
QX700™ ddPCR™ System Analysis Software, Premium Edition

User Guide

Version 1.5

BIO-RAD

QX700 ddPCR System
Analysis Software
Premium Edition
User Guide

Version 1.5

BIO-RAD

Bio-Rad Technical Support Department

The Bio-Rad Technical Support department in the U.S. is open Monday through Friday, 5:00 AM to 5:00 PM, Pacific time.

Phone: 1-800-424-6723, option 2

Email: Support@bio-rad.com (U.S./Canada Only)

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.

Legal Notices

No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopy, recording, or any information storage or retrieval system, without permission in writing from Bio-Rad Laboratories, Inc.

Bio-Rad reserves the right to modify its products and services at any time. This guide is subject to change without notice. Although prepared to ensure accuracy, Bio-Rad assumes no liability for errors or omissions, or for any damage resulting from the application or use of this information.

Bio-Rad, ddPCR, Droplet Digital, Droplet Digital PCR, and QX700 are trademarks of Bio-Rad Laboratories, Inc. in certain jurisdictions.

Purchase of Digital PCR and/or Single-Cell NGS Sample Preparation products (the "Products") from Bio-Rad Laboratories is subject to Bio-Rad Laboratories, Inc. Standard Terms and Conditions of Sale, which can be accessed at <https://bio-rad.com/en-us/terms-conditions>. Unless we expressly state otherwise in additional Terms and Conditions, no rights are granted for you to distribute or resell the Products. Unless we expressly state otherwise in additional Terms and Conditions, no rights are granted for the development or commercialization of diagnostic assays for use with the Products without a license from Bio-Rad. It is the user's obligation to obtain a commercial license from Bio-Rad for (i) all commercial uses (not just diagnostic uses) and (ii) sale of assays for use on Bio-Rad's dPCR and ddSEQ instruments. The Products and/or their use are covered by U.S. and foreign patents and/or pending patent applications owned by or under license to Bio-Rad Laboratories, Inc. See <https://bio-rad.com/en-us/trademarks> for trademark information.

All trademarks used herein are the property of their respective owner.

Copyright © 2026 by Bio-Rad Laboratories, Inc. All rights reserved.

Revision History

Document	Date	Description of Change
QX700 ddPCR System Analysis Software User Guide, Premium Edition, Version 1.5 DIR No. 10000258611, Ver A	April 2026	Create a standalone guide for premium 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

This page intentionally left blank.

Table of Contents

FrontCover	a
Revision History	iii
Chapter 1 Droplet Digital PCR Overview	9
ddPCR Advantages	9
ddPCR Workflow	10
Finding Out More	10
Chapter 2 System Overview	11
Intended Use	11
QX700 ddPCR System Models	12
Associated Software Applications	13
Chapter 3 Using Premium Edition	15
21 CFR Part 11 Compliance	15
QX700 Data Service Permissions	19
Individual User Authentication	20
Sample Validation	21
Electronic Signatures	21
Experiment Audit Trail	23
Creating the Regulatory Report PDF	24
Chapter 4 Experiment Analysis Overview	29
Analysis Process Workflow	30
Opening an Experiment and Logging In	31
Accepted File Types and File State Indicators	33
General Navigation Options	35
Channel Fluorophores and Colors	35
Chambers Pane	36
Analysis Software Views	37
Analysis Software Menus	37
File Menu	38

Table of Contents

I/O Menu	39
Save Images Menu	40
Help Menu	41
Chapter 5 Performing Quality Control	43
Droplet Image Quality Control	46
Excluding Droplets	48
Chapter 6 Modifying the Experiment Setup	51
Editing General Experiment Parameters	53
Pooling Replicate Chambers	54
Combining Graphical Data for Multiple Chambers	57
Adding or Removing Chambers	59
Reordering Chambers	60
Applying Spillover Compensation	60
Automated Method	63
Template-Based Method	67
Manual Method	68
Chapter 7 Data Analysis	73
Plots and Populations	73
Plot Types	74
Line Thresholding Color View	76
Polygon Thresholding Zones and Populations	79
Changing Dot Plot Axis Values	80
Navigating 3D Dot Plots	83
Reading Histograms	85
Adjusting Fluorescence Thresholds	86
Using the Lines Thresholding Mode	87
Using the Polygon Thresholding Mode	89
Exploring and Analyzing Droplets	100
Chapter 8 Viewing Results	105
Viewing the Result Table	109
Quantifying Fluorescence Separability	111
Interpreting Uncertainty Curves	114

Chapter 9 Post-Processing Changes	117
Performing Post-Processing Analysis	118
Chapter 10 Exporting System and Analysis Data	123
Data That is Always Exported	124
Data Available for Optional Export	128
Appendix A Software Installation	133
PC System Requirements	133
Installing QX700 ddPCR System Analysis Software	134
Downloading the Software	134
Installing Premium Edition	134
Appendix B Remote Maintenance	139
BackCover	141

Table of Contents

This page intentionally left blank.

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 $\pm 10\%$ 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](https://www.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 Premium 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 data service application is also installed with Premium Edition to meet regulatory requirements. For more information, see [Using Premium Edition on page 15](#).

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 134](#).

Premium Edition requires the user to log into the data service and applications, and reverify login credentials when using auditable functionality.

Table 2. Supporting software applications

Software	Used to...
QX700 ddPCR System Control Software, Premium Edition	Set up and run ddPCR experiments on the instrument, with added functionality to support CFR 21 Part 11
QX700 ddPCR System Analysis Software, Premium Edition	Analyze droplet data acquired by the instrument and the calculated absolute concentrations of the targeted nucleic acids, with added functionality to support CFR 21 Part 11
QX700 Data Service	Manage user accounts and ensure compliance with 21 CFR Part 11; <i>required for Premium Edition</i>

This page intentionally left blank.

Chapter 3 Using Premium Edition

This chapter explains the additional functionality included in the Premium Edition of the QX700 ddPCR System Analysis Software. Premium Edition can enable your organization to meet the Food and Drug Administration (FDA) regulations imposed by good laboratory practices (GLP) and good manufacturing practices (GMP) in pharmaceutical and biotechnology environments. It also provides the necessary features for the QX700 Droplet Digital PCR System to operate in compliance with Title 21 of the U.S. Code of Federal Regulations Part 11 (21 CFR Part 11), within a closed system¹.

21 CFR Part 11 Compliance

In Premium Edition, QX700 ddPCR System Analysis Software operates in conjunction with QX700 Data Service, which functions as the system user account manager and ensures compliance with all 21 CFR Part 11 operations. For information and instructions regarding the QX700 Data Service, refer to the QX700 Droplet Digital PCR System Instrument Guide (catalog no. 10000171493).

Important: To be secure and in compliance with 21 CFR Part 11, the security controls built into QX700 ddPCR System Analysis Software must be properly configured and administered by the system administrators in your organization. Bio-Rad Laboratories, Inc. makes no claim that the QX700 ddPCR System Analysis Software is 21 CFR Part 11 compliant in and of itself, nor does the company guarantee compliance for the user. Your organization must establish policies and standard operating procedures (SOPs) that work in conjunction with the QX700 instrument and software provided by Bio-Rad to ensure compliance with 21 CFR Part 11.

[Table 3 on page 16](#) describes how the QX700 ddPCR System Analysis Software complies with each applicable section of 21 CFR Part 11.

¹A closed system is defined as “an environment in which system access is controlled by the persons who are responsible for the content of electronic records that are on the system”.

Table 3. 21 CFR Part 11 compliance features

Section	Function	Customer responsibility	Compliance
11.10 (a)	Validation	Yes	System validation, as well as experiment results validation, is supported by QX700 ddPCR System Control Software and QX700 ddPCR System Analysis Software. Validation SOPs for the customer organization are required.
11.10 (b)	Human-readable records	N/A	Electronic records in human-readable format are supported throughout QX700 ddPCR System Control Software and QX700 ddPCR System Analysis Software.
11.10 (c)	Protection of records	Yes	All electronic records are maintained within the QX700 ddPCR System Control Software and QX700 ddPCR System Analysis Software environment until you transfer them to external electronic archives that comply with customer organization policies.
11.10 (d)	Limited system access	Yes	Access to QX700 ddPCR System Control Software and QX700 ddPCR System Analysis Software is controlled through individual user authentication. The customer organization is responsible for establishing and maintaining the appropriate list of user accounts.
11.10 (e)	Audit trails	Yes	QX700 ddPCR System Control Software and QX700 ddPCR System Analysis Software tracks changes in an audit trail that does not expire. The customer organization is responsible for creating and controlling backups.

Table 3. 21 CFR Part 11 compliance features, continued

Section	Function	Customer responsibility	Compliance
11.10 (f)	Operating system checks	Yes	<p>QX700 ddPCR System Control Software and QX700 ddPCR System Analysis Software provide guidance and checks for setting up experiments.</p> <p>QX700 ddPCR System Control Software and QX700 ddPCR System Analysis Software allow you to create custom standard experiments that can, following validation by the user, be released for routine workflows.</p>
11.10 (g)	Authority checks	Yes	<p>Access to QX700 ddPCR System Control Software and QX700 ddPCR System Analysis Software is controlled by individual authentication and user role assignments.</p> <p>Users cannot modify electronic records; modification requires specified permissions and validation before release into the routine workflow.</p>
11.10 (h)	Device checks	Yes	<p>Experiment configuration and parameters are checked by QX700 ddPCR System Control Software and QX700 ddPCR System Analysis Software.</p> <p>The customer organization is responsible for sample ID input and standard experiments validation.</p>
11.10 (i)	Determination of education	Yes	<p>User guides and training documentation are provided by Bio-Rad Laboratories, Inc.</p> <p>The customer organization is responsible for establishing and maintaining the appropriate training levels.</p>
11.10 (j)	Written policies	Yes	<p><i>Not available</i></p> <p>The customer organization is responsible for establishing and maintaining SOPs to comply with the regulations.</p>

Table 3. 21 CFR Part 11 compliance features, continued

Section	Function	Customer responsibility	Compliance
11.10 (k)	System documentation	Yes	Users cannot change QX700 ddPCR System Control Software and QX700 ddPCR System Analysis Software documentation. The customer organization is responsible for distribution to users and version control .
11.100 (a)	Electronic signature, general requirements	Yes	Unique to individual users
11.100 (b)		N/A	Verification of identify
11.100 (c)		N/A	Certification of equivalence
11.200 (a/b)	Electronic signature, components and control	N/A	Signature with username and password ensure a genuine owner
11.300 (a)	Controls for identification (user names and password	Yes	Uniqueness of username and password
11.300 (b)		Yes	Periodic check of issuance
11.300 (c)		Yes	Loss management
11.300 (d)		Yes	Safeguards and detection of unauthorized attempts

QX700 Data Service Permissions

Each user role in the QX700 Data Service provides authorized users with access to a set of predefined permissions for the QX700 Droplet Digital PCR System and QX700 ddPCR System Analysis Software. Permissions are managed in the Roles/Permission tab on the QX700 Data Service user interface, where all available permissions are displayed in a list format. Granted permissions for a user role are indicated by a selected checkbox.

QX700 ddPCR System Analysis Software provides the permissions explained in [Table 4](#).

Table 4. QX700 Data Service permissions

User Role Permission	Description
Assay Edition	Provides access to assay editing
Export Data	<p>In addition to analytical data included in the 21 CFR Part 11 Regulatory Report, the Export Data permission allows the authorized user to export all data files listed using QX700 ddPCR System Analysis Software. The preselected data elements (greyed out) are always part of the data export. You can select checkboxes to export additional information.</p> <p>Important: Once exported, you can edit the data in Excel and .csv files outside the QX700 ddPCR System Analysis Software environment, without being recorded in the experiment audit trail. Therefore, only Sample Results reported within the generated PDF report are compliant with 21 CFR Part 11.</p>
Generate PDF Report	<p>The Generate PDF report permission allows the authorized user to create and export a 21 CFR Part 11-compliant PDF report of an electronically signed experiments using QX700 ddPCR System Analysis Software. The user can include the experiment audit trail information as part of the PDF report.</p> <p>Important: To electronically sign an experiment, the Validate Experiments permission is required.</p>

Table 4. QX700 Data Service permissions, continued

User Role Permission	Description
Invalidate Experiments	<p>The Invalidate Experiments permission allows the user role to remove an existing electronic signature that was executed by a different user. After the signature is removed, the user role can edit the data.</p> <p>Important: In GMP and GLP environments, where a high level of security is necessary, results must be reviewed by a second person as a control mechanism. Under this rule, access to data and actions to modify data results must always be available to more than one authorized user (two-person, four-eyes principle). The Invalidate Experiments permission supports the second reviewer hierarchy with full compliance for 21 CFR Part 11.</p> <p>For more information, see Electronic Signatures on page 21.</p>
Validate Experiments	<p>The Validate Experiments permission allows the authorized user to verify the result of the experiment for each individual sample in the experimental run in QX700 ddPCR System Analysis Software Result Table.</p> <p>The Validate Experiments permission further allows the user to electronically sign the validated result of the entire experiment in the Regulatory Report page of the QX700 ddPCR System Analysis Software Export menu.</p> <p>For more information, see Sample Validation on page 21 and Electronic Signatures on page 21.</p>

Individual User Authentication

To be compliant with 21 CFR Part 11, your organization must ensure that only authorized individuals are able to access Premium Edition. Therefore, you must log in to the software using your individual user authentication credentials (username and password).

Important: Failed logins might occur due to typing errors, but can also occur because of a connectivity issue to the QX700 Data Service. After three failed attempts to log in, the account is locked and only an IT administrator can reset and reactivate your Windows account.

If your session expires due to inactivity, Premium Edition requires a login to re-enable the screen. If another user logs in before you return, or if the application is closed, any unsaved data is lost. Automatic logout requirements are managed by your organization, but Bio-Rad recommends that you save your work and then close the QX700 applications if you must leave them unattended.

Sample Validation

Before you can sign and export the experiment results, QX700 ddPCR System Analysis Software software requires each individual sample to be reviewed and flagged as valid or invalid. Sample acceptance criteria might vary, depending on the specific SOP for an application or assay. Therefore, your organization must define sample conformity criteria using the QX700 ddPCR System within the application area.

Individual samples are flagged in the Valid Sample or Invalid Sample columns in the Results Table in the View Result display. By default, the status of all samples is undefined, so you can apply the same status to a selection of samples.

- ▶ Press the SHIFT or ALT key and select the samples, and then click the desired validity status.

Note the following for sample validation for pooled chambers:

- When using the Pooled Chamber feature, the experiment validity status of the individual sample chambers cannot differ from the pooled chamber sample result.
- If the validity status of all individual sample chambers composing a pooled chamber sample result are consistent, the validity status of the pooled chamber sample is implicitly set to the same value.
- If the validity status of the individual sample chambers selected for pooling differ, the validity status of the pooled chamber sample remains undefined.

You can also assign a validity status for the pooled chamber sample directly. This overwrites the individual sample validity status to match the pooled chamber sample validation status.

Electronic Signatures

In QX700 ddPCR System Analysis Software Premium Edition, you can electronically sign all validated experiment results, provided that you are assigned the required permissions. Electronic Signature is available in the Regulatory Report section of the Export view and is structured as follows:

- Electronic Signature Status displays a summary of the electronic signature status for the individual experiment file (*signed file record, signature user identification record, signature meaning record, and signature date and time record*)
- Sample Validity displays the record (summary) of the concluded sample results for the experiments (*valid samples, invalid samples, and unflagged samples*)

Important: To electronically sign an experiment, you must first flag the result of all samples in the experiment file as valid or invalid in the Result Table. If the samples are not flagged, an advisory message appears in the Perform Electronic Signature section and you cannot proceed with electronic signature.

- Perform Electronic Signature (*execution of the electronic signature for the experiment file*)

- Meaning of Signature

- Default value is set to display "n.a."

Important: In GMP and GLP environments, results must be reviewed by a second person as a control mechanism for a higher level of data security. Use the Meaning of Signature field to establish a result release hierarchy, tailored for your organization and fully compliant with 21 CFR Part 11.

- Electronic signature definitions must be stated within the specific organization process.

- Sign

- To electronically sign the experiment file, you must log in with your user name and password to confirm and authenticate. The user acknowledges full accountability and responsibility for all actions initiated to generate the reported ddPCR results

Note: Once the experiment is signed, a signature icon appears to the right of the experiment name in the top left corner of the experiment. The signature icon is a visual to affirm that the experiment has been electronically signed and is locked to prevent any further modifications. Subsequently, all QX700 ddPCR System Analysis Software features that allow modifications to the experiment file are disabled throughout the software menu.

- Unsign

The user can click Unsign, and then log in to confirm removal of the electronic signature

Important: To unsign an experiment that was electronically signed by a different author, you must be assigned the Invalidate Experiment user permission; otherwise, a "permission denied" message appears.

Experiment Audit Trail

The experiment audit trail records all events for an experiment file in QX700 ddPCR System Control Software and QX700 ddPCR System Analysis Software. Events included in the experiment audit trail record are:

- Scanning template parameters
- Cartridge IDs
- Scanning parameter modifications
- Droplet recognition modifications
- Sample modifications
- Experiment loading
- Spill-over compensation modifications
- Threshold or polygon position modifications
- Post-processing modifications
- Electronic signature record of the experiment file including user identification and date and timestamp.
- Experiment design (pooling chambers, importing, setting, or removing assays and protocols, and enabling or disabling chambers)
- Starting or canceling a run
- Rescanning chambers
- Changing a run priority

The experiment audit trail does not track events related to the Export functionality, since export events are not in the scope of the Regulatory Report page. To produce a consistent experiment audit trail, all computers running the software must be configured with the correct date and time.

You can review the experiment audit trail in QX700 ddPCR System Analysis Software.

To open the experiment audit trail viewer

1. Click Export in the menu bar, and then click the Regulatory Report tab.
2. In the Regulatory Reporting section click Display (below Audit Trail).

Creating the Regulatory Report PDF

Important: The experiment Regulatory Report can only be created if the experiment file is signed electronically. All date and time stamps are displayed with the ISO 8601 format. For information, see [Electronic Signatures on page 21](#).

To export the Regulatory Report:

1. In the menu bar, tap/click Export > Regulatory Report.
2. In the Regulatory Reporting section under PDF Report, tap/click Generate Report.
3. (Optional) Below Generate Report, select the applicable checkboxes to include the audit trail information and individual chamber report.
4. Choose the location to save the file and click OK.

When the report is exported, the file is automatically opened in the default PDF viewer. You can print the report in letter-size or A4 format.

The Regulatory Report is **not** editable and includes the following information:

- Experiment name
- User and software traceability, including
 - Electronic signature operator, date, and time
 - Electronic signature meaning (used for the second signature)
 - Scan operator, date, and time
 - Report generation operator, date, and time
 - Software name and version used to create the Regulatory Report
 - Software name and version used to scan the chambers
- Experiment and analysis setup information section, including
 - Instrument type identification
 - Instrument serial number
 - Cartridge type
 - Mix type
 - Standard experiment status
 - Number of analyzed chambers

- Number of analyzed samples (considering pooled chambers)
- Spillover compensation matrix
- Thresholding scope (common to all chambers or individually per chamber)
- Thresholding type (lines or polygons)
- Scanned channel information
 - Channel name
 - Fluorophore name
 - Target name
 - Time exposure
- Thermal cycling program information, including
 - Protocol ID
 - Protocol name
 - Altitude profile
 - PCR program steps
 - Layout of the protocols in the cartridges of the cartridge plate
- Cartridge layout for each cartridge plate
 - Cartridge ID
 - Chamber ID
 - Sample name
 - Pooling ID (if chambers are pooled, the pooling ID is written between brackets next to the Chamber ID)
 - Protocol ID
 - Post-processing setup, describing the type of post-processing analysis and the target and reference populations

- QC Indicators, including (for each individual chamber)
 - Chamber ID
 - Sample name
 - Chamber context
 - Image sharpness quality for a chamber
 - Number of analyzable droplets for a chamber
 - Number of saturated objects for a chamber
 - Quality flag for a chamber (green flag = High, green flag with eye = Medium, and yellow flag = Low)

- Result Table (for each individual chamber and pooled chamber)
 - Cartridge ID
 - Chamber ID
 - Chamber context
 - Sample name
 - Sample validity status
 - Protocol tag
 - Population name
 - Dilution factor
 - Concentration
 - Number of positive droplets per population
 - Number of negative droplets per population
 - Separability score
 - Minimum concentration
 - Maximum concentration
 - Relative uncertainty

Note: Chambers in the QC Indicators Table and the Results Table appear in the same order, as they appear in the QX700 ddPCR System Analysis Software. Populations that appear in the results table correspond to the populations that visible in the software.

- Footer section including
 - Report generation operator, date, and time
 - Experiment name
 - Current page and total number of pages

The Regulatory Report can also include the following two optional sections if they are selected:

- Individual and pooled chamber level information, including (for each individual chamber and each pooled chamber)
 - Pooling/chamber name
 - Pooling/Chamber Details section, including
 - Chamber ID
 - Sample name
 - Chamber/Pooling context
 - Pooling ID
 - General quality flag for pooling
 - Image sharpness quality for chamber
 - Number of analyzable droplets for chamber
 - Number of saturated objects for chamber
 - Sample type per channel
 - U = unknown
 - P = positive
 - N = negative
 - S = standard
 - Dilution factor

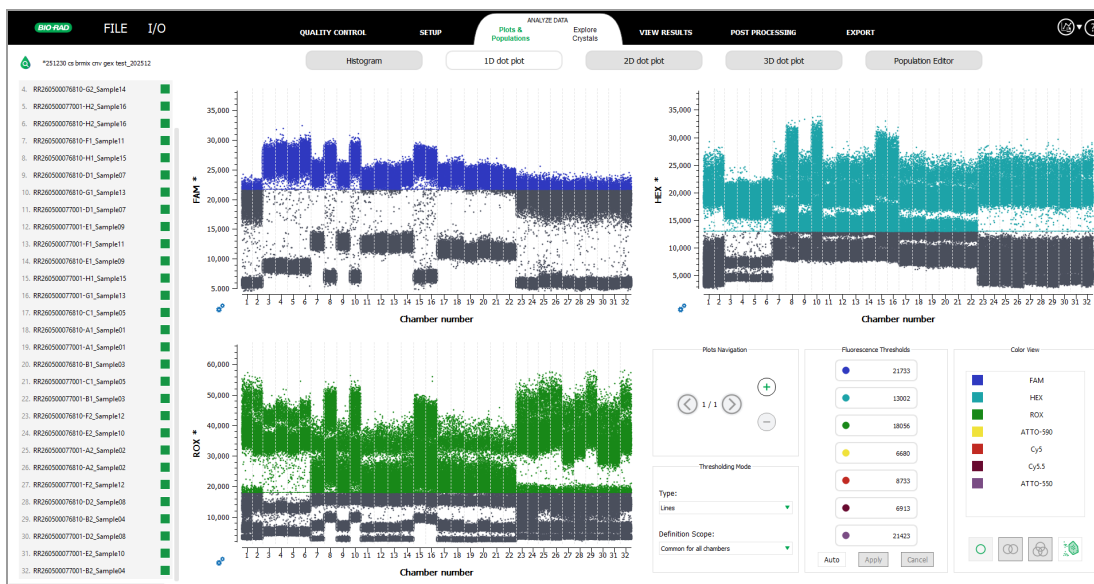
- Chamber/Pooling Result section, including
 - Sample validity status
 - Total number of droplets per chamber / pooling per channel
 - Dilution factor
 - Concentration
 - Number of positive droplets
 - Number of negative droplets
 - Minimum concentration
 - Maximum concentration
 - Relative uncertainty
 - Separability score
- 2D plots, as configured in the 2D dot plot tab in the Plots & Populations tab.
- Experiment audit trail

Chapter 4 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.

