HELP

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QuantStudio™ 3D AnalysisSuite™ Software: Relative Quantification

for use with QuantStudio™ 3D Digital PCR System and QuantStudio™ 3D AnalysisSuite™ Server System

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Revision A.0

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The information in this guide is subject to change without notice.

Contents

QuantStudio™ 3D AnalysisSuite™ Software Help: Relative Quantification

Welcome to the *QuantStudio™ 3D AnalysisSuite™ Software Help: Relative Quantification*.

The Relative Quantification module allows you to quantify the amount of a target relative to total genetic material interrogated using digital PCR (dPCR). This analysis module is ideal for ratio analysis or low-fold detection to perform applications such as copy number variation on the QuantStudio™ 3D Digital PCR 20K Chip. Up to 100 chips can be combined and analyzed in a single project, to automatically display a scatter plot based on the observed FAM™ dye,VIC® dye, and undetermined dye signal events. For further quality control, you can manually define the boundaries of these events and use the software to calculate a ratio of rare to wild-type events across the chips in a project.

Use the **Table of Contents** in the left navigation pane to find your topic of interest, or enter your search term(s) in the **Search** field above (see "Obtain information from the Help system" on page 7).

About this Help system

Revision history

Revision	Date	Description	Pub. no.
A.0	February 2014	New Product- help documentation for use and navigation of the server-based QuantStudio™ 3D AnalysisSuite™ Software designed to analyze Relative Quantification digital PCR experiments.	MAN0009636

Purpose

This Help system provides step-by-step instructions for using the server-based QuantStudio™ 3D AnalysisSuite™ Software to analyze Relative Quantification digital PCR experiment data generated by the QuantStudio™ 3D Digital PCR System and describes how to troubleshoot the data.

Prerequisites

This Help system assumes that you have access to and are familiar with the procedures provided in the *QuantStudio™ 3D Digital PCR System User Guide* (see "Related documentation" on page 54).

This Help system also assumes that you have a general understanding of:

- Data storage, file transfers, and copying and pasting.
- The Red Hat® Enterprise Linux® and Microsoft® Windows® operating systems, the Internet, and Internet-based browsers.

Obtain information from the Help system

The QuantStudio™ 3D AnalysisSuite™ Software features a Help system that describes how to use each feature of the user interface. You can use the Help system to find topics of interest by:

- Reviewing the table of Contents in the sidebar.

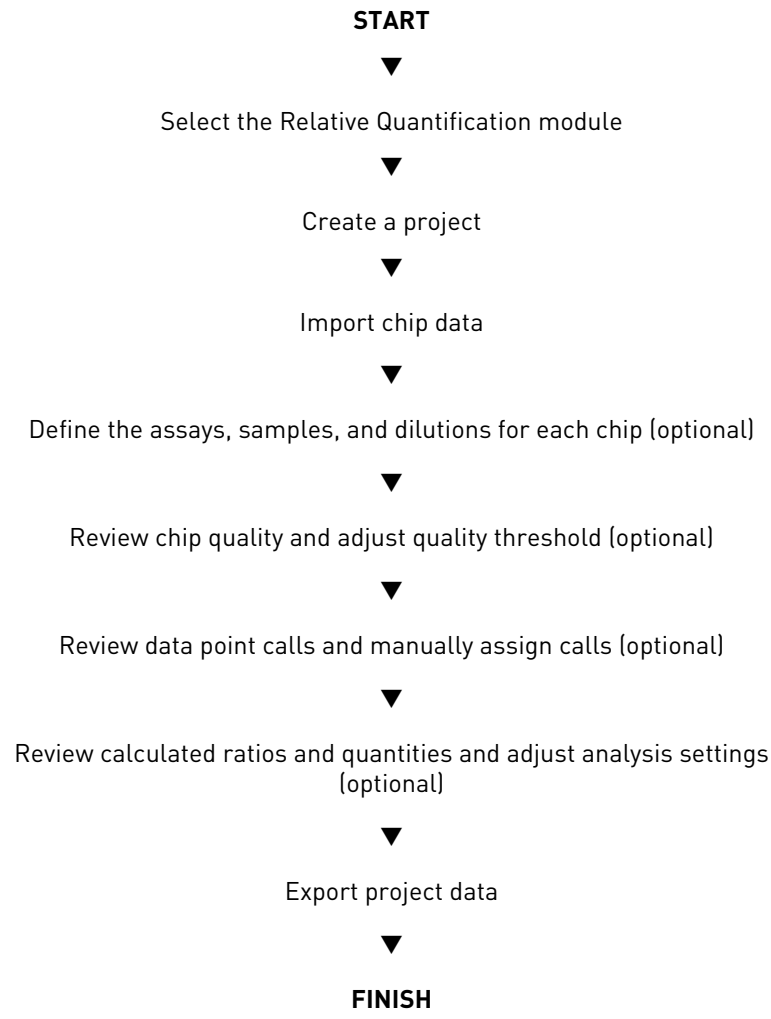
- Searching for a specific topic – Enter your search term in the Search field in the upper right, then click **Enter**. The search results will appear under the Search tab in the sidebar.

To access the Help system click Help from any screen.

1 Analysis workflow

Relative Quantification analysis workflow

The following figure shows the workflow for analyzing dual reporter digital PCR experiments using the Relative Quantification module of the AnalysisSuite™ Software.



Note: If you cannot view the results of an open project, then contact Technical Support (see "Obtaining support" on page 54).

2 Create a project

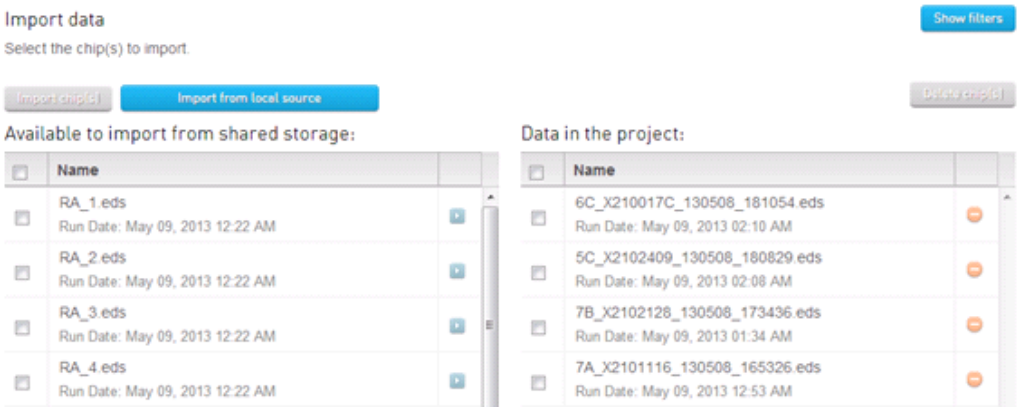
Create a project

1. In the Project Listing screen, click **Create project**.
2. Enter a unique project name up to 100-characters in length.
   The project name:
   - Should be descriptive and easy to remember.
   - Cannot contain the following characters:
     % * ? | ; : , ! @ # $ ( ) < > / \ " ' ` ~ [ ] { } = & ^ -
   - Cannot end with a period (.).
3. Click **OK**. The software saves the project.
4. In the Import Data tab, import data into the project as needed.

3 Import chip data

A project can include experiment data (.eds) files from up to 100 chips. You can import chips from the shared storage on the QuantStudio™ 3D AnalysisSuite™ Server System or from a local source.

Note: Refer to the *QuantStudio™ 3D Digital PCR System User Guide* (Pub. no. MAN0007720) for information on transferring data files from the QuantStudio™ 3D Digital PCR Instrument to another location (shared storage on the AnalysisSuite™ Server System , a network file server, an external storage device such as a USB drive, or the Life Technologies™ cloud data storage service).



Import from shared storage

If you transferred completed experiment (.eds) files from chips run on a QuantStudio™ 3D Instrument to the shared storage on the QuantStudio™ 3D AnalysisSuite™ Server System, they are listed in the Import Data tab and are available for import into the current project.

In the Import Data tab, select from the chip(s) available to import from shared storage:

- **Individual chip** – Select a row in the table, then click (Import into project).
- **Multiple chips** – Click-drag or press **Shift** to select continuous rows, or press **Ctrl** to select discontinuous rows, then click **Import chip(s)**.

The selected chip(s) are moved to the project list on the right. You can select to delete () any chip before continuing with project setup.

Note: If a transferred experiment file is not visible in the Import Data tab, then contact Technical Support (see "Obtaining support" on page 54).

Import from local source

To import chip data from your computer or other local source (network file server or USB drive):

1. In the Import Data tab, click **Import from local source**.
2. Navigate to and select one or more experiment (.eds) files to import, then click **Open**.

   Note: Click-drag or press **Shift** to select continuous files, or press **Ctrl** to select discontinuous files.

The selected files are added to the project list on the right. You can select to delete (⊖) any file before continuing with project setup.

