

**Luminex®**

complexity simplified.

# Guava® easyCyte™ HT System User Guide

For Use with GuavaSoft Software 4.0



EC REP

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

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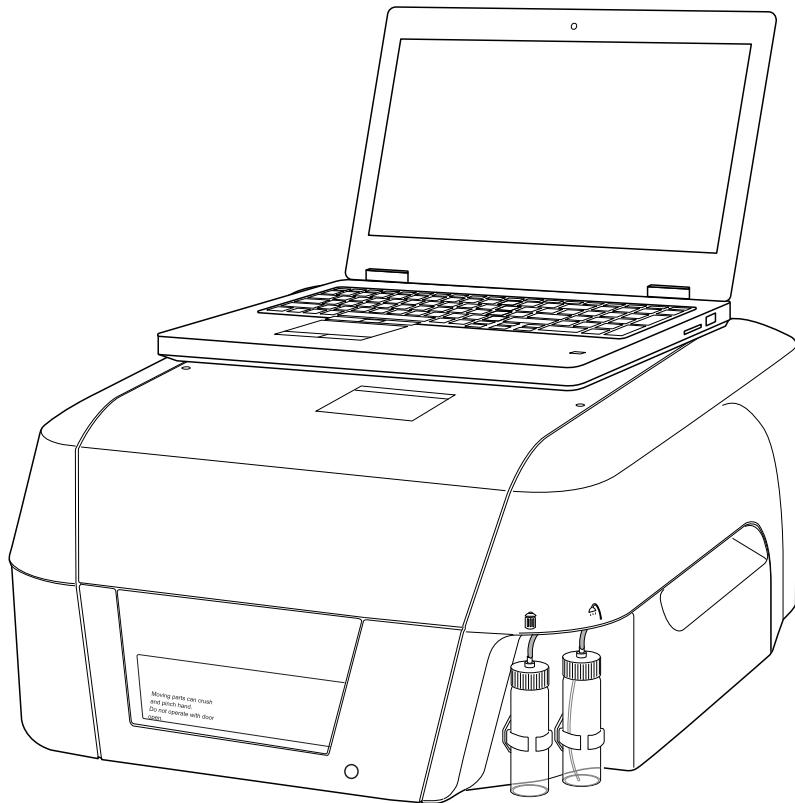
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# Chapter 1: System and Software Introduction

## Introduction

The Guava® easyCyte™ HT System streamlines drug discovery and cell culture monitoring and screening by providing turnkey assays for a wide range of single-cell-based applications. The system includes the Guava easyCyte Instrument, a laptop computer with pre-installed software for data acquisition and analysis, and optimized reagents and protocols.

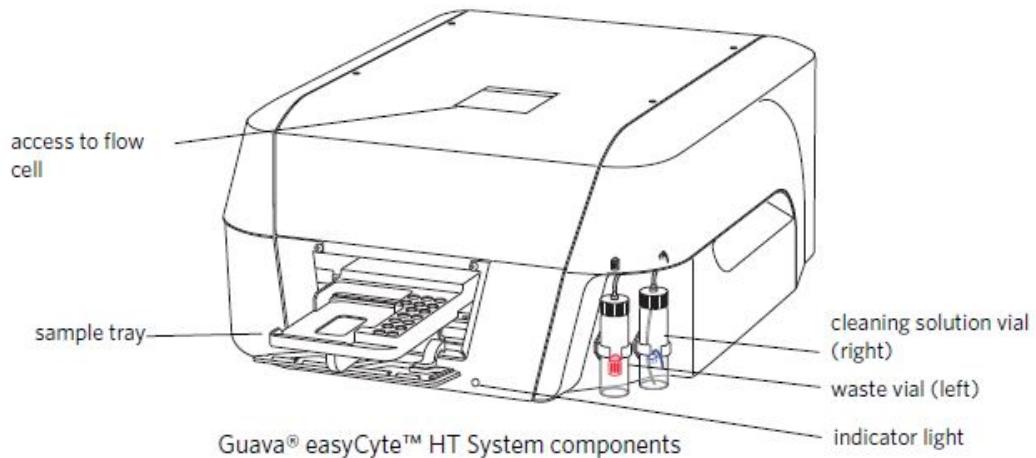
GuavaSoft Software includes dedicated modules for cell- and bead-based assays, minimizing training requirements. In addition, the software includes the Guava easyCheck™ module, which verifies that the system is performing optimally, and the Guava Clean module allows you to run an automated cleaning procedure prior to shutdown.



GuavaSoft Software automatically saves data files, which can be recalled later for offline analysis. In addition, results are exported to a spreadsheet file.

# Instrument Overview

The Guava® easyCyte™ HT System was designed for easy operation and minimal maintenance. The power switch, sample tray, and waste and cleaning solution vials are the only instrument components that you will routinely handle, and most of your interaction is through the software via the laptop. The power switch is located on the back-right side of the instrument.



# Software Overview

Use GuavaSoft Software for the acquisition and analysis of data.

GuavaSoft Software is used to set up and maintain the system, offers a number of assay-specific acquisition and analysis modules, depending on your applications.

The Guava® InCyte™ module was developed to be an open assay module providing all the tools for instrument setup, sample acquisition, and data analysis. The Guava InCyte module allows you to acquire and analyze up to 12 fluorescence parameters in combination with forward scatter (FSC) and side scatter (SSC), as well as area, width, and time. It provides automated compensation and an instant update feature, making it easier to perform complex analysis.

The Guava easyCyte™ HT is configured to detect fluorochromes or fluorochromes with similar fluorescence. For example, Blue- Violet (BLU-V) is the filter for the blue fluorescence emission off the violet laser.

# Consumables Overview

The Guava® easyCyte™ HT System requires three kinds of reagents:

- Instrument maintenance reagents
- Pre-optimized reagents/reagent kits for Luminex instruments
- General flow cytometry reagents

# Luminex Technical Support

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

Contact outside the U.S. and Canada by calling: +1 512-381-4397

International: + 800-2939-4959

Fax: 512-219-5114

Email: [support@luminexcorp.com](mailto: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

Symbol	Meaning	Symbol	Meaning
<b>RUO</b>	For Research Use Only. Not for use in diagnostic procedures.		WEEE Symbol
<b>CE</b>	Conformite Europeenne (EU CE Marking of Conformity) CE conformity marking		Manufacturer
<b>EAC</b>	EurAsian Conformity (EAC) Mark		CSA mark
	Hand Crush / Force From Above		

# Chapter 2: Regulatory and Safety Considerations

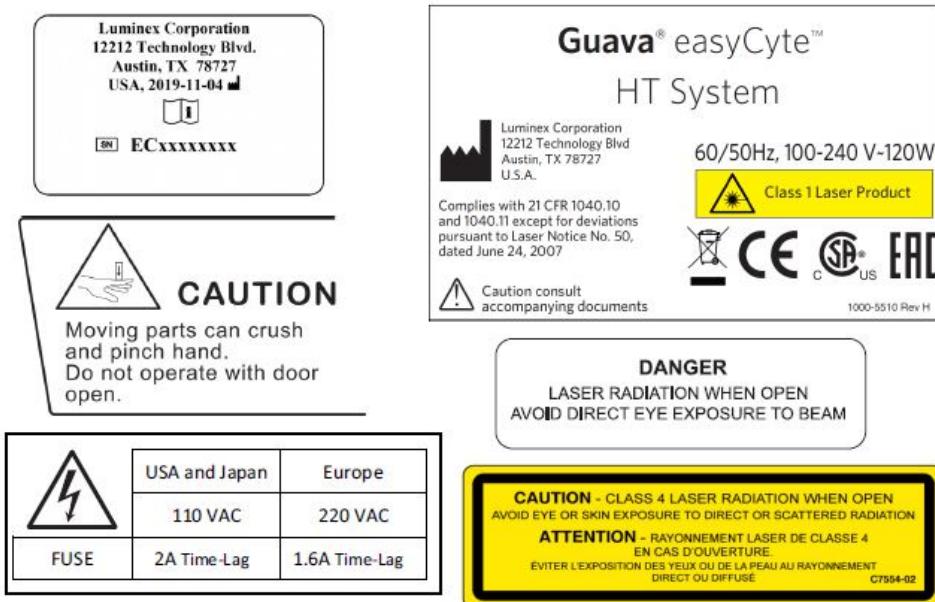
The Guava® easyCyte™ HT System is equipped with safety features for your protection. Use the system only as directed in this user guide. The interior of the instrument contains potential electrical, mechanical, and laser radiation risks. For this reason, access to the interior of the instrument is possible only with a tool and is limited to Luminex trained service personnel. There are no user serviceable or accessible parts in the interior of the instrument necessary for operation. If the user believes the instrument is exhibiting anomalous behavior, do not open the instrument, contact Luminex Technical Support.

## Safety Labels

Do not perform instrument maintenance or service except as specifically stated. Please read the following safety information before using the system.

The following are examples of the labels affixed to the Guava® easyCyte™ HT instrument.

**NOTE:** The following caution label is visible when the flowcell access panel is removed. The panel is to be removed when the system is powered off. Even when powered on, the accessible laser radiation falls within Class I limits.



# Warnings and Precautions

## 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.

The use of tubes other than those specified may result in damage to the instrument.

Do not run any other programs, including Internet Explorer®, on the laptop while using GuavaSoft Software to acquire data. GuavaSoft Software requires the full resources of your laptop during data acquisition. Running other programs during a run may interfere with acquisition or interrupt the run.

The results of the assays are dependent upon the proper use of the reagents and instrument. Refer to the appropriate reagent kit user guide for specific instructions and limitations.

## Electrical Safety

The instrument must be connected per the instructions in the user guide. The power conditioner is required to meet electrical compliance.



Before changing a fuse, turn off the instrument and unplug the power cord to avoid any danger of electrical shock.

## Biological Safety

All biological specimens and materials that come into contact with biological specimens can transmit potentially fatal disease. To prevent exposure to biohazardous agents, follow these guidelines:



Where exposure to potentially biohazardous material, including aerosol, exists, follow appropriate biosafety procedures and use personal protective equipment (PPE). PPE includes gloves, gowns, laboratory 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 biohazardous waste material.

Dispose of waste in accordance with federal, state, and local regulations.

## Laser Safety

The Guava® easyCyte™ HT System is classified under FDA 21 CFR 1040.10 and 1040.11 as a Class 1 laser product. The Guava easyCyte HT complies with IEC 60825-1:2014 and 21 CFR 1040.10 and 1040.11 except for deviations pursuant to Laser Notice No. 50, dated June 24, 2007.

No laser radiation is accessible to the user during normal instrument operation.

The following lasers are used in the easyCyte HT System:

Laser	Wavelength (nm)	Maximum Power (mW)
Violet	405 ±5	110
Blue	488 ±5	160
Green	532 ±5	110
Red	641 ±6	110

**NOTE:** These are the maximum powers for each laser.

	To avoid exposure to laser radiation or electric shock do not open the instrument or attempt to perform any internal maintenance. There are no user-serviceable parts.
	Turn off all power to the system before attempting to remove the flowcell.
	Use of controls or adjustments or performance of procedures other than those specified herein can result in hazardous radiation exposure.

# Chapter 3: Performance Specifications and System Components

## Environmental Conditions

- Temperature: 16°C to 35°C (60°F to 86°F)
- Humidity: 10% to 90% relative humidity (non-condensing)
- Pollution degree: 2
- Altitude: 2000m
- Installation category: II

## System Specifications

### General System Specifications

- Power: 100-240 VAC, 50/60 Hz, 120W
- Fuse rating:
  - USA and Japan (110 V): 2.5A, 250 V Time-lag
  - Europe: (220-240 V): 1.6A, 250 V Time-lag (x2)
- Instrument size: 10 in (25 cm) H x 20 in (51 cm) W x 24 in (61 cm) D
- Instrument weight: 70 lb (31.8 kg)
- Laptop size: 11.5 in (29.2 cm) when open H x 14.2 in (36.1 cm) W x 10.4 in (26.4 cm) D
- Laptop weight: 8 lbs (3.6 kg)
- counting accuracy: ±10%
- counting precision: ≤10% CV

### Optics Specifications

- Lasers:
  - Violet laser (405 nm), 100 mW
  - Blue laser (488 nm), 50 mW or 150 mW

- Green laser (532 nm), 100 mW
- Red laser (642 nm), 100 mW
- Forward scatter detector: photodiode
- Side scatter detector: photodiode

## Laser Specifications

### Guava® easyCyte™ HT Lasers and Fluorescent Filters

The following table lists the laser(s) and power for each of the Guava® easyCyte™ HT Instruments and the filters used for each parameter.

Instrument Name	eC 5&5HT	eC 5HPL & 5HT HPL	eC 6-2L & 6HT-2L	eC 8 & 8HT	eC BG & HT	eC 11 &	eC BGR &	eC 12 &	eC BGV &
# of Channels	5	5	6	8	BG	11HT	HT BGR	12HT	HT BGV
Laser Combo	B	BHPL	B/R	B/R	B/G	B/V	B/G/R	B/R/V	B/G/V
Item Number SL	0500-5005B	0500-5009B	0500-5007B	0500-5008B	0500-5015B	0500-5020B	0500-5025B	0500-5012B	0500-5030B
Item Number HT	0500-4005B	0500-4009B	0500-4007B	0500-4008B	0500-4015B	0500-4020B	0500-4025B	0500-4012B	0500-4030B
Laser	488nm (Blue)	488nm (Blue)	488nm (Blue)	488nm (Blue)	488nm (Blue)	488nm (Blue)	488nm (Blue)	488nm (Blue)	488nm (Blue)
Power	50mW	150mW	50mW	150mW	150mW	150mW	150mW	150mW	150mW
FSC	N/A	✓	✓	✓	✓	✓	✓	✓	✓
SSC	488/16	✓	✓	✓	✓	✓	✓	✓	✓
Green-B	525/30	✓	✓	✓	✓	512/18	✓	512/18	✓
Yellow-B	583/26	✓	✓	✓	✓	575/25	✓	575/25	✓
Red-B	695/50	✓	✓	✓	✓	✓	✓	✓	✓
NIR-B	785/70				✓	✓	✓	✓	✓
Laser					532nm (Green)		532nm (Green)		532nm (Green)
Power					100mW		100mW		100mW
Yellow-G	583/26				575/25		575/25		575/25
Orange-G	620/52				✓		609/30		✓
Red-G	695/50				✓		✓		✓
NIR-G	785/70				✓		✓		✓
Laser					405nm (Violet)		405nm (Violet)		405nm (Violet)
Power					100mW		100mW		100mW
Blue-V	450/45				✓		✓		✓
Green-V	525/30				✓		✓		512/18
Yellow-V	583/26				✓		✓		575/25
Orange-V	620/52								✓
Red-V	695/50				✓		✓		
NIR-V	785/70				✓		✓		
Laser			642nm (Red)	642nm (Red)			642nm (Red)	642nm (Red)	
Power			100mW	100mW			100mW	100mW	
Red-R	661/14		✓	✓			✓	✓	
NIR-R	785/70			✓			✓	✓	

## Signal Processing Specifications

- parameter dynamic range:
  - 5.0 decades (Guava® InCyte™ software module)
  - 4.0 decades (Guava ExpressPro software module, Guava ViaCount™, Guava Nexin®, and Guava Cell Cycle Modules)
- time: every particle time stamped
- pulse processing: digital signal processing

## Fluidics Specifications

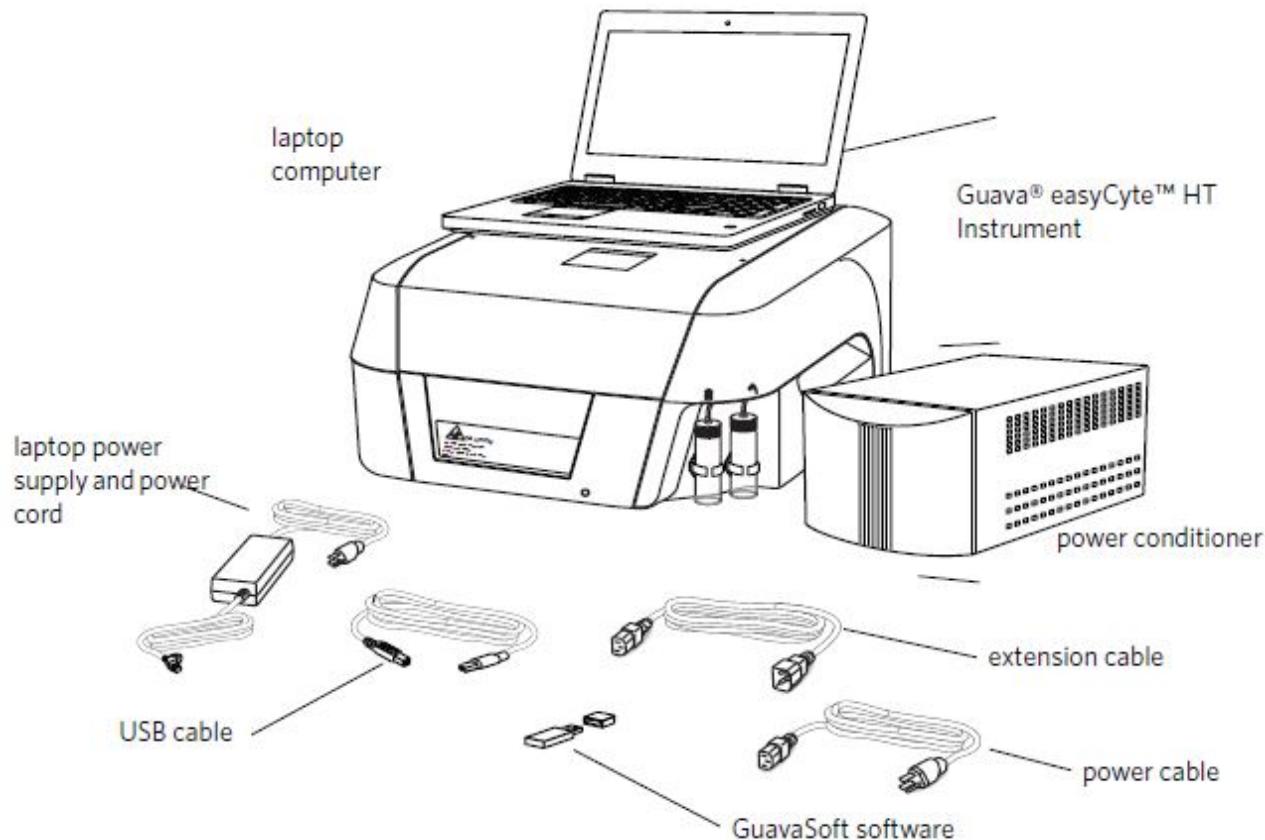
- flowcell dimension: standard square capillary with ID of 100 µm
- pump: positive displacement
- sample flow rate: 7 µL/min to 72 µL/min
- cleaning and waste vials: 30 mL glass vials with screw tops
- waste generation: typically <40 mL in 8 hours of continuous use
- dead volume: 50 µL (for 96-well microplate); 75 µL (for 0.5 mL microcentrifuge tubes)
- sample concentration: final particle concentration of  $10^4$  to  $10^6$  particles/mL for accurate results
- sample requirement: as few as 2,000 cells/test; typically 25,000 to 100,000 cells/test depending on the assay

## Data Management Specifications

- computer: Dell™ laptop running Windows® 10 Professional (64-bit only), and including Microsoft® Excel. Minimum configuration: Intel® Core™ i5- 3210M processor (2.5 GHz, 3M cache); 4 GB, DDR3-1600 MHz SDRAM; 320 GB hard drive; 2 USB ports
- data file structure: Output data file formats:
  - binary data storage in Flow Cytometry Standard (FCS) 3.0 format
  - spreadsheet results file in comma-separated value (CSV) format
  - optional export of binary data in FCS 2.0 or 3.0 format
  - optional CSV list-mode data

# System Components

The Guava® easyCyte™ HT System is shipped with the following components.



## Software Components

A USB drive contains GuavaSoft Software. The Guava® easyCyte™ HT System User Guide is installed with the software.

## Hardware Components

### Sample Tray

Sample aliquots are placed in a microplate or sample tube, then loaded in the sample tray. The tray holds one 96-well microplate and up to 10 sample tubes. A minimum sample volume of 100 µL is required for wells, 150 µL for 0.5-mL microcentrifuge tubes, and 900 µL for 1.5-mL microcentrifuge tubes.

**NOTE:** Snap-cap tubes can be used in place of the 1.5-mL screw-cap tubes (for washing and cleaning) if the caps are cut off.

For a list of supported microplates and tubes, refer to the Order Information chapter.

Do not attempt to open the sample tray door with your fingers. Always use the Eject Tray button in GuavaSoft Software to open the door, pausing the system first, if necessary. If you attempt to manually open the door, a warning message appears and the worklist automatically stops. If this happens, exit GuavaSoft Software, then restart the program.



On rare occasions, the software and instrument may pause to resynchronize with each other. If this occurs, the system will pause for 20–30 seconds and a message will appear in the status bar at the bottom of the screen indicating a “Tray Hold-Off.” Although this is not serious and the system will resume again after synchronizing, be aware that after the tray hold-off occurs, the tray may move without warning.

The use of tubes or plates other than those specified may result in damage to the instrument.

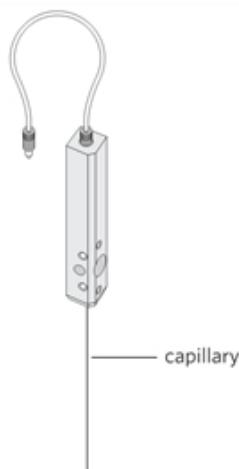
## Cleaning Solution and Waste Vials

The cleaning solution vial (located on the right ) can be filled with Guava® Instrument Cleaning Fluid (ICF), for easy system cleaning.

The waste vial (located on the left ) captures the sample fluid after it exits the fluid system. Empty the waste vial at the end of each day or more often, if necessary. Add 5 mL of 100 % bleach to the vial after you empty it.

## Fluid System

Sample uptake occurs through a capillary and is regulated by a variable-speed fluid pump. The pump does not require sheath fluid or other supplementary fluids for operation.



Because the system's sampling precision depends on the integrity of the fluid pathway, it is important to maintain a clean system.

## Laser

The Guava® easyCyte™ HT System has up to three lasers out of a possible four options—blue, violet, red, and green. During acquisition using Guava InCyte™ or easyCheck™ modules, all three lasers turn on. During acquisition using the ExpressPro module, the blue and red lasers turn on. During acquisition using any of the other Guava Software modules, only the blue laser turns on. The lasers turn off when acquisition is complete.

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# Consumable Components



Adhere to standard laboratory safety practices when handling hazardous, toxic, or flammable reagents and chemicals. Contact Luminex Technical Support when in doubt about compatibility of cleaning and decontamination agents or materials.

Only use reagents, assays, or other consumables that are within their expiration date. Dispose of all expired reagents, assays, or consumables in the appropriate waste fluid container.

- Guava® Instrument Cleaning Fluid (ICF), 100 mL
- Sample tubes
- easyCheck™ Kit Reagent
- Stained samples, per user specific protocols
- 96-well plate
- microcentrifuge tubes

## Required Laboratory Reagents

- Deionized (DI) water
- Bleach containing between 5-6% hypochlorite

**NOTE:** For cleaning the flow cell Luminex corporation recommends using a solution of <0.6% hypochlorite. For decontamination of the biological waste Luminex recommends using 5 mL of 5-6% hypochlorite, unless otherwise stated by your safety guidelines.

## Optional Reagents

- Assay specific reagents, as needed.

## Fluorochromes

The following table lists the fluorochromes that can be used with the Guava® easyCyte™ HT System, the laser that excites the dye, and the parameter/detector that detects the emitted signal. For additional information, contact Luminex Technical Support.

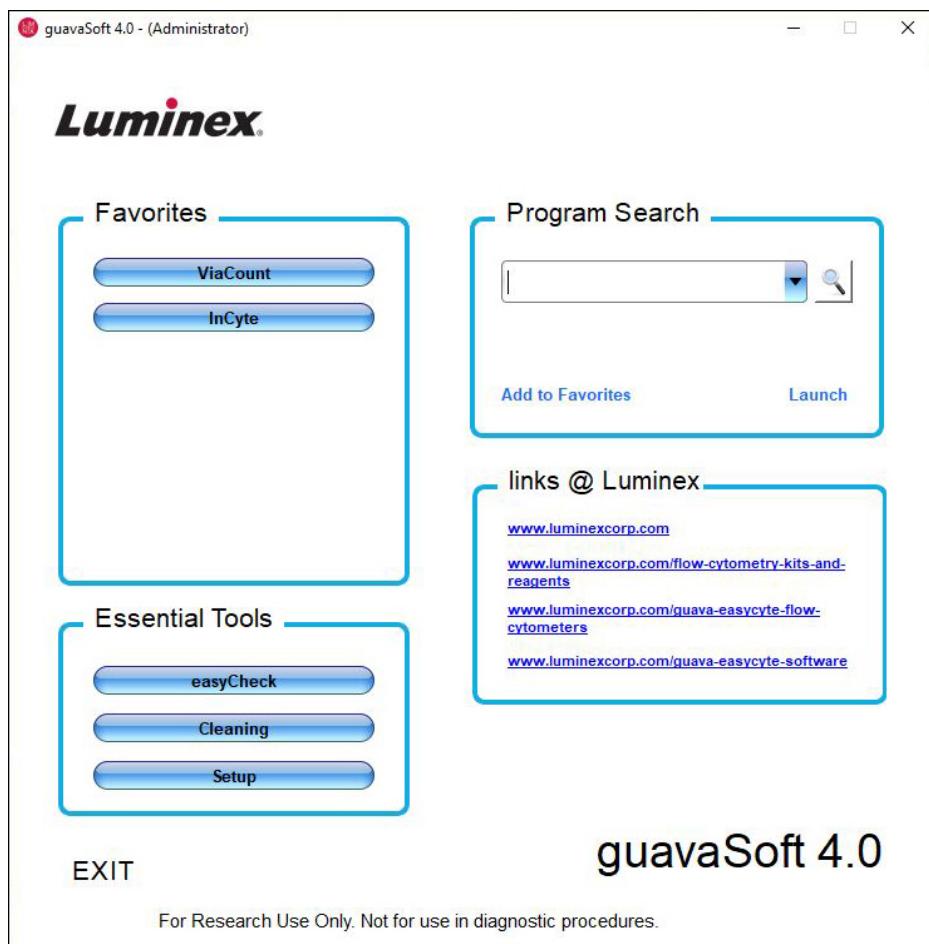
Blue (450/45 nm)	Green (525/30 nm) (512/18 nm)*	Yellow (583/26 nm) (575/25 nm)*	Orange (620/52 nm) (609/30 nm)**	Red (661/15 nm)	Red (695/50 nm)	NIR (785/70)
AlexaFluor® 405	Alexa Fluor® 430	BV 570®	BV 605®	BV 650®	BV 711®	BV 785®
BV 421®	BV 510®	Cascade Yellow	QD 625	eFluor 650	QD 705	eFluor 750
Cascade Blue	Cascade Yellow	Pacific Orange	Ethidium bromide	QD 655	7-AAD	QD 800
DAPI	Pacific Green	QD 565	PE-Dazzle™ 594	7-AAD	PE-AlexaFluor® 647	PE-AlexaFluor® 750
DyLight™ 405	Pacific Orange	QD 585	PE-Texas Red	Acridine Orange (RNA)	PE-AlexaFluor® 700	PE-Cy7
eFluor 450	QD 525	DsRED	7-AAD	Ethidium bromide	PE-Cy5.5	FM® 5-95
Hoescht 33258	QD 545	Ethidium bromide	Alexa Fluor® 568	PE-Cy5	PerCP	PE-Cy7
LIVE/DEAD Violet	Zombie Aqua	JC-1 (aggregate)	EthD-1, EthD-2	Propidium Iodide (PI)	PerCP-Cy5.5	APC-H7
Marina Blue	Acridine Orange (DNA)	R-PE	Ethidium bromide	7-AAD	7-AAD	APC-Alexa Fluor® 750
Pacific Blue	Alexa Fluor® 488	RFP	mCherry	Ethidium bromide	PE-Cy5.5	APC-Cy7
	BODIPY-FL	Alexa Fluor® 532	MitoTracker® Red	Nile Red	APC-Cy5.5	
	Calcein	Alexa Fluor® 555	Nile Red	PE-Cy5	DRAQ5	
	CFSE	Cy3	PE-Dazzle™ 594	Propidium Iodide (PI)	DyeCycle™ Ruby	
	CF™ 488	Dil	PE-eFluor 610	Alexa Fluor® 647		
Cy2	DsRED	PE-Texas Red	Alexa Fluor® 660			
DyLight™ 488	dTomato	Propidium Iodide (PI)	APC			
FAM	DyLight® 550	Rhodamine Red-X	BODIPY 650/665			
FITC	Ethidium bromide		Cy5			
GFP/eGFP	mOrange		DilC1(5)			
JC-1 (monomer)	PO-PRO™-3 Iodide		DyLight™ 650			
Oregon Green	R-PE		eFluor® 660			
Rhodamine 110 & 123	RFP		MitoSense Red			
SYBR Gold	SYTOX® Orange		Sytox® Red			
SYBR Green			TO-PRO® 3			
SYTOX Green			TOTO®-3			
Thiazole Orange						
TO-PRO-1						
YFP/eYFP						
YO-PRO-1						
YOYO-1						

Excitation:  
 Violet laser (405 nm)  
 Blue laser (488 nm)  
 Green laser (532 nm)  
 Red laser (642 nm)

# Chapter 4: Software Functionality

## Main Menu

The GuavaSoft Software Main Menu allows you to select an assay from either a list of favorites or the program search list. Essential Tools allows you to run Guava® easyCheck™, clean the instrument, or click Setup, where you can customize your list of favorites. Use links @ Luminex to quickly access Luminex websites for information.



## Favorites

Favorites allows you to quickly select an assay. You can have up to seven assays at a time in your Favorites list. To add a favorite assay, select the assay from the Program Search list and click **Add to Favorites**. The new assay will be added to the bottom of the list. And if the list already consists of seven assays, the first assay in the list will be removed.



To remove a favorite assay, click **Setup** under Essential Tools. Remove the check from the check box for the assay you wish to remove. Click **DONE**.

## Program Search List

The Program Search List allows you to select an assay to run or open a Guava® modules in the InCyte™ module. You can create a Favorites list to quickly select an assay later. The main menu allows you to select from the following assays:

- Guava ViaCount™ Assay for performing cell counting and viability assays
  - GuavaViaCount™ (Legacy) Assay - can only be opened in analysis mode
- Guava® ExpressPro Assay for performing assays with up to six colors, or where time, area, and/or width parameters are necessary
- Guava® InCyte for performing assays with up to 12 colors and 2 scatter parameters, or where time, area, and/or width parameters are necessary, or if you want to analyze any Guava FCS 3.0 data files
- Guava Nexin® and Guava® TUNEL Assays for performing apoptosis assays
  - GuavaNexin® (Legacy) Assay - can only be opened in analysis mode
- Guava® Cell Cycle Assay for performing DNA cell cycle assays
  - Guava®Cell Cycle (Legacy) Assay - can only be opened in analysis mode

- Guava® **Cytochrome c** for mitochondrial cytochrome c loss
- Guava® **MitoDamage** for apoptosis, mitochondrial membrane depolarization, and cellular death detection
- **Human CD4/CD8 T Cell**

Additionally, Guava® modules are available for acquisition and/or analysis using the InCyte module.

## Essential Tools

Guava® easyCheck™ allows you to check the system's counting, fluorescence, and scatter performance prior to running samples. The easyCheck Bead is a standard particle used with this tool.

**Cleaning** allows you to run Guava Clean, an automated system cleaning cycle that cleans the fluid system.

**Setup** allows administrators to:

- configure certain software features for specific users
- remove assays from your Favorites list
- enter assay registrations codes

**NOTE:** The Setup button is available only when an administrator is logged onto the system.

## links @ Luminex

This section allows you to access commonly used Luminex websites. You must have internet access.

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## Request an Unlock Key

Guava® InCyte™ requires an unlock key. When the instrument is shipped, the unlock code is automatically entered. If you get a message containing your computer code and prompting for an unlock key, e-mail support@luminexcorp.com and we will provide you with an unlock key for software purchased.

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## Guava® Modules

Software modules are available for all Guava® assays. The modules can be used for acquisition and/or analysis. The instrument settings, plots, gates, and markers for the assay are contained in the module. If you already acquired data using either InCyte or another GuavaSoft Software module, open the data file in the module and perform analysis using the Analysis Method provided.

## Using a Guava® Module

For more information on using the modules, refer to the appropriate assay kit package insert.

1. From the GuavaSoft Main Menu, choose an assay module from the Program Search list and click **Launch** or choose an assay module from the Favorites list. The assay module opens in the GuavaSoft Software.

2. Select the **Analyse** or **Acquire** button at the top of the control panel. Perform acquisition or analysis according to the instructions outlined in the assay kit package insert.

**NOTE:** The instrument must be turned on for the Acquire button at the top of the control panel to be active.

- If you are acquiring samples, the instrument settings should be close to what you need. Check the settings and adjust, if necessary.
- If you are analyzing InCyte-acquired data, but you acquired the data without the use of a module, drag the template Analysis Method to the Analysed Group. Check the gates and markers and adjust, if necessary.
- If you are analyzing a data file acquired using a program other than InCyte, open the data file. The assay module will contain the appropriate Analysis Method. Create an Analysed Group and drag the data file and the Analysis Method to the Analysed Group. Check the gates and markers and adjust, if necessary.

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## GuavaSoft Software Overview

**NOTE:** Some software features may not be present for all assay modules. Please see the appropriate assay kit package insert for details specific to the assay you are working on.

### Key Features of the Guava® InCyte™ Module

A single Method (or analysis strategy) can be applied to multiple data sets in parallel, or multiple Methods can be applied to a single data set to obtain statistics to be displayed in the heat map. The process of creating equation-based gating schemes has been simplified through the use of drag-and-drop regions; "draggable" features are used throughout Guava® InCyte™.

The HeatMap allows you to visually compare results using a plate map that displays varying shades of blue to represent relative statistical values. Results from up to six parameters or six data sets can be displayed simultaneously.

The Single Sample Analysis feature allows you to append to or analyze large FCS files or files with many statistics. The calculation of all statistics for all sample in a data set can take time. In Single Sample Analysis mode, only the statistics for the current sample will be calculated. When acquisition or analysis is complete, you can select when you wish to update the group stats for all samples in the data set.

Additionally, all channels can be used with reagents that fluoresce in appropriate channels.

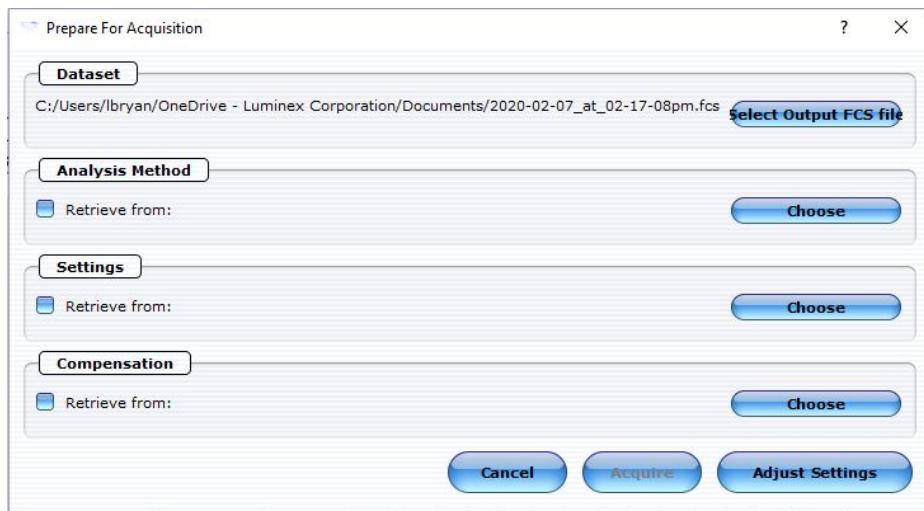
### Components of GuavaSoft Software Files

- Data files acquired using GuavaSoft module software contain a raw FCS file (Dataset) and automatically paired default analysis method (predefined regions gates markers statistics)

**NOTE:** At the start of acquisition within the modules, you have the option to select and retrieve instrument settings and name your data file.

- Data files acquired using Guava® InCyte™ contain raw FCS data (Dataset), the analysis components also known as Analysis Methods (regions, gates, markers, statistics, etc), instrument settings, and compensation settings. The raw data combined with the Analysis Method make up the InCyte Analysed Group.

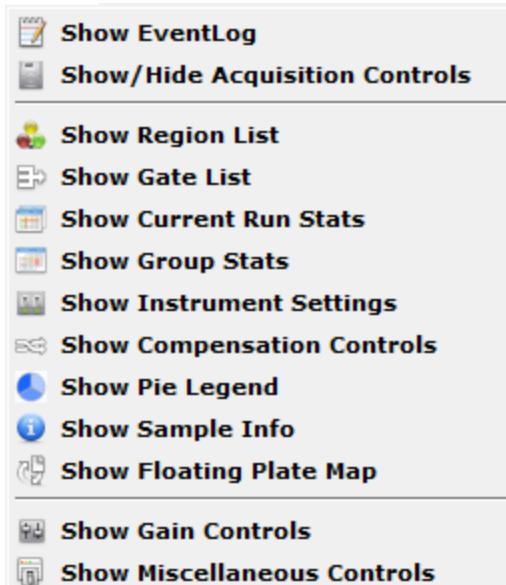
**NOTE:** At the start of acquisition, you have the option to select a Method, instrument settings, and compensation settings. These components can be retrieved from a saved InCyte FCS file. You can also retrieve the Method and/or instrument settings from individual saved Method and instrument settings files. If you do not retrieve a Method, a default Method will be applied.



## GuavaSoft Software Tool Bar

The tool bar appearing on the far left side of the window provides additional features for acquisition and analysis. The features are also accessible from the Tools menu. Other than the Pie Legend, the tools are described in detail at the point in the workflow where they are used.

**NOTE:** In some GuavaSoft Software modules, some of the features shown below may be disabled.



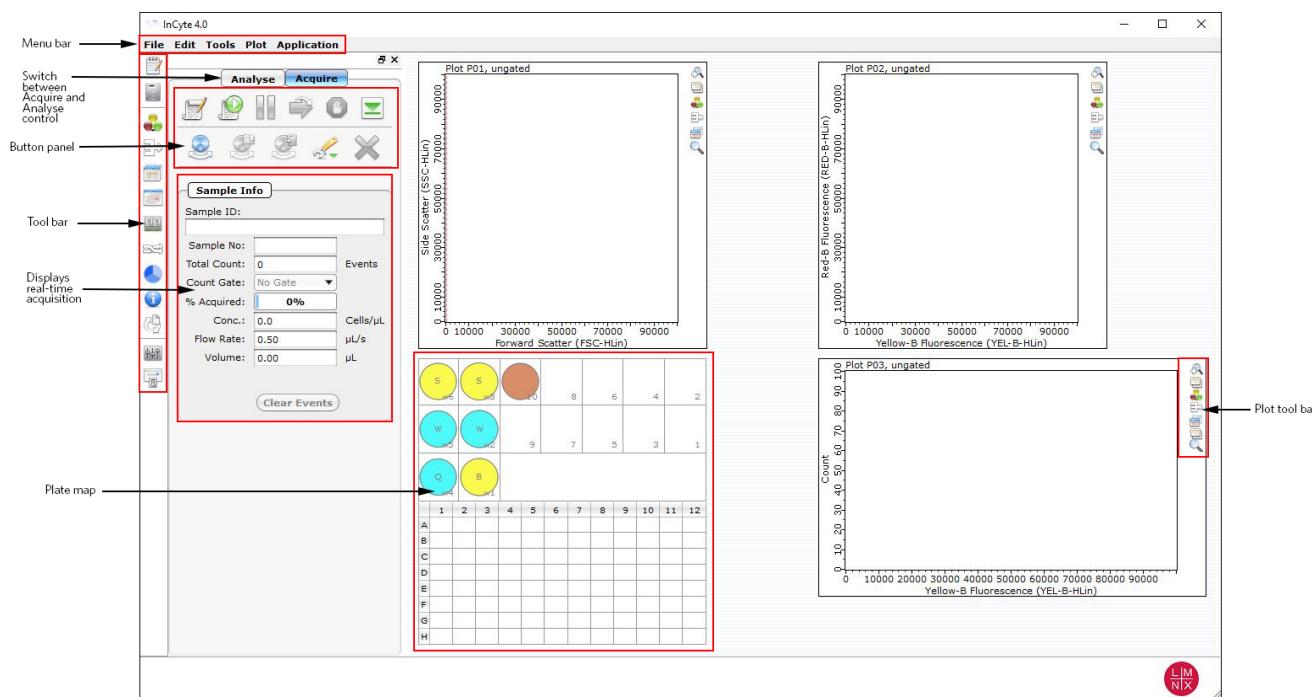
## GuavaSoft Software Acquisition Screen



Do not run Excel® software, Internet Explorer® browser, or any other program on the laptop while using GuavaSoft Software to acquire data from the Guava® easyCyte™ System. GuavaSoft Software requires the full resources of your laptop during data acquisition. Running other programs (even if you are not actively using them) during a run may interfere with acquisition or interrupt the run.

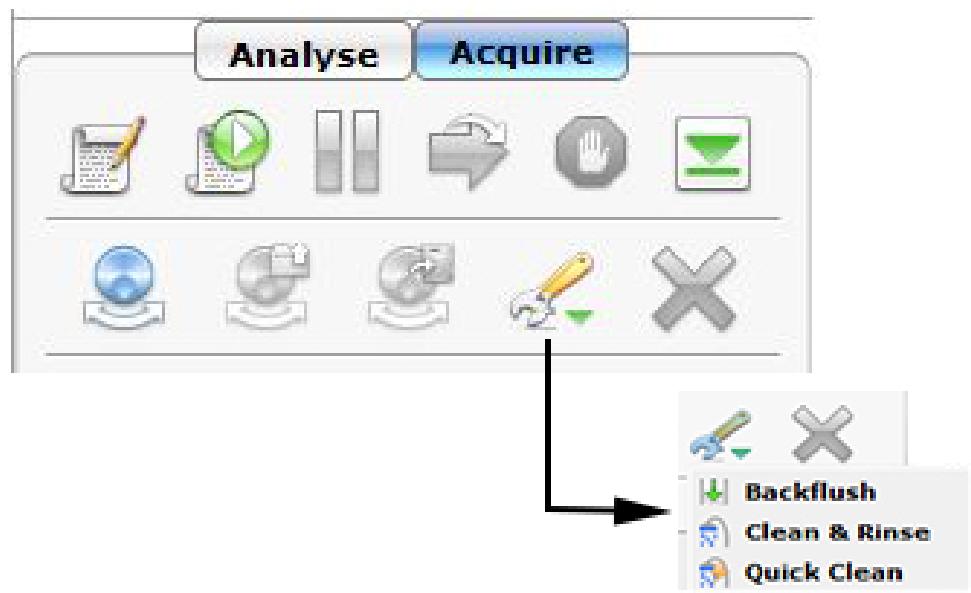
**NOTE:** Always save GuavaSoft Software data files directly to the laptop's hard drive during acquisition. Saving data to a network or location other than the computer's hard drive may result in data loss. You may copy the file(s) to another location when acquisition is complete.

The Acquisition screen displays when you enter the assay. Use the Acquisition screen to acquire data from samples you run on the Guava® easyCyte HT System. You can also perform data analysis from the Acquisition screen immediately following acquisition.



## GuavaSoft Software Acquisition Buttons

Place the cursor over the icon to display text describing each button.



The following table describes the acquisition buttons.

Table 1. Acquisition Buttons and Definitions

Acquisition Button	Description
Edit Worklist	Launches Worklist Editor Software.
Start Worklist	Opens the sample tray, allows you to select the worklist file, the Method, instrument settings, and compensation, prompts you to load tubes, and select the sample for adjust settings, if applicable.
Pause / Resume	Pauses the assay allowing you to select Eject Tray, Settings, Quick Clean, Clean & Rinse, or Backflush, or to access the Analysis screen where you can view data from previously acquired samples. The acquisition of the current sample will finish before the system pauses, and the button will read Pause requested. Resume restarts the assay where it left off.
Next Step	Proceeds to the next step in the data acquisition process. The data already acquired is saved. Next is not available during certain functions, for example during a washing or mixing step.
Stop Worklist	Stops the assay after finishing the acquisition for the current sample. After stopping you cannot resume the worklist.

Acquisition Button	Description
Eject Tray / Load Tray	<b>Eject Tray</b> opens the sample tray allowing you to add or remove a plate or tube. <b>Load Tray</b> retracts the sample tray.
Settings	Three buttons allow you to adjust instrument settings, and save and retrieve settings. <b>Adjust Settings</b> allows you to adjust instrument settings using the appropriate sample. <b>Save Settings</b> allows you to save the current instrument settings to a separate file. <b>Retrieve Settings</b> allows you to recall instrument and analysis settings from a settings file and download the settings to the easyCyte™ HT instrument. If this button is disabled, your system administrator may have chosen to not allow the retrieval of Methods and instrument settings from individual Method and instrument settings files. These settings can be retrieved only from an FCS file.
Capillary Cleaning Tools	To use any of the following functions during acquisition, click <b>Pause</b> , then click the cleaning button. Click <b>Resume</b> to restart the assay. <b>Backflush</b> reverses the flow of fluid out of the flow cell. Perform a backflush if the acquisition rate declines and you suspect a clog. Follow a backflush with a Quick Clean. <b>Clean &amp; Rinse</b> thoroughly cleans the fluid pathway with a series of Quick Clean cleaning cycles and Backflush cycles. <b>Quick Clean</b> cleans the fluid pathway. The system automatically performs a Quick Clean at the end of each Guava easyCheck™ Cycle. You can also select Quick Clean in Worklist Editor to automatically run Quick Clean during or after the assay.
Abort Worklist	Stops the worklist and ejects the sample tray. The data for the current sample is not saved. You cannot resume an assay after you abort. You must reload the sample tray and start the assay from the beginning.

