates Dialog

- The “Select Plates” dialog can be used to choose plates to add to the Quick Select plates list that is used to populate the Plate type list in a New Experiment.
- It can also be accessed when setting up a new experiment by selecting Browse under the Plate type in the New Experiment Dialog

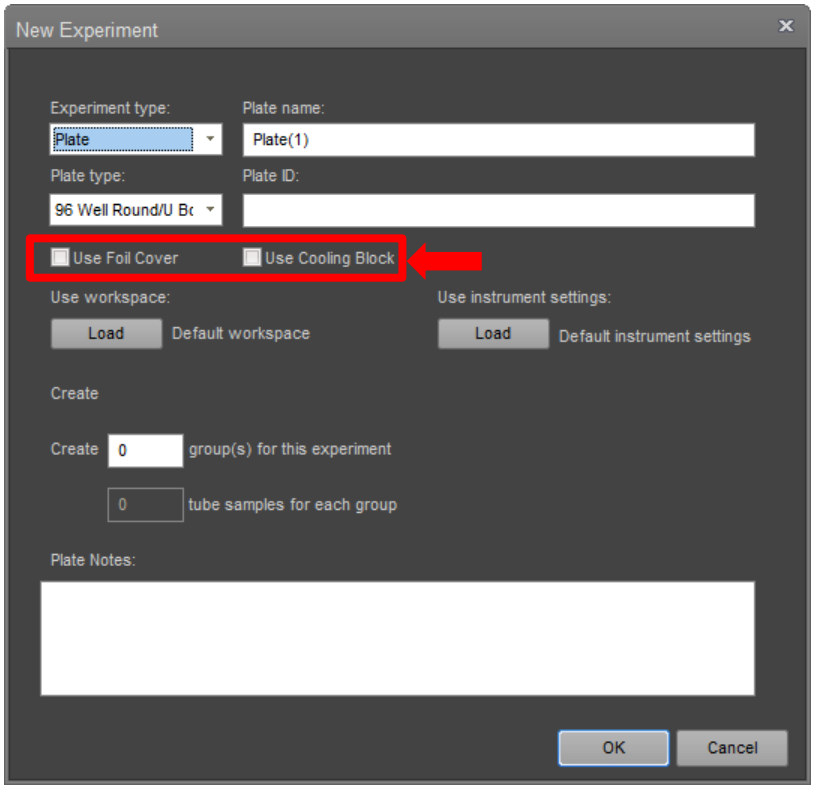




- Using the Select Plates dialog, new, customizable plates, with plate specific dimensions, can be added and existing plates can be edited.
- Custom plates can be exported and plates that have been created in a different version of Attune NxT software can be imported by appropriate option from the Import drop down



Selection of cooling block or foil cover: New Experiment Dialog box



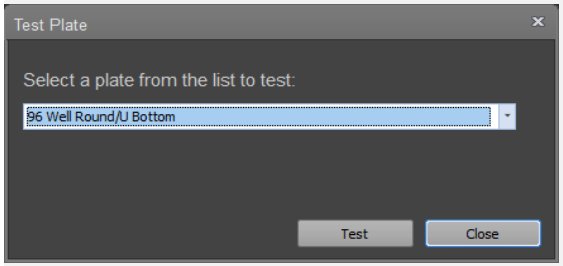
- New checkboxes are visible when set to CytKick Max that allow the user to use a foil cover or cooling block
- The cooling block option is enabled for 96 well plates For 96-well plates there is a universal cooling block for all plate types and also a cooling block for Thermo Fisher 2205 plate type

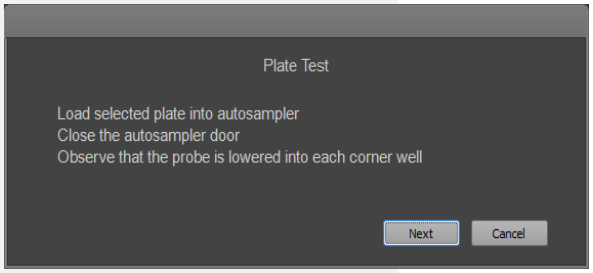
- To use the cooling rack for 1.5 mL and 2 mL tubes users should select the 16 well eppendorf rack for the plate type.
- NOTE: to use the cooled 16 well tube rack it is not necessary to select Use Cooling Block option

- NOTE: If using a Foil Cover it is important to indicate this in the experiment set up as the Mixing algorithm will behave differently if a foil cover is present.

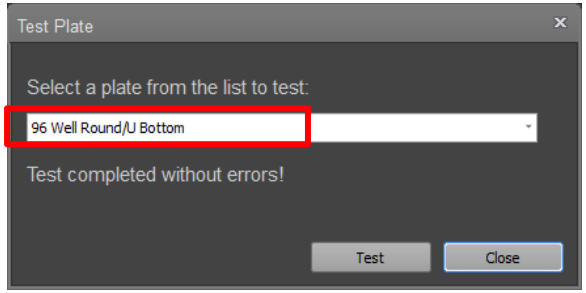
Test plate function

- The *Test Plate* function ensures that the CytKick probe position is in the correct location in all four corners of the plate.

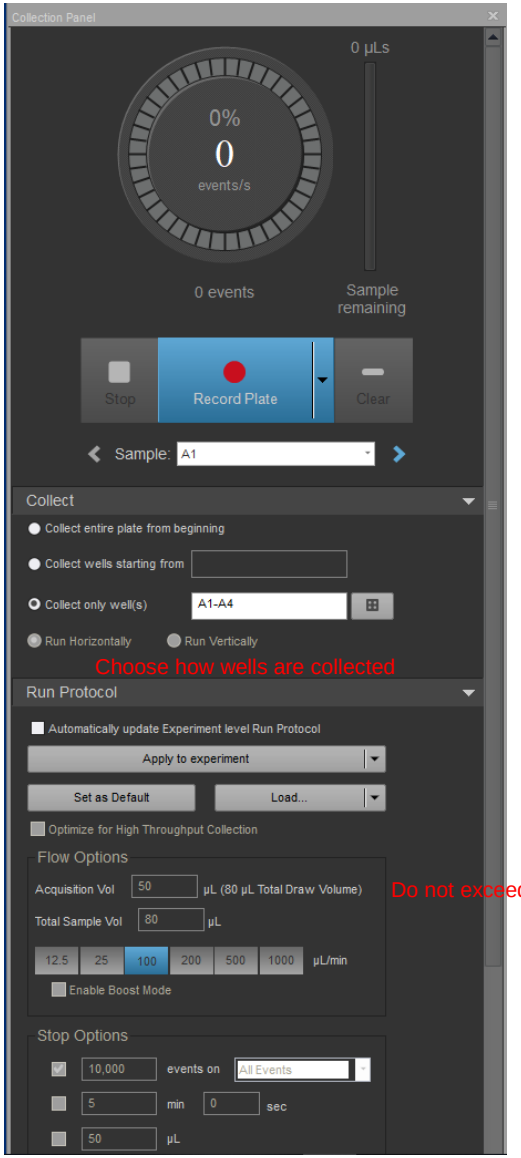




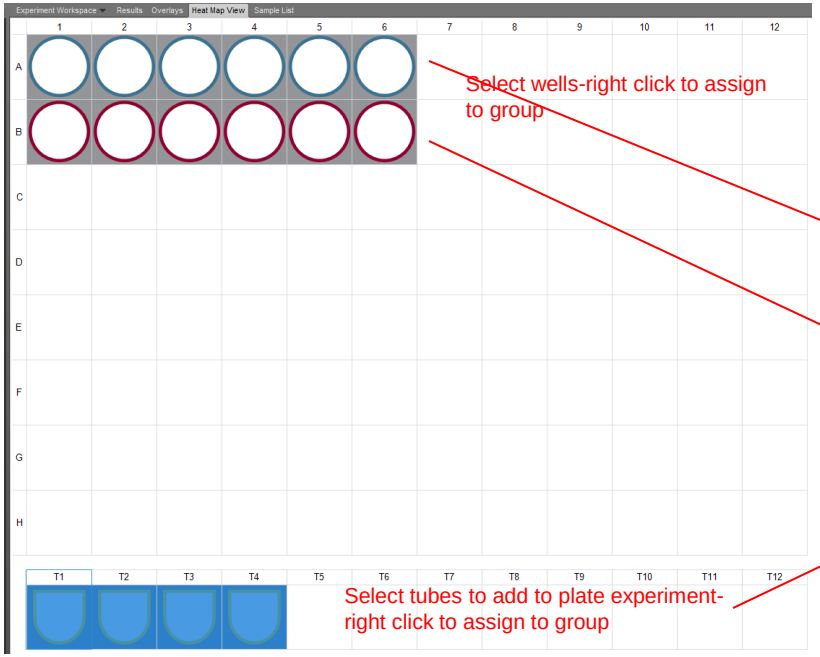
- The CytKick will move through each corner of the selected plate or tube rack and lowers the probe to the well depth as defined in the selected plate definition. This cycle is repeated three times.

