Celigo® Cytometer
User Guide

Celigo® Software Version 5.2

8001619 Rev. I

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Appropriate Use	For research and development uses only. Not for use in diagnostic or therapeutic procedures. The Celigo cytometer is designed to perform both user-interactive and automated functions for the measurement and analysis of cells. The Celigo cytometer is designed to be used in a temperature and humidity controlled laboratory environment that is free from aerosols, liquid sprays or spills, and excessive airborne dust. Use of the Celigo instrument and software is governed by the *Celigo Terms and Conditions* and the *Nexcelom Bioscience, LLC. Software End User License Agreement*.
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Contents

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1. About this Guide

This chapter provides a brief description of this guide and how to use it.

1.1 Purpose

The purpose of this guide is to describe the Celigo cytometer operation procedures, consisting of image capture (scanning) and analysis. For the administrative procedures, see the *Celigo Cytometer Administrator Guide* (8001620). Also see the applicable application guide.

	**NOTE: The displays you see onscreen may differ from the figures in this document, depending on the application you have selected in Current Application.**

1.2 Conventions Used in this Guide

This section describes the symbols and signal words used in this guide.

1.2.1 Hazard Symbols

This guide uses symbols and associated signal words to communicate safety hazards. The hazard symbols (Table 1) allow you to easily recognize the hazard type. The signal word definitions (Table 2) comply with ANSI Z535.4.

Table 1. Hazard Symbols

Symbol	Hazard
	Voltage or electrical current
	Moving parts (pinch point hazard)
	General

Table 2. Hazard Severities

Signal Word	Severity
DANGER	Indicates an imminently hazardous situation that will result in severe personal injury or death if it is not avoided.
WARNING	Indicates a potentially hazardous situation that could result in severe personal injury or death if it is not avoided.
CAUTION	Indicates a potentially hazardous situation that may result in minor or moderate personal injury. It may also alert against unsafe practices. These include practices that may result in system damage, data corruption, data loss, or settings loss.

1.2.2 Other Symbols

This guide uses the additional symbols and signal words shown in Table 3.

Table 3. Additional Symbols

Symbol	Meaning
	A note. Indicates helpful information for the topic or step being described.

1.3 Safety Precautions

This section describes the precautions for safe operation of the Celigo cytometer.

1.3.1 Electrical Safety

The Celigo cytometer contains voltages and electric currents that are potentially hazardous. Under normal circumstances, the user and other persons in the vicinity of the Celigo cytometer are protected from accidental contact with these electrical hazards by physical barriers (exterior panels and access doors) and by electrical grounding of the instrument.

To reduce the risk of electric shock:

Do not remove instrument covers without proper training.

Ensure that all three-pronged power cords from the Celigo cytometer (total of 3 for instrument, workstation and monitor) are plugged into properly grounded 100 – 240 VAC receptacles only.

Use only 10A SLO-BLO 250 V, ceramic 3AB fuses (PN C0003892).

In the event of a spill of an aqueous or other conductive solution within the instrument, power down the system and unplug the power cords before attempting to clean up the spill.

In the event of a foreign object falling into the instrument through an opening (such as the access door or one of the air vents), power down the system using the main on/off switch in the back of the instrument and unplug the power cords before attempting to retrieve the foreign object.

Do not perform repairs on or within the Celigo cytometer. Only qualified service personnel should perform repairs.

If you are uncertain about any of the safety issues highlighted in this manual or have additional safety concerns, do not hesitate to contact Nexcelom Customer Service directly, with your questions or concerns, before attempting to service the Celigo cytometer.

	**WARNING: Use this product in the manner described in this document, and while observing all specified safety precautions. When used other than as specified, the safety features may be impaired or defeated. Failure to adhere to the safety precautions and/or procedures outlined in this document may result in system failure, personal injury, or death, for which Nexcelom shall not be held liable under any circumstances.**

	**WARNING: Risk of electric shock! The Celigo cytometer contains voltages and electric currents that are potentially hazardous. To reduce the risk of electric shock, do not remove instrument covers without proper training. Qualified service personnel should perform all repairs.**

1.3.2 Moving Part Hazards

The Celigo cytometer contains mechanical components that move within the instrument. Some of these components move in a linear fashion (for example, the x-axis and y-axis movements of the specimen stage), and some of these components have a rotational motion (for example, the galvanometer motors).

The Celigo cytometers moving components can pose risks of pinching, crushing, cutting, twisting or entrapping body parts, particularly hands and fingers. To avoid injury by the instruments moving components, you must observe the following precautions.

While an application is executing, keep the access door closed.

While an application is executing, the various mechanical components of the instrument can move at any time, without warning. Do not reach into the instrument while an application is executing, unless the application specifically requests that a new specimen plate be loaded onto the stage at a particular time.

Do not reach into the instrument to remove or load a specimen plate while the stage is still moving. Wait until the stage has come to a complete stop, before reaching into the instrument.

Keep clothing, jewelry, hair, and other loose materials clear of the instrument's mechanical components. Moving components can catch hold of such loose materials, thereby forcing a body part into a dangerous position.

	**CAUTION: The Celigo cytometer contains moving mechanical components that are capable of causing bodily harm. Do not reach into the instrument while parts are moving. Keep clothing, jewelry, hair, and other loose materials clear of mechanical components.**

1.4 Disposal Compliance

Nexcelom Bioscience complies with European Union Waste Electrical and Electronic Equipment (WEEE) Directive 2002/96/EC. The Celigo cytometer, as supplied by Nexcelom, contains Electrical and Electronic Equipment (EEE) and is suitable for recycling, provided it is not contaminated with hazardous substances.

If disposal is needed, contact Nexcelom Customer Service for further information, assistance, and costing for disposal.

1.5 Technical Assistance

Contact Nexcelom Customer Service for further information:

Nexcelom Bioscience, LLC.
Customer Service
360 Merrimack St. Building 9
Lawrence, MA 01843, USA

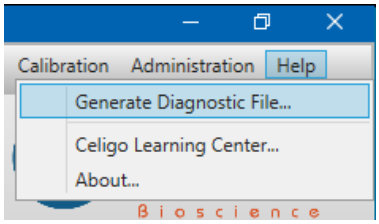
From the United States:
email: support@nexcelom.com
phone: +1 978-327-5340

From Europe:
e-mail: support@nexcelom.co.uk
phone: +44 (0) 161 232 4593

If you have a technical question that you are unable to answer after consulting the documentation provided with the Celigo cytometer, please contact Nexcelom for assistance. So that we may assist you in the most expeditious manner possible, please generate and send an error report as follows:

1. At the upper right corner of the Start tab, select **Help > Generate Diagnostic File** (Figure 1).

Figure 1. Generate Diagnostic File



2. Perform the prompt instructions.
3. Save the resulting error report to your desktop.
4. Attach the error report to an e-mail and send it to the applicable e-mail address, per the above list.

2. Product Description

This chapter provides a brief description of the Celigo cytometer.

2.1 Introduction

The Celigo adherent cell cytometer is a benchtop *in situ* cellular analysis system that rapidly provides high quality whole-well images for routine brightfield and fluorescent cellular analysis with a simple workflow.

The ability to analyze cells directly, in the tissue-culture flasks and microplates where they are grown and stored, provides users with the confidence that their analysis of cells is representative of *in situ* conditions.

Features of the Celigo cytometer include the following:

- Operates with a variety of T-flasks, slides, petri dishes, and multi-well plates
- Provides data from all cell types, adherent and non-adherent
- Brightfield and fluorescence cell imaging, identification, and characterization
- Fluorescence detection for up to four fluorochromes
- Analyzes every cell in every well, due to consistent illumination out to the edge of the well, eliminating the brightfield edge effect
- Fast full-well and partial-well imaging
- Requires minimal sample manipulation
- Easy-to-use interface

The Celigo cytometers fast imaging speed is largely due to its proprietary optical pathway, which employs a large field-of-regard (FOR) F-theta lens with high-speed galvanometer mirrors to rapidly scan large areas without moving the sample. The large FOR allows the scanning mirrors to obtain a series of images (fields-of-view or FOV) with fewer movements of the plate carrier.

The Celigo cytometer is designed for a wide variety of cell-based assays in both label-free and fluorescence applications. The instruments brightfield applications perform cell counting and growth tracking, accurately counting adherent and non-adherent cells without the use of stains, while rejecting plate artifacts. The ability of the Celigo cytometer to count individual cells without labeling or cell destruction represents an enabling technology for areas of life science research such as drug discovery and clone validation.

The Celigo cytometer is also capable of detecting and analyzing a wide range of fluorescent cell stains in assays for DNA synthesis, viability, apoptosis, cell counting, and for detection of highly secreting clones for protein production. The Celigo cytometer enables users to multiplex assays with up to four independent fluorescent channels in addition to the brightfield channel.

2.2 Hardware and Optics

The Celigo cytometer contains several hardware and optical subsystems:

LED-based brightfield illumination

Four-channel fluorescent illumination, with LED-based excitation

AVT PIKE camera

Rapid auto-focus

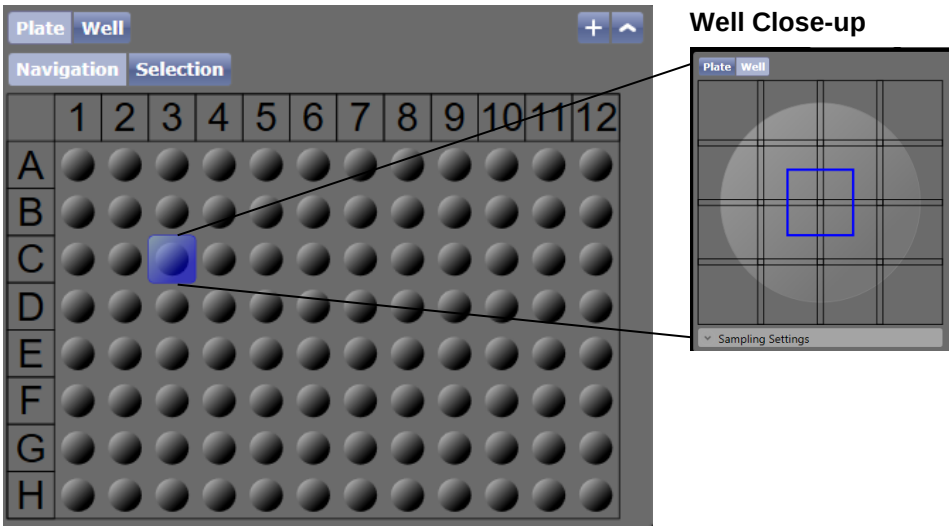
F-theta lens, which defines the FOR, with scanning galvanometer mirrors

XYZ stage system for plate loading and accurate positioning

The Celigo cytometer possesses a unique optical system that enables it to image cells at exceptional speeds. The large FOR lens views a significantly larger area than conventional microscope imaging systems. The FOR allows the direct viewing of 4 complete wells of 384-round-well plates and 1 complete well of a 96-well plate. Within the FOR, multiple images (FOVs: fields-of-view) are collected to provide full coverage of the well.

Figure 2 shows the scanned image of a 96-well plate. The well close-up view (displayed in the Scan tab) shows that the Celigo cytometer uses 16 FOVs for one 96-well plate.

Figure 2. Scanned Image of 96-Well Plate



3. Startup and Shutdown

This chapter provides the procedures for starting up and shutting down the Celigo cytometer.

3.1 Startup

Perform the following steps to start up the Celigo cytometer.

To start up the Celigo cytometer

1. Turn on the main power button on the front of the instrument.
2. Turn on the Celigo computer.
3. Log in to the computer.
4. Launch the Celigo application.

   The Celigo program opens and the HOME tab appears.

3.2 Shutdown

Perform the following steps to shut down the Celigo cytometer.

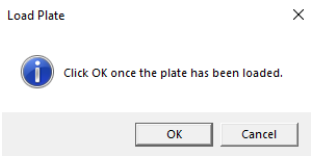
To shut down the Celigo cytometer

1. In the Celigo application, in any tab, click the Unload Plate button (Figure 3)

Figure 3. Unload Plate Button

An Unload Plate window appears (Figure 4).

Figure 4. Unload Plate Window



2. Remove the plate or flask from the instrument.
3. In the Unload Plate window, click **OK** or **Cancel**.
4. Exit the Celigo program by clicking the X icon in the upper right corner of the window.
5. Shut down the computer using the Windows Start/Shutdown menu.

NOTE: It is recommended that you only *periodically* shut down the computer. Keeping it on will allow for defragmentation, which helps Celigo cytometer performance.

6. Switch off the instrument power button.

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4. Celigo Cytometer Workflow Overview

This chapter describes the main tasks performed after Celigo cytometer (Celigo) startup.

For the Celigo cytometer startup and shutdown procedures, see chapter 3.

	NOTE: Hovering over the Celigo user interface screens with the cursor brings up dialog boxes explaining various settings.

Initial screen is the HOME tab, where after logging in, user can select different tasks. Depending on what task you choose, the workflow tabs at the top of the screen will update with the updated workflow items for that task.

For example, to perform a new scan of a new plate, you make entries in the following tabs (Figure 5), using them from left to right. The orange-brown tab is the current active tab location. Blue tabs with white text are available tabs user can access, where grey text is an inactive tab.

Figure 5. Celigo Operation Workflow Tabs

HOME tab: Select create a new scan

SETUP tab: Enter plate details and load plate

SCAN tab: Enter application and image acquisition details

ANALYZE tab: Enter object identification analysis details

GATE tab: Enter gating and classification details (optional)

RESULTS tab: View and export results

	NOTE: After loading a plate or previous scan, the Plate ID, Scan ID, Scan Result ID, application selected, current experiment, and Celigo status are displayed on top of the tabs.

Chapters 5 through 10 guide you through the tabs to perform a new scan and the follow-up tasks, which consist of analysis, gating, and then viewing results.

The tabs that users will be able to access or display for the follow-up tasks depend on your user permissions. See Administration Guide for user permission details.

	NOTE: The displays you see onscreen may differ from the figures in this document, depending on the application you have selected in Current Application.

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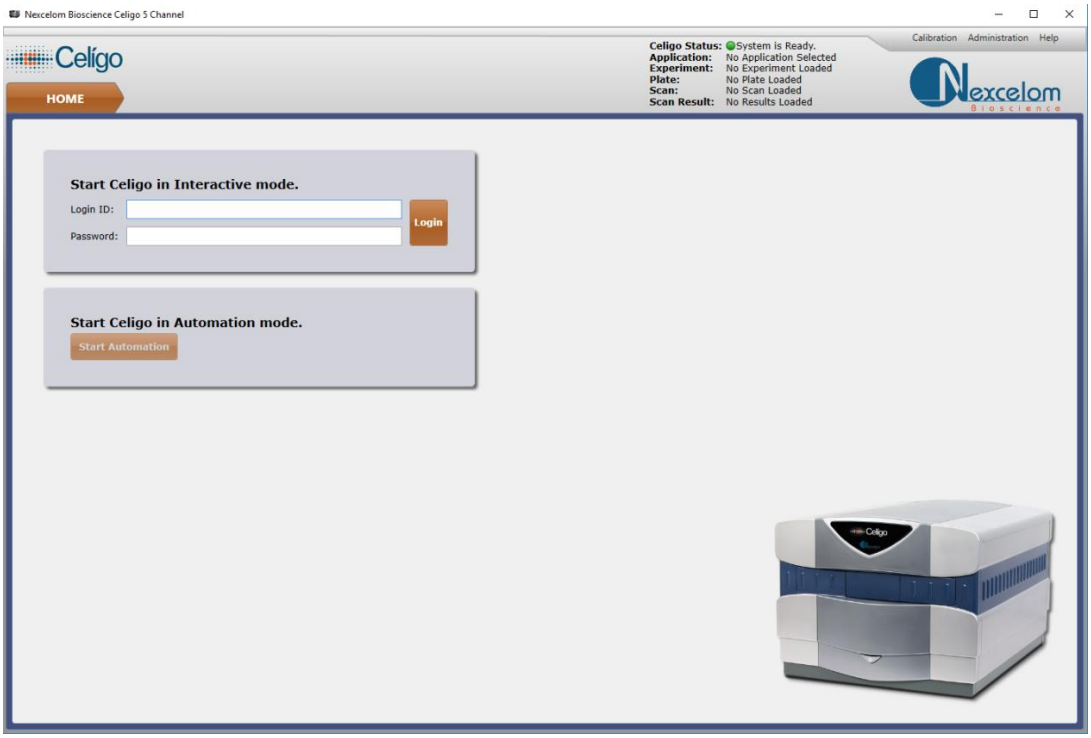
5. HOME and SETUP Tabs

The HOME tab (Figure 6) is used for the following tasks:

- Logging in to the Celigo application
- Running a project
- Starting a new scan
- Selecting an individual existing scan to analyze and/or view
- Selecting multiple existing scans for batch analysis
- Selecting multiple existing scans for batch export
- Managing data
- Starting Automation mode (optional, requires a license)

This chapter describes how to perform these tasks.

Figure 6. HOME Tab Log in screen



5.1 Logging In

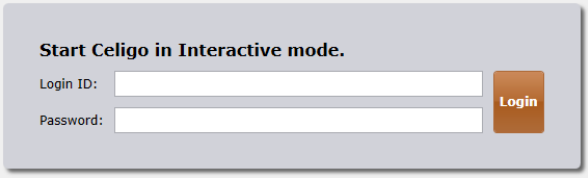
Log in to the Celigo application as follows.

1. In the HOME tabs Enter Login… dialog box (Figure 7), enter your user name and password. The default login entries are as follows:

   Login ID – Type **Ladmin**

   Password – Leave blank

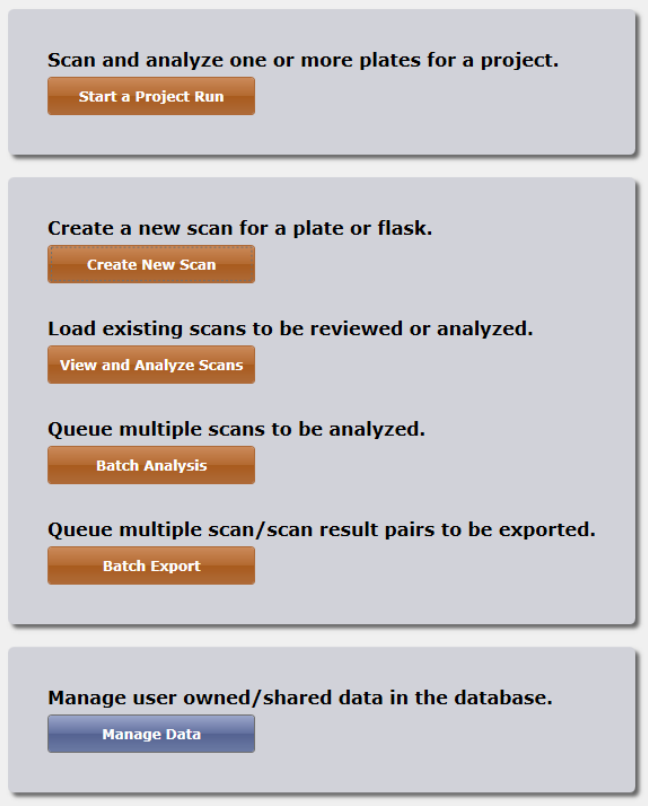
Figure 7. Enter Login... Dialog Box



2. Click **Login**.

   In the HOME tab, the task list for the Celigo application appears (Figure 8).

Figure 8. HOME tab Task List for the Celigo Application



3. Do one of the following:

   If running a project, continue to **Start a Project Run** section 5.2

   If starting a new scan, continue to **Creating a New Scan** section 5.3.

   If selecting an individual existing scan to work with, continue to **Selecting an Individual Existing Scan** section 5.4. This method will allow you to see the images and analysis overlays of the scan.

If performing batch analysis, continue to **Selecting Multiple Scans for Batch Analysis** section 5.5. This method will allow you to analyze multiple scans, but you will not be able to see the images and overlays of the scans.

If performing batch export, continue to **Selecting Multiple Scans for Batch Export** section 5.6.

If exporting, importing or deleting scans or saved settings, continue to **Manage Data** in the Administration Guide.

5.1.1 Returning to the HOME Tab

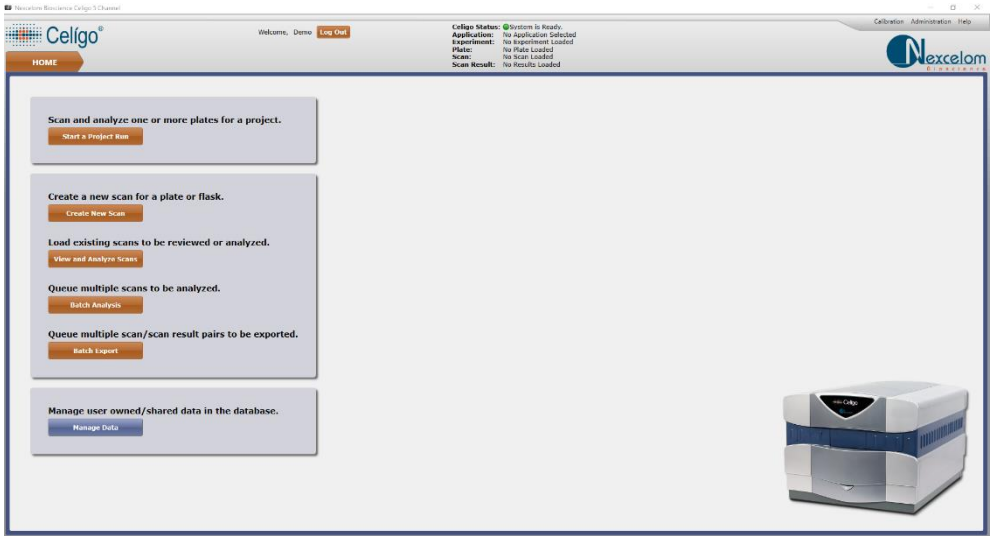
You can go back to the HOME tab from the other tabs. The plate or flask remains in the instrument.

	**CAUTION: Returning to the HOME tab removes your entered selections. If you want to retain your entered selections then adjust them for another scan, click the SCAN tab.**

To return to the HOME tab

Click the HOME tab when in any other tab (Figure 9) returns to HOME tab.

Figure 9. Back-to-HOME Tab



The system displays the HOME tab. The plate or flask remains in the instrument.

5.2 Starting an Existing Project Run

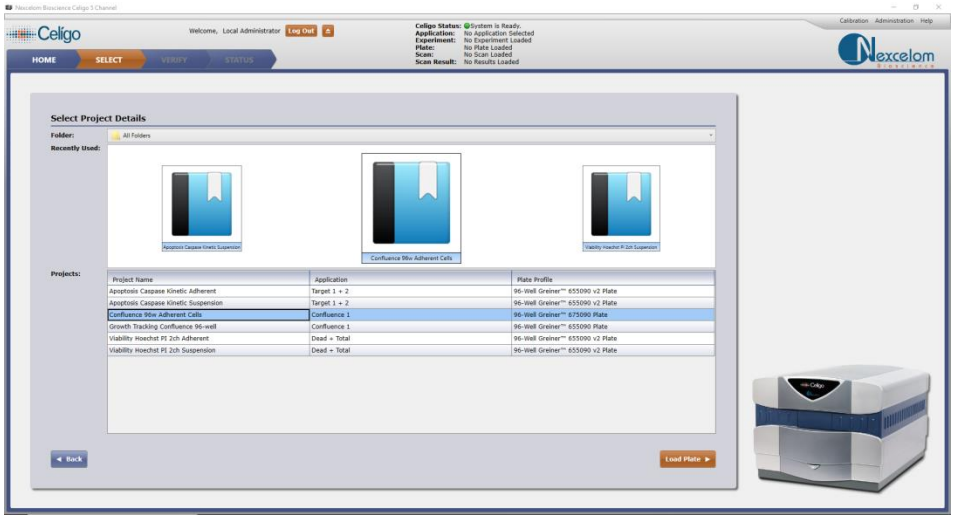
Perform the following steps to start a project run in Project Mode. This requires a saved project to exist in database. To save a Project in an active session, see section 6.10.2 or the Administration Guide from the Data Manage page.

To start a new project run

1. In the HOME tabs task list (Figure 8 in section 5.1), click **Start a Project Run**.

The Select Project Details screen appears (Figure 10).

Figure 10. Select Project Details



2. Select a Project from the list or icon in the carousel.

   Note: The carousel will display the last ten icons used, therefore newly created icons may not display immediately. Please select the Project using the Project list.

3. Click **Load Plate**.

4. After plate holder is ejected, place plate on holder and enter a Plate ID (Figure 11)

   Note: If after entering in the Plate ID and clicking X the system does not move forward; alter the Plate ID and try again. Plate IDs must have original names per plate type.

Figure 11. Enter Plate ID

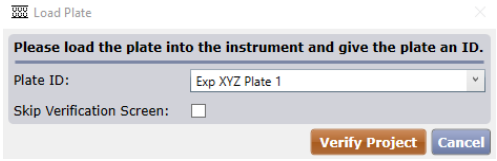
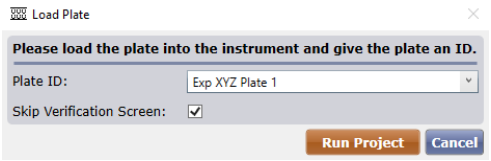


Figure 12. Enter Plate ID with checked box



(Optional) Check box for Skip Verification Screen (Figure 12) to skip this verification step and proceed to step 7 to run project.

5. Click **Verify Project**.

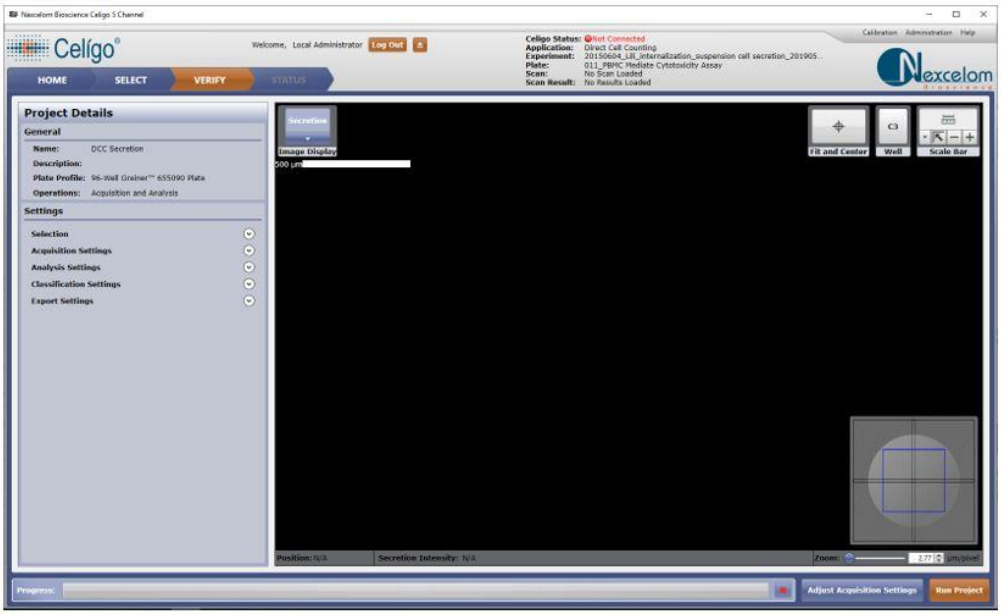
   The plate holder enters the system and focus registration begins using the experiment settings from the selected project.

   The system will move to the VERIFY tab (Figure 13) and display the focus registration well after it completes registering.

The left column parameters allow the user to see the details of the project. Clicking on the down arrows expands the details for each section.

The image display area shows the image after focus registration, allowing the user to verify the focus before scanning.

Figure 13. VERIFY Tab Screen



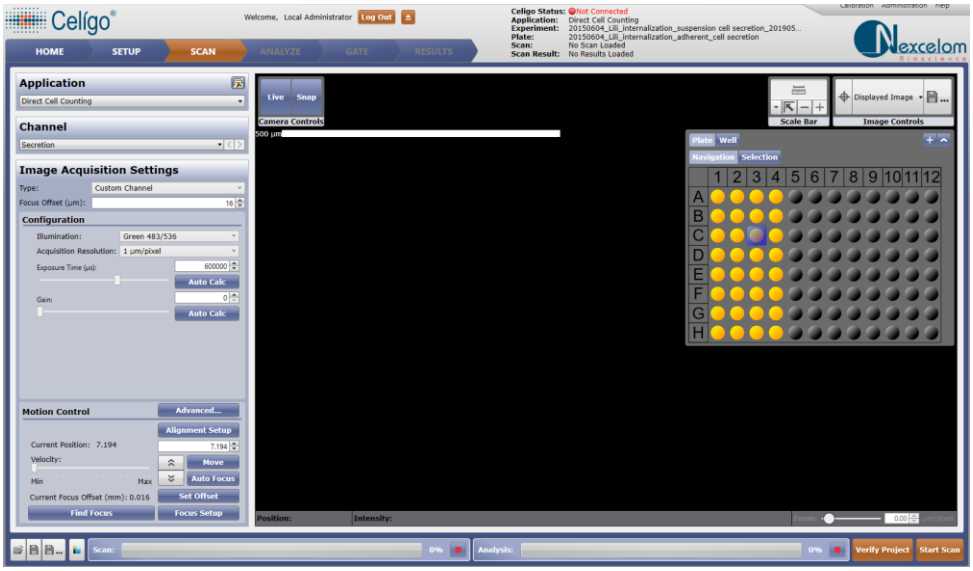
6. Proceed with one of the following:

   - Click **Run Project** (Figure 13) in lower right corner to proceed with the scan according to the project details, or
   - Click **Adjust Acquisition Settings,** in lower right corner, to make adjustments to focus and illumination in the SCAN tab (Figure 14).

     Note: If the adjustments are not saved, they will not be applied to future projects and will only be applied to the current workflow. Switching between tabs will not require resetting the adjustments.

     - If no adjustments are made, click **Verify Project** to return to VERIFY tab to continue in Project Mode.
     - Alternatively, click **Start Scan** and proceed to acquire images in Interactive mode. Refer to SCAN tab details in section 6, for further instructions.

Figure 14. Adjust acquisition settings in SCAN tab



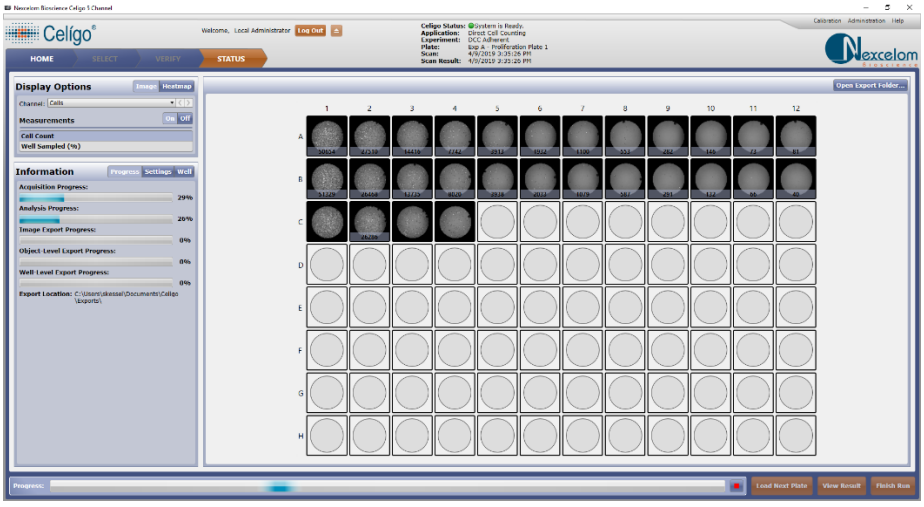
7. Click **Run Project** to proceed with the scan according to the project details.

   The system proceeds to acquire and/or analyze the images according to project details and presents them in the STATUS tab (Figure 15). Once completed, exports will begin automatically to the projects defined directory.

   If user chose to skip verification step, the system will focus register, then proceed to acquire images accordingly and present them in the STATUS tab (Figure 15).

   User can view thumbnails or double click thumbnails to view full resolution images. Reference RESULTS tab section 9 for interactive details.

Figure 15. STATUS tab in Project Mode



8. Choose to do one of the following (Figure 15):
   - Click **Load Next Plate** to scan another plate with same project.
   - Click **View Result** to view high resolution images and graphic overlays
   - Click **Finish Run** to return to the HOME tab

- Click **HOME** tab to exit the current project mode and return to HOME.
- Click **SELECT** tab to return to Select Project Details.

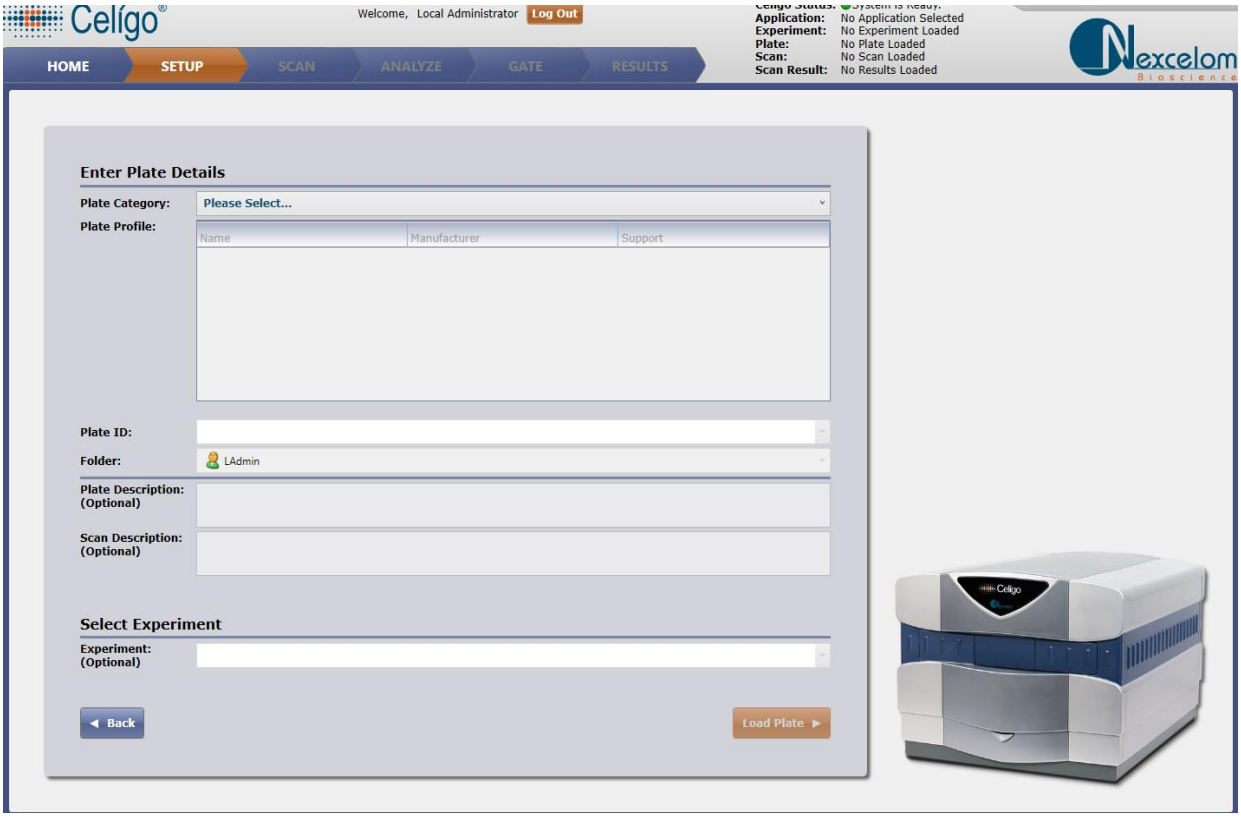
5.3 Creating a New Scan

Perform the following steps to create a new scan.

To create a new scan

1. From the HOME tab task list (Figure 8), click **Create New Scan**.
2. The Enter Plate Details screen appears (Figure 16) in the SETUP tab.

Figure 16. Enter Plate Details Screen



3. In the Enter Plate Details screen, make the following entries:
   - Plate Category: Choose plate type and/or format
   - Plate Profile: Choose plate vendor & part number

     Note: If a specific plate vendor is not visible contact Nexcelom Bioscience to request a recent list or have a template created.
   - Plate ID: Enter new plate ID or a previously entered plate ID

     Note: If the system is not accepting the new plate ID, that plate ID may already exist. Alter the ID slightly and try again.

	NOTE: To edit the Plate ID after performing a scan, use the Manage Data function per the Administrator Guide section Changing Plate Information.

- Folder: Destination for the plate details file, default is the signed in user folder
- Plate Description (Optional): Describe the plate
- Scan Description (Optional): Describe the scan
- Experiment (Optional): Load a previously saved experiment for use in a new scan

	NOTE: Only the experimental settings are compatible with the chosen plate category are displayed for selection.

4. Click **Load Plate**.

	NOTE: If using a network database setup, if the plate is already in use by another user, a prompt will be displayed notifying the user that the plate is already in use.

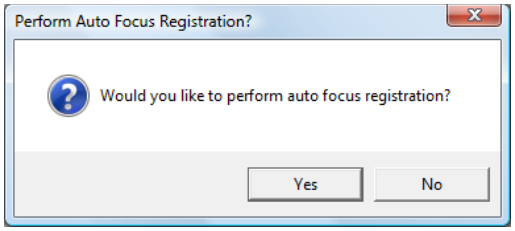
5. Do one of the following:

   If you left the Experiment menu blank in step 3, skip to step 8.

   If you previously entered an Experiment menu selection in step 3 with Focus Type in Focus Setup set to **None**, no registration occurs; skip to step 8.

   If you previously entered an Experiment menu selection in step 3 and you defined a Focus Type in Focus Setup in the experiment, a Would you like to perform auto focus registration? message box appears (Figure 17). Continue to step 6.

Figure 17. Perform Auto Focus Registration Message Box



6. Click **Yes** or **No** as follows:

   Yes – All of the image acquisition settings, including any offsets and focus registration settings that had been made in the Focus Setup dialog box, will be applied to the upcoming scan.

   No – All of the image acquisition settings *except* any offsets and focus registration settings made in the Focus Setup dialog box will be applied to the upcoming scan. Selecting No will allow you to manually select

Focus Setup settings to be applied instead of those in the Focus Setup dialog box.

	NOTE: If you do not change the Focus Setup settings, you can apply the saved settings later by clicking Start Scan in the Scan tab and clicking Yes in the resulting Your current focus has not been registered message box. This will create new settings that are close to or that match the saved settings, depending on the well registered.

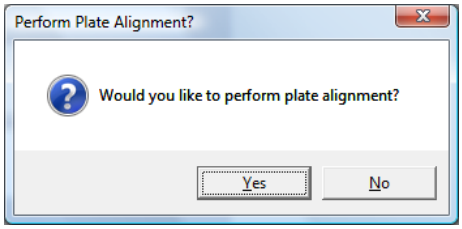
7. Do one of the following:

   If you entered an Experiment menu selection in step 3 without Plate Alignment Setup, skip to step 8.

   If you entered an Experiment menu selection in step 3 with the Plate Alignment Type in Plate Alignment Setup set to **None**, skip to step 8.

   If you entered an Experiment menu selection in step 3 and you defined the Plate Alignment Type in Plate Alignment Setup in the experiment, select one of the following in the resulting Plate Alignment message box (Figure 18):

Figure 18. Perform Plate Alignment Message Box



   – **Yes** – A plate alignment will be performed, using the settings in the experiment file.

   – **No** – A plate alignment will not be performed.

   If you clicked Yes, the system now performs a plate alignment.

	NOTE: Plate Alignment works for 96-well and 384-well black walled clear bottom plates. Reference Section 6.7.3 for further details on plate alignment.

8. A Load Plate message box (Figure 19) appears.

Figure 19. Load Plate Message Box

9. Carefully place a plate or flask onto the stage, using the following practices:

Note: An adapter may be required to secure the T-25 and T-75 flasks onto the stage.

For a plate:

- Manually push the plate into the inner left corner of the plate carrier. Ensure that the plate is seated flat in the stage, with well position A1 in the upper-left hand corner of the plate as you face the front of the instrument.

For a T25 flask, petri dish, or slide:

- Insert the appropriate holder onto the stage. Load the sample into the holder by pulling back the spring mechanism. Ensure that the sample is seated flat. If the sample is a flask, ensure that the flask is inserted with the cap toward the left side in relation to an operator in the front of the instrument.

For a T-75 flask:

- Load directly onto the stage with the cap toward the left side.

	**CAUTION: Do not reach into the Instrument while the stage is in motion!** **Keep clothing, jewelry, hair, and other loose materials clear of mechanical components.** **The Celigo cytometer contains moving mechanical components that are capable of causing bodily harm.**

10. Click **OK** in the Load Plate message box.

    The access door closes. If you had entered an Experiment menu selection, the settings in the selection will be used.

    The screen progresses to the SCAN tab.

11. Continue to SCAN Tab Chapter 6.

5.4 Selecting an Existing Scan to Analyze and/or View

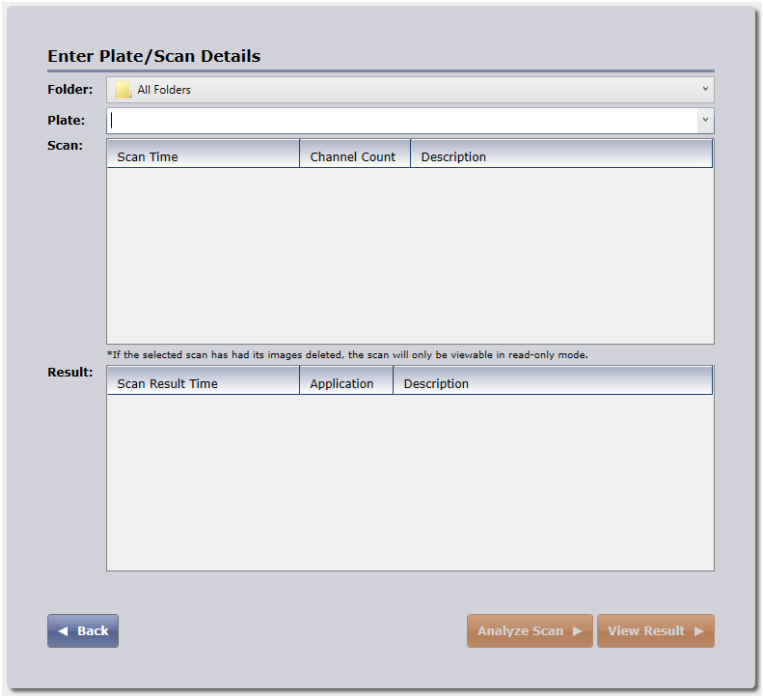
Perform the following steps to select an existing scan to analyze, re-analyze, or review.

To select an existing scan to analyze and/or view

1. In the HOME tabs task list (Figure 8 in section 5.1), click **View and Analyze Scans**.

   The Enter Plate/Scan Details screen appears (Figure 20) in the SETUP tab.

Figure 20. Enter Plate/Scan Details Screen Before Selections

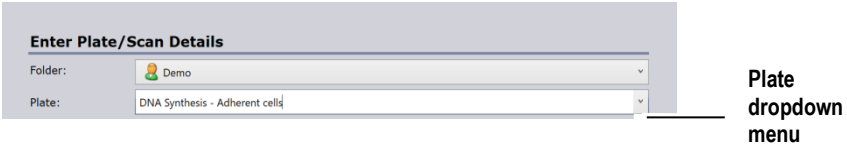


2. In Folder, select the name of the folder where the scan you want to view is located. (Optional)

	**NOTE: If you do not remember the folder name where the scan is located, you can click the HOME tab and then click Manage Data to search for a keyword.**

3. In Plate, select the name of the plate associated with the desired scans by doing one of the following (Figure 21):
   - Click the dropdown menu arrow.
   - Type the name of an existing plate to display matching names.

Figure 21. Plate Selection

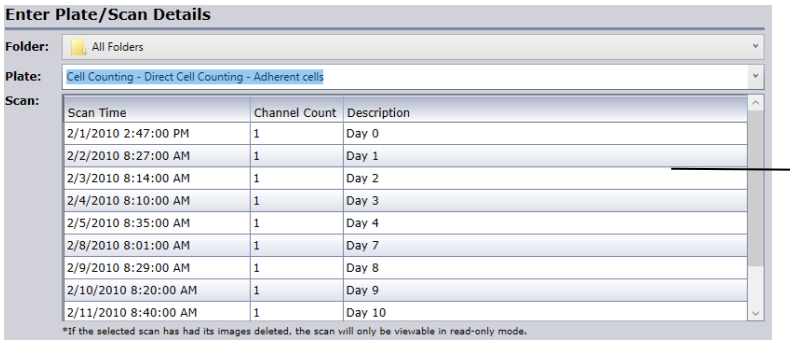


The list of scan times associated with the selected plate appears in the Scan section.

4. In the Scan section (Figure 22), select the scan time for the previously scanned plate.

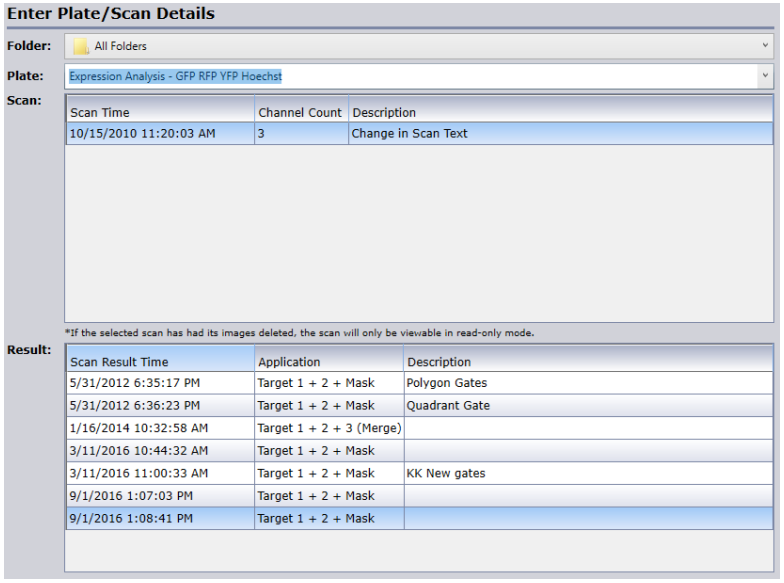
NOTE: Multiple image acquisitions of the same plate with the same Plate ID will be saved with unique scan times, according to the scanning start time.

Figure 22. Scan Selection



5. In Result (Figure 23), select a scan result intending to view a result.

Figure 23. Scan Result Type Selection



6. Do one of the following:

   – If you want to analyze the scan with the same or new application, click the **Analyze Scan** button (Figure 24).

     If you want to analyze the scan, continue working in the ANALYZE tab by going to ANALYZE Tab chapter 7.

   – If you want to view a scan with an existing result, click the View Result button (Figure 24).

     For additional information when viewing scan and scan result details, go to Results Tab chapter 9.

Figure 24. Analyze Scan and View Result Buttons

The ANALYZE, GATE, or RESULTS tab become enabled, depending on your permissions, as follows:

If you have full read/write/delete permissions for the existing selected plate, the ANALYZE and GATE tab become enabled.

If you have read-only permissions for the existing selected plate, only the RESULTS tab becomes enabled.

If the selected scan has its images deleted, only the RESULTS tab becomes enabled.

If the application for the scan result is not active, only the RESULTS tab becomes enabled.

	**NOTE: If using a network database setup, if the plate is already in use by another user, a prompt will be displayed notifying the user that the plate is already in use.**
	**NOTE: To select a group of existing scans for re-analyzing in batches with saved analysis and gate settings, see Selecting Multiple Existing Scans for Batch Analysis section 5.5.**
	**NOTE: To select a group of existing scans for exporting in batches, see Selecting Multiple Existing Scans for Batch Export section 5.6.**

5.5 Selecting Multiple Existing Scans for Batch Analysis

Batch Analysis allows user to analyze multiple scans with previously saved analysis settings (section 7.10) and, optionally, classification settings (section 8.21) from one or more scans as a group that you queue.

Batch analysis prevents the need to select and analyze scans individually in the ANALYZE tab. User can select batch analysis to analyze a group of scans for the first time or to re-analyze a group of scans using the same application or a new application. Users are able to apply a different application to each scan.

Batch analysis consists of a dual function of analysis and then an export of the results from the new results.

This section describes how to select multiple scans for batch analysis and how to start the batch analysis.

