QX700™ ddPCR™ System Analysis Software, Standard Edition

User Guide

Version 1.5

BIO-RAD

QX700 ddPCR System
Analysis Software
Standard Edition
User Guide

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Revision History

Document	Date	Description of Change
QX700 ddPCR System Analysis Software User Guide, Standard Edition, Version 1.5 DIR No. 10000258610, Ver A	April 2026	Create a standalone guide for standard edition; realign chapters and sections for more efficient flow; update the content to include new and enhanced functionality for v1.5
QX700 ddPCR System Analysis Software User Guide, Version 1.4 DIR No.10000171494, Ver C	August 2025	Remove registered copyright symbol from all instances of naica; add new analysis option for pooled chambers; add section on chamber panel and saves; minor fixes to content for correction, style, and clarity
QX700 ddPCR System Analysis Software User Guide, Version 1.4 DIR No.10000171494, Ver B	July 2025	Update with SME edits
QX700 ddPCR System Analysis Software User Guide, Version 1.4 DIR No.10000171494, Ver A	June 2025	New Bio-Rad document, adapted from existing content

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Chapter 1 Droplet Digital PCR Overview

Droplet Digital™ PCR (ddPCR™) is a digital polymerase chain reaction method based on water-oil emulsion droplet technology. Using reagents and workflows that are similar to Taqman probe-based design, ddPCR employs a combination of microfluidics and proprietary surfactant chemistries to divide each sample into a high volume of water-in-oil droplets per well.

In general, ddPCR can be performed on the instrument with all types of DNA sample. However, individual sample type compatibility for digital PCR applications might require a dedicated assay validation by the end-user. The extraction method used, as well as sample purity, can influence sample compatibility for digital PCR applications.

ddPCR Advantages

ddPCR achieves high performance in the following areas:

- Absolute quantification of target DNA copies in samples to determine precise concentrations without using standard curves; this technique is ideal for nucleic acid target sequence measurements, viral titer concentrations, or microbial load determination
- More precise measurement of differences in gene copy number to better identify genomic alterations that indicate phenotypic variability, complex behavioral traits, and disease
- Detection of rare mutations or sequences in complex samples, such as a few tumor cells in a wild-type background
- Absolute quantification of gene expression levels, particularly involving low-abundance microRNA
- Next-generation sequencing (NGS) quantification to increase sequencing accuracy, validate variations/mutations, and reduce run repeats
- Low copy number quantification and gene expression of individual cells required by the high degree (10-fold to 100-fold) of cell-to-cell variation in gene expression and genomic content among homogeneous post-mitotic, progenitor, and stem cell populations
- Fast, precise, and cost-effective assessment of HDR (Homology directed repair) and NHEJ (non-homologous end joining) generated by CRISPR-Cas9 or other genome editing tools to detect genome edits
- Partitioning of the sample in ddPCR enables higher precision and more reliable measurement of small differences in the target DNA sequences.

- High-copy samples and background are diluted, effectively enriching the concentration in partitions with positive targets. Increased signal-to-noise ratio allows for the sensitive detection of rare targets and enables a ±10% precision in quantification.
- Error rates are reduced by removing the amplification efficiency reliance of qPCR, enabling accurate quantification of targets to near zero.
- For absolute quantification, there is no requirement for a standard curve.

ddPCR Workflow

The ddPCR process adheres to the following workflow:

- Experiment prep — samples are prepared by combining DNA or RNA with primers, probes dye, and Bio-Rad ddPCR supermix.
- Droplet generation — samples are fractionated into thousands of droplets in each well, with target and background DNA distributed randomly into the droplets during the partitioning process.
- Thermal cycling — following droplet generation, the droplets are run through the thermal cycler, which performs PCR amplification of the nucleic acid target in each individual droplet.
- Droplet reading — Each droplet is scanned (read) to determine the fraction of positive droplets in the original sample. Poisson statistical formulas are used to determine the absolute quantity.

  Note: Positive droplets containing at least one copy of the target DNA molecule exhibit increased fluorescence, as compared to negative droplets. Poisson statistical formulas are used to determine the absolute quantity.

Finding Out More

You can visit bio-rad.com to download product information, technical notes, videos, and user manuals related to ddPCR instruments and technology.

Chapter 2 System Overview

This chapter briefly describes the QX700™ Droplet Digital PCR System components and the intended use of the system. For comprehensive information on each instrument model, see the QX700 Droplet Digital PCR System Instrument Guide (catalog no. 10000171493).

Intended Use

Important: The instrument and software are intended for **Research Use Only** and are NOT for use in diagnostic procedures.

The QX700 ddPCR System is used to configure, run, and analyze experiments that produce DNA target data. The system is intended for qualified personnel who are trained in molecular biological techniques, as well as system care and use. *Before using the QX700 ddPCR System, you must complete all required training provided by Bio-Rad*.

This user guide contains information on analysis features for the Standard Edition. For information on the setup and run execution features in the QX700 ddPCR System Control Software, refer to the QX700 Droplet Digital PCR System User Guide (catalog no. 10000171493).

QX700 ddPCR System Models

To support your organization's throughput needs, the QX700 ddPCR System is available in the configurations described in Table 1. For detailed information on instrument capabilities, refer to the QX700 Droplet Digital PCR System User Guide (catalog no. 10000171493).

Each model of the QX700 ddPCR System performs a three-step process to produce experiment data on DNA and RNA targets. Droplets are generated, DNA is amplified using PCR, and then droplet data is scanned (read) in up to seven fluorophore channels.

Table 1. Instrument models

Category	QX700 E (Essential)	QX700 S (Standard)	QX700 HT (High Throughput)
Throughput (samples per run)	Up to 48	Up to 192	Up to 384
Time to results	~2.5 hrs	~4.5 hrs	~8 hrs
Description	Entry-level model designed for basic throughput needs and cost-sensitive labs	Balanced option that offers higher sensitivity and moderate throughput	Premium model optimized for high-throughput environments
Recommended for...	Smaller-scale applications or labs just beginning to use digital PCR	Labs requiring more robust performance, but without the scale of HT	Large-scale screening, biopharma, and CRO/ CDMO applications

Associated Software Applications

The QX700 Droplet Digital PCR System includes the software applications described in Table 2, which are designed to support experiment setup, instrument operations, and data analysis.

The applications are installed on the instrument and accessible from the touch screen but you can also install the software on one or more PCs to maximize capabilities for setting up and analyzing experiments while instruments are in use for run processing. For information on installing the software, see Installing QX700 ddPCR System Analysis Software on page 120.

No login is required for Standard Edition.

Table 2. Supporting software applications

Software	Used to...
QX700 ddPCR System Control Software, Standard Edition	Set up and run ddPCR experiments on the instrument
QX700 ddPCR System Analysis Software, Standard Edition	Analyze droplet data acquired by the instrument and the calculated absolute concentrations of the targeted nucleic acids; no login required

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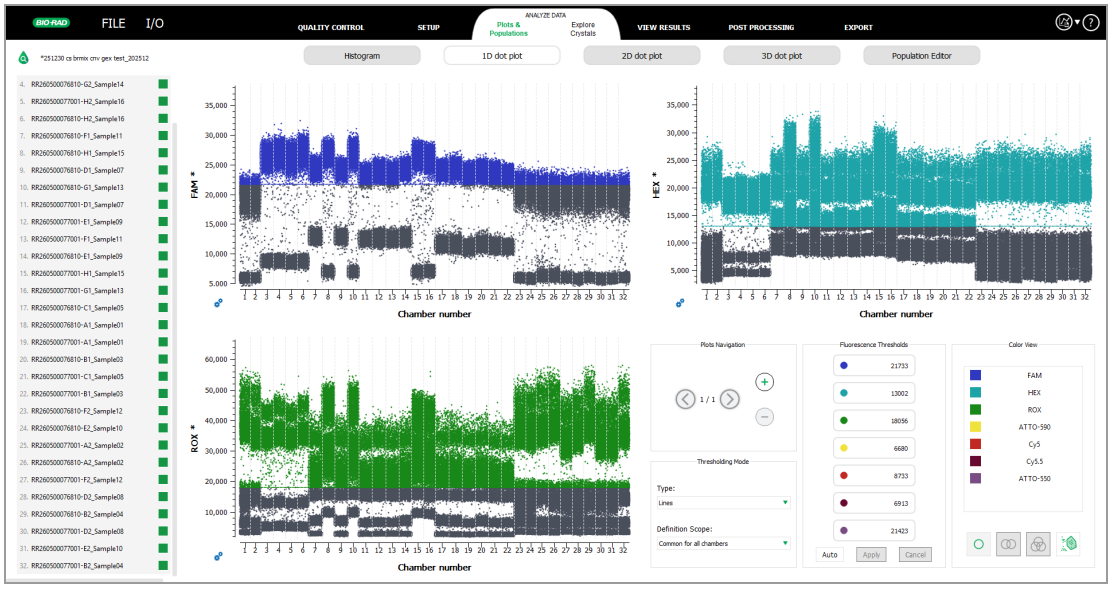
Chapter 3 Experiment Analysis Overview

In this guide, an *experiment* is defined as a set of samples that are processed by the QX700 Droplet Digital PCR System using the droplet generation, thermal cycling, and droplet scanning (reading) ddPCR functions.

Samples are run through the instrument in a cartridge plate containing up to three RDG16 cartridges. During the experiment run, the system applies the scanning parameters, embedded file instructions, and experiment details to the samples in each chamber. When the run concludes, the system creates a .niodata file for analysis in the QX700 ddPCR System Analysis Software.

When you open the experiment file, the Plots & Populations view in the Analyze Data tab appears by default.

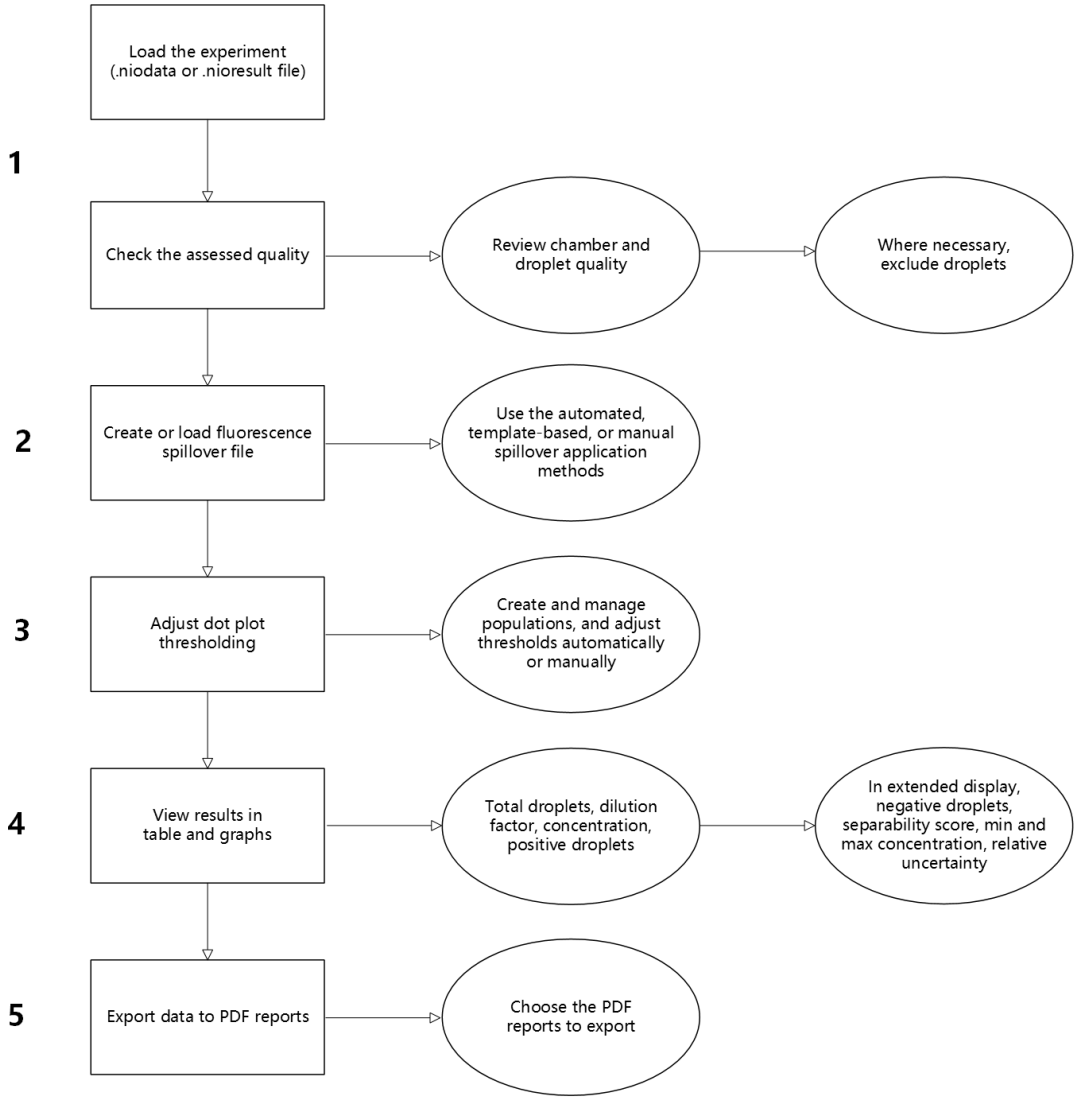


Important: When you save a .niodata file in the analysis software, it is always saved as a .nioresult file (whether or not you make changes) so the .niodata file remains in its original state.

Analysis Process Workflow

User analysis in the QX700 Analysis Software typically adheres to the following general workflow.

General Workflow Steps for QX700 Data Analysis



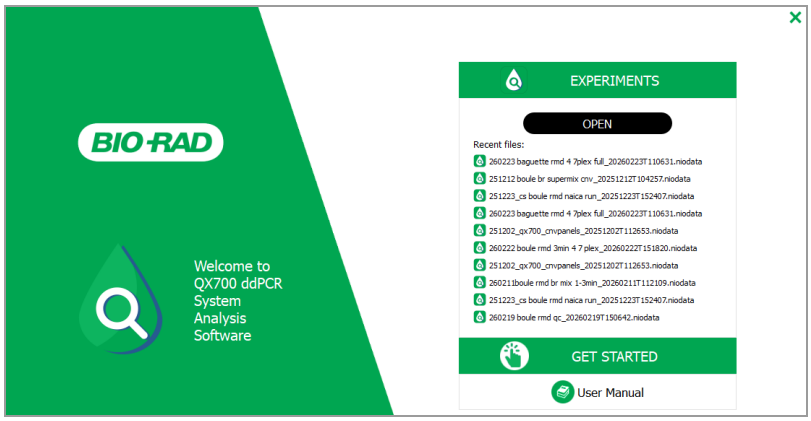
Opening an Experiment

You can open the QX700 ddPCR System Analysis Software from the instrument touch screen or a Windows computer. For information on installing the software on computers, see Appendix A, Software Installation.

To open an experiment

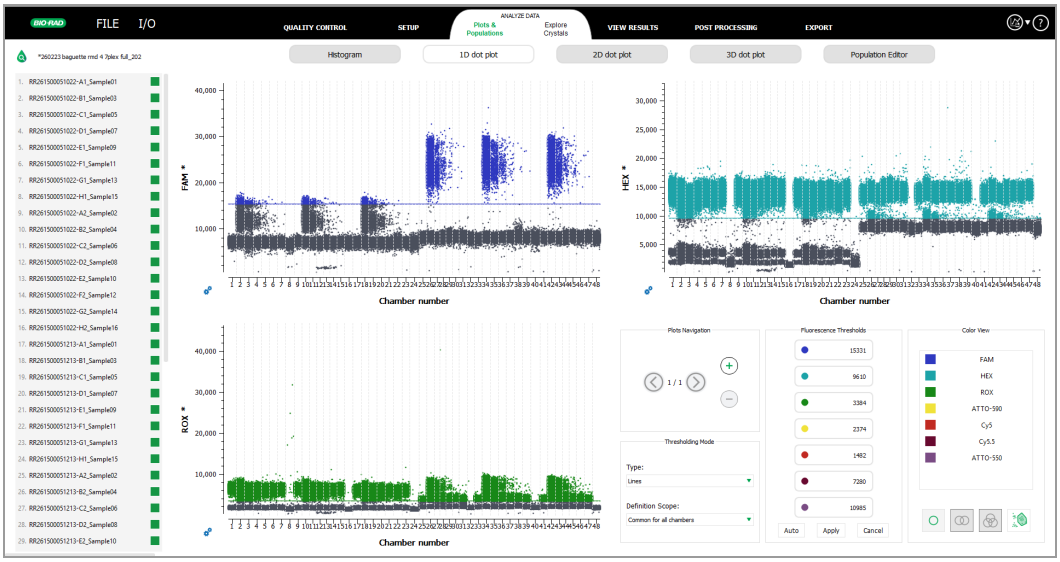
1. Tap/click or double-click the software shortcut ( ).
2. When the Experiments dialog appears, do one of the following to open a .niodata or .nioresult file:
   - Tap/click Open and navigate to the data file storage location to select the file
   - Select a recent file from the list.

     Note: After first use, up to ten most recently opened files appear in the Recent files list to facilitate subsequent access. If you close the Open file dialog before you select a file, the blank experiment layout remains. You can tap/click File > Home to reopen the Experiments dialog.



The .niodata and .nioresult files open to the Populations & Plots display in the Analyze Data tab. *Data files can take a few minutes to load*.

The 1D dot plot view appears by default, and all chambers in the left pane are automatically selected.



Note: After the file opens, you can use the I/O menu to load compatible configuration files for spillover compensation matrices, plots, and analysis. For information on the file types, see I/O Menu on page 25.

Accepted File Types and File State Indicators

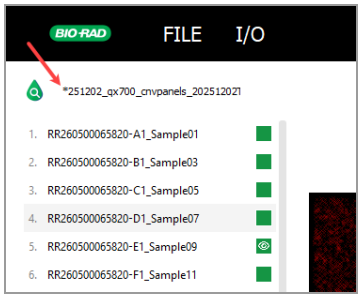
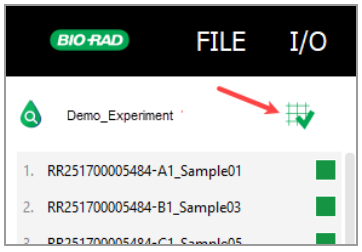
The QX700 ddPCR System Analysis Software accepts the file types specified in Table 3.

Table 3. QX700 data and configuration file formats

File type	Description
.niodata	*Original data file generated by the QX700 ddPCR System Control Software* .niodata files contain the original high-quality images required for image analysis. Experiments that are opened for first-time analysis open in .niodata format. **Note:** The .niodata file type has been reduced in size but might still take a few minutes to open.
.nioresult	*Post-analysis file generated by the QX700 ddPCR System Control Software when the original .niodata file is saved.* When you save a .niodata file after making changes, the analysis software creates a .nioresult file with the changes and keeps the .niodata file in its original state so the initial data is preserved.
.ncp	*Plot configuration file* .ncp files include axis labels, scale type (linear or logarithmic), and the plot page sequence in the histogram, 1D dot plot, and 2D dot plot configurations.
.nca	*Analysis configuration file* .nca files include the following information for all data analysis parameters: ■ Manual or automated thresholding strategy ■ Thresholding mode and scope ■ List of the populations (default or custom) with their name, color, and zones ■ Coordinates defining each zone, such as threshold values for lines or vertex coordinates for polygons).
.ncm	*Compensation matrix format* .ncm files contain the compensation parameters applied to the signal for each channel; they are specific to the assay and assay conditions, but are independent of exposure time.

Table 4 contains information on symbols that appear next to the experiment name.

Table 4. File state indicators

File state	Symbol	Description
Modified, but not yet saved	*	When an asterisk (*) appears next to the file name, the file has unsaved changes. Otherwise, no changes were applied to the file since it was opened.  When an asterisk (*) is used in a plot (next to fluorophore names), the experiment is NOT compensated. Asterisks can appear the x-axis and y-axis.
Compensated		When a grid icon with a check mark appears after the experiment name (above the Chamber Pane), spillover compensation has been applied. Validation requires that a compensation file is loaded in the QX700 ddPCR System Control Software. 

General Navigation Options

If you are using the instrument touch screen you can tap the different screen elements and options. If you are using a Windows PC with a keyboard and mouse, you can use the following navigation and selection options:

- Scroll a list using the mouse roller
- Zoom in and out of a graph using the mouse roller
- CTRL-click to select multiple files
- SHIFT-click to select multiple sequential files
- Right-click and drag to rotate a graph *(3D amplitude plots only)*
- Left-click and drag to move the graph and the rotation center *(3D amplitude plots only)*
- Double-click to reset the view icon description
- Click to drag and drop

Note: Some sections of this guide cite specific navigation options for the specified functions.

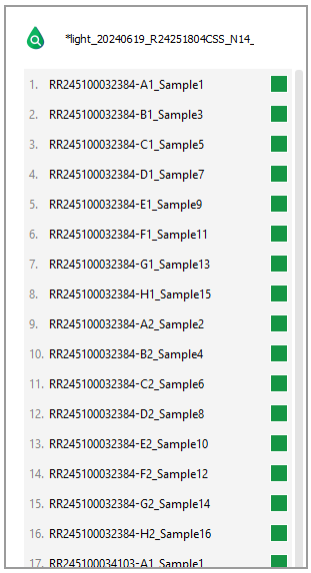
Channel Fluorophores and Colors

In the QX700 ddPCR System Analysis Software, the fluorophores cited below correlate to the specified color and channel.

Fluorophore	Color		Channel
FAM		Blue	Channel 1
HEX		Teal	Channel 2
ROX		Green	Channel 3
Atto-590		Yellow	Channel 4
Cy5		Red	Channel 5
Cy5.5		Infra-Red	Channel 6
Atto-550		Purple	Channel 7

Chambers Pane

The term "chamber" indicates a sample well in the RDG16 cartridge. The QX700 ddPCR System can accept up to three RDG16 cartridges per run, for a total of 48 chambers. The left pane, which contains the list of scanned chambers in each cartridge, in the order they were processed, is accessible from each of the functional tabs in the top menu bar. A quality indicator appears to the right of each chamber. For information on the quality indicators, see Performing Quality Control on page 29. The experiment name is also shown above the chamber list.



Chambers are named per the following naming convention:

<RDG16 cartridge ID>-<chamber ID>_<sample name>

Each segment of the chamber name is explained in Table 5.

Table 5. Chamber naming segments

Component	Description
Cartridge ID	Unique barcode of the cartridge containing the chamber; this information is typically filled in the QX700 ddPCR System Control Software as the cartridge barcode.
Chamber ID	Position of the chamber in the cartridge (A, B, C, and so forth from top to bottom) followed by the column index of the chamber on the cartridge (1 or 2 from left to right); this information is automatically defined by the software (for example, A1 or D2).
Sample Name	Name of the sample in the chamber

Analysis Software Views

The QX700 ddPCR System Analysis Software provides the main views specified in Table 6.

Table 6. Main software views

Tab	General Purpose	For more information, see...
Quality Control	View droplet quality	Performing Quality Control on page 29
Setup	View/edit experiment parameters	Modifying the Experiment Setup on page 37
Analyze Data	Analyze plot data and droplet images	Data Analysis on page 59
View Results	Review results table; analyze concentration, uncertainty curve charts	Viewing Results on page 91
Post Processing	Apply post-processing calculations	Post-Processing Changes on page 103
Export	Export selected details, data, and chart graphics	Exporting System and Analysis Data on page 109

Analysis Software Menus

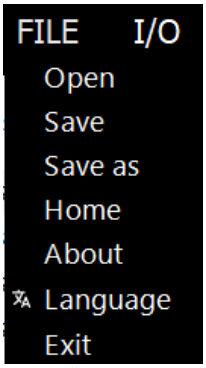
The QX700 ddPCR System Analysis Software provides tabs and menus that allow you to

- Open and save experiments
- Load and save compensation and configuration information
- View experiment data quality
- Edit experiment setup
- Analyze experiment data
- View results (data tables and graphs)
- Apply post-processing calculations
- Export selected details, data, and chart graphics
- Save images
- View context-sensitive help pop-ups

File Menu

To display the File menu

▶ Click File in the menu bar. The menu expands to show all options.



Using the File menu, you can

- Open .niodata and .nioresult experiment files
- Save experiments

  When you save a .niodata file, it is saved as a .nioresult file. The original .niodata remains unchanged to preserve data integrity.
- Return to the Home dialog containing the recent files list
- View software information
- Change the language
- Exit the software

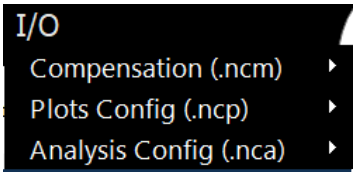
I/O Menu

Using the I/O menu, you can load and save the following file types to apply to the experiment:

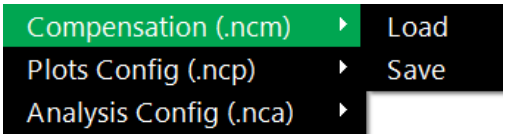
- Spillover compensation (.ncm)
- Custom plot configuration (.ncp)
- Custom analysis configuration (.nca)

To open the I/O menu

1. Select I/O in the menu bar.



2. Select an option to open the submenu, and then select Load or Save. Follow the prompts to navigate to the file or save the file, respectively. *Each submenu contains the Load and Save options*.



Save Images Menu

The Save menu allows you to save or copy plots and other graphics.

Table 7 describes the available options. You can save the file with the existing file name or a new file name.

Table 7. Save menu options

Icon	Option	Description
	Save image	Saves the image in .png format
	Save vector image	Saves the image in .svg format
	Copy image	Copies the image to the clipboard, from which you can paste it into another resource (graphics program, document, and so forth)

To save or copy an image

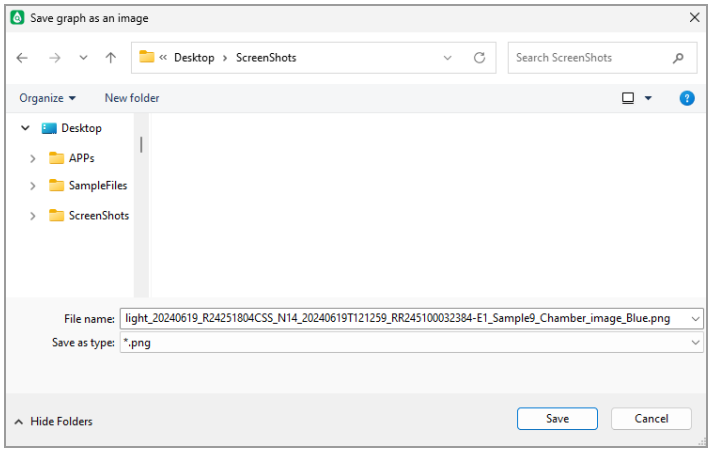
1. Open the tab containing the image.
2. Tap/click the icon in the upper-right corner to open the menu.

