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Introduction

Welcome to Q-View Software

Q-View™ Software is a tool for the quantitative analysis of multiplexed chemiluminescent planar-based arrays. Analysis is performed in three easy steps (Figure 1) which are covered in detail in this manual:

- **Image Processing**: Capture or import images, and align the plate overlay.
- **Well Assignment**: Group, name, and apply dilution factors to wells.
- **Data Analysis**: Customize and export concentrations, statistics, and charts.

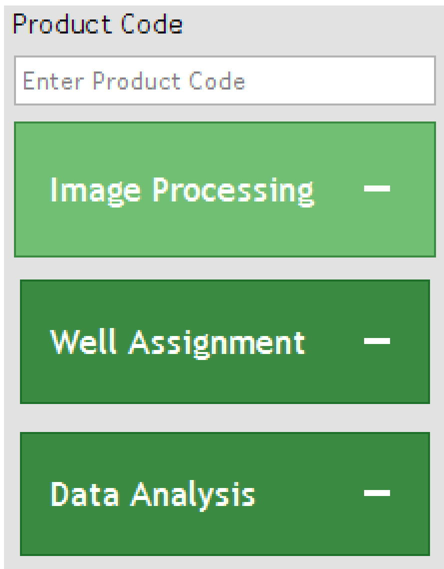


Figure 1. Q-View Software workflow.

Terminology

- **Q-Plex™**: The brand name of the multiplex ELISA kits manufactured by Quansys Biosciences.
- **Q-View™ Imager LS**: The brand name of the life science chemiluminescent imager produced by Quansys Biosciences.
- **Q-View™ Imager Pro**: The brand name of the first clinical-grade chemiluminescent imager produced by Quansys Biosciences.
- **Q-View™ Imager Plus**: The brand name of the latest clinical-grade chemiluminescent imager produced by Quansys Biosciences.
- **Q-View™ Software**: The brand name of the multiplex ELISA analysis software produced by Quansys Biosciences.
- **Project**: A file with the ".Q-View" extension. Projects contain images, plate overlays, well assignments, and data for one or more plates.
- **Plate Overlay**: The well diagram of the plate that is aligned by the user over the plate image comprising the spots within the well.
- **In-Kit Certificate of Analysis**: An In-Kit Certificate of Analysis is included with every Q-Plex kit. This card includes information such as the software product code, the calibrator lot number, and the calibrator and/or control reconstitution volumes. It also includes a

table of the analytes tested by the kit, the associated calibrator concentrations, and a graphical representation of the spotted array.

- **Software Product Code**: The alphanumeric code that defines parameters such as the number and position of the spots in each well, the concentration of each analyte in the calibrator, the optimal curve fit settings, and control ranges when applicable.

Projects

Q-View Software stores the images and analyses of related plates together in a file, called a project, with the ".Q-View" extension. As you work, changes to these projects are saved automatically. Multiple projects can be open at the same time (Figure 2).

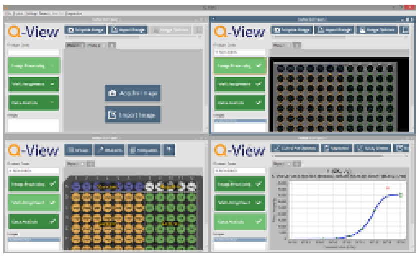


Figure 2. Multiple projects opened.

Before version 3.1, projects were stored as folders. Project folders can still be opened by the software but they will be upgraded to the project file format when opened. Project files are specific to Q-View Software and cannot be viewed or manipulated by 3rd party software.

Project details can be accessed by opening **Project > Project Details**. The project creator, creation date, and a list of users that have opened the project along with associated timestamps are recorded.

Project notes can be recorded and viewed by opening **Project > Project Notes**. These notes allow users to record read-only messages related to the project.

Whenever a project is closed or a report is exported, the full report is recorded if it has changed in any way. **Project history** can be viewed by opening **Project > Project History**.

Projects saved on a network need to be accessed through mapped network drives (e.g. Z:\myProject.Q-View) and not network locations (e.g. \\server\myProject.Q-View).

Quick Start Guide

1. Installation
   a. Install Q-View Software on any number of computers.
   b. Set up your imager and any other equipment to be used for the experiment.
   c. Using a Q-View imager, ensure the imager has been focused, dark field images have been captured, and set-up is complete before proceeding. All of these

procedures can be found in the Q-View Imager LS, Pro, and Plus manuals.

2. Process and image the Q-Plex kit
    a. Process the Q-Plex kit according to the instructions in the kit manual.
    b. **Capture the image(s)** using a Q-View imager or other compatible, third-party imager.
    With third-party imagers, only grayscale, TIFF and CR2 image files of at least 12 bits per pixel should be imported for scientific reporting purposes. Images with a white background must be inverted within Q-View Software by clicking **Image Options > Invert Image** .
3. Analyze images