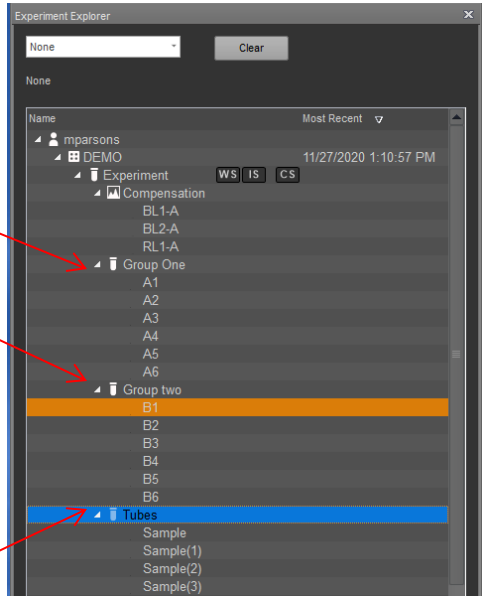


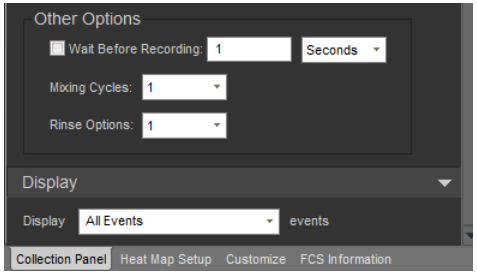
Collection panel for CytKick



Do not exceed minimum draw volume

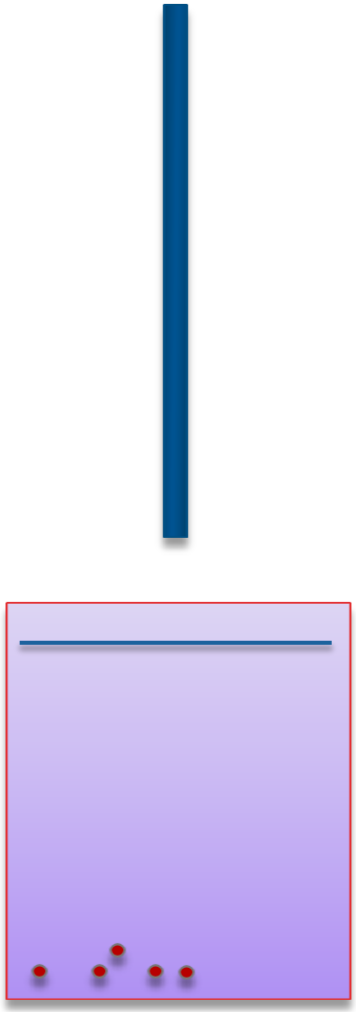






Choose mix settings

Attune® Autosampler Mixing procedure



The user sets:

- The plate type
- The total sample volume
- The number of mixes

  (Max. 3 mixes, recommended at this time)

The system defines:

- The liquid level in well
- The probe position
- The mixing method

Mixing sample by aspiration instead of shaking ensures homogeneity of the sample and maintains cell viability

Mixing Algorithm on the CytKick Max

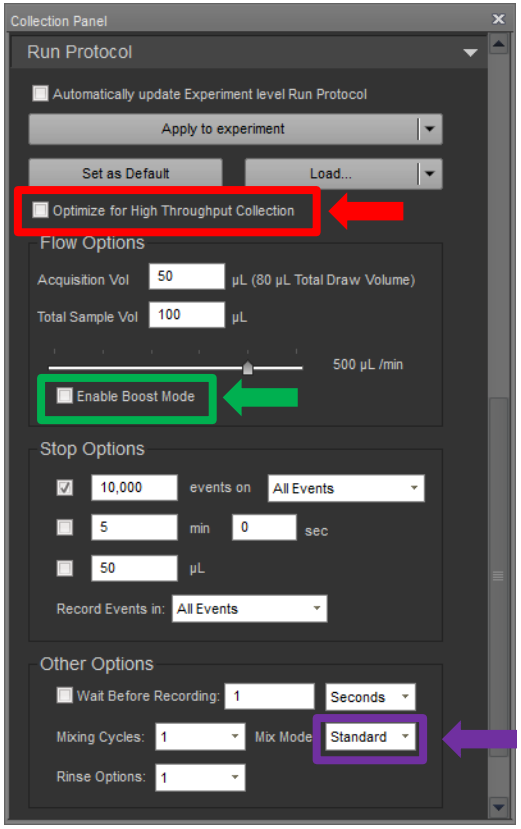
- Mixing with the CytKick and CytKick Max involves a combination of mixing by aspiration and stirring.

- The volumes that are used for aspiration are dependent on the plate format and the sample volume defined in the software.

- Three different algorithms are incorporated into the system for mixing and which algorithm is used is defined by the plate type automatically:
  - SquareMoveAndBlast: Probe moves in a square pattern (including a center point, 1 mm above the bottom of the well
  - CircularMoveAndBlast: Probe moves in a circular pattern, aspirates and dispenses at 5 locations, 1 mm above the bottom of the well using mix volumes specified for well volume
  - CircularMove: Probe moves around well at specified radius without aspirating or dispensing (ie. Stirring the well)

*If foil cover option is selected, there is no stirring, only mixing up and down

Run Protocol Options



- **Optimize for High Throughput Collection:**
  - 20 µL Acquisition Volume
  - 1000 µL/min
  - Enable Boost Mode checked
  - Stop on 20 µL
  - 1 Mix Cycle
  - 1 Rinse
  - **Only used for rapid yes or no criteria**

- **Boost mode** is only enabled at 500 and 1000 µL/min and is used to increase plate processing speed which reduces the processing time for a standard 96-well plate from 45 minutes to 22 minutes
- **Increases carry-over between wells**

- **Mix mode:**
- When the option is set as **Standard**, the Sample is mixed using the normal mixing speed
- When the option is set to **Gentle**, the Sample is mixed and aspirated at a slower speed. For fragile cells, viscous samples, or samples prepared in viscous buffers, use the Gentle mix mode

Optimize for High-Throughput Collection

- When selected, the **Optimize for High-Throughput Collection** option optimizes the Run protocol for high-throughput data collection as follows:

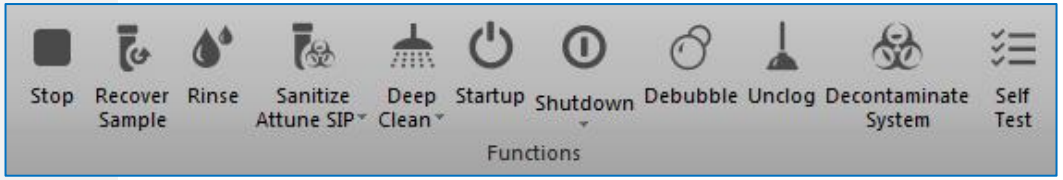
Run protocol parameter	Attune™ NxT and CytKick™ Autosampler	CytKick™ MAX Autosampler
Stop volume	40 µL	20 µL
Acquisition volume	40 µL	20 µL
Flow rate	500 µL/minute	1000 µL/minute
Mixing cycles	1	1
Rinse between samples	1	1
Wait before recording	Unchecked	Unchecked

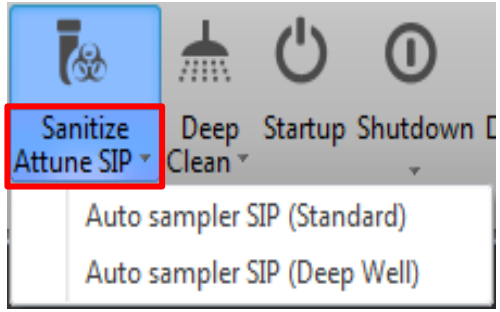
If “Optimize for High-Throughput Collection” is checked then automatically acquisition run and flow rate will be adjusted and “Enabled boost mode” will be checked as well