4 Define chip settings

The Define Chips tab is automatically populated with the default values from chips imported into a project. You can edit the default chip settings applied to the chips in a project, select the default chip settings to use at chip import, import new chip settings from an existing experiment (.eds) file, and apply the same chip settings to multiple chips in the project.

Note: During analysis, chips with the same sample, target, and dilution settings are considered technical replicates, while chips with the same sample and target but different dilution settings are considered a dilution series.

- To edit default chip settings:
  - Click a field in the table and enter a value.

    Note: Optionally, enter an editable comment for a chip.
  - As needed, use the options below the table to continue to set up (add or edit) chip settings and select the default chip settings to use at chip import.

- To assign the same chip settings to multiple chips:
  a. Select the top checkbox to select all rows, or select individual checkboxes. Click-drag or press **Shift** to select continuous rows. Press **Ctrl** to select discontinuous rows.

  b. Click **Assign settings to multiple chips**, select the chip settings to use, then click **Save**.

- To import new chip settings into the project:
  a. Click **Import settings**.

  b. Navigate to and select an existing experiment (.eds) file, then click **Open**.

Set up assays

After you create a project, you can use the Define Chips tab to edit existing assays, add new assays, and select the default assays for the project.

The default assay in the software uses the following settings:

Assay	Target Dye
assay(default)	FAM

Note: The software automatically assigns FAM™ dye as the Target Dye to each new chip that is imported into a Relative Quantification project. The software uses the Target Dye as the numerator in reported ratio results.

- To edit existing assays:
  - In the chip settings table, click a field in the **Assay** column, then:
    - Enter a new assay name (do not use spaces) and optionally select a new Target Dye. A new assay is added to the assay details table and is available for selection in the chip settings table.
    - Select a new assay from the drop-down menu. The Target Dye defined for the selected assay is automatically shown.
  - Below the chip settings table, click **Show details** next to the Assay type. Optionally, edit the assay settings.

  Note: Changes to assay settings are automatically shown in the chip settings table.

- To add and define new assays:
  a. Below the chip settings table, click **Add** next to an Assay type.
  A new assay is added to the assay details table and is available for selection in the chip settings table.

  b. (Optional) Edit the assay settings:
    - Enter a new **Assay** name (do not use spaces).
    - Select a **Target Dye** from the drop-down menu.
    - Enter a **Description** for the assay.

  c. (Optional) Click **Delete** to remove a setting.

  Note: You cannot delete settings that are currently assigned to one or more chips.

  d. (Optional) Select a default assay.

- To select a default assay:
  a. Below the chip settings table, click **Show details** next to the Assay type.

  b. Select the **Default value** option for the assay you wish to apply to each new chip upon import into the project. This assay will be shown by default in the chip settings table. Click **Clear** to remove your selection.

Set up samples

After you create a project, you can use the Define Chips tab to edit existing samples, add new samples, and select the default samples for the project.

- To edit existing samples:
  - In the chip settings table, click a field in the **Sample** column, then:
    - Enter a new sample name (do not use spaces). A new sample is added to the sample details table and is available for selection in the chip settings table.
    - Select a new sample from the drop-down menu.
  - Below the chip settings table, click **Show details** next to the Sample type. Optionally, edit the sample settings.

  Note: Changes to sample settings are automatically shown in the chip settings table.

- To add and define new samples:
  a. Below the chip settings table, click **Add** next to the Sample type.
  A new sample is added to the sample details table and is available for selection in the chip settings table.

  b. (Optional) Edit the sample **Name**.

  c. (Optional) Click **Delete** to remove a setting.

  Note: You cannot delete settings that are currently assigned to one or more chips.

  d. (Optional) Select a default sample.

- To select a default sample:
  a. Below the chip settings table, click **Show details** next to the Sample type.

  b. Select the **Default value** option for the sample you wish to apply to each new chip upon import into the project. This sample will be shown by default in the chip settings table. Click **Clear** to remove your selection.

Set up dilutions

After you create a project, you can use the Define Chips tab to edit existing dilutions, add new dilutions, and select the default dilutions for project.

- To edit existing dilutions:
  - In the chip settings table, click a field in the **Dilution** column, then:
    - Enter a new dilution. A new dilution is added to the dilution details table and is available for selection in the chip settings table.

      Note: You can enter the dilution as numeric values (for example, 0.01), alphanumeric values (for example, 1 to 100), or in scientific notation (for example, 1E-2).

  - Select a new dilution from the drop-down menu.
- Below the chip settings table, click **Show details** next to the Dilution type. Optionally, edit the dilution settings.

Note: Changes to dilution settings are automatically shown in the chip settings table.

- To add and define new dilutions:
  a. Below the chip settings table, click **Add** next to the Dilution type.
  The software computes the next dilution based on the default dilution factor of 10. This new dilution is added to the dilution details table and is available for selection in the chip settings table.

  b. (Optional) Edit the dilution value shown in the **Name** column.

  c. (Optional) Click **Delete** to remove a setting.

  Note: You cannot delete settings that are currently assigned to one or more chips.

  d. (Optional) Select a default dilution.

- To select a default dilution:
  a. Below the chip settings table, click **Show details** next to the Dilution type.

  b. Select the **Default value** option for the dilution you wish to apply to each new chip upon import into the project. This dilution will be shown by default in the chip settings table. Click **Clear** to remove your selection.

Import chip setup

You can import existing chip setup information from a saved comma-separated (.csv) file instead of entering chip settings into the Define Chips tab.

1. Select the **Define Chips** tab of an open project.

2. Click **Upload setup**, then navigate to and select the chip setup (.csv) file you wish to import in to the project.

   Note: Make sure to select the correct chip setup type for your analysis module.

3. Click **Open** to add the setup information to the project.

4. (Optional) Continue to define the chip settings.

5 View the data quality for each chip

You can use the Review Quality tab to perform a quality check on the digital PCR data from each chip in a project and conduct further analysis, if necessary. The data views in the Review Quality tab:

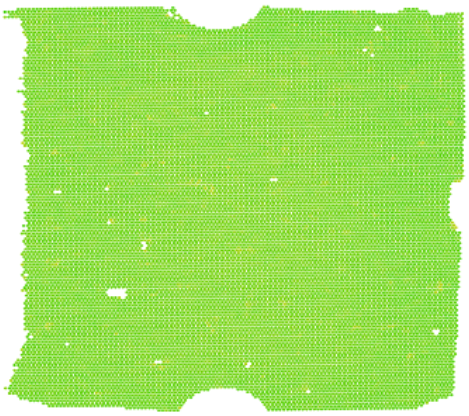
- Visualize the spatial distribution of data across the chips.
- Provide an overview of the observed dye intensities for reaction wells (data points) that exceed the user-defined quality threshold (see "About chip quality" on page 44).
- Are color-coded by the assigned call by default.

Note: The information displayed and the activities you can perform in the Review Quality tab will vary based on your experiment type.

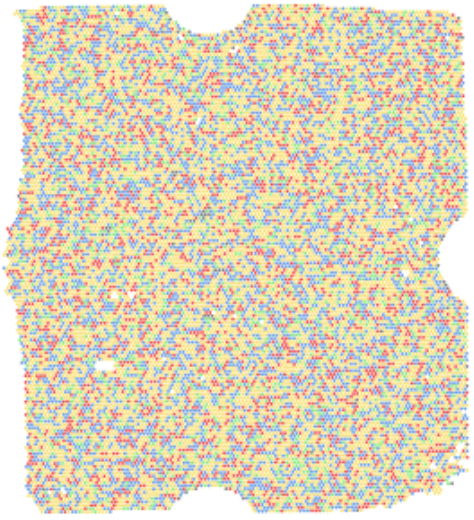
Review chip quality

1. Select the **Review Quality** tab of an open project.
2. Select a chip to view:
   - Select a row in the Table view tab.
   - Navigate to and select an image in the Chip view thumbnails tab. Move the pointer over an image to view the experiment name.

   Note: Use the flag indicators to determine which chips to view. We recommend performing a quality check of the data for chips that display a or flag (see "About data quality flags" on page 47).
3. Select the data point color options:
   - **Color by quality** – Displays each data point by quality, on a continuous color scale from red (low quality) to green (high quality). You can use this option to include or exclude reaction wells from the results. For example:



- **Color by calls** (default) – Displays each data point by the assigned call, based on the target (dye) signal detected in a reaction well. You can use this option to verify the uniformity of calls across a chip. For example:



Note: Data points that do not meet the default quality threshold are automatically filtered out (removed) by the software and displayed in white. You can lower the quality threshold to include these reaction wells in the results. Each chip fiducial (area of attachment of the chip to the chip base) is displayed in the software as a white semi-circle at the chip edge(s).

4. Review the data points in each view and adjust the quality threshold or omit chips from the results as needed.

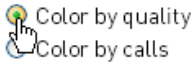
Set the quality threshold

In the Review Quality tab, select a chip to view and adjust the quality threshold until you have an acceptable balance of data quantity and quality.

