Luminex®
complexity simplified.

CellStream® User Manual

RUO For Research Use Only. Not for use in diagnostic procedures.

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CE

EC REP

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Chapter 1: System Introduction

System Overview

The CellStream® system is used to quantify physical and fluorescence properties for single cells and other particles at high throughput. The CellStream system uses a single time-delay integration (TDI) charge-coupled device (CCD) camera for photon collection. This maximizes sensitivity and enables camera-based features and advanced morphological features to be calculated. The CellStream system also allows for cellular imagery to be visualized in real-time in the Event Gallery.

The CellStream system can achieve 20 colors of detection, depending on the number of lasers in the system. The system can be equipped with 1 laser (5 colors) or up to 7 lasers (20 colors). A minimum of 5 lasers is needed to measure 20 colors. Installation of additional lasers can be performed in the field.

Luminex Technical Support

Contact Luminex Technical Support by telephone in the U.S. and Canada by calling: 1-877-785-2323

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International: + 800-2939-4959

Fax: 512-219-5114

Email: *support@luminexcorp.com*

Additional information is available on the Luminex website. Search on the desired topic, navigate through menus. Also, review the website's FAQ section. Enter *http://www.luminexcorp.com* in your browser's address field.

This manual can be updated periodically. To ensure that you have a current version, contact Technical Support.

Symbols Glossary

You will encounter these symbols throughout this manual. They represent warnings, conditions, identifications, instructions, and regulatory agencies.

Symbol	Meaning	Symbol	Meaning
	Caution.		Caution possibility of electric shock.
	Biological risks.		Serial Number.
	Warning Laser Beam.		Consult instructions for use.
	For Research Use Only. Not for use in diagnostic procedures.		WEEE Symbol Separate collection for electrical and electronic equipment.
	Conformite Europeenne (EU CE Marking of Conformity) CE conformity marking		Manufacturer Indicates the medical device manufacturer, as defined in EU Directives 90/385/EEC, 93/42/EEC and 98/79/EC.
	China RoHS		SGS mark
	Hand Crush / Force From Above		Authorized representative in the European Community. Indicates the Authorized representative in the European Community.
	FCC Compliance Mark	_	_

Chapter 2: Regulatory and Safety Considerations

Warnings and Precautions

	Consult safety documentation where the caution symbol is marked.
	This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to part 15 of the FCC rules. These limits were designed to provide reasonable protection against harmful interference when the equipment is used in a commercial environment. This equipment generates, uses, and can radiate radio-frequency energy and, if not installed and used in accordance with the user manual, can cause harmful interference to radio communications. The operation of this equipment in a residential area is likely to cause harmful interference—in which case the user will be required to correct the interference at the user's own expense.

In any situation that you encounter a symbol shown below, consult this manual or other Luminex documentation to determine the nature of the potential hazard and any necessary actions you should take.

General Safety

	The protection provided by the equipment can be impaired, or the warranty voided, if the system is used in a manner not specified by the Luminex documentation or by Luminex Corporation.
	Always observe standard laboratory safety practices.

Electrical Safety

	Electrical hazards are present in the system, particularly in the main power supply. To protect against electrical shock, the instrument must be connected to a properly grounded receptacle in accordance with the electrical code that is in force in your region.

Laser Safety

The CellStream® system is a Class 1 laser device and complies with the U.S. FDA Center for Devices and Radiological Health 21 CFR Chapter 1, Subchapter J.

The CellStream system complies with IEC 60825-1:2014, 21 CFR 1040.11 and 1040.11 except for deviations pursuant to Laser Notice No. 50, dated June 24, 2007.

The CellStream system may have the following lasers:

Wavelength	Maximum Power
375 nm	70 mW
405 nm	175 mW
488 nm	200 mW
532 nm	150 mW
561 nm	150 mW
642 nm	150 mW
730 nm	40 mW
785 nm	70 mW

NOTE: No laser radiation is accessible to the user during normal instrument operation. If the enclosure is opened (or disassembled) or the top tray cover is open, redundant interlocks on the instrument turn the lasers off.

Use of controls or adjustments or performance of procedures other than those specified in this manual can result in hazardous radiation exposure.

Voltage or current hazard is sufficient to cause shock, burn, or death. Disconnect before servicing.

Biological Safety

The CellStream® system is rated at BSL1. Do not load or flush samples containing infectious agents without first exposing the sample to inactivating conditions. Luminex recommends that samples be fixed in 2% Formalin in PBS for at least 10 minutes before running the samples on the CellStream.

Where exposure to potentially biohazardous material, including aerosol, exists, follow appropriate biosafety procedures and use personal protective equipment (PPE). PPE includes gloves, gowns, labor-atory coats, face shields or mask and eye protection, respirators, and ventilation devices. Observe all local, state, federal and country-specific biohazard handling regulations when disposing of bio-hazardous waste material.

Fluids Safety

Sensors behind the bottles trigger a pop-up window to alert the user that the reservoir volumes are low or the waste volume is high. The triggers are set such that any sample run or script currently running can finish in the event that the pop-up window appears during acquisition.

Do not remove any bottles while any scripts are running (e.g., sample acquisition, debubble, etc.). Once the script has finished, remove the bottle and refill with the appropriate solution. Waste can be emptied at any time. Dispose of the waste in accordance with local regulations.

Debubbler reagent is 70% Isopropanol and is flammable in its liquid or vapor states. The reagents bottle lids must be securely fastened during operation. Any heat, sparks, open flames, or hot surfaces must be kept away from the Isopropanol bottle during operation or refilling. Do not smoke when Isopropanol is present.

- Store Isopropanol (Debubbler reagent) that is not in the instrument in a flammable safety cabinet.
- Refill the Debubbler reagent bottle with Isopropanol, in a well ventilated area or a fume hood.
- Take precautionary measures against static discharge while refilling or handling Isopropanol (Debubbler reagent).
- Ensure any spills are cleaned up immediately to prevent flammable vapor hazards. Avoid breathing reagent vapor.

Mechanical Safety

The system has parts that move during operation. Risk of personal injury is present. The moving parts present puncture, hand-crushing hazards, and pinching hazards..

Access to moving parts under the hood of CellStream® system is intended only for Luminex service personnel.

Chapter 3: Performance Specifications and System Components

Environmental Conditions

- For indoor use at an altitude of less than 2000 m (6561.68 feet).
- Operating temperature: 16°C to 25°C (60.8°F to 77°F).
- Maximum relative humidity of 80%, non-condensing.
- Operating ambient temperature: within +/-2.5 °C (36.5°F).
- Pollution Degree: II.
- The CellStream system has a rated voltage of 100 VAC to 240 VAC, a rated frequency of 50/60 Hz, and a rated current of 3.0 A

System Specifications

- The noise level of the CellStream® system is less than 70 dB(A).
- Weight: 48 kg (<105 lbs)
- Dimensions: 440 mm x 625 mm x 495 mm (17 in x 24 in x 19 in)
- Provide at least 3 inches of clearance behind the instrument for proper ventilation.
- The main power supply may not fluctuate more than +/-10% and must meet transient over voltage category (II).

Disconnect the Instrument

NOTE: Do not position the instrument so that disconnecting the power cord is difficult.

To disconnect the instrument from the power supply, turn off the power to the instrument by pushing the switch on the back of the instrument. Unplug the power source from the wall, which must be located near the instrument and in view of the operator.

Transportation

The CellStream® system relies on many delicate alignments for proper operation. The system should only be moved by a Luminex representative.

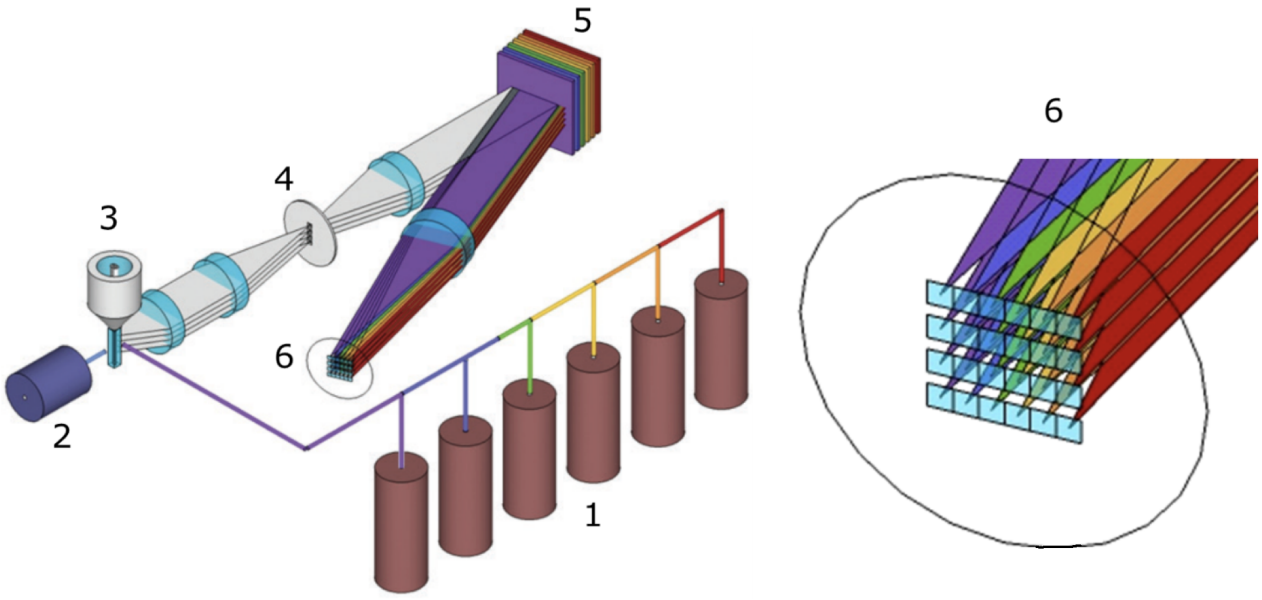
NOTE: If movement of the CellStream® system is required, use proper lifting techniques to avoid injury. Keep the following points in mind while lifting:

- Lift with a second person, as the CellStream system weighs 105 lbs.
- Make sure that you have a secure, comfortable grip when lifting.
- Make sure that the path from where the object 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.

21 CFR Part 11 Compliance

21 CFR Part 11 compliance in the CellStream® system is controlled by a separate application called the Compliance Manager. To learn how to install and use the Compliance Manager, please contact Luminex Technical Support.

CellStream® Optical Specifications



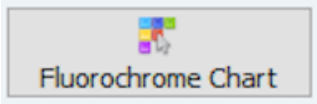
- **Fluorescence and side scatter lasers**: The instrument can be equipped with 1 to 7 fluorescence excitation lasers (375 nm, 405 nm, 488 nm, 532 nm, 561 nm, 642 nm, 730 nm) and a dedicated 785 nm side scatter laser.
- **FSC illumination**: FSC (forward scatter) is measured using a dedicated 450 nm LED (light-emitting diode).
- **Flow cell**: Samples are hydrodynamically focused using sheath fluid into a 250 µm flow cell.
- **Collection optics**: A series of lenses and filters focuses and projects photons onto the filter stack. Each illumination source is spatially separated into 4 zones for projection onto the CCD (charge-coupled device) camera.
- **Filter stack**: A single filter stack is used to spatially separate photons into 6 channels: 456/51, 528/46, 583/24, 611/31, 702/87, and 773/56. They are the same for all configurations, however, they are modified when the 532 nm and/or the 730 nm lasers are enabled.

- **22-channel TDI CCD detector**: The CellStream® system TDI (time delay integration) CCD camera uses patented Amnis® technology to image objects in flow with unparalleled sensitivity. TDI collects photons the entire time an object is in front of the CCD detector and integrates these photons in exact synchrony with the object to produce in-focused imagery. A single CCD detector is capable of measuring up to 22-parameters (FSC, SSC (side scatter), 20 colors). The camera is divided into 4 zones with 6 channels each. Of the 24 available channels, 2 are not available for photon collection.

CellStream® Sample Preparation

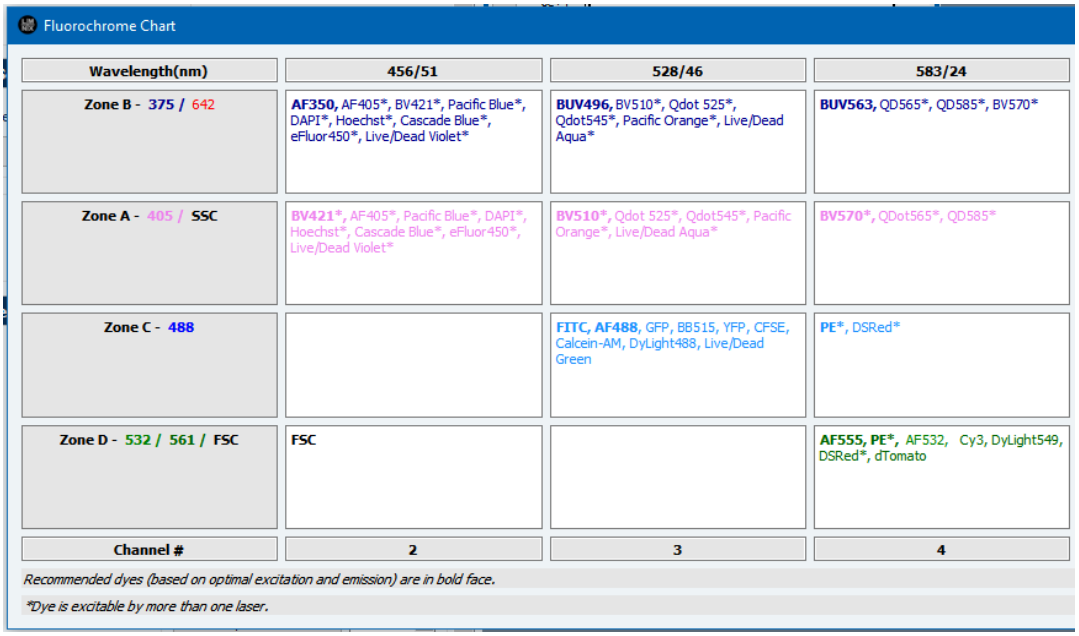
Fluorochrome Chart

View the recommended dyes, based on optimal excitation and detection channels, by clicking **Fluorochrome Chart**.



NOTE: Turning on the 532 nm laser will add a 532/17 nm bandpass filter. Turning on the 730 nm laser will add a 730/32 nm bandpass filter.

The recommended dyes are displayed in bold.

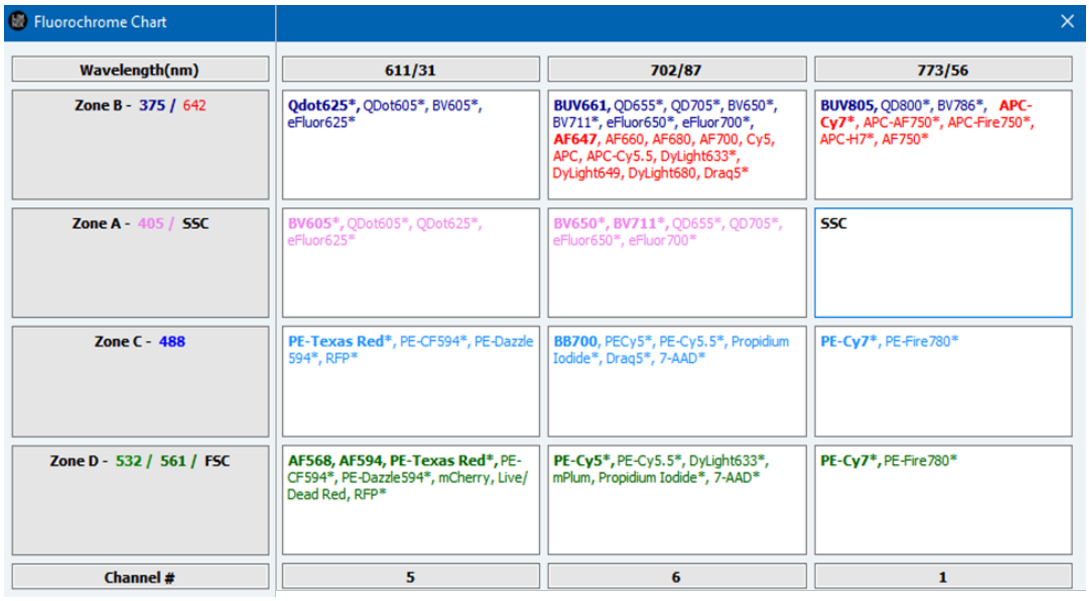


Fluorochrome Chart

Wavelength(nm)	456/51	528/46	583/24
Zone B - 375 / 642	**AF350,** AF405*, BV421*, Pacific Blue*, DAPI*, Hoechst*, Cascade Blue*, eFluor450*, Live/Dead Violet*	**BUV496,** BV510*, Qdot 525*, Qdot545*, Pacific Orange*, Live/Dead Aqua*	**BUV563,** QD565*, QD585*, BV570*
Zone A - 405 / SSC	**BV421***, AF405*, Pacific Blue*, DAPI*, Hoechst*, Cascade Blue*, eFluor450*, Live/Dead Violet*	**BV510***, Qdot 525*, Qdot545*, Pacific Orange*, Live/Dead Aqua*	**BV570***, QDot565*, QD585*
Zone C - 488		**FITC, AF488,** GFP, BB515, YFP, CFSE, Calcein-AM, DyLight488, Live/Dead Green	**PE***, DSRed*
Zone D - 532 / 561 / FSC	**FSC**		**AF555, PE*,** AF532, Cy3, DyLight549, DSRed*, dTomato
Channel #	2	3	4

Recommended dyes (based on optimal excitation and emission) are in bold face.

**Dye is excitable by more than one laser.*



Fluorochrome Chart

Wavelength(nm)	611/31	702/87	773/56
Zone B - 375 / 642	**Qdot625***, QDot605*, BV605*, eFluor625*	**BUV661,** QD655*, QD705*, BV650*, BV711*, eFluor650*, eFluor700*, **AF647,** AF660, AF680, AF700, Cy5, APC, APC-Cy5.5, DyLight633*, DyLight649, DyLight680, Draq5*	**BUV805,** QD800*, BV786*, **APC-Cy7***, APC-AF750*, APC-Fire750*, APC-H7*, AF750*
Zone A - 405 / SSC	**BV605***, QDot605*, QDot625*, eFluor625*	**BV650*, BV711***, QD655*, QD705*, eFluor650*, eFluor700*	**SSC**
Zone C - 488	**PE-Texas Red***, PE-CF594*, PE-Dazzle 594*, RFP*	**BB700,** PECy5*, PE-Cy5.5*, Propidium Iodide*, Draq5*, 7-AAD*	**PE-Cy7***, PE-Fire780*
Zone D - 532 / 561 / FSC	**AF568, AF594, PE-Texas Red*,** PE-CF594*, PE-Dazzle594*, mCherry, Live/Dead Red, RFP*	**PE-Cy5*,** PE-Cy5.5*, DyLight633*, mPlum, Propidium Iodide*, 7-AAD*	**PE-Cy7*,** PE-Fire780*
Channel #	5	6	1

Successful Panel Design

The CellStream® system measures 425 nm to 800 nm emission and accommodates the same fluorescence dyes as most commercial flow cytometers. Refer to the fluorochrome chart for setting up a fluorescent panel on the CellStream system.

While the CCD (charge-coupled device) camera offers excellent sensitivity, there are additional considerations for successfully designing a fluorochrome panel.

The CCD detector does not allow control over individual channel gains, instead laser power must be set to scale signals appropriately. Consequently, data quality is enhanced when the brightness levels of all probes excited off a single laser are balanced to within one log of each other. Probe balancing avoids the saturation of bright stains when they are combined with dim stains in the same sample and excited by the same laser.

System Components

CellStream® System Components

- Single Load Portal
- Autosampler Portal
- Fluidic Reservoir
- Computer running the Windows® platform
- Computer running Linux

Consumable Components

The use of the recommended reagents is required for proper operation of the instrument. The Sterilizer, Cleanser, and Debubbler reagents are used in the Sterilize and Debubble scripts.

Table 1. Reagents and Consumables

Reagents and Consumables	Description	Source*	Catalog#
Sterilizer	10% bleach with no additives **NOTE:** 10% bleach is defined as 0.6-0.8% sodium hypochlorite. Using 0.22um filtered bleach will prevent particulate detection by the system.	VWR	JT9416-1
Cleanser	Coulter Clenz®	Beckman Coulter	8546929
Debubbler	70% Isopropanol	Fisher Scientific	A459
Sheath	HyClone D-PBS, without: calcium and magnesium	Cytiva Life Sciences	SH30028.03
Rinse	Milli-Q® Deionized water, at least 0.22 µm filtered	Varies	N/A
Calibration Beads	CellStream® Calibration Reagent	Luminex Corporation	CS-400104
Tubes	1.5 mL microcentrifuge tubes	Fisher Scientific	02-681-320
Plates	Corning 96 well clear polystyrene microplate (round bottom)	Sigma-Aldrich	CLS3798
Plate Cover	X-pierce film, Excel Scientific	Sigma-Aldrich	Z722502

NOTE: Traditional FACS tubes cannot be used on the CellStream system.

*This table is provided for information only; other sources of the same reagent may be used. Please contact Luminex Technical Support to obtain the SDS of reagents.

Reagent Bottle Locations

From left to right the bottle locations are Rinse, Sterilizer, Cleanser, Debubbler, Waste, and Sheath. Ensure that each reagent bottle is refilled with the correct approved reagent.

NOTE: Take the bottles out of the instrument in order to empty or refill them. All of the reagent bottles are non-pressurized, and therefore, the bottles can be opened while the instrument is powered on.

NOTE: Use proper personal protective equipment when handling reagents such as gloves, goggles, and/or lab coats.

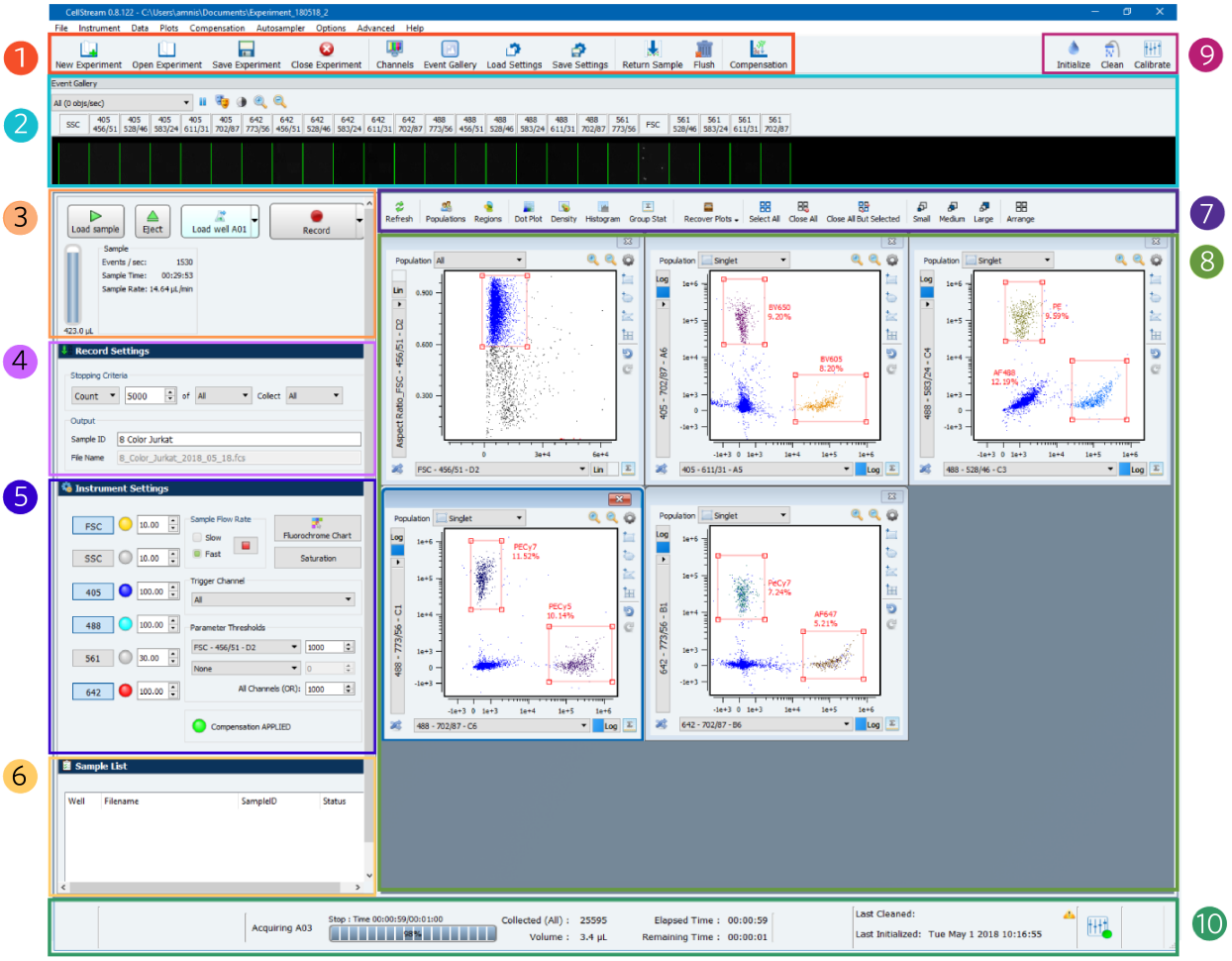
Chapter 4: Software Functionality

The CellStream® system software is divided into Acquisition and Analysis software programs. Both software programs have a similar user interface.

Main Menu

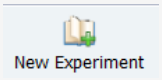
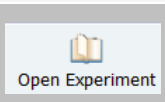
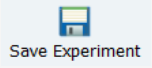
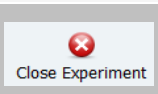
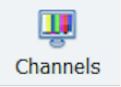
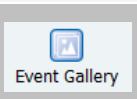
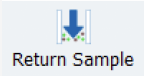
The CellStream® Acquisition software shares common interface features shared with CellStream Analysis software. The CellStream Acquisition software controls the operation of the system and acquisition of data. CellStream Analysis software is used to analyze data that were recorded in CellStream Acquisition software and allows for multi-file analysis.

Table 2. CellStream Acquisition Software User Interface



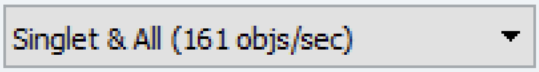
1. Experiment Toolbar	6. Sample List
2. The Event Gallery	7. Analysis Toolbar
3. Load/Record Data	8. Analysis Workspace
4. Record Settings	9. Startup/Shutdown
5. Instrument Settings	10. Instrument Status

Experiment Toolbar

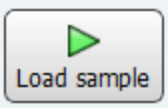
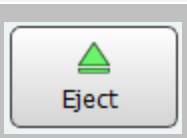
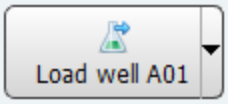
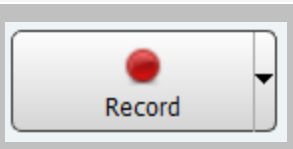
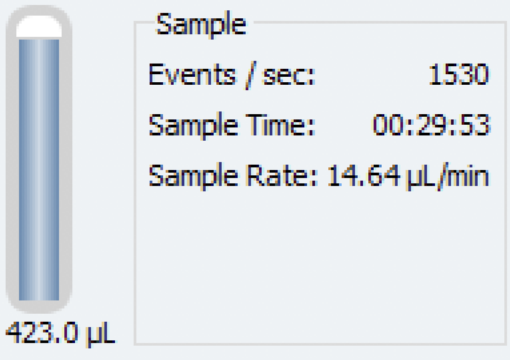
Icon	Function
	Creates a new experiment (.exp).
	Opens an existing experiment (.exp).
	Saves the current experiment (.exp).
	Closes the current experiment.
	Enables/disables collection of specific channels.
	Displays the Event Gallery.
	Loads previously saved instrument settings.
	Saves the current instrument settings.
	Returns the sample to the provided tube or well plate.
	Flushes the current sample into the waste collector.
	Displays the Compensation panel.