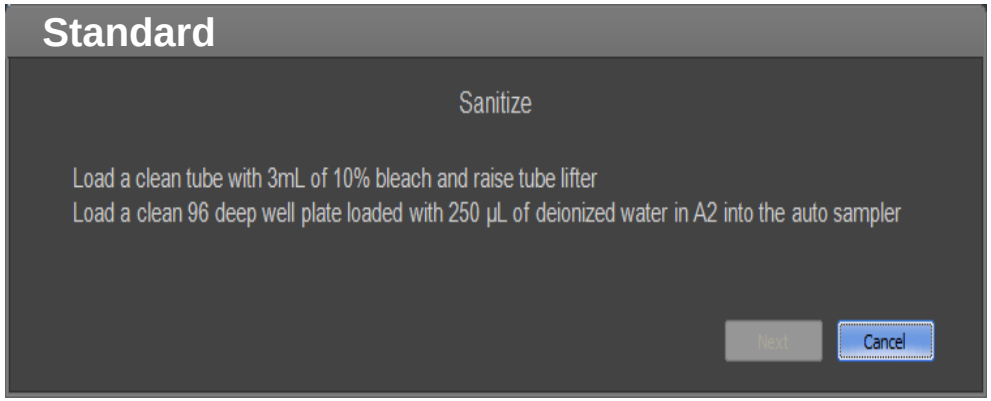
*boost mode is only applicable for 500ul/min and 1000ul/min

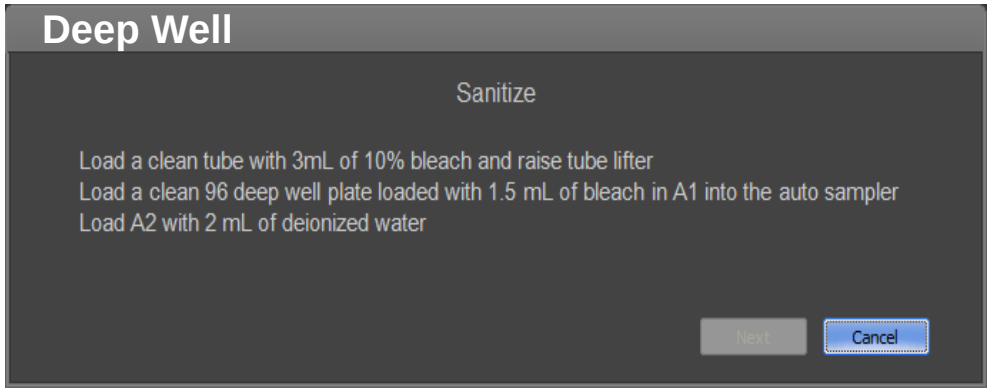
Sanitize Autosampler SIP

- Between experiments and user
- After running beads
- If running DNA dyes
- As part of shutdown procedure



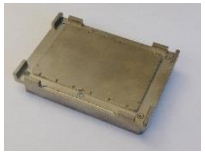






Cooling block specifications

- The CytKick Max comes with three cooling blocks included:
  - Universal well plate holder: Expected duration of chill at 2-15˚ C, 64 minutes
    Part number 100081492

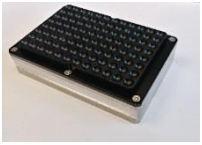


  - Microtube cooling block: Expected duration of chill at 2-15˚ C, 108 – 120 minutes
    Part number 100076823
    Holds 1.5 mL and 2 mL Eppendorf tubes

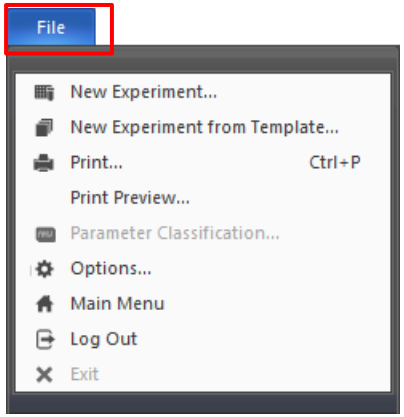
  - Delrin microtube Holder: Expected duration of chill at 2-15˚ C, 27 minutes
    Part number 100064241

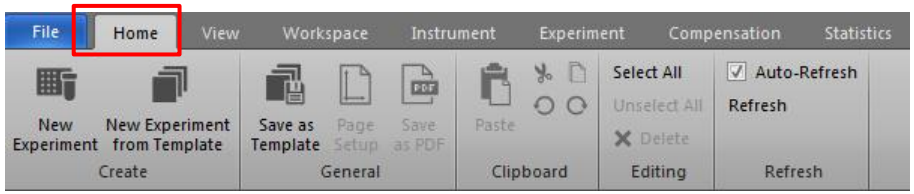
Note: This item is not intended specifically for cooling and is more generally of use for running tubes without cooling

  - 96-well round bottom plate holder: Expected duration of chill at 2-15˚ C, 95 – 100 minutes
    Part number 100063482
    For use with Thermo 2205 96-well U bottom plate