   Note: The displayed Save menu icon represents the last selection from the menu.
3. Tap/click Save Image, Save Vector Image, or Copy Image.

4. Pause the arrow on the image or area to capture and then click the mouse button.

   One of the following occurs:

   - If you are copying, then the image is copied to the clipboard. You can paste the image into a different resource (image editor program, document, and so forth).
   - If you are saving, a Save as dialog opens. The default file name appears in the File name field.



5. To save the file:

   a. (Optional) Navigate to a new storage location.

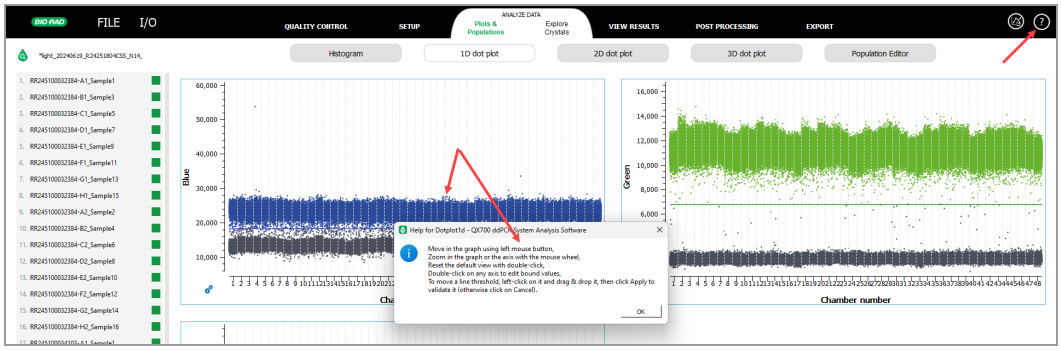
   b. Enter a new file name or keep the default entry.

   c. Click Save.

   The image is saved as a .png or .svg file.

Help Menu

Use the Help menu to access context-sensitive tool tips that explain the selected functionality.



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Chapter 4 Performing Quality Control

The Quality Control tab provides the tools required to perform a visual inspection of the droplets in each chamber and identify higher object saturation, which can result in high exposure time or artifacts.

A quality flag appears to the right of the chamber name. A flag is applied to each chamber to indicate the assessed overall quality of the droplets. Each quality flag is described in Table 8.

Table 8. Chamber quality flags

Quality flag	Description
	A green flag indicates that all quality indicators are within expected specifications.
	A green flag with an eye icon means that Bio-Rad recommends inspecting the droplets visually to decide whether the result should be considered.
	A yellow flag indicates that the system identified one or both of the following conditions: ■ Too many artifacts were identified in the droplet image ■ Too few (less than 1,000) detected droplets were countedin the chamber

Options and information appear in the right pane are described in Table 9.

Table 9. Quality Control options

Option	Description
Channel selection	Tap/click a colored circle to view an image of the droplets in the corresponding channel LED.
Contrast adjustment	Tap/click the + or – icons to raise or lower the image contrast and brightness for each selected channel. You can also tap/click Auto to automatically adjust the contrast and tap/click Reset to return to the original display.
Droplet exclusion	Tap/click Exclude to exclude droplets in the chamber. You can also cancel the action or restore the excluded droplets.
Legend	The legend pane allows you to show or hide droplets as they are annotated (artifact, analyzed, positive). Legend items correspond to the selected channel.
Number of analyzable droplets	The higher the number the better the confidence in predicted concentration results.
Number of oversaturated objects	Te lower the number the better the chamber quality. **Important:** Object oversaturation can apply to droplets subjected to high exposure time, or to artifacts. To correct oversaturation, you can check exposure times in QX700 ddPCR System Control Software to ensure they are not too high, or you can clean the cartridge with an appropriate wipe to remove dust or fluorescent elements that might have accumulated on the foil. For information, refer to the QX700 Droplet Digital PCR System Instrument Guide.

The following graphics illustrate a droplet image for each indicator.

Fig. 1: Example of a droplet image in a chamber with acceptable data quality

Fig. 2: Example of a droplet image in a chamber flagged with the green eye

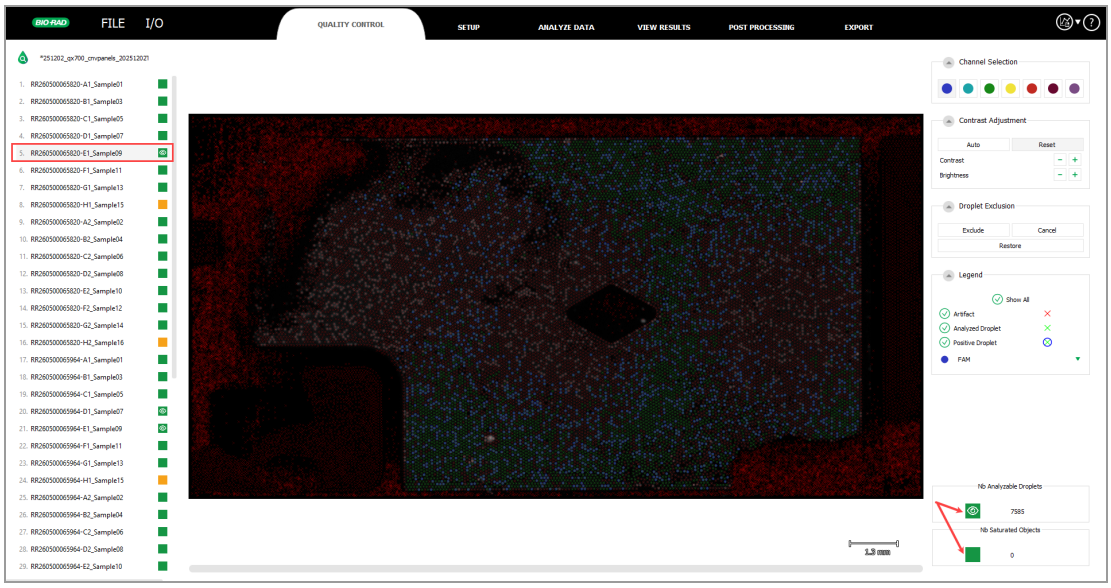
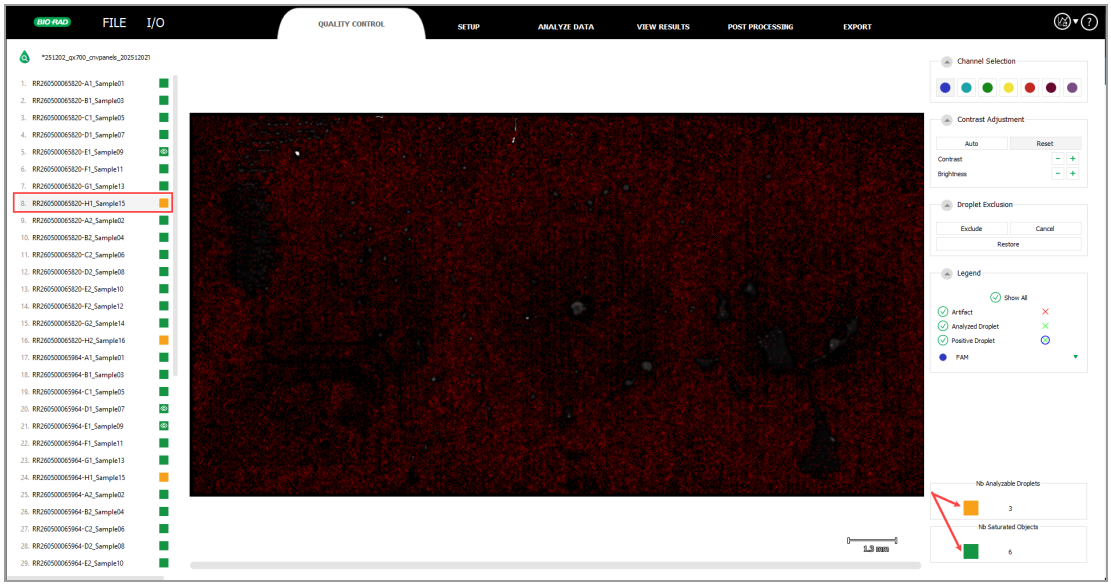
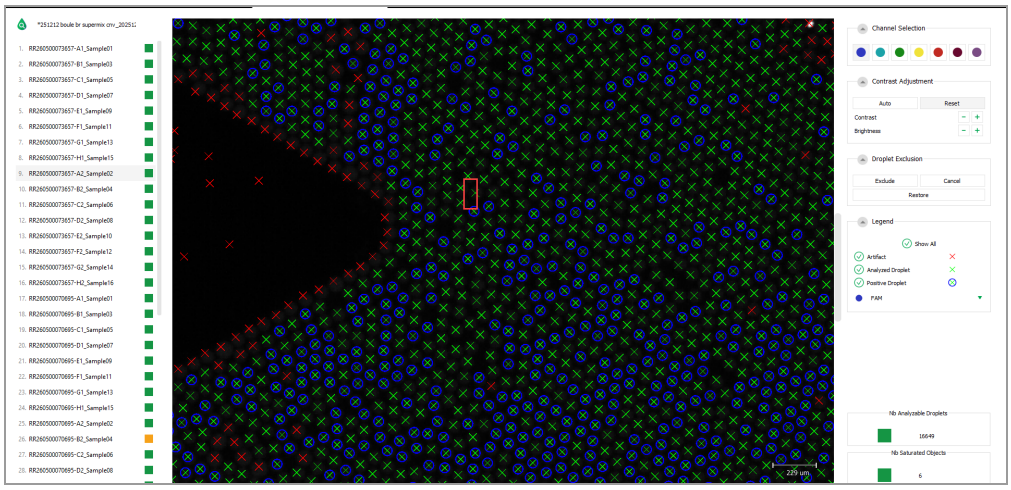


Fig. 3: Example of a droplet image in a chamber with a yellow flag



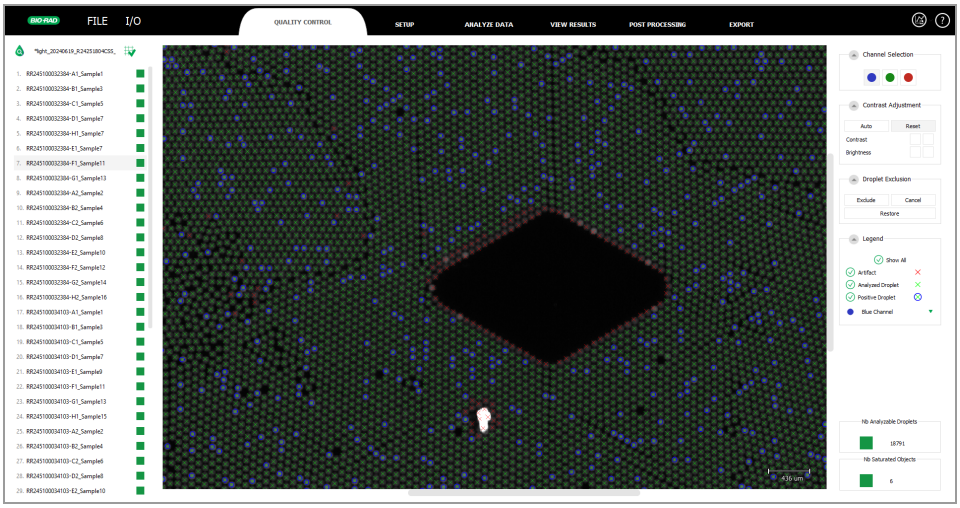
Droplet Image Quality Control

Use the Quality Control display to analyze the droplet quality in each chamber. You can use the mouse scroller to enlarge the droplet icons.

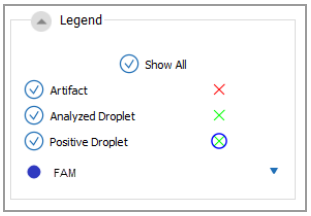


To perform quality control

1. To access the quality control display and features, tap/click Quality Control in the menu bar.
2. Tap/click a chamber to display the image.
3. Tap/click a channel under Channel Selection.
4. Use the mouse scroller to zoom the image and identify artifacts, analyzed droplets, and positive droplets for the selected channel.



The following legend identifies the indicator symbols:



5. Show All is selected by default. To reduce the items in the display, clear Show All and then select one or two of the items.

   The indicators are described as follows:

   - Red cross — Artifact (dust, undersized or oversized droplet, irregularly shaped droplet, boundary droplet, and so forth. Artifacts are not counted as analyzable droplets
   - Green cross — Negative droplet, which is counted as an anayzable droplet
   - Green cross in a blue circle — Positive droplet, which is counted as an analyzable droplet

6. To navigate in and around the image, see General Navigation Options on page 21.
7. To select a different channel, tap/click a colored circle under Channel Selection.
8. To automatically adjust the image contrast, tap/click Reset under Contrast Adjustment.

Excluding Droplets

Oversegmentation (two or more droplet crosses instead of one) or undersegmentation (one droplet instead of two or more) of the droplets might exist, which can lead to false positive or negative results. Bio-Rad recommends that you manually inspect the droplets, as follows:

- Where there are red crosses, the software automatically rejects the droplets, and no action is required.
- Where aberrant crosses are green, you must exclude them manually.

Note: Artifacts are detected in the channel but some artifacts might exist and be visible only in other channels, depending on the experiment/assay/chemistry used. Bio-Rad recommends that you check all channels available in the experiment in case there are droplets that must be manually excluded.

To exclude droplets

1. To exclude one or several droplets considered as false positives (such as artifacts):
   a. Right-click on each droplet to be excluded.
   b. Tap/click Exclude under Droplet Exclusion.

      Note: Excluded droplets are marked with yellow hexagons.

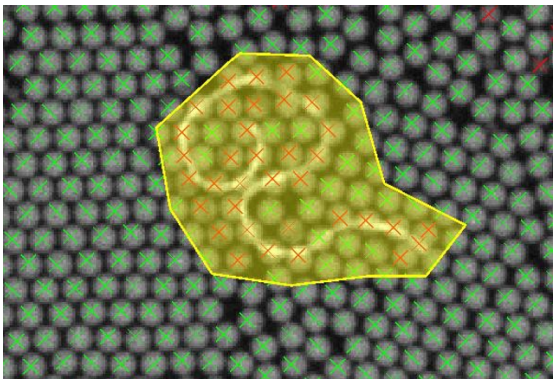
2. To exclude all droplets in an image region:

   a. Press CTRL, and then right-click several times to define the polygon image region to exclude (one click = one vertex).

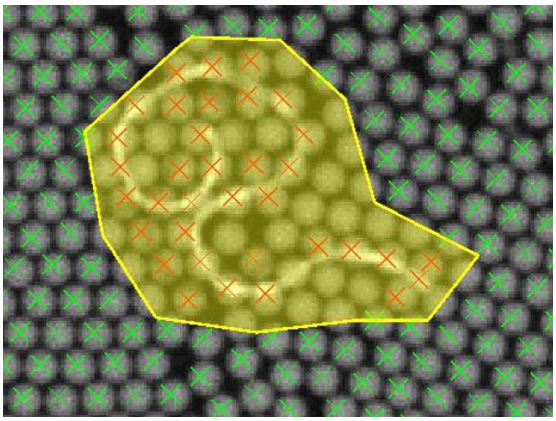
   b. Release CTRL to define the last polygon vertex.

   c. Tap/click Exclude under Droplet Exclusion.

   The following graphic shows a selection of droplet or polygonal image regions to be excluded. Before you tap/click Exclude, droplet annotations are still visible in the yellow shape.



   The following graphic reflects the removal of all selected droplet or image regions. After you tap/click Exclude, droplet annotations are no longer visible in the yellow shape. All relevant droplets in the polygonal region are removed from the analysis and marked with yellow color.



3. To cancel the current selection of droplets to be removed, tap/click Cancel under Droplet Exclusion.

4. To restore all the manually excluded droplets, tap/click Restore under Droplet Exclusion.

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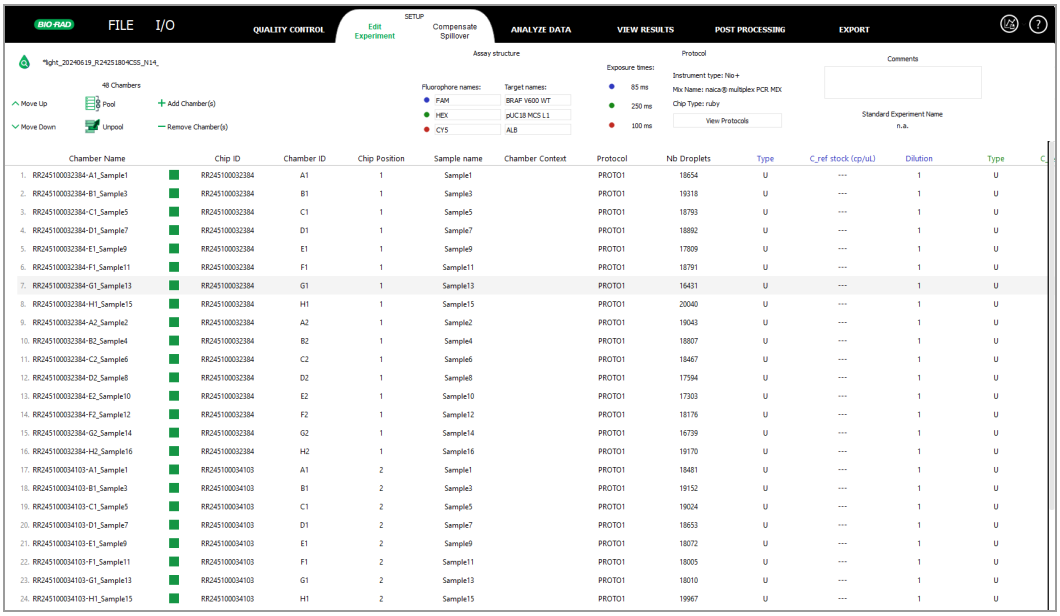
Chapter 5 Modifying the Experiment Setup

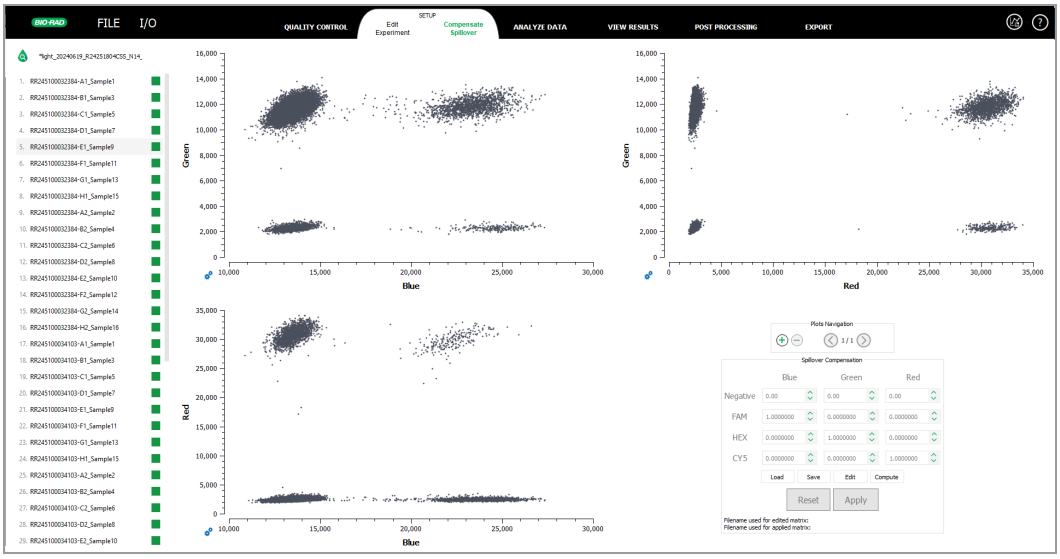
The Setup tab includes the Edit Experiment and Compensate Spillover displays, which are briefly described in the following bullet points:

- **Edit Experiment** — view data from the run and change configuration elements in the table. You can also edit fluorophore and target names, add or remove chambers, and pool (merge) chambers for higher detection sensitivity.



- **Compensate Spillover** — load an existing compensation matrix that matches the experiment parameters and apply it to the results. You can also manually edit the spillover compensation in the grid that appears in the lower-right corner.

  Important: Bio-Rad recommends that only expert users manually modify spillover compensation values.

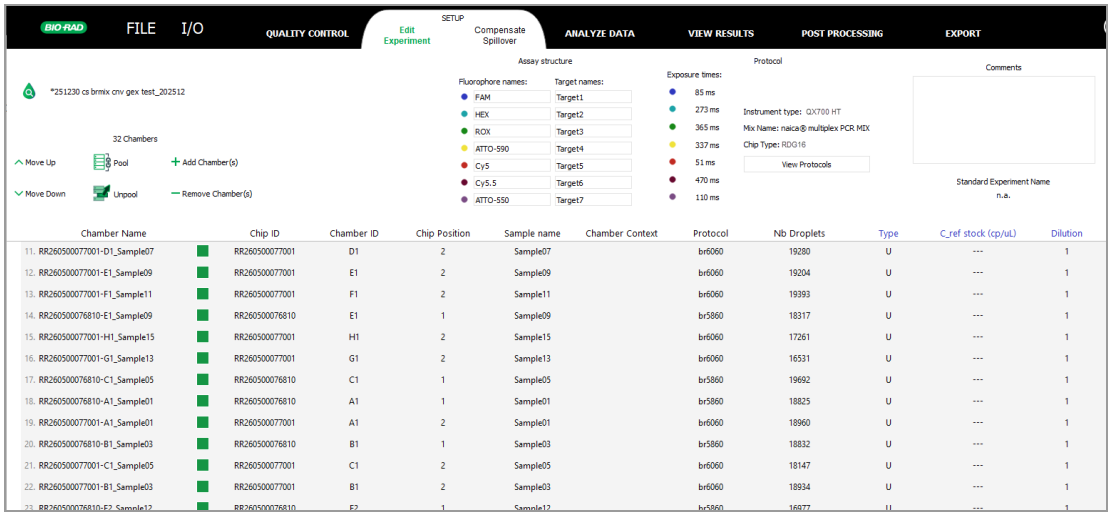


Editing General Experiment Parameters

In the Edit Experiments view, the top pane contains the fluorophore names and target names, as well as a Comments field. and the table contains chamber settings. Editable values are typically defined in the QX700 ddPCR System Control Software. but they can be modified in the QX700 Analysis Software.

The assay and thermal cycling protocol that were specified in the QX700 ddPCR System Control Software are also identified in the top pane and are not editable.

Note: If all chambers do not share the same assay and protocol, Bio-Rad recommends dividing the experiment into chamber subsets using the pooling option and add/remove chambers option.



To edit global parameters in the top pane

1. In the Experiment Setup view, do any or all of the following:
   - Tap/click in a Fluorophore name field and replace the existing entry.
   - Tap/click in a Target name field and replace the existing entry with the name of a target of interest.
   - Tap/click in the Comments field to enter information about the experiment.

   Important: Other information that appears in the top pane is read-only.

To modify chamber settings

1. Press CTRL-click or SHIFT-click to select one or more chambers.

   In the table, the column headings appear in the fluorophore (channel) color (Blue = FAM, Teal = HEX, Green = ROX, and so forth)

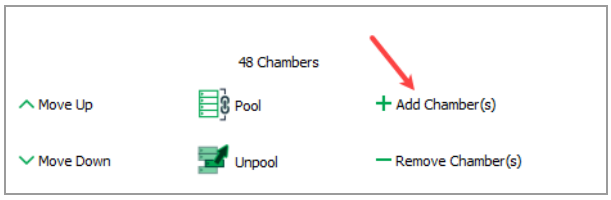
2. Edit the the entries in the following columns:

   - Sample type *(U = unknown, N = negative, P = positive, and S = standard)*
   - Dilution factor *(a value of 10 equals a 10-fold dilution)*

     Note: If the dilution factor is modified from 1 to 10, then the estimated stock concentration is multiplied by 10. The updated dilution factor is considered in post-processing data calculations, except for Linkage Analysis, which is always calculated at a dilution factor of 1.

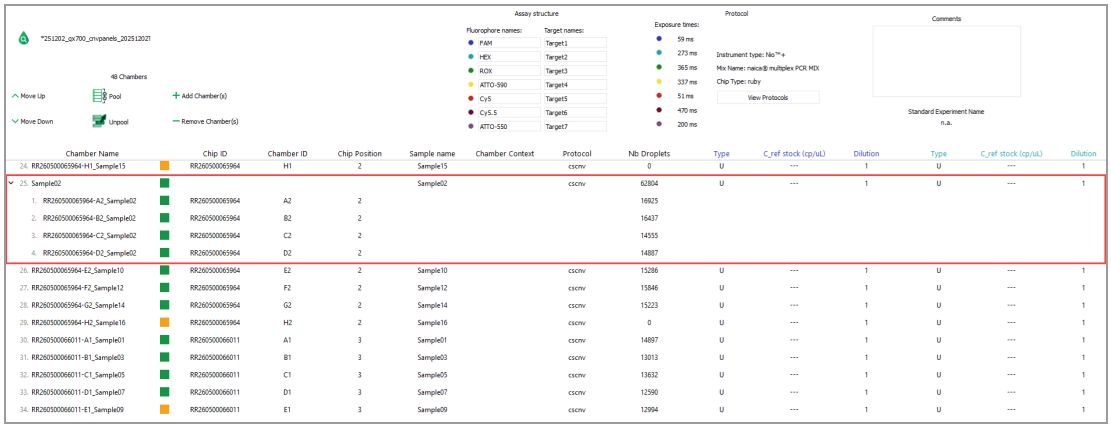
Pooling Replicate Chambers

You can pool (merge) chambers containing the same sample (replicates) to increase detection sensitivity and quantification precision.



By considering each set of pooled chambers as one large chamber, pooling increases the analyzed sample volume and quantification accuracy. The higher the number of pooled chambers, the higher the total number of analyzed droplets. This reduces sampling and partitioning errors, thereby increasing the quantification certainty.

After the chambers are pooled, QX700 ddPCR System Analysis Software automatically computes the concentration of each target in the parent chamber and updates the predicted concentration data in View Results > Results Table. One line is added for the parent chamber and the individual chamber identifiers appear below.