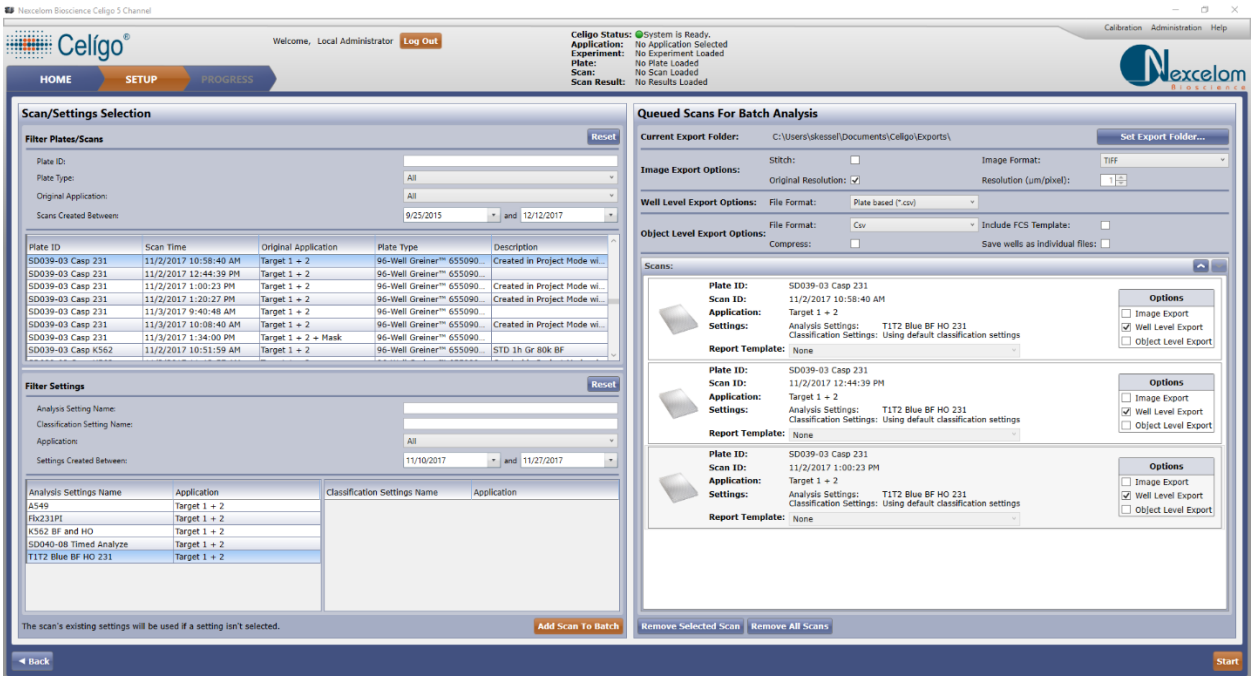
	**NOTE: Scans with images that were deleted (scan image deletion can occur in data management) cannot be analyzed and will not appear in the scan selection.**

To select multiple existing scans for batch analysis

1. In the HOME tabs task list (Figure 8 in section 5.1), click **Batch Analysis**.

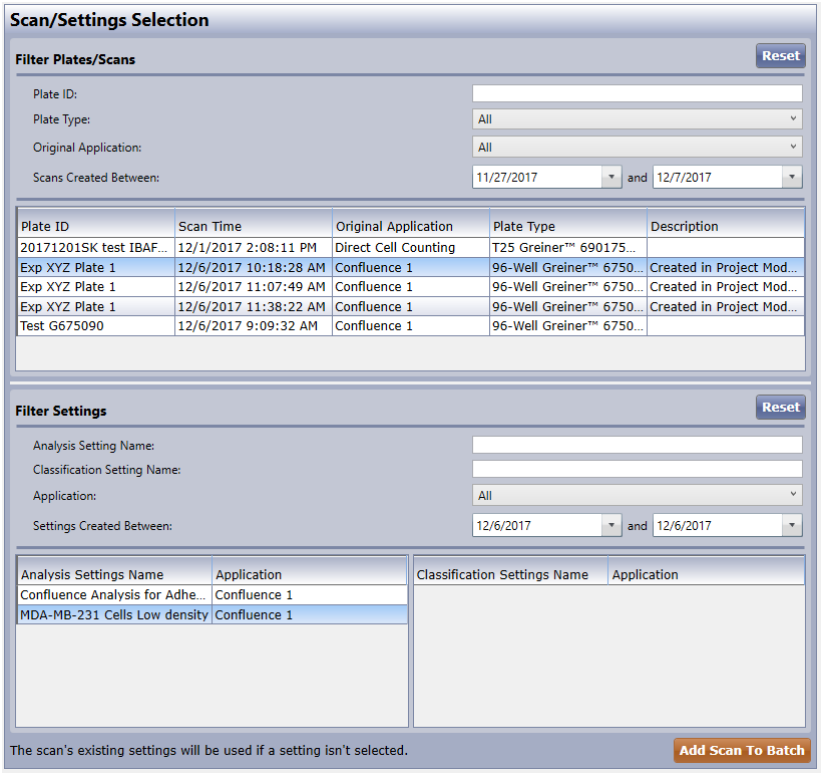
   The Scan/Settings Selection/Queued Scans for Batch Analysis window appears (Figure 25) in the SETUP tab.

Figure 25. Scan/Settings Selection and Queued Scans for Batch Analysis Window



2. In the Filter Plates/Scans section, enter search criteria in Plate ID so that the wanted scan appears in the scan search results list (Figure 26).

Figure 26. Scan Search Results List



3. In the scan search results list, select a scan line item to be analyzed so that it is highlighted in blue.

   Each scan start time from the same Plate ID is listed individually.

   You can select only one scan at a time to queue for batch analysis.

   The analysis settings that have been previously saved in the database and that are compatible with the selected scan are displayed in the bottom left pane for selection.

   To undo the selection in the Filter Settings pane, click **Reset**.

4. In the Filter Settings pane, select an analysis settings line item to be used for analysis of the selected scan so that it is also highlighted in blue.

   Enter in search criteria in either Analysis Setting Name or Classification Setting Name to narrow down the selection

   You can use this pane for re-analyzing the selected scan using a different application than originally used. To do this, select an analysis settings line item that has a different application in the Application column.

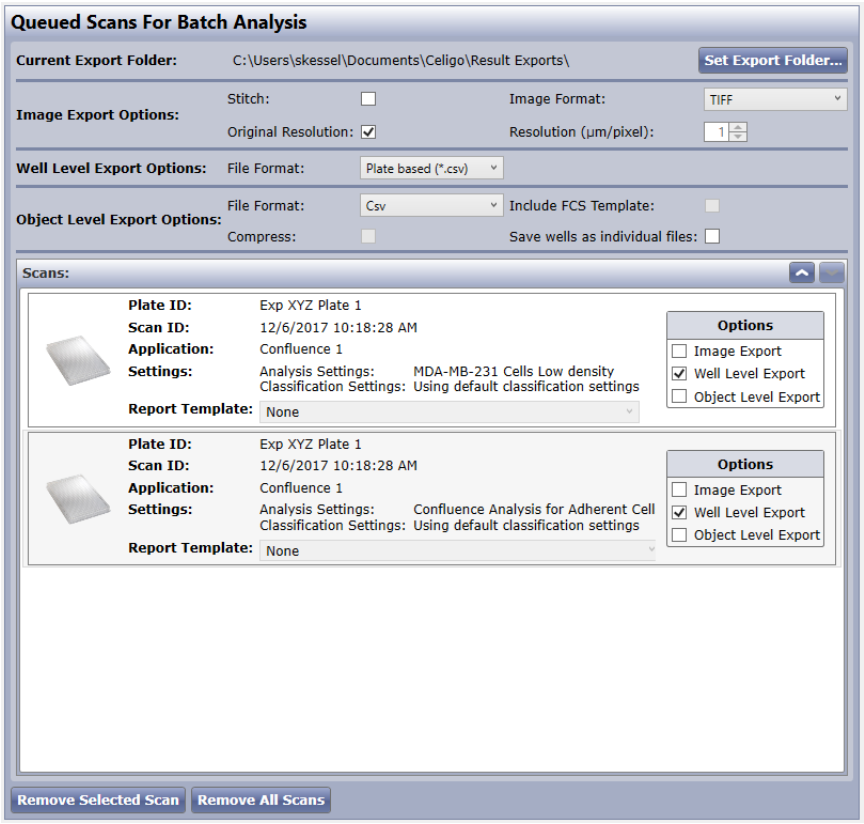
   If you do not make a selection in the Filter Settings pane, the scans existing settings will be applied during analysis.

   To undo the selection in the Filter Settings pane, click **Reset**.

5. Click **Add Scan to Batch** or press the Alt and A keys to add selection.

   Selected scans and assigned analysis settings appear in the Queued Scans for Batch Analysis pane (Figure 27).

Figure 27. Queued Scans for Batch Analysis



6. In Queued Scans for Batch Analysis (Figure 27), select one or more of the following, depending on the option selections available:

   Current Export Folder:

   – Select **Set Export Folder** to change to alternative location from default location.

   Image Export Options:

   – Check the Stitch checkbox if you would like the images to be assembled so that you can view the entire well at one time instead of each of the images separately.
   – Use the Image Format option to select from JPG, Bitmap, and TIFF file formats for exported images.
   – Select whether to export at the original, acquired resolution or at a lower resolution in order to speed up the export process.

   Well Level Export Options:

   – Select file format for CSV file to be displayed in, either Plate based layout or Tabular layout.
      - Plate – Measurements are grouped with the data appearing in consecutive grids.
      - Tabular – Each well and measurements are recorded in individual rows.

   Object Level Export Options:

   – Select file format for either CSV, FCS, ICE with or without images

CSV – Comma Separated Value format.

FCS – Flow Cytometric Standard format.

ICE – Image Cytometer Experiment data format.

- To include FCS Express Template if FCS or ICE (with or without images) is selected as format
- To compress if ICE (with or without Images) is selected as format
  - When compression is turned on, the extension of the file will be ACS instead of ICE.
- To save wells as individual files or as one file.

	**NOTE: ICE and ACS file formats require DeNovo FCS Image or Plus 6.0 or higher.**
	**NOTE: If using a resolution other than the original resolution, the numeric range to select will automatically change based on if the scans/scan results that have been selected for export are too large for the currently selected file format.**

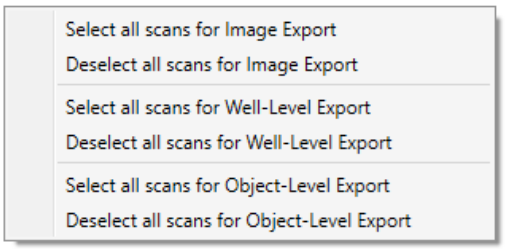
7. In Scans section (Figure 27), for each Scan listed, select one or more of the following, depending on the option selections available:
   - In the Options menu, select the file format that you want the system to use for exporting line items.

     Image Export

     Well Level Export

     Object Level Export
   - Right-clicking your mouse in this area, opens dialog to check or uncheck all items at once (Figure 28).

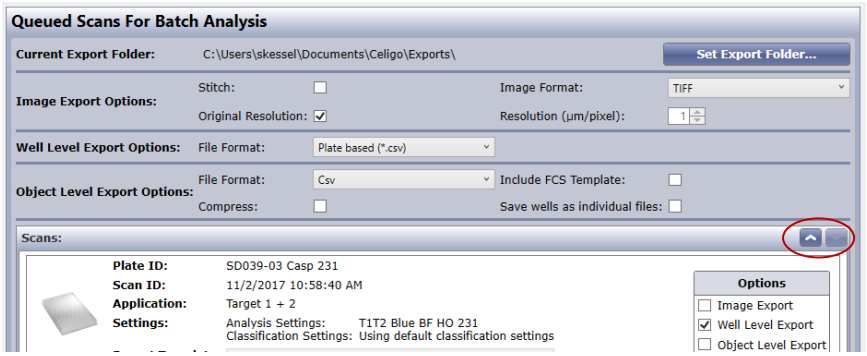
Figure 28. Right-Click for multiple selections dialog



8. Do the following as needed:
   - To change the queue order, click the Up and Down arrows in the Queued Scans for Batch Analysis pane (Figure 29).
   - To remove scans to be analyzed, select the scan line item in the upper left pane and click **Remove Selected Scans** (Figure 28)**.**
   - To remove all scans to be analyzed, click **Remove All Scans**.
   - To specify which Report Template to use for each scan (if Include FCS Template option is selected), select a report template to use in the Report

Template dropdown. If no report template is desired, leave the selection as None.

Figure 29. Up and Down Arrows for Changing Batch Analysis Queue Order



9. To start batch analysis, click **Start** (Figure 25)**.**

   The Scans for Batch Analysis progress window (Figure 30) appears with four types of progress indicators:

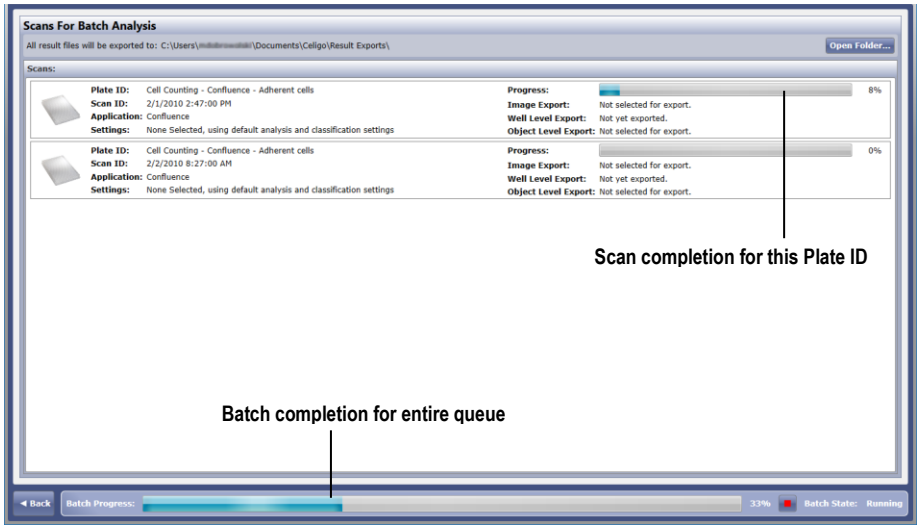
   During processing of each export, a circular progress bar appears (not shown in Figure 30).

   To the right of each scan, a progress bar shows the percent completion of the individual scan.

   At the bottom of the window, a progress bar shows the percent completion of the entire queue.

   When the batch analysis is complete, the progress bar reads 100% and the Batch State reads Finished.

Figure 30. Scans for Batch Analysis Progress Window



10. Once batch analysis task is complete. Do one of the following:

   – If you want to see the resulting CSV, FCS, ICE, or ACS files, click **Open Folder**.

   – Click **Back** or **HOME tab.**

5.6 Selecting Multiple Existing Scans for Batch Export

Batch export allows you to export multiple existing scans as a group that you queue. Batch export prevents the need to select and export scans individually.

You can batch export data files and image files.

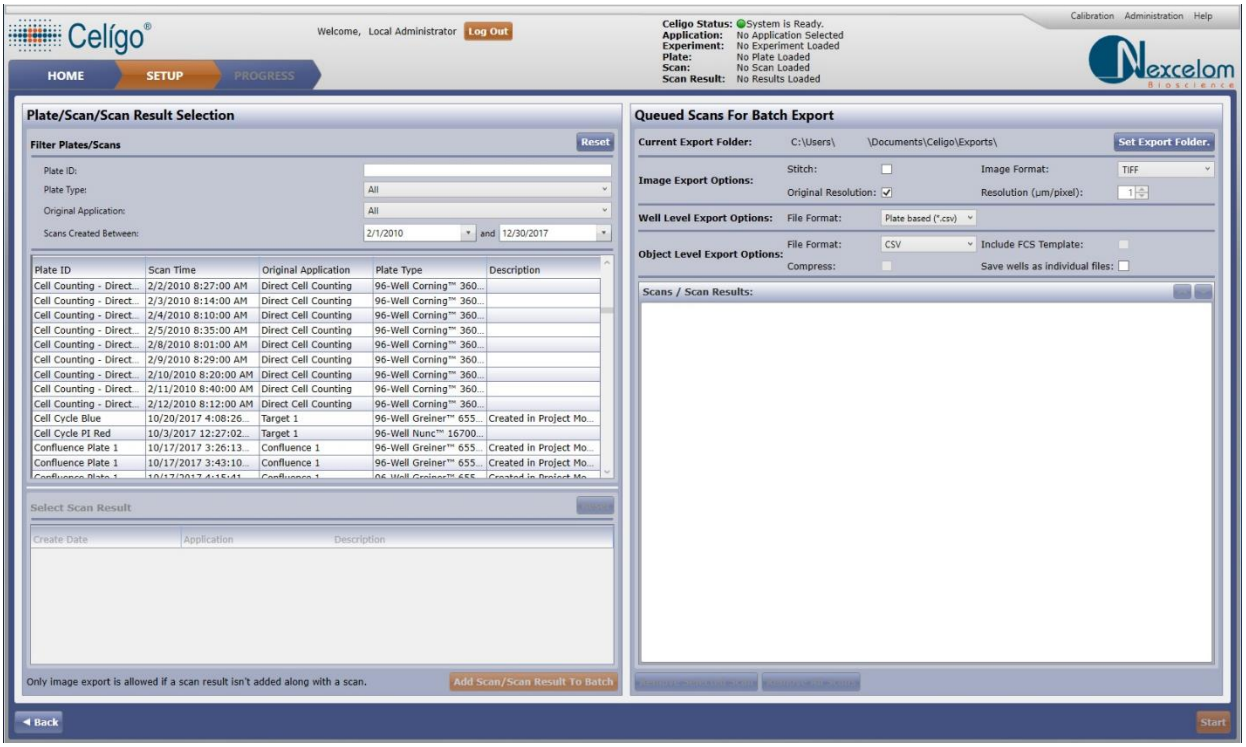
This section describes how to select multiple scans for batch export and how to start the batch export.

To select multiple existing scans for batch export

1. In the Start tabs task list (Figure 8 in section 5.1), click **Batch Export**.

   The Plate/Scan/Scan Result Selection/Queued Scans for Batch Export window appears (Figure 31).

Figure 31. Plate/Scan/Scan Result Selection/Queued Scans for Batch Export Window



2. In the upper left table of the Plate/Scan/Scan Result Selection pane (Figure 31), select a scan line item for which you want to export images (and optionally any associated data) so that it is highlighted in blue.

   Each scan start time from the same Plate ID is listed individually.

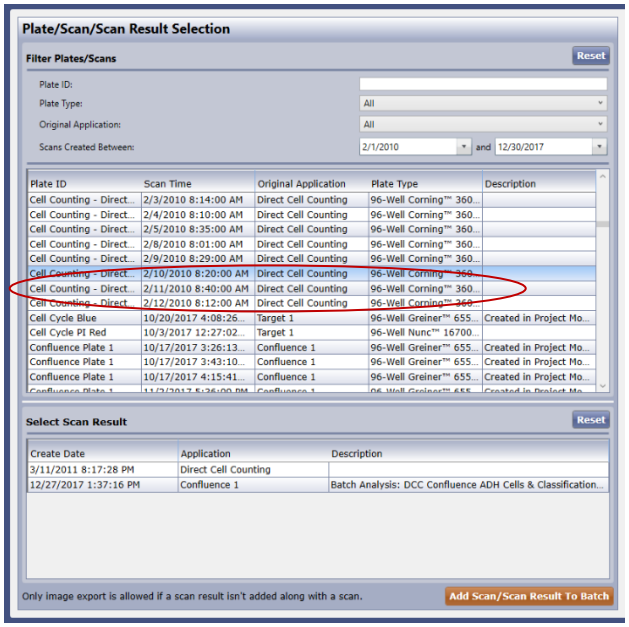
   You can select only one scan at a time to queue for batch export.

   The Select Scan Result pane at the bottom left shows the scan results associated with the selected scan.

	NOTE: Scans with images that were deleted (scan image deletion can occur in data management) cannot be added to the queue without a result also selected since images cannot be exported for this scan.

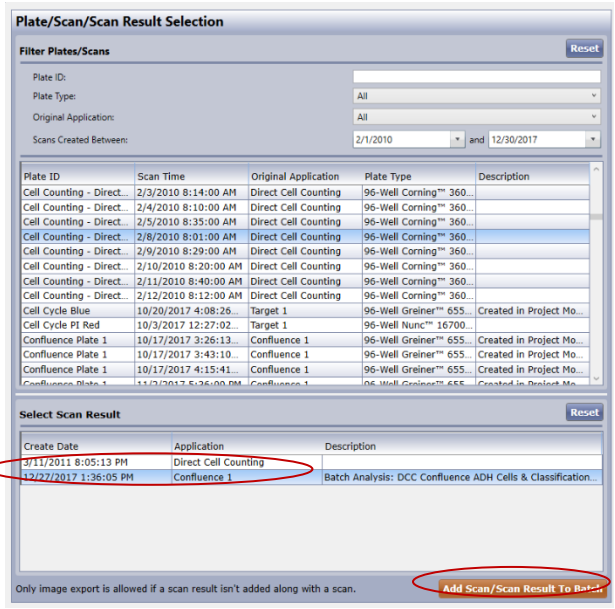
3. Do one of the following:
   - If you want to export only an image (scan), skip to step 5, without making a selection in the Select Scan Result pane (Figure 32).
   - If you want to export data (also called a scan result) associated with the Scan ID selected in the upper left table (as well as export the image), select a line item in the Select Scan Result pane. Continue to step 4.

Figure 32. Selecting a Scan and Image for Batch Export



4. In the Select Scan Result pane at the bottom left, select the scan result (data) that is associated with the selected scan (Figure 32).

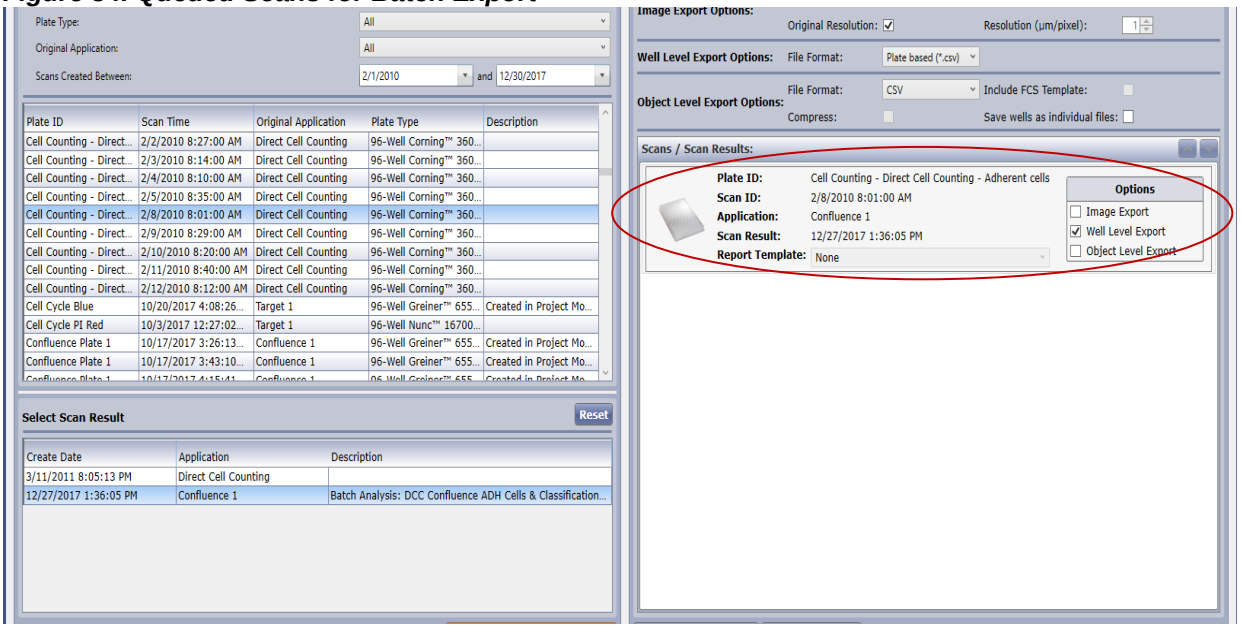
Figure 33. Add Scan/Scan Results to Batch



5. Click **Add Scan/Scan Result to Batch** (Figure 33)**.**

   The scans selected for batch export appear in the Queued Scans for Batch Export pane. Options checkboxes also appear for the selected scans (Figure 34).

Figure 34. Queued Scans for Batch Export



6. In the Queued Scans for Batch Export pane (Figure 34), you can do one or more, depending on the Option selections available:

   Current Export Folder:

   Select **Set Export Folder** to change to alternative location from default location.

Image Export Options:

- Check the Stitch checkbox if you would like the images to be assembled so that you can view the entire well at one time instead of each of the images separately.
- Use the Image Format option to select from JPG, Bitmap, and TIFF file formats for exported images.
- Select whether to export at the original, acquired resolution or at a lower resolution in order to speed up the export process.

Well Level Export Options:

- Select file format for CSV file to be displayed in, either Plate based layout or Tabular layout.
    - Plate – Measurements are grouped with the data appearing in consecutive grids.
    - Tabular – Each well and measurements are recorded in individual rows.

Object Level Export Options:

- Select file format for either CSV, FCS, ICE with or without images

    CSV – Comma Separated Value format.

    FCS – Flow Cytometric Standard format.

    ICE – Image Cytometer Experiment data format.

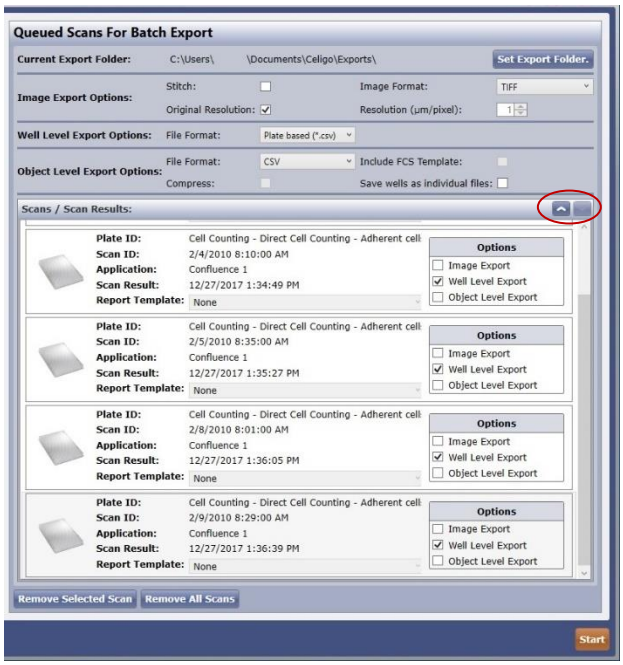
    Note: Check the Compression box when selecting ICE formats for ACS output.
- To include FCS Express Template if FCS or ICE (with or without images) selected as format
- To save wells as individual files or as one file.

	**NOTE: If using a resolution other than the original resolution, the available, numeric range to select from will automatically change based on whether any of the scans/scan results which have been selected for export are too big for the currently selected file format.**
	**NOTE: ICE and ACS file formats require DeNovo FCS Image or Plus 6.0 or higher.**

7. Do the following as needed:
    - To select or deselect all scans for image, well-level, or object-level export, right-click a scan line item in the upper right pane and select the **Select all scans...** or **Deselect all scans...** menu selection. See Figure 25 displaying the select and deselect all dialog window.
    - To remove individual scans queued for export, select the scan line item in the upper right pane and click **Remove Selected Scans**.
    - To remove all scans to be analyzed, click **Remove All Scans**.
    - To change the queue order, click the Up and Down arrows in the Queued Scans for Batch Export pane (Figure 35).

– To specify which Report Template to use for each scan (if Include FCS Template option is selected), select a report template to use in the Report Template dropdown. If no report template is desired, leave the selection as None.

Figure 35. Up and Down Arrows for Changing Batch Export Queue Order



8. To start batch export, click **Start** (Figure 35)**.**

   The Scans for Batch Export progress window appears with three types of progress indicators:

   – To the right of each scan, a progress bar shows the percent completion of the individual export.

   – At the bottom of the window, a progress bar shows the percent completion of the entire queue.

   – When the batch export is complete, the progress bar reads 100% and the Batch State reads Stopped.

9. If you want to see the exported files, click **Open Folder**.

   The batch export task now is complete.

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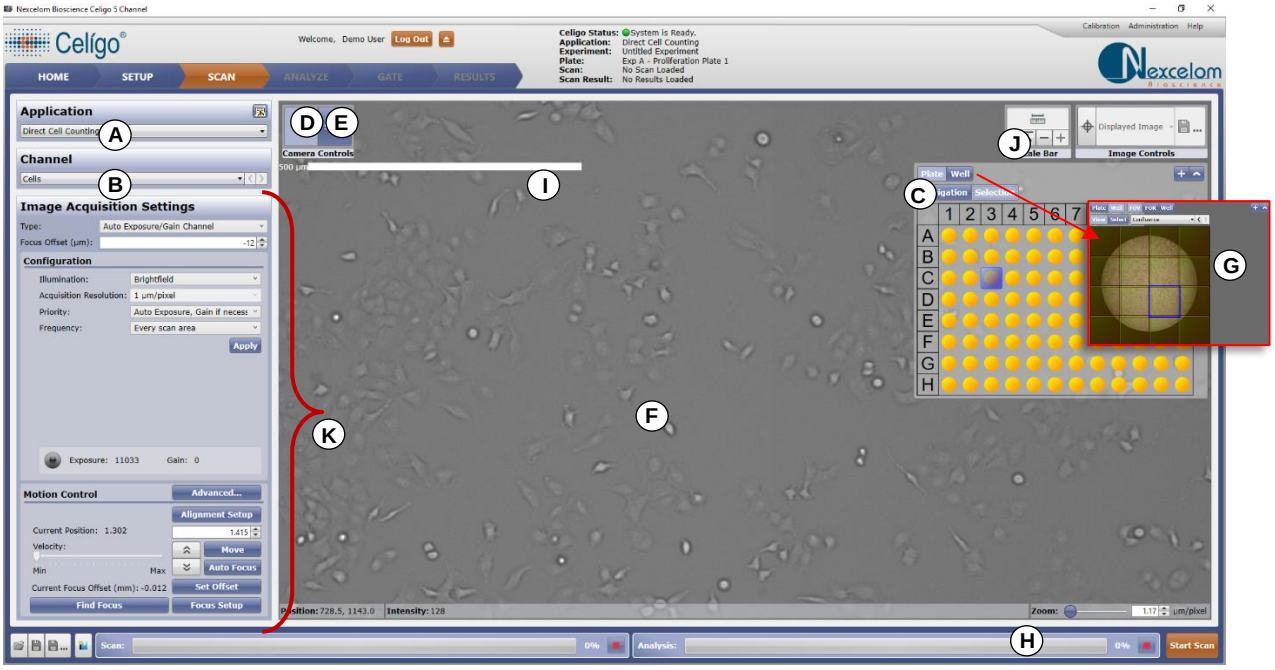
6. SCAN Tab

This chapter describes how to perform a scan. You do this task in the SCAN tab (Figure 36).

	NOTE: The displays you see onscreen may differ from the figures in this document, depending on the application you have selected in Current Application.

Figure 36. SCAN Tab



6.1 Selecting an Application

Applications are used to set the parameters needed for data acquisition for specific assays. Common components such as configuration settings are constant across all the applications. Components specific to each application are available only when that specific application is selected.

For instructions that are specific to each of the Celigo applications, see the applicable Celigo Application Guide.

	NOTE: The displays shown in the remainder of this document are typically for the Expression Analysis Application.

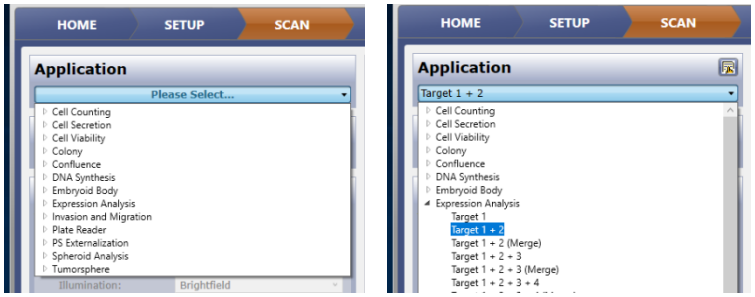
To select an application

In the Application section (Figure 36 and Figure 37 item A), in Current Application, select the application to be used for the scan.

1. Select a Suite, then application with appropriate number of channels

   In this example (Figure 36), Expression Analysis: Target 1+2 is selected.

Figure 37. Select Application Drop Down Menu



6.2 Selecting a Channel for Image Acquisition Settings

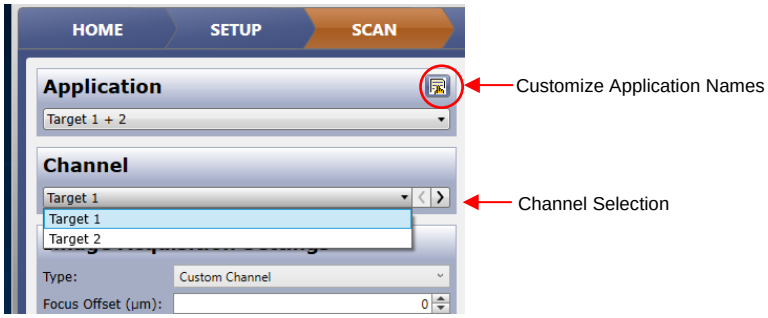
Some applications are associated with more than one channel. For example, the Cell Viability application is associated with three channels – Live, Dead, and Total. If you have selected such an application, a Current Channel menu appears in the Scan tab.

Current Channel is used to select a channel to then set its image acquisition settings.

To select a channel for image acquisition settings

In the Channel section (Figure 38), in Current Channel, select the channel for which you want to enter image acquisition settings.

Figure 38. Channel Selection



6.2.1 Changing Channel and Class Names in the SCAN Tab

You can change the default channel and class names to customized names. Features names are inherited from channel names. Your entries will appear as follows:

	NOTE: All applications allow for channel renaming. Class renaming is available only in some applications.

Channel names will appear in the following displays:

Current Channel menu in the SCAN tab

Image Display buttons in the ANALYZE, GATE, and RESULTS tabs

Feature names (inherited from Channel names) will appear in:

Graphic Overlay buttons in the ANALYZE and RESULTS tabs

Class names will appear in:

All views in the GATE tab

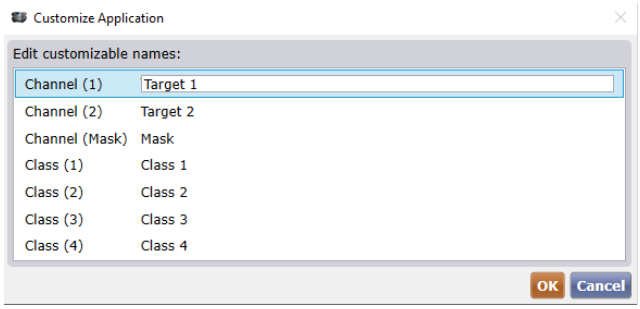
Results displayed in the ANALYZE, GATE, and RESULTS tabs

To change the channel and class names in the SCAN tab

1. In the SCAN tab, click the Customize Application button (Figure 38).

   The Customize Application dialog box appears (Figure 39).

Figure 39. Customize Application Dialog Box



2. In the column on the right, click the line item that you want to change. In the following example (Figure 40), all the default channel and class names have been changed except the last two classes.

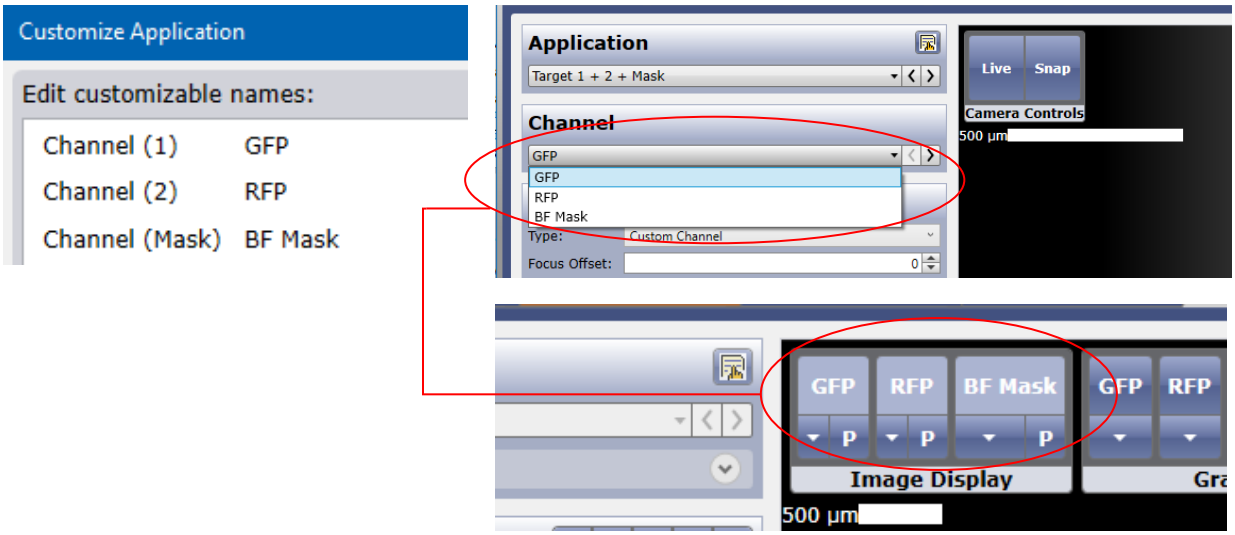
Figure 40. Changing Channel and Class Names

The channel names that you entered in the right-hand column of the Customize Application dialog box appear in the following displays (Figure 41):

SCAN tabs Current Channel menu selections

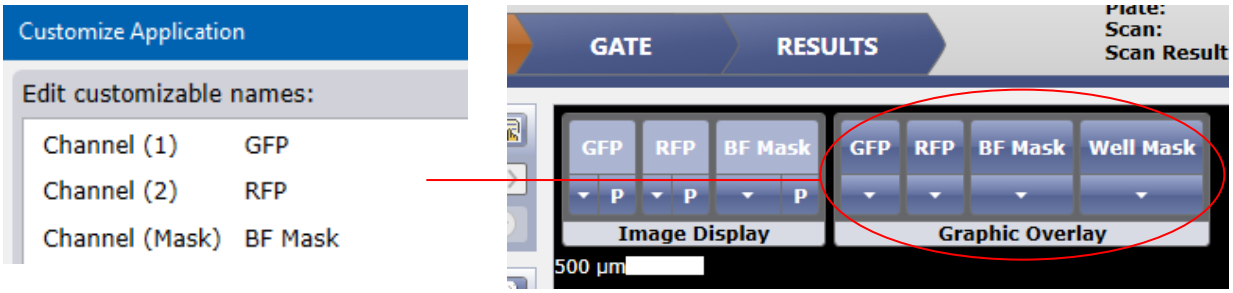
ANALYSIS tabs Image Display buttons

Figure 41. Customized Current Channel Names



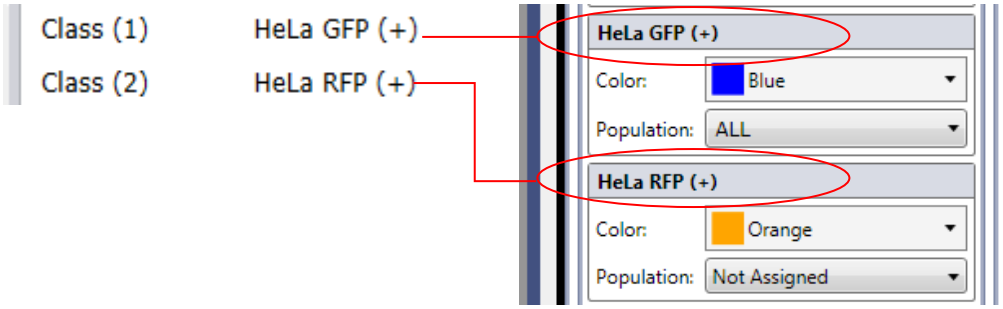
The features names are inherited from the channel names that you entered in the right-hand column of the Customize Application dialog box appear as Image Display and Graphic Overlay buttons in the ANALYZE tab (Figure 42).

Figure 42. Customized Feature Names



The class names that you entered in the right-hand column of the Customize Application dialog box appear as Classes in the GATE tabs Classes view (Figure 43).

Figure 43. Customized Class Names



6.3 Viewing Wells in the SCAN Tab

Perform the following steps to view wells. When you do this, you are navigating to a well and viewing it, as opposed to viewing a scanned image. The well displayed is the portion of the well within the field of view (FOV).

To view wells

1. In the Navigate/Select Wells section, click **Navigation** (Figure 36 item C).
2. Click the desired well with the pointer to move to that location.
3. In the Camera Controls pane, click **Live** (Figure 36 item D) or **Snap** (Figure 36 item E) to select it.

   The center of the well appears in the SCAN tab image display area (Figure 36 item F).

   Click on the FOV button to display the field-of-view (FOV, Figure 36 item G).

   To reset to the center FOV at any time, click **Navigation** and then click a well.
4. To reduce photobleaching, turn off Live camera view.

   To do this, click **Live** to de-selected it. Alternatively, capturing an image by clicking **Snap** only exposes the sample to light for the set exposure time.

	**NOTE: For scanning with fluorescence illumination, it is recommended that you keep Live on for the shortest period possible. Keeping Live on for an extended period can result in bleached samples.**

5. In the small well view (Figure 36 item G), navigate as needed:

   To display the details for a FOV in the center display, click the FOV square.

   To zoom in, use the magnification slide bar (Figure 36 item H) or click and scroll with the mouse.

6.4 Scale Bar – Overview for All Tabs

A draggable scale bar is provided for the Image Displayer across all tabs of the application. The scale bar by default is displayed in the top left area of the image displayer (Figure 36 item I). The user can drag the scale bar by clicking on it with the left mouse button and dragging the scale bar to a newly desired position.

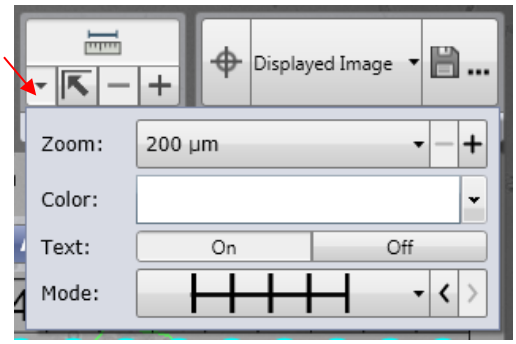
The scale bar can be customized and adjusted in various ways using the Scale Bar Options control located in upper right area of image display area (Figure 36item J). It is outlined below.

Figure 44. Scale Bar Options control

- On/Off Toggle Button - The button at the top of the scale bar options control toggles on and off the display of the scale bar.
- Additional Options - The down arrow opens up a menu for more options (discussed below).
- Move to Default Position - The diagonal arrow pointing to the top left corner will set scale bar to its default location. This can be useful if you cannot grab the scale bar easily or if it was dragged behind another control on the image displayer.
- Increase/Decrease Size - The plus and minus buttons will adjust the size of the scale bar. The buttons are disabled if the scale bar would be smaller than 50 pixels on the screen or larger than half the size of the image displayer. The valid sizes are 10 µm, 20 µm, 50 µm, 100 µm, 200 µm, 500 µm, 1 mm, 2 mm, 5 mm, and 10 mm.

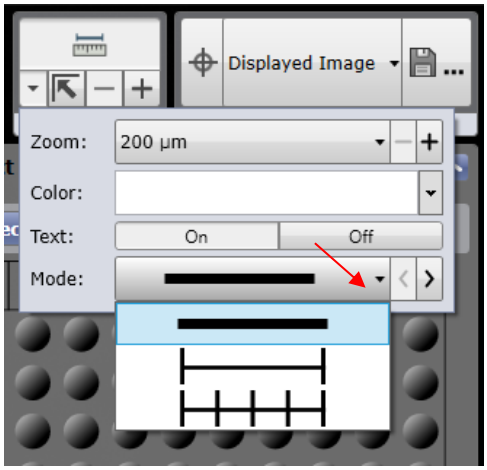
When the down arrow button is pressed (Figure 45), additional options appear to adjust the zoom value more precisely. This brings up a dialog to adjust the color of the scale bar, units label, buttons to toggle the units label on and off, and a dropdown to allow the user to adjust the look of the scale bar.

Figure 45. Additional scale bar options



The scale bar modes look like the following (Figure 46):

Figure 46. Scale bar modes



6.5 Zoom Resolution – Overview for All Tabs

The zoom resolution control is in the bottom right corner of each image. The zoom control will allow users to jump to a specific displayed zoom value in microns per pixel resolution (Figure 47).

The user can do so by typing in the micron per pixel value that is desired and the image displayer will automatically move to that zoom level.

The value will automatically update to the current scale whenever the image is zoomed in or out.

Figure 47. Zoom resolution control

6.6 Quick Zoom – Overview for All Tabs

Double-clicking on an image in any Tab displaying whole well view (whole well display mode in the ANALYZE tab, the GATE tab, and the RESULTS tab) will perform a one-step quick zoom so that the segmentation outlines are visible.

If the current zoom value is such that the feature overlays are not yet displayed, then the software will zoom to a scale at which a user can start seeing those outlines.

It will also center the image displayed on the position/coordinate that the user double-clicked of that well image.

If a user is currently zoomed in at a zoom level where the feature outlines are displayed, then the software will zoom out so that the whole well is visible and centered.

6.7 Selecting Image Acquisition Settings

The Image Acquisition Settings (Figure 36 item K) allow you to adjust the illumination, channel, exposure, gain, acquisition resolution, motion control, focus, sampling, and off-axis settings for scanning. Perform the following tasks to select these settings:

Select the Illumination Type

Select the Configuration Settings

Select the Motion Control Settings (optional)

Select the Focus Settings (optional, resulting in Focus Type default None being used, although not recommended)

Select the Sampling and Off-Axis Settings (optional)

6.7.1 Selecting the Channel Type

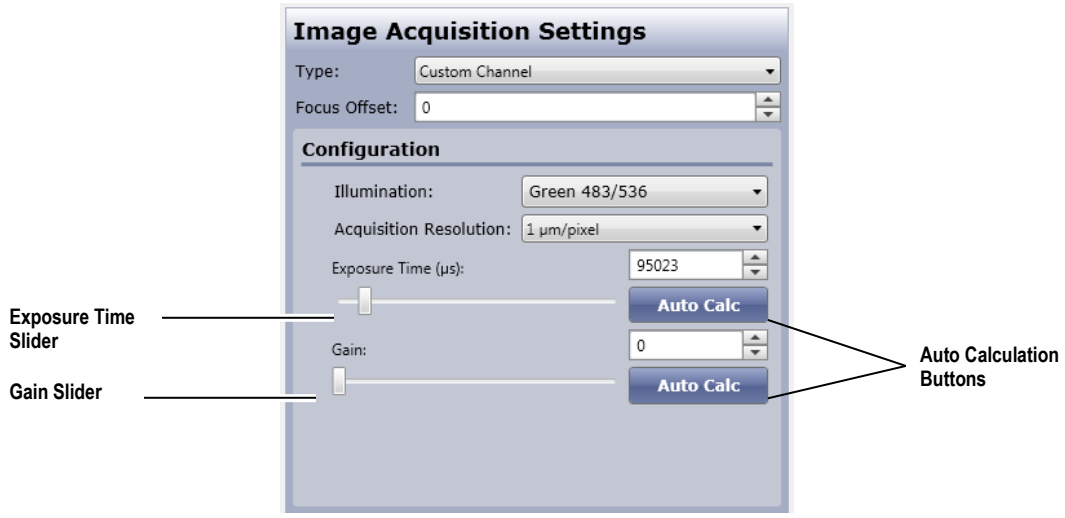
When you make a Type selection, you are selecting whether you want to manually make the image lighting adjustments or allow the software to make them.

To select the channel type

1. In Type (Figure 48) select one of the following:
   - **Custom Channel** – Allows you to specify the light source to use for that channel and to select a desired exposure and gain settings (not pre-determined settings), which will be applied to the entire plate.
   - **Auto Exposure/Gain Channel** – The software will automatically set the brightfield lighting by adjusting exposure and gain. Not recommended for Fluorescent illumination.

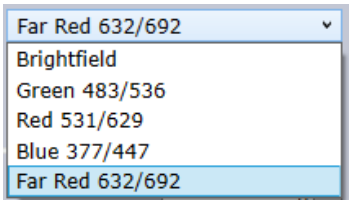
6.7.1.1 Customizing the Custom Channel Type

Figure 48. Custom Channel



1. In Illumination, select **Brightfield**, **Red**, **Green**, **Blue, or Far Red** (depending on Celigo configuration) (Figure 49)**.**

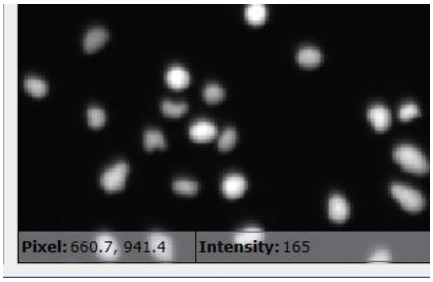
Figure 49. Menu of Illumination Choices for a 5-channel Instrument



2. If Exposure Time and Gain selections appear in the application you have selected, adjust Exposure Time and then Gain using one of the following methods (Figure 48)
   - Type an entry in the Exposure Time and Gain fields
   - Click the Up and Down arrows to the right of the fields
   - Move the Exposure Time and Gain slide bars
   - Click **Auto Calc** to the right of the fields for the system to automatically calculate the exposure time and gain for brightfield illumination to return image pixel values between 125-150.

Pixel Intensity can be observed by moving the mouse over the fluorescent object or background and reading the pixel intensity read out at the bottom of the screen (Figure 50).

Figure 50. Pixel XY Location and Intensity Readout



	NOTE: For fluorescent cell images, ideal object pixel intensity should be between 125-175, with background pixels between 01-50. **If dyes are weak, and excessive exposure is required, background pixel intensity may increase. This is not desirable and may require user to use a stronger dye with a better signal to noise ratio.**
	NOTE: Adjust Exposure Time first because a higher Gain value will increase the background noise level. If a large amount of light exposure is needed to view the well objects, you can improve (reduce) image capture time by increasing the Gain, then lower Exposure times to obtain faster image acquisition time.

3. Select **Acquisition Resolution** as needed

   Increases the pixel size while also increasing sensitivity.

   In 2 micron (µm) resolution, a 2x2 area (4 adjacent pixels) is read as one, with the resulting average intensity for brightfield and additive intensity for fluorescence.

   When values of acquisition resolution greater than 1 µm/pixel are used, acquisition times will be reduced.

   Acquisition resolution greater than 1 is not recommended for small objects such as cells, but is good for larger objects such as in the confluence, tumorsphere and colony applications.