## GuavaSoft Acquire Control Panel

**NOTE:** In some GuavaSoft Software modules, some of the features on the control bar may be disabled.

**Acquisition Buttons**

Place the cursor over the icon to display text describing the button.

**Sample Info**

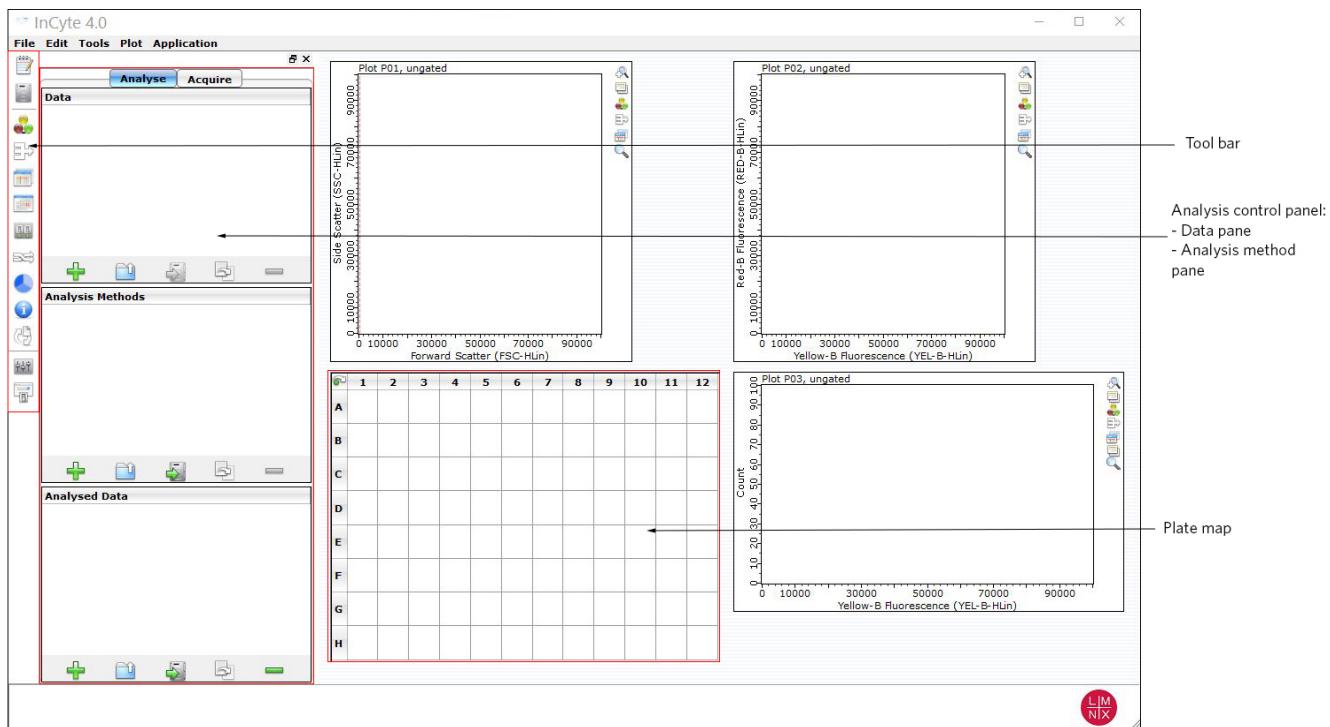
- **Sample ID:** Displays the Sample ID for the individual sample. Defaults to the plate well number.
- **Sample No:** Displays the sample #. This number defaults to 1 and advances at the completion of sample acquisition.
- **Total count:** Displays the number of events to acquire. The default is 5000. Total count changes to Gated count if you apply a count gate
- **Count Gate:** allows you to select a gate used as a counting gate. All events above the threshold are saved to the file whether they are in the gate or not.
- **% Acquired:** A progress bar provides an estimate of the target event count during acquisition.
- **Conc.:** Displays the cells/µL that have exceeded the threshold.
- **Flow Rate:** Displays the flow rate selected during the adjust settings step (µL/s).
- **Volume:** Displays the sample volume that is acquired.
- **Clear Events:** Click to clear the display and restart acquisition during adjust settings.

## GuavaSoft Software Analysis Screen

The Analysis screen allows you to analyze data from samples that were previously acquired. When you open a data set, the data for the first sample displays. The samples within the file are listed in the Data pane. Click the + in front of the FCS file to display the list of samples. Click any sample to view the data for that sample. You can also click the up/down arrows on the keyboard to select samples.

You can access the Analyse screen by clicking **Analyse** at the top of the control panel. If you display the Analyse screen at the completion of acquisition, the samples you just ran are listed in the Data pane. Any gates and marker you set during acquisition will display.

**NOTE:** A collapsed version of the Analysis control panel is seen in some of the assay specific modules. For more information, see the appropriate assay packet insert.



## GuavaSoft Software Plots

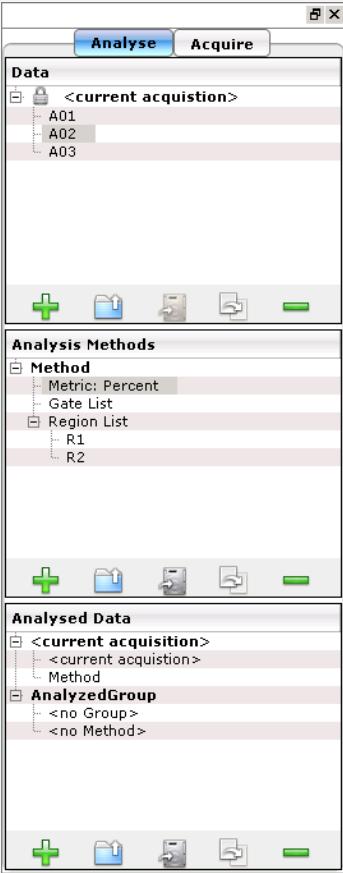
The plots area of the window displays three plots by default. Use the Plots menu to change the number of plots displayed. Use the tool bar on the right to change the plot type and set regions and gates.

- Undock
- Plot type
- New Region
- Plot gate
- New Stat
- Marker
- Edit Overlay
- List
- Zoom

## GuavaSoft Software Plate Map

The plate map provides a visual representation of the samples contained in the dataset. During analysis, click a well in the plate map to view the data for that sample in the plots. Place the cursor over a well to display the results for that sample. If you are using the HeatMap feature, the plate map provides a visual representation of the relative values for each well, or well-to-well variations in varying shades of blue.

## GuavaSoft Software Analyse Control Panel

	<p><b>Analysis Buttons</b></p> <p>Place the cursor over the icon to display text describing the button. The buttons have the same function for each pane but apply specifically to that pane.</p>  <p>New      Open      Save      Duplicate      Delete</p> <p><b>Data</b></p> <p>Displays the open data files, as well as any user-created subsets of these files or groups and allows you to select a data set or group for analysis.</p> <p><b>Analysis Methods</b></p> <p>Displays the Analysis Methods for the current experiment. Each Analysis Method contains a gate list, a region list, and a metric (statistical parameter). InCyte-acquired files contain Methods. For data files acquired using a program other than Guava® InCyte™, you must create a new Method or open an existing Method before you can perform analysis.</p> <p><b>Analyzed Data</b></p> <p>Displays the FCS file and the associated Method. Created by pairing a non-InCyte-acquired data file with a new or existing Method during analysis, or created automatically during acquisition using Guava® InCyte.</p>
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## GuavaSoft Files

GuavaSoft Software automatically saves a flow cytometry standard (FCS) 3.0 data file, which contains the data for all samples within a data set.

- a worklist file, which you create before you can run an assay. The worklist file is a .xml file that is automatically saved with the same name as the data set.

Additionally, you can optionally choose to save (or export) the following files:

- a separate instrument settings file
- FCS 2.0 or 3.0 files for individual samples for analysis using third-party analysis programs
- list-mode data files

**NOTE:** When using InCyte™, you can additionally save a separate Method file.

**NOTE:** To keep your computer performing optimally, periodically clear old files from your hard drive by archiving the files to a back-up storage location.

### Flow Cytometry Standard (FCS) 3.0 Data Files

Guava® InCyte™ saves a single FCS file containing all the samples within a data set. The extension .fcs is automatically appended to the file name.

FCS files are data files saved in a format compatible with standard flow cytometry analysis applications as defined by the Society for Analytical Cytology [Cytometry. 1990;11(3):323-332]. One FCS 3.0 file is saved for all samples acquired within a data set.

**NOTE:** Always save GuavaSoft Software's data files directly to the laptop's hard drive during acquisition. Saving acquisition data to an outside network may result in data loss. You may copy the file(s) to another location when acquisition is complete.

### Worklist Files

A worklist file contains all of the information for each well/tube entered at the WorkList Editor screen. The extension .xml is appended to the worklist file name.

### Method Files

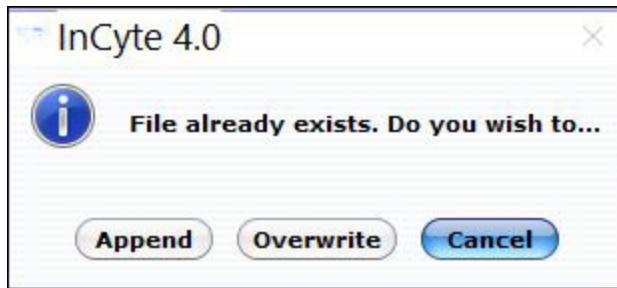
A Method contains all the analysis components (gates, regions, markers, plots, parameters, and statistical setup). When you acquire data using Guava® InCyte™, Methods are part of the FCS file. Methods can also be saved to a separate file (.gsy). Data files acquired using a program other than InCyte will not have Methods associated with them.

### Instrument Settings Files

Instrument settings are automatically saved with the FCS file. You can also save instrument settings to a separate file. The extension .gst is automatically appended to the file name you enter.

## Append or Overwrite Existing Files

If you select the name of an existing dataset name at the start of acquisition, you will be prompted to either append or overwrite it. If you append, the sample number defaults to the next available number in the existing data file.



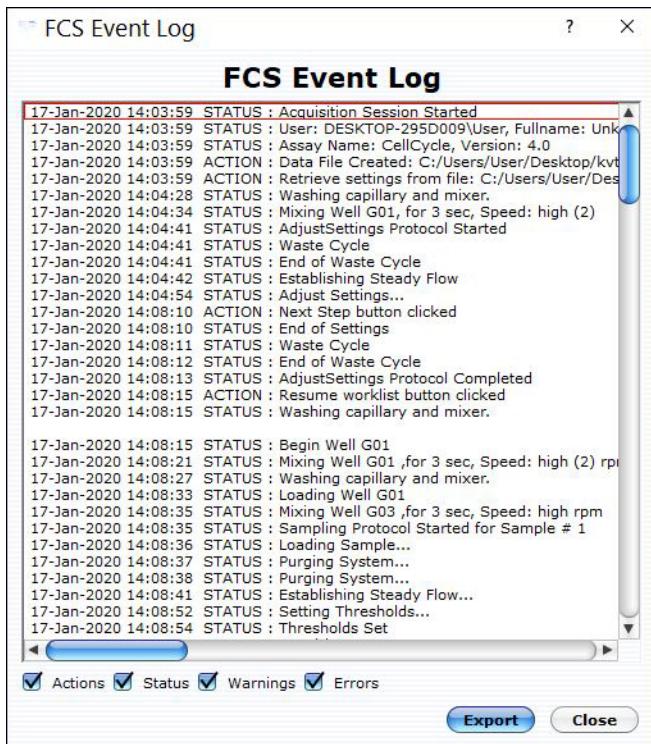
If you append data to an existing file, the instrument settings and analysis gates and markers are automatically updated to reflect the settings for the last sample in the file.

**NOTE:** Your system administrator may have configured GuavaSoft Software to disable appending and/or overwriting files. If appending only is disabled, you may create a new file or overwrite an existing file. If overwriting only is disabled, you may create a new file or append to a copy of an existing file. If both appending and overwriting are disabled, you must create a new file.

**NOTE:** When appending to large files, consider using Single Sample Analysis. This feature allows you to open large files more quickly, by eliminating the wait time that results from the calculation of statistics for all samples before and after acquisition.

## FCS Event Log

Each time you run an assay, GuavaSoft Software saves a log containing a list of all events that occurred during the assay. This information is contained within the FCS data file. To view this list, click the **Event Log** icon in the tool bar, or select **Tools > Show Event Log**. A list of all events appears with the date and time the event occurred.



You can filter the list to view errors, warnings, statuses, and/or actions. Select the appropriate check box(es) to display the types of events you wish to view.

During acquisition, every step the instrument performs, independent of the operator (for example, priming, setting thresholds, performing calculations) is logged. Every step the operator performs (for example, key presses, selections, changes to gates and markers, logging comments) is also logged.

If errors or warnings occur during a run, a message appears in red in the status bar indicating that errors/warnings have been logged and how many times they have occurred.

Warnings include:

- Less than 10 particles/ $\mu\text{L}$ . Sample is too dilute. Accuracy may be compromised.
- More than 500 particles/ $\mu\text{L}$ . Sample is too concentrated. Please dilute or accuracy may be compromised.

**NOTE:** The default value for Guava® InCyte™ can be changed in WorkEdit Software, and the warnings will reflect the new user-defined concentrations. However, Luminex does not recommend entering values higher than the default values.

- The run timed out before enough events were acquired.
- Adjust Settings timed out. Please re-enter Adjust Settings if necessary to complete the instrument set-up.
- Maximum velocity exceeded for “x” events (applies only to area/width parameters)

Errors include:

- The tray door was opened. When you click **OK**, worklist will be halted but your acquired data will be saved. To continue, either restart the worklist or create a new worklist beginning from the well that was aborted when the door was opened.

During data analysis, any local or global changes are logged along with the sample name to which changes were made. Names are saved for the Dataset, Analysis Method, and Analysed Group, whether retrieved prior to adjust settings or for the final Analysed Group when analysis is complete. Overwriting an Analysed Group is also logged.

## Access Instrument Settings

Guava® InCyte™ saves the instrument settings for each sample acquired.

1. From the Guava® InCyte™ module, open the FCS needed for analysis.
  2. Choose **File > Open** from the menu bar.
    - a. From the **Open File** dialog box, navigate to the FCS file for analysis and click **Open**.
  3. Choose the .fcs file from the **Analysed Data** pane.
  4. Click the **Show Instrument Settings** icon from the tool bar, or select **Tools > Show Instrument Settings**.
- NOTE:** In addition to the threshold, gains, and compensation, all of the acquisition information, such as date, time, number of events, total volume, concentration, and flow rate, along with sample-specific information, like sample ID and number, dilution factor, and original volume are saved.
5. To copy the window to the clipboard, right-click in the window and select **Copy to Clipboard**.
  6. To export the settings to a .csv file for use in a spreadsheet program, click **Export to CSV**. Select the folder where you want to store the file, enter a file name, and click **Save**.
  7. To print the settings, click **Print Stats**.

The screenshot shows a software interface titled "Instrument Settings". It displays a table of acquisition parameters for three samples. The columns are labeled: Sample Number, Sample ID, Number of Events, Termination Count, Count Gate, Dilution Factor, Original Volume, Cells/µL, Total Volume (µL), and Acquisition Time (s). The data is as follows:

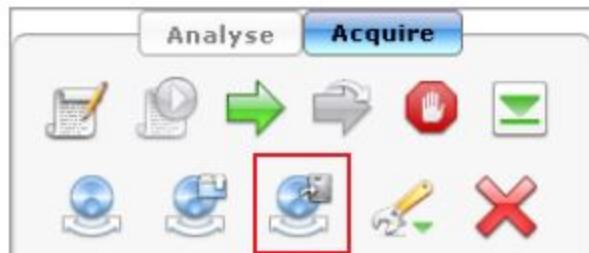
Sample Number	Sample ID	Number of Events	Termination Count	Count Gate	Dilution Factor	Original Volume	Cells/µL	Total Volume (µL)	Acquisition Time (s)
1	Sample #1	5000	5000	All Events	10.00	10.00	839.15	5.96	10.10
2	Sample #2	5000	5000	All Events	10.00	10.00	839.15	5.96	10.10
3	Sample #3	5000	5000	All Events	10.00	10.00	839.15	5.96	10.10

At the bottom of the dialog box are two buttons: "Export To CSV" and "Print Stats".

## Save Instrument Settings

Although the instrument settings are automatically saved with the FCS file, GuavaSoft Software allows you to save the current instrument settings and analysis gates and markers to a separate file. You can recall this file later to:

- download the instrument settings to the Guava® easyCyte™ System for acquisition
  - apply the gates and markers to data during acquisition
1. Perform the adjust setting step before you save a settings file. Click **Start New Session File**. The Prepare For Acquisition dialog box displays.
  2. Add any files needed and click **Adjust Settings**.
  3. Once the Adjust Settings step is complete, click the **Save Settings** button. The **Save Instrument Settings Dialog** box displays.



4. Enter a **File name** for the file and select the location to save it.
5. Click **Save**.

## Retrieve Instrument Settings

1. From the Guava® InCyte™ module, click the **Retrieve Settings** button from the Acquisition screen. The Retrieve Instrument Settings Dialog box displays.



2. Locate the file you need to retrieve and click **Open**. The settings are downloaded to the Guava® easyCyte™ HT System.

**NOTE:** If you retrieve instrument settings after you perform the adjust settings step, you will be prompted to repeat the adjust settings step. You can choose to repeat the adjust settings if you want.

- a. Load the control sample and click **OK** in the InCyte 4.0 message box, or click **Cancel** to skip repeating the adjust settings step.

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## Worklists

Worklists allow you to set up the acquisition commands to acquire samples from a microplate and/or 10 tube locations in the sample tray. You can also programs the cleaning cycles you wish to perform during the run. Use Worklist Editor software to create worklists. Worklists can be saved as templates for the Guava® InCyte™ assay. The templates can be saved as a CSV file and modified/updated using Microsoft Excel or similar spreadsheet program.

## Worklists Editor Software

Worklist Editor Software allows you to set up the acquisition commands to acquire samples from a microplate and/or 9 tube locations in the sample tray. The right side of the screen displays a map for the microplate, allowing you to select wells for acquisition. Above the plate map are locations for up to nine tubes for samples and seven tubes for washing and cleaning functions. The left side of the screen allows you to select the assay, the number of events to acquire for each selected well/tube, the number of acquisition replicates, and the acquisition order. You can also select to save FCS 2.0 files for each sample, wash the mixer and/or capillary, and choose to mix samples before acquisition.

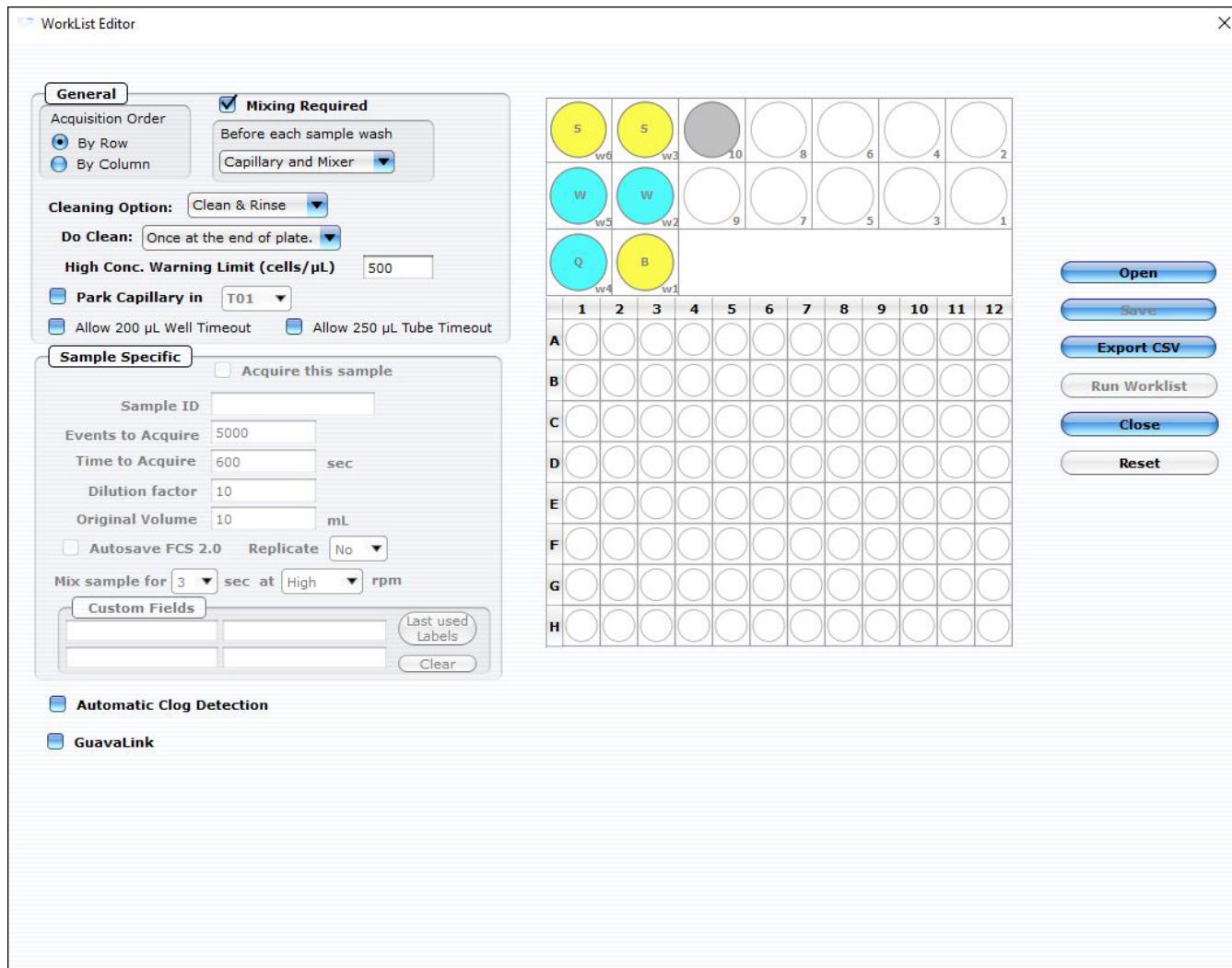
**NOTE:** Tube locations w1 through w6 are designated for capillary/mixer washes, instrument cleaning, and back-flushing. Tube location 10 is designated for washing if you select **Clean & Rinse**.

**NOTE:** The options you select within Worklist Editor Software will always supersede the settings from a file that you retrieve settings from or append.

You can open Worklist Editor Software directly from the Guava® InCyte™ assay screen by clicking Worklist Editor in the acquisition button panel. The Worklist Editor launches within the software module. You can also open the Worklist Editor from the Analysis screen, however, only to generate, modify, and save worklists.

**NOTE:** To run a worklist, you must be in Acquisition mode.

The information entered in Worklist Editor will display in the Guava InCyte Sample Information control panel during the assay. However, you cannot modify the information once you start the worklist.



## Worklists Editor Software Fields and Buttons

Field	Description
Acquisition Order	Choose <b>by Row</b> or <b>by Column</b> to acquire selected wells by row or column. The recommended acquisition order is by Row. Acquiring by row starts with row A (wells A1, A2, etc) and ends with row H. Acquiring by column starts with column 1 (wells A1, B1, etc) and ends with column 12. Only the wells selected in the worklist will be acquired. If tubes and wells are selected for acquisition, samples in tubes will be acquired in numerical order before samples in wells.
Mixing Required	Select <b>Mixing Required</b> if you wish to mix, then at the bottom of the Sample Specific information, choose how many seconds (1-10) and at what speed (Low, Med, or High). You can choose mixing times and speeds for individual samples.

Field	Description
Before each sample wash	Select <b>Only Capillary</b> to wash the capillary between samples. Select <b>Capillary and Mixer</b> if you choose to mix samples and wish to wash the capillary and mixer between samples. Select <b>None</b> if you do not want to wash the capillary or mixer. All washing functions apply to the entire run (tubes and wells).
Cleaning	Select a cleaning option—either Quick Clean or Clean & Rinse—to perform during the run. Choose <b>Once at the end of plate</b> , <b>Every 48 samples</b> , <b>Every 24 samples</b> , or <b>Every 12 samples</b> (Quick Clean only). Regardless of the option selected, the system will always perform a Quick Clean after the last sample.
High Conc. Warning Limit	The software will display a warning message during acquisition if the cell concentration exceeds the concentration value entered for the selected flow rate. You may lower the high concentration warning limits from the default values shown, if necessary. Luminex does not recommend entering values higher than the default values.
Park Capillary in	Select the tube (T01-T09) where you want the capillary parked when the system is not in use. Place a tube of DI water in that location.
Allow 200 µL Well Timeout Allow 250 µL Tube Timeout	Select <b>Allow 200uL Well Timeout</b> or <b>Allow 250uL Tube Timeout</b> if a larger acquisition volume is needed.
Sample Specific	<ul style="list-style-type: none"> <li>Click <b>Acquire this sample</b> after selecting a well/tube for acquisition. Wells/tubes selected for acquisition appear in blue.</li> <li>Enter an optional <b>Sample ID</b> for each individual well or tube. If you do not enter an ID, the ID defaults to the well/tube number.</li> <li>Enter the number of <b>Events</b> to acquire for the selected well/tube. The default is 5000. The range is 100 to 200,000. You may also set an acquisition duration (1–600 seconds), depending on the flow rate selected. Acquisition ends when the first limit (number of events or time limit) is reached.</li> <li>Enter the dilution factor and original volume (defaults are 1 and 10, respectively).</li> <li>Check <b>Autosave FCS 2.0</b> files to save files in FCS 2.0 format.</li> <li>Select the number of <b>Replicates</b> (2–8 for wells; 2–15 for tubes). If you wish to acquire the sample only once, select <b>No</b>. <b>NOTE:</b> All Sample Specific features can be applied to selected wells.</li> <li><b>Custom Fields:</b> Editable fields you can use to add additional sample characteristics.</li> </ul>
Buttons	<ul style="list-style-type: none"> <li><b>Open</b> to open a saved worklist file</li> <li><b>Save</b> to save the worklist to a file</li> <li><b>Export CSV</b> to export a CSV file</li> <li><b>Run this Worklist</b> to start the worklist</li> <li><b>Close</b> to close the window and return to the assay</li> <li><b>Reset</b> to clear all changes made and revert back to the original screen when Worklist Editor was first opened</li> </ul>

Field	Description
Automatic Clog Detection	Select the Automatic Clog Detection check box to specify the Clog Threshold (cells/ $\mu$ L) and the action taken after a clog, such as Backflush, QuickClean, or Abort.

## Create a Worklist

Use Worklist Editor software to select wells and/or tubes for acquisition, and program specific acquisition commands.

1. Open the Guava® InCyte™ module.
  2. Click the **Worklist Editor** icon from the Button panel or click **Application > Worklist Editor**. The Worklist Editor dialog box displays.
  3. Click to select wells/tubes for acquisition. Then, click the **Acquire this sample** check box to mark the selected wells/tubes. The wells/tubes selected for acquisition appear blue. You may select wells/tubes all at once, or you can select groups of wells that will have the same acquisition criteria applied to them.
- NOTE:** Tube locations w1 through w6 are designated for capillary/mixer washes, instrument cleaning, and back-flushing. Tube location 10 is designated for washing if you select **Clean & Rinse**.
- Click an individual well/tube to select it. To select groups of wells/tubes, click and drag, or press the Shift key and click. To select non-adjacent wells/tubes, press the Ctrl key and click the wells/tubes.
  - Click letters A-H on the microplate map to select a row.
  - Click numbers 1-12 on the microplate map to select a column.
  - Click the white box in the upper-left corner of the plate map to select the entire plate.
  - To deselect, press the Ctrl key while clicking the well/tube, column, or row.
4. Enter the number of **Events to Acquire** for the selected wells/tubes. The default is 5000 for InCyte. The range is 100 to  $2 \times 10^5$ .



Acquiring events significantly above these recommended ranges, especially with a high sample number, may cause the software to crash and/or a loss of data.

5. Enter a **Time to Acquire** limit. This time is the maximum time that sample will be acquired, up to the maximum time allowed for a given flow rate.

Flow Rate	Times out
Very low	600 seconds
Low	420 seconds
Medium	210 seconds
High	105 seconds

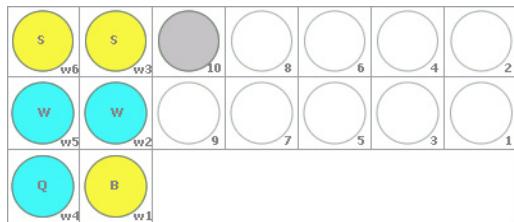
6. Check the **Autosave FCS 2.0** files check box if you want to save files in FCS 2.0 format for the selected wells/tubes.

**NOTE:** Keep in mind FCS files can take up significant space on your hard drive. Back up data regularly to keep your computer performing optimally. You can also export data.

7. Enter the number of acquisition replicates for the selected wells/tubes. To select only one acquisition per well/tube, select **No**.
8. Select the Acquisition Order. Choose either by Row or by Column. If you selected tubes for acquisition, samples in tubes will be acquired before the samples in wells. The recommended acquisition order is by Row.
9. Select the mixing features. If you wish to mix each sample before acquiring it. Then, select the length of time and speed you wish to mix each sample. The mixer speed and mix time can be applied to individual wells or tubes.
- NOTE:** Luminex recommends mixing at high speed for 3 seconds; although certain cell types may require a longer mixing time at the same or lower speed.
10. Select the appropriate (capillary/mixer) wash features. The options you select apply to the entire run (tubes and wells).
  - If you choose to wash the capillary only, place a 1.5-mL microcentrifuge tube filled with deionized water in tube position w5.
  - If you choose to wash the capillary and the mixer, place two 1.5-mL microcentrifuge tubes filled with deionized water in tube positions w2 and w5, and place two empty tubes in positions w3 and w6.

**NOTE:** To minimize carryover, Luminex recommends changing the wash water in w2 after every plate.

**NOTE:** When washing the capillary and mixer, change the tube in w3 at the end of every plate if it is more than a third full.



- a. For washing the capillary and mixer, load tubes filled with water in positions w2 and w5.
- b. Load empty tubes in positions w3 and w6.
- c. Load a tube with 100 µL 100% bleach in position w1 for backflushing.
- d. Always load a tube filled with water in position w4 for the Quick Clean, which is run automatically at the completion of the assay.
- e. When running Clean & Rinse, load tubes in the following positions:
  - filled with Guava® ICF in position 10
  - filled with water in position w4
  - containing 100 µL bleach in position w1

11. Select the cleaning option and frequency. If you choose Clean & Rinse, place a 1.5-mL microcentrifuge tube filled with Guava® Instrument Cleaning Fluid (ICF) in position 10. Ensure that the tube in w1 contains 100 µL of bleach for backflushing and the tube in w4 is filled with DI water for both Clean & Rinse and Quick Clean.

**NOTE:** You can use Clean & Rinse a total of four times before you need to refill the w4 tube with water and tube 10 with ICF, and empty then add 100 µL of bleach to the w1 tube for backflushing. Always remember to fill and/or empty these tubes after every four Clean & Rinse cycles or at the end of each run.

12. Lower the high concentration warning limits from the default values shown, if necessary. The software will display a warning message during acquisition if the cell concentration exceeds the concentration value you enter for the selected flow rate.



A screenshot of a software interface showing a text input field labeled "High Conc. Warning Limit (cells/µL)" with the value "500" entered. The input field is part of a larger panel with a light gray background.

**NOTE:** If your samples are heterogeneous in size or granularity, such as lysed whole blood, or do not contain cells simultaneously positive for two fluorochromes, such as PBMCs stained with CD3 and CD19, consider lowering the default high concentration warning limits (and acquiring your cells at lower concentrations). This will minimize the number of coincident events, which could adversely affect your results. Luminex recommends a concentration of less than or equal to 200 cells/µL for these types of samples.

13. Select the tube for parking the capillary.
  14. If applicable, proceed to the assay specific parameters. Enter the dilution factor (up to 200,000) and the original volume. The original volume is the volume of the cell suspension from which you took your sample aliquot, before you diluted or stained it.
- NOTE:** The dilution factor and original volume can be used when creating custom Expressions. Otherwise, they do not have an effect on Stat marker statistics.
15. (Optional) Click **Save** to save the file. You can save worklist files as .xml or .csv files.
  16. Click **Run Worklist** if you are ready to begin the assay.

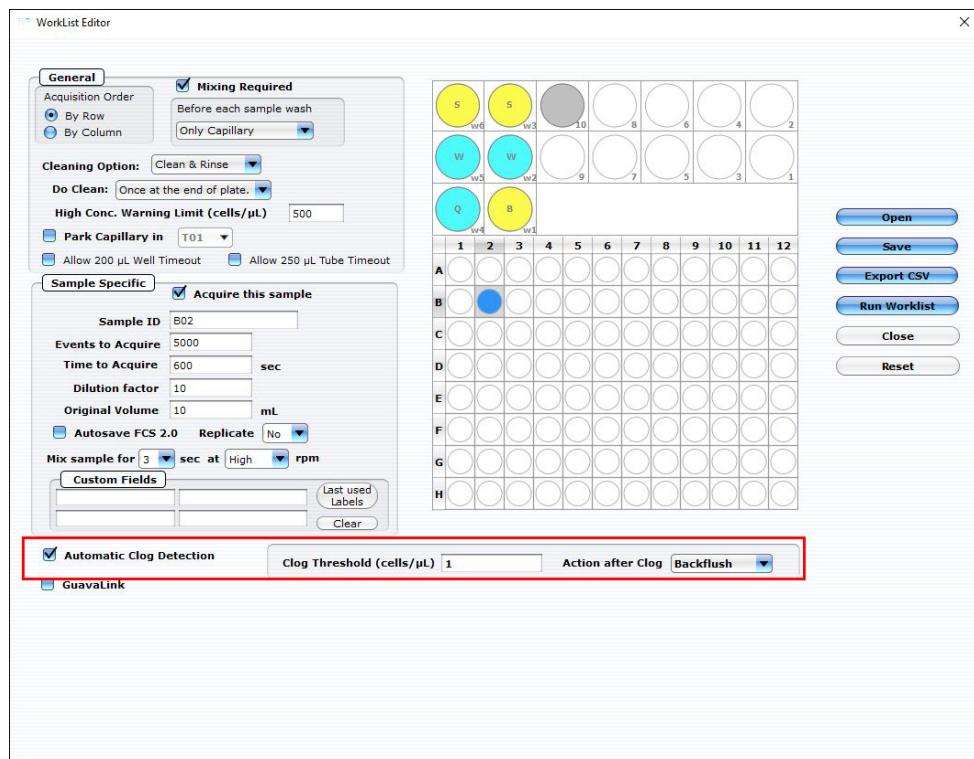
## Use Automatic Clog Detection

The Automatic Clog Detection feature allows you to specify the Clog Threshold (cells/µL) and the action taken after a clog, such as Backflush, QuickClean, or Abort.

After sample acquisition, if the cell concentration is below the Clog Threshold, the system beeps three times and the selected action is performed.

**NOTE:** The system will check for frequency of a clog. If a clog is detected on three consecutive sample runs, the Worklist is aborted.

1. From the Guava® InCyte™ module, click **Application > Worklist Editor**.
2. Select the **Automatic Clog Detection** check box.

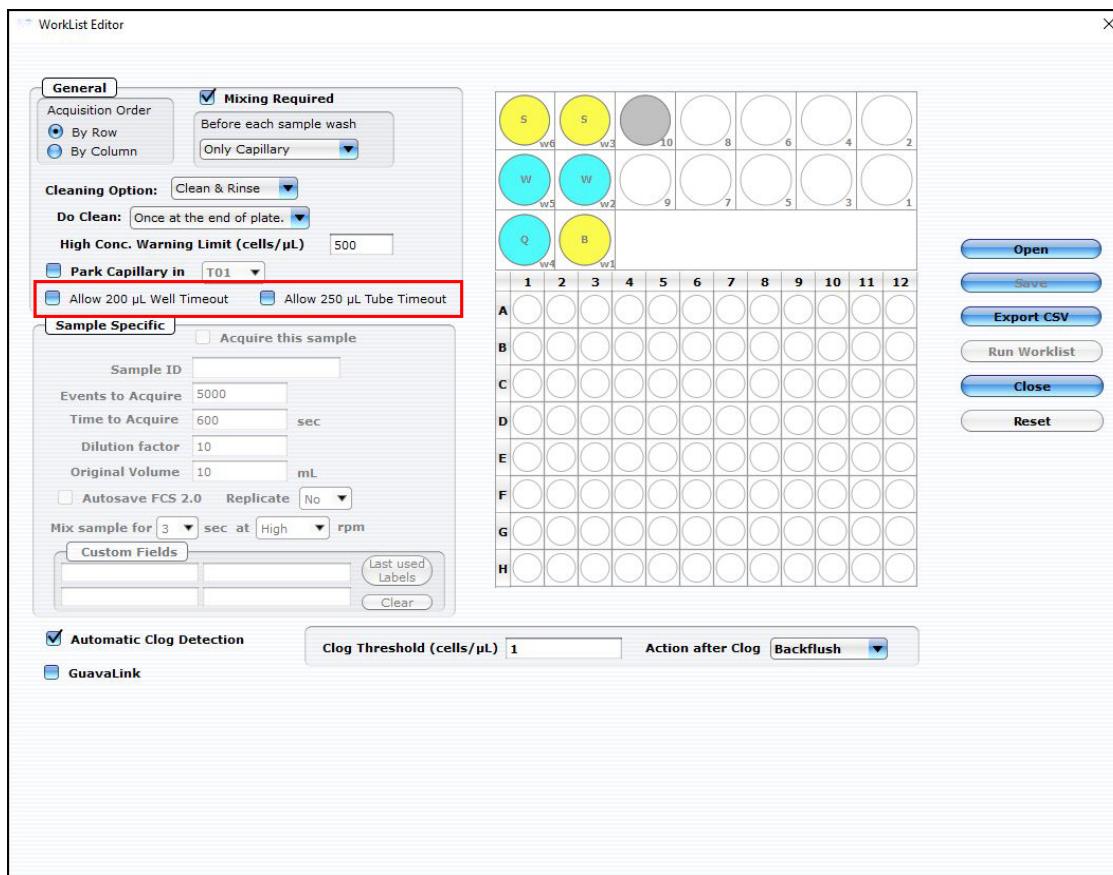


3. Enter the **Clog Threshold** in the **Clog Threshold (cells/μL)** field.
4. Choose the action needed after the clog is detected by clicking the **Action after Clog** drop-down menu. You can choose from **Backflush**, **QuickClean**, or **Abort**.

## Use Volume Timeout

If you have more events than can be reached in a normal volume timeout (150 μL in normal u-bottom plates), then the Volume Timeout feature allows you to run larger volumes (200 to 250 μL) and use the full pump stroke.

1. From the Guava® InCyte™ module, click **Application > Worklist Editor**.
2. Select the **Allow 200 μL Well Timeout** or the **Allow 250 μL Tube Timeout** check box.



## Create a Worklist Template

You can create a worklist template for InCyte only. The template can be modified and updated using Microsoft Excel or similar spreadsheet program. To create the template, you will export a worklist, then use Excel or other spreadsheet program to edit the fields. Then, simply import the file back into Worklist Editor for InCyte to run your worklist.

1. From the Guava® InCyte™ module, click **Application > Worklist Editor** to open the Worklist Editor.
2. Enter the desired settings into the WorkList Editor. This CSV file will serve as your template.
3. Click **Export CSV**. The Save WorkList File dialog box displays.
  - a. Choose a location to save the file.
  - b. Enter a **File name**.
  - c. Click **Save**.
4. Open the CSV file in Excel or compatible spreadsheet software.
  - Edit the Sample ID column, as necessary (up to 40 characters). A sample position can be designated for acquisition by making any alpha-numeric entry in the Sample ID field.
  - Do not modify the Sample Well column. This information pertains to the sample well or tube location.
  - The Sample ID, Sample Well, and General settings headers are required. If fields are blank or values are erroneous, a default value will appear and a message will indicate this.
  - To designate a sample for acquisition, enter a value in the Sample ID field.
  - If values are not entered for certain fields, default values may apply.

- If all values for a particular column are identical, the item and value can be added to General settings. If an item is mentioned in both the column and General settings, the column values are given preference.
  - If values entered are not valid (for example, non-numerical values in fields that require numbers), default values will apply. For InCyte, an error message appears, indicating the applicable cells.
  - Columns positions (headings) and the values in General settings can be reordered.
  - Templates for InCyte are unique in that they include columns for Mix speed and Mix time, allowing for sample-specific mix speed and mix time settings.
5. Save the file as a .csv in Excel or compatible spreadsheet software.
- NOTE:** Do not save it in Excel format (for example, .xls).
6. From the WorkList Editor dialog box, click **Open** to re-import the template that you saved above. The Open WorkList file dialog box displays.
- a. Navigate to the location where the .csv file was saved.
  - b. Click **Open**.

# Chapter 5: Preparing the System

## Connect the Guava® easyCyte™ HT System

Although the Guava® easyCyte™ HT System is a portable unit, it contains precisely aligned optical components that are sensitive to jarring movements. If you need to move the instrument, place the instrument on a stable surface in a dedicated location in the laboratory and allow at least 4 inches between the back of the instrument and the wall for proper ventilation. Maintain easy access to the power cord in case the instrument needs to be disconnected in an emergency. A Luminex service representative will perform the initial installation.



To avoid damage to the instrument, be sure to remove the shipping restraint before plugging in the instrument.

**NOTE:** If the instrument needs to be moved to a new location in the lab area or building, always use two people to lift and a sturdy transport such as a cart. If a longer distance move requiring pickup is needed, contact Luminex. A Relocation and Installation service is available for a fee.

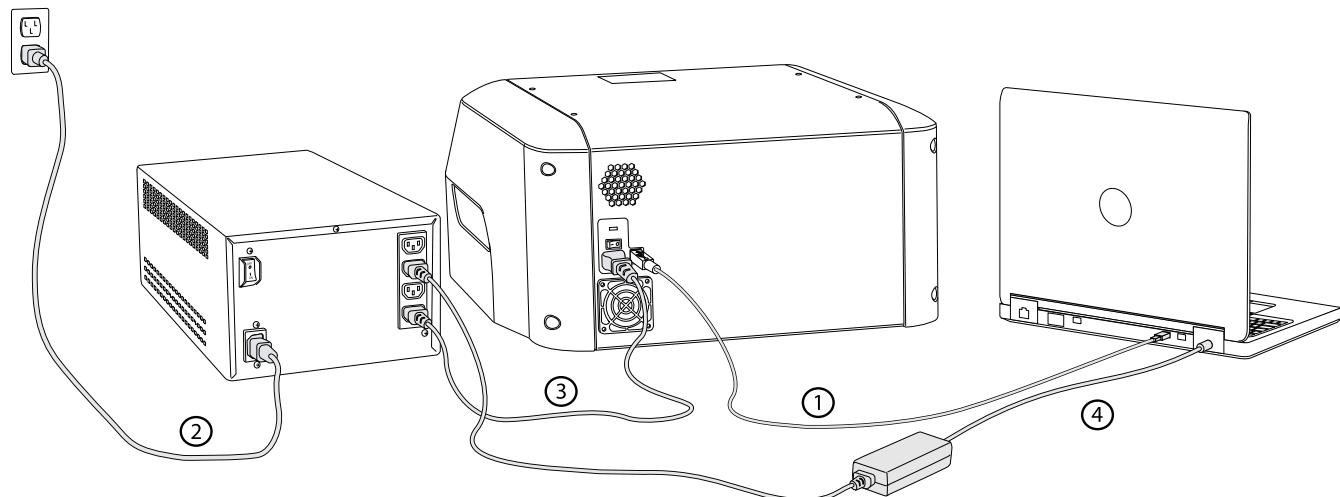
1. Connect the easyCyte Instrument to the laptop with the USB cable.
2. Plug the power cord from the power conditioner into a grounded (three-prong) AC power outlet.
3. Connect the extension cable to an available power outlet on the back of the power conditioner to the power input on the instrument.

**NOTE:** The power conditioner is required to meet electrical compliance.

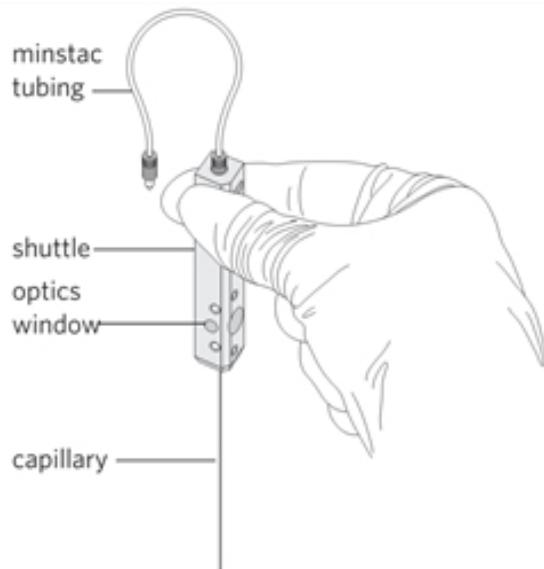
4. Connect the laptop power supply to the laptop. Plug the power supply into the power cord, then plug the power cord into the power conditioner.



The power conditioner is not a continuous power supply. Ensure that the instrument is powered on during acquisition.