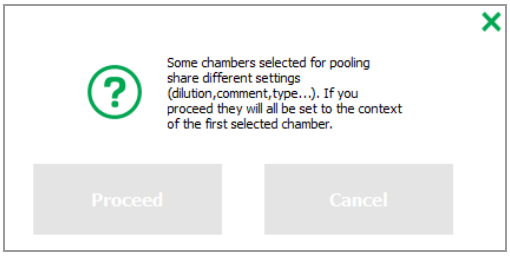
For each detection channel, the sample type, reference concentration, and dilution factor appear in the parent row.When you export the data, these new lines are exported in the same order in the Results sheet of the exported Excel file, and the pooled chamber IDs appear in the Chamber_Details sheet.

To pool chambers containing the same sample

1. Tap/click Setup > Edit Experiment.
2. From the list of chambers on the left, select the chambers to be pooled.

   The selected chambers are highlighted in the list.
3. Tap/click Pool.

   If the chambers to be pooled do not contain the same settings, an advisory message appears per the following example.

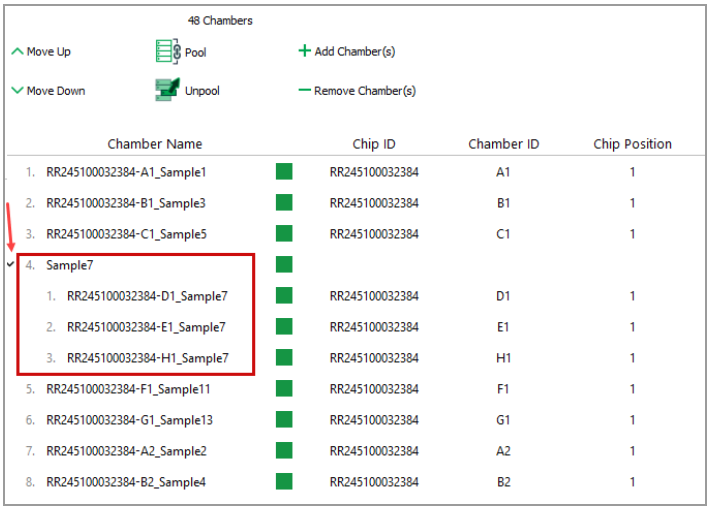


4. Read the message and do one of the following:

- If the chamber selection is as intended, tap/click Proceed.
- To select a different set of chambers, tap/click Cancel and repeat steps 2 and 3.

A parent row is added to the table (Sample 7 in the following graphic).

5. Tap/click the arrow to the left of the parent ID to expand and display the individual chambers that were pooled. The parent ID is appended to each.



6. To unpool the chambers, tap/click Unpool.

Note: Unpooled chambers return to the single-chamber format, but retain the third segment of the chamber name assigned to the pooled chambers (such as Sample7).

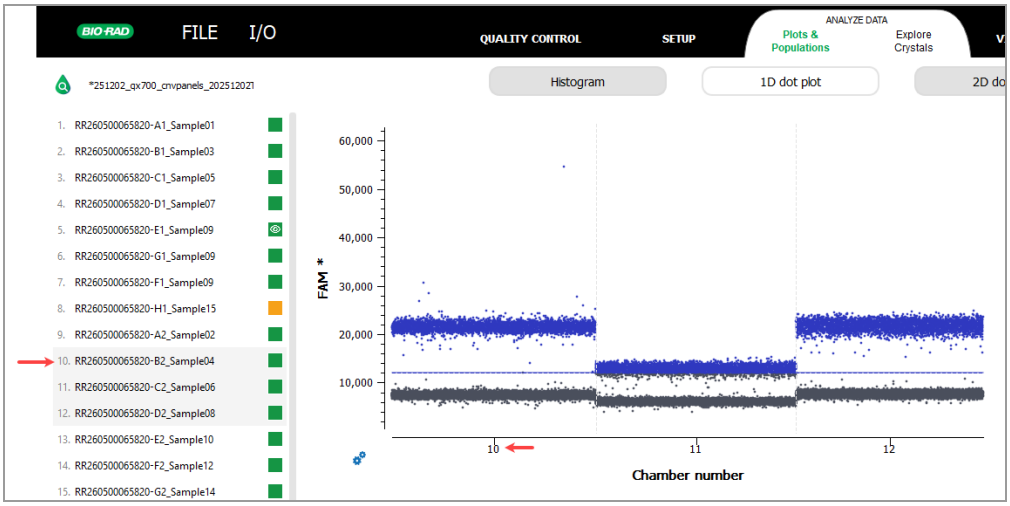
Combining Graphical Data for Multiple Chambers

Use the Plots & Populations tab to display selected chamber data side-by-side (1D dot plots) or collectively.

To display 1D dot plots side-by-side

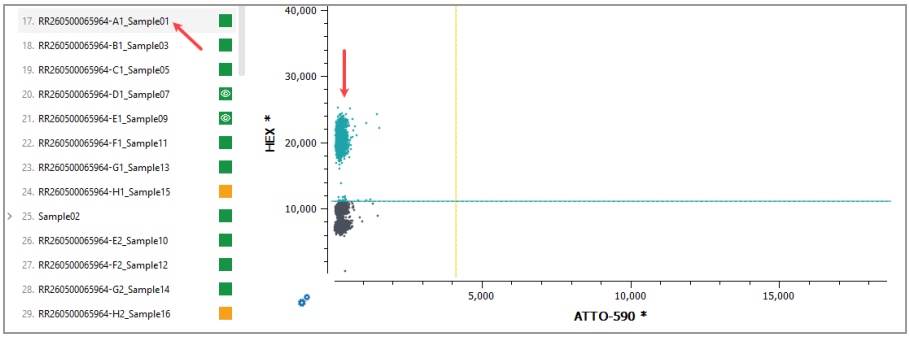
1. Tap/click the 1D Dot Plot tab.
2. In the left pane, press CTRL-click and select the chambers.

   The plots for each selected chamber appear sequentially.

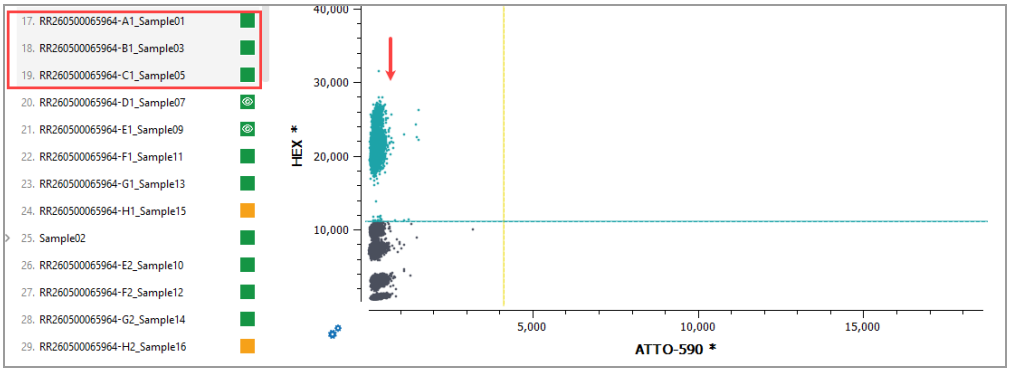


To combine chambers in 2D or 3D dot plots

1. Tap/click the 2D dot plot or 3D dot plot tab.



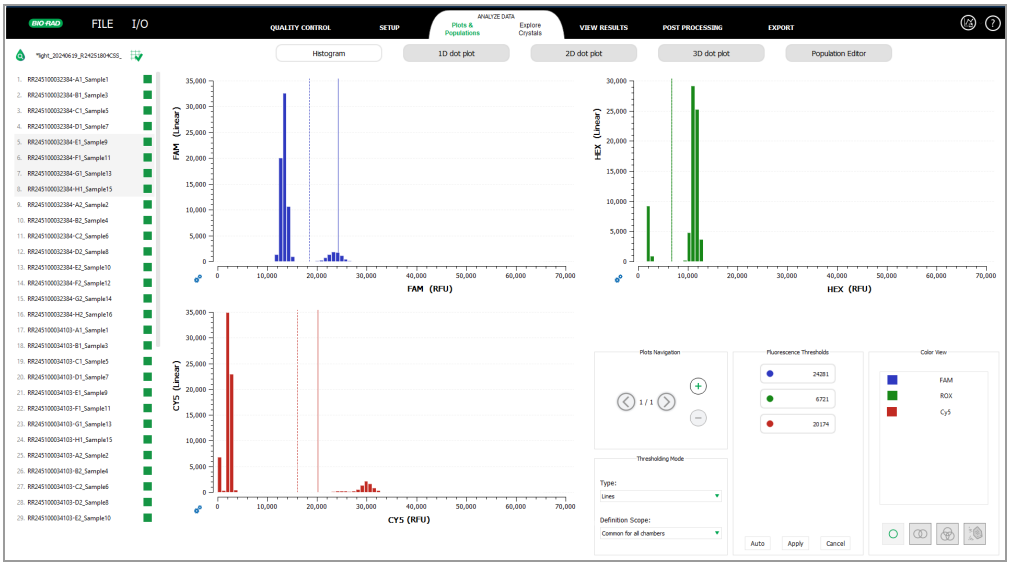
2. In the left panel, press CTRL-click and select the chambers to be merged.



To combine the histograms

1. Tap/click the Histogram tab.
2. In the left panel, press CTRL-click and select the chambers to be pooled.

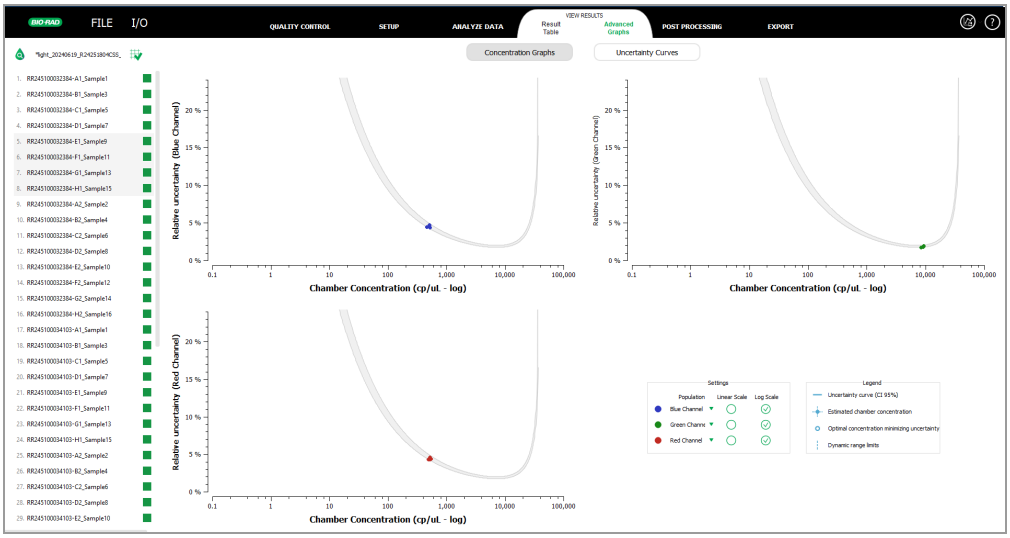
   QX700 ddPCR System Analysis Software displays histograms according to pooled chambers.



To combine the uncertainty curves

1. Tap/click the Histogram tab.
2. In the left panel, press CTRL-click and select the chambers to be pooled.

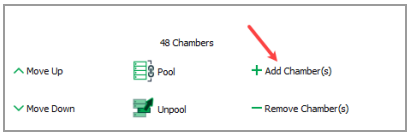
   QX700 ddPCR System Analysis Software displays the uncertainty curves of the pooled chambers.



Adding or Removing Chambers

To add chambers from another experiment

1. Click the +Add Chambers icon and navigate to the experiment.



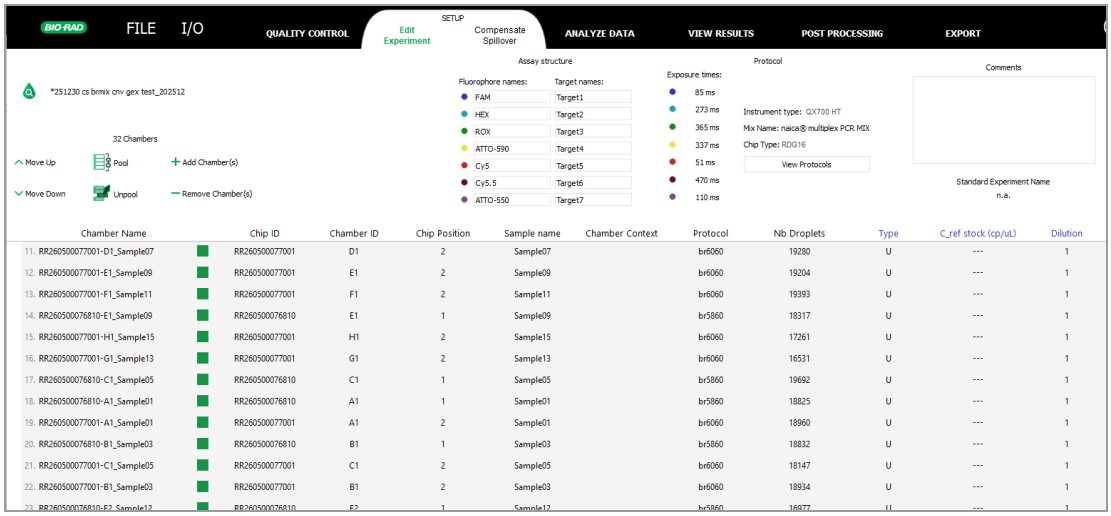
2. Select the .niodata or .nioresult file to be imported and tap/click OK.

   Important: The parameters of the files (exposure times, compensation, image processing) must match or an error message appears. Mismatched parameters can compromise concentration and other results.

To remove chambers

1. Select the applicable chambers in the Chamber pane.
2. Tap/click Remove Chambers.

Reordering Chambers



To reorder the chambers in the list

1. Select one or more chambers.
2. Tap/click the Move Up and Move Down arrows in the top pane, or tap/click a column heading to sort in ascending or descending order.

Applying Spillover Compensation

When using different types of fluorophores on the QX700 ddPCR System, spectral overlap can sometimes occur between two or more detection channels. To make proper adjustments for this fluorescence spillover, you must apply a spillover compensation matrix for each assay using the Compensate Spillover function in the Setup tab.

A compensation matrix corresponds to specific thermal cycling conditions and a specific multiplex assay composed of oligonucleotides (probes and primers) and target sequence DNA. You should create a new compensation matrix if either or both of the following changes are made to the experiment before the chambers are processed:

- Primers, probes, or DNA target sequences are modified (such as a change in primer or probe sequence or concentration)
- The thermal cycling program is modified

In *uncompensated* experiments, the Spillover Compensation dialog appears per the example in Fig. 4. Negative fields appear in the top row and contain a zero for each color. By default, all remaining values are set to zero (0.0000000) except in each field where the fluorophores intersect, which contains 1.0000000.

Fig. 4: Matrix for an uncompensated experiment

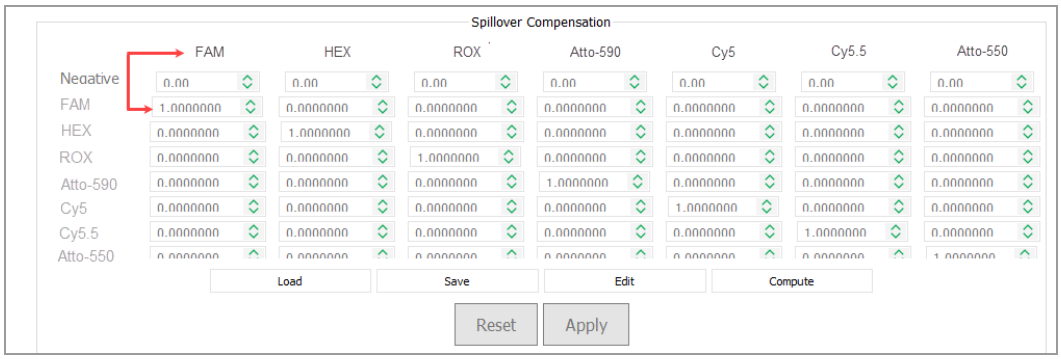
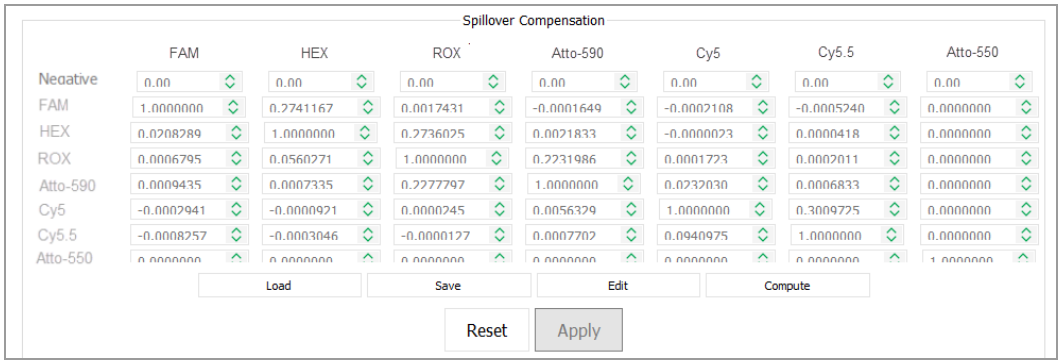


Fig. 5: Matrix for a compensated experiment



After you apply the compensation matrix to the data, the spillover signal is removed from the dot plot. and distance between clusters should be better defined to improve the the separability score. For more information, see Quantifying Fluorescence Separability on page 97.

Important: The correction is not performed in the droplet image, since it was acquired by the instrument. Therefore, a droplet can appear as positive in a channel without actually being present in the corresponding dot plot.

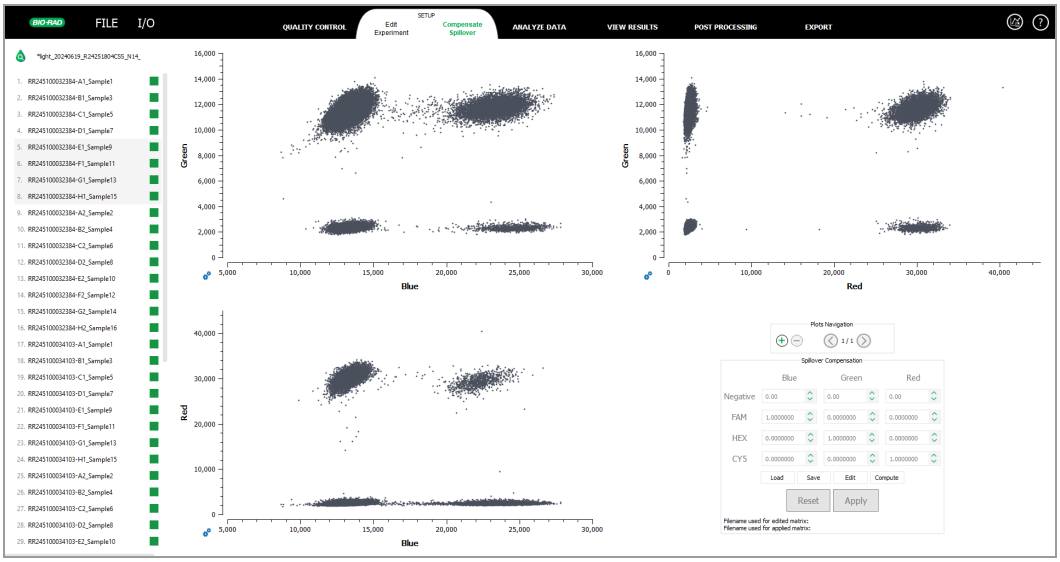
After you compute a compensation matrix using one of the methods cited in this chapter to adjust the thresholding in the chart, the fields that previously contained zeros are populated with the excitation matrix coefficients where the fluorophores intersect with the other channels (Fig. 5).

To compensate the fluorescence spillover

1. Tap/click Setup > Compensate Spillover.

   Note: To change the matrix fluorophore labels, open the Edit Experiment tab and rename the fluorophores in the top pane.

   The QX700 ddPCR System Analysis Software displays the 2D amplitude plot. The following graphic shows uncompensated data in the Compensate Spillover view.



2. To create or edit a compensation matrix, see Automated Method on page 49, Template-Based Method on page 53, or Manual Method on page 54. Additional graphics illustrate how the dot plots change in accordance with the spillover compensation.

   Important: Unless you are an expert user, Bio-Rad recommends using the automated method or the template-based method to compensate the spillover of an assay.

Automated Method

To achieve more accurate thresholding of a multiplex assay, Bio-Rad recommends using the automated method to create a compensation spillover matrix.

Important: When using the automated method, you should perform a monocolor control run first, to produce one fluorescent signal per channel in each chamber. The control is composed of all PCR reaction reagents for a given assay, with *only one* DNA target sequence present, as shown in Table 10.

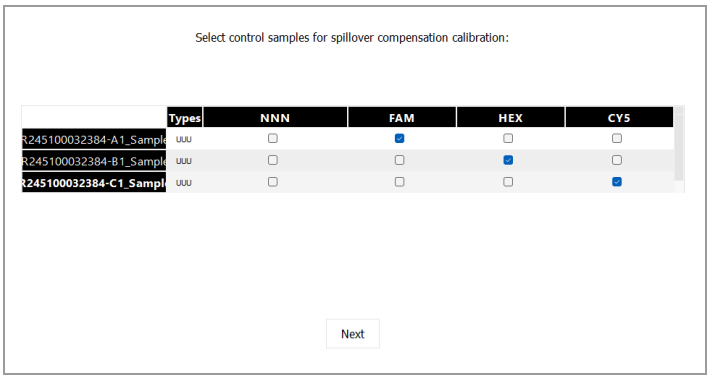
Table 10. Mono-color compensation controls

Fluorophore	Channel LED	Target	Description
Negative control	N/A	NNNNNNN	No positive signal (no target)
FAM	Blue	**P**NNNNNN	Target for FAM only
HEX	Teal	N**P**NNNNN	Target for HEX only
ROX	Green	NN**P**NNNN	Target for ROX only
Atto-590	Yellow	NNN**P**NNN	Target for Atto-590 only
Cy5	Red	NNNN**P**NN	Target for Cy5 only
Cy5.5	Infra-Red	NNNNN**P**N	Target for Cy5.5 only
Atto-550	Purple	NNNNNN**P**	Target for Atto-550 only

You must remove all but one target from the mix, leaving only the target of the desired fluorescence in any given chamber. If you repeat for each fluorophore, then only a signal target is amplified, producing a fluorescence signal that is higher than the background signal generating from the probes. One chamber per channel, plus a negative, is required to compute a monocolor compensation matrix.

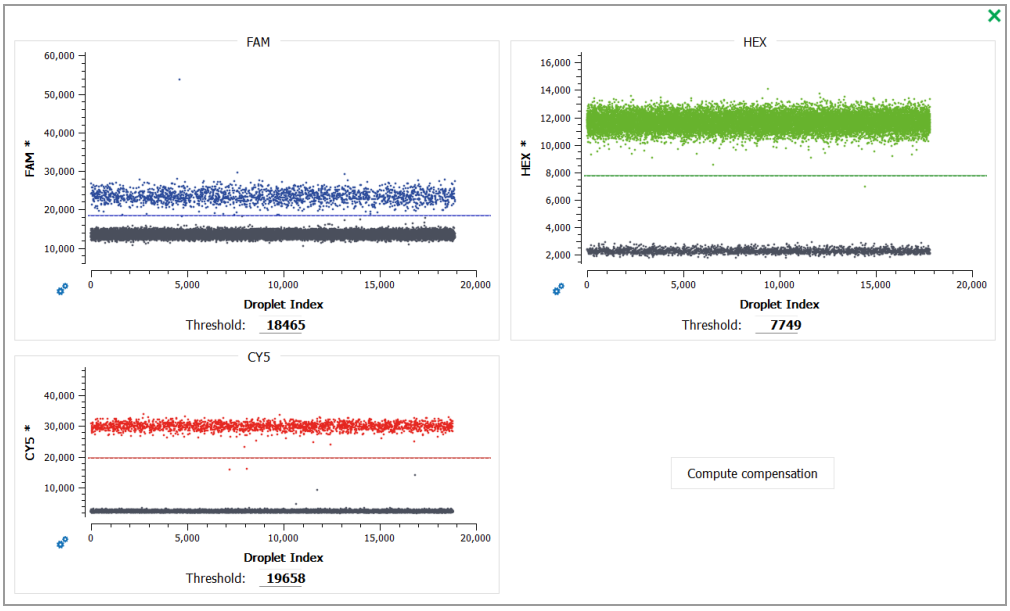
To automatically compute a compensation matrix

1. After the data is generated from the monocolor experiment, tap/click Setup > Compensate Spillover, and then tap/click Compute.
2. In the resulting dialog, tap/click the checkboxes to match the appropriate monocolor controls to the chambers.

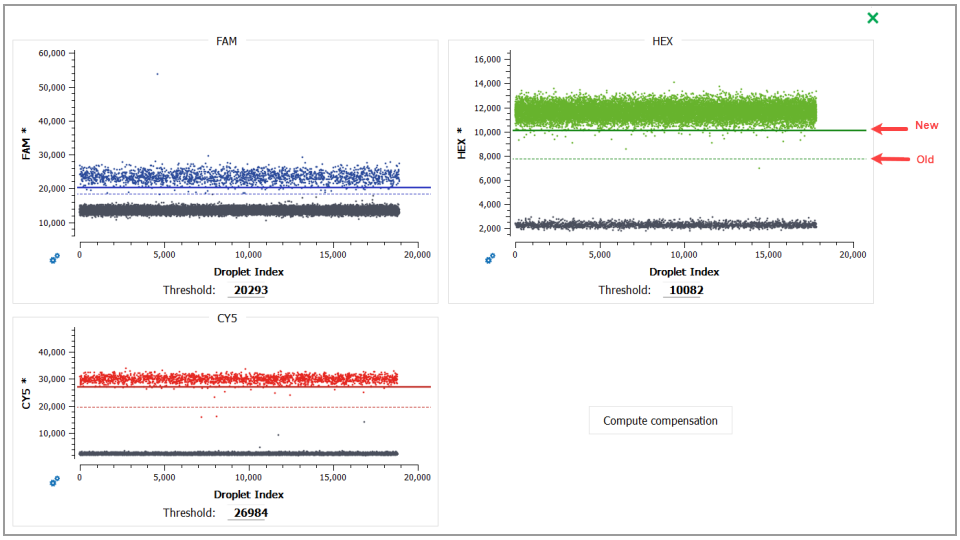


3. Tap/click Next.

   QX700 ddPCR System Analysis Software automatically places a threshold in the 1D plots.