The Event Gallery

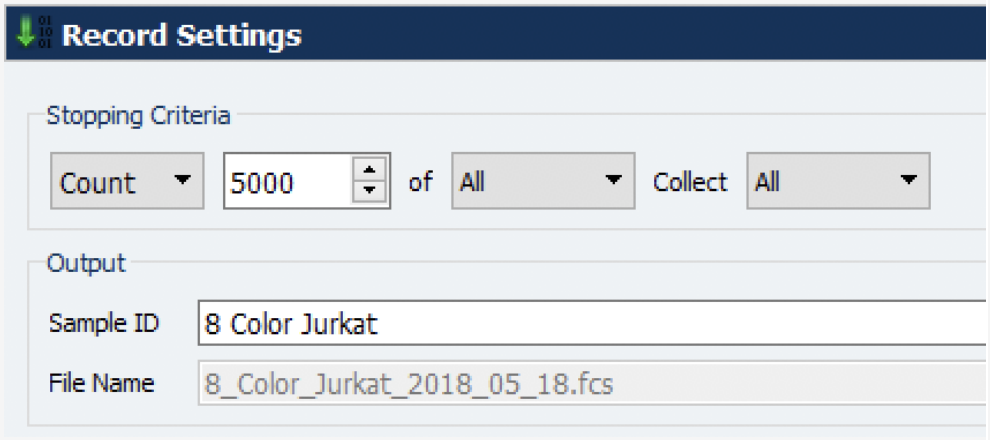
Once a sample is loaded, the Event Gallery displays the live flow of the sample in channels.

Icon/Item	Function
	Selects a population to view.
	Pauses the Event Gallery.
	Turns the mask on or off. The mask shows how the objects are being identified.
	Sets the display contrast.
	Zooms in or out on the Event Gallery images.

Load/Record Data

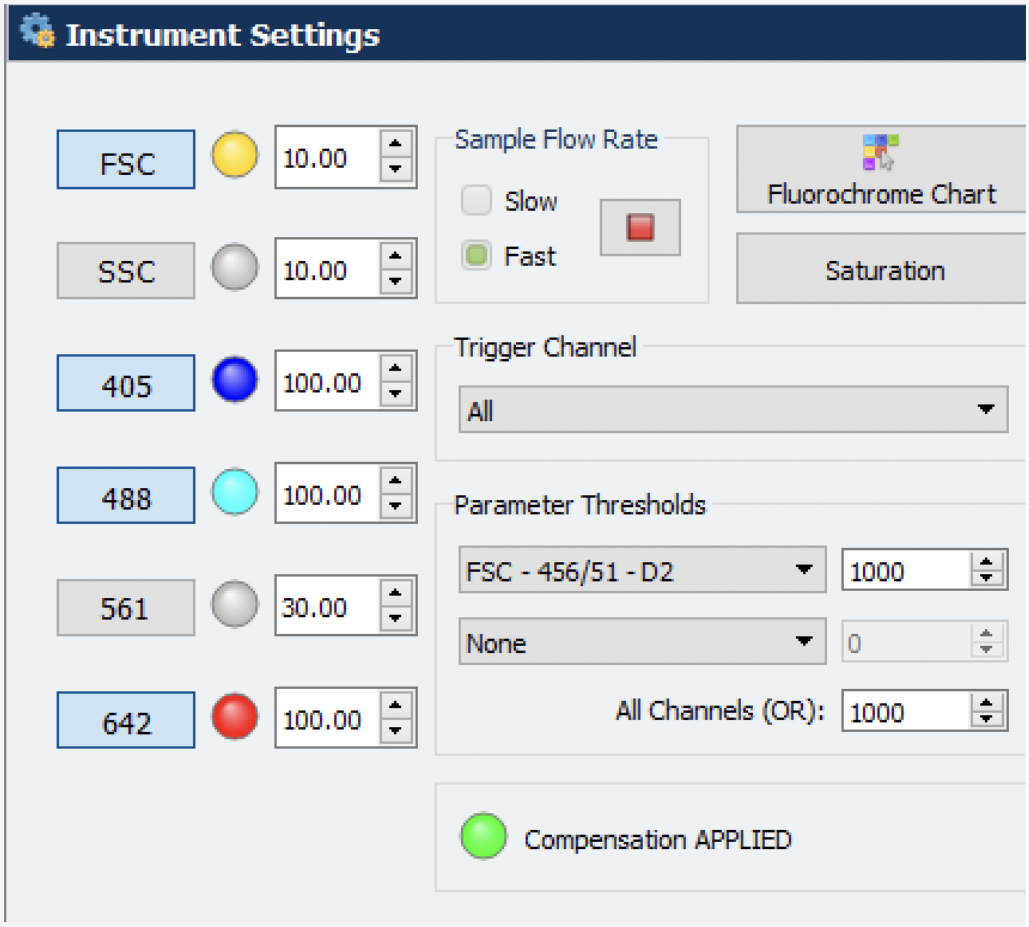
Icon/Item	Function
	Loads the sample.
	Opens or closes the well plate holder.
	Loads a selected well. The drop-down menu specifies the well(s).
	Records the acquisition. The drop-down menu specifies the record settings.
	Displays the sample volume remaining, events/sec, time remaining, and sample flow rate.

Record Settings



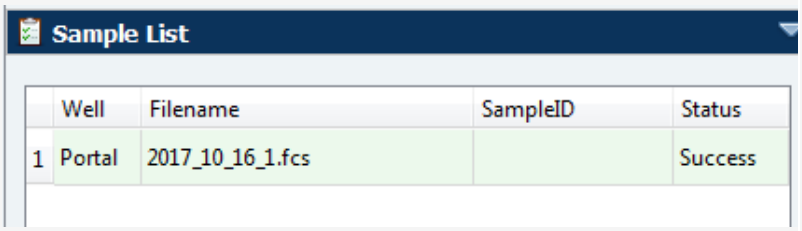
Sets the stopping criteria, (e.g., count, volume, or time), collection population, and names the sample file.

Instrument Settings



Sets the laser powers, sample flow rate, trigger channel, and parameter thresholds. Displays the fluorochrome chart and saturation percentages.

Sample List



Displays the sample files and collection status within an experiment and renames the files.

Analysis Toolbar

The analysis area displays dot plots, histograms, density plots, and a group statistics table. Multiple regions can be created and modified on the plots.

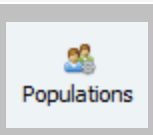
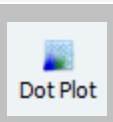
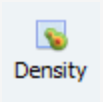
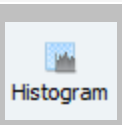
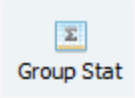
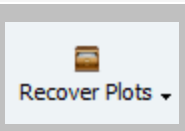
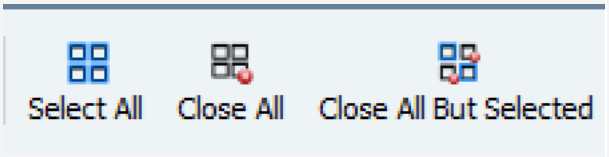
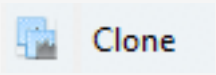
Icon	Function
	Refreshes all the data on the plots.
	Opens the Populations Manager.
	Opens the Regions Manager.
	Creates a dot plot.
	Creates a density plot.
	Creates a histogram.
	Generates a statistics window to display statistics for desired populations.
	Recovers plots that have been previously closed.

Table 3. Selecting and Sizing the Plots

	Select All: Selects all plots/charts displayed. Close All: Closes all plots/charts displayed. Close All But Selected: Closes all other plots/charts except the selected ones.
	Small: Resizes charts to the smallest display. Medium: Resizes charts to normal display. Large: Resizes charts to the largest display. Arrange: Aligns the plots in a grid.

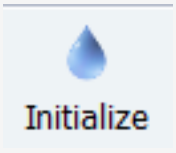
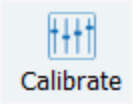
Under the Plots menu, there is one additional option:

	Generates a duplicate of the selected plot.

Analysis Workspace

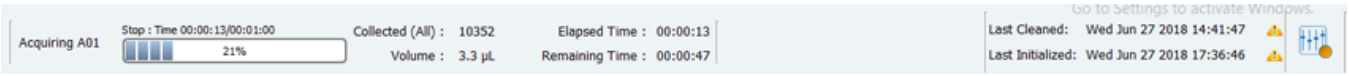
The display and working area for plots/charts.

Startup/Shutdown

Icon	Function
	Initializes the fluidics of the instrument.
	Cleans the system.
	Runs a series of system calibrations and tests.

Instrument Status

The instrument status region shows the instrument's current status, such as the last time the instrument was cleaned, initialized, and calibrated as well as any ongoing acquisition events.



Chapter 5: Preparing the System

Fluidics

Fill System Fluidics Reservoirs

Sensors behind the bottles trigger a pop-up window to alert the user that the reservoir volumes are low or the waste volume is high. The triggers are set such that any sample run or script currently running can finish in the event that the pop-up window appears during acquisition.

Do not remove any bottles while any scripts are running (e.g., sample acquisition, debubble, etc.). Once the script has finished, remove the bottle and refill with the appropriate solution. Waste can be emptied at any time. Dispose of the waste in accordance with local regulations.

1. Fill the Sheath bottle with phosphate buffered saline (PBS with no surfactants) for running samples.
2. Fill the labeled Rinse bottle with deionized (DI) water for rinsing the instrument during shutdown.

Fluid is drawn from the bottles into the sheath and flush syringe pumps. The sheath pump helps to control the speed of the core stream and the size of the core stream diameter. The flush pump is used to clean and flush the system.

Empty the Waste Fluid

Sensors behind the bottles trigger a pop-up window to alert the user that the reservoir volumes are low or the waste volume is high. The triggers are set such that any sample run or script currently running can finish in the event that the pop-up window appears during acquisition.

Do not remove any bottles while any scripts are running (e.g., sample acquisition, debubble, etc.). Once the script has finished, remove the bottle and refill with the appropriate solution. Waste can be emptied at any time. Dispose of the waste in accordance with local regulations.

If biological samples have been tested with the system, use your standard laboratory safety practices when handling system waste. Wear appropriate personal protective equipment such as gloves and eyewear during the waste disposal procedure.

The waste bottle holds all of the fluids that have been run through the CellStream® system. Add 200 mL of bleach to the empty waste bottle. Luminex recommends that the waste bottle contain 10% bleach when full.

NOTE: 10% bleach is defined as 0.6-0.8% sodium hypochlorite. Using 0.22um filtered bleach will prevent particulate detection by the system.

NOTE: Biological samples are potentially dangerous.

1. Disconnect the two quick connect lines from the waste bottle.
2. Slide the bottle out and tip it up to remove lid.
3. Dispose of waste according to local regulations.
4. Add approximately 200 mL of 10% bleach to the empty waste bottle.

   NOTE: 10% bleach is defined as 0.6%-0.8% sodium hypochlorite.
5. Replace the lid and screw it on tightly.
6. Slide the waste bottle back into the instrument and attach both quick connect lines to the top of the bottle ensuring both lines “click” in.

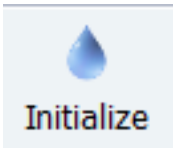
Power on the System and Log into the Software

1. Turn on the CellStream® computers running Windows® and Linux operating systems.
2. Turn the CellStream system on by pushing the power switch located on the back of the instrument, adjacent to the power cable. The sample portal will be illuminated by a blue LED when the instrument is on.
3. Log onto the computer and double-click the CellStream Acquisition icon to start the Acquisition software.

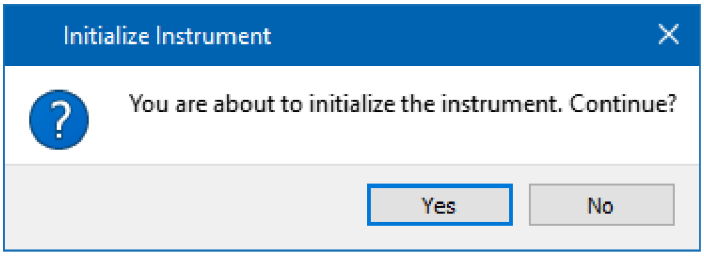
Initialize the System

The CellStream® system initialization script is fully automated using on-board fluidics, and takes approximately 10 minutes to complete. Initialization is required after the instrument has been cleaned, to prepare the instrument for data acquisition.

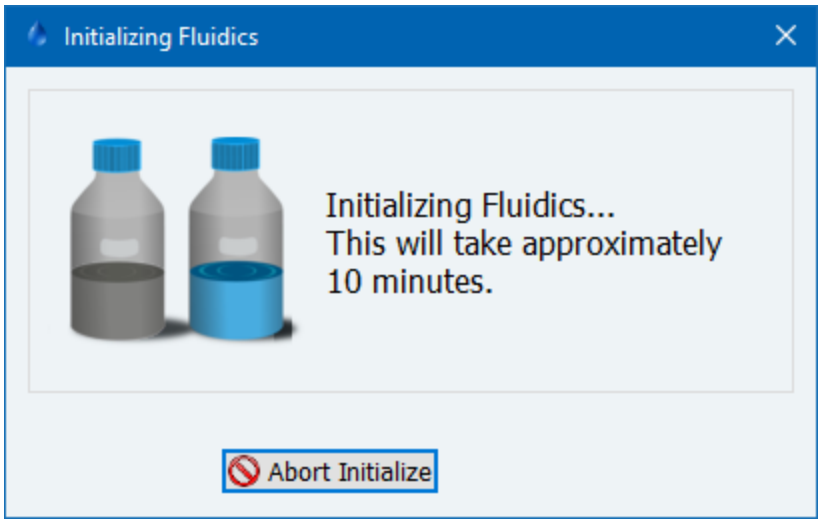
1. Ensure the CellStream system fluidic reservoirs are full and waste bottle is empty.
2. Click the **Initialize** button on the top right corner of the user interface.



3. Click **Yes** to confirm.



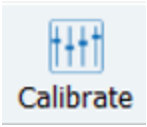
4. The dialog box below will close when the initialization is complete. If the initialization was run by mistake, click **Abort Initialize**.



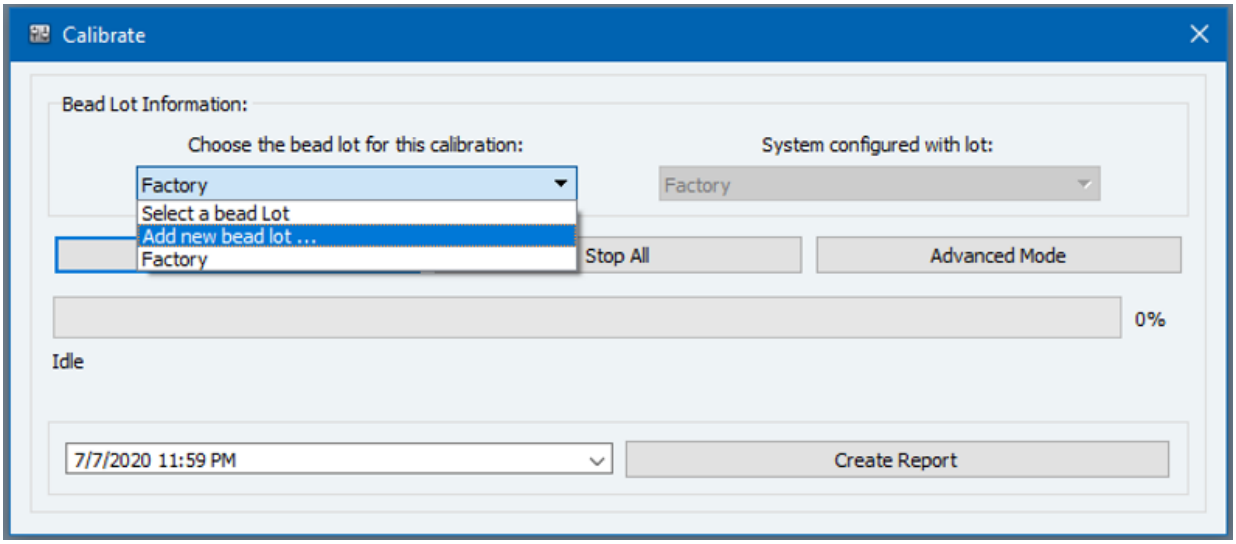
Add New Bead Lot

The Master Bead Lot file is a simple text file to upload into the Acquisition software when a new lot of calibration beads is obtained. The Master Bead Lot file ensures that during calibration, the lasers are using the fluorescent intensity values for the calibration beads in use.

1. Open the Acquisition software and click **Calibrate** in the upper-right corner.

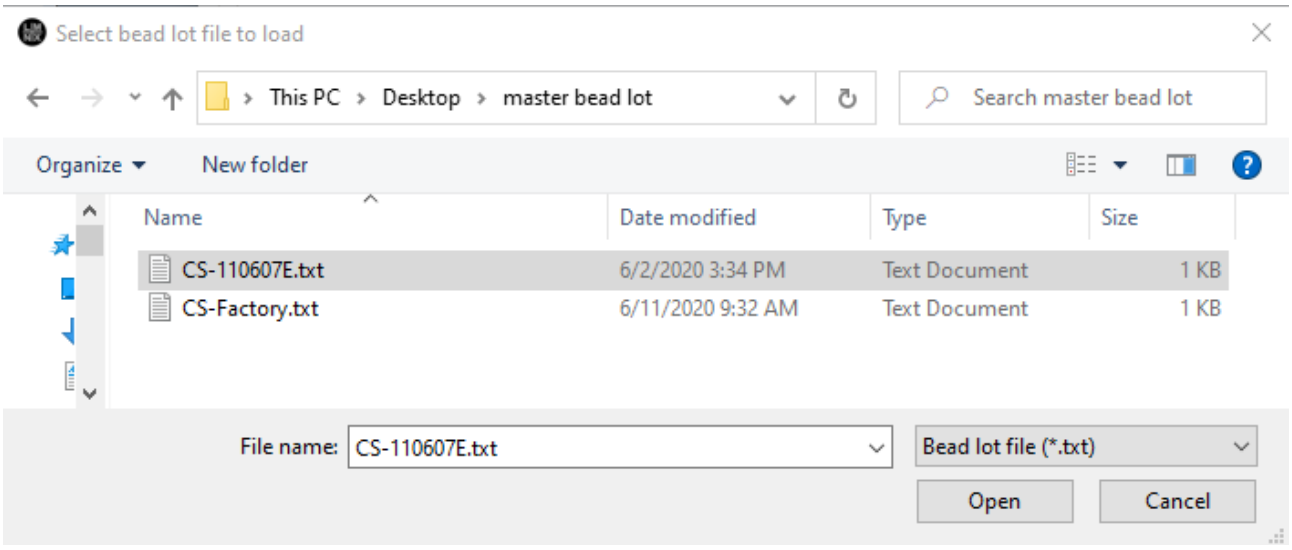


2. Click **Choose the bead lot for this calibration:** drop-down menu and choose **Add new bead lot...**.



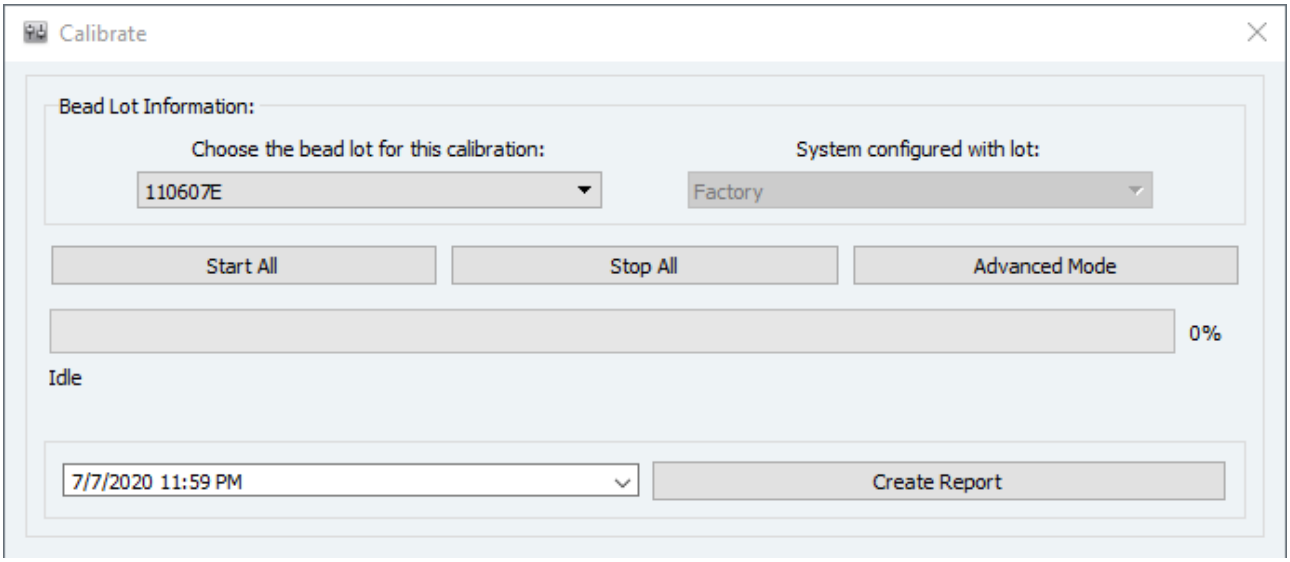
3. In the **Select bead lot file to load** dialog box, locate the new bead lot file and click **Open**. The bead lot will now be selected as the bead lot for calibration.

   NOTE: Depending on the instrument and software settings, you may be directed to Local Disk > Program Files > CellStream > INSPIRE when Select bead lot file to load is chosen. Navigate to Desktop > master bead lot to locate the bead lot file.



   NOTE: The Master BeadLot file is a text file that can be downloaded from the Luminex website . Alternately, call Luminex Technical Support.

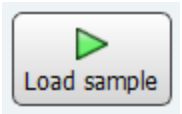
   NOTE: If the bead lot has already been added to the instrument, you only need to select the bead lot that matches the beads from the Choose the bead lot for this calibration: drop-down menu.



Calibrate the System

The CellStream® Calibration script runs a series of calibrations and tests using CellStream Calibration Reagent to ensure the instrument is meeting performance specifications. The Calibration script is fully automated and takes approximately 5 minutes to complete. Luminex recommends to calibrate daily before data acquisition.

1. Click **Load Sample**.



2. Add 125 µL or 4 drops of CellStream® Calibration Reagent into a 1.5 mL Eppendorf tube.
3. Add the Eppendorf tube to the single sample loading area and click **OK** in the Insert New Sample Tube dialog box.
4. Click **Calibrate**.

NOTE: The system will check the fluid levels and run an automated quality control program.

5. In the **Calibrate** dialog box, choose the appropriate option in the drop-down menu and then click **Start All**.

NOTE: If the correct bead lot info is not available in the drop-down menu, contact Luminex Technical Support.

NOTE: If any calibration tests fail, refer to troubleshooting.

NOTE: Optionally, to view individual results from the Calibration, click Advanced Mode. To see the history of a test, double-click on the test. Choose different dates in the drop-down menu at the top of the window to see the test results.

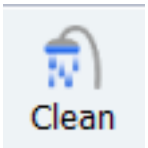
Run the Clean Procedure

Debubbler reagent is 70% Isopropanol and is flammable in its liquid or vapor states. The reagents bottle lids must be securely fastened during operation. Any heat, sparks, open flames, or hot surfaces must be kept away from the Isopropanol bottle during operation or refilling. Do not smoke when Isopropanol is present.

- Isopropanol (Debubbler reagent) that is not in the instrument must be stored in a flammable safety cabinet.
- When refilling the Debubbler reagent bottle with Isopropanol, do so in a well ventilated area or a fume hood.
- Take precautionary measures against static discharge while refilling or handling Isopropanol (Debubbler reagent).
- Ensure any spills are cleaned up immediately to prevent flammable vapor hazards. Avoid breathing reagent vapor.

The Clean procedure automatically turns off all illumination sources and rinses the entire fluidic system with water, sterilizer, cleanser, debubbler, and water again. The sterilizer is held in the system for 10 minutes to ensure decontamination.

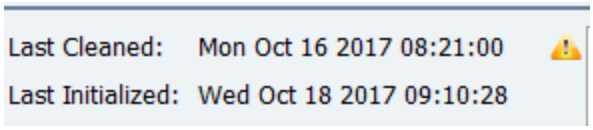
1. Fill the Rinse, Cleanser, Sterilizer, and Debubbler bottles, if necessary.
2. Empty the waste bottle.
3. Remove tubes from the single load portal.
4. Click **Clean**.



NOTE: Optionally, select the **Shutdown after clean** check box to shut down the CellStream® system and computers after the Clean procedure is complete.

Time required: ~30 minutes (unattended)

A warning icon will appear if the instrument has not been initialized or cleaned within the past 24 hours.



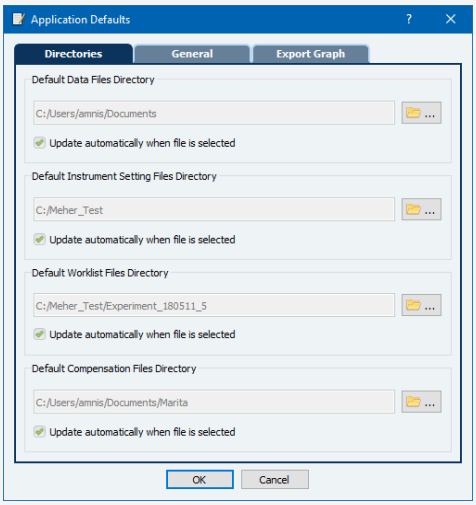
Check Fluid Levels

1. To check fluid levels, navigate to **Instrument** > **Advanced** > **View Tank Levels**.
2. The software will generate a pop-up to indicate if a fluidic reservoir must be emptied or filled. Luminex recommends to empty the waste and fill each bottle before an autosampler experiment.

Chapter 6: Running the Samples

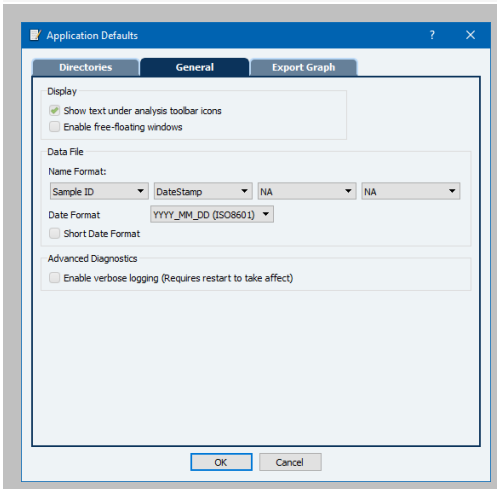
Set Application Defaults

Under **Options** > **Application Defaults**, there are several system settings that can be adjusted.