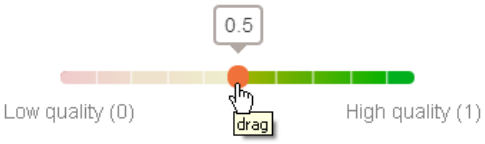
Note: Thermo Fisher Scientific has validated the system using the default quality threshold of 0.5. Increasing the quality threshold may not necessarily improve the accuracy of your results. We recommend adjusting the quality threshold if you observe obvious artifacts (such as debris, bubbles, or excess PCR reaction) which

produce lower quality data points that can be excluded from analysis by increasing the quality threshold. See Chapter 16, "Troubleshooting" for more information.

1. Select the **Color by quality** data point color option.



2. Drag the slider (below the Chip view) to the desired quality threshold, in 0.02 increments from 0 to 1. A higher quality threshold will filter out (remove) more data points from the results.



   The software automatically reanalyzes the chip results and updates the following:

   - Color and number of data points in each view that are above the quality threshold.
   - Quality Threshold value shown in the Table view tab.
   - Flag indicator, if applicable. For example, a or flag indicates that a user has modified the analysis results originally generated by the instrument software.

3. (Optional) To apply the new quality threshold to all of the chips in the project, select **Apply to all chips**.

After you review the chip level results, you can continue to adjust the quality threshold, review the project level results, or omit the chip results from the project.

Omit chip results

If you determine that the data quality for a chip is unacceptable after reviewing the data in the Review Quality tab, you can omit the chip results from the project.

Note: Omitting a chip does not delete the chip from the project. You can select to include data from an omitted chip in the project results at any time.

- To omit a chip from the results:
  - Select a row in the Table view tab, then select the **Omit** checkbox.
  - Select an image in the Chip view thumbnails tab, then click **Omit this chip from results**.

  The software automatically removes the chip data from the project results and disables the data views for the selected chip.

- To include an omitted chip in the results:
  - Select a row in the Table View tab, then deselect the **Omit** checkbox.

– Select an image in the Chip view thumbnails tab, then click **Include this chip in results**.

The software automatically includes the chip data in the project results and enables the data views for the selected chip.

6 View the Relative Quantification calls

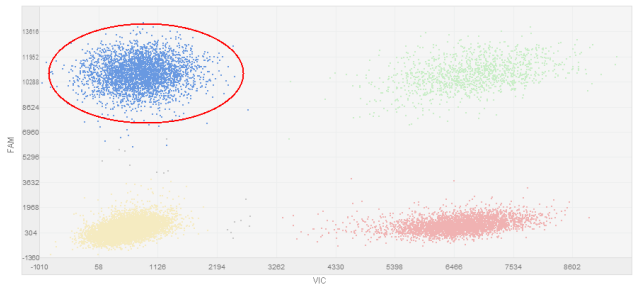
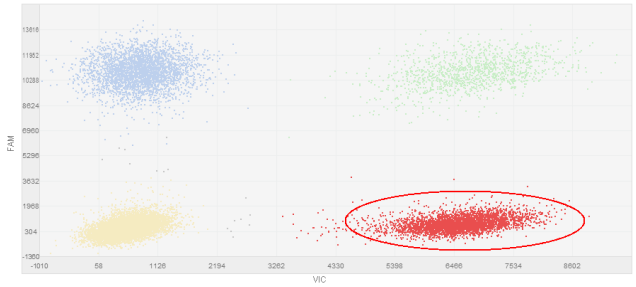
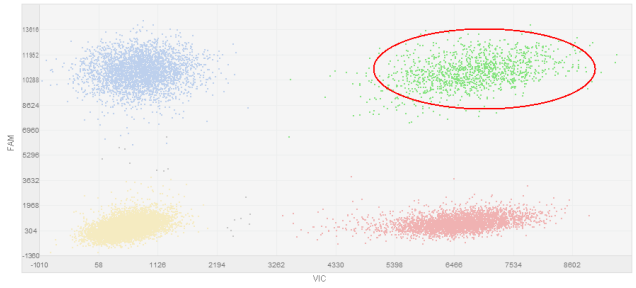
For Relative Quantification experiments, the software automatically assigns a call to each data point in a chip based on single dye thresholds that define each call type. You can use the data viewing tools in the Relative Quantification module to review the automatic call assignments for each chip in a project and manually assign calls as needed.

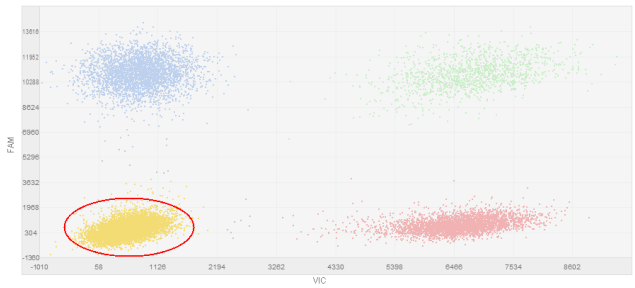
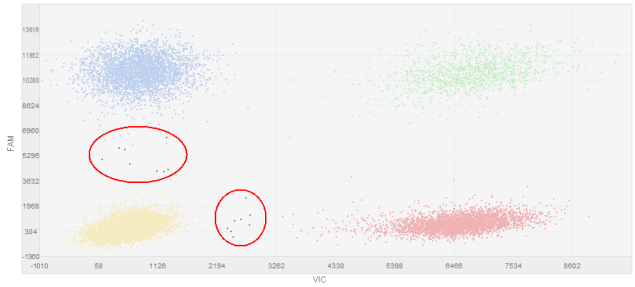
Note: Adjusting the quality threshold in the Review Quality tab will not override any manual call assignments.

About the Relative Quantification calls

The Review Calls tab displays the call assigned to each data point in the selected chip(s) of a Relative Quantification project.

- **Scatter plot** – Displays the signal from the FAM™ reporter dye on the Y-axis against the signal from the VIC® reporter dye on the X-axis. By default, all data points are shown in the plot. The data points in the plot are color-coded according to the following call types:

Call (Color)	Description
FAM (blue) 	Data points with only FAM™ reporter dye signal, generally located closer to the Y-axis of the plot, away from the origin
VIC (red) 	Data points with only VIC® reporter dye signal, generally located closer to the X-axis of the plot, away from the origin
FAM + VIC (green) 	Data points with both FAM™ and VIC® reporter dye signal, generally located midway between the FAM™ and VIC® data point clusters in the plot, away from the origin

Call (Color)	Description
No Amp (yellow) 	Data points with no signal, generally located near the origin of the plot
Undetermined (dark grey) 	Data points with unresolved signal, manual call, or originally below the default quality threshold then included at a lower quality threshold, generally located anywhere on the plot

- **Results table** – Includes the following:

Column	Description
Show	When selected, shows data points in the scatter plot for the associated chip
Assay	Assay name
Sample	Sample name
Dilution	Sample dilution factor entered for the chip
Chips	Chip containing the data group
Omit	When selected, omits the call results for the associated chip

Review the Relative Quantification calls

Use the data viewing tools present in the Review Calls tab of a Relative Quantification project to verify the automatic call assignments and edit calls as needed.

- In the scatter plot:
  - Change the plot display settings as needed:
    - **To move within the plot display** – Click , click , then drag to reposition the plot. Click to return to the original zoom level.

  - **To change the plot display** – Click , then drag a slider to the desired X-axis, Y-axis, or data point size to show in the plot. Click anywhere in the Adjust Axis dialog to close the dialog.

    IMPORTANT! The software automatically scales the plot to display all data points in the selected chip(s). To easily view particular data points in the plot, increase the data point size as needed.

  - Review the call for each data point and edit as needed (see "Edit calls" on page 24).
  - View the plot as an image file (see "Scatter plot" on page 27).

- In the results table:
  - Use the table display settings to show, sort, or group the data (see "Change the display of table data" on page 42).
  - Use the Show checkboxes to show or hide data in the scatter plot. Click and drag or press **Shift** to select continuous rows, or press **Ctrl** to select discontinuous rows. Use the top checkbox to select or deselect all rows.
  - Omit the call results for a chip (see "Omit chip data" on page 26).

Edit calls

In the Review Calls tab for Relative Quantification projects, select one or more chips to view and confirm that data points cluster as expected. Review the call for each data point and edit as needed.

IMPORTANT! Due to the high number of data points present in a chip, there may be some overlap in the display of individual data points. We strongly recommend that you use the following procedure to review and verify the assigned call for *each* data point.

1. Use the results table to show or hide the data from one or more chips in the scatter plot, and adjust plot settings as needed (see "Review the Relative Quantification calls" on page 23).

2. Use the following methods to review each cluster in the scatter plot and select data points for editing:
   - **Data points in a cluster** – This is an ideal method for reviewing data points from multiple chips.
     a. Click a call type (FAM, VIC, and so on) to bring those data points to the foreground.