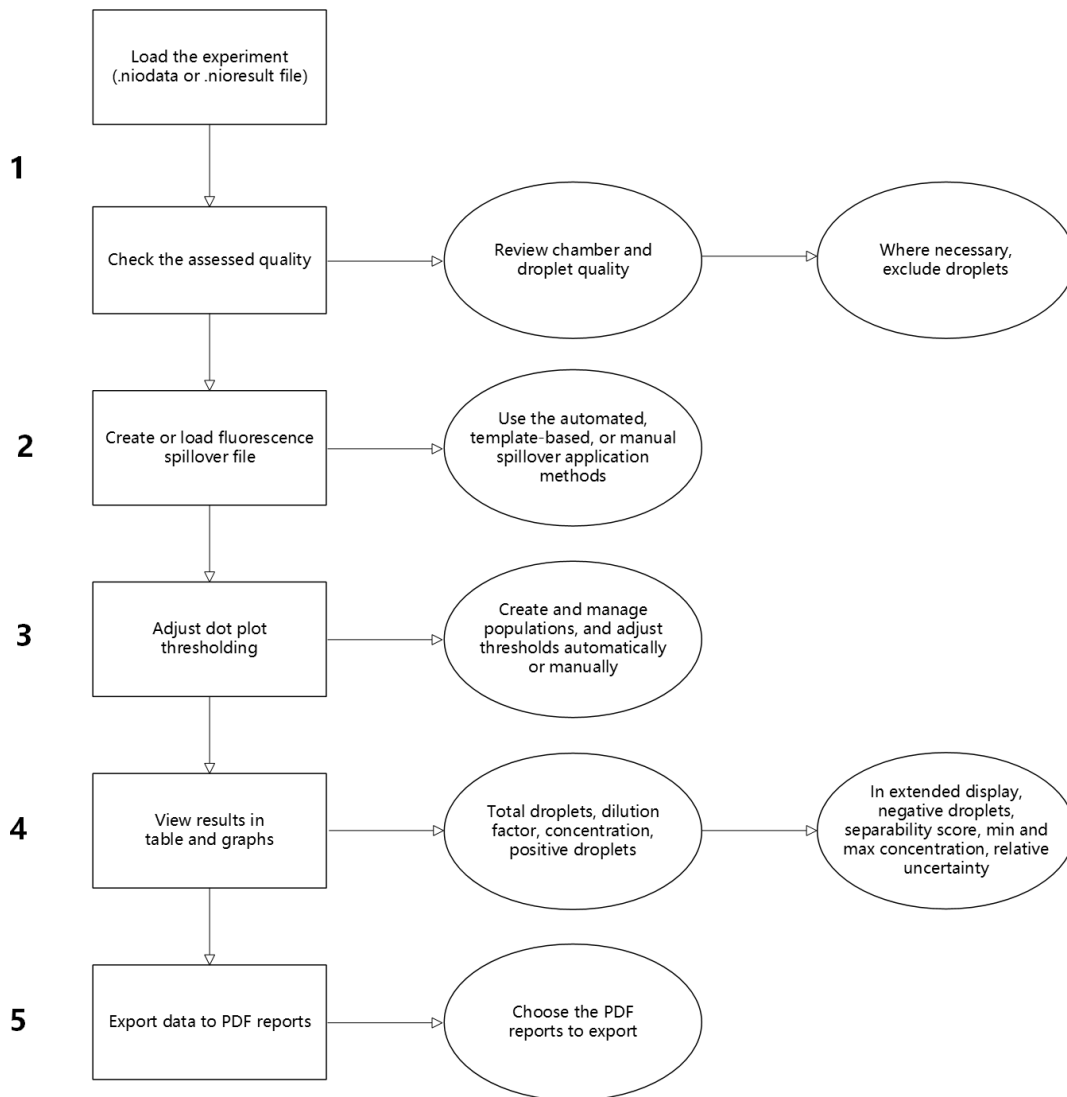


Important: When you save a .niodata file in the analysis software, it is always saved as a .nioresult file (whether or not you make changes) so the .niodata file remains in its original state.

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 and Logging In

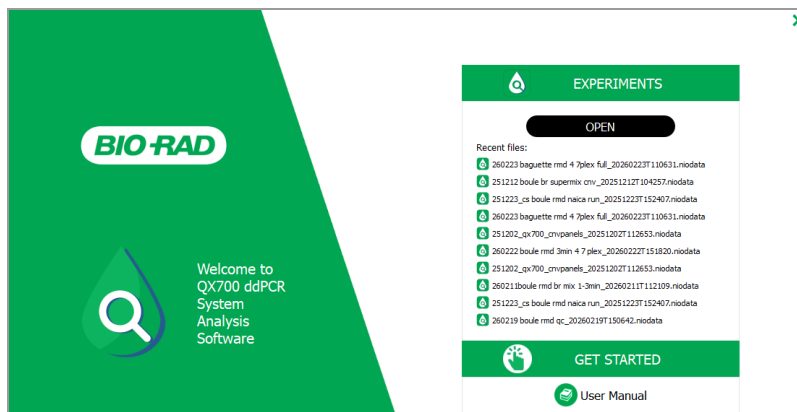
You can open the QX700 ddPCR System Analysis Software from the instrument touch screen or a Windows computer. For information on installing the software on computers, see [Appendix A, Software Installation](#).

Premium Edition requires a login to open the software, and also to confirm auditable actions. For more information on using Premium Edition, see [Using Premium Edition on page 15](#).

To open an experiment

1. Tap/click or double-click the software shortcut ().
2. Enter your user ID and password, and then tap/click Log in.
3. 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.

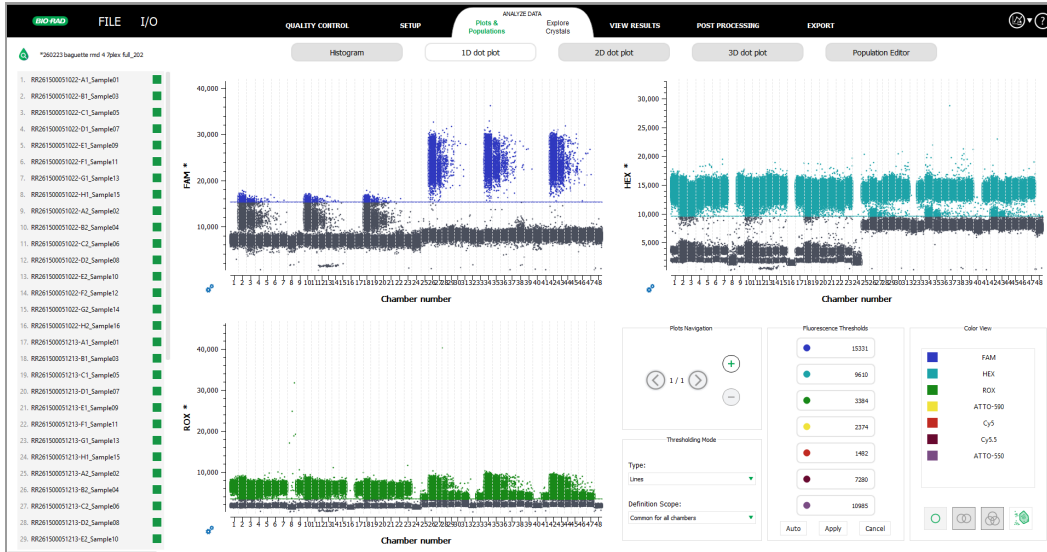
Note: After first use, up to ten most recently opened files appear in the Recent files list to facilitate subsequent access. If you close the Open file dialog before you select a file, the blank experiment layout remains. You can tap/click File > Home to reopen the Experiments dialog.



Chapter 4 Experiment Analysis Overview

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 39](#).

Accepted File Types and File State Indicators


The QX700 ddPCR System Analysis Software accepts the file types specified in [Table 5](#).

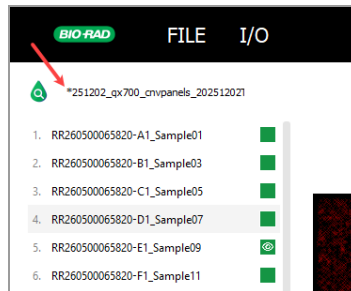
Table 5. QX700 data and configuration file formats

File type	Description
.niodata	<p><i>Original data file generated by the QX700 ddPCR System Control Software</i></p> <p>.niodata files contain the original high-quality images required for image analysis. Experiments that are opened for first-time analysis open in .niodata format.</p> <p>Note: The .niodata file type has been reduced in size but might still take a few minutes to open.</p>
.nioreult	<p><i>Post-analysis file generated by the QX700 ddPCR System Control Software when the original .niodata file is saved.</i></p> <p>When you save a .niodata file after making changes, the analysis software creates a .nioreult file with the changes and keeps the .niodata file in its original state so the initial data is preserved.</p>
.ncp	<p><i>Plot configuration file</i></p> <p>.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.</p>
.nca	<p><i>Analysis configuration file</i></p> <p>.nca files include the following information for all data analysis parameters:</p> <ul style="list-style-type: none"> ■ 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	<p><i>Compensation matrix format</i></p> <p>.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.</p>

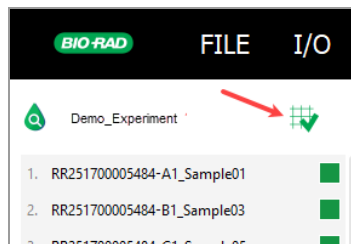
Table 6 contains information on symbols that appear next to the experiment name.

Table 6. 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.
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.



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.



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 43](#). The experiment name is also shown above the chamber list.

The screenshot shows a window titled "Flight_20240619_R24251804CSS_N14_". Below the title is a list of 17 chambers, each with a number, a unique ID, and a green square quality indicator.

Number	Chamber ID	Quality Indicator
1.	RR245100032384-A1_Sample1	■
2.	RR245100032384-B1_Sample3	■
3.	RR245100032384-C1_Sample5	■
4.	RR245100032384-D1_Sample7	■
5.	RR245100032384-E1_Sample9	■
6.	RR245100032384-F1_Sample11	■
7.	RR245100032384-G1_Sample13	■
8.	RR245100032384-H1_Sample15	■
9.	RR245100032384-A2_Sample2	■
10.	RR245100032384-B2_Sample4	■
11.	RR245100032384-C2_Sample6	■
12.	RR245100032384-D2_Sample8	■
13.	RR245100032384-E2_Sample10	■
14.	RR245100032384-F2_Sample12	■
15.	RR245100032384-G2_Sample14	■
16.	RR245100032384-H2_Sample16	■
17.	RR245100034103-A1_Sample1	■

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 7](#).

Table 7. 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 8](#).

Table 8. Main software views

Tab	General Purpose	For more information, see...
Quality Control	View droplet quality	Performing Quality Control on page 43
Setup	View/edit experiment parameters	Modifying the Experiment Setup on page 51
Analyze Data	Analyze plot data and droplet images	Data Analysis on page 73
View Results	Review results table; analyze concentration, uncertainty curve charts	Viewing Results on page 105
Post Processing	Apply post-processing calculations	Post-Processing Changes on page 117
Export	Export selected details, data, and chart graphics	Exporting System and Analysis Data on page 123

Analysis Software Menus

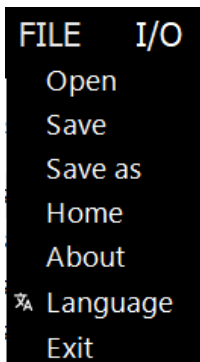
The QX700 ddPCR System Analysis Software provides tabs and menus that allow you to

- 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

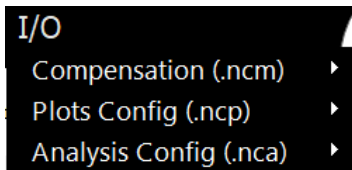
I/O Menu

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

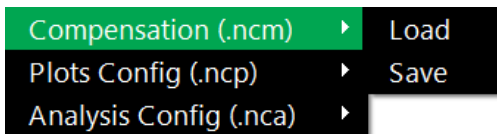
- Spillover compensation (.ncm)
- Custom plot configuration (.ncp)
- Custom analysis configuration (.nca)

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

Table 9. 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.

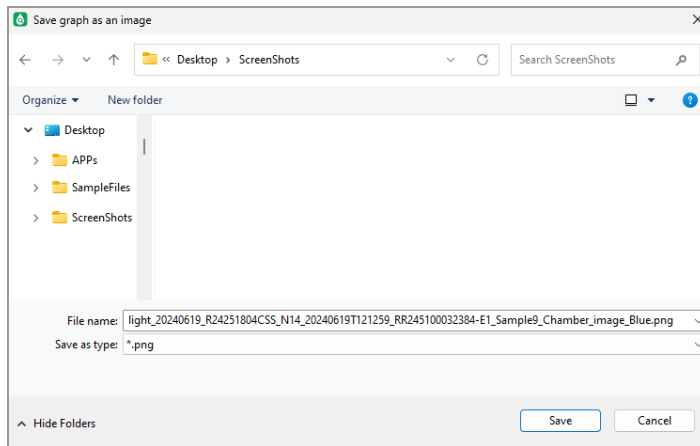
Note: The displayed Save menu icon represents the last selection from the menu.

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.

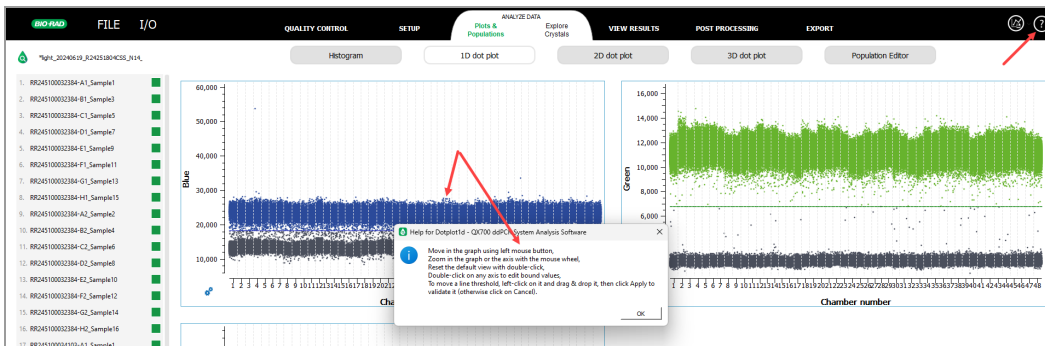


5. To save the file:
 - a. (Optional) Navigate to a new storage location.
 - b. Enter a new file name or keep the default entry.
 - c. Click Save.

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.



This page intentionally left blank.




Chapter 5 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 10](#).

Table 10. 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: <ul style="list-style-type: none"> Too many artifacts were identified in the droplet image Too few (less than 1,000) detected droplets were counted in the chamber

Options and information appear in the right pane are described in [Table 11](#).

Table 11. 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	To 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

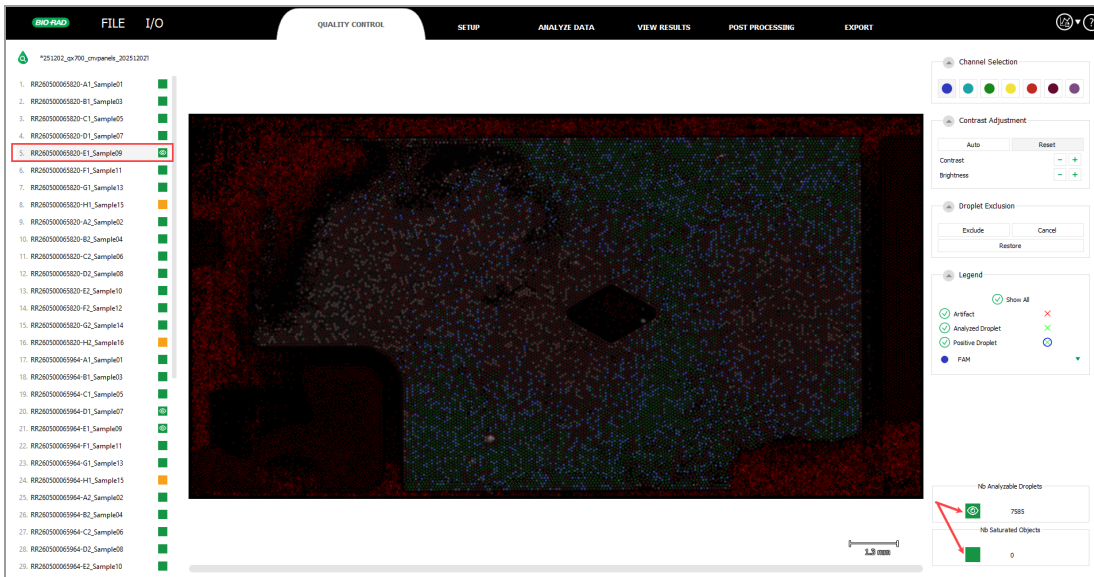
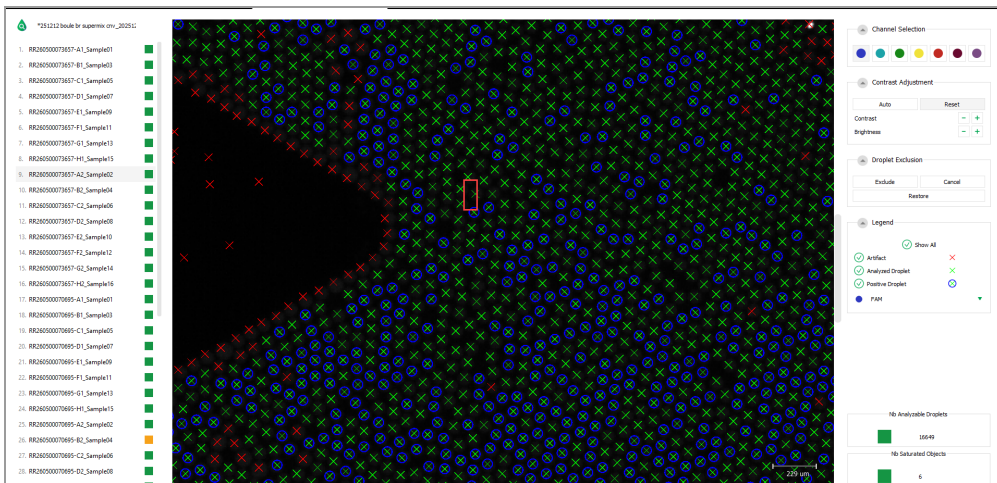


Fig. 3: Example of a droplet image in a chamber with a yellow flag



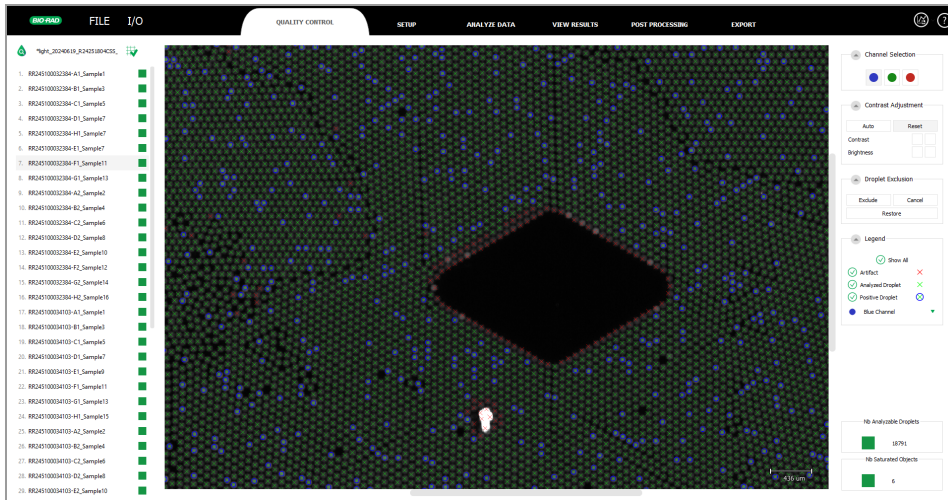
Droplet Image Quality Control

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

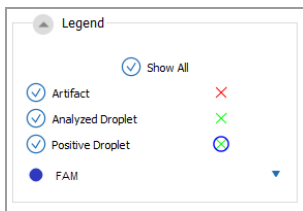


To perform quality control

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 analyzable 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 35](#).
 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.

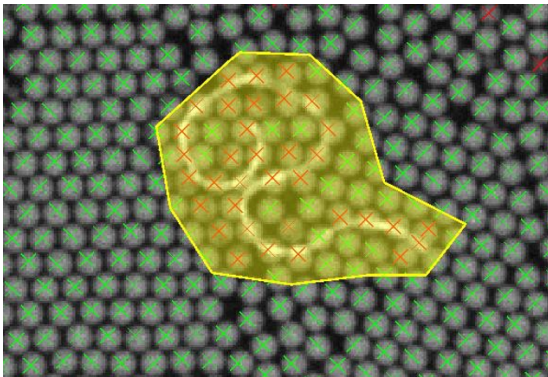
To exclude droplets

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.