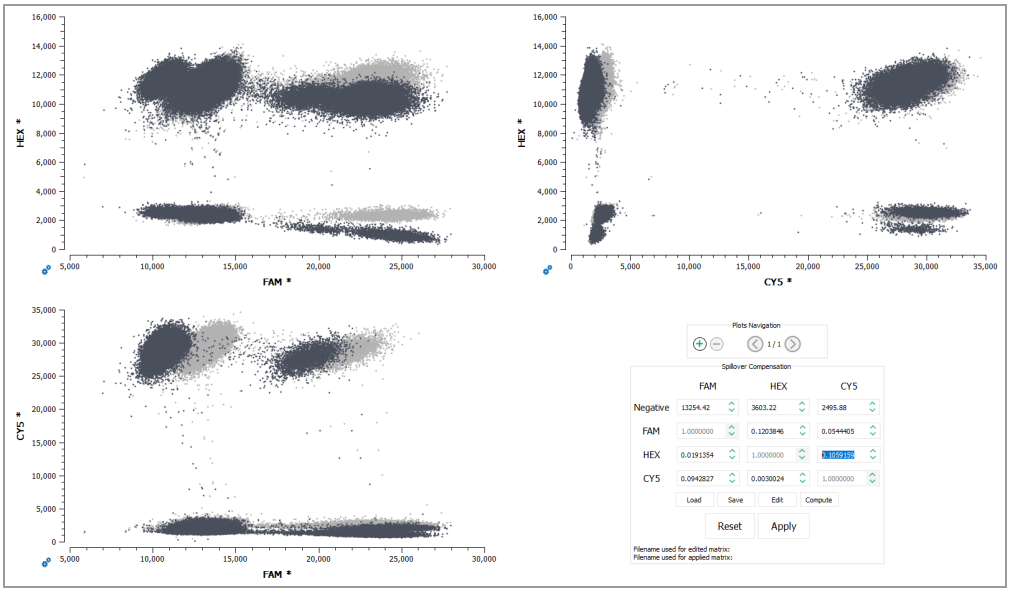


4. (Optional) To adjust the values, drag and drop the threshold lines.

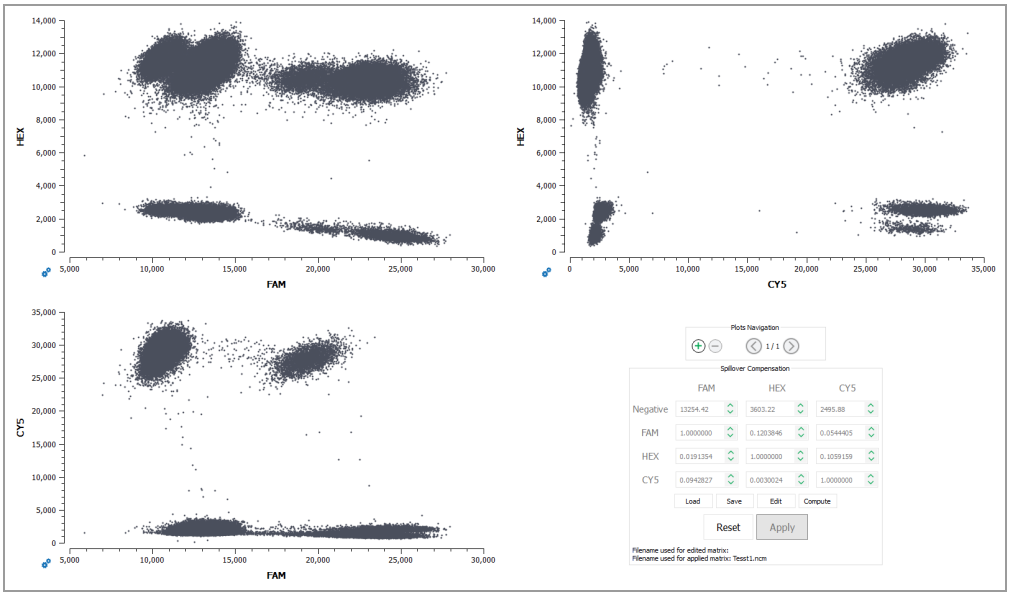


5. Tap/click Compute compensation.

   QX700 ddPCR System Analysis Software displays the 2D dot plot, with the spillover compensation applied to the data in a comparative view with the original clusters. The difference between the uncompensated data (light gray) and the compensated data (dark grey) is displayed as a preview.



6. If the cluster positions are correct (orthogonal to each other), tap/click Apply to apply the compensation matrix to all the chambers.



7. Tap/click Save.

   The compensation matrix is generated as a .ncm file, which you can reuse the file as a template for other experiments if the same assay and thermal cycling conditions apply. For more information, see Template-Based Method on page 53.

Template-Based Method

If you already have a compensation matrix calculated and saved that matches the current experiment conditions (same fluorophores, targets, and so forth), then you can use a template to resolve fluorescence spillover.

Important: Two experiments with different scanning times can use the same compensation matrix, since compensation matrices are normalized by the exposure time. However, the two experiments *must* share the same assay conditions and thermal cycling program.

To use the template-based spillover compensation method

1. Below the compensation matrix layout, tap/click Load.
2. Navigate to and select an .ncm file and then click Apply.

   The compensation values are imported into the matrix.
3. If necessary, edit existing values and click Save to save the matrix as a new .ncm file.

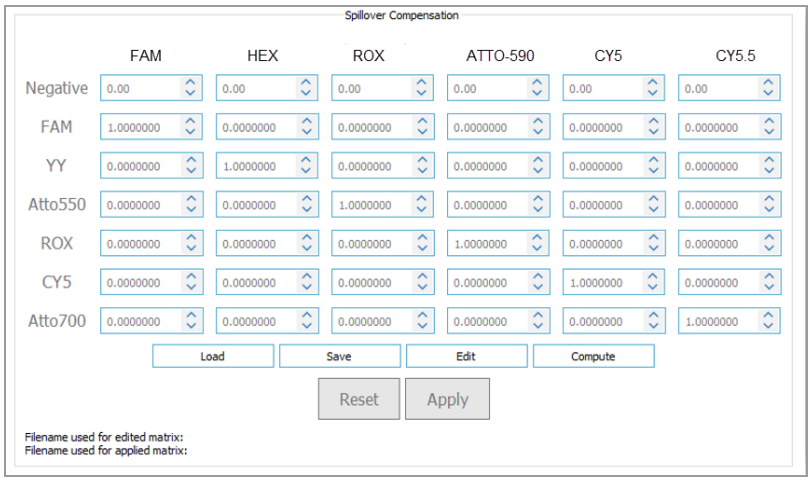
   Note: You can also export the spillover compensation file using the Export function. For information, see Exporting System and Analysis Data on page 109.

Manual Method

This method can be applied if neither the template-based method (see below) nor the automated method (above) can be applied.

Important: The manual spillover compensation method is an expert function designed to address non-specificity issues. It is not recommended unless you are an expert user and understand its impact.

The following graphic illustrates the default compensation matrix view.

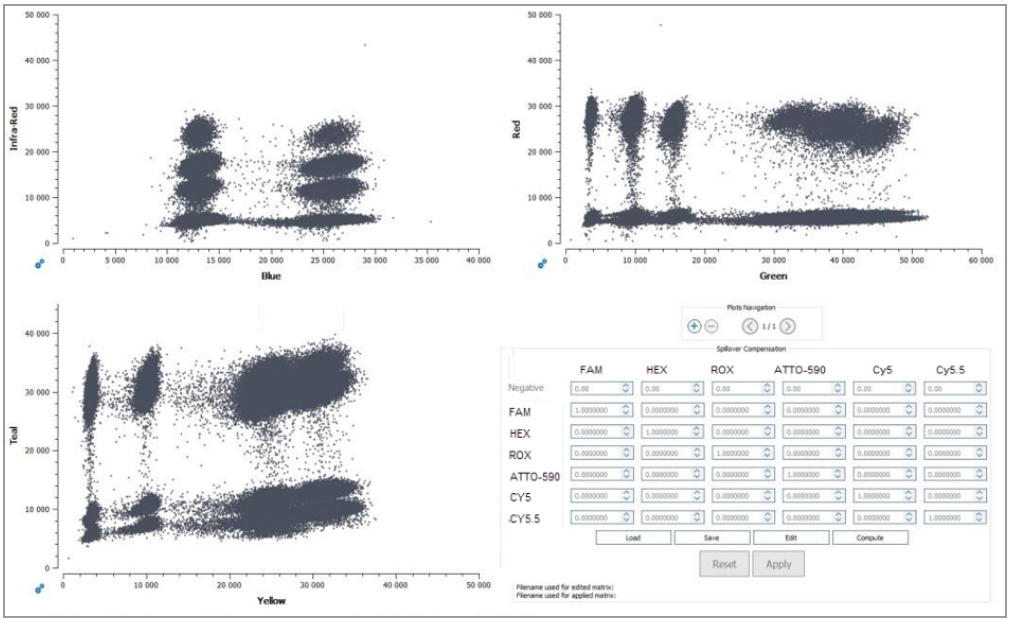


To use the manual spillover compensation method

1. Tap/click Compensation Spillover, and then tap/click the Edit button below the matrix.

   The uncompensated clusters appear in the 2D dot plot view.

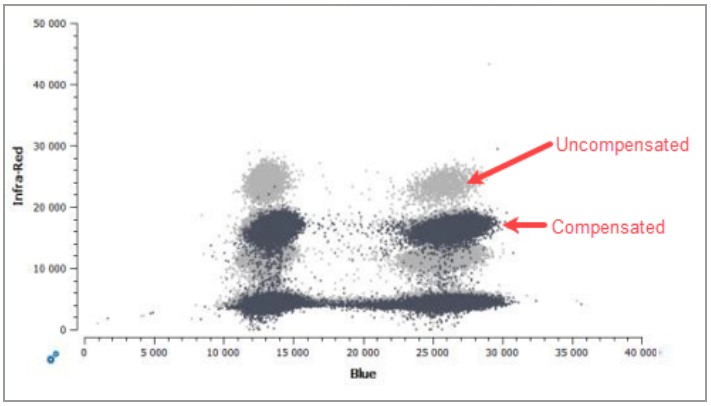
   Important: As with the automated compensation method, manual compensation is meant to place the clusters in positions that are orthogonal to each other.



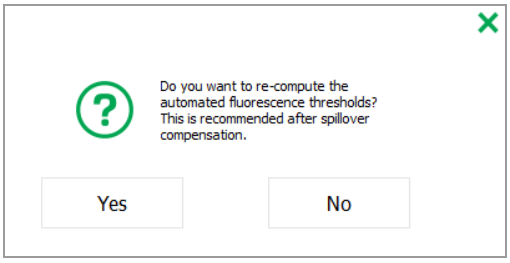
2. Enter coefficient values individually in the Spillover Compensation matrix or click the up and down arrows. *The fields containing 1.000000, in which the same fluorophore intersects, are not editable*.

3. Set the negative values in the first line by entering the mean RFU value of the negative cluster in each channel.

After the compensation matrix has been manually modified, the compensated (dark gray) and uncompensated (light gray) droplet populations overlap in the graph.



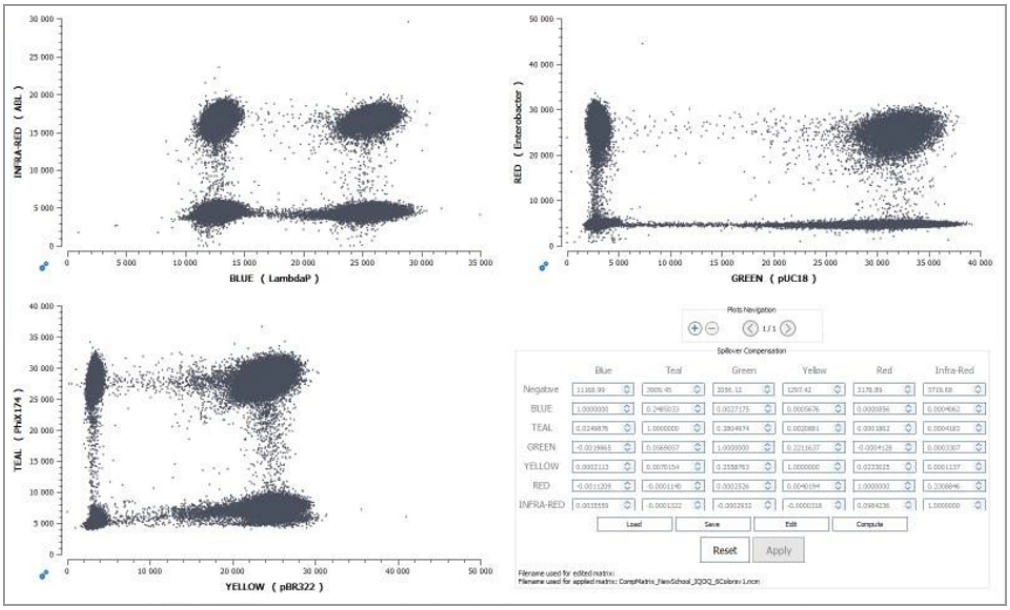
4. To apply the spillover compensation to the full experiment, tap/click Apply.
5. When the following message appears, tap/click Yes.



After the spillover compensation is completed, the spillover signal is removed from the dot plot. The correction is not performed on the droplet image, since it was acquired by the instrument. Therefore, a droplet can appear as positive in one channel (red in this example, due to spillover) without being present in the red dot plot, since the compensation matrix removed the spillover signal.

6. To reset the spillover compensation and return to a non-compensated experiment, tap/click Reset.

The following graphic shows the correctly compensated 2D dot plots.



The compensation matrix is generated as a .ncm file, which you can reuse the file as a template for other experiments if the same assay and thermal cycling conditions apply. For more information, see Template-Based Method on page 53.

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Chapter 6 Data Analysis

The Analyze Data tab contains the following analysis views:

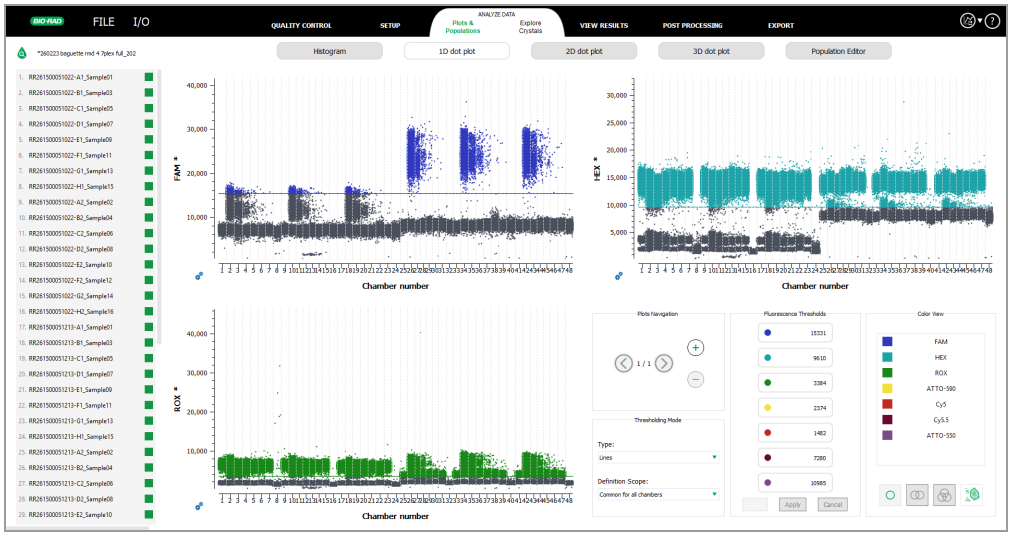
- **Plots & Populations** — The Plots and Populations view illustrates the droplet data for each fluorophore channel in 1D, 2D, 3D, and histogram dot plots. The default view is the initial three fluorophores but you can prompt additional screens to show the dot plots for the remaining fluorophores (up to seven) in larger experiments. You can also add and edit zones and populations for different targets and change the thresholding type and scope.
- **Explore Crystals** — view the dot plots and droplet images using the options at the bottom of the display. QX700 ddPCR System Analysis Software reloads the amplitude plot settings, which include the displayed channel and the axis min/max values.

Continue to the following sections for information on all droplet analysis functionality.

Plots and Populations

When you open a .niodata file, the Analyze Data tab displays the Plots & Populations view by default.

All chambers are selected in the left pane and the software uses a range of up to seven (7) fluorescence channels to display the droplets.

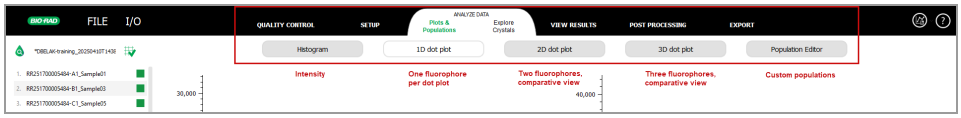


For information on fluorophores and matching LED colors, see Channel Fluorophores and Colors on page 21.

By default, thresholds are applied to the data automatically. If the fluorescence of the droplet is higher than the defined threshold, then a droplet is considered positive; otherwise it is considered negative.

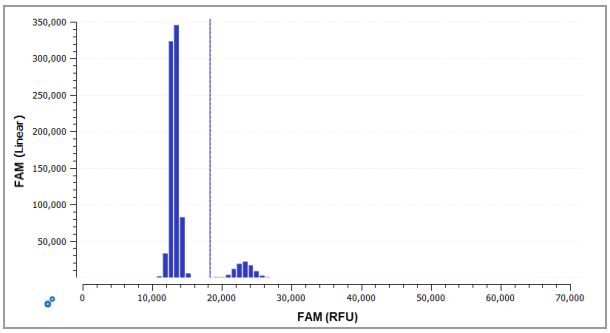
Plot Types

Droplets are measured by Relative Fluorescence Unit (RFU) and represented graphically in a variety of plot displays (1D, 2D, and 3D dot plots, and histogram). A population editor is also available where you can define custom populations within zones.

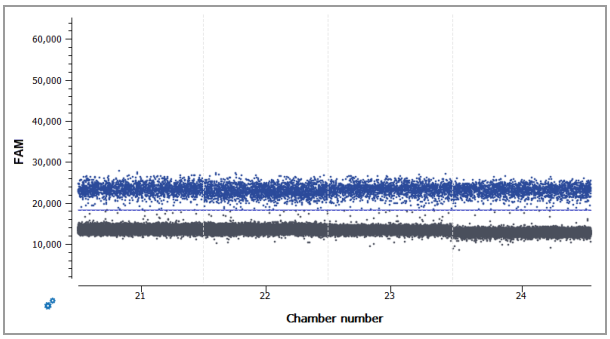


The following graphics display each plot type:

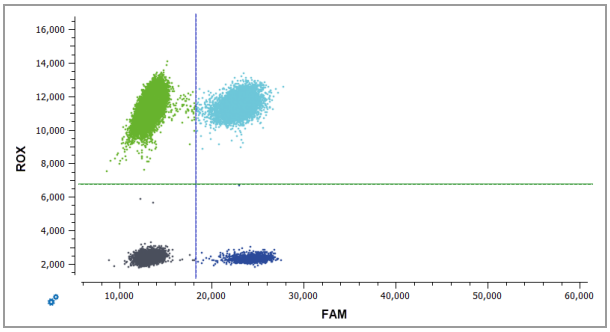
- Histogram



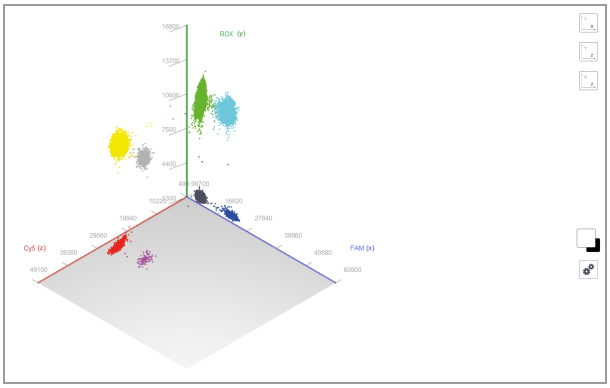
- 1D dot plot



- 2D dot plot



- 3D dot plot



Line Thresholding Color View

The Color View pane appears in Lines thresholding mode, and shows all fluorophores in the experiment matched to the LED color. Below the fluorophores, the color display combination icons (two, four, and eight) and the population selector icon appear. Each icon is described in Table 11 on page 62. All negative droplets are colored gray in the charts.

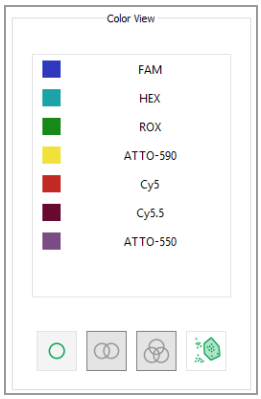


Table 11. Color View icons

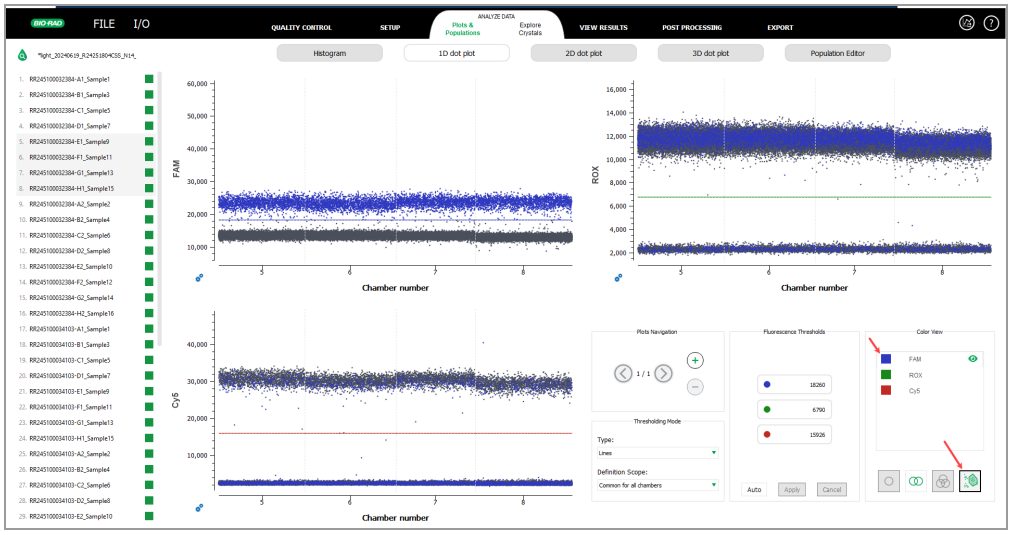
Icon	Description
	*Two-channel display*. For each fluorophore represented by a dot plot, the two-color view mode displays positive droplets in the corresponding color and negative droplets in gray.
	*Four-channel display*. For the two selected fluorophores in each dot plot, the four-color view mode displays positive droplets as follows: ■ Color A (for example, blue LED or FAM) ■ Color B, (for example, teal LED or HEX) ■ Colors A and B (blue and teal t represent FAM and HEX) All negative droplets appear in gray.

Table 11. Color View icons, continued

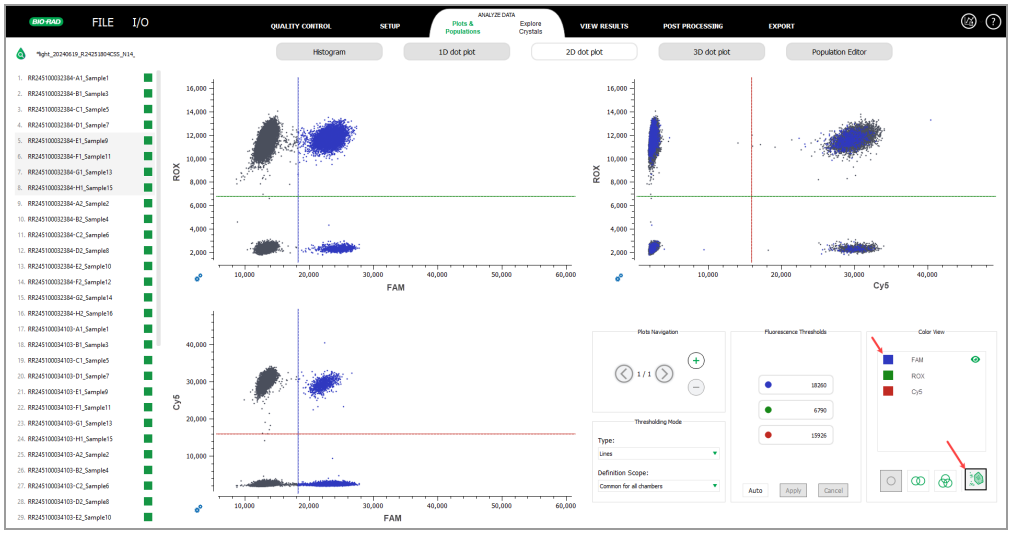
Icon	Description
	Eight-channel display. This view mode is only available for three-channel (FAM/Blue-ROX/Green-Cy5/Red/) experiments and provides the different colors for single-positive, double-positive, or triple-positive droplets, according to the corresponding color code in the Venn diagram[1]. The dark background represents negative droplets across all channels. 
	Population selector. This mode allows you to visualize the droplets that are included in a dot plot population to identify issues with clusters, compensation, and so forth. To illustrate the negative and positive droplets for the defined populations (or population combinations) in Population Selector mode, the 1D and 2D dot plots provide a color for each channel by default. For example, if the user is viewing the FAM channel, all positive droplets appear in *blue* and all negative droplets appear in *gray*.

[1] To help the user understand the classes of droplet positivity combinations in a triplex experiment, QX700 ddPCR System Analysis Software uses the 8-color Venn-style (rosace) diagram.

The following example graphic shows positive droplets in the 1D dot plot when FAM is selected under Color View and the Population Selector icon is selected.



The following example graphic shows positive droplets in the 2D dot plot when FAM is selected under Color View and the Population Selector icon is selected.



Note: If the.niodata or .nioresult file contains data for more than three fluorophore channels, you can use the plus and arrow icons under Plots Navigation to add and navigate to the remaining plots.