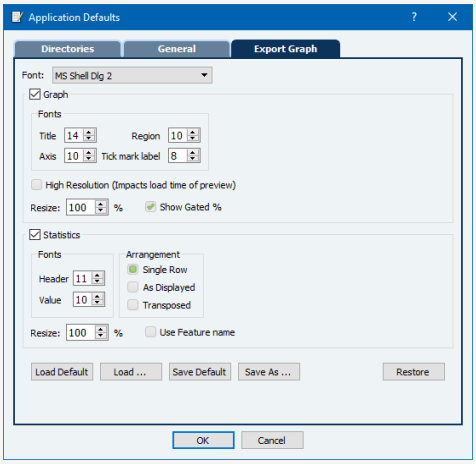
On the **Directories** tab, the following items can be adjusted:

- Default Data Files Directory
- Default Instrument Setting Files Directory
- Default Worklist Files Directory
- Default Compensation Files Directory



On the **General** tab, the following settings can be adjusted:

- Show text under analysis toolbar icons
- Enable free-floating windows
- Data File naming and date format
- Logging options for advanced diagnostics



On the **Export Graph** tab, the following settings can be adjusted:

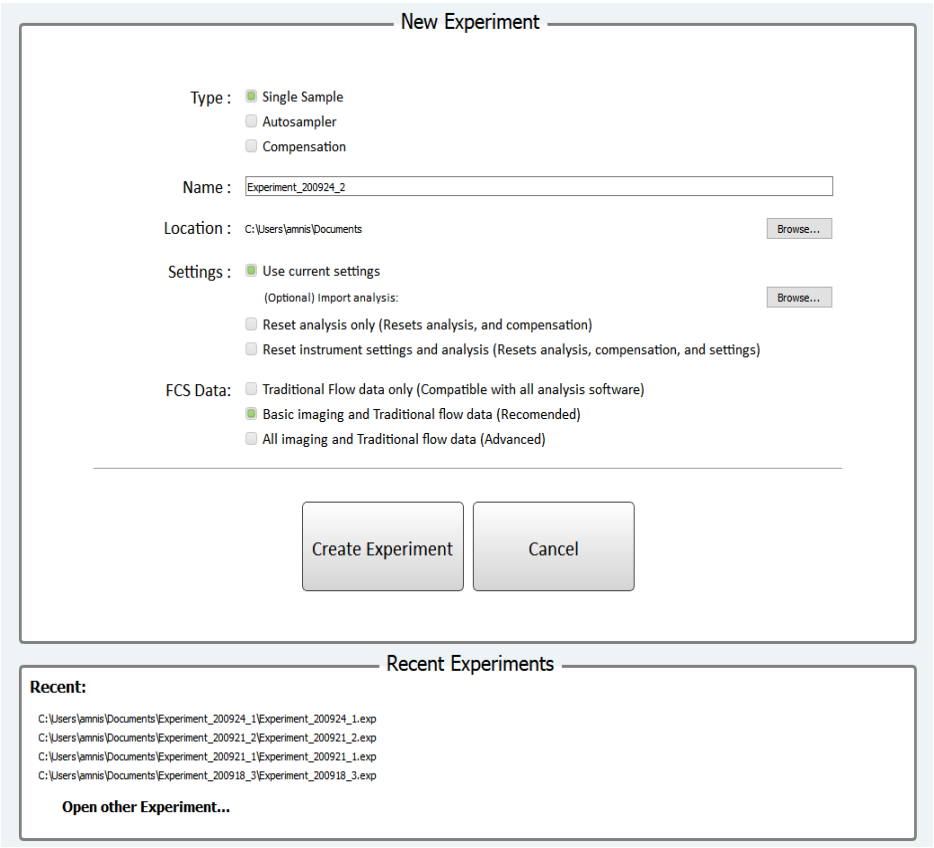
- Font type and size
- Image resolution
- % Gated display on plots
- Statistics display options
- Save and load graph export options

Create a New Experiment

Creating a new experiment creates an Experiment folder which contains the Experiment file (.exp) as well as the .fcs data collected within the experiment. The experiment file (.exp) includes the analysis (plots, statistics, etc.), compensation matrix, and sample list and can be opened in CellStream® Acquisition or Analysis software.

1. Click **New Experiment**.

   NOTE: The interface offers several options for creating a new experiment.



2. Select the following options for the experiment:

   Type:

   - **Single Sample** – Collects experimental samples manually.
   - **Autosampler** – Defines an autosampler plate to collect samples automatically.
   - **Compensation** – Collects single-color compensation controls using an automated workflow.

   Name: Creates a name for the experiment. The default name is Experiment_DayMonthYear_# where # is the number of experiments created on a particular day.

   Location: The default location where the experiment will be saved. Click **Browse** to specify a different location.

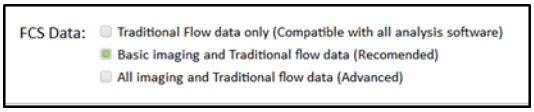
   Settings:

   - **Use current settings** – Uses current instrument and experiment settings.
     Optional: Use **Import analysis** to load a different Experiment.
   - **Reset analysis only** – Uses current instrument settings and resets analysis to default (clears plots, regions, compensation).
   - **Reset instrument settings and analysis** – Loads default settings and resets analysis to default (clears plots, regions, compensation).

   FCS Data: Chooses the feature set for acquisition and exporting of data.

   This option allows the user to collect data with three different levels of feature sets.

   - **Traditional Flow data only** - Reduces the feature set that excludes the morphological features such as raw maximum pixel, area, and aspect ratio of the fluorescent intensities. The aspect ratio of FSC is included in this set so that doublet discrimination can be analyzed. This option is useful to analyze CellStream data in third party software.
   - **Basic imaging and Traditional flow data** - Includes some of the more useful morphological parameters (area, raw max pixel, and aspect ratio).
   - **All imaging and traditional flow data** - Includes all morphological features available in CellStream as well as the traditional flow parameters.
     - For FCS Data, choose one option by selecting the appropriate box:

     - Data will be exported for analysis with the chosen feature set.
     - For a complete list of features for each option, see the Appendix B topic.

   Recent Experiments area: Lists the previously run experiments and presents an option to **Open other Experiment...**.

3. Click **Create Experiment**. The main screen of the data acquisition interface appears.

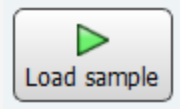
   NOTE: If there is already an experiment open, you will be asked if you want to save the open experiment before creating a new one.

Load Single Sample Experiments

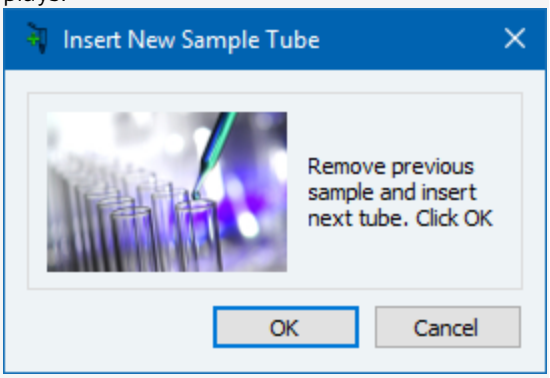
Related topics: Alternate method for an unattended run: *Run the Autosampler*

From an Eppendorf tube

1. Click **Load sample**.



   The **Insert New Sample Tube** dialog box displays.

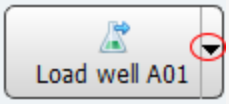


2. Add the sample to the single-sample portal with a volume of at least 35 µL.
3. Place the tube in the single sample loading area.
4. Click **OK** on the **Insert New Sample Tube** dialog box.
5. The instrument will load the sample and start running the sample.

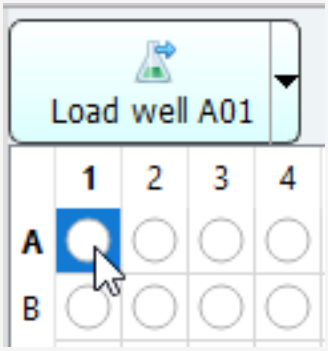
From a well

1. Click **Eject**.

2. Load a 96-well plate.
3. Click **Eject** again to reload the plate.
4. Click on the drop-down arrow on the right side of the **Load well A01** icon.

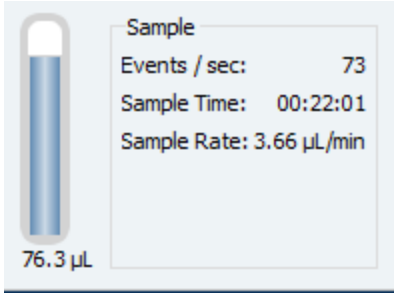


5. Select the desired well (A01, A02, etc.) from the drop-down.



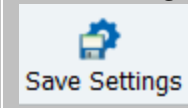
6. With the selected well listed on the button, click the **Load well** icon again. The sample from the selected well will load.

After loading a sample, the system displays the volume remaining in the sample run, the event rate (events/sec), time remaining in sample run, and sample flow rate (µL/min).



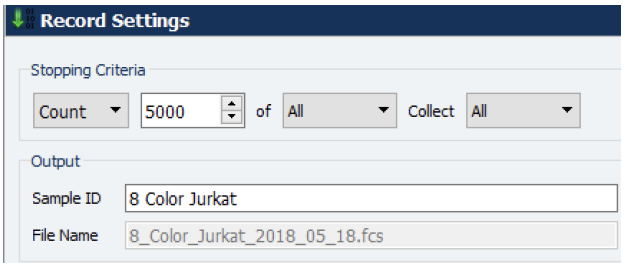
Define the Instrument Settings

An Instrument Settings File (*.ist) includes the record settings, instrument settings, channels enabled, compensation, and analysis (plots, statistics, etc).

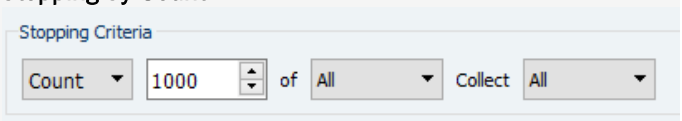
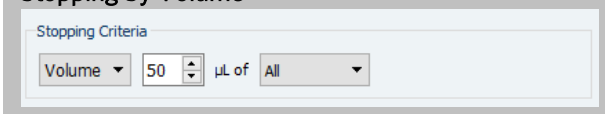
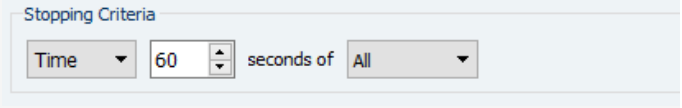
Loading a Setting	Click the **Load Settings** icon.  Select the .ist file and click **OK**.
Saving a Setting	After creating the desired instrument settings and controls, click the **Save Settings** icon.  Enter a file name and click **Save**. An Instrument Settings File (*.ist) will be saved. The settings can be loaded and applied to single loader or well plate experiments.

Use the Record Settings

The Record Settings control panel is used to specify when to stop acquisition of the sample.

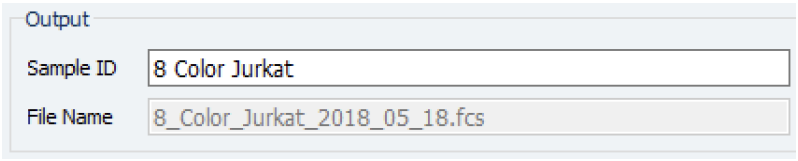


There are three options for Stopping Criteria:

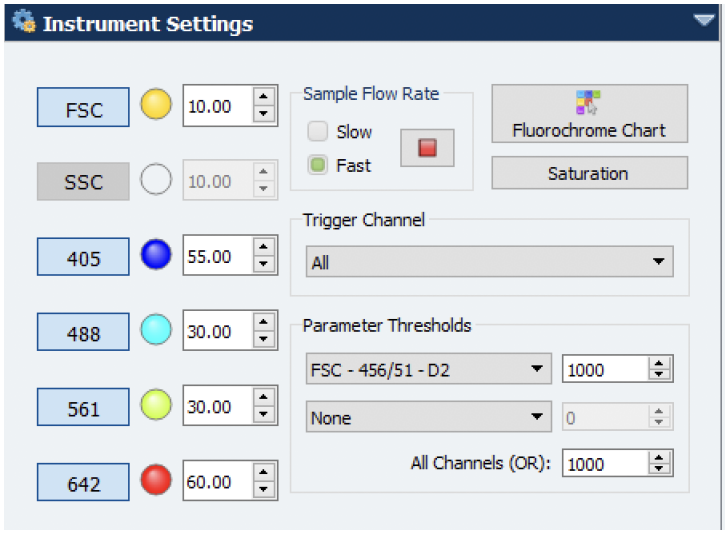
Stopping by Count 	Set the stop criteria by defining a number of events for the selected population. In the **Collect** drop-down menu, choose the population to collect. This collects data from a desired population while setting the stopping criteria based on a different population.
Stopping by Volume 	In the **Volume** textbox, enter the sample volume to be collected in microliters (µL). In the **All** drop-down menu, choose the population to collect.
Stopping by Time 	In the **Time** text-box, enter the sample time to be collected in seconds. In the **All** drop-down menu, choose the population to collect.

Optionally, in the Output section, a Sample ID can be entered.

NOTE: Use application defaults (Options > Application Defaults) to set the desired file name structure.



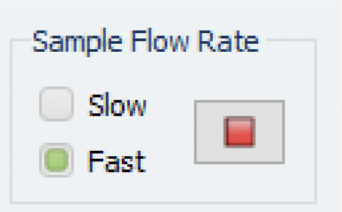
Instrument Settings



In the Instrument Settings panel, many tasks can be performed:

- Adjust the Sample Flow Rate
- Set Laser Power to Avoid Saturation
- View the Fluorochrome Chart
- Adjust Parameter Thresholds
- Adjust the Trigger Channel
- Enable or Disable Channels

Adjust the Sample Flow Rate



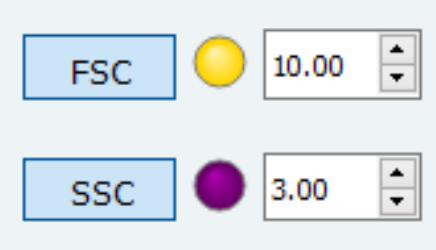
Select from two sample flow rates:

- Slow: 3.66 µL/min
- Fast: 14.64 µL/min

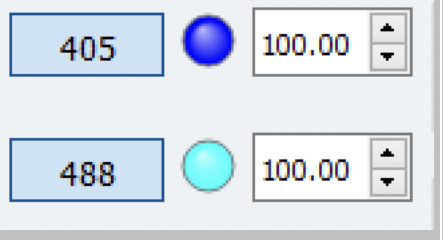
NOTE: Use fast to maximize throughput (recommended for most applications), or slow to maximize sensitivity (recommended for experiments such as extracellular vesicle detection).

Set Laser Power to Avoid Saturation

There are several options for setting up and adjusting the lasers.



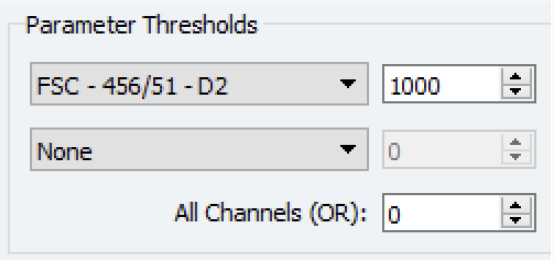
- When not using Small Particle Detection (SPD), always have the FSC (forward scatter) and SSC (side scatter) lasers on. Before running an experiment, determine the appropriate FSC and SSC laser power levels with a manual run (i.e., a "set-up" sample).



- Select the button with the laser name to turn the laser on or off.
- Set the laser intensities by typing a value in the text box or use the up/down arrows.

  NOTE: Laser power is shown as a percentage of maximum (100%).

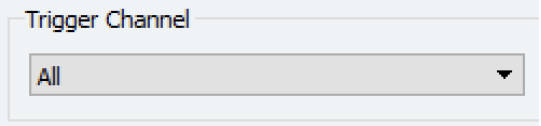
Adjust Parameter Thresholds



Set the parameter threshold to eliminate unwanted debris from acquisition. The first two drop-down menus allow individual channels to be chosen. Choosing two individual channels applies AND logic: both thresholds must be satisfied for an event to be acquired.

The **All Channels (OR)** threshold uses OR logic on all channels: an event will be acquired if any one channel exceeds the OR threshold. All set thresholds must be satisfied for an event to be acquired.

Adjust the Trigger Channel

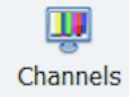


The default settings for the CellStream® system are to trigger on All channels simultaneously; Luminex recommends this setting is NOT changed with the exception of samples with large amounts of fluorescent background (i.e., free fluorescent antibody has not been washed out) where using the All trigger is resulting in much fewer events than expected. An example of this is lysed unwashed blood samples.

NOTE: For lysed unwashed blood samples, Luminex recommends triggering on a CD45 or nuclear stain.

Enable or Disable Channels

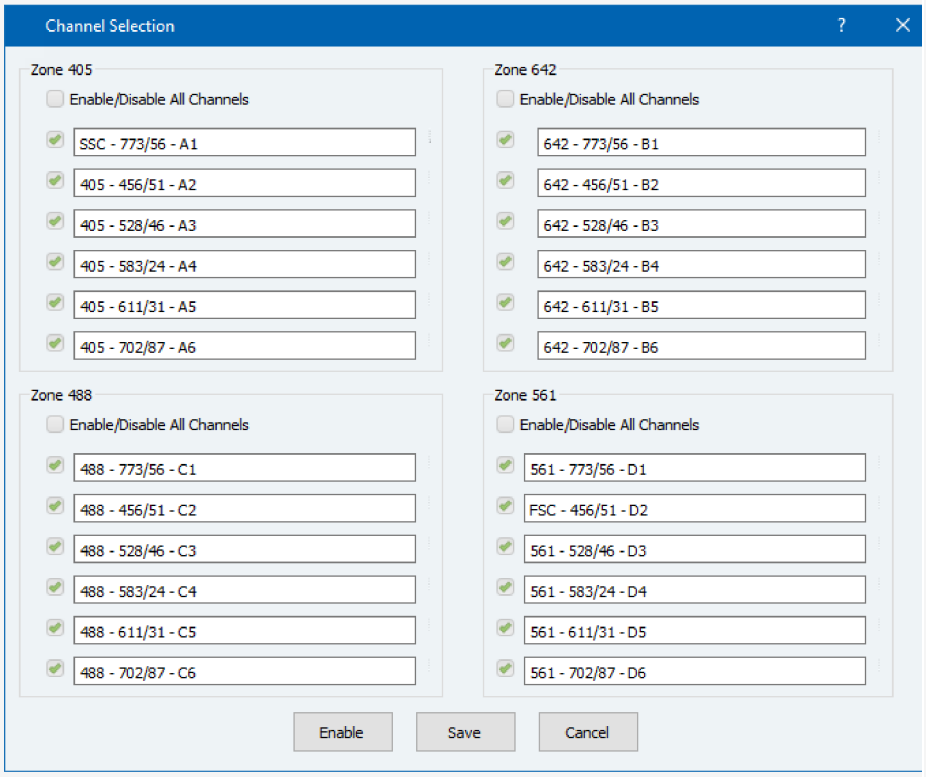
If needed, channels can be disabled during acquisition to reduce file size and the number of parameters displayed during analysis.



Click the Channels icon to enable/disable any channels based on the needs of the experiment. Any channels selected will display as options to select in the x and y axes of the plots. (The Channels icon is only available when a sample is loaded and running.)

NOTE: If a channel is disabled, no data for this channel will be acquired. Be certain that no data are required from any disabled channels.

When clicking the Channels button, the Channel Selection pop-up window will be displayed and specific channels can be enabled or disabled.



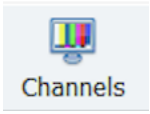
Select **Enable/Disable All Channels** to turn on/off all the channels, or select individual channels to enable or disable. Click **Save** to save any changes made to the individual channels.

Rename Channels

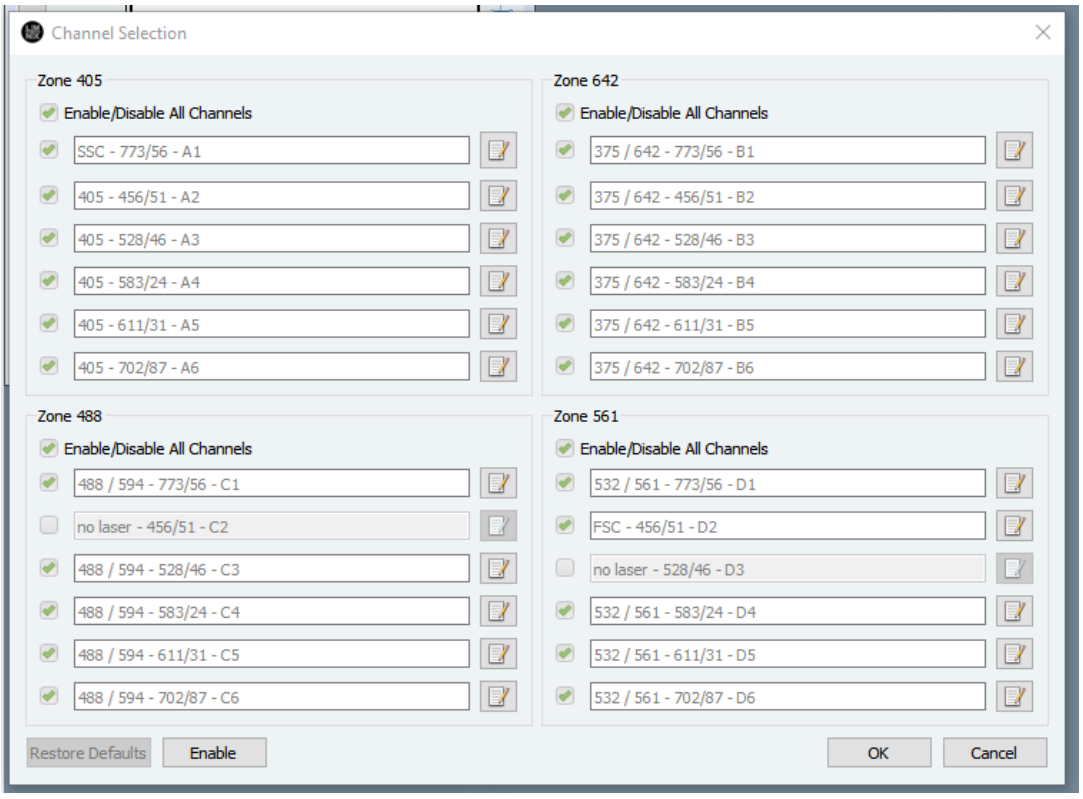
Rename Channels at the Session/Experiment Level

Use this workflow to edit channel names at the session or experiment level. These names will be retained on the specific session or experiment you created them in.

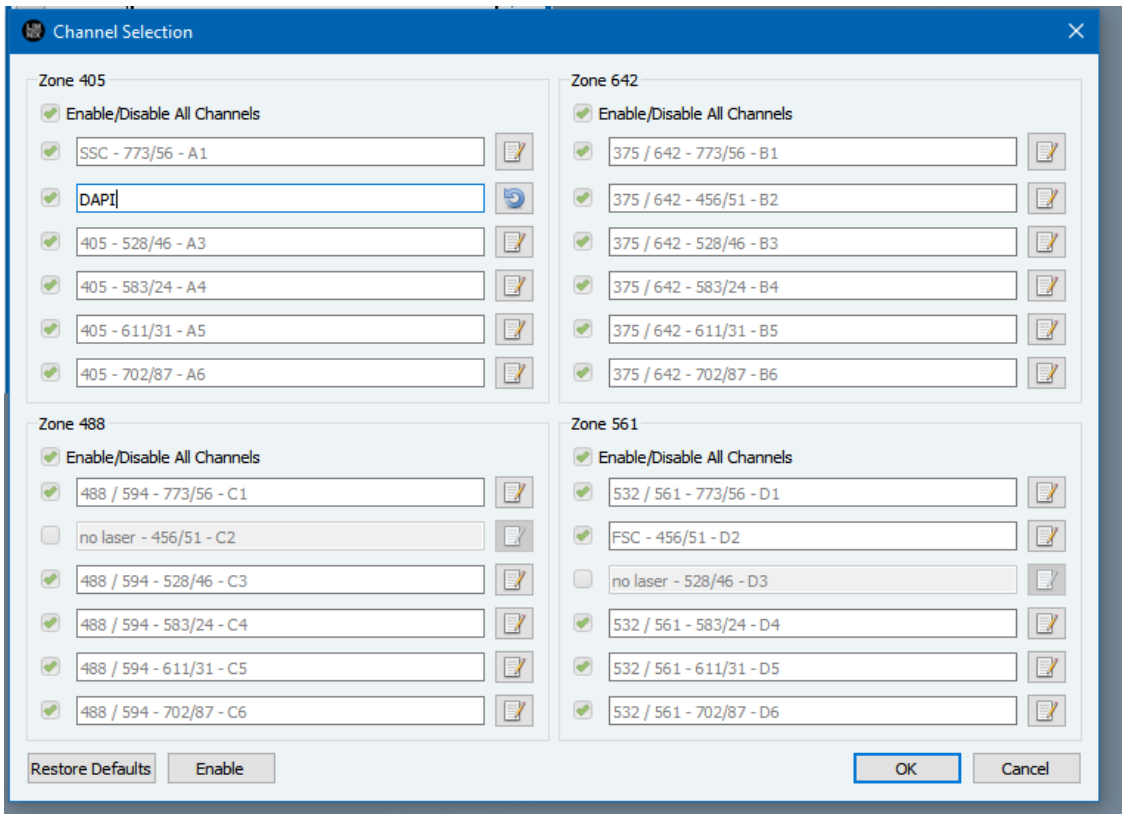
1. From the main page of the acquisition software, click the **Channels** button in the top panel. The **Channel Selection** window displays.



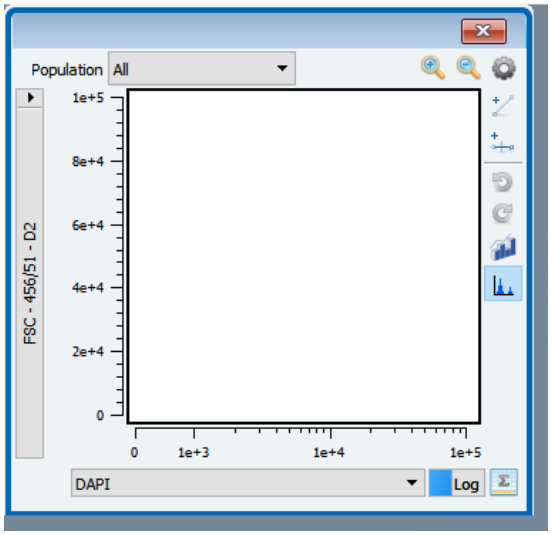
2. To edit the Channel name and subsequent plots and histograms, click the **Edit channel name** icon ( ) to the right of the channel.



3. Type in the new Channel name and click **OK** (for example "DAPI").



Renamed channels now display in the drop-down menu for all plots.