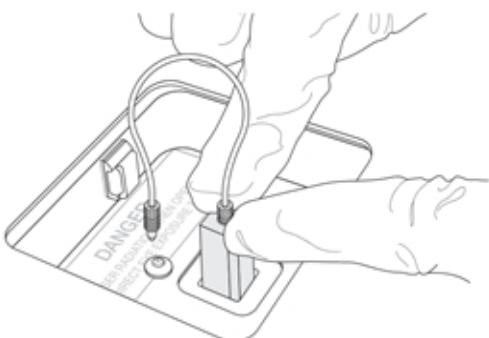


- To connect the laptop to a local network or the internet, contact your network administrator for assistance.
  - If connecting to a printer, ensure that you install the appropriate print drivers.
5. Connect the mouse cord to an open USB port on the laptop.
  6. Locate the waste vial and the cleaning solution vial.
    - a. Screw the waste vial to the cap assembly and snap the vial into the holder on the instrument.
    - b. Screw the cleaning solution vial to the cap assembly and snap the vial into the holder on the instrument.
  7. Remove the flowcell hatch and place it on top of the instrument.
  8. Install a new flowcell by correctly positioning it above the instrument and carefully lowering it into the receptacle.

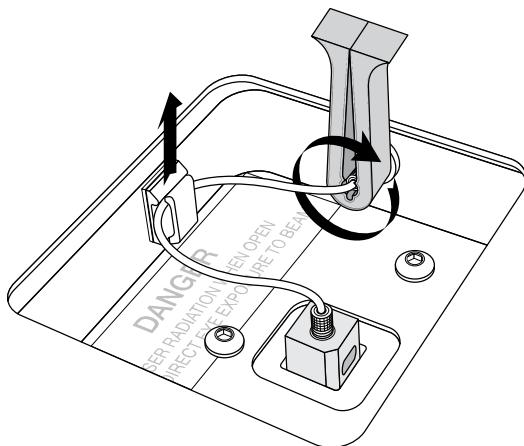


**NOTE:** The flowcell fits only one way into the receptacle. Keep the flowcell completely vertical and avoid bumping the capillary against the instrument or sides of the receptacle.

9. Use your fingers to press down on the top of the flowcell on either side of the minstac tubing until the flowcell clicks into place. Do not press down on the minstac tubing at the top of the shuttle.



10. Connect the tubing to the instrument. Make sure the tubing is screwed ion tightly. If necessary, use the tightening tool. Then insert the tubing into the clamp.



11. Replace the flowcell hatch.
12. Power on the power conditioner, the laptop, and the instrument. Then, start GuavaSoft software.
13. Prime the fluid system by clicking **Cleaning** from the GuavaSoft Software main menu and following the instructions to load tubes of deionized water and cleaning solution on the Guava easyCyte HT System.

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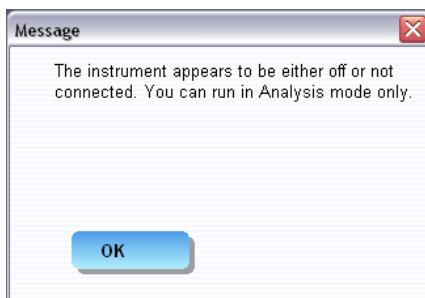
## Power on the Instrument

1. Turn on the power conditioner if it is not already on. Once it is powered on, it can remain on.
2. Turn on the laptop computer.
3. Turn on the Guava® easyCyte™ HT System. The power switch is located half-way up on the right side at the back of the instrument.
4. When the indicator lights turn on, double-click the **guavaSoft 4.0** application icon on the desktop.

**NOTE:** You can also navigate to the **Start** button > **Programs** > **Luminex** > **guavaSoft 4.0** > **guavaSoft**.

5. Ensure the cleaning solution vial is filled with Guava Instrument Cleaning Fluid (ICF) and the waste vial is filled with 5 mL of 100% bleach.

**NOTE:** If the software detects a communication problem with the easyCyte HT System or that the system is not turned on, the following message appears.



GuavaSoft Software will start but you will only be able to access an assay's analysis mode. If you wish to perform acquisition, exit GuavaSoft Software then restart it. If the message appears again, shut down the system. Ensure that the USB cable between the laptop and the instrument is securely connected before restarting the laptop. When the laptop is finished booting up, turn on the Guava easyCyte HT System. When the indicator lights are on, start GuavaSoft Software.

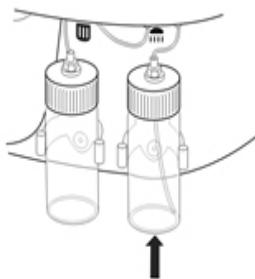
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## Maintain the System Fluids

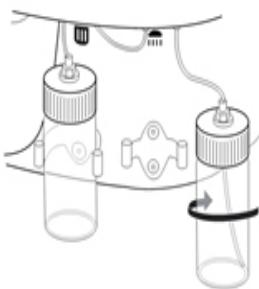
### Fill the Cleaning Solution Vial

Fill the cleaning solution vial with Guava® Instrument Cleaning Fluid (ICF) when it is approximately 1/4 full. The cleaning solution is aspirated through the tubing in the vial. Do not allow the vial to empty. Check the vial frequently to ensure it does not run dry. Letting the vial run dry will create air bubbles in the fluid system and require you to prime the system with water. One vial of cleaning solution will allow you to perform approximately 15 cleaning cycles.

1. Gently pull up on the cleaning solution vial to remove the vial from the bracket. The cleaning solution vial is on the right.



2. Unscrew the cleaning solution vial from the cap.



3. Fill the cleaning solution vial with Guava ICF to just below the bottom of the cap. Do not overfill the vial.
4. Screw the cleaning solution vial back to the cap assembly and install the vial on the Guava easyCyte™ HT System.

**NOTE:** Ensure the tubing that goes into the cleaning solution vial is still attached to the cap.

## Empty the Waste Vial

Empty the waste collection vial at the end of each day, or as needed.



Where exposure to potentially biohazardous specimens and materials, including aerosol, exists, follow appropriate biosafety procedures and use personal protective equipment (PPE). PPE includes gloves, gowns, laboratory 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 biohazardous material.

1. Gently pull up on the waste vial to remove the vial from the bracket. The waste vial is located on the left.
2. Unscrew the waste vial from the cap. Twist the luer-lock connector to remove the waste tubing from the vial cap.

**NOTE:** Fluid may seep from the cap while it is disconnected from the vial. To prevent waste fluid from dripping on the work surface, place the cap in a small container or on a disposable, absorbent pad.

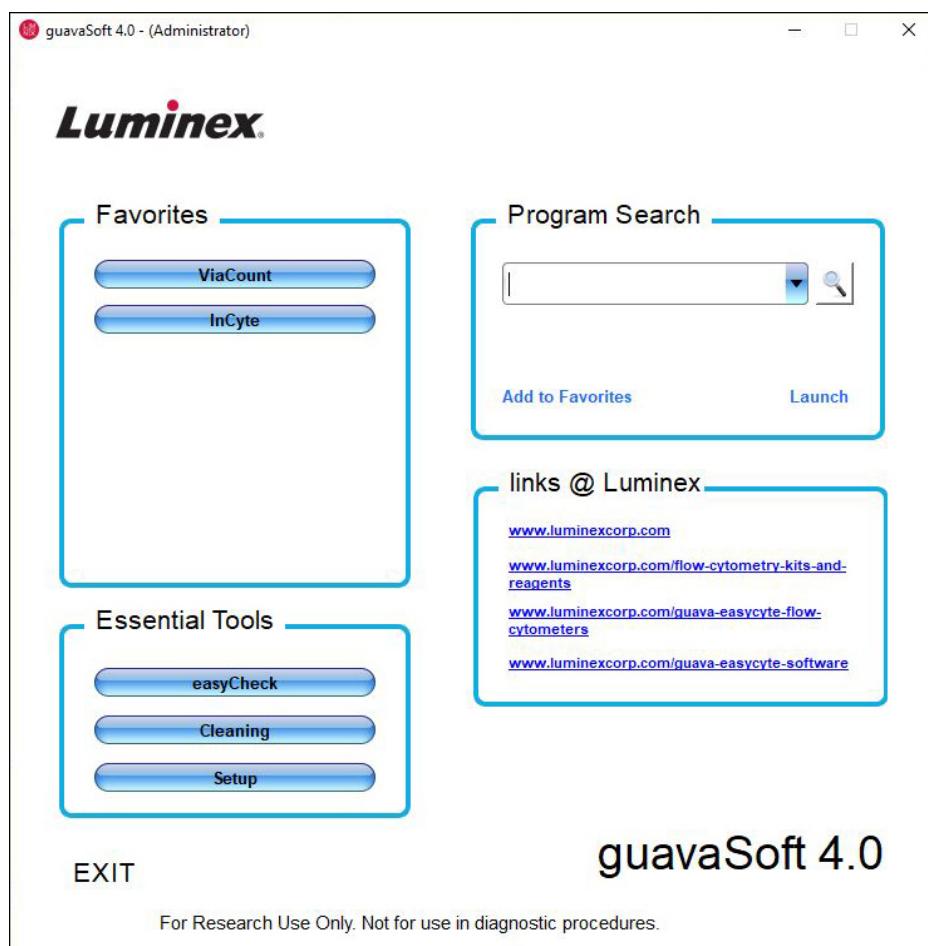
**NOTE:** If you notice a leak on top of the waste vial cap, tighten the connector on the cap.

3. Dispose of the contents according to your local and state biohazardous waste disposal guidelines.
4. Rinse the waste vial with water.
5. Add approximately 5 mL of 100% bleach to the waste vial, screw the vial back to the cap assembly and install the waste vial on the Guava® easyCyte™ HT System.

**NOTE:** Ensure the tubing that goes into the waste vial is still attached to the cap.

# Main Menu

The GuavaSoft Software Main Menu allows you to select an assay from either a list of favorites or the program search list. Essential Tools allows you to run Guava® easyCheck™, clean the instrument, or click Setup, where you can customize your list of favorites. Use links @ Luminex to quickly access Luminex websites for information.

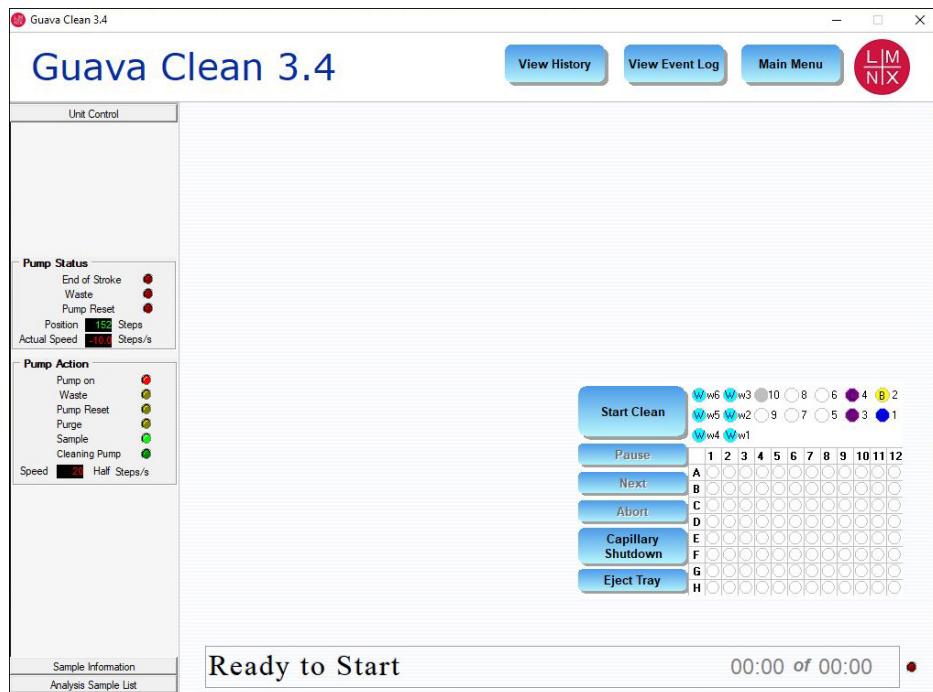


## Perform the Guava® Clean Procedure

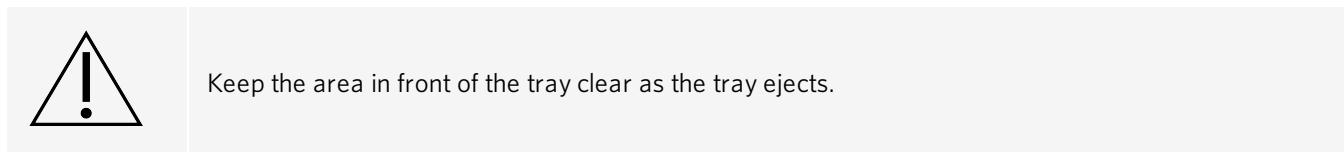
Run Guava® Clean to clean the instrument at the beginning and end of the day, as well as between assays if a thorough cleaning is needed. You can also use Guava Clean to prime the fluid system or if you suspect there is air in the fluid lines. Fill the cleaning solution vial and sample tube with deionized (DI) water and run Guava Clean to prime. Guava Clean takes approximately 15 minutes to complete. While the procedure is running, the lasers are turned off.

**NOTE:** If running samples that have high background, such as lysed whole blood, Luminex recommends running two complete cleaning cycles to flush the system of any residual sample.

1. From the GuavaSoft Software Main Menu, click **Cleaning**. The Guava Clean screen displays.

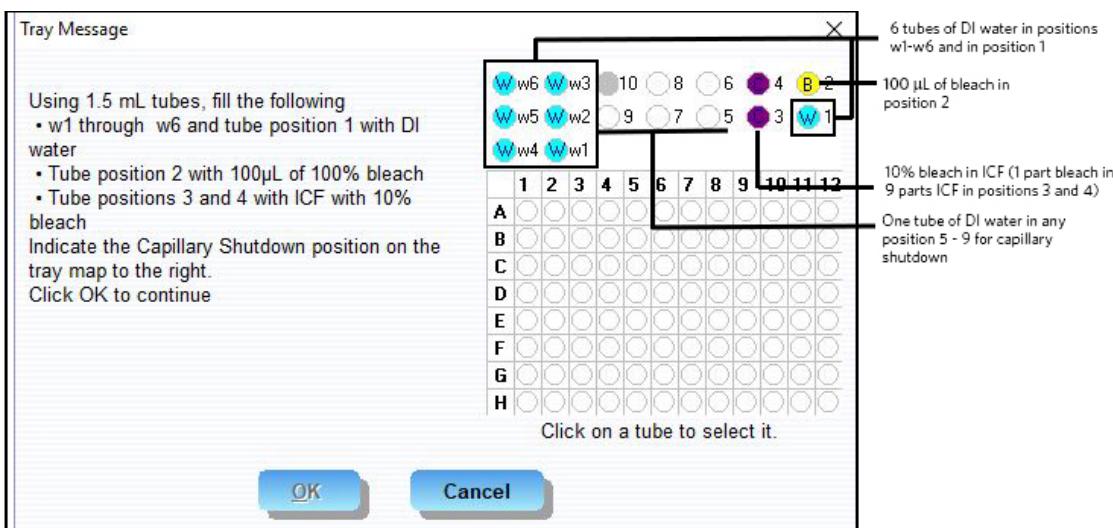


2. Click **Start Clean**. The plate is ejected.



3. Load the following tubes on the instrument:

- Load seven tubes filled with DI water in positions w1, w2, w3, w4, w5, w6, and tube position 1.
- Load a tube containing 100 µL of 100% bleach in position 2, indicated by the yellow well.
- Load two tubes with a mixture of 10% bleach solution and Guava ICF (1 part 100% bleach in 9 parts Guava Instrument Cleaning fluid (ICF)) in tube positions 3 and 4.
- Load a tube filled with DI water in any tube position 5–9 for the capillary shutdown. Then, click to select this position on the plate map.



- After selecting the tube position (5–9) for DI water for the capillary shutdown, click **OK**.



If you click **OK** or **Cancel** in the dialog box, the tray will automatically load. Keep the area clear as the trayloads.

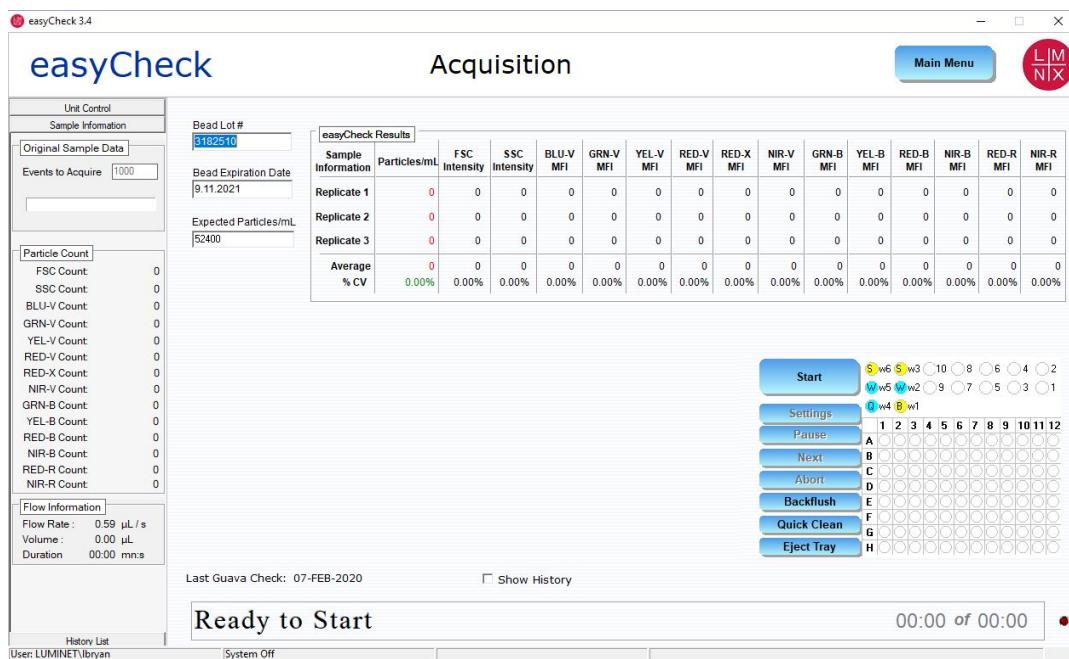
- If you are finished using the instrument, click **Eject Tray** and dispose of the used plate and tubes.
  - Perform the Shut Down the Capillary procedure to shut down the capillary with a tube of DI water to keep it moist while the instrument is not in use.
- NOTE:** If you will not be using the instrument for an extended time, perform the capillary shutdown procedure periodically using a fresh tube of DI water to ensure that the capillary does not dry out. Check the tube of water to ensure it has sufficient volume.
- Click **Main Menu** to return to the GuavaSoft Software main menu and continue working, or click **Exit** to close GuavaSoft Software.
- NOTE:** Do not reuse the tubes that were previously used for the Guava Clean procedure. After the cleaning cycle is complete, dispose of the tubes according to your local regulations.

## Run the Guava® easyCheck™ Procedure

Run the Guava® easyCheck™ procedure at the start of each day you use the Guava® easyCyte™ HT System to ensure the system is performing properly. easyCheck averages the results from three acquisitions of a Guava easyCheck Bead sample to determine if the results are within the expected range.

**NOTE:** Before running the Guava easyCheck procedure, perform a Quick Clean using deionized (DI) water to prime the fluid system. If you have not used the system in more than one day, perform two Quick Clean procedures using water to prime the fluid system.

- Prepare a 1:20 dilution of the Guava easyCheck Bead Reagent. Refer to the easyCheck Kit package insert for information.
- From the GuavaSoft Main Menu, click **easyCheck**. The easyCheck screen displays.



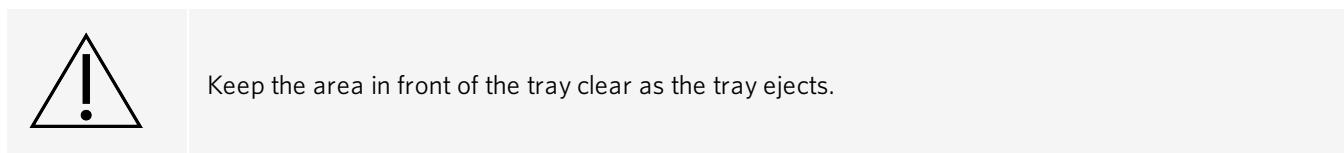
3. The first time you run the easyCheck procedure, enter the following in the appropriate fields:

- the easyCheck **Bead Lot #**, which can be found on the easyCheck card,
- the **Bead Expiration Date** (found on the easyCheck Beads Reagent box),
- the **Expected Particles/mL**, which can be found on the easyCheck card. Thereafter, enter any necessary changes to these values.

**NOTE:** The Expected Particles/mL is typically around 50,000, however check the information card that comes with the easyCheck Kit for the actual particle count for each new lot. The particles/mL corresponds to the concentration of beads in your prepared sample where the Guava easyCheck Bead Reagent was diluted 1:20 with Guava Check Diluent.

**NOTE:** Your system administrator may have configured GuavaSoft Software to require that you enter values in these fields each time you run the easyCheck procedure. If the fields are blank when you access the easyCheck screen, you must enter the current information.

4. Click **Start**.



5. The sample tray opens and a dialog box displays prompting you to load DI water, empty tubes, and to select the tube/well containing the beads.

- Load tubes filled with DI water in w2, w4, and w5.

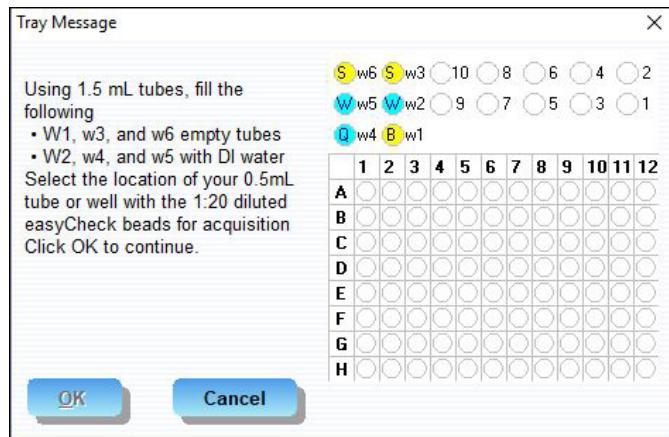
**NOTE:** Always load tube position w4 with water (for Quick Clean).

- Load empty tubes in w3 and w6.
  - Load one tube containing 100 µL of bleach in position w1 (for disinfecting backflushed fluid)
  - Load the easyCheck sample in a 0.5-mL tube or microplate.
  - Click to select the tube/well with beads.
6. Click **OK**.



If you click **OK** or **Cancel** in the dialog box, the tray will automatically load. Keep the area clear as the trayloads.

**NOTE:** If you are using a microplate, make sure well A1 of the plate is in the top-right corner of the tray.



**NOTE:** The system then automatically performs the adjust settings function and acquires three replicates of the bead sample and displays the Particles/mL, FSC Intensity, SSC Intensity, and mean fluorescence intensity (MFIs) for each replicate. The averages and %CVs for all results are also displayed.

## Results

### Guava® easyCheck™ Results

The easyCheck™ Software displays the %CVs and the averages for the particles/mL (bead count), FSC and SSC intensities, and all mean fluorescence intensities (MFIs) for the three replicates.

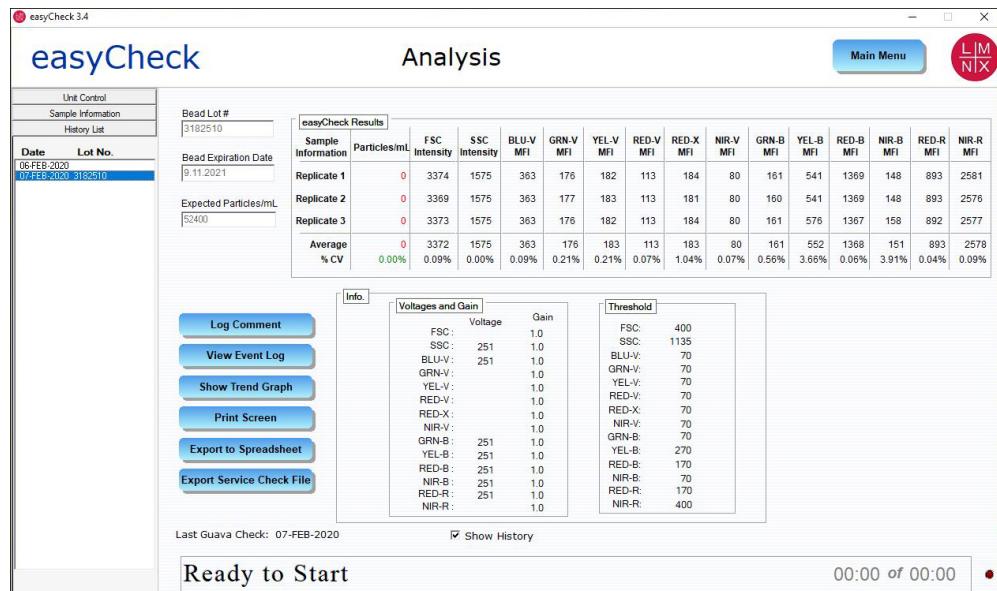
- If any result for Particles/mL falls outside  $\pm 10\%$  of the expected value, the result is outside the acceptable range and appears in red. For example, if the actual particle count is 50,000, the acceptable Particles/mL range ( $\pm 10\%$ ) is 45,000 to 55,000.
- If any result for MFI falls outside  $\pm 15\%$  of the expected value, the result is outside the acceptable range.
- If the %CV for Particles/mL is  $> 10\%$ , it appears in red.
- The %CV for FSC and SSC Intensities and MFIs for the three replicates should be  $< 5\%$ .

easyCheck Results													
Sample Information	Particles/mL	FSC Intensity	SSC Intensity	BLU-V MFI	GRN-V MFI	YEL-V MFI	RED-V MFI	GRN-B MFI	YEL-B MFI	RED-B MFI	NIR-B MFI	RED-R MFI	NIR-R MFI
Replicate 1	48913	1232	3427	3090	1021	1029	1001	811	1627	1011	505	1034	2531
Replicate 2	48406	1230	3440	3085	1019	1027	991	810	1625	1009	501	1019	2482
Replicate 3	48710	1230	3431	2982	1027	1021	982	818	1644	1007	475	1015	2377
Average	48676	1230	3433	3052	1022	1025	991	813	1632	1009	493	1023	2463
% CV	0.52%	0.09%	0.19%	1.98%	0.44%	0.41%	0.94%	0.54%	0.63%	0.17%	3.31%	0.98%	3.20%

- Refer to the information card that comes with the Guava® easyCheck™ Kit for the acceptable intensity ranges for each parameter. This information may change from lot to lot.
- To monitor the instrument performance, look at the average and %CV values for FSC and SSC Intensity, and mean fluorescence intensities (MFIs) for each channel for each replicate.
- If the Particles/mL (count) for a replicate or the average falls outside the acceptance range, or if an intensity value is outside the acceptable range, run Guava easyCheck Quick Clean or Clean & Rinse. Rerun the easyCheck Procedure after cleaning is complete.
- If issue persists, refer to topic Guava® easyCheck™ Procedure Troubleshooting.
- If the signal intensity for any of the parameters shows significant drift over time beyond the range listed, and this change is not correlated to a change in the bead lot, a new flowcell, or instrument service, contact Luminex Technical Support.

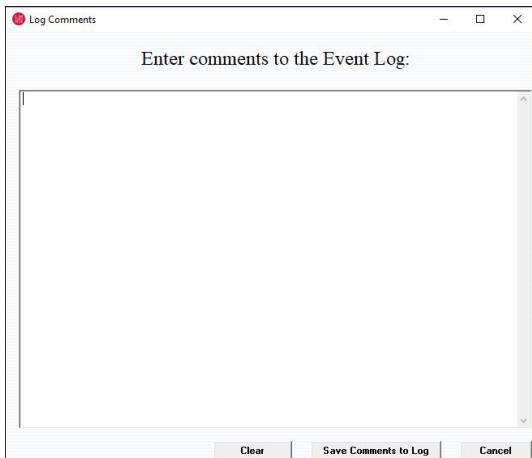
## View the Guava® easyCheck™ Results

- To display a history of all Guava® easyCheck™ runs and view the results for individual runs, select the **Show History** check box at the bottom of the easyCheck screen. The **History List** panel opens showing a list of all easyCheck Procedure runs. To display the results for a particular run, click on the run in the list.

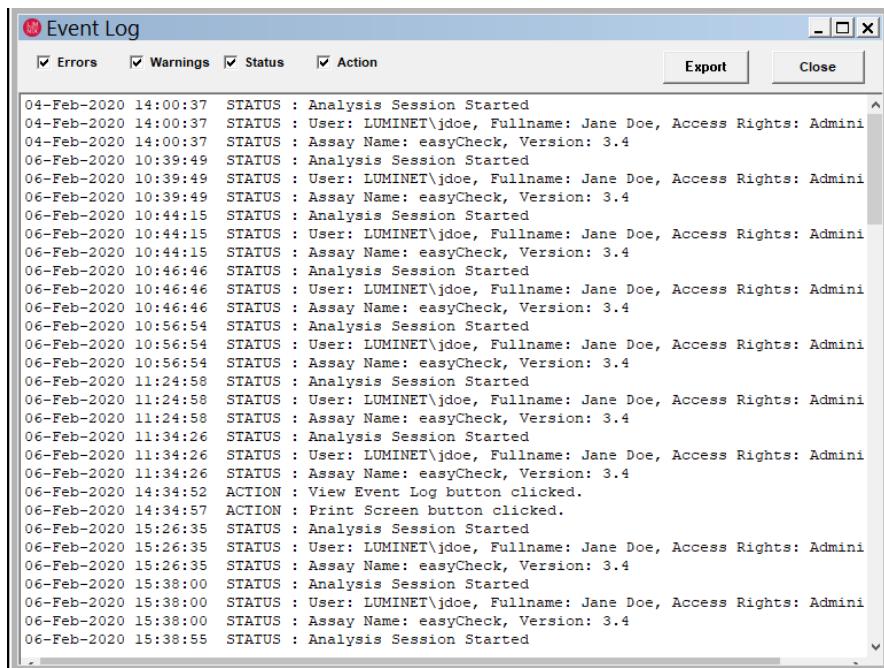


- Click on a run in the **History List** panel to display the results for that run.
- Click **Log Comment**. The Log Comments dialog box displays.
  - Enter comments related to the run.

b. Click **Save Comments to Log**.



4. Click **View Event Log** to display the Event Log dialog box, which lists **Errors**, **Warnings**, **Status**, and **Action** that occurred during the easyCheck run.
5. Once you are done viewing the results, click **Close**.



6. Click **Show Trend Graph** to display the **Check Trend Graph** of the Particles/mL value from the last 30, 60, or 90 runs.

## Export the Guava® easyCheck™ Results

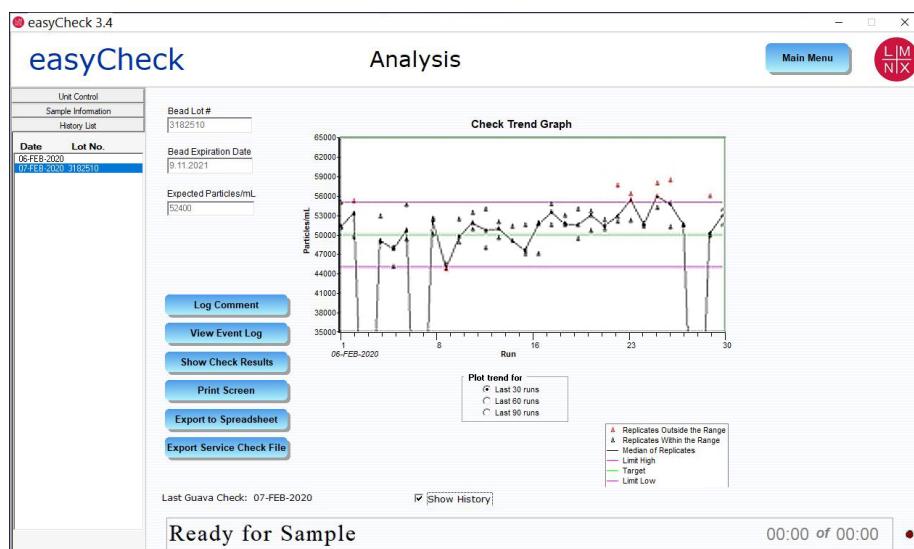
- From the Guava® easyCheck™ Analysis screen, click **Export to Spreadsheet** to export the data from all easyCheck runs to a spreadsheet file. The **Export History List Dialog** box displays.  
**NOTE:** The file contains the average and %CV for each parameter, as well as the details for each replicate of all runs.
- Choose a location to export the file to, enter the **File name**, and click **Save**.

## Print the Guava® easyCheck™ Results

- From the Guava® easyCheck™ Analysis screen, click **Print Screen** to print the results shown on the screen. A **Print** dialog box displays.
- Choose the printer you want to print to and click **OK**.  
**NOTE:** Ensure the printer you want to print to is already setup.

## View and Generate Trend Data

- From the GuavaSoft Software Main Menu, click **easyCheck**.
- From the Guava® easyCheck™ Acquisition screen, select the **Show History** check box at the bottom of the screen. The easyCheck screen will switch to Analysis mode.
- Then click **Show Trend Graph**. A trend graph displays showing the Particles/mL results from the last 30, 60, or 90 runs. A data point appears for each of the three replicate values. The date appears for every seventh or eighth time that the Guava easyCheck procedure was run.



A legend in the lower-right corner of the window lists the information found on the graph. A description of the items in the legend appears in the following table.

Legend Item	Description
Replicates Outside Range	data point appears as a red triangle (value falls outside the high or low, 10% limit lines)
Replicates Within Range	data point appears as a black triangle
Median of Replicates	a black line connects the median values from each triplicate
Limit High	pink line that appears 10% above expected particle count
Target	green line at the expected particle count entered
Limit Low	pink line that appears 10% below expected particle count

# Chapter 6: Acquiring the Samples

## Guava® InCyte™ Introduction

The Guava® InCyte™ Software module was developed to be an open assay module providing all the basic tools for sample acquisition and data analysis. The Guava InCyte module allows you to acquire and analyze up to 12 fluorescence parameters in combination with forward scatter (FSC) and side scatter (SSC), as well as area, width, and time.

The Guava easyCyte™ Instrument is configured to detect the following fluorochromes or fluorochromes with similar fluorescence. The filter is listed first, followed by the laser. For example, Blue-Violet (BLU-V) is the filter for the blue fluorescence emission off the violet laser.

Filters off the violet laser:

- Blue-Violet (BLU-V) parameter - DAPI, Brilliant Violet 421, Pacific Blue, Cascade Blue, or Alexa Fluor™ 405
- Green-Violet (GRN-V) parameter - Brilliant Violet 510 or Pacific Green
- Yellow-Violet (YEL-V) parameter - Brilliant Violet 605 or QDot 565
- Orange-Violet (ORG-V) parameter - Brilliant Violet 605
- Red-Violet (RED-V) parameter - Brilliant Violet 650 or eFluor 650
- NIR-Violet (NIR-V) parameter - Brilliant Violet 750

Filters off the blue laser:

- Green-Blue (GRN-B) parameter - FITC, GFP, or Alexa Fluor™ CF-488
- Yellow-Blue (YEL-B) parameter - Phycoerythrin (PE)-based, Cy3, Alexa Fluor™ 532, propidium iodide (PI), TRITC, and DS Red reagents
- Red-Blue (RED-B) parameter - PE-Cy5, PerCP, 7-AAD, PE-Cy5.5, and PI
- NIR-Blue (NIR-B) parameter - PE-Cy7

Filters off the green laser:

- Yellow-Green (YEL-G) parameter - Cy3, RFP
- Orange-Green (ORG-G) parameter - mCherry, PE-Texas Red
- Red-Green (RED-G) parameter - PE-Cy5
- NIR-Green (NIR-G) parameter - PE-Cy7

Filters off the red laser:

- Red-Red (RED-R) parameter - Allophycocyanin (APC), Cy5, or Alexa Fluor™ CF-647.
- NIR-Red (NIR-R) parameter - APC-Cy7, or APC-Alexa Fluor™ 750

# Guava® InCyte™ Acquisition Screen

The Guava® InCyte™ Acquisition Screen opens displaying three plots. A tool bar at the left edge of the screen allows you to access most of the acquisition controls. You can find these same options in the Tools menu in the menu bar.

The Analysis and Acquire control panel displays the controls for acquiring and analyzing data.

- Click **Analyse** or **Acquire** to display the desired control panel.
- Click in the top bar (above the Analyse and Acquire buttons) to drag the floating control panel to any area of the screen or dock it on the right side of the window.
- To redock it on the left, double-click the bar at the top of the panel.
- To hide the control panel, click the X in the top-right corner.
- To redisplay the panel, choose **Tools > Show/Hide Acquisition Controls** from the menu bar, or click the **Acquisition Controls** icon in the tool bar on the left edge of the acquisition screen. Place your cursor over any button in the Analyse or Acquire button panel to see text describing the button.

## Set Up the Plot Layout

Use the Guava® InCyte™ Plot menu in the menu bar to set up the plot layout. Up to 24 plots can be displayed at one time.

1. From the Guava InCyte Acquisition screen, navigate to **Plot** on the menu bar.
2. Choose the number of plots to display.

**NOTE:** 2x2 to 5x5 represent the number of plots displayed, down vs. across.

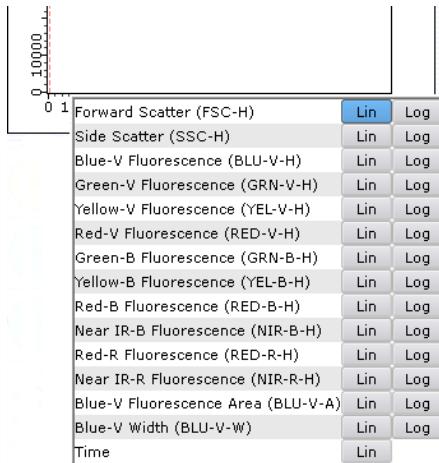
## Plot Tool Bar

- Use the **Undock** icon to detach and enlarge the plot. The plot will detach in its own floating window. Drag the bottom-right corner to increase the size of the plot window. Copy the zoomed plot to the clipboard for higher resolution plots. To reattach the plot, click the **Undock** icon again or the X in the top-right corner.



- Use the **Plot type** icon to select **Dot Plot**, **Histogram**, or **Contour Plot**, and to adjust **Plot Settings** such as smoothing histograms and contours and selecting the dots to display in dot plots. Contour plots can be used only during analysis.
- Use the **New region** and **Plot gate** icons to create regions and apply gates, respectively.

- Use **New Stat Marker** to obtain statistics for any existing regions in the plot. Or, you can create a new region and obtain statistics for that region. The **Edit overlay list** allows you to superimpose histograms from multiple samples derived from one data file.
- To change the plot parameters once data is displayed, click the parameter name and select the new parameter from the dialog box.



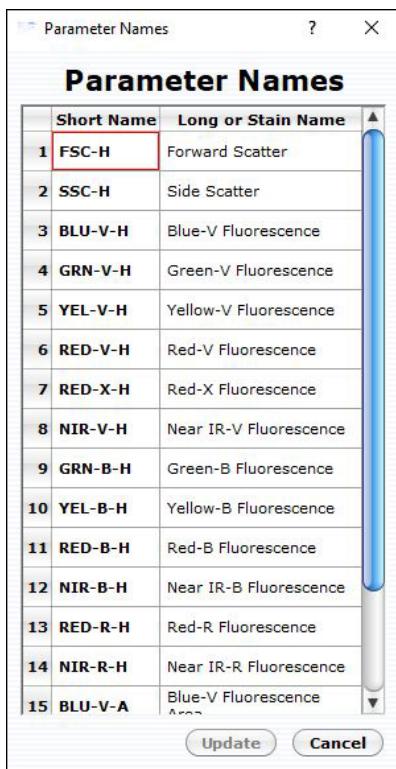
- To copy a plot to the clipboard, right-click the plot and select **Copy Plot to Clipboard**.
- Use the **Zoom** icon to zoom an area of the plot. Click and drag a rectangle in any direction to encompass the population you wish to zoom in on. To scroll the data in the plot, click the icon, then hold down the **Shift** key while you click and drag. To unzoom, click the **Zoom** icon again, then press and hold the **Ctrl** key while you click anywhere in the plot.

## Change Parameter Names

You can change the default long (or stain) name of any parameter. The short name, for example, GRN-B-H, is fixed and will still appear in the plots in parentheses after the new long name.

1. From the GuavaSoft Software Main Menu, navigate to **Favorites > InCyte**. The InCyte Acquisition screen displays.
2. Click **Application > Change Parameter Names** from the menu bar. The **Parameter Names** dialog box displays.

**NOTE:** Area and width are listed only if they were selected during the adjust settings step prior to acquisition.



3. Double-click the existing name in the **Long or Stain Name** text field. Enter in the new name, or use the tab key to highlight the desired field and begin entering the new name.
4. Click anywhere in the **Parameter Names** dialog box to activate the **Update** button. Click **Update**.

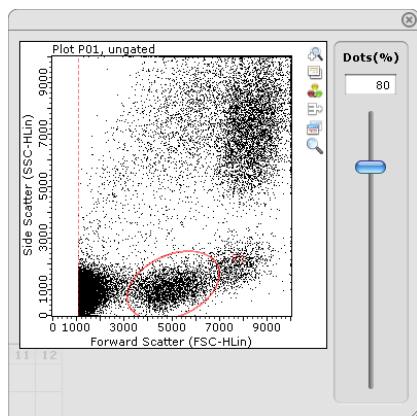
**NOTE:** You can now see the new name display under the plots.

## Change the Display of Data in Plots

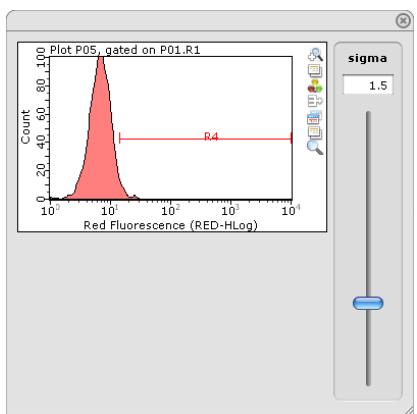
You can make the following changes to the display of data in these plots. The settings are saved with the Analysed Group.

1. From the Guavasoft Software Main Menu, navigate to **Favorites > InCyte**. The InCyte Acquisition screen will display.
2. Locate the plot layout you want to change the display of data in. Click the **Plot Type** icon from the Plot tool bar.
3. Choose **Plot Settings**.

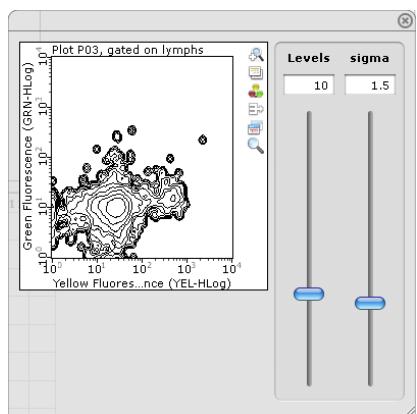
- Use the **Dots(%)** slider to set the number of dots to display from 1 to 100%. The default is 100%.



- Smooth histograms. Use the **sigma** slider to set the amount of smoothing (sigma) from 0 to 5.0, where 0 is no smoothing. The default is 1.5.



- Smooth contour plots. Use the **Levels** slider to choose the number of levels from 5 to 20. Use the **sigma** slider to set the amount of smoothing (sigma) from 0 to 5.0, where 0 is no smoothing. The defaults are 10 and 1.5, respectively. Contour plots can be created during analysis only.

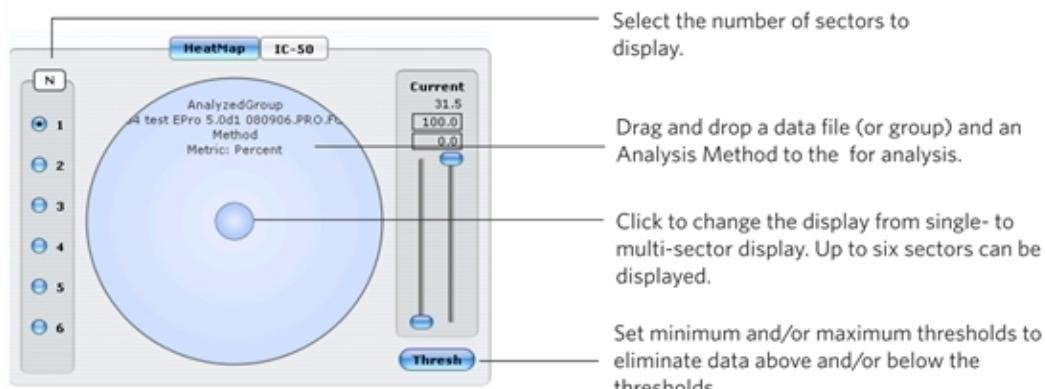


## Pie Legend

The Pie Legend contains the HeatMap legend and the IC-50 application. The HeatMap legend (circle) is used to display the data results for each Analysed Group (data + Method) in the plate map. Use the **Show Pie Legend** icon in the tool bar to display this workspace.



The HeatMap legend shows one sector by default. You can create up to six sectors for six experiments. Each sector will have a data file (or group) paired with an Analysis Method. To divide the legend into multiple sectors click N=2, 3, etc. To clear a sector, right-click it and choose **Clear Sector**.