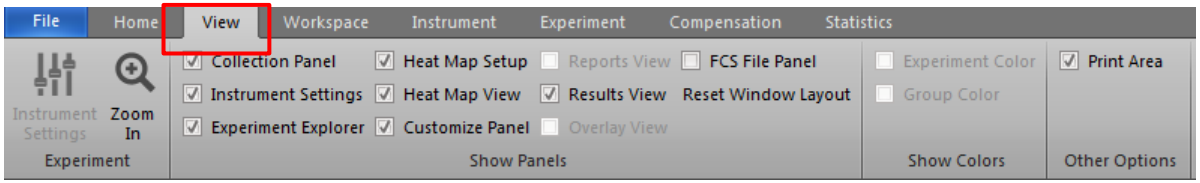


Ribbons and Tabs



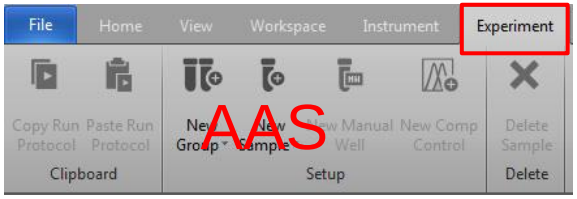


Home



View

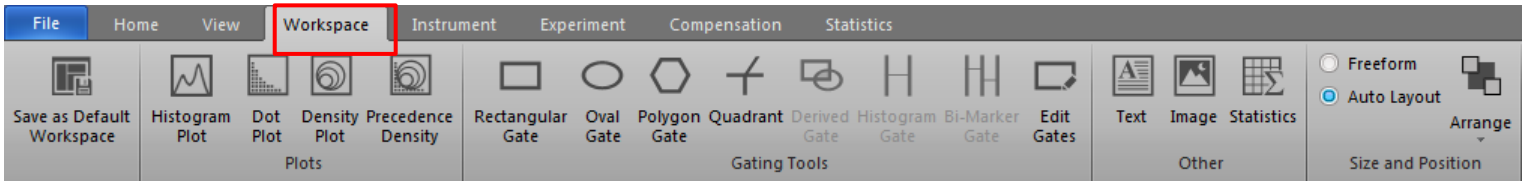
Instrument



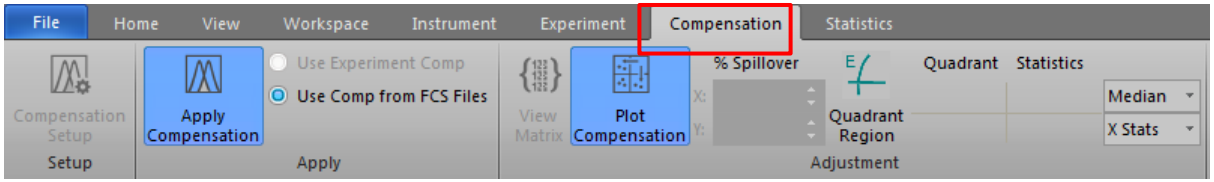
Autosampler

More information in the SW user guide

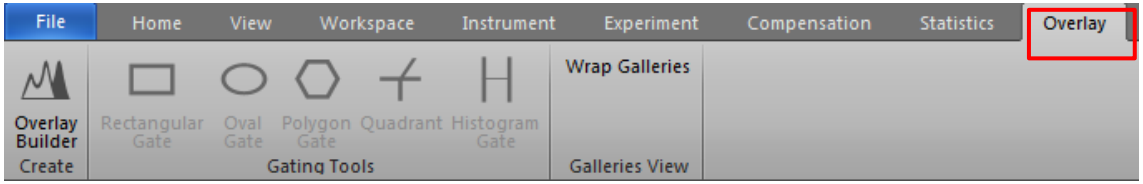
Ribbons and Tab - continued



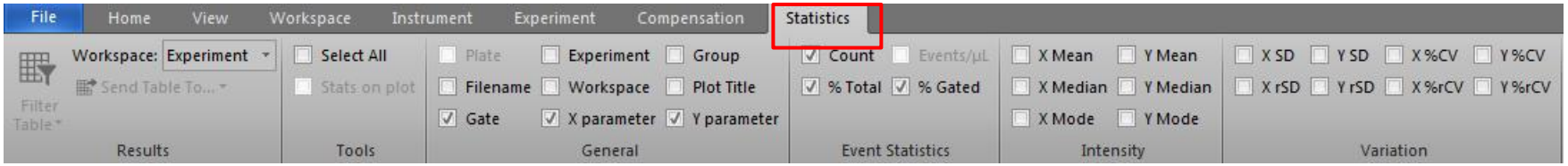
Workspace



Compensation



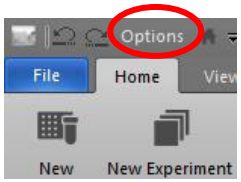
Overlay builder

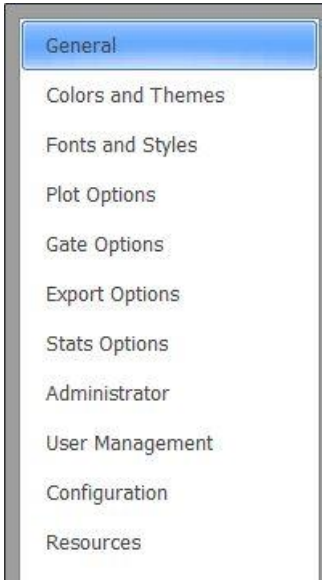


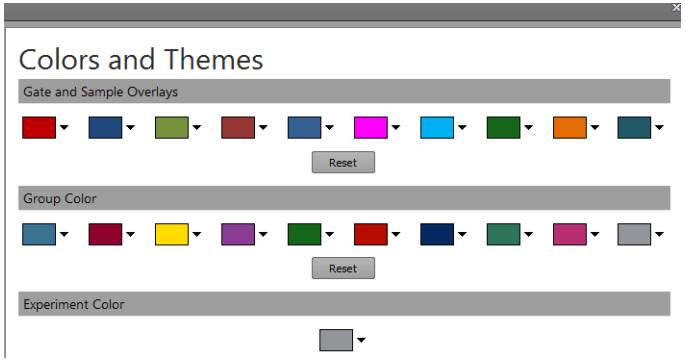
Statistics

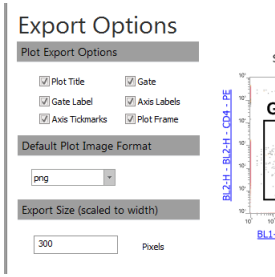
More information in the SW user guide

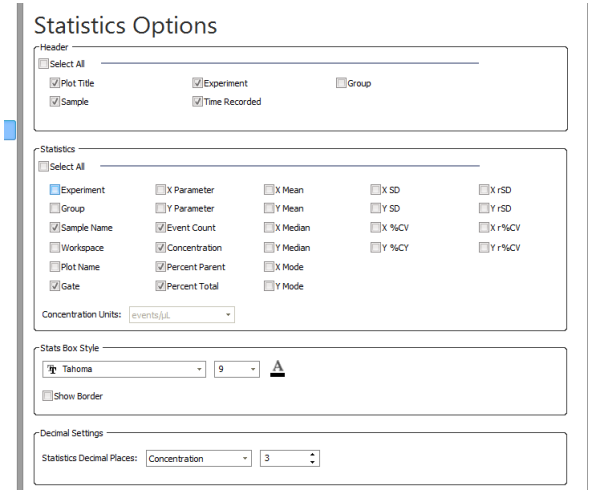
Setting User Preferences –under options

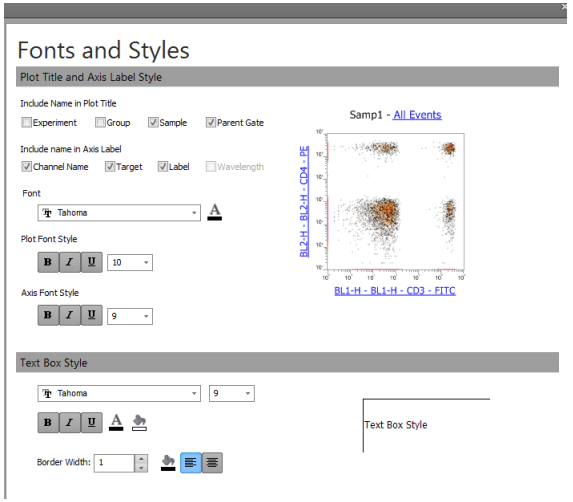


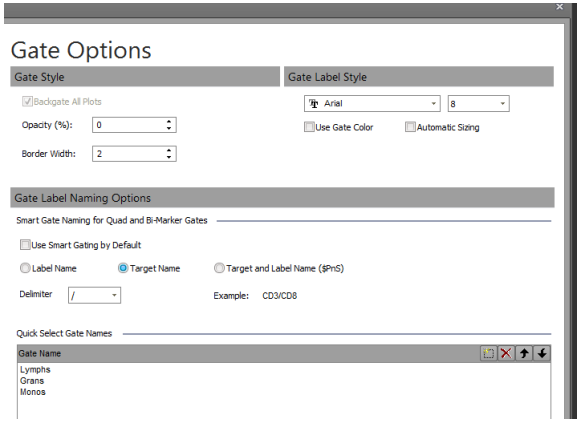






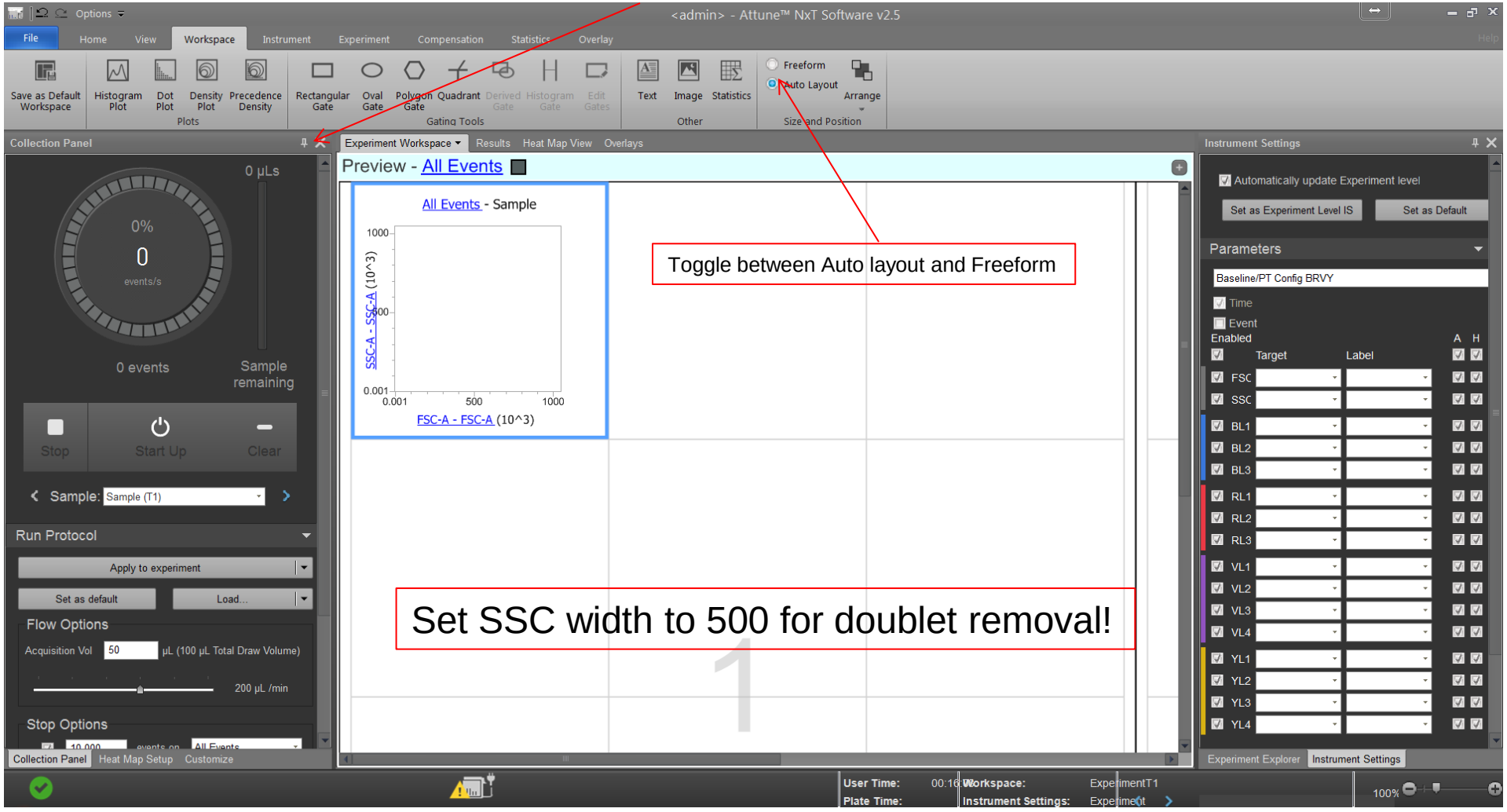


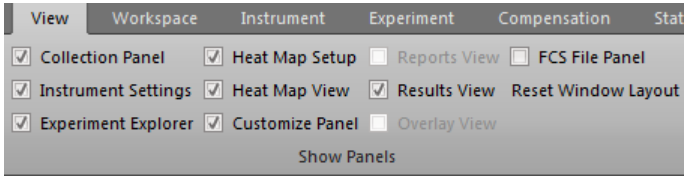




Data analysis – Managing the Workspace

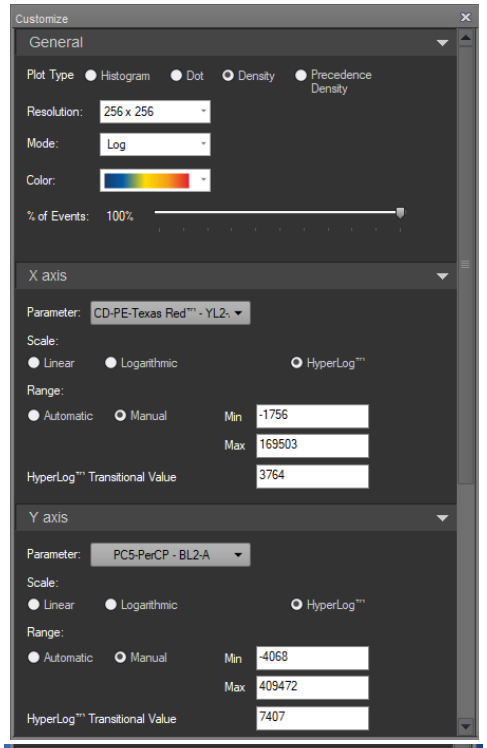