	**NOTE: For applications using acquisition resolution: To change the selection or deselection, Live must first be turned off. If Live is on, click Live so that it is turned off, make the acquisition resolution change, and then turn Live on again.**
	**NOTE: Selecting or deselecting acquisition resolution for one channel automatically applies the setting to the other channels.**

6.7.1.2 Customizing the Auto Exposure/Gain Channel Type

If you selected Auto Exposure/Gain Channel (Figure 50in the Type field and therefore Priority appears in the application you have selected, select one of the following Priority selections to apply the exposure and gain for obtaining the desired lighting level:

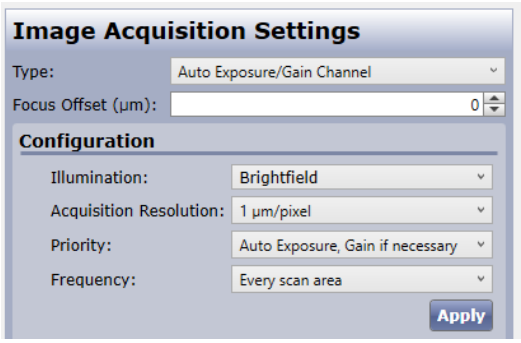
Auto Exposure Only – Gain will not be adjusted.

Auto Gain Only – Exposure will not be adjusted.

Auto Exposure, Gain if necessary – Exposure will be adjusted first, with Gain being used only if needed to increase light level. (Default)

Auto Gain, Exposure if necessary – Gain will be adjusted first, with Exposure being used only if needed to increase light level.

Figure 51. Auto Exposure/Gain Channel



If you selected Auto Exposure/Gain Channel (Figure 51) in the Type field and therefore Frequency appears in the application you have selected, select one of the following:

Every Scan Area – Applies the selected settings on an individual well basis. (Default)

Once for the Sample – Applies the selected settings to the sample (plate) as a whole, using the first selected well as the reference point for the remainder of the selected wells.

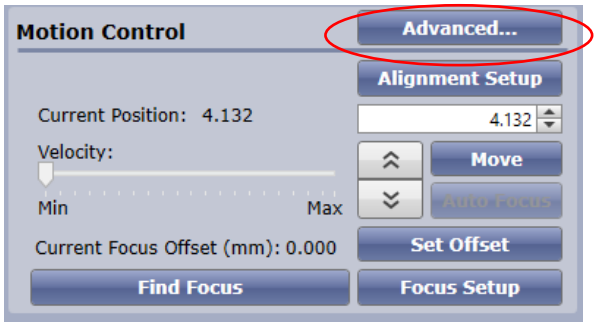
6.7.2 Selecting Motion Control Settings

Motion control settings are selections related to the settling time (scan delay) and the stage motion speed. Adjusting the settling time and stage motion speed are useful for embryoid body counting and suspension cells in larger-well-size formats, such as 6-well plates.

To select motion control settings

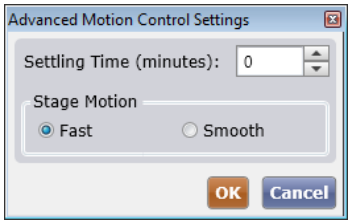
1. In the Motion Control section (Figure 52), click **Advanced**.

Figure 52. Motion Control Section



The Advanced Motion Control Settings dialog box (Figure 53) appears.

Figure 53. Advanced Motion Control Settings



Select the following as needed:

Settling Time – Select the number of minutes that you want the system to wait before it begins the scan.

Stage Motion – Select one of the following:

- **Fast** – The stage moves in a fast motion, causing faster scanning but more movement of liquid within the vessel.
- **Smooth** – The stage moves in a gradual increase speed then decreases in S-curve motion to reduce movement of liquid within the vessel. Smooth is the default in the Colony Counting: EB application and when using larger volume plates or flasks.

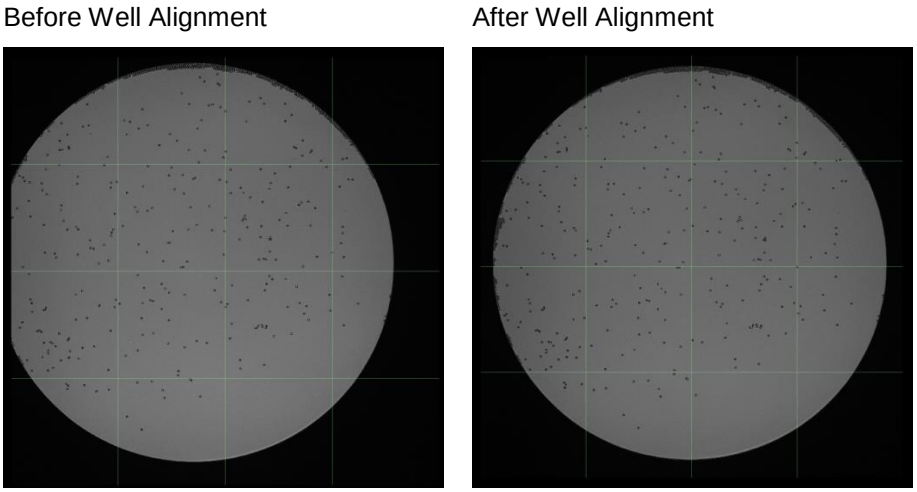
6.7.3 Plate Alignment Setup

For slightly misaligned 96- and 384-well black walled plates, where wells are not perfectly centered in image, well alignment procedure can be applied prior to the image acquisition. See Figure 54 for an example of before and after well alignment.

Alignment Setup scans the currently loaded plate and evaluates the well centers for better XY alignment of wells in the image acquisition. The system performs the alignment on one or four wells and then applies those settings for scanning the remaining wells. The resulting alignment will stay in effect until you eject the current plate.

This feature is only available for 96-well or smaller black walled flat-bottomed plates.

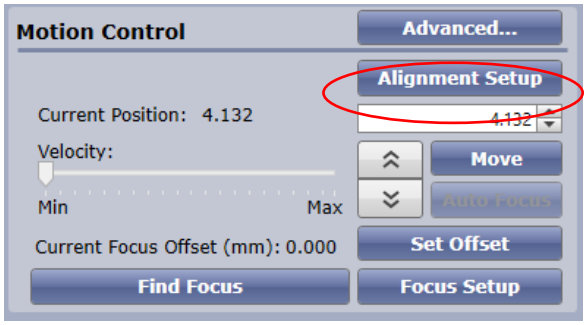
Figure 54. Well Alignment Improvement



To select alignment settings

2. In the left-hand pane, click **Alignment Setup** (Figure 55)**.**

Figure 55. Alignment Setup Button



3. Select one of the following (Figure 56):

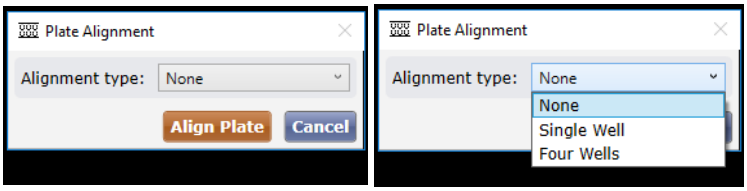
   None: (Default) no alignment performed prior to scan

   Single Well: Centers the plate so that the wells are in the center of the image, using one well for the centering measurements. The registration well used for Hardware Auto Focus or Image Based Auto Focus is the well evaluated for well alignment. If None is selected, then Well A1 is the well evaluated. This method is faster than Four Wells alignment, but is less accurate.

   Four Wells: Centers the plate so that the wells are in the center of the image, using four wells for the centering measurements. The wells that the system centers are the wells near each of the four plate corners. This method is more accurate than Single Well alignment, but takes slightly more time.

4. Click **Align Plate**.

Figure 56. Plate Alignment Selection Options



When loading an experiment in the load plate screen, load next plate dialog, automation, or project modes, alignment will occur on the focus registration well of the associated experiment.

6.7.4 Selecting Focus Settings

Select the focus settings to be used during scanning by performing either of the following procedures. The procedures are comprised of two types of focus registration. Focus registration sets the method to be used for determining the focal positions during image acquisition.

Focusing with **Manual Registration** proceed to section 6.7.4.1– The manual focus adjustment determines and sets a focal position for image acquisition, using the current well. The system then will use that focal position as the baseline to determine the different focal positions for the other wells.

Focusing with **Auto Registration** (recommended) proceed to section 6.7.4.2– The *system* will determine and set a focal position for image acquisition, using the current well. The system then will use that focal position as the baseline to determine the different focal positions for the other wells.

	**NOTE: Experiment settings saved after Auto Registration can be used to automatically define focus registration for future image acquisitions (such as under robotic control).**

For a list of focusing button, field, and menu descriptions, see section 6.7.4.3.

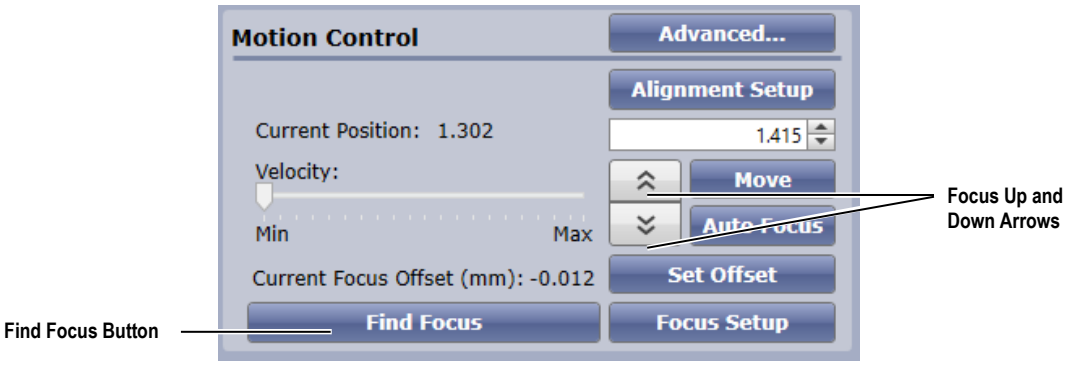
6.7.4.1 Focusing with Manual Registration

Perform the following steps to focus with manual registration.

To focus with manual registration

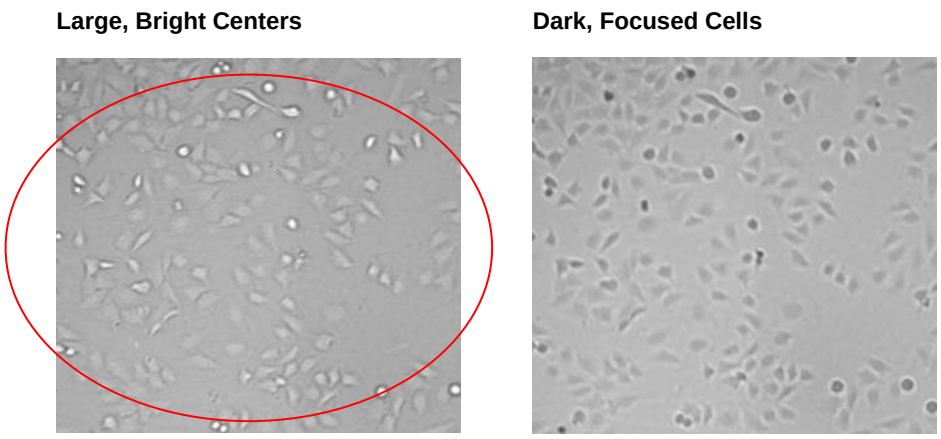
1. Click **Live** in the Camera Controls section.
2. Navigate to a well by clicking on a well in the Navigate/Select Scan Areas (Figure 36 item C) to view it in the plate view display area.
3. In the focus selections (Figure 57), located immediately below Motion Control, do the following:
   a. Adjust the focus by using either the Focus Up and Down arrows or the Find Focus button.
      – Up arrow moves the Z motor (scan lens) up, closer to the sample.
      – Down arrow moves the Z motor (scan lens) down, away from the sample.

Figure 57. Focus Selections



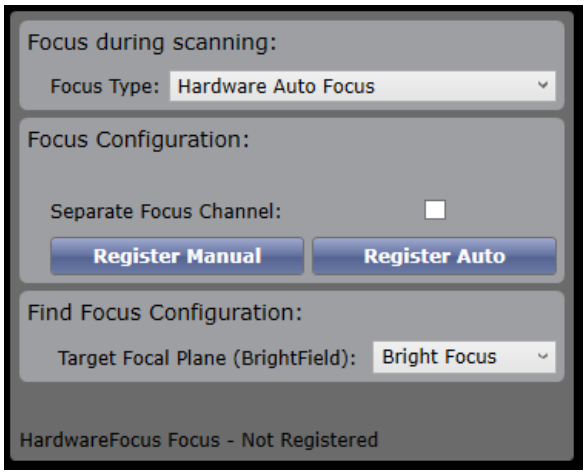
NOTE: For brightfield, a useful practice is to set a focus position that slightly defocuses the cells so that they appear bright in the center with dark edges (Figure 58). This will allow for more accurate segmentation.

Figure 58. Cell Signature with Large, Bright Center vs. Dark, Focused Cells



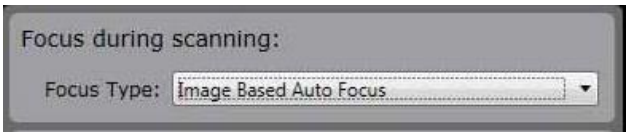
b. Click **Focus Setup**.
The Focus Setup dialog box (Figure 59) appears.

Figure 59. Focus Setup Dialog Box



4. In Focus Type (Figure 60), select one of the following:

Figure 60. Focus Type



None – No autofocus will be applied during each capture. Instead, the current scan lens z position (displayed in Current Position) will be used.

Hardware Auto Focus – A software focus algorithm will be used during initial capture in the setup well to determine the best scan lens z position (focal position). The system will then measure the plate bottom position and focus based on this positional setting for each well area. This method uses the plate bottom, not the cells, to determine focal plane. However, Hardware Auto Focus is a faster scanning method.

Image Based Auto Focus – The system will use a software focus algorithm to determine the best scan lens position (focal position) to perform image capturing. Image Based Auto Focus is a slower scanning method, than Hardware Auto Focus, but does focus on the cells for each focus attempt (instead of the plate bottom)

Plate Map – The system will use a (previously created by user) calibrated focus map for the plate to move to the calibrated z position for the plate at the specified x/y coordinate. This feature is only available if the plate type had been calibrated for focus using the plate focus map wizard. For details on how to create a focus map for your plate type, see the Administration Guide.

	**NOTE: You do not make a selection in the Target Focal Plane (Brightfield) menu when focusing with Manual Registration.**

5. Click **Register Manual**.

The system focuses using the current scan lens position, executes the focus type method if selected (hardware or image-based focus), and then displays the results in the live screen.

6. Close the Focus Setup dialog box by clicking **Focus Setup**.

6.7.4.2 Focusing with Automatic Registration

Perform the following steps to focus with automatic registration.

To focus with automatic registration

1. Click **Live** in the Camera Controls section.
2. Navigate to a well by clicking on a well in the Navigate/Select Scan Areas (Figure 36 item C) to view it in the plate view display area.
3. (Optional) In the focus selections (Figure 57), located immediately below Motion Control, visually verify that an object is visible in the well by using the Focus Up and Down arrows.

   If the plate loaded is the same as the plate selected in the SETUP tab for manufacture and catalog number, the plate should load close to where the cells can be seen and focus registration can occur within range.

4. Click **Focus Setup**.

   The Focus Setup dialog box (Figure 59) appears.

5. In Focus Type (Figure 60), select one of the following:

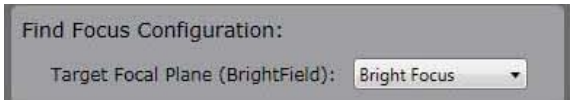
   Hardware Auto Focus – The system will measure the plate bottom position relative to the scan lens of the selected contrast position (focal position) and will execute this focus method based on this positional setting for each well. This method uses the plate bottom, not the cells, to determine focal plane. Hardware Auto Focus is a faster scanning method than Image Based Auto Focus.

   Image Based Auto Focus – The system will use a software focus algorithm to determine the selected contrast position (focal position) and will apply this algorithm during each well area capture. Image Based Auto Focus is a slower scanning method than Hardware Auto Focus; but does focus on the cells for each focus attempt (instead of the plate bottom).

   Plate Map – The system will use a calibrated focus map for the plate to move to the calibrated z position for the plate at the specified x/y coordinate. This feature is only available if the plate type had been calibrated for focus.

6. For Brightfield only: In the Find Focus Configuration section (Figure 61), select one of the Target Focal Plane options to set the criteria that the system will use during the scans for determining focus:

Figure 61. Find Focus Configuration Section



Bright Focus – (Default for Brightfield) The software algorithm calculates the scan lens position that results in the bright focus of the cells

Dark Focus – The software algorithm calculates the scan lens position that results in the darkest focus of cells

Best Contrast – Select for Fluorescence Illumination only. The software algorithm calculates a scan lens position that results in the best contrast.

	**NOTE: The Target Focal Plane menu is associated with the focus of brightfield images only. For fluorescence illumination, the system will automatically use Best Contrast, regardless of menu selection.**

7. Click **Register Auto**.

   The software algorithm profiles the image, a focus is determined using the selected focus output, focus type method (hardware or image-based focus) is executed, and the results are presented in the live screen.

8. Close the Focus Setup dialog box by clicking **Focus Setup**.

9. Adjust the current registered focus position as needed. To do this:
   a. Click the Focus Up and Down arrows to focus the displayed image in Live Camera Mode.
   b. Click **Set Offset**.

	**NOTE: A useful practice is to set a focus position that slightly defocuses the cells so that they appear bright in the center with dark edges (Figure 58). This will allow for more accurate segmentation.**

Determine the next step by continuing to section 6.8.

For further information on the focus section, continue to next section.

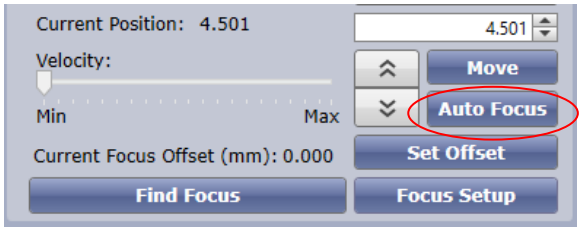
6.7.4.3 Focusing Button, Field, and Menu Descriptions

The following are descriptions for the various buttons, fields, and menus used when setting up focusing. They are listed alphabetically.

Auto Focus (Figure 62)– Activated after registering focus in Focus Setup. The system will find the best focus, based on the Target Focal Plane (Brightfield), using the Focus Type selected in the Focus Setup dialog box. The selection is executed immediately when you click this button. To test the effect of this button, defocus the displayed image using the Focus Up and Down arrows, and then click **Auto Focus** to verify that the resulting display returns to being in focus.

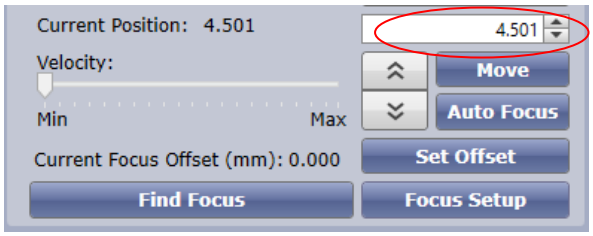
	**NOTE: The Auto Focus button is disabled if None was selected in Focus Type.**

Figure 62. Auto Focus Button



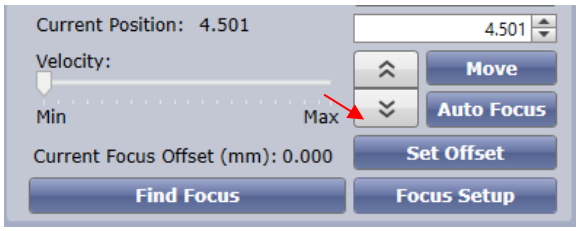
Current Position Field (Figure 63) – Used to enter an absolute value that you then commit by clicking **Move**.

Figure 63. Current Position Field



Down Arrow (Figure 64) – Decreases the scan lens position value (in Current Position field), moving the scan lens downward, away from the plate. The selection is executed immediately when you click this button, as opposed to most of the other focusing buttons, which are executed after clicking **Snap**.

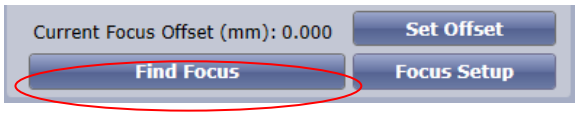
Figure 64. Down Arrow



Down Arrow moves the scan lens downward, away from the plate

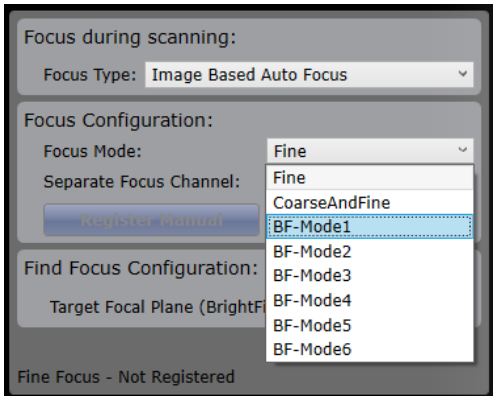
Find Focus (Figure 65) – Uses an image-based focus algorithm to determine the focal position. This does not program the focus method for scan acquisition. Cells must be at least partially visible for the system to correctly perform image-based auto focus.

Figure 65. Find Focus Button



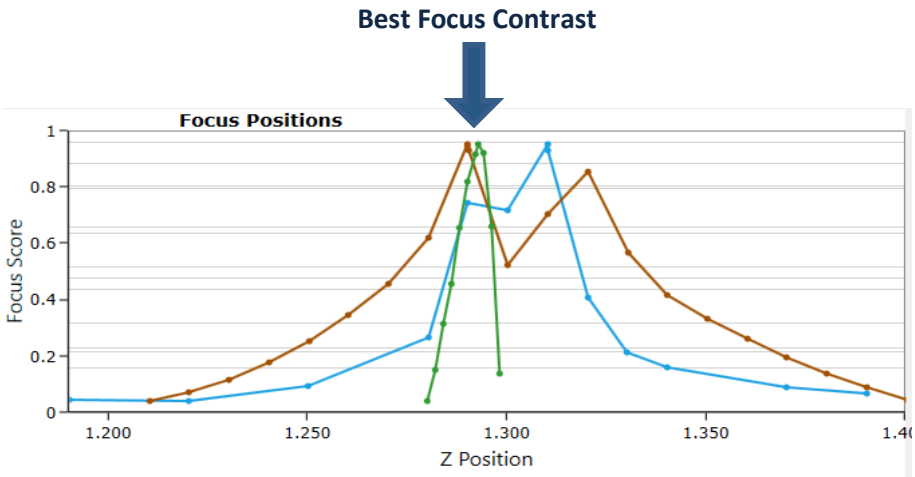
Focus Mode Field (Figure 66) – Active for Focus Type of Image Based Auto Focus only. Used to select the range in which the system will attempt to find the focus.

Figure 66. Focus Mode Field



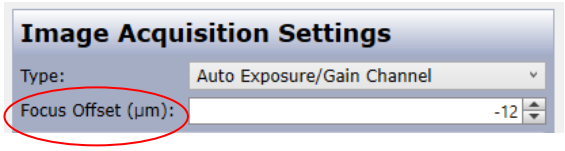
- **Fine** – The system will attempt to find the focus within a narrow range of focus. This method is faster than CoarseAndFine, but may not result in finding the cells at the bottom of the plate if well-to-well focus positions are significantly different.
- **CoarseAndFine** – The system will attempt to find the focus over a full range and then, once in the general area of focus, will perform fine focusing. This method is slower than Fine, but is more likely to result in finding the cells at the bottom of the plate if well-to-well focus positions are significantly different.
- **BF Modes**: Bright Field (BF) Modes offer a combination of different algorithms to enhance focus performance during image scan. These modes should be evaluated for what best works with the plate content.

Focus Mode	# Algorithms	Time (seconds)	Max Z Range (µm)	Total # focus positions
Fine	1	3	77 µm	9
CoarseAndFine	2	9	200 µm	22
BF-Mode1	1	5	190 µm	22
BF-Mode2	2	8	190 µm	33
BF-Mode3	1	3	18 µm	11
BF-Mode4	3	14	200 µm	46
BF-Mode5	2	11	200 µm	35
BF-Mode6	1	6	200 µm	13



Focus Offset Field (Figure 67) – The value that you want the system to use as the focus difference between the autofocus-registered position (0 position, registered by clicking **Register Manual** or **Register Auto**) and the position of the current channel being set. (For the automated method of entering the Focus Offset fields value, see **Set Offset**).

Figure 67. Offset Focus



If the focus settings were imported, the Focus Offset field is already populated.

Focus Setup Button (Figure 68)– Displays a dialog box for selecting autofocus settings that the system will use during the scan.

Figure 68. Focus Setup Button

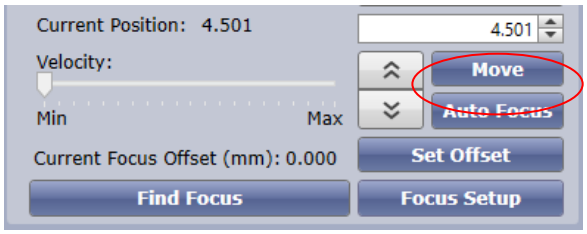
Focus Type Menu (Figure 60).

- **None** – No autofocus will be applied during each capture. Instead, the current scan lens position (displayed in Current Position) will be used.
- **Hardware Auto Focus** – A software focus algorithm will be used during initial capture in the setup well to determine the best scan lens position (focal position). The system will then measure the plate bottom position and focus based on this positional setting for each well. This method uses the plate bottom, not the cells, to determine focal Hardware Auto Focus is a faster scanning method than Image Based Auto Focus.
- **Image Based Auto Focus** – The system will use a software focus algorithm to determine the best scan lens position (focal position) to perform image capturing. Image Based Auto Focus is a slower scanning method, but does focus on the cells for each focus attempt (instead of the plate bottom).
- **Plate Map** – The system will use a calibrated focus map for the plate to move to the calibrated z position for the plate at the specified x/y

coordinate. This feature is only available if the plate type had been calibrated for focus.

Move Button (Figure 69) – Commits the value that you enter in the Current Position field, moving the scan lens to the position.

Figure 69. Move Button

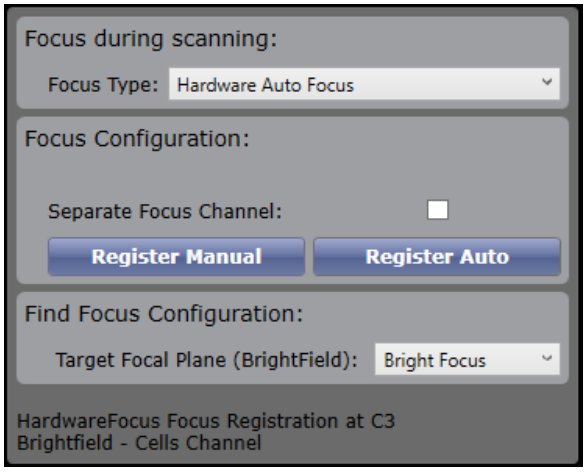


Register Auto Button (Figure 70) – The system records both of the following sets of selected information:

- Target Focal Plane, Brightfield, selection options are available as part of selecting Image Based Auto Focus.
- Current Channel, Current Position (called the Autofocus Position), and Focus Type. The system defines this information, as opposed to the Register Manual button case, in which the user defines this information.

  The registered Current Position will serve as the baseline (position 0) that the system will use when assigning offset positions for the channels. The registered information appears at the bottom of the Focus Setup window.

Figure 70. Register Auto and Register Manual Buttons in Focus Setup Window

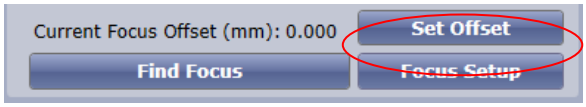


Register Manual Button (Figure 70) – The system records the Current Channel, Current Position (called the Autofocus Position), and Focus Type. The user defines this information, as opposed to the Register Auto button case, in which the system defines this information.

The registered Current Position will serve as the baseline (position 0) that the system will use when assigning offset positions for the channels. The registered information appears at the bottom of the Focus Setup window.

Set Offset Button (Figure 71) – Sets a focal position that is offset from the previous autofocus. The system or user may determine the offset value. The selected offset value appears in the Focus Offset field.

Figure 71. Set Offset Button



Target Focal Plane (**Brightfield**, Figure 72)– Sets the criteria that you want the system to use for determining the focal position to be used for scanning each well. These selections are used only if you are using Register Auto, which uses Image Based Auto Focus.

- **Bright Focus** – Select for Brightfield illumination only. The system finds the scan lens focus position that results in the highest amount of bright cells (finds the bright focus peak).
- **Dark Focus** – Select for Brightfield illumination only. The system finds the scan lens focus position that results in the highest amount of dark cells (finds the dark focus peak)
- **Best Contrast** – Select for Fluorescence Illumination only. The system focuses by finding a scan lens focus position that results in the best contrast.

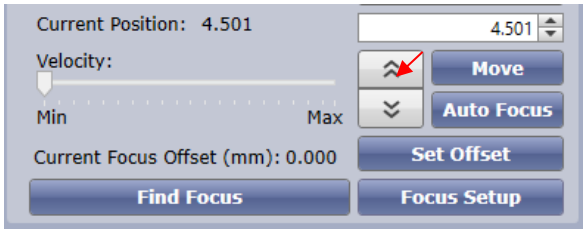
  Best Contrast is not recommended for Brightfield illumination.

	NOTE: A Target Focal Plane selection matters only for Brightfield illumination. For Fluorescence illumination, the system will automatically use Best Contrast, regardless of your selection.

Figure 72. Target Focal Plane (Brightfield)

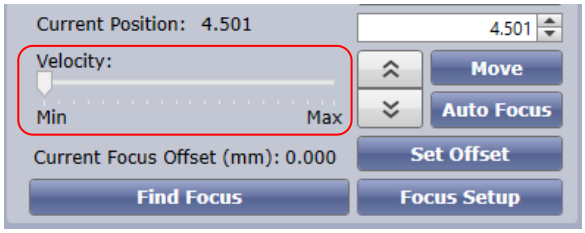
Up Arrow (Figure 73) – Increases the scan lens position value (in Current Position field), moving the scan lens upward, closer to the plate. The selection is executed immediately when you click this button, as opposed to most of the other focusing buttons, which are executed after clicking **Snap**.

Figure 73. Up Arrow



Velocity Percentage Slide bar (Figure 74) – Selects the degree to which each click of the Up and Down arrows moves the scan lens (Current Position value).

Figure 74. Velocity Percentage Slide bar



Velocity Percentage Slider changes the speed of focus up and down moves with manual arrows

6.7.4.4 Focusing with Separate Focus Channel

When using an application, you may determine that you get better focusing using an illumination that is not associated to one of the channels in your application. If you reach this situation, you can use Separate Focus Channel. Enabling this feature will create a new channel that is only used during the focus operation of acquisition. Any images captured or evaluated with this channel are not saved.

To use Separate Focus Channel:

1. Check the Separate Focus Channel checkbox in Focus Setup (Figure 75).

The resulting action will create a Focus channel that is added to your channels list and the application will automatically switch to this new channel. The Focus channel will inherit all settings of the current channel at the time the Separate Focus Channel checkbox was checked (Figure 76).

Figure 75. Separate Focus Channel Selection

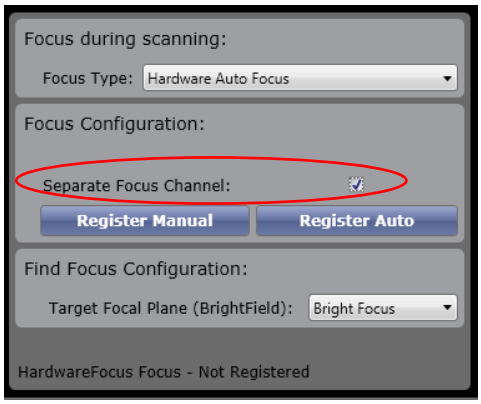
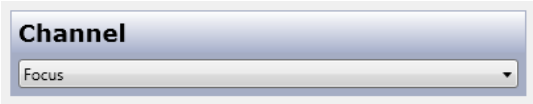


Figure 76. Separate Focus Channel



2. Change the illumination of the Focus channel to the illumination you get the better focus on.
3. Register the focus using the desired well and focus type.

	NOTE: When registering, if Separate Focus Channel is selected, the separate focus channel is always switched to automatically perform the registration. If it is desired to register on a different channel, the separate focus channel setting would need to be turned off.

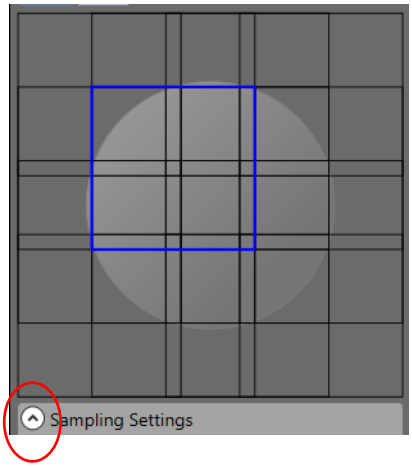
4. Then, for setting up your original application channels, you change to those channels and setup your focus offsets as described in section 6.9.

6.7.5 Selecting Sampling and Off-Axis Settings

You have the option to select sampling and off-axis settings to speed up scan and analysis time. These image acquisition settings are in a separate location from the Image Acquisition Settings pane, in a section called Sampling/Off-Axis Settings (Figure 77). These settings are saved in the image acquisition settings you enter in the Image Acquisition Settings pane.

	**NOTE: Some plate formats do not support sampling or off-axis scanning. For off-axis scanning, for example, the plate must be a 384-well or 1536-well plate because the well must fit in a single FOV.**

Figure 77. Sampling/Off-Axis Settings



Sampling Slider

Sampling/Off-Axis Settings provides the following functions:

- Sampling Settings – Captures a smaller well smaller than the whole well.
- Off-Axis Settings – Captures multiple wells during a single stage position.

These concepts and the associated procedures are described further below.

6.7.5.1 Selecting Sampling

Figure 78 and Figure 79 show examples of sampling and off-axis selections. The 96-well plate example shows sampling patterns from one to four FOV images. The light gray squares mark the well area that will be scanned. The blue square is the current position of the well being viewed.

Figure 78. 96-Well Plate Sampling Selections

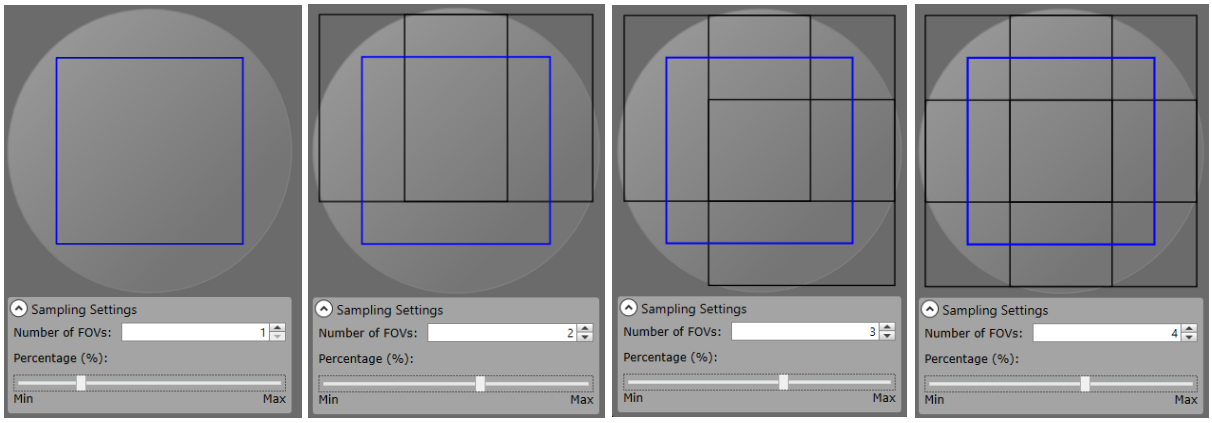


Figure 79. 384-Square-Well Plate Sampling and Off-Axis Selections

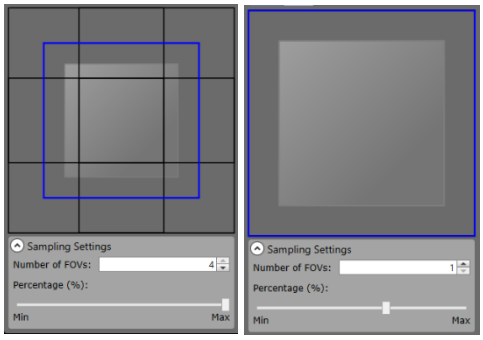
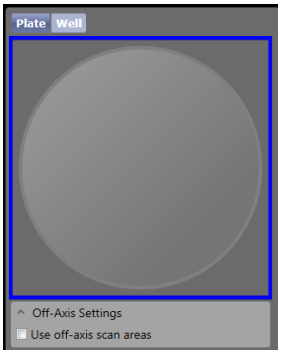


Figure 80. 384-Round-Well Plate Sampling and Off-Axis Selections



To select Sampling

	**NOTE: If a Sampling/Off-Axis Settings line item does not appear in the small well view, the selected plate format does not support sampling or off-axis.**

1. In the small well view at the bottom right (Figure 36 item E above), select the arrow to the left of Sampling/Off-Axis Settings.
2. Do one of the following:

   Slide bar method (recommended) – With the mouse, drag the sampling slide bar (Figure 77 above) until the Number of FOVs field displays the number of images you want to be scanned in each well.

   When you use this entry method, the Number of FOVs field automatically displays only numbers that will result in square images (1 image, 2 images x 2 images, 3 images x 3 images, etc.), preventing blank image areas.

   Numerical entry method – In Number of FOVs, type a number to display the number of images you want to be scanned, and then press **Enter**.

   When you use this entry method, non-square images are possible, resulting in blank image areas.

   If you type a number higher than the amount possible for scanning the selected plate format, the system automatically displays the full-well FOV number (turns off sampling).
3. To turn off sampling after it is turned on, move the sampling slide bar back to the farthest **Max** position.
4. Save the settings if desired (section 6.10.1) Saving an Experiment in the Scan Tab and then begin the image capture by continuing to Selecting Wells for Scanning (section 6.14)

6.7.5.2 Selecting Off-Axis

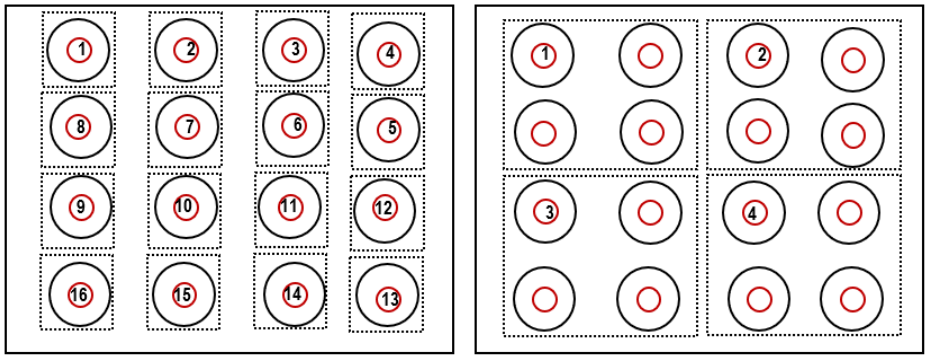
On-axis scanning uses one focal point per well. Off-axis scanning uses one focal point per group of wells (4 wells for 384-well plates, 16 wells for 1536-well plates).

Figure 81. On-axis versus Off-Axis Scanning – 384-well provides a simplified illustration of on-axis versus off-axis scanning, using a 384-well plate as an example. It shows that, using on-axis scanning of a 384-well plate, each well would be captured individually; whereas off-axis scanning, four wells would be captured for each stage movement.

For off-axis scanning, at scan start time, the system will move to one well to perform autofocus and then move between a group of wells to capture the image, repeating this process for the remainder of the plate.

Figure 81. On-axis versus Off-Axis Scanning – 384-well illustrates on-axis versus off-axis scanning. The red numbered center circle approximately indicates the scanned focal point and the order in which the image is acquired. The dotted outlines indicate the wells acquired during a single image acquisition. In this example, during on-axis scanning, the instrument captures one well at a time, whereas during off-axis scanning, the instrument captures four wells at a time.

Figure 81. On-axis versus Off-Axis Scanning – 384-well



NOTE: Selecting Off-Axis will result in a slightly lower scan quality than when the setting is not selected, due to plate bottom inconsistencies from well to well. This is the tradeoff for the faster scanning speed that this setting allows.

To select Off-Axis

1. Select the **Off-Axis** checkbox.
2. To turn off Off-Axis after it is turned on, deselect the **Off-Axis** checkbox.

Determine the next step by continuing to section 6.8.

6.8 Determining the Next Step

Do one of the following:

For a single-channel scan, you are done selecting the image acquisition settings. Save the settings if desired (section 6.10) Saving an Experiment in the Scan Tab) and then begin the image capture by continuing to Selecting Wells for Scanning (section 6.14).

For two- or three-channel scans, select image acquisition settings for the remaining channels by continuing to section 6.9.

6.9 Selecting Image Acquisition Settings for any Remaining Channels

Perform the following steps to select image acquisition settings for any channels that you have not yet set up.

To select image acquisition settings for any remaining channels

1. In Channel, select the next channel to be used.
2. In Type, make selections per section 6.7.1.
3. In the Configuration section, make selections per section 6.7.1.1
4. Do one of the following:

For manual control, click the Up and Down arrows.

For automatic control, make sure that cells are at least partially visible, and click **Find Focus**.

Focus Offset populates with the Z position that the system automatically determined, based on the focus position you registered in section 6.7.4.

5. Click **Set Offset**.
6. If you want to confirm the focal plane that will be used for the scan, defocus the current view and select **Auto Focus**.
7. If the focus position is not acceptable to you, repeat steps 4 through 6.
8. To select image acquisition settings for subsequent channels, repeat steps 1 through 7.
9. Save the settings if desired (section 6.10.1 Saving An Experiment in the Scan Tab) and then begin the image capture by continuing to Selecting Wells for Scanning section 6.14.

6.10 Saving Settings – Overview for all Tabs

You can save various types of settings in the Celigo tabs to either the database or your hard drive (export). You save the set of selections for potential use when later scanning a plate that is the same plate type. The types of save buttons are

- Save Experiment
- Analysis Settings
- Classification Settings
- Project

The types of information recorded by the various Save buttons, where it gets recorded (into the database or onto the hard drive), and their locations are as follows:

Save Experiment buttons (Figure 82 and Figure 83) – These buttons, located in the SCAN, ANALYZE, GATE, and RESULTS tabs, save the experiment to the database. For details on how to save an experiment, see section 6.10.1.

An experiment refers to the compilation of settings that currently exist throughout the SCAN (including Channel name), ANALYZE, and GATE tabs. Using an experiment save button takes a sort of snapshot of the settings in all three tabs simultaneously, even though only a single tab is currently displayed.

When an experiment is saved in the ANALYZE tab, for example, this records the settings from all three tabs simultaneously. You may wait to see the RESULTS tab, for instance, to then record the settings. You can choose to overwrite the previous saved experiment file or add a new file.

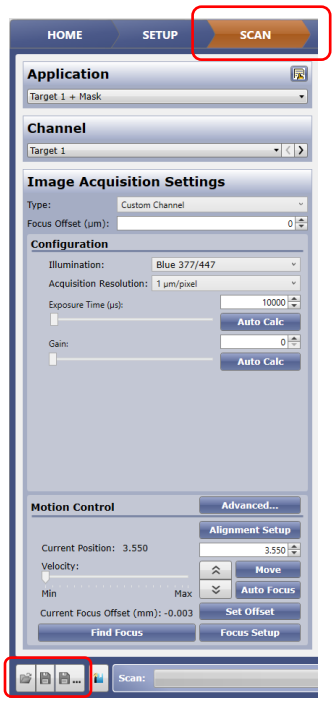
Loading an experiment can only be performed in the SCAN, ANALYZE and CLASSIFY tab. If saved analysis or classification settings were loaded on the ANALYZE or CLASSIFY tab, then a saved experiment was loaded in either tab, the experiment settings overwrite the previously loaded analysis or classification settings.

Once a setting has been saved, you can modify and save settings with the Save button. After loading settings, you can modify them and then do one of the following:

– Click Save to overwrite the previously saved settings file, or
– Click Save As to create a new saved setting file with a new name.

Figure 82. Experiment Save Buttons in SCAN and ANALYZE Tab

SCAN Tab



ANALYZE Tab

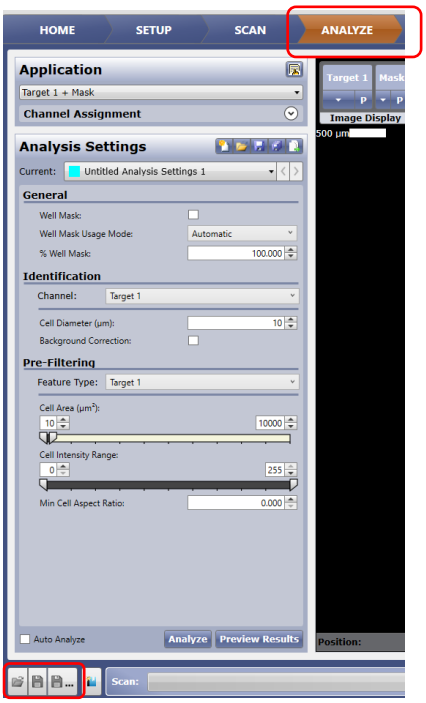
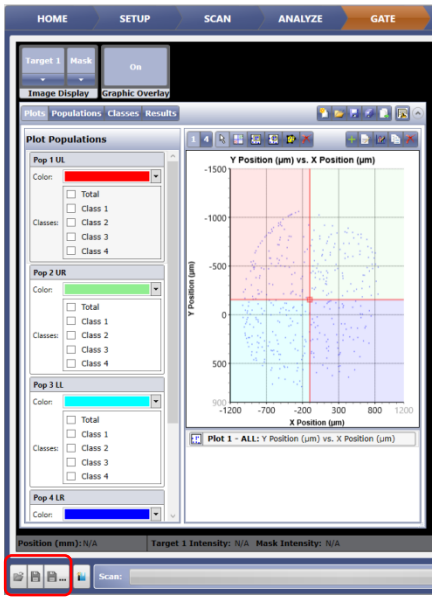
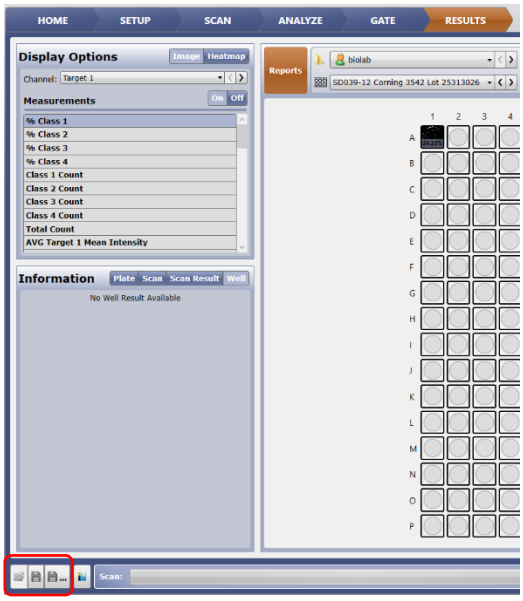


Figure 83. Experiment Save-to-the-Database Buttons in GATE and RESULTS Tab

GATE Tab



RESULTS Tab



If you have not yet entered experiment settings at the time that you use the experiment save-to-the-database buttons, the system automatically enters default settings. The default experiment settings differ among applications.

Analysis Settings Save buttons (Figure 84 and **Error! Reference source not found.**) – These buttons, located in the ANALYZE tab, save the analysis settings to either the database or the hard drive (performs an export), allowing you to reuse the analysis settings later, independent of the experiment (group of settings). Once a setting has been saved, you can modify and save settings with the Save button. After loading settings, you can modify them and then do one of the following:

- Click Save to overwrite the previously saved settings file, or
- Click Save As to create a new saved setting file with a new name.

Figure 84. Analysis Settings

Classification Settings Save buttons (Figure 85 and **Error! Reference source not found.**) – These buttons, located in the GATE tab, save the classification settings to the database or hard drive (performs an export), allowing you to reuse the classification settings later, independent of the experiment (group of settings). Once a setting has been saved, you can modify and save settings with the Save button. After loading settings, you can modify them and then do one of the following:

- Click Save to overwrite the previously saved settings file, or
- Click Save As to create a new saved setting file with a new name.

Figure 85. Classification Settings

Create Project button (Figure 86) – This button, located in the SCAN, ANALYZE, GATE, and RESULTS tabs, creates a project and saves it to the database. Create Projects button opens a dialog box for the user to bundle specific plate profile, saved experiment and export details into one selectable project file.

For details on how to create a Project, see section 6.10.2.

For details on how to run a Project, see section 5.2.

Figure 86. Create Project button

Projects are created in any tab, but only editable in the Data Manage mode.

Projects reference the plate profile and application (via saved Experiment).

For instructions on modifying projects or importing projects, experiments, analysis or classification settings, see the *Celigo Cytometer Administrator Guide*.

6.10.1 Saving an Experiment

When you save an experiment to the database in any tab, you are recording a snapshot of all the settings that currently exist in the SCAN, ANALYZE, and GATE tabs.

When you save an experiment, you are saving it to the database, not to the hard drive (export).

For an overview of the save button locations and functions in the various tabs, see section 6.10.

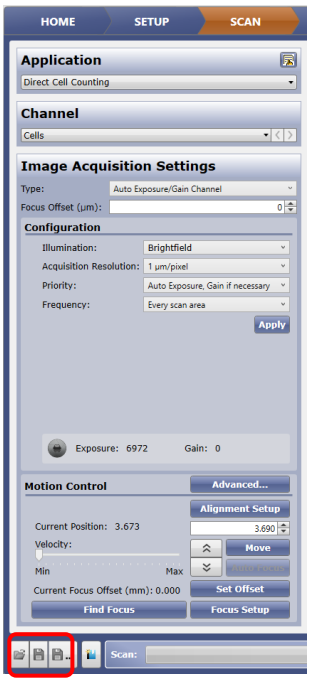
6.10.1.1 Saving an Experiment to the Database

To save an experiment to the database

	**NOTE: Before saving an experiment, at least one well must be selected in the plate view display. If not, all the wells will be auto selected in the experiment that will be saved.**

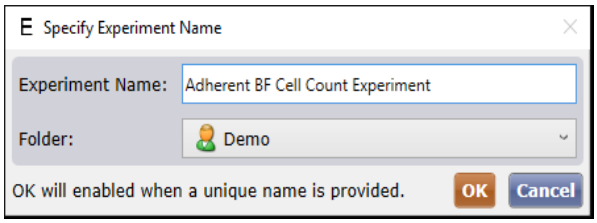
1. At the bottom of the tab, in the Experiment section, click the **Save** or **Save As** button (Figure 87).