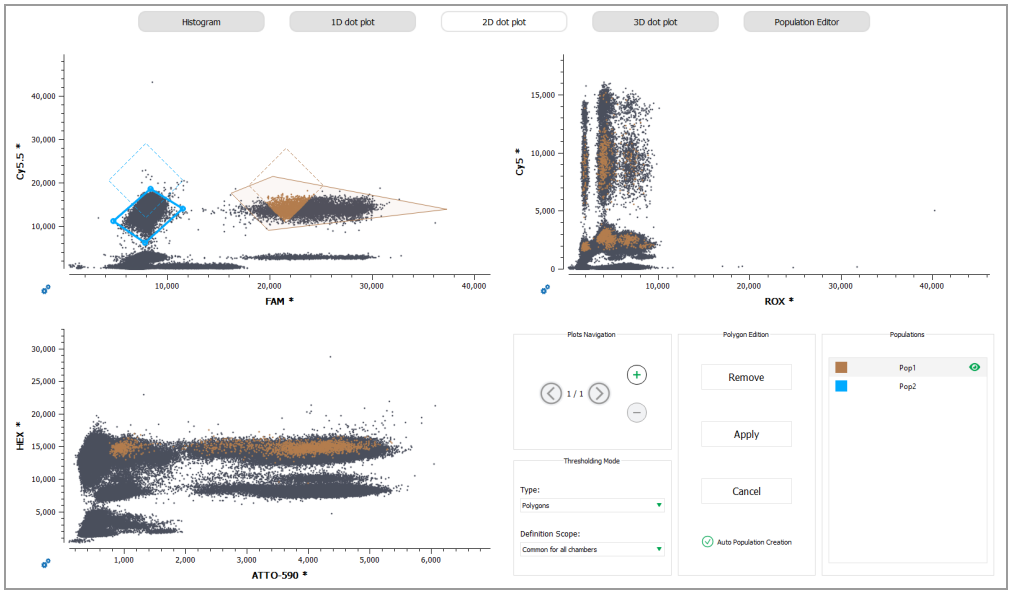
Polygon Thresholding Zones and Populations

For 2D and 3D dot plots, you can define and apply polygon thresholding. You can change the thresholding mode and scope, and then draw your polygons to create each population identifier.

Important: If you define polygons for your thresholding method, all previous thresholding (including automatic line thresholding) is overwritten.

Use the specified population colors to define your polygons in the 2D or 3D dot plots.

Fig. 6: Defined polygons

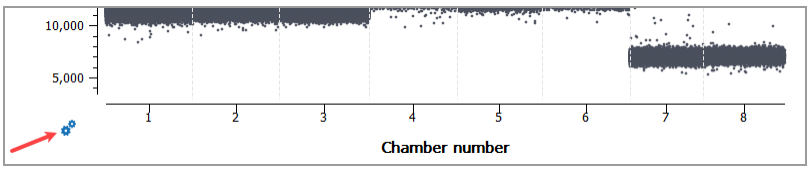


Tip: If the.niodata or .nioresult file contains data for more than three fluorophore channels, you can use the plus and arrow icons under Plots Navigation to add and navigate to the remaining plots.

The polygons appear in the Zones pane after they are defined.

Changing Dot Plot Axis Values

▶ To edit defined axis boundary ranges, tap/click the icon in the lower-left corner of a graph (or double-click on an axis).

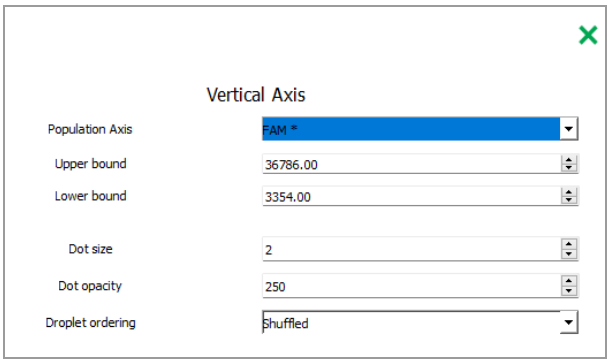


In a 1D dot plot, only the *vertical* axis values, which represent the range of relative fluorescence intensity (RFU) in the droplets, are editable. The *horizontal* axis label is read only and depends on the number of chambers selected, as follows:

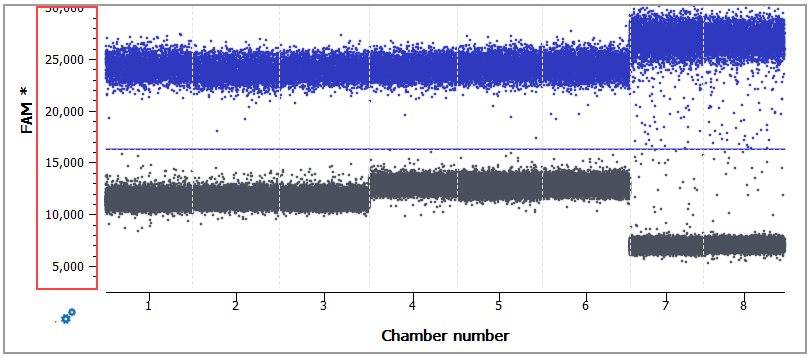
- If only one chamber is selected, "Droplet Index" appears
- If multiple chambers are selected, "Chamber Number" appears (1, 2, 3, and so forth).

To modify axis values

1. Select a fluorophore from the Population Axis dropdown.
2. Enter new vertical axis values to define the upper and lower bounds.



The axis range is modified in the dot plot.



3. (Optional) In the remaining fields, change the dot size, dot opacity, and droplet order.
4. Repeat to modify the values in other 1D dot plots.

In a 2D dot plot, both the vertical axis and the horizontal axis are editable.

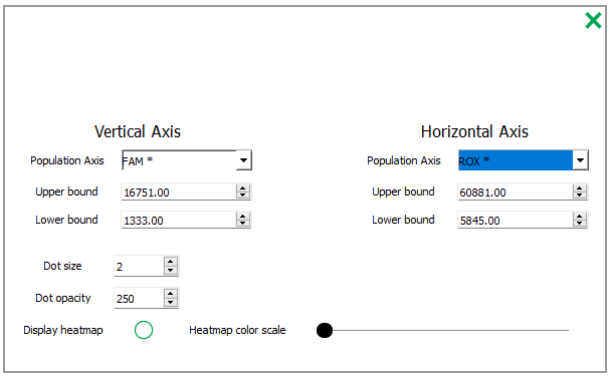
- The vertical axis displays the RFU of the droplets in one selected channel
- The horizontal axis displays the RFU of the droplets in another selected channel

1. Under Vertical Axis, do the following:
   a. Select a fluorophore from the Population Axis dropdown.
   b. Enter new vertical axis values to define the upper and lower bounds.

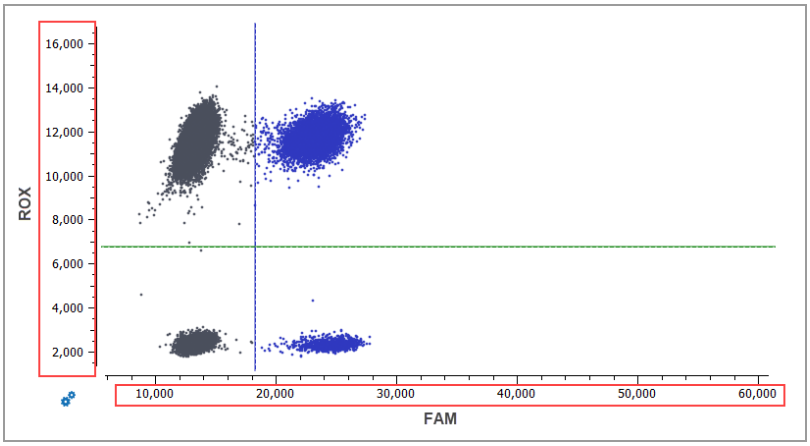
2. Under Horizontal Axis, do the following:

   a. Select a fluorophore from the Population Axis dropdown.

   b. Enter new horizontal axis values to define the upper and lower bounds.



The axis range is modified in the dot plot.

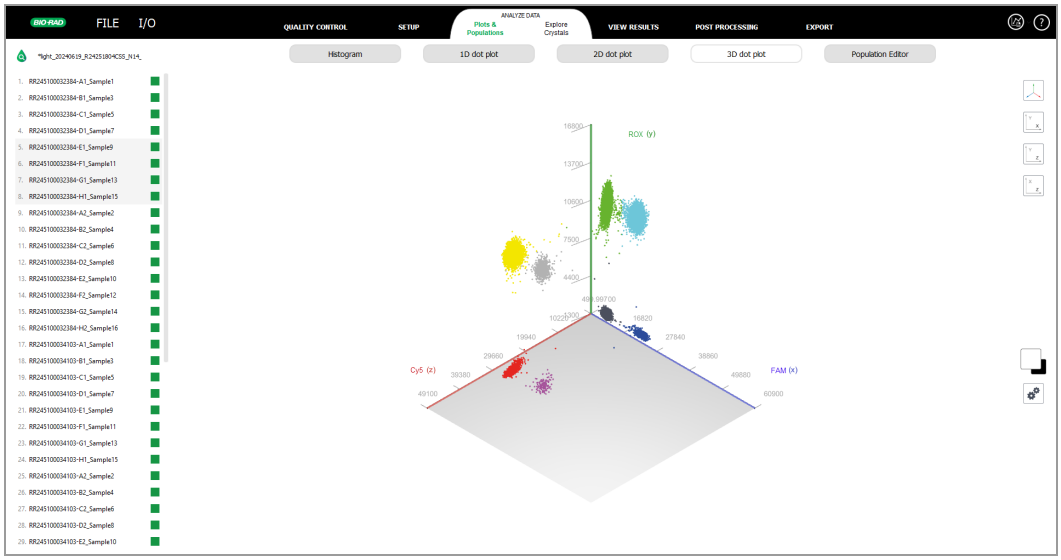


3. (Optional) In the remaining fields, change the dot size, dot opacity, and select the checkbox to display the heat map.

4. Repeat to modify the values in other 2D dot plots.

Navigating 3D Dot Plots

In 3D plots, each axis represents the fluorescence value of the droplets in any given channel.



To display the 3D dot plots

▶ Tap/click Analyze Data > Plots & Populations, and then select the 3D dot plot tab to display the plots.

Note: To navigate in the 3D plot, see General Navigation Options on page 21.

You can also use the following icons:

Reset the view.

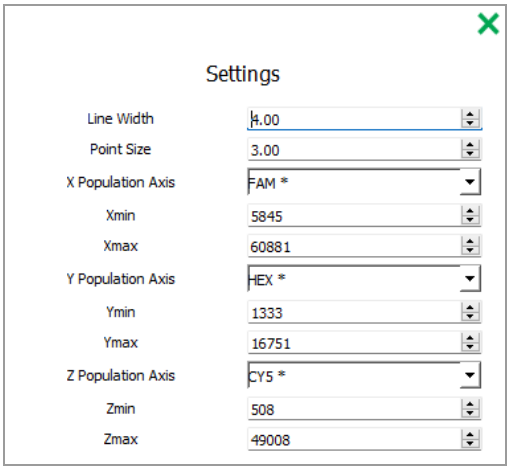
Project the view on the first and second axis.

Project the view on the second and third axis

Project the view on the first and third axis

Switch between black and white backgrounds

Modify the point size, the line width, the grid step or the population axis



Reading Histograms

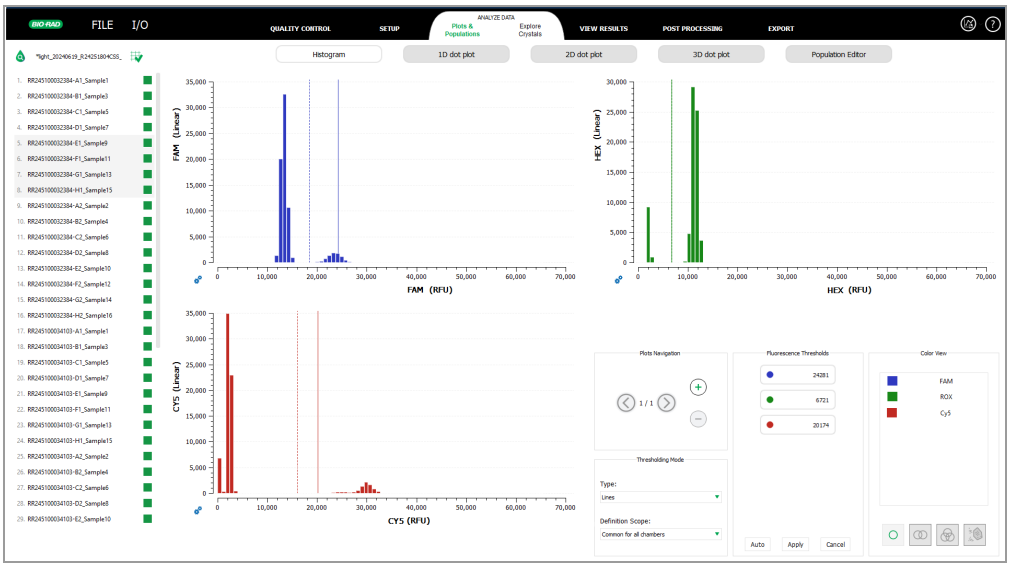
Use the histogram to view the negative population on the left and the positive droplet population on the right, as well as to check and adjust the fluorescence thresholds. If you have selected multiple chambers, then the number of droplets in each fluorescence bar are combined.

To view the histograms of the droplet fluorescence values

1. Tap/click Analyze Data > Plots & Populations > Histogram.

   The histograms of the currently selected chambers are displayed for each channel, as follows:

   - The x-axis shows the fluorescence value (in RFU)
   - The y-axis shows the number of associated droplets.



Adjusting Fluorescence Thresholds

QX700 ddPCR System Analysis Software considers the raw data from all samples in the experiment to automatically estimate and apply fluorescence thresholds that separate positive and negative droplets. Thresholds are redefined if you import a compatible spillover compensation matrix. For information on compensation spillover matrices, see Applying Spillover Compensation on page 46.

Line thresholds are applied automatically in 1D and 2D dot plots by default, but you can switch to polygon thresholding in the 2D plots. Thresholding considers the fluorescence points from all chambers to maximize the *inter*-class variance (among the groups) and minimize the *intra*-class variance (within the groups).

Important: The automated threshold estimation is more accurate if both positive and negative droplet populations are represented in the experiment and in each channel; therefore, Bio-Rad recommends that you include at least one negative control chamber and one positive control chamber for each of the channels in the experiment (up to seven).

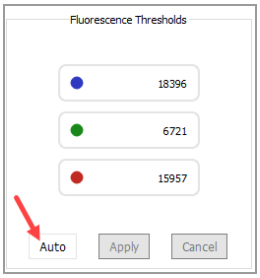
You can move (click and drag) the thresholds applied by the software to adjust the number of droplets recognized as positive and negative. For more information on thresholds, see Using the Lines Thresholding Mode on page 73 and Using the Polygon Thresholding Mode on page 75.

Using the Lines Thresholding Mode

Line thresholding is the default thresholding method for 1D and 2D dot plots, and is applied automatically when an experiment is loaded. For information on polygon thresholding (2D plots only) see Using the Polygon Thresholding Mode on page 75.

Automatically Calculating Line Thresholds

1. Click Analyze Data and select the 1D dot plot tab.
2. Click Auto, as shown in the following graphic:



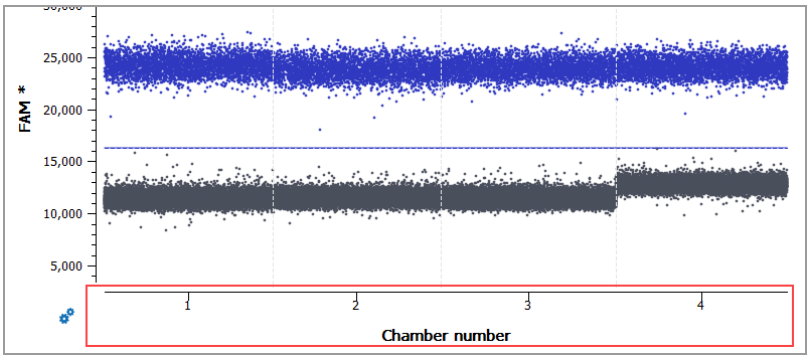
Each displayed channel value represents the estimated number of positive droplets.

Modifying Line Thresholds

As you review the automatically calculated thresholds for selected chambers in each fluorescence plot, you can drag the lines to adjust the thresholds where necessary.

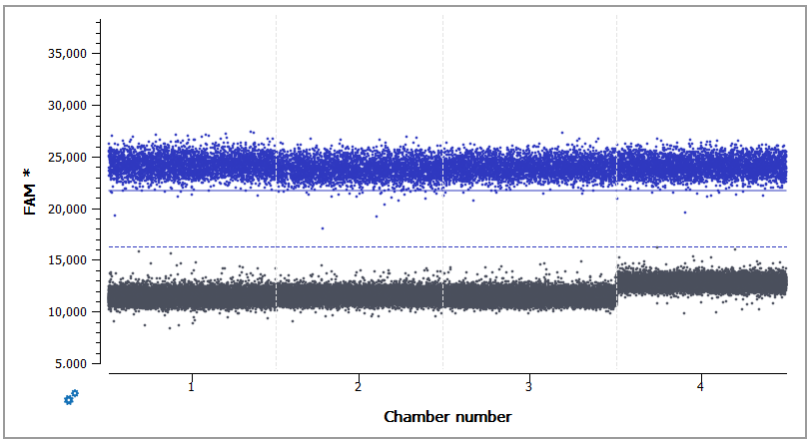
1. In the left pane, select the applicable chambers and then select the 1D dot plot tab.

   Note: The multi-chamber view displays a separator between each chamber in the plot on the horizontal axis (per the following graphic), and the original calculated collective threshold is displayed.

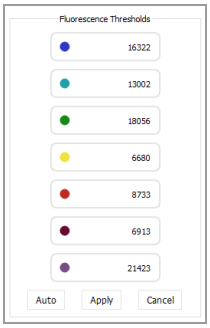


2. To manually adjust the fluorescence thresholds, select a threshold in one of the plots to drag and drop the threshold line.

   Note: When you click and drag the threshold line in one chamber, the threshold is redrawn for all chambers. The dotted line represents the original threshold and the solid line represents the new threshold.



   When you adjust the threshold, the value changes for the corresponding channel under Fluorescence Thresholds.



3. To apply the modified values, tap/click Apply under Fluorescence Thresholds.

   Positive and negative droplets are recalcuated into a separability score for each population of interest, and then exported as an advanced quality control file. Separability scores are used to evaluate amplification efficiency. For more information on separability scores, see Quantifying Fluorescence Separability on page 97.

   Note: To discard the values before applying, click Cancel. To restore the automatic fluorescence thresholds, click Auto.

Using the Polygon Thresholding Mode

Polygons provide a flexible way in 2D plots to define or edit individual droplet *zones*, which are used to identify a broader set of characteristics. Within a zone, you can build *populations* for additional granularity in sample and target results. Populations can include positive and negative droplets, concentration, confidence interval, and so forth. You can also define negative droplet zones.

Important: When you switch to Polygon thresholding, the existing zones and populations are removed. Polygon thresholding is available for 2D dot plots only.

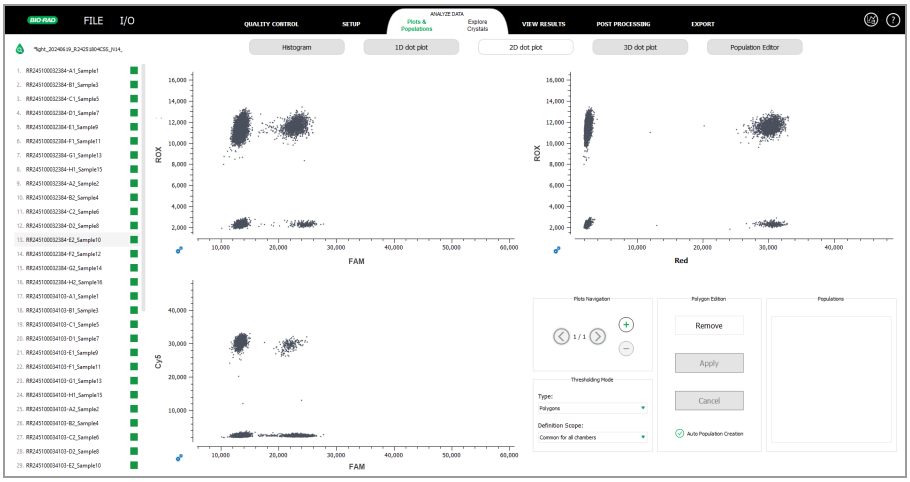
Use the following keyboard commands and mouse options to create, modify, move, and delete polygons:

- Press SHIFT and right-click to create a basic diamond polygon in one click.
- Left-click on an edge of the polygon to add a new vertex in the middle of this edge.
- Left-click on an edge of the polygon then drag and drop to move the polygon.
- Left-clicking inside a polygon does not move the polygon, but does move the 2D view.
- Left-click on any vertex of the polygon to modify its position.
- Right-click on a vertex of the polygon to remove the vertex.
- Right-click outside the polygon to remove it.

To create a polygon

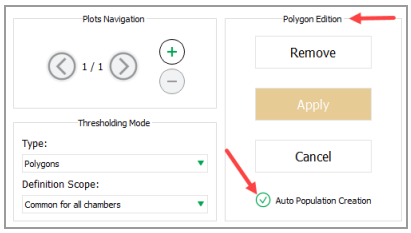
1. Tap/click Analyze Data > Plots & Populations > 2D dot plots.
2. Under Thresholding Mode, select Polygons from the Type dropdown list.

   All dots appear in gray before you apply polygon thresholds.



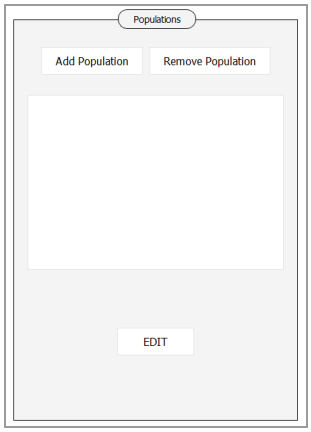
3. Do one of the following:

   - If the Auto Population Creation checkbox under Polygon Edition is selected (default setting), use SHIFT right-click in the 2D dot plot to create a polygon by the droplet cluster to be segregated.

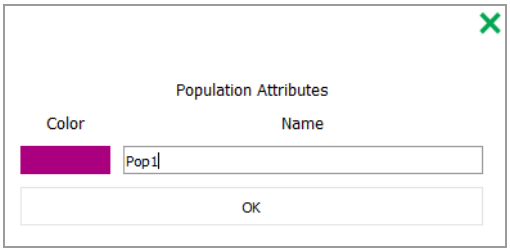


     The Population Attributes pop-up opens immediately, and you can use this method if you prefer to create a polygon identifier and then immediately edit the polygon. Continue to Step 4.

   - If the checkbox is cleared, you can tap/click Population Editor and then click Add Population. You can use this method if you prefer to create all polygon identifiers first, and then create the polygons. Continue to Step 4.

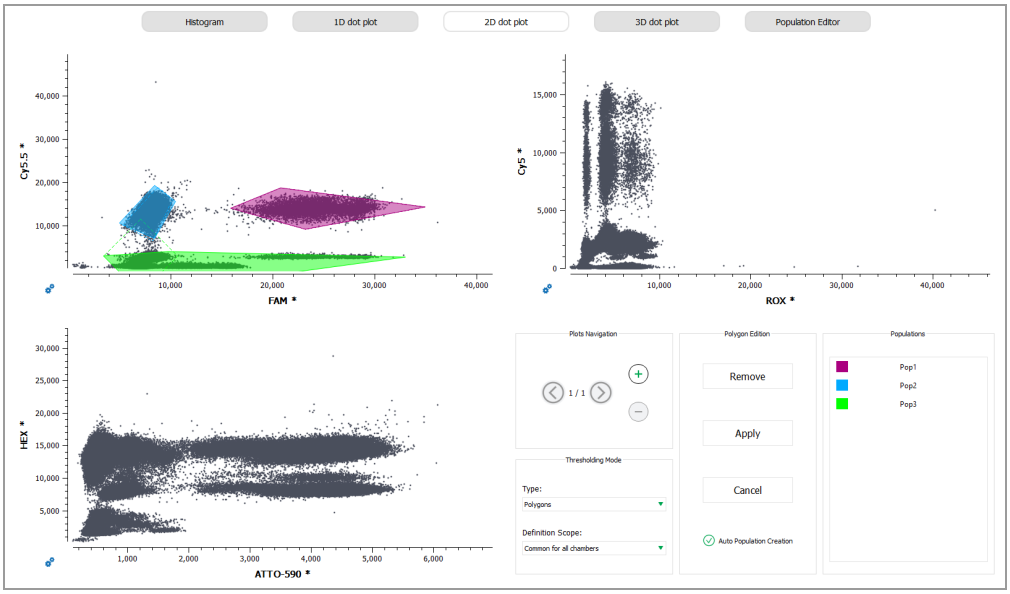


4. In the Population Attributes pop-up, select a color to identify the population and then enter a population name and click OK.



5. Tap/click OK to create the polygon identifier.

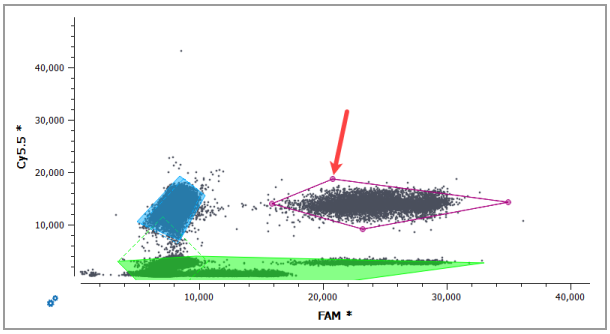
6. Repeat for each population identifier that you need for plot segregation.



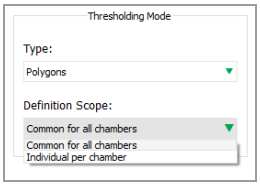
Modifying or Removing Polygon Thresholds

After you create polygons, you can edit them according to your needs and apply the changes:

1. To edit a polygon, click a polygon in the dot plot to display the vertex points and adjust the polygon shape and size.

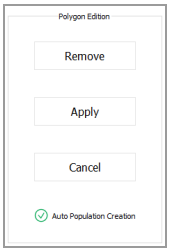


2. In the 2D plot view, you can localize polygons using the dropdown list under Definition Scope:



   - To set the polygon thresholds uniformly across the whole experiment, ensure Common for all chambers is selected for Definition Scope.
   - To make adjustments to each polygon zone, chamber by chamber, ensure Individual by chamber is selected.

3. Under Polygon Edition, you can
   - Select a polygon and click Remove.
   - Edit a polygon and click Apply.
   - Cancel your changes.
   - Select or clear the Auto Population Creation checkbox.