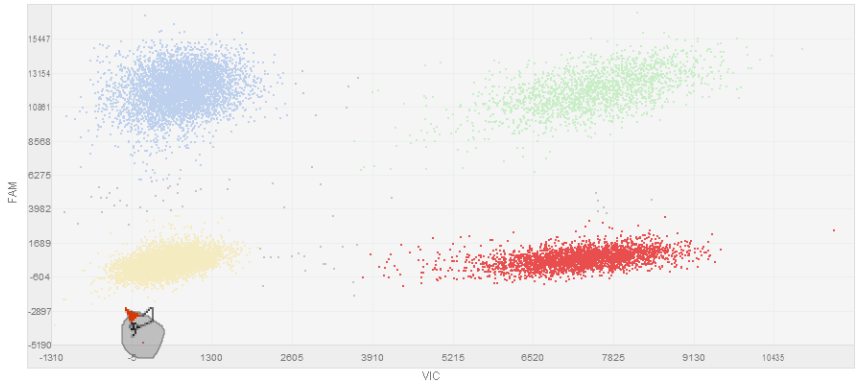
     

     Note: By default, all data points are shown in the plot. Data points in the background (faded color) may be hidden from view by data points in the foreground (saturated color). You can only edit data points that are in the foreground.

     b. Confirm that the data points are shown in the expected location in the plot (see "About the Relative Quantification calls" on page 21).

Note: For example, normally the data points for the VIC® call type should cluster closer to the X-axis of the plot, away from the origin. The presence of data points near the origin among the VIC® data points may indicate that samples failed to amplify. See Chapter 16, "Troubleshooting" for more information.

c. Click (lasso tool), click-drag around one or more data points in the foreground that you wish to edit, then release the mouse button.



d. Go to step 3 to manually assign the expected call type to all points in the selected region.

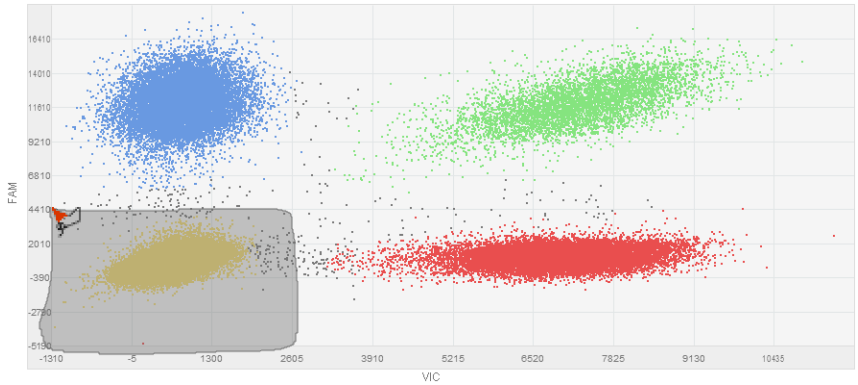
e. Repeat this process for each call type.

IMPORTANT! Data points with incorrect calls may be hidden from view by data points with correct calls. Make sure to bring the data points for each call type to the foreground and review and verify the assigned call for each data point.

- **All data points in a region** – This is an ideal method for reviewing data points from a single chip.

a. Click **All** to bring all data points in the plot to the foreground.

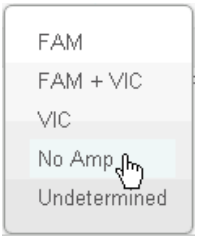
b. Click (lasso tool), click-drag around a region in the plot that you wish to edit, then release the mouse button.



c. Go to step 3 to manually assign the expected call type to all points in that region.

3. Manually assign a call:
   - Click a call type.

   - Right-click in the plot and select a call type from the drop-down menu.



   The software automatically reassigns the new call type to your selection.

   Note: Adjusting the quality threshold in the Review Quality tab will not override any manual call assignments. However, after you adjust the quality threshold we recommend that you review the call for each data point to verify if there are any new undetermined calls in the plot that are hidden from view by other data points.

4. As needed, click **Undo** or **Reset** to reverse one or more manual calls (see "Undo or Reset manual calls" on page 26).

5. If required, omit the chip data from the results (see "Omit chip data" on page 26).

Undo or Reset manual calls

To reverse one or more manual calls for the selected chip(s) in a project, you can click:

- **Undo** one time to reverse the last manual call applied to the chip(s), or multiple times to reverse multiple call edits in sequence.

  IMPORTANT! If you import or delete chip(s) from a project, you cannot Undo any of the manual calls that were applied before this action.

- **Reset** to return all manual calls applied to the chip(s) to the original calls made by the software.

Omit chip data

If you determine that the calls for a chip in a Relative Quantification project are unacceptable after reviewing the data in the Review Calls tab, you can omit the chip data from the project results.

Note: Omitting chip data does not delete the chip from the project. You can select to include omitted chip data in the project results at any time.

- **Omit chip data** – In the data table, select the **Show** checkbox to show the calls for a chip in the scatter plot, then select the **Omit** checkbox for the selected chip. The software automatically removes the chip data from the project results and disables the data views for the selected chip.

- **Include omitted chip data** – In the data table, select the **Show** checkbox to show the calls for a chip in the scatter plot, then deselect the **Omit** checkbox for the selected chip.
  The software automatically includes the chip data in the project results and enables the data views for the selected chip.

Scatter plot

Save the Scatter plot

You can save the plot shown in the Review Calls tab as a Portable Network Graphic (PNG) file.

1. Select the **Review Calls** tab of an open project.
2. Adjust the plot display settings as needed.
3. Click **View As Image**, then click **Save As** in the image dialog box.
   The software automatically saves the image as a PNG file using the default file name (Scatter_Plot_`<project name>`.png) and browser downloads location.
4. Close the image dialog box.

Print the Scatter plot

You can print an image of the plot shown in the Review Calls tab.

1. Select the **Review Calls** tab of an open project.
2. Adjust the plot display settings as needed.
3. Click **View As Image**, then click **Print** in the image dialog box.
   The software automatically opens the image in a new Untitled tab.
4. In the Print dialog box, select the printer and print options, then click **Print**.

   Note: Make sure to close the Untitled tab when the print is complete.
5. Close the image dialog box.

7 View the analysis results for a project

To view the analysis results for all of the chips (experiments) in a project:

1. Select the **See Results** tab of an open project.
2. If needed, select the **Results** tab if it is not already shown by default.

About the Relative Quantification results

For Relative Quantification experiments, the Results tab displays the aggregate results for each unique sample and assay combination, which may include replicates and/or multiple dilutions.

- **Bar graph** – Displays the data group (Sample-Assay) on the X-axis and dye signal ratio (Target/Total) on the Y-axis.

  Note: You can use the available data display and table display settings to change the default data group shown in the graph (see "Review the Relative Quantification results" on page 29).
- **Results table** – Includes the following:

Column	Description
Color	Color assigned to the data group
Assay	Assay name
Sample	Sample name
Target/Total	Calculated ratio of target dye signal over total dye (FAM™ + VIC®) signal from the target gene(s)
CI Target/Total	Upper and lower confidence interval for Target/Total dye signal ratio
Copies/µL (FAM and VIC)	Quantity of sample in copies/µL for each dye
CI Copies/µL (FAM and VIC)	Upper and lower confidence interval for quantity of sample in copies/µL for each dye
Precision (FAM and VIC)	Calculated precision (%) of each dye for the data group (see "About precision" on page 34) **Note:** You can improve (lower) the calculated precision by combining multiple chips into one "virtual" chip. To do this, apply the same sample name to all chips of interest.
Chips	Number of chips containing the data group
Recommendation	Suggestion for further dilutions as generated by the software if the set precision is exceeded

Note: The calculated sample quantity (Copies/µL) for each dye represents the concentration of the sample in the PCR reaction mix, not the concentration of the original sample before dilution (stock concentration), unless you have set the appropriate dilution in the software.

Review the Relative Quantification results

Review the calculated ratios and quantities shown in the Results tab for a Relative Quantification project, and use the display settings to show and group the data as needed.

- In the bar graph:
  - Move the pointer over a bar in the graph to view a summary of the results for that data group.
  - Adjust the data display and analysis settings (see Chapter 9, "View the Relative Quantification analysis and display settings").
  - View the graph as an image file.
- In the results table, you can:
  - Use the checkboxes to show or hide data in the bar graph. Click-drag or press **Shift** to select continuous rows, or press **Ctrl** to select discontinuous rows. Use the top checkbox to select or deselect all rows.
  - Select a color square in the Color column to replace the color associated with the data group.

– Use the table display settings to show, sort, or group the data (see "Change the display of table data" on page 42).

8 View the analysis results for each chip

To view the analysis results for each chip in a project:

1. Select the **See Results** tab of an open project.
2. If needed, select the **Replicates** tab if it is not already shown by default.