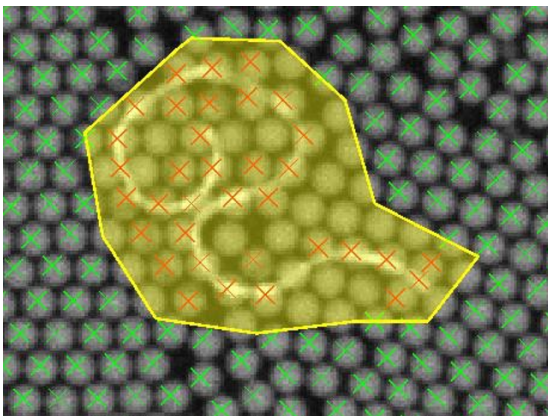
Note: Excluded droplets are marked with yellow hexagons.

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.

This page intentionally left blank.

Chapter 6 Modifying the Experiment Setup

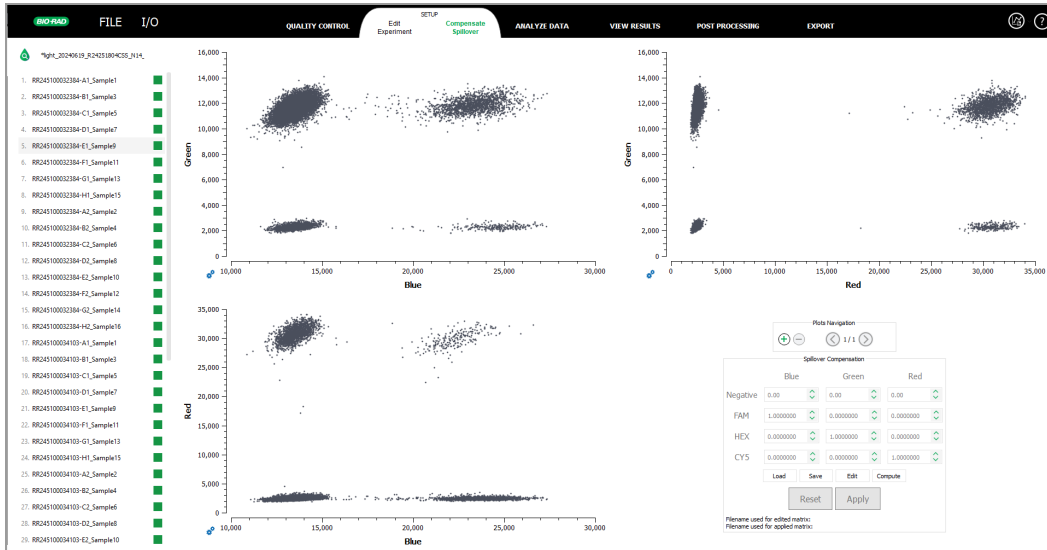
The Setup tab includes the Edit Experiment and Compensate Spillover displays, which are briefly described in the following bullet points:

- 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.

Chamber Name	Chip ID	Chamber ID	Chip Position	Sample name	Chamber Context	Protocol	NB Droplets	Type	C_ref stock (cp/mL)	Dilution	Type
1. RR245100032384-A1_Sample1	RR245100032384	A1	1	Sample1		PROT01	18654	U	---	1	U
2. RR245100032384-B1_Sample3	RR245100032384	B1	1	Sample3		PROT01	19318	U	---	1	U
3. RR245100032384-C1_Sample5	RR245100032384	C1	1	Sample5		PROT01	18793	U	---	1	U
4. RR245100032384-D1_Sample7	RR245100032384	D1	1	Sample7		PROT01	18892	U	---	1	U
5. RR245100032384-E1_Sample9	RR245100032384	E1	1	Sample9		PROT01	17809	U	---	1	U
6. RR245100032384-F1_Sample11	RR245100032384	F1	1	Sample11		PROT01	18791	U	---	1	U
7. RR245100032384-G1_Sample13	RR245100032384	G1	1	Sample13		PROT01	16431	U	---	1	U
8. RR245100032384-H1_Sample15	RR245100032384	H1	1	Sample15		PROT01	20040	U	---	1	U
9. RR245100032384-A2_Sample2	RR245100032384	A2	1	Sample2		PROT01	19043	U	---	1	U
10. RR245100032384-B2_Sample4	RR245100032384	B2	1	Sample4		PROT01	18807	U	---	1	U
11. RR245100032384-C2_Sample6	RR245100032384	C2	1	Sample6		PROT01	18467	U	---	1	U
12. RR245100032384-D2_Sample8	RR245100032384	D2	1	Sample8		PROT01	17594	U	---	1	U
13. RR245100032384-E2_Sample10	RR245100032384	E2	1	Sample10		PROT01	17303	U	---	1	U
14. RR245100032384-F2_Sample12	RR245100032384	F2	1	Sample12		PROT01	18176	U	---	1	U
15. RR245100032384-G2_Sample14	RR245100032384	G2	1	Sample14		PROT01	16739	U	---	1	U
16. RR245100032384-H2_Sample16	RR245100032384	H2	1	Sample16		PROT01	19170	U	---	1	U
17. RR245100034103-A1_Sample1	RR245100034103	A1	2	Sample1		PROT01	18481	U	---	1	U
18. RR245100034103-B1_Sample3	RR245100034103	B1	2	Sample3		PROT01	19152	U	---	1	U
19. RR245100034103-C1_Sample5	RR245100034103	C1	2	Sample5		PROT01	19024	U	---	1	U
20. RR245100034103-D1_Sample7	RR245100034103	D1	2	Sample7		PROT01	18653	U	---	1	U
21. RR245100034103-E1_Sample9	RR245100034103	E1	2	Sample9		PROT01	18072	U	---	1	U
22. RR245100034103-F1_Sample11	RR245100034103	F1	2	Sample11		PROT01	18005	U	---	1	U
23. RR245100034103-G1_Sample13	RR245100034103	G1	2	Sample13		PROT01	18010	U	---	1	U
24. RR245100034103-H1_Sample15	RR245100034103	H1	2	Sample15		PROT01	19967	U	---	1	U

- 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.

Chamber Name	Chip ID	Chamber ID	Chip Position	Sample name	Chamber Context	Protocol	Nb Droplets	Type	C _{ref} stock (cp/uL)	Dilution
11. RR260500077001-D1_Sample07	RR260500077001	D1	2	Sample07		br6060	19280	U	---	1
12. RR260500077001-E1_Sample09	RR260500077001	E1	2	Sample09		br6060	19204	U	---	1
13. RR260500077001-F1_Sample11	RR260500077001	F1	2	Sample11		br6060	19393	U	---	1
14. RR260500076810-E1_Sample09	RR260500076810	E1	1	Sample09		br5860	18317	U	---	1
15. RR260500077001-H1_Sample15	RR260500077001	H1	2	Sample15		br6060	17261	U	---	1
16. RR260500077001-G1_Sample13	RR260500077001	G1	2	Sample13		br6060	16531	U	---	1
17. RR260500076810-C1_Sample05	RR260500076810	C1	1	Sample05		br5860	19662	U	---	1
18. RR260500076810-A1_Sample01	RR260500076810	A1	1	Sample01		br5860	18825	U	---	1
19. RR260500077001-A1_Sample01	RR260500077001	A1	2	Sample01		br6060	18960	U	---	1
20. RR260500076810-B1_Sample03	RR260500076810	B1	1	Sample03		br5860	18832	U	---	1
21. RR260500077001-C1_Sample05	RR260500077001	C1	2	Sample05		br6060	18147	U	---	1
22. RR260500077001-B1_Sample03	RR260500077001	B1	2	Sample03		br6060	18934	U	---	1
23. RR260500076810-E2_Sample12	RR260500076810	E2	1	Sample12		br5860	16977	U	---	1

To edit global parameters in the top pane

- 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)

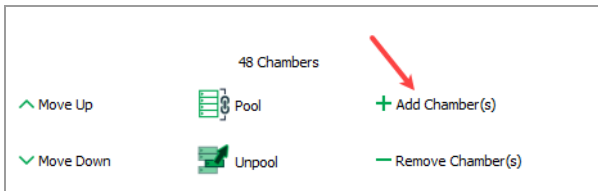
2. Edit the the entries in the following columns:

- 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.

The screenshot shows the software interface with a 'Results Table' at the bottom and an 'Assay Structure' panel at the top. The 'Assay Structure' panel includes 'Fluorophore names' (FAM, HEX, ROX, ATTO-590, Cy5, Cy5.5, ATTO-650) and 'Target names' (Target1-7). The 'Protocol' panel shows 'Exposure times' (59 ms, 275 ms, 365 ms, 337 ms, 51 ms, 470 ms, 200 ms) and 'Instrument type: No™+'. The 'Results Table' has columns for Chamber Name, Chip ID, Chamber ID, Chip Position, Sample name, Chamber Context, Protocol, Nb Droplets, Type, C_ref stock (cp/uL), Dilution, and a final Dilution column. A red box highlights a parent row (Sample15) and its four child rows (Sample15-A2, B2, C2, D2).

Chamber Name	Chip ID	Chamber ID	Chip Position	Sample name	Chamber Context	Protocol	Nb Droplets	Type	C_ref stock (cp/uL)	Dilution	Type	C_ref stock (cp/uL)	Dilution
RR260500069964-H1_Sample15	RR260500069964	H1	2	Sample15		cscrv	0	U	---	1	U	---	1
Sample15				Sample15									
1. RR260500069964-A2_Sample15	RR260500069964	A2	2	Sample15		cscrv	16925	U	---	1	U	---	1
2. RR260500069964-B2_Sample15	RR260500069964	B2	2	Sample15		cscrv	16437	U	---	1	U	---	1
3. RR260500069964-C2_Sample15	RR260500069964	C2	2	Sample15		cscrv	14555	U	---	1	U	---	1
4. RR260500069964-D2_Sample15	RR260500069964	D2	2	Sample15		cscrv	14887	U	---	1	U	---	1
16. RR260500069964-E2_Sample10	RR260500069964	E2	2	Sample10		cscrv	15296	U	---	1	U	---	1
17. RR260500069964-F2_Sample12	RR260500069964	F2	2	Sample12		cscrv	15846	U	---	1	U	---	1
18. RR260500069964-G2_Sample14	RR260500069964	G2	2	Sample14		cscrv	15223	U	---	1	U	---	1
19. RR260500069964-H2_Sample16	RR260500069964	H2	2	Sample16		cscrv	0	U	---	1	U	---	1
20. RR260500066011-A1_Sample01	RR260500066011	A1	3	Sample01		cscrv	14897	U	---	1	U	---	1
21. RR260500066011-B1_Sample03	RR260500066011	B1	3	Sample03		cscrv	13013	U	---	1	U	---	1
22. RR260500066011-C1_Sample05	RR260500066011	C1	3	Sample05		cscrv	13632	U	---	1	U	---	1
23. RR260500066011-D1_Sample07	RR260500066011	D1	3	Sample07		cscrv	12590	U	---	1	U	---	1
24. RR260500066011-E1_Sample09	RR260500066011	E1	3	Sample09		cscrv	12994	U	---	1	U	---	1

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.

Important: If you are using Premium Edition, individual chambers are automatically validated when the pool is validated. Bio-Rad recommends that you corroborate the results.

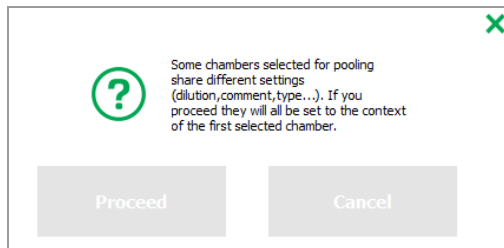
To pool chambers containing the same sample

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.

48 Chambers				
Move Up	Pool	Add Chamber(s)		
Move Down	Unpool	Remove Chamber(s)		
	Chamber Name	Chip ID	Chamber ID	Chip Position
1.	RR245100032384-A1_Sample1	RR245100032384	A1	1
2.	RR245100032384-B1_Sample3	RR245100032384	B1	1
3.	RR245100032384-C1_Sample5	RR245100032384	C1	1
4.	Sample7			
1.	RR245100032384-D1_Sample7	RR245100032384	D1	1
2.	RR245100032384-E1_Sample7	RR245100032384	E1	1
3.	RR245100032384-H1_Sample7	RR245100032384	H1	1
5.	RR245100032384-F1_Sample11	RR245100032384	F1	1
6.	RR245100032384-G1_Sample13	RR245100032384	G1	1
7.	RR245100032384-A2_Sample2	RR245100032384	A2	1
8.	RR245100032384-B2_Sample4	RR245100032384	B2	1

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).

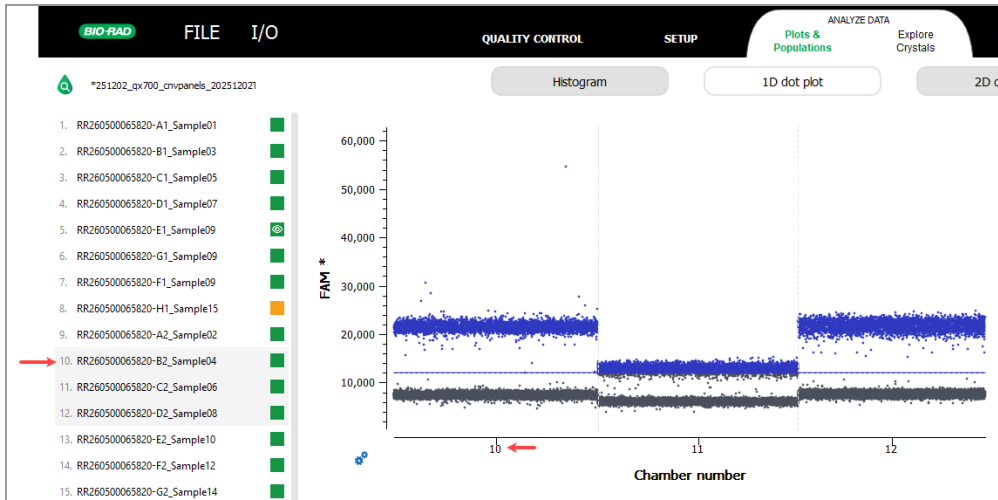
Combining Graphical Data for Multiple Chambers

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

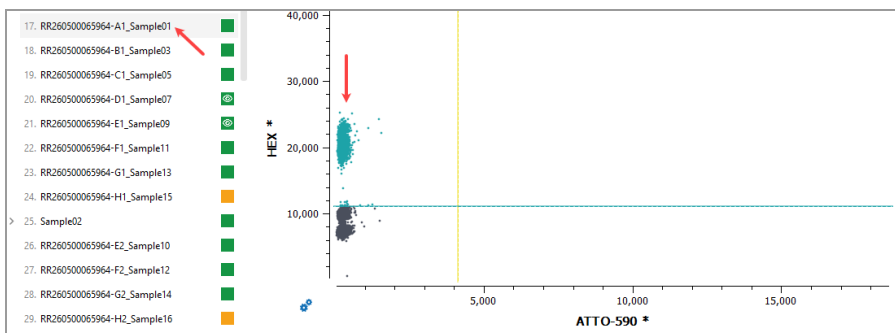
1. Tap/click the 1D Dot Plot tab.
2. In the left pane, press CTRL-click and select the chambers.

The plots for each selected chamber appear sequentially.



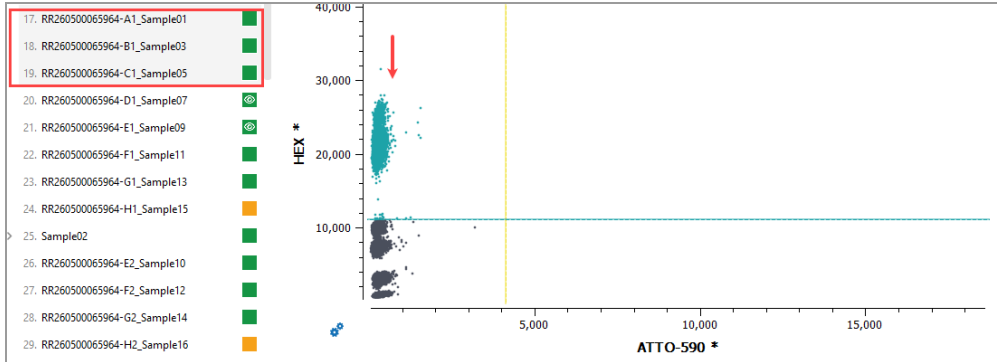
To combine chambers in 2D or 3D dot plots

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



Chapter 6 Modifying the Experiment Setup

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.

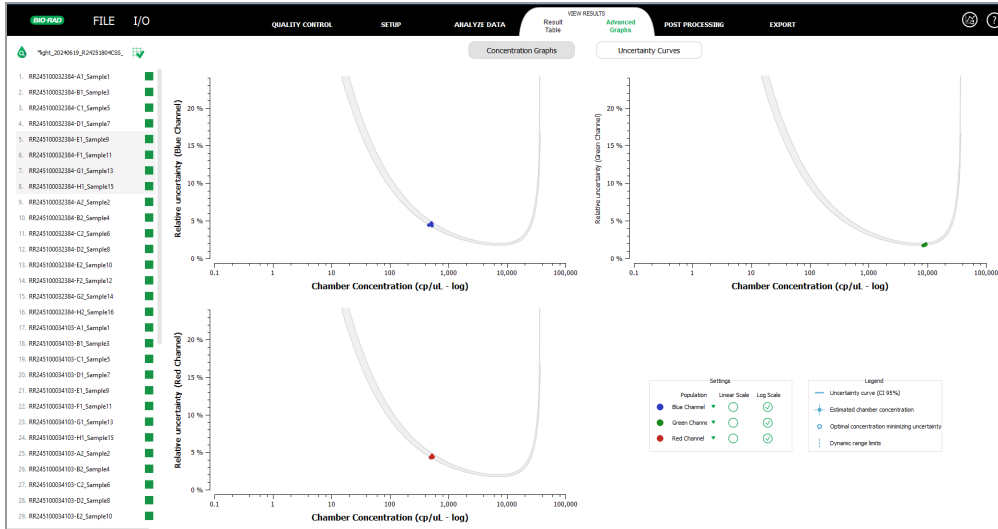
QX700 ddPCR System Analysis Software displays histograms according to pooled chambers.



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.

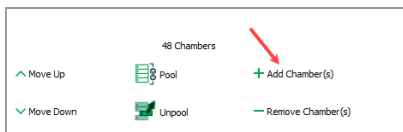
QX700 ddPCR System Analysis Software displays the uncertainty curves of the pooled chambers.



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

Chamber Name	Chip ID	Chamber ID	Chip Position	Sample name	Chamber Context	Protocol	Nb Droplets	Type	C_ref stock (cp/ul)	Dilution
11. RR260500077001-D1_Sample07	RR260500077001	D1	2	Sample07		br5060	19280	U	---	1
12. RR260500077001-E1_Sample09	RR260500077001	E1	2	Sample09		br5060	19204	U	---	1
13. RR260500077001-F1_Sample11	RR260500077001	F1	2	Sample11		br5060	19393	U	---	1
14. RR260500076810-E1_Sample09	RR260500076810	E1	1	Sample09		br5860	18317	U	---	1
15. RR260500077001-H1_Sample15	RR260500077001	H1	2	Sample15		br5060	17261	U	---	1
16. RR260500077001-G1_Sample13	RR260500077001	G1	2	Sample13		br5060	16531	U	---	1
17. RR260500076810-C1_Sample05	RR260500076810	C1	1	Sample05		br5860	19662	U	---	1
18. RR260500076810-A1_Sample01	RR260500076810	A1	1	Sample01		br5860	18825	U	---	1
19. RR260500077001-A1_Sample01	RR260500077001	A1	2	Sample01		br5060	18960	U	---	1
20. RR260500076810-B1_Sample03	RR260500076810	B1	1	Sample03		br5860	18832	U	---	1
21. RR260500077001-C1_Sample05	RR260500077001	C1	2	Sample05		br5060	18147	U	---	1
22. RR260500077001-B1_Sample03	RR260500077001	B1	2	Sample03		br5060	18934	U	---	1
23. RR260500076810-F2_Sample12	RR260500076810	F2	1	Sample12		br5860	16677	U	---	1

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 (0.000000) except in each field where the fluorophores intersect, which contains 1.000000.

Fig. 4: Matrix for an uncompensated experiment

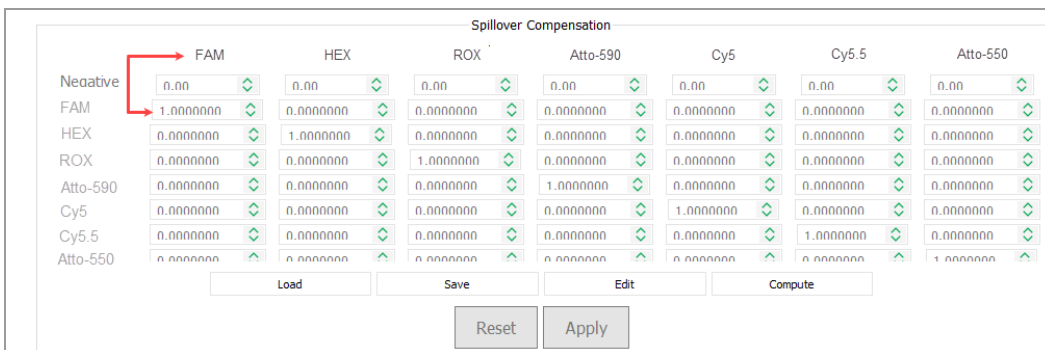
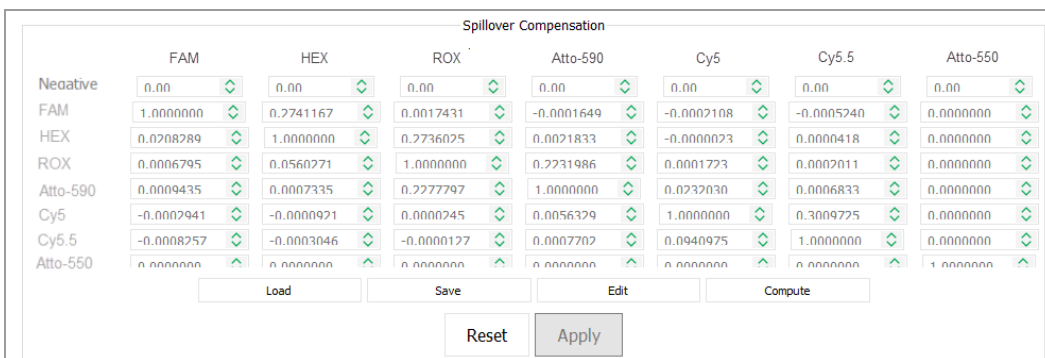


Fig. 5: Matrix for a compensated experiment



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 111](#).