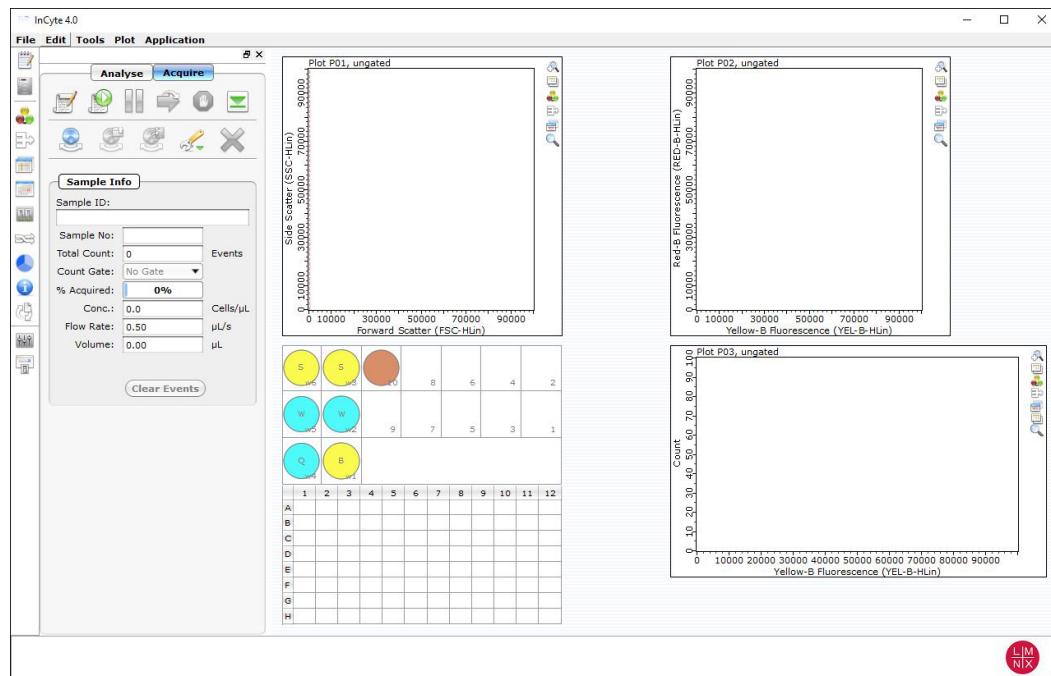


- **N** allows you to select the number of sectors you want to display. Sectors are numbered 1 to 6 with each new sector entering the upper-left portion of the circle.
- **Thresh** allows you to apply upper and/or lower limits on statistical values to eliminate data above or below the thresholds. The **Current** panel is only functional when one sector is being displayed, or when multiple sectors are used but the HeatMap legend is in single-mode display.
- The IC-50 feature allows you to rapidly convert time course or dose response studies into standard curves for the calculation of compound IC50/EC50 values.

## Acquire a New Dataset

1. From the Guavasoft Software Main Menu, navigate to **Favorites > InCyte**. The InCyte Acquisition screen displays.

**NOTE:** The Guava InCyte window opens in Acquisition mode, if the easyCyte HT System is turned on. The top portion of the control panel contains 10 buttons used to control acquisition, adjust, retrieve and save instrument settings, and select cleaning options.



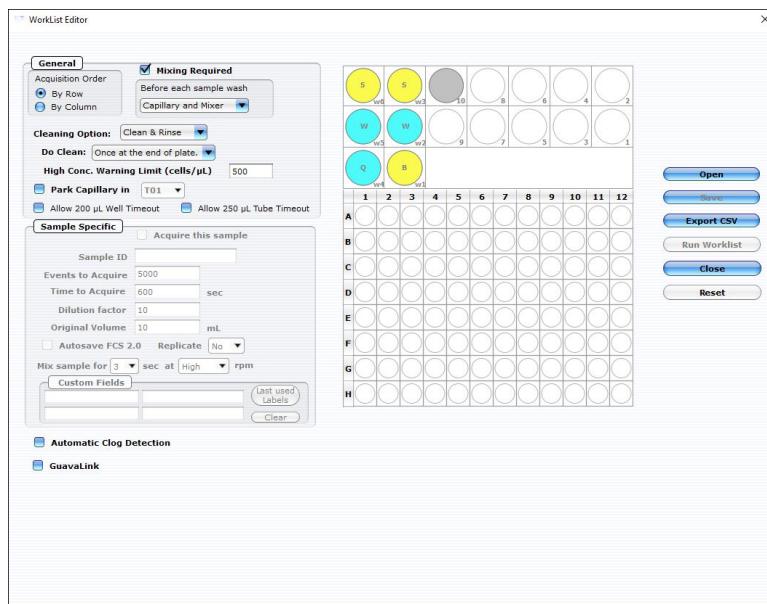
2. Prepare samples for acquisition in a microplate or sample tubes.
3. Click the **Edit Worklist** button, the first button in the Button panel, or select **Application > Worklist Editor** to open Worklist Editor and define the worklist parameters.

**NOTE:** You can also open an existing worklist.

- a. To open an existing worklist, click the **Start Worklist** button from the Button panel. The **Open WorkList File** dialog box displays.
- b. Choose between opening a saved worklist or running the currently loaded worklist. InCyte worklists are .xml files.



- c. Or, from the WorkList Editor, click **Open**.
- d. Choose the WorkList file and click **Run Worklist**. The tray ejects and a dialog box displays prompting you to enter a data set file name.



4. Define the worklist parameters in WorkList Editor software and click **Run Worklist**. When you start a worklist, the sample tray ejects. The **Prepare For Acquisition** dialog box displays allowing you to select a data file name and location, Analysis Method, and instrument and compensation settings.



- a. Click **Select Output FCS file** to save the file. The **Create New Session File** dialog box displays. Navigate to the location you want to save the file to, and click **Save**.

**NOTE:** The default storage location is in My Documents. The default file name is the date and time.

- b. For **Analysis Method**, click **Choose**. The **Open Method File** dialog box will display. Navigate to the .gsy or .fcs file you want to open, and click **Open**. The Prepare For Acquisition dialog box will populate Settings and Compensation from the file you chose for Analysis Method.
- The FCS or Method file must be from InCyte, version 3.0, or later. If you do not choose a Method file, a default one will be created automatically.

- If you select an FCS file to obtain the Method, you can also retrieve the instrument settings and compensation settings that were saved with that FCS file.
- c. For **Settings**, click **Choose**. The **Read Settings** from dialog box displays. Navigate to the .gst or fcs file you want to open, and click **Open**.

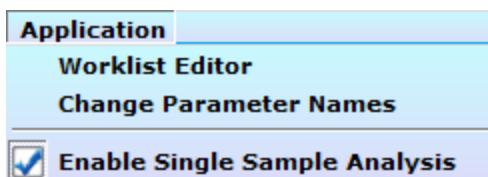
**NOTE:** The FCS or instrument settings file must be from InCyte, version 3.0, or later..

- d. For **Compensation**, click **Choose**. The **Read compensation settings from** dialog box displays. Navigate to the .gst or fcs file you want to open, and click **Open**.

**NOTE:** The FCS or instrument settings file must be from InCyte, version 3.0, or later.

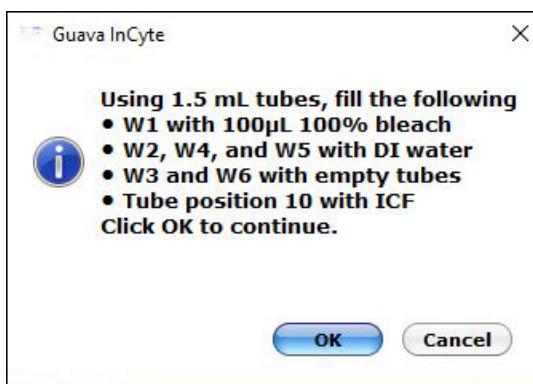
**NOTE:** Always save GuavaSoft data files directly to the system's hard drive during acquisition. Saving data to a network or location other than the computer's hard drive may result in data loss. You may copy the files to another location when acquisition is complete.

**NOTE:** When appending to a file, you can use the Single Sample Analysis mode. This allows you to quickly open large files that have been analyzed without having to calculate the group stats for each sample in the data set. Select **Enable Single Sample Analysis** from the Application menu prior to opening the data file.



5. Click **Adjust Settings**. If you retrieved instrument settings and you want to skip the adjust settings step, click **Acquire** to begin acquiring the first sample.

**NOTE:** Whether you are ready to acquire or adjust settings, a dialog box displays prompting you to load your samples.



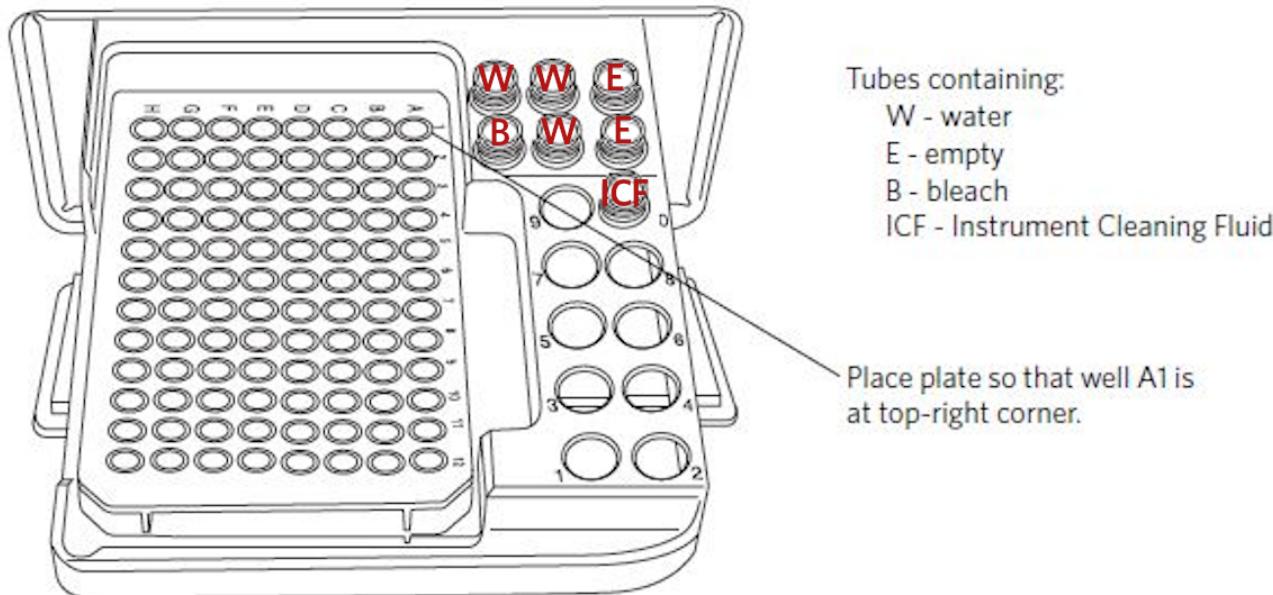
6. Place the microplate or sample tubes, as well as the cleaning tubes in the tray. Make sure well A1 of the plate is in the top-right corner. Load the following 1.5-mL microcentrifuge tubes in these positions:

- Load tubes containing 1.5 mL of water in positions w2, w4, and w5 (for Quick Clean and washing the capillary and mixer).

**NOTE:** Ensure that the tube in position w4 (for Quick Clean) is filled with water.

- Load empty tubes in positions w3 and w6 (for spinning/drying the mixer).
- Load a tube containing 100 µL of 100% bleach in position w1 (for a backflush) to disinfect material deposited from a backflush.

- Load a tube containing 1.5 mL of ICF in position 10 for Clean & Rinse.
- Load a tube for parking the capillary.



7. Click **OK** in the dialog box after you are finished loading samples and cleaning tubes to load the sample tray.

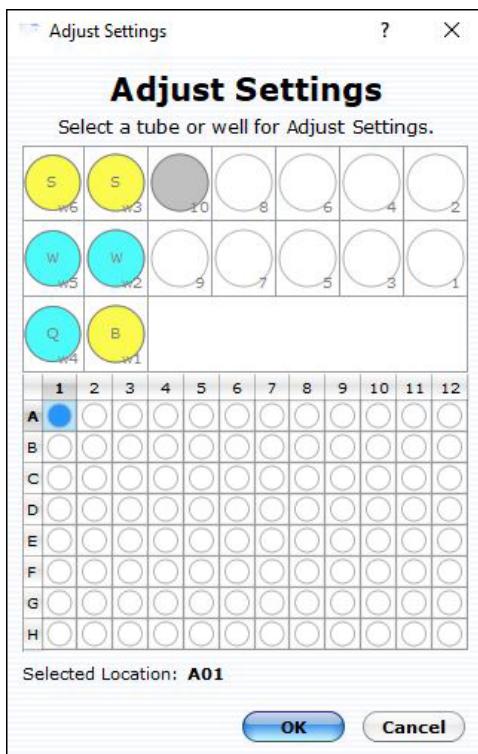


Keep the area clear as the tray loads.

Always use the **Eject Tray** button in the InCyte software module to open the door. Click **Pause** first, if necessary. Never open the door with your fingers.

## Adjust the Instrument Settings and Acquire Data

1. A dialog box displays prompting you to select the sample for adjusting settings. Click to select the well or tube (1-9) used to adjust settings, then click **OK**. Luminex recommends using a stained negative or isotype control sample for the initial adjustments. You can choose to skip the adjust settings step even if you did not retrieve settings, however Luminex recommends always performing the adjust settings step.



- Check the **Conc** (Cells/ $\mu$ L) value in the **Sample Info** pane and ensure that it is less than or equal to 500.

**NOTE:** If the value is greater than the high limit for the corresponding flow rate, dilute the sample with the appropriate buffer to lower the concentration and minimize the risk of coincident events. For optimal performance, Luminex recommends a concentration of 250 cells/ $\mu$ L or lower.

- To fine tune the settings, you can make the following adjustments using the Gain, Compensation, and Miscellaneous Controls. Open each instrument adjustment window using the Tools menu or the icons in the tool bar (left edge of the application window). If necessary, click the window's title bar to drag the window to a new location.



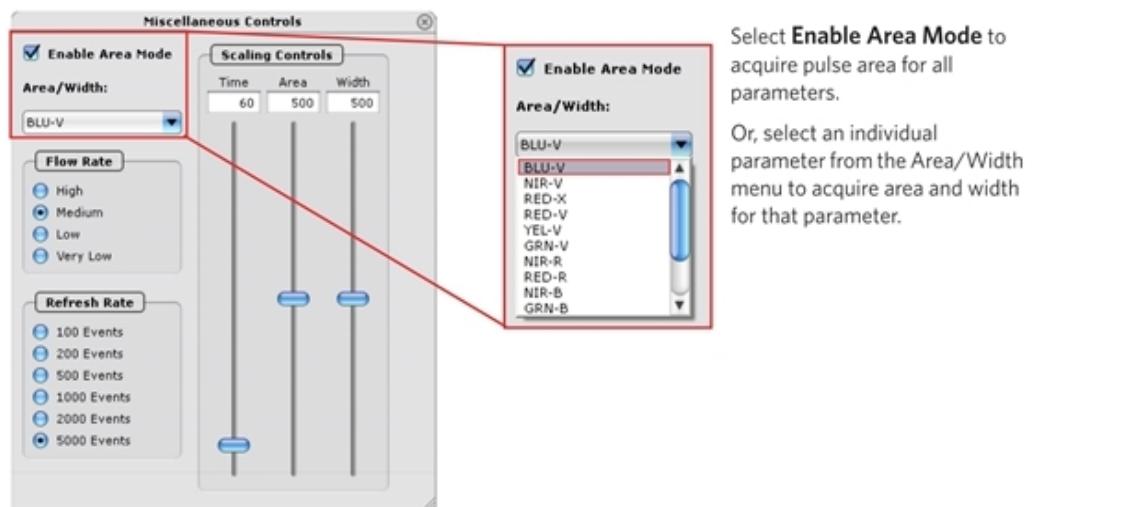
- Click the **Miscellaneous Controls** icon. The **Miscellaneous Controls** dialog box displays.
  - Select **Very Low** (0.12  $\mu$ L/s), **Low** (0.24  $\mu$ L/s), **Medium** (0.59  $\mu$ L/s), or **High** (1.2  $\mu$ L/s) to set the Flow Rate. The default flow rate is Medium.
  - Select the maximum number of events radio button to set the **Refresh Rate**. The maximum number of events (100–5,000) you selected will display.

**NOTE:** If you change the flow rate during the adjust settings step, Luminex recommends that you repeat the adjust settings step at the new flow rate to ensure that the markers and threshold are set correctly.

- iii. If you want to save area measurements for all parameters, select **Enable Area Mode**. Area will be acquired for all parameters. The threshold parameter, which is always height, will display in height (H) at the bottom of the plot parameters list. Use the Area/Width menu if you want to acquire width for one parameter.

If you want to acquire area for only one parameter, do not select the **Enable Area Mode** check box.

Instead, select the parameter from the Area/Width menu. The area and width for the selected parameter will be acquired. Use the Area and/or Width Scaling Control sliders to reduce or amplify the signal so that the cells are visible and on scale.



- iv. The time parameter is saved automatically. Use the **Time Scaling Controls** slider to set the maximum time (in seconds) to record. Adjust the y-axis (count) scale on the plot, if necessary.
- v. Set up the type of plots and parameters you wish to display. If you retrieved a Method file, the plots are configured with the regions and gates defined in that Method. If modifications are necessary to accommodate the new data, navigate to the **Plot** menu in the menu bar. Choose the number of plots to display.

**NOTE:** 2x2 to 5x5 represent the number of plots displayed, down vs. across.

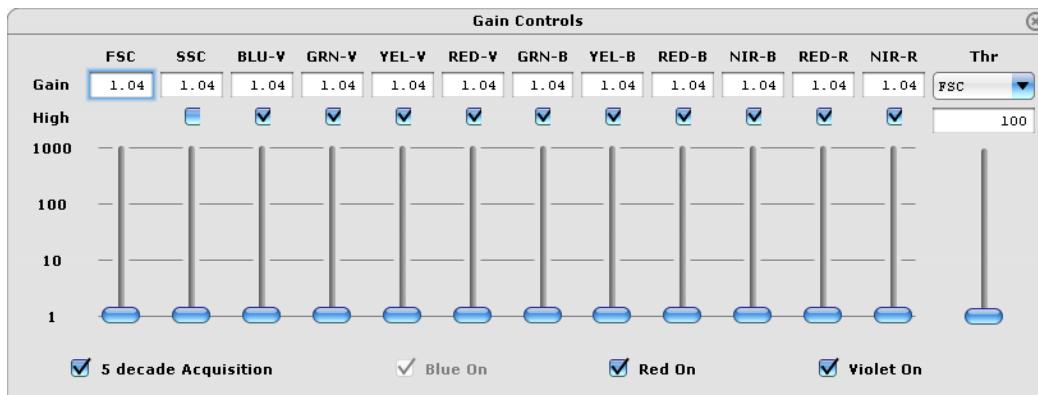
- b. Click the **Gain Controls** icon. The **Gain Controls** dialog box will display.
  - i. Choose the **Thr** drop-down menu to choose the threshold parameter.
  - ii. Use the sliders or the arrow keys on the keyboard to adjust a value from 1 to 1024. You can also enter a numerical value in any of the Gain text boxes to adjust the settings. Adjust the gain controls so that the negative population (or isotype) is positioned in the lower-left corner of the fluorescence plot and the cells are evenly distributed in the lower-left quadrant. Start from a lower gain setting and gradually increase the value. For greater detection and sensitivity across a broad range of fluorescence, the overall gain range can be adjusted using the **High** check box. By default, all boxes are checked. If further adjustment is needed or the signal is at its maximum, remove the checks from the High Calibration check boxes and allow the gain to stabilize for 2 to 5 seconds.

**NOTE:** Use the **Clear Events** button in the Sample Info panel to clear the display.

- iii. To acquire 4 log decades, clear the **5 decade Acquisition** check box.
- iv. If the red and/or violet laser is not needed for your application, clear the check box.

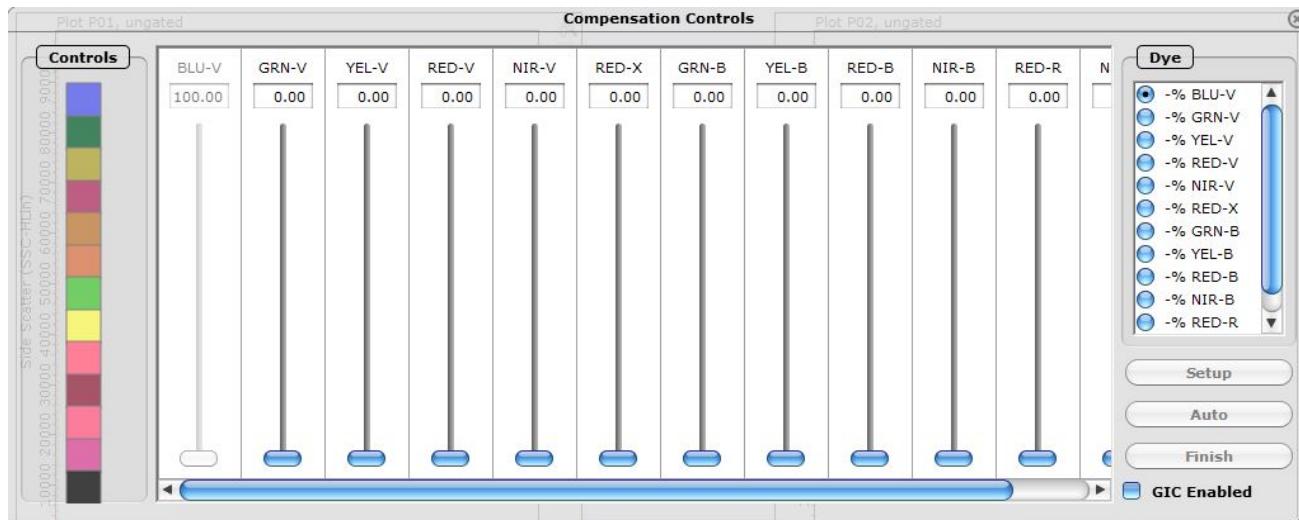
**NOTE:** Selecting and deselecting lasers will result in changes in laser power. Additionally, %CVs may vary slightly depending on the laser mode selected for acquisition.

- v. To adjust the threshold, use the slider or click and drag the threshold marker (dotted red line) up or down the axis of the dot plot displaying the threshold parameter until the desired amount of debris or other unwanted events are eliminated below the threshold. You can also enter a numerical value in the text box and press **Enter** on the keyboard.



- To adjust compensation, click the **Compensation** icon. The **Compensation Controls** dialog box displays. Select the radio button for the parameter you want to subtract, then use the slider to adjust the percentage of the overlapping signal to be removed from the detector.
- If the **Gain Independent Compensation (GIC) Enabled** check box is not selected and if adjustments to gain are made after compensation settings have been applied, the compensation will automatically be updated. Refer to the topic Perform Sample Compensation for additional information.

**NOTE:** If changes to gain are made when the GIC compensation check box is selected, compensation percentages will not change. A factor is applied to these values. For more information, contact Luminex Technical Support.



4. You may set regions and gates prior to acquiring the samples.

If you want to apply a count gate, create and define the gate, then select it from Count Gate drop-down menu under Sample Info. You can select the count gate from the Adjust Settings screen only.

You can also view real-time statistics during the adjust settings step. See for information on creating stat markers and viewing the real-time statistics.

5. When you are finished adjusting settings, click the **Next Step** button.

- If necessary, you can repeat the adjust settings step to ensure that other samples (such as another positive control) are on scale, appropriately positioned, and compensated, by clicking **Adjust Settings**, loading the sample, and clicking **OK**.
- If you want to save the instrument settings, click the **Save Settings** icon in the control panel. Enter a file name and click **Save** to save a .gst file.

**NOTE:** Once the worklist is complete, you can no longer save the instrument settings.

6. Click the **Resume Worklist** button. The system acquires the first sample.

**NOTE:** You may click the **Pause Worklist** button at any time during the run to select **Eject Tray** or **Capillary Cleaning Tools** then select **Backflush**, **Clean & Rinse**, or **Quick Clean**. The system will complete the current step before pausing. Click **Resume Worklist** to continue.

**NOTE:** If you want to adjust the instrument settings during the run, click **Pause Worklist**, then **Adjust Settings**. When the settings are set, click **Next Step**, then **Resume Worklist**.

**NOTE:** The % Acquired progress bar provides an estimate of the target event count during the acquisition period, which times out after 1.75 minutes (high flow rate), 3.5 minutes (medium flow rate), 7 minutes (low flow rate), or 10 minutes (very low flow rate).

**NOTE:** The plate map in the control panel provides a visual status of acquisition. The well currently being acquired appears with an open blue circle. Wells acquired appear as a solid blue circle.

**NOTE:** If you are acquiring more than 125,000 events per sample (gated or ungated) or a file size greater than 750 MB, InCyte analysis may be slow. Luminex recommends batching sample acquisition to reduce file size.

The system automatically performs a Quick Clean or a Clean & Rinse at the end of the assay.

At the completion of the worklist, a copy of the Data, Method, and AnalysedGroup are automatically loaded into the Analysis control panel. Guava InCyte saves the data for all samples as a single FCS 3.0 file to the specified location.

The FCS file contains:

- the acquired data for all tubes in the run
- the Method (plots, regions, gates, and metrics, if applicable)
- an AnalysedGroup (the data paired with the Method)
- instrument settings (gains, compensation, miscellaneous settings)

7. If necessary, back up data files, to free up hard disc space.

# Chapter 7: Analyzing the Data

## Guava® InCyte™ Analysis

Guava® InCyte™ Software allows you to open and analyze any Guava FCS 3.0 data file, regardless of the software module used for acquisition. You can proceed to analysis from the acquisition screen.

### Enable Single Sample Analysis

Guava® InCyte™ Software provides a single-sample analysis feature which allows you to analyze (or modify the analysis for) one sample within an FCS file and generate the stats for that sample without updating the stats for the remaining samples.

**NOTE:** You must enable Single Sample Analysis mode before opening a data file.

1. Open the **InCyte** module. The InCyte Acquisition screen will display.
2. Choose **Application** from the menu bar and select **Enable Single Sample Analysis** before opening the data set. After gating and analysis is complete, statistics are generated only for the selected sample.



3. To calculate and update the stats for the remaining samples, click the **Show Group Stats** icon from the tool bar. The **Group Stats** window displays.
4. Click **Calculate Stats for All**.

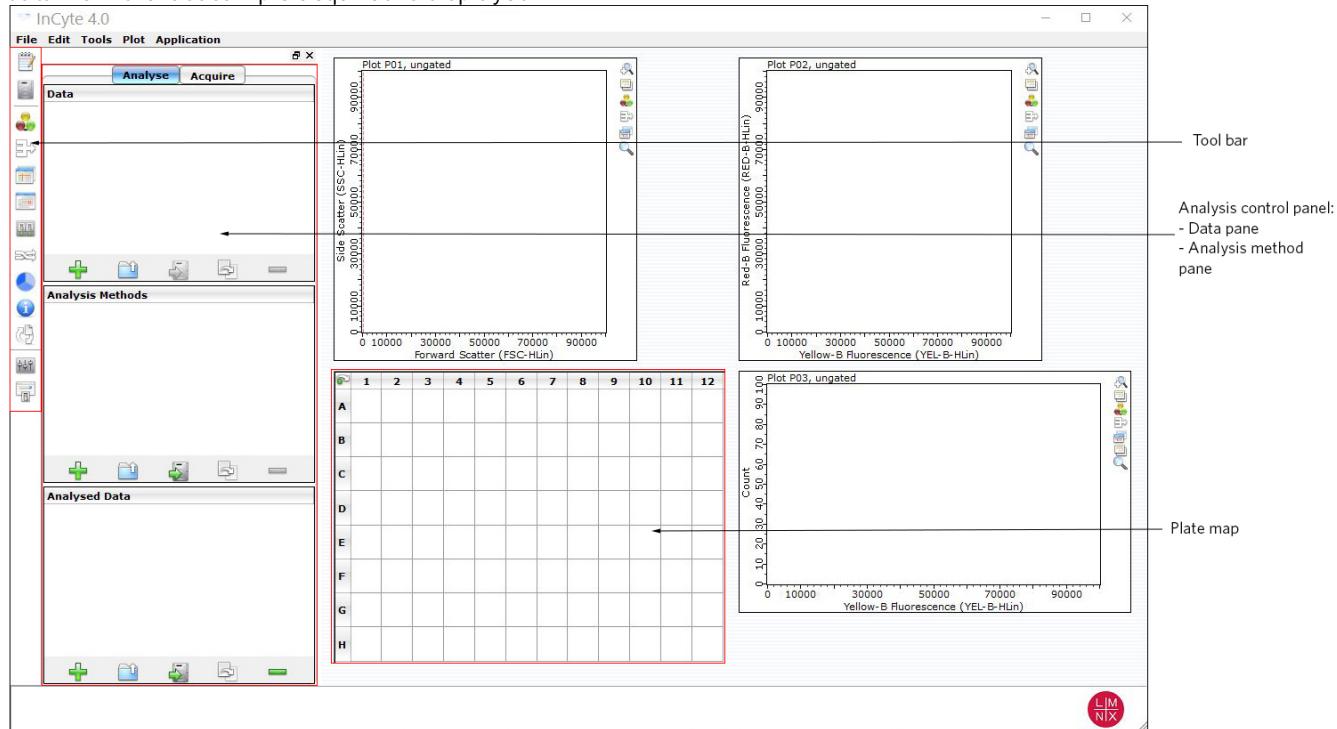
The screenshot shows a 'Group Stats' window with a table of data. The table has columns for Well, Sample ID, Date, R2.Percent.UL Percent for R2 gated by P01.R1 (%), and R2.Percent.UR Percent for R2 gated by P01.R1 (%). The data rows are as follows:

Well	Sample ID	Date	R2.Percent.UL Percent for R2 gated by P01.R1 (%)	R2.Percent.UR Percent for R2 gated by P01.R1 (%)
A01	A01	03.01.2017	0.42	0.03
A02	A02	03.01.2017	0.39	0.06
A03	A03	03.01.2017	0.42	0.03
A04	A04	03.01.2017	66.20	0.00
A05	A05	03.01.2017	64.36	0.00
A06	A06	03.01.2017	66.44	0.00
A07	A07	03.01.2017	0.00	67.29
A08	A08	03.01.2017	0.00	64.41

At the bottom of the window, there are four buttons: 'Calculate Stats for All' (disabled), 'Setup' (highlighted in blue), 'Export To CSV', and 'Print Stats'.

## The Guava® InCyte™ Analyse Panes

Click the **Analyse** button at the top of the InCyte™ control panel to access the Analyse screen. You can proceed to analysis directly from the acquisition screen. If you click **Analyse** from the Acquisition screen after acquiring a sample, the data from the last sample acquired is displayed.



The Analyse control panel provides a working outline for each analysis session. It contains three panes—the Data, Analysis Methods, and Analysed Data. An FCS file generated using InCyte™ will automatically contain all three elements.

- The **Data** pane lists all open FCS files, as well as any groups (FCS file subsets) created.
- The **Analysis Methods** pane contains all Method(s), each comprised of its associated plots, regions, gates, and metrics. Methods can be thought of as analysis strategies.
- The **Analysed Data** pane lists all user-defined AnalysedGroups. Analysed Groups are created by pairing the data file with Method—each consisting of a data file (or group) with its associated Method. When you close an Analysed Group, the associated FCS file and Method will close automatically.

Place your cursor over an icon in any of the three panes to see text describing the icon

## Analyze Files Acquired Using Guava® InCyte™

Data files acquired using InCyte™ will already have the necessary components for each of the three Analysis panes—the data file, the Method, and the Analysed Group. Simply open the FCS file.

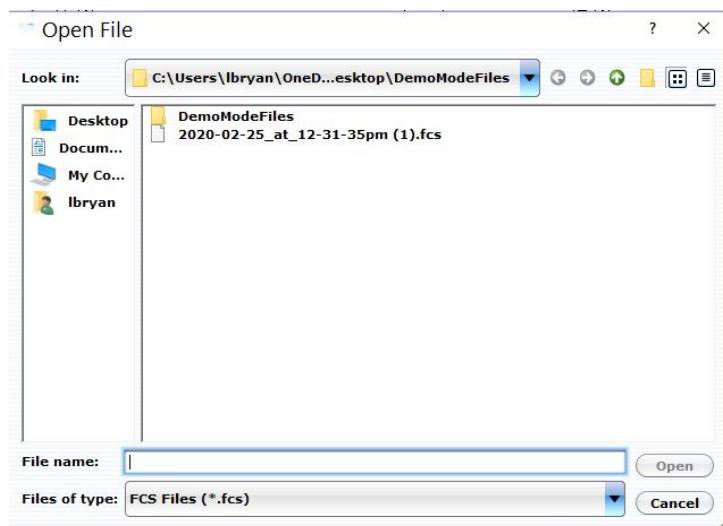
During analysis, the original FCS file and any analysis settings (plots, parameters, gates) will not be affected. If you wish to save any changes to the newly analyzed InCyte file, simply resave the file using the Analysed Data pane (Save Analysed Group icon).

You can proceed to analysis from the acquisition screen.

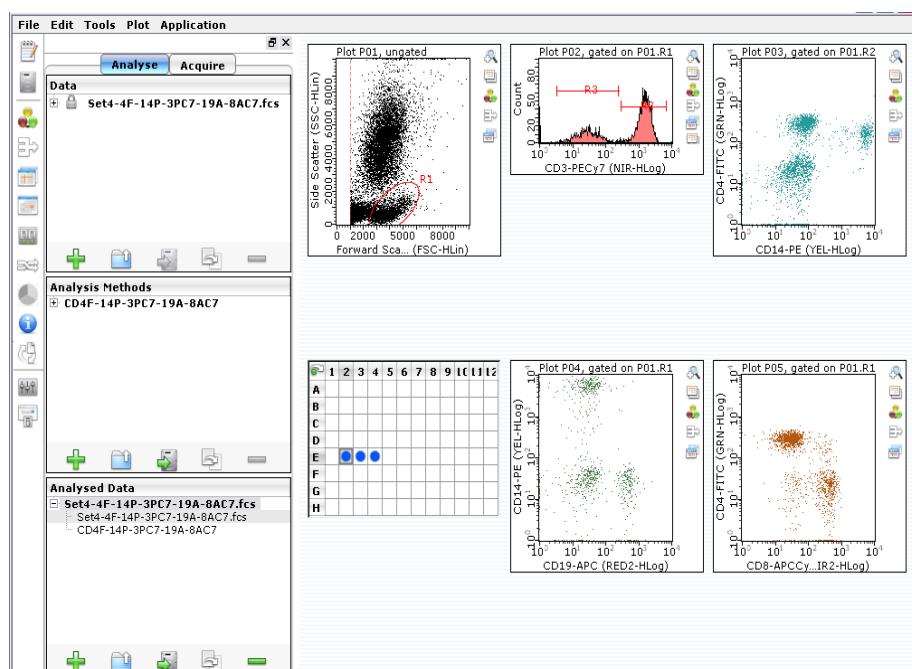
**NOTE:** You can analyze tube-based data but the results cannot be displayed in the plate map. Additionally, the gates and markers do not carry over from one tube to the next. Tubes must be analyzed individually (tube-by-tube basis). Use the Stat Setup feature to obtain results for tube data.

**NOTE:** If you open and analyze multiple files within a session, all the FCS data files, Methods, and Analysed Groups will remain in the panes from all files that were opened. If they have already been saved, you can remove them from the pane by selecting the item and clicking the **Delete** icon at the bottom of that pane.

1. Open the **InCyte** module. The InCyte Acquisition screen will display
2. Click **Analyse**, then choose **File > Open** from the menu bar.
  - a. From the **Open File** dialog box, navigate to the FCS file for analysis and click **Open**. The data for the first sample displays.

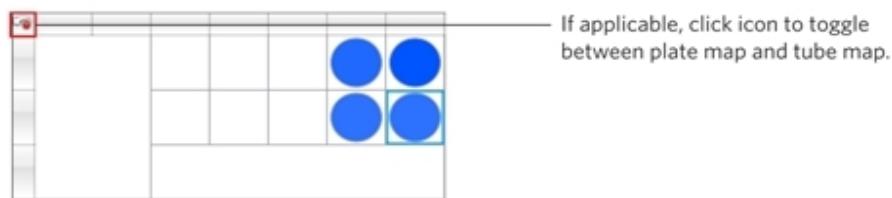


3. Click the **+** to the left of the data file name to display a list of all samples contained in the file. All wells with acquired data appear as open circles in the corresponding locations in the plate map.



4. Click on a sample in the Data pane to display the unanalyzed data for that sample in the plots, however the information in the plate map will be lost. Click the Analysed Group (fcs file in the Data pane) to redisplay the information in the plate map.
  - a. Select the individual wells in the plate map to display the analyzed data for that well in the plots, or use the arrow keys to quickly scroll through the wells, displaying the data.

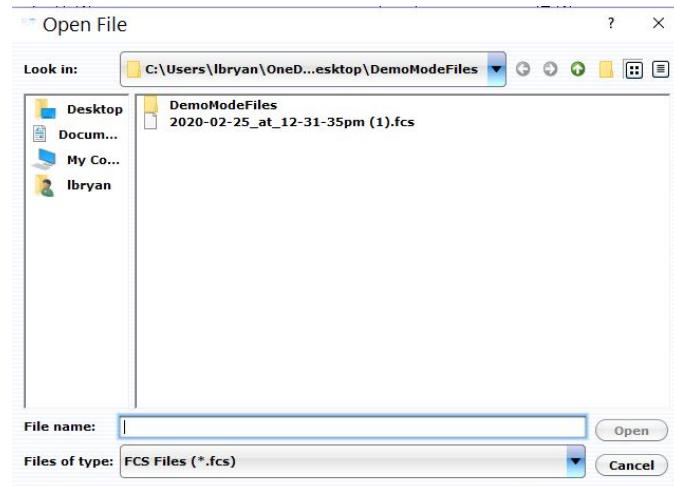
**NOTE:** An icon displays in the upper-left corner of the plate map. This icon allows you to toggle between the tube map and plate map. Samples from tubes acquired on your Guava® easyCyte™ HT System will always display in the plate map. If the data set was acquired on an easyCyte HT System, which allows you to acquire from both tubes and a microplate, the samples acquired from tubes will show up in the tube map.



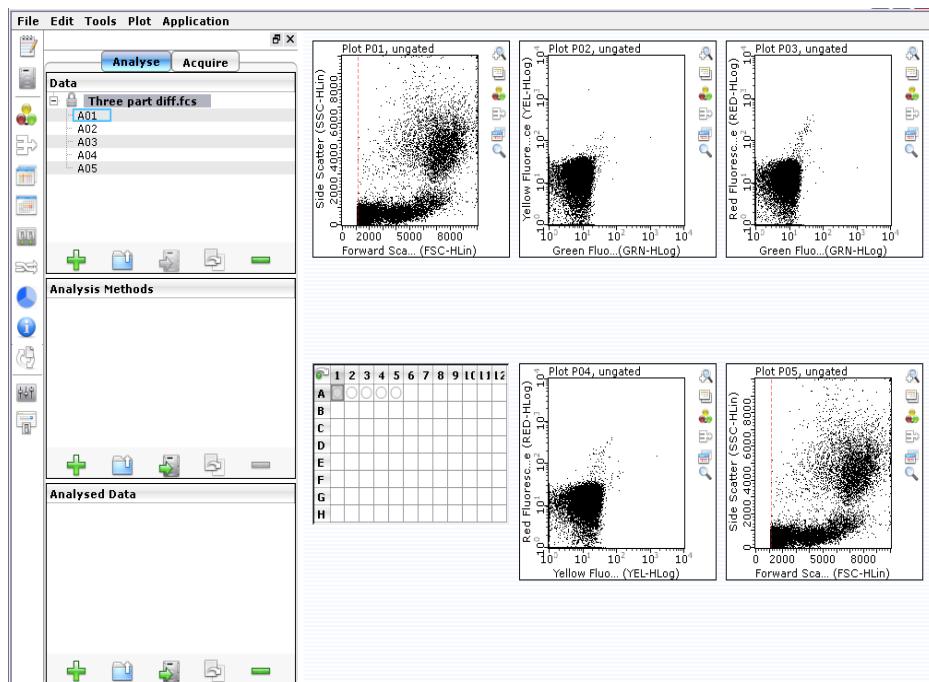
## Analyze Files Acquired Using Other GuavaSoft Modules

Data acquired using any GuavaSoft module other than InCyte™, for example, ExpressPro, will not have an Analysis Method associated with it. However, when you open the file in InCyte, a default Method will automatically be created.

1. Open the **InCyte** module. The InCyte Acquisition screen will display.
2. Click **Analyse**, then choose **File > Open** from the menu bar.
  - a. From the **Open File** dialog box, navigate to the FCS file for analysis and click **Open**. The data for the first sample displays.



3. Click the + to the left of the data file name to display a list of all sample wells and tubes contained in the file. All wells with acquired data appear as open circles in the corresponding locations in the plate map.
4. Select the sample from the file list or click on a well in the plate map to display data in the plots.

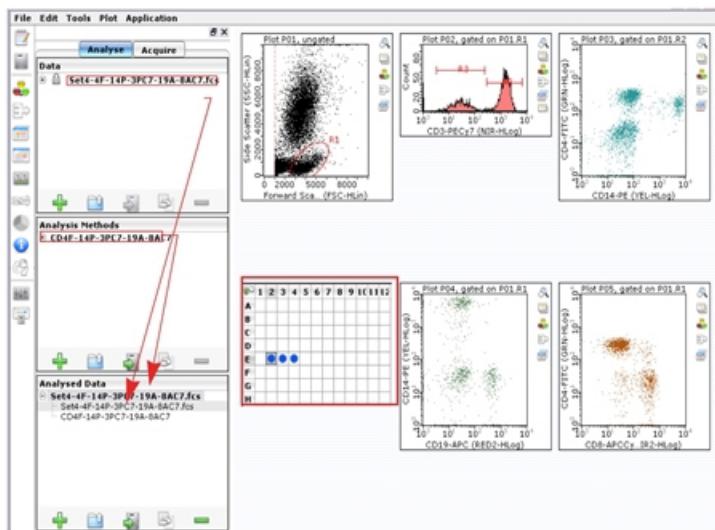


- When a data file is generated in a Guava® Software module other than InCyte, InCyte will automatically create a default Analysis Method and Analysed Group for the data file/set. The Method will contain a percent (%) metric, initially. If you want to open an existing Method click the **Open Method** icon from the Analysis Methods pane. Method files have the extension .gsy.

**NOTE:** To rename the Method, double-click it and enter a new name related to this analysis.



- Choose a .gsy file and click **Open**. If you opened an existing Method, you will need to drag it to the Analysed Group.

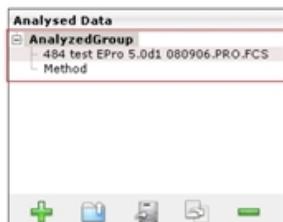


Data and Method are automatically paired to create an Analysed Group. If you open a saved Method, you will need to drag it to the Analysed Group.

**NOTE:** Notice the sample wells in the plate map become dark blue (if the Method was newly created) or various shades of blue (if a previously defined Method is used). You can place the cursor over any well in the plate map to view results for that sample.

7. Click on a sample in the Data pane to display the unanalyzed data for that sample in the plots, however the information in the plate map will be lost. Click the Analysed Group to redisplay the information in the plate map.
  - a. Select the individual wells in the plate map to display the analyzed data for that well in the plots, or use the arrow keys to quickly scroll through the wells, displaying the data.

**NOTE:** Any data file or Method file can be replaced by choosing it from the respective pane and dragging to the Analysed Group. This allows you to quickly interchange data files and Methods.



AnalysedGroup containing a data file and Analysis Method.

8. To save an AnalysedGroup, click on the file in the Analysed Data pane , then click the **Save Analysed Group** icon.
  - a. From the **Save Analysed Group** dialog box, navigate to the location you want to save the file, enter a name, and click **Save**. The AnalysedGroup will be saved as an FCS file and will contain the method (regions, gates, metrics, and stats), as well as the data and the instrument settings.

You are now ready to start customizing your Analysis Method using regions and gates.

## Create Analyzed Group

A group is a user-defined set of samples from one or more Flow Cytometry Standard (FCS) files (or data sets). Grouping allows you to extract and analyze a subset of samples from the data set(s). You can also use grouping to apply different Methods to subsets of data that are part of a single FCS file, to get different statistics, for example.

To create a group:

1. From the **InCyte** module, click the **Analyse** control panel, then choose **File > Open** from the menu bar.
  - a. From the **Open File** dialog box, navigate to the FCS file for analysis and click **Open**. The data for the first sample displays.
2. Drag the cursor across the appropriate wells in the plate map, right-click the selected wells, and choose **Create group** from the menu. To select non-consecutive wells, press the **Ctrl** key while clicking to select wells. The order that you select and add the wells to the group is the order that they will be in the group.



- The group name appears under the original FCS file in the Data pane. To rename the group, double-click the name and enter in the new name.
- To add additional samples to an existing group, select the wells and drag them to the group in the Data pane.

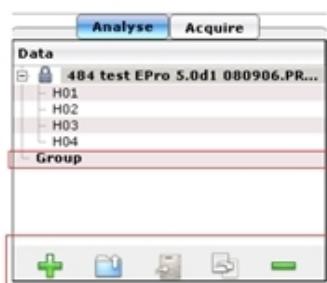
**NOTE:** You can combine samples from different FCS files; however, if you select the same/overlapping wells, the plate map and plots will display only the first of the overlapping well(s) selected (eg, the first A01 selected).