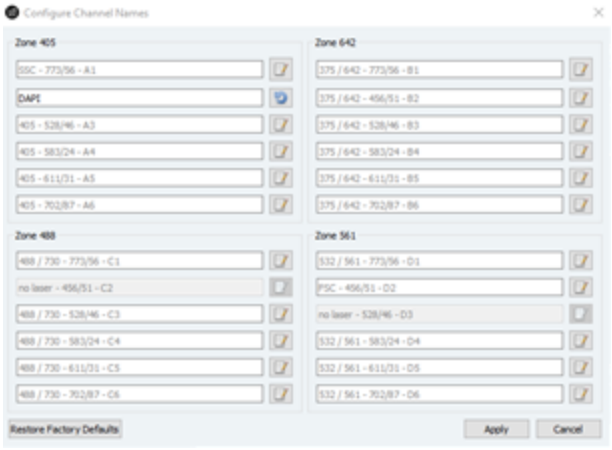
NOTE: The renamed channel will also display in the CellStream® analysis software.

4. To restore channel names to factory settings, click the **Restore system name** icon () and click **OK**.

Rename Channels at the System Level

Use this workflow to edit channel names at the system level. These names will be retained on the system each time the CellStream® instrument is used.

1. From the CellStream software, select **Instrument > Configure Channel Names**.
2. In the **Configure Channel Names** box, click the Edit Channel Name button: 



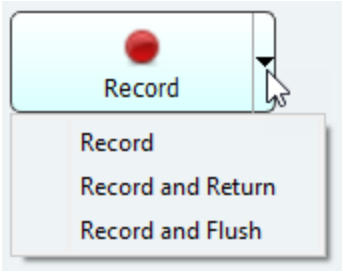
3. Enter the desired channel name in the text box.
4. Click **Apply**. Renamed channels now appear in the drop-down menu for all plots.

   NOTE: To restore channel names to factory settings, click the Restore System Name button: 

The new names created using this workflow are retained for each session or experiment. These can be overwritten for an individual session using the "Rename Channels: Session/Experiment Level" procedure.

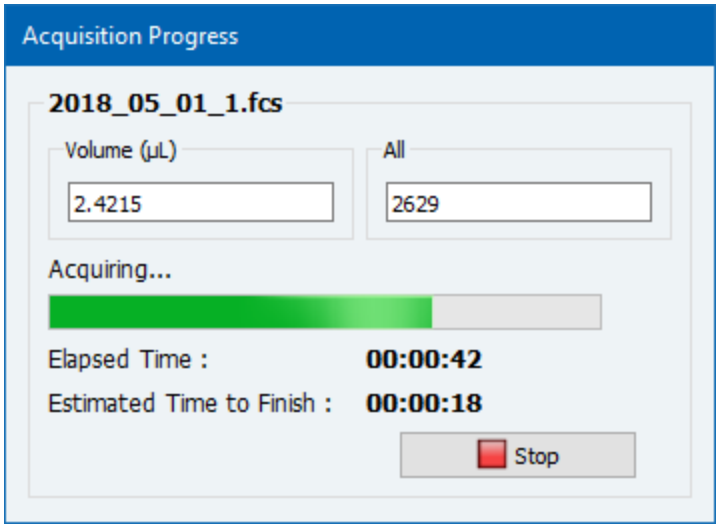
Record the Data

Choose one of the three options from the Record drop-down menu to choose the Record setting.

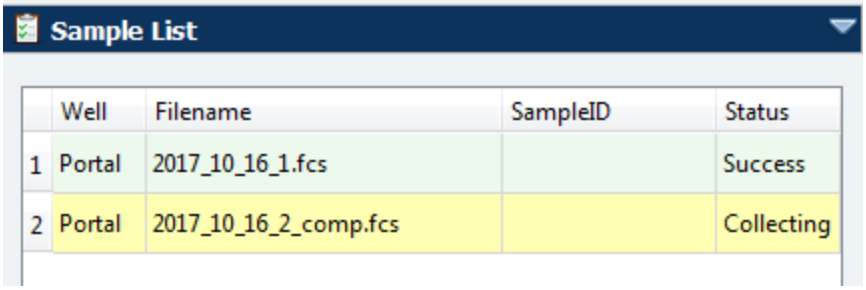


- **Record**: Record the data file and continue to run the sample.
- **Record and Return**: Record the data file, then return the remaining sample.
- **Record and Flush**: Record the data file, then flush the remaining sample.

1. Click **Record** to start recording. The system will display the Acquisition Progress:



The Sample List area will display the status of the processed sample(s).



NOTE: If Stop is clicked during acquisition, the system will provide a prompt to either keep the partially recorded data file or discard the data file. The Sample List area will show the Status as Incomplete.

2. Save and close the experiment once all samples have been collected.

Run the Autosampler

After you created a new experiment and defined the instrument settings, as needed, samples can be loaded into a well plate to use the Autosampler feature. The Autosampler enables unattended operation of samples in 96-well plates loaded into the CellStream® system.

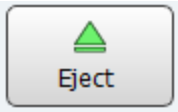
Prior to running the plate, the wells in the plate can be defined with instrument settings, output file name, and other parameters.

The instrument can also sterilize and shutdown at the completion of the plate.

Load a Well Plate and Select Wells

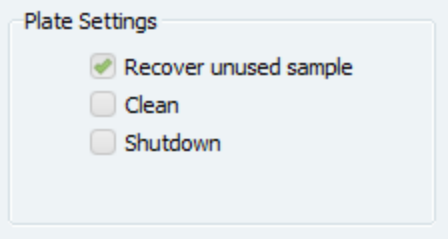
1. Prepare samples in a 96-well plate.

2. To load the plate, click **Eject**.



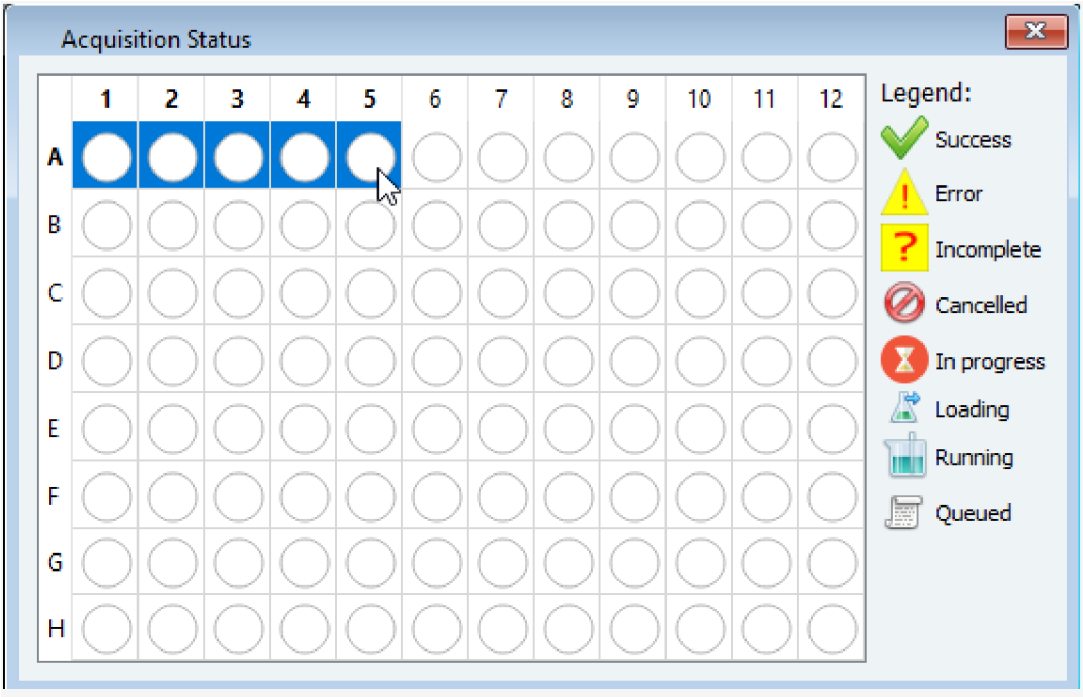
3. Load the plate with the samples into the nest.
4. Click **Eject** again to load the plate in the instrument.

5. Specify the **Plate Settings**.



- **Recover unused sample:** Returns any unused sample.
- **Clean:** Sterilizes the instrument after the plate is run.
- **Shutdown:** Shuts down the instrument after the plate is run.

The plot area will display a plate with the Acquisition Status.



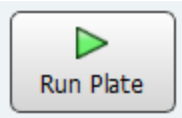
Select the desired wells to run by dragging the cursor over the plate.

With a well or wells highlighted (click and drag), right-click to view the options. In the table below, A01 represents the Well ID, a value that updates based on the selected well(s).

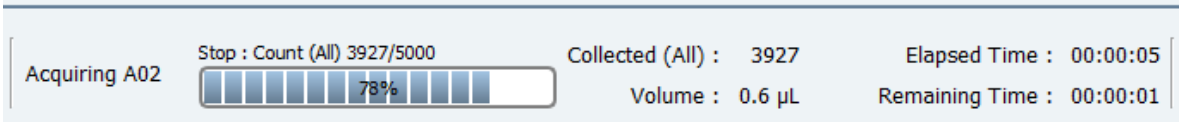
Menu Option	Function
Eject/Load Tray	Ejects/Loads tray
Manual Run > Load from well A01	Specifies to run from the selected well. From here, Record can be clicked.
Unattended Run > Collect using current settings	Collects data using the current settings.
Unattended Run > Collect using instrument settings	Loads an .ist file containing the desired instrument settings.
Unattended Run > Skip A01/samples	Skips the selected well(s) during the run.
Unattended Run > Remove A01/samples from list	Removes the selected well(s) from the run and from the sample list.
Unattended Run > Re-Collect A01 (Note: This option displays only after collection.)	Allows for re-collection of any remaining sample from the select well A01. If recollecting, the details for this well can be edited, if needed.

Optional: Editing the details before running the plate.

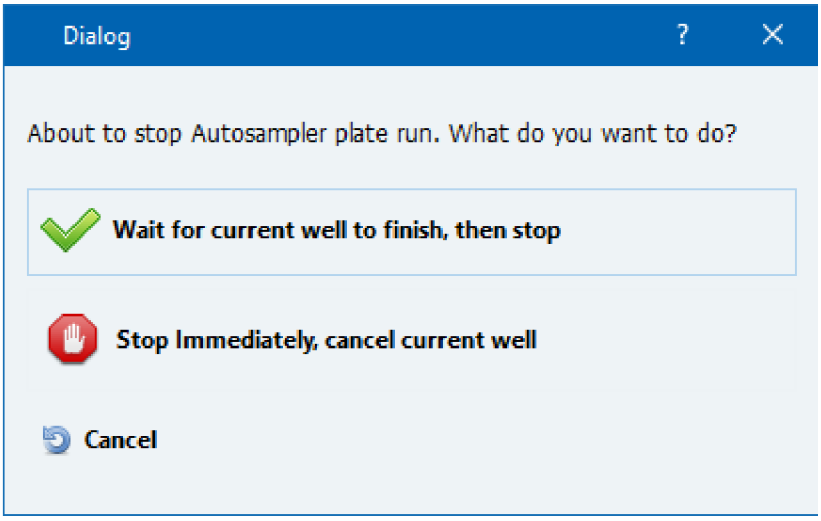
1. Begin Autosampler data acquisition by clicking **Run Plate**.



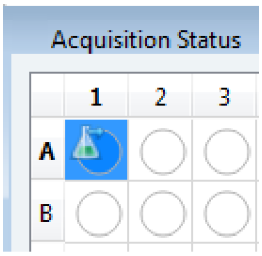
- The status indicator at the bottom of the screen will show the progress of each well.



NOTE: If the Autosampler is stopped during its run, the following options are displayed:



- The plate will display the Acquisition Status of each well.

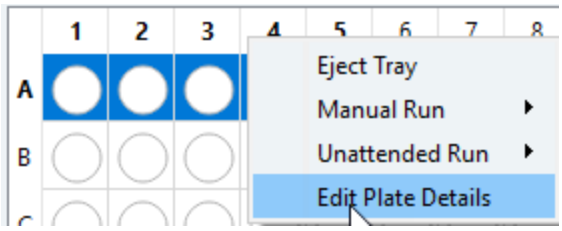


Legend: Success	**Success**: Data collection/recording completed successfully
Error	**Error**: Data collection/recording failed due to some error; no .fcs file was created
Incomplete	**Incomplete**: Partial collection of the data, .fcs file has been created
Cancelled	**Cancelled**: Recording of data canceled by the user
In progress	**In progress**: Data collection/recording process is in progress
Loading	**Loading**: Sample loading is in progress
Running	**Running**: Sample is loaded and running in the instrument
Queued	**Queued**: Wells are in queue to be processed (sample loading, running, and recording)

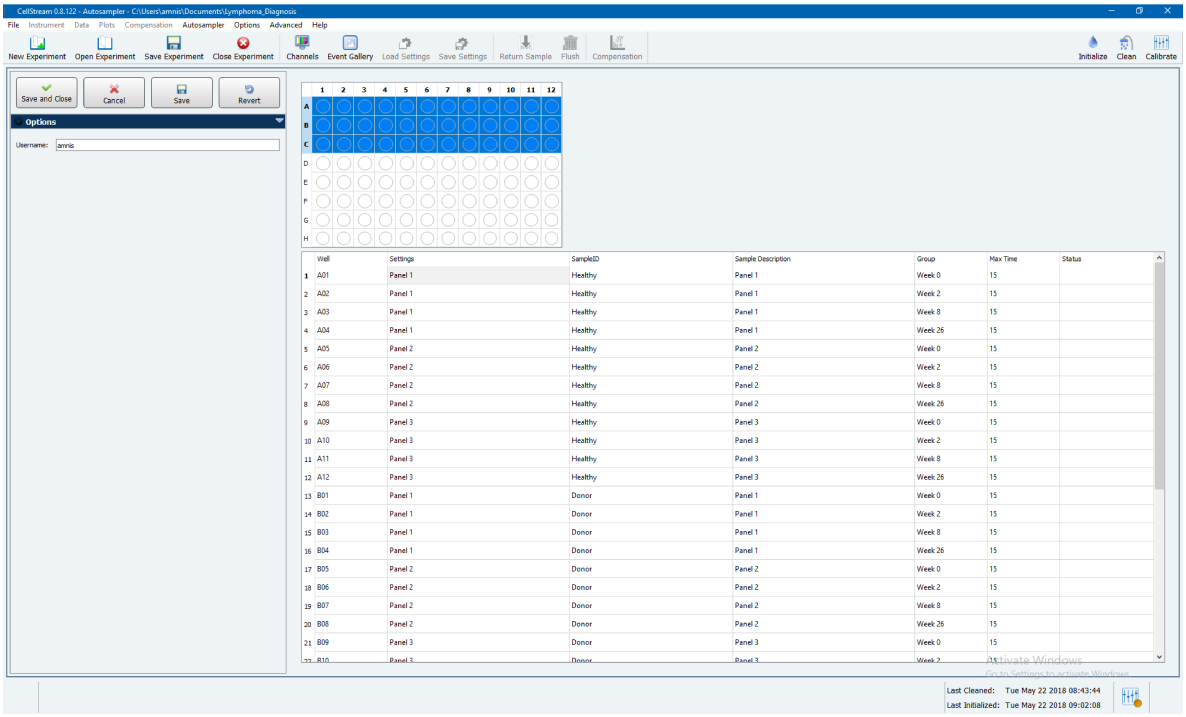
Edit Plate Details

The Plate Details area allows for the selection of settings and modifying well information (sample ID, sample description, etc).

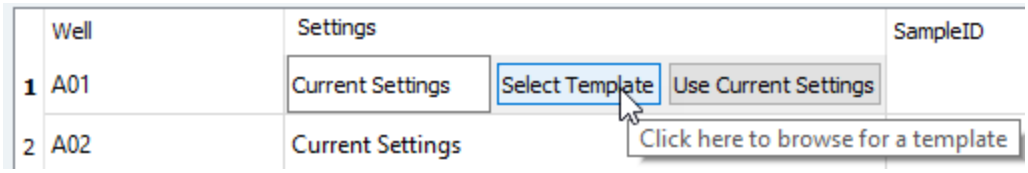
1. Right-click on any well and choose **Edit Plate Details**.



2. In the Edit Plate area, highlight one or more wells to see their details. All pertinent information will display in the table.



3. To edit instrument settings, double-click on **Current Settings** to **Select Template** (to modify the settings) or keep the current settings (**Use Current Settings**).

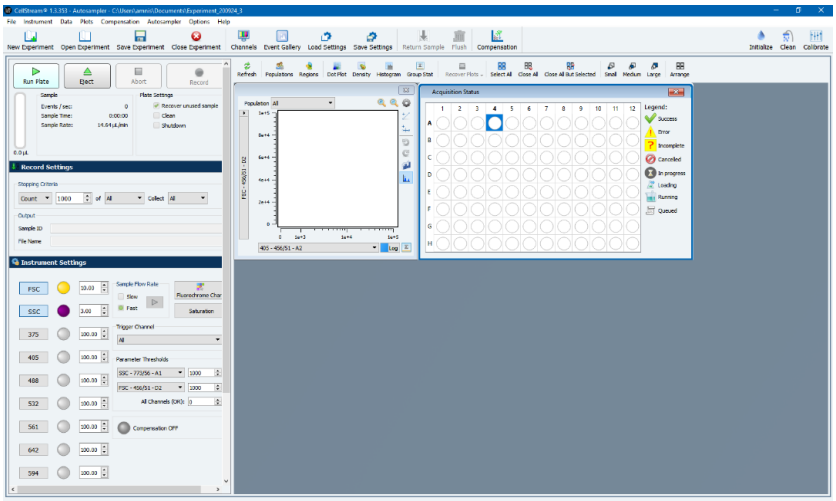


- Copy/paste values into the table's cells (SampleID, Sample Description, Group) or enter them manually.
- **Revert** removes all changes.
- **Save and Close** to exit and save changes.
- **Cancel** cancels all changes.
- **Tip**: Navigate to **Options** > **Application defaults** > **General** to automatically name .fcs files using plate details (Sample ID, Well Number, Date, etc.)

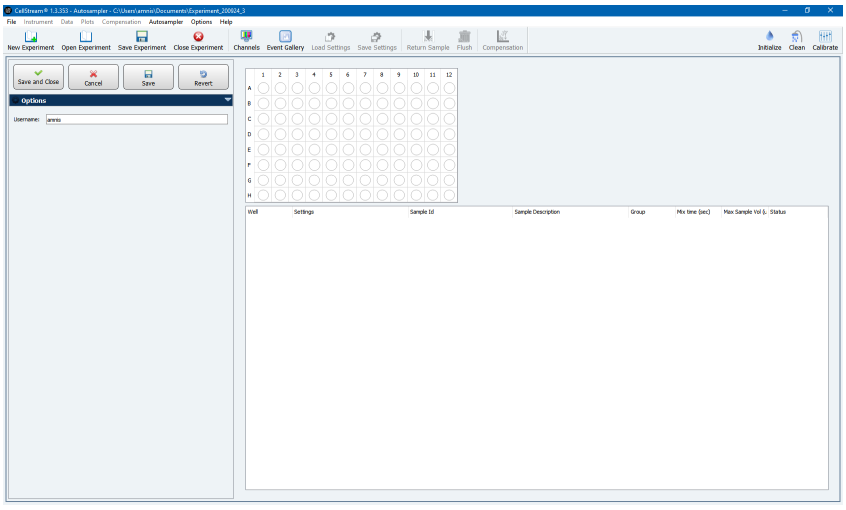
Optional Sample Load Parameters (Sample Load Volume and Mixing Time)

Single Loader and Autosampler experiments have advanced options for configuration of the sample load volume, and the mixing time. These options can be configured during set up of the experiment and applied to each well as the sample loads into the CellStream® instrument. For Single Loader samples, the values are preserved and reused for each sample in the experiment. However, if the experiment is closed or a new experiment is created, the options are reset to their default values.

1. Click **New Experiment** and create an Autosampler experiment.
2. Click **Create Experiment**. A plate map will display on the main acquisition interface.

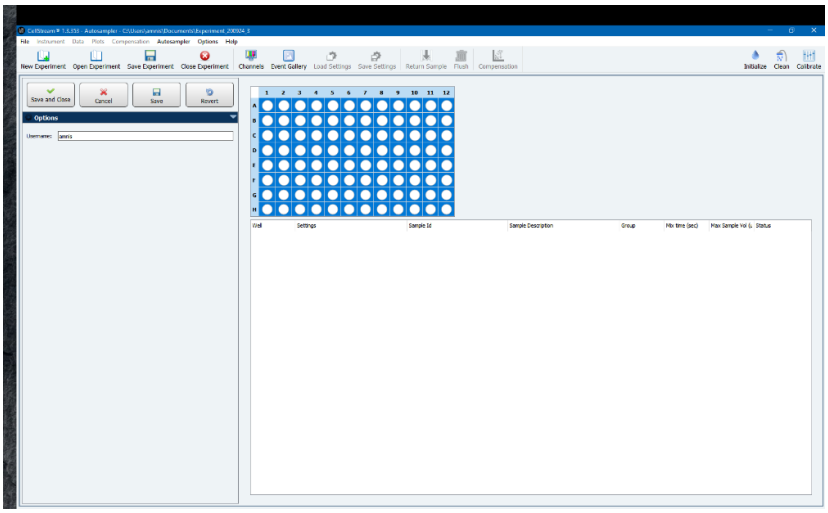


3. Right-click any well to edit the plate.
4. Choose **Edit Plate Details** from the drop-down menu. The plate map setup displays.

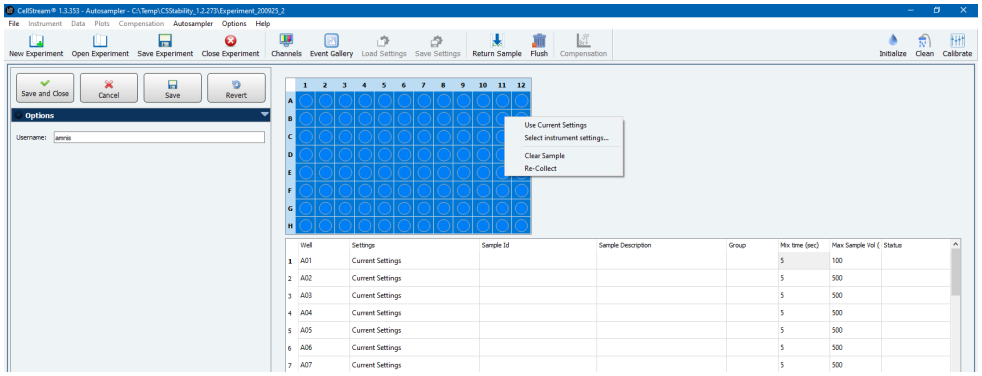


5. Select the number of wells to configure by dragging the mouse over the wells. The selected wells will be highlighted in blue.

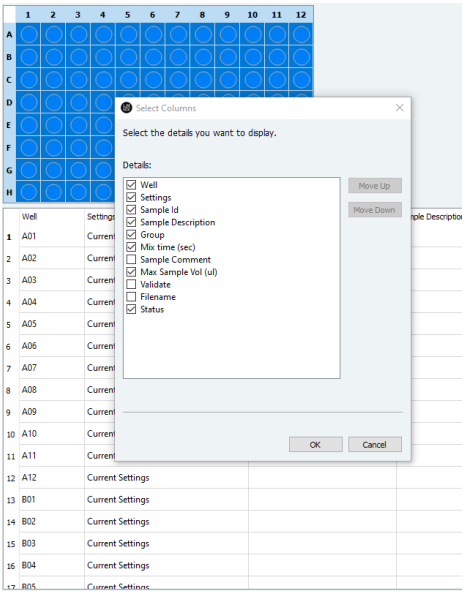
   NOTE: Control + selecting well using the mouse can be used to choose wells in different locations on the plate (i.e., not consecutive).



6. Right-click any well to open the drop-down menu.
7. Choose **Use Current Settings** to configure the wells.

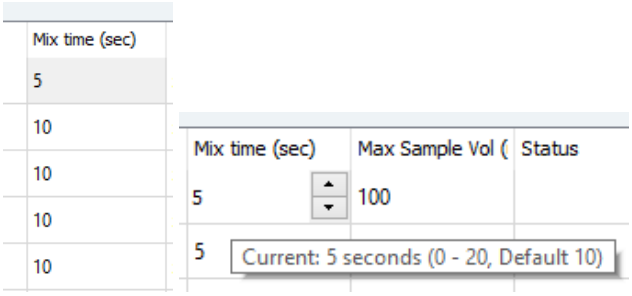


8. Right-click any column header and click **Select Columns...** to rearrange the columns.



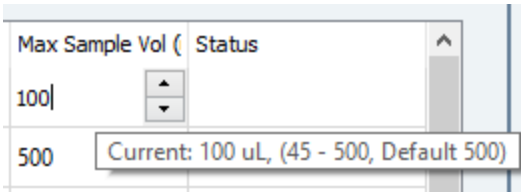
   a. Select the desired boxes to set the available options for the wells.
   b. Click the option to **Move Up** or **Move Down**. This re-orders the option (column) in the configurator from left to right.

9. A Sample ID and Sample Description can be added to each well. Double-click the appropriate row for each well to add the information.
10. To change the Mix time, double-click the **Mix time (sec)** cell to configure the well. Enter a number from 0 to 20 or use the arrows. The default mix time is 10 seconds.



11. Right-click on the cell to access Cut, Copy, and Paste options, allowing you to copy and paste values to other selected wells.

12. To change the Max Sample Volume, double-click the **Max Sample Vol** cell to configure the load volume for the well. Enter a number from 45 to 500. The default is 500 µL.

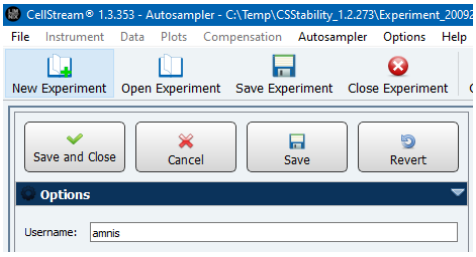


13. Right-click on the cell to access Cut, Copy, and Paste options, allowing you to copy and paste values to other selected wells.
14. To use a template (.ist file) for the selected wells, right-click on a well in the plate map and choose **Select instrument settings**, then click **Template**. This allows you to upload an .ist file to apply to the experiment. The columns will autopopulate.

    NOTE: Alternately, upload an .ist file by right clicking a cell in the Settings column and choosing Select file...

15. To clear well settings, right-click on the well and choose **Clear Sample**. This removes all well settings for all of the selected wells and reverts them back to default settings.
16. To rerun a well, right-click on the well and click **Re-collect**.
17. Click **Save and Close** to save and return to the main acquisition page. You can also:

    - Click **Cancel** to return to the main acquisition page.
    - Click **Save** to save and stay in the plate editor.
    - Click **Revert** to cancel all changes made in the current screen since the last save or opening.



Small Particle Detection

The CellStream® system must perform object synchronization, where the signal from each object is aligned across all channels to produce multi-parametric flow cytometry data. In standard operation, the CellStream synchronizes the FSC (forward scatter) and SSC (side scatter) signals using a specialized image analysis algorithm. However, small objects such as extracellular vesicles and small bacteria may not generate a sufficient FSC or SSC signal to allow synchronization, resulting in desynchronized data. For these sample types, Luminex recommends using Small Particle Detection, which enables synchronization of objects generating little to no FSC/SSC signal.