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.

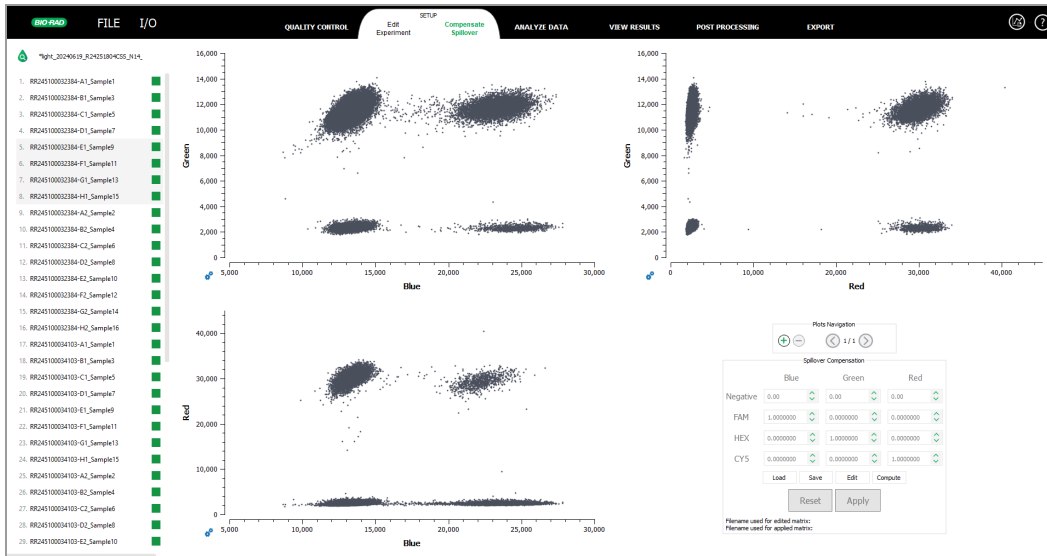
After you compute a compensation matrix using one of the methods cited in this chapter to adjust the thresholding in the chart, the fields that previously contained zeros are populated with the excitation matrix coefficients where the fluorophores intersect with the other channels (Fig. 5).

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 63](#), [Template-Based Method on page 67](#), or [Manual Method on page 68](#). 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 monochrome 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 12](#).

Table 12. Mono-color compensation controls

Fluorophore	Channel LED	Target	Description
Negative control	N/A	NNNNNNN	No positive signal (no target)
FAM	Blue	PNNNNNN	Target for FAM only
HEX	Teal	NPNNNNN	Target for HEX only
ROX	Green	NNPNNNN	Target for ROX only
Atto-590	Yellow	NNNPNNN	Target for Atto-590 only
Cy5	Red	NNNNPNN	Target for Cy5 only
Cy5.5	Infra-Red	NNNNNP	Target for Cy5.5 only
Atto-550	Purple	NNNNNNP	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 monochrome 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.

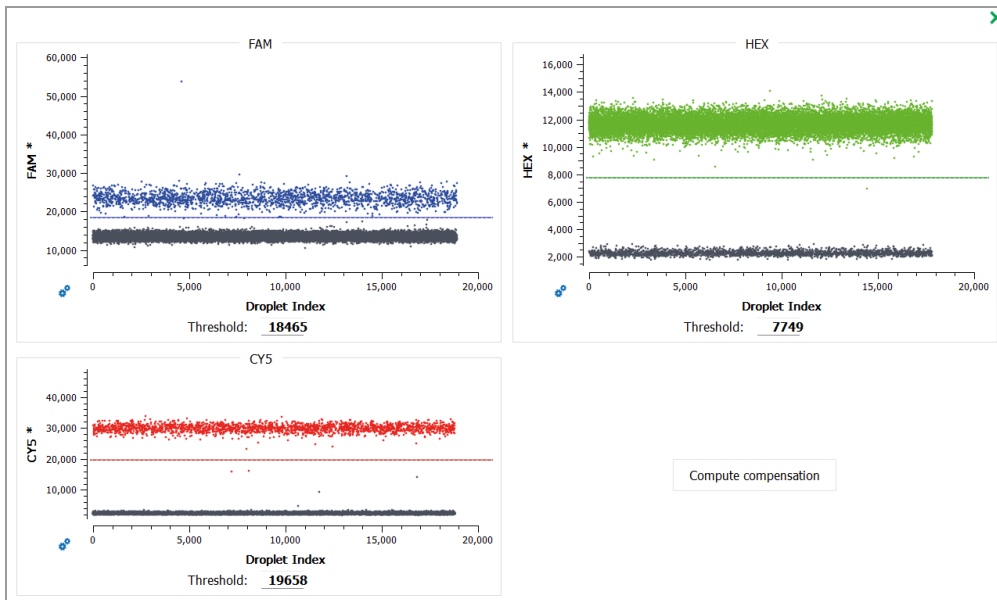
Select control samples for spillover compensation calibration:

	Types	NNN	FAM	HEX	CY5
245100032384-A1_Sample	uuu	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
245100032384-B1_Sample	uuu	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
245100032384-C1_Sample	uuu	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>

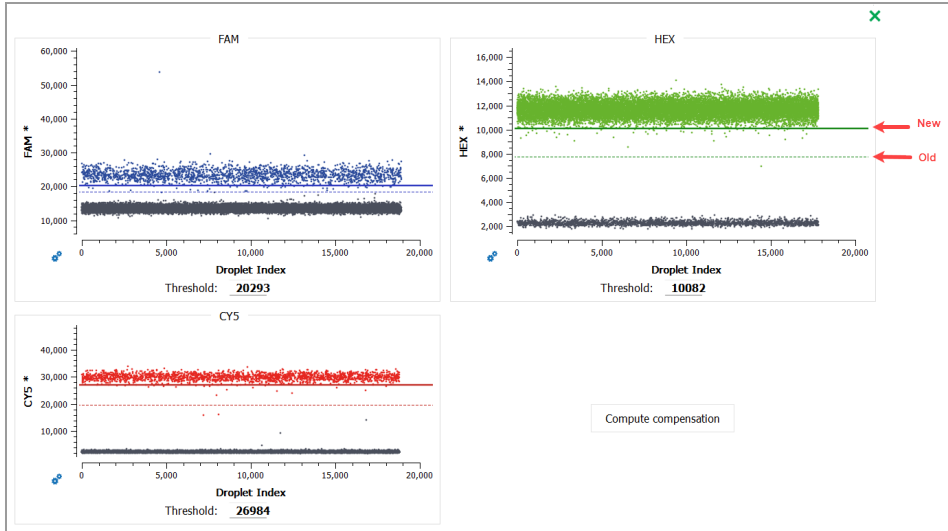
Next

3. Tap/click Next.

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

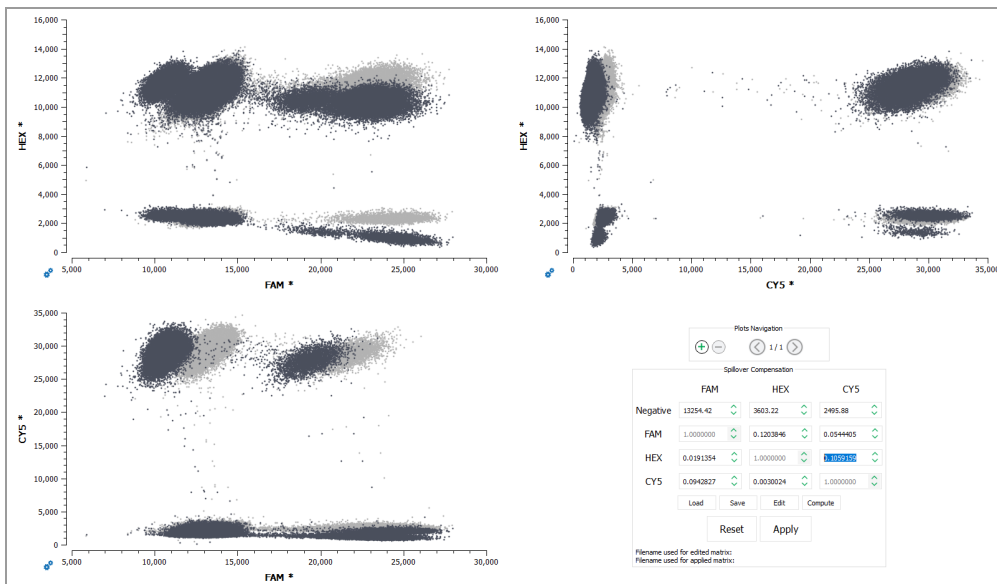


- (Optional) To adjust the values, drag and drop the threshold lines.

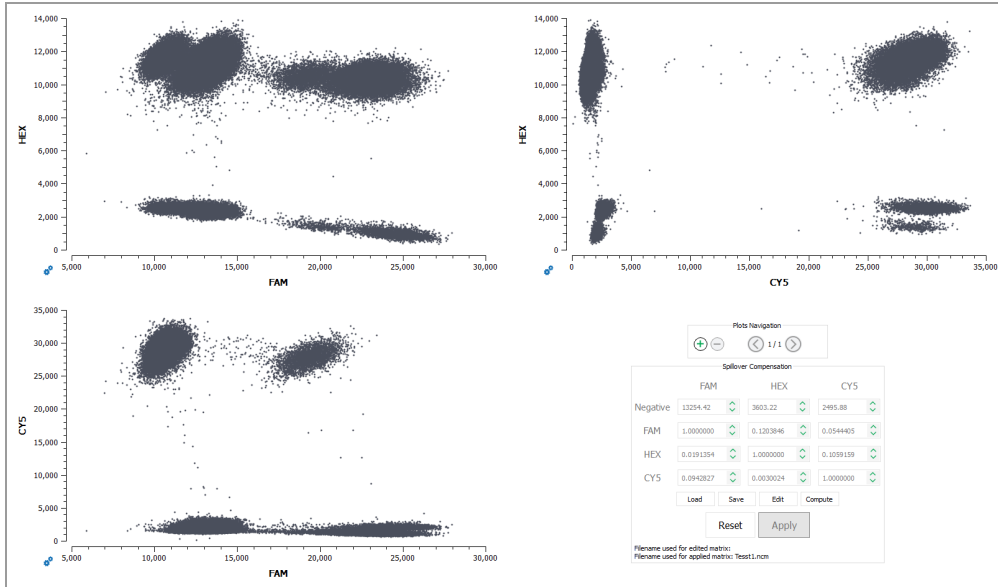


- Tap/click Compute compensation.

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 67](#).

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 123](#).

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.

The image shows a software interface titled "Spillover Compensation". It features a grid of 7 rows and 6 columns. The columns are labeled FAM, HEX, ROX, ATTO-590, CY5, and CY5.5. The rows are labeled Negative, FAM, YY, Atto550, ROX, CY5, and Atto700. Each cell in the grid contains a numerical value (0.00 or 1.0000000) and a small blue arrow icon. Below the grid are several buttons: Load, Save, Edit, Compute, Reset, and Apply. At the bottom left, there are two lines of text: "Filename used for edited matrix:" and "Filename used for applied matrix:".

	FAM	HEX	ROX	ATTO-590	CY5	CY5.5
Negative	0.00	0.00	0.00	0.00	0.00	0.00
FAM	1.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
YY	0.0000000	1.0000000	0.0000000	0.0000000	0.0000000	0.0000000
Atto550	0.0000000	0.0000000	1.0000000	0.0000000	0.0000000	0.0000000
ROX	0.0000000	0.0000000	0.0000000	1.0000000	0.0000000	0.0000000
CY5	0.0000000	0.0000000	0.0000000	0.0000000	1.0000000	0.0000000
Atto700	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	1.0000000

Buttons: Load, Save, Edit, Compute, Reset, Apply

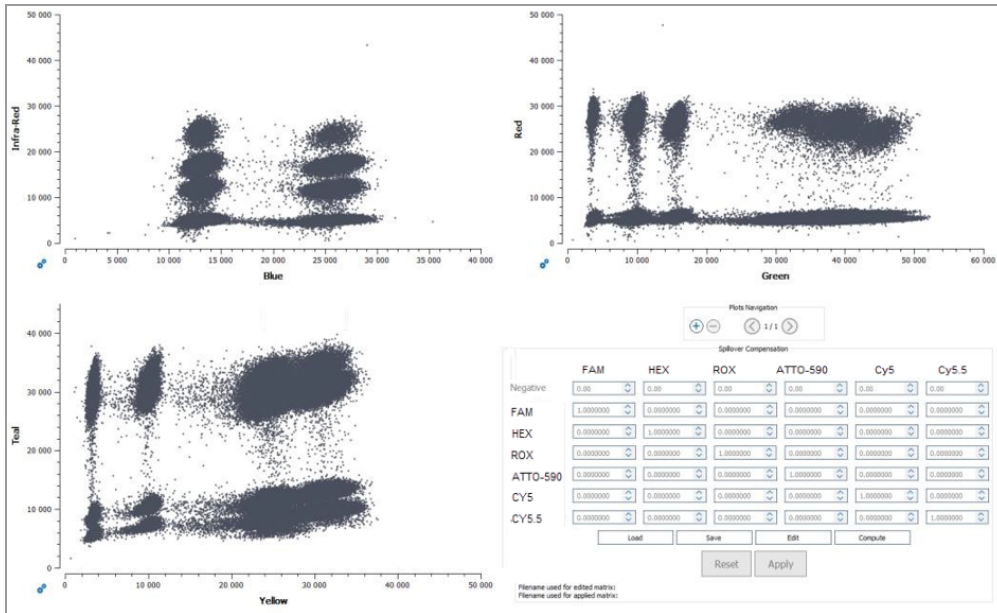
Filename used for edited matrix:
Filename used for applied matrix:

To use the manual spillover compensation method

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

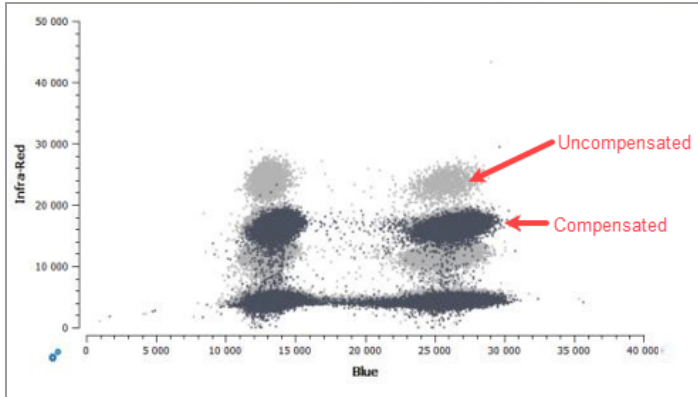
The uncompensated clusters appear in the 2D dot plot view.

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

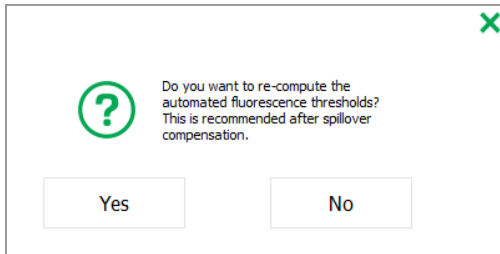


2. Enter coefficient values individually in the Spillover Compensation matrix or click the up and down arrows. *The fields containing 1.000000, in which the same fluorophore intersects, are not editable.*
3. Set the negative values in the first line by entering the mean RFU value of the negative cluster 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.



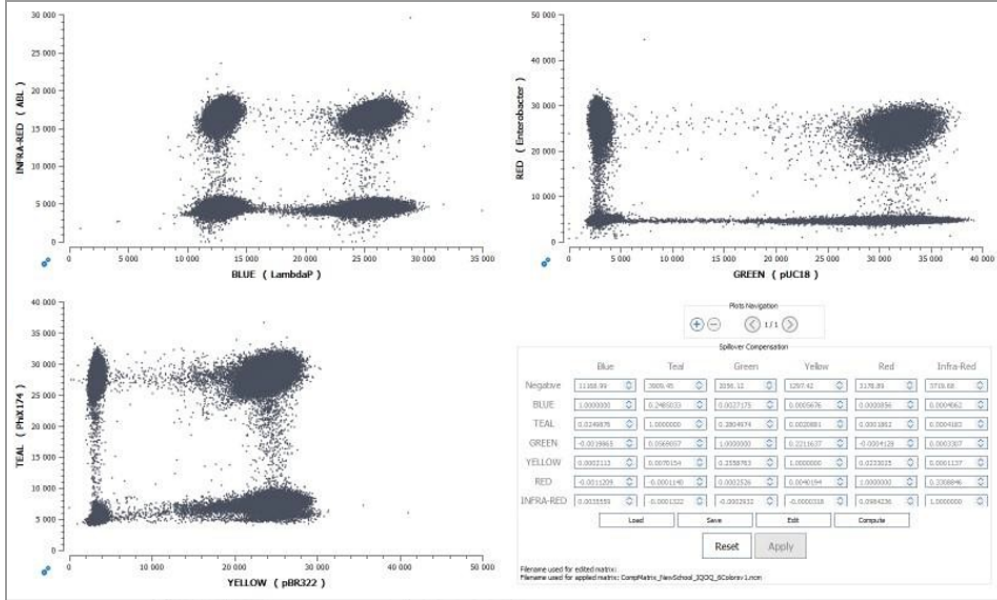
4. To apply the spillover compensation to the full experiment, tap/click Apply.
5. When the following message appears, tap/click Yes.



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 67](#).

This page intentionally left blank.

Chapter 7 Data Analysis

The Analyze Data tab contains the following analysis views:

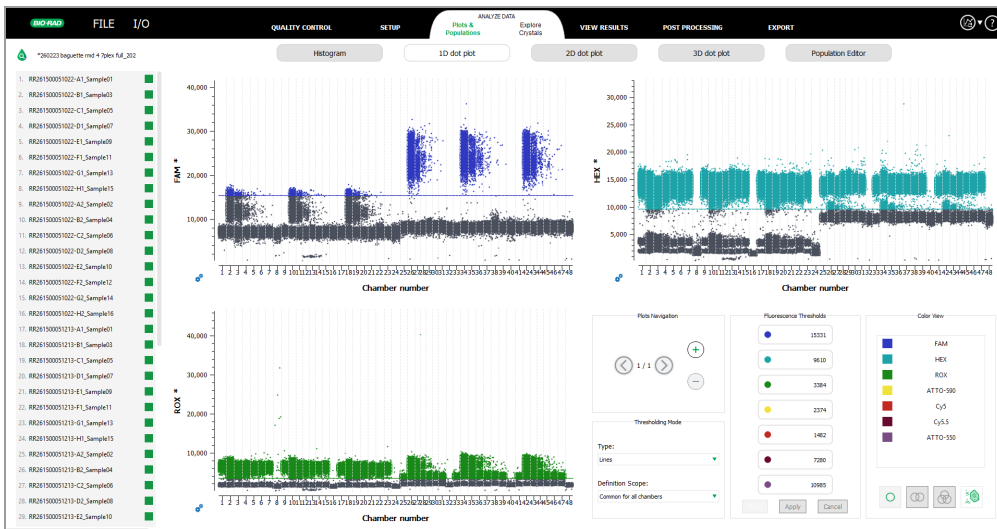
- **Plots & Populations** — The Plots and Populations view illustrates the droplet data for each fluorophore channel in 1D, 2D, 3D, and histogram dot plots. The default view is the initial three fluorophores but you can prompt additional screens to show the dot plots for the remaining fluorophores (up 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.

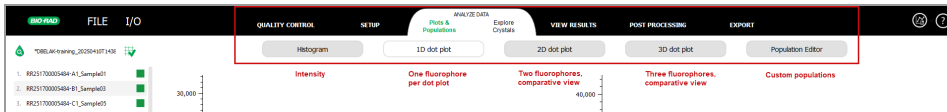


For information on fluorophores and matching LED colors, see [Channel Fluorophores and Colors on page 35](#).

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.

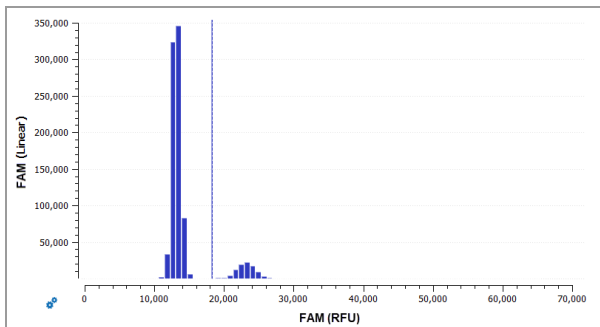
Plot Types

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.