Figure 87. Experiment Save-to-the-Database Button in the SCAN Tab



The Specify Experiment Name dialog box (Figure 88) appears, with the Folder field displaying the default entry (logged-in user folder).

Figure 88. Specify Experiment Name Dialog Box



2. In the Experiment Name field, enter a name for the experiment (group of settings).

   You can use these settings for later experiments if the plate type is the same.

3. In the Folder field, select a different destination folder for the settings file as needed.

6.10.2 Creating a Project

When a user creates a project in any tab, it is recording a snapshot of all the settings that currently exist in the SCAN, ANALYZE, and GATE tabs.

Projects will save Plate Profile, Experiments Settings, Export options and locations for easy recall and use from the HOME tab. User will just have to type in a Plate ID for the current scan when using projects.

6.10.2.1 Steps to Creating a Project

Projects can be created in a current session in the SCAN, ANALYZE, GATE and RESULTS tabs. For details on running a Project, see section 5.2.

1. Clicking on the **Create Project** icon (Figure 89)

Figure 89. Create Project button

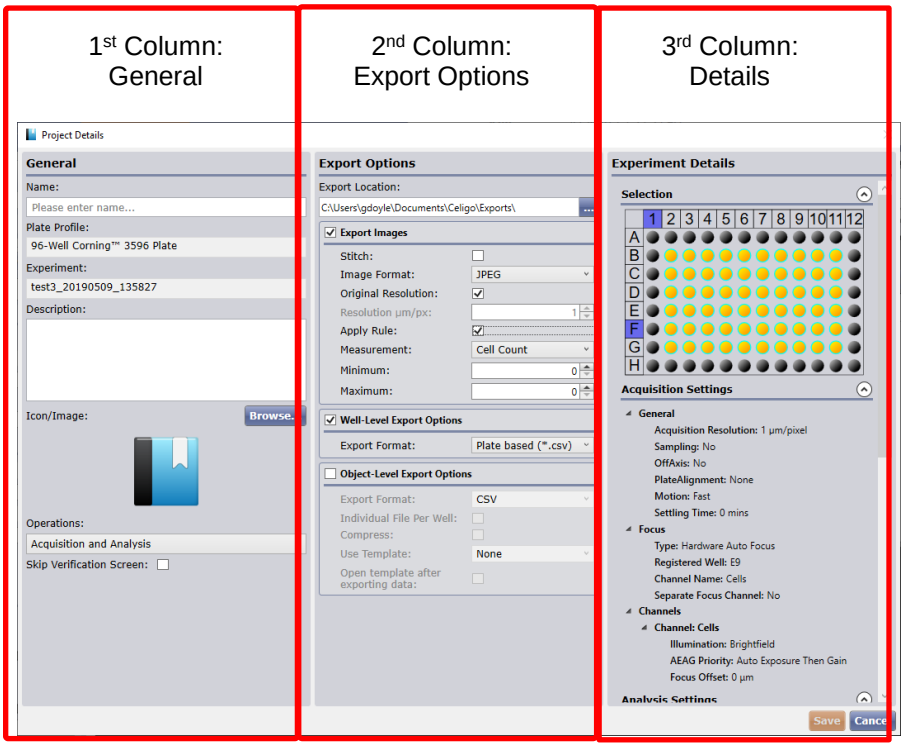
   The Project Details dialog opens a window (Figure 90).

2. Enter a **Name** in the first dialog box in first column.
3. Check desired **Export Options** and the **Export Location** in middle column.

   Apply a rule (i.e., establishing cell count min and max as shown in Figure 231) to only export images that fit within specific parameters.

4. Review **Experiment Details** in the third column.
5. Click **Save**.

Figure 90. Project Details Dialog



To Add an Image Icon to the Project Details

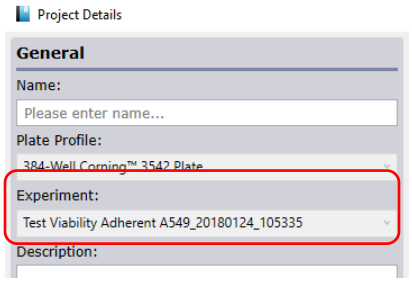
1. Click the Browse button in the General section
2. Browse to a previously stored image

Note: It is recommended to use a screen capture snipping tool to capture a screen-shot of cells.

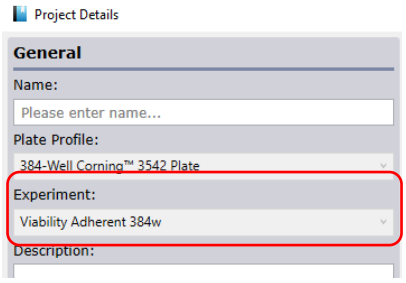
If the Experiment in the workspace exists in the database and is not modified/edited then the Create Project button will use that Experiment. However, for all other cases (unsaved experiment) the Create Project button will create a new Experiment with an auto-generated name (Figure 91).

Figure 91. Create Project with or without Saved Experiment

No Experiment Saved



Saved Experiment



6.11 Loading an Experiment – Overview for All Tabs

Users can load previously saved experiments from the database on all tabs except for the RESULTS tab. To load an experiment from the SETUP tab when creating a new scan, see section 5.3. The user can load an experiment using the button in the bottom left hand corner of any tab.

6.11.1 Loading an Experiment

When you load an experiment from the database in any tab, you are loading a sort of snapshot of all the settings that will be loaded into the SCAN, ANALYZE, and GATE tabs.

6.11.1.1 Loading an Experiment from the Database

To load an experiment from the database

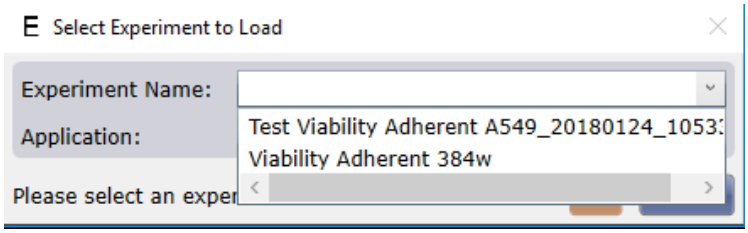
	**NOTE: The list of available experiment settings in the SCAN Tab will be filtered based on the currently loaded plates number of rows and columns (they need to be the same as the experiment being loaded).**
	**NOTE: The list of available experiment settings in the ANALYZE and GATE Tabs will be filtered based on the same constraint as the SCAN Tab and to the constraint that the experiment has the same number of channels or less as the current scan.**

1. At the bottom of the tab, in the Experiment section, click the **Load** button (Figure 92).

Figure 92. Experiment Load-from-the-Database Button in the Scan Tab

A Select Experiment to Load dialog box (Figure 93) appears.

Figure 93. Select Experiment to Load Dialog Box



2. In the Experiment Name field, enter a name for the experiment (group of settings). The Application field will auto populate with the name of the application of the selected experiment.

6.12 Centering an Onscreen Image in the Scan Tab

You can center an onscreen image in the SCAN tab. Doing this does not change the raw images.

To center an onscreen image in the SCAN tab

In the Image Controls section (Figure 36 item K above and Figure 94), click the **Center Image** icon.

Figure 94. Image Controls in Scan Tab

6.13 Exporting an Onscreen Image in the Scan Tab

In the Scan tab, you can export a selected well image as seen onscreen.

To export an onscreen image in the Scan tab

In the Image Controls section (Figure 36 item K and Figure 94 above), click the **Save Image** icon.

The remaining steps for exporting the image are the same as exporting the same type of image in the RESULTS tab. For detailed instructions, see Exporting an Onscreen Image in the RESULTS Tab in section 9.7.3.

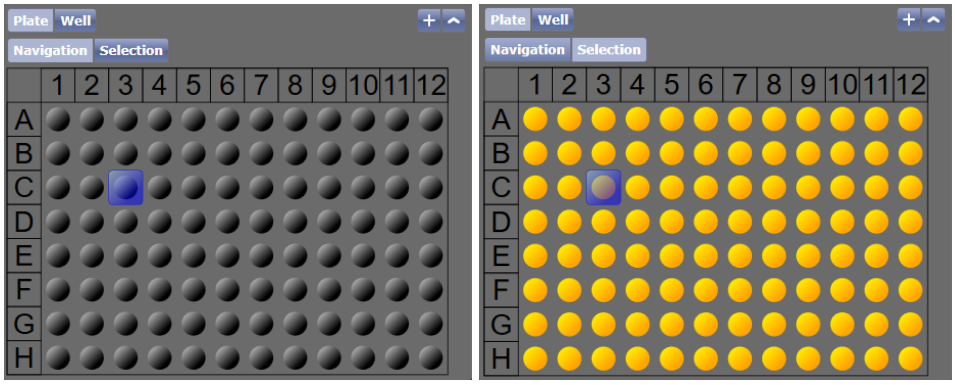
6.14 Selecting Wells for Scanning

When you select wells for scanning, you are identifying which wells you want the system to scan.

To select wells

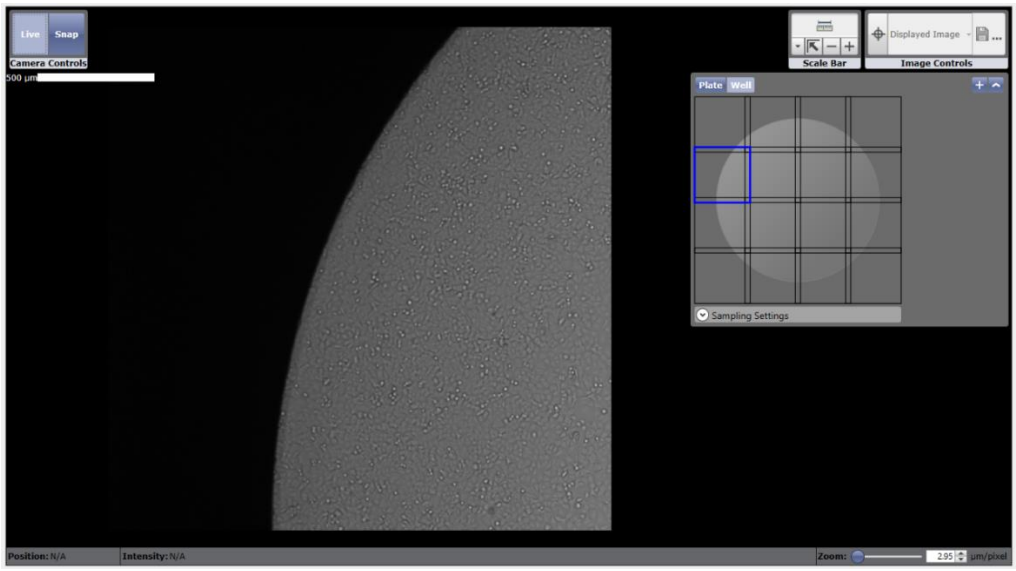
1. In the Navigate/Select Scan Areas section, click **Selection** (Figure 95).
2. Do one of the following:
   - To select individual wells, click each desired well or click and drag over each well. (To deselect, click the well again).
   - To select the entire plate, click the upper left corner of the plate view display. (To deselect, click the upper left corner again).
   - To select individual rows or columns, click the corresponding number or letter in the plate view display.
   - The displayed well colors indicate well scanning status as follows:
     - Black – Not selected for scanning
     - Yellow – Selected for scanning

Figure 95. Navigate/Select Scan Areas



To sample a section of the well, click on Well button (Figure 36 G) and select a segment to be scanned (Figure 96).

Figure 96. Select a Well Segment



6.15 Starting the Scan

Now that you have selected an application, acquisition settings, and wells, (sections 6.1 through 6.15), perform the following steps to start the scan.

To start the scan

1. Click **Start Scan** in lower right corner to begin scanning/imaging the plate. The ANALYZE tab immediately opens to allow you to adjust the image analysis settings. See chapter 7 for image analysis instructions.

   If the message box Your current focus has not been registered appears, this is because your entries in the SCAN tab included an Experiment menu selection and you clicked **No** in the resulting Would you like to perform auto focus registration message box. You did this because you wanted to manually select the well to be scanned instead of using the Focus Setup selections in the Experiment file.

To skip analysis, and view thumbnail images and full resolution images that display as each well is scanned, click the RESULTS tab when in the ANALYZE Tab. You can view scan results (images) at any time once they have been imaged/saved to database. For more instructions on viewing scan results, see Viewing Scan Details section 9.1.

If you want to analyze scans at another time, load the scanned images from the HOME tab by clicking View and Analyze Scans.

The plate can be removed after scanning is complete. For instructions on removing the plate from the instrument, see chapter 10.

	**NOTE: After removing the plate, you cannot adjust the image acquisition settings.**

6.15.1 Stopping and Restarting a Scan

If you need to stop a scan that is in process, a progress bar displays current progress at bottom of screen with a red box (Figure 97. Scan Progress Bar and Stop button).

To stop a scan

Click the red stop button at the bottom middle of the tab.

Figure 97. Scan Progress Bar and Stop button

After stopping a scan, you can only start a new scan, not resume the existing scan.

To restart a scan

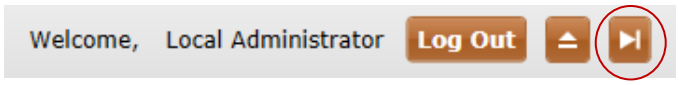
Click **Start Scan** in the Scan tab. The new scan will be created with a new timestamp.

Continue to Analyze Tab chapter 7.

6.16 Acquiring the Next Plate

After acquisition of a scan completes (and analysis if analysis of the scan was performed), an acquire next plate option will appear at the top of the screen in the RESULTS tab, allowing you to acquire another plate of the same plate profile definition with the same experiment settings. This option allows you to skip steps for loading additional plates if the user has several plates to acquire with the same settings.

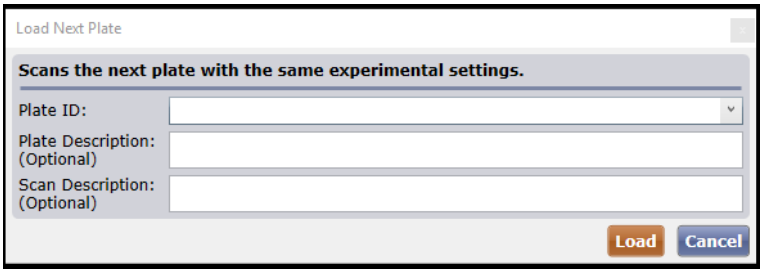
Figure 98. Acquire Next Plate



To acquire the next plate

1. Click **Acquire Next Plate** button (Figure 98). The Acquire Next Plate dialog will appear.

Figure 99. Load Next Plate Dialog



2. In the Load Next Plate dialog (Figure 99), make the following entries:

   Enter Plate Details section:

   – Plate ID – Enter plate ID

	To edit the Plate ID after performing a scan, use the Manage Data function per the Administrator Guide section Changing Plate Information.

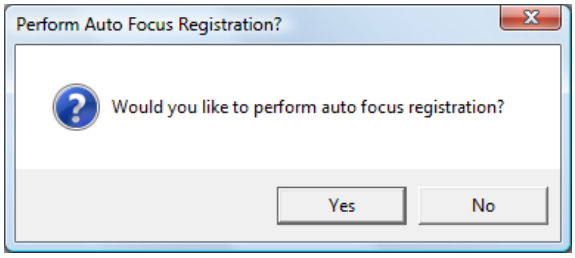
   – Plate Description (Optional) – Describe the plate
   – Scan Description (Optional) – Describe the scan

3. In the Load Next Plate dialog, click **Load**.

	If using a network database setup, if the plate is already in use by another user, a prompt will be displayed notifying the user that the plate is already in use.

4. If the experiment the previous plate used defined a Focus Type in Focus Setup, a Would you like to perform auto focus registration? message box appears (Figure 100) and continue to step 7, otherwise continue to step 8.

Figure 100. Perform Auto Focus Registration Message Box



5. Click **Yes** or **No** as follows:

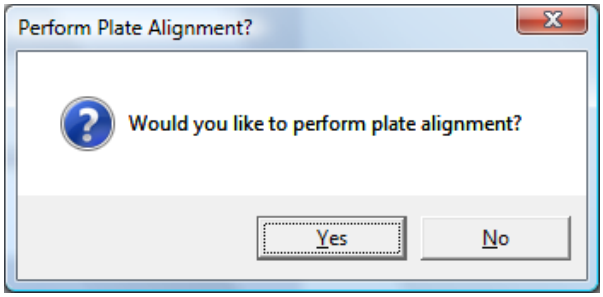
   Yes – All of the image acquisition settings, including any offsets and focus registration settings that had been made in the Focus Setup dialog box will be applied to the upcoming scan.

   No – All of the image acquisition settings *except* the focus registration settings made in the Focus Setup dialog box will be applied to the upcoming scan. Selecting No will allow you to manually select Focus Setup settings to be applied instead of those in the Focus Setup dialog box. For details on setting up focus refer to section 6.7.4.

	NOTE: If you do not change the Focus Setup settings, you can apply the saved settings later by clicking Start Scan in the Scan tab and clicking Yes in the resulting Your current focus has not been registered message box. This will create new settings that are close to or that match the saved settings, depending on the well registered.

6. If the experiment the previous plate used a defined Plate Alignment Type in Plate Alignment Setup, a Plate Alignment message box appears (Figure 101) and continue to step 9. Otherwise continue to step 10.

Figure 101. Perform Plate Alignment Message Box



7. Click **Yes** or **No** as follows:
   - Yes – A plate alignment will be performed, using the settings in the experiment file.
   - No – A plate alignment will not be performed.

   If you clicked Yes, the system now performs a plate alignment.
8. A Load Plate message box (Figure 102) appears.

Figure 102. Load Plate Message Box

9. Carefully place a plate or flask onto the stage, using the following practices:

   For a plate:
   - Manually push the plate into the inner left corner of the plate carrier.
   - Ensure that the plate is seated flat in the stage, with well position A-1 in the upper-left hand corner of the plate as you face the front of the instrument.

   For a T25 flask, petri dish, or slide:
   - Insert the appropriate holder onto the stage. Load the sample into the holder by pulling back the spring mechanism. Ensure that the sample is seated flat. If the sample is a flask, ensure that the flask is inserted with

the cap toward the left side in relation to an operator in the front of the instrument.

For a T-75 flask:

- Load directly onto the stage with the cap toward the left side.

10. Click **OK** in the Load Plate message box.

    The access door closes. If you had entered an Experiment menu selection, the settings in the selection will be used.

    The SCAN tab appears.

	**CAUTION: Do not reach into the Instrument while the stage is in motion!** **Keep clothing, jewelry, hair, and other loose materials clear of mechanical components.** **The Celigo cytometer contains moving mechanical components that are capable of causing bodily harm.**

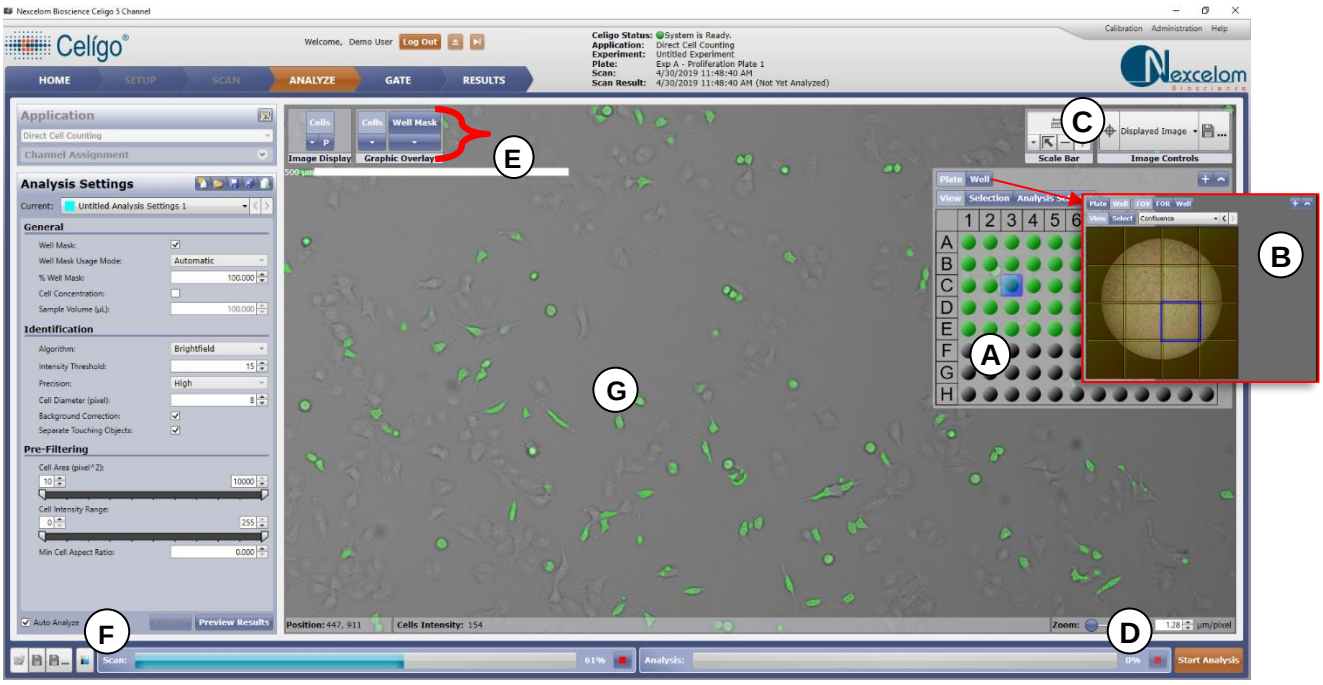
Continue to Scan Tab Chapter 6.

7. ANALYZE Tab

This chapter describes how to analyze scans to properly identify an object from the background. You do this task in the ANALYZE tab (Figure 103).

	NOTE: After selecting Start Scan in the SCAN tab, you can make analysis selections for wells in the ANALYZE tab, without waiting for the scanning of all wells to complete.
	NOTE: The displays you see onscreen may differ from the figures in this document, depending on the application you have selected in Current Application.

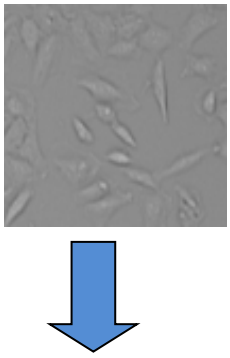
Figure 103. ANALYZE Tab



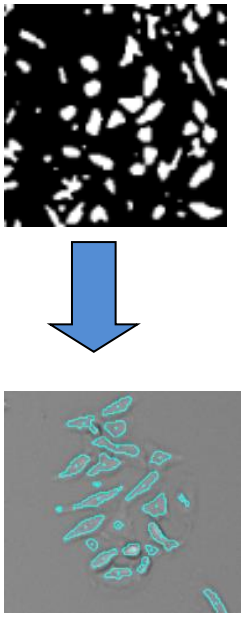
The image analysis workflow consists of two components: identification and pre-filtering (Figure 104).

The identification parameters determine image segmentation – the process of locating objects and separating them from the background into individual entities. The pre-filtering parameters are used to filter the found objects to differentiate cells based on their characteristics, such as size, shape, and intensity.

Figure 104. Image Analysis Workflow



Raw Image Scan



General settings and Identification:

Locate objects and boundaries

The figure at the left shows a binary (black and white) image of the segmented objects (white) on the background (black)

Pre-filtering:

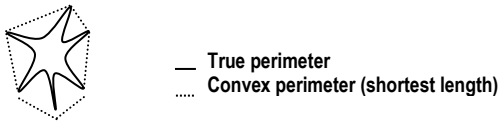
Filter objects to include and exclude based on specific image-based criteria

7.1 ANALYZE Tab Definitions

Table 4 shows ANALYZE tab terms that are common to all of the Celigo applications. For terms that are used in only some of the applications, see the applicable Application Guide.

Table 4. Analyze Tab Definitions

Term	Definition
AOI	Area of Interest, typically a cell. An AOI is made up of features (see Feature definition below).
Area	Total area of all the AOI features in microns squared (utilized sub pixel accuracy).
Feature	Represents one source of measurement and is the combination of two channels: • Channel from which segmentation mask is taken • Channel from which we measure intensity In most applications, by default, these two channels are the same one and there is a one to one match between channels and features.
Field of Regard	The total area that can be seen without moving the plate, instead uses the mirror galvanometers to pan around the area. This usually consists of multiple fields of view. Max number of images is four by four.
Field of View	The total area that can be captured by the camera without any movement from the plate or mirror galvanometers.

Term	Definition
Form Factor	A shape descriptor measuring how close together (compact) portions of the AOI are from one another. It is derived from the perimeter and area. The value ranges between 0 and 1. A circular blob is most compact and is defined to have a form factor measure of 1.0 (the maximum); a more convoluted shape has a lower value.
Aspect Ratio	A ratio of the AOIs breadth to the AOIs length. The value ranges between 0 and 1.
Intensity Average	Average of the pixel intensities. Will differ from Total Intensity / Area.
Smoothness	A measure of the evenness of an AOIs contour. It is a ratio of the convex perimeter to the true perimeter of a blob. The convex perimeter of any rough blob is always shorter than its true perimeter. The smoothness value therefore shows the degree to which a blobs convex perimeter is smaller than its true perimeter. The value ranges between 0 and 1. A completely smooth blob is defined to have a smoothness value of 1.0 (the maximum), whereas any other blob has a value less than 1.0 because its convex perimeter is shorter than its true perimeter. 
Scan Area	A generic term referring to wells in well plates or predetermined areas in flasks.
Total Intensity	Sum of the intensities of all pixels in the object.
X, Y Position	Coordinates within the well, reported in microns. The center of the well marks the origin.

7.2 Viewing Scanned Images

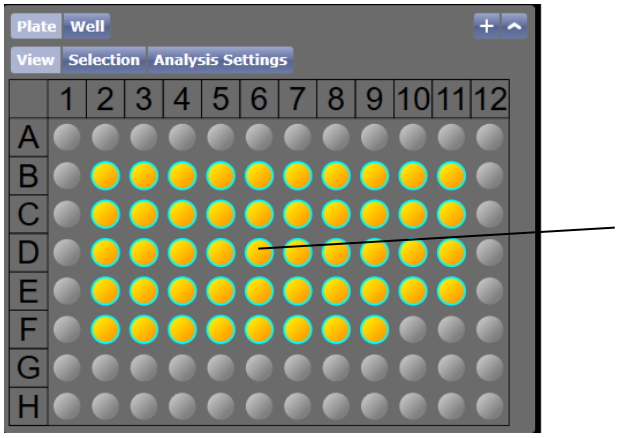
When you view a scanned image, the image displayed is the image within a single FOV (not an entirely stitched well, all or part of a well image fits within a single FOV), or a single FOR (only certain applications enable support for viewing a FOR). You view scanned images by using several of the same interface navigation methods you used to view wells in section 6.3.

During this analysis phase, you also change the displays to show the effect of your analysis settings on the scanned image.

To view scanned images

1. If a plate to be analyzed is not already displayed, display it per Selecting an Individual Existing Scan section 5.4.
2. Change the image displayed in the right-hand pane as needed by clicking a well in the View/Select Scan Areas section (Figure 103 item A and Figure 105). The resulting image appears at the same relative position and at the same magnification level as the previously displayed image.

Figure 105. View/Select Scan Areas Section



To hide or show the View/Select Scan Areas section

In the View/Select Scan Areas section (Figure 103 item A and Figure 105), do any of the following as needed:

- Up arrow (^) – minimizes the plate map to see more of the image display area.
- Plus sign (+) – increases the plate map size, useful for small well formats
- View button – when selected then select a well, will display the images from that well in image display area
- Selection – when selected then select wells, will highlight wells yellow to identify which wells will be analyzed

To change the appearance of the displayed image

Do any of the following as needed:

- To display the details for a FOV in the center display area, click the FOV square in the small well view (Figure 103 item B)
- To re-center the FOV, click the target button in Image Controls (Figure 103 item C). See Centering an Onscreen Image in the ANALYZE Tab in section 7.12 for additional details.
- To zoom in, use the magnification slide bar (Figure 103 item D) or scroll with the mouse.
- In the display image area (Figure 103 item G) move the image around on the screen by clicking and dragging it.

7.2.1 Using the Image Display and Graphic Overlay Buttons in the ANALYZE Tab

The Image Display and Graphic Overlay buttons (Figure 103 item E and Figure 106) are two sets of buttons that allow you to view a scanned image in different ways, as follows:

Image Display – Display or hide a channel, such as Live or Dead.

Graphic Overlay – Display or hide color overlays that highlight the objects that the system has identified as cells in response to your analysis settings.

Using the Image Display and Graphic Overlay buttons does not change the raw images.

7.2.1.1 Turning the Image Displays and Graphic Overlays ON and OFF

When the ANALYZE tab first appears after scanning, the Image Display and Graphic Overlay buttons are off (channels and segmentation not showing), indicated by dark blue buttons in those two sections.

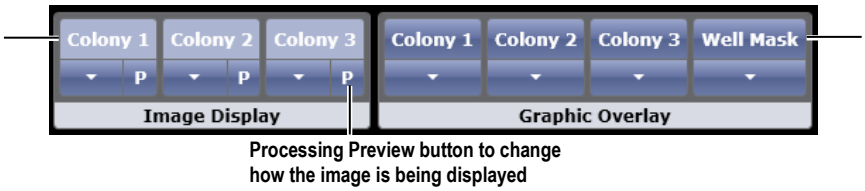
To turn the image displays and graphic overlays on and off

Click the Image Display and Graphic Overlay buttons to toggle them to the alternate position.

Figure 106 shows examples of Image Display and Graphic Overlay buttons.

	**NOTE: The Image Display and Graphic Overlay button names will differ according to the application you have selected in Current Application, and in the Expression Analysis application, depends on your Image Display and Graphic Overlay customized settings**

Figure 106. Image Display and Graphic Overlay Buttons



7.2.1.2 Selecting Colors and Overlays

Light blue button (shown here) indicates that the button is selected (ON)

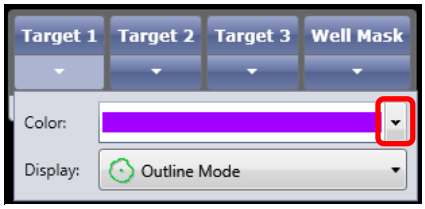
You can select the color and shape of the graphic overlay that the system displays around cells, using the Graphic Overlay section. You can also change the pseudo-coloring of the images, using the Image Display section. You can change the Graphic Overlay and Image Display selections for each application channel and well mask.

To select the color of a graphic overlay

1. In the Graphic Overlay section (Figure 107), click the menu down arrow for the feature (for example, Live or Target 1) you want to apply color to.

   The Graphic Overlay Settings appear (Figure 107).

Figure 107. Graphic Overlay Settings

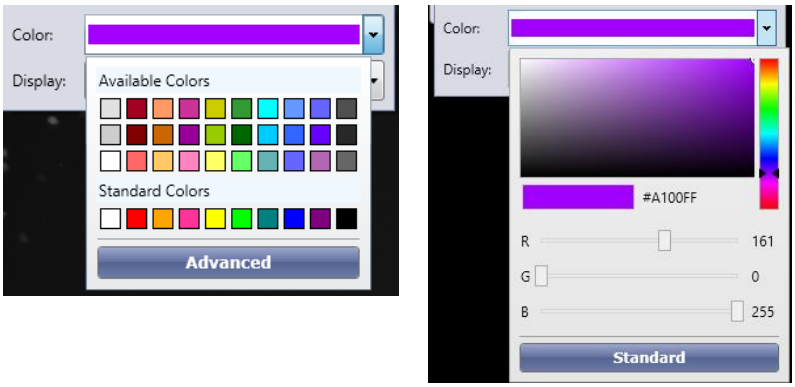


2. Select the Color menu.

   The color tools appear (Figure 108 A).

Figure 108. Color Tools

(A) Standard Colors **(B) Advanced custom colors**

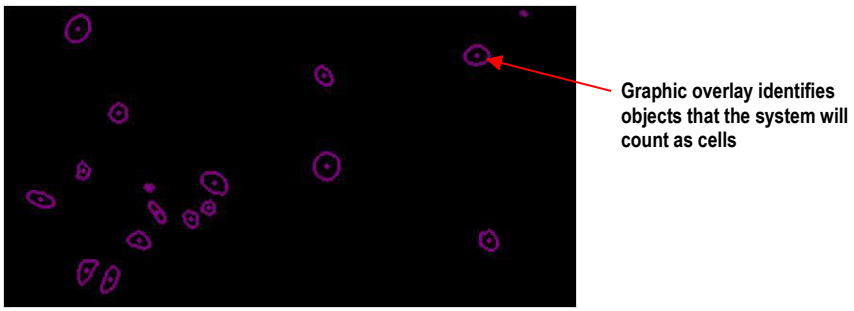


3. Use the color tools as needed:
   - Select a color.
   - Select more colors with Advance button, Figure 108 A.

     RGB (Red, Green, Blue) slide bars – Slide to increase color saturation.

   The graphic overlay changes to the selected color (Figure 108 B).

Figure 109. Purple Graphic Overlay



To select the shape of a graphic overlay

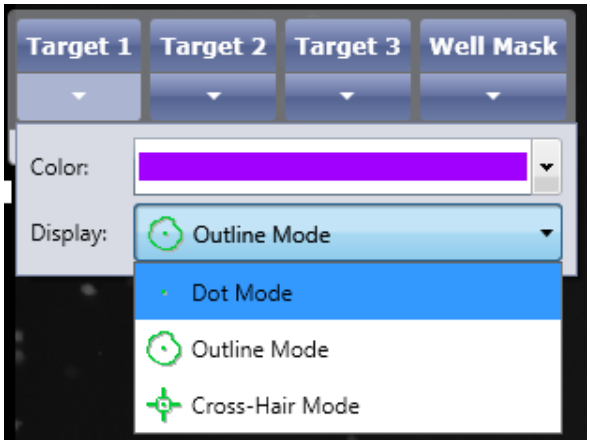
1. In the Graphic Overlay section (Figure 109 above), click the menu down arrow for the feature (for example, Live or Target 1) you want to apply the graphic overlay shape.

   The Graphic Overlay Settings appear (Figure 107 above).

2. Select the Display menu.

   The Display menu appears (Figure 110).

Figure 110. Display Menu

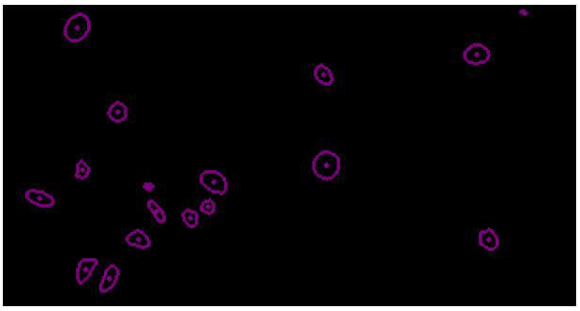


3. In the Display menu, select the type of display mode (shape) you want to apply to the overlay.

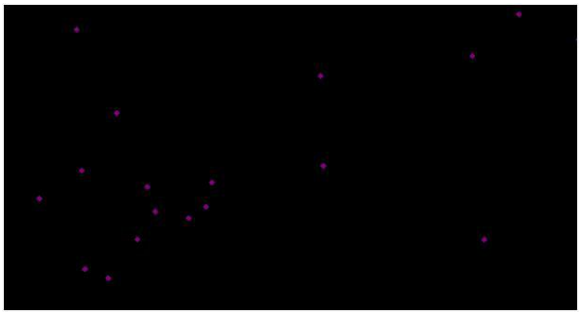
Figure 111 shows an example of the changing of a Graphic Overlay from Outline Mode to Dot Mode.

Figure 111. Selecting the Shape of a Graphic Overlay

Before Changing Graphic Overlay



After Changing Graphic Overlay

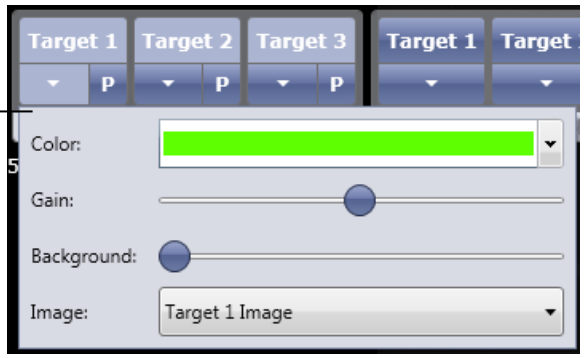


To select the color of an image display

1. In the Image Display section, click the menu down arrow for the feature that you want to apply a color to.

   The Image Display Settings appear (Figure 112).

Figure 112. Image Display Settings

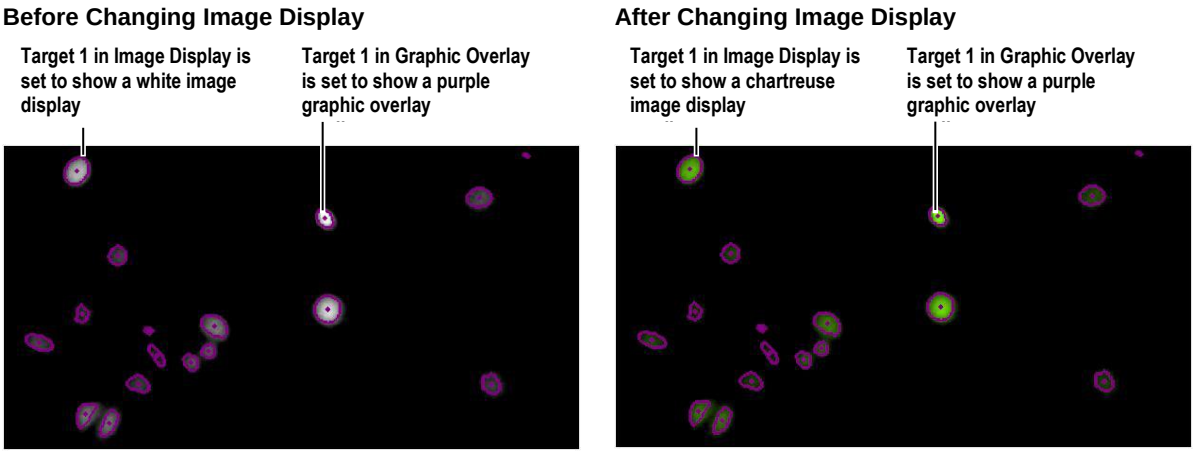


2. Select the Color menu.

The color tools appear. The color tools for the Image Display section are similar to the color tools in the Graphic Overlay section (Figure 107). For details on how to use the color tools, see To select the color of a graphic overlay and Figure 106 above.

Figure 113 shows an example of the changing of an image display from White to Chartreuse. In the example, Target 1 was selected in the Graphic Overlay as Purple, and therefore you see a purple Graphic Overlay both before and after.

Figure 113. Selecting the Color of an Image Display



To select other image display settings

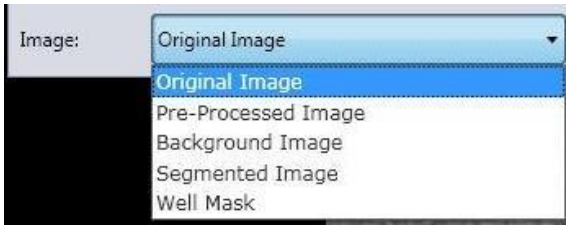
1. In the Image Display section (Figure 106 above), click the menu arrow for the feature that you want to apply image display settings.
2. Make the following selections as needed:

   Gain – Slide the slide bar to change the overall brightness of the image, including background and objects.

   Background – Slide the slide bar to reduce the background brightness of the image.

   Image menu – Selections vary according to the application selected in Current Application. Original Image, Pre-Processed Image, Background Image, Segmented Image (also known as binary image), and Well Mask (Figure 112) are available in all applications. Additional selections are available in specific applications.

Figure 114. Image Menu



Whenever you adjust an analysis parameter, it is useful to click on and off the **P** (Processing Preview) button (shown in Figure 103 item E) to toggle between the segmented (P turned on) and standard view (P

turned off). This helps you note the change in the segmented image to better discriminate cells.

To select update settings

To update the segmented image and overlays as you make each selection, displaying a progress wheel after you make each field entry, checkmark Auto Analyze (Figure 103 item F).

To update the segmented image and overlays only when you click Analyze, deselect Auto Analyze.

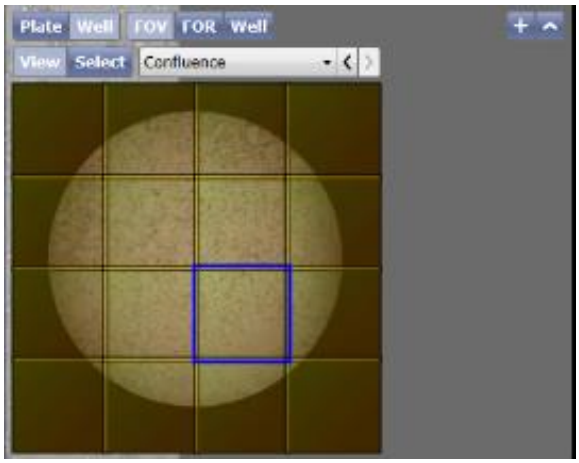
The Auto Analyze checkbox is *not* check marked by default.

7.2.2 Using the different display modes

The Analyze tab allows users to switch between Field of View, Field of Regard, and Whole Well display modes for all confluence-based applications (e.g., Confluence, Colony, and Tumorsphere applications).

	NOTE: Viewing of Pre-Processed Images in the ANALYZE Tab will only work when viewing wells in FOV or FOR mode. If Well mode is selected, this selection is not displayed and the Pre-Processed Images button will turn off and become disabled.

Figure 115. Display modes



7.3 Changing the Names of the Image Display and Graphic Overlay Buttons in the Analyze Tab

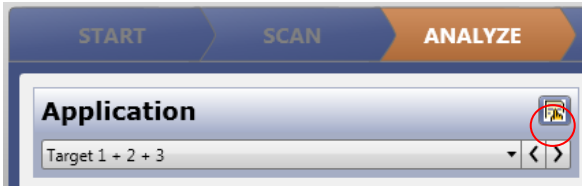
You can change the Image Display and Graphic Overlay button names to customized names in the ANALYZE tab.

	NOTE: This functionality is available in the Expression Analysis application only.

To change the names of the Image Display and Graphic Overlay buttons in the Analyze tab

1. In the Analyze tab, click the Customize Application button (Figure 116).

Figure 116. Customize Application Button in the Analyze Tab



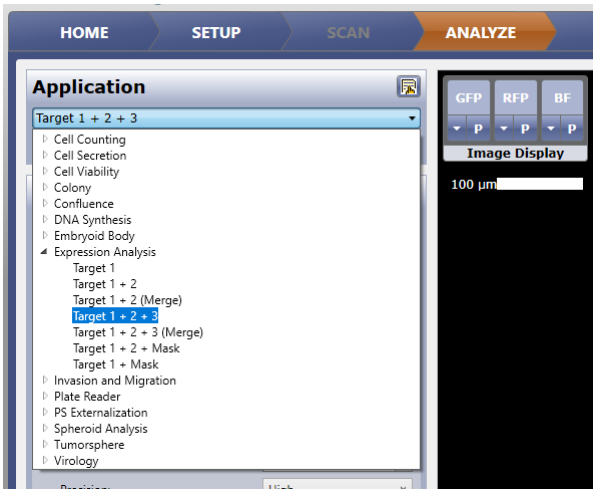
The Customize Application dialog box appears.

2. The remaining steps are the same as in the SCAN tab. For instructions, see section 6.2.1.

7.4 Changing Application in the ANALYZE Tab

Users can change applications in the analyze tab to another application with the same number of channels or less as the current scan. Just select from the dropdown list which is already filtered to show the valid applications and then the analysis settings will update as well as the Image Displayers header.

Figure 117. ANALYZE Tab Application Selection Change



	NOTE: If switching to an application with fewer channels, the channels at the end of the list are removed. Only those channels that are used (assigned) are displayed to the user in the image display area. Channels that were removed can be added back in by changing channel assignments. **For instructions, see section 7.5**

7.5 Changing Channel Assignments

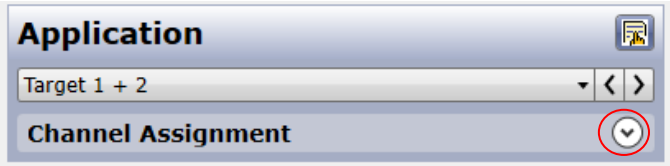
When you change channel assignments, you are selecting which channels to use and in what order. For example, if a scan was acquired with 3 channels, but you realized your mask channel is not your last channel, channel assignment can correct this. With channel assignment, you can change the order of those

channels. Likewise, if you switched to a 2-channel application, you can specify which 2 of the 3 channels from the scan you want to analyzed. You do this in the Analyze tab.

To change the channel assignment for analysis

1. In the Application section (Figure 118 item A), expand the Channel Assignment section by clicking on the expanding arrow.

Figure 118. Channel Assignment Control



Item A

2. For each channel listed for the application (left side), select the scan channel you want to use (right side) by expanding the dropdown menu and selecting the scan channel to use for the application channel.

Figure 119. Application Channel Assignments

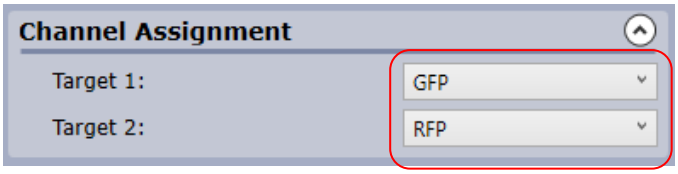
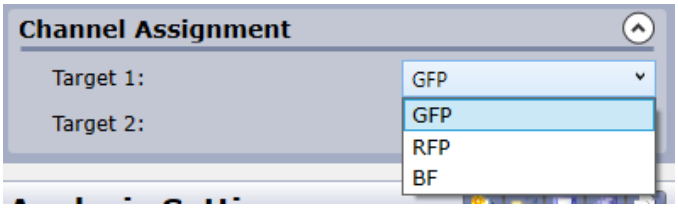


Figure 120. Application Channel Assignment Selection



When the channel assignments change, the channel images, color, and other display settings change in the image display area of the screen.

	**NOTE: After making a channel assignment change, analysis settings are not reordered and remain in place. The settings used to determine the identification of objects in the images should be re-evaluated when and if a channel assignment is performed.**

7.6 Selecting Wells for Analysis

When you select wells for analysis, you are identifying which wells you want the system to analyze. You do this in the Analyze tab.

To select wells for analysis

1. In the View/Select Scan Areas section (Figure 103 item A), click **View**.
2. Click **Selection**.

3. Click the wells that you want the system to analyze.

   The displayed well colors indicate well scanning and analysis status as follows:

   Black – Scan has not yet completed (system is currently in the process of scanning)

	**NOTE: After selecting Start Scan in the Scan tab, you can make analysis selections for wells in the Analyze tab, without waiting for the scanning of all wells to complete.**

   Gray – Not yet selected for scanning

   Green – Scan has completed, image saved to database

   If a well is already selected, you must deselect it (click again to deselect) to see the green indicator that image of that well is completed and saved

   Yellow – Selected for analyzing

To select FOV within well for analyses

When you select the specific FOV within the well for analysis, you are identifying which segment you want the system to analyze. You do this in the Analyze tab.

1. In the View/Select Scan Areas section (Figure 121), click **Select**.

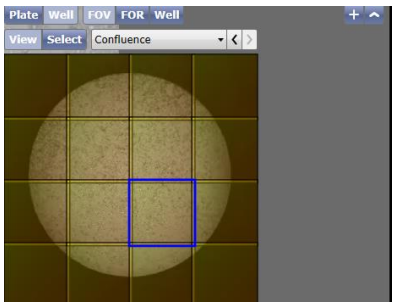
   Note: The yellow overlay indicates the FOV has been selected. By default, the entire well is selected.

2. Click on well segment you DO NOT want to scan.

   Note: Deselected segments will turn grey. Deselected segments will not be analyzed. Click on the segment again to reselect it, a yellow overlay will appear over segment.

3. Click Start Analysis, when ready to analyze

Figure 121. Well FOV Selection



7.7 Selecting Analysis Settings

Perform the following steps to select analysis settings.

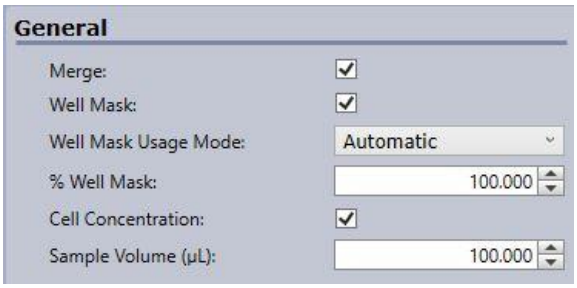
7.7.1 Selecting General Analysis Settings

When you select general analysis settings, you are defining how the system will determine the area to be analyzed (the well mask).

To select general analysis settings

In the General section (Figure 122), make the following selections:

Figure 122. General Section

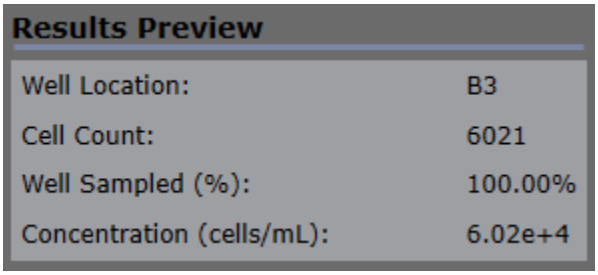


1. Well Mask – (recommended for Brightfield segmentation) – Applies a boundary at the well edge so that the well edge (subject to distortion or plate artifacts) is excluded from segmentation.
2. Well Mask Usage Mode – Select one of the following to identify the area to be analyzed:

   Automatic (Default) – The system looks at the well to find the edge of the well (looks for the local minima). This method is more accurate than Original, but slower.