About the Relative Quantification replicate results

For Relative Quantification experiments, the Replicates tab reports the results for each chip in the project. Results can be grouped by replicate (chips assigned with the same sample, assay, and dilution) to help identify any outliers.

The Replicates table includes the following:

Column	Description
Assay	Assay name
Sample	Sample name
Dilution	Sample dilution factor entered for the chip
Chip	Chip assigned with the same sample, assay, and dilution
Target/Total	Calculated ratio of target dye signal over total dye (FAM™ + VIC®) signal from the target gene(s)
CI Target/Total	Lower and upper confidence interval for Target/Total dye signal ratio
Copies/Rxn (FAM and VIC)	Quantity of sample in copies/reaction well, for each dye
CI Copies/Rxn (FAM and VIC)	Lower and upper confidence interval for quantity of sample in copies/reaction well, for each dye
Copies/µL (FAM and VIC)	Quantity of sample in copies/µL, for each dye
CI Copies/µL (FAM and VIC)	Lower and upper confidence interval for quantity of sample in copies/µL, for each dye

Column	Description
# of Neg (FAM and VIC)	Total number of negative calls for each dye in the chip, as determined from the Review Calls scatter plot **Note:** Negative call means the software determines that a well does not contain any copy of the target labeled with the assigned target dye.
# of Undetermined	Total number of undetermined calls in the chip, as determined from the Review Calls scatter plot **Note:** Undetermined calls are those data points with unresolved signal, manual call, or originally below the default quality threshold then included at a lower quality threshold, generally located anywhere on the plot.
# qualified by QT	Total number of reaction wells in the chip that exceed the selected quality threshold
# of Filled	Total number of filled reaction wells in the chip
Run Date	Date and time the chip run was completed on the instrument
Flag	Quality flag for the chip, if present
Comment	Chip level comment, if entered on the Define Chips tab

Note: The calculated sample quantity (Copies/Rxn and Copies/µL) represents the concentration of the sample loaded into the chip, not the concentration of the original sample before dilution (stock concentration).

Review the Relative Quantification replicate results

Review the calculated ratios and quantities and the assigned calls shown in the Replicates tab for a Relative Quantification project, and omit any outliers.

- Select the results to display in the table (see "Change the display of table data" on page 42):
  - Drag a column heading above the table to group the table data by the selected attribute. To create a hierarchical grouping, repeat this process with additional columns.
  - Adjust additional table display settings to show or sort the table data.
- Select an experiment (.eds) file in the **Chip** column to review the chip quality and omit the chip from the results, if necessary.

9 View the Relative Quantification analysis and display settings

The See Results tab also contains the data analysis and display settings for the project, which you can select in the settings view at the top of the tab.

1. Click **Show settings** to open the settings view.
2. Select **Color by** to assign the bar graph with one of the default color categories (**Sample** or **Assay**), or by the **User defined** color selected in the Results table.
   The software automatically updates the data display in the bar graph, and in the Color column of the Results table.
3. Select the data analysis settings:
   - **Confidence level (%)** – Select the confidence level to use for the project, as shown in the bar graph and Results table. The default value is 95%.
   - **Desired precision (%)** – Select the desired precision for the software to use to generate recommended actions for the project. The default value is 10%.

     Note: If the calculated value shown in the Precision column of the Results table exceeds the desired precision entered here, the software may provide a suggestion for further dilutions of the PCR reaction mix to achieve the desired precision (see the Recommendation column for more information).

   The software automatically updates the data display in both the bar graph and Results table.
4. Click **Hide settings** to close the settings view.

About the confidence interval (CI)

The confidence interval (CI) calculations for the Absolute Quantity (AQ) in the AnalysisSuite™ Software only account for the Poisson error and result in a CI that is consistent with the random distribution of molecules across the chip assuming that the deposition of the molecules follows a Poisson process. For the CI around the Relative Quantity (RQ), the AQ of each target is first determined along with the CI around the AQ. The RQ is then calculated along with a CI around the RQ, consistent with the CI expected for the ratio of two types of target molecules distributed by two independent Poisson processes. If replicate chips are run, the combined RQ result

across the replicate chips is calculated using a weighted average of the RQ result from individual chips, where the weighting factor is derived from the inverse of the CI around the RQ value from each chip.

About precision

Precision refers to the ability to distinguish between two measurements (such as sample concentrations) with a certain confidence. The AnalysisSuite™ Software calculates precision as the ratio of the maximum deviation of the confidence bound or interval (numerator) to the mean value (denominator). For example:

$$\text{Precision} = \frac{\max\left(\text{abs}\left(\lambda_{\text{UpperCBound,LowerCBound}} - \lambda\right)\right)}{\lambda}$$

where λ `(lambda)` is the value you wish to estimate. In the software, λ is the sample concentration measured in copies/µL. The maximum deviation is used since the confidence interval (CI) is asymmetric.

Precision is used to express the tightness of the CI (see "About the confidence interval (CI)" on page 33). The lower the precision, the tighter the CI. For example, if the sample concentration estimate is 1 copy/µL and the 95% CI for this estimate is 0.9 to 1.1, the calculated precision would be 10% with a 5% chance that the true concentration is outside of the 0.9 to 1.1 CI, and assuming the only source of variation comes from the random distribution of molecules across a chip assuming that the deposition of the molecules follows a Poisson process.

Note: For the Relative Quantity (RQ) calculations in the AnalysisSuite™ Software, the Absolute Quantity (AQ) of each target is first determined along with the precision around the AQ, then the RQ is calculated. Precision around the RQ is not calculated by the software.

10

Export data

Export chip setup

You can export the setup information for each chip in a project into a comma-separated (.csv) file, for use with other projects and that you can open in a spreadsheet program such as Microsoft® Excel®.

1. Select the **Define Chips** tab of an open project.
2. Click **Export settings**.
   The software automatically exports all of the chip settings in the project to a comma-separated (.csv) file using the default file name (<project name>_Setup) and browser downloads location.

Export project level results

The Export tab contains an overview of the project data that you can export and open in a spreadsheet program such as Microsoft® Excel®.

1. Select the **Export** tab of an open project.
2. Review the information shown in each section:

Section	Description
Summary	Displays the setup properties and analysis settings for the project **Note:** The software automatically appends this summary to the top of each exported file.
Results	Displays the results for all chips that are assigned to the same sample group, which may include replicates and/or multiple dilutions
Replicates	Displays the results for all chips that are assigned to the same sample group and dilution
Digital calls	Displays the digital calls for each chip in the project

3. Adjust the table display settings as needed.

   Note: While you can adjust the display of data in each table for review purposes, the software will export all data using the default table display settings.

4. Click **Export**.
   The software automatically exports the project data to a comma-separated (.csv) file using the default file name (<project name>_export) and browser downloads location.

Export projects

Transfer one or more projects out to another work station running AnalysisSuite™ Software or into files that you can archive or pass to another user.

1. Select an analysis module from the QuantStudio™ 3D AnalysisSuite™ Software homepage.
2. In the Project Listing screen, use the checkboxes to select one or more projects to transfer out of the software.

   Note: Click-drag or press **Shift** to select continuous rows, or press **Ctrl** to select discontinuous rows. Use the top checkbox to select or deselect all rows.
3. Click **Export project(s)**.
   The software automatically exports a single project to a .las file or multiple projects to a .zip file using the project name and browser downloads location.

11 Manage the Results plot

Save the Results plot

You can save the plot shown in the See Results tab as a Portable Network Graphic (PNG) file.

1. Select the **See Results** tab of an open project.
2. Adjust the plot display settings as needed.
3. Click **View as image**, then click **Save As** in the image dialog box.
   The software automatically saves the image as a PNG file using the default file name (Result_Plot_<project name>.png) and browser downloads location.
4. Close the image dialog box.

Print the Results plot

You can print an image of the plot shown in the See Results tab.

1. Select the **See Results** tab of an open project.
2. Adjust the plot display settings as needed.
3. Click **View as image**, then click **Print** in the image dialog box.
   The software automatically opens the image in a new Untitled tab.
4. In the Print dialog box, select the printer and print options, then click **Print**.

   Note: Make sure to close the Untitled tab when the print is complete.
5. Close the image dialog box.

12 Manage projects

You can create new projects and manage existing projects in the software from the Project Listing screen, which shows all of the projects that are present in a selected analysis module.

View the list of projects

To view the Project Listing screen, first log into the software then:

- Select an analysis module from the QuantStudio™ 3D AnalysisSuite™ Software homepage.
- Click (**Home**) at the top of any screen in an open project.

  Note: To select a different analysis module, click **QuantStudio™ 3D AnalysisSuite™** at the top of any screen in the software.

Create a project

1. In the Project Listing screen, click **Create project**.
2. Enter a unique project name up to 100-characters in length.