Set up the Instrument for Small Particle Detection

In normal operation mode, FSC (forward scatter) and SSC (side scatter) are used to synchronize the four zones on the camera. Small particles like extracellular vesicles, viral particles, and nanoparticles do not give off enough FSC and SSC signal to perform this operation; therefore, a constant line rate is used to synchronize the four zones of the camera in Small Particle Detection.

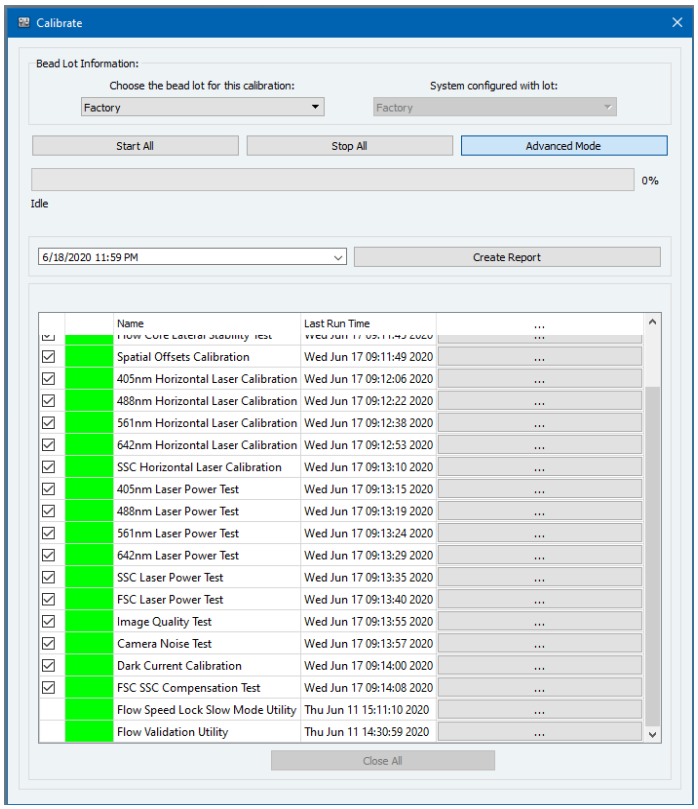
The Flow Speed Lock Slow Mode Utility verifies that the sample and sheath flow rates are stable. The Flow Validation Utility checks the stability of the sheath flow speed and calculates/sets the camera line rate for Small Particle Detection.

NOTE: Flow Speed Lock Slow Mode Utility, Flow Validation Utility, and all other calibration tests, must be run with Small Particle Detection mode disabled.

Flow Speed Lock Slow Mode Utility and Flow Validation Utility are not automatically run during calibration. However, Luminex recommends to run these utilities prior to running an experiment in Small Particle Detection mode. The Flow Speed Lock Slow Mode Utility verifies the sheath flow rate is converting to the target rate within the required lock on time and the Flow Validation Utility checks the stability of the sheath flow speed over 5 minutes to ensure synchronized data and calculates/sets the camera line rate for Small Particle Detection.

Complete the Flow Speed Lock Slow Mode Utility and Flow Validation Utility using the following steps:

1. Load 4 drops of CellStream® Calibration Reagent into a 1.5 mL Eppendorf tube.
2. Set the instrument to slow speed (NOT Small Particle Mode).
3. Load the sample.
4. Click **Calibrate** to open the Calibration window.
5. Click **Advanced Mode** to open the complete list of calibrations.



6. Navigate to the **Flow Speed Lock Slow Mode Utility** near the bottom of the list and select the green box to open the utility in a separate window.
7. Click **Start** to run the utility. The **Flow Speed Lock Slow Mode Utility** window minimizes.

NOTE: If the utility fails, verify the calibration sample is running on slow speed (do not have Small Particle Detection enabled for the utilities) and run the utility again. If the utility fails again, confirm the lab is at the approved temperature for Small Particle Detection mode and then contact Luminex Technical Support. Small Particle Detection can operate in the full CellStream operation temperature range; however, for proper synchronization the temperature should not exceed ± 2.5°C from the installation temperature.

8. Once the utility is complete, close the window.
9. To verify the stability of the flow speed and calculate/set the line rate for Small Particle Detection, navigate to the **Flow Validation Utility** at the bottom of the list and select the green box to open the utility in a separate window.
10. Click **Start** to run the utility.

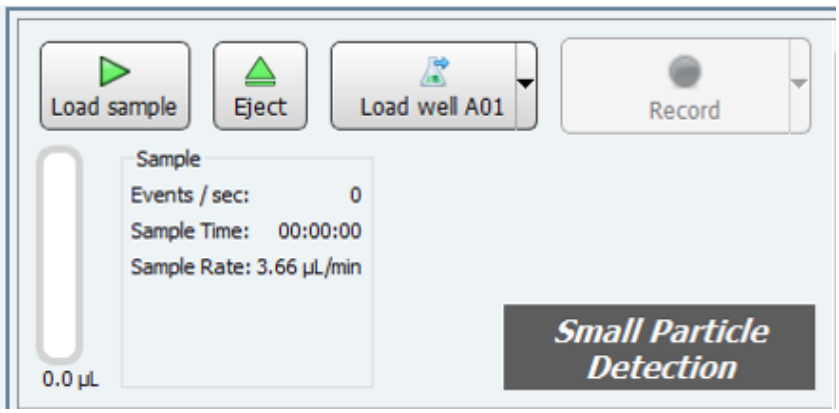
    NOTE: If the utility fails, verify the calibration sample is running on slow speed (do not have Small Particle Detection enabled for the utilities) and run the utility again. If the utility fails again, confirm the lab is at the approved temperature for Small Particle Detection mode and then contact Luminex Technical Support. Small Particle Detection can operate in the full CellStream operation temperature range; however, for proper synchronization the temperature should not exceed ± 2.5°C from the installation temperature.

11. Once the utility is complete, close the **Utility and Calibration** windows.

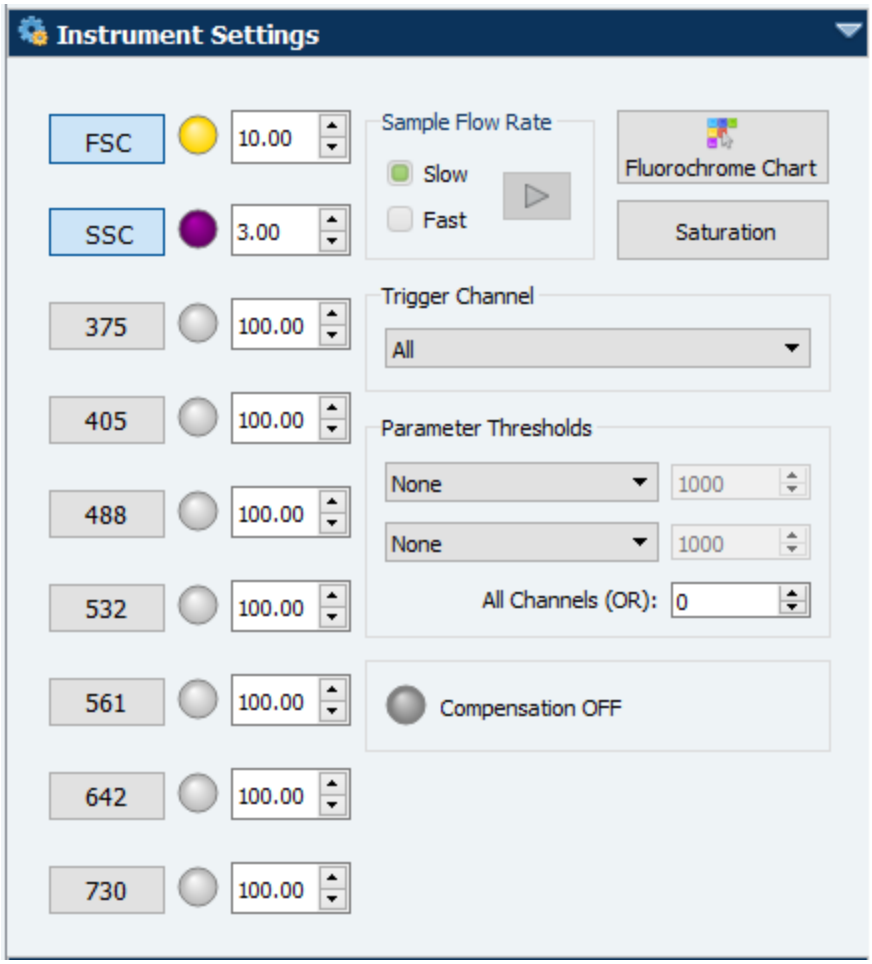
Activate Small Particle Detection for Single Sample or Autosampler

1. Open or create a new experiment.
2. Navigate to **Instrument** > **Advanced** > **Set Up Small Particle Detection**.

    - Small Particle Detection is now activated, indicated by the gray text box in the lower right corner of the sample load/record panel.



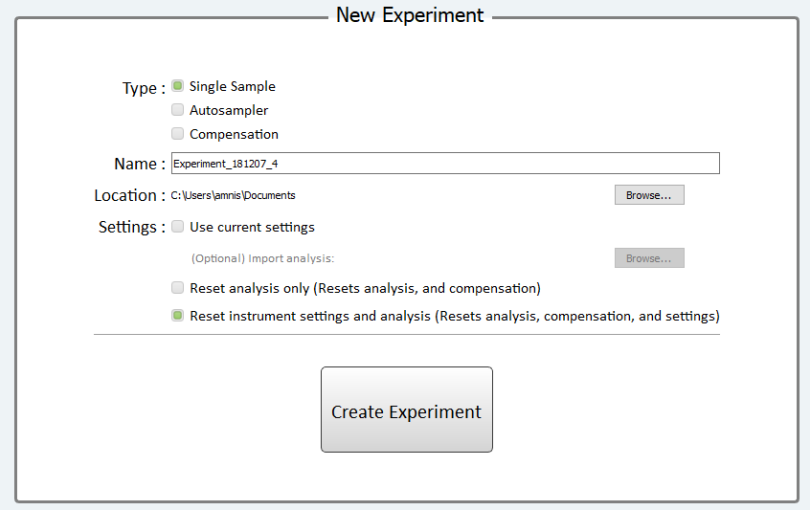
- The following default settings have been applied for Small Particle Detection:
    - Small Particle Detection requires the Sample Flow Rate to be set to Slow.
    - Trigger Channel is set to All; this can be set to a single trigger, if desired.
    - The Parameter Thresholds are set to zero or None.



- **Object detection**: Use a fluorescence stain(s) to identify small particles of interest and allow removal of non-fluorescent background using gates. Maximize sensitivity to the smallest objects by setting lasers used for fluorescence excitation to 100%.
- **Thresholds**: If thresholds are turned on (above zero), be careful to not set them so high that particles of interest are excluded. For preliminary experiments where an appropriate threshold is unknown, Luminex recommends to keep the default threshold settings at None to ensure collection of all particles of interest, and background can be gated out during analysis.
- **Background**: Unfiltered sheath fluid and/or high FSC/SSC settings will produce a significant amount of non-fluorescent background when thresholds are set to zero. If reduction in background signal is desired, Luminex recommends to use 0.1 µm filtered sheath and to keep the SSC (side scatter) and FSC (forward scatter) laser powers low (at default) or turn them off.

Reset Settings to Exit Small Particle Detection

1. Start a new experiment and select **Reset instrument settings and analysis**.



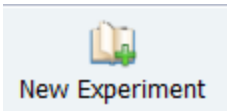
- Or navigate to **Instrument** > **Reset Settings**.

- Or choose **Load Settings** and load a template that was not using Small Particle Detection.

Chapter 7: Performing Sample Compensation

Collect Sample Compensation Data in the Acquisition Software

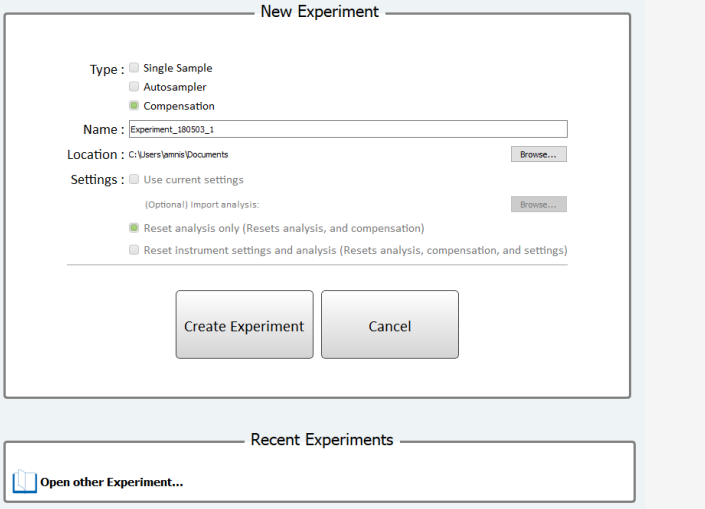
1. Prepare single-color compensation controls using cells or beads for each fluorochrome in the staining panel, as well as an unstained control.
2. Click **New Experiment**.



3. Select **Compensation** as the Type of experiment.

NOTE: Optionally, enter the Name of the experiment and click Browse to select a Location to save the experiment.

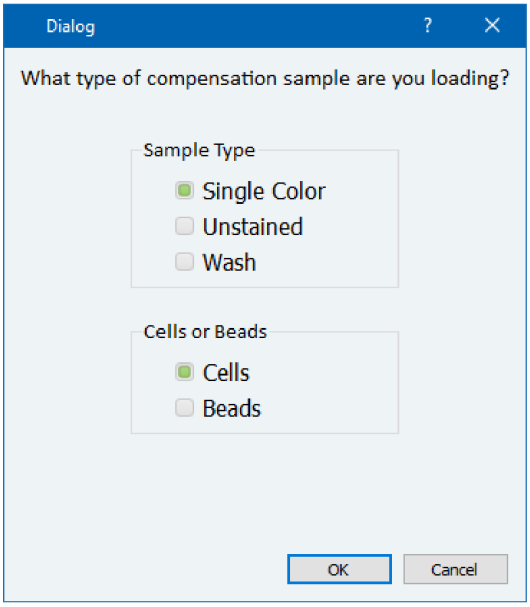
NOTE: Reset analysis only is the only option available for the instrument settings.



4. Click **Create Experiment**.

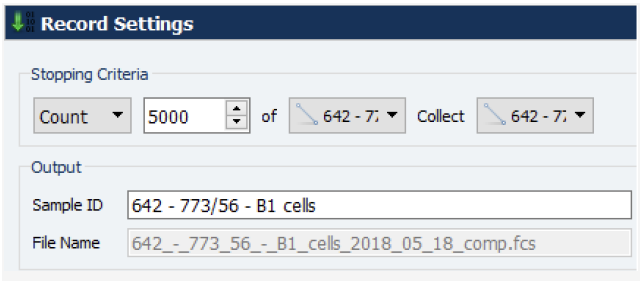
5. In the **Instrument Settings** panel, the same laser powers must be used to collect single-color compensation controls as were used for the experimental samples.
   - If the laser powers have changed, they must be manually adjusted to match the settings used during collection of the experimental samples.

     NOTE: For compensation experiments, the SSC (side scatter) laser will be disabled to allow the calculation of crosstalk into the SSC channel.
   - All channels will be enabled and the parameter thresholds will be set to default values used for compensation control collection.
   - If collecting experiment data after compensation, be sure to reload experiment settings to revert the changes applied while collecting compensation coefficients.
6. Click **Load Sample** and add a compensation sample from a tube or well plate.
7. Select the **Sample Type** and specify the type of sample loaded (cells or beads).



8. Click **OK**.

NOTE: Compensation samples can be acquired in any order. The peak channel will be detected automatically within the Record Settings panel, but those settings can be changed, if needed.

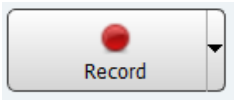


An FSC (forward scatter) histogram and region will be created to gate on a cell or bead population.

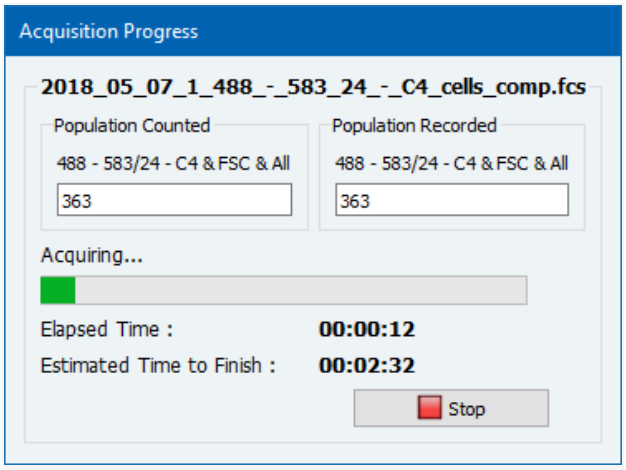
Adjust the region to gate on the appropriate population as needed.
For single-color controls, a histogram of the peak channel is plotted and a region is created to gate on the fluorescence population.

- Verify the correct peak channel has been identified.
- Adjust the region to gate on fluorescence positive population.
- Optional: rename the compensation control. Luminex recommends naming the compensation control the name of the fluorochrome, which can simplify data troubleshooting.

9. Choose one of the three options from the **Record** drop-down menu to choose the Record settings.



The Acquisition Progress dialog box displays.
The default number of events to be collected from each compensation control is 5000. This can be adjusted in the Stopping Criteria area.



10. Repeat the process, as needed, for additional compensation control samples.

    NOTE: If the correct peak channel is not identified, it will be necessary to replicate the steps performed by the CellStream® software.

    a. Generate a histogram for the correct peak channel.
    b. Apply a region to the peak channel to gate on the fluorescence positive events.
    c. Set the stop criteria to the desired number of events and collect data for the peak channel only.
    d. Provide a sample ID.

11. Save and close the experiment when finished.
12. Open CellStream Analysis software to calculate the compensation matrix.

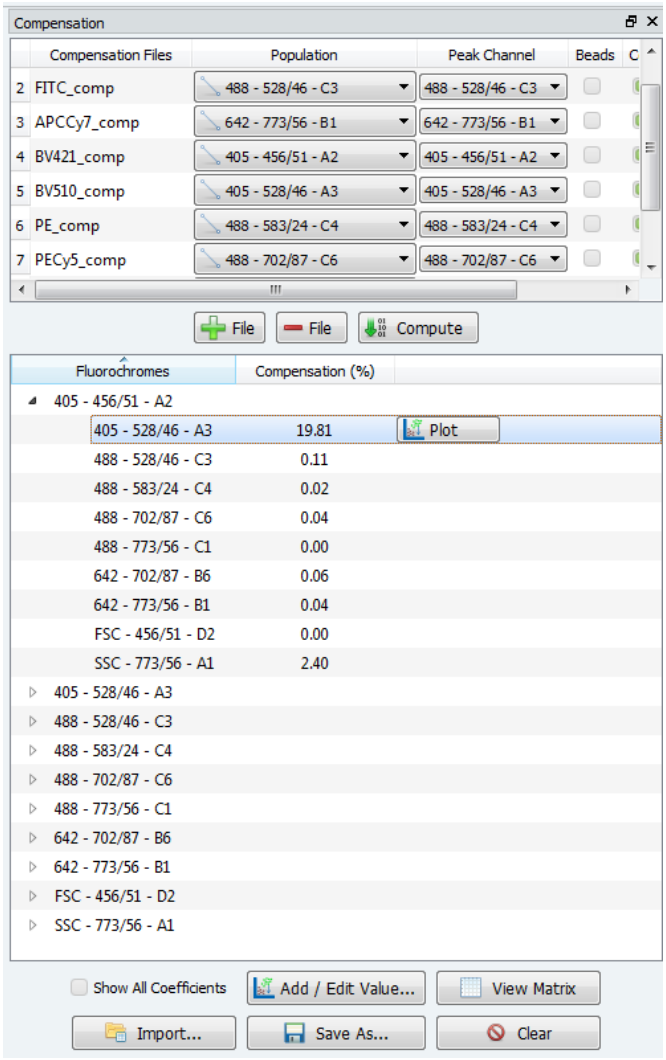
Calculate a Sample Compensation Matrix in the Analysis Software

1. Click **Open Experiment** to open the compensation experiment.

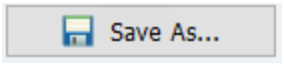
2. Click **Compensation** if the compensation panel is not displayed.

   NOTE: The peak channels should be correctly detected for each single-color control. If any channels are not automatically detected, use the drop-down menu to select the proper channel.

3. Click **Compute**. The compensation coefficients display.



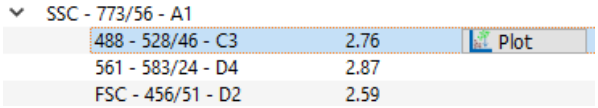
4. Click **Save As** to save the compensation matrix. The compensation matrix can now be applied to an experiment within CellStream® Acquisition or Analysis software.



5. Save and close the experiment when finished.

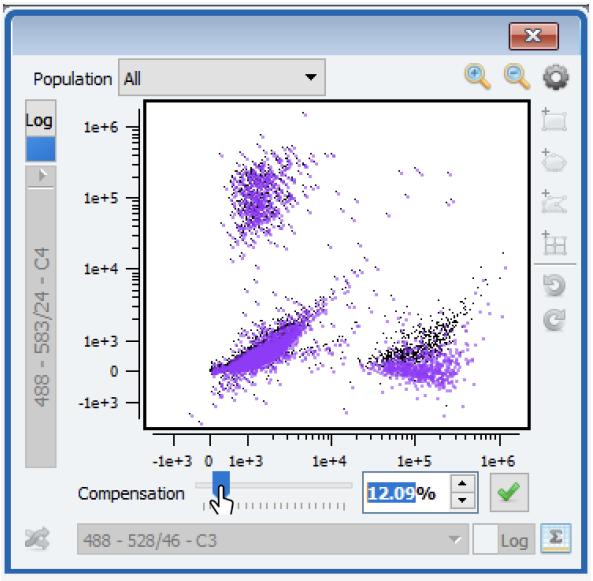
Adjust Sample Compensation Results

1. Adjust the compensation coefficient by clicking the **Plot** button in the Compensation table.

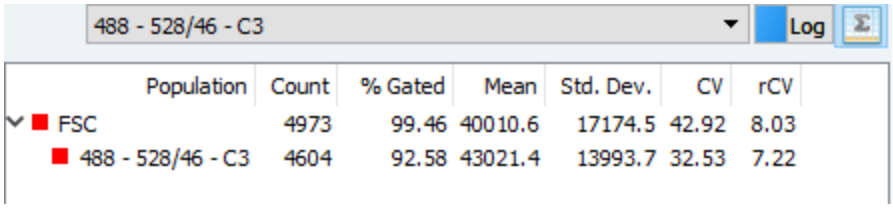


2. In the plot, use the **Compensation** slider bar to adjust the compensation.

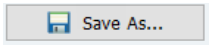
   - The black dots represent the original population and the purple dots represent the adjusted compensation.
   - Click the green check mark to apply the compensation coefficient.



3. Click the **Σ** icon to view the statistics.



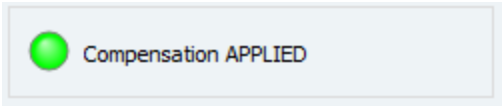
4. To save the matrix (.ctm) and the calculated coefficients, click **Save As**.



Apply a Sample Compensation Matrix

If a compensation matrix (.ctm file) has already been created, it can be applied within CellStream® Acquisition or Analysis software.

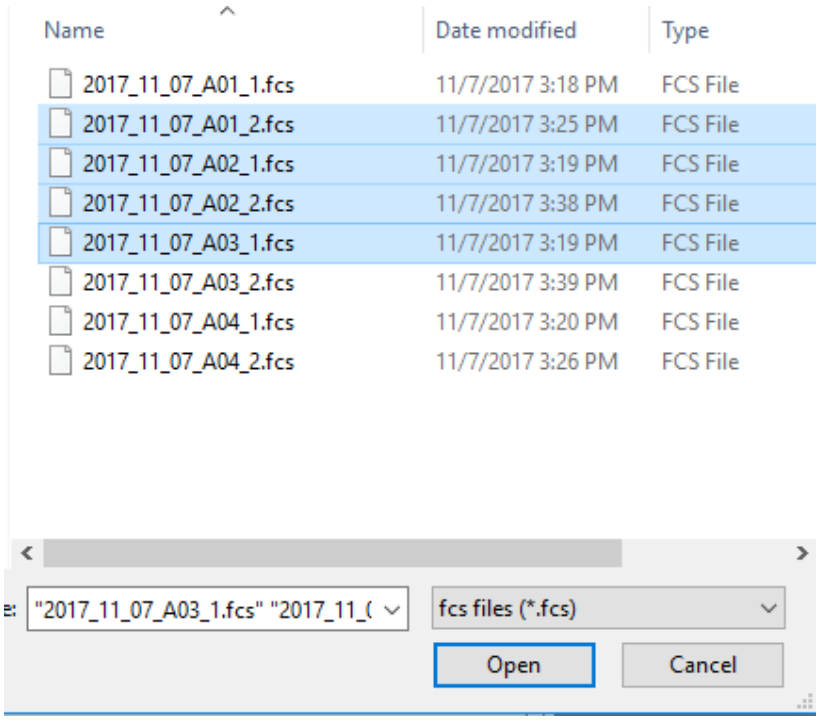
1. Click **Compensation**.
2. Click **Import** at the bottom of the panel that is displayed to add a compensation file (.ctm or .exp).
   The compensation data are displayed in the right side panel.
   If applying compensation during data acquisition, the **Compensation APPLIED** status displays in the Instrument Settings panel within CellStream Acquisition software. (The Compensation APPLIED status doesn't appear in the Analysis software.)



Add Samples to an Experiment

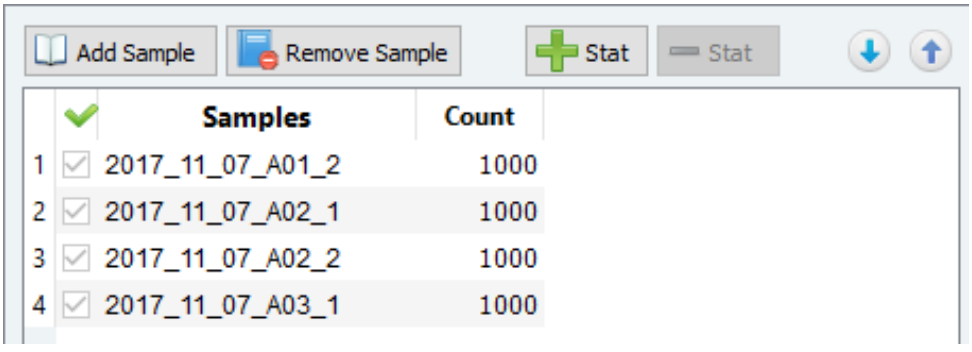
An experiment is a collection of one or more samples recorded in CellStream® Acquisition software that can be evaluated in CellStream Analysis software. The software automatically computes coefficients for compensation.

1. Click **Open Experiment**.
2. Choose a .exp file and click **Open**. If creating a new experiment, first add previously acquired samples (.fcs and _comp.fcs).
3. Click **Add Sample**.
4. In the popup window, choose one or more samples to add and click **Open**.

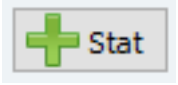


The samples display in the list.