panels can be unpinned and moved to different screen

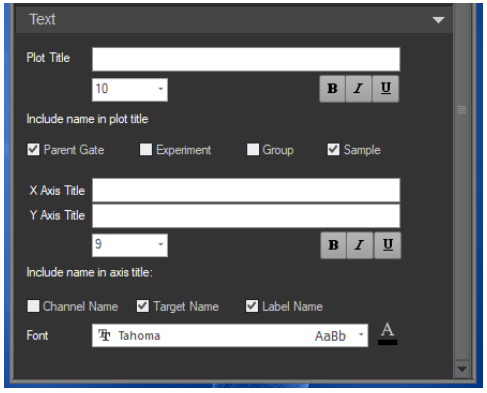




Under the View tab different panels can be displayed

Workspace - Plots



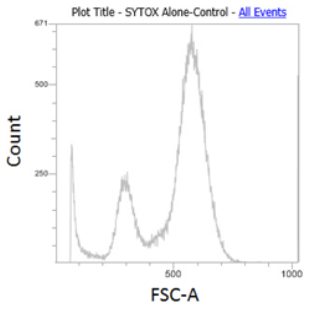


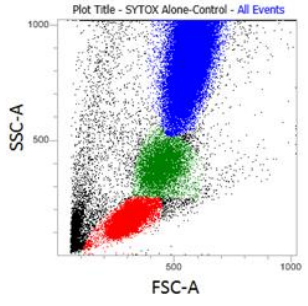
Histogram – single parameter plot showing number and distribution of events

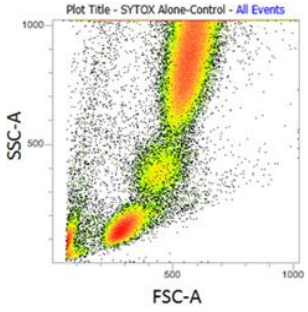
Dot plot – two parameters plot where each axis represents the signal intensity of one parameter

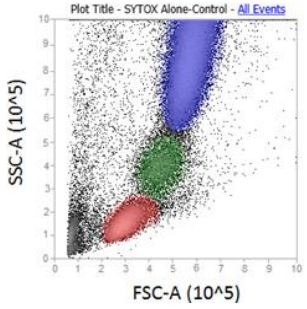
Density plots – two parameters plot where colors represent the density of a population of events with the same intensity

Density precedence – a combination of Dot and Density display. A gradient is used to indicate the number of events within each of the plot bins and color is used to display the parent gate of events present.









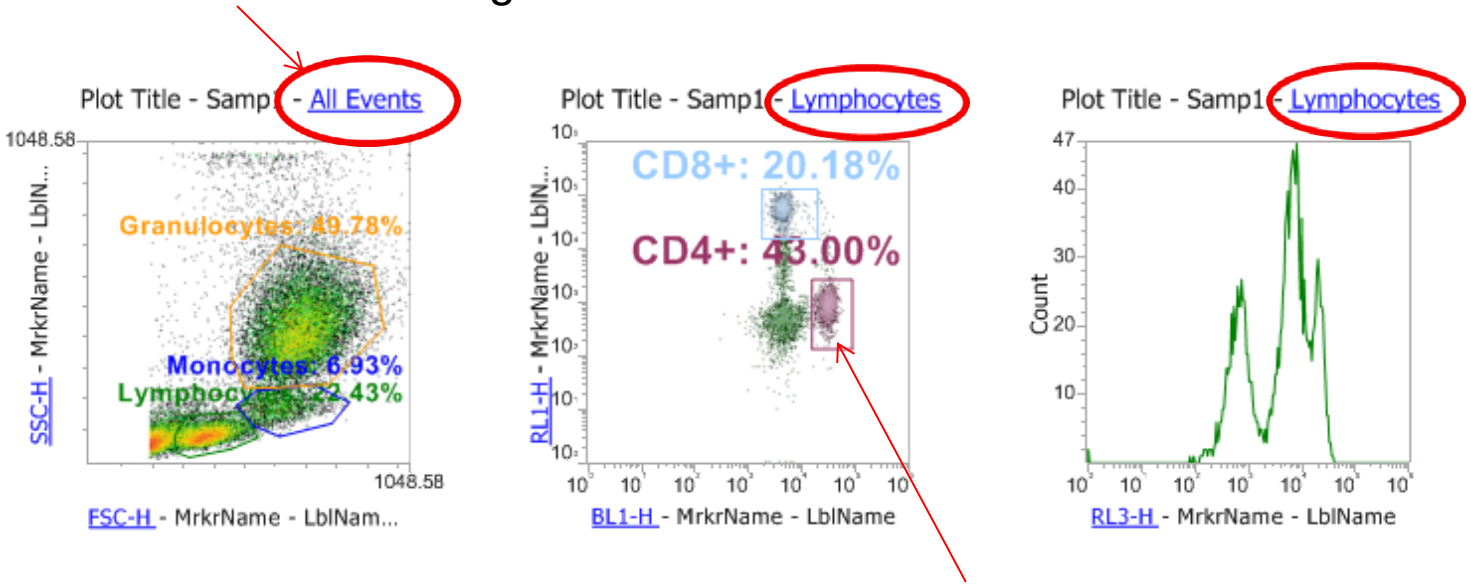
When a plot is highlighted the *Customize* Panel can be used to change plot titles, axis labels, axis scaling, plot type etc.