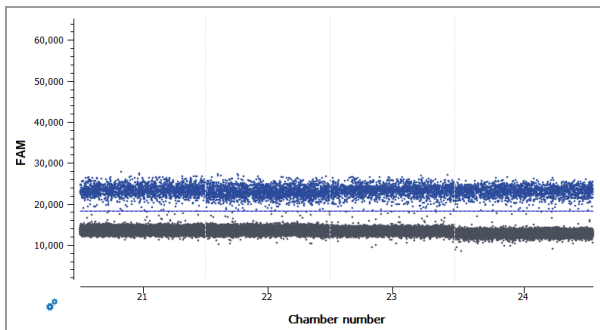


The following graphics display each plot type:

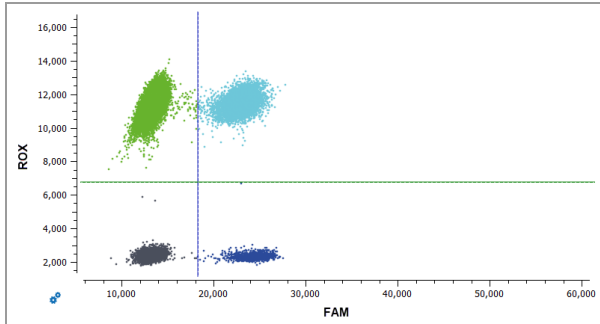
■ Histogram



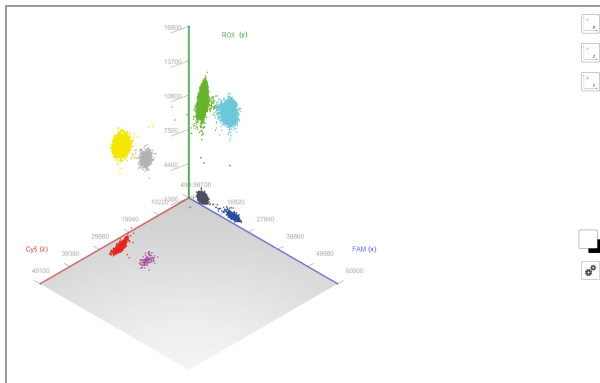
■ 1D dot plot



■ 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 13 on page 76](#). All negative droplets are colored gray in the charts.

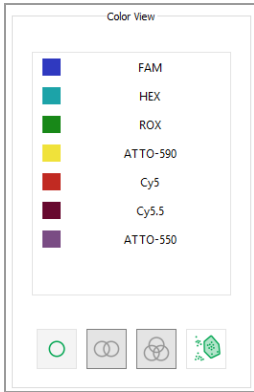


Table 13. Color View icons





Icon	Description
	<i>Two-channel display.</i> 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.
	<i>Four-channel display.</i> For the two selected fluorophores in each dot plot, the four-color view mode displays positive droplets as follows: <ul style="list-style-type: none"> ■ 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.

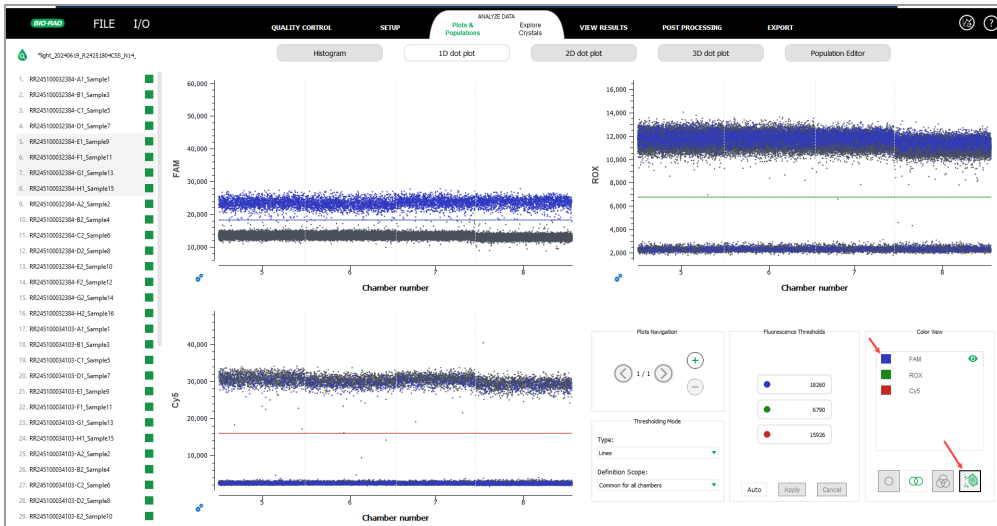
Table 13. Color View icons, continued

Icon	Description
	<p><i>Eight-channel display.</i></p> <p>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 diagram¹. The dark background represents negative droplets across all channels.</p>
	<p><i>Population selector.</i> 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.</p> <p>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 <i>blue</i> and all negative droplets appear in <i>gray</i>.</p>

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.

Chapter 7 Data Analysis

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.

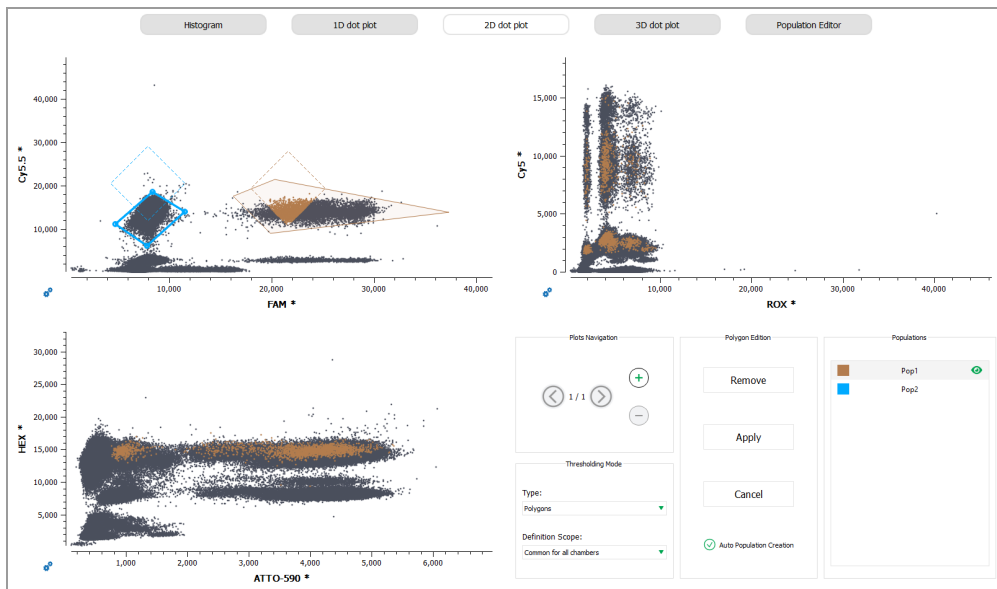
Polygon Thresholding Zones and Populations

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.

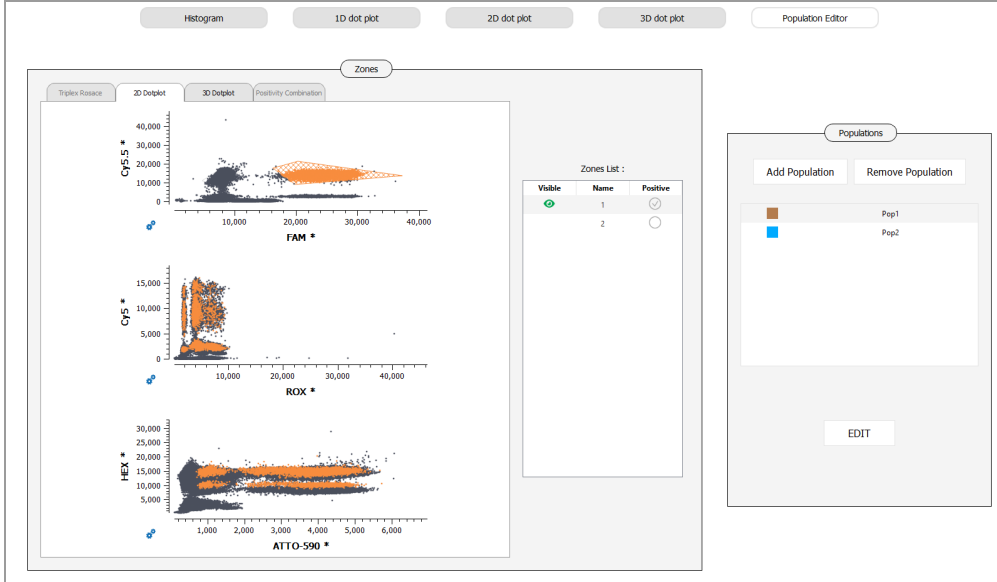
Use the specified population colors to define your polygons in the 2D or 3D dot plots.

Fig. 6: Defined polygons




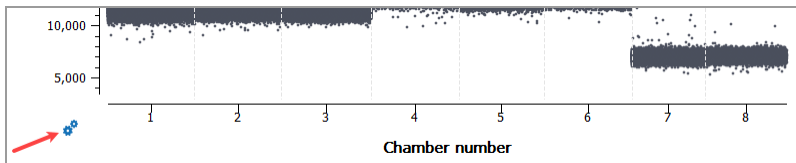
Tip: If the .niodata or .nioreresult file contains data for more than three fluorophore channels, you can use the plus and arrow icons under Plots Navigation to add and navigate to the remaining plots.

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:

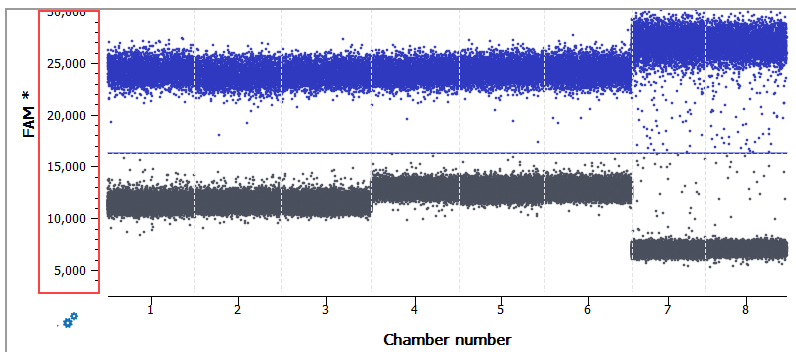
- If only one chamber is selected, "Droplet Index" appears
- If multiple chambers are selected, "Chamber Number" appears (1, 2, 3, and so forth).

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.

Vertical Axis	
Population Axis	FAM *
Upper bound	36786.00
Lower bound	3354.00
Dot size	2
Dot opacity	250
Droplet ordering	Shuffled

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.

Vertical Axis

Population Axis: FAM *

Upper bound: 16751.00

Lower bound: 1333.00

Dot size: 2

Dot opacity: 250

Display heatmap:

Heatmap color scale:

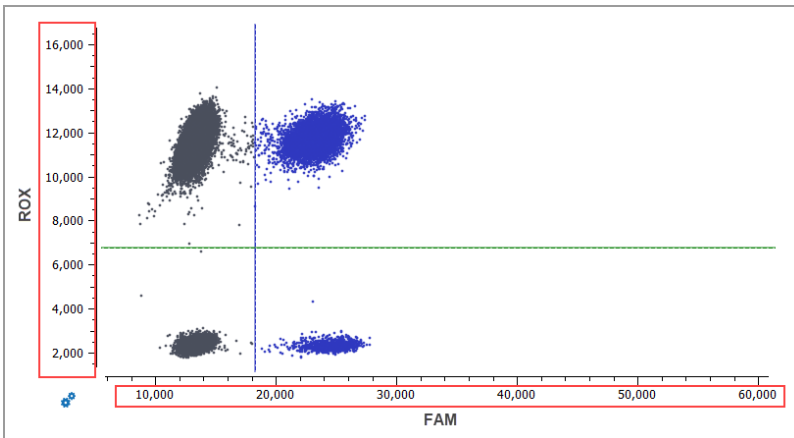
Horizontal Axis

Population Axis: ROX *

Upper bound: 60881.00

Lower bound: 5845.00

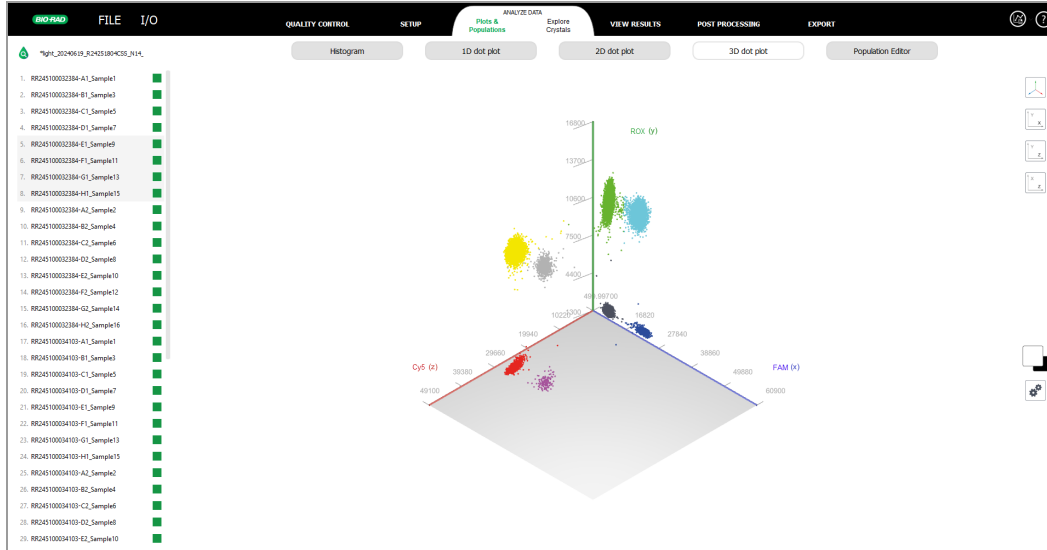
The axis range is modified in the dot plot.



3. (Optional) In the remaining fields, change the dot size, dot opacity, and select the checkbox to display the heatmap.
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 35](#).

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

X

Settings

Line Width	<input type="text" value="4.00"/>
Point Size	<input type="text" value="3.00"/>
X Population Axis	<input type="text" value="FAM *"/>
Xmin	<input type="text" value="5845"/>
Xmax	<input type="text" value="60881"/>
Y Population Axis	<input type="text" value="HEX *"/>
Ymin	<input type="text" value="1333"/>
Ymax	<input type="text" value="16751"/>
Z Population Axis	<input type="text" value="CY5 *"/>
Zmin	<input type="text" value="508"/>
Zmax	<input type="text" value="49008"/>

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 60](#).

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).

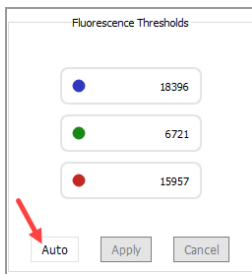
You can move (click and drag) the thresholds applied by the software to adjust the number of droplets recognized as positive and negative. For more information on thresholds, see [Using the Lines Thresholding Mode on page 87](#) and [Using the Polygon Thresholding Mode on page 89](#).

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 89](#).

Automatically Calculating Line Thresholds

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



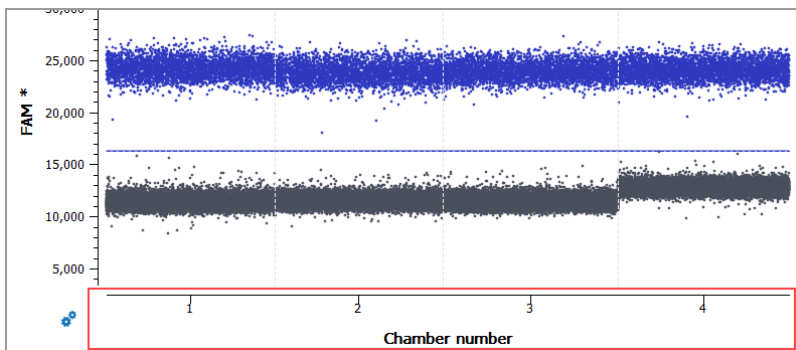
Each displayed channel value represents the estimated number of positive droplets.

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.

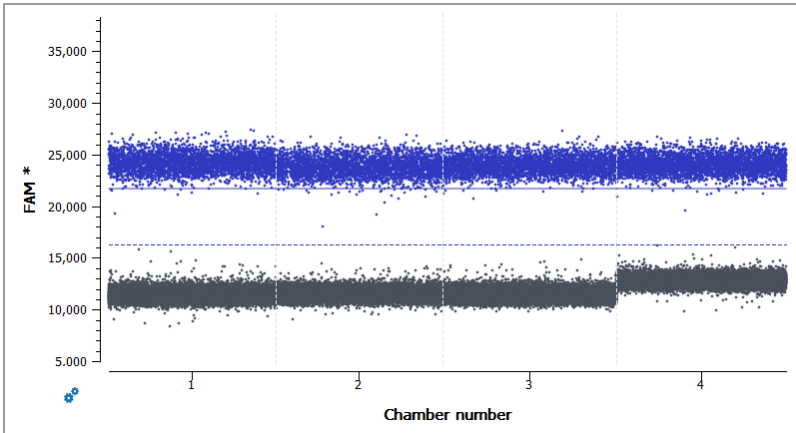
1. In the left pane, select the applicable chambers and then select the 1D dot plot tab.

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.

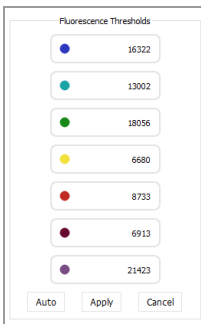


- To manually adjust the fluorescence thresholds, select a threshold in one of the plots to drag and drop the threshold line.

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.



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

Positive and negative droplets are recalculated 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 111](#).

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.

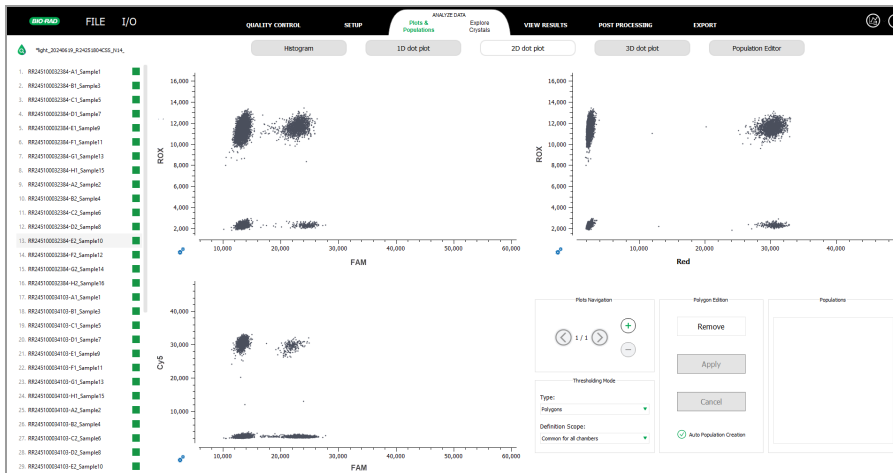
Use the following keyboard commands and mouse options to create, modify, move, and delete polygons:

- 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

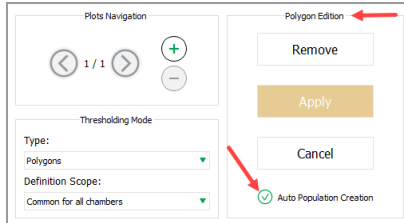
1. Tap/click Analyze Data > Plots & Populations > 2D dot plots.
2. Under Thresholding Mode, select Polygons from the Type dropdown list.

All dots appear in gray before you apply polygon thresholds.



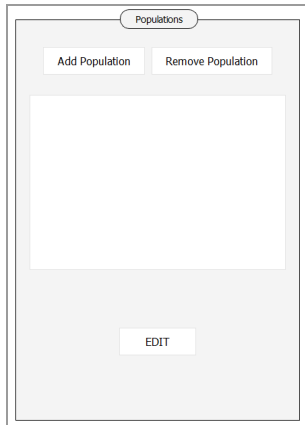
3. Do one of the following:

- 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.

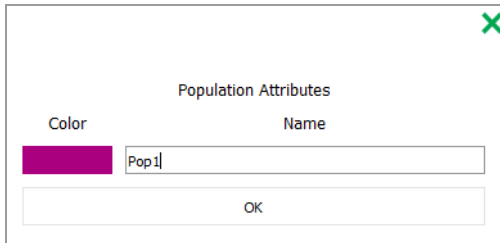


The Population Attributes pop-up opens immediately, and you can use this method if you prefer to create a polygon identifier and then immediately edit the polygon. Continue to Step 4.

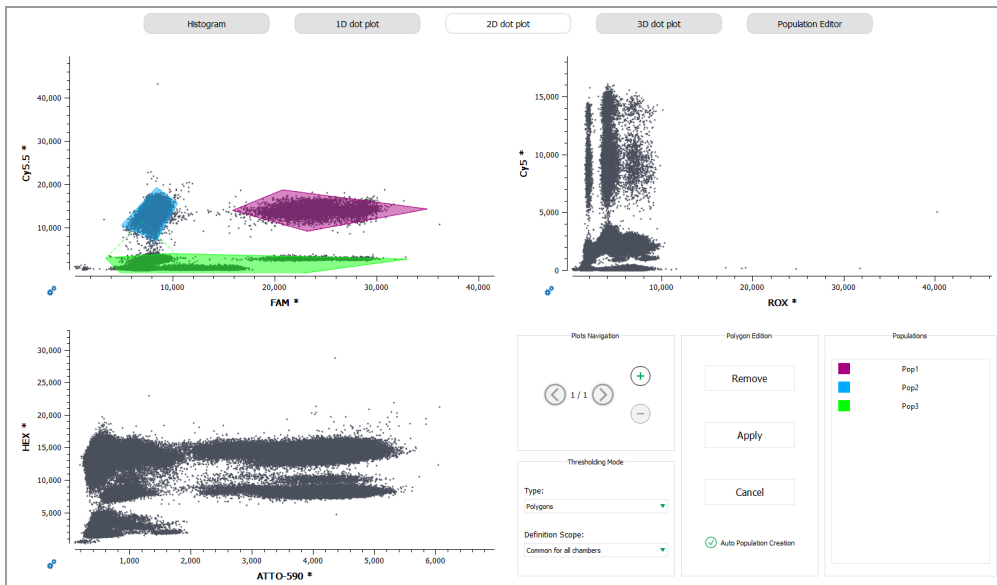
- If the checkbox is cleared, you can tap/click Population Editor and then click Add Population. You can use this method if you prefer to create all polygon identifiers first, and then create the polygons. Continue to Step 4.