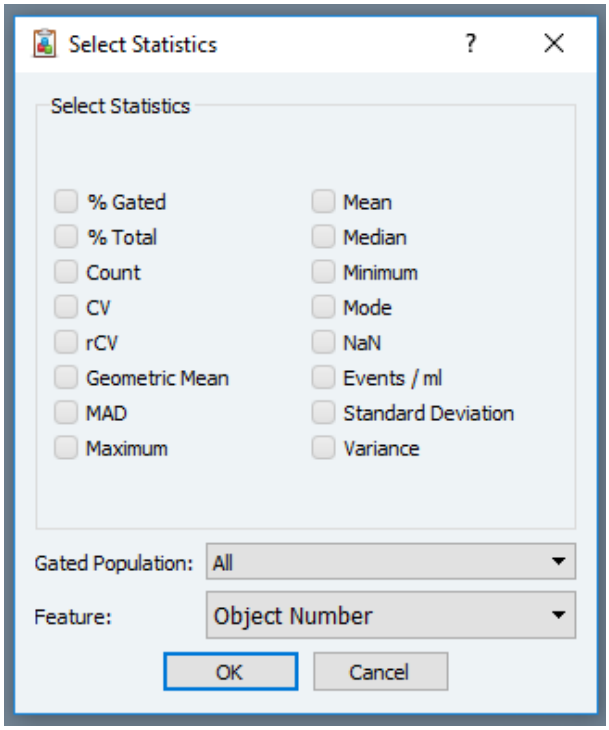
- **Option 1**: Remove any unwanted samples by selecting a sample and clicking the **Remove Sample** button.
- **Option 2**: Reorder samples in the list by selecting a sample and clicking the up/down arrows.



5. To add statistics, click the **Stat** button.

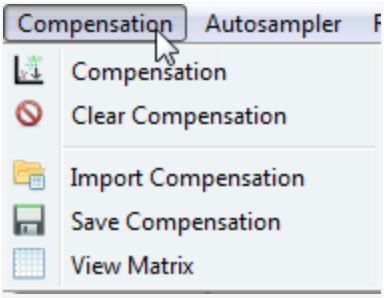


6. Select the statistics to add to the table and click **OK**.



NOTE: To remove a statistic, click the remove statistic button and select the statistics to remove.

Sample Compensation Toolbar



Compensation: View the compensation panel

Clear Compensation: Delete the current compensation

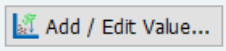
Import Compensation: Import an existing compensation matrix (.ctm or .exp file) to apply it to an experiment

Save Compensation: Save the current compensation

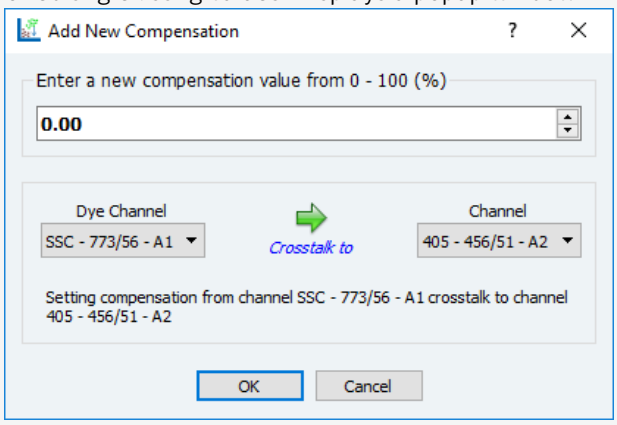
View Matrix: View the compensation matrix

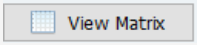
Compensation Matrix Functions

Displays all coefficients in the compensation tree.

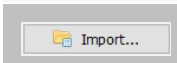


Allows for adding a new coefficient to the compensation matrix or editing existing values. Displays a popup window:

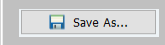




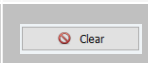
Views the matrix as a grid.



Imports an existing compensation matrix.



Saves the current compensation matrix.

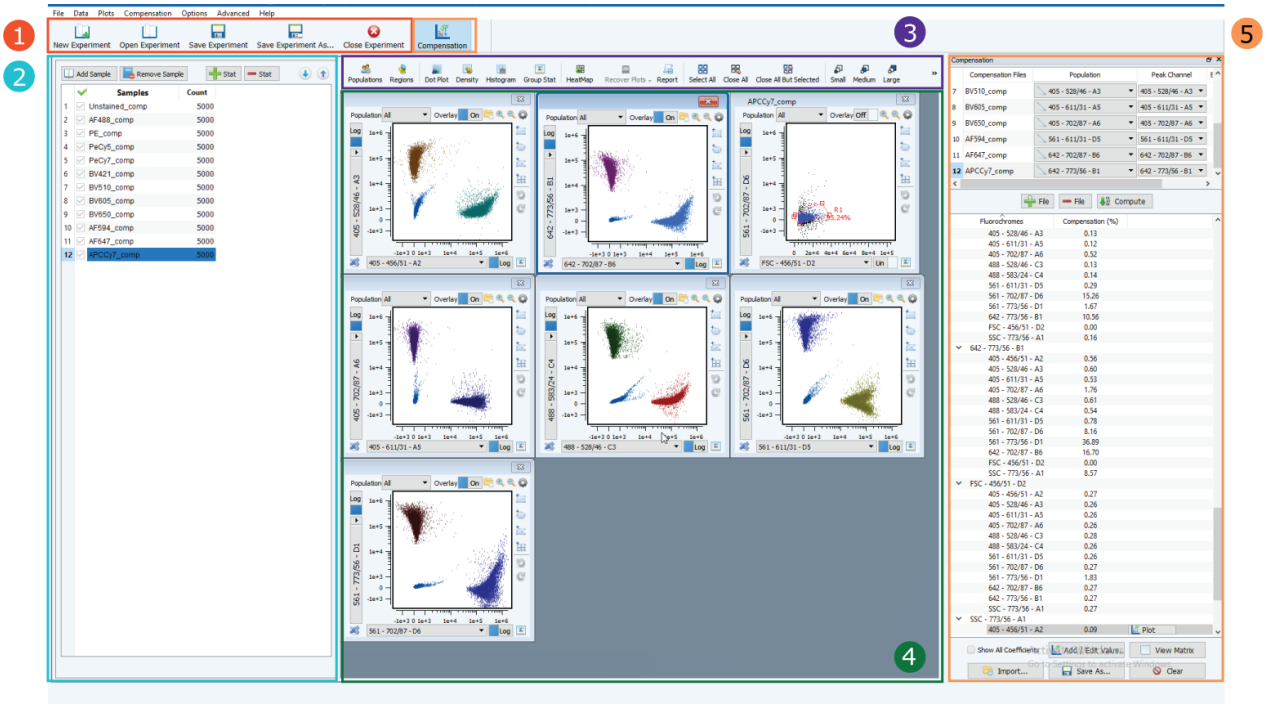


Clears all values from the matrix.

Chapter 8: Analyzing Data

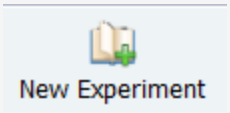
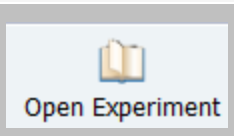
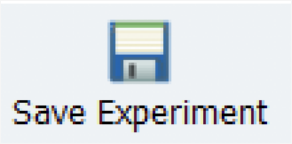
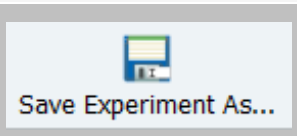
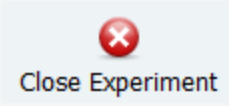
CellStream® Analysis Software

The CellStream® Analysis software shares common interface features shared with CellStream Acquisition software. This image labels the user interface areas in the CellStream Analysis software.

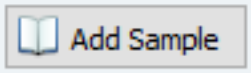
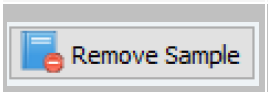
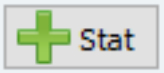
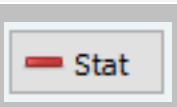


1. Experiment Toolbar
2. Sample List
3. Analysis Toolbar
4. Analysis Workspace
5. Compensation Panel

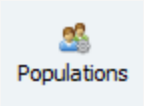
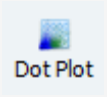
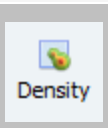
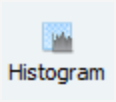
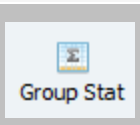
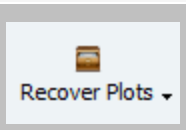
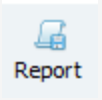
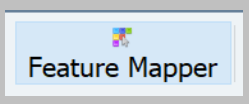
Experiment Toolbar

Icon	Function
	Creates a new experiment (.exp).
	Opens an existing experiment (.exp).
	Allows for the creation of a new Experiment folder to save .exp and .fcs files.
	Creates a new Experiment folder and saves current experiment (.exp) and .fcs files within the folder.
	Closes the current experiment.
	Displays the Compensation panel.

Sample List

Icon	Function
	Adds a sample to the experiment.
	Removes a sample from the experiment.
	Adds selected statistics to the display.
	Removes selected statistic from the display.
	Reorders samples within the sample list.

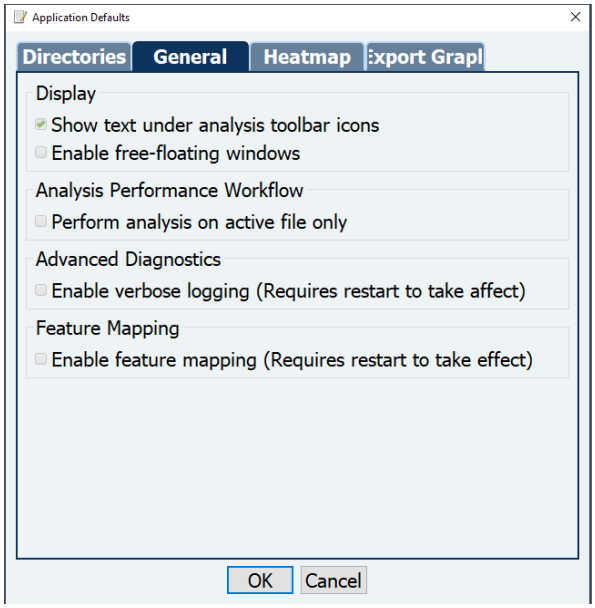
Analysis Toolbar

Icon	Function
	Opens the Population Manager.
	Opens the Regions Manager.
	Creates a new dot plot chart.
	Creates a density plot.
	Creates a new histogram.
	Opens a table that can be customized to view specific statistics for all populations (or selected populations).
	Displays a heatmap for the select samples.
	Recovers plots that have been previously closed.
	Generates an analysis report of the current experiment as a .pdf or .odt file.
	If you did not rename any channels during acquisition, use this option to rename features for analysis plots, histograms, and graphs in the analysis software. Feature Mapper must be enabled before any data is opened.

Rename Features Using the Feature Mapper

To rename features, enable the Feature Mapper before opening any data files in the analysis software.

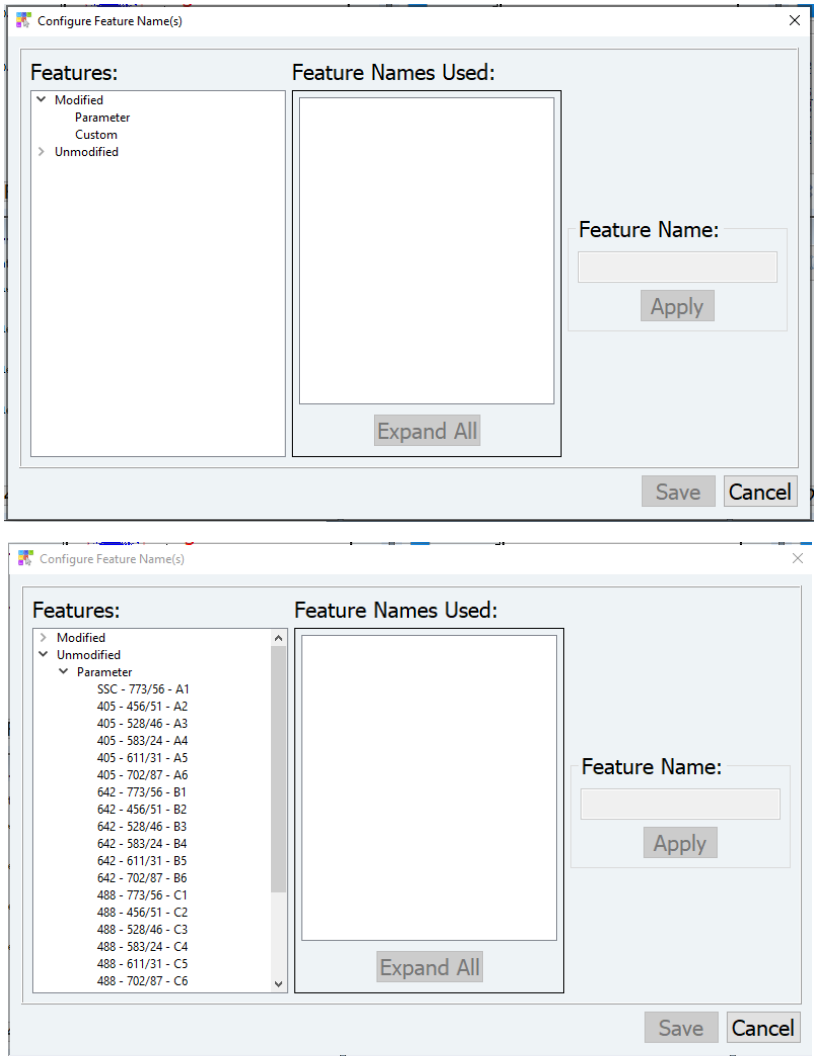
1. From the CellStream® analysis software, click **Options**> **Application Defaults**. The **Application Defaults** menu displays:



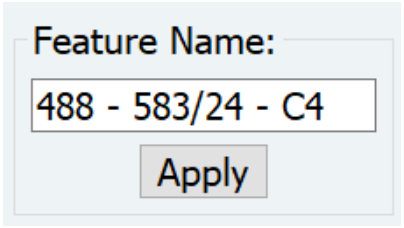
2. Click the **General** tab.
3. Select **Enable feature mapping**, then click **OK**.
4. Close and restart the analysis software.
5. Open the experiment from a saved file.
6. Click **Feature Mapper**.

   NOTE: A sample must be added to the experiment to enable the Feature Mapper button.

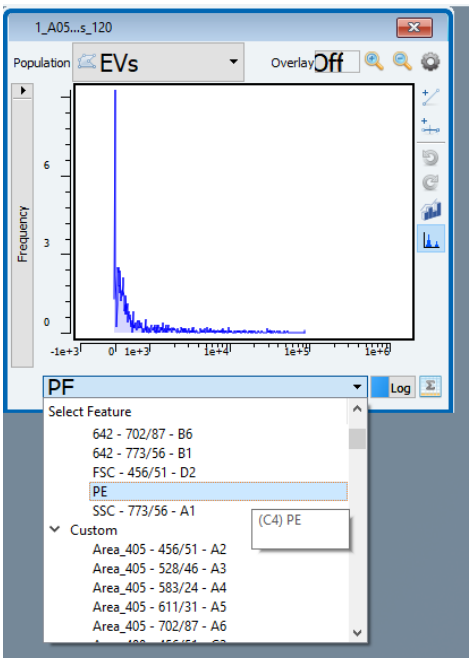
7. The **Configure Feature Names(s)** menu displays. Click the drop-down arrow to the left of **Unmodified**, and then click the drop-down arrow to the left of **Parameter**.



8. Choose the Feature to rename. This will display in the **Feature Name** box. Type the new name in the box and click **Apply**.



9. Click **Save**. The new name for the feature will now display in the drop-down menu for each plot and histogram (all graphs).

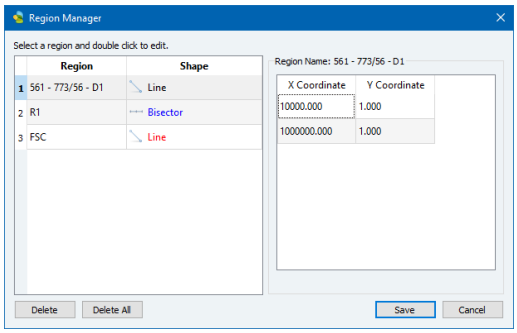


NOTE: The zone affiliated with the newly named feature will still display in the tooltip (e.g., (C4) PE).

Adjust Regions

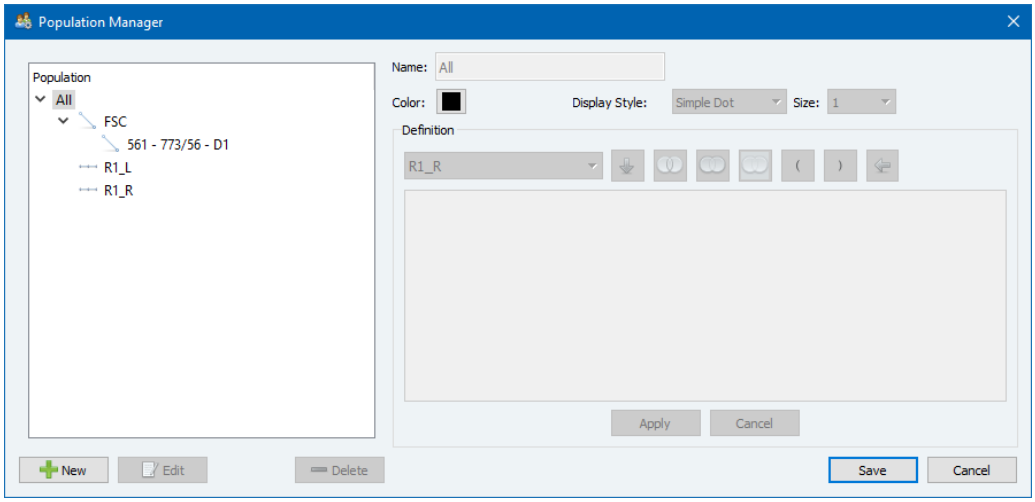
A region is a defined set of coordinates. The Region Manager allows for fine tuning of coordinates for very minor adjustments of regions.

1. Click **Regions**.
2. Make the necessary changes in the Region Manager and click **Save**.



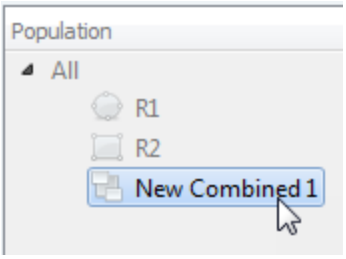
Population Manager

The Population Manager allows the customization of colors, names, and dot style for each population, exclusion of unwanted populations, and creation of combined populations using Boolean operators.

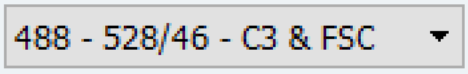


Create a New Population

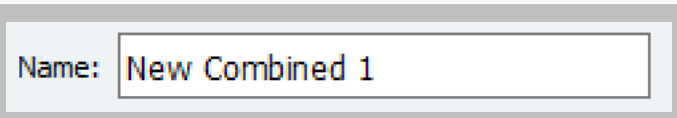
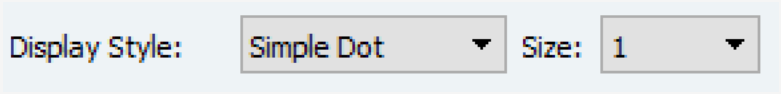
1. Click **New** to create a new population. A **New Combined** population is created.



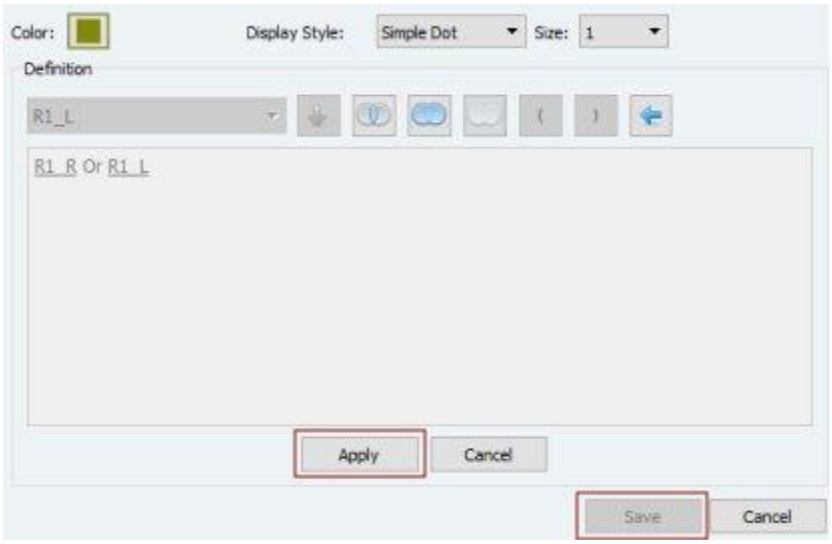
2. Define the combined population.

Feature/Icon	Function
	Choose a population using the drop-down menu.
	Inserts a population.
	Adds the AND operator.
	Adds the OR operator.
	Adds the NOT operator.
	Adds left or right brackets.
	Removes one operator.

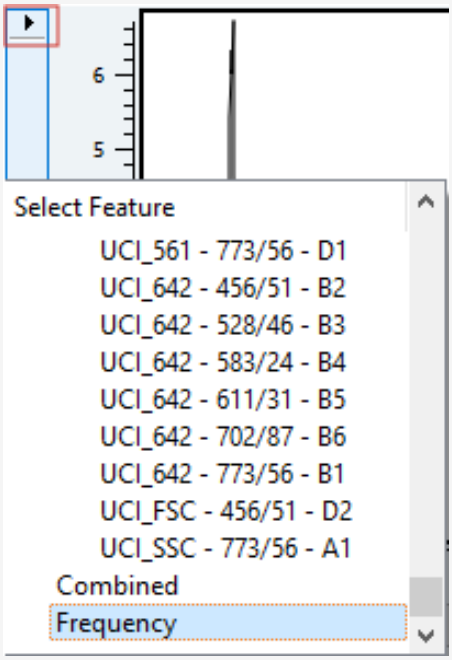
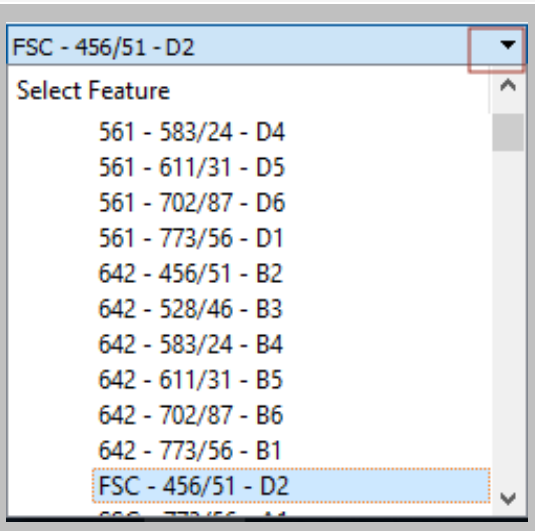
Editing options include:

Feature/Icon	Function
	Changes the population color.
	Field for specifying the population name.
	Specifies the population display style.
	Edits the population; deletes the population.

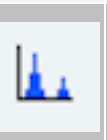
3. Click **Apply**, then **Save** to save any changes.



Analysis Workspace

Feature	Function
	Displays choices for the y axis in the drop-down menu.
	Displays choices for the x axis in the drop-down menu.
	Zooms in or out on the graph.
	Performs undo or redo of one previous action.
	Switches between a Linear or Log scale.
	Displays the graph statistics.

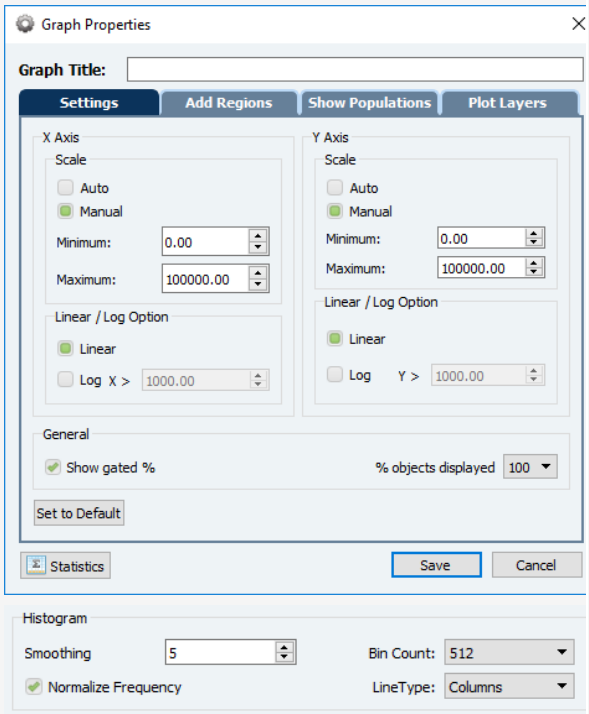
On the right side of the plots, several tools can be used to specify regions on a plot.

Dot Plot and Density Options		Histogram Options	
	Creates a rectangular region.		Creates a line region.
	Creates an elliptical region.		Creates a bisector region.
	Creates a polygonal region.		Switches between a column or line histogram.
	Creates a quadrant to make four regions.		Switches between a raw or smooth histogram.

Graph Properties

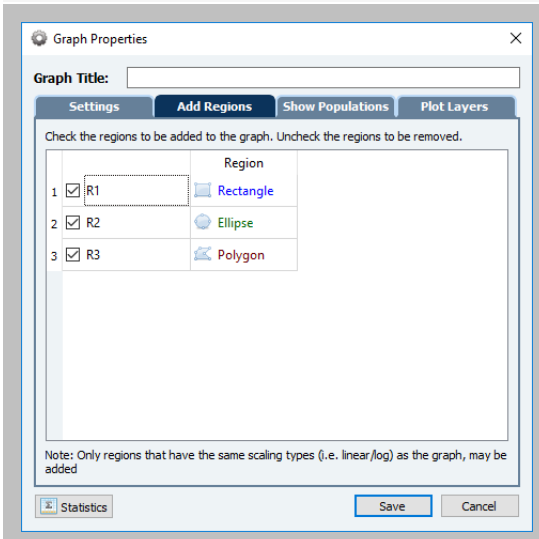
Within Graph Properties, plots can be edited for scaling and object display, regions displayed, populations displayed, and plot layers ordered.

Click the **Graph Properties** icon: 



Settings

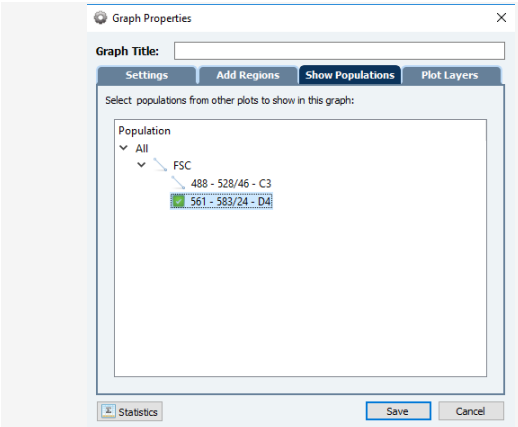
Adjust the scales of the X and Y Axes, Linear/Log options and show gated % and % of objects displayed.
The Settings for a histogram include options to change the scale of the smoothing, bin count, and line type.