Workspace – Gating Tools

Regions are used to define areas to which statistics can be applied

Gates are derived from regions used to establish population hierarchy's

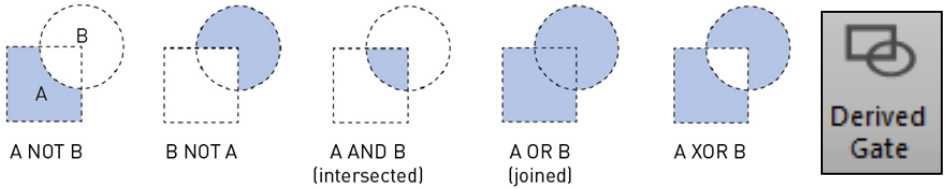
Pull-down menu to select gate

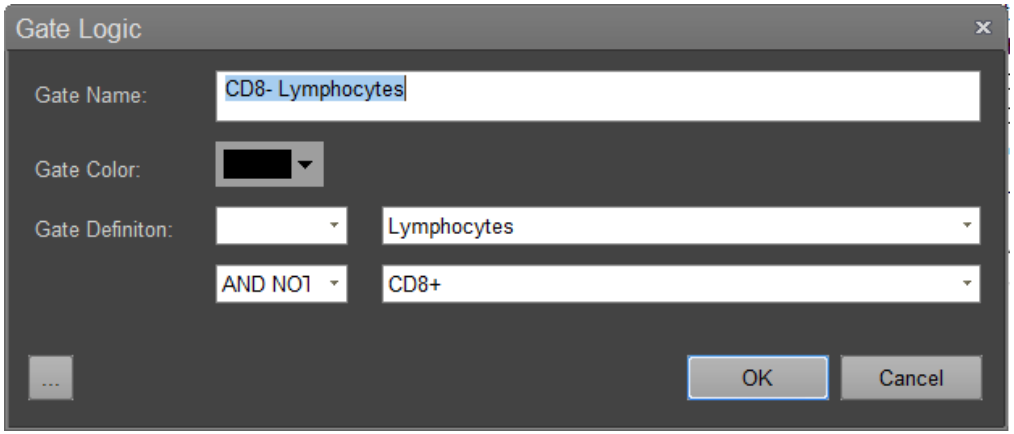


Right click on region to create daughter plot

Workspace - Derived gates

- Gates can be customized by using Boolean logic (OR, AND, NOT, XOR) to link multiple gates together





AND gates = all events that are shared

OR gates = all events found within 2 or more individual gates

NOT gates = all events found outside the gate

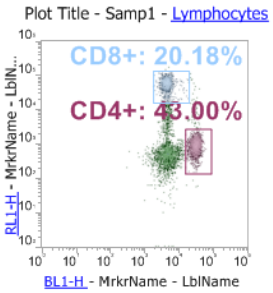
XOR gates = unique events found within an individual gates

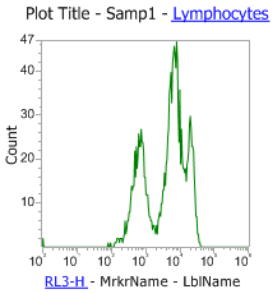
Note:	*When naming a derived gate with two words, use parentheses to enclose both words.*
	Derived gates can only be created using regions.

Results displays

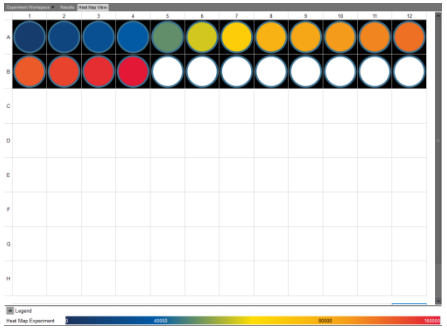
Visual

Workspace plots





Heat Map

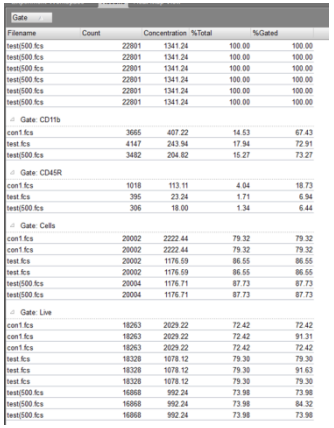


Statistics

Statistics box (on plot)

Name	Count	%Total	%Gated	Concentration
All Events	25,218	100.000	100.000	2,802.000
Cells	20,002	79.316	79.316	2,222.444
Live	18,263	72.420	91.306	2,029.222
RBC	12,163	48.231	66.599	1,351.444
live wbc	5,435	21.552	29.760	603.889
CD45R	1,018	4.037	18.730	113.111
CD11b	3,665	14.533	67.433	407.222

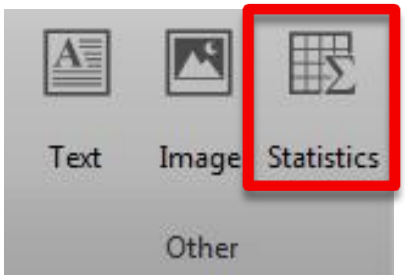
Results table



Statistics export

- CSV spreadsheet

Statistics Table

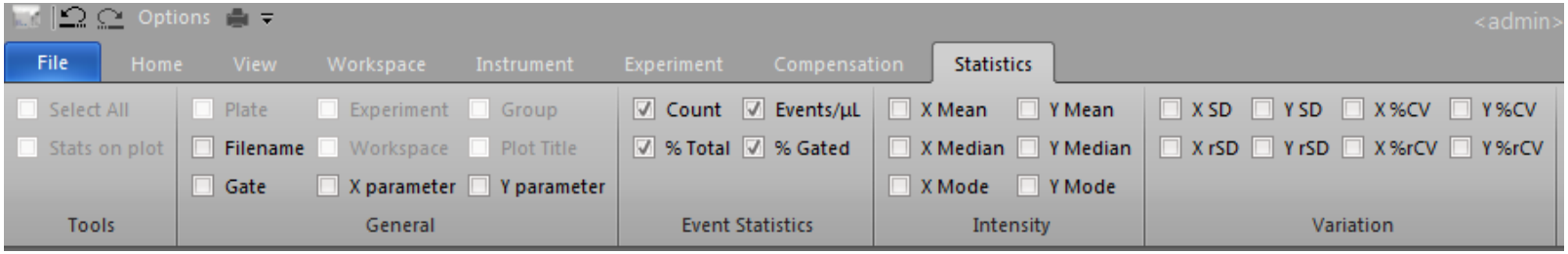


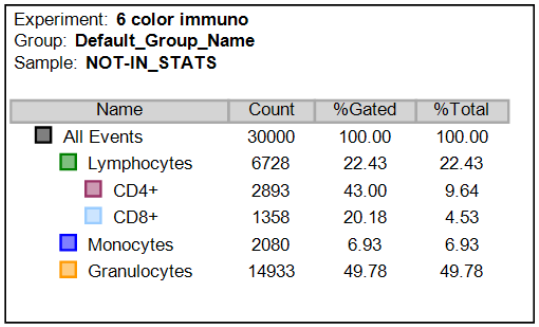
Experiment: **6 color immuno**
Group: **Default_Group_Name**
Sample: **NOT-IN_STATS**

Name	Count	%Gated	%Total
All Events	30000	100.00	100.00
Lymphocytes	6728	22.43	22.43
CD4+	2893	43.00	9.64
CD8+	1358	20.18	4.53
Monocytes	2080	6.93	6.93
Granulocytes	14933	49.78	49.78

- To display **Workspace Statistics Table**, click Statistics without selecting a plot. Workspace statistics contains data of all the gates in the Workspace.
- To display **Plot Statistics Table**, select a plot in the Workspace and then click Statistics. Local statistics only displays data pertaining to the selected plot.
- Alternatively, you can insert Statistics table by right-clicking on a plot or on the workspace, and select Insert Statistics
- Prior to adding a statistics box, make sure the workspace has at least one plot.