3. To remove an individual well, click the **+** sign for the **Group** folder in the **Data** pane and right-click the item that you want to remove.
4. Choose **Remove Item**. The deleted well is removed from the group and appears as an empty square in the plate map.

**NOTE:** Wells can only be deleted from groups, not from the original FCS file.

5. To remove an entire group, right-click the group in the **Data** pane and choose **Remove Item**.

**NOTE:** Use the **Duplicate Group** icon in the Data pane to make a copy of a group.



Group under the data file from which it was created.

You can also use the icons at the bottom of the Data pane to create, open, save, duplicate, and delete a group.

---

## Perform Sample Compensation

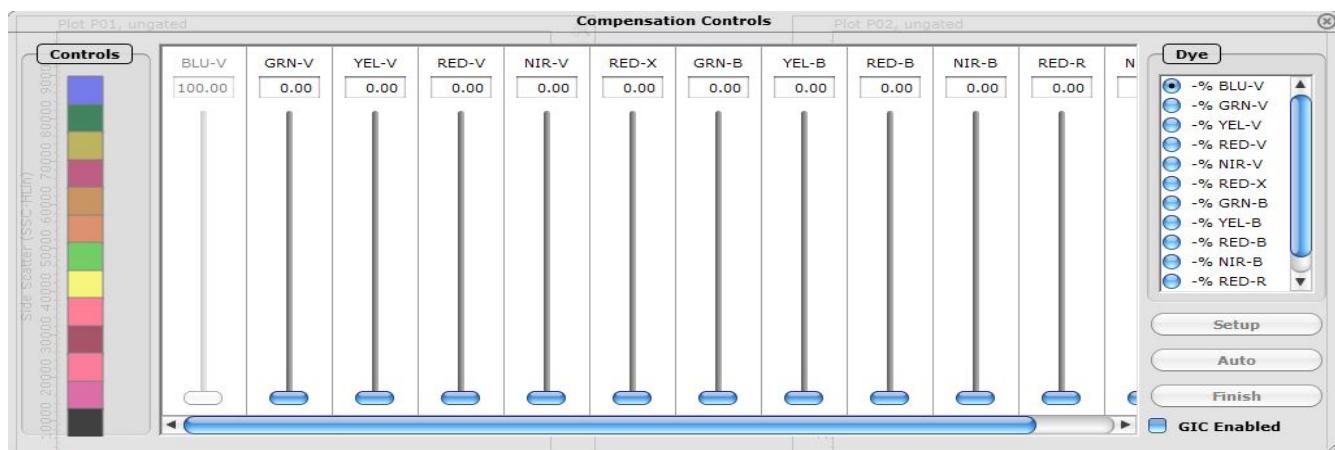
**NOTE:** If GIC Compensation is activated during acquisition or analysis, a GIC factor is applied to the compensation matrix. For more information, contact technical support.

### Perform Manual Compensation

You can perform compensation before or after acquiring your samples.

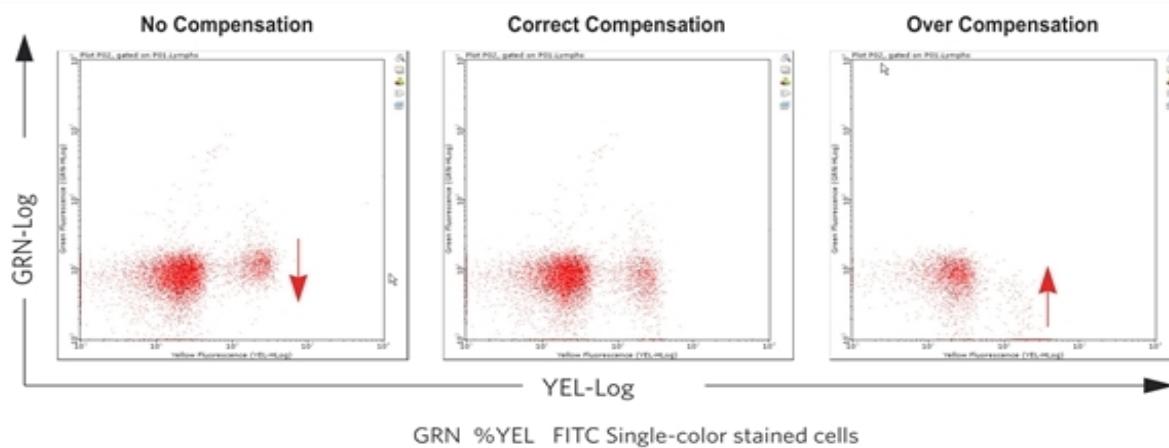
1. Open the **InCyte** module. The InCyte Acquisition screen will display
2. Click **Analyse**, then choose **File > Open** from the menu bar.
- a. From the **Open File** dialog box, navigate to the FCS file for analysis and click **Open**. The data for the first sample displays.
3. Choose **Tools > Show Compensation Controls** from the menu bar to open the Compensation Controls window.

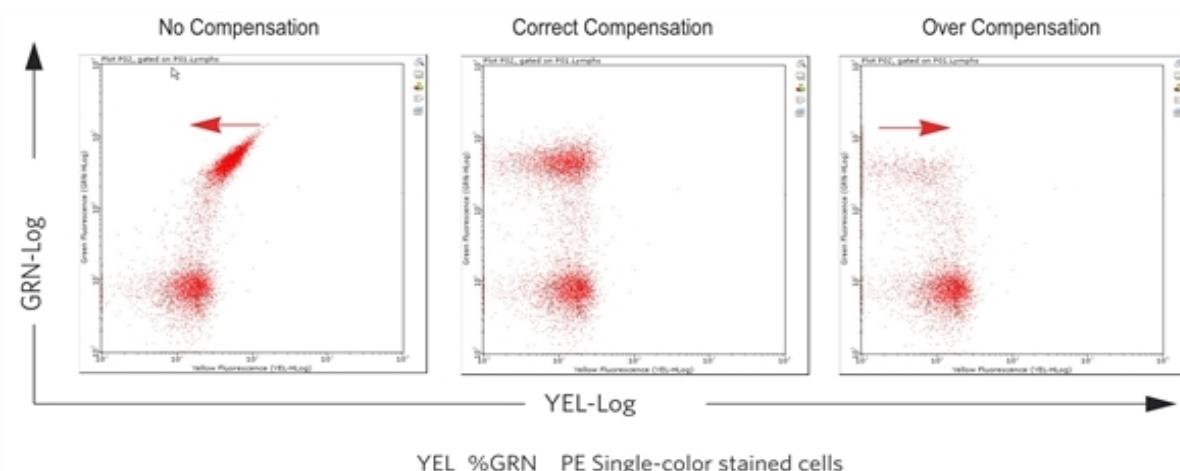
**NOTE:** The controls at the far left are for semi-automated compensation only.



- a. Under the **Dye** pane, select the radio button for the channel corresponding to the single color control.
- b. Use the slider to adjust how much signal to remove from the other detectors/channels.
- c. Check the compensation for each fluorochrome combination you are using. Compensation settings are correct when the center of the stained population is aligned with the center of the isotype or negative control population. Avoid having too many cells touching the axis (overcompensated).

**NOTE:** Some fluorochromes require very little compensation because they have little overlap, such as PE into the GRN channel (GRN-B - % YEL-B), as shown in the first three plots below; whereas others require much more compensation because they have more overlap, such as FITC into the YEL channel (YEL-B - % GRN-B), as shown in the last three plots below.





- Exit the **Compensation Controls** dialog box by clicking the **x** in the top-right corner.

## Perform Compensation During Acquisition

- From the Guavasoft Software Main Menu, navigate to **Favorites > InCyte**. The InCyte Acquisition screen will display
- Choose **File > Open** from the menu bar to set up the plots.
  - From the **Open File** dialog box, navigate to the FCS file for analysis and click **Open**. The data for the first sample displays.
- Check the **Conc (Cells/ $\mu$ L)** value in the **Sample Info** pane and ensure that it is less than or equal to 500.
 

**NOTE:** If the value is greater than the high limit for the corresponding flow rate, dilute the sample with the appropriate buffer to lower the concentration and minimize the risk of coincident events. For optimal performance, Luminex recommends a concentration of 250 cells/ $\mu$ L or lower.
- Choose **Tools > Show Compensation Controls** from the menu bar to open the Compensation Controls window.
  - Under the **Dye** pane, select the radio button for the channel corresponding to the single color control.
  - Use the slider to adjust how much signal to remove from the other detectors/channels.
  - Repeat the remaining compensation adjustments for each control in your experiment.

**NOTE:** For example, to remove the FITC signal from the YEL detector, select the **-% GRN-B** radio button under Dye. Then adjust the YEL slider to remove the FITC signal from the YEL channel (YEL-B - % GRN-B). Next, using the PE single-color control, select the **-% YEL-B** button, and adjust the GRN-B slider to remove the PE signal from the GRN channel (GRN-B - % YEL-B).
- Exit the **Compensation Controls** dialog box by clicking the **x** in the top-right corner

## Perform Compensation Post-Acquisition

Guava® InCyte™ allows you to perform post-acquisition compensation on data files acquired using software modules that have the compensation feature and allow you to adjust compensation during acquisition, such as ExpressPlus, ExpressPro, and InCyte.

**NOTE:** You can adjust compensation only; you cannot adjust gain settings.

- Open the **InCyte** module. The InCyte Acquisition screen will display

2. Choose **File > Open** from the menu bar to set up the plots.
    - a. From the **Open File** dialog box, navigate to the FCS file for analysis and click **Open**. The data for the first sample displays.

**NOTE:** Luminex recommends using single-color samples, then checking the compensation again with a sample stained with all fluorochromes to fine-tune, if necessary.
  3. Choose **Tools > Show Compensation Controls** from the menu bar to open the Compensation Controls window.
    - a. Under the **Dye** pane, select the radio button for the signal you want to subtract, then use the slider to adjust the percentage of the signal you want to subtract from the detector.

**NOTE:** For example, to remove the FITC signal from the YEL detector, select the **- % GRN-B** radio button under Dye. Then adjust the YEL slider to remove the FITC signal from the YEL channel (YEL-B - % GRN-B). Next, using the PE single-color control, select the **-% YEL-B** button, and adjust the GRN-B slider to remove the PE signal from the GRN channel (GRN-B - % YEL-B).
  4. Repeat for the remaining compensation adjustments for your samples. Settings are applied to all samples in the data set, as well as all groups derived from the data set. Adjustments made to a sample within a group, are applied to all samples in the originating data file.
- NOTE:** The controls at the left of the window are for semi-automated compensation only.
5. Exit the **Compensation Controls** dialog box by clicking the **x** in the top-right corner

## Perform Semi-automated Compensation

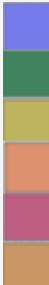
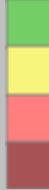
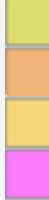
Guava® InCyte™ allows you to perform semi-automated compensation on data files acquired using software modules that have the compensation feature and allow you to adjust compensation during acquisition, such as ExpressPlus, ExpressPro, and InCyte.

**NOTE:** You must have acquired single-color control samples to use the semi-automated compensation feature.

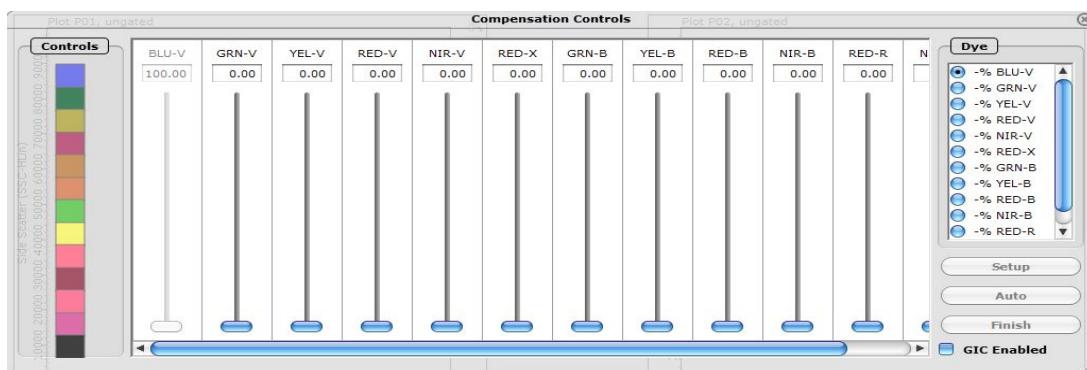
**NOTE:** You can adjust compensation only; you cannot adjust gain settings.

1. Open the **InCyte** module. The InCyte Acquisition screen will display
2. Choose **File > Open** from the menu bar.
  - a. From the **Open File** dialog box, navigate to the FCS file for analysis and click **Open**. The data for the first sample displays.

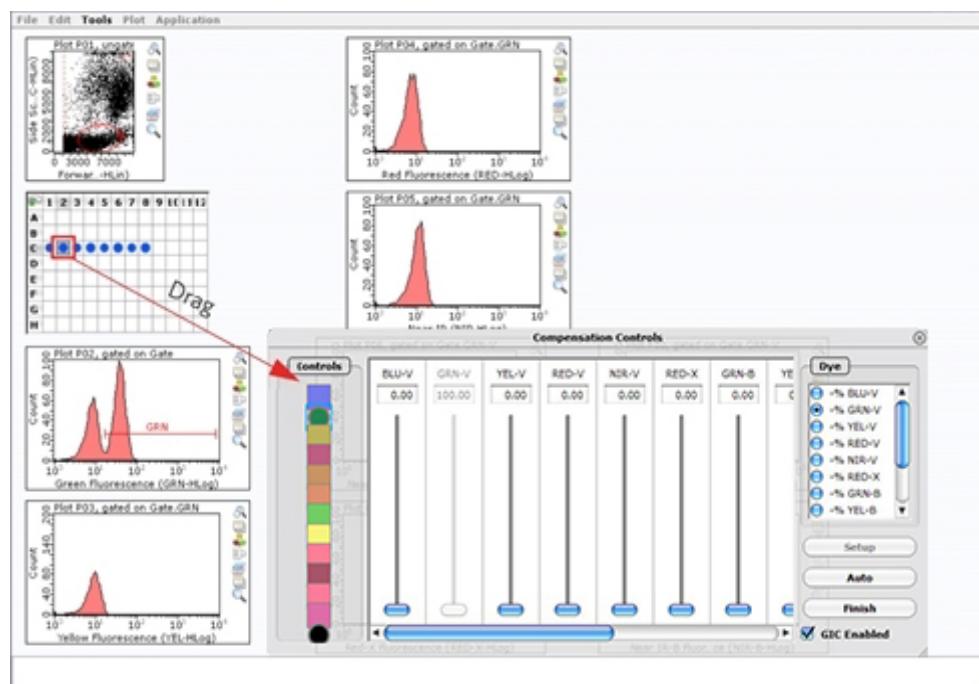
**NOTE:** Luminex recommends using single-color samples, then checking the compensation again with a sample stained with all fluorochromes to fine-tune, if necessary.
3. Choose **Tools > Show Compensation Controls** from the menu bar to open the Compensation Controls window. This window is specific for compensation during analysis. The Compensation Controls window contains a slider for each parameter and a list of controls in the far left panel. These controls correspond to each parameter and the fluorochrome used:

Comp Color	Channel	Laser
	BLU-V	405 nm (Violet)
	GRN-V	
	YEL-V	
	ORG-V	
	RED-V	
	NIR-V	
	GRN-B	488 nm (Blue)
	YEL-B	
	RED-B	
	NIR-B	
	YEL-G	532 nm (Green)
	ORG-G	
	RED-G	
	NIR-G	
	RED-R	642 nm (Red)
	NIR-R	
	negative or isotype control	

**NOTE:** If it is necessary to gate the data, pair a Method with the data file to create an Analysed Group and set regions and gates. Otherwise, it is not necessary to create an Analysed Group to adjust post-acquisition compensation.



- Click **Setup** in the **Compensation Controls** dialog box. The acquisition screen changes to display two dot plots on the left with the plate map below, and up to six histograms for each fluorescence channel.
- Adjust the default region in plot 1 so that it encompasses the events of interest.
- Drag the isotype (or negative) control from the plate map to the black square at the far left in Compensation Controls window
- Drag the next control, for example the GRN control (FITC-positive control) from the plate map to the green square at the far left in the Compensation Controls window (see example in figure below). For this example, the green square turns into a circle and the FITC control data is displayed in the GRN histogram.
- Adjust the GRN histogram marker to include the positive population.



- Drag the next control, for example YEL control (PE-positive control) to the yellow square. Continue dragging the remaining controls to the corresponding Controls squares and adjusting the markers to include the positive population.
- Check that the markers are correct for the isotype control.
- Click **Auto**. The software automatically calculates the compensation values for each parameter. Settings are applied to all samples in the data set, as well as all groups derived from the data set. Adjustments made to a sample within a group are applied to all samples in the originating data file.
- If you need to adjust compensation further, Perform Compensation Post-Acquisition.

- j. Click **Finish** to return to the analysis display.

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# Regions

## Create New Regions

Guava® InCyte™ offers six types of regions/markers—ellipses, rectangles, octagons, polygons, and quadrant markers for dot plots, and histogram markers for histograms. All of these regions are created using either the New Region icon in the plot tool bar, except the quadrant marker, or the New Stat Marker icon in the tool bar, which allows you to select statistics for the region/marker. A single Analysis Method can contain a maximum of 24 regions or stat markers, and you can have multiple stats per marker.

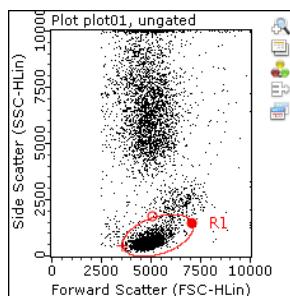
InCyte regions are associated with one (histogram) or two (dot plot) parameters in which they were created. If you change the x- or y-axis parameter on the plot, the region is no longer displayed. However, it is still contained within the Method.

1. From the Guava® InCyte™ module, open the FCS needed for analysis.
2. Choose **File > Open** from the menu bar.
  - a. From the **Open File** dialog box, navigate to the FCS file for analysis and click **Open**.
3. Once an AnalysedGroup is created, either by acquiring InCyte data or by dragging a data file and a Method to an AnalysedGroup, click the **New region** icon in the plot tool bar and choose a region from the menu.
4. In the **New Region Name** dialog box, enter a name for the region and click **OK**. You can leave the default name, but a unique region name can be helpful in differentiating Analysis Methods. The newly created region appears on the plot with the region name. Click and drag the name to move it. The region is listed in the Region List under the Method (see below) and in the Region List table (Region List icon in the main tool bar or **Tools > Show Region List**).

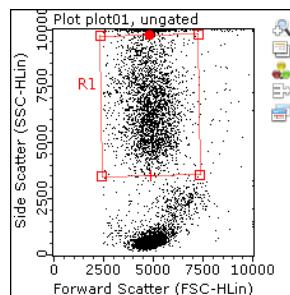


5. Adjust the region to encompass the data. Or, for a polygon, create the region.

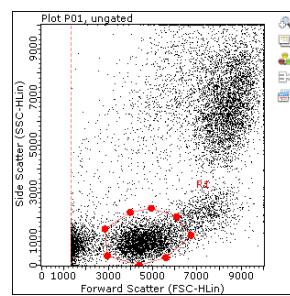
- To adjust an **elliptic region**, click anywhere on the edge of the ellipse, except on a handle, and drag it to a new location. The ellipse has two handles. The open circle allows you to narrow/widen the ellipse. The solid circle allows you to lengthen, as well as rotate the ellipse around a point opposite the solid circle.



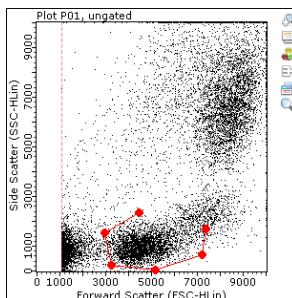
- To adjust a **rectangle region**, click anywhere on the edge of the rectangle, except on a handle, and drag it to a new location. The rectangle has five handles. The open squares allow you to extend at the corresponding corner. The solid square allows you to rotate and resize.



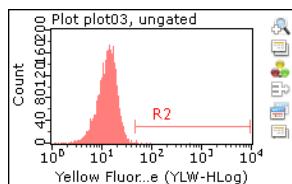
- To adjust an **octagonal region**, click anywhere on the edge of the octagon, except on a handle, and drag it to a new location. The octagon has eight handles. Click and drag the handles to adjust the shape of the octagon. To add or remove segments, right-click on a handle and select **Insert Line Segment** or **Delete Line Segment**.



- To create a **polygon region**, click to add the first handle, then move the cursor to the next point and click. Continue creating up to 32 segments until the shape is complete. Finish by clicking again at the first handle to close it. To move it, click anywhere on the edge, except on a handle, and drag it to a new location. To adjust the shape, click and drag the handles. To resize or rotate, press the Shift key and click and drag a handle.



- To adjust a **histogram region**, click either of the two handles. The histogram marker can be moved vertically as well as horizontally.



## View the Region List

When you create a region, it appears in the Region List.

- From the Guava® InCyte™ module, open the FCS needed for analysis.
- Choose **File > Open** from the menu bar.
  - From the **Open File** dialog box, navigate to the FCS file for analysis and click **Open**.
- Choose **Tools > Show Region List**. The **Region List** dialog box displays a list of all regions and quad stat markers in the selected Analysis Method, their type, and plots axes.
  - To display all the columns in the Region List dialog box, click **Resize**.

The Region List dialog box contains a table with the following data:

Name	Type	X-Par	Y-Par	Color LL	Color UL
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					

On the right side of the dialog box, there are three buttons: **Delete**, **Close**, and **Resize**. Below the table is a horizontal scroll bar.

## Adjust Local and Global Regions

You can make adjustments to regions for specific samples within a data set. For example, you may want to adjust a region for the data in one or more samples to compensate for a shift in the data.

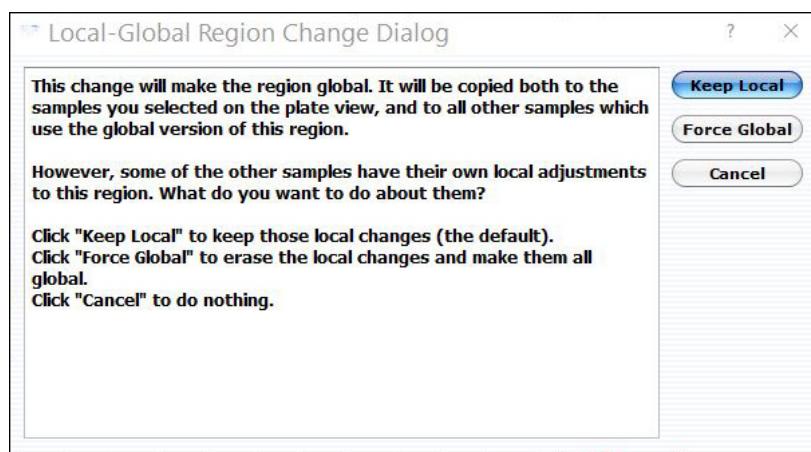
1. From the Guava® InCyte™ module, open the FCS needed for analysis.
2. Choose **File > Open** from the menu bar.
  - a. From the **Open File** dialog box, navigate to the FCS file for analysis and click **Open**.
3. To change a region for an individual sample, press and hold the **Ctrl** key while manipulating the region (elliptic, rectangle, octagon, or histogram).
4. To change the region for multiple samples, highlight the desired wells, then hold the **Ctrl** key while manipulating the region. The change will be applied to all the highlighted samples.

The region will turn from red to black, indicating that it is a local region—it applies only to the sample data displayed in the plots.

**NOTE:** If you manipulate a global region without holding down the **Ctrl** key, the change will automatically apply globally (to the data for all samples in the run).

Once a local region is created:

- if you have only one local region in the data set and you attempt to change it without holding down the **Ctrl** key, the change will become global and apply to all regions in the data set.
- if you have multiple local regions and you attempt to change a single local or global region without holding down the **Ctrl** key, the following dialog box appears:



- To apply the change to the region you are adjusting and all global regions, but keep any other local regions unchanged, click **Keep Local**.
- To apply the change to all samples in the run, click **Force Global**.
- To cancel the change and not apply it to any regions, click **Cancel**.

If you want to make an adjustment to a local region to change it further, hold down the **Ctrl** key to keep the change local and not affect any other regions—local or global.

After making a region(s) local, you can still make adjustments to global regions without holding down the **Ctrl** key. When the dialog box appears, click **Keep Local**. The adjustment will apply to all global regions. The local regions will remain local and unchanged.

## Delete Region

1. From the Guava® InCyte™ module, open the FCS needed for analysis.
2. Choose **File > Open** from the menu bar.
  - a. From the **Open File** dialog box, navigate to the FCS file for analysis and click **Open**.
3. Choose the .fcs file in the **Analysed Data** pane.
4. From the plot, click the region you want to delete, then right-click and choose **Delete Region**. You can also select the region in the Region List window and click **Delete**.
5. Click **OK** in the Delete Region dialog box. The region will be deleted from all associated plots. The region name in the Analysis Method will appear as ???.

## Hide Region

Hide Region(s) allows users to look at one marker across multiple sub-populations. If you have multiple plots with the exact same x and y axis, you can choose to hide regions.

1. From the Guava® InCyte™ module, open the FCS needed for analysis.
2. Choose **File > Open** from the menu bar.
  - a. From the **Open File** dialog box, navigate to the FCS file for analysis and click **Open**.
3. Choose the .fcs file in the **Analysed Data** pane.
4. Click the **New Stat Marker** from the plot tool bar.
5. Choose **Hide Region(s)**.
6. Select the region of interest to display from the drop-down menu.

---

## Gates

A gate can be as simple as a single region, or a complex combination of regions and multiple parameters. Gates allow you to further characterize and isolate populations based on the regions. A maximum of 32 gates can be created for one Analysis Method.

You can create gates for analysis and/or acquisition. Count gates used during acquisition allow you to acquire specific populations of interest. All events above the threshold are saved to the file whether they are in the gate or not. However, the number of Events to Acquire is applied to events that fall within the gate.

## Define Gate

### Apply a Gate

The gating process has been simplified by dragging and dropping regions. Any region can be dragged from one plot to any other plot. This process allows you to create gates defined with the "AND" operator. You can also create gates using the OR and NOT operators.

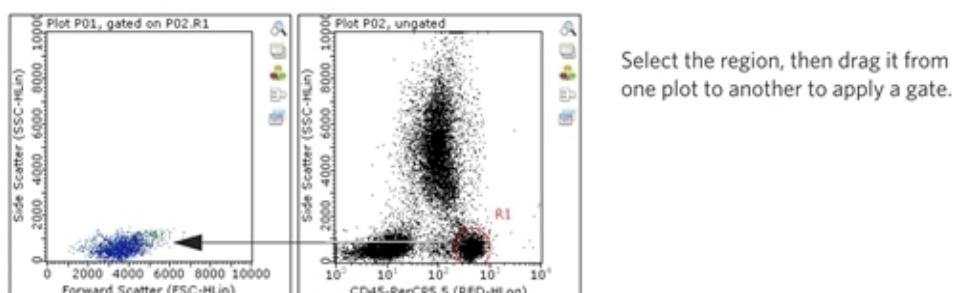
1. From the Guava® InCyte™ module, open the FCS needed for analysis.
2. Choose **File > Open** from the menu bar.

- a. From the **Open File** dialog box, navigate to the FCS file for analysis and click **Open**.
  3. Choose the .fcs file from the **Analysed Data** pane.
  4. From the plot, click to select the region (handles appear), then click in the center of a region and drag it to another plot.
- NOTE:** This “applies” the data within the boundaries of the region to the second plot. For example, if you created a region on live cells in plot 1 and drag the live-cell region to plot 2, only the live cells will be displayed in plot 2.
5. If prompted, a **New Gate Name** dialog box displays allowing you to rename the newly created gate. You can enter a more meaningful name or use the default. Click **OK**.



**NOTE:** You can also define a gate by typing the regions and the operators AND, OR, and NOT.

**NOTE:** If the Analysis Method has gates already defined, you can also select a gate from the Plot gate menu in the plot tool bar.



When you apply a gate, the following changes occur:

- The events displayed in Plot 2 will automatically change to reflect only those defined by the region that was dragged in. To view the ungated data again, click the **Plot gate** icon to the right of the plot and select **<ungated>**.
- The color of the newly gated data will reflect the color of the gate defined in the Gate List window. The default color for the first gate is red.
- The plot heading will reflect the gate applied (for example, *Plot P01, gated on P02.R1*).
- The newly created gate will display in the Analysis Methods pane under the Method, in the Gate List , and the Plot gate pop-up menu to the right of the plot.

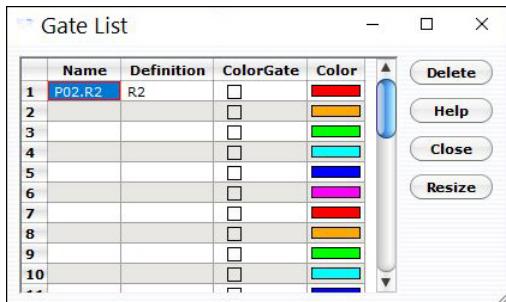
**NOTE:** Changes made to the position or size of any region(s) used to define a gate will automatically be reflected in all plots gated by that region.

## Use the Gate List Feature

When you create a gate, it appears in the Gate List.

1. From the Guava® InCyte™ module, open the FCS needed for analysis.

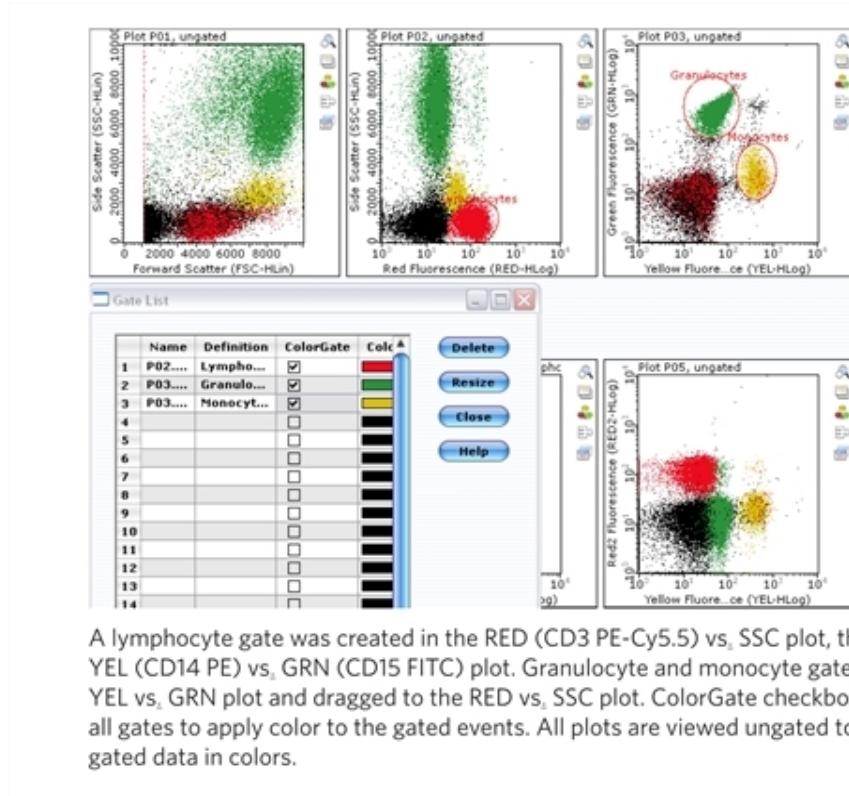
2. Choose **File > Open** from the menu bar.
  - a. From the **Open File** dialog box, navigate to the FCS file for analysis and click **Open**.
3. Choose the .fcs file in the **Analysed Data** pane.
4. Click the **Gate List** icon in the tool bar or choose **Tools > Show Gate List**. The Gate List dialog box displays a list of all gates in the selected Analysis Method, their Definition, and Color.



5. To display all the columns in the window, click **Resize**.
6. To delete a gate from the list, select the gate and click **Delete**. Click **OK** in the confirmation box.
7. To change the gate color, double-click the color in the Color column. The ColorPickerDialog displays.
8. To turn a region into a gate by entering a name for the gate in the Name field and entering the region name (for example, R1) in the Definition field.
9. To define a gate using the operators AND, OR, and NOT, create regions to identify the sub-populations of interest. Open the gate list and enter an appropriate name for the gate. Enter a definition, for example:
  - R1 AND R2 means the event must be in both the R1 and R2 regions to be included in the gate.
  - R1 OR R2 means the event must be in either the R1 or R2 region to be included in the gate.
  - R1 AND (NOT R2) can be used if R1 and R2 overlap and you want to include events in R1 but not in R2.
 The gate can now be applied to any plot by clicking the **Plot gate** icon from the plot tool bar and selecting the gate from the list.

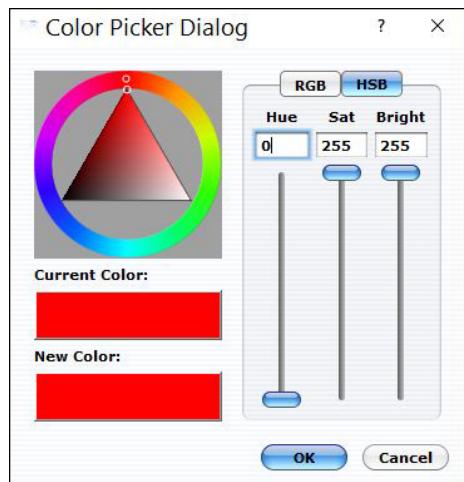


- The ColorGate box allows you to view a backgate. Create a gate in one plot, then drag that gate to the plot of interest. This applies the gate to the second plot and shows only the gated events. Select **Tools > Show Gate List** and check the ColorGate box for the gate. The gated events appear in both plots in the selected color. View the second plot without a gate (select <ungated> from the plot tool bar) to see the color gated events in relationship to all events.



## Change the Color of Gated Data

You can change the color of data within a gate.



- From the Guava® InCyte™ module, open the FCS needed for analysis.
- Choose **File > Open** from the menu bar.
  - From the **Open File** dialog box, navigate to the FCS file for analysis and click **Open**.
- Choose the .fcs file from the **Analysed Data** pane.

4. Click the **Gate List** icon in the tool bar and double-click the color in the **Color** column. The **Color Picker Dialog** box displays. Use this dialog to change the color, hue, saturation, and brightness.
5. To change the color, click the **RGB** button and use the sliders to adjust each color. Or, click to select a color in the outer circle. Drag the pointer to change the color.
6. To adjust the hue, saturation, or brightness, click the **HSB** button and use the sliders to make the appropriate adjustments. Or, click a shade in the inner triangle.
7. Click **OK** when you are finished.

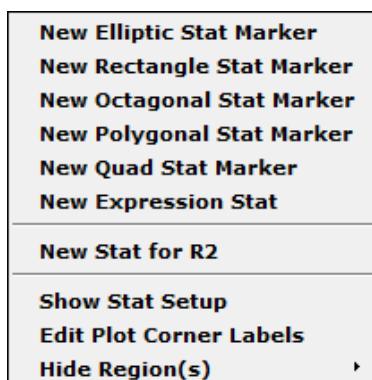
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## Statistics

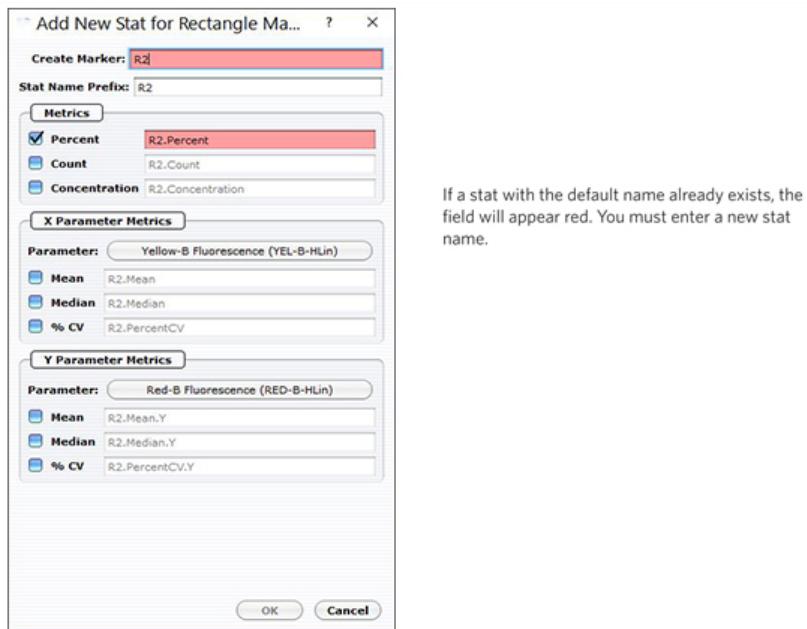
### Create Stat Markers

The Stats feature allows you to derive, display, and export statistics for single samples, groups, or whole data sets. Each stat is derived from a region/marker. Stats can be assigned to existing regions, or new regions can be created. A single Analysis Method can contain a maximum of 24 regions/markers.

1. From the Guava® InCyte™ module, open the FCS needed for analysis.
2. Choose **File > Open** from the menu bar.
  - a. From the **Open File** dialog box, navigate to the FCS file for analysis and click **Open**.
3. Choose the .fcs file from the **Analysed Data** pane.
4. Click the **New Stat Marker** icon in the plot tool bar and select the appropriate option.  
You can obtain statistics for any existing regions in the plot. Or, you can create a new region and obtain statistics for that region.
  - Select **New Elliptic/Rectangle/Octagonal/Quad/Histogram Stat Marker** to add a new region and obtain stats for it. Histogram Stats appear only in a histogram plot.
  - Select **New Stat for R1** (or name of existing region) to obtain stats for an existing region.
  - Select **New Expression Stat** to create an expression statistic.
  - Select **Show Stat Setup** to display the Current Run Stats.
  - Select **Edit Plot Corner Labels** to add custom labels to your plots.
  - Select **Hide Region(s)** to look at one marker across multiple sub-populations.



5. The **Add New Stat for...** dialog box displays for the stat type you selected. The default base stat name is the same as the gate name. Base stat implies it is the base name before you selected a specific metric (stat), then the stat name becomes BaseStatName.Metric.

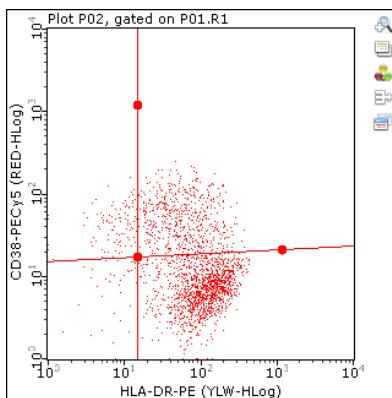


6. Select the metric—**Percent**, **Count**, **Mean**, and **Median**, **Concentration**, or **%CV**.
7. For quad stats only, select the quad region(s) of interest. Select **Show on plot** if you wish to see the stat for each quadrant appear in the corners of the plot. If multiple parameters are selected, the count appears by default. Quadrant markers will appear on the plot after you click **OK**.
8. Click **OK**. The Current Run Stats dialog box displays with the newly created stat. The value listed corresponds to the sample well highlighted in the plate map. Each stat is automatically applied to all samples in a given data set.
9. To see the results for any well, click on the well in the plate map. If you adjust the marker/region or any region that is part of the gating strategy, the statistics are automatically updated.

## Adjust Quadrant Markers

1. From the Guava® InCyte™ module, open the FCS needed for analysis.
2. Choose **File > Open** from the menu bar.
  - a. From the **Open File** dialog box, navigate to the FCS file for analysis and click **Open**.
3. Choose the .fcs file from the **Analysed Data** pane.
4. To adjust quadrant markers, click the plot to select the markers. The handles will display solid.
5. Position the cursor over the handle at the intersection and drag to the desired location. You can adjust the angle of the markers  $\pm 44^\circ$  from their original locations.
6. Drag the handle (solid circle) towards the end of the marker and tilt it to the desired location.

**NOTE:** You can add color to the different quadrants to help make populations easier to identify.



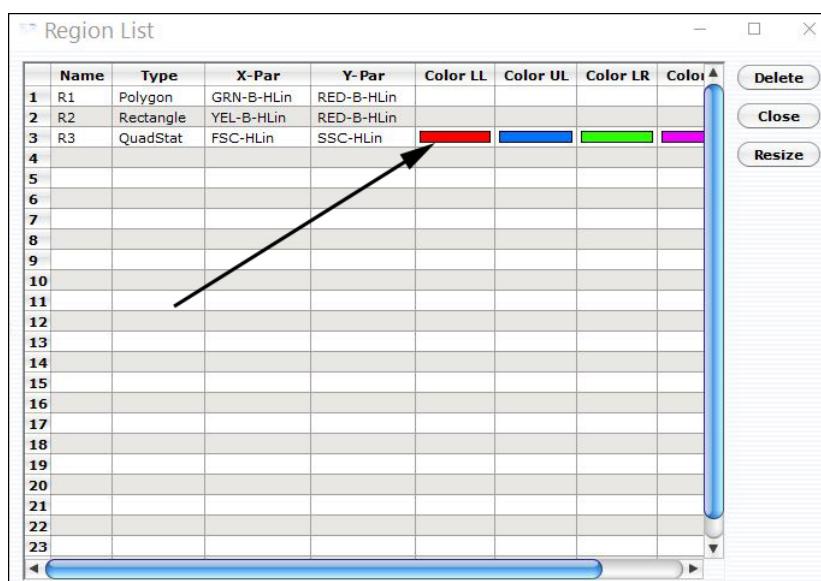
## Add Color to Quadrants

Add color to quadrants to help make populations easier to identify.

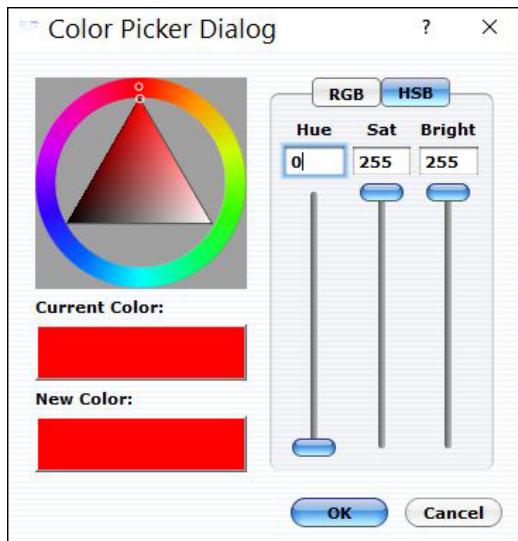
1. From the Guava® InCyte™ module, open the FCS needed for analysis.
2. Choose **File > Open** from the menu bar.
  - a. From the **Open File** dialog box, navigate to the FCS file for analysis and click **Open**.
3. Choose the .fcs file in the **Analysed Data** pane.
4. Click the **Region List** icon from the tool bar. The Region List dialog box displays.

**NOTE:** If you do not see a QuadStat in the Type column of the Region List, create one by clicking the **New Stat Marker** icon in the plot tool bar.

- a. Click the **New Stat Marker** icon in the plot tool bar and choose **New Quad Stat Marker**. The Add New Stat for QuadStat Marker dialog box displays.
- b. Make adjustments as needed and click **OK**.
5. Double-click the cell in the column for the quadrant you want to change the color for. The Color Picker Dialog displays.



6. Adjust the color using the outer circle or click the RGB button and use the sliders to adjust the color.



- To adjust the hue, saturation, or brightness, click the HSB button and use the sliders to make appropriate adjustments.

## Current Run Stats Window

The Current Run Stats window displays when you create a stat. You can also click the **Show Current Run Stats** icon in the main tool bar or select **Tools > Show Current Run Stats** from the menu bar to display the window. For each Analysis Method, all stats for all plots are listed in this window. The Values displayed correspond to the sample well highlighted in the plate map. Simply click any well to see the value for that sample. Stats are listed in the order they were created. You can reorder them during setup, if needed.

For each Analysis Method, all stat markers are listed in the Region List, as well as the Analysis Methods pane. All stats are saved with the Method.

Current Run Stats								
Stat Name	Value	Metric	Units	Marker Name	Quad	Parameter	Gate	Expression
R2.Count.UL	637	Count		R2	Upper Left	---	P01.Lymphs	
R2.Count.UR	3	Count		R2	Upper Right	---	P01.Lymphs	
R2.Count.LL	3166	Count		R2	Lower Left	---	P01.Lymphs	
R2.Count.LR	3	Count		R2	Lower Right	---	P01.Lymphs	

[Setup](#)   [Print Stats](#)
  
[customize stats](#)

- Setup** - you can customize the stats listed in the Current Run Stats window.
- Print Stats** - you can print the statistics listed in the table.
- To copy the window to the clipboard, right-click in the window and select **Copy to Clipboard**.

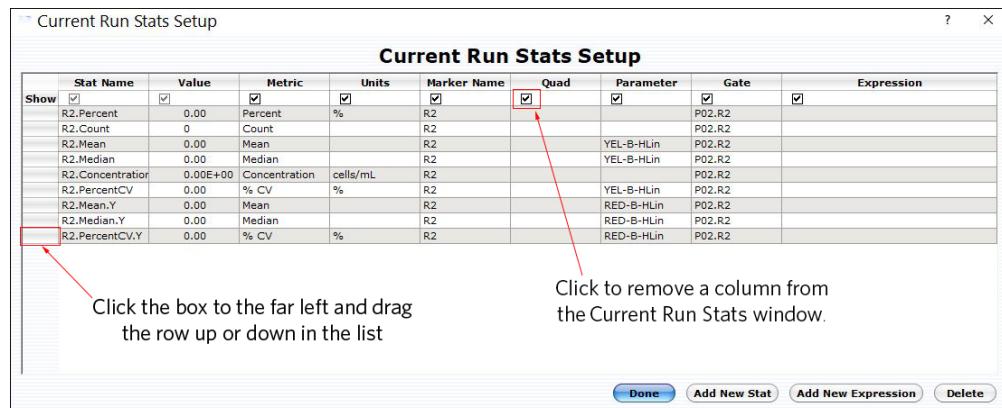
## Customize Current Run Stats

- From the Guava® InCyte™ module, open the FCS needed for analysis.