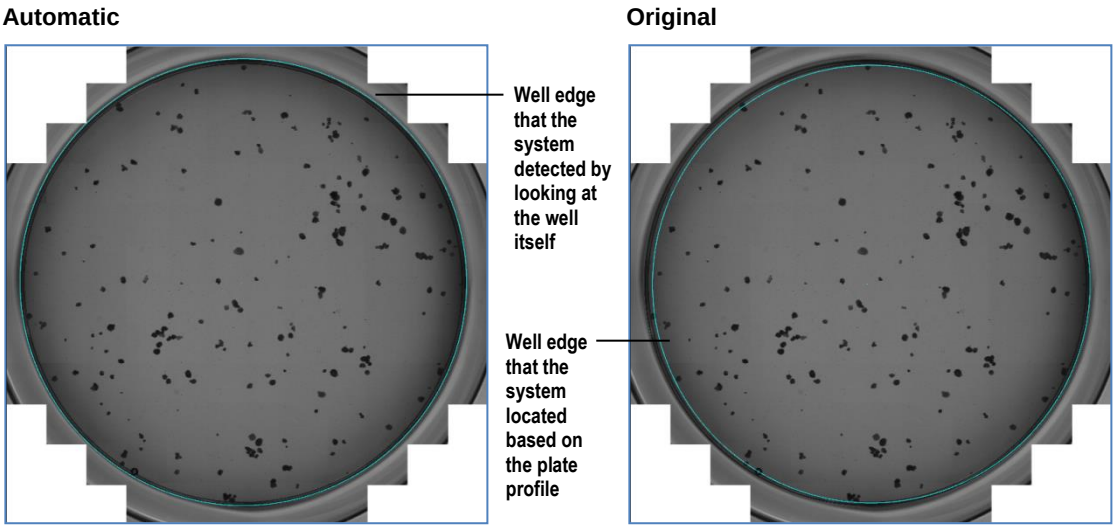
   Original – The system uses the set mapped position for the edge of the well, specified in the plate profile. This position is used for each well. Therefore, if a well is not correctly aligned in the image area, parts of it might undesirably be excluded from analysis. This method is faster than Automatic, but less accurate.
3. % Well Mask – Sets the percentage of the well to be analyzed. Select 0 (none of the well is to be analyzed) through 150. Default is 100% (entire well is to be analyzed).
4. Cell Concentration (cells/mL) – The system will display the cells per mL (Figure 124). If unchecked the system will read NaN. In order to activate this option the % Well Mask (Figure 122) must also be selected.

   Figure 123. Results Preview



Examples of the Well Mask Usage Mode settings for a 6-well plate are shown in Figure 124.

Figure 124. Well Mask Usage Mode Selections



7.7.2 Selecting Identification Analysis Settings

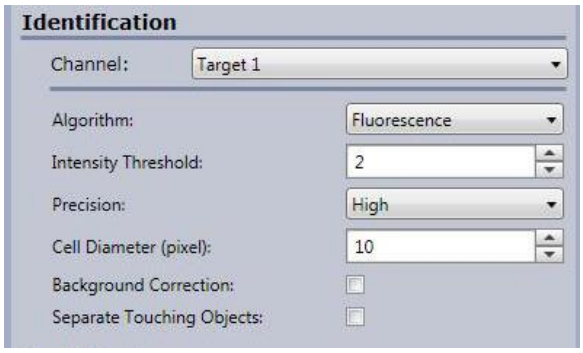
When you select identification analysis settings, you are defining, for each channel, which objects in an image are to be included in the analysis.

In most applications, the identification analysis settings are applied separately to each channel scanned.

To select identification analysis settings

In the Identification section (Figure 125), make the following selections, referring to the applicable Application Guide for starting suggestions:

Figure 125. Identification Section



1. Channel (field appears for applications that use multiple channels) – Select the channel for which you want to enter analysis settings.

	NOTE: Make sure that the Image Display and Graphic Overlay selections match the Channel selection for which you are setting identification analysis settings. This will ensure that the entries you make in the identification section will be applied to the correct channel (the channel being displayed).

The system automatically changes the Feature Type menu selection to the appropriate selection, based on the Channel selection. An exception is if you have renamed either the Channel name or Feature Type name and the names do not match.

2. Algorithm – Select **Brightfield**, **Fluorescence**, **FL Confluence**, **Dark Object**, or **Texture** as follows:

   Brightfield – The algorithm looks for objects with a bright center and dark edges

   Fluorescence – The algorithm looks for fluorescent objects (bright pixels over darker background)

   FL Confluence – The algorithm looks for large fluorescent objects (bright pixels over darker background)

   Dark Object – The algorithm looks for dark objects with no bright center

   Texture - The algorithm looks for texture differences between the objects found and the background areas.

	**NOTE: The available selections in the Algorithm menu differ depending on the application you have selected in Current Application.**

3. Intensity Threshold:

   This feature marks individual pixels as object or background pixels, resulting in a binary (segmented) image.

   Smaller threshold values allow the identification of lower contrast cells but increase the background noise that is detected.

   Larger threshold values can be used for higher contrast objects, such as non-adherent cells, and reduce the noise detected. Fewer pixels per object are identified as threshold values are increased.

4. Precision – The highest precision setting uses the full resolution image for segmentation, while lower precision values use increasingly smaller images. Lower precision settings decrease analysis time but may not be appropriate for very small objects.
5. Cell Diameter – Select a suggested approximate diameter (in pixels) of the cell to be identified. Larger values will cause larger cells to be enhanced. Smaller values will cause smaller cells to be enhanced.
6. Background Correction (For Brightfield Illumination only; background correction using Fluorescence Illumination is only available in the Expression Analysis applications) – Minimizes background variations by applying an average value.
7. Separate Touching Objects – Applies a watershed algorithm to separate touching objects. This setting is effective at distinguishing discrete cells within tightly grouped clusters of cells.

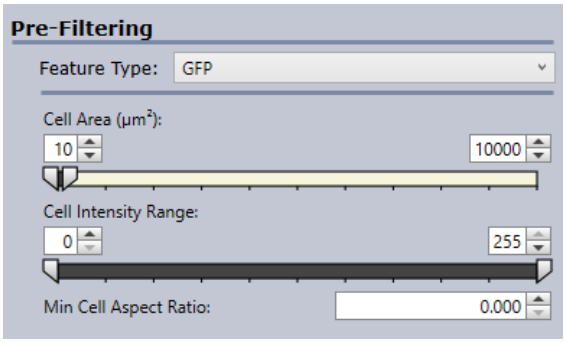
7.7.3 Selecting Pre-Filtering Analysis Settings

When you select pre-filtering analysis settings, you are applying filter parameters to objects that the Identification settings located. You do this for each channel.

To select pre-filtering analysis settings

In the Pre-Filtering section (Figure 126), make the following selections, referring to the applicable Application Guide for suggested initial parameters:

Figure 126. Pre-Filtering Section



	NOTE: The system automatically selects the appropriate Feature Type if you have selected a Channel in the Identification section. An exception is if the Channel and Feature Type names do not match (which does exist for specific applications).

1. Under Feature Type, select the appropriate application feature (channel) that you are currently segmenting (for example, **Live**, **Dead**, or **Total**).
2. Do one of the following:

   For individual cells, make the following selections:

   - Cell Area – Defines (in squared pixels) the minimum and maximum size of a cell in the plate. This option can be used for filtering out debris and artifacts or filtering different cell types.
   - Cell Intensity Range – Defines the minimum and maximum of intensity values used to filter found objects. This option is most useful in rejecting darker artifacts in high contrast cell counting, such as non-adherent cells.
   - Min Cell Aspect Ratio – Ratio of the AOIs breadth to the AOIs length. A Min Cell Aspect Ratio of 1 is equal to a perfect circle; a value lower than 1 corresponds to a more oval shaped object, such as an adherent cell type of object.

   For confluence: In the Cell Counting application, select the Min Cluster Size. For details about Min Cluster Size, see *Celigo Cytometer Cell Counting and Growth Tracking Application Guide* (CS-017).
3. (Optional) Save the settings if desired (Reference section 7.8 Saving an Experiment in the Analyze Tab) and then determine the next step by continuing to section 7.14.

7.8 Previewing Results

After having analyzed a selected region of a well, you can view what the end measurement results would be for the analyzed area. To view these results, press the Preview Results button near the bottom of the screen (Figure 127).

Pressing this button will toggle on the results preview (Figure 128). Pressing the button again will hide the results preview.

Figure 127. Preview Results Button

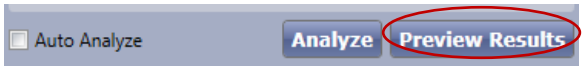
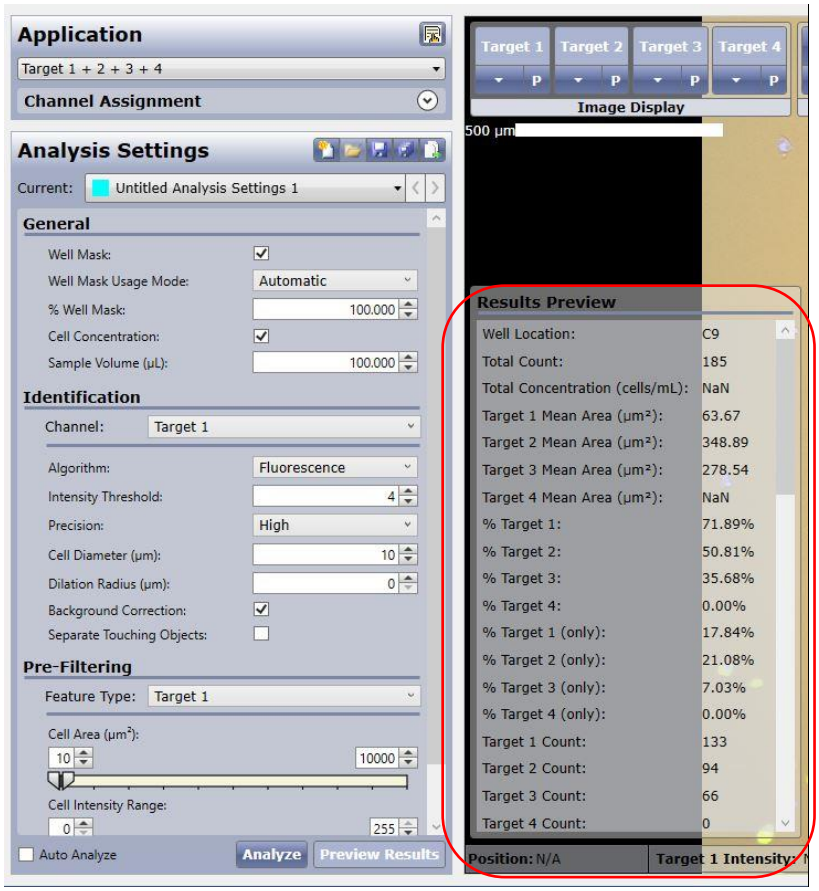


Figure 128. Results preview



7.9 Saving an Experiment or Project in the ANALYZE Tab

When you save an experiment to the database in the Analyze tab, you are recording a snapshot of all the settings that currently exist in the Scan, Analyze, and Gate tabs.

When saving Projects in the ANALYZE tab, it records from the current session the experiment settings, plate type and export options into a file for later re-use in the HOME tab.

In the Analyze tab, when you save an experiment or project, you are saving it to the database (not to your hard drive).

For an overview of the save button locations and functions in the various tabs, see section 6.10.

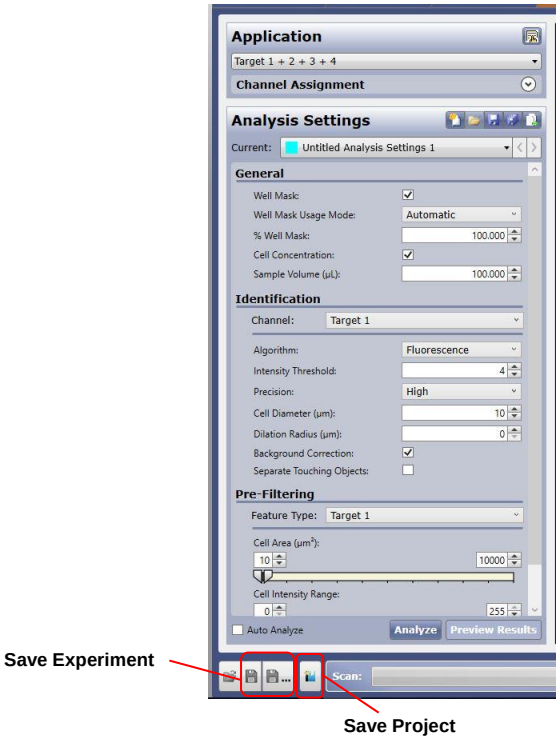
7.9.1 Saving an Experiment to the Database in the ANALYZE Tab

Save an experiment to the database in the ANALYZE tab using the Experiment Save-to-the-Database button (Figure 129). The same way as in the SCAN and RESULTS tabs. For detailed instructions, see Saving an Experiment to the Database in the SCAN Tab, section 6.10.1.1.

7.9.2 Saving a Project to the Database in the ANALYZE Tab

Save a Project to the database in the ANALYZE tab using the Project Save-to-the-Database button (Figure 129). The same way as in the SCAN and RESULTS tabs. For detailed instructions, see Saving a Project to the Database in the SCAN Tab, section 6.10.1.1

Figure 129. Save-to-Database Buttons in the ANALYZE Tab



7.10 Saving Analysis Settings in the ANALYZE Tab

In the ANALYZE tab, you can save analysis settings to either the database or the hard drive. You can also create a set of new analysis settings.

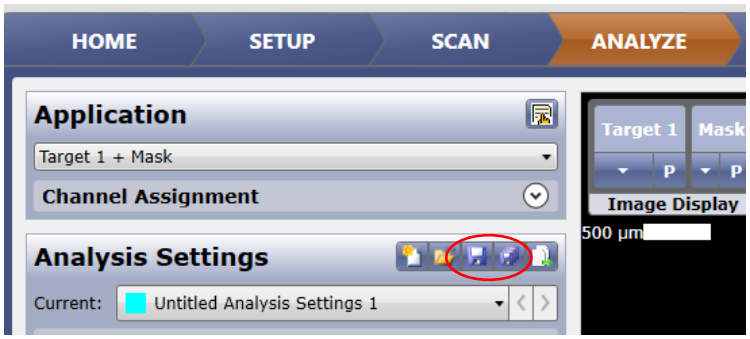
For an overview of the save button locations and functions in the various tabs, see section 6.10.

7.10.1 Saving Analysis Settings to the Database in the ANALYZE Tab

To save analysis settings to the database in the ANALYZE tab

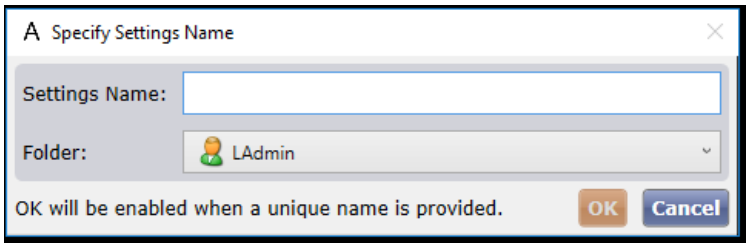
1. In the ANALYZE tab, in the Analysis Settings section, click the **Save** or **Save As** button (Figure 130).

Figure 130. Analysis Settings Save-to-the-Database Buttons



A Specify Settings Name dialog box appears (Figure 131), with the Folder field displaying the default entry (logged-in user folder).

Figure 131. Specify Settings Name Dialog Box



2. In the Settings Name field, enter a name for the analysis settings.

   If the settings name is unique, the OK button becomes available.

3. In the Folder field, select a different destination folder for the settings file as needed.

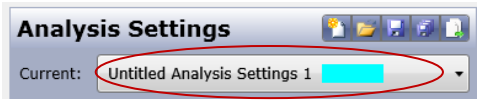
4. Click **OK**.

   The system saves the settings name.

   The system saves the analysis settings that you have entered at this point and saves them under the Analysis Settings name you created. The Analysis Settings name now appears in Current for later possible selection.

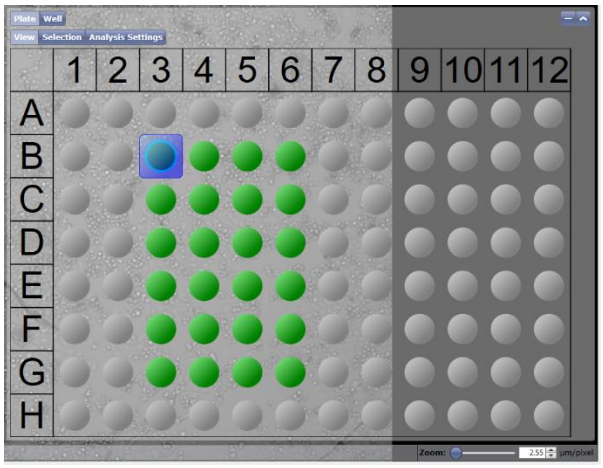
   A color outline appears around the selected wells. The color of the outline is associated with the current Analysis Settings (Figure 132).

Figure 132. Analysis Settings Current Field Color Setting



Individual wells can be analyzed using different settings, as illustrated in Figure 133. The figure shows an example of wells marked with analysis setting colors. You can select the analysis setting for wells by selecting the Analysis Settings button and then click on any well. This will associate the well with the current analysis setting.

Figure 133. View/Select Scan Areas Section with Analysis Setting Colors



7.11 Exporting Analysis Settings to the Hard Drive in the ANALYZE Tab

When you use the ANALYZE tab to export analysis settings, you are exporting the analysis settings to a hard drive in XML format.

	NOTE: You can also use the Data window to export analysis settings in XML format. For instructions, see the Celigo Cytometer Administrator Guide section titled Exporting Settings.

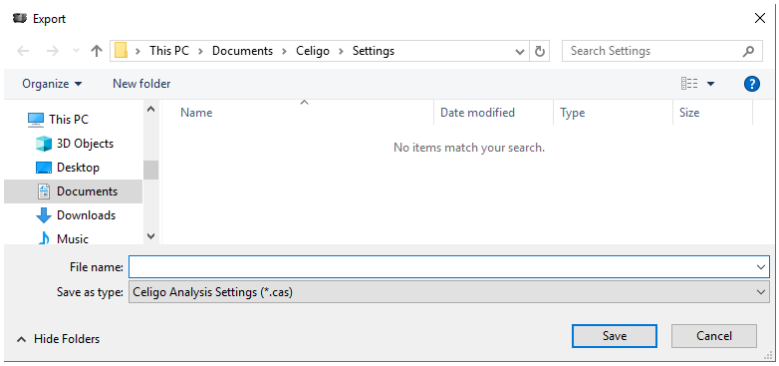
To export analysis settings to the hard drive in the ANALYZE tab

1. In the Analysis Settings buttons at the top of the ANALYZE tab, click the **Analysis Settings Export** button (Figure 134).

Figure 134. Analysis Settings Save-to-the-Hard-Drive (Export) Button

2. The Export dialog box appears (Figure 135).

Figure 135. Export Dialog Box



3. In File name, type a name for the file you are exporting.
4. Click **Save**.

7.11.1 Creating New Analysis Settings

You can create a new set of analysis settings to use for analysis. A listing for the new settings will be available in the Current menu.

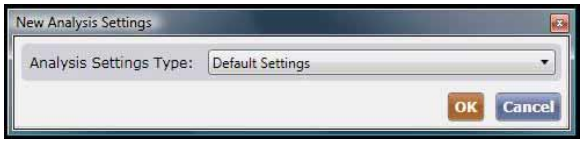
To create new analysis settings

1. In the Analyze tab, in the Analysis Settings section, click the **New Analysis Settings** button (Figure 136).

Figure 136. New Analysis Settings Button

The New Analysis Settings dialog box appears (Figure 137).

Figure 137. New Analysis Settings Dialog Box



2. In Analysis Settings Type, make one of the following selections:

   Default Settings – Creates a set of analysis settings with the applications default values.

   Settings Copied from Current Analysis Settings – Creates a set of analysis settings that is a duplicate of the current settings.

3. Click **OK**.

   In the Current menu, a listing appears for the newly created settings.

7.12 Centering an Onscreen Image in the ANALYZE Tab

You can center an onscreen image in the ANALYZE tab. Doing this does not change the raw images.

To center an onscreen image in the Analyze tab

In the Image Controls section (Figure 103 item C above and Figure 138), click the **Center Image** icon.

Figure 138. Image Controls in Analyze Tab

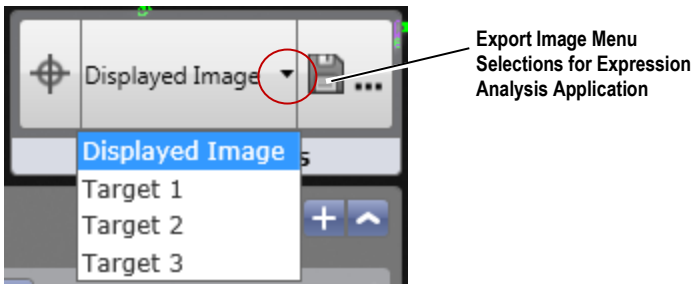
7.13 Exporting an Onscreen Image or Individual Channel Image in the ANALYZE Tab

In the ANALYZE tab, you can export a selected well image as seen onscreen (do a screenshot) or for an individual channel. The export includes any applied pseudo-coloring and/or graphic overlays displayed.

To export an onscreen image in the ANALYZE tab

1. In the Image Controls section (Figure 103 item C and Figure 138 above), click the Export Image Menu (defaults to Displayed Image) to the left of the Save Image icon, and select either **Displayed Image** or a channel (Figure 140).

Figure 139. Export Image Menu in the ANALYZE Tab



2. Click the **Save Image** icon.

The remaining steps for exporting the image are the same as exporting the same type of image in the RESULTS tab, with the following exception: In the ANALYZE tab, select from multiple selections in the Displayed Image menu to the left of the Save Image icon (Figure 138). For detailed instructions, see Exporting an Onscreen Image in the Results Tab in section 9.7.3.

7.14 Determining the Next Step

Do one of the following:

If you want to select a user-defined population within the scanned population, perform this gating task by continuing to GATE Tab chapter 8.

If you do not want to perform gating now, start the analysis by clicking **Start Analysis** at the bottom right of the ANALYZE tab and then skip to RESULTS Tab chapter 9. For more details on starting analysis, see section 7.14.1.

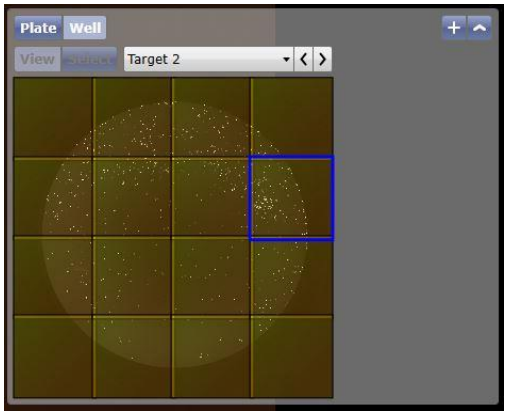
7.14.1 Analyzing a Scan in the ANALYZE Tab

You analyze a scan in the ANALYZE tab.

To analyze well results in the ANALYZE tab

To perform analysis and then create a new scan result, click the **Start Analysis** button (Figure 140).

Figure 140. Start Analysis Button in the ANALYZE Tab



The RESULTS tab appears with the results from the analysis. You can delete scan results at any future date. For instructions on deleting scan results, see the *Celigo Cytometer Administrator Guide*.

7.14.1.1 Stopping Analysis

If you need to stop analysis or re-analysis that is in process (green progress bar displayed), you can stop it in any tab.

To stop analysis

Click the red stop button at the bottom right side of the tab (Figure 141).

After stopping analysis, you can only start a new analysis.

Figure 141. Analysis Progress Bar

7.14.1.2 Restarting Analysis

After stopping analysis, you can only start a new analysis. You can start analysis in the ANALYZE or GATE tabs.

To restart an analysis

Click **Start Analysis** (Figure 141)**.** A new scan result will be created with a new timestamp.

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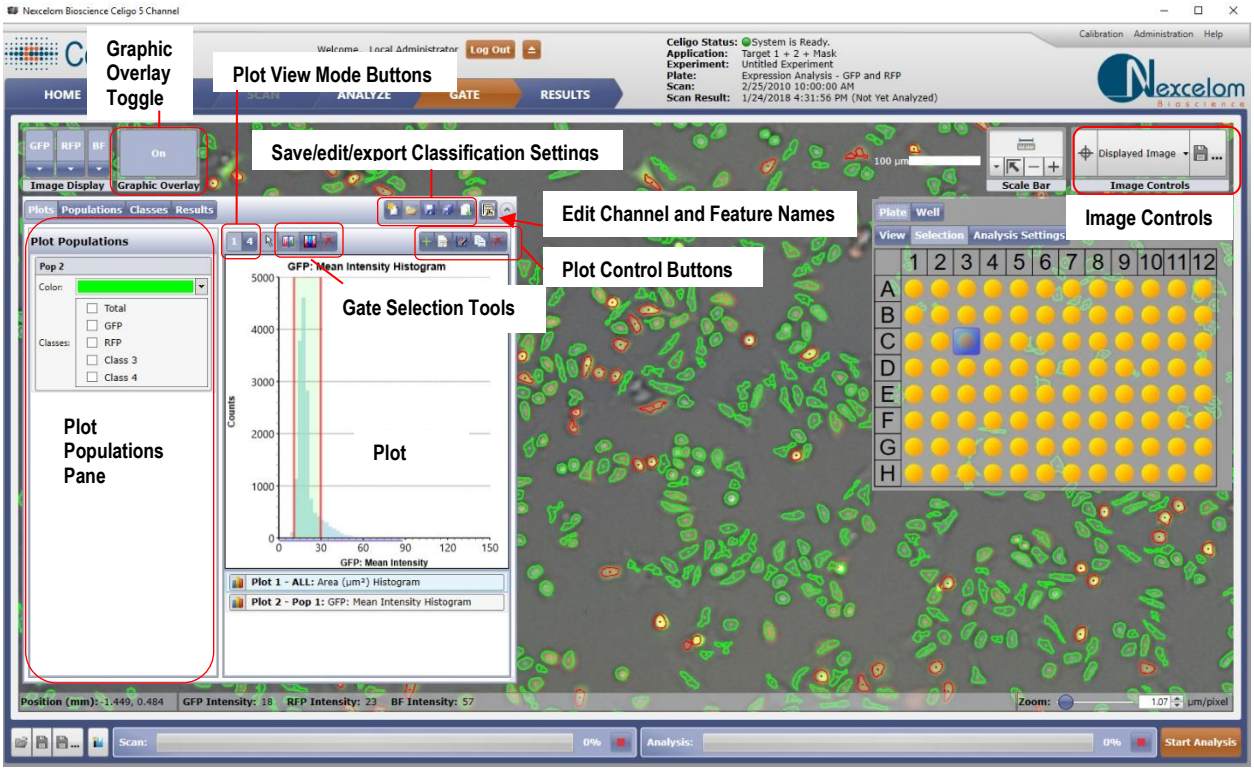
8. GATE Tab

The GATE tab (Figure 142) allows you to specify classification settings that are used after feature analysis. After making these selections, you can view cells belonging to various classes for an entire well (one at a time) for evaluation purposes. For correct analysis, some applications require that you make Gate tab entries.

This chapter is in the order in which a user typically performs the tasks.

Figure 142. Gate Tab



8.1 GATE Tab Definitions

There are four main elements used during GATE tab tasks:

Plot – A graphic representation of the data the system collects during image acquisition and analysis.

Gate – Region that a user defines within a plot, for the purpose of system analysis.

Population – The outcome of a gate.

Class – A population for which data will be reported on the Results tab.

For an additional list of GATE tab terms that are common to all of the Celigo applications, see ANALYZE Tab Definitions in section 7.1. The list of terms is the same for both tabs. For terms that are used in only some of the applications, see the applicable Application Guide.

8.2 Selecting Wells for Classification

When you select wells for classification, you are displaying wells for creating plots, gates, and assigning classes.

To select wells for classification

1. In the View/Select Scan Areas section (Figure 142 above), click **View**.
2. The remaining steps are the same as in the ANALYZE tab. For instructions, see section 7.2.

8.3 Creating a Plot

Create plots to isolate subsets of data for analysis. A plot can be either a histogram or scatter plot.

	**NOTE: While displaying plot selections, toggle the Image Display and Graphic Overlay buttons in the GATE tab as needed to view the populations you want to select. Use these buttons the same way as in the ANALYZE tab, with the exception that a Processing Preview (P) button is not used in the GATE tabs Image Display buttons. For details about using the Image Display and Graphic Overlay buttons, see section 7.2.1. Using these buttons does not change the raw images.**

To create a plot

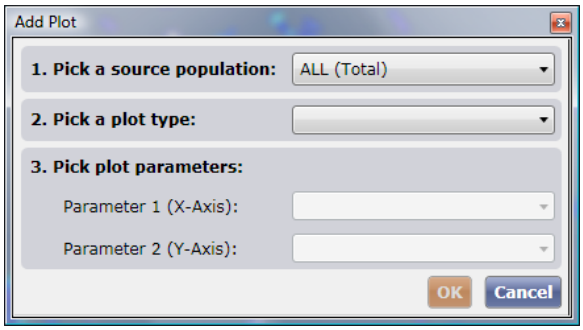
1. Select a well per section 8.2.
2. Click the **Plots** button.

   The Plot Populations pane appears.
3. In the GATE tabs Plots view, in the Plot Controls, click the green (**+)** Add Plot button (Figure 143).

Figure 143. Add Plot Button

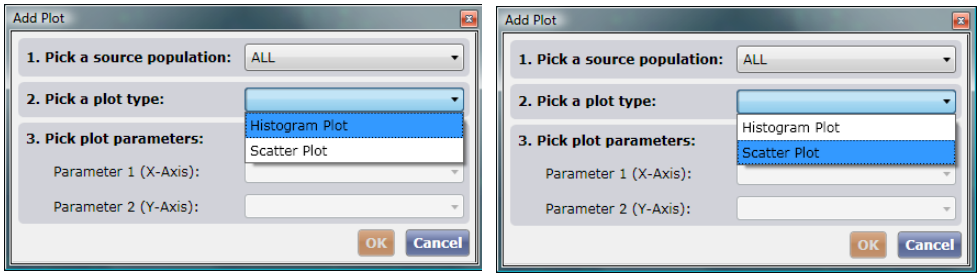
The Add Plot dialog box appears (Figure 144).

Figure 144. Add Plot Dialog Box



4. Make selections in the following fields (Figure 145):

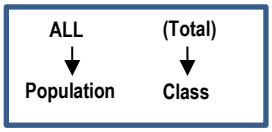
Figure 145. Selecting a Plot Type



Pick a source population – Select the name of a population that you want to display. A population is the outcome of a gate. The population names are ALL, Pop 1, Pop 2, etc.

- ALL means all data points (objects). ALL is the default population that the system assigns to all the data points (objects) in the segmentation result from the Analyze tab.
- For the first plot, only ALL (Total) is available for selection. This means that the class called Total has been assigned to the population called ALL. In other words, ALL (Total) means that the ALL population is called Total. The syntax of ALL (Total) is shown in Figure 146.

Figure 146. ALL (Total) Syntax



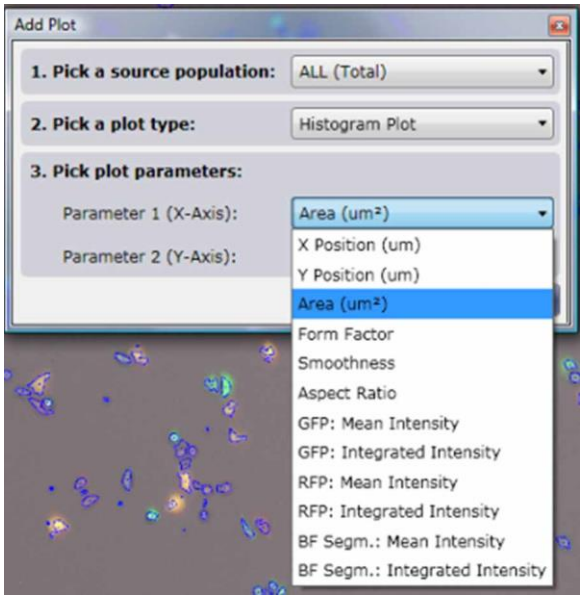
Pick a plot type:

- Histogram Plot
- Scatter Plot

Pick plot parameters:

- If you want a histogram plot to display, make a selection in the Parameter 1 field (Figure 147) only
- If you want a scatter plot to display, make a selection in both the Parameter 1 field and Parameter 2 field

Figure 147. Add Plot Dialog Box Parameter 1 Menu Selections



5. Click **OK**.

   A histogram plot (Figure 148) or scatter plot (Figure 149) appears, and is listed in the clickable plot selection list below the plot.

   The plot selection list below the plot shows the name of plot, source population for the plot, and the axes visible on the plot.

	NOTE: The time required for the system to categorize each object and/or cell is determined by the size of the well and the number of segmented objects in each well.

Figure 148. Histogram Plot

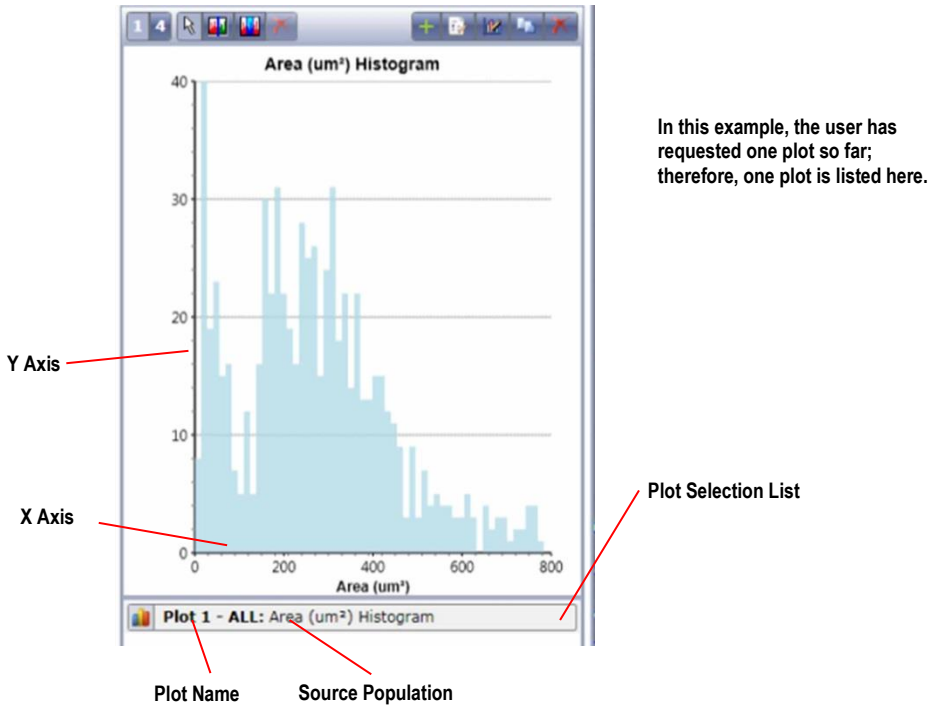
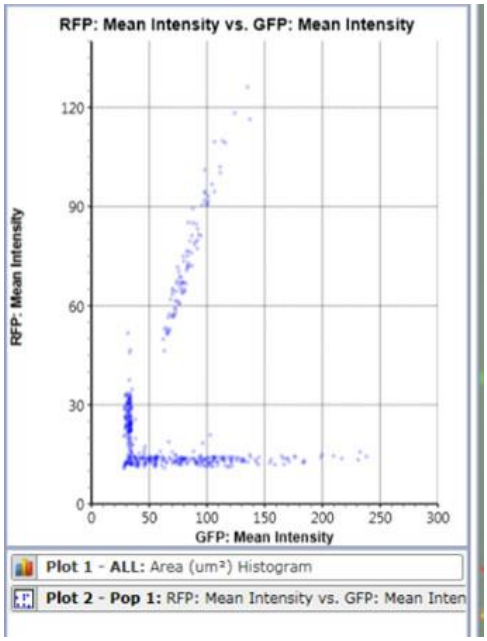


Figure 149. Scatter Plot



8.3.1 Creating a Second or Subsequent Plot

You can create a new plot at any time after creating the first one.

To create a second or subsequent plot

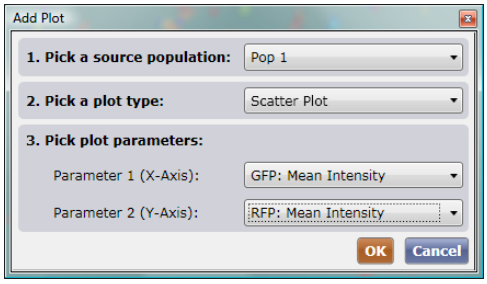
Perform the same steps as described in Creating a Plot section 8.3, with the following exception: When completing the Pick a Source population field:

If you have not yet selected any gates for the existing plot (described in Creating a Gate 8), only ALL will be available for selection.

If you have selected any gates for the existing plot, by default the system selects the most recent population you created.

In the following example (Figure 150), the most recent gate that the user created has output called Pop 1. Therefore, when creating a second plot, the system has defaulted to Pop 1. In the Pick a source population menu, select any listed population.

Figure 150. Add Plot Dialog Box



The plot name, color, and class information appears in the Plot Populations pane. The system assigns a plot number using the next consecutive number available.

8.3.2 Resizing the Display Panes

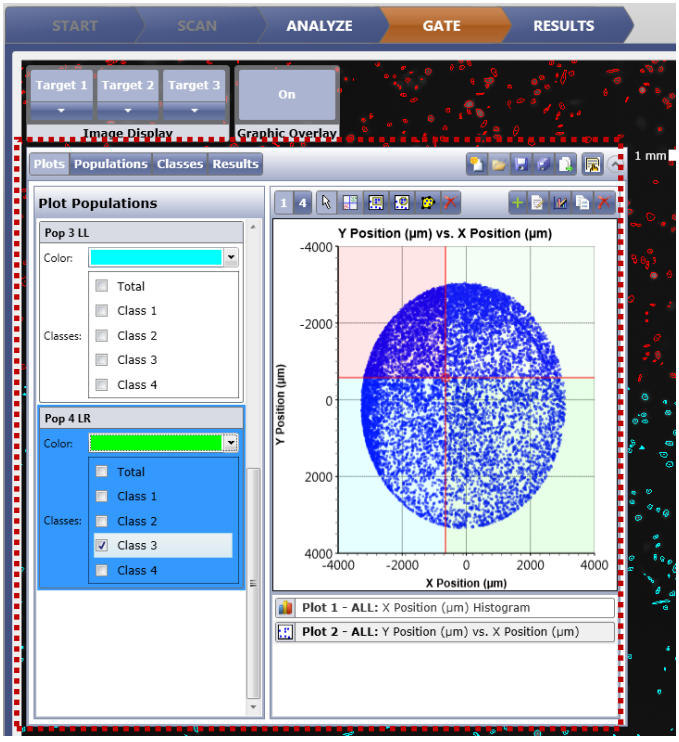
You can resize the display panes located to the left of the View / Select Scan Areas section. Resizing the display pane will not change the plotted data, it is only for viewing purposes.

To resize a plot

1. Place the mouse cursor over the lower right corner of the display panes until an angled double-headed arrow appears ( ) in circled area in Figure 151.

   In Figure 151, the display pane is indicated by a red dashed outline.

Figure 151. Resizing the Display Panes



2. Drag the corner of the display pane to adjust plot viewing size and shape.

8.3.3 Magnifying a Plot Size (Zoom)

You can magnify the appearance of a plot (zoom in on it) for easier viewing. This action is not to change the plot data, but is only for viewing purposes.

To magnify a plot size (zoom)

Left-click the plot and then scroll with the mouse scroll wheel.

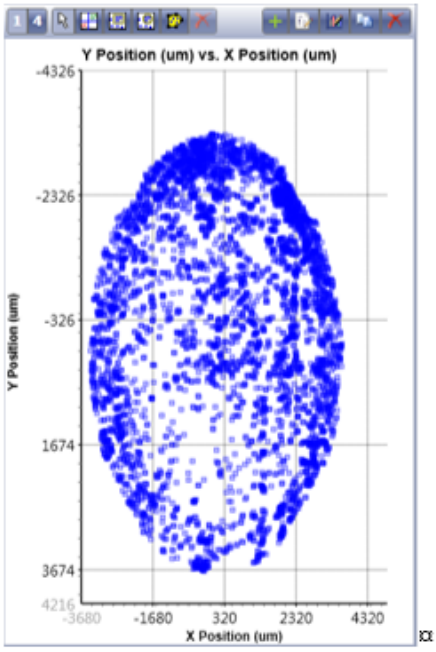
The displays include the following indicators:

A purple line appears along each plot axis to indicate which portion of the sample (AOIs) you are viewing (Figure 152). When the plot shows the entire sample, the purple lines do not appear.

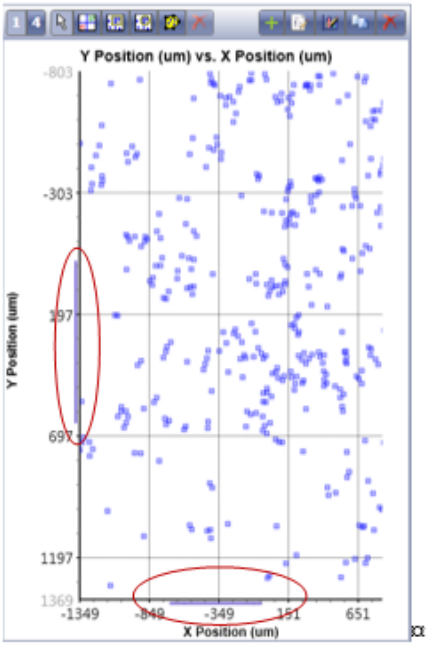
In histogram plots, the closer that you magnify (zoom), the lower the bin count and therefore the lower the height of the histogram plot bars.

Figure 152. Area of Interest Indicator Lines

Entire sample is in view (no indicator lines)



Portion of sample is in view (indicator lines) zoomed in area



8.3.4 Panning (Dragging) a Plot

When you pan a plot, you are dragging it to control which area of data is currently in view. This action is not to change the plot data, but is only for viewing purposes.

To pan a plot

Left-click anywhere inside the axes anywhere on the plot, and then press the mouse scroll wheel down and drag the cursor.

8.4 Changing a Plot

You can change various properties of a plot. When you perform these tasks, you are changing the display of the plot, not the listed selections themselves.

8.4.1 Changing Plot Parameter Selections

You can change the plot parameter selections that you had made when creating a plot. If you do this, any existing gates on the plot will be deleted and any plots or logical populations that are dependent on the output populations that are defined by the gate will be reassigned to the source population of the plot.

To change plot parameter selections

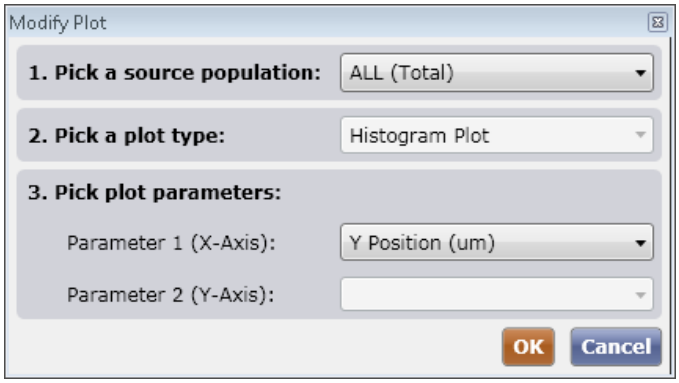
1. In the GATE tabs Plots view, click the plot for which you want to change the parameters.

2. In the plot control buttons (Figure 142 above), click the **Modify Plot Input Parameters** button (Figure 153).

Figure 153. Modify Plot Input Parameters Button

The Modify Plot dialog box appears (Figure 154).

Figure 154. Modify Plot Dialog Box



3. In Pick plot parameters, select the new parameters to be plotted.

   If you are changing the parameters of a histogram plot, you can make a selection in the Parameter 1 field only

   If you are changing the parameters of a scatter plot, you can make a selection in both the Parameter 1 field and Parameter 2 field

4. Click **OK**.

	**NOTE: If the parameters are changed, any existing gates on the plot will be deleted and any plots or logical populations that are dependent on the output populations that are defined by the gate will be reassigned to the source population of the plot.**

8.4.2 Modifying Plot Display

You can change the plot settings that you had made when creating a plot. You may change things like the plot axes for both histograms and scatter plots, but you may also adjust the size, shape, and color of the data points in scatter plots.

To change plot settings

1. Display the plot by clicking the **Plots** button.

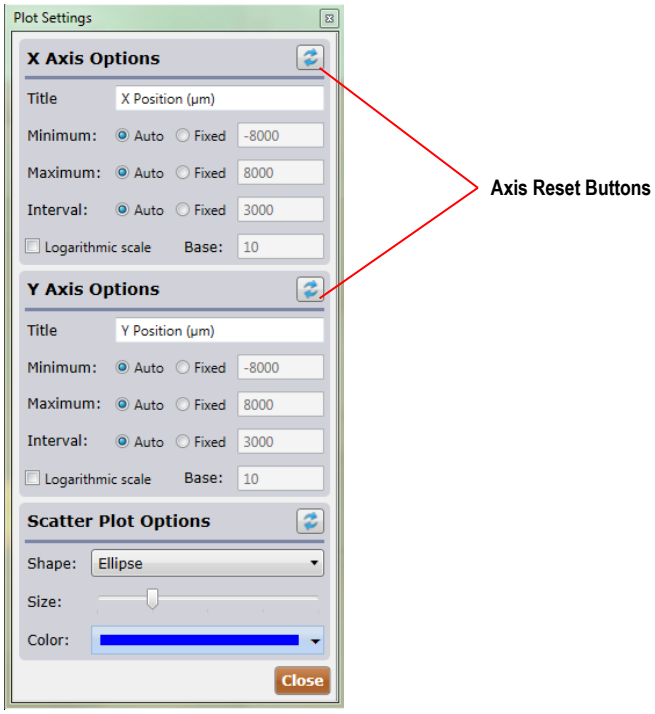
   The Plot Populations pane appears.

2. Display the plot for which you want to change the plot settings.
3. Click the plot.
4. In the plot control buttons (Figure 142 above), click the **Modify Plot Settings** button (Figure 155).

Figure 155. Modify Plot Settings Button

The Plot Settings dialog box appears (Figure 156).

Figure 156. Plot Settings Dialog Box



5. Make the following changes as needed in the X Axis Options and/or Y Axis Options sections (Figure 156):

   Title – Changes the plot Axis title

   Minimum and Maximum values:

   – Auto – Resizes the plot to the minimum and maximum automatic values
   – Fixed – Resizes the plot to the minimum and maximum fixed values
   – Field entry – Enter a value for resizing

   Interval – Specifies the axis intervals as follows:

   – Auto – Displays minimum and maximum automatic values
   – Fixed – Displays minimum and maximum fixed values
   – Field entry – Enter a value for resizing

   Logarithmic scale – Displays a logarithmic plot view

   – Base – Enter a base value for the logarithmic scale

   Axis Reset button – Click to reset the axis to automatically calculate the bounds

6. Make any changes as needed in the Scatter Plot Options section (Figure 156):

   Shape – Changes the shape of the individual plot data points between an Ellipse and a Rectangle

Size – Changes the size of the plot data points from smallest (far left) to largest (far right)

Color – Changes the color of the plot data points

8.4.3 Copying a Plot to the Clipboard

You can copy the current content of all plots in the plot view onto the clipboard. The image of the plots can be pasted into other applications. This activity will copy exactly what is displayed, at the current magnification and resolution. This including any orange selection border.

To copy a plot view to the clipboard

1. Click the **Plots** button.
2. In the plot view mode buttons (shown in Figure 142), click the **1** or **4** to display the content you want to copy as follows:

   Click **1** to view/copy a single plot

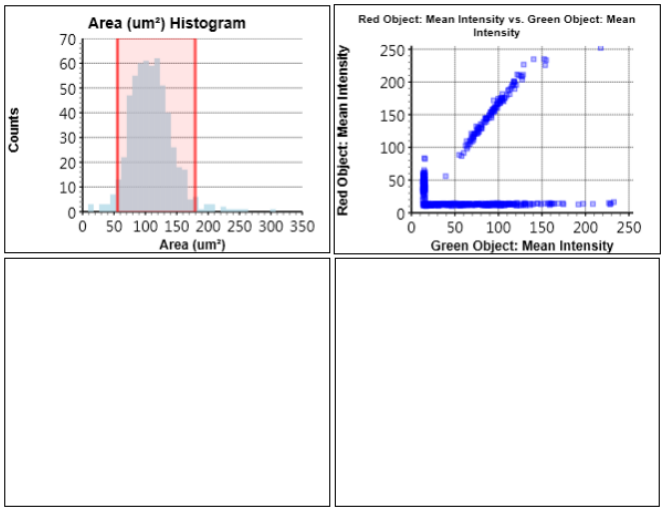
   Click **4** to view/copy all of the displayed plots (4 plots are visible at any one time)

   For additional instructions on changing the plot view, see Changing the Plot View in section 8.17.
3. In the plot control buttons (Figure 142 above), click the **Copy Plot View to Clipboard** button (Figure 157). Now you can paste the image into a program that will accept images, such as Microsoft Word.