Customize Statistics





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Name	Count	%Gated	%Total
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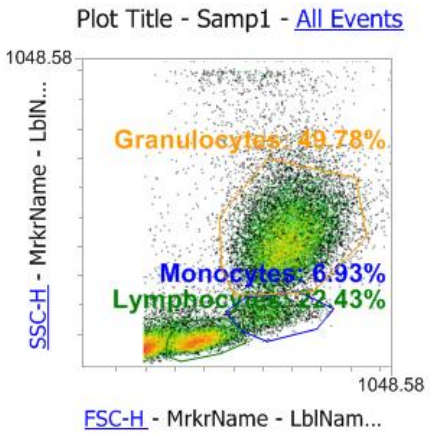
CD8+	1358	20.18	4.53
Monocytes	2080	6.93	6.93
Granulocytes	14933	49.78	49.78

- To customize Statistics table, select the Table and check statistics to display in the *Statistics* Tab



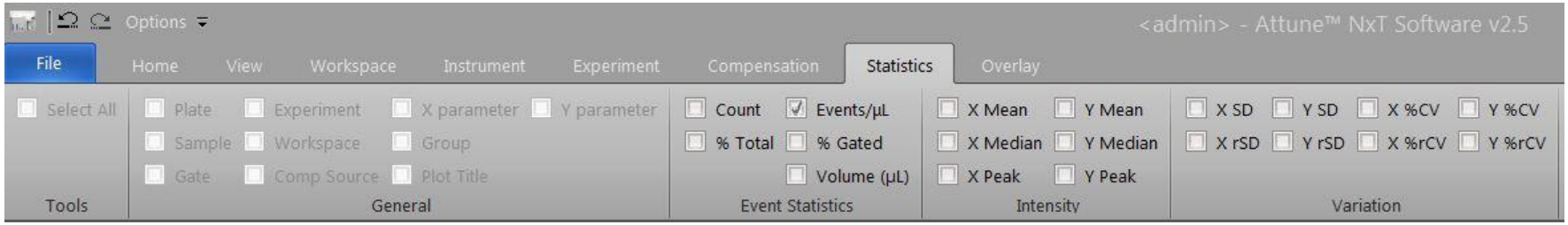
- To customize Statistics value displayed on a plot, select the plot and choose the statistic

Statistical Values

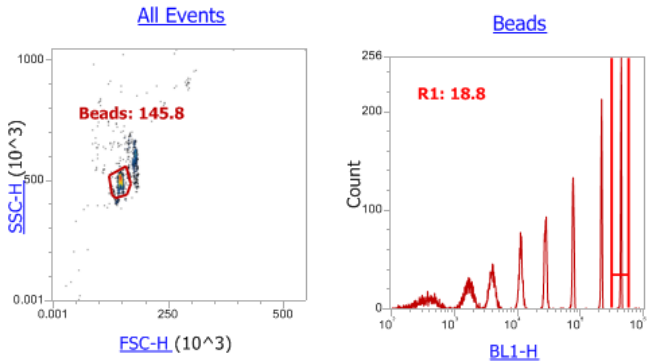
- **Count**: Number of events collected
- **Events/µl**: Concentration of events/µl in the gated region
- **% Total**: Percentage of total events collected
- **% Parent**: Percentage of a population based on the number of events collected in the parent gate
- **Mean**: Sum of the signal intensities of a gate divided by the number of values
- **Median** (50th percentile): signal intensity of a gate separating the higher half of a data population
- **Mode**: signal intensity that appears most often in a set of data
- **SD**: Standard Deviation, amount of dispersion of signal intensity around the Mean
- **rSD**: Robust Standard Deviation, amount of dispersion of signal intensity around the Median
- **%CV**: Percent coefficient of variation, Standard Deviation of the peak divided by the Mean of the peak, times 100
- **%rCV**: Percent Robust coefficient of variation, Standard Deviation of the peak divided by the Median of the peak, times 100

Sample Concentration

The **Concentration Statistic** can be selected from the Statistics Ribbon



- Values are displayed as Events/µL

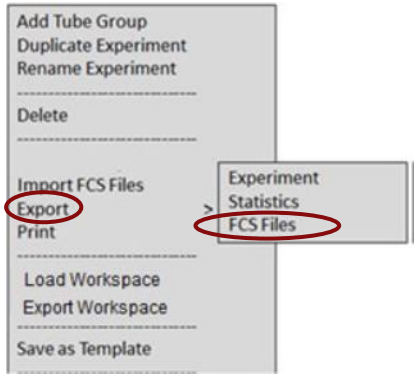


Parameters: **BL1-H**
Gate: **All Events**
Experiment: **8 peak beads**
Group: **Group**
Time Recorded: **10:45:51**

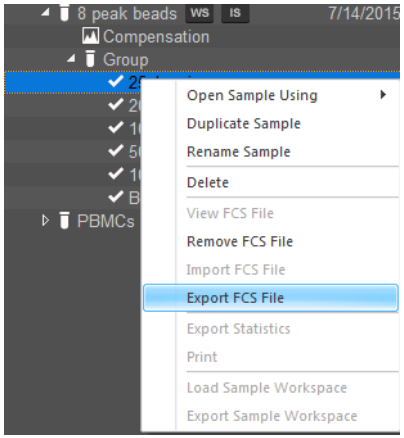
Name	Gate	Count	%Total	%Gated	X Median	Concentration
All Events	All Events	10,000	100.000	100.000	26,705	161.3
Beads	Beads	9,038	90.380	90.380	27,084	145.8
R1	R1	1,166	11.660	12.901	467,501	18.8

Export/Save of files as FCS 3.0 and FCS 3.1

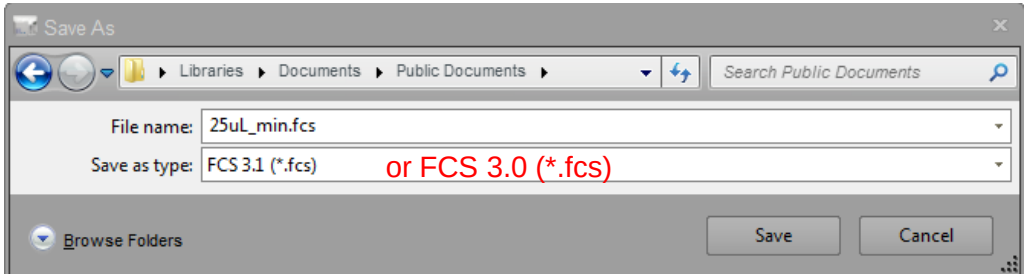
All experiment samples



Selected experiment samples



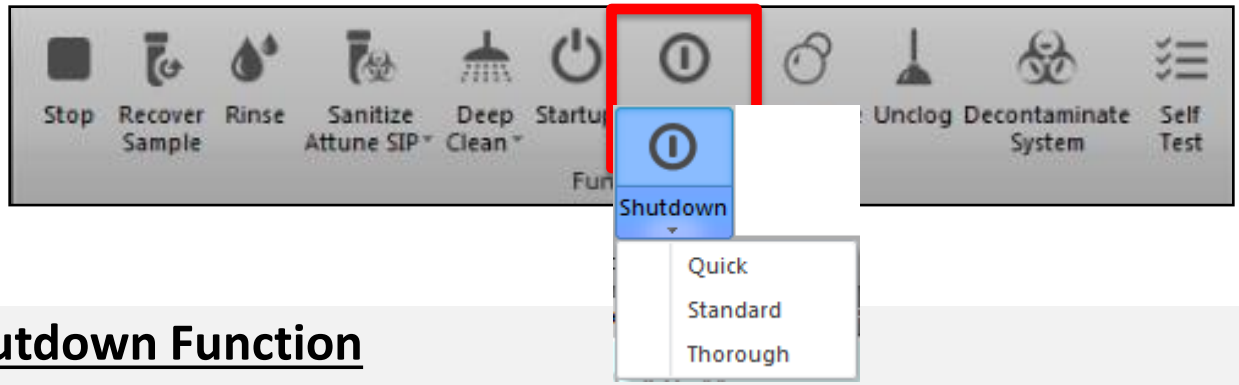
Right click on the experiment name or selected sample(s) and select “Export FCS file” from the drop down menu



FCS 3.1 files are compatible with: FlowJo V10+, Kaluza, FCS Express V4+

FCS 3.0 files are compatible with: FlowJo V7+, FCS Express V3+ and Kaluza

Instrument Shutdown



Shutdown Function

- Sanitizes the instrument
- Cleans and rinses the fluid lines
- Refills fluid lines with shutdown solution
- Uses 3ml Coulter Clenz

Ensures that:

- Fluid lines refilled with a solution that prevents crystal formation and bubbles.
- Once *Shutdown starts, can log out of Attune software*
- *CytKick will go to standby upon completion*

Instrument Cleaning Guide

Between samples	• **Rinse** – automatically initiated when SIP is lowered (for tubes), or set in *run protocol* for plates • **Sanitize SIP** between sticky samples or cell counts
Between users / experiments **USE:** **1) if there is ≥30 min between users.** **2) If there is <30 min between users.**	**1) Unclog** then **Quick Deep Clean** - 30mins cleaning routine (click on the arrow below the Deep Clean icon to select ***Quick***) or **2) Unclog** then **2X Sanitize SIP** / **Sanitize Autosampler SIP** (plate experiments) – 1st time with 3 mL 10% Bleach 2nd time with 3 ml Wash or De-bubble solutions
End of day (3 steps)	• ***Unclog*** • *****SIP Sanitize with 1:3 dilution of Attune Flow Cell Cleaning solution*** ***(*** Daily - if system runs ≥ 6 hr/day otherwise once a week) • ***Thorough*** **Shutdown** (click on the arrow below the Shutdown icon to select *Thorough*)