2. Choose **File > Open** from the menu bar.

  - a. From the **Open File** dialog box, navigate to the FCS file for analysis and click **Open**.

3. Choose the .fcs file in the **Analysed Data** pane.
4. Click the **Show Current Run Stats** icon in the tool bar. The Current Run Stats dialog box displays.
5. Click **Setup**. The **Current Run Stats Setup** dialog box displays.



6. To change the Stat Name, double-click the **Stat Name** field and enter a new name.
7. To change the metric, gate, quadrant, marker name, or parameter, double-click the item and select the new one from the drop-down list.

**NOTE:** Expression stats cannot be modified. They can only be deleted.

**NOTE:** If you want to change the parameter, double-click to display the parameters for that plot. If you want to see all available parameters for the data set, press Ctrl and double-click the parameter value. For a parameter to be displayed, you must select it in the **Add New Stat Marker** dialog.

8. To remove a column, deselect the Show check box for the selected parameter(s) and click **Done**. The Current Run Stats window is updated.
9. To create stats directly from the Current Run Stats Setup window, click **Add New Stat** to display the Add New Stat window.

**NOTE:** This window is similar to the window that displays when you select **Show Stat Setup** from the New Stat Marker icon in the plot tool bar. However, this stat marker window allows you to create a stat independent of a plot and based on any gate.

- a. Enter a stat name, then select the marker/region, gate, parameter, and metric, and click **OK**.
  10. To reorder the rows and/or columns in the Current Run Stats Setup window, click the far-left box of the row and drag the row up or down, or click the column header and drag the column to the left or right.
- NOTE:** You cannot move the Stat Name or Value column. They are fixed.
11. To delete a stat, select the stat from the list and click **Delete**.

## Create Expression Statistics

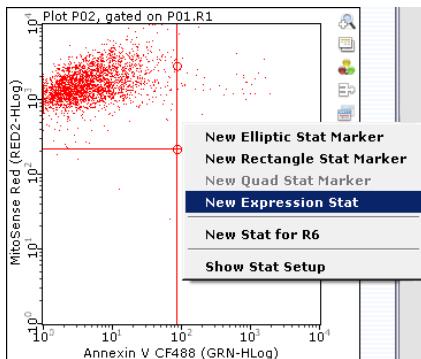
Expression stats allow you to create custom statistics. For example, you can apply the dilution factor to a count or concentration value of a specific population to get the actual value for your diluted sample. Or, you can create an expression statistic that adds the events in two quadrants of quad markers.

1. From the Guava® InCyte™ module, open the FCS needed for analysis.

2. Choose **File > Open** from the menu bar.

  - a. From the **Open File** dialog box, navigate to the FCS file for analysis and click **Open**.

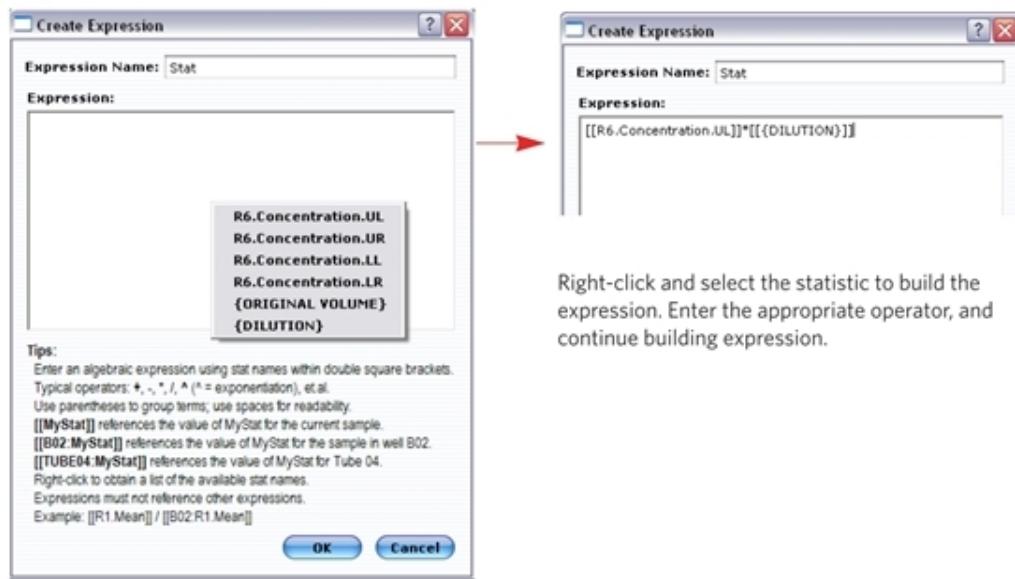
3. Choose the .fcs file from the **Analysed Data** pane.
4. Click the **New Stat Marker** icon in the plot tool bar and choose **New Expression Stat**. The **Create Expression** window displays.



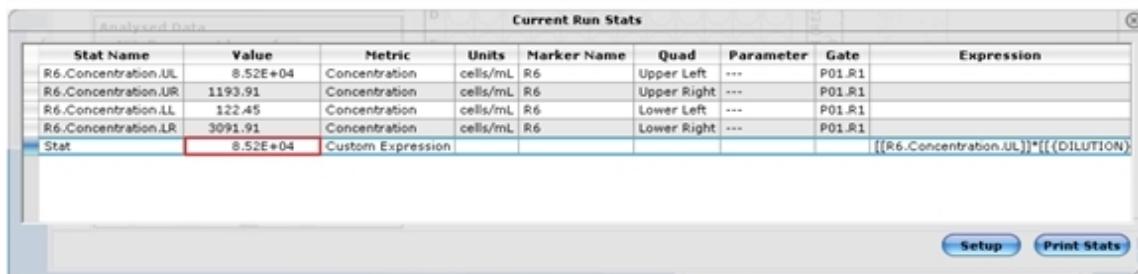
5. Enter a stat name in the **Expression Name** field.
6. Right-click in the **Expression** area of the window and select the stat. Type an operator (+, -, \*, /, or ^ for exponentiation). Complete the expression.

**NOTE:** To create an expression for a specific tube, press **Ctrl** and right-click the Expression area. The pop-up menu allows you to select a specific tube.

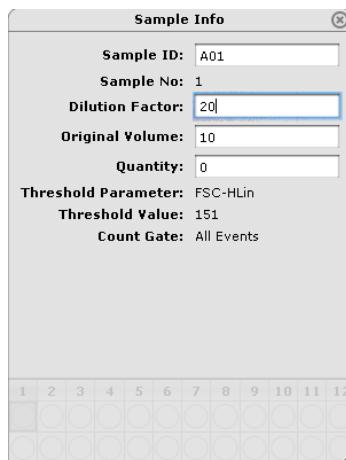
The following example shows an expression created to apply the dilution factor of 20 to events in the upper-left quadrant of a plot.



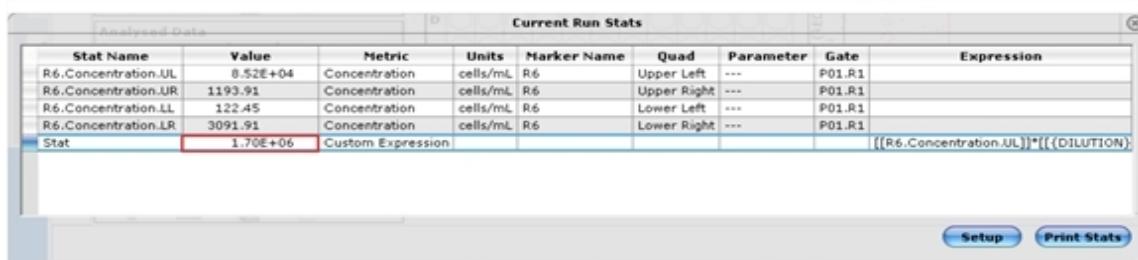
7. Click **OK**. The expression displays in the Current Run Stats window.



- Click the **Show Sample Info** icon in the tool bar to the left of the data panes. The **Sample Info** dialog box displays. Enter the dilution factor (up to 200,000) for the sample and press **Enter**.



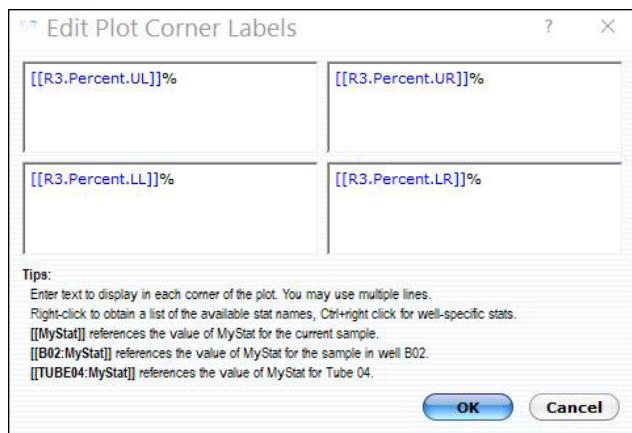
**NOTE:** The updated concentration value, based on the new dilution factor, displays in the Current Run Stats window.



## Label Plot Corners

You can add custom labels to the corners of any plots. Default labels already appear in plots with quadrant markers, if you select Show on plot when setting up the quadrant using the New Quad Stat Marker option.

- From the Guava® InCyte™ module, open the FCS needed for analysis.
- Choose **File > Open** from the menu bar.
  - From the **Open File** dialog box, navigate to the FCS file for analysis and click **Open**.
- Choose the .fcs file in the **Analysed Data** pane.
- Click the **New Stat Marker** icon in the plot tool bar and choose **Edit Plot Corner Labels**. The **Edit Plot Corner Labels** dialog displays.



5. If you are adding labels to a plot with quadrant markers, click the default [count] label and enter a new label. If you are adding labels to a plot without quadrant markers, simply type the label in the corresponding box.
6. Click **OK**. The labels display in the corners of the plot.

## Group Stats Window

The Group Stats window displays the statistical values for all samples in the data set (or group).

1. From the Guava® InCyte™ module, open the FCS needed for analysis.
2. Choose **File > Open** from the menu bar.
  - a. From the **Open File** dialog box, navigate to the FCS file for analysis and click **Open**.
3. Choose the .fcs file in the **Analysed Data** pane.
4. To display the Group Stats window, click the **Show Group Stats** icon in the main tool bar or select **Tools > Show Group Stats** from the menu bar.

Group Stats				
Well	Sample ID	Date	R2.Percent.UL Percent for R2 gated by P01.R1 (%)	R2.Percent.UR Percent for R2 gated by P01.R1 (%)
A01	A01	03.01.2017	0.42	0.03
A02	A02	03.01.2017	0.39	0.06
A03	A03	03.01.2017	0.42	0.03
A04	A04	03.01.2017	66.20	0.00
A05	A05	03.01.2017	64.36	0.00
A06	A06	03.01.2017	66.44	0.00
A07	A07	03.01.2017	0.00	67.29
A08	A08	03.01.2017	0.00	64.41

- Click **Calculate Stats for All** if you want to update the stats for all samples in the data set after you have analyzed or updated the analysis for a single sample.

**NOTE:** You must be in Single Sample Analysis mode for this feature, which is under **Application > Enable Single Sample Analysis**. This mode can only be activated before you open the data file for analysis.

- Click **Setup** to customize the stats listed in the Group Stats window.
- Click **Export To CSV** to export the group stats to a comma-separated values file for analysis using a spreadsheet program. Select the location, enter a file name, and click **Save**.

- Click **Print Stats** to print the statistics listed in the table.
- To copy the window to the clipboard, right-click in the window and select **Copy to Clipboard**.

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## Export Results

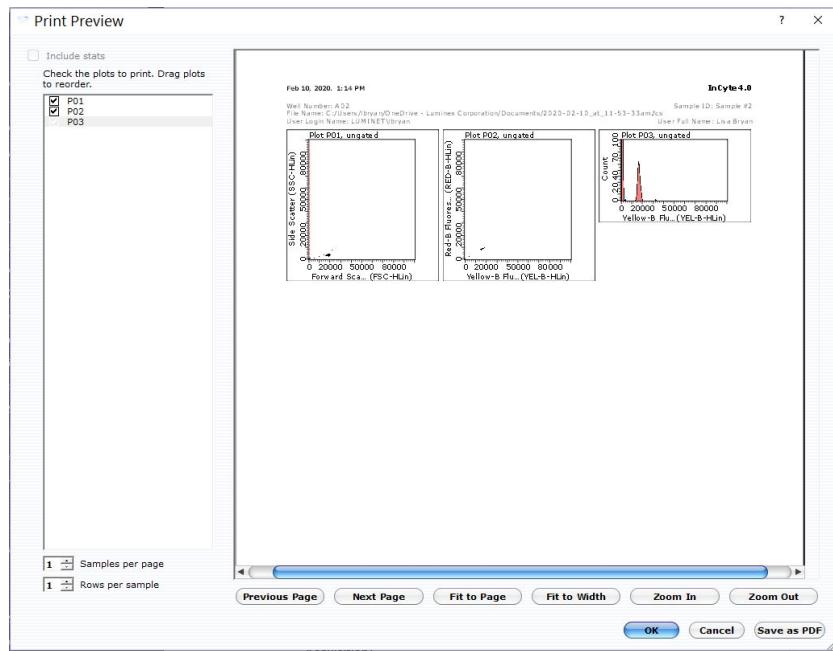
### Export Guava® InCyte™ Results to FCS and List-Mode Files

You can export Guava® InCyte™ results from the current run or any sample run to both FCS 2.0 and FCS 3.0 files. The data must have an AnalysedGroup associated with it. One FCS file is saved for each sample acquired. You can analyze both FCS 2.0 and 3.0 files using a third-party flow cytometry analysis application. FCS 3.0 files are exported with all parameters included. FCS 3.0 files with semi-automated or post-acquisition compensation may be compatible with some third-party applications. For more information, contact Luminex Technical Support. You can also export list-mode data files.

1. From the Guava® InCyte™ module, open the FCS needed for analysis.
  2. Choose **File > Open** from the menu bar.
    - a. From the **Open File** dialog box, navigate to the FCS file for analysis and click **Open**.
  3. Choose the .fcs file in the **Analysed Data** pane.
  4. Navigate to **File > Export** and choose the type of file you want to export.
- NOTE:** You can also choose a specific AnalysedGroup from the Analysed Data Pane and choose the appropriate export option from the File menu.
5. Choose the folder where you want to save the file, and enter a file name.
  6. Click **Save**. The samples are numbered sequentially and a number is automatically appended to the file name. For example, if the sample number is A01, the file will be named *filename*-1.FCS, A02 is *filename*-2.FCS, etc.

### Print the Results

1. From the Guava® InCyte™ module, open the FCS needed for analysis.
2. Choose **File > Open** from the menu bar.
  - a. From the **Open File** dialog box, navigate to the FCS file for analysis and click **Open**.
3. Choose the .fcs file in the **Analysed Data** pane.
4. (Optional) Set up the page orientation, paper size, and margins by selecting **File > Page Setup**.
5. Before printing results, use the **Print Preview** option to customize the information that prints. Navigate to **File > Print Preview**. The Print Preview window displays.



6. Select how you want the results to print:

- Choose the plots to print by selecting the check box next to the plots. The selected plots will print in the order they are listed. If you want to reorder the plots in the list, click and drag a plot to a new location in the list.
- Select the **Include stats** radio button to include the statistics. The stats will appear in a table at the end of the printout.
- Choose how many samples you want to print per page.
- Choose how many rows you want to print per sample.
- To scroll through the pages, place the cursor on the page, then click and drag (the hand) to move the page or click the **Previous Page** or **Next Page** buttons.

7. Click **OK**. The results will print to the default printer.

8. (Optional) You can click **Save as PDF** and print from there.

# Chapter 8: Troubleshooting

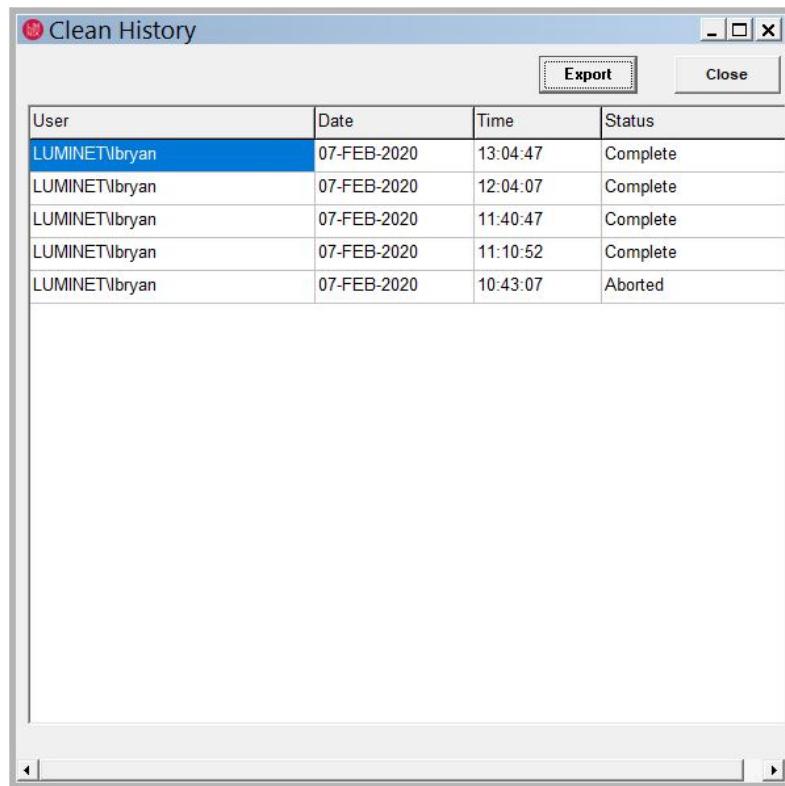
## History and Event Logs

GuavaSoft automatically saves a log of all Guava® Clean runs performed, as well as a log of all events that occurred during each run.

### Clean History Log

The cleaning history log contains a record of each time Guava® Clean is run.

1. From the GuavaSoft Software Main Menu, click **Cleaning**. The Guava Clean screen displays.
2. To open the log, click **View History** at the top of the Guava Clean screen. The Clean History log displays a list of Guava Clean runs by date and time, whether the procedure was completed or aborted, and the operator who was logged on when the procedure was performed.



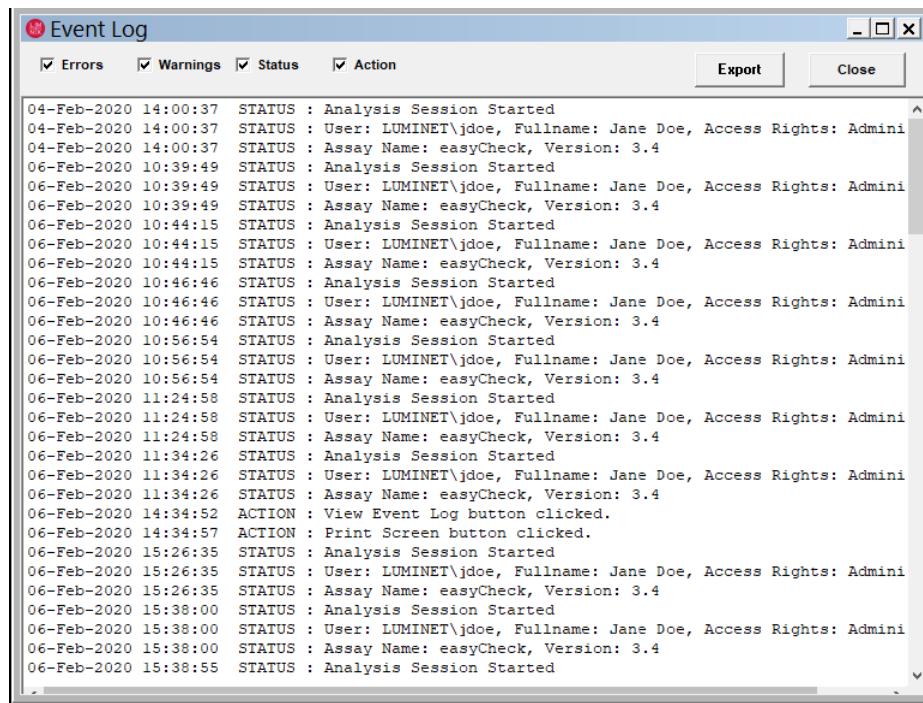
A screenshot of a Windows-style dialog box titled "Clean History". The dialog has a standard title bar with minimize, maximize, and close buttons. Below the title bar is a toolbar with "Export" and "Close" buttons. The main area is a table with four columns: "User", "Date", "Time", and "Status". The table contains five rows, each representing a cleaning run. The first row is highlighted with a blue background. The data in the table is as follows:

User	Date	Time	Status
LUMINET\rbryan	07-FEB-2020	13:04:47	Complete
LUMINET\rbryan	07-FEB-2020	12:04:07	Complete
LUMINET\rbryan	07-FEB-2020	11:40:47	Complete
LUMINET\rbryan	07-FEB-2020	11:10:52	Complete
LUMINET\rbryan	07-FEB-2020	10:43:07	Aborted

## View and Export Event Log

The Event Log displays a list of all the events that occurred during a Guava® Clean run.

- From the GuavaSoft Software Main Menu, click **Cleaning**. The Guava Clean screen displays.
- To view the Event Log, click **View Event Log** at the top of the Guava Clean screen. The **Event Log** lists every event/step that the operator and instrument performed by date and time.



**NOTE:** You can filter the list to view **Errors**, **Warnings**, **Status**, and **Actions**. Select the appropriate check box(es) to display the types of events you want to view.

- To export the list as a .csv file, click **Export**. The **Save Log Dialog** box displays.
- Select the location where you want to save the file and click **Save**.

## Export Service Check File for Technical Support

- From the GuavaSoft Software Main Menu, click easyCheck.

**NOTE:** The easyCheck™ screen opens in Acquisition mode. You must switch to Analysis mode to export the Service Check file.

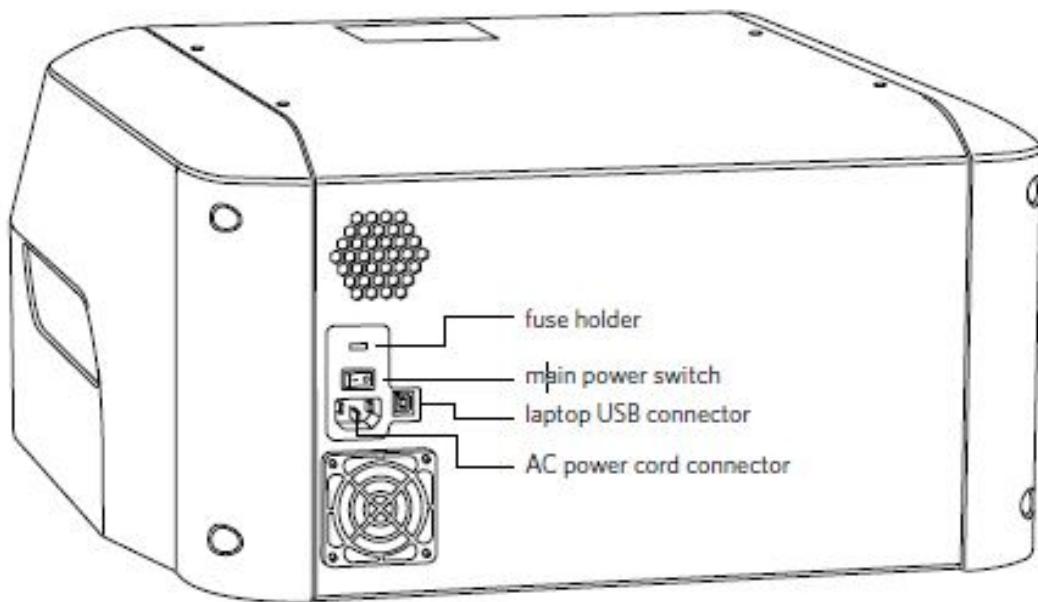
- Click **Export Service Check File** to export the Service Check file. The Service Check file is a zipped file containing the detailed results from the most recent easyCheck run. This file is used by service personnel to troubleshoot your system. If your easyCheck results continue to fail. Use this export feature to export the file so that you can send it to Luminex Technical Support. The Export Service Check File dialog box displays.
- Select the storage location and click **Save**.

## Replace the Fuses

The AC fuse is located on the rear panel to the right of 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.



1. Remove the fuse holder cover using a small screwdriver and pivot to expose the fuse carrier.
2. Pull out the fuse carrier.
3. Carefully remove the fuses and replace with new fuses.

- USA and Japan (110 V): 2.5 A, 250 V Time-lag
- Europe: (220-240 V): 1.6 A, 250 V Time-lag (x2)

**NOTE:** If you have a 110 V supply you will need to replace a single fuse. If you have a 220 V supply you will need to replace two fuses.

4. Insert the fuse carrier back into the fuse holder. The fuses 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.

## Guava® easyCyte™ System Troubleshooting

Problem	Possible Causes	Solutions
Laptop prompts for user ID or password.	Laptop is set up for authorization.	Do not enter password. Click <b>OK</b> or <b>Cancel</b> to continue. Contact your IT department for assistance with any modifications. The original laptop setup does not require a password.
During start-up or operation, software freezes on a particular screen.	1. System may be searching for directory during startup. 2. There may be a computer software driver incompatibility with the Guava software or firmware.	1. Press <b>Enter</b> to continue. Reboot system, if necessary. 2. Contact Luminex Technical Support
Message: <i>The instrument appears to be either off or not connected. You can run in Analysis mode only.</i>	1. Guava® easyCyte™ HT System is not turned on or is not getting power. 2. Cable connection between easyCyte HT System and laptop is loose. 3. easyCyte HT System and laptop were not powered on in correct sequence or have lost communication. 4. There is an electrical or software compatibility problem preventing communication with the instrument.	1. Ensure easyCyte HT System power cord is properly plugged in and system is turned on. 2. Ensure USB cable is securely connected to laptop. Use USB 2.0 ports if possible. Reboot computer, if necessary. 3. Turn off easyCyte HT System, exit GuavaSoft Software, restart laptop, turn on easyCyte HT System, wait for indicator light before starting the software. 4. Contact Luminex Technical Support
GuavaSoft Software launches, but only Analysis mode is available when an assay is launched.	Registration code not entered or not entered correctly.	Enter registration code and ensure all characters are correct.
Instrument will not power on.	Loose power cable.	Ensure instrument is plugged. Check all power connections.

Problem	Possible Causes	Solutions
Laptop keeps shutting down.	1. Power supply to laptop is faulty. 2. Screen saver is interfering. 3. Laptop overheating.	1. Ensure laptop is plugged in correctly. Use surge protector and ensure it is plugged in and turned on. 2. Adjust power scheme screen saver options. Click <b>Start&gt;Settings&gt;Control Panel</b> . Double-click <b>Display</b> , select <b>Screen Saver</b> tab, click <b>Settings</b> under <i>Energy saving features of monitor</i> . Make sure “Setting for Always On power scheme” are all set to Never. Laptop should not be allowed to “sleep.” GuavaSoft Software will stop acquiring data until the laptop is woken up. 3. Ensure cooling vents are clean and unobstructed.
For InCyte only  Message: Sorry - this unlock is invalid.	Incorrect or no unlock key.	Ensure the correct unlock key is entered. If necessary, contact Luminex Technical Support to obtain unlock key.
Noise occurring during sampling.	1. Mixer paddle is making contact with the plate. 2. Mixer paddle may be misaligned or bent.	1. Ensure you are using a compatible plate. 2. Contact Luminex Technical Support.
Message:  The tray door is open. appears when the door is shut.	The door sensor switch is damaged.	Contact Luminex Technical Support.
Message:  TRAY HOLD OFF STATE	1. The program is waiting for the automation to reset. 2. The tray door was opened. 3. The program lost detection of automation position or status. 4. There is a mechanical or electrical problem with the automation or mixer function.	1. Wait approximately 30 seconds for reinitialization. 2. Keep tray door closed during acquisition. 3. Exit GuavaSoft, turn the instrument off, then on again. Restart GuavaSoft, then open the worklist. 4. Contact Luminex Technical Support.



Do not run Excel® software, Internet Explorer® browser, or any other program on the laptop while using GuavaSoft Software to acquire data from the Guava easyCyte HT System. GuavaSoft Software requires the full resources of your laptop during data acquisition. Running other programs (even if you are not actively using them) during a run may interfere with acquisition or interrupt the run

## Guava® easyCheck™ Procedure Troubleshooting

Problem	Possible Cause	Solutions
No event counts appear for RED-R and NIR-R.	<ol style="list-style-type: none"> <li>Wrong beads used.</li> <li>Red laser not operating or problem with the signal.</li> </ol>	<ol style="list-style-type: none"> <li>Use easyCheck™ beads. Do not use Guava® Check beads.</li> <li>Contact Luminex Technical Support.</li> </ol>
One or more Particles/mL results falls outside the acceptance range (appears in red).	<ol style="list-style-type: none"> <li>System is not clean.</li> <li>Incorrect information entered in easyCheck fields.</li> <li>Bead suspension incorrectly prepared.</li> </ol>	<ol style="list-style-type: none"> <li>Run <b>Quick Clean</b>. If results are still outside range, run Clean Only.</li> <li>Ensure correct Bead Lot # and Expected Particles/mL are entered. Refer to easyCheck Beads vial label and information card for values.</li> <li>Prepare fresh bead sample and rerun easyCheck Procedure. Refer to easyCheck Kit package insert for preparation instructions.</li> </ol>
FSC, SSC, GRN-B, YEL-B, RED-B, NIR-B, RED-R, and/or NIR-R intensity is >15% outside the acceptable range.	<ol style="list-style-type: none"> <li>System is not clean.</li> <li>Problem with detector or laser.</li> </ol>	<ol style="list-style-type: none"> <li>Run Quick Clean. If results are still outside range, run Guava Clean.</li> <li>If problem persists, contact Luminex Technical Support.</li> </ol>
Particle counts for FSC, SSC, GRN-B, YEL-B, RED-B, NIR-B, RED-R, and/or NIR-R channels is not within 100 events of each other.	<ol style="list-style-type: none"> <li>If FSC count is zero or low, capillary may not be seated correctly.</li> <li>If any of the counts is low, possible problem with detector.</li> </ol>	<ol style="list-style-type: none"> <li>Remove metal plate on top of the instrument. Firmly push down on the top of the flow cell assembly to ensure it is seated all the way down in the optical tower. If problem persists, contact Luminex Technical Support.</li> <li>Rerun easyCheck Procedure. If counts are still low, contact Luminex Technical Support.</li> </ol>

Problem	Possible Cause	Solutions
<b>Few</b> events, as indicated in Particle Count section of Sample Information control panel.	1. Clogged flowcell. 2. Insufficient sample volume.	1. Remove sample, load bleach, click Backflush. Follow with Quick Clean using DI water. 2. Minimum sample volume is 100 µL for round-bottom wells and 150 µL for 0.5-mL tubes. Use 0.5-mL tubes in the tube position, as a higher volume (1 mL) is required for 1.5-mL tubes.
<b>No</b> events, as indicated in Particle Count section of Sample Information control panel.	1. Sample tube or plate not properly loaded. 2. Insufficient sample volume. 3. No beads in sample. 4. Clogged flowcell. 5. Broken flowcell. 6. Sample pump not working. 7. Laser is not operational. 8. Loose fitting on minstac tubing (under metal plate).	1. Ensure tube or plate is in place and tray is loaded. 2. Minimum sample volume is 100 µL for round-bottom wells and 150 µL for 0.5-mL tubes. Use 0.5-mL tubes in the tube position, as a higher volume (1 mL) is required for 1.5-mL tubes. 3. Ensure correct sample is loaded. 4. Remove sample, load bleach, click <b>Backflush</b> . Follow with Quick Clean using DI water.. 5. Remove flowcell and inspect for damage to the capillary end. Replace, if necessary. 6. Run Quick Clean and watch for fluid in waste vial. 7. Contact Luminex Technical Support. 8. Ensure tubing connector is secure.
Message:  <i>The login user does NOT have read/write permission to the file GuavaCheckLog.csv in the Log folder. Contact the system administrator for assistance.</i>	The user was not assigned access control to the system.	Contact your system administrator for user access to the software.

## Guava® InCyte™ Troubleshooting

Problem	Possible Cause	Solutions
InCyte™ will not launch; prompts you for an unlock code.	An unlock code was not entered or was entered incorrectly.	Contact Luminex Technical Support for the unlock code.
InCyte starts in Analysis mode. Acquisition mode is not available.	Communication problem between instrument and laptop.	Ensure USB cable is connected between instrument and laptop.
Gates and/or events in plots disappear.	<ol style="list-style-type: none"> <li>1. Data file or Method is selected.</li> <li>2. Data was acquired in tubes.</li> <li>3. More than 96 samples were acquired.</li> </ol>	Click on the AnalyzedGroup in the Analysed Data pane to display data and gates.
Message: Maximum velocity exceeded for "x" events.	The maximum velocity for x number of events exceeded the event velocity algorithm. Applies only to area/width parameters.	If the number of events that exceeded the maximum velocity is >10% of the total event count, try lowering the flow rate or diluting the sample.
Few events, as indicated in Cell Count section of Sample Information control panel.	<ol style="list-style-type: none"> <li>1. Clogged flowcell.</li> <li>2. Insufficient sample volume.</li> <li>3. Cells in suspension have settled.</li> </ol>	<ol style="list-style-type: none"> <li>1. Remove sample, load bleach, click <b>Backflush</b>. Follow with Quick Clean.</li> <li>2. Minimum sample volume is 100 µL for round-bottom wells, 150 µL for 0.5-mL tubes, and 900 µL for 1.5-mL tubes.</li> <li>3. Ensure sample mixing option was selected in WorkEdit Software.</li> </ol>
Unexpected events appearing in plots.	Instrument settings not optimal. Acquiring debris.	Adjust settings so debris is below threshold.
FSC Count under Cell Count shows events, but the events appear in the wrong places in plots.	<ol style="list-style-type: none"> <li>1. Sample was not stained.</li> <li>2. Cell lysis.</li> </ol>	<ol style="list-style-type: none"> <li>1. Check sample. If necessary, restain sample from original suspension.</li> <li>2. Check buffers used to process cells.</li> </ol>
Data analysis slow.	Analyzing large data files.	Use Single Sample Analysis mode to analyze individual samples more quickly.

Problem	Possible Cause	Solutions
<b>No events</b> , as indicated in Particle Count section of Sample Information control panel.	<ul style="list-style-type: none"> <li>1. Sample tube or plate not loaded.</li> <li>2. Insufficient sample volume.</li> <li>3. Clogged flowcell.</li> <li>4. Broken flowcell.</li> <li>5. Sample pump not working.</li> <li>6. Laser not operational.</li> <li>7. Loose fitting on minstac tubing (under metal plate).</li> </ul>	<ol style="list-style-type: none"> <li>1. Ensure tube is loaded and loader assembly is up.</li> <li>2. Minimum sample volume is 100 µL for round-bottom wells, 150 µL for 0.5-mL tubes, and 900 µL for 1.5-mL tubes.</li> <li>3. Remove sample, load bleach, click <b>Backflush</b>. Follow with Quick Clean using DI water.</li> <li>4. Remove flowcell and inspect for damage. Replace if necessary.</li> <li>5. Run Quick Clean and watch for fluid in waste vial.</li> <li>6. Allow laser to warm up for 15 min. If laser is not on, contact Luminex Technical Support.</li> <li>7. Ensure tubing connector is secure.</li> </ol>
Events appear in some plots but not in others.	Ensure correct gate is selected for plot in question.	<ol style="list-style-type: none"> <li>1. Open plot menu, point to Apply Gates and select gate.</li> <li>2. Check gate definition to ensure it includes the correct regions and operators.</li> </ol>
Events appear off scale in dot plots or histograms.	Gains set incorrectly, or samples staining brightly.	Adjust gain setting so positive populations appear on scale. Repeat Adjust Settings with negative sample. Adjust compensation settings.
Cannot resolve dim positive staining from background signal.	Dirty flowcell.	<ol style="list-style-type: none"> <li>1. Perform at least one cycle of Guava® Clean.</li> <li>2. While acquiring samples, select <b>Clean &amp; Rinse</b> instead of Quick Clean, and if necessary, run it frequently.</li> </ol>

Problem	Possible Cause	Solutions
Poor resolution between positive and negative populations.	<ol style="list-style-type: none"> <li>1. Gains too low to detect fluorescent signals.</li> <li>2. Incomplete staining with fluorescent probe, or fluorescent probe inappropriate for cell type.</li> <li>3. Fluorescent probes over-exposed to light, stored improperly, or expired.</li> <li>4. Non-specific binding of fluorescent probes.</li> <li>5. Background noise too high.</li> </ol>	<ol style="list-style-type: none"> <li>1. Adjust settings to increase fluorescent signal. Adjust compensation settings.</li> <li>2. Ensure positive control is staining adequately and with correct reagent.</li> <li>3. Refer to reagent package insert for proper storage instructions. Do not expose reagent to excessive light. Do not use expired reagents.</li> <li>4. If using antibody-based probes, try Fc blocking reagent during staining to minimize non-specific binding. Otherwise, titer the fluorescent probes down to reduce the nonspecific staining.</li> <li>5. Adjust settings to increase FSC threshold to remove debris. Or, wash stained sample and reacquire.</li> </ol>
Poor resolution between positive populations.	<ol style="list-style-type: none"> <li>1. Incomplete staining with reagent(s).</li> <li>2. Too much reagent in staining tube.</li> <li>3. Fluorescence background too high.</li> <li>4. Gain too high causing signal to bleed into other parameters.</li> <li>5. Gain too low to optimally detect positive signal.</li> <li>6. Background noise too high.</li> </ol>	<ol style="list-style-type: none"> <li>1. Check expiration date and amount of reagent(s) used in staining.</li> <li>2. Washing cells may remove residual reagent.</li> <li>3. Washing cells may remove residual reagent.</li> <li>4. Adjust settings to reduce gain. Adjust compensation settings.</li> <li>5. Adjust settings to increase gain. Adjust compensation settings.</li> <li>6. Adjust settings to increase FSC threshold to remove debris. Or, select one of the fluorescence parameters as the threshold.</li> </ol>

# Chapter 9: Maintaining the System

To ensure accurate test results, properly clean and maintain the Guava® easyCyte™ System. Read and follow all instructions in this chapter.



When analyzing potentially infectious biological samples with the Guava easyCyte System, follow standard laboratory safety practices. These safety precautions should also be taken when cleaning or maintaining the system.

## General Maintenance Precautions

Observe the following general maintenance precautions.



Personnel who use, maintain, or clean the Guava® easyCyte™ System should be trained in standard laboratory safety practices and should follow those practices when handling the system. Samples and waste fluid can contain bio-hazardous material. Where exposure to bio-hazardous material, including in an aerosol form, exists, follow appropriate biosafety procedures, use personal protective equipment, and use ventilation devices. The interior of the instrument contains potential electrical, mechanical and laser radiation risks. For this reason, access to the interior of the instrument is possible only with a tool and is limited to Luminex trained service personnel. There are no user serviceable or accessible parts in the interior of the instrument necessary for operation.

## Perform Preventative Maintenance

Luminex recommends performing a preventative maintenance on the instrument at least yearly depending on instrument usage. For more information contact Luminex Technical Support.

## Run the Guava® Quick Clean

The Guava® Quick Clean is a short cleaning cycle that allows you to clean the fluid system during or after an assay. The Quick Clean feature is accessible from each GuavaSoft Assay screen, as well as from the Guava® easyCheck™ screen. At the completion of an assay, the system automatically performs a Quick Clean.

For the Guava InCyte™ and ExpressPro assays, you can choose between Quick Clean or Clean & Rinse. You can also perform additional Quick Clean and Clean & Rinse cycles as often as you like during an assay.

1. Place a tube filled with deionized (DI) water in tube position w4 when you load the tray at the start of a run.
2. If you want to perform a Quick Clean using a cleaning solution other than water, you can add one of the following to another tube or well. The system will prompt you to select the tube/well.
  - undiluted Guava Instrument Cleaning Fluid (ICF) to clean the system, followed by water to rinse
  - a 10% bleach solution in Guava ICF (1 part bleach in 9 parts Guava ICF; for example, 100 µL bleach plus 900 µL Guava ICF) to clean and sanitize, followed by water to rinse

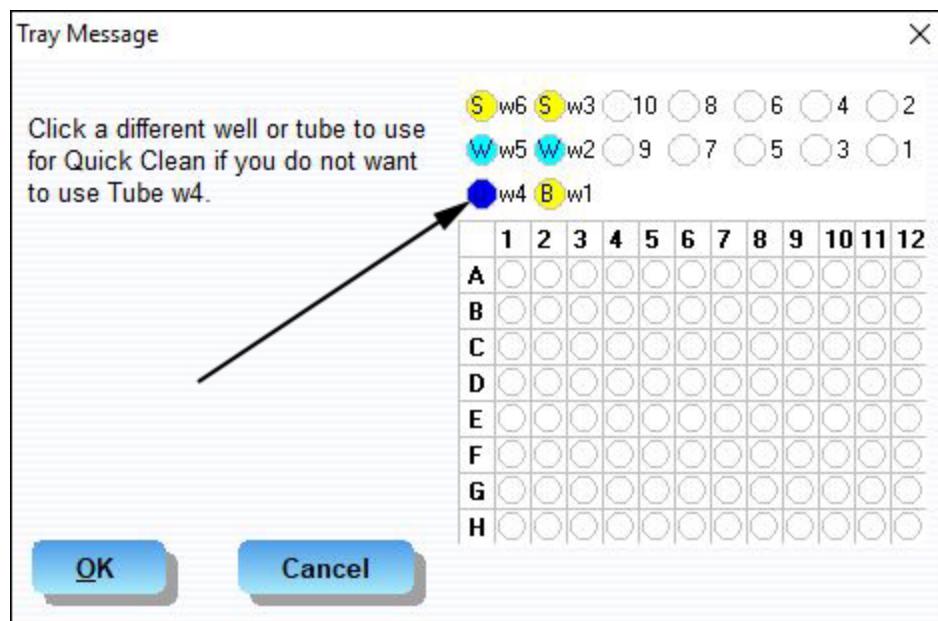
**NOTE:** You can perform approximately three Quick Clean cycles from a single well and seven Quick Cleans from a 1.5-mL tube containing 1.5 mL of fluid.

3. Click **Quick Clean** from the easyCheck screen or any Guava screen.

**NOTE:** A message displays prompting you to select the well or tube used for the Quick Clean. The default tube for Quick Clean is position w4.

**NOTE:** If the system is acquiring samples, click **Pause**, then click **Quick Clean**.

4. Leave the default position w4 selected, or click to select a different well/tube. Click **OK**.



5. If you ran water, you are finished. If you ran Guava ICF, either straight or diluted with bleach, click **Quick Clean** again to rinse, select the well/tube containing water, and click **OK**.
6. If you paused the run to perform the Quick Clean, click **Resume**.

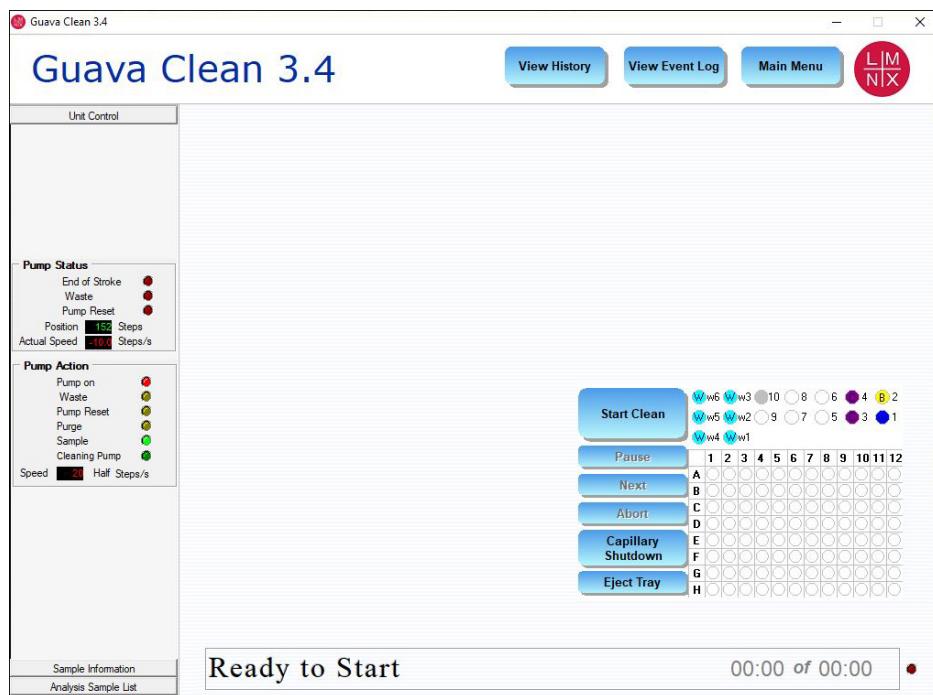
**NOTE:** If you will not be using the system for 30 minutes or more, leave the instrument on and leave GuavaSoft at the current assay. After a Quick Clean, the capillary tube is left in distilled water to prevent it from drying out.