Figure 157. Copy Plot View to Clipboard Button

Figure 158 shows the portion of the displayed Gate tab that will be copied to the clipboard (when 4 plots are visible)

Figure 158. Plot View Copied to Clipboard



8.5 Deleting a Plot

You can delete a plot. Plots or logical populations that are dependent on the output populations defined on the deleted plot will be reassigned to the source population of the plot.

To delete a plot

1. Click once on the plot to select it.

   If a 4-plot view is displayed, an orange border appears around the selected plot.

2. In the plot control buttons (Figure 142 above), click the Delete Plot (**X)** button (Figure 159).

Figure 159. Delete Plot Button

	**CAUTION: Be sure to select the correct Delete button. The Delete *Plot* button is on the right-hand side of the Plot. The Delete *Gate* button is further left.**
	**NOTE: Any plots or logical populations that are dependent on the output populations that are defined by the gate you are deleting will be reassigned to the source population of the plot.**

8.6 Creating a Gate

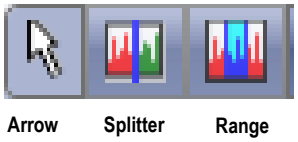
A gate is an area that you define to isolate subsets of data. You can create one or more gates on a plot.

The output of a gate on a plot is a concrete population, also simply called a population. In addition to concrete populations, you can define logical populations based on combinations of concrete and/or logical populations. For instructions on defining a logical population, see section 8.11.1.

8.6.1 Creating a Gate on a Histogram Plot

Create a gate on a histogram plot using the gate selection tools for histogram plots (Figure 160).

Figure 160. Gate Selection Tools for Histogram Plots



Arrow tool – Use to select the current gate to modify its position, size, or shape, or to delete it

Splitter tool – Adds boundary lines between populations

Range tool – Creates one or more areas to select a range of values

To create a gate on a histogram plot

1. Click the splitter or range tool.
2. If you selected the **splitter tool**: Left-click the cursor arrow over plot area of interest.

   When you have completed the selection process, a single red line appears in the middle, two (or more if more splits are used) colors are displayed, and a Pop section appears for that population in the Plot Populations pane (Figure 161).
3. If you selected the **range tool**: Left-click and drag the cursor arrow over plot area of interest.

   When you have completed the selection process, a colored, shaded area appears with a red colored line on each side of the selected area, and a Pop section appears for that population in the Plot Populations pane (Figure 161).

The shading color on the plot corresponds to the Color field for that population in the Plot Populations pane. The same color is shown in the color overlay on the image.

The data in the Populations view updates in real time as you select or change the selection area in the Plots view.

Figure 161 shows a histogram plot with four subpopulations selected using the splitter tool multiple times.

Figure 161. Splitter Tool Selections Made on a Histogram

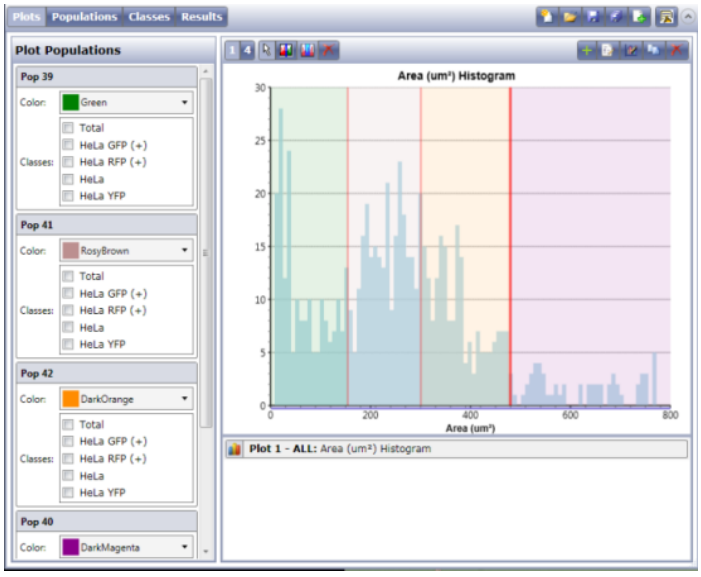
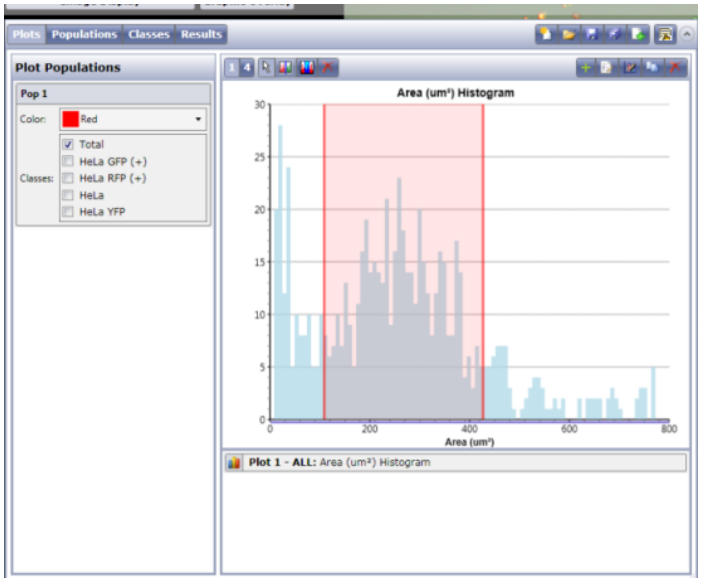


Figure 162 shows a histogram plot with one subpopulation selected using the range tool.

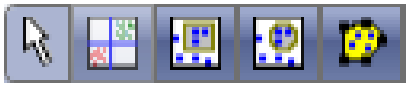
Figure 162. Range Tool Selections Made on a Histogram



8.6.2 Creating a Gate on a Scatter Plot

Create a gate on a scatter plot using the gate selection tools for scatter plots (Figure 163).

Figure 163. Gate Selection Tools for Scatter Plots



Arrow tool – For selecting the current gate to modify its position, size, or shape, or to delete it

Quadrant tool – Creates a four-quadrant gate

Rectangle tool – Creates one or more square or rectangular gates

Ellipse tool – Creates one or more circular or elliptical gates

Polygon tool – Creates a free-form gate

To create a gate on a scatter plot

1. Click one of the four tools (Quadrant, Rectangle, Ellipse, or Polygon).
2. Left-click while dragging the cursor over area of interest on plot.

   When you have completed the selection process, a colored, shaded area appears, and a Pop section appears for that population in the Plot Populations pane.

   The shading color on the plot corresponds to the Color field for that population in the Plot Populations pane. The same color is shown in the color overlay on the image.

   The data in the Populations view updates in real time as you select or change the selection area in the Plots view.

3. In the Plots tab, in each Pop section, click a Classes checkbox to identify the class you want to associate with that population.

   Figure 164 shows a scatter plot with three subpopulations selected using the rectangle, ellipse, and polygon tools. The figure also shows the Classes checkbox selected for each population.

Figure 164. Rectangle, Ellipse, and Polygon Tool Selections Made on a Scatter Plot

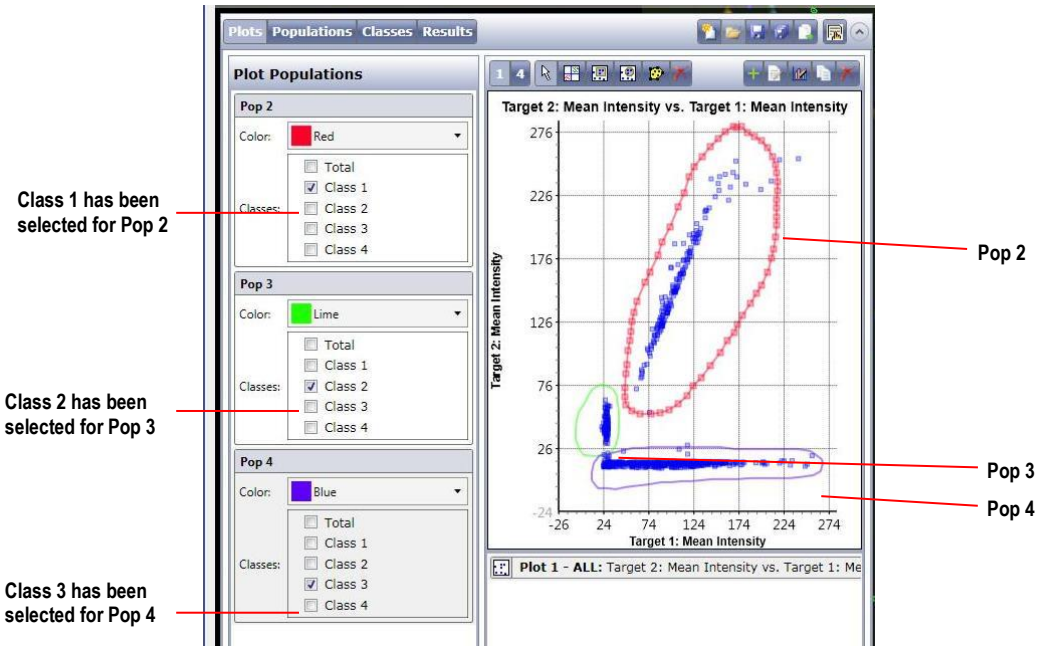
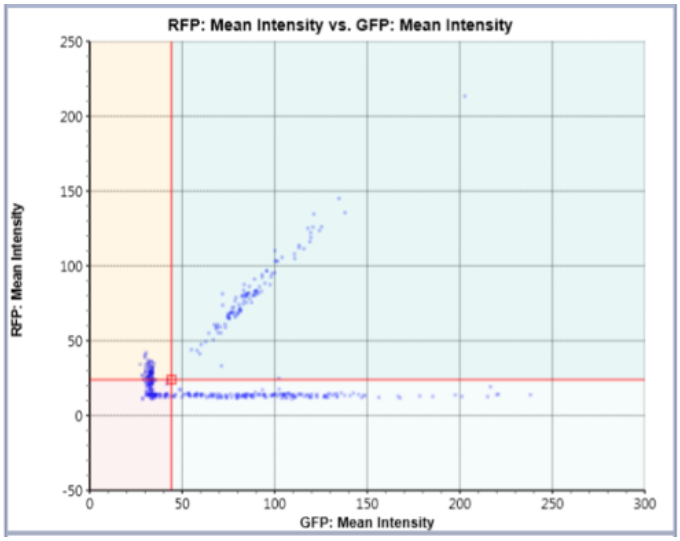


Figure 165 shows a scatter plot with four subpopulations selected using the quadrant tool.

Figure 165. Quadrant Tool Selections Made on a Scatter Plot



8.7 Changing a Gate

8.7.1 Changing a Gate with a Mouse

You can move or change the shape of a gate with the use of a mouse.

To change a gate

1. In the gate selection tools (Figure 142 above), make sure that the Arrow tool is selected.
2. Click once on any part of the plot (histogram plot or scatter plot) to select it.

   A thick border appears around the plot.
3. Do any of the following as needed:

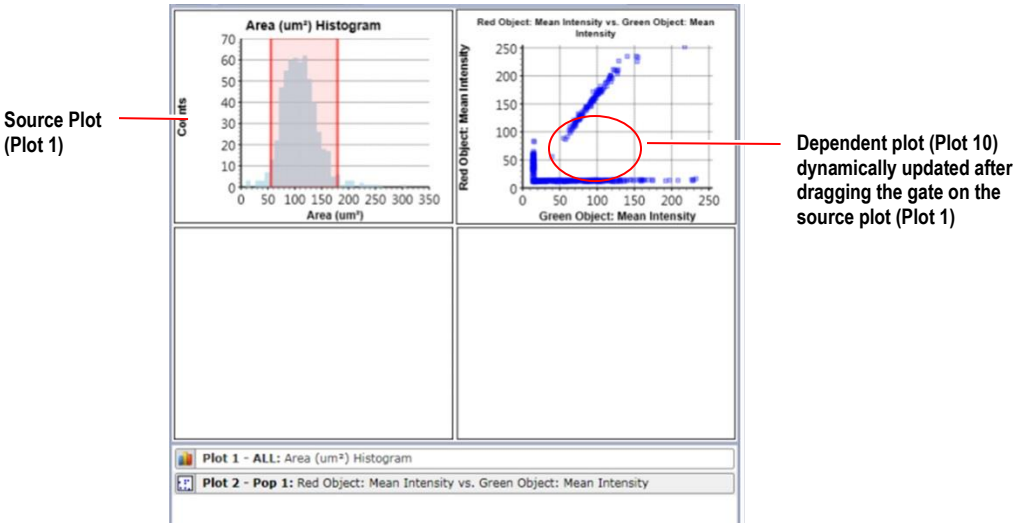
   To resize an area, click the gate border and then click and drag.

   To move an area without resizing it, click and drag inside the gate border.

   Changing the gate area of a plot automatically changes the plots, populations, and classes that you have associated with it (made dependent on it).

   In the example in Figure 166, dragging the gate on Plot 1 caused the system to update Plot 10 in real time.

Figure 166. Changing the Gate on a Source Plot Changes Any Dependent Plots



8.7.2 Changing a Gate with Explicit Values

You can move or change the shape of a gate with explicit values.

To change a gate

1. In the gate selection tools (Figure 166 above), make sure that the Arrow tool is selected.
2. Double click on the area in the plot covered by the gate you want to modify.

3. An Edit Gate Bounds dialog will appear. Modify the values in this dialog to the new values you want to have for the gate and click **OK** to perform the modification. Click **Cancel** to keep the gate bounds at the current settings.

   Each gate will have a different dialog appear to modifying the gate bounds. The following section summaries the options available for each gate:

   Splitter – The dialog for modifying splitter gates allows you to modify all splitters for the plot at once. Each splitter contains a single X value that is limited by the bounds of the splitter before and after it. (See Figure 167 and Figure 168 below)

Figure 167. Splitter

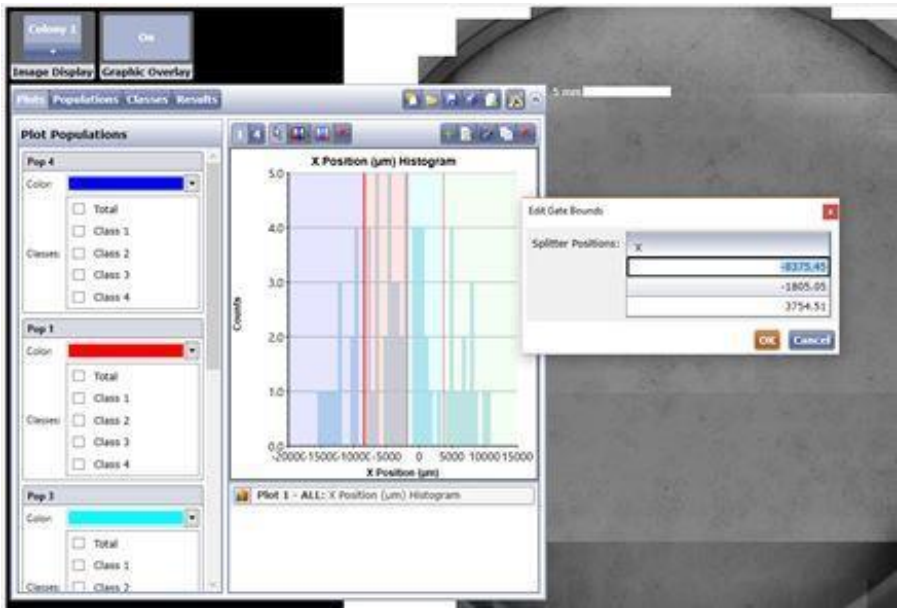
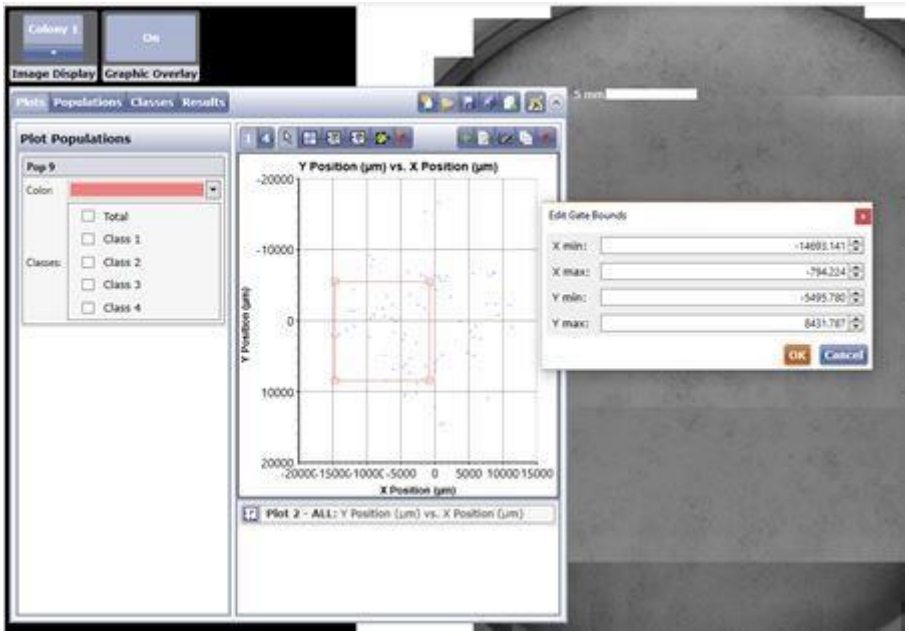


Figure 168. Rectangle



Range – The dialog for modifying a range gate allows you to modify the X minimum and maximum values of the gate.

Quadrant – The dialog for modifying a quadrant gate allows you to modify the X and Y coordinate of the gate.

Rectangle – The dialog for modifying a rectangle gate allows you to modify the X minimum and maximum and Y minimum and maximum values of the gate.

Ellipse – The dialog for modifying an ellipse gate allows you to modify the X minimum and maximum and Y minimum and maximum values of the gate.

	**NOTE: Explicit value modification of gates is not supported for polygon gates.**

8.8 Deleting a Gate

You can delete a gate from a plot. When you do this, any plots or logical populations that are dependent on the output populations that are defined by the gate being deleted will be reassigned to the source population of the plot.

To delete a gate

1. In the gate selection tools (Figure 142 above), make sure that the Arrow tool is selected.
2. Click once on the gate to select it.

   If you are changing a gate on a histogram plot, a thick border appears around the plot. If a scatter plot, a border with squares appears around the plot.
3. In the gate selection tools, click the Delete Gate (**X)** button (Figure 142 above, Figure 169, and Figure 170).

Figure 169. Delete Gate Button for a Scatter Plot

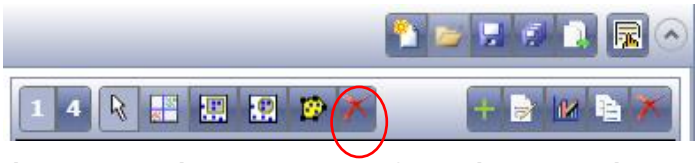


Figure 170. Delete Gate Button for a Histogram Plot

	**CAUTION: Be sure to select the correct Delete button. The Delete *Plot* button is further right.**

	**NOTE: Any plots or logical populations that are dependent on the output populations that are defined by the gate you are deleting will be reassigned to the source population of the plot.**

8.9 Viewing Population Details

You can view the following types of population details:

Name – Name of the plot. Allows for editing by clicking in the text box and changing the name.

Source – If the population was created by a gate, the name of the plot that the gate is defined within is displayed. If the population is a logical population, the logical expression that defines the population is displayed.

Statistics – Average, Standard Deviation, Min (Low) and Max (High) value for each of the available parameters in a single well.

Count – The number of items in the population.

Object Level Data – The values for each of the available parameters for each object in the population.

To view population details

1. Click the **Populations** button.

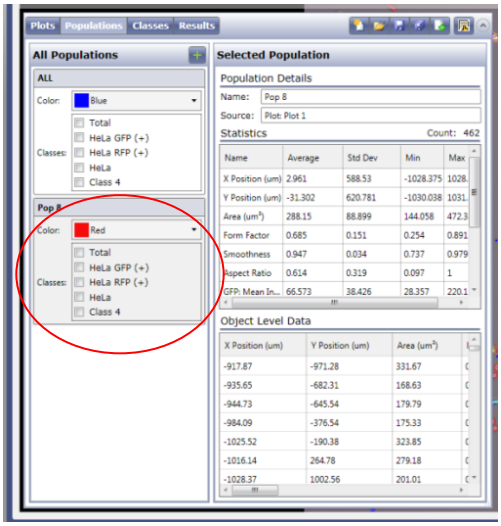
   The All Populations pane appears with a list of all existing populations (outputs of gates).

2. In the All Populations pane, locate the population name for which you want to view the details, and click anywhere on the title bar for that population bar or inside that section.

   The selection highlights and a Selected Population pane will appear for the selected pane, with the Population Details.

   In the example in Figure 171, the user wants to view the details for Pop 8 population and therefore has selected the Pop 8 pane.

Figure 171. Viewing Population Details



For instructions on viewing the data associated with a selected object (cell) and vice versa, see Viewing Object-level Data in section 8.14.

8.10 Changing the Color for a Population

You can change the color that represents the population on the plot and in the Graphic Overlay display.

To change the color for a population

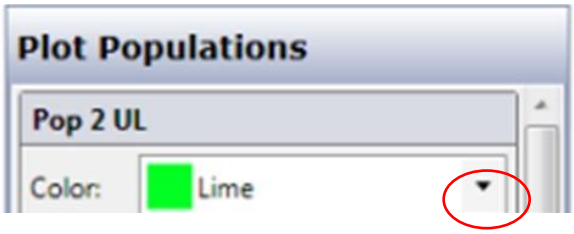
1. Click the **Populations** button.

   The All Populations pane appears with a list of all existing populations (outputs of gates).

2. In the All Populations pane, locate the population name that has the color you want to change.

3. Click the Color menu (Figure 172) next to the color you want to change.

Figure 172. Changing the Color for a Plot Population



4. In the resulting color tools display, select a different color.

   Use the color tools for selecting the color of a population the same way that you use them for selecting the color of an image display in the Gate tab and other tabs. For the details on using the color tools, see Selecting Colors and Overlays in section 7.2.1.2.

8.11 Changing a Population Name

You can change the name of a population (the output of a gate) to help you distinguish population names from each other.

To change a population name

1. Click the **Populations** button.

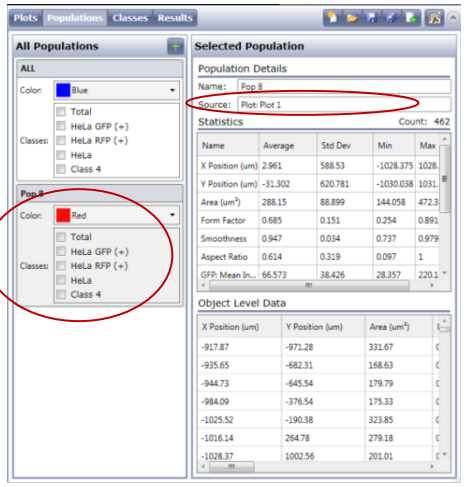
   The All Populations pane appears with a list of all existing populations (outputs of gates).

2. In the All Populations pane, locate the population name you want to change, and click anywhere on the title bar for that population bar or inside that section.

   The selection highlights and a Selected Population pane will appear for the selected pane, with editable fields.

   In the example in Figure 173, the user wants to change the name of the Pop 8 population and therefore has selected the Pop 8 pane.

Figure 173. Changing a Population Name



3. In the Name field, type the desired name for the population.

8.11.1 Defining a Logical Population

You can assign an identity to a population set, called a logical population. To do this, you make syntax selections, such as AND, OR, XOR, and NOT. The newly defined logical population is added to the list of existing populations.

One use for logical populations is to define a logical population as the sum of multiple populations. You can then assign a class to that group of populations.

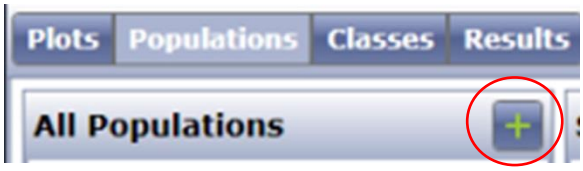
To define a logical population

1. Click the **Populations** button.

   The All Populations pane appears with a list of all existing populations (outputs of gates).

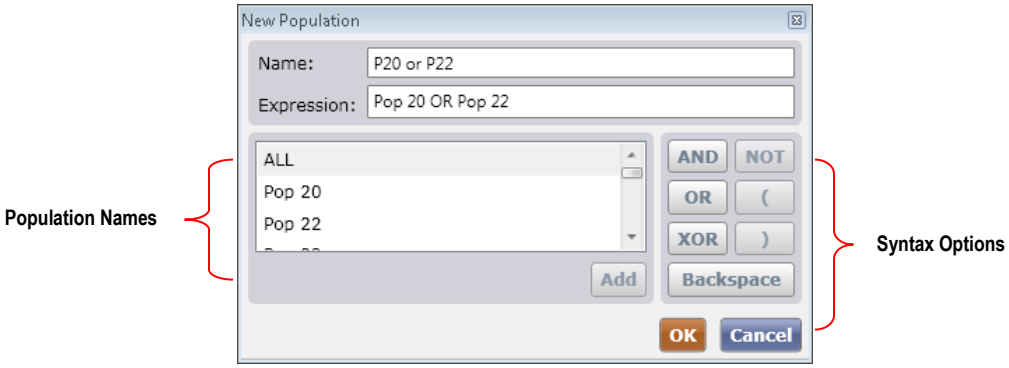
2. At the top of the All Populations pane, click the **Add Logical Population** button (**+**) (Figure 174).

Figure 174. Add Logical Population Button



The New Population dialog box appears (Figure 175).

Figure 175. New Population Dialog Box



3. Fill in the Name field by typing a name for the logical population, such as **P20 or P22**.
4. Fill in the Expression field as follows:
   a. Click in the Expression field.
   b. In the bottom left pane, double-click a population name.
   c. In the right-hand pane, click a syntax option as needed.
5. Click **OK**.

   A new population is then generated and its name appears in the All Populations pane.

8.11.2 Deleting a Logical Population

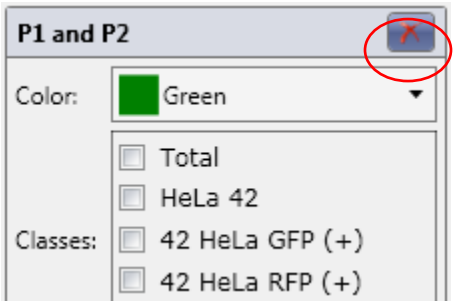
You can delete a logical population. If you do this, any plots that are dependent on this logical population will be reassigned to the source population(s) of the logical population.

To delete a logical population

Do one of the following:

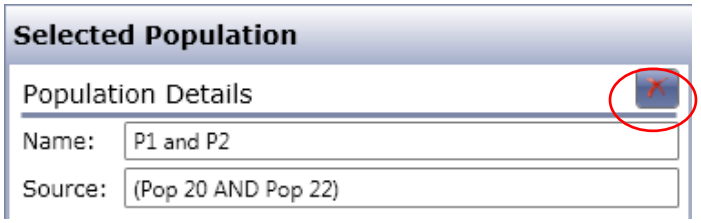
In the All Populations Pane, in the pane for the logical population, click the (**X**) button, deletes the Population (Figure 176).

Figure 176. Delete Button in the All Populations Pane



In the Selected Populations pane, click the logical population and then click the (**X**) button next to Population Details, deletes the Population (Figure 177).

Figure 177. Delete Button in the Selected Population Pane with Logical Population Selected



NOTE: An X appears in a Selected Population pane only if the population is a logical population.

8.12 Viewing Class Displays

You can view the following type of class displays:

A list of classes associated with all of the populations

A class details display for a selected class

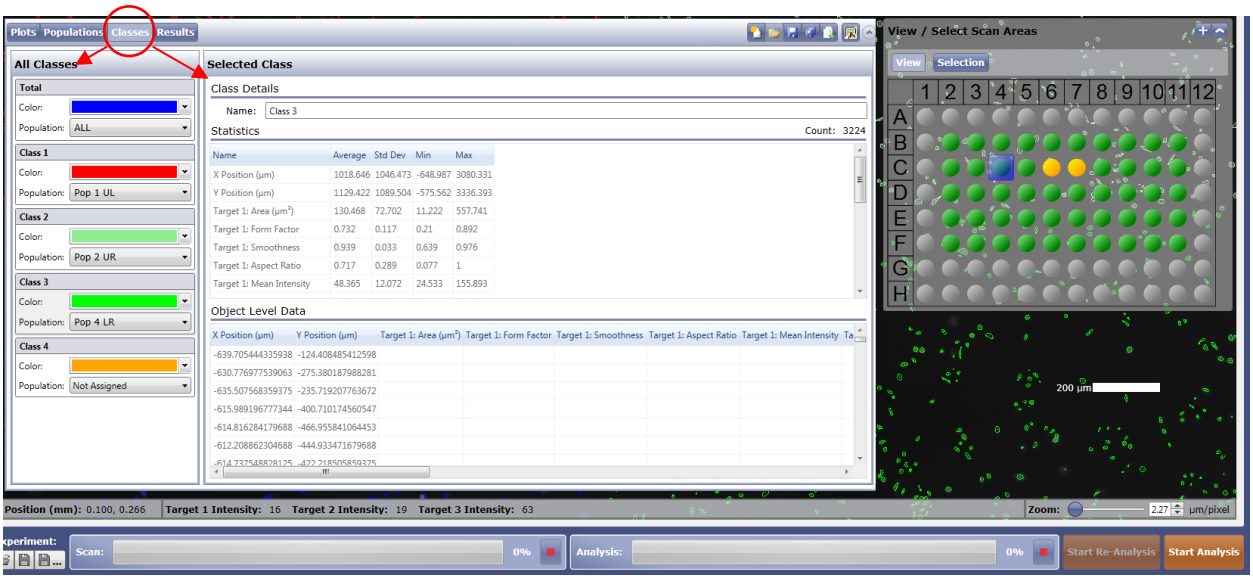
- Name – Name of the class
- Statistics – Shows the Average, Standard Deviation, Min (Low) and Max (High) value for each of the available parameters in a single well
- Object Level Data – Shows the number of items in the class (Count) and the values for each of the available parameters for each object in the class.

To view class displays

1. Click the **Classes** button.

   The Classes view appears (Figure 178).

Figure 178. Classes View



2. In the All Classes pane, click the population for which you want to view class information.

   The class information for the selected population appears in the Selected Class pane.

3. In the Statistics or Object Level Data sections, if you want to sort the columns, click the heading of a column by which you want the others to sort.

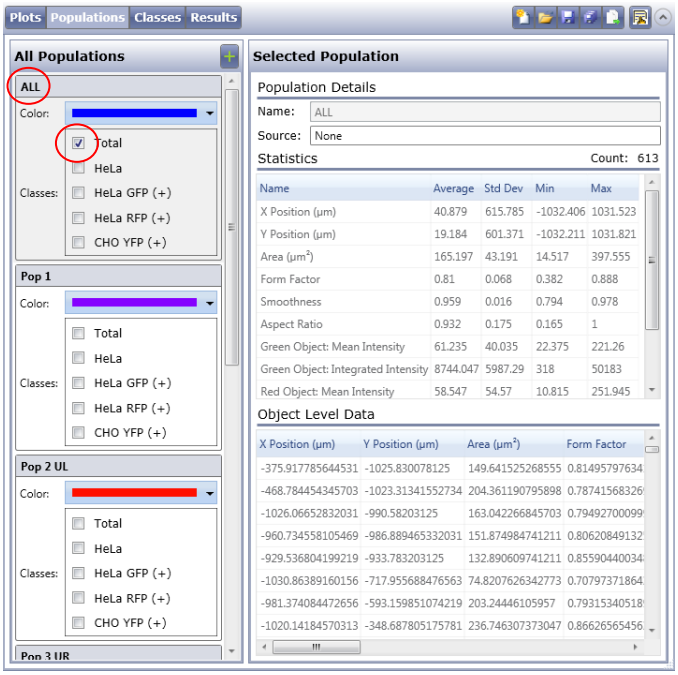
For instructions on viewing the data associated with a selected object (cell) and vice versa, see Viewing Object-level Data in section 8.14.

8.13 Assigning a Class to a Population

You can assign which population (outcome of a gate) is the source for a class. You can do this in the Plots, Populations, or Classes views. After processing the plate, the Results tab reports the parameters for only those classes you have assigned.

When you first create a plot, the system by default assigns the class called Total to the population for the entire well, called the ALL population, as shown in Figure 179. The syntax of ALL (Total) is shown in Figure 146 in section 8.3.

Figure 179. Total Class



You can reassign any class to any population. You can assign multiple classes to a single population.

8.13.1 Assigning a Class in the Plots View

To assign a class in the Plots view

1. If the plot of interest is not displayed, display it by clicking the **Plots** button.

   The Plot Populations pane appears.

2. Click the gate that contains the objects that you want to classify.

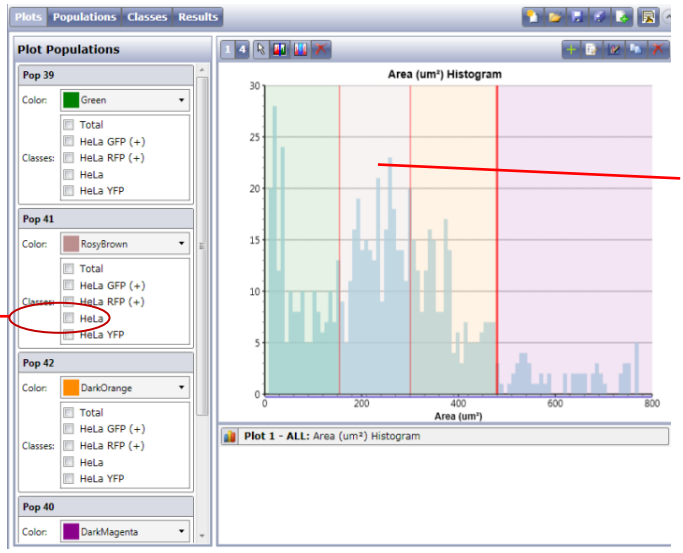
   A border appears around the selected gate and the associated Population name highlights in the Plot Populations pane.

3. In the Plot Populations pane, locate the section for the population that you want to classify.

4. Checkmark the Classes checkboxes class that you want to assign to the population.

   The example in Figure 180 is for assigning a class to Pop 41.

Figure 180. Assigning a Class in the Plots View



8.13.2 Assigning a Class in the Populations View

To assign a class in the Populations view

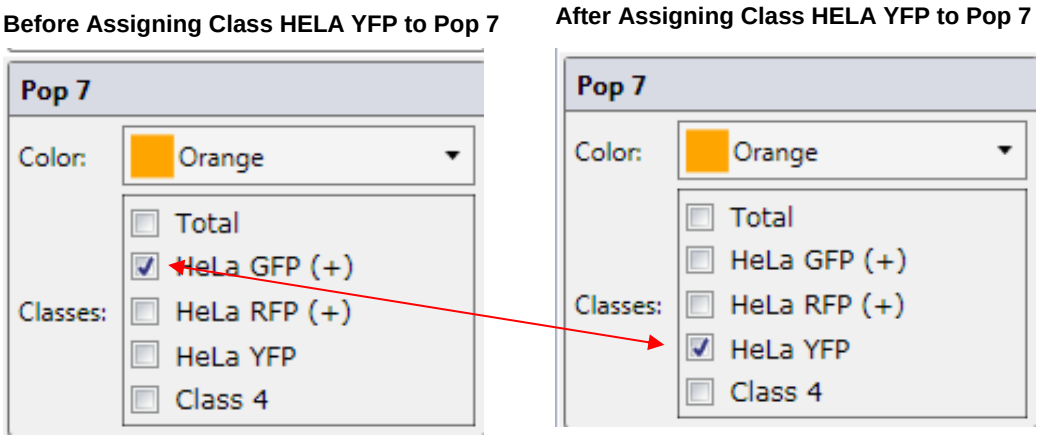
1. Click the **Populations** button.

   The All Populations pane appears.

2. In the All Populations pane, locate the name of the population to which you want to assign one or more classes.

3. In the population section, checkmark the Classes checkboxes that you want to assign to the population (Figure 181).

Figure 181. Assigning a Class in the Populations View



8.13.3 Assigning a Class in the Classes View

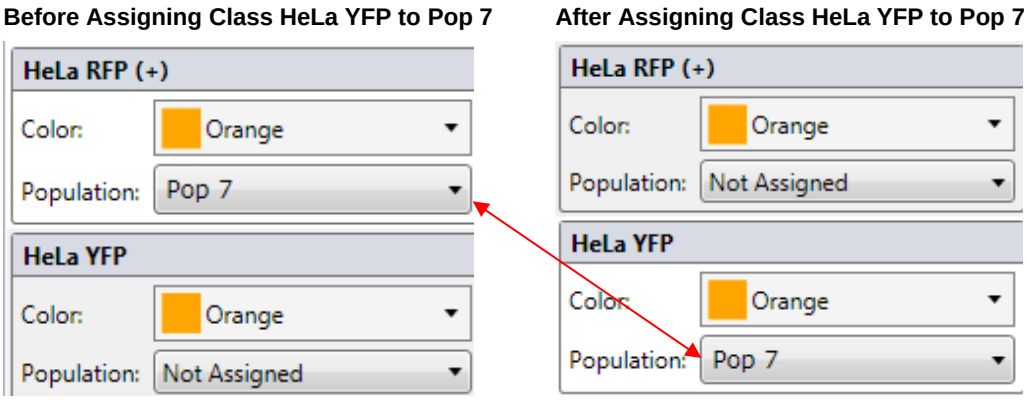
To assign a class in the Classes view

1. Click the **Classes** button.

The All Classes pane appears.

2. In the All Classes pane, locate the section for the class that you want to assign to a population.
3. In the Population menu for the class, select the population to which you want to assign the class (Figure 182).

Figure 182. Assigning a Class in the Classes View



8.14 Viewing Object-level Data

You can select a cell (object) for viewing its associated data and vice versa. You select a population for which you want to view the object-level (cell) data.

8.14.1 Viewing an Object (Cell) Associated with Selected Data

You can view an object (cell) associated with selected data

To view an object (cell) associated with selected data

In the Populations or Classes view, do the following as needed:

1. In the All Populations or All Classes pane, click a population (such as Pop 1).

   A border appears around the selection.

2. In the Object Level Data pane, click a data line item.

   A border appears around the selected data line item. In the displayed image, a square appears around the object associated with the selected population and data.

8.14.2 Viewing Data Associated with a Selected Object (Cell)

You can view data for a selected object (cell).

To view data associated with a selected object (cell)

In the Populations or Classes view, do the following as needed:

1. In the All Populations or All Classes pane, click a population (such as Pop 1).

   An orange border appears around the selection.

2. In the displayed image, click an object (cell).

   An orange border appears around the selected object. If the selected object is within the population selected in step 1, in the Object Level Data pane an orange border appears around the data line item associated with the selected object.

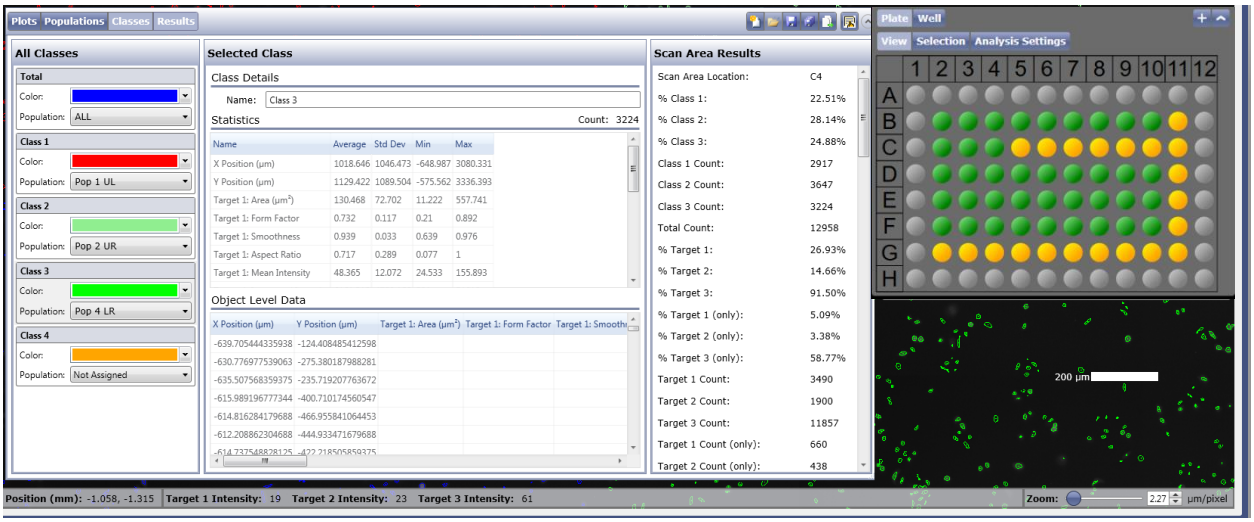
8.15 Viewing Scan Results at the Well Level

You view scan results at the well level in the Gate tabs Results view. When you do this, you are viewing the results of your classification settings. In the Gate tabs Results view, the term scan results refer to the data you are viewing at the well level in real-time, as you select each well. In the other Results tab (described in chapter 9), you view scan results at the plate level after scanning is complete.

To view scan results at the well level

1. Click the Gate tabs Results button (Figure 183).

Figure 183. Gate Tabs Results View



The plots remain displayed on screen and a Scan Area Results pane appears.

2. In the View/Select Scan Areas section, make sure that the View button is selected.
3. Click the well for which you want to view scan area results (data).

   The Scan Area Results pane data changes to reflect the new well selection, and displays the scan results associated with the selected well. Data is presented for each class (including the Total class) that you assigned to a population in section 8.13

8.16 Resetting Classification Settings

You can reset classification settings to the applications default classification settings.

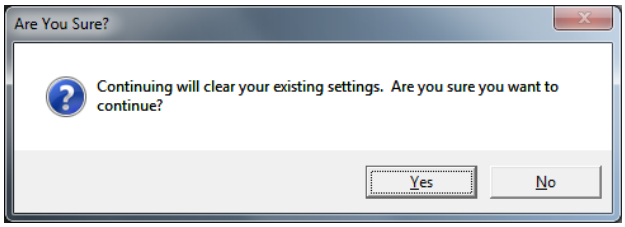
To reset the classification settings

In the Classification settings buttons click the **New Classification Settings** button (Figure 184).

Figure 184. New Classification Settings Button

Clicking the New Classification Settings button will present the new classification settings warning dialog to ensure that the requested operation was desired and not accidental. Click Yes to confirm.

Figure 185. New Classification Settings Warning Dialog



Any gate selections will be removed from the display, and the associated classification settings are reset to the default that the current application specifies.

8.17 Changing the Plot View

You can toggle between a single plot view and multiple-plot views. The multiple-plot view displays up to four plots at a time. You can replace any of the four displayed plots with any in the plot list.

To change the plot view

1. In the plot view mode buttons (Figure 186) above the plot, click one of the following:

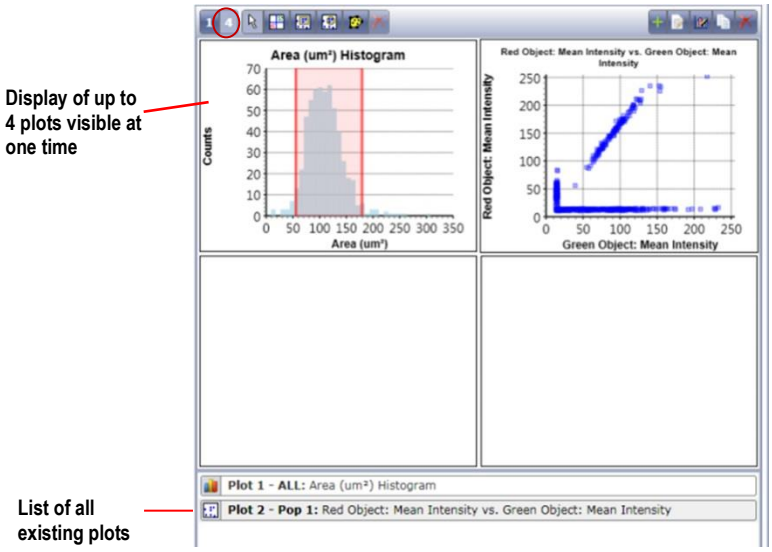
Figure 186. Plot View Mode Buttons

1 – Single-plot view

4 – Multiple-plot view

Up to four plots appear. Figure 187 shows a multiple-plot view.

Figure 187. Multiple-Plot View Selected



2. If more than four plots are listed in the plot list below the plots, view the remaining plots as follows:
   a. Click any displayed plot.
      An orange border appears around the selected plot.
   b. In the plots list, click the name of a plot that you want to display.
      The plot whose name you selected appears in the display, replacing the plot with the orange border.

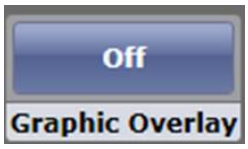
8.18 Using the Image Display and Graphic Overlay Buttons in the Gate Tab

You use the Image Display and Graphic Overlay buttons in the Gate tab the same way as in the Analyze tab, with the exception that the Gate tab has a single Graphic Overlay button that toggles on or off (Figure 188) instead of an on/off button for each feature. For detailed instructions on using the Image Display buttons, see Using the Image Display and Graphic Overlay Buttons in the Analyze tab, section 7.2.1.

Figure 188. Graphic Overlay Button in the Gate Tab

Graphic Overlay On

Graphic Overlay Off



8.19 Changing the Names of the Image Display and Graphic Overlay Buttons in the Gate Tab

You can change the Image Display and Graphic Overlay button names to customized names in the Gate tab.

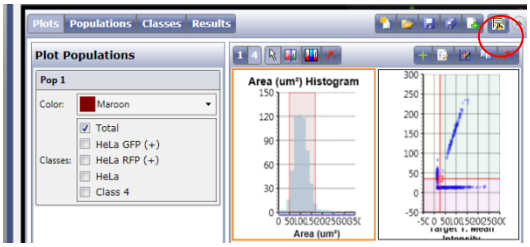
	NOTE: This functionality is available in the Expression Analysis application only.

To change the names of the Image Display and Graphic Overlay buttons in the Gate tab

1. In the Gate tab, click the Customize Application button (Figure 189).

	NOTE: This button is available in the Expression Analysis application only.

Figure 189. Customize Application Button in the Gate Tab



The Customize Application dialog box appears.

2. The remaining steps are the same as in the Scan tab. For instructions, see section 6.2.1.

8.20 Saving an Experiment or Project in the GATE Tab

When you save an experiment or project to the database in the GATE tab, you are recording a sort of snapshot of all the settings that currently exist in the Scan, Analyze, and Gate tabs. This file can be re-called for another scan.

A project includes the saved experiment, plate type and export options selected.

In the GATE tab, when you save an experiment or project, you are saving it to the database (not to the hard drive).

For an overview of the save button locations and functions in the various tabs, see section 6.10.

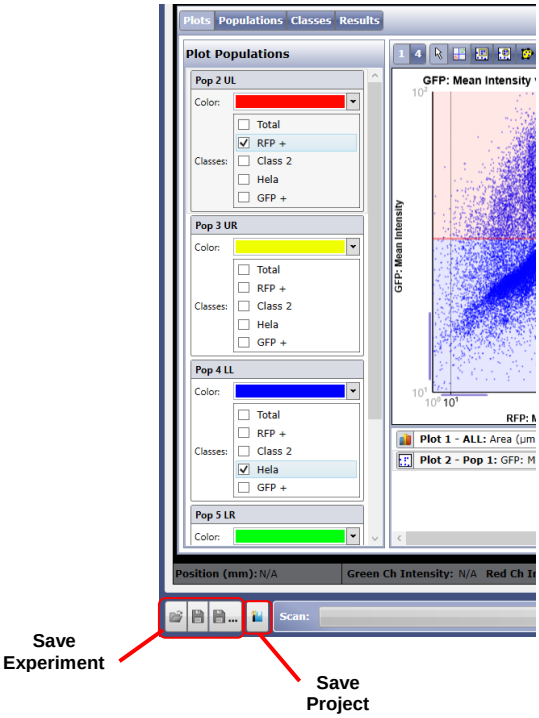
8.20.1 Saving an Experiment to the Database in the GATE Tab

You save an experiment to the database in the GATE tab using the Experiment Save-to-the-Database button (Figure 190). This is the same way as in the SCAN and ANALYZE tabs. For detailed instructions, see Saving an Experiment to the Database in the SCAN Tab, section 6.10.1.1.

8.20.2 Saving a Project to the Database in the GATE Tab

You save a project to the database in the GATE tab using the Project Save-to-the-Database button (Figure 190). This is the same way as in the SCAN and ANALYZE tabs. For detailed instructions, see Saving a Project to the Database in the SCAN Tab, section 6.12.

Figure 190. Save-to-the-Database Buttons in the GATE Tab



8.21 Saving Classification Settings in the GATE Tab

In the GATE tab, you can save classifications settings to the database or the hard drive.

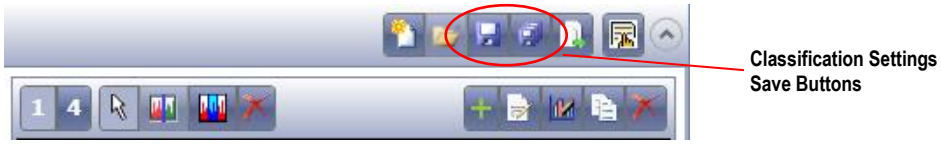
For an overview of the save button locations and functions in the various tabs, see section 6.10.

8.21.1 Saving Classification Settings to the Database in the GATE Tab

To save classification settings to the database in the Gate tab

1. In the Classification Settings buttons at the top of the Gate tab, click the **Save** or **Save As** button (Figure 191).

Figure 191. Classification Settings Save-to-the-Database Buttons in the Gate Tab



A Specify Settings Name dialog box appears (Figure 131), with the Folder field displaying the default entry (logged-in user folder).

2. In the Settings Name field, enter a name for the classification settings.

   If the settings name is unique, the OK button becomes available.