Note: Always wipe the outside of the SIP after doing a SIP Sanitize

Maintaining computer efficiency & data quality

- **Operator/user**

  De-select parameters not required to minimize file size

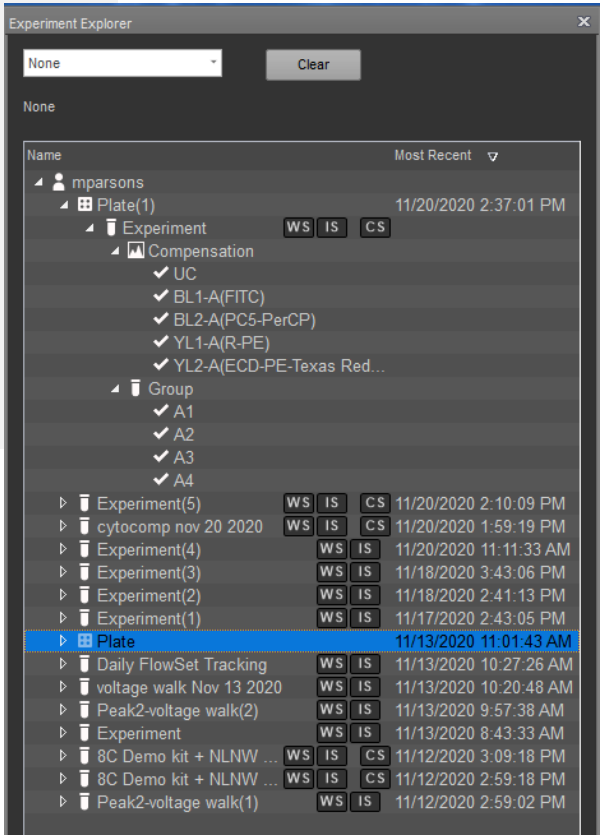
  ***Do not clutter the Experiment Explorer

  Close experiments not currently active

  Export then delete experiments from the browser

  Export experiments - .atx or .apx

  FCS files – 3.0 or 3.1 format



Concentration and Flow Rates

The event rate will approach maximums stated in the column header when samples of stated concentrations are run at the flow rates below.

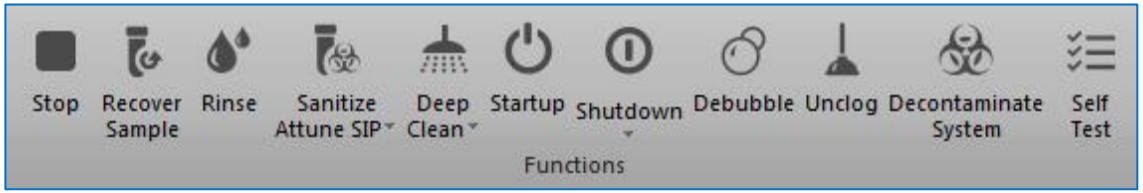
When acquiring large event files (i.e files with > 10^6 events), plot parameters should not be changed while recording.

Sample flow rate	***Fastest*** **(35,000 ev/sec)** ***maximum*** **sample concentration**	***Accurate counts*** **(8,000 ev/sec) maximum sample concentration**	**Cell size and flow rate recommendation**
1000 µL/ minute	2.1 x 10^6 cells/mL	0.48 x 10^6 cells/mL	- Particles > 4 µm - Predominantly acoustic focusing
500 µL/ minute	4.2 x 10^6 cells/mL	0.96 x 10^6 cells/mL	- Particles > 2 µm - Predominantly acoustic focusing
200 µL/ minute	6.7 x 10^6 cells/mL	1.5 x 10^6 cells/mL	
100 µL/ minute	1.3 x 10^7 cells/mL	3 x 10^6 cells/mL	
25 µL/ minute	5.4 x 10^7 cells/mL	1.2 x 10^7 cells/mL	- Small particles < 2 µm - Predominantly hydrodynamic focusing - Smallest sample core - Best resolution from background for dimly positives assays
12.5 µL/ minute	1.0 x 10^8 cells/mL	2.4 x 10^7 cells/mL	

Let your biology and data quality be your guide. *If good data is obtained while running at 2-8,000 ev/sec, adjust the sample concentration and flow rate to maintain that.*

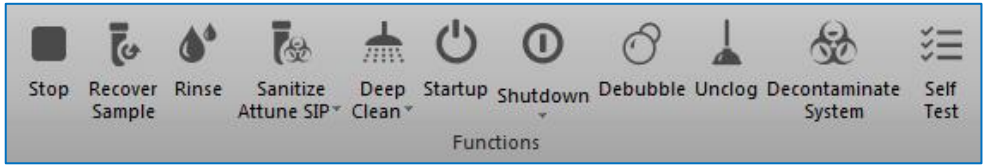
Note: Higher flow rates may show some loss of sensitivity

Fluidics Functions (on the Instrument tab)



- **Stop** - click to end any running routine.
- **Recover Sample** - returns unused sample volume back to the well or the tube.
- **Rinse -** flushes system between samples to minimize carryover. Rinse runs automatically every time the SIP is lowered, but it can also be user-initiated.
- **Sanitize Attune SIP** - sanitizes the SIP and sample lines between sticky/dirty samples or experiments; requires 10% bleach solution. Use wash solution instead if use beads.
- **Deep Clean** - thoroughly washes the system sample lines and flow cell between sticky/dirty samples or experiments; requires 10% bleach solution (can also use debubble solution)
- **Startup -** primes the instrument fluidics with Attune® Focusing Fluid.
- **Shutdown** - automatically cleans, sanitizes and shuts down the instrument.
- **Debubble** - clears bubbles from the fluidics lines of the cytometer; Attune® Debubble solution required.
- **Unclog** - back flush operation to remove clogs from the sample line.
- **Decontaminate System -** semi-automated decontamination of the Cytometer and the Auto Sampler fluidics.

Cleaning Functions



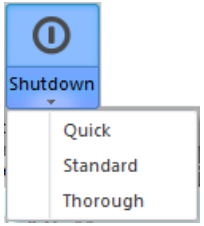
Sanitize SIP

Quick wash/sanitize of sample line.
Duration: 1 min. cycle time.
Requires 3 mL 10% Bleach. Use wash solution instead if run beads.
Run between users especially after sticky samples, DNA stains or beads.

Deep Clean

Sanitize system with bleach and wash solutions for selectable period of time.
Three levels: Quick (10 cycles/ 25 min), Standard (20 cycles/50 min, Thorough 30 cycles/75 min.

Shutdown



System clean and flush with bleach, wash, and shutdown.

Quick	10 cycles /30 minutes	Few samples
Standard	20 cycles/60 minutes	Immunophenotyping, apoptosis
Thorough	30 cycles/75 minutes	Samples with sticky dyes (PI), NLNW

Instrument placed in stand-by (dream state) upon completion

Located under the instrument tab

Plate experiments
Can add tubes to plate experiment usually for compensation – these are indicated below the plate as T1, T2 etc
Under the collection window, beside the record button is a pull down menu which allows the collection of either certain wells to all of plate or tubes within the plate experiment
Can collect entire plate or selected wells
To select wells click on the square box to the right of “collect wells starting from” – you can also use this to collect selected wells along with holding down the “ctl” key to select certain wells
When using heat map with plates or tubes remember to select the “apply changes’ tab in the upper right area of the window. There is also the ability to create a default
Can run comp from wells or plate.
When select well or group of wells can right click to create a group. Can select a different group of wells –right click and create a different group- In the collection panel window can adjust collection settings and under the “apply to experiment tab” and select the group to which it will be applied or select as default collection settings.
Can also save and export plate settings if you want to repeat

Optimize for high throughput can only be used at 500-1000ul/min (note 1000ul/min is too high to be meaningful) –boost mode affects rinsing between washes and may result in some carryover

Heat maps are useful when creating a visual reference for detecting change of a particular value for example above a certain percentage, in each well of a plate.

Other stuff
Very important- note when selecting draw volume be very careful to ensure that there is enough volume in the tube to accommodate the dead volume. In parenthesis to total vulume that is required to draw a selected amount is shown- best practice is to add 10% to that value
Adding custom fluorochromes or filters to config- export new config to file to be imported by new user. Fluorescence protein config already sent to desk top folder “Attune”. baseline config, mcherry, no lyse,no wash.

Possible to drag and drop IS,WS,CS from one experiment to next

After voltage walk new voltage settings have been saved to optimal config along with a template that includes an experiment for adjusting target channels using flow set to set optimal MFI using new voltages.

Experimental templates are found in manage templates tab