## Perform the Guava® Clean Procedure

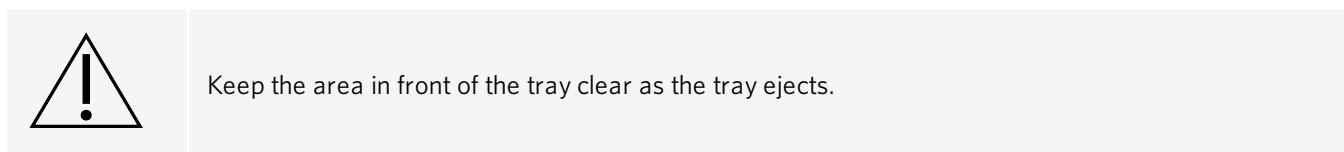
Run Guava® Clean to clean the instrument at the beginning and end of the day, as well as between assays if a thorough cleaning is needed. You can also use Guava Clean to prime the fluid system or if you suspect there is air in the fluid lines. Fill the cleaning solution vial and sample tube with deionized (DI) water and run Guava Clean to prime. Guava Clean takes approximately 15 minutes to complete. While the procedure is running, the lasers are turned off.

**NOTE:** If running samples that have high background, such as lysed whole blood, Luminex recommends running two complete cleaning cycles to flush the system of any residual sample.

- From the GuavaSoft Software Main Menu, click **Cleaning**. The Guava Clean screen displays.

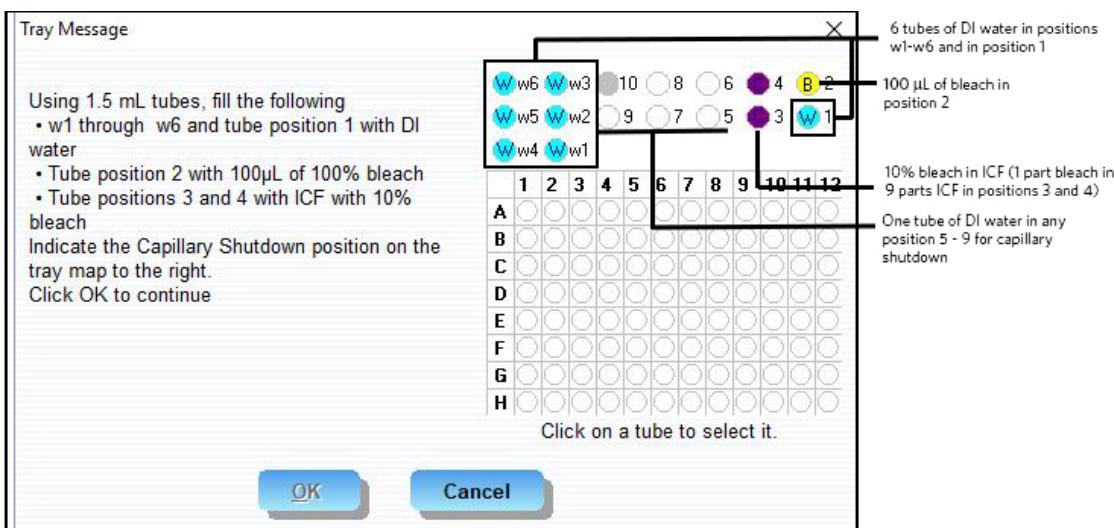


- Click **Start Clean**. The plate is ejected.



- Load the following tubes on the instrument:

- Load seven tubes filled with DI water in positions w1, w2, w3, w4, w5, w6, and tube position 1.
- Load a tube containing 100 µL of 100% bleach in position 2, indicated by the yellow well.
- Load two tubes with a mixture of 10% bleach solution and Guava ICF (1 part 100% bleach in 9 parts Guava Instrument Cleaning fluid (ICF)) in tube positions 3 and 4.
- Load a tube filled with DI water in any tube position 5–9 for the capillary shutdown. Then, click to select this position on the plate map.



- After selecting the tube position (5–9) for DI water for the capillary shutdown, click **OK**.



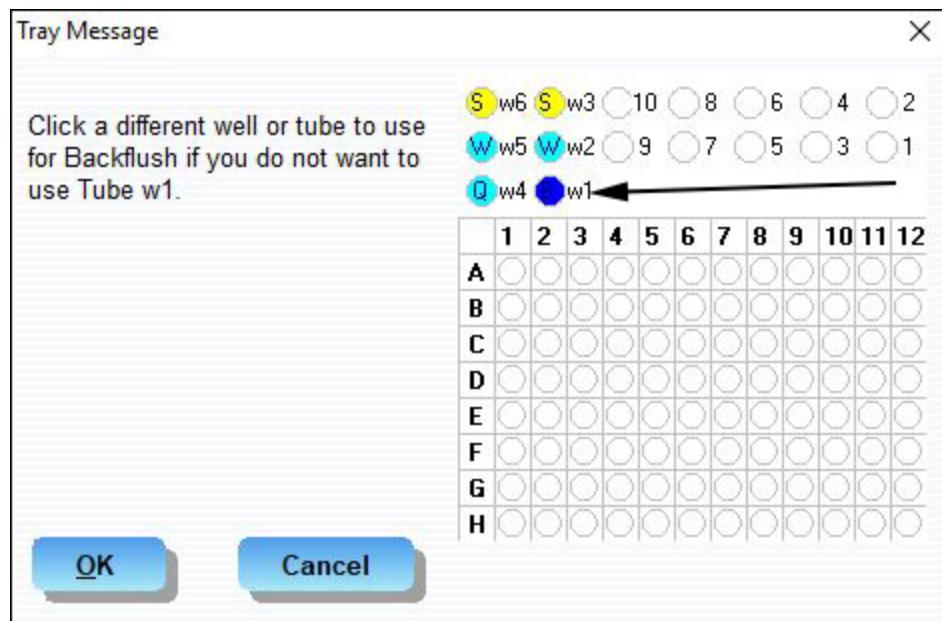
If you click **OK** or **Cancel** in the dialog box, the tray will automatically load. Keep the area clear as the trayloads.

- If you are finished using the instrument, click **Eject Tray** and dispose of the used plate and tubes.
  - Perform the Shut Down the Capillary procedure to shut down the capillary with a tube of DI water to keep it moist while the instrument is not in use.
- NOTE:** If you will not be using the instrument for an extended time, perform the capillary shutdown procedure periodically using a fresh tube of DI water to ensure that the capillary does not dry out. Check the tube of water to ensure it has sufficient volume.
- Click **Main Menu** to return to the GuavaSoft Software main menu and continue working, or click **Exit** to close GuavaSoft Software.
- NOTE:** Do not reuse the tubes that were previously used for the Guava Clean procedure. After the cleaning cycle is complete, dispose of the tubes according to your local regulations.

## Perform the System Backflush

The backflush feature reverses the flow of fluid out of the flow cell. If you suspect that the fluid pathway is clogged or blocked, perform a Backflush. You can find the Backflush button on any of the assay screens or on the Guava® easyCheck™ screen.

- Click **Backflush** from the easyCheck screen or any Guava assay screen. A message displays prompting you to select the well or tube used for backflushing. The default tube for backflushing is position w1, containing 100  $\mu$ L of bleach.
- NOTE:** If the system is acquiring samples, click **Pause**, then click **Backflush**.
- Leave the default position w1 selected, or click to select a different well/tube. Click **OK**.

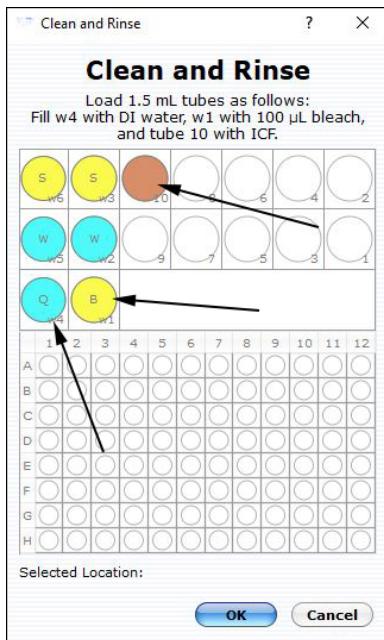


3. When the backflush is complete, click **Quick Clean** to rinse the bleach from the capillary.
4. Click **Resume** if you paused the run to perform a backflush.

## Run a Clean & Rinse

The Clean & Rinse feature automatically performs a series of Quick Clean cleaning cycles with one backflush to thoroughly clean the fluid system. Use Clean & Rinse when optimum system sensitivity is needed.

1. From the Guava InCyte module or ExpressPro acquisition screen, click **Clean & Rinse**. You can also select it as a cleaning option in the Worklist Editor Software for these applications. A Clean and Rinse dialog box displays prompting you to load tubes.
2. Load the following tubes:
  - a tube filled with deionized (DI) water in position w4
  - a tube of 100 µL bleach in position w1
  - a tube filled with Guava ICF in position 10



- Click OK.

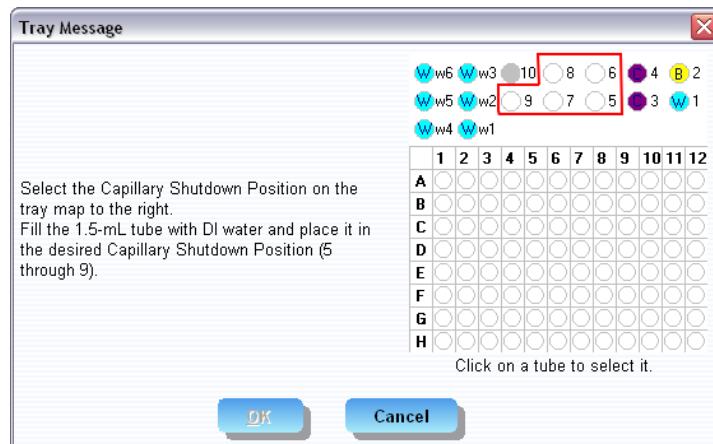
**NOTE:** You can perform approximately four Clean & Rinse cycles before you will need to refill the fluid in tubes w4 and 10, and empty then refill tube w1 with bleach.

## Shut Down the Capillary

At the completion of Guava® Clean, eject the tray and dispose of the used plate and tubes. Then, to keep the capillary wet when you turn off the system, use the Capillary Shutdown feature to place the capillary in a tube of water.

**NOTE:** If you will not be using the instrument for an extended time, perform the capillary shutdown procedure periodically, using a fresh tube of deionized (DI) water to ensure that the capillary does not dry out. Check the tube of water to ensure it has sufficient volume.

- From the Guava Clean 3.4 screen, click **Capillary Shutdown**. A Tray Message dialog box displays prompting you to select the tube for the capillary shutdown position.

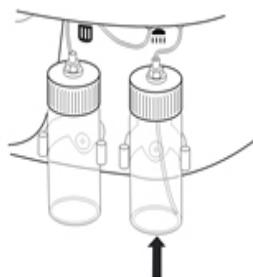


2. Click to select a tube position 5 through 9 at the top of the plate map.
3. Place a tube containing 1.5 mL of deionized (DI) water in that location and click **OK**.

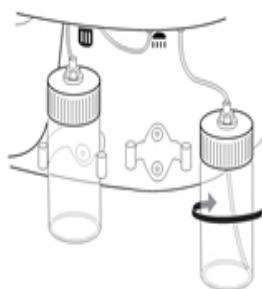
## Fill the Cleaning Solution Vial

Fill the cleaning solution vial with Guava® Instrument Cleaning Fluid (ICF) when it is approximately 1/4 full. The cleaning solution is aspirated through the tubing in the vial. Do not allow the vial to empty. Check the vial frequently to ensure it does not run dry. Letting the vial run dry will create air bubbles in the fluid system and require you to prime the system with water. One vial of cleaning solution will allow you to perform approximately 15 cleaning cycles.

1. Gently pull up on the cleaning solution vial to remove the vial from the bracket. The cleaning solution vial is on the right.



2. Unscrew the cleaning solution vial from the cap.



3. Fill the cleaning solution vial with Guava ICF to just below the bottom of the cap. Do not overfill the vial.
4. Screw the cleaning solution vial back to the cap assembly and install the vial on the Guava easyCyte™ HT System.

**NOTE:** Ensure the tubing that goes into the cleaning solution vial is still attached to the cap.

## Empty the Waste Vial

Empty the waste collection vial at the end of each day, or as needed.

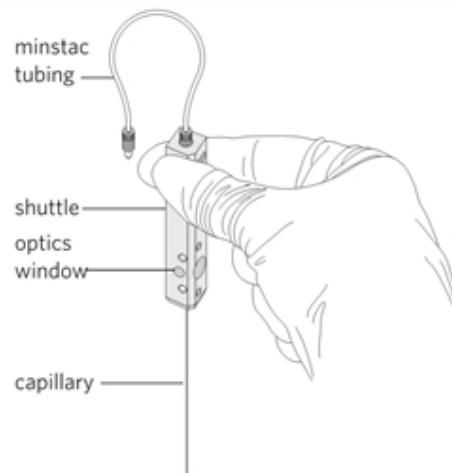


Where exposure to potentially biohazardous specimens and materials, including aerosol, exists, follow appropriate biosafety procedures and use personal protective equipment (PPE). PPE includes gloves, gowns, laboratory coats, face shields or mask and eye protection, respirators, and ventilation devices.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

1. Gently pull up on the waste vial to remove the vial from the bracket. The waste vial is located on the left.
2. Unscrew the waste vial from the cap. Twist the luer-lock connector to remove the waste tubing from the vial cap.  
**NOTE:** Fluid may seep from the cap while it is disconnected from the vial. To prevent waste fluid from dripping on the work surface, place the cap in a small container or on a disposable, absorbent pad.  
**NOTE:** If you notice a leak on top of the waste vial cap, tighten the connector on the cap.
3. Dispose of the contents according to your local and state biohazardous waste disposal guidelines.
4. Rinse the waste vial with water.
5. Add approximately 5 mL of 100% bleach to the waste vial, screw the vial back to the cap assembly and install the waste vial on the Guava® easyCyte™ HT System.  
**NOTE:** Ensure the tubing that goes into the waste vial is still attached to the cap.

## About the Flowcell

The flowcell assembly consists of the shuttle, the capillary, and the minstac tubing. The shuttle houses the optical window, where the laser beam intersects the sample. When handling the flowcell assembly, grasp it towards the top of the shuttle, where the minstac tubing is attached, to avoid getting fingerprints on the optics window. When installing a flowcell, ensure this tubing connection is tight to avoid fluid leaking from the top of the shuttle.



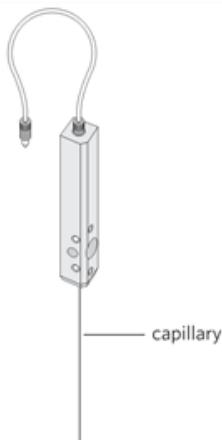
A flowcell removal tool is provided and can be used to remove the flowcell. A tightening tool is also provided and can be used to tighten the minstac tubing to the instrument during flowcell installation.

## Clean the Flowcell Assembly

Sample uptake occurs through a capillary and is regulated by a variable speed fluid pump. The pump does not require sheath fluid or other supplementary fluids for operation. Because the system's sampling precision depends on the integrity of the fluid pathway, it is important to maintain a clean system. Follow these guidelines to maintain a clean system:

- Do not allow samples to remain in the capillary for extended periods of time.
- Perform frequent cleaning cycles to prevent the build-up of cellular debris that may restrict sample flow.
- Always keep a tube of water loaded when the instrument is not in use.
- If a clog does occur, clear it by using the backflush feature, which reverses the flow of fluid and flushes it out of the flowcell at a high speed. Some assays allow you to select the sample flow rate for acquisition

**NOTE:** For most assays, the Medium flow rate is the default. This works well when the sample concentration is approximately 500 particles/ $\mu\text{L}$  or less. If the sample concentration is higher, dilute the sample or use a lower flow rate. For assays where the peak CV is critical, such as Cell Cycle, use the Low or Very Low flow rate. Always use the same flow rate for acquisition that you use for adjusting the settings.

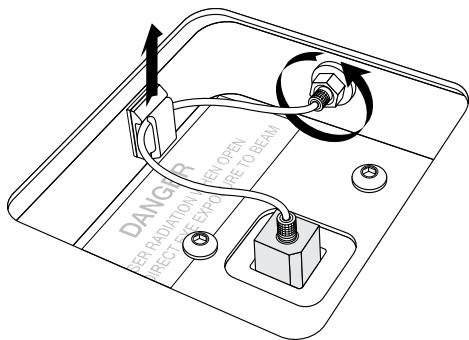


Use the syringe assembly tool to clean the flowcell. When removing the flowcell, handle it with care. The capillary tube is fragile; avoid touching it unnecessarily. Do not force the flowcell into the receptacle. However, slight pressure may be required to properly seat the flowcell once it has been inserted into the receptacle.

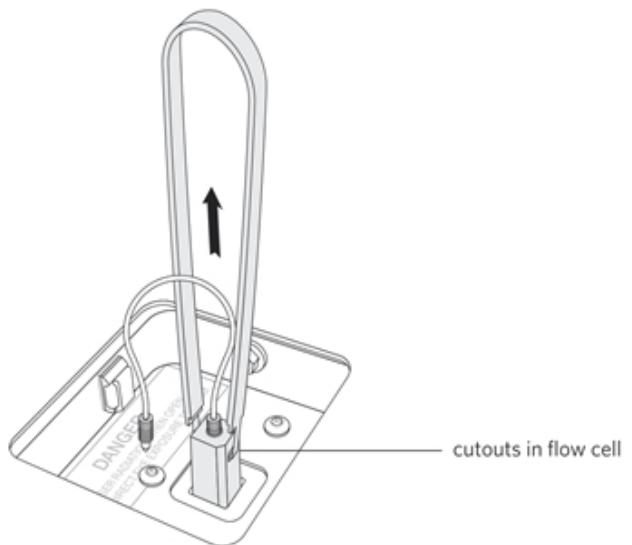


To avoid exposure to laser radiation, turn off the power to the Guava® easyCyte™ HT System before attempting to remove the flowcell.

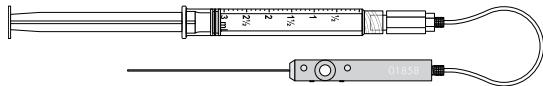
1. Turn off all power to the system.
2. Remove the flowcell hatch from the top of the instrument.
3. Remove the minstac tubing from the clamp and disconnect it from the instrument.



4. Remove the flowcell by grasping it at the top with your fingers and pulling straight up. Do not pull up on the tubing. You can also use the flowcell removal tool to remove the flowcell.
  - a. Grasp the flowcell with the removal tool by placing the clamps into the cutouts at the top of the flowcell, as shown.
  - b. Pull the tool straight up to remove the flowcell.



5. Fill the syringe cleaning tool with water or Guava ICF. Connect the syringe to the minstac tubing on the flowcell. Ensure the fitting is tight.

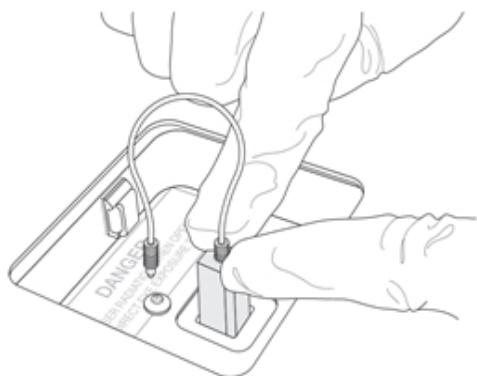


6. Use a Kimwipe® wipe to hold the flowcell at the top of the shuttle to capture fluid that may leak. Apply gentle, steady pressure to the plunger and watch as the fluid flows from the tip of the capillary.
  - Make sure the fluid stream is straight. If it is not straight, the tip of the flowcell may be chipped or there may be a partial clog in the flowcell.
  - Check the capillary to ensure there are no leaks along the length of it.
  - Make sure fluid is not leaking where the tubing is connected at the top of the flowcell.
7. Unscrew the syringe from the minstac tubing. Leave the minstac tubing attached to the flowcell.
8. Use a Kimwipe wipe to dry the end of the tubing that you disconnected from the syringe.

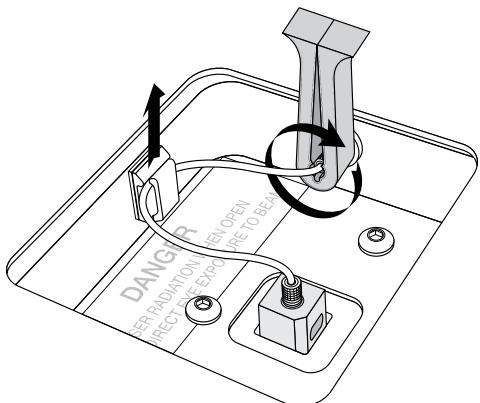


Dry connector before reinstalling the flow cell.

9. If the flowcell is clean and intact, reinstall it. If it is damaged or not functioning properly, discard it and replace it with a new flowcell.
  - Install the clean (or new) flowcell by correctly positioning it vertically above the instrument and carefully lowering it into the receptacle. The flowcell fits only one way into the receptacle. Avoid bumping the capillary as you install it.
  - Use your fingers to push down on the top of the flowcell on either side of the tubing until the flowcell clicks into place. Do not press down on the tubing at the top of the shuttle. Press down until the flowcell stops and won't go any farther.



10. Connect the tubing to the instrument. Make sure the tubing is screwed on tightly. If necessary, use the tightening tool. Then insert the tubing into the clamp.



11. Turn the power back on to the system.
12. Ensure that the cleaning solution vial is full, then run Quick Clean to prime the system. If starting the instrument after it has been shut down, run Guava Clean to prime. Watch the tubing during the Quick Clean to ensure no bubbles appear in the tubing.
13. To ensure that the flowcell was correctly installed, run the easyCheck procedure. While the first replicate is being acquired, watch for bubbles in the minstac tubing. If bubbles or leaks are visible, the tubing may not be adequately tightened.
14. Replace the flowcell hatch.

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## Replace the Flowcell

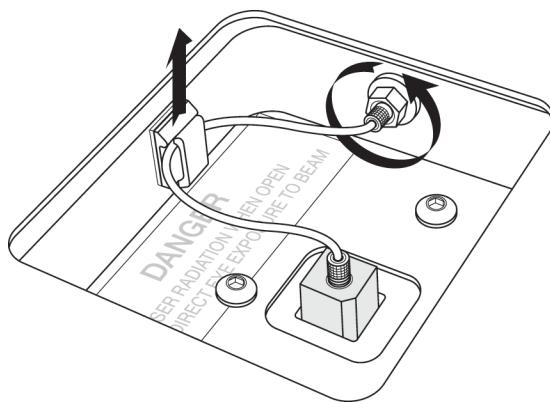
You can replace the flowcell if it becomes damaged or clogged so severely that backflushing and cleaning the system do not fix the problem. When replacing the new flowcell, handle it with care. The capillary tube is fragile; avoid touching it unnecessarily. Do not force the flowcell into the receptacle. However, slight pressure may be required to properly seat the flowcell once it has been inserted into the receptacle.



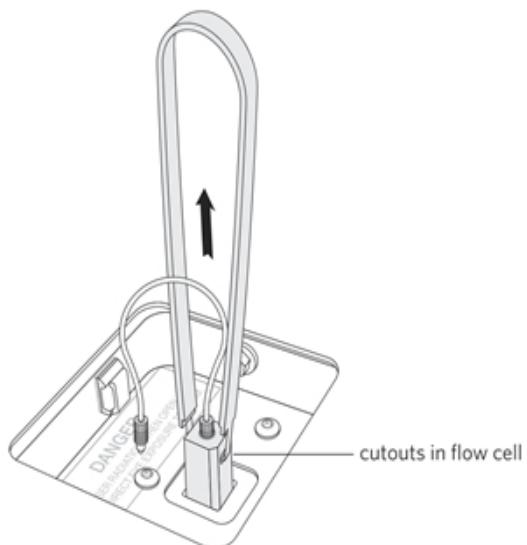
To avoid exposure to laser radiation, turn off the power to the Guava® easyCyte™ HT System before attempting to remove the flowcell.

Turn off all power to the system before attempting to remove the flowcell.

1. Turn off all power to the system.
2. Remove the flowcell hatch from the top of the instrument.
3. Remove the minstac tubing from the clamp and disconnect it from the instrument.



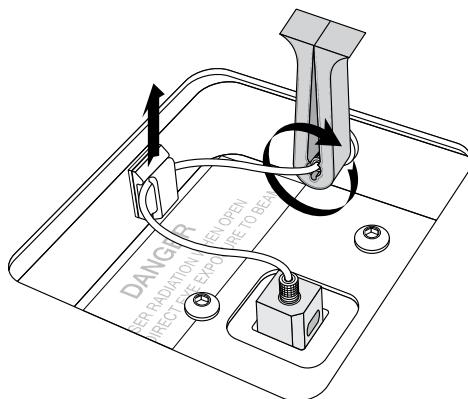
4. Remove the flowcell by grasping it at the top with your fingers and pulling straight up. Do not pull up on the tubing. You can also use the flowcell removal tool to remove the flowcell.
  - a. Grasp the flowcell with the removal tool by placing the clamps into the cutouts at the top of the flowcell, as shown.
  - b. Pull the tool straight up to remove the flowcell.



5. Install a new flowcell by correctly positioning it above the instrument and carefully lowering it into the receptacle. The flowcell fits only one way into the receptacle. Keep the flowcell completely vertical and avoid bumping the capillary against the instrument or sides of the receptacle. Use your fingers to press down on the top of the flowcell on either side of the tubing until the flowcell clicks into place. Do not press down on the tubing at the top of the shuttle.



6. Connect the tubing to the instrument. Make sure the tubing is screwed on tightly. If necessary, use the tightening tool. Then insert the tubing into the clamp.



7. Turn the power back on to the system.
8. Ensure that the cleaning solution vial is full, then run Quick Clean to prime the system. If starting the instrument after it has been shut down, run Guava Clean to prime.
9. To ensure that the flowcell was correctly installed, run the Guava® easyCheck™ procedure. While the first replicate is being acquired, watch for bubbles in the minstac tubing. If bubbles or leaks are visible, the tubing may not be adequately tightened.
10. Replace the flowcell hatch.

## Run the Instrument Shutdown Procedure

1. Run the Guava Clean procedure at the end of the day.
  - a. After the cleaning procedure is complete, return to the **Main Menu** and exit GuavaSoft Software.
2. To shut down the Guava easyCyte HT System, press the power switch located half-way up on the right edge at the back of the instrument.

**NOTE:** Do not shut down the Guava easyCyte HT System while GuavaSoft Software is running.

# Chapter 10: Configuring the System Settings (Admin Only)

Read and follow this chapter if:

- you want to use access control in your laboratory or environment, or
- you want to create new user accounts, or
- you are a network administrator and need information about the Guava® System environment in order to create Guava user accounts in a manner consistent with your local security policy.

Luminex recommends that you contact your local network administrator for help in setting up user accounts and access control.

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## Setting up Access Control

GuavaSoft Software, version 4.0, supports features which allow a laboratory administrator in controlled environments to restrict users from access to certain features of the Guava® easyCyte™ HT System to help ensure that standard procedures are followed correctly. Users whose access has been restricted cannot change instrument or analysis settings, and may only operate the Guava easyCyte HT System according to previously saved Settings files or the instrument default settings.

By default, after installation there are no access control restrictions, and the default user account has access to all features of the software. If such unrestricted access is sufficient for your needs, then no further steps are necessary and you may begin using the software.

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## Access Control Levels

GuavaSoft Software, version 4.0, supports three levels of access control, *Administrator*, *Supervisor*, and *Operator*. Each user account under the Windows® operating system has one of these levels, which is assigned by the lab administrator on a case-by-case basis. A higher access control level always has privileges of all access control levels below.

*Administrator*-level user accounts have unrestricted access to all features of the software including the “Administration Configuration,” and are assumed to be GuavaSoft Software Administrators who know how to properly configure all features of the software for supervisor-level and operator-level users.

*Supervisor*-level user accounts have unrestricted access to all features of the software except the “Administration Configuration” and are assumed to be expert users who know how to properly use all features of the software at the supervisor and operator levels.

**All user accounts can:**

- perform the Adjust Settings step
- retrieve instrument settings files
- start new data sets
- run the Guava® easyCyte™ HT System and acquire data (entering all relevant information such as sample IDs, number of events to acquire, and dilution factor and original volume, when applicable)
- perform Quick Clean, Backflush, and Cleaning procedures
- run the easyCheck™ procedure
- abort an acquisition, when necessary
- export data to FCS 2.0, 3.0, or CSV format
- add comments to event log
- view event log file

**Operator-level user accounts cannot:**

- change instrument settings (FSC gain, PM1 or PM2 voltages, pump speed)
- adjust markers or gates
- make any adjustments during the Adjust Settings step
- use Next Step, except to (a) finish Adjust Settings, or (b) terminate a normal acquisition
- save instrument settings

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## Before Setting Up Access Control

The GuavaSoft Software, version 4.0, installer automatically creates the three different Guava® User Groups: under Windows, namely, GuavaAdmin, GuavaSupervisor, and GuavaOperator. The correct access to the various Guava files needed to function properly are also automatically set up by the installer.

**NOTE:** If you plan to connect the system to a network, be sure to contact your local network administrator for help, since the procedures described here may need to be modified in order to comply with security policies in effect for your local network.

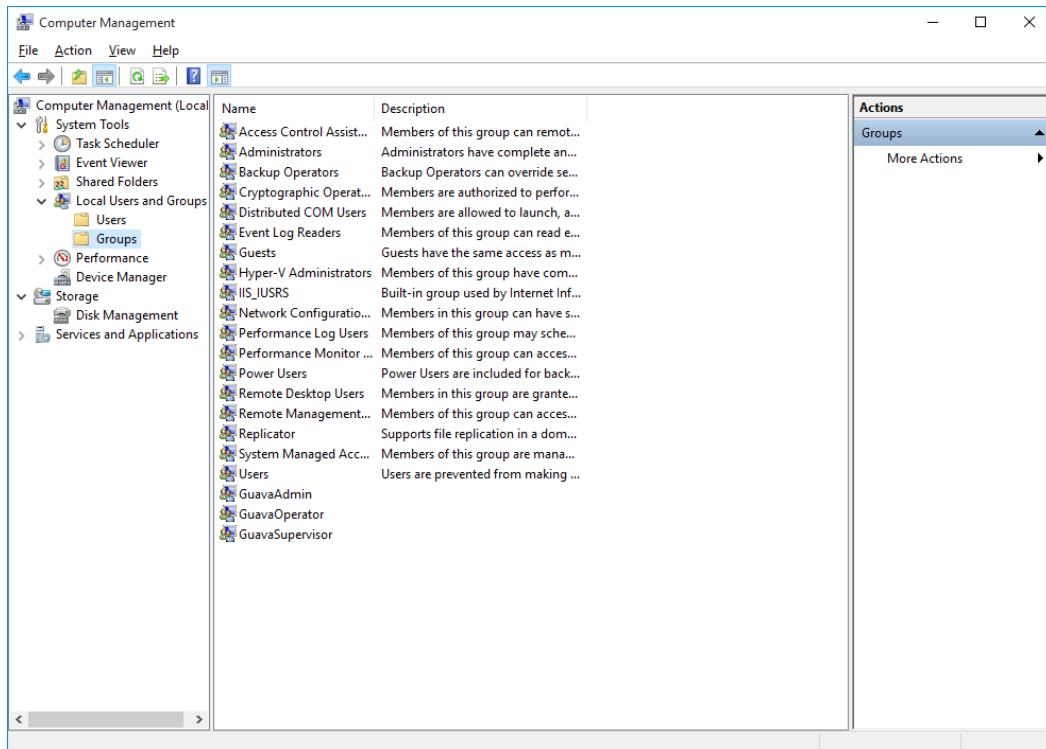
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## View the System User Groups

After installation, the three User Groups, GuavaAdmin, GuavaSupervisor, and GuavaOperator are automatically created by the software.

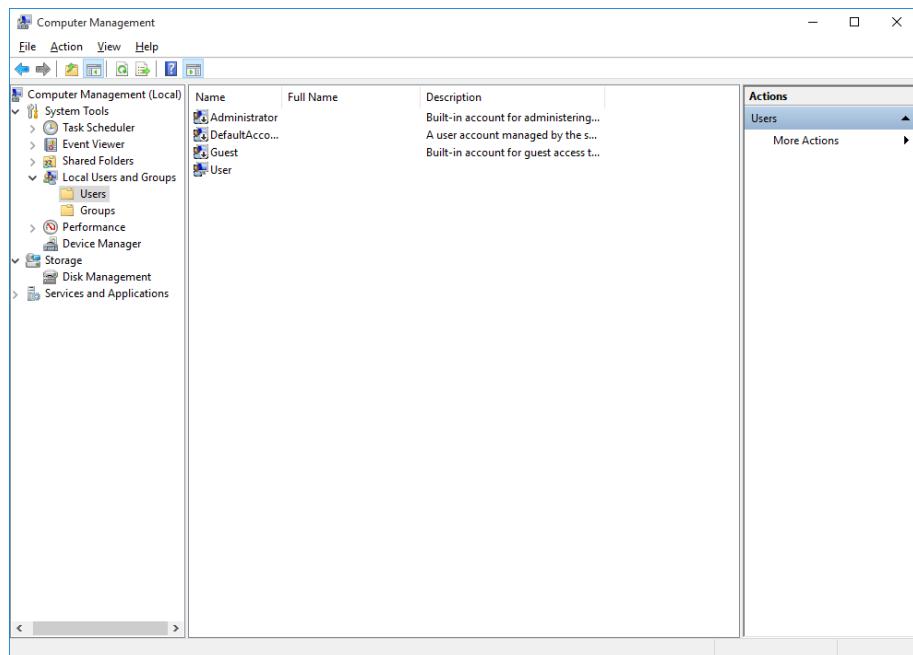
The GuavaAdmin group is used by GuavaSoft Software to allow the Guava Administrator to configure the "Administration Configuration." This option is available from the GuavaSoft Software Main Menu to a Guava Administrator only. The GuavaAdmin group consists of only those users who are to share responsibility for administering Access Control. All members of the GuavaAdmin group should also be a Windows Administrator. Only these users (or a Windows administrator) will be able to assign or change the access control levels for other users.

1. Log on as administrator. (No password is required by default.) If needed, contact your IT department for admin access.
2. Click **File Explorer** from the Windows taskbar. The **File Explorer** window displays.
3. From the left column, right-click **This PC** and choose **Manage** from the menu. The **Computer Management** window displays.
4. Expand the content of the **Local Users and Groups** folder and click on the **Groups** folder.

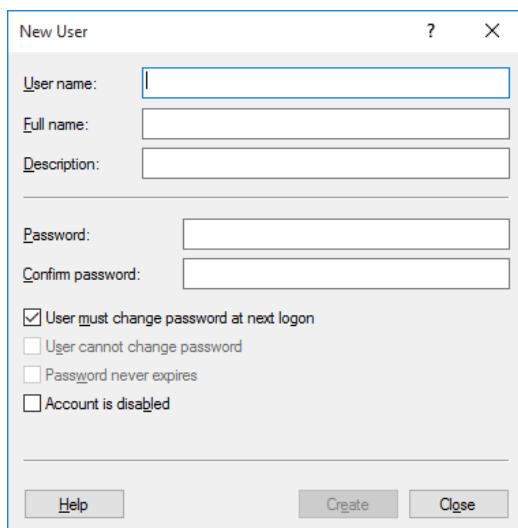


## Create a New User Account

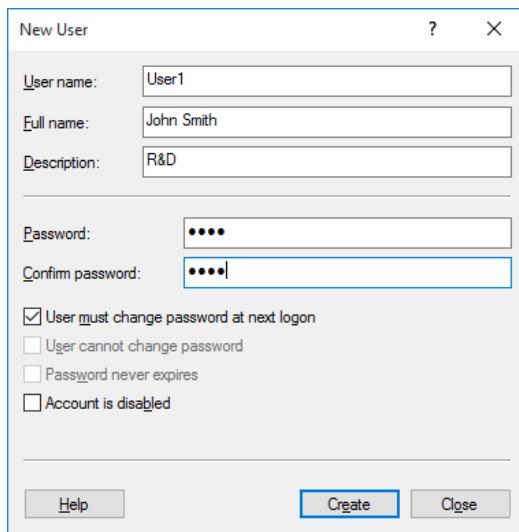
1. Log on as administrator. If needed, contact your IT department for admin access.
2. Click **File Explorer** from the Windows taskbar. The **File Explorer** window displays.
3. From the left column, right-click **This PC** and choose **Manage** from the menu. The **Computer Management** window displays.
4. Locate the **Local Users and Groups** folder and click the arrow to expand its contents. Click the **Users** folder.



5. Click **Action** from the tool bar, then **New User**. The **New User** dialog box displays.



6. Enter a User name to be added to the system. Enter a full name (first and last), a password and confirmation, and a description. In the example below, a user account "User1" for "Joe Smith" in "R&D" is created.



7. Click **Create**. You can continue adding more users by repeating these steps.
8. Click **Close** when you are finished adding users. You will see the new user name display in the list of users.

**NOTE:** You will need to assign each user to the proper access control group.

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## Assign Access Control Level to a User

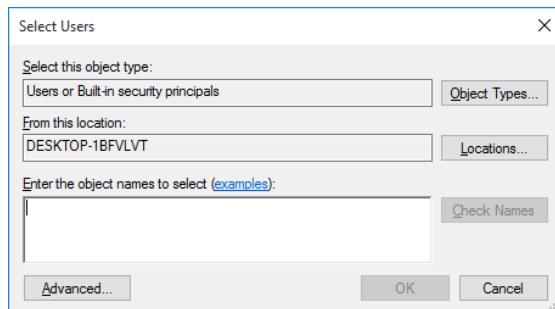
If you want the user to be able to administer Access Control for other users or to be able to configure the "Administration Configuration," (normally, very few users should have this access), add the user to the GuavaAdmin group. Also make sure that the user you have added to the GuavaAdmin group is also a Windows® Administrator.

In a similar manner, a user account can be assigned to have either Supervisor- or Operator-level access control simply by adding the user to either the GuavaSupervisor or GuavaOperator group, respectively.

Be careful not to assign a user to more than one Guava® group. The higher Group privilege takes precedence, which may not be what you want.

## Assign a User Account GuavaAdmin-Level Access

1. Log on as a Windows Administrator. If needed, contact your IT department for admin access.
2. Click **File Explorer** from the Windows taskbar. The **File Explorer** window displays.
3. From the left column, right-click **This PC** and choose **Manage** from the menu. The **Computer Management** window displays.
4. Locate the **Local Users and Groups** folder and click the arrow to expand its contents. Click the **Groups** folder.
5. Right-click on the **GuavaAdmin** group and choose **Properties**. The GuavaAdmin Properties window displays.
6. Click **Add**. The **Select Users** window displays.



7. Enter the user login name for the administrator and click **Check Names**.
8. Click **OK**. The user login name will display in the **Members** list.
9. Repeat these steps to add additional administrators, if needed.
10. Click **Apply**, then click **OK**.
11. Reboot the system.

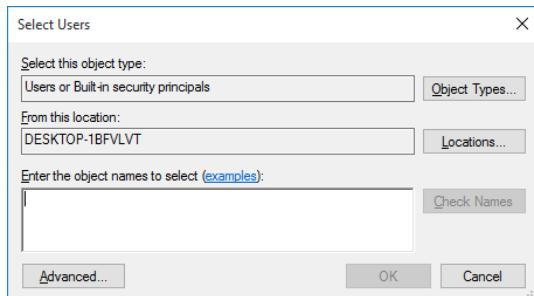
## Add User to Windows Administrator Group

If a user in the GuavaAdmin group is not a Windows® Administrator, you can add the user to the Administrators group by completing the following:

1. Click **File Explorer** from the Windows taskbar. The **File Explorer** window displays.
2. From the left column, right-click **This PC** and choose **Manage** from the menu.
3. Locate the **Local Users and Groups** folder and click the arrow to expand its contents. Click the **Groups** folder.
4. Right-click on the **Administrators** group in the right side of the window and choose **Add to Group**. The **Administrators Properties** window displays.
5. Click **Add**. The **Select Users** window displays.
6. Enter the user names in the **Enter the object names to select** field.
7. Click **Check Names**.
8. Click **OK**.
9. Click **Apply**, then click **OK**.
10. Reboot the system.

## Assign a User Account GuavaSupervisor-Level Access

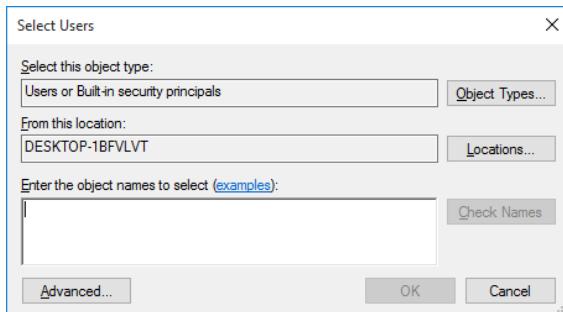
1. Log on as a Windows Administrator. If needed, contact your IT department for admin access.
2. Click **File Explorer** from the Windows taskbar. The **File Explorer** window displays.
3. From the left column, right-click **This PC** and choose **Manage** from the menu. The **Computer Management** window displays.
4. Locate the **Local Users and Groups** folder and click the arrow to expand its contents. Click the **Groups** folder.
5. Right-click on the **GuavaSupervisor** group and choose **Properties**.
6. Click **Add** in the GuavaSupervisor Properties window. The **Select Users** window displays.



7. Enter the user login name for the supervisor and click **Check Names**.
8. Click **OK**. The user login name will display in the Members list.
9. Click **Apply**, then click **OK**.
10. Repeat these steps to add additional supervisors, if needed.
11. Reboot the system.

## Assign a User Account GuavaOperator-Level Access

1. Log on as a Windows Administrator. If needed, contact your IT department for admin access.
2. Click **File Explorer** from the Windows taskbar. The **File Explorer** window displays.
3. From the left column, right-click **This PC** and choose **Manage** from the menu. The **Computer Management** window displays.
4. Locate the **Local Users and Groups** folder and click the arrow to expand its contents. Click the **Groups** folder.
5. Right-click on the **GuavaOperator** group and choose **Properties**.
6. Click **Add** in the GuavaOperator Properties window. The **Select Users** window displays.



7. Enter the user login name for the operator and click **Check Names**.
8. Click **OK**. The user login name will display in the Members list.
9. Click **Apply**, then click **OK**.
10. Repeat these steps to add additional operators.
11. Reboot the system.

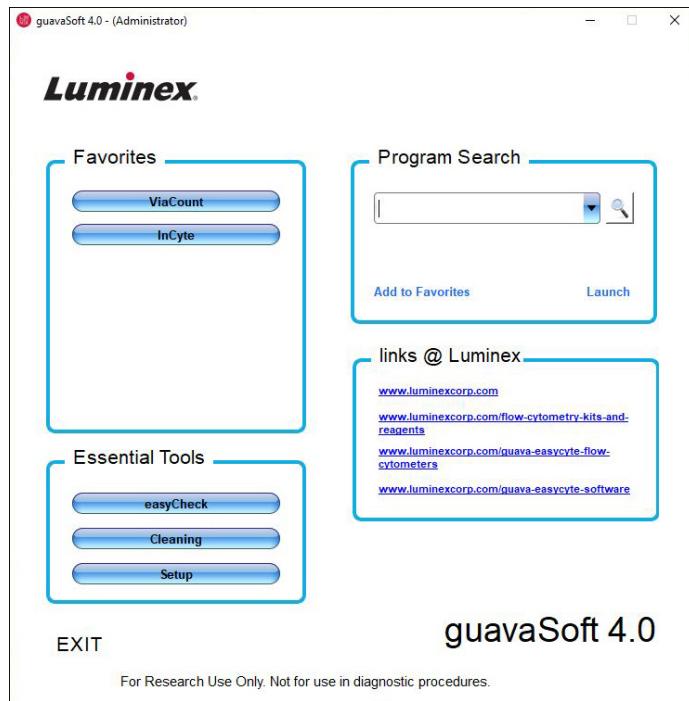
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## Configure Software Features by User Role

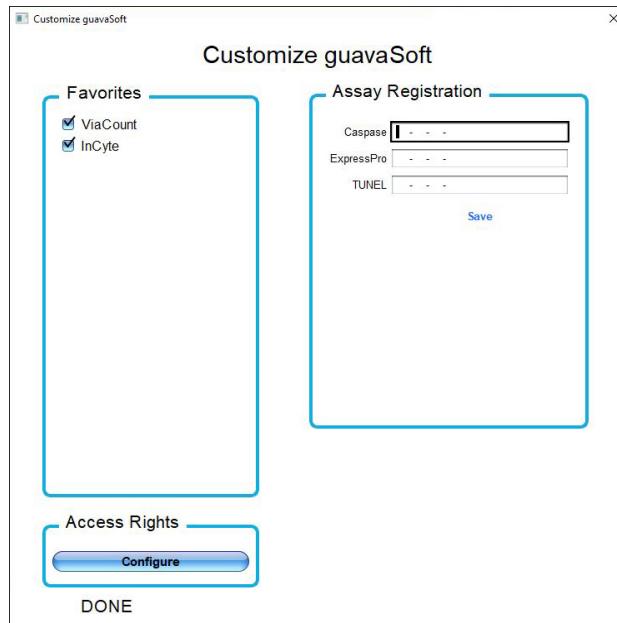
Administrators can configure or customize certain software features for supervisors and/or operators.

**NOTE:** You must be logged on as an administrator to gain access to the Setup button on the GuavaSoft Software Main Menu. If needed, contact your IT department for admin access.