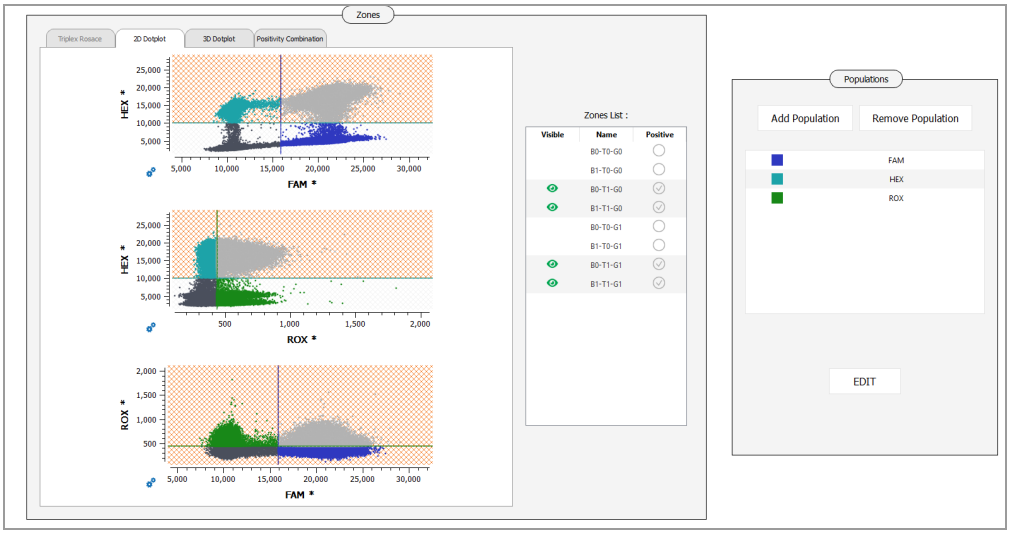
Defining a Population in a Zone

A population is an entity defined by a zone or several zones. You can use the Population Editor to specify which zones comprise a population.

Note: If the Automatically create population checkbox is selected, the Population Attributes pop-up appears automatically after you draw your polygon. After you complete the information and tap/click OK, the population appears in the Results table.

To edit your populations in the Population Editor

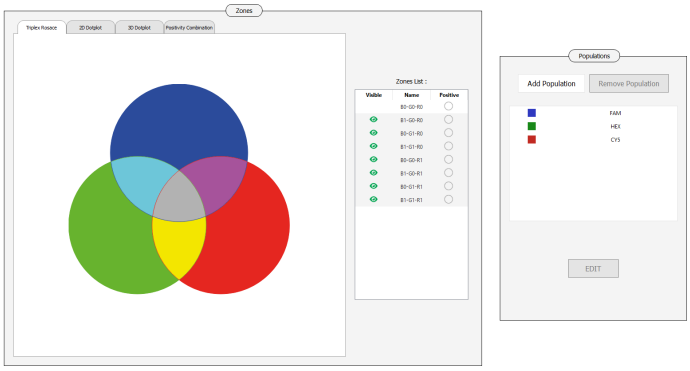
1. Tap/click Analyze Data > Plots & Populations > Population Editor.
2. For each population created, tap/click the population name to display the associated zones in the list and in the plots, Venn diagram plots, Venn diagram view, and positivity combination view.



In the Zones panel, several tabs provide different way of viewing the zones:

- In the Triple Rosace tab, a Venn diagram that provides a selection of single, double, or triple-positive zones.

  Note: The Triple Rosace tab is only enabled for experiments using the Cy5-FAM-ROX (Red-Blue-Green) channels.



- Use the 2D Dot plot to view thresholds and polygons in a 2D space
- Use the 3D dot plot to view the channels that are related to the thresholds or polygons. In the 3D view you can see the projection of a 2D polygon along the 3D axis of the 3D dot plot.
- Positivity Combination (only available) in Lines thresholding mode)

3. To change the color and population name, tap/click Edit.
4. To update the zones that are included in the population, change the checkbox associated to a zone.

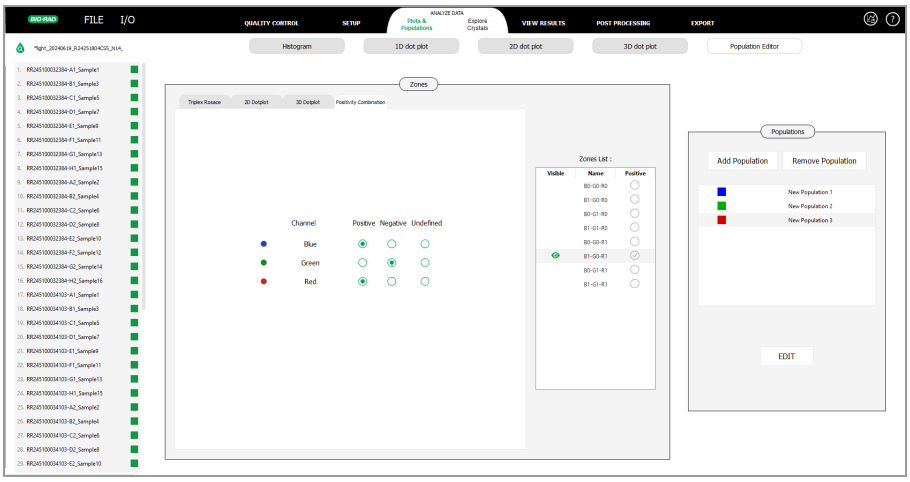
   Note: When viewing the zones in the 2D dot plot, select the 2D dot plot tab and then use one of the following methods to select a zone:

   - Tap/click a zone in the 2D plot display.
   - Tap/click a row under Zones List. The selected zone is shown as selected in the table and appears in the dot plot.
   - Select the zone checkbox to include it in the currently edited population.

     Note: You must tap/click Edit in the population list to select a zone for a population.

5. To display the desired population, tap/click Population Editor in the menu bar and then select a quadrant in the 2D dot plot tab.

6. Tap/click the Positive Combination widget to display the status of the populations (positive, negative, or undefined).



Note: This display is useful for creating and displaying populations. For example, you set the FAM (Blue) channel to Positive and the other channels to Negative to create a Simple FAM (Blue) Positive population.

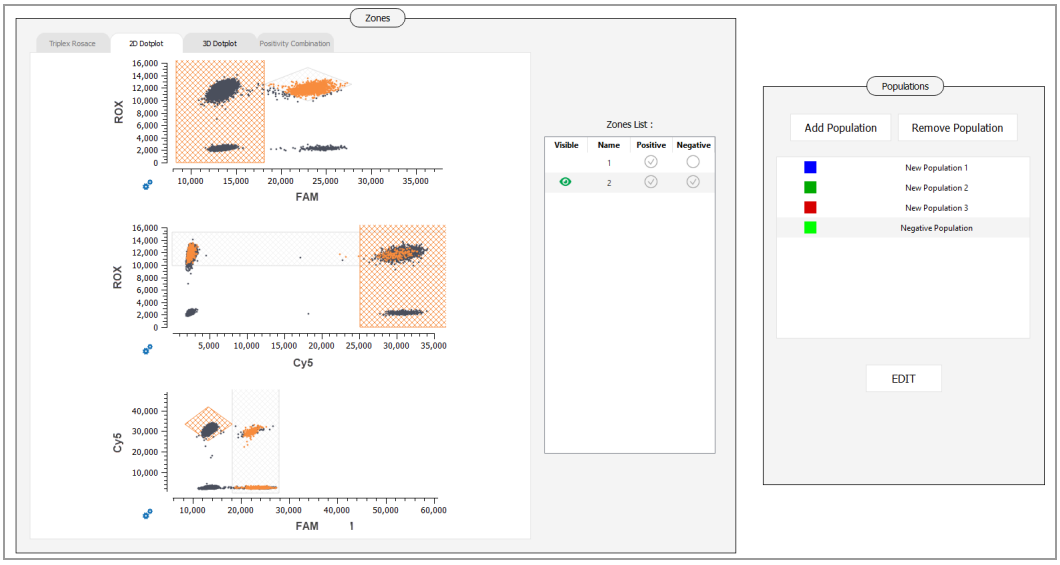
By definition, the droplets included in the population are counted as positive droplets. By default, all the droplets that are not included in the population are counted as negative droplets for this population. Continue to the next section to learn how to define negative zones in a population.

Defining Negative Zones

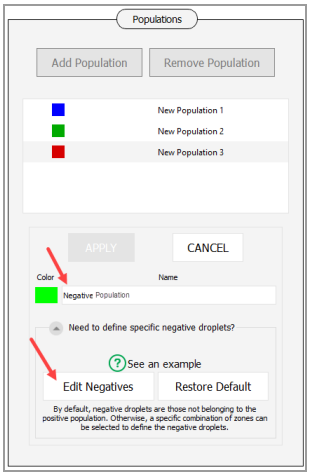
For some applications, such as Drop-Off, you might need to choose a specific droplet population and define a negative zone.

To define a negative zone for a population

1. Select a specific zone and then select the 2D dot plot tab.
2. Select Polygons as the thresholding type and then create a new polygon.
3. Select the Population Editor tab and then select the population of interest.
4. In the Populations display, tap/click Edit > Need to define specific negative droplets.



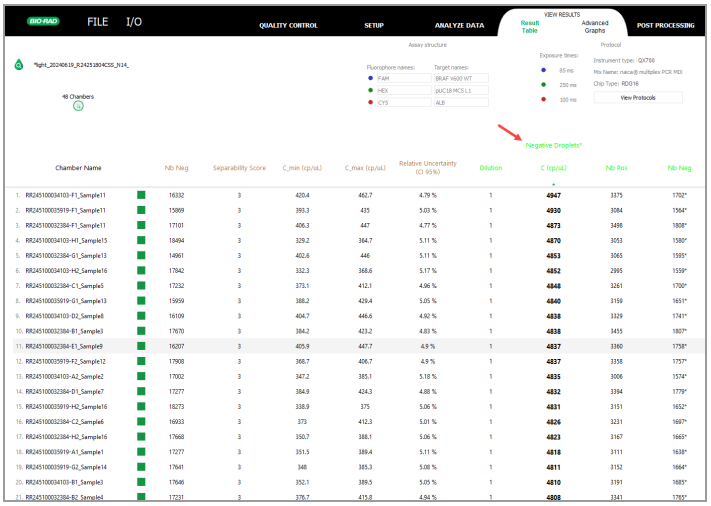
5. Select the negative droplets polygon and select the Negative checkbox, and then tap/click Apply.



The concentration calculation for the polygon of interest only considers the droplets detected in the negative droplet polygon.

Populations for which specific negative droplets have been defined are indicated with an asterisk symbol (*) appended to the population name, which indicates that the sum of positive and negative droplets for the populations might not be equal to the total number of droplets. The asterisk is displayed throughout the Result Table.

The column set appears in the View Results table.



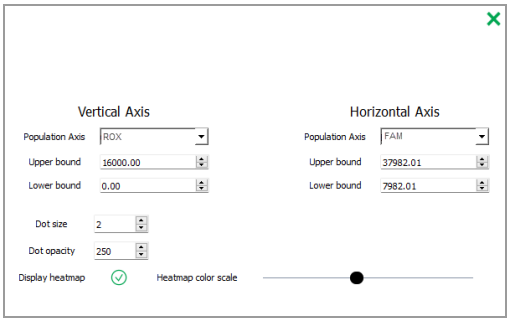
Heatmaps

Heatmaps are convenient analysis tool to delimit 2D zones. In heatmap mode, the color map represents the different droplet density levels in the fluorescence populations.

Droplet densities utilize a color gradient that is displayed on the second y axis. Low droplet densities are represented by shades of gray on the lower axis (light grey) while increasing droplet densities are color coded in shades of blue, green, yellow and red to scale to the highest droplet density.

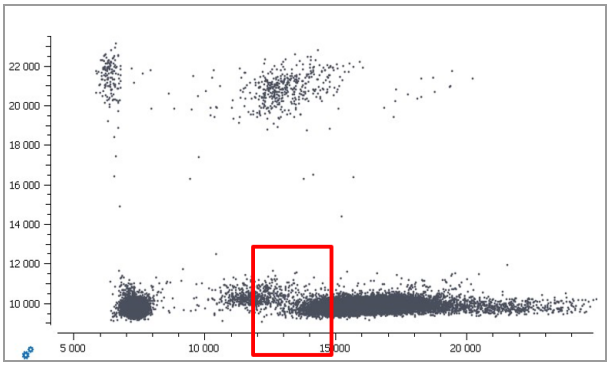
To activate the heatmap visualization in 2D dot plots

▶ Open plots settings and tap/click the icon to opne the dialog.

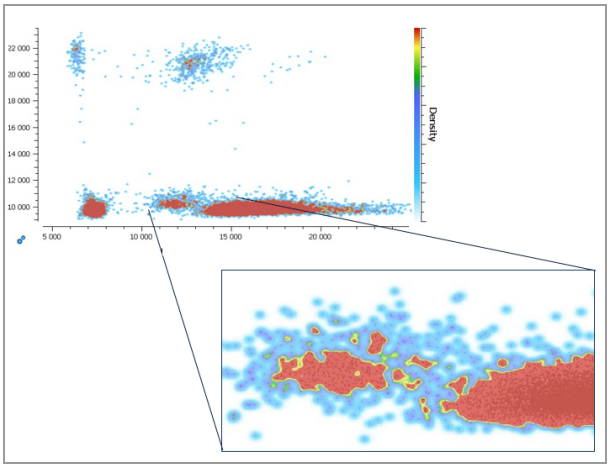


The color scale on the right of the graph gives the complete color range that is used to represent the different density levels.

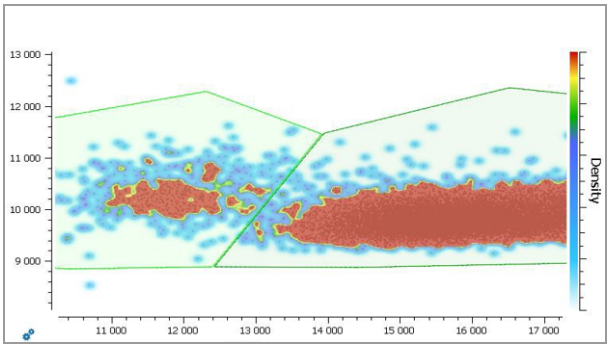
Negative populations are typically very dense, which can hide smaller density variations between less dense areas. You can modify the heatmap color scale parameter to highlight populations of lower density levels. For example, when droplet clusters are not well separated, the heatmap feature allows the definition of distinctive droplet clusters, as shown in the following graphic.



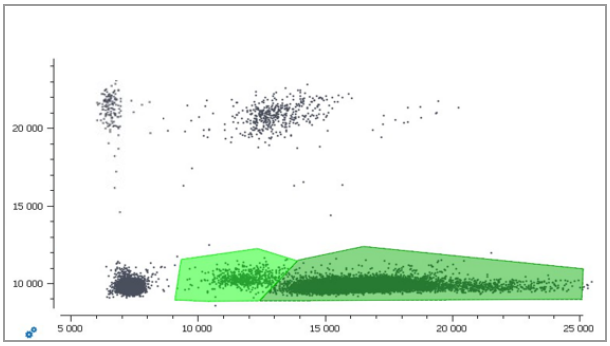
The following graphic shows the heatmap activated.



The following graphic zooms into the 2D-dot plot and shows the placement of polygons in the intersection of the two clusters guided by the heatmap color gradient (red indicating the most dense population).

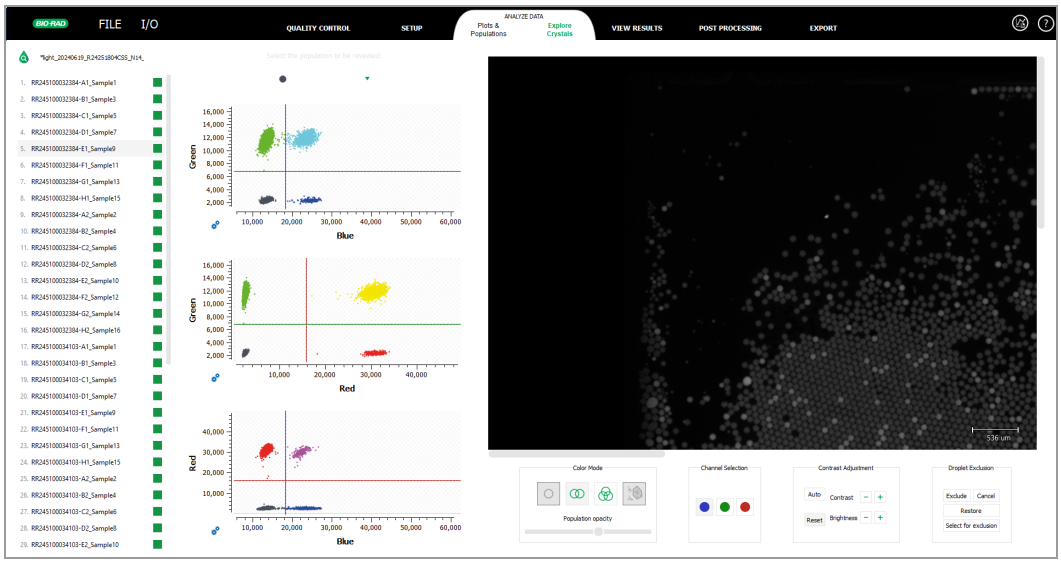


You can zoom out to deactivate the heatmap feature.



Exploring and Analyzing Droplets

The Explore Crystals view provides multiple droplet displays, including 2D dot plots and droplet images, that you can use for droplet analysis.

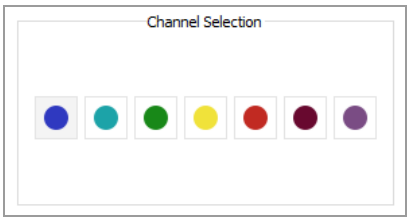


To analyze a droplet population using Explore Crystals

1. Tap/click Analyze Data > Explore Crystals, and then select a chamber from the list. *You can analyze one chamber at a time.*

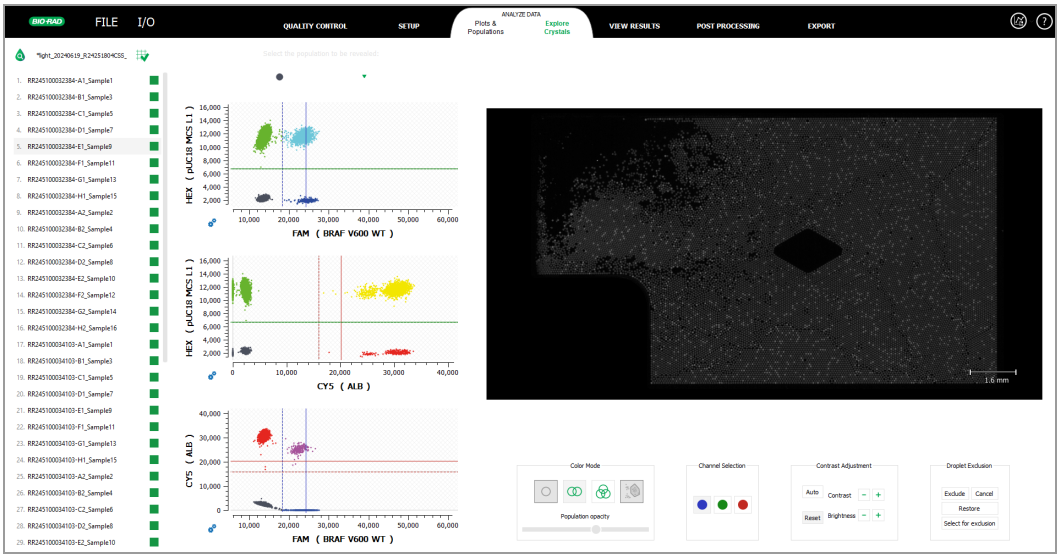
   Note: To navigate in the chamber image, see General Navigation Options on page 21.

2. To change the detection channel, tap/click a colored circle under Channel Selection.

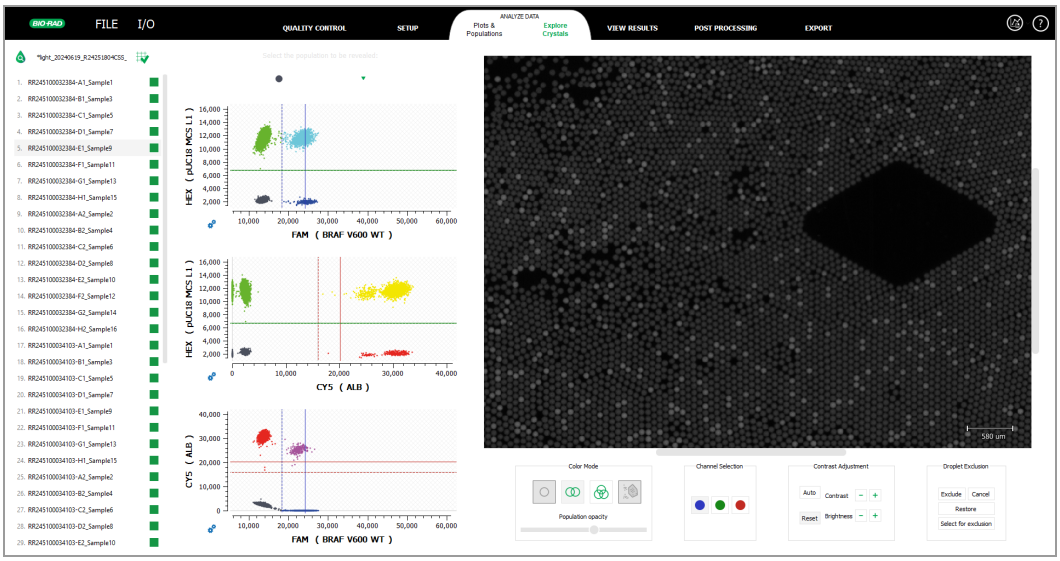


3. To automatically adjust image contrast, tap/click Auto under Contrast Adjustment.

The following graphic displays the Explore Crystals view in 2D simplified color mode.



The following graphic displays a zoomed view of the droplet image.



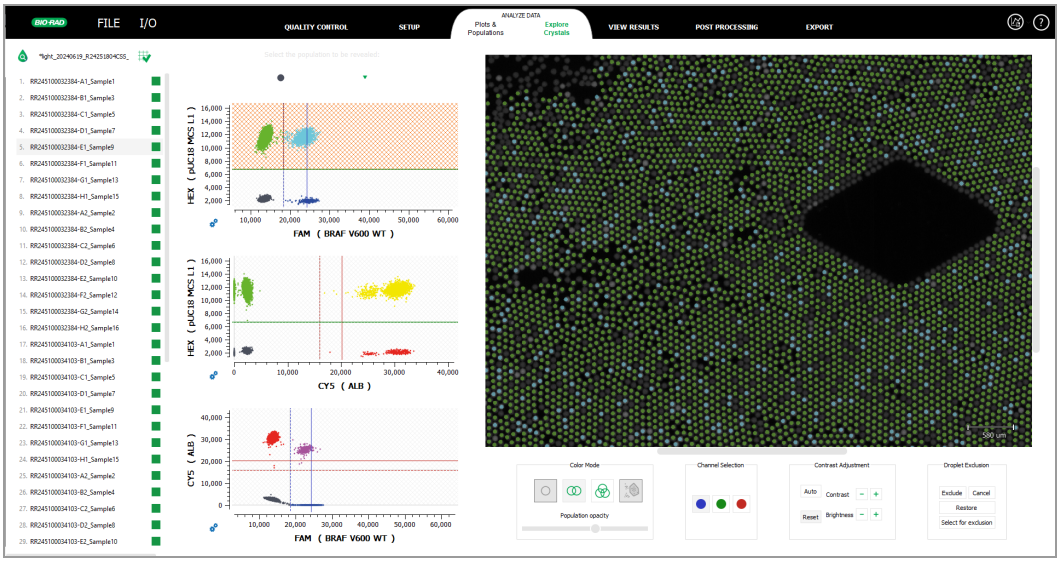
To highlight a specific droplet in the crystal image

1. Left-click on the quadrant of interest to activate or deactivate the highlighted droplets included in one quadrant of a 2D graph,

   The activated quadrant fill is transparent orange in the graph and the droplets included in this quadrant are highlighted in their current color codes in the image.

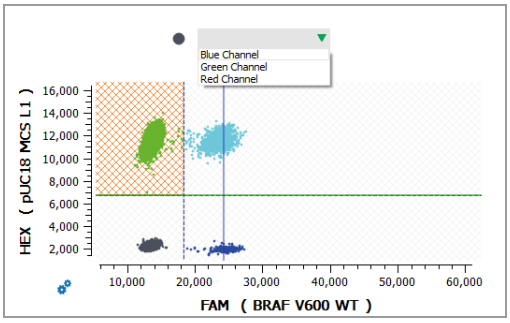
2. Use the Population opacity cursor to adjust the opacity.

3. Press SHIFT > left-click to select multiple quadrants in a 2D graph and highlight multiple droplet populations, as shown in the following graphic:

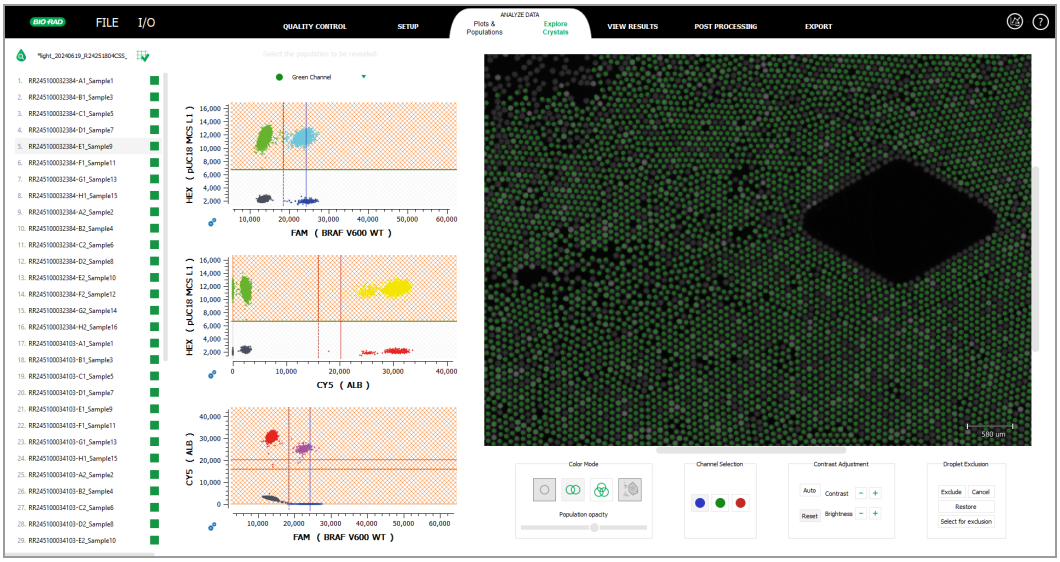


To highlight the droplets belonging to a given population

▶ Select the population name in the drop-down menu above the 2D dot plots.