3. In the Folder field, select a different destination folder for the settings file as needed.

4. Click **OK**.

   The system saves the settings name.

8.21.2 Exporting Classification Settings to the Hard Drive in the GATE Tab

When you use the GATE tab to export classification settings, you are exporting to a hard drive in XML format.

	**NOTE: You can also use the Data Management window to export classification settings in XML format. For instructions, see the Celigo Cytometer Administrator Guide section titled Exporting Classification Settings in the Data Management Tab.**

To export classification settings to the hard drive in the Gate tab

1. In the Classification Settings buttons at the top of the Gate tab, click the **Classification Settings Export** button (Figure 192).

Figure 192. Classification Settings Export Button

2. The remaining steps are the same as when exporting analysis settings in the Analyze tab. For instructions, see Analyze Tab section 7.11.

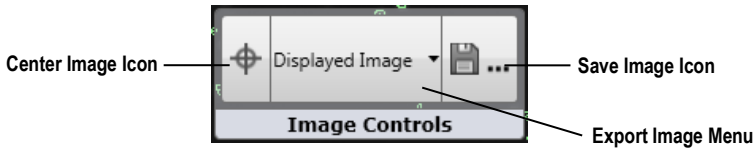
8.22 Centering an Onscreen Image in the GATE Tab

You can center an onscreen image in the GATE tab. Doing this does not change the raw images.

To center an onscreen image in the Gate tab

In the Image Controls section (Figure 142 above and Figure 193) click the Center Image icon.

Figure 193. Image Controls in Gate Tab



8.23 Exporting an Onscreen Image in the GATE Tab

In the Gate tab, you can export a selected well image as seen onscreen (do a screenshot), including any applied pseudo-coloring and/or graphic overlays displayed.

To export an onscreen image in the Gate tab

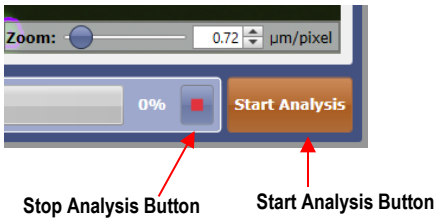
In the Image Controls section (Figure 193 above), click **Save Image**.

The remaining steps for exporting the image are the same as when exporting the same type of image in the Results tab, with the following exception: In the Gate tab, select from channel color selections in the Displayed Image menu to the left of the Save Image icon (Figure 193). For detailed instructions, see Exporting an Onscreen Image in the Results Tab in section 9.7.3.

8.24 Analyzing a Scan in the GATE Tab

You can analyze a scan using the GATE tabs Start Analysis button (Figure 194) the same way as in the Analysis tab. For detailed instructions, see Analyzing a Scan in the Analysis Tab in section 7.14.1.

Figure 194. Start Analysis Button in the Gate Tab



8.24.1 Stopping Analysis in the GATE Tab

If you need to stop analysis that is in process (green progress bar displayed), you can stop it in any tab. The instructions are the same as described for the Analyze tab. They are repeated here for your convenience.

To stop analysis

Click the red stop button at the bottom right side of the tab (Figure 194).

After stopping analysis, you can only start a new analysis, not resume the existing analysis.

8.24.2 Restarting Analysis in the GATE Tab

After stopping analysis, you can only start a new analysis, not resume the existing analysis. You can restart analysis in the ANALYZE or GATE tabs. The instructions are the same as described for the ANALYZE tab.

To restart a stopped analysis in the GATE tab

Click **Start Analysis** (Figure 194). A file will be created with the same filename as the stopped scan, and will have a new timestamp.

Continue to Results Tab chapter 9.

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9. Results Tab

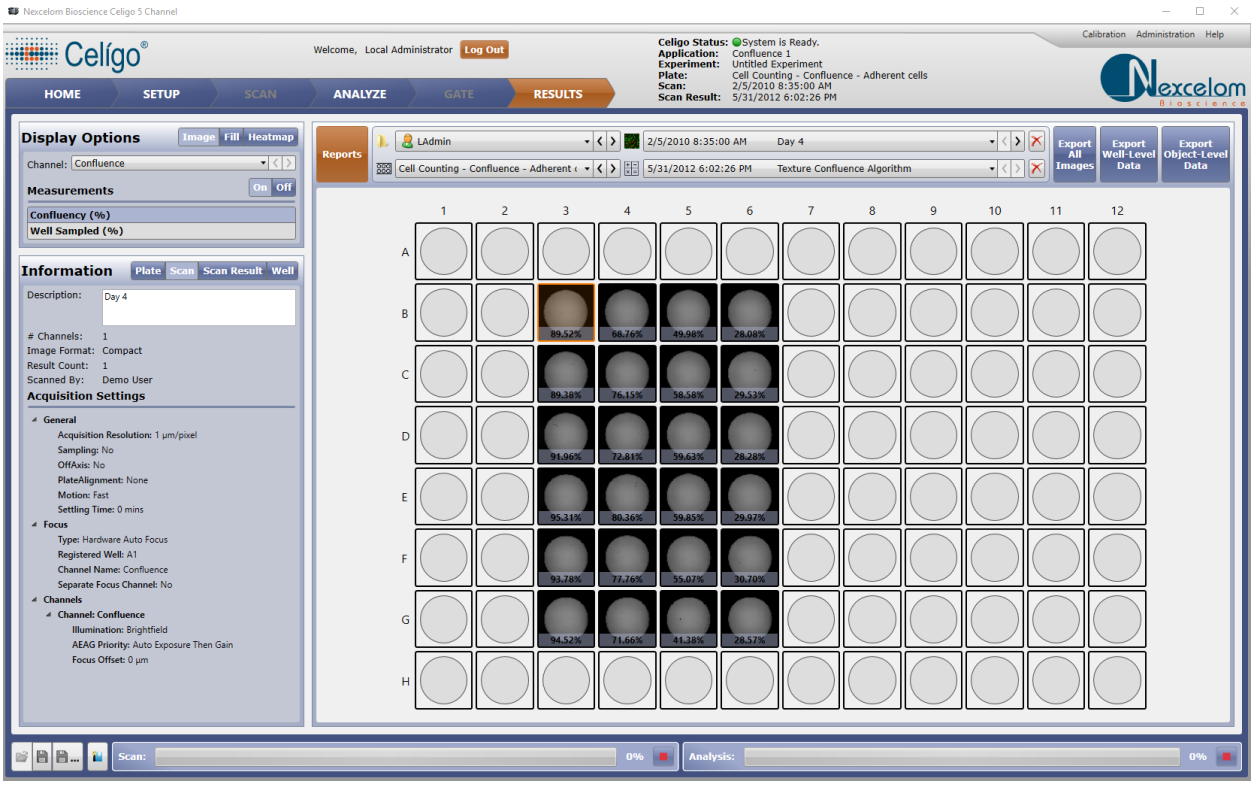
Whenever the Results tab is selected (Figure 195), the currently loaded scan appears by default. A plate-level view appears with thumbnail images of the wells as the plate is being scanned. Data can be selected for view in this tab, even as scanning continues.

From the plate-level view, you can choose to view a list of all scans (view all scans) and/or investigate the scan results for each scan. You can also investigate the scan results for a scan from the list or change to any other plate the user has access to.

Each plate ID is associated with one or more scan IDs. Each scan ID is associated with one or more sets of data, called scan results.

NOTE: The displays you see onscreen may differ from the figures in this document, depending on the application you have selected in Current Application.

Figure 195. Results Tab Plate-Level View



9.1 Viewing Scan Results

When you view scan results, you are viewing the results of an analyzed scan. The results are in the form of scans (images) and analyzed data (scan results) from the scans. Scans have timestamps independent of the scan result timestamps, reflecting the time of scanning versus data analysis.

When you display the Results tab, the currently loaded scan appears by default. You then make selections as needed to change displayed images.

Viewing scan results consists of selecting scans for viewing, and selecting display options for changing the displays. You can view scan results in an overview display containing thumbnail images, called a plate-level view, and in a magnified, close-up display, called a well view.

9.1.1 Viewing Scans and Scan Results in the Plate-Level View

To select a different scan and/or scan result in the plate-level view

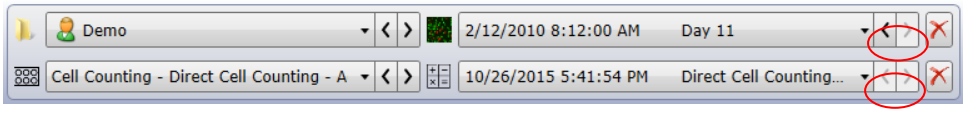
Do either of the following:

Select the Scan menu and/or Scan Result menu pulldown menu arrow (Figure 196).

Click the **<** or **>** arrow to the right of the Scan menu and/or Scan Result menu (Figure 196) for a sequential display in timestamp order. If an arrow is disabled, that means there are now additional items to view in that direction (indicating that you are at the first, last, or only item in the list).

In this figure, the selections are for a plate scanned at 2/12/2010 8:12:00 AM start time and then analyzed at 10/26/2015 5:41:54 PM start time.

Figure 196. Selecting a Scan and/or Scan Result

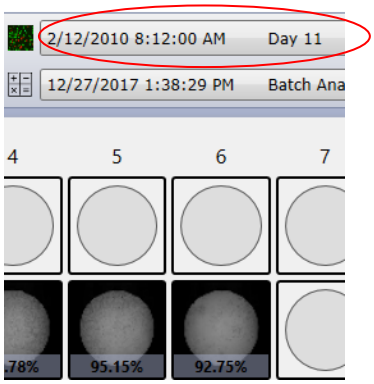


	NOTE: Selecting a different Scan automatically defaults to the associated Scan Results with the most recent timestamp for the current application being viewed. If no Scan Result exists with the same application, then the most recent timestamp is viewed.
	NOTE: The < and > arrows will be greyed out if only a single scan or scan result is available for selection.

Figure 197 shows an example of selecting a scan.

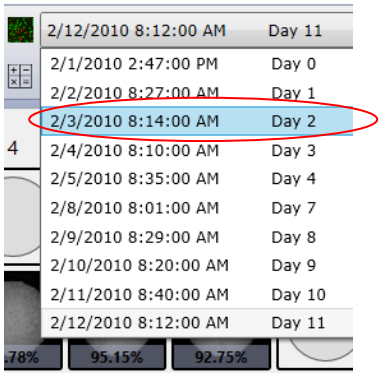
Figure 197. Changing Scan Selection in RESULTS tab

Before Scan Menu Selection



The displayed images were *scanned* at this start time.
The displayed images were *analyzed* at this start time.

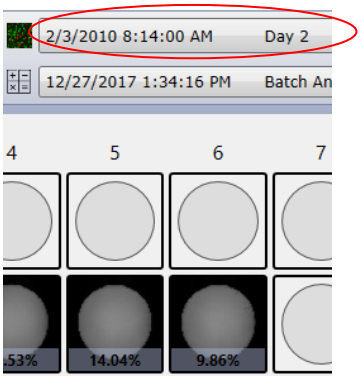
Making Scan Menu Selection



Drop down menu displays all scan time.

Timestamps from multiple scans of the same plate

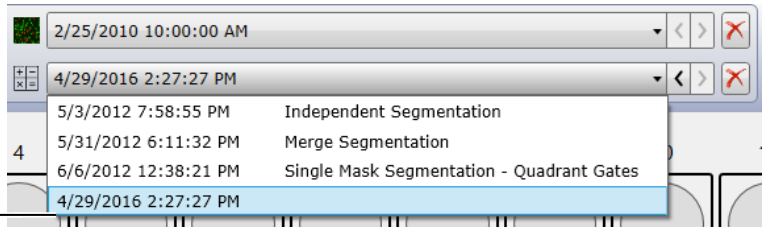
After Scan Menu Selection



The new Scan time selected is displayed and thumbnails updated with image and data.

Figure 198 shows an example of selecting scan results (data associated with a scan).

Figure 198. Scan Result Menu and Arrows in the Plate-Level View



The system automatically then displayed the most recent analysis timestamp (Scan Result) for the Scan you selected.

9.1.2 Viewing a Different Plate in the Plate-Level View

To select a different plate to view in the plate-level view

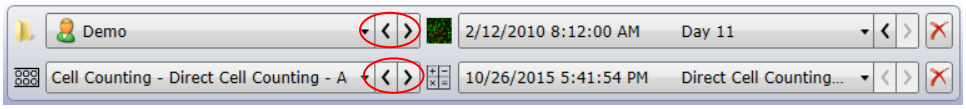
Do either of the following:

Select the Plate menu and/or Folder menu pulldown menu arrow (Figure 199).

When selecting the down arrow for a plate, you are viewing all the plates currently located in the currently selected Folder in alphabetical order. When selecting the down arrow for a folder, you are viewing all folders the user has access to in alphabetical order. Click the **<** or **>** arrow to the right of the Plate or Folder menu (Figure 199) for a sequential display in alphabetical order. If an arrow is disabled, that means there are now additional items to view in that direction (indicating that you are at the first, last, or only item in the list).

In this figure, the selections are for a plate is Cell Counting – Direct Cell Counting followed by a – with the plates current description and for a Folder the folder name of Demo.

Figure 199. Selecting a Plate and/or Folder



	NOTE: Selecting a different Plate automatically defaults to the last analyzed Scan of the Plate and the associated Scan Results with the most recent timestamp for the current application being viewed. If no Scan Result exists with the same application, then the most recent timestamp is viewed.
	NOTE: Selecting a different Plate that is currently in use by another user (locked), a message prompt will appear to the user and the selection will revert.
	NOTE: Selecting a different Folder automatically defaults to the first Plate not currently in use by another user (locked). If all plates in the folder are locked or there are no plates in the folder, a message prompt will appear to the user and the selection the revert.

9.1.3 Viewing Scans and Scan Results in the Well Detail View

To display the well detail view for a scan

Do any of the following:

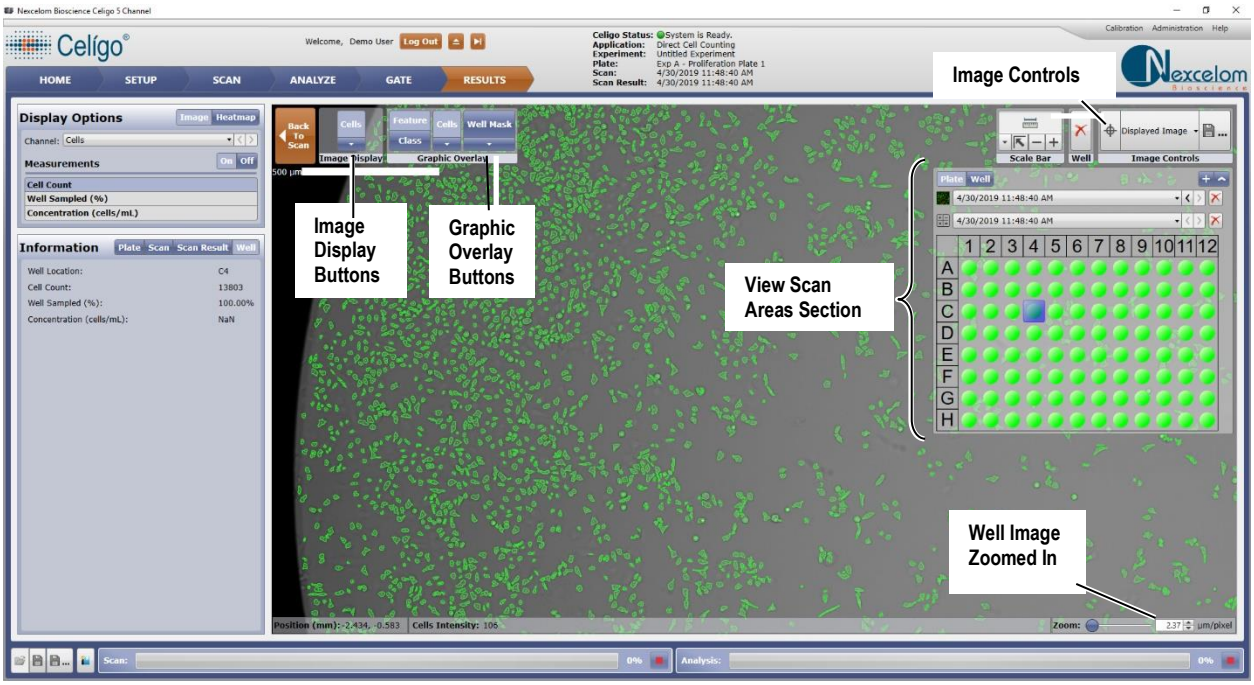
In the plate-level view (Figure 195 above), double-click the thumbnail image for the scan you want to view in detail.

In the plate-level view (Figure 195 above), right-click the thumbnail image and select View.

In the well detail view (Figure 200 below), click a well in the View Scan Areas section.

The entire well image appears in the right-hand side of the Results tab. Figure 200 (below) shows a well image that has been magnified (zoomed). A magnified image allows you to see a graphic overlay if the Graphic Overlay is turned on.

Figure 200. Results Tab Well Detail View



Viewing a scan that had been processed using version 2.0 or older, the classes overlays are merged and displayed as one overlay, and therefore show only On and Off in the Graphic Overlay section (Figure 201 on the left). If viewing scans that had been processed using version 2.1 or newer, the Graphic Overlay section will display all available classes (Figure 201).

Figure 201. Graphic Overlay Buttons

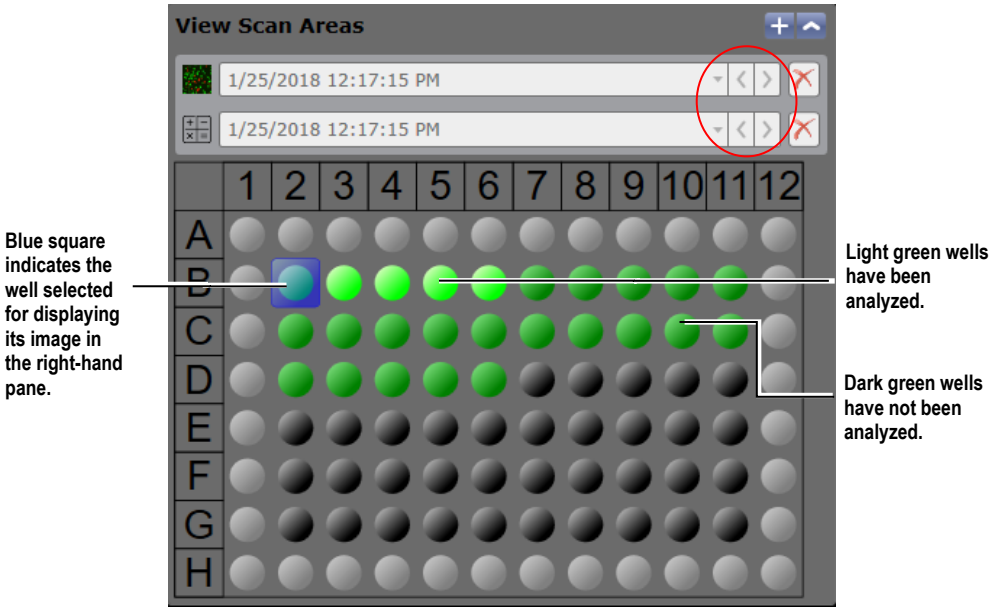
Classes for a scan analyzed on Celigo Software Version 2.0 or older

Classes for a scan analyzed on Celigo Software Version 2.1 or newer

To select a different scan and/or scan result in the well detail view

In the View Scan Areas section (Figure 202), use the Scan menu, Scan Result menu, and < and > arrows. The resulting image appears at the same relative position and at the same magnification level as the previously displayed image. These tools function the same way as in the plate-level view. For instructions, see section 9.1.1.

Figure 202. View Scan Areas Section



To hide or show the View Scan Areas section

In the View Scan Areas section (Figure 202 above), click  , double-click the title bar, or click  (if shown) as needed.

To select a different well for display

In the View Scan Areas section (Figure 202 above), click a well to display the associated scanned image in the right-hand pane. The resulting image appears at the same relative position and at the same magnification level as the previously displayed image.

To change the appearance of the displayed image

Do any of the following as needed:

Magnify (zoom) the well detail display to see the objects more closely and to see a graphic overlay in the resulting detail view (Figure 200 above). For zoom instructions, see section 6.3. The overlay will show only if the Graphic Overlay button is turned on.

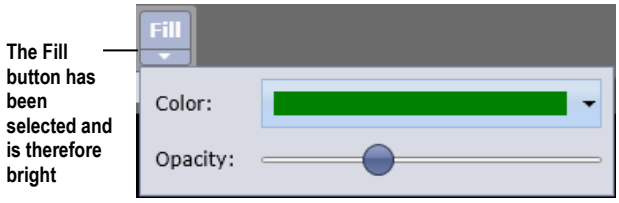
Turn the display of channels (such as Live and Dead) on and off using the Image Display buttons (Figure 200 above). For more information, see section 9.1.4.

Use the Graphic Overlay buttons to turn the graphic overlays (Features and Classes) on, off, change the color, or shape (Figure 200 above). For more information, see section 7.2.1.

For scans containing more than 1 channel, change the displayed gain, background, and color for each channel as needed (originally chosen in the Analyze tab) using the Graphic Overlay buttons. For more information, see section 7.2.1.

For confluence-based applications, such as Cell Counting, Colony Counting: Single Colony Verification, or Wound Healing, change the color and opacity of the confluence fill by clicking the Fill button and using the resulting Color menu and Opacity slide bar (Figure 203).

Figure 203. Fill Color and Opacity Settings



To return to the Results tabs plate-level view

In the Results tabs well detail view, click the **Back to Scan** button (Figure 204).

Figure 204. Back to Scan (to Plate-Level View) Button

9.1.4 Using the Image Display and Graphic Overlay Buttons in the Results Tab

The Image Display and Graphic Overlay buttons in the Results tab are used the same way as in the Analyze tab. The following exceptions apply:

A Processing Preview (P) button is not used in the Results tabs Image Display buttons.

The Results tabs Graphic Overlay buttons include a Feature button and a Class button.

For details about using the Image Display and Graphic Overlay buttons, see section 7.2.1. Using these buttons does not change the raw images.

9.2 Selecting a Well

You can select a well.

To select a well

In the Results tabs plate-level view (Figure 195), single-click any thumbnail image.

An orange border appears around the well, indicating that it has been selected.

When a thumbnail image is selected, the well results will appear in the Information section (Figure 195) at the left side of the Results tab if the Information section has the Well mode selected. When a thumbnail image is not yet selected, the left side of the Results tab displays a message No Scan Area Selected. If no scan results exist for the well, a message states No Scan Area Results.

9.3 Viewing Information

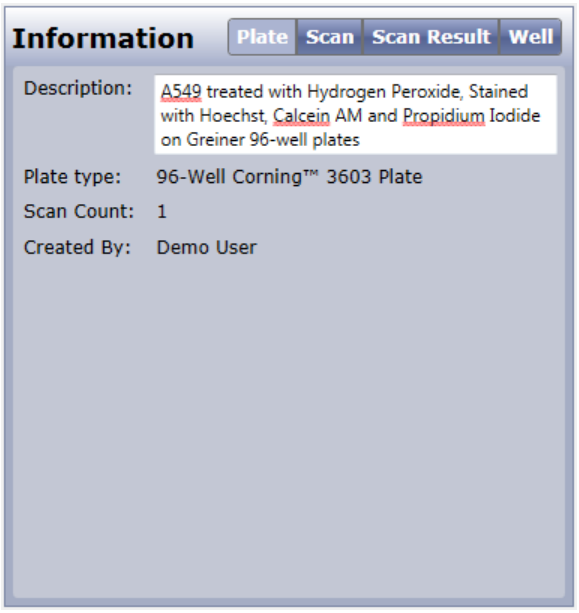
You can view information for the currently selected plate, scan, scan result and well.

9.3.1 Viewing and Modifying Plate Details

To view plate details

In the plate-level view (Figure 205), in the Information pane, select the Plate button.

Figure 205. Plate Details



The plate details show you the following:

- Description – The Plates description
- Plate type – The plate profile of the plate.
- Scan Count - Number of times the plate was acquired.
- Created By - The user who created the plate.

To modify the plate description

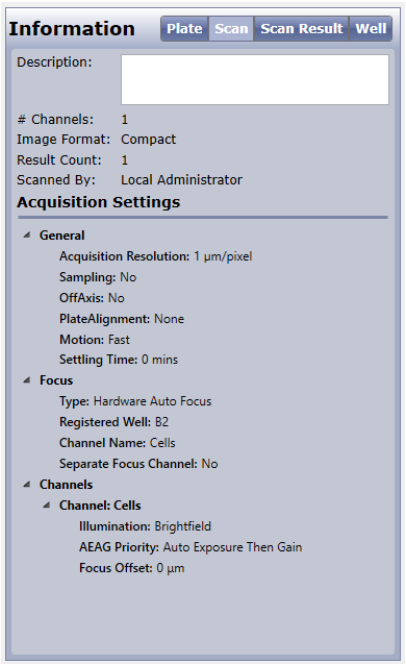
In the Information pane, click the Plate button and type the description that help distinguish the plate from the others.

9.3.2 Viewing and Modifying Scan Details

To view scan details

In the plate-level view, in the Information pane, select the Scan button (Figure 206).

Figure 206. Scan Details



The scan details show you the following:

- Description – The Scans description
- # of Channels – The number of channels the scan was acquired with.
- Image Format – The image format that was used when the scan was acquired.
    - Compact – Image compression was used.
    - Raw – No image compression was used.
- Result Count - # of times the scan was analyzed.
- Scanned By - The user who scanned the plate.
- Acquisition Settings – All the acquisition settings used to create the scan.

To modify the scan description

In the Information pane, click the Scan button and type the description that help distinguish the scan from the others.

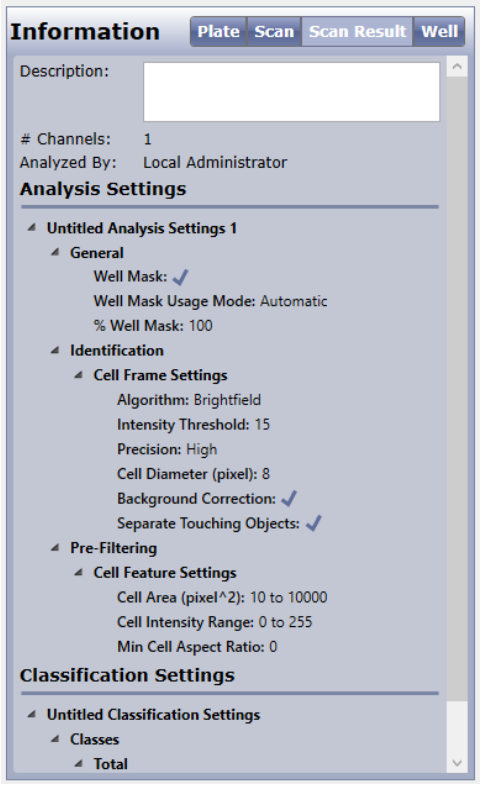
	**NOTE: Acquisition settings for the plate being viewed are summarized. They allow the user to find the how the plate was acquired with focus type, exposure settings, channels names and color.**

9.3.3 Viewing and Modifying Scan Result Details

To view scan result details

In the plate-level view, in the Information pane, select the Scan Result button (Figure 207).

Figure 207. Scan Result Details



The scan result details show you the following:

- Description – The Scan Results description
- # of Channels – The number of channels the scan result used for analysis.
- Analyzed By - The user who analyzed the scan.
- Analysis Settings – The analysis setting(s) used to create the scan result.
- Classification Setting – The classification setting used to create the scan result.

To modify the scan result description

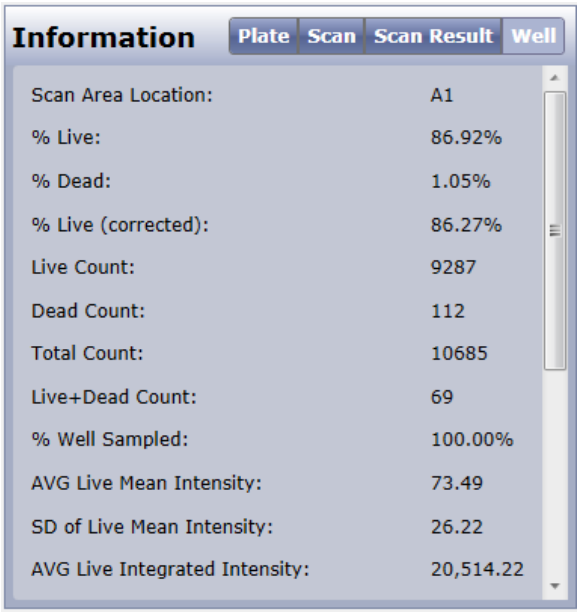
In the Information pane, click the Scan Result button and type the description that help distinguish the scan result from the others.

9.3.4 Viewing Well Details

To view well details

In the plate-level view, in the Information pane, select the Well button (Figure 208).

Figure 208. Well Details



The well details show the well location and all the well level measurements for the currently selected well.

If no well is selected, a message No Scan Area Result Available will appear in the pane until a well becomes selected.

9.4 Selecting Display Options

Make the following selections in the Display Options section as needed.

9.4.1 Selecting a Display Mode

To select a display mode

In the plate-level view, in the Display Options section, select the mode you want to display.

Figure 204. Result Tab Display Modes

There are 3 modes to select from:

- Image – This mode displays a thumbnail image for each well.
- Fill – This mode displays a colored segmented image for each well.

- Heatmap – This mode shows a colored shape for each well representing the current well measurement in relation to the other wells in the scan result.

9.4.2 Selecting a Channel for Displaying Thumbnails in Image Mode

To select a channel for displaying thumbnails

In the plate-level view, in the Display Options section (Figure 195 above and *Figure* 209.), click the **Image** button. Then, select the channel you want to display.

Figure 209. Images Section

9.4.3 Viewing and Modifying a Colored Fill Display

You are able to select a colored fill display in some applications; more specifically those that identify and analyze larger areas (non-individual cells).

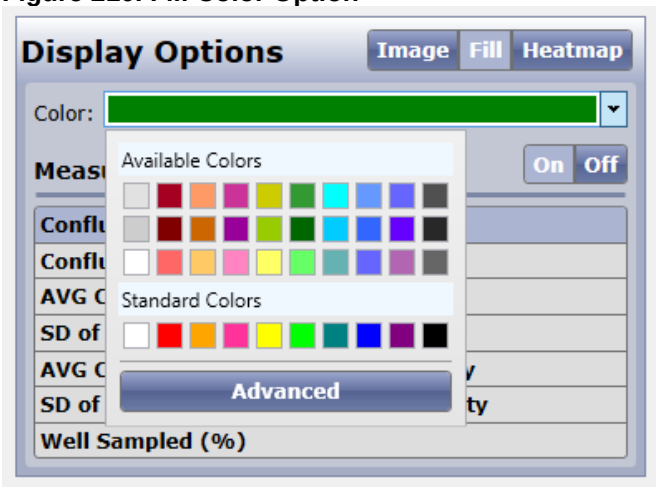
To view a colored fill display

In the plate-level view, in the Display Options section (Figure 195 above and Figure 210), click the **Fill** button.

To change the color of fill display

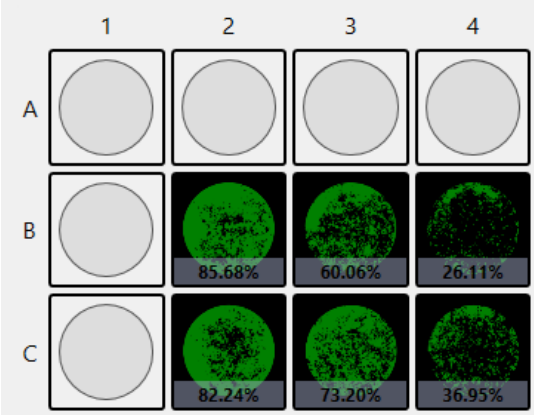
In the plate-level view, in the Display Options section (Figure 195 above and Figure 210), click the **Fill** button. Then, select the color you want the fill thumbnails to appear in.

Figure 210. Fill Color Option



Changing the fill color will refresh the thumbnails on the screen to appear in the selected color (Figure 211).

Figure 211. Colored Fill Thumbnails



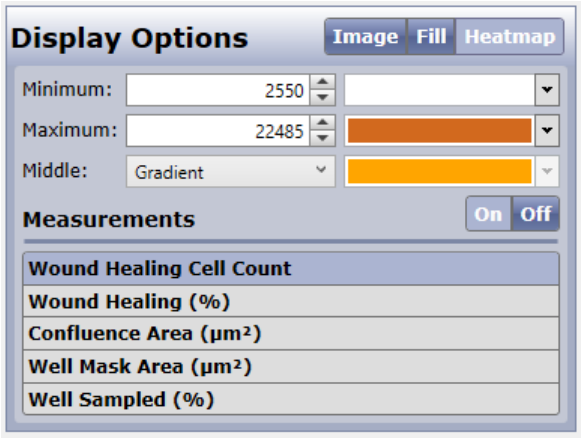
9.4.4 Viewing Results in a Heatmap

You can display a heatmap. A heatmap indicates which measurements fall below, within, and above a user-defined value. When Heatmap is on, the system highlights the thumbnail images that are below (<), within (=), and above (>) a user-defined heatmap value with color overlays.

To display a heatmap

In the plate-level view, in the Display Options section (Figure 212), click the **Heatmap** button.

Figure 212. Heatmap Display Options



To change heatmap values

In the plate-level view, in the Display Options section (*Figure 213*), click the **Heatmap** button. Then, update the minimum and maximum values as desired.

To change heatmap colors and modes

In the plate-level view, in the Display Options section (*Figure 214*), click the **Heatmap** button. Then, modify the colors seen in the right column next to the minimum and maximum textboxes.

If the Middle dropdown menu is set to Gradient, you will only be allowed to select two colors that get blended as a gradient between minimum and maximum.

Figure 213. Gradient Heatmap Color Options

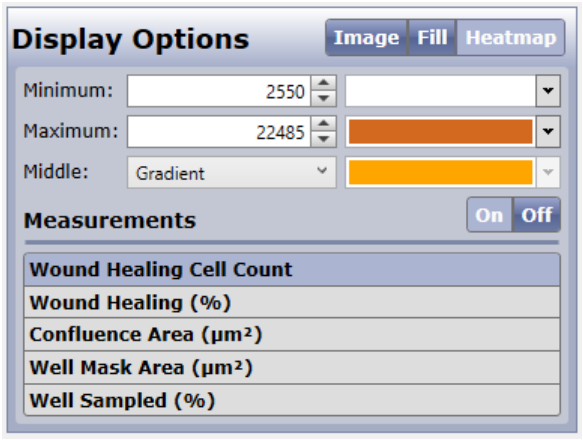
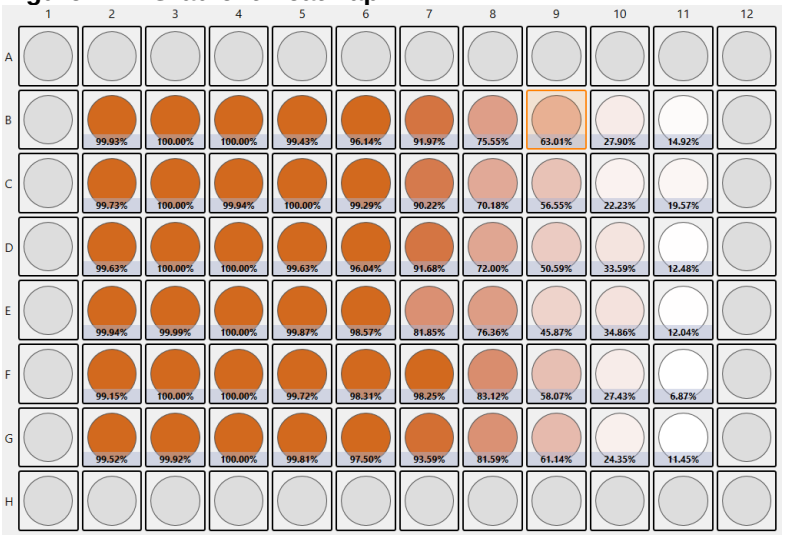


Figure 214. Gradient Heatmap



If the middle dropdown menu is set to Solid (Figure 215), a third color becomes selectable. Then all values below the minimum are set to the minimum color, all the values higher than the maximum are set to the maximum color, and all the values in between are set to the middle color.

Figure 215. Solid Heatmap Color Options

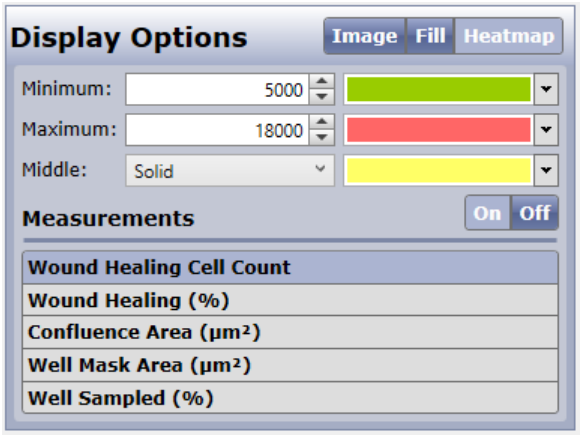
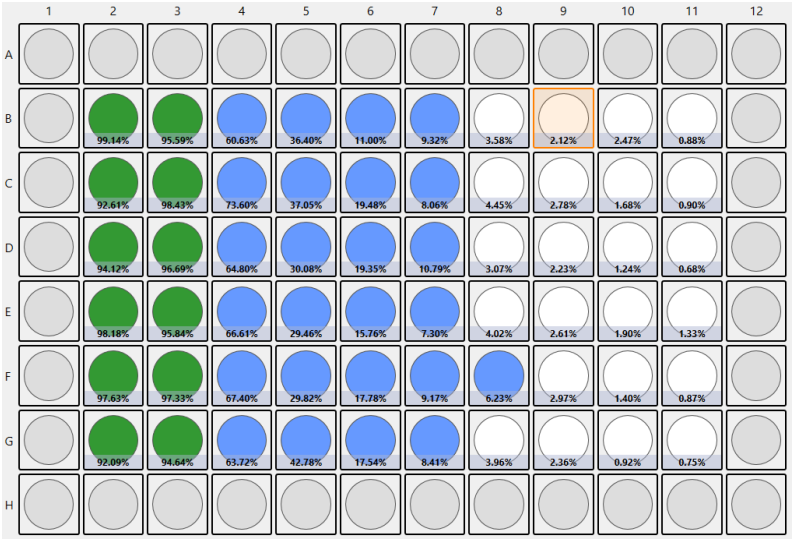


Figure 216. Solid Heatmap



9.4.5 Displaying Measurements Over Well

You can turn on and off measurements for display over each well.

To display measurements over wells

In the plate-level view, in the Display Options section (Figure 198 and Figure 217), the On button to the right of the Measurements section.

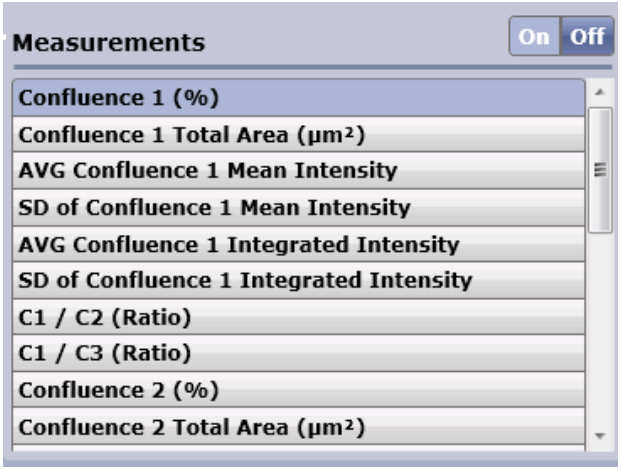
To remove measurements over wells

In the plate level view, in the Display Options section (Figure 198and Figure 217), the Off button to the right of the Measurements section.

To select which measurement to display over wells

In the list of parameters below the Measurements section, click a parameter, such as Confluence 1 (%) (Figure 217) so that it highlights in blue. The selected parameter will appear over each well.

Figure 217. Measurements Section



9.5 Generating Reports

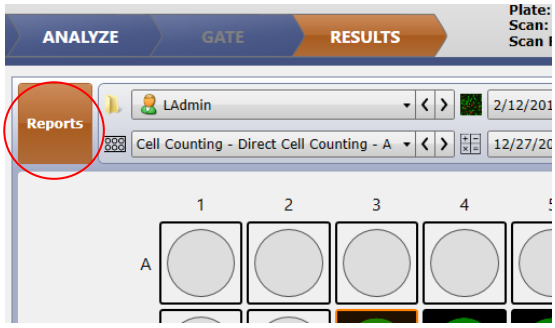
Perform the following steps to generate reports.

	NOTE: A scan must be analyzed first in order to be included in a generated report.

To generate reports

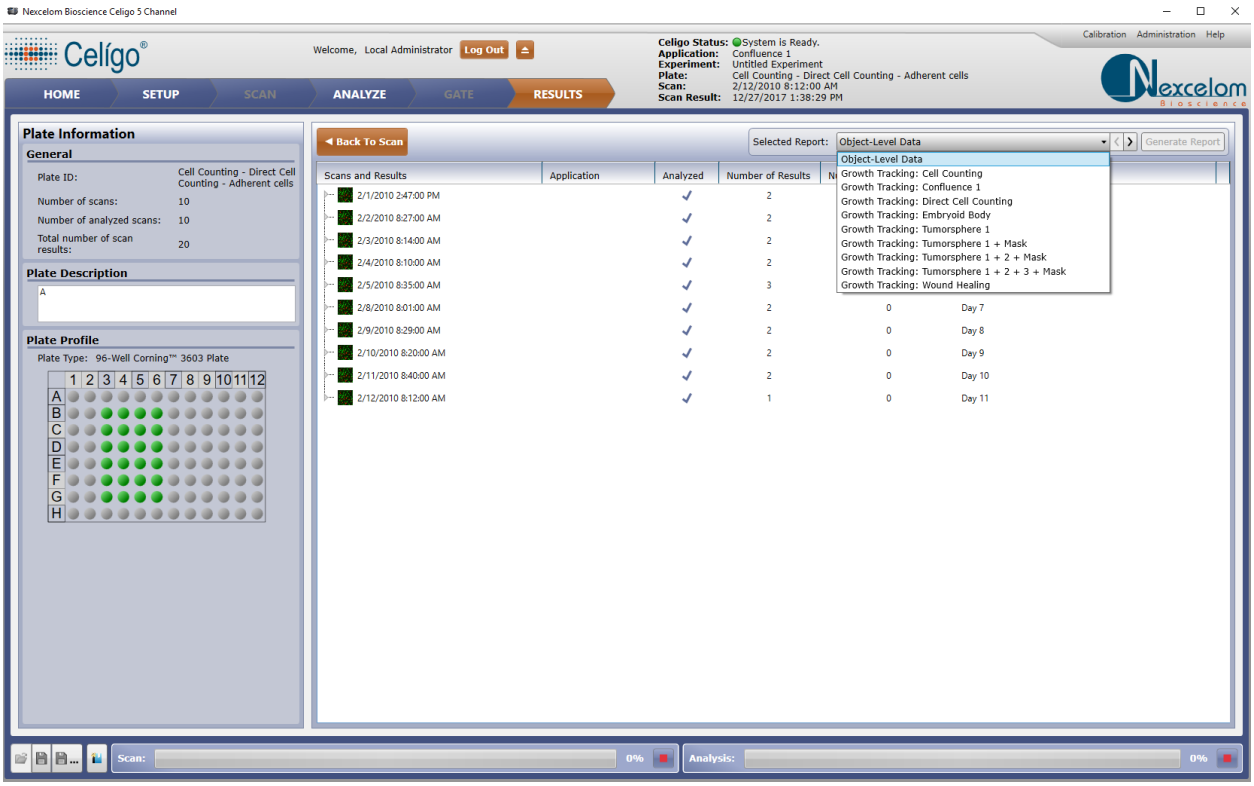
1. In the Results tab, at the plate-level view, click **Reports** (Figure 218).

Figure 218. Displaying Scans for *Reporting*



The report selection screen appears. This screen lists the existing scans and associated scan results for the plate ID (Figure 219).

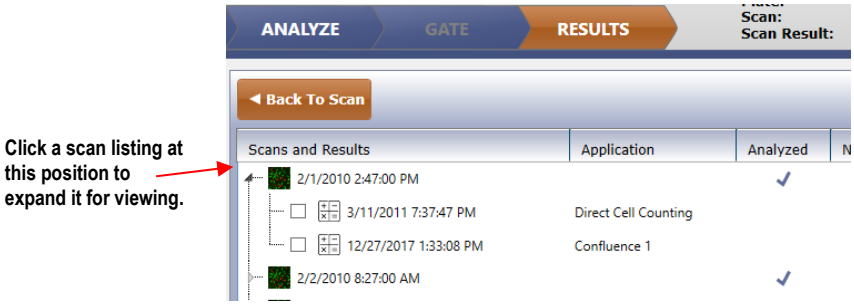
Figure 219. Report Selection Screen



2. View the scan results for other listed scans as needed by clicking the scans in the Scan Time list.

To expand a scan listing for viewing in the Scan list, click its listing below the Scan Result list (Figure *220*).

Figure 220. Viewing Scan Results



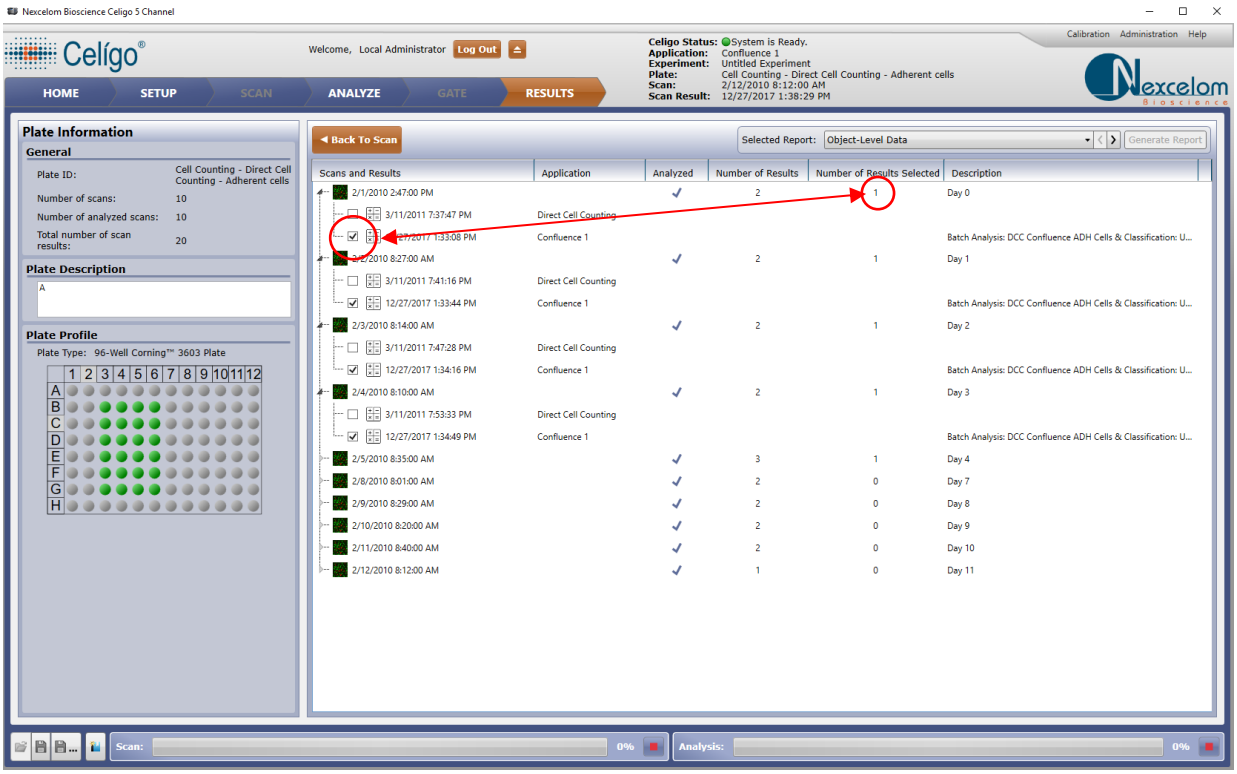
3. Checkmark the Select For Report checkbox (Figure 221) next to each scan result for which you want to generate a report. You can also right-click a scan result and make the following additional selections:

   Select all scan results

   Deselect all scan results

   Select last scan result for each scan for the specified application

Figure 221. Selecting Scan Results for Reporting



4. In the Selected Report menu, located at the top of the right-hand pane, select the main type of report you want to generate:

   Growth Tracking Reports – To view growth tracking reports (see Figure 222 for an example).

For the list of the applications that support growth tracking and object-level data reports, see the Selected Report menu. For the instructions on growth tracking reporting, see the applicable Application Guide.

Object-Level Data Reports – To view graphs of measured cell parameters such as intensity and area (see Figure 222 for an example).

All applications support object-level data reports. For the instructions on generating object-level data reports, see the applicable Application Guide.