Add Regions

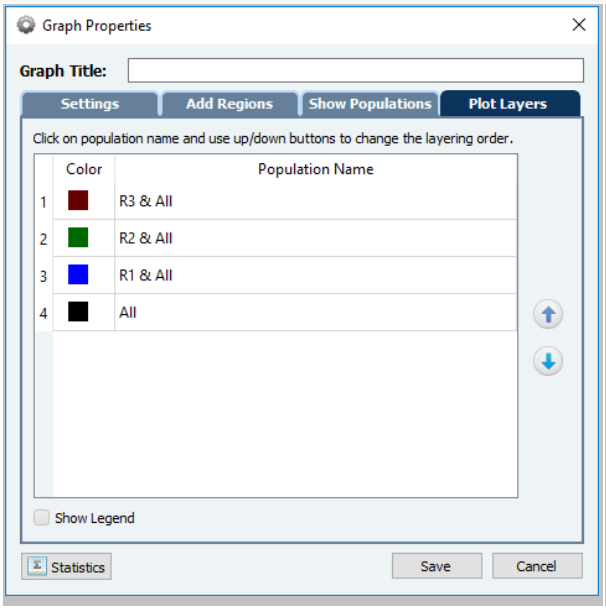
Add regions to or remove regions from the graph.

Only regions created with the same scaling can be added to graphs (i.e., linear regions cannot be added to log plots).



Show Populations

Show a population on a graph by clicking on the population. A green check box appears for displayed populations.



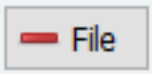
Plot Layers

Adjust the layering order of the populations and change their colors.

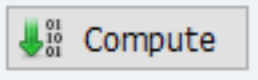
Compensation Panel

Click the Compensation icon at the top of the screen to bring up the Compensation panel.

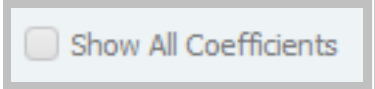
Adds a compensation file (.fcs).



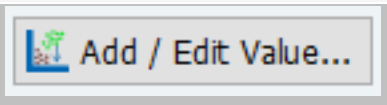
Removes a compensation file (.fcs).



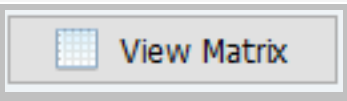
Computes compensation coefficient using all control files.



Displays all coefficients.

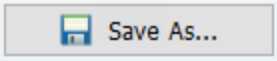


Adds a new coefficient to the compensation matrix.

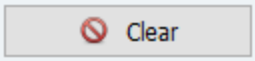


Views the matrix as a grid, instead of as a tree.

Imports an existing compensation matrix.



Saves the current compensation matrix.

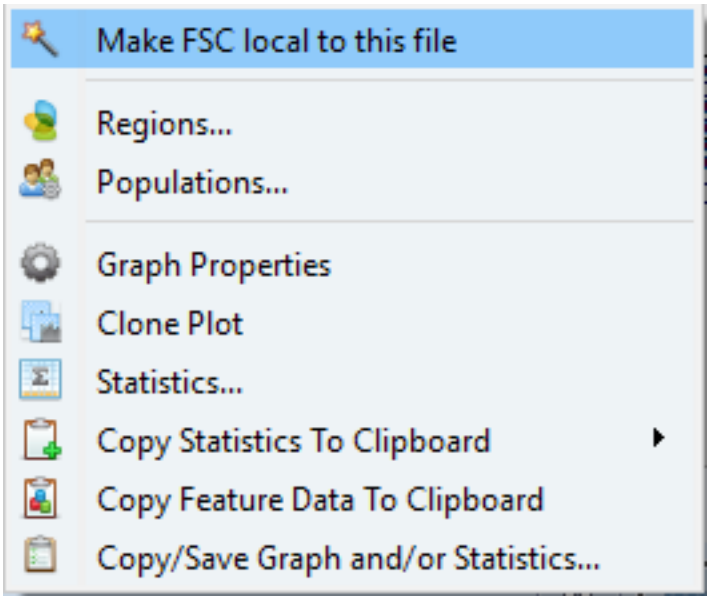


Clears all values from the matrix.

Multi-file Analysis Tools

Global and Local Regions

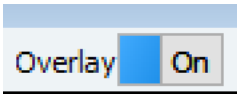
Regions are set to global as the default. To set a local region for an individual file, right-click on the region and select the option to make the region local to the file.



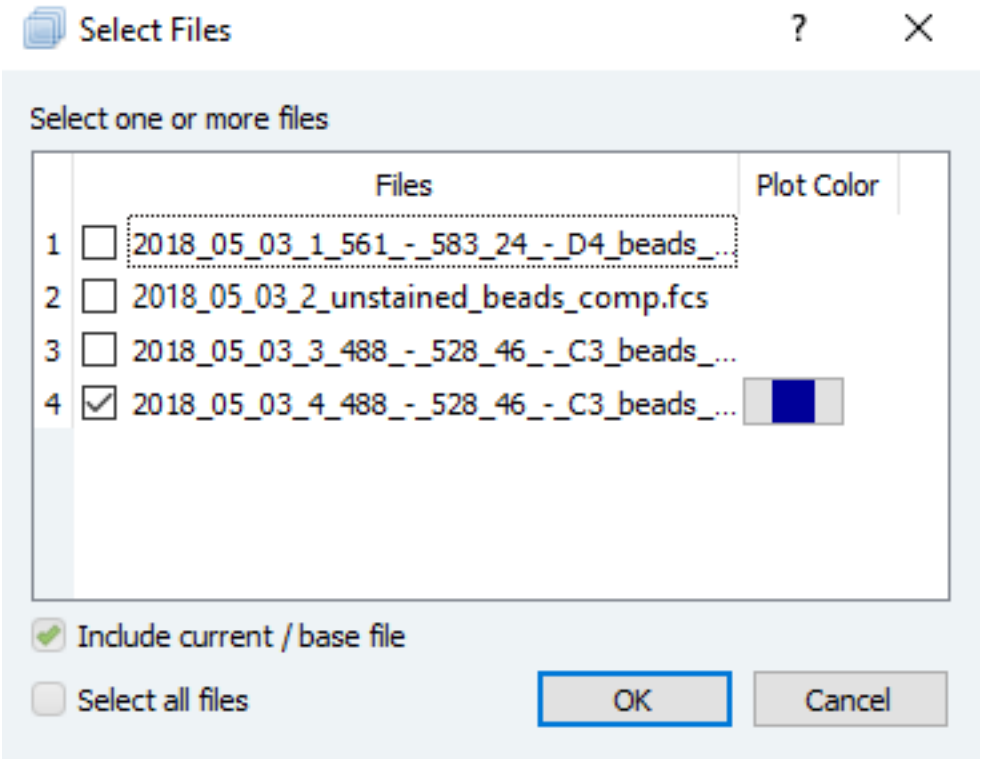
Plot Overlays

The overlay features allow the display of one or more samples on the same plot. This is available for both dot plots and histograms.

1. Next to **Overlay** in the graph or plot of acquired data, slide the button to **On**.

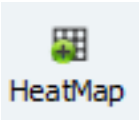


2. In the **Select Files** dialog box, choose the files to display on the graph or plot and the plot colors (optional) and click **OK**.



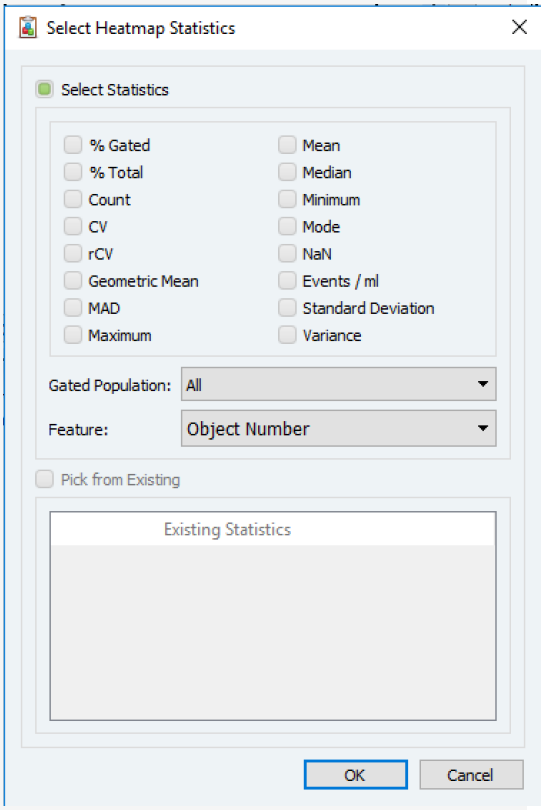
Create Heatmaps

1. To generate a heatmap for any statistic, click **HeatMap** from the row of icons at the top of the graph area.

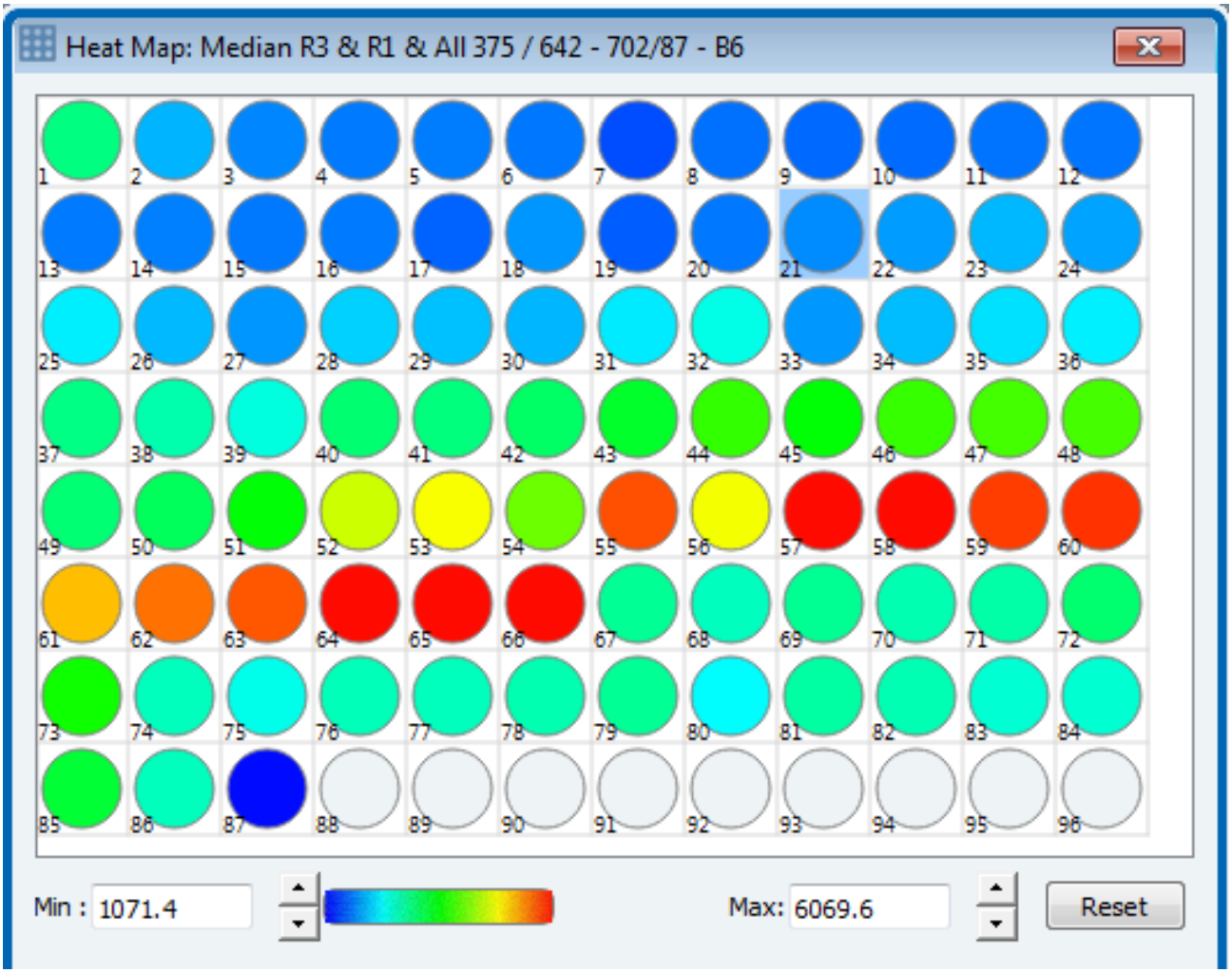


2. Select one statistic to display, choose a desired population from the **Gated Populations** drop-down menu and choose a Feature from the **Feature** drop-down menu.

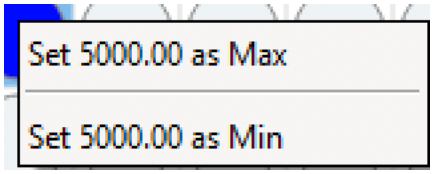
Alternatively, the **Pick from Existing** check box allows for the display of an existing statistic in the heatmap.



3. Click **OK** to display the heatmap in the analysis workspace. The heatmap will be displayed according to the selected settings.



4. Optional: Right-click on a well to set the Max or Min.

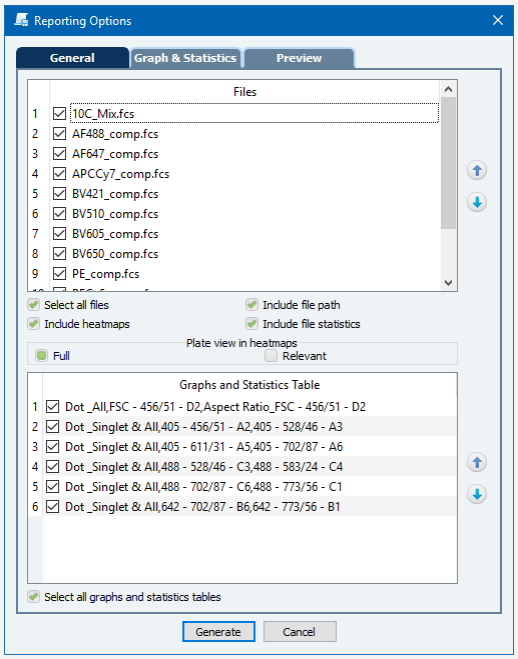


Export Data

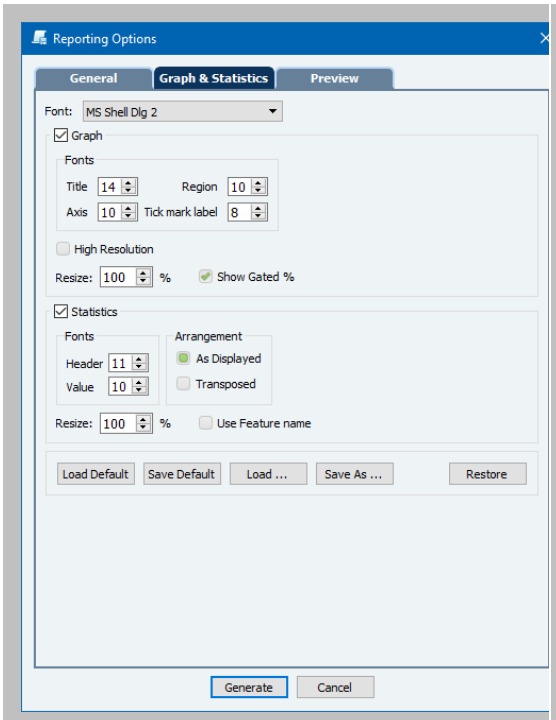
Generate Reports

CellStream® Analysis software offers several options for creating customized reports with plots and stastistics.

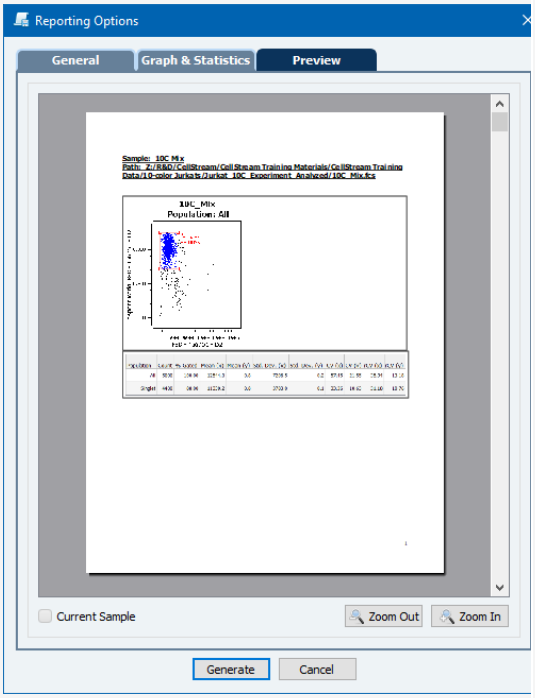
1. Click **Report** to view the Reporting Options.



On the **General** tab, select files to include, plots to include, and statistics to display.



On the **Graphs & Statistics** tab, the font sizes on all elements of graphs (titles, axes, tick marks, regions) and statistics tables can be modified. In addition, once selected, desired settings can be saved to a .ini file and applied to other experiments.

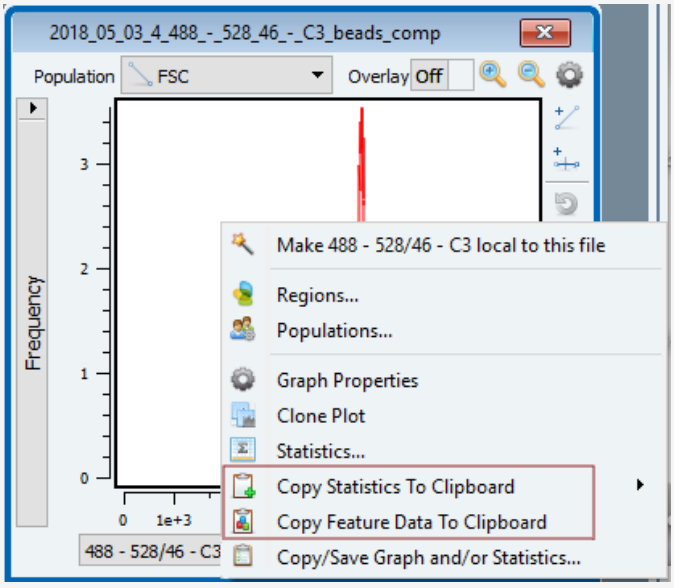


On the **Preview** tab, preview the report.

2. Click **Generate** to create the report. Save the report as a .pdf or .odt.

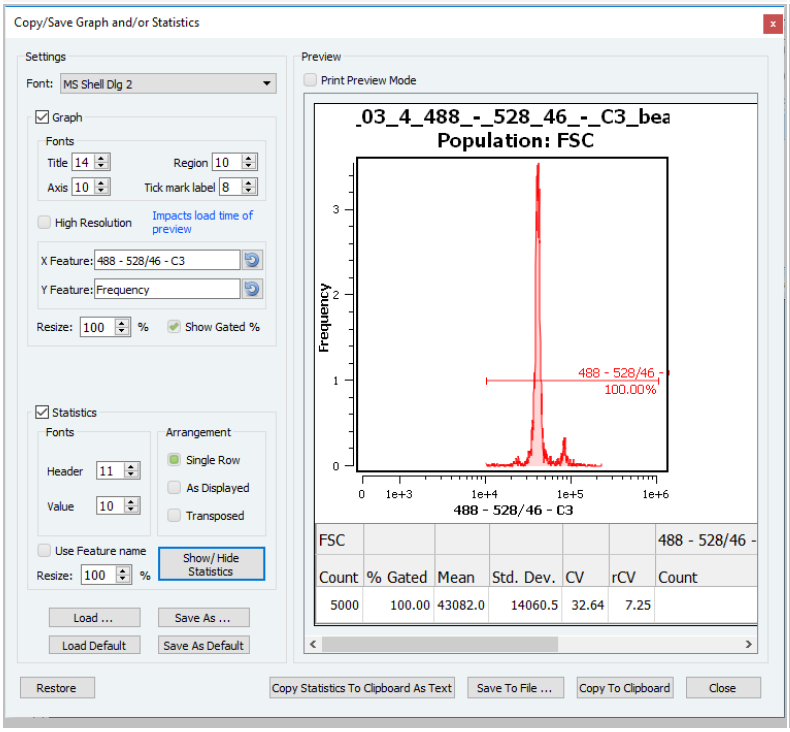
Export Statistics, Feature Data, or Graphs

Right-click on a plot to export data to the clipboard, an image file, or a .pdf. The options related to exporting include:



Copy Statistics To Clipboard:
Copy the plot statistics to the clipboard.

Copy Feature Data To Clipboard:
Copies feature value for each event in the data file.



In the **Copy/Save Graph and/or Statistics** window, select the desired graph and statistics settings. The check boxes allow graphs and statistics to be displayed together or individually. A previously saved .ini file can be loaded or a .ini file can be created to save the current settings for future use. The graph or statistics can then be copied to the clipboard or saved to a file (.pdf, .bmp, .jpeg, .jpg, .png, .ppm).

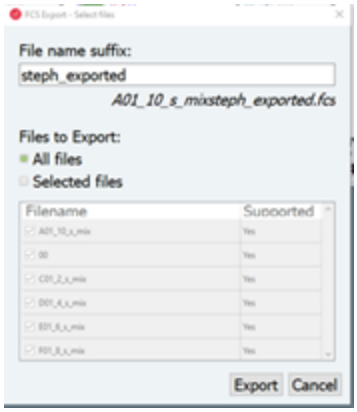
Export Reduced Feature Set for Third Party Software Analysis

To analyze data in a third party software program without the morphological feature, use the Export function to export a reduced feature set. The reduced feature set includes the following features:

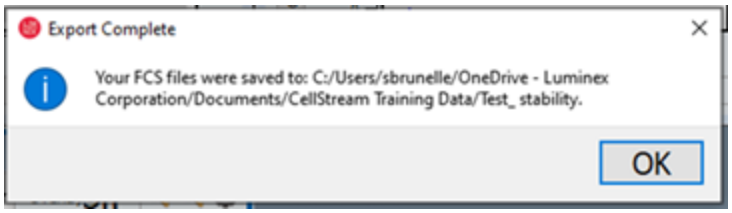
- Aspect ratio of FSC
- Aspect ratio of SSC
- Uncompensated intensity
- Compensated intensity

This minimal feature set will be recognized by other software programs that do not use morphological data.

1. From the CellStream® Analysis program, select **File** > **Export FCS files...**
2. Rename the files to be exported.
3. Choose **All Files** to export all files, or **Selected Files** to export only the files you select.

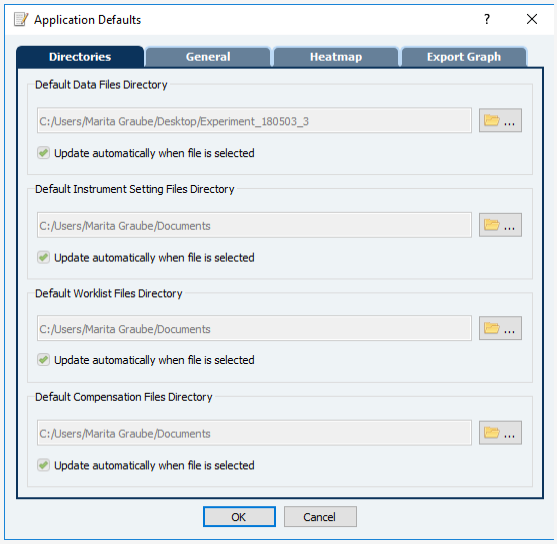


4. Confirm the files you want to export are supported by looking in the right column of the **FCS Export** window.
5. Click **Export**.
6. The **Select a Folder** dialog box appears. Select the path to export the FCS files.
7. The **Export Complete** box appears if the export completed successfully. The **Export Complete** window also displays the path of the FCS files. Click **OK** to continue.



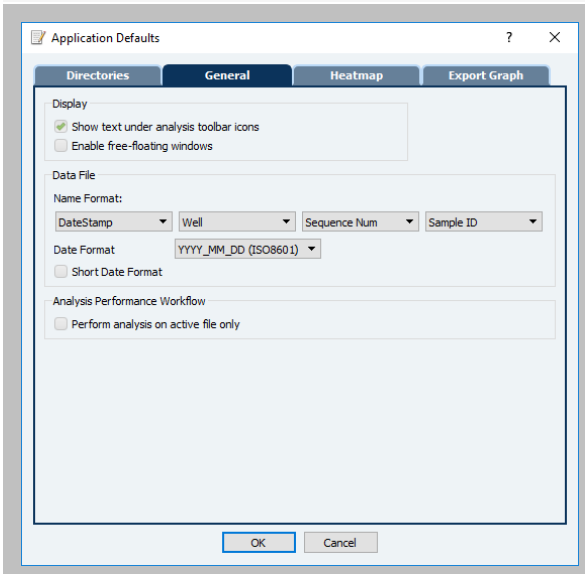
Set Analysis Application Defaults

Under Options > Application Defaults, there are several system settings that can be adjusted in the CellStream® Analysis software.



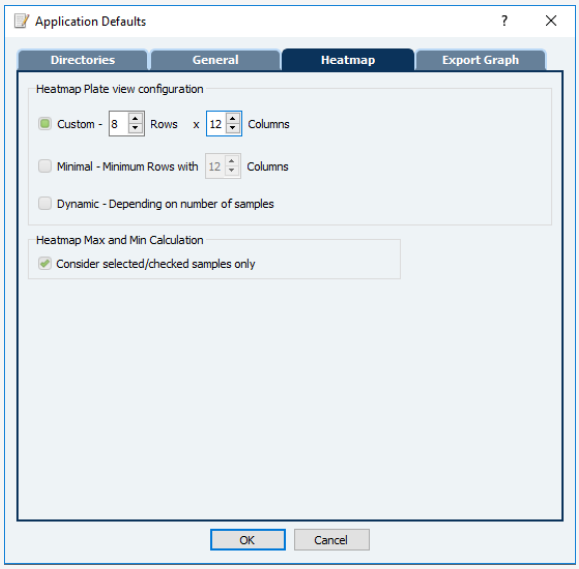
On the **Directories** tab, the following settings can be adjusted:

- Default Data Files Directory
- Default Instrument Setting Files Directory
- Default Worklist Files Directory
- Default Compensation Files Directory



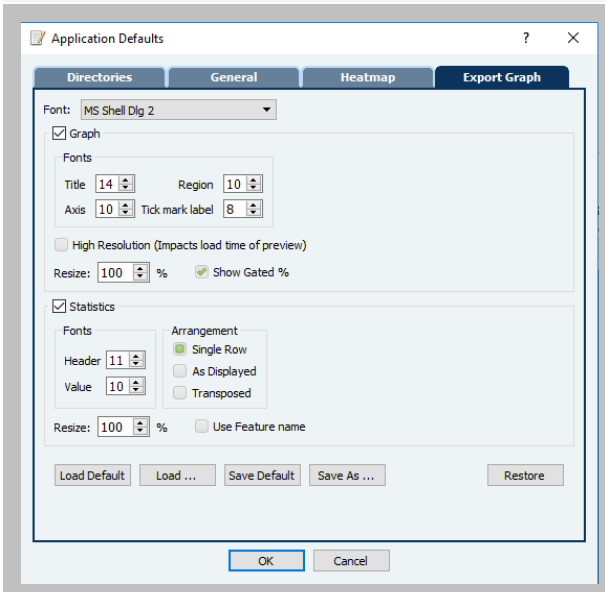
On the **General** tab, the following settings can be adjusted:

- Show text under analysis toolbar icons
- Enable free-floating windows
- Name Format and Date Format
- Perform analysis on active file only



On the **Heatmap** tab, the following settings can be adjusted:

- Select default heatmap display options
- Display heatmap information for selected samples only



On the **Export Graph** tab, the following settings can be adjusted:

- Font size for graph labels
- Resolution and size of the graph
- Statistics display (including font size and arrangement)
- Options to save settings as a default, as a .ini file, or load a previously saved .ini settings file.