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



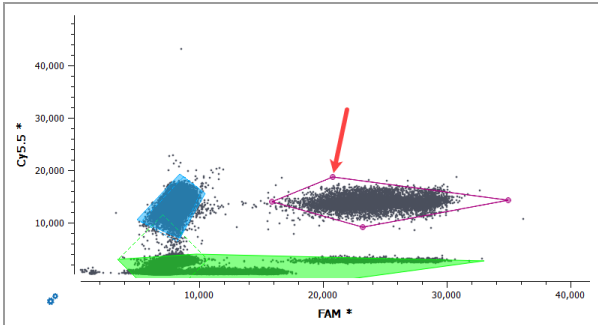
- Tap/click OK to create the polygon identifier.
- Repeat for each population identifier that you need for plot segregation.



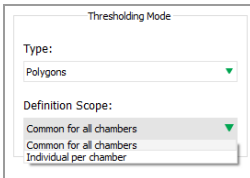
Modifying or Removing Polygon Thresholds

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.



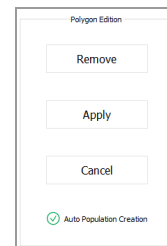
2. In the 2D plot view, you can localize polygons using the dropdown list under Definition Scope:



- 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.



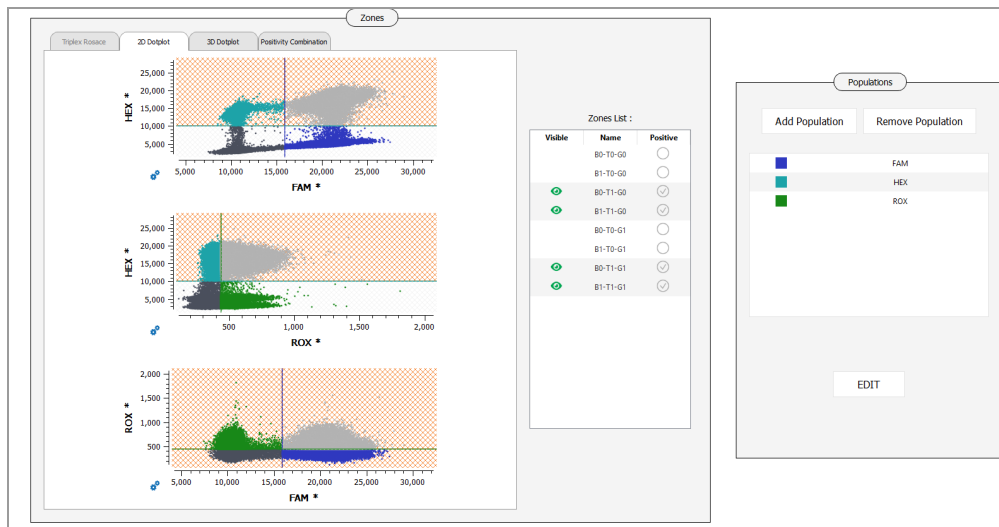
Defining a Population in a Zone

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

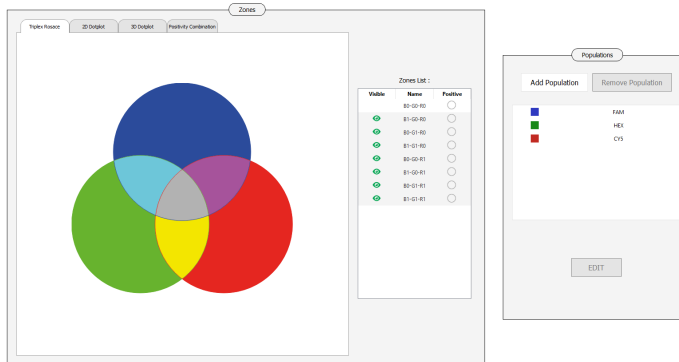
1. Tap/click Analyze Data > Plots & Populations > Population Editor.
2. For each population created, tap/click the population name to display the associated zones in the list and in the plots, Venn diagram plots, Venn diagram view, and positivity combination view.



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:

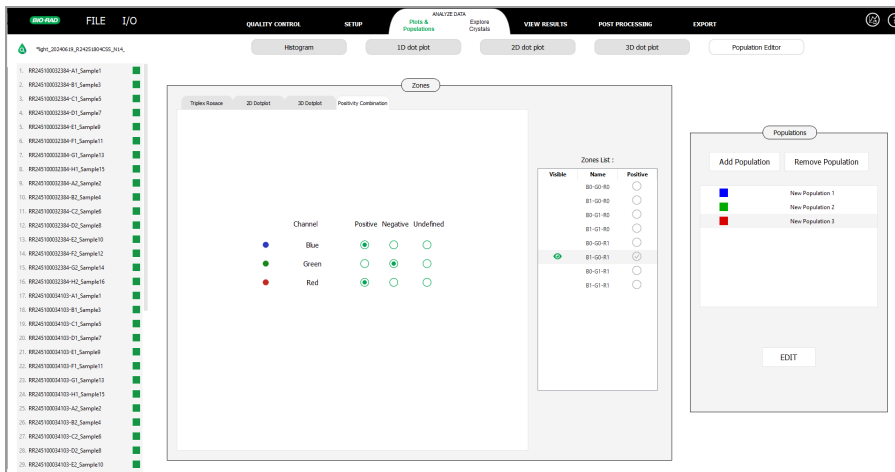
- Tap/click a zone in the 2D plot display.
- Tap/click a row under Zones List. The selected zone is shown as selected in the table and appears in the dot plot.
- Select the zone checkbox to include it in the currently edited population.

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

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



- Tap/click the Positivity 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.

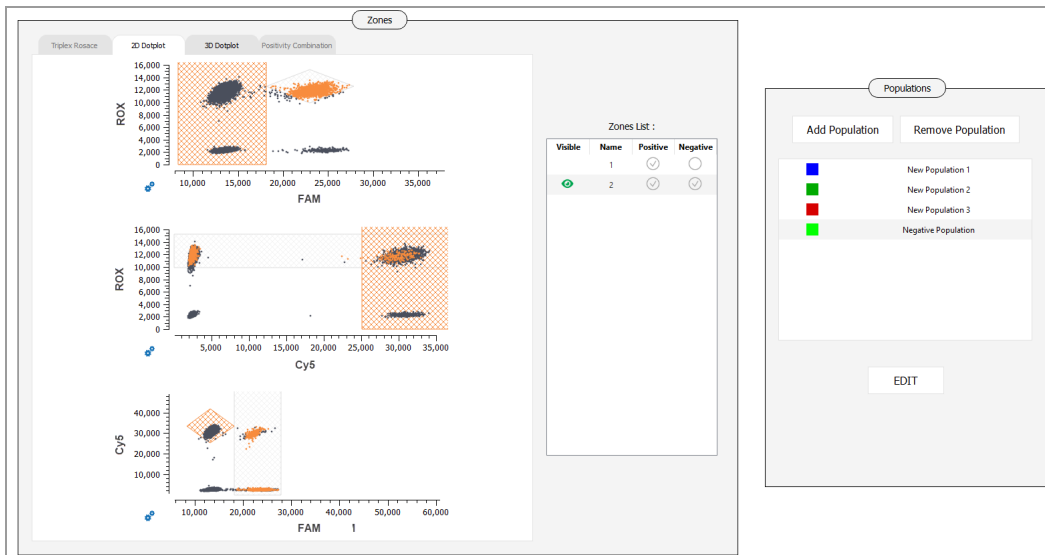
By definition, the droplets included in the population are counted as positive droplets. By default, all the droplets that are not included in the population are counted as negative droplets for this population. Continue to the next section to learn how to define negative zones in a population.

Defining Negative Zones

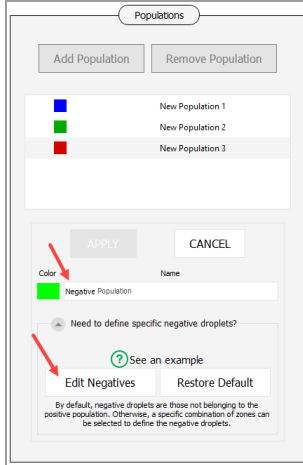
For some applications, such as Drop-Off, you might need to choose a specific droplet population and define a negative zone.

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.



- 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.

Chamber Name	Nb Neg	Separability Score	C _{min} (top%)	C _{max} (top%)	Relative Uncertainty (SD 95%)	Dilution	C (top%)	Nb Pos	Nb Neg
1. RR245100034103-F1_Sample11	16332	3	425.4	462.7	4.79 %	1	4947	3375	1732*
2. RR245100035919-F1_Sample11	15989	3	393.3	431	5.03 %	1	4930	3044	1594*
3. RR245100032384-F1_Sample11	17101	3	406.3	447	4.77 %	1	4873	3498	1808*
4. RR245100034103-H1_Sample15	18484	3	329.2	364.7	5.11 %	1	4870	3553	1580*
5. RR245100032384-G1_Sample15	14961	3	402.6	446	5.11 %	1	4853	3065	1595*
6. RR245100034103-H2_Sample16	17542	3	332.3	366.6	5.17 %	1	4852	2995	1559*
7. RR245100032384-C1_Sample15	17232	3	373.1	412.1	4.96 %	1	4848	3281	1700*
8. RR245100035919-G1_Sample13	15959	3	388.2	429.4	5.05 %	1	4840	3159	1651*
9. RR245100034103-G2_Sample16	16109	3	404.7	446.6	4.82 %	1	4838	3329	1741*
10. RR245100032384-B1_Sample16	17670	3	364.2	423.2	4.83 %	1	4838	3455	1802*
11. RR245100032384-E1_Sample19	16207	3	405.9	447.7	4.9 %	1	4837	3380	1758*
12. RR245100035919-F2_Sample12	17908	3	368.7	406.7	4.9 %	1	4837	3358	1752*
13. RR245100034103-A2_Sample12	17002	3	347.2	385.1	5.18 %	1	4835	3006	1574*
14. RR245100032384-D1_Sample17	17277	3	384.9	424.2	4.88 %	1	4832	3384	1759*
15. RR245100035919-H2_Sample16	16273	3	338.9	375	5.06 %	1	4831	3151	1622*
16. RR245100032384-C2_Sample16	16932	3	373	412.3	5.01 %	1	4829	3231	1687*
17. RR245100032384-H2_Sample16	17668	3	350.7	386.1	5.06 %	1	4823	3147	1645*
18. RR245100035919-A1_Sample14	17277	3	351.5	389.4	5.11 %	1	4818	3111	1638*
19. RR245100035919-G2_Sample14	17641	3	348	385.3	5.08 %	1	4811	3152	1664*
20. RR245100034103-B1_Sample16	17646	3	352.1	389.5	5.05 %	1	4810	3191	1685*
21. RR245100032384-B2_Sample14	17211	3	376.7	415.9	4.94 %	1	4808	3241	1705*

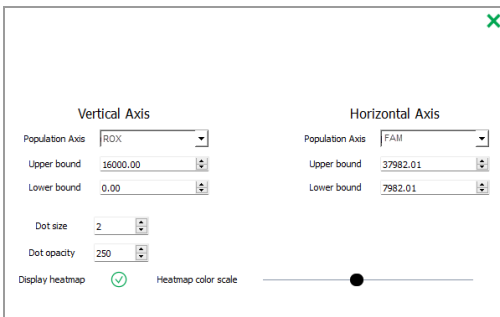
Heatmaps

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.

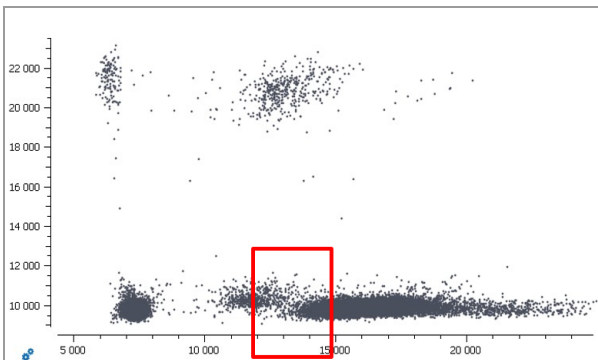
To activate the heatmap visualization in 2D dot plots

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

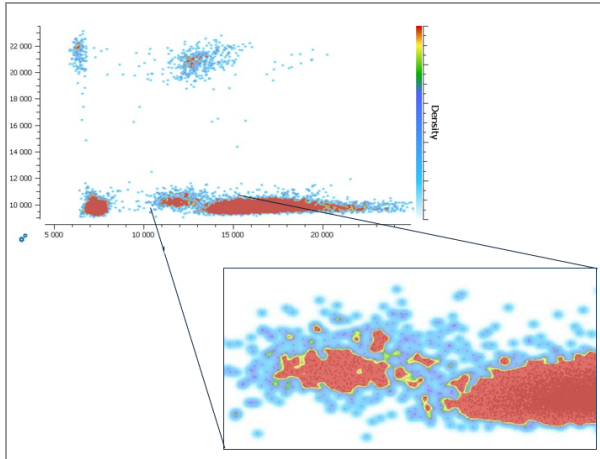


The color scale on the right of the graph gives the complete color range that is used to represent the different density levels.

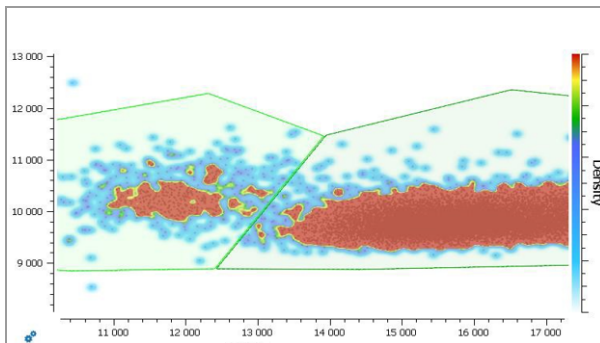
Negative populations are typically very dense, which can hide smaller density variations between less dense areas. You can modify the heatmap color scale parameter to highlight populations of lower density levels. For example, when droplet clusters are not well separated, the heatmap feature allows the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



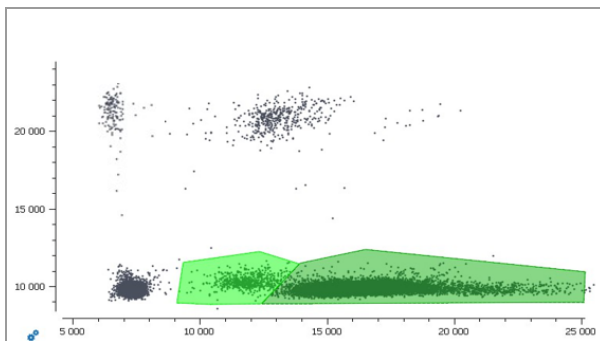
The following graphic shows the heatmap activated.



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).

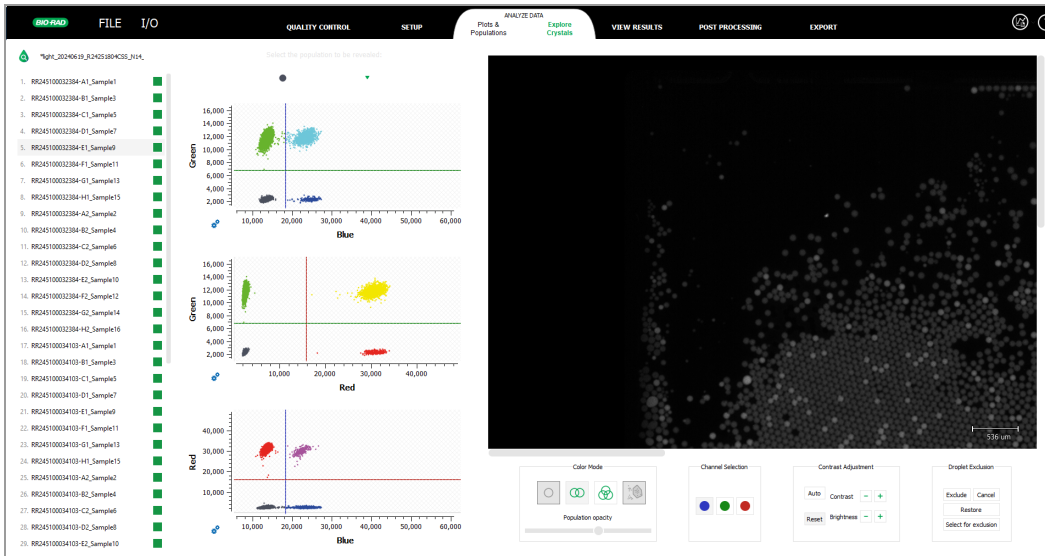


You can zoom out to deactivate the heatmap feature.



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.

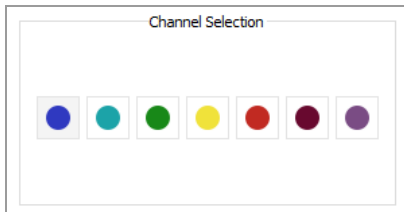


To analyze a droplet population using Explore Crystals

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

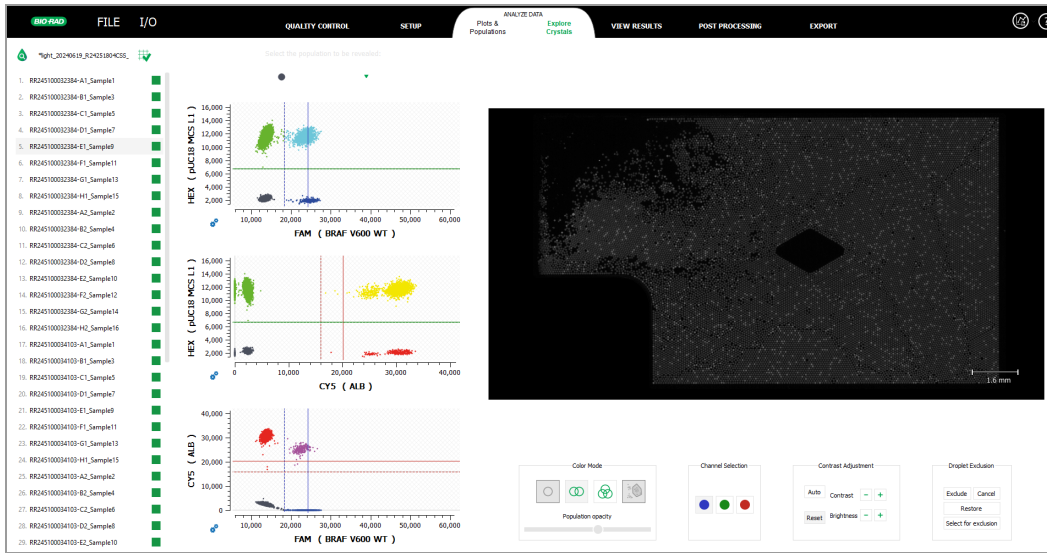
Note: To navigate in the chamber image, see [General Navigation Options on page 35](#).

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

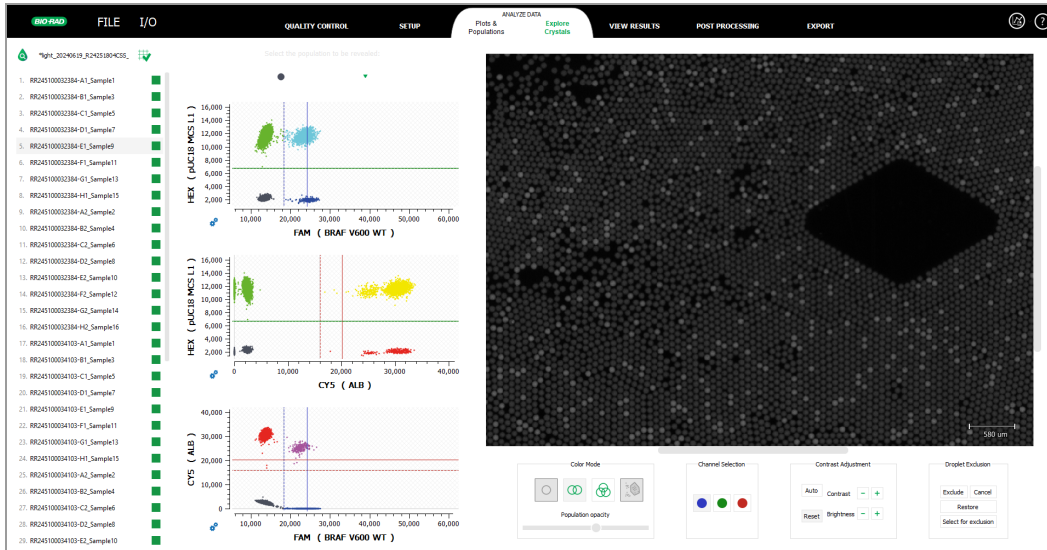


- To automatically adjust image contrast, tap/click Auto under Contrast Adjustment.

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



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

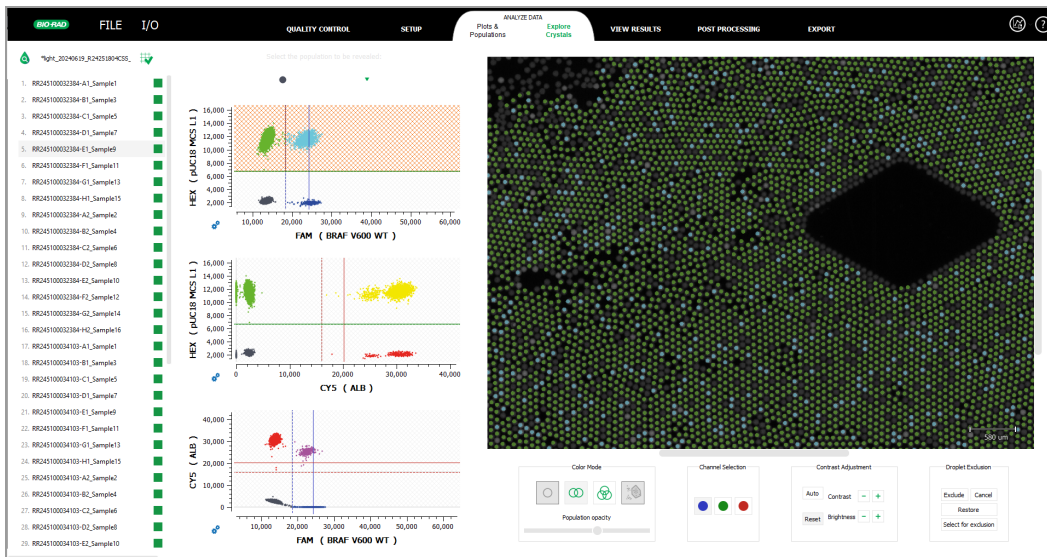


To highlight a specific droplet in the crystal image

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

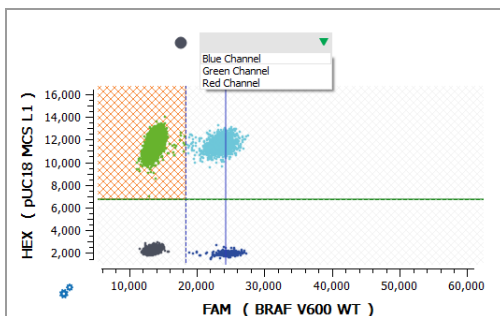
The activated quadrant fill is transparent orange in the graph and the droplets included in this quadrant are highlighted in their current color codes in the image.