5. Click **Generate Report**.
6. Click Export Report button. (Figure 222 upper right corner).

Figure 222. Growth Tracking Report

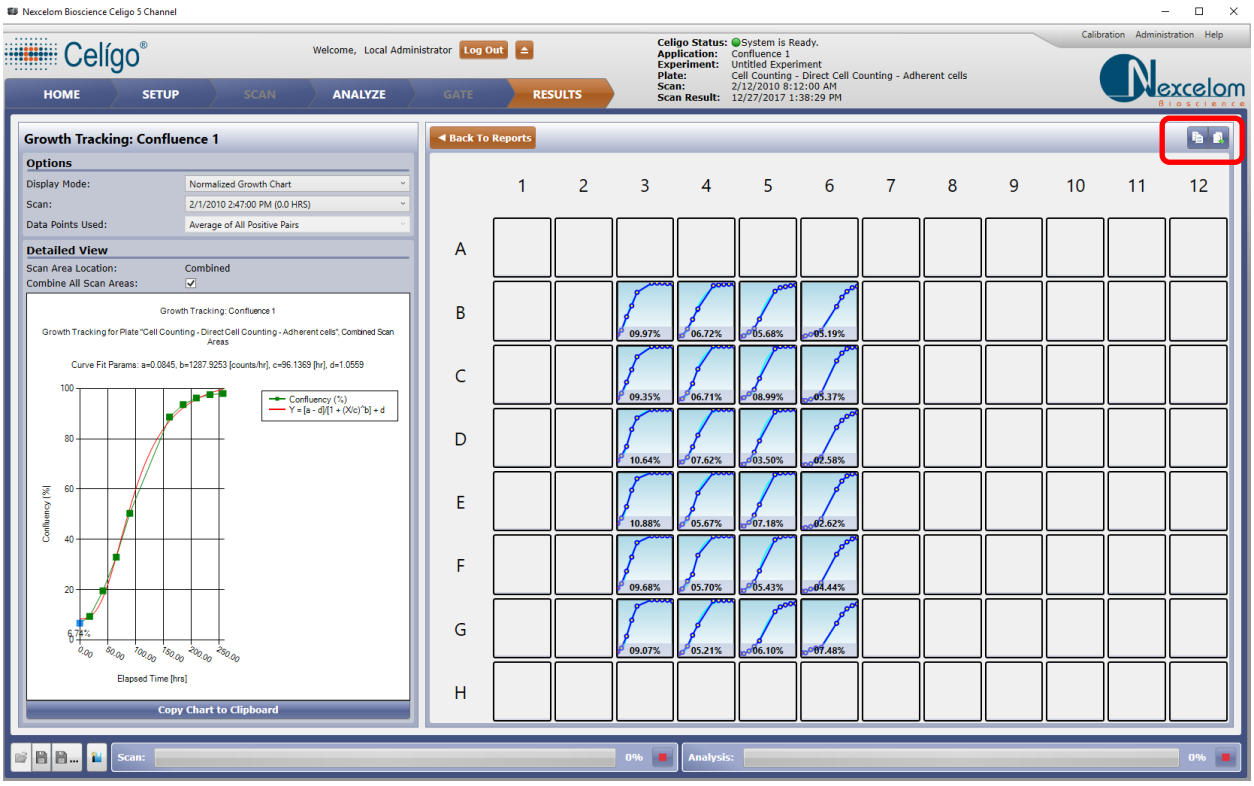
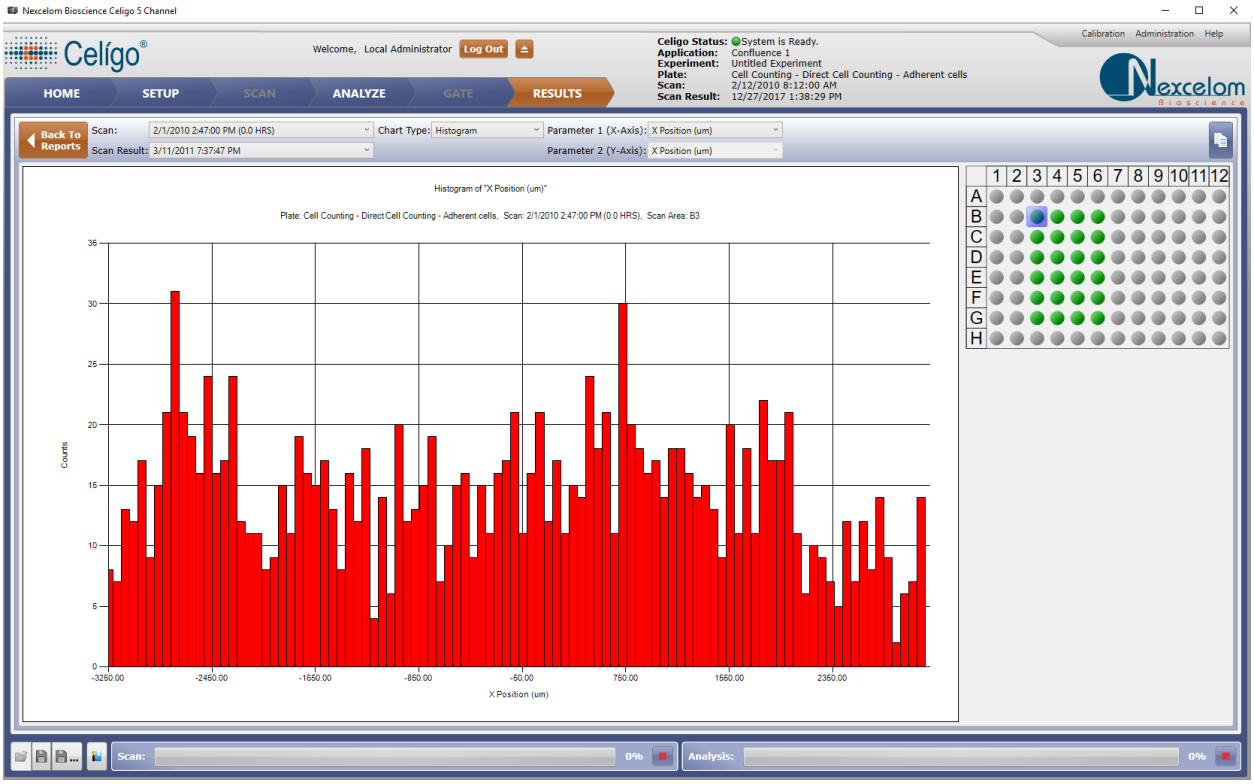


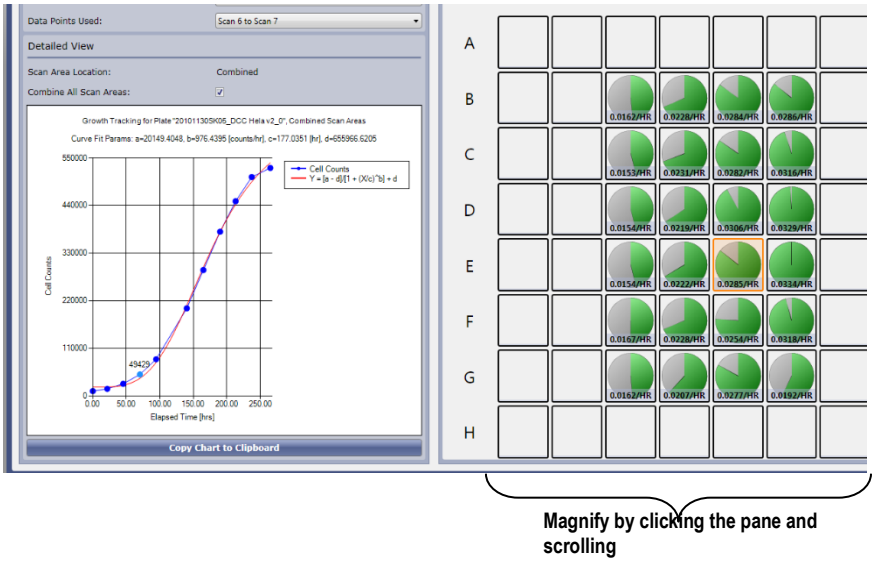
Figure 223. Object-Level Data Report



9.5.1 Magnifying a Pie Chart Size (Zoom)

You can magnify the appearance of a pie chart pane (not a growth curve) (Figure 224) by left-clicking the pie chart and then scrolling with the mouse scroll wheel.

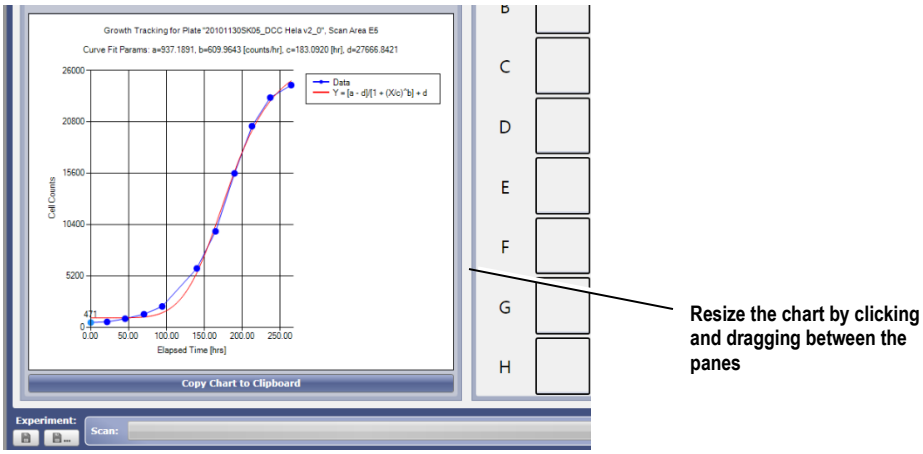
Figure 224. Magnifying a Chart Size



9.5.2 Re-Sizing a Chart

Increase or decrease the size of a chart by clicking and dragging the vertical line between the left and right panes (Figure 225). This action is not to change the chart data, but is only for viewing purposes.

Figure 225. Re-Sizing a Chart



9.5.3 Saving an Experiment in the RESULTS Tab

To save an Experiment to the database in the RESULTS tab use the Experiment buttons (Figure 226) in the same way as in the SCAN and ANALYZE tabs. For detailed instructions, see Saving an Experiment to the Database in the SCAN Tab, section 6.10.1.1.

In the RESULTS tab, when you save an experiment, you are saving it to the database, not to the hard drive (export).

When you save an experiment to the database in the RESULTS tab, you are recording a sort of snapshot of all the settings that currently exist in the Scan, Analyze, and Gate tabs.

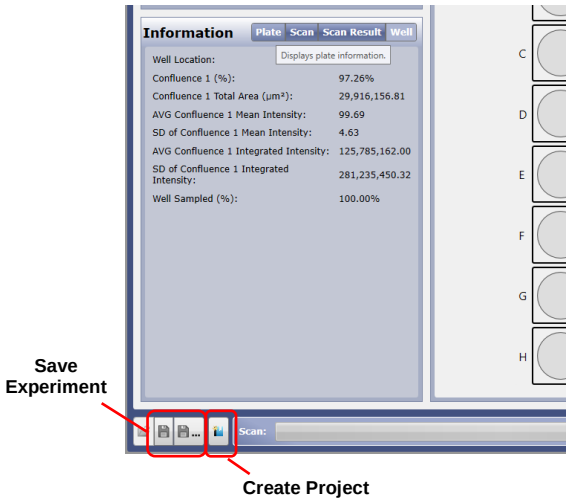
Saved Experiments creates a file the records all the parameters from the current SCAN, ANALYZE, and GATE tabs. This file can be recalled in for later reuse on another plate scan.

For an overview of the save button locations and functions in the various tabs, see section 6.10.

9.5.4 Creating a Project in the RESULTS Tab

Create Projects button (Figure 226) creates a file the uses the current settings in the saved experiment, additionally saved are the plate type and format, and user selected export options. This file can be recalled in for later reuse on another plate scan. For additional details on creating a project, see section 6.10.2.

Figure 226. Experiment and Projects Save-to-the-Database Buttons in the RESULTS Tab



9.6 Centering an Onscreen Image in the RESULTS Tab

You can center an onscreen image in the RESULTS tab. Doing this does not change the raw images.

To center an onscreen image in the Results tab

In the Image Controls section (Figure *200* above and Figure 227), click the **Center Image** icon.

Figure 227. Image Controls in RESULTS Tab

9.7 Exporting Images or Data from the RESULTS Tab

You can export images and data in the RESULTS tab. You can export data for individual wells or multiple wells, and in both the plate-level views and well detail views.

9.7.1 Exporting Images or Data for a Single Well

You can export the following types of images or data for a single well:

Export a single wells object-level data

Export all wells raw images associated with a well (FOV/channel)

Export a wells onscreen image (do a screenshot)

9.7.1.1 Exporting a Single Wells Object-level Data

You can export a single wells object-level data from the RESULTS tabs plate-level view or well detail view. The export options for doing this are Comma Separated Value (CSV), Flow Cytometric Standard (FCS) format, Image Cytometer Experiment data format (ICE), and Archival Cytometer Standard format. Information gathered from each segmented object (cell) is collated in one CSV file (one CSV file for a 96-well plate, for example) and includes:

x, y position of object (location in well)

Data from analysis settings for each channel: For example, area (µm), integrated intensity, mean intensity, form factor, smoothness, and aspect ratio.

9.7.1.1.1 Exporting a Single Wells Object-level Data in the Plate-level View

To export a single wells Object-level Data in the plate-level view

1. In the RESULTS tabs plate-level view (Figure 195 above), right-click the desired well (thumbnail).
2. Select **Export Well Object-Level Data** in the resulting menu.

   A Windows Save dialog box appears.
3. In the Save dialog box, enter a name for the CSV, FCS, ICE (with or without images) file.
4. Click **Save**.

9.7.1.1.2 Exporting a Single Wells Object-level Data in the Well Detail View

To export a single wells Object-level Data in the well detail view

1. If the well detail view is not displayed, display it by double-clicking the thumbnail image in the plate-level view. For details on displaying the well detail view, see Viewing Scans and Scan Results in the Well Detail View section 9.1.2.

   The well detail appears.
2. At the bottom of the Scan Area Results pane, click the **Export Object Level Report** button.

   A Windows Save dialog box appears.

3. Perform section 9.7.1.1.1 steps 3 and 4.

9.7.1.2 Exporting All Raw Images for a Single Well

You can export all raw images associated with a single well in the plate-level view or well detail view. The export options for doing this are .jpg, .bmp, and .tif formats, and whether you want separate or stitched images. The resulting file is grayscale and high-resolution. The grayscale result is because files exported with this method are raw images. The camera records raw images in grayscale. The resolution is higher than the images from the onscreen image method described in section 9.7.3.

The scan of a single well is comprised of multiple FOVs. You are given the option to export the images for a single well as a stitched whole-well image (multiple FOVs combined into a single image).

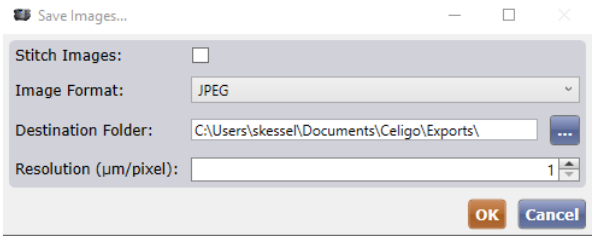
9.7.1.2.1 Exporting All Raw images for a Single Well in the Plate-Level View

To export all raw images in the plate-level view

1. In the Results tabs plate-level view (Figure 195 above), right-click the desired well (thumbnail) and then select **Export Images** in the resulting menu.
2. Double-click the well to display the single-well image and click **Export Images** at the bottom of the Scan Area Results pane.

   A Save Images Dialog box appears (Figure 228).

Figure 228. Save Images Dialog Box



3. In the Save Images dialog box, do any of the following:

   Checkmark **Stitch Images** if you want to combine all FOV images for a well into a single well image.

   Select the desired image format (*.jpg, *.tif, *.bmp).

   Select the location where you want the single image to be saved.

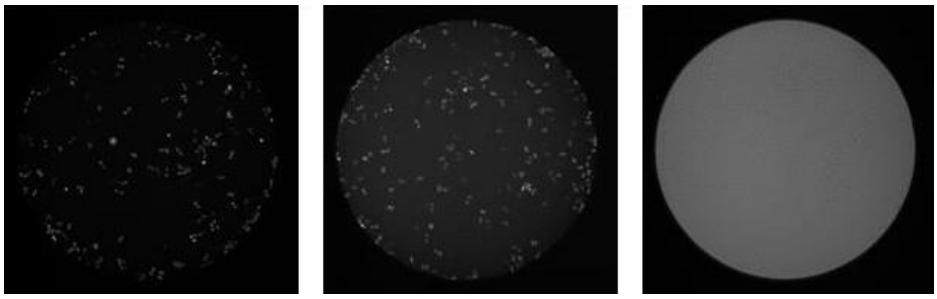
   Specify the output resolution (microns per pixel) to use.

	NOTE: Stitching is not available for non-sampled and/or non-binned 6-well wells at 1 micron per pixel resolution.
	NOTE: If you are exporting images comprised of multiple FOVs (larger than 384 wells) and you want to combine all FOV images for a well into a single well image, be sure to checkmark Stitch Images. Otherwise, each FOV for a well will be exported as an image.

	NOTE: Image resolution will be limited by the resolution of the acquisition resolution (the resolution at which the scan images were acquired).

Figure 229 shows examples of thumbnails of exported raw image files, which automatically appear in the user defined location. The examples are single whole-well images for different channels, exported by selecting **Export Images**.

Figure 229. Exported Raw Image File Examples



9.7.1.2.2 Exporting All Raw Images for a Single Well in the Well Detail View

To export all raw images in the well detail view

1. If the well detail view is not displayed, display it by double-clicking the thumbnail image in the plate-level view. For details on displaying the well detail view, see Viewing Scans and Scan Results in the Well Detail View section 9.1.2.

   The well detail appears.

2. At the bottom of the Scan Area Results pane, click the **Export Images** button.

   A Save Images dialog box appears (Figure 228 in section 9.7.1.2.1).

3. Perform section 9.7.1.2.1 step 3.

9.7.2 Exporting Images or Data for an Entire Scan (Multiple Wells)

You can export all raw images for an entire scan (multiple wells). You can also export data for an entire scan.

9.7.2.1 Exporting All Raw Images for Multiple Wells

You export all raw images for multiple wells in the plate-level view.

To export all raw images for multiple wells

In the Results tabs plate-level view (Figure 195 above), click **Export All Images** (Figure 230).

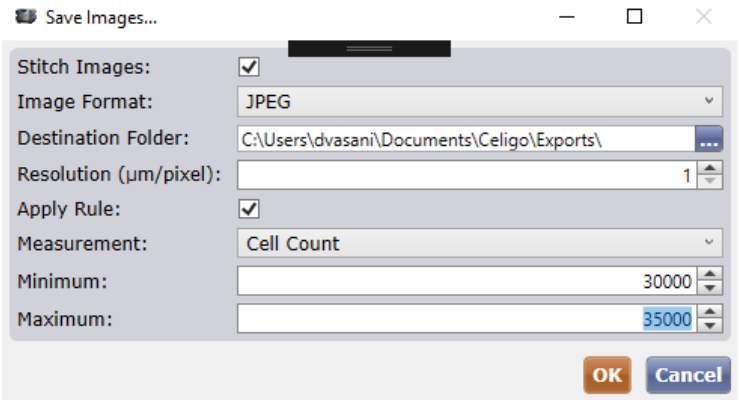
Figure 230. Export All Images Button

The export options for doing this are .jpg, .bmp, and .tiff formats, and whether you want separate or stitched images.

The Save Images dialog box (Figure 231) appears for selecting the desired format and destination folder for the files.

Specify a rule (i.e., establishing cell count min and max as shown in Figure 231) to only export images that fit within specific parameters.

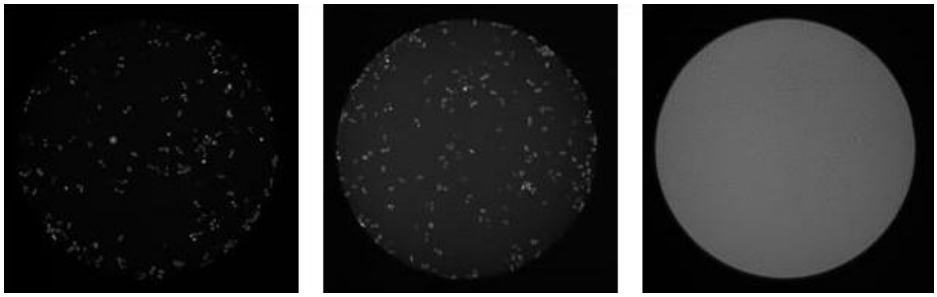
Figure 231. Save Images Dialog Box



The resulting export is one image per FOV per channel. For example, if you have scanned one complete 96-well plate and used three channels, 4608 image files will be exported (96 x 16 FOV x 3 channels = 4608 image files).

The resulting files using the **Export All Images** method are grayscale. This is because files exported using this method are the raw images. The camera records raw images in grayscale. The files using this method are high-resolution. Figure 232 shows examples of raw image files that have been opened in a graphic application.

Figure 232. Exported Raw Image Files



9.7.2.2 Exporting All Data for Multiple Wells

To export all well level data or object level data in the plate-level view.

To export all data for multiple wells

In the Results tabs plate-level view (Figure *195* above), click one of the following data export buttons (Figure 233), depending on the type of data you want to export:

	NOTE: Export Object-Level Data is a valid option for the Confluence Protocol application.

Figure 233. Data Export Buttons

To export all well-level data – Click **Export Well-Level Data**

The export option for doing this comes with two CSV options: Plate based and Tabular based. In the Plate based format, the data appears in plate format in the Scan Results tab and contains all information specified in the Analyze tab and Gate tab settings, including Current Scan Area Results. In the tabular based format, the data is presented in a table form that facilitates analysis in 3rd party software. In both cases, the data is collated into one file containing scan data and all data associated with the specific application. This operation locks the scan and marks it as Reported On.

To export all object-level data – Click **Export Object-Level Data**

This selection will gather and export the data from every object counted in the plate. The export options for doing this are CSV, FCS, Multiple FCS, ICE, and ACS format. The data is collated into one file and includes:

- Well number (row and column)
- Row only
- Column only
- XY position of object (location in well)
- Data from the analysis settings: Area (µm), integrated intensity, mean intensity, form factor, smoothness, and aspect ratio
- Classification settings

9.7.3 Exporting an Onscreen Image in the RESULTS Tab

In the RESULTS tab, you can export a selected well image as seen onscreen (do a screenshot), including any applied pseudo-coloring and/or graphic overlays displayed. The resulting file is in color and lower resolution. In the Results tab, you export an onscreen image for a single well (in the well detail view). The image export options are .jpg, .bmp, and .tif image formats.

To export an onscreen image for a single well in the Results tab

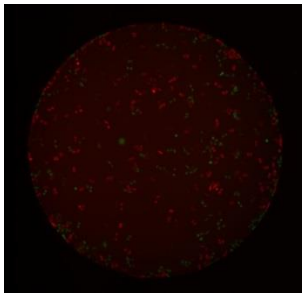
1. If the well detail view is not displayed, display it by double-clicking the thumbnail image in the plate-level view. For details on displaying the well detail view, see Viewing Scans and Scan Results in the Well Detail View section 9.1.2.

2. In the Image Controls section at the top right of the Results tab (Figure 227 above):
   a. Keep the default **Displayed Image** selection as is.
   b. Click the **Save Image** icon.
3. In the resulting **Save Displayed Image** dialog box, select the desired image format (*.jpg, *.tif, *.bmp) and the location where you want the single image to be saved.

   The resulting file using the **Save Image** method is in color, showing any pseudo-coloring and/or graphic overlays that you had applied onscreen, and is low-resolution. As it is essentially a screenshot, if you have zoomed on the image, the saved image will be the enlarged image. The file is a composite image, with the pseudo-coloring and/or graphic overlays applied for the fluorescent channels and the brightfield channel layered on top of each other. (Graphic overlays are not shown in Figure 234). The images from this method, though in color, are a lower quality image than the images from any of the other export image methods.

   Figure 234 shows an example of the same scanned well as in Figure 229, except after applying pseudo-coloring onscreen and then using the **Save Image** export method.

Figure 234. Exported Pseudo-Colored Image File Example



4. To modify the displayed image, toggle on/off the Image Display and Graphic Overlay buttons.

   Your selection of Image Display and Graphic Overlay buttons are applied onscreen and in files exported using the **Save Image** method only, not to files exported using any of the **Export Images** methods. Files exported using the **Export Images** methods are raw images and therefore do not reflect any onscreen changes.

   In the Results tab, use the Image Display and Graphic Overlay buttons the same way as in the Analyze tab. For details about using the Image Display and Graphic Overlay buttons, see section 7.2.1. Using these buttons does not change the raw images.

9.8 Deleting Data

Scans, Scan Results, and Wells may be deleted from various sections of the Results tab now.

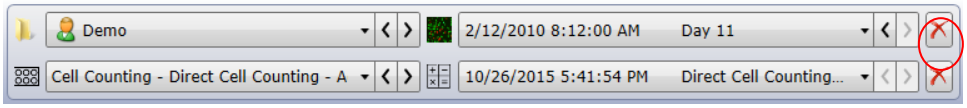
9.8.1 Deleting Scans

Scans may be deleted with the button located to the right of the Scan navigation arrows. When deleting a scan, all associated scan results will also be deleted.

To delete a Scan from the Results tab

1. Left-click the red X to the right of the Scan selection combo box.
2. Click Yes when the Are you sure? dialog appears.

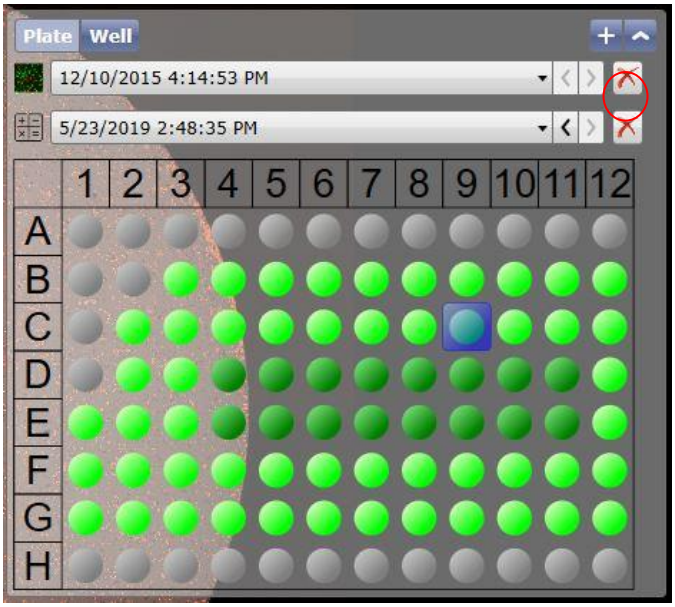
Figure 235. Delete a scan from the Plate Level View



To delete a Scan from the Well Detail View

1. Left-click on the red X to the right of the Scan selection combo box.
2. Click Yes when the Are you sure? dialog appears.

Figure 236. Delete a scan in the Well Detail View



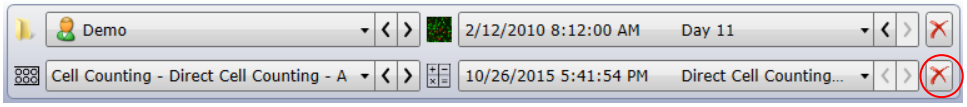
9.8.2 Deleting Scan Results

Scan Results may be deleted with the button located to the right of the Scan Result navigation arrows.

To delete a Scan Result from the Results tab

1. Left-click the red X to the right of the Scan Result selection combo box.
2. Click Yes when the Are you sure? dialog appears.

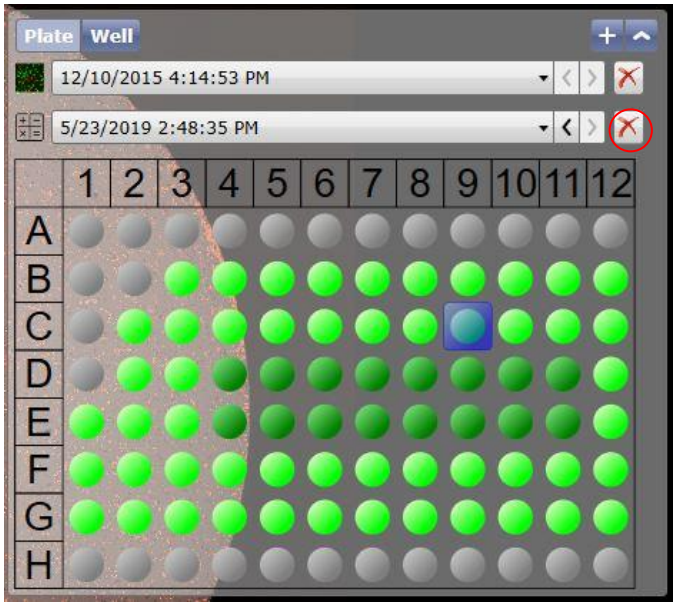
Figure 237. Delete a scan result in the Plate Level View



To delete a Scan Result from the Well Detail View

1. Left-click on the red X to the right of the Scan Results selection combo box.
2. Click Yes when the Are you sure? dialog appears.

Figure 238. Delete a scan result in the Well Detail View



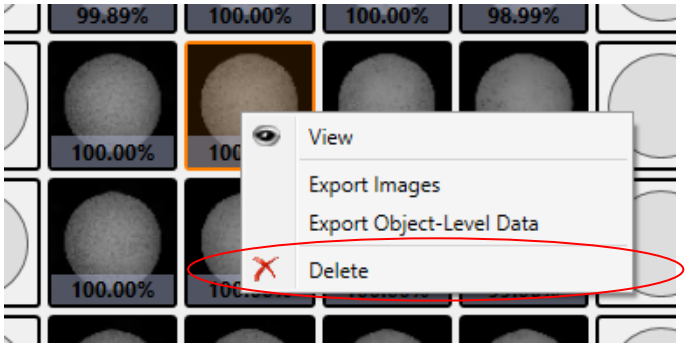
9.8.3 Deleting Wells

Wells may be deleted in a couple of different ways from the Results tab and the Image Displayer.

To delete a Well from the Results tab

1. Right-click on the Well you would like to delete.
2. Select the Delete option.
3. Click Yes when the Are you sure? dialog appears.

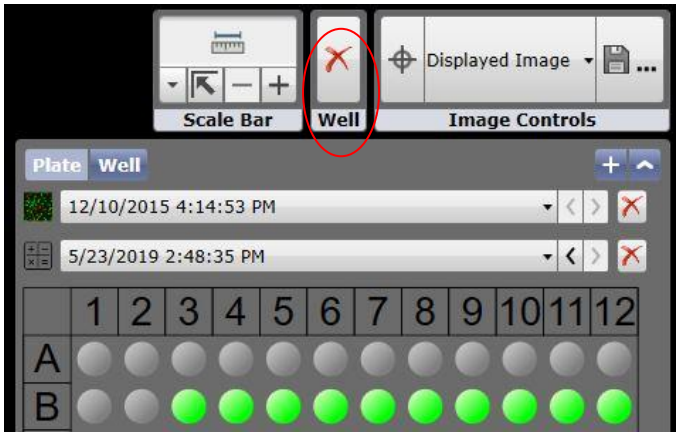
Figure 239. Right-click to delete single well



To delete a Well from the Well Detail View

1. Left-click on the red X to the left of the Image Controls near the top of Image Displayer.
2. Click Yes when the Are you sure? dialog appears.

Figure 240. Delete Well from Well view



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10. Removing the Plate

Whenever you are finished scanning and/or analyzing a plate or flask, unload (remove) it as follows.

NOTE: If you want to clear the entered settings and return to the Start tab without removing the plate or flask, see Returning to the Start Tab section 5.1.1.

To remove the plate

1. Click the unload Plate button in any tab (Figure 3 in section 3.2).

   The gripper door opens and the plate appears.

2. Remove the plate from the plate carrier.

   The image capture (scanning) and follow up tasks now are complete. To perform further analysis on the scanned images later, you can return to the Analyze and Gate tabs.

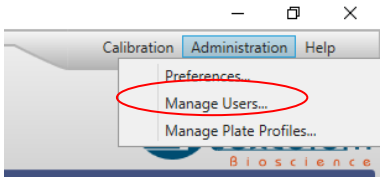
11. Preferences

Whenever you are at the START tab after having logged into the system, you can access system preferences to alter software behavior.

To access system preferences

In the START tab, after logging in, at the top of the Celigo user interface, select **Administration > Preferences… (**Figure 241**)**.

Figure 241. Accessing System Preferences



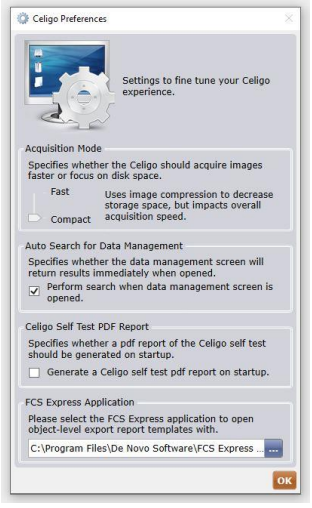
To modify system preferences

Once the preferences dialog is open (Figure 242), you can modify any of preferences by performing the following:

- Acquisition Mode – This setting specifies whether or not to use image compression when acquiring images during acquisition.
    - Fast – No image compression is used during acquisition.
    - Compact – Image compression is used during acquisition. Recommended.
- Auto Search for Data Management – This setting specifies whether or not data management will display results immediately when opened or wait for the user to first make a selection and refresh the search.
- Celigo Self Test PDF Report – Specifies whether a PDF report of the Celigo Self Test should be generated on startup.

	**NOTE: Acquisition Mode setting is only available for Celigo (acquisition). This setting will not be present when using the Celigo Satellite software.**

Figure 242. Celigo Preferences



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12. Glossary

The following is a glossary of terms that are commonly used with the operation of a Celigo cytometer.

For the definitions of parameters shown in displays (for example, AOI, Intensity Average, and Scan Area), see the section or Application Guide that describes the display.

Term	Definition
Experiment	The settings that currently exist throughout the Scan, Analysis, and Gate tabs.
Field of Regard	The total area that can be seen without moving the plate, but instead, using the mirror galvanometers to capture more area. This usually consists of multiple fields of view.
Field of View	The total area that can be captured by the camera without any movement from the plate or mirror galvanometers.
Image Display	A set of buttons used to show or hide a channel, such as Live or Dead.
Import Plates	Import scans.
Live image	The real-time view of the selected well, as opposed to the scanned image.
Off-Axis	Off of the well center. Selecting this image acquisition setting will capture multiple wells from a single stage position.
On-Axis	On the well center. This default image acquisition setting will capture one well during scanning of a single stage position.
Report	Chart that illustrates data for a user-specified scan or multiple scans.
Sampling	An image acquisition setting that captures a well smaller than the whole well.

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Appendix A Celigo Satellite Application

This appendix describes the Celigo Satellite application (from here on referred to as the Satellite application). This application is installed on Celigo satellite workstation PCs. Celigo satellite workstation PCs are not connected to Celigo instruments.

The Satellite application provides the same functionality as the Celigo application, with the exception that the Satellite application provides more streamlined importing functionality instead of scanning functionality.

NOTE: In the Celigo application, you perform imports in only the Data window; in Satellite, you also perform imports in the IMPORT tab. Instructions for importing using the Celigo applications Data window are in the Celigo Cytometer Administrator Guide.

For instructions on performing the Satellite tasks, see the following:

All chapters in this User Guide except:

- Start tabs task list described in chapter 5.
- Scan Tab chapter 6.

All chapters in the Administrator Guide.

The remainder of this appendix.

A.1 Start Tab in the Satellite Application

The Start tabs task list for the Satellite application (Figure 243) provides the following buttons:

View and Analyze Scans – to view and analyze scans saved in the database.

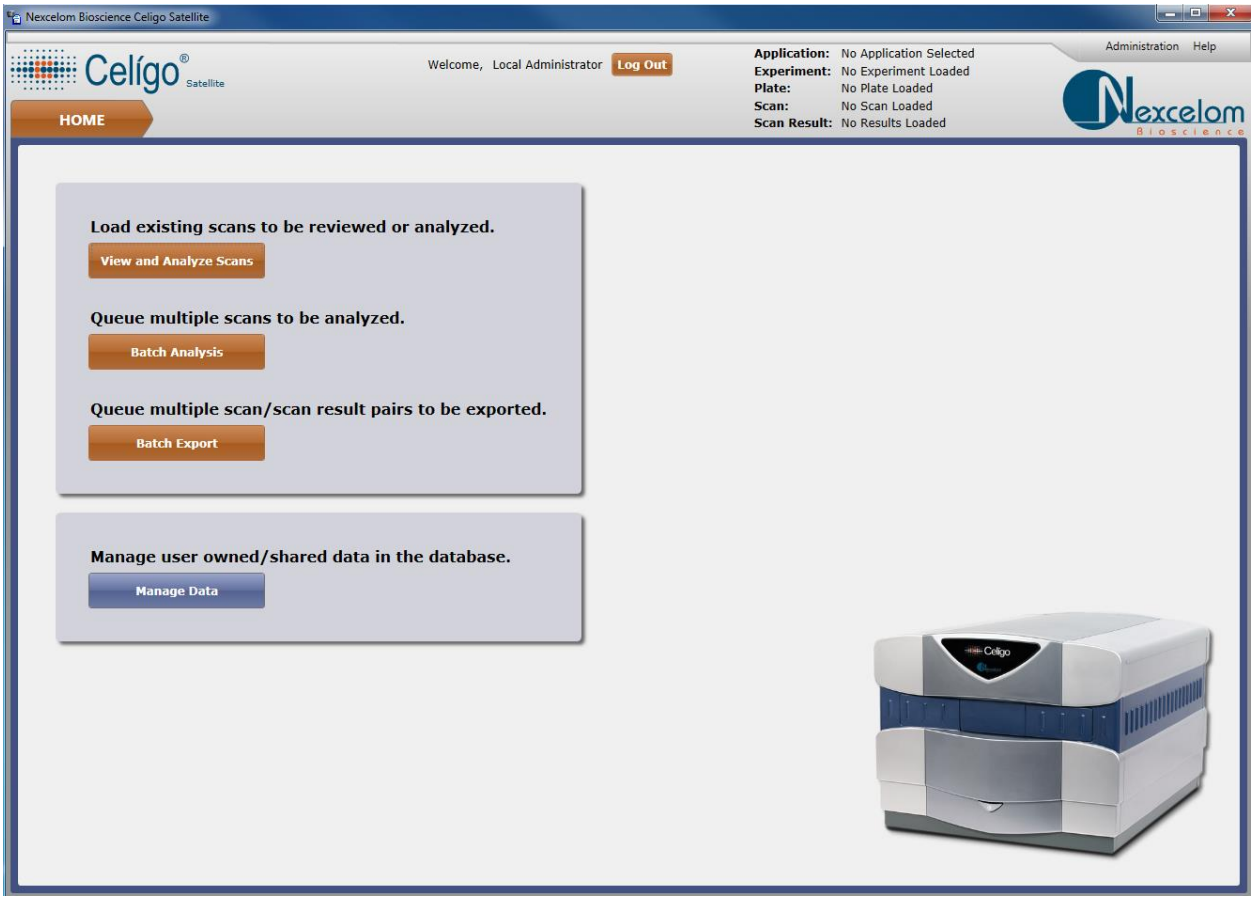
Batch Analysis – set up batch analysis of multiple scans.

Batch Export – set up batch export of data: scan results and images

Manage Data – access and manage the scans, scan results, analysis settings, classification settings, experiment settings, and projects

Additionally, the Administration and Help buttons are available.

Figure 243. Task List for the Satellite Application



Appendix B Automation Feature

This appendix describes the optional automation feature and how to use it.

B.1 Feature Description

The automation feature enables users to connect the Celigo cytometer with scheduling software for automated control of a plate handling robot. Users are able to automatically load, scan, and analyze plates and flasks, save and export the results.

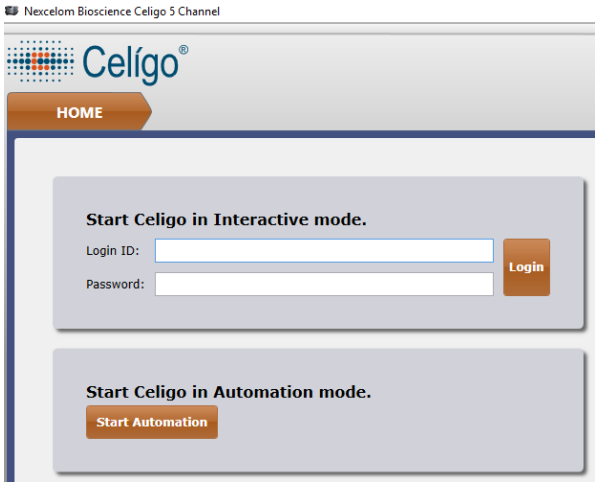
B.2 Starting Automation

After the hardware and Applications Programming Interface (API) have been set up for using automation, you can start the automation function.

To start automation

In the HOME tab, before logging in, select **Start Automation (**Figure 244**)**.

Figure 244. Starting Automation Mode



The Automation Mode is active message (Figure 245) appears and the automation software starts operating according to the programmed actions.

Figure 245. Automation Mode Message

B.3 Stopping Automation

You do not typically need to stop the automated tasks; the tasks will stop automatically after their scheduled completion. You can stop the automation function at any time.

To stop automation

Click **Exit Automation Mode**.

Appendix C Technical Specifications

The Celigo cytometer technical specifications are listed in Table 5.

Table 5. Technical Specifications

Parameter	Specification
Instrument Dimensions	20 inch wide x 25 inch long x 19 inch high (without PC)
Instrument Weight	50 kg (110 lbs) without PC
Electrical Power	100-240 VAC, 50/60 Hz, 600 W
Fuse Rating	10 A SLO-BLO 250 V, Ceramic 3AB, PN C0003892
Operating Temperature	15 ºC to 25 ºC
Operating Humidity	10% to 90% RH, non-condensing
Instrument Shipping & Storage Temperature	-18 ºC to 65 ºC
Instrument Shipping & Storage Humidity	10% to 90% RH, non-condensing
Manufacturer	Nexcelom Bioscience, LLC. 360 Merrimack St., Building 9 Lawrence, MA 01843
Distributor	Nexcelom Bioscience, LLC. 360 Merrimack St., Building 9 Lawrence, MA 01843

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Appendix D Plate Profiles

The following sections list the plate profiles that are provided with software release v5.2.

Support Status	Definition of Support Status
Supported	Supported plates are plates that have been tested, verified and are known to work with the Celigo instrument. They have been shown to work with most applications.
Unsupported	Unsupported plates are plate profiles that are available for the Celigo instrument that work but have been known to have imaging and/or focus issues.

Table 6. Plate Profiles

6-Well	Manufacturer	Wells	Well Type	Support Status	Compatibles
6-Well BD Falcon™ 353046 Plate	Corning	6	Clear	Supported	353224, 353934, 353846, 351146, 353502
6-Well Corning™ 3516 Plate	Corning	6	Clear	Supported	3471, 3506, 3335
6-Well CytoOne® CC7682-7506 Plate	CytoOne	6	Clear	Supported	---
6-Well Greiner™ 657160 Plate	Greiner	6	Clear	Supported	657185, 657165
6-Well Nunc™ 140675 Plate	Thermo	6	Clear	Supported	---

12-Well	Manufacturer	Wells	Well Type	Support Status	Compatibles
12-Well BD Falcon™ 353043 Plate	Corning	12	Clear	Supported	353224, 351143, 353503
12-Well Corning™ 3513 Plate	Corning	12	Clear	Supported	3336, 3512
12-Well CytoOne® CC7682-7512 Plate	CytoOne	12	Clear	Supported	---
12-Well Nunc™ 150628 Plate	Thermo Scientific	12	Clear	Unsupported	---

24-Well	Manufacturer	Wells	Well Type	Support Status	Compatibles
24-Well BD Falcon™ 353047 Plate	Corning	24	Clear	Supported	353226, 353935, 353847, 351147, 358115, 354723, 356723, 354775, 356775, 353504
24-Well Corning™ 3524 Plate	Corning	24	Clear	Supported	3337, 3526, 3527, 3573
24-Well CytoOne® CC7682-7524 Plate	CytoOne	24	Clear	Supported	---
24-Well Greiner™ 662160 Plate	Greiner	24	Clear	Supported	662102, 622165
24-Well Nunc™ 142475 Plate	Thermo Scientific	24	Clear	Unsupported	---
24-Well PE Visiplate™ 1450606 Plate	Perkin Elmer	24	Black	Supported	---
24-Well Seahorse™ XF24 Plate	Seahorse Biosciences	24	Clear	Supported	---

48-Well	Manufacturer	Wells	Well Type	Support Status	Compatibles
48-Well Corning™ 3548 Plate	Corning	48	Clear	Supported	---
48-Well Greiner™ 677180 Plate	Greiner	48	Clear	Supported	677102, 677165
48-Well Nunc™ 150687 Plate	Thermo Scientific	48	Clear	Unsupported	---

96-Well	Manufacturer	Wells	Well Type	Support Status	Compatibles
96-Well BD Falcon™ 351177 Plate	Corning	96	U-bottom	Supported	---
96-Well BD Falcon™ 353072 Plate	Corning	96	Clear	Supported	353916, 353936, 353075, 351172, 354407, 354429, 354461, 356461, 354516, 356516, 354607, 356698, 356690, 354689, 356689, 354409, 354410, 354670, 354596, 354657
96-Well BD Falcon™ 353219 Plate	Corning	96	Black, White	Supported	353377
96-Well BD Falcon™ 354640 Plate	Corning	96	Black, White	Supported	354650, 356650, 354651, 356651, 356701, 356693, 354649, 356649, 356640, 356700, 356692, 356717
96-Well BD Falcon™ 356717 Plate	Corning	96	Black	Supported	354717
96-Well Corning™ 3596 Plate	Corning	96	Clear	Supported	3300, 3474, 3595, 3598, 3599, 3585, 3595, 3628, 3841
96-Well Corning™ 3603 Plate	Corning	96	Black, White	Supported	3604, 3610, 3631, 3632, 3651, 3843, 3842, 3903, 3904, 3601, 3635, 3340, 3842, 3843
96-Well Corning™ 3603 Plate	Corning	96		Supported	
96-Well Corning™ 3696 Plate	Corning	96	Half Area, Black	Supported	3686, 3688, 3690, 3693, 3694, 3695, 3696, 3697
96-Well Corning™ 7007 Plate	Corning	96	U-bottom	Supported	---
96-Well Greiner™ 650185 Plate	Greiner	96	U-bottom	Supported	---
96-Well Greiner™ 655087 Plate	Greiner	96	Black	Supported	655088
96-Well Greiner™ 655090 Plate	Greiner	96	Black, White	Supported	655087, 655097, 655946, 655948, 655936, 655956, 655098, 655094, 655944
96-Well Greiner™ 655161 Plate	Greiner	96	Clear	Supported	655101, 655192
96-Well Greiner™ 655180 Plate	Greiner	96	Clear, chimney	Supported	655182, 655185, 655940, 655930, 655950
96-Well Greiner™ 675986 Plate	Greiner	96	Half Area, Black	Supported	67509x

96-Well	Manufacturer	Wells	Well Type	Support Status	Compatibles
96-Well Nexcelom3D ULA-96U Plate	Nexcelom Bioscience	96	Clear, U-bottom	Supported	ULA-96U
96-Well Nunc™ 167008 Plate	Thermo	96	Clear	Supported	---
96-Well PE Isoplate™ 6005050 Plate	Perkin Elmer	96	Black	Supported	---
96-Well PE Viewplate™ 6005225 Plate	Perkin Elmer	96	Black	Supported	---
96-Well Seahorse™ FX96 Plate	Seahorse Bioscience	96	Black	Supported	---

384-Well	Manufacturer	Wells	Well Type	Support Status	Compatibles
384-Well BD Falcon™ 353962 Plate	Corning	384	Clear	Supported	---
384-Well Corning™ 3542 Plate	Corning	384	Low vol, Black	Supported	3540
384-Well Corning™ 3680 Plate	Corning	384	Clear	Supported	3640, 3844, 3700, 3701, 3702, 3844
384-Well Corning™ 3764 Plate	Corning	384	Black, White	Supported	3653, 3655, 3846, 3845, 3706, 3707, 3711, 3683, 3845, 3846, 3712
384-Well Corning™ 3827 Plate	Corning	384	Low attach	Supported	---
384-Well Greiner™ 781091 Plate	Greiner	384	Black	Supported	781098, 781095, 781094, 781944, 781090, 781096, 781097, 781946, 781948, 781936, 781956
384-Well Greiner™ 781182 Plate	Greiner	384	Clear	Supported	781185, 781186, 781061, 781940, 781930, 781950
384-Well Nexcelom3D ULA-384U Plate	Nexcelom Bioscience	384	U-bottom	Supported	---

1536-Well	Manufacturer	Wells	Well Type	Support Status	Compatibles
1536-Well BD Falcon™ 356771 Plate	Corning	1536	Black	Supported	---
1536-Well Corning™ 3838 Plate	Corning	1536	Black, White	Supported	3833, 3836, 3893
1536-Well Greiner™ 789866 Plate	Greiner	1536	Black	Supported	789896

1-Well	Manufacturer	Wells	Well Type	Support Status	Compatibles
1-Well Nunc™ Omnitray	ThermoFisher	1	Clear	Supported	242811

TC Flasks	Manufacturer	Wells	Well Type	Support Status	Compatibles
T25 BD Falcon™ 353014 Flask	Corning	480	Clear	Supported	---

T25 BD Falcon™ 353014 Flask - Single View	Corning	1	Clear	Supported	---
T25 Corning™ 430639 Flask	Corning	441	Clear	Unsupported	---
T25 Greiner™ 690175 Flask	Greiner	480	Clear	Supported	---
T25 Greiner™ 690175 Flask - Single View	Greiner	1	Clear	Supported	---
T75 BD Falcon™ 353136 Flask	Corning	168	Clear	Supported	---
T75 BD Falcon™ 353136 Flask - Single View	Corning	1	Clear	Supported	---
T75 Corning™ 430641 Flask	Corning	168	Clear	Unsupported	---

Holders for	Manufacturer	Wells	Well Type	Support Status	Compatibles
1-Slide holder (2/3 cover slip)	Nexcelom Bioscience	1	---	Supported	---
1-Slide Holder (square cover slip)	Nexcelom Bioscience	1	---	Supported	---
4-Slide Holder (2/3 cover slip)	Nexcelom Bioscience	4	---	Supported	---
4-Slide Holder (square cover slip)	Nexcelom Bioscience	4	---	Supported	---
BD Falcon™ 353003 Dish	Corning	1	---	Supported	353803

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