   The project name:
   - Should be descriptive and easy to remember.
   - Cannot contain the following characters:
     % * ? | ; : , ! @ # $ ( ) < > / \ " ' ` ~ [ ] { } = & ^ -
   - Cannot end with a period (.).
3. Click **OK**. The software saves the project.
4. In the Import Data tab, import data into the project as needed.

Open a project

1. In the Project Listing screen, navigate to and select a project name in the Projects table to open it.

   Note: By default, a project opens to the Project Dashboard tab. Click the **Import Chips** link to import chips to an empty project.

2. Manage the project properties as needed.

Copy a project

You can create a copy of an existing project for use with the same Life Technologies™ user account.

1. Open the project to be copied (see "Open a project" on page 39).

   Note: Make sure to note down the original name for later use.

2. Rename the project (see "Rename a project" on page 40).

3. Export the renamed project from the software (see "Export projects" on page 36).

4. Rename the open project using the original project name noted in step 1.

   Note: Project names in the software must be unique.

5. Import the renamed project into the software (see "Import a project" on page 39).
   The software shows both project names (the original and the copy) in the Project Listing screen for the logged in user.

Import a project

You can use the Project Listing screen to import projects from another location or under a different name. Only files of type .las can be transferred to the AnalysisSuite™ Software.

1. In the Project Listing screen, click **Import project**, then navigate to and select the project (.las) file you wish to import into the software.

   IMPORTANT! Make sure the project you select to import has a unique project name (see "Rename a project" on page 40).

2. Click **Open** to add the project to the Projects table.

13 Manage project properties

View the project summary

The Project Dashboard tab contains a summary of the latest information about a project. You can review the project level notifications and results, and navigate to detailed setup and results screens from this tab.

1. Select the **Project Dashboard** tab of an open project, if not already selected.

   Note: By default, a project opens to the Project Dashboard tab. Click the **Import Chips** link to import chips to an empty project.

2. View a summary of the latest information about the project:
   - **Notifications** – Displays the data flags and comments for the project. From here, you can add new comments or reply to existing comments.
   - **Results to date** – Plots the analysis results for all chips in the project. Click **See details** to navigate to the See Results tab and review the results in more detail.
   - **Description** – Optionally, click to enter a description for the project if none is displayed in this field. The project description is included in the export of project data.
   - **Targets** or **Assays**, **Samples**, **Dilutions**, and **Chips** – Displays the chips and chip settings associated with the project. Click **See details** next to a setting to navigate to the Define Chips tab and review the settings in more detail.

3. Optionally, edit the following project properties from this tab:
   - Add a comment, or read and reply to comments in the project.
   - Enter a description for the project.

Rename a project

You can rename a project at any time (for example, if you wish to create a copy of an existing project).

1. Click the project name or **Edit project name** from any screen of an open project.

2. Enter a unique project name up to 100-characters in length.

   The project name:
   - Should be descriptive and easy to remember.
   - Cannot contain the following characters:

% * ? | ; : , ! @ # $ ( ) < > / \ " ' ` ~ [ ] { } = & ^ -

- Cannot end with a period (.).

3. Press **Enter**.

Add comments to a project

The Add Comments dialog box allows you to enter detailed information about the project (for example, observations about the data, reasons why you made specific decisions, and so on). You can add comments to a project at any time. You may prefer to enter comments after viewing and analyzing the data.

1. Click **Add comment** in the Notifications section of the Project Dashboard tab, or at the top of any other screen in an open project.

2. Enter up to 255 characters to associate with the project, then click **POST**.

   Note: After you click POST, the comment is time stamped and permanently recorded in the project, and it cannot be modified or removed.

3. (Optional) In the Notifications section of the Project Dashboard tab, click **Read/ Reply** and repeat step 2 to respond to a posted comment.

   Note: You can also add an editable comment to any chip in the project from the Define Chips tab.

14 Manage project display settings

Apply a filter

You can filter the chip data shown in the Import Data and Review Quality tabs as follows (for example, to show fewer chips or to view specific chip results):

- **Filter the chips to import** – In the Import Data tab, click **Show Filters**, then:
  - **Show by import state** – Select **Chips not imported yet** to view the remaining chips available for import into the project. Select **All Chips** to remove the filter criteria.
  - **Filter by run date** –
    - Select the **Run in the past** checkbox, then enter the run date settings for the chips to import. Deselect the checkbox to remove the filter criteria.
    - Select the **Date range of run** checkbox, then click to select a data range, or enter a data range for the chips to import. Deselect the checkbox to remove the filter criteria.

  The software automatically updates the chip data shown in the Import Data tab according to your filter criteria.

- **Filter the chip quality data** – In the Review Quality tab, click **Show Filters**, then:
  - **Search for a specific chip** – Enter all or part of the chip name you wish to view, then click **Go**. Click **Clear** to remove the filter criteria.
  - **Sort the chip results** – Select to sort by **Flagged chips** or **Recently ran chips**.

  The software automatically updates the chip data shown in the Review Quality tab according to your filter criteria.

Change the display of table data

You can use any of the following settings to change the display of table data in an open project, if available:

- **Show/hide columns** – Click to the right of a column heading, then select the contents to show or hide in the table from the **Columns** drop-down menu.

- **Sort column entries** – To sort column content:
  - Click once on a column heading to sort the rows in the table according to the content in the column. You can change the sorting order with a second click. A third click will clear the sorting order.

  - Click to the right of a column heading, then select to **Sort Ascending** or **Sort Descending** from the drop-down menu.

- **Change column order** – Click a column heading and drag the column to the desired position.

- **Group column entries** – Drag a column heading above the table to group the table data by the selected attribute. To create a hierarchical grouping, repeat this process with additional columns, then:
  - Click to expand all entries for a group and click to collapse the entries.
  - Click to sort the table data according to the selected group. You can change the sorting order with a second click. A third click will clear the sorting order.
  - Click to remove the group.

- **Adjust the viewable area of a table section** – Click-drag the split line to adjust the viewable area of each table section.

15 About the Software Quality Metrics

About chip quality

The software assesses whether the data on a chip is reliable based upon loading, signal, noise, and other data analysis characteristics for each reaction well (see "Factors contributing to quality value assessment" on page 44) and displays quality indicators for each chip in a project:

- **Quality threshold** – A measure of the individual well quality for a chip, on a continuous scale from 0 (low quality) to 1 (high quality). The default quality threshold is 0.5 (see "About the quality threshold" on page 46). You can adjust this scale to filter the wells (data points) to include or exclude from analysis (see "Set the quality threshold" on page 18).
- **Quality flag** – A measure of the overall chip quality, based on well quality thresholds and other data analysis characteristics. Use to determine which chips to review (see "About data quality flags" on page 47).

Factors contributing to quality value assessment

The quality value for each reaction well in a Digital PCR 20K Chip can help to eliminate areas of the chip compromised by faulty loading, presence of debris particles, or poor imaging.

The AnalysisSuite™ Software calculates a quality value for each reaction well based on the following factors, assessed in order:

1. Contrast between each reaction well and its surroundings (see "Contrast assessment" on page 45).
2. Spatial uniformity of positive and negative wells across the chip (see "Spatial uniformity assessment" on page 45).

After the software calculates a quality value for each reaction well in a chip, it applies a default quality threshold of 0.5 (see "About the quality threshold" on page 46), resulting in the automatic exclusion of reaction wells with quality values < 0.5 from further analysis. This allows the inclusion of unaffected areas of the chip in the results and helps to eliminate false positives from non-biochemical sources.

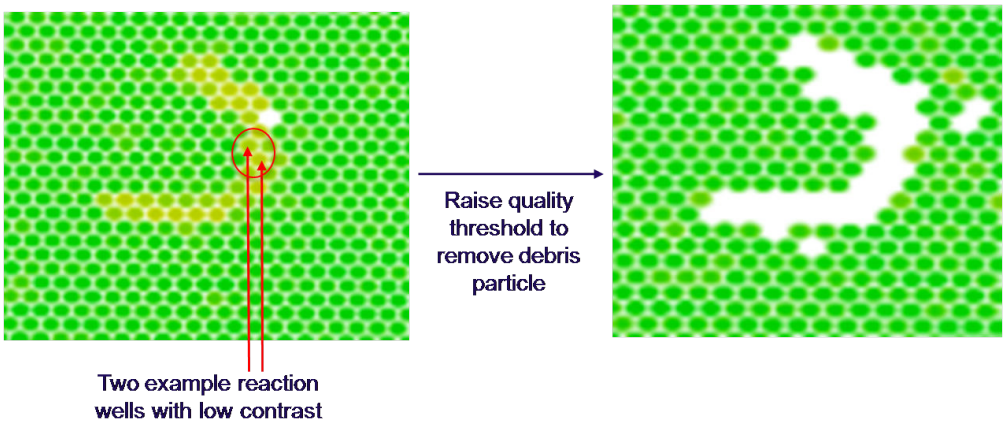
Note: Thermo Fisher Scientific has validated the system using the default quality threshold of 0.5. Increasing the quality threshold may not necessarily improve the accuracy of your results. We recommend adjusting the quality threshold if you observe obvious artifacts (such as debris, bubbles, or excess PCR reaction) which produce lower quality data points that can be excluded from analysis by increasing the quality threshold. See Chapter 16, "Troubleshooting" for more information.