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:

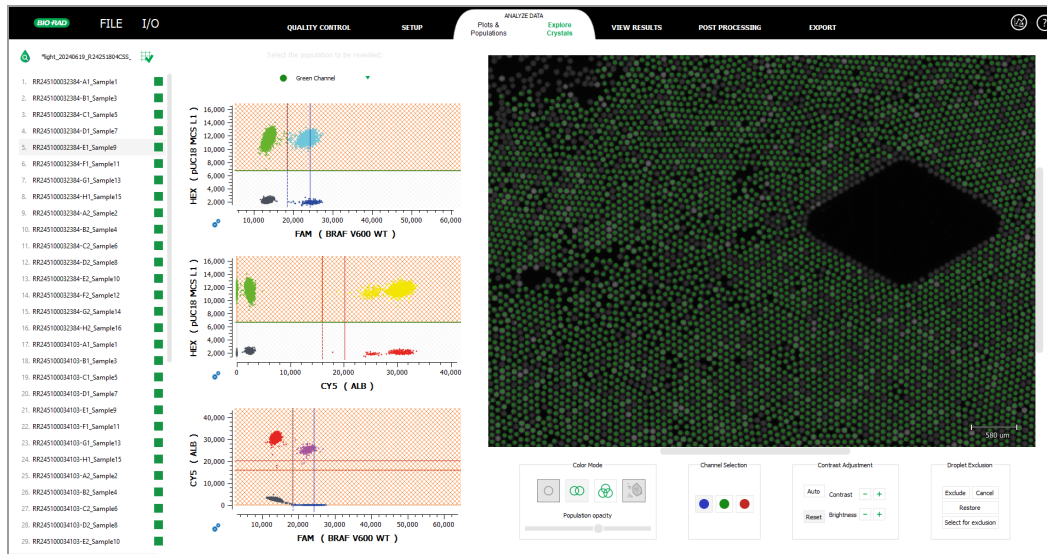


To highlight the droplets belonging to a given population

- ▶ 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.

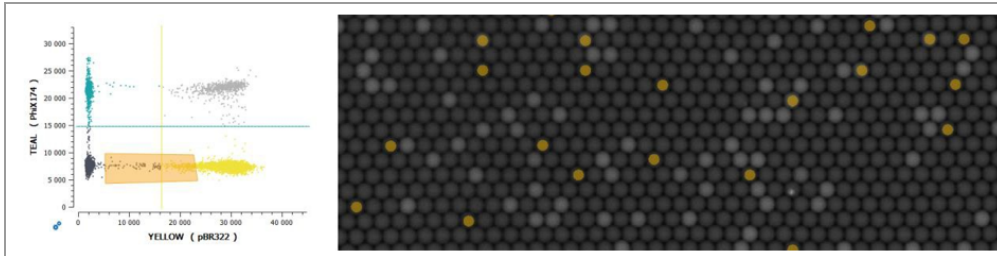


To highlight droplets included in any user-defined polygonal region of a 2D graph

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.

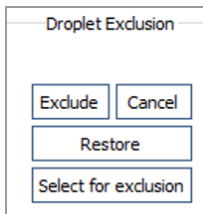
QX700 ddPCR System Analysis Software draws the polygon with orange edges on the 2D graph, and the droplets included in the polygon are highlighted in orange in the image.

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.



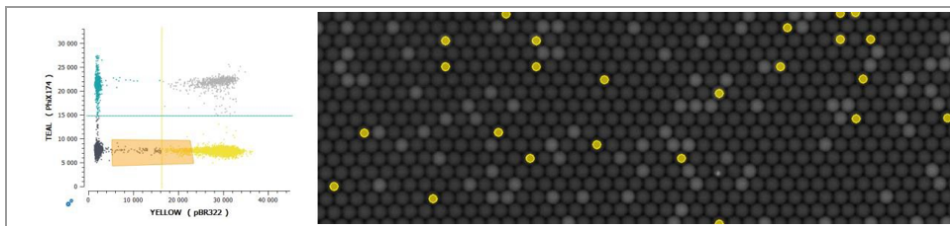
To exclude highlighted droplets from the analysis

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 8 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.

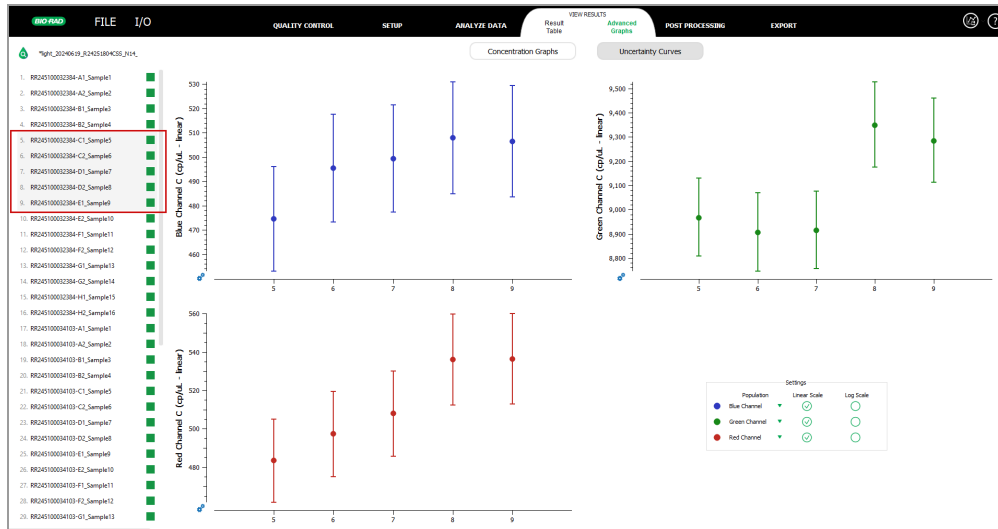
Chamber Name	Chamber Context	Protocol	Nb Droplets	Dilution	Blue Channel		Green Channel		Red Channel		Nb Pos	
					C (cp/μL)	Nb Pos	Dilution	C (cp/μL)	Nb Pos	Dilution		C (cp/μL)
1. RR24510002384-A1_Sample1	■	PR0701	18654	1	477.7	1868	1	8922	16055	1	511.2	1992
2. RR24510002384-B1_Sample3	■	PR0701	19318	1	488.5	1976	1	8975	16658	1	510	2058
3. RR24510002384-C1_Sample5	■	PR0701	18793	1	474.5	1870	1	8966	16200	1	483.3	1903
4. RR24510002384-D1_Sample7	■	PR0701	18892	1	499.3	1973	1	8914	16255	1	507.9	2005
5. RR24510002384-E1_Sample9	■	PR0701	17809	1	506.5	1885	1	9283	15518	1	536.4	1990
6. RR24510002384-F1_Sample11	■	PR0701	18791	1	514.3	2018	1	9142	16297	1	521.3	2044
7. RR24510002384-G1_Sample13	■	PR0701	16431	1	503	1728	1	9238	14296	1	521.9	1789
8. RR24510002384-H1_Sample15	■	PR0701	20040	1	459.4	1934	1	8451	16842	1	487.8	2047
9. RR24510002384-I1_Sample17	■	PR0701	19043	1	468.7	1873	1	8464	16107	1	489.6	1952
10. RR24510002384-J1_Sample19	■	PR0701	18807	1	491.5	1935	1	8627	16010	1	501.5	1972
11. RR24510002384-K1_Sample21	■	PR0701	18407	1	495.3	1914	1	8904	15884	1	497.3	1921
12. RR24510002384-L1_Sample23	■	PR0701	17594	1	507.8	1867	1	9348	15363	1	536.1	1965
13. RR24510002384-M1_Sample25	■	PR0701	17303	1	489.7	1774	1	8999	14933	1	518.4	1872
14. RR24510002384-N1_Sample27	■	PR0701	18176	1	489.6	1863	1	9039	15708	1	534.7	2025
15. RR24510002384-O1_Sample29	■	PR0701	16739	1	470.1	1651	1	8664	14270	1	491.8	1723
16. RR24510002384-P1_Sample31	■	PR0701	19170	1	460.7	1855	1	8481	16226	1	474.1	1906
17. RR24510004103-A1_Sample1	■	PR0701	16481	1	484.8	1877	1	8614	15725	1	484.2	1926
18. RR24510004103-B1_Sample3	■	PR0701	19152	1	472.2	1897	1	8551	16256	1	478.8	1907
19. RR24510004103-C1_Sample5	■	PR0701	19234	1	467.4	1866	1	8627	16195	1	480.9	1955
20. RR24510004103-D1_Sample7	■	PR0701	18852	1	484.7	1894	1	8942	16008	1	517.5	2015
21. RR24510004103-E1_Sample9	■	PR0701	18272	1	493.6	1867	1	8974	15583	1	515.8	1946
22. RR24510004103-F1_Sample11	■	PR0701	18935	1	544.7	2041	1	9134	16611	1	514.7	2021

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

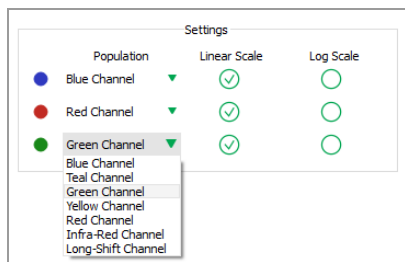
The default view contains the chamber name, quality control flag, protocol identifier, total (Nb) droplet count, dilution factor, target concentration (copies per μl), and positive (Nb Pos) droplet count. You can also extend the view to include negative droplets, separability score, minimum and maximum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

Chamber Name	Chamber Context	Protocol	Nb Droplets	Dilution	Blue Channel		Green Channel						
					C (cp/μL)	Nb Pos	Nb Neg	Separability Score	C_min (cp/μL)	C_max (cp/μL)	Relative Uncertainty (CI 95%)	Dilution	C (cp/μL)
1. RR24510002384-A1_Sample1	■	PR0701	18654	1	477.7	1868	16786	7	456.1	499.4	4.54%	1	8922
2. RR24510002384-A2_Sample2	■	PR0701	19043	1	468.7	1873	17170	7	447.5	480	4.51%	1	8464
3. RR24510002384-B1_Sample3	■	PR0701	19318	1	488.5	1976	17342	6	467	510.1	4.41%	1	8975
4. RR24510002384-B2_Sample4	■	PR0701	18807	1	491.5	1935	18672	7	489.7	515.5	4.46%	1	8627
5. RR24510002384-C1_Sample5	■	PR0701	18793	1	474.5	1870	18623	6	455	486.1	4.51%	1	8966
6. RR24510002384-C2_Sample6	■	PR0701	19487	1	461.3	1914	18553	6	472.2	517.6	4.48%	1	8451

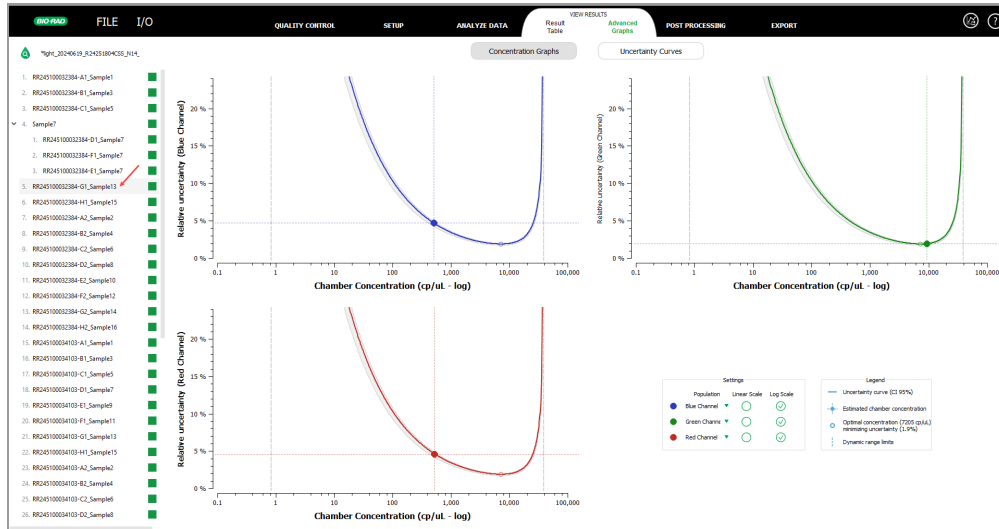
- **Advanced Graphs** — contains graphics for concentration and uncertainty curves (confidence intervals).
- Concentration graphs illustrates the copies per μL using a line and a data point for each selected chamber. You can choose to display the data in linear or logarithmic scale.



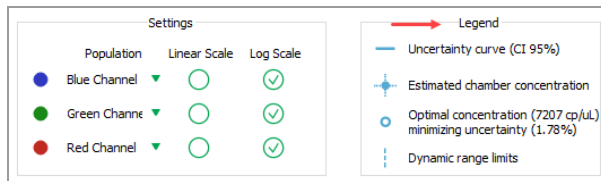
Important: In the Settings widget, the Population section displays a maximum of three channels simultaneously. Click in a channel field to display the dropdown list, and select a different channel if applicable.



- 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.

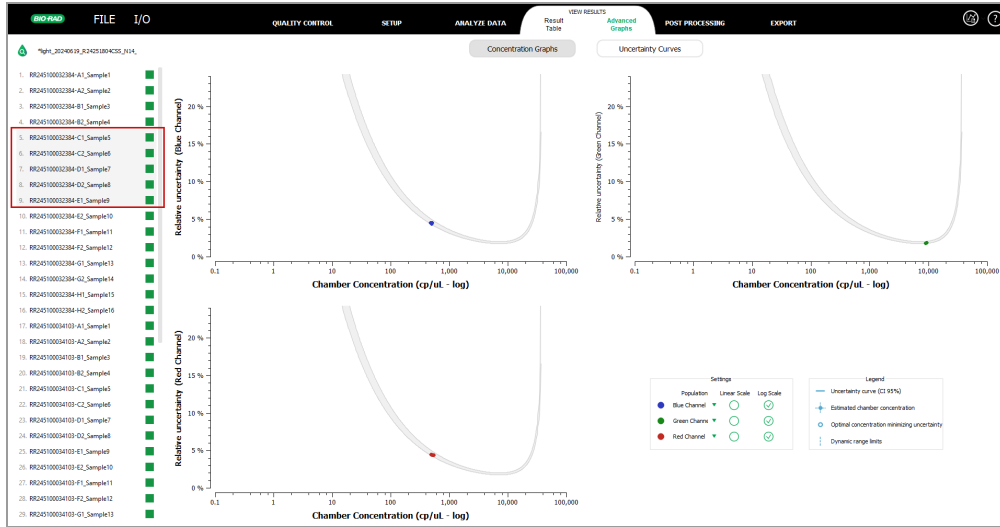


A legend also identifies elements in the display (uncertainty curve line, estimated chamber concentration, optimal concentration to minimize uncertainty, and dynamic range limits).

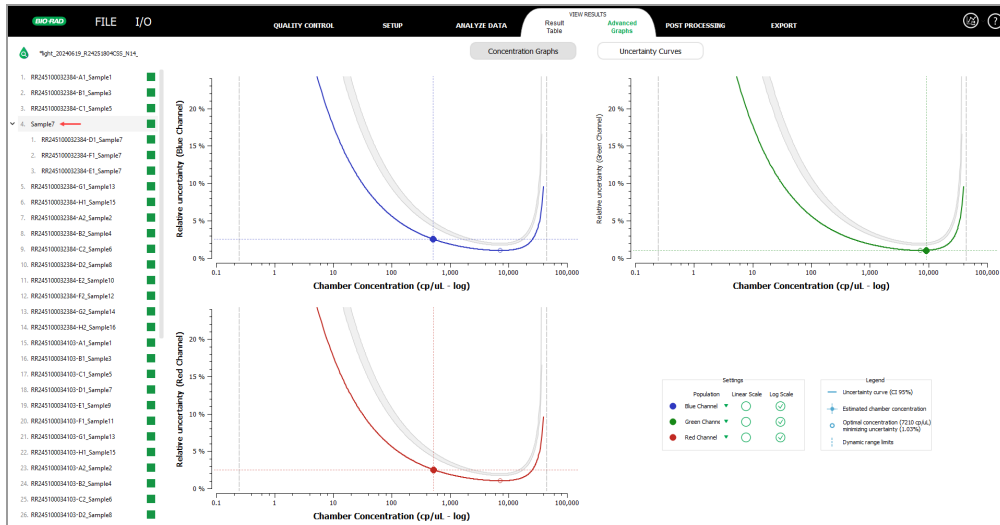


Chapter 8 Viewing Results

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.

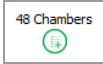
2. Click Result Table to view the concentration data.

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).

Chamber Name	Chamber Context	Protocol	Nb Droplets	Blue Channel			Green Channel			Red Channel		
				Dilution	C (cp/μL)	Nb Pos	Dilution	C (cp/μL)	Nb Pos	Dilution	C (cp/μL)	Nb Pos
1. RR24510002284-A1_Sample1		PR0T01	1864	1	477.7	1868	1	892.2	1695	1	511.2	1992
2. RR24510002284-B1_Sample3		PR0T01	19318	1	488.2	1975	1	897.5	16958	1	510	2058
3. RR24510002284-C1_Sample5		PR0T01	18793	1	474.5	1870	1	896.6	16200	1	483.3	1903
4. RR24510002284-D1_Sample7		PR0T01	18892	1	499.6	1974	1	891.4	16255	1	507.9	2005
5. RR24510002284-E1_Sample9		PR0T01	17809	1	506.5	1885	1	928.3	15518	1	536.4	1990
6. RR24510002284-F1_Sample11		PR0T01	18791	1	514	2017	1	914.2	16287	1	521.1	2043
7. RR24510002284-G1_Sample13		PR0T01	16431	1	503	1728	1	923.8	14296	1	521.9	1789
8. RR24510002284-H1_Sample15		PR0T01	20040	1	459.4	1934	1	845.1	16942	1	487.8	2047
9. RR24510002284-A2_Sample2		PR0T01	19043	1	469	1874	1	846.4	16107	1	489.6	1952
10. RR24510002284-B2_Sample4		PR0T01	18807	1	491.5	1935	1	862.7	16010	1	501.5	1972
11. RR24510002284-C2_Sample6		PR0T01	18467	1	495.1	1913	1	890.4	15884	1	497.3	1921
12. RR24510002284-D2_Sample8		PR0T01	17394	1	507.8	1887	1	934.8	15383	1	536.1	1965
13. RR24510002284-E2_Sample10		PR0T01	17303	1	489.7	1774	1	899.9	14933	1	518.4	1872
14. RR24510002284-F2_Sample12		PR0T01	18176	1	489.6	1863	1	903.9	15708	1	534.5	2024
15. RR24510002284-G2_Sample14		PR0T01	16739	1	470.1	1651	1	864.4	14270	1	491.8	1723

Chapter 8 Viewing Results

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



- 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.

Chamber Name	Chamber Content	Protocol	Nb Droplets	Dilution	C (cp/uL)	Nb Pos	Nb Neg	Separability Score	C_min (cp/uL)	C_max (cp/uL)	Relative Uncertainty (CI 95%)	Dilution	C (cp/uL)
1: 89245100023384-A1_Sample1		PROTO1	18654	1	477.7	1868	16786	7	456.1	498.4	4.54 %	1	8922
2: 89245100023384-A2_Sample2		PROTO1	19043	1	466.7	1873	17170	7	447.5	490	4.53 %	1	8464
3: 89245100023384-B1_Sample3		PROTO1	19318	1	485.5	1976	17342	6	467	510.1	4.41 %	1	8975
4: 89245100023384-B2_Sample4		PROTO1	18807	1	491.5	1935	16872	7	469.7	513.5	4.46 %	1	8627
5: 89245100023384-C1_Sample5		PROTO1	18793	1	474.5	1870	16929	6	453	496.1	4.53 %	1	8966
6: 89245100023384-C2_Sample6		PROTO1	19407	1	485.1	1914	16053	6	473.0	517.6	4.48 %	1	8964

- Use the bottom scroll bar to scroll right or left and view result for all populations.

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

- 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.

VIEW RESULTS

Protocol: 3PlexValidation_NO_v29.3

Altitude profile: Low

Thermocycling program

95°C for 180sec, 1°C/sec	
95°C for 10sec, 1°C/sec	x45
58°C for 15sec, 1°C/sec	
25°C for 60sec, 1°C/sec	

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.

The screenshot shows the software interface with the 'ANALYZE DATA' tab selected. The 'VIEW RESULTS' section is active, displaying a 'Result Table' with a 'Separability Score' column highlighted by a red arrow. The table contains 19 rows of assay data for the 'Blue Channel'.