The associated zones (quadrants or polygons) are filled in transparent orange in the graph. The droplets included in these zones are highlighted with the population color in the image graph quadrants.

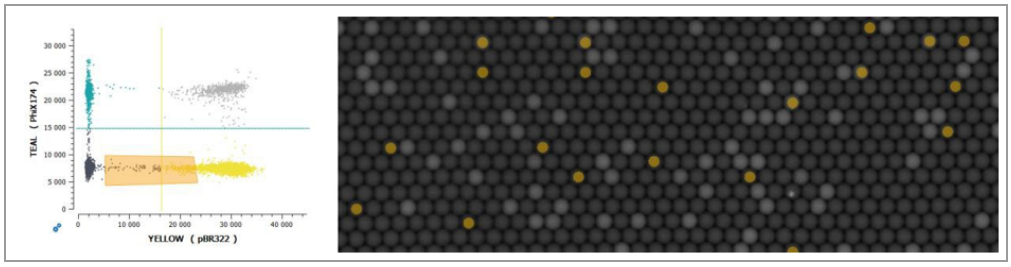


To highlight droplets included in any user-defined polygonal region of a 2D graph

1. Press CTRL right-click enough times to define the polygon region shape to be drawn (one click = one vertex).
2. Release the CTRL key after defining the final polygon vertex.

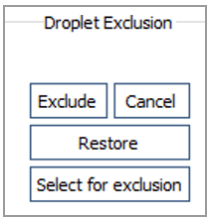
   QX700 ddPCR System Analysis Software draws the polygon with orange edges on the 2D graph, and the droplets included in the polygon are highlighted in orange in the image.

The following graphic shows a highlighted droplet population using a polygon drawn in the 2D graph. Included droplets are highlighted in orange in the droplet image.



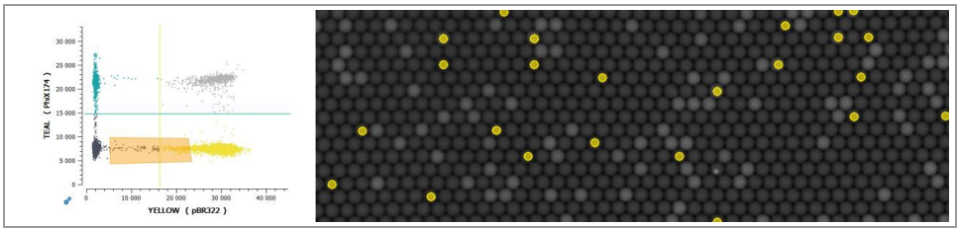
To exclude highlighted droplets from the analysis

1. In the Droplet Exclusion widget, tap/click Select for exclusion.



2. To exclude droplets in the highlighted polygon, right-click on droplets in the droplet image to select them.
3. Tap/click Exclude to remove the droplets from the analysis.

   As shown in the following image, manually excluded droplets are identified with yellow hexagons in the crystal images and are removed from the dot plots.

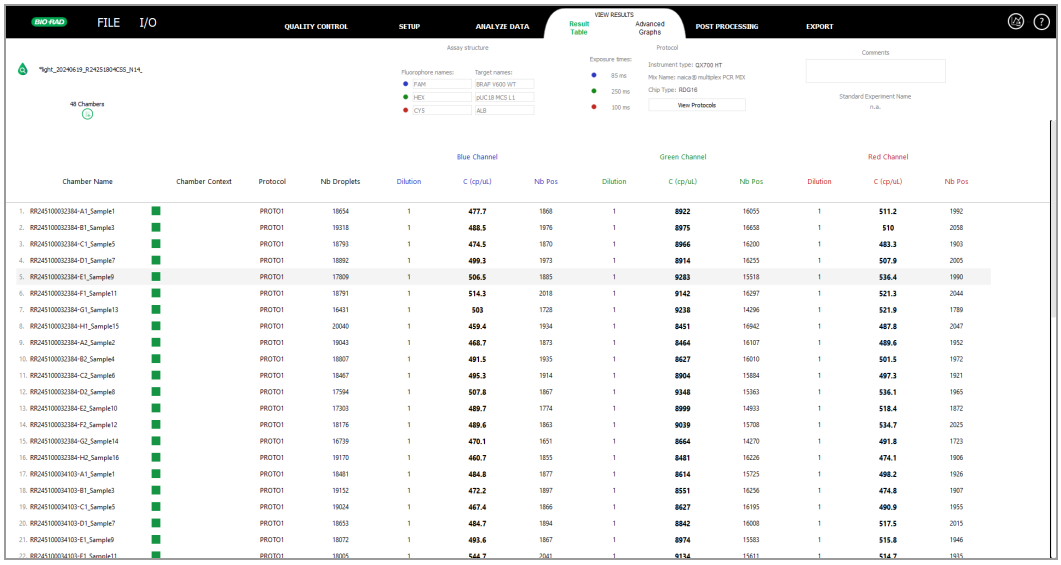


To restore all manually excluded droplets

▶ In the Droplet Exclusion widget, tap/click Restore.

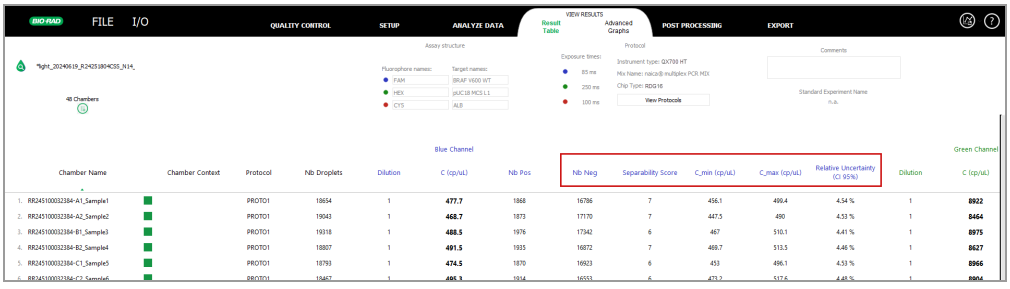
Chapter 7 Viewing Results

The View Results view contains the Results Table (results collected and calculated from each RDG16 cartridge chamber in the QX700 experiment run) and Advanced Graphs, which contain concentration and uncertainty curve graphical displays.



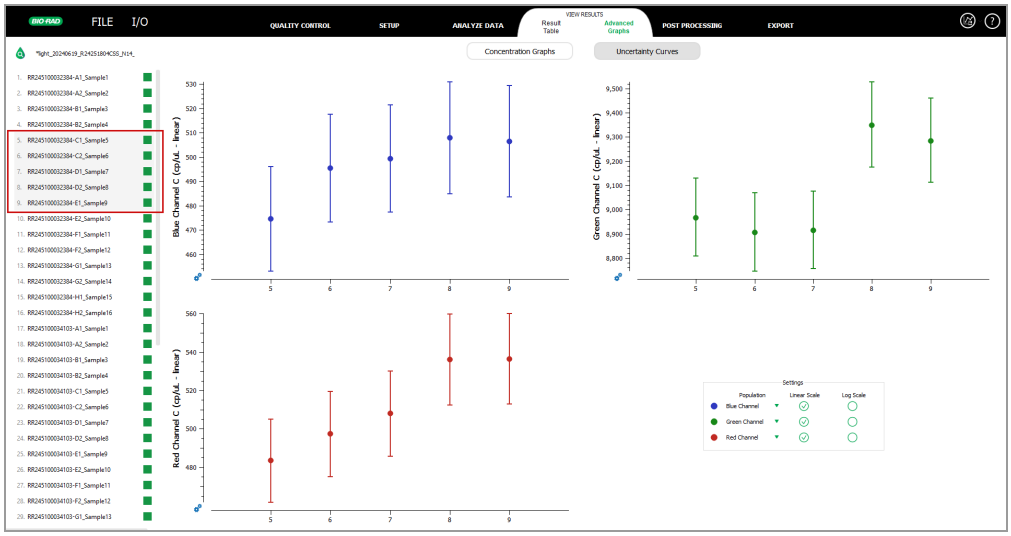
- **Results Table** — contains experiment details and results.

  The default view contains the chamber name, quality control flag, protocol identifier, total (Nb) droplet count, dilution factor, target concentration (copies per µl), and positive (Nb Pos) droplet count. You can also extend the view to include negative droplets, separability score, minimum and maximum copies per µL, and relative uncertainty at a confidence interval of 95%.

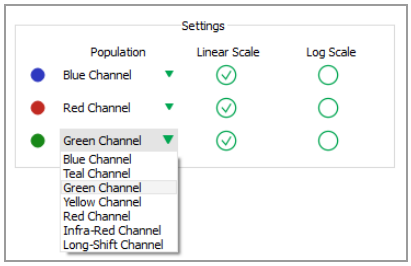


- **Advanced Graphs** — contains graphics for concentration and uncertainty curves (confidence intervals.

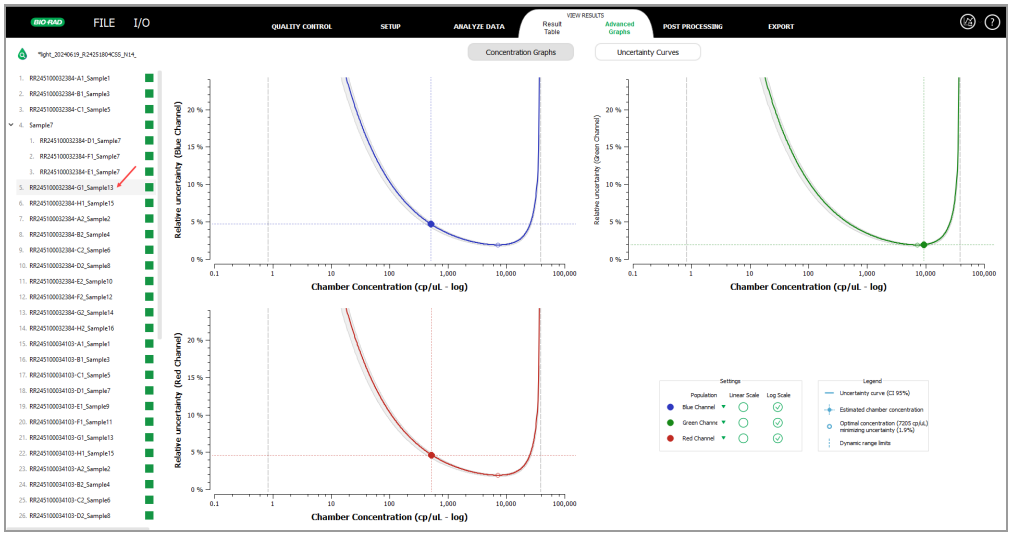
  - Concentration graphs illustrates the copies per µL using a line and a data point for each selected chamber. You can choose to display the data in linear or logarithmic scale.



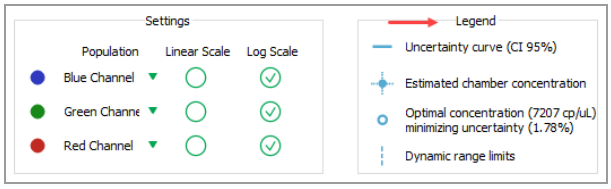
Important: In the Settings widget, the Population section displays a maximum of three channels simultaneously. Click in a channel field to display the dropdown list, and select a different channel if applicable.



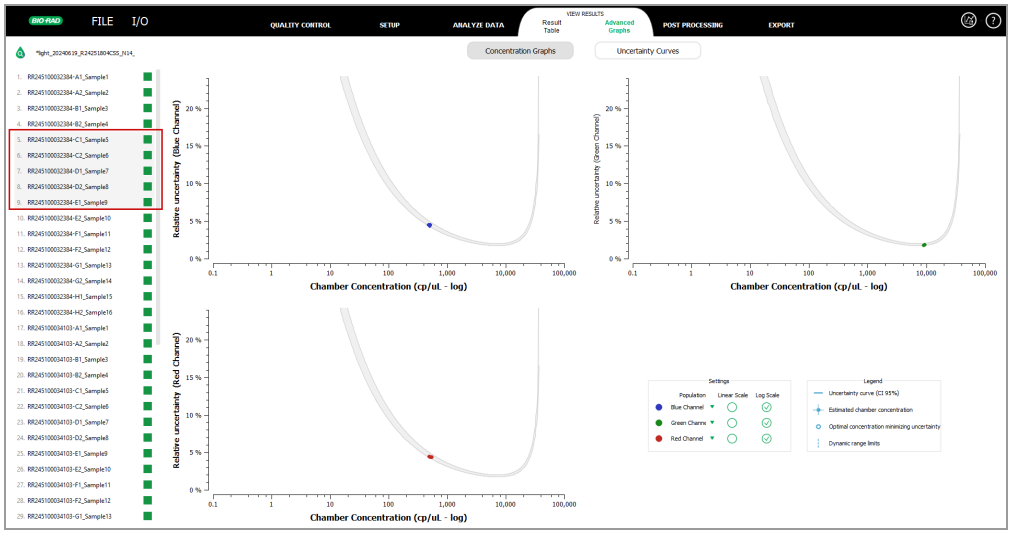
- Uncertainty Curves illustrate the variability associated with quantification of targeted nucleic acids at different concentrations. The data appears in a single arc for individual, multiple, and pooled selections.



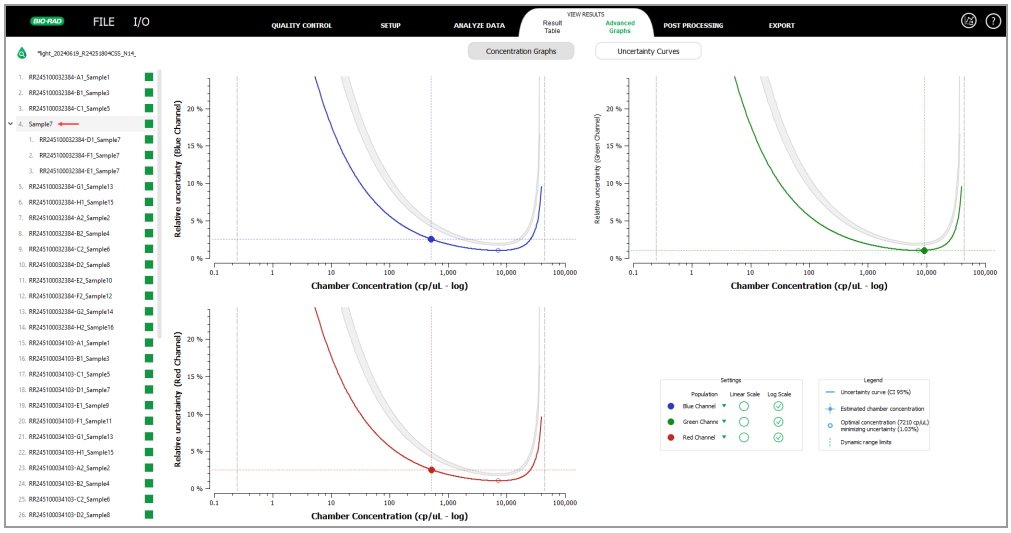
A legend also identifies elements in the display (uncertainty curve line, estimated chamber concentration, optimal concentration to minimize uncertainty, and dynamic range limits).



You can select a single chamber, or you can select merged chambers that appear as a collective curve. The following graphic shows the uncertainty curves with multiple chambers selected.



You can also select parent or subordinate pooled chambers. When you select the parent, as shown in the following graphic, the arcs are adjusted to show the data points for the pooled data.



Viewing the Result Table

For each chamber, the Result Table view contains the total number of droplets, dilution factor, concentration, and positive droplets. You can also expand the table to show the negative droplets, separability score, minimum and maximum copies per µL, and relative uncertainty at a 95% confidence interval.

If the sample dilution factor in the PCR mix is specified, the estimated concentrations are those of the DNA target molecule in the initial stock, in copies per µL. For example, if the sample has been diluted 10x in the PCR mix, then the field should contain 10 as the dilution factor and the stock concentration is equal to the chamber concentration, multiplied by 10.

Important: If the dilution factor has not been specified, then it is set to 1 by default. This indicates that the estimated concentration is the concentration in the loaded mix (the concentration of the target molecule in the chamber) and **not** the initial (stock) concentration.

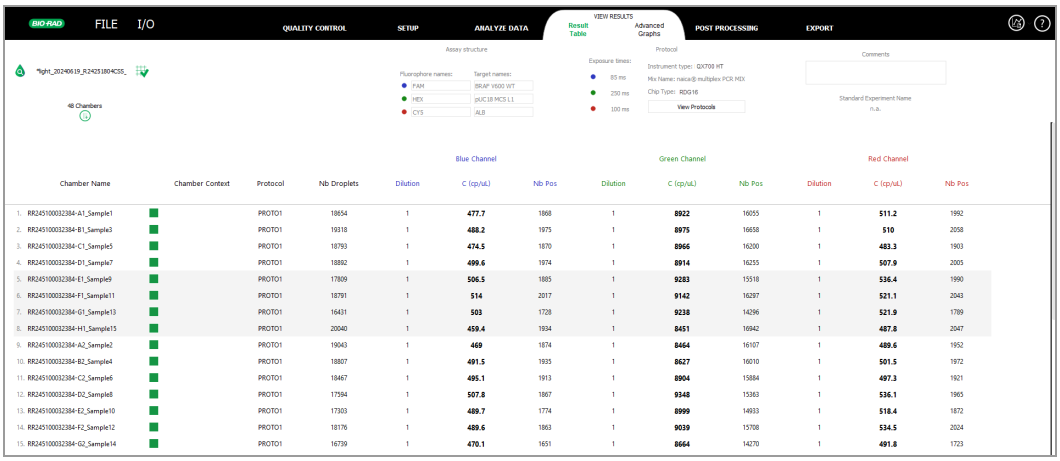
To obtain the concentration results

1. Click View Results > Result Table.

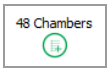
   QX700 ddPCR System Analysis Software computes the absolute nucleic acid concentrations based on the standard Poisson statistics that are commonly used in ddPCR.

2. Click Result Table to view the concentration data.

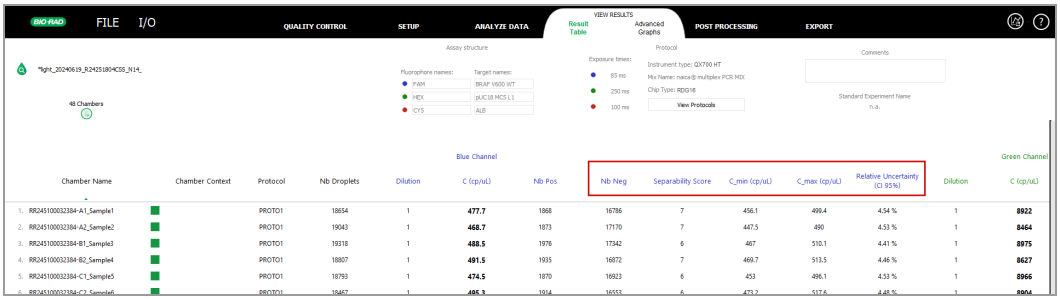
   Column titles are color-coded to coordinate with the corresponding fluorophore (channel) color (Blue for the FAM channel, Teal for the HEX channel, and so forth).



3. To expand the table to display additional data columns, click the chamber icon.



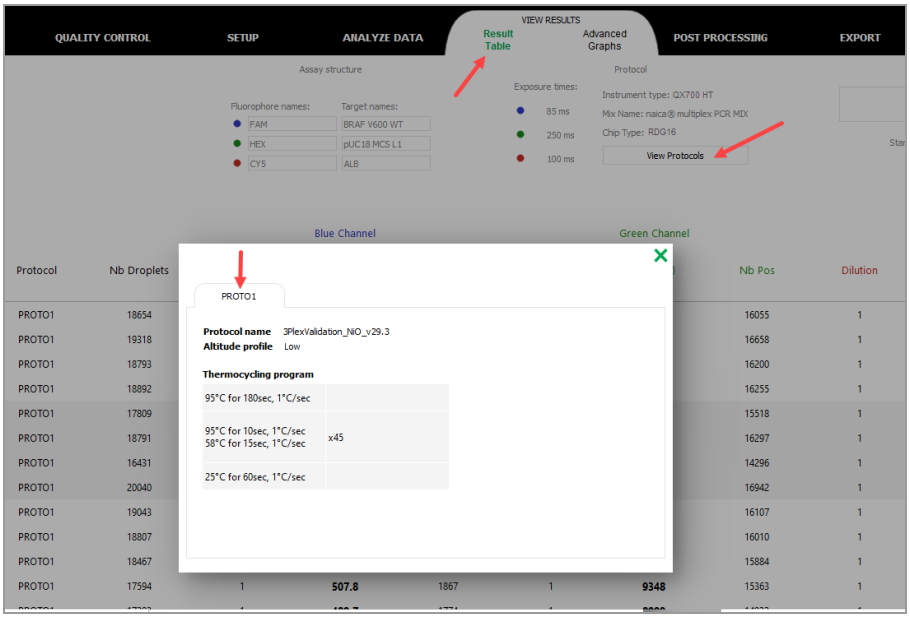
4. The total number of chambers belonging to the experiment is specified above the left pane. You can also click the icon to display additional data columns.



5. Use the bottom scroll bar to scroll right or left and view results for all populations.

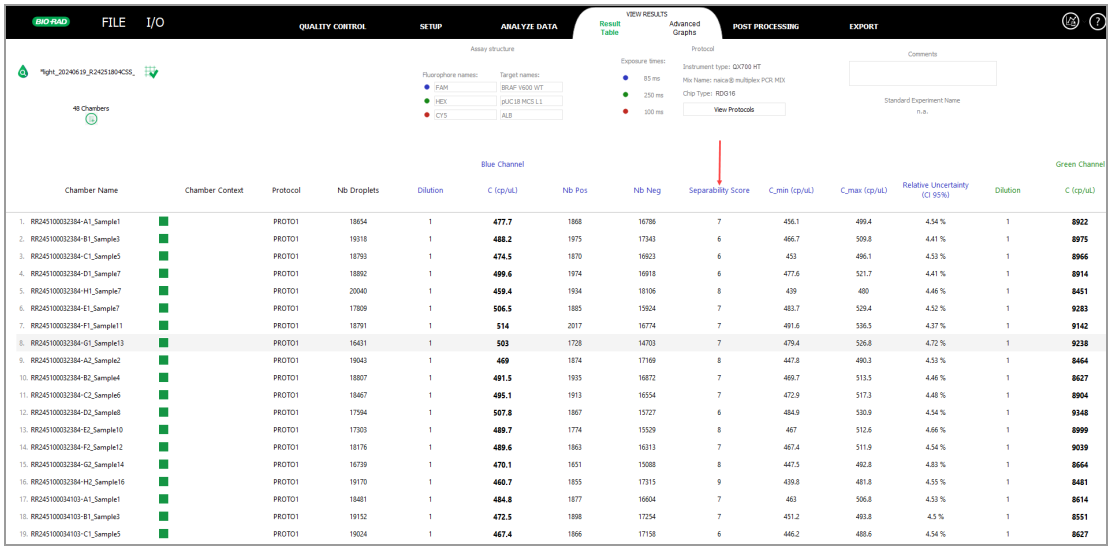
   Tip: You can copy and paste the results to an Excel spreadsheet.

6. Click View Protocols to display a pop-up containing the thermal cycling protocol information. If more than one protocol is assigned, a tab appears for each.



Quantifying Fluorescence Separability

QX700 ddPCR System Analysis Software automatically computes the separability score, which is provided for each detection channel and displayed in the Results Table extended display.

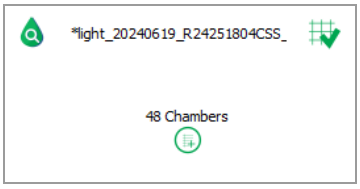


Separability scores are used for both quality control and assay optimization. The higher the score for the droplet population, the better the amplification efficiency for the target of interest. A low separability score, or a decrease in a previously characterized separability score, can signal a quality issue that is impacting the PCR amplification efficiency; for example, using an incorrect PCR program, sample degradation, or incorrect probe/primer concentrations.

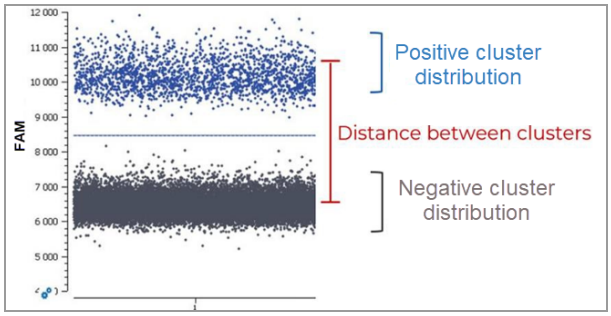
Note: The separability score is only one parameter that is evaluated when optimizing assays. You must confirm proper quantification using known quantities of positive controls. For example, the separability score for any given channel could be high, but if the reaction is not specific to the desired target, the target quantification might be incorrect. For more information, see Using the Separability Score for Assay Optimization on page 99.

To expand the column display

▶ In the View Results > Results Table display, tap/click the Chambers icon to show the column containing the separability scores.



The separability score is an objective measure of the separation of positive and negative clusters of any given amplified target in a sample. The score is based on the distance between the positive and negative clusters and the positive and negative cluster spreads, as shown in the following graphic:



In this example

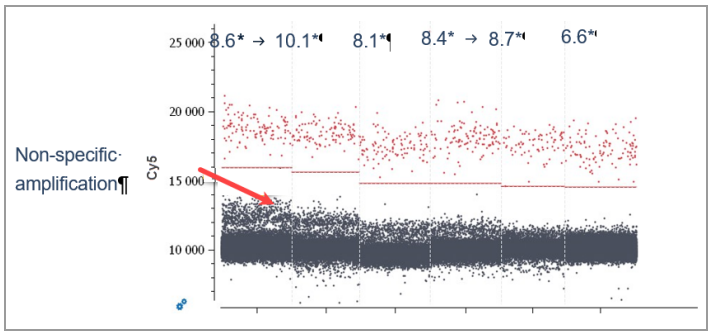
- The positive droplet cluster includes the amplified target of interest, so a high fluorescence intensity is displayed.
- The target of interest is absent from the negative droplet cluster, so the fluorescence intensity is low compared to the positive cluster.