Contrast assessment

The AnalysisSuite™ Software identifies the contrast between each reaction well in a chip and its surroundings by comparing the signal level in the reaction well to the signal level of the surrounding area.

A high contrast is expected *regardless of well concentration*, while areas with low contrast receive low quality values and are used to identify and remove the effects of debris on the chip or chip lid.

In the example below, a chip is shown in the Review Quality tab using the **Color by quality** option, then a higher quality threshold is applied to remove the debris particle from further analysis (see "Set the quality threshold" on page 18).



Spatial uniformity assessment

The factors contributing to spatial uniformity across the chip are assessed in the following order:

1. Identification of positives and negatives for each dye using the distribution in a fluorescence histogram (see "Positive and negative uniformity" on page 45).
2. Spatial distribution of FAM™ and/or VIC® dye signal (see "Dye signal uniformity" on page 46).

Positive and negative uniformity

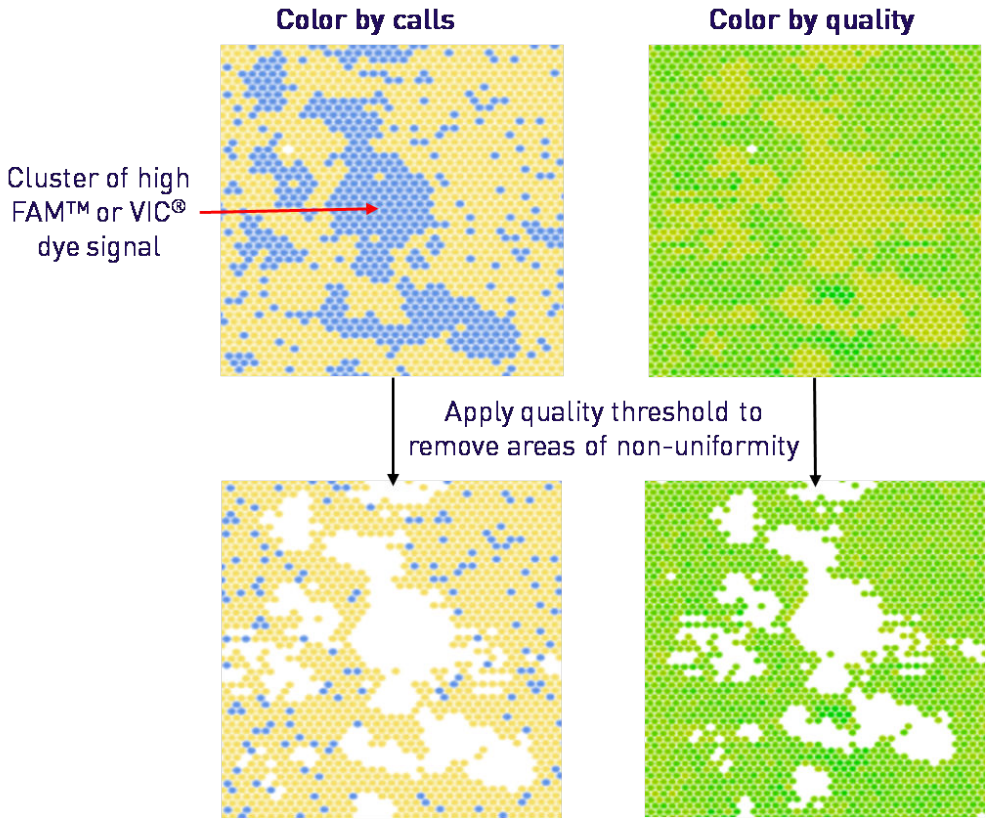
To identify the positive and negative calls in a chip, the AnalysisSuite™ Software uses a histogram of the dye fluorescence values to automatically set a call discrimination threshold for the chip, then determines the positive or negative call for each dye in each reaction well on the chip. The location of the reaction well on the chip and the call it receives is used to assess the spatial uniformity of the distribution of the calls across the chip (see "Dye signal uniformity" on page 46).

Note: The positive and negative call determination is based upon an automatic call threshold of the unfiltered data. For example, the threshold assessment is most effective with a bi-modal (two peak) distribution of fluorescent values for each dye.

Dye signal uniformity

To assess the spatial distribution of positives and negatives across the chip, the AnalysisSuite™ Software uses an algorithm to compare the expected degree of clusters (connectivity) of high FAM™ or VIC® dye signal *at a given concentration* against the observed connectivity to calculate the uniformity component of the per well quality value. A uniform distribution of positive calls is expected. Reaction wells forming groups of positives that cluster differently from what is expected at the estimated concentration are assigned lower quality values. Note that this algorithm is only applicable at concentrations *under a certain value*, where predicting the degree of connectivity makes sense.

Among other artifacts, this algorithm helps to identify the unexpected transfer of fluid across wells (bridging) on a chip. In the example below, a chip is shown in the Review Quality tab using the **Color by calls** option to identify areas of non-uniformity. From the **Color by calls** option, a higher quality threshold is applied to remove the areas of non-uniformity from further analysis (see "Set the quality threshold" on page 18).



About the quality threshold

The quality threshold in the AnalysisSuite™ Software is set at 0.5 by default. This default was empirically set by Thermo Fisher Scientific using a representative sample of chips with higher and lower quality images (including clean and dirty chips, bridging, and loading failures) and was selected to automatically exclude reaction wells with quality values below the threshold from further analysis.

To use the quality threshold in your analysis, we recommend that you review the data in the Review Quality tab for any chips that display the (yellow) flag (see Chapter 5, "View the data quality for each chip"), then use the:

- **Color by calls** view to assess the spatial uniformity of the positive and negative calls across the chip (see "Spatial uniformity assessment" on page 45).
- **Color by quality** view to adjust the quality threshold as needed to exclude wells that are of lower quality than surrounding higher quality wells but are not automatically filtered from the analysis using the default threshold of 0.5. For example, you may wish to exclude wells that contain obvious artifacts (such as debris), that are within clusters of positive or negative wells, or that lie between the main positive and negative call distributions.

Note: Thermo Fisher Scientific has validated the system using the default quality threshold of 0.5. Increasing the quality threshold may not necessarily improve the accuracy of your results. We recommend adjusting the quality threshold if you observe obvious artifacts (such as debris, bubbles, or excess PCR reaction) which produce lower quality data points that can be excluded from analysis by increasing the quality threshold. See Chapter 16, "Troubleshooting" for more information.

About data quality flags

Data quality flags generated and displayed by the software are a measure of the overall chip quality, based on well quality thresholds and other data analysis characteristics.

In order, from high quality to low quality:

- (green) – Review of the analysis results is not required. This flag is shown if the data meets all quality thresholds.
- (yellow) – Review of the analysis results is suggested. This flag is shown if the:
  - Instrument cannot clearly identify the population of unamplified wells.
  - Distribution of unamplified wells on the chip is not uniform.
  - Sample concentration is outside the optimal range (200-2000 copies/µL) for the system as currently defined by the Thermo Fisher Scientific chip quality metrics.

    Note: For more information on sample concentration, see the *QuantStudio™ 3D Digital PCR System User Guide*.
- (red) – Review of the analysis results is strongly suggested. If necessary, reimage the Digital PCR 20K Chip or rerun the sample. This flag is shown if the total number of filled wells is < 5000 or the percentage of low quality wells (those with well quality < 0.5) is > 15%.
- (green), (yellow), or (red) – Indicates that a user has modified the analysis results originally generated by the instrument software.

  Note: To revert an edited flag to its default state, delete the associated chip from the project, then import it back into the project.

Note: For single reporter experiments, the software displays a quality flag if it is associated with the selected target dye. For dual reporter experiments, the software displays the lowest quality flag associated with the target dyes.

16 Troubleshooting

Troubleshooting quality flags in the software

The following table summarizes some of the more common problems that can trigger a data quality flag as viewed in the AnalysisSuite™ Software. See Chapter 15, "About the Software Quality Metrics" for more information about the data quality assessment process used by the software.