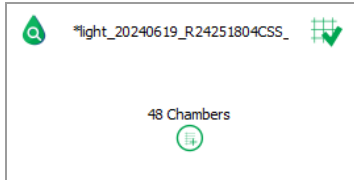
Chamber Name	Chamber Context	Protocol	Nb Droplets	Dilution	C (cp/µL)	Nb Pos	Nb Neg	Separability Score	C _{min} (cp/µL)	C _{max} (cp/µL)	Relative Uncertainty (CI 95%)	Dilution	C (cp/µL)
1. RR245100032384-A1_Sample1	■	PROTO1	1804	1	477.7	1868	16786	7	456.1	499.4	4.34 %	1	8922
2. RR245100032384-B1_Sample3	■	PROTO1	19318	1	488.2	1975	17343	6	466.7	509.8	4.41 %	1	8975
3. RR245100032384-C1_Sample5	■	PROTO1	18793	1	474.5	1870	16823	6	453	496.1	4.33 %	1	8966
4. RR245100032384-D1_Sample7	■	PROTO1	18892	1	499.6	1974	16918	6	477.6	521.7	4.41 %	1	8914
5. RR245100032384-E1_Sample7	■	PROTO1	20040	1	459.4	1934	18106	8	439	480	4.46 %	1	8451
6. RR245100032384-F1_Sample7	■	PROTO1	17809	1	506.5	1885	15824	7	483.7	529.4	4.32 %	1	9283
7. RR245100032384-G1_Sample11	■	PROTO1	18791	1	514	2017	16774	7	491.6	536.5	4.37 %	1	9142
8. RR245100032384-H1_Sample13	■	PROTO1	16431	1	503	1728	14703	7	479.4	526.8	4.72 %	1	9238
9. RR245100032384-A2_Sample2	■	PROTO1	19043	1	469	1874	17169	8	447.8	490.3	4.33 %	1	8464
10. RR245100032384-B2_Sample4	■	PROTO1	18807	1	491.5	1933	16872	7	469.7	513.5	4.46 %	1	8627
11. RR245100032384-C2_Sample6	■	PROTO1	18467	1	495.1	1913	16554	7	472.9	517.3	4.48 %	1	8904
12. RR245100032384-D2_Sample8	■	PROTO1	17594	1	507.8	1867	15727	6	484.9	530.9	4.34 %	1	9348
13. RR245100032384-E2_Sample10	■	PROTO1	17303	1	489.7	1774	15329	8	467	512.6	4.66 %	1	8999
14. RR245100032384-F2_Sample14	■	PROTO1	18176	1	489.6	1863	16313	7	467.4	511.9	4.34 %	1	9039
15. RR245100032384-G2_Sample14	■	PROTO1	16739	1	470.1	1651	15088	8	447.5	492.8	4.83 %	1	8664
16. RR245100032384-H2_Sample16	■	PROTO1	19170	1	460.7	1855	17315	9	439.8	481.8	4.35 %	1	8481
17. RR245100034103-A1_Sample1	■	PROTO1	18481	1	484.8	1877	16604	7	463	506.8	4.33 %	1	8614
18. RR245100034103-B1_Sample3	■	PROTO1	19132	1	472.5	1896	17254	7	451.2	493.8	4.3 %	1	8551
19. RR245100034103-C1_Sample5	■	PROTO1	19024	1	467.4	1866	17138	6	446.2	488.6	4.34 %	1	8627

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.

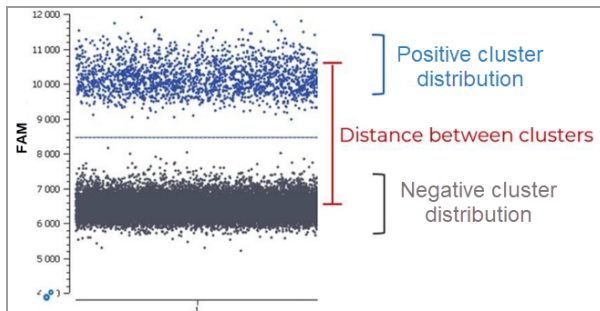
Note: The separability score is only one parameter that is evaluated when optimizing assays. You must confirm proper quantification using known quantities of positive controls. For example, the separability score for any given channel could be high, but if the reaction is not specific to the desired target, the target quantification might be incorrect. For more information, see [Using the Separability Score for Assay Optimization on page 113](#).

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

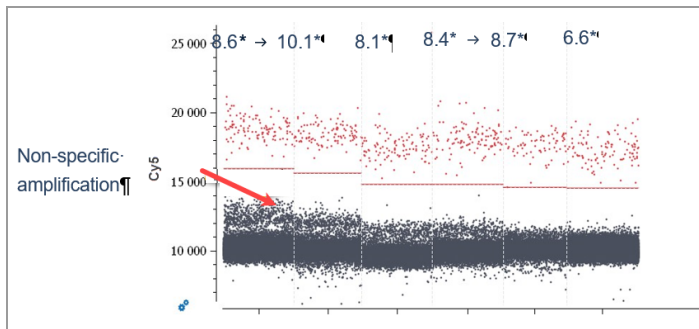
- The positive droplet cluster includes the amplified target of interest, so a high fluorescence intensity is displayed.
- The target of interest is absent from the negative droplet cluster, so the fluorescence intensity is low compared to the positive cluster.

The following bullet points describe the separability score values:

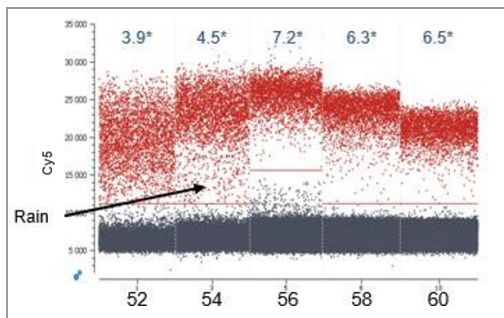
- For the FAM (Blue) channel, a separability score of 4 is the minimum acceptable value to distinguish clearly and set the threshold between the positive and negative clusters on a 1D dot plot.
- The separability score is low for the FAM (Blue) channel because of the addition of the reference dye, fluorescein. 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 with no performance loss. To reduce assay complexity and the overall 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.

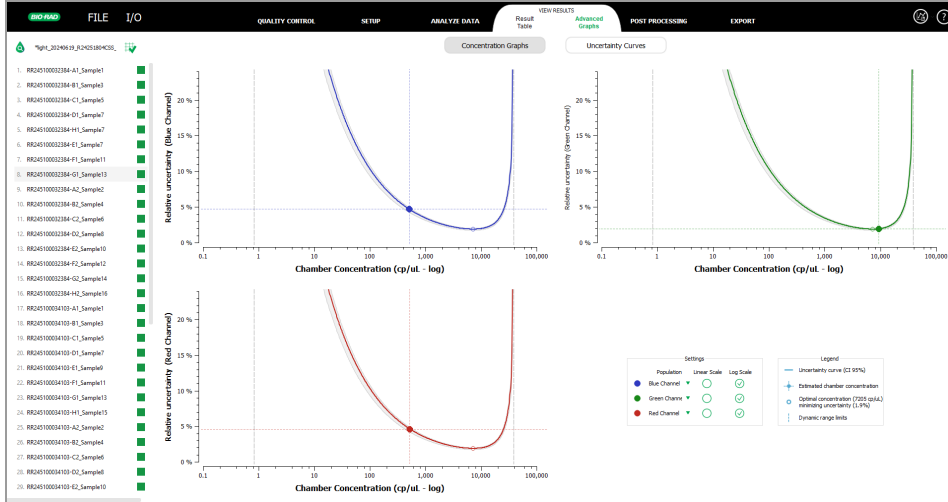
Interpreting Uncertainty Curves

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/ μ L).
- 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 $\sim 79.7\%$ of the droplets are positive, whatever the number of droplets and the confidence level.

This page intentionally left blank.

Chapter 9 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.

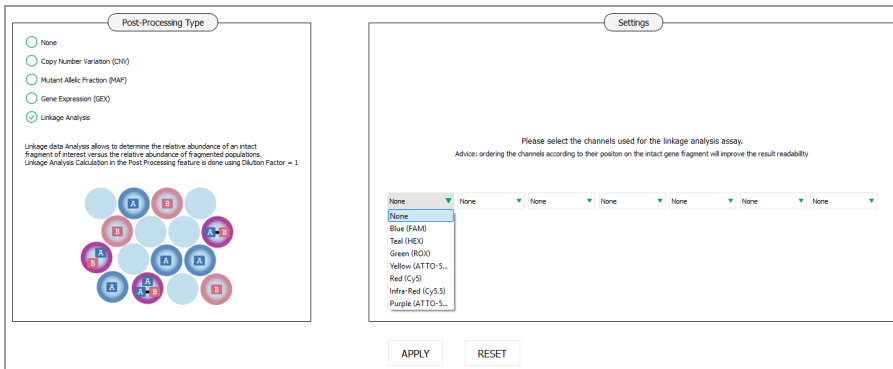
Performing Post-Processing Analysis

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 14 on page 120 describes the options available for each experiment type, and Linkage Analysis, to perform post-processing analysis.

Table 14. Post-processing options

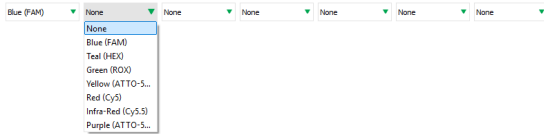
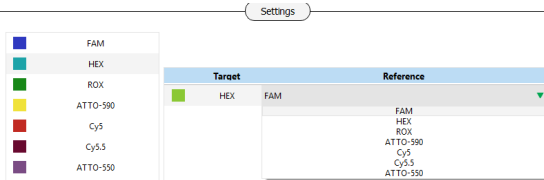
View	CNV	MAF	GEX	LINK
<p>Under Settings, you can select and add populations sequentially; select a population in the left column, and then tap/click Add population to processing.</p> <div style="border: 1px solid gray; padding: 5px; width: fit-content; margin: 10px auto;">Add population to processing</div>	X	X	X	
<p>Under Settings, you can select the channels used for the linkage analysis assay.</p> <p>Tap/click each drop-down arrow to select a different channel.</p> <div style="text-align: center; margin: 10px 0;"> <small>Please select the channels used for the linkage analysis assay. Advice: ordering the channels according to their position on the intact gene fragment will improve the result readability</small> </div> 				X
<p>Under Settings, the selected color is also used in the Results Table.</p>	X	X		
<p>By default, the FAM channel appears in the Reference column.</p> <p>1. To modify, tap/click FAM and select a reference from the dropdown list.</p> <div style="margin: 10px 0;">  </div>	X	X		
<p>Note: If you select the <i>Use same for all targets</i> checkbox, then the reference chosen first is applied to the other populations.</p>				

Table 14. Post-processing options, continued

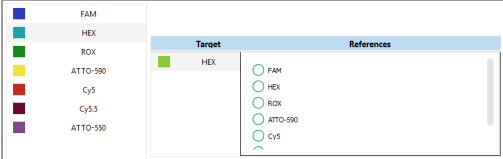
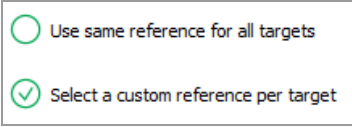
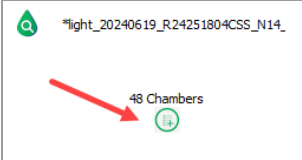
View	CNV	MAF	GEX	LINK
<p>Under Settings, to select one or more reference genes for the same target gene, double-click the Reference column.</p> 			X	
<p>Note: If several reference genes are selected, the GEX ratio is calculated using the geometric mean of the selected reference genes.</p>				
<p>To specify 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.</p>	X			
<p>You can select a custom reference per target or use the same reference for all targets.</p> 	X	X	X	
<p>When you click Apply, post-processing calculations are launched.</p>	X	X	X	X
<p>Values are displayed in the Results tab. In addition to the experiment parameters, only the following appear:</p> <ul style="list-style-type: none"> ■ 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 14. Post-processing options, continued

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

Chapter 10 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

Please select the file type you wish to export to:

.csv .xlsx

Please select the elements you wish to export:

- Experiment details (.csv / .xlsx)
- Chamber details (.csv / .xlsx)
- Results (.csv / .xlsx)
- Zoning And Population Data (.csv / .xlsx)
- Spillover compensation file (.ncm)
- QC indicators (.csv / .xlsx)
- Droplet-Level Data
- Crystal Images (.png)
- Dot Plots (.png)
- Plot Settings File (.ncp)
- Populations Analysis Result (.nca)

Chamber choice: RR245100032384-B1_Sample3 ▾

[U:104232|OneDrive - Bio-Rad Laboratories Inc|Desktop|1]

Browse

Export

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 124](#) and [Data Available for Optional Export on page 128](#). For information on exporting the Regulatory Report in .pdf format, see [Using Premium Edition on page 15](#).

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/ μ L 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 100](#) and [Performing Quality Control on page 43](#).

- 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/ μ L), using the following rounding rules:
 - If $c > 1000$ cp/ μ L, the estimate is truncated
 - If $c > 10$ cp/ μ L, the estimate is rounded to one digit
 - If $c < \text{or} = 10$ cp/ μ L, 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/ μ L)
 - 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/ μ L)
 - Min and max limits of the 95% confidence interval for the estimated stock concentration (in copies/ μ L)
 - 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 fragment or all the other combinations of channels:
 - Estimated stock concentration (in copies/ μ L)
 - Min and max limits of the 95% confidence interval for the estimated stock concentration (in copies/ μ L)
 - 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.

This page intentionally left blank.

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 Premium 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 133](#) for the minimum system requirements. Beginning with Version 1.5, each software edition is installed from its own installer file.

Premium Edition is designed to provide more validation rigor if configured correctly, and requires the QX700 Data Service and additional configuration to satisfy regulatory requirements associated with CFR21 Part 11. Instructions are included in this chapter but you should contact Bio-Rad Technical Support for assistance and to answer any questions.

Important: *Correct configuration for CFR21 Part 11 is the customer's responsibility. For information, see [21 CFR Part 11 Compliance on page 15](#). The QX700 Droplet Digital PCR System is for research use only and is NOT intended for diagnostics.*

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 15](#).

Table 15. 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](https://www.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. If you are using Premium Edition, you can only open files that were created while you were logged in.

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](https://www.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 Premium Edition

Premium Edition requires the installation and configuration of the QX700 Data Service in addition to the analysis software, and should be managed by a Bio-Rad FSE. If you prefer to install it yourself, contact Bio-Rad Technical Support.

For Premium Edition, you must install and configure the , as well as the QX700 ddPCR System Analysis Software.

- Locate and unzip the downloaded installation files, and then complete the steps to install the data service and premium edition of the analysis software.

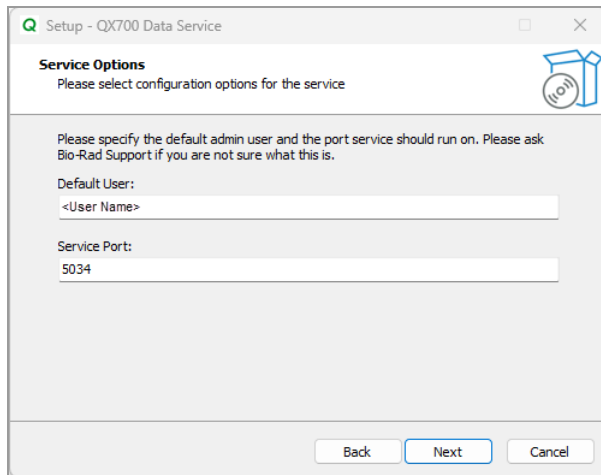
An installation Wizard is prompted when you tap/click the software installation executable file.

To install the data service software

1. Right-click the installation executable file for the QX700 Data Service and select Run as administrator.
2. Accept the license agreement and continue through the Wizard dialogs.

Note the following:

- The default folder is C:\Program Files\Bio-Rad\QX700DataService. You can keep the default folder location (recommended) or tap/click Browse to navigate to a storage different folder.
- If you are performing an upgrade rather than a new installation, you must either confirm that you are installing to the existing folder or designate a different folder.
- In the Service Options dialogs:
 - Select Local, to authorize user accounts that are set up in Windows or Active Directory to authorize users on your organization's network; if more than one PC will be set up to connect to the instrument, then Active Directory is recommended.
 - Tap/click Yes to import an existing Data Service database or No to skip the step.
 - Identify or confirm the initial user and ensure Port 5034 is specified for the data service.



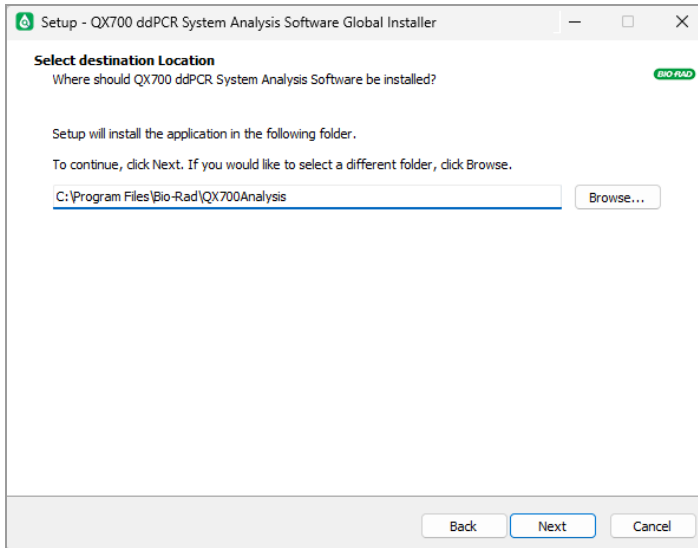
3. Click Install to begin the installation and display a progress bar.
4. When the installation concludes, click Finish.
5. (Optional) Restart the computer now or later.

To install the Premium Edition analysis software

6. Right-click the installation .exe file and select Run as administrator.

Appendix A Software Installation

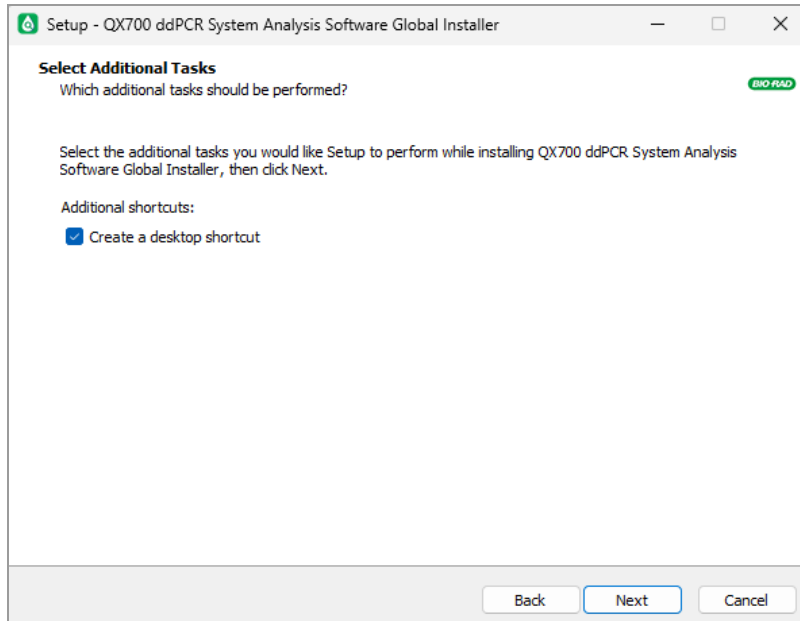
7. The default folder path is C:\Program Files\Bio-Rad\QX700Analysis. You can keep the default (recommended) or you can click Browse and navigate to a different folder location.
8. Click Next.



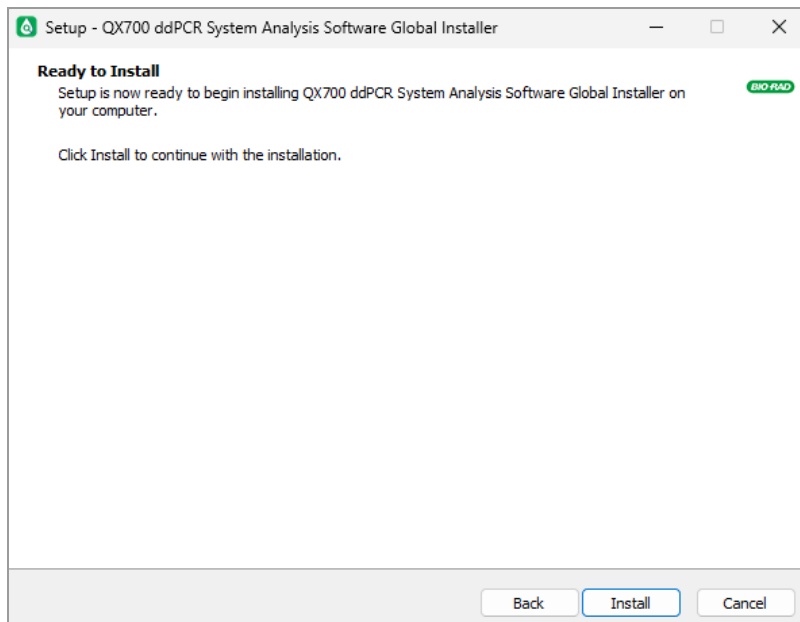
If you are performing an upgrade rather than a new installation, a message appears asking you to confirm that you are installing to the existing folder.

9. Click Yes to confirm.
10. If you are installing Premium Edition, ensure the Host and Port fields contain localhost and 5034, respectively. Otherwise, continue to the next step.

11. Select Create a desktop shortcut, and then click Next.

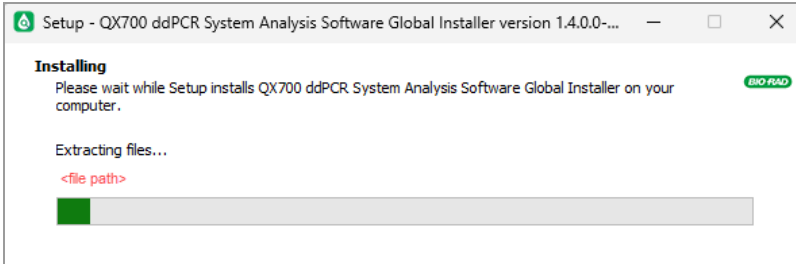


12. Click Install.



Appendix A Software Installation

The wizard displays an installation dialog with a progress bar.



13. When the installation concludes, click Finish.
14. (Optional) Restart the computer.

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\\logs

Appendix B Remote Maintenance



**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
France 00 800 00 24 67 23 **Germany** 00 800 00 24 67 23 **Greece** 30 210 7774396 **Hong Kong** 852 2789 3300 **Hungary** 00 800 00 24 67 23
India 91 124 4029300 **Israel** 000 800 00 24 67 23 **Italy** 00 800 00 24 67 23 **Japan** 81 3 6361 7000 **Korea** 82 080 007 7373
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