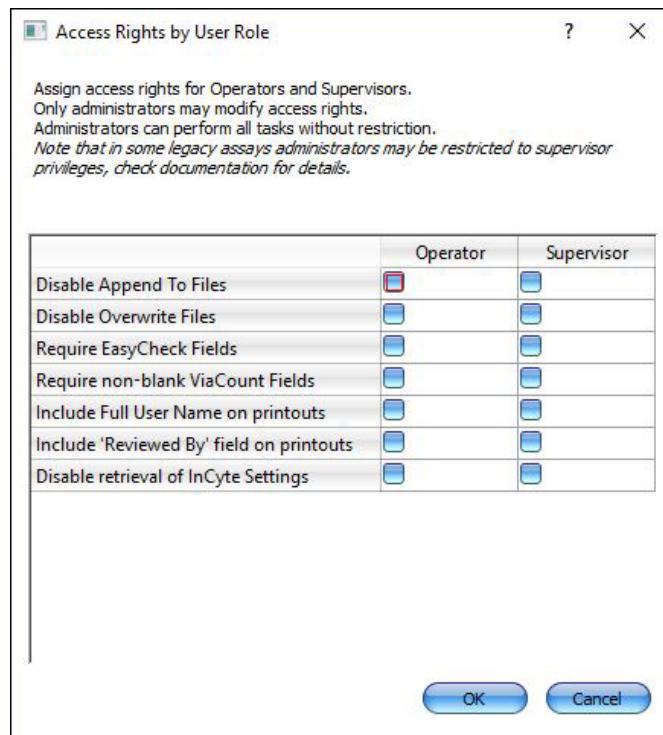
- From the GuavaSoft Software Main Menu, click **Setup**. The Customize GuavaSoft screen displays.



- Click **Configure** under **Access Rights**. The Access Rights by User Role dialog box displays.



- Select the check boxes for the features you want to allow for supervisors and/or operators.



The following table describes each feature.

Feature	Description
Disable Append to Files	If selected, users will not be allowed to append to an existing FCS 3.0 file. Users may overwrite the existing file or create a new file. This applies to a data file when you click <b>New Data Set</b> (or <b>Start New Data Session File</b> for Guava® InCyte™) at the start of a session.  If overwriting is disabled, you will be appending to a copy of the existing file since you cannot overwrite it. If overwriting is allowed, you will be appending to the end of the existing file.
Disable Overwrite Files	If selected, users will not be allowed to overwrite an existing FCS 3.0 file. Users may append to the existing file or create a new file. This applies to a data file when you click <b>New Data Set</b> (or <b>Start New Data Session File</b> for Guava® InCyte) at the start of a session, as well as to a data file when you make changes during analysis.

Feature	Description
<b>Require EasyCheck Fields</b>	If selected, the user must enter values for the Bead Lot #, Bead Expiration Date, and Expected Particles/mL before starting every easyCheck run. If this option is not selected, the information will default to the last information entered.
<b>Require non-blank ViaCount Fields</b>	If selected, the user must enter values for the Reagent Lot # and Reagent Expiration Date before a ViaCount™ Assay can be started. If this option is not selected, the reagent information is not required.
<b>Include Full User Name on printouts</b>	If selected, the user's full name is included on all printouts generated by that user. This information is taken from the "Full Name" field in the user's Windows account record.
<b>Include 'Reviewed By' field on printouts</b>	If selected, places a <i>Reviewed by:</i> _____ field on all printouts generated by the user.
<b>Disable retrieval of InCyte Settings</b>	If selected, users cannot adjust instrument settings or retrieve InCyte Methods, instrument settings, and compensation settings from individual Method or instrument settings files. Methods, instrument settings, and compensation settings can only be retrieved from a single FCS file.

4. Click **OK** to save the settings.

# Chapter 11: Shipping

## Prepare Unit for Shipment

Contact Luminex Technical Support for the Decontamination Form and instructions. The form must be returned via e-mail before you can return the instrument. A return authorization (RMA) number will be issued to you. Write this number on the outside of the shipping box. If you did not save the original shipping box, you can order one.

1. Perform the Guava® Clean procedure.
2. Remove all tubes and plates from the tray.
3. Clean and disinfect the cleaning solution vial and waste vial.
4. Wipe down the outside of the instrument with 70% isopropyl alcohol.
5. Power off the laptop and instrument.
6. Disconnect the laptop, instrument, and power conditioner.
7. Remove the flowcell from the instrument. Use the syringe cleaning tool to flush the flowcell with water, and then flush it with air. Store the flowcell in the capillary box.
8. Put the shipping restraint on the instrument plate tray. For instructions on installing the shipping restraint, contact Luminex Technical Support.
9. Tape the capillary hatch closed.
10. Tape the tray door closed.
11. Place the instrument in the shipping box. Ensure you include the cleaning and waste vials. Always use two people to lift the instrument.
12. Place the blue shipping foam over the instrument.
13. Place the USB cable, extension cable, and power cord in the sides around the foam insert.

**NOTE:** If requested to return the laptop computer, wipe down the laptop with 70% isopropyl alcohol and place it on top in the center of the foam insert.
14. Place the cover on the box and insert the white locks into the holes on the sides.

# Chapter 12: Ordering Information

For ordering information, please contact Luminex technical support, or visit our website at [www.luminexcorp.com](http://www.luminexcorp.com).

Luminex and its distribution network will provide Guava® products to all sectors of life science research in certain countries outside North America and Europe.

## Instrument

Parts	Catalog Number
Guava® easyCyte™ 5	0500-5005
Guava® easyCyte™ 5 HPL	0500-5009
Guava® easyCyte™ 5HT	0500-4005
Guava® easyCyte™ 5HT HPL	0500-4009
Guava® easyCyte™ 6-2L	0500-5007
Guava® easyCyte™ 6HT-2L	0500-4007
Guava® easyCyte™ 8	0500-5008
Guava® easyCyte™ 8HT	0500-4008
Guava® easyCyte™ 10 BG	0500-5015
Guava® easyCyte™ 10HT BG	0500-4015
Guava® easyCyte™ 11 BV	0500-5020
Guava® easyCyte™ 11HT BV	0500-4020
Guava® easyCyte™ 12 BGR	0500-5025
Guava® easyCyte™ 12HT BGR	0500-4025
Guava® easyCyte™ 13 BRV	0500-5012

Parts	Catalog Number
Guava® easyCyte™ 13HT BRV	0500-4012
Guava® easyCyte™ 14 BGV	0500-5030
Guava® easyCyte™ 14HT BGV	0500-4030

## System Components

Parts	Catalog Number
laptop computer	CN-0475-01
cleaning solution vial assembly (40 mL)	CN-0450-01
waste vial assembly (40 mL)	CN-0451-01
flowcell assembly	CN-0448-01
flowcell tightening tool	CN-0478-01
flowcell removal tool (tweezer model)	CN-0479-01
syringe assembly cleaning tool	0110-0210
fuse 110 V (2.5 A, 250 V)	CN-0476-01
fuse 220-240 V (1.6 A, 250 V) X2	CN-0477-01
Guava® easyCyte™ System User Guide	89-00002-00-705
instrument shipping box	CN-0449-01

## Essential Kits

Kit	Catalog Number
Guava® easyCheck™ Kit (50 tests)	4500-0025
Guava® ICF (Instrument Cleaning Fluid) [100 mL]	4200-0140

## Training

On-Site Instrument Training	Part Number
Guava® easyCyte™ Installation and Basic Training (half day)	0500-1690
Guava® easyCyte™ Installation and Advanced Training (full day)	0500-1680

Installation Qualification/Operation Qualification (IQ/OQ)	Catalog Number
Guava® easyCyte™ HT IQ/OQ (Luminex performed)	8000-1998

## Compatible Microplates

Following is a list of microplates compatible with the Guava® easyCyte™ HT System. Plates are listed by manufacturer.

### Porvair

Plate Type	Catalog No.	Description
Flat-bottom/clear	208004 208003	polysterene, clear, 350 µL polypropylene, no rim, 350 µL
Round-bottom/clear	209004 209003	polysterene, clear, 270 µL polypropylene, raised rim, 270 µL
V-bottom/clear	210004 210003	polysterene, clear, 220 µL polypropylene, raised rim, 220 µL

### Eppendorf

Plate Type	Catalog No.	Description
Polypropylene plates Flat-bottom	0030 601.106 0030 602.102	clear, PCR clean clear, sterile

Plate Type	Catalog No.	Description
U-bottom	0030 601.203 0030 602.200 0030 601.807 0030 601.572	clear, PCR clean clear, sterile black, PCR clean white, PCR clean
V-bottom	0030 601.300 0030 602.307 0030 601.904 0030 601.670	clear, PCR clean clear, sterile black, PCR clean white, PCR clean

## Techno Plastic Products (TPP)

Plate Type	Catalog No.	Description
Flat-bottom	92096 92696	tissue culture, polystyrene tissue culture, polystyrene
U-bottom	92097 92697	tissue culture, polystyrene tissue culture, polystyrene

## Greiner Bio-One

Plate Type	Catalog No.	Description
Flat-bottom/standard	655 161 655 101 655 001 655 061	sterile non sterile ELISA, MICROLON 200, med binding ELISA, MICROLON 600, high binding
Flat-bottom/chimney well solid-bottom/clear	655 160 655 162 655 180 655 182 655 185 655 080 655 081 655 940 655 930 655 950	sterile, cell culture treated sterile, cell culture treated sterile, cell culture treated, with lid sterile, cell culture treated, with lid for suspension, sterile, cell cultured treated, with lid ELISA, MICROLON 200, med binding ELISA, MICROLON 600, high binding Poly-D-Lysine, with lid Poly-D-Lysine, with lid Collagen Type I, with lid

Plate Type	Catalog No.	Description
solid bottom/white	655 073 655 083 655 075 655 074	sterile, cell cultured treated sterile, cell cultured treated, with lid non-sterile, LUMITRAC 200 med binding sterile, LUMITRAC 600 high binding
solid bottom/black	655 079 655 086 655 076 655 077	sterile, cell cultured treated sterile, cell cultured treated, with lid non-sterile, FLUOTRAC 200 med binding sterile, FLUOTRAC 600 high binding
uClear bottom/white	655 088 655 098 655 095 655 094 655 944	sterile, cell cultured treated sterile, cell cultured treated, with lid non-sterile, med binding sterile, high binding Poly-D-Lysine, with lid
uClear bottom/black	655 087 655 090 655 096 655 097 655 946 655 948 655 936 655 956	sterile, cell cultured treated sterile, cell cultured treated, with lid non-sterile, med binding sterile, high binding Poly-D-Lysine, with lid Poly-D-Lysine, with lid Poly-D-Lysine, with lid Collagen Type I, with lid
lumox bottom/black	9612 0096 9600 0024	sterile, cell cultured treated, with lid sterile, cell cultured treated, with lid
glass bottom/black	655 892	sterile, with lid
uclear/clear	655 801	non-sterile
U-bottom/chimney well solid bottom	650 261 650 201 650 207 650 209	natural, sterile natural, non-sterile white, non-sterile black, non-sterile
V-bottom/chimney well solid bottom	651 201 651 207 651 209	natural, non-sterile white, non-sterile black, non-sterile
Flat-bottom/chimney well solid bottom	655 201 655 207 655 209	natural, non-sterile white, non-sterile black, non-sterile

## Corning Life Sciences

Plate Type	Catalog No.	Description
Flat-bottom	3361	clear, polystyrene, high bind, with lid
	3590	clear, polystyrene, high bind, without lid, non-sterile
	9018	clear, polystyrene, high bind, without lid, non-sterile
	3591	clear, polystyrene, not treated, without lid, non-sterile
	9017	clear, polystyrene, not treated, without lid, non-sterile
	3628	clear, tissue culture treated, without lid, sterile
	3596	clear, polysterene, tissue culture treated, with lid, sterile
	3598	clear, polysterene, tissue culture treated, with lid, sterile
	3599	clear, polysterene, tissue culture treated, with lid, sterile
	3585	clear, tissue culture treated, with lid, sterile
	3595	clear, polysterene, tissue culture treated, with low-evaporation lid, sterile
	3474	clear, ultra low attachment, with lid, sterile
U-bottom or round-bottom	3360	clear, tissue culture treated, without lid, sterile
	3367	clear, polysterene, not treated, without lid, sterile
	3788	clear, polysterene, not treated, with lid, sterile
	3795	clear, polystyrene, not treated, without lid, sterile
	3798	clear, polystyrene, not treated, without lid, not sterile, special process
	3799	clear, tissue culture treated, with lid, sterile
	3356	black, polypropylene, not treated, without lid, not sterile
	3359	clear, polypropylene, not treated, without lid, sterile
	3365	clear, polypropylene, not treated, without lid, not sterile (suggested replacement for 3371)
	3371	see 3365 above
V-bottom	3896	clear, polystyrene, not treated, without lid, sterile
	3897	clear, polystyrene, not treated, without lid, not sterile
	3898	clear, polystyrene, not treated, without lid, not sterile, special process

## Thermo Fisher Scientific

Plate Type	Catalog No.	Description
Flat-bottom	269620	clear, polysterene, not treated, not sterile
	269787	clear, polysterene, not treated, sterile
	439454	clear, polysterene, Maxisorp, not sterile
	442404	clear, polysterene, Maxisorp, not sterile
	475094	clear, polysterene, Polysorp, not sterile

Plate Type	Catalog No.	Description
U-bottom	143761	clear, polystyrene, cell culture treated, without lid, sterile
	163320	clear, polystyrene, ncell culture treated, with lid, sterile
	168136	clear, polystyrene, cell culture treated, with lid, sterile
	262162	clear, polystyrene, not treated, without lid, sterile
	268152	clear, polystyrene, not treated, without lid, not sterile
	268200	clear, polystyrene, not treated, with lid, sterile
	449824	clear, polystyrene, Maxisorp, without lid, not sterile
	475434	clear, polystyrene, Polysorp, without lid, not sterile
	267245	natural, polypropylene, not treated, without lid, not sterile
	267334	natural, polypropylene, not treated, without lid, not sterile
	267342	black, polypropylene, not treated, without lid, not sterile
	267350	white, polypropylene, not treated, without lid, not sterile
	267369	red, polypropylene, not treated, without lid, not sterile
	267385	blue, polypropylene, not treated, without lid, not sterile
	267407	yellow, polypropylene, not treated, without lid, not sterile

## Falcon

Plate Type	Catalog No.	Description
Flat-bottom	353936	Falcon® Microtest - clear, TC-treated, polystyrene, with lid
	353075	Falcon® Microplate - clear, TC-treated, polystyrene, with lid, sterile, individually wrapped
	351172	Falcon® Assay - clear, not treated polystyrene, with lid, sterile, individually wrapped
	353872	Corning® Primaria Microtest - clear, surface-modified polystyrene for enhanced cell culture, low evaporation lid, sterile, individually wrapped
	353072	Falcon® Cell - clear, TC-treated, with lid, sterile, individually wrapped
	353916	Falcon® clear, TC-treated, with lid
	353936	Falcon® Microtest - clear, TC-treated polystyrene, with lid
U-bottom	353077	Falcon® Microplate - clear, TC-treated polystyrene, with lid, sterile, individually wrapped
	353910	Falcon® Microplate - clear, not treated polystyrene, without lid
	351190	Falcon® Microplate - polypropylene, not treated
	351177	Falcon® Assay - clear, not treated, polystyrene, with lid, individually wrapped, sterile

## Incompatible Microplates

Following is a list of microplates not compatible with the Guava® easyCyte™ HT System.

Manufacturer	Catalog No.	Description
Thermo Fisher Scientific	249662	Nunc, conical bottom PS
	249570	Nunc, clear PS
	442587	Nunc, PP

## Microcentrifuge Tubes

Following is a list of microcentrifuge tubes compatible with the Guava® easyCyte™ HT System.

Microcentrifuge Tubes	Catalog No.
0.5-mL microcentrifuge tubes (free-standing with screw cap) <ul style="list-style-type: none"> <li>used for samples</li> <li>dead volume of ~75 µL</li> </ul>	VWR 16466-036 (Guava 1000-2990)
1.5-mL microcentrifuge tubes (conical bottom, no screw cap)	VWR 16466-030(Guava 1000-0780)
1.5-mL microcentrifuge tubes (conical bottom, screw cap)	VWR 89004-288
1.5-mL microcentrifuge tubes (round bottom, screw cap) <ul style="list-style-type: none"> <li>used for cleaning and when washing the capillary and mixer</li> </ul>	VWR 60872-324
screw caps	VWR 89004-346

**NOTE:** Snap-cap tubes can be used in place of the 1.5-mL screw-cap tubes (for washing and cleaning) if the caps are cut off.

## Microplate Minimum Assay Volumes

Dead Volumes:

- round-bottom plate: ~50 µL
- flat-bottom plate: ~75 µL
- V-bottom plate: ~25 µL

Assay Volumes:

- dead volume + 50 µL (at 200 cells/µL)

# Chapter 12: Additional Features

## IC-50/EC-50

Understanding the kinetic characteristics of a given compound's mode of action is important for determining efficacy, toxicity, and dosage. Assays measuring parameters such as apoptotic status or protein expression are commonly used to obtain this information. Use the IC-50 application to create curves based on the relationship between concentration and response, where response is measured by changes in the statistical parameter for the applied Analysis Method. IC-50 (or EC-50) values are derived directly from the calculated curves.

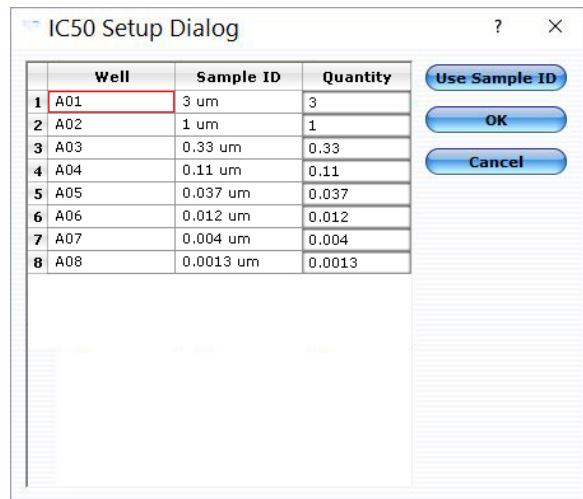
- The EC50 value is the concentration of a drug, antibody, or toxicant which induces a response halfway between the baseline and maximum following a specified exposure time.
- The IC50 is a measure of the effectiveness of a compound to inhibit or reduce a measurable response by 50% relative to the baseline (which in this case is a maximum value) and absolute minima.

### Create an EC-50 or IC-50 Curve

To determine the EC-50 value for a given compound, pair a data file with a Method, then define the regions and gates. If the compound-specific sample set is part of a larger data set, create a group to define this specific subset. Then drag the group to the legend sector replacing the original FCS data file.

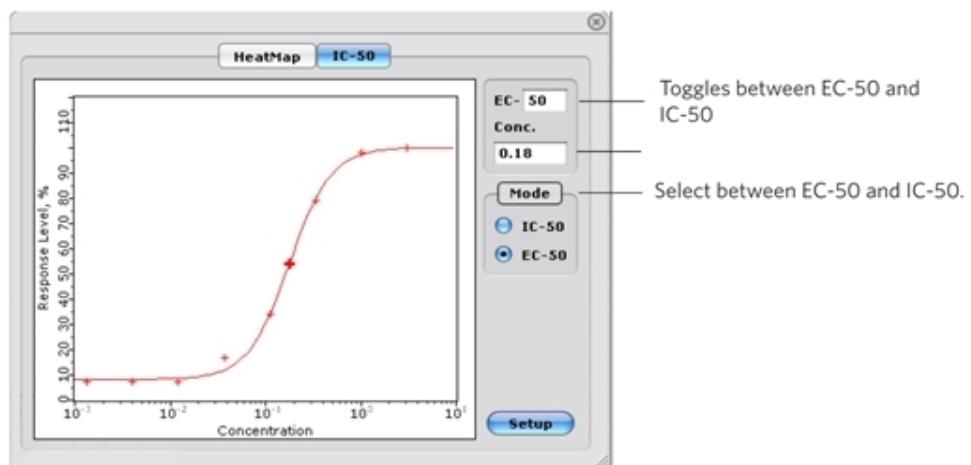
1. From the Guava® InCyte™ module, open the FCS needed for analysis.
2. Choose **File > Open** from the menu bar.
  - a. From the **Open File** dialog box, navigate to the FCS file for analysis and click **Open**.
3. Click the **Show Pie Legend** icon in the tool bar.
4. Drag the **Analysed Data** to the HeatMap legend.
5. Click **IC-50** at the top of Pie Legend. A graph displays where x and y axes are defined as Concentration (log10 scale) and response level % (linear), respectively.
  - The y coordinates correspond to the numerical values derived from the AnalysedGroup. Values are converted to a relative scale where the maximum derived value is set to 100% on the y axis.
  - The x coordinates represent the actual compound concentrations used at each step in the titration. The axis display is open and set automatically from the range of concentrations used.
6. To set the x-axis values for the curve, click the **Setup** button. The IC50 Setup Dialog box displays, showing the wells used in the analysis, the Sample ID, and Quantity.

**NOTE:** The Sample ID is derived from the original FCS file. If a sample ID was not entered for each well, the default well name displays (A01, A02).
7. Enter the Quantity (concentration) for each well. Quantity defines the x-axis values for the graph. These are user-defined and must be entered for each curve.



**NOTE:** If, during acquisition, you enter a concentration as the sample ID, you can click the **Use Sample ID** button to enter the Quantity. Characters before the “space” in the ID are used for the quantity. For example, if your sample ID is “3 um staurosporine,” the 3 is used for the quantity. You can also type the values into the Quantity fields. Enter the actual number (.01, .001, .0001) or use scientific notation (1e-2, 1e-3, 1e-4).

- Click **OK** to display the graph. The EC-50 (IC-50) value is marked by a bold + on the curve.



You can make the following adjustments to the data:

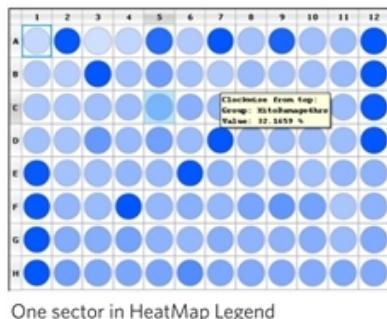
- Change the target value (from 20–80). For example, instead of viewing a graph where 50% of the effect is observed, set the EC or IC value to 25 to see where 25% of the effect is observed.
- The Conc field provides the numerical value for the EC-50 point, in this case 0.16.
- Select between EC-50 and IC-50.

# HeatMap

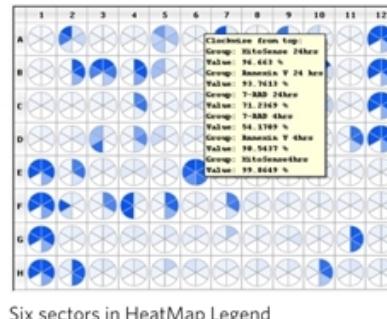
HeatMapping provides a colorimetric representation of comparative results at the experiment level. Each Analysed Group is represented by an individual sector in the HeatMap legend. After the completion of sample analysis, individual parameters can be compared over the entire experimental run. A visual representation of the data and the results will appear in the plate map. Well-to-well variations in blue (dark blue = maximum value, white = minimum value) are based on relative differences in the data as measured on a linear scale.

The plate map can simultaneously display results for up to six Analysed Groups—the same number of sectors that the legend is displaying. Place the cursor over any well to see the result for that Analysed Group. When the HeatMap legend displays more than one sector, the results for every sector will appear within the well pop-up. If the HeatMap is displaying multiple sectors, the threshold feature is disabled.

**NOTE:** If the Analysis Method used to create the Analysed Group is new (no regions and/or gates have been defined), the plate map will initially display all wells as dark blue. Perform the analysis on the data before proceeding to HeatMapping.



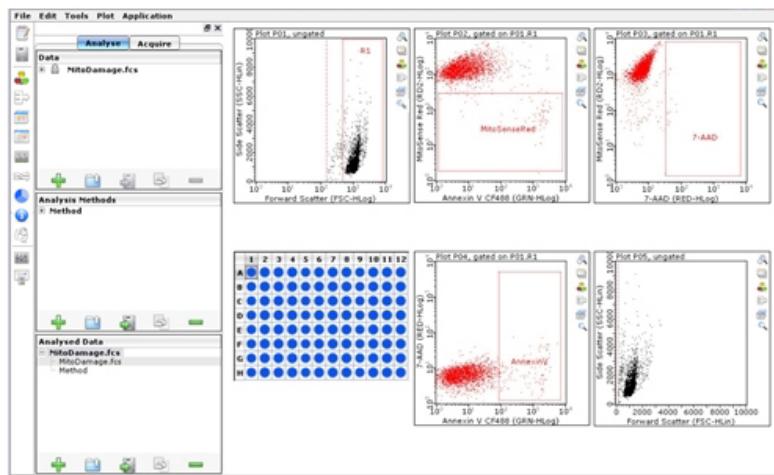
One sector in HeatMap Legend



Six sectors in HeatMap Legend

## Create a Single-Parameter HeatMap

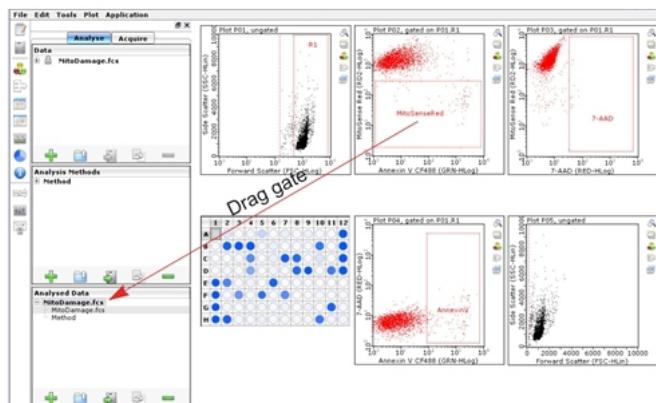
- From the Guava® InCyte™ module, open the FCS needed for analysis.
- Choose **File > Open** from the menu bar.
  - From the **Open File** dialog box, navigate to the FCS file for analysis and click **Open**.
- Choose the .fcs file in the **Analysed Data** pane.



Jurkat cells were stained with MitoDamage kit to look at mitochondria membrane potential (MitoSense Red), apoptosis (Annexin V CF488), and cell death (7-AAD). R1 was created for cell population, then applied to plots 2-4. Three regions were created for each population of interest.

4. Click the **Plot gate** icon from the plot tool bar to select the gate of interest.
5. Select the region of interest on the plot (solid handles will display) and drag it to the Analysed Group in the Analysed Data pane.

**NOTE:** As you start to drag the gate, a gray box appears at the cursor. Drag the gate to the Analysed Group and a plus sign (+) appears on the cursor. Drop the gate in the Analysed Group.



MitoSense Red region is dragged to the Analysed Group. Plate map displays well-to-well variation of relative percentage of mitochondrial membrane potential among samples.

6. If prompted, the **New Gate Name** dialog box displays where you can change the name if you want or click **OK** to accept the default gate name.

**NOTE:** After the gate is applied to the Analysed Group, the wells will change to varying shades of blue, depending on the population percentages. Place the cursor over any well to see value for that well.

**NOTE:** You can also use the HeatMap legend by dragging the Analysed Group and the gate to the legend. Then, to update the analysis, simply drag a new gate to the legend. The plate map will update automatically.

7. If needed, change the metric and/or threshold. Above the threshold panel is the maximum derived value for a given data set. Each slider tool is based upon a % scale where the minimum value (0 is lower limit) is Zero and the maximum value (100 in upper limit) is defined by the maximum value derived from the analysis. Simply slide the bars up or down to set boundaries for data display.
8. To save the HeatMapped Analysed Group, select it and click the **Save Analysed Group** button (3rd) in the pane.
  - a. Navigate to the location where you want to save it and enter a file name.
  - b. Click **Save**. The Analysed Group will be saved as an FCS file and will contain the Method (regions, gates, metrics, and stats), as well as the data and the instrument settings.

## Create a Multi-Parameter HeatMap

The following example is the same as the single-parameter HeatMap. In this example, three Analysed Groups are created to view and compare the results for three different populations simultaneously.

1. From the Guava® InCyte™ module, open the FCS needed for analysis.
2. Choose **File > Open** from the menu bar.
  - a. From the **Open File** dialog box, navigate to the FCS file for analysis and click **Open**.
3. Choose the .fcs file in the **Analysed Data** pane.
4. To compare multiple parameters from one FCS file, duplicate the Analysed Group. Select the **Analysed Group**, then click the **Duplicate Analysed Group** button (4th button) in the Analysed Data pane.

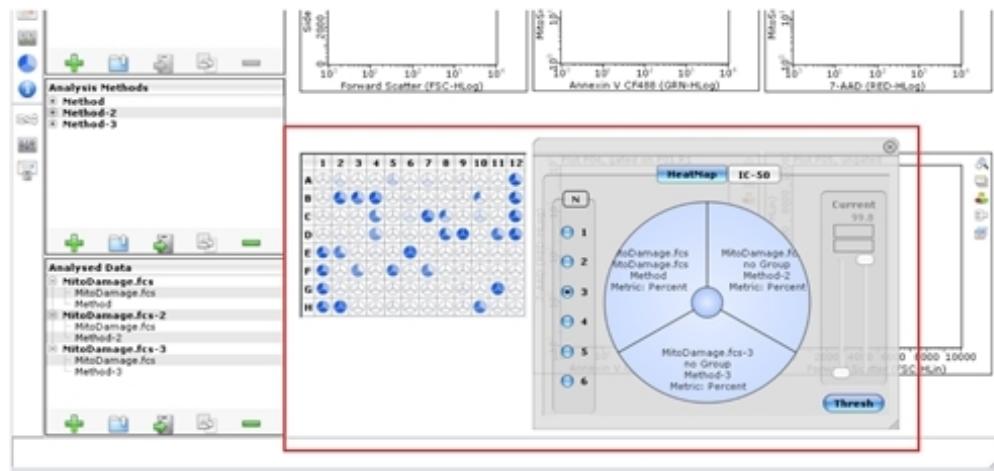
**NOTE:** For this example, the Analysed Group was duplicated twice to obtain results on three populations.

5. Select the first **Analysed Group**
6. Click the **Plot gate** icon from the plot tool bar to select the gate of interest.
7. Select the region of interest on the plot (solid handles will display) and drag it to the Analysed Group in the Analysed Data pane.
8. If prompted, the **New Gate Name** dialog box displays where you can change the name if you want or click **OK** to accept the default gate name.

**NOTE:** You can also change the name of an Analysed Group by double-clicking it and entering a new name.

9. Repeat the above steps for the remaining Analysed Groups..
10. Click the **Show Pie Legend** icon in the tool bar on the left side of the application window to display the HeatMap legend.
11. Select the number of sectors (N) to display.
12. Click and drag the Analysed Groups to the appropriate sectors.
13. Click the center circle in the legend to display the results for all populations of interest in the plate map.

**NOTE:** When multiple sectors are displayed, no data is shown in the plots and you cannot modify any Methods (add regions, move gates, etc). To modify individual Methods, click on the sector you wish to update.



14. The plate map shows each well with the number of sectors used for analysis. Place the cursor over any well to see all the values for that well.
15. (Optional) To clear a sector, right-click on the sector in the HeatMap legend and select **Clear sector**.
16. If needed, change the metric and/or threshold. Above the threshold panel is the maximum derived value for a given data set. Each slider tool is based upon a % scale where the minimum value (0 is lower limit) is Zero and the maximum value (100 in upper limit) is defined by the maximum value derived from the analysis. Simply slide the bars up or down to set boundaries for data display.
17. To save the HeatMapped Analysed Groups, select one and click the **Save Analysed Group** button (3rd) in the pane.
  - a. Navigate to the location where you want to save it, and enter a file name.
  - b. Click **Save**. The Analysed Group will be saved as an FCS file and will contain the Method (regions, gates, metrics, and stats), as well as the data and the instrument settings. Repeat for the remaining groups.

## Create a Single-Parameter HeatMap Using a Statistic or Expression Stat

The following example shows how to use the HeatMap to get a visual and numerical representation of results for a single or multiple parameters from one data file.

1. From the Guava® InCyte™ module, open the FCS needed for analysis.
2. Choose **File > Open** from the menu bar.
  - a. From the **Open File** dialog box, navigate to the FCS file for analysis and click **Open**.
3. Choose the .fcs file in the **Analysed Data** pane.
4. If not already done, create a stat region or a statistic for any region created. When you create a new stat, the **Current Run Stats** window displays, keep this window open.
5. Click the **Show Pie Legend** icon in the tool bar on the left side of the application window to display the HeatMap legend.
6. Select the statistic of interest from the **Current Run Stats** window. If the Current Run Stats window is not open, you can find it by clicking the **Show Current Run Stats** icon in the tool bar.
7. Drag the statistic to the HeatMap Legend.
8. If prompted, click **OK** to accept the new gate name, or you can change the name.

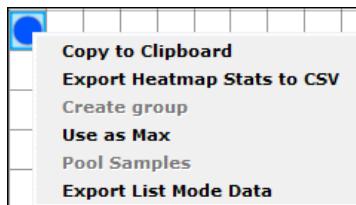
## Export Guava® InCyte™ HeatMap Results to a Spreadsheet

For each AnalysedGroup, you can export the results to a comma-separated values (CSV) file for analysis using a spreadsheet program such as Microsoft® Excel. The CSV file will contain the following statistics: Group name, Stat Type, Parameter, Stat gate, Control gate, Log Display, Max, Threshold Max%, Threshold Max, Threshold Min%, Threshold Min. In addition, each sample well will include a derived statistical value, its unit of measurement, a control well (if applicable), the well's sample ID, and the FCS 3.0 file from which it was derived.

1. From the Guava® InCyte™ module, open the FCS needed for analysis.
2. Choose **File > Open** from the menu bar.
  - a. From the **Open File** dialog box, navigate to the FCS file for analysis and click **Open**.
3. Choose the .fcs file in the Analysed Data pane.
4. Right-click on any well and select **Export Heatmap Stats to CSV**. The Export CSV dialog box displays.

**NOTE:** If the HeatMap legend is divided into multiple sectors, then data from each sector will be exported. If you want to export results for a single sector (or AnalysedGroup),

- a. Click on the specific sector in the legend.
- b. Right-click the well and choose **Export Heatmap Stats to CSV**.



**NOTE:** You can copy and save any of the plots, HeatMap, or IC-50 graphs by right-clicking, then selecting **Copy To Clipboard**.

5. Choose the location for the .csv file.
6. Enter a **File name** and click **Save**.

## Metrics

Metrics are the statistical parameters applied to a set of gated events. Applying a metric results in the calculation of a numerical value for each well/sample. These values are compared across a given data set and determine the well-to-well variability in color displayed in the plate map. There are six metrics from which to choose:

- **Percent (%)** - Percentage of events within a given gate relative to the total number of events displayed within the originating plot.
- **Count** - Number of events within a given gate.
- **Concentration** - Event count for a gate as a function of the volume of sample acquired (events/uL).
- **Mean** - Mean fluorescent intensity (MFI) for a set of gated events. If derived from a dot plot, the mean value applies to the parameter associated with the x axis. (For log scale, the mean is geometric; for linear scale, the mean is arithmetic.)
- **Median** - Median fluorescence for a set of gated events. If derived from a dot plot, the median value applies to the parameter associated with the x axis.
- **Mean Ratio** - A measure of the fluorescence intensity for a set of gated events as defined by the following:  

$$\text{ratio} = \text{MFI}_{\text{well X}} / \text{MFI}_{\text{control well}}$$
 The control MFI may be derived from a single sample or represent the average

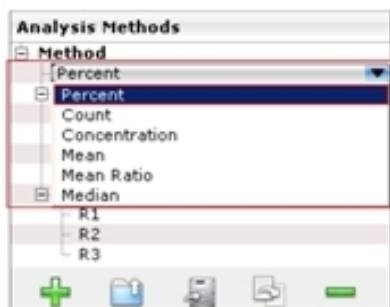
value from a set of control samples. To apply a mean ratio, select **Mean Ratio**. Using the plate map, select the appropriate control well(s) and drag to the corresponding legend sector. This well's value is used as the denominator in the above equation. You must select a control well as the denominator to get a mean ratio.

## Change the Metric

The default metric for a new Method is percent. To select a different metric:

1. From the Guava® InCyte™ module, open the FCS needed for analysis.
2. Choose **File > Open** from the menu bar.
3. From the **Open File** dialog box, navigate to the FCS file for analysis and click **Open**.
4. Choose the .fcs file in the **Analysed Data** pane.
5. Double-click the **Metric** in the **Analysis Methods** pane, then open the drop-down menu to display the Metric list.
6. Select a metric to apply it as the statistical parameter for that Method. Once selected, the newly chosen metric will replace the previous one.

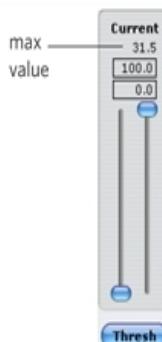
**NOTE:** If you are HeatMapping and the corresponding Method occupies a legend sector, the plate map will automatically update to reflect the change.



To change the metric, double-click the metric in the Method and select the new metric.

## Thresholds

The Workspace contains a dual-barred threshold tool for setting upper and lower limits on statistical values. The threshold bars can be used to set limits on a single AnalysedGroup at a time (the one that is displayed in the plate map).



## Set Threshold Max Value

Any well can be selected to represent the max value.

1. From the Guava® InCyte™ module, open the FCS needed for analysis.
2. Choose **File > Open** from the menu bar.
  - a. From the **Open File** dialog box, navigate to the FCS file for analysis and click **Open**.
3. Choose the .fcs file in the **Analysed Data** pane.
4. To set a max threshold value, right-click on a well that contains a sample from the plate map.
5. Choose **Use as Max** from the menu. The new max value will also be displayed at the top of the threshold panel.
6. To deselect as max threshold value, right-click on the well again from the plate map and choose **Don't Use as Max**.

---

## Show Floating Plate Map

You can undock the plate map for a more detailed analysis and to increase/decrease the size of the plate map.

1. From the Guava® InCyte™ module, click the **Show Floating Plate Map** icon from the tool bar. This undocks the plate map allowing you to move it around the screen or to adjust the size of the plate map.
2. Click the **x** in the top-right corner to return the plate map to its original position.

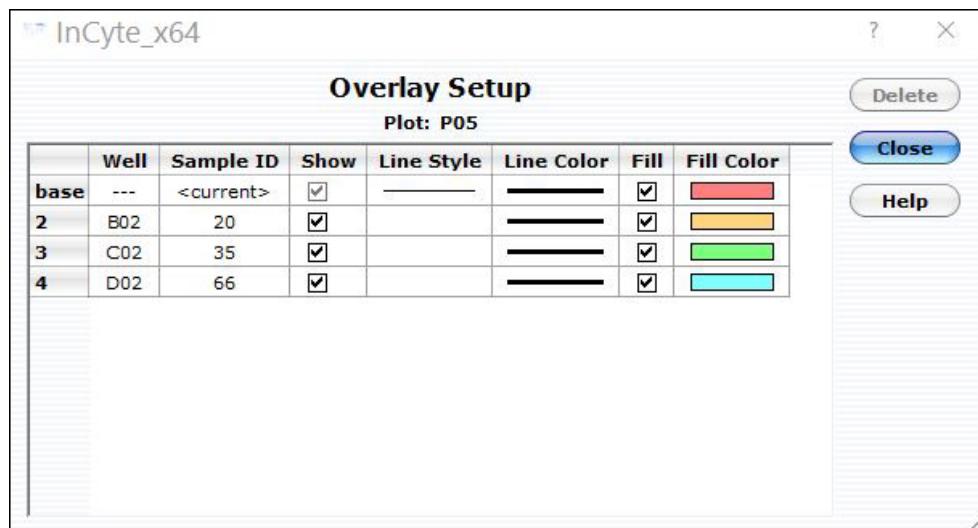
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## Histogram Overlay

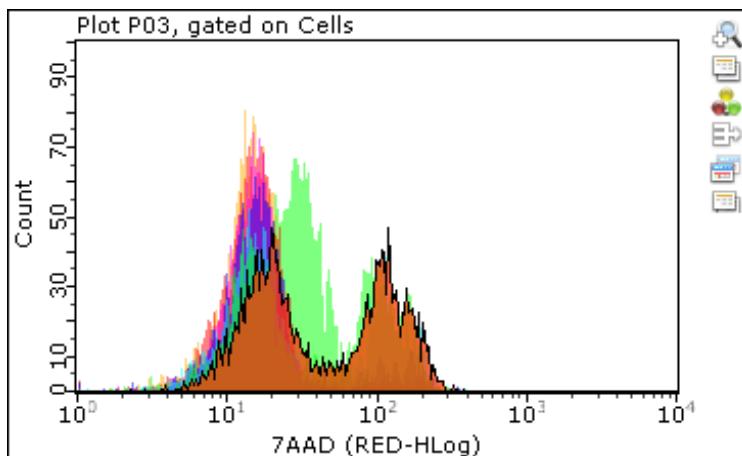
The histogram overlay feature allows you to superimpose histograms from multiple samples derived from one data file. By selecting a different color for each sample, you can see the different sample data overlaid within the same plot. Each histogram plot has its own overlay list.

1. From the Guava® InCyte™ module, open the FCS needed for analysis.
2. Choose **File > Open** from the menu bar.
  - a. From the **Open File** dialog box, navigate to the FCS file for analysis and click **Open**.
3. Choose the .fcs file in the **Analysed Data** pane.
4. Click the **Edit Overlay List** icon from the histogram plot (the second to last icon in the tool bar of the histogram plot). The Overlay Setup dialog box displays with the currently displayed sample well listed as base in the far-left column and <current> under Sample ID.
5. To add samples to the overlay list (and plot), drag a well directly from the plate map to either the histogram plot or the Overlay Setup dialog box.

**NOTE:** To select multiple wells, drag to select wells or use the **Ctrl** key to select non-consecutive wells.



**NOTE:** The plot will display the selected sample data in the designated colors. When you overlay plots, any markers you had previously set, as well as the stats, apply to the base plot only. The overlays are derived from the same gate as the initial plot. You can also change the parameter displayed by clicking the x-axis label and choosing a different parameter.



6. To change the overlay order, click the number (or "base") in the left column and drag it up or down in the list.
7. To remove data for an individual sample, click to select the well in the list and click **Delete**.

**NOTE:** You cannot delete the base sample.

8. To hide the data for an overlay from the plot, click to remove the check mark from the Show check box.
9. Click **Line Style**, **Line Color**, and **Fill Color** to change the appearance of the overlay.

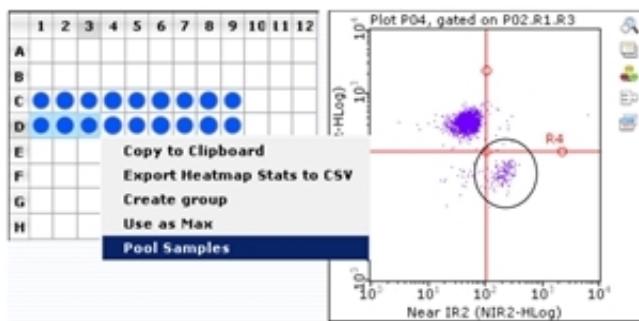
**NOTE:** You cannot overlay sample data with different plot parameters. A message will display informing you that some samples do not have the required parameters.

# Data Pooling

During analysis you can combine the data from multiple samples into a single sample. This can be useful when analyzing rare events.

**NOTE:** To pool samples, you must first pair a data file with a method to create an analyzed group.

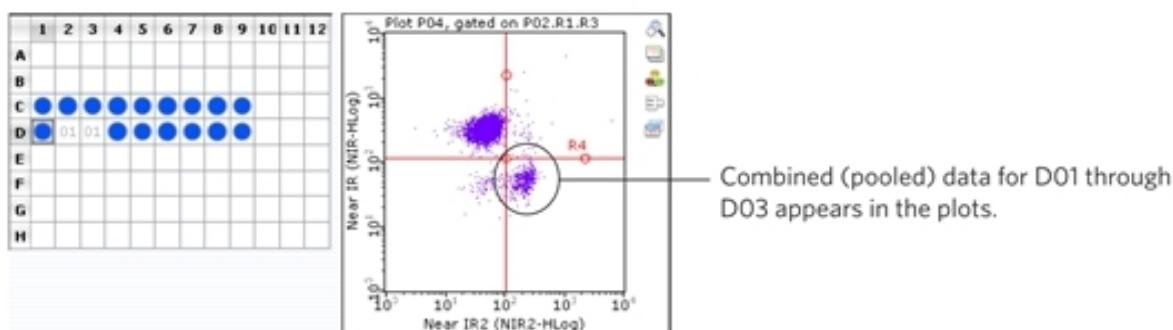
1. From the Guava® InCyte™ module, open the FCS needed for analysis.
2. Choose **File > Open** from the menu bar.
  - a. From the **Open File** dialog box, navigate to the FCS file for analysis and click **Open**.
3. Choose the .fcs file in the **Analysed Data** pane.
4. Click a well that you want to pool data from other wells into and then drag to select the other wells.
5. Right-click the selected wells and choose **Pool Samples** from the menu.



#### Example:

D01 is selected and cursor is dragged through D03. Right-click and select Pool Samples. Wells D02 and D03 now appear with 01 label.

**NOTE:** The data combined from all selected wells displays in the plots. The wells display in the plate map with the label number of the first selected well.



6. To see the new combined number of events for a given population, open the **Current Run Stats** window by selecting **Tools > Show Current Run Stats**.
  - a. If stats have not been created, click the **New Stat Marker** icon to the right of the plot and select **New Stat for R [x]**.

**NOTE:** The [x] in New Stat for R[x] will change based on what you name the new stat.

  - b. Select the **Count** as the Metric and click **OK**.
7. To unpool the data, right-click the selected wells and choose **Cancel Pooled Samples**.

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