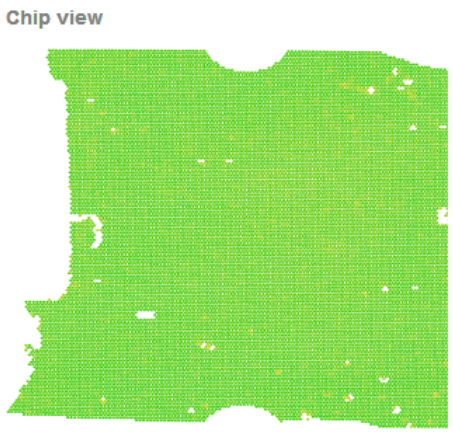
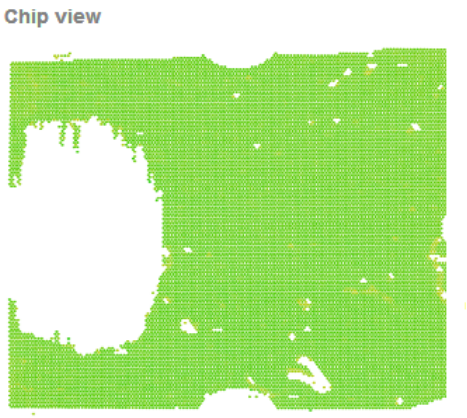
Observation	Possible Cause	Action
Chip displays flag.	The total number of filled wells is below the default threshold of 5000 or the percentage of low quality wells is above the default threshold of 5%.	Look for any visible chip failures (for example, leaking or a large bubble, liquid on the chip lid, cracked chip, and so on): • If present – Discard the chip and run the sample again. • If not present – Re-image the chip and import the new experiment file into the software. If the chip still displays a flag, discard the chip and run the sample again.
Chip displays flag.	The sample concentration is outside of the optimal range (200-2000 copies/µL) for the system as currently defined by the Thermo Fisher Scientific chip quality metrics.	Run the sample again at a higher dilution. If the chip still displays a flag, then troubleshoot the chip image in the software (see "Troubleshooting chip images using the Chip View" on page 49). **Note:** Refer to the dilution recommendation shown in the Recommendation column of the Results tab.

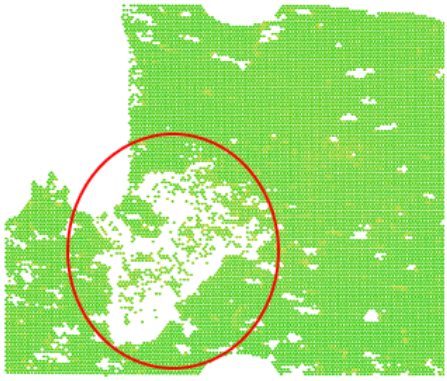
Observation	Possible Cause	Action
Chip displays flag. *(continued)*	The instrument cannot clearly identify the population of unamplified wells.	Review the data in the Review Quality tab of the Relative Quantification module. In the Histogram view, if you observe: • A single peak (mono-modal) – Confirm that the call threshold is on the appropriate side of the peak based on the chip settings (for example, NTC or low dilution). Manually adjust the call threshold to improve the call assignment accuracy, if necessary. • Two peaks (bi-modal) with poor separation or more than two peaks – – Increase the quality threshold to create a more bi-modal distribution, if possible. Make sure to balance the number of data points included in the analysis against the desired data quality and call separation. – Manually adjust the call threshold to improve the call assignment accuracy. If this is not possible, omit the chip from the results. **Note:** If you set up both target dyes for the chip, review the data in the scatter plot view of the Relative Quantification module and verify that calls are clearly separated in the plot.
	The distribution of unamplified wells on the chip is not uniform.	Review the data in the Chip view of the Review Quality tab. Select the **Color by calls** data point color option, then: 1. Verify that calls are randomly distributed across the chip. 2. Increase the quality threshold to adjust the negative call distribution across the chip, if possible. Make sure to balance the number of data points included in the analysis against the desired data quality and call distribution. 3. (Optional) If calls are still not randomly distributed across the chip, omit the chip from the results.

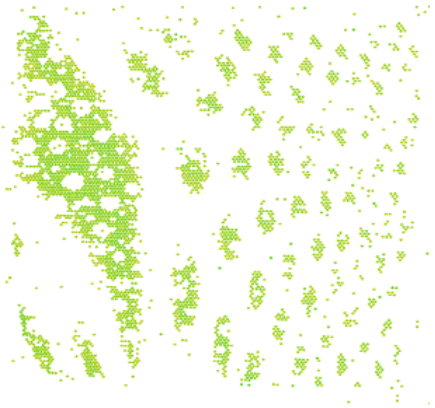
Troubleshooting chip images using the Chip View

The following table summarizes some of the more common problems that can affect imaging of Digital PCR 20K Chips.

Note: In the examples shown below, the conditions triggered a data flag in the Digital PCR 20K Chip and the conditions were diagnosed using the Chip View of the AnalysisSuite™ Software.

Observation	Possible Cause	Action
	Debris or other contaminants are present on the Chip Sealant during imaging.	No action required. The AnalysisSuite™ Software can compensate for small quantities of debris or other contaminants on the Digital PCR 20K Chip.
	A bubble was present in the Sample Loading Blade when it was used to apply the PCR reaction to the Digital PCR 20K Chip.	If possible, use the AnalysisSuite™ Software to filter the low quality data points, or discard the chip and run the sample again. When loading the Sample Loading Blades: • If you are using a manual pipette, pipette to the first stop. • If you are using an electronic pipette, decrease your pipetting speed. • If a bubble does form in the Sample Loading Blade, gently tap it to remove bubble before loading.

Observation	Possible Cause	Action
Chip view  Chip view 	• Excess PCR reaction was present on the Digital PCR 20K Chip after loading it with the Sample Loading Blade. • The Sample Loading Blade was drawn across the chip too quickly or at an angle shallower than 70-80°.	If possible, use the AnalysisSuite™ Software to filter the low quality data points, or discard the chip and run the sample again. To prevent leaving excess PCR reaction on the Digital PCR 20K Chips: • Confirm that the heated block used to load Digital PCR 20K Chips is set to 40±1°C. • Make sure to wait 20 seconds after loading a Digital PCR 20K Chip before pre-wetting it with Immersion Fluid. • Make sure to install theTilt Base to the GeneAmp® PCR System 9700. You must thermal cycle the Digital PCR 20K Chips at an 11° angle. • Make sure to draw the Sample Loading Blade across the chip slowly (>10 seconds) and at a 70-80° angle.

Observation	Possible Cause	Action
Chip view  Chip view 	• The Digital PCR 20K Chip leaked during thermal cycling or imaging. • A large bubble was present in the chip (insufficient Immersion Fluid). • Immersion Fluid was not applied to the chip immediately after loading (evaporation of the PCR reaction). • Excess Immersion Fluid is present on the Chip Case Lid.	If present, remove excess Immersion Fluid from the chip lid and run the chip again. If possible, use the AnalysisSuite™ Software to filter the low quality data points. Make sure to apply Immersion Fluid to each chip immediately after loading it with PCR reaction. To minimize leakage, when sealing each Digital PCR 20K Chip: • Wear correctly fitted gloves to prevent the glove material from snagging during lid application. • Make sure that the Chip Case Lid is correctly aligned to the Chip Case. • Firmly press all four corners when applying the Chip Case Lid. • Cure the Chip Sealant under ultraviolet light for at least 30 seconds.

Troubleshooting analysis results

The following table summarizes some of the more common problems that can affect the analysis results for a project as viewed in the AnalysisSuite™ Software. In some of the examples shown below, the conditions triggered a data quality flag.

Observation	Possible Cause	Action
The See Results tab does not display numerical results after combining multiple chips with the same data group (sample and target or assay combination).	The software cannot calculate numerical results for one or more of the chips in the data group. For example, the sample concentration is either too high or too low.	Omit the chip(s) that do not contain numerical results from the project.
The calculated call threshold does not update in the Review Quality tab after increasing the quality threshold.	The number of data points is less than the minimum threshold of 5000.	Decrease the quality threshold to include more than 5000 data points.

Documentation and support

Related documentation

Document	Publication number	Description
QuantStudio™ 3D Digital PCR System User Guide	MAN0007720	Describes the QuantStudio™ 3D Digital PCR System hardware and software and provides information on preparing, maintaining, and troubleshooting the system.
QuantStudio™ 3D Digital PCR System Quick Reference	MAN0008159	Describes the QuantStudio™ 3D Digital PCR System hardware and software and provides brief, step-by-step procedures for common tasks.
QuantStudio™ 3D AnalysisSuite™ Server System Quick Reference	4486248	Describes the AnalysisSuite™ Server System hardware and software and provides information on installing the system and brief, step-by-step procedures for common tasks.

Portable document format (PDF) versions of related documentation are available at **http://www.lifetechnologies.com/quantstudio3d**.

Note: To open the user documentation, use the Adobe® Reader® software available from **www.adobe.com**

Note: For additional documentation or if you cannot access the user documentation, see "Obtaining support" on page 54.

Obtaining support

For the latest services and support information for all locations, go to:

www.lifetechnologies.com/support

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)

- Submit a question directly to Technical Support (**techsupport@lifetech.com**)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

Headquarters
5791 Van Allen Way | Carlsbad, CA 92008 USA | Phone +1 760 603 7200 | Toll Free in USA 800 955 6288
For support visit **lifetechnologies.com/support** or email **techsupport@lifetech.com**
lifetechnologies.com

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