Chapter 9: Maintaining the System

General Maintenance Precautions

	Observe the following general maintenance precautions.
	Personnel who use, maintain, or clean the CellStream® system should be trained in standard laboratory safety practices and should follow those practices when handling the instrument.
	Samples and waste fluid can contain biohazardous material. Where exposure to biohazardous material, including in an aerosol form, exists, follow appropriate biosafety procedures, use personal protective equipment, and use ventilation devices.
	Avoid contact with moving parts. Disconnect the instrument from the power source when the procedure instructs you to do so.
	The movement of mechanical parts within the instrument can cause injury to fingers and hands. Access to moving parts under the hood of the CellStream system is intended only for Luminex service personnel.
	Using controls or making adjustments other than those specified in this manual can result in hazardous exposure to laser radiation, in exposure to biohazards, or in injury from the mechanical or electrical components.

Clean the Exterior of the System

Clean spills on the instrument with a mild detergent. Using gloves, clean the sample portal and sample elevator with 10% bleach. Dispose of waste using proper precautions and in accordance with local regulations.

NOTE: 10% bleach is defined as 0.6-0.8% sodium hypochlorite.

Preventative Maintenance

The CellStream® system contains no serviceable parts. Only Luminex-trained technicians are authorized to align the laser beams or otherwise repair or maintain the instrument. The instrument's fluidic system should be cleaned after each day's use. Tubing and valves are replaced by Luminex service personnel as part of a routine preventive maintenance schedule.

12-Month Preventative Maintenance

12-month preventative maintenance is scheduled and performed by a Luminex Technician only if a service contract has been purchased.

If there is any problem with the instrument or instance that requires immediate repair or service, please contact Luminex Technical Support.

Replace the Fuse

The AC fuse is located on the rear panel above the AC power cord connector.

Turn off the main power switch at the back of the instrument and disconnect the power cord before replacing fuses.

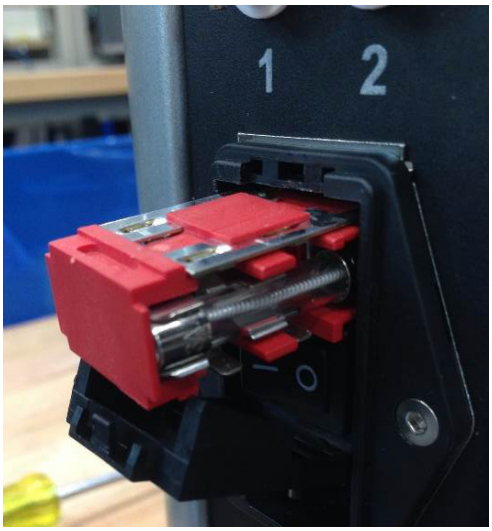
CellStream® system Fuse Rating: 250 VAC 1.5 A Time Lag, 5x20 mm Glass Cartridge

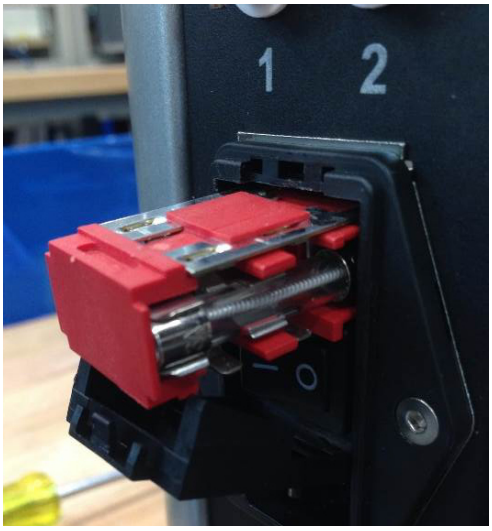
Only use fuses that comply to the specified rating.

1. Remove the fuse holder cover using a small screwdriver and pivot to expose the fuse carrier.


1
2

2. Pull out the fuse carrier using a flat screwdriver.





3. Carefully remove both of the fuses and replace with new fuses. Use the same type and rating as installed in your system.

4. Insert the fuse carrier back into the fuse holder. The prongs of the fuse holder will face the inside of the fuse holder.

5. Replace the fuse holder cover.
6. Reconnect the power cord and turn on the main power switch.

Chapter 10: Troubleshooting

Unstable Fluidics or Sample Fails to Load

Possible Causes	Recommended Solutions
Air bubbles in the sample	Make sure a sufficient sample volume is loaded, 50 µl is recommended. Detergents and foaming agents (such as fetal bovine serum) can cause bubbles to form in the lines. Avoid using detergents or buffers during sample prep if possible.
Air bubbles in fluid lines	Run the initialize script. Load calibration beads and verify the system runs normally.
Clog in fluid lines	Filter the sample with a 70 µm nylon cell strainer. Run the clean script, followed by the initialize script. Load calibration beads and verify the system runs normally.
96-well plate pierceable plate cover is not properly placed	Verify the recommended pierceable plate cover is being used (X-Pierce™ film) and that the wells of the plate are carefully aligned with the wells drawn on to the plate film.
Sample is too concentrated	Clumpy and viscous samples cause cavitation in the fluidic lines and create bubbles. Dilute the sample to at least $1x10^7$ cells/mL and, if necessary, filter the cells through a 70 µm nylon mesh.

Sample Synchronization Fails After Load

Possible Causes	Recommended Solutions
FSC (forward scatter) and SSC (side scatter) illumination is too low to measure signal (e.g., for small particle detection applications)	Increase FSC and SSC illumination powers.
Too few objects present to perform FSC/SSC synchronization.	Increase the concentration of the sample or spike in beads to allow for synchronization of extremely clean samples.
Illumination not enabled	Verify SSC and FSC illumination is on and check that objects are visible in the event gallery. Load calibration bead sample to verify system performance.
Inappropriate sheath solution	Verify the sheath is dPBS. Third party sheath buffers cannot be used on the CellStream® system.

Cross-contamination from Previous Samples

Possible Causes	Recommended Solutions
CellStream® Calibration beads are appearing in current sample	Load a sample containing 0.1% Triton™-X in deionized water (DI) water to wash residual CellStream Calibration beads from the sample line.
DNA dye from previous sample is labeling current sample	DNA dyes must be thoroughly flushed from the sample lines, to prevent residual dye from labeling subsequent samples. Load a sample of 0.4% to 0.7% hypochlorite (sterilizer) followed by a phosphate buffered saline (PBS) wash, to remove all traces of the DNA dye in the instrument, or run the clean script (~30 min).
Cells from the previous sample are appearing in current sample	This suggests a minor clog. Load a sample of 10% bleach followed by a PBS wash to remove most contaminating cells, or run the clean script (~30 min). **NOTE:** 10% bleach is defined as 0.6-0.8% sodium hypochlorite. Using 0.22um filtered bleach will prevent particulate detection by the system.

Advanced Cleaning (in case of a clog or extensive debris)

1. Make a solution of 10% bleach in clean deionized (DI) water.

   NOTE: 10% bleach is defined as 0.6-0.8% sodium hypochlorite. Using 0.22um filtered bleach will prevent particulate detection by the system.

2. Heat the 10% bleach/DI water solution to 100°C.

   NOTE: Microwave 1 mL of the solution for 1 minute.

3. Pipette 200 µL of the bleach/DI water solution into an Eppendorf tube and load the sample.
4. After running the 10% bleach/DI water solution, run a clean DI water sample and check for clogging or debris.
5. If debris is present, run another 10% bleach/DI water mixture.
6. Repeat the 10% bleach/DI water cycle until no debris is present.

Advanced Cleaning of the System Bottles

When contamination is a problem and does not clear with running hot bleach as a sample through the system, cleaning the system bottles might be necessary. Cleaning the bottles may be needed if the laboratory where the CellStream® system is located has an overall contamination issue (including contamination such as mold in the HVAC system of the building). The system is highly sensitive and is able to detect foreign objects in the system bottles (i.e., debris and contaminants). Luminex recommends using filtered sheath fluid, 0.1 µm for Small Particle Detection (SPD) and 0.2 µm for normal mode.

1. Open the two lower front doors of the system.
2. Disconnect the waste and sheath bottles from their tubing and remove the bottles from the system.
3. Unscrew the Rinse, Sterilizer, Cleanser, and Debubbler bottles from their caps and remove the bottles from the system (caps stay attached to fluidics lines).
4. Dump all liquid from the six bottles according to biosafety and environmental practices.
5. Fill each bottle to ~95% capacity with 10% bleach and cap tightly.

   NOTE: 10% bleach is defined as 0.6-0.8% sodium hypochlorite. Using 0.22um filtered bleach will prevent particulate detection by the system.

6. Incubate all bottles with the bleach solution for 10 minutes. Shake or invert the waste and sheath bottles every couple of minutes. Gentle shake the four attached bottles every couple of minutes.
7. Initialize the system.
8. After Initialization is complete, discard the bleach solution from the bottles.
9. Rinse all bottle with ultrapure deionized water (0.22 µm filtered) three times.
10. Refill the sheath bottle with the recommended sheath fluid, cap, and place back into the CellStream system. Reattach the tubing connections.
11. Cap and place the empty waste bottle back into the CellStream instrument. Reattach the tubing connections.

12. Fill the Sterilizer, Cleanser, Debubbler, and Rinse bottles with the appropriate fluids and place back into the CellStream instrument.
    - Sterilizer bottle with 10% bleach

      NOTE: 10% bleach is defined as 0.6% sodium hypochlorite.
    - Cleanser bottle with Coulter Clenz®
    - Debubbler bottle with 70% isopropanol
    - Rinse bottle with deionized water (at least 0.22 µm filtered)
13. Attach fluidic lines and tighten caps.
14. Click **Clean** to run the Clean Procedure.
15. Initialize the system.
16. Run multiple water samples (3 to 5) to determine if contamination is still present.
17. If no contamination is present, load a bead sample and calibrate the system.
18. Run samples as usual.

Erroneous Fluid Level Indicator

Possible Causes	Recommended Solutions
Tank has moved away from the sensor	Open the buffer compartment and move the tank closer to the sensor until the fluid level indicator is correct.

Instrument Will Not Pass Calibration

Symptoms	Possible causes	Recommended Solutions
If multiple tests fail	Calibration beads are not running properly	The beads must be running >500 events per second, and without significant clumping. If the beads are diluted or clumped, try running a fresh tube of beads and re-run calibration.
Laser power test continues to fail after previous troubleshooting.	CellStream® Calibration Reagent has not been stored properly (proper storage condition is 4°C to 8°C, protected from light).	Repeat calibration with CellStream Calibration Reagent that has been properly stored.
One or a few tests fail	-	Select individual tests and rerun.

Software Issues

Symptoms	Possible causes	Recommended Solutions
Instrument software fails to start	Instrument power not turned on	Verify blue light in sample load portal is illuminated. Toggle power switch on back-left corner of instrument.
	Computer running Linux not on or not fully booted	Verify the power is on the computer running Linux. Wait for computer to boot. If this fails, press power button on computer running Linux and wait for the computer to shut down. Press power button to restart the computer running Linux and wait for computer to reboot.
Software fails to start after previous troubleshooting steps	-	Shutdown the computer running the Windows® platform. Turn off power to instrument. Press power button to shutdown the computer running Linux. Restart the computer running Linux, the computer running the Windows platform and turn on power to instrument. Start the instrument software.

Appendix A:

Install the CellStream® System

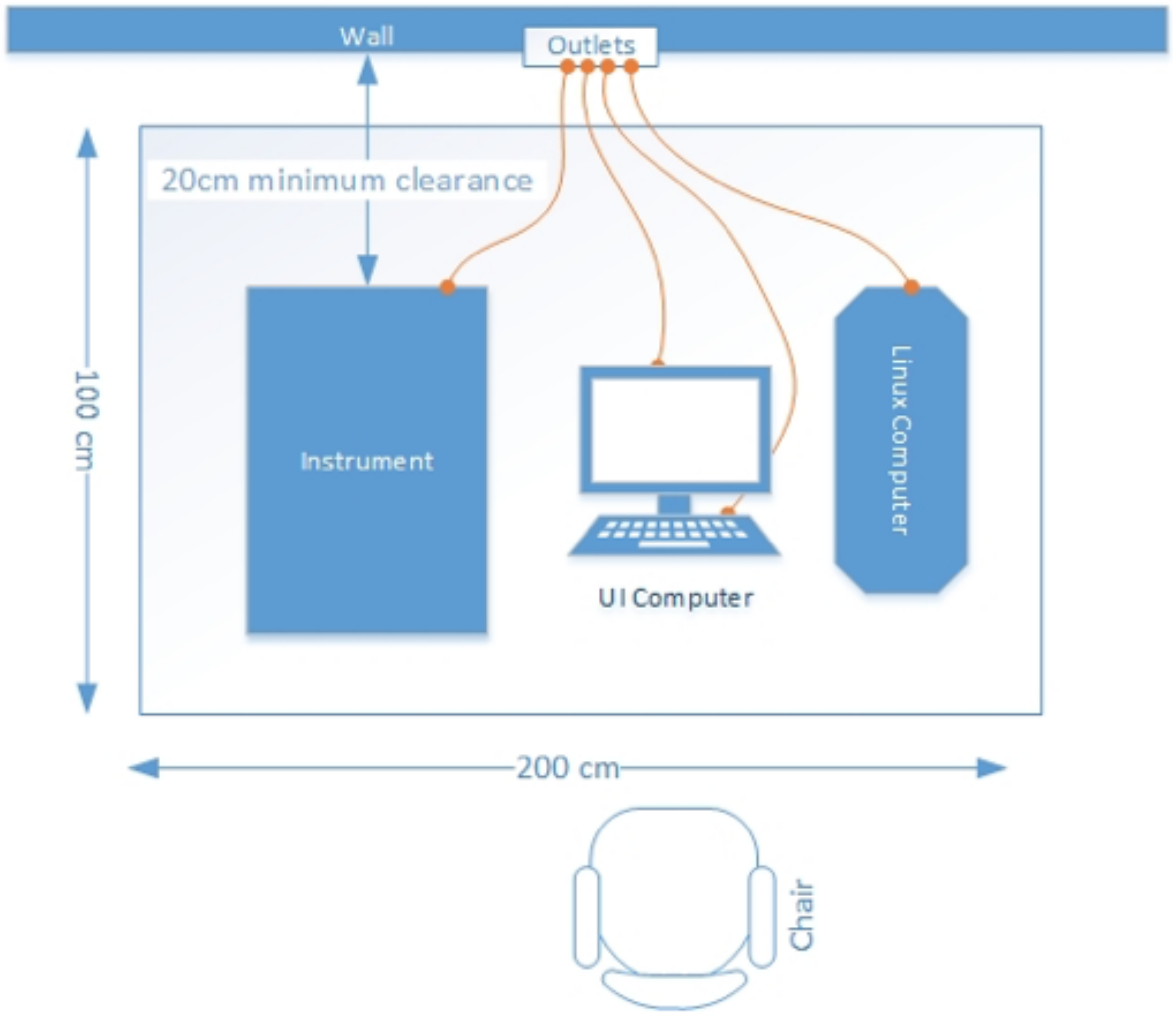
NOTE: Only Luminex authorized personnel can install the CellStream® system. Contact Luminex Technical Support.

The CellStream® system weighs approximately 48 kg (105 lbs) and should be installed on a sturdy table or lab bench so that all 4 feet of the instrument are securely positioned. The table or bench must be capable of supporting at least 136 kg (300 lbs). The area of operation should be clean, dry, level, and free of persistent vibrations. The ideal table dimensions are approximately 100 cm in depth by 200 cm wide in order to accommodate the space required for the instrument, monitor, and computers.

	Do not place instrument in an unstable position. Do not install it in a location where it might fall, even if the surface is level.
	Do not block the vents. Inadequate ventilation can result in overheating possibly causing damage to the instrument. The instrument must be placed at least 20 cm (7.87 in) away from a wall or any obstruction to allow adequate ventilation and sufficient space for the power cord to be connected without being stressed.
	Do not place anything on top of the instrument. Clearance of at least 50 cm (19.685 in) should be provided above the instrument to permit the instrument to be opened freely in case of servicing.

Access to 100 to 240 50/60 Hz properly grounded electrical outlets is required and Luminex recommends that the instrument is connected to a surge protector. These electrical outlets should be directly behind the table or bench that the instrument is placed on. The CellStream system must be connected to a properly grounded electrical outlet to protect against electric shock. The instrument can be connected to a 300+ Watt uninterruptible power supply, but it is not required.

When plugging into mains, ensure there is sufficient slack in the power cables so the cables are not stressed. Place the instrument table/bench near the outlets or secure power cables to ground with gaffer tape so the power cables do not pose a trip hazard. Refer to the diagram below for instrument placement and how to connect instrument to electrical supply mains:

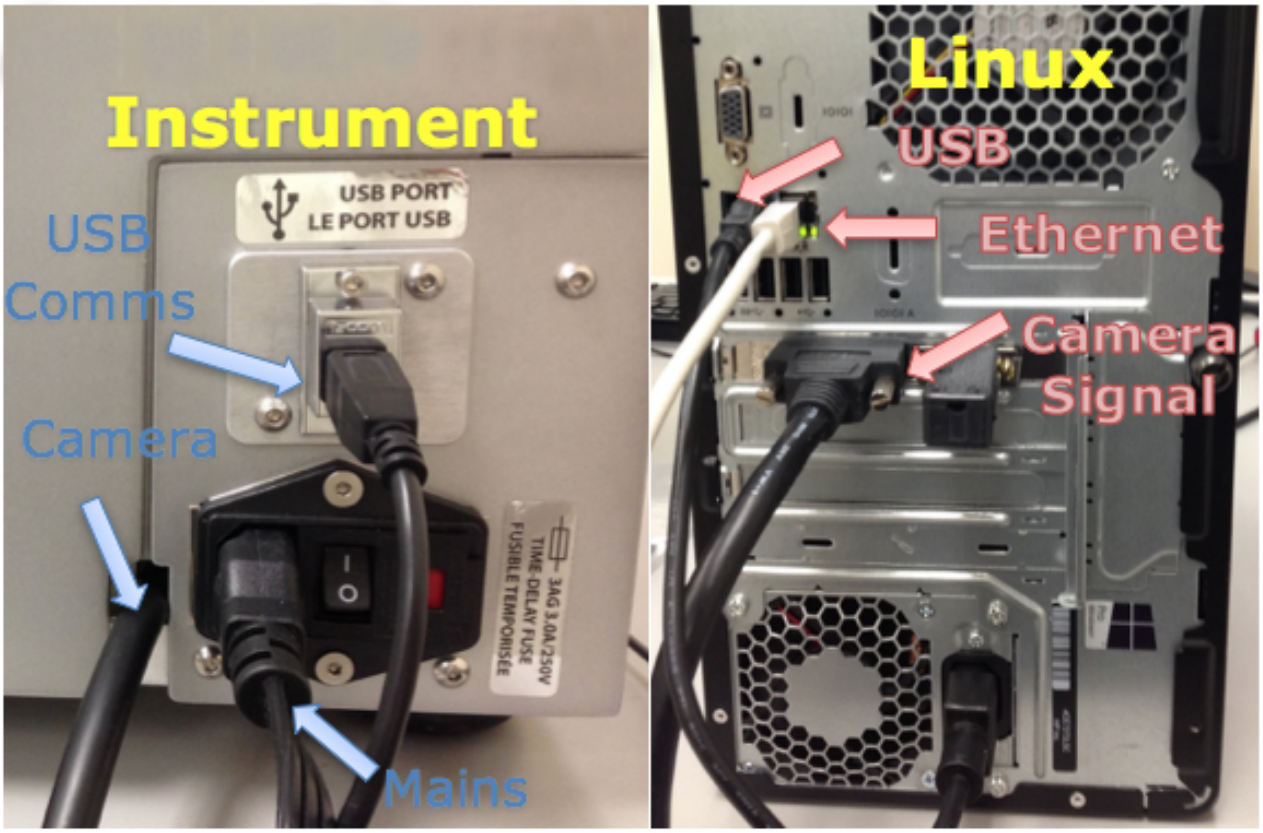


Instrument Connections

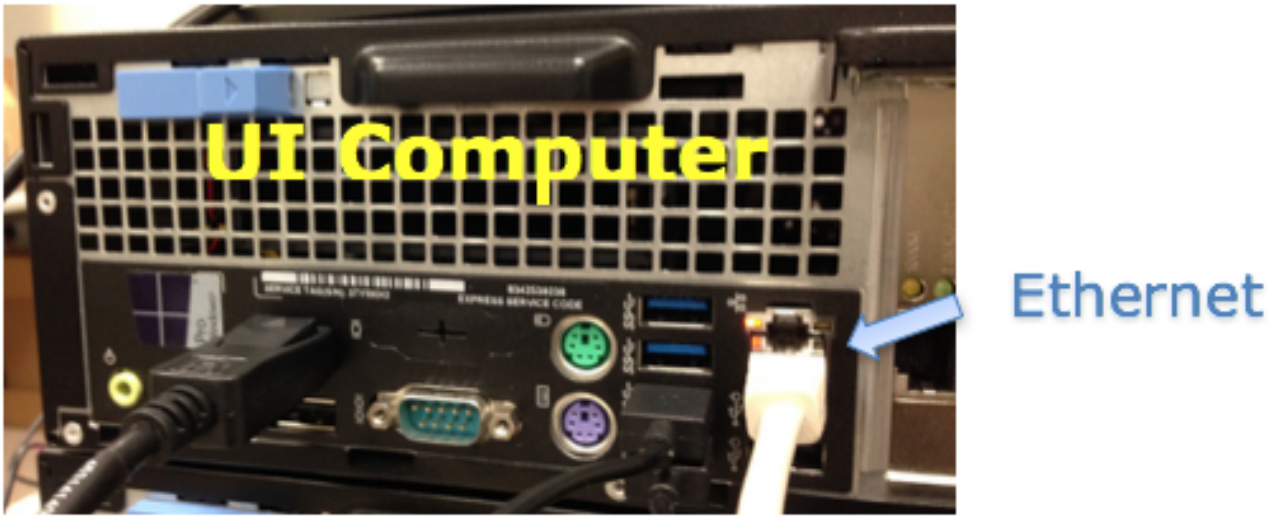
The CellStream system has two communications ports located at the back panel:

- The Camera Signal cable
- The USB Comms cable

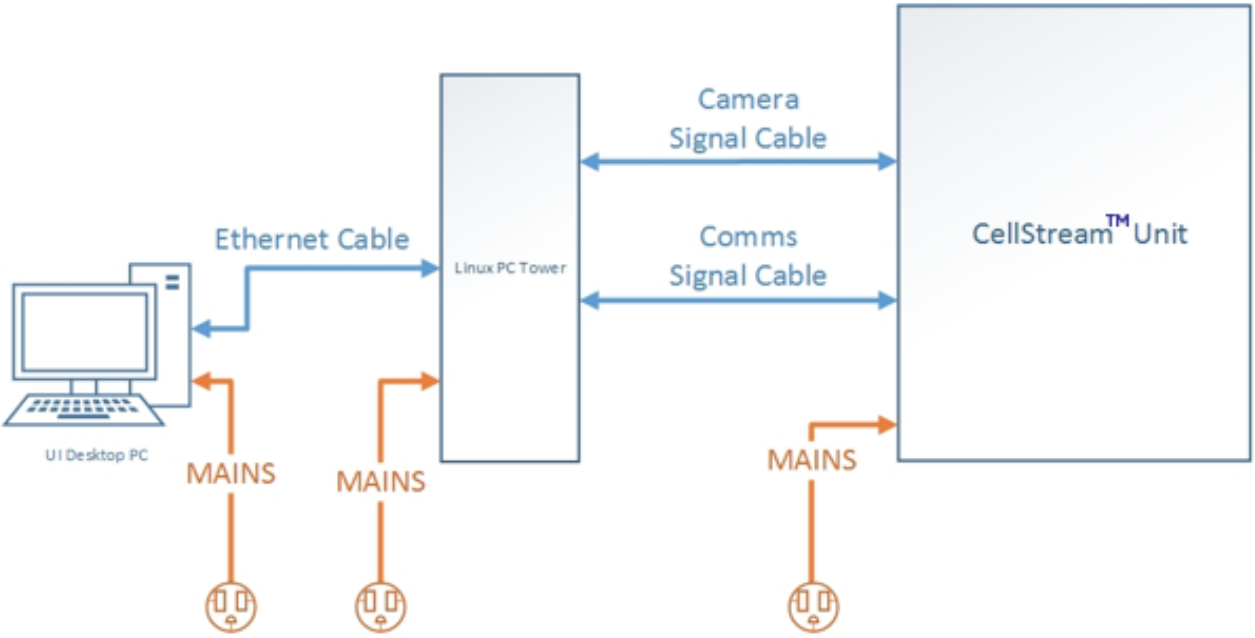
The Camera Signal cable and the USB Comms cable must be connected from the back of the CellStream system to the back of the Linux Computer for proper instrument function. These cables must be properly plugged in and anchored with accompanying screws. Ensure the Linux Computer and UI Computer is placed near the CellStream system to allow communication cables sufficient slack and to avoid stressing the cables.



Additionally, the Linux Computer is connected to the UI Computer via an Ethernet cable.



Refer to the diagram below for CellStream system connections.


Camera
Signal Cable
Ethernet Cable
Linux PC Tower
Comms
Signal Cable
CellStream™ Unit
UI Desktop PC
MAINS
MAINS
MAINS

Appendix B:

List of Features for each option

Traditional Flow Data Only (Minimal):

- Area of FSC
- Area of SSC
- Aspect Ratio of FSC
- Aspect Ratio of SSC
- Object Number
- Compensated Intensity
- Uncompensated Intensity

Basic imaging and Traditional flow data (User):

- Area (all channels)
- Aspect Ratio (all channels)
- Object Number
- Compensated Intensity
- Uncompensated Intensity
- Time
- Raw Maximum Pixel
- Raw Centroid X

All imaging and traditional flow data (All):

- Area (all channels)
- Aspect Ratio (all channels)
- Object Number
- Compensated Intensity
- Uncompensated Intensity
- Time
- Raw Maximum Pixel
- Raw Centroid X
- Camera Time Stamp
- Camera Line Number
- Raw Centroid Y
- FlowSpeed
- Y Centroid
- Raw Minimum Pixel

- Mean Pixel
- Background Mean
- Background Standard Deviation
- Gradient RMS
- Major Axis
- Minor Axis
- Saturation Count
- Saturation Percent

Disclaimers

The screen shots presented in this manual may vary in appearance from those on your computer, depending on your display settings.

The CellStream® system is for research use only and not for use in diagnostic procedures.