The following bullet points describe the separability score values:

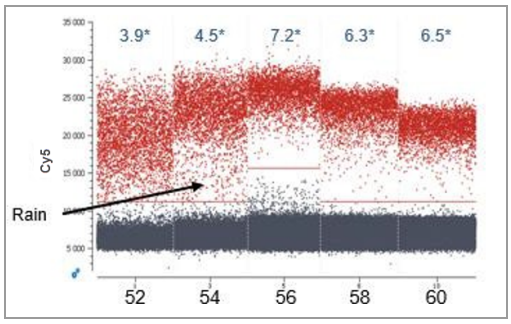
- For the FAM (Blue) channel, a separability score of 4 is the minimum acceptable value to distinguish clearly and set the threshold between the positive and negative clusters on a 1D dot plot.
- The separability score is low for the FAM (Blue) channel because of the addition of the reference dye, fluorescein. Fluorescein allows detection of the droplets by the analysis software, but increases the background signal (negative population fluorescence) in the FAM channel.
- For all other channels, a separability score above 8 is acceptable, depending on the assay and the fluorophore used.

Using the Separability Score for Assay Optimization

Unoptimized PCR conditions can yield more than one distinct positive population, which can make defining a threshold difficult and cause inaccurate quantification of the target of interest.



Sub-optimal PCR conditions can result in "rain," which are droplets of intermediate fluorescence due to inefficient amplification, as shown in the following graphic:



You can use the separability score to

- Determine the optimal elongation temperature common to all target amplifications. The score is critical for assay optimization because unoptimized probes and primers can cause undesired probe and primer interactions, such as non-specific amplification that can lead to a distinct second population.
- Optimize assays and help determine the ideal PCR conditions that are common for all primers and probes used in an assay.
- Determine the optimal probe/primer concentration at which there is good separability between the positive and negative clusters witgh no performance loss. To reduce assay complexity and the overeall quantities of reagents required for the reaction, it can be especially useful to determine the lowest concentration of required probes/primers.
- Evaluate the assay performance. For example, if the Separability Score was 4 in the FAM channel, but rose to 6 after changing the elongation temp from 59 °C to 61° C, then a potential improvement in the assay is indicated.

  Note: To evaluate the impact on assay performance, a comparison of the Separability Scores before and after making a reaction change should be done for all channels.

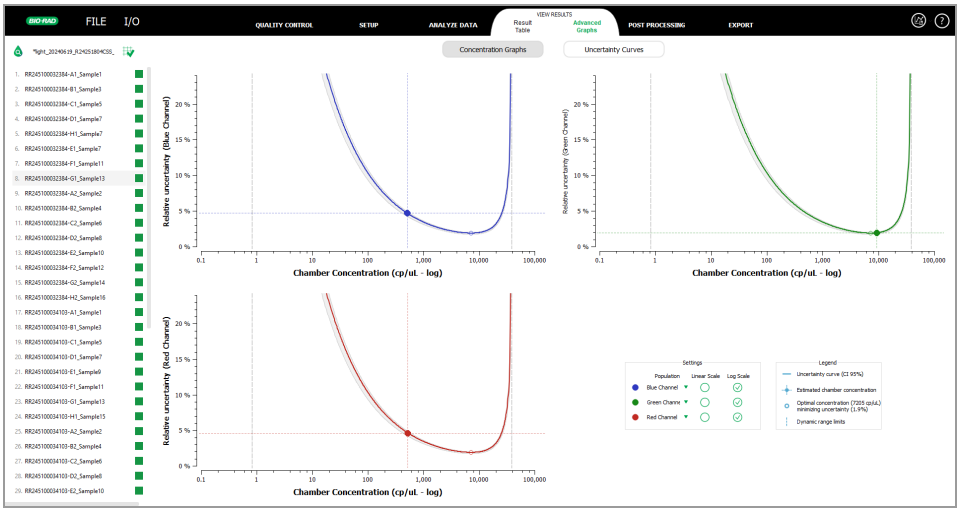
Interpreting Uncertainty Curves

For individual, multiple, and pooled chambers, you can view the curve of relative uncertainty at a 95% confidence level in the Advanced Graphs tab.

To view the graph

1. Select chambers, as follows:
   - Single chamber
   - Multiple chambers
   - Pooled chambers, parent chamber or subordinate chambers

2. Tap/click View Results >Advanced Graphs, and then select the Uncertainty Curves tab.



The 95% relative uncertainty curves (as shown in the above graphic) are displayed for each channel, where

- The x-axis shows all possible chamber concentrations (in copies/µL).
- The y-axis shows the 95% relative uncertainty of the chamber concentration, which is based on the total number of droplets analyzed in the currently selected chamber.
- The colored curve corresponds to the relative uncertainty curve of the currently selected chamber. The higher the total number of droplets in the chamber, the lower the curve.
- The upper gray curve corresponds to the relative uncertainty curve of the chamber with the fewest droplets.
- The lower gray curve corresponds to the relative uncertainty curve of the chamber with the most droplets.

  Note: If the two gray curves are the same, they can be obscured under the colored curve of the selected chamber.
- The filled points located on the curve correspond to the predicted concentrations in the selected chambers. You must distinguish between the chamber concentration and the stock concentration, which is equal to the chamber concentration multiplied by the dilution factor.
- The white point located on the curve represents the chamber concentration, which minimizes the relative uncertainty. This indicative value can help to estimate optimal dilution for the experiment.

  Note: The white point is reached when ~79.7% of the droplets are positive, whatever the number of droplets and the confidence level.

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Chapter 8 Post-Processing Changes

The following bullet points describe the Setup and Results submenus in the Post Processing tab:

- **Setup** — allows the user to specify the reference gene for each type of post-processing assay and for each target population or gene. This can be identical for all target genes, or unique to each target gene. Copy Number Variation (CNV), Mutant Allele Fraction (MAF), Gene Expression (GEX) and Linkage Analysis are available for selection.

  Important: Post-processing for large data sets can take more time. A "waiting" animation (spinner) appears to alert you that the data is still being processed.

- **Results** — contains the total droplet count for each chamber, plus recalculated experiment details from the post-processing analysis.

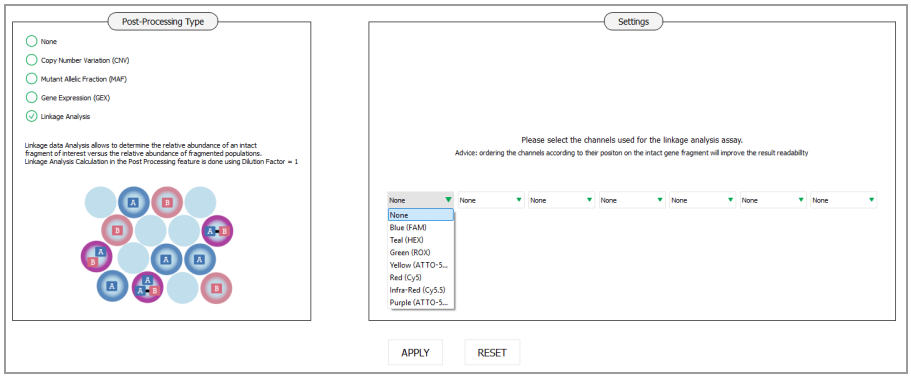
  Note: The post-processing Results table is populated with data from one experiment type at a time. If you select a different experiment type and recalculate, the existing columns and data are overwritten with the recalculated data.

Performing Post-Processing Analysis

In the Setup view, you can specify settings to recalculate data for the Copy Number Variation (CNV), Mutant Allelic Fraction (MAF), and Gene Expression (GEX) experiment types, and also for Linkage Analysis. When you select an experiment type on the left, the calculation formula appears with a brief explanation. The Settings dialog, where you can add populations and define references, appears on the right. Each Settings dialog is tailored to the experiment type selected.

Fig. 7: Post-processing example

Fig. 8: Post-processing example, Linkage Analysis



Important: Linkage analysis is always calculated at a dilution factor of 1.

The Results view contains the updated data after the post-processing algorithm recomputes the data for your selected target and reference inputs.

Important Notes:

- If you select Gene Expression, you must calculate the geometric mean manually.
- Post-processing calculations can take a few minutes. The QX700 ddPCR System Analysis Software displays a processing animation to alert the user that the software is computing data.
- Linkage analysis is not available when you define thresholds using polygons.

To perform a post-processing analysis of the results

1. Select Post Processing > Setup, and then select an experiment type.

2. In the Settings dialog on the right, choose a population, and then tap/click Add population to processing.
3. Repeat to add more populations.

Table 12 on page 106 describes the options available for each experiment type, and Linkage Analysis, to perform post-processing analysis.

Table 12. Post-processing options

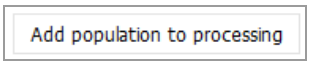
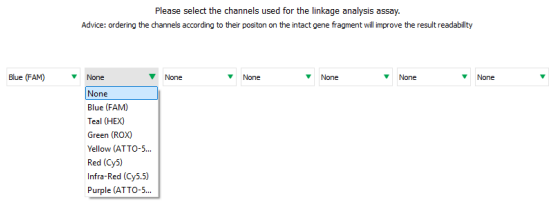
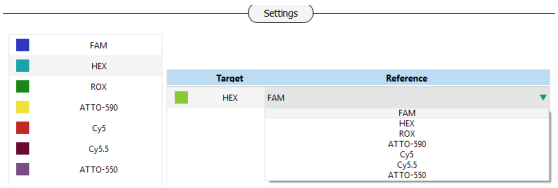
View	CNV	MAF	GEX	LINK
Under Settings, you can select and add populations sequentially; select a population in the left column, and then tap/click Add population to processing. 	X	X	X	
Under Settings, you can select the channels used for the linkage analysis assay. Tap/click each drop-down arrow to select a different channel. 				X
Under Settings, the selected color is also used in the Results Table.	X	X		
By default, the FAM channel appears in the Reference column. 1. To modify, tap/click FAM and select a reference from the dropdown list.  **Note:** If you select the *Use same for all targets* checkbox, then the reference chosen first is applied to the other populations.	X	X		

Table 12. Post-processing options, continued

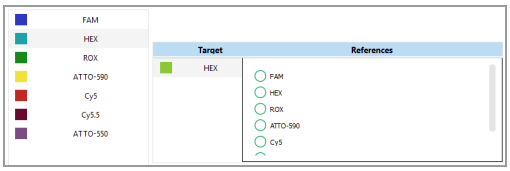
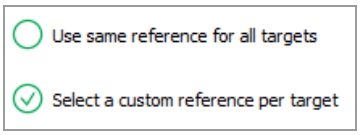
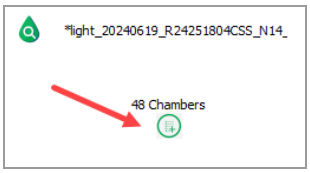
View	CNV	MAF	GEX	LINK
Under Settings, to select one or more reference genes for the same target gene, double-click the Reference column.  **Note:** If several reference genes are selected, the GEX ratio is calculated using the geometric mean of the selected reference genes.			X	
To specify the Cn reference value (set at 1 by default), double-click a line in the Cn Ref column and enter a value, and then press Enter.	X			
You can select a custom reference per target or use the same reference for all targets.  	X	X	X	
When you click Apply, post-processing calculations are launched.	X	X	X	X
Values are displayed in the Results tab. In addition to the experiment parameters, only the following appear: ■ For CNV, only the Cn ref and CNV values ■ For MAF, only the MAF values ■ For GEX, only the GEX value ■ For Linkage Analysis, only the concentration and values of relative abundance in %	X	X	X	X

Table 12. Post-processing options, continued

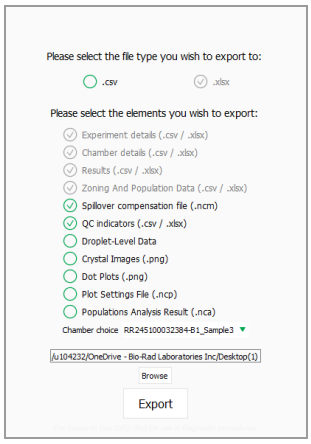
View	CNV	MAF	GEX	LINK
To extend the Results table and display confidence interval and uncertainty percentage columns, tap/click the chamber count icon to extend the display. 	X	X	X	X
To improve results readability, respect the order of the different targeted genes on the intact gene fragment from 5' to 3'.				X

Chapter 9 Exporting System and Analysis Data

Using the Export tab, you can select chambers and export corresponding default and selected experiment data to selected file types and formats, as shown in the following graphic. QX700 ddPCR System Analysis Software can export to .csv and .xlsx formats.

Note the following:

- You can select enabled data types from the list
- The grayed out data types are always exported



For information on the fields and other information that is always exported, as well as optional exports, see Data That is Always Exported on page 110 and Data Available for Optional Export on page 114.

To export the data

1. In the menu bar, tap/click Export.
2. Select a file type (.csv or .xlsx).
3. Select each experiment element to export.
4. Tap/click Browse and navigate to the desired output folder.
5. Tap/click Export.

When the export concludes, the output folder opens by default.

Data That is Always Exported

Important: if samples from different experiments (.niodata or .nioresult) are merged, the output details might differ from this section.

The following data is always exported:

- Experiment details (csv file, Excel Experiment_Details sheet), including:
  - Fluorophore and target names
  - User comments
  - Scanning parameters (exposure times in ms for each channel)
  - Spillover compensation status (compensated or uncompensated)
  - The QX700 ddPCR System Analysis Software edition used to export the data
  - Timestamp of data export
  - Thresholding type for the experiment (line vs polygon) and definition scope (common for all chambers or Individual per chamber)
  - Cartridge type
  - Mix name
  - Kit name
  - Instrument configuration
  - Standard experiment name (if applicable)
- Chamber details for each chamber (.csv file, Excel Chamber_Details sheet), including:
  - Cartridge barcode, chamber position (A, B, C, and so forth), sample name
  - Pooling ID (empty, if not applicable)
  - Protocol tag
  - Protocol name
  - Sample type (U, N, P, or S) for each detection channel

    Note: For sample type S, reference concentration in copies/µL is also included.
  - Original experiment name
  - QX700 ddPCR System Control Software edition used to export (standard or premium)

- Scanning timestamp
- Serial number of the QX700 ddPCR System used to scan
- Index of cartridge position (slot 1, 2, 3 from left to right on the cartridge plate) and index of cartridge plate at the time of the scan
- Cartridge model
- Droplet diameter (estimated average droplet diameter in microns)
- Number of manually removed droplets

  Note: For information, see Exploring and Analyzing Droplets on page 86 and Performing Quality Control on page 29.

- Results for each chamber (.csv file, Excel Results sheet), including:
  - Cartridge barcode, chamber position, sample name
  - Total number of analyzed droplets
  - For each population:
    - Sample type (only in line thresholding mode)
    - Dilution factor (N/A if not applicable)
    - Estimated stock concentration (in copies/µL), using the following rounding rules:
      - If c > 1000 cp/µL, the estimate is truncated
      - If c > 10 cp/µL, the estimate is rounded to one digit
      - If c < or = 10 cp/µL, the estimate is rounded to two digits
    - Number of positive droplets
    - Number of negative droplets (if not specifically defined as negatives in the population)
    - Separability score
    - Min and max limits of the 95% confidence interval for the estimated stock concentration (in copies/µL)
    - 95% relative uncertainty for the estimated stock concentration (equal to the 95% confidence interval divided by 2x the estimated concentration)

- Post-processing
  - Post-processing setup for the experiment (.csv file and PP_setup sheet in Excel file):
  - Post-processing analysis type
    - Target population
    - Reference population
    - Cn reference value (CNV analysis)
    - Channels used in linkage analysis
- Post-processing results for each chamber (.csv file and PP_setup sheet in Excel file):
  - Chamber name
  - Pooling ID
  - Protocol name
  - Total number of analyzed droplets
  - For each CNV calculation:
    - CN ref value
    - CNV value
    - Min and max limits of the 95% confidence interval for the CNV value and 95% relative uncertainty for the estimated CNV value
  - For each MAF calculation
    - MAF value
    - Min and max limits of the 95% confidence interval for the MAF value and 95% relative uncertainty for the estimated MAF value
  - For each GEX calculation:
    - GEX value
    - Min and max limits of the 95% confidence interval for the GEX value and 95% relative uncertainty for the estimated GEX value

- Linkage analysis:
  - For the total fragment:
    - Estimated stock concentration (in copies/µL)
    - Min and max limits of the 95% confidence interval for the estimated stock concentration (in copies/µL)
    - 95% relative uncertainty for the estimated stock concentration (equal to the length of 95% confidence interval divided by 2 times the estimated concentration)
  - For the the intact fragrment or all the other combinations of channels:
    - Estimated stock concentration (in copies/µL)
    - Min and max limits of the 95% confidence interval for the estimated stock concentration (in copies/µL)
    - 95% relative uncertainty for the estimated stock concentration
    - Relative abundance (%)
    - Min and max limits of the 95% confidence interval for the relative abundance and 95% relative uncertainty for the relative abundance

- Zone Details (.csv file Excel Zone Details sheet), including details for each fluorescence zone
  - Chip barcode and chamber position, sample name
  - Zone ID

    Note: In line thresholding mode, there are always two fluorescence zones where N is the number of detection channels in line thresholding mode, where there is one zone per user-defined polygon in polygon thresholding mode.
  - Zone type, (quadrant in line mode, polygon in polygon mode)

    Applicable to 3-color experiments only, as follows:
    - The x and y channels are used to define the zone and the z channel (where Chan1 = Blue, Chan2 = Green, Chan3 = Red)
    - List of x–y coordinates of the 2D points defining the zone (in line thresholding, there are always five (5) points defining a square)
    - Zone coordinates for the z boundaries (In polygon thresholding mode the z-values for the boundaries are always 0–65535 RFU)

- Population Details (.csv file or Excel Population_Details sheet), including details for each population
  - Population name
  - Population color (RGB values)
  - List of the zone IDs where the populations have been built
  - If applicable, list of Zone IDs, specifically defined as negatives for the population

Data Available for Optional Export

Optionally, you can export the following data:

- QC Indicators (.csv file or Excel QC_Indicators sheet), including the following for each chamber:
  - Cartridge barcode (chamber position and sample name)
  - Quality flags
- Advanced QC Indicators (.csv file or Excel QC_Advanced sheet), including the following for each chamber:
  - Cartridge barcode (chamber position and sample name)
  - In line thresholding mode only:
    - Automated threshold values for each channel
    - Manual threshold values for each channel
  - In line and polygon thresholding modes, for each droplet population
    - Dimensions (channel ID to define the zone, where 1 = Blue, 2 = Green, 3 = Red, and so forth)
    - Separability score
    - μ pos (mean of the fluorescence values of the positive droplet population computed in 1D, 2D or 3D, depending on the number of channels used to define the population)
    - μ neg (mean of the fluorescence values of the negative droplet population, computed in 1D, 2D or 3D, depending on the number of channels used to define the population)

- std-dev pos
  - For 1D defined populations, L1 standard deviation of the positive droplet fluorescence values in 1D
  - For 2D or 3D defined populations, mean of the projected distances between the positive droplet fluorescence points and their center in 2D or 3D, along the axis defined by the positive population center and the negative population center in 2D or 3D
- std-dev neg
  - For 1D-defined populations, L1 standard deviation of the negative droplet fluorescence values in 1D
  - For 2D- or 3D- defined populations, mean of the projected distances between the negative droplet fluorescence points and their center in 2D or 3D along the axis defined by the positive population center and the negative population center in 2D or 3D

- Droplet-level data (.csv) files for each chamber, each population – by default, the FAM (Blue) channel, ROX (Green) channel, and Cy5 (Red) channel populations – and each compensation status (compensated and not compensated)

  Each file includes one line per droplet and for each droplet the file contains

  - The x and y coordinates of the droplet center in the chamber
  - Fluorescence values (in RFU) of the droplet in each channel
  - Numerical index of the droplet

  Each chamber includes

  - One general file which contains the information of all the droplets analyzed in the chamber.
  - Two population-specific files for each population (named XXX), as follows:
    - File name for the first file = XXX_POS, which contains the information for the droplet subset defined as positive for the population of interest
    - File name for the second file = XXX_NEG, which contains the information for the droplet subset defined as negative for the population of interest
- Spillover compensation file (.ncm format), including:
  - Translation vector for the fluorescence background in each channel
  - Excitation matrix for the degree of excitation of each fluorophore by each LED

- Droplet images (.png format in 8 bits), including the images of each chamber in each channel
- Dot plots (.png format); in the relevant channels, this includes
  - 1D dot plots for each chamber
  - 2D dot plots for each chamber
  - Concatenated (side-by-side) 1D graphs with all the chambers in sequential view
  - Concentration graphs
  - Uncertainty curves
  - Droplet fluorescence histograms
- Plots configuration file, exported in .ncp format (includes the histogram, 1D and 2D dot plot configurations
- Analysis template file (exported in .nca format); including the following information as data analysis parameters):
  - Manual or automated thresholding strategy
  - Thresholding mode and scope
  - List of the populations (default or custom) with their name, color, and zones
  - Coordinates defining each zone – only the coordinates associated with the first chamber of the current experiment are saved; these coordinates will apply to each new chamber of any further experiments
- If the zoning mode is set to Individual per chamber, then you can specify the chamber with the zoning configuration to be exported in the .nca file

Note the following:

- Exporting all chamber images or graphs extends the data export timeframe.
- The indicative size for the exported chamber images in .png format is 3 MB per channel per chamber
- From each display, you can save image and plot views using the following icons:
  - Save as an image file (.png)

  - Save as a vector file (.svg)

  - Copy to clipboard

To save the file

1. Click the applicable image or plot.
2. Click File > Save or Save as and follow the prompts.

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Appendix A Software Installation

As part of the Bio-Rad qualification protocols, a field service engineer (FSE) sets up the instrument, installs or upgrades your Standard Edition software, and activates the software licensing. You can also install the software on additional PCs to facilitate plate setup and experiment data analysis while the instrument is executing ddPCR runs. See PC System Requirements on page 119 for the minimum system requirements. Beginning with Version 1.5, each software edition is installed from its own installer file.

Standard Edition is sufficient if your organization does NOT need to operate in compliance with 21 CFR Part 11

PC System Requirements

To utilize the experiment setup and analysis capabilities while runs are being performed on the QX700 Droplet Digital PCR System, Bio-Rad recommends installing the software applications on one or more PCs that meet the minimum requirements specified in Table 13.

Table 13. Minimum PC requirements

Component	Minimum requirement
Operating system	Windows 10 or Windows 11 (64-bit), Professional Edition
RAM	16 GB (minimum)
Processors	Intel Core i5 or higher, 2GHz or higher (minimum)
Graphics card	NVidia GeForce GT 1030 or higher (or equivalent, recommended)
Screen resolution	1920 x 1080, aspect ratio 16:9 (minimum)
Storage	2 GB required for software installation

Installing QX700 ddPCR System Analysis Software

You can upgrade QX700 ddPCR System Analysis Software on the instrument, and you can install the edition you are using on a Windows PC. A Bio-Rad website user account is required to access the installation zip file. If you do not have an account, go to bio-rad.com and click Register in the upper-right corner to open the Sign in page. Scroll to and complete the required information in the New Customer section.

Important: If you are installing the software on additional PCs to expand your run setup and data file analysis capabilities, you must install the same software edition on each PC that is installed on your instrument or the run files generated on the instrument will be incompatible with the software.

Note: If your instrument or PC does not have a wireless connection enabled, you can install the software from a USB drive.

Downloading the Software

To download the software

1. Open bio-rad.com and sign in with your customer account.
2. In the website landing page, type QX700 ddPCR System Analysis Software in the Search field and then click the Search icon.
3. When the results appear, click the Software checkbox on the left, under Products.
4. Click Download Software to download the zip file to the specified location.
5. Continue to Installing the Software.

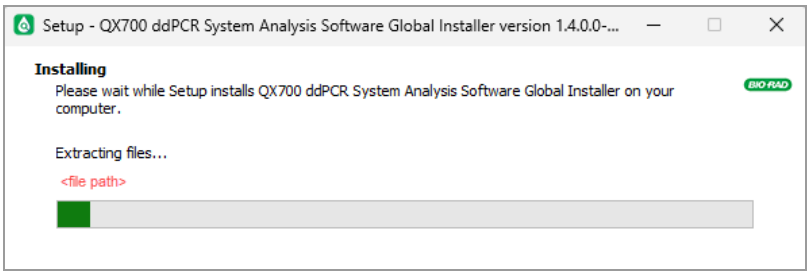
Installing Standard Edition

To install the software

1. Locate and unzip the downloaded installation file.
2. Right-click the installation executable file and select Run as administrator.
3. In the initial dialog, keep the default folder location (recommended) or tap/click Browse to navigate to a storage different folder.

   The default folder is C:\Program Files\Bio-Rad\QX700Analysis. If you are performing an upgrade rather than a new installation, a message asking you to confirm that you are installing to the existing folder.
4. Tap/click Next, and then tap/click Yes to confirm.

5. (Optional) Select the checkbox to create a desktop shortcut and click Next.
6. Select Create a desktop shortcut, and then click Next.
7. Click Install to begin the installation and display a progress bar.



8. When the installation concludes, click Finish.
9. (Optional) Restart the computer now or later.

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Appendix B Remote Maintenance

To enable remote software maintenance on the QX700 Droplet Digital PCR System, ensure that

- The PC is turned on and connected to the internet
- Team Viewer is installed on the instrument

To start a remote maintenance session

1. Double-click the Team Viewer icon on the PC desktop.

2. Send the user ID and password by email to support@bio-rad.com (U.S./Canada Only)

   Note: For technical assistance outside the U.S. and Canada, contact your local technical support office or click the Contact us link at www.bio-rad.com.

You must have the following information available:

- Software version (available in File > About)
- Log files that have been generated in the following directory:

  %USERPROFILE%\Public\Bio-Rad\<software name>\logs

Bio-Rad
Laboratories, Inc.

Life Science
Group

Website bio-rad.com **USA** 1 800 424 6723 **Australia** 61 2 9914 2800 **Austria** 00 800 00 24 67 23 **Belgium** 00 800 00 24 67 23 **Brazil** 55 11 3065 7550
Canada 1 800 361 1808 **China** 86 21 6169 8500 **Czech Republic** 00 800 00 24 67 23 **Denmark** 00 800 00 24 67 23 **Finland** 00 800 00 24 67 23
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Luxembourg 00 800 00 24 67 23 **Mexico** 52 55 5488 7670 **The Netherlands** 00 800 00 24 67 23 **New Zealand** 64 9 415 2280 **Norway** 00 800 00 24 67 23
Poland 00 800 00 24 67 23 **Portugal** 00 800 00 24 67 23 **Russian Federation** 7 495 721 14 04 **Singapore** 65 6415-3170 **South Africa** 27 21 531 7504
Spain 00 800 00 24 67 23 **Sweden** 00 800 00 24 67 23 **Switzerland** 00 800 00 24 67 23 **Taiwan** 886 2 2578 7189 **Thailand** 662 651 8311
United Arab Emirates 971 4 818 7300 **United Kingdom** 00 800 00 24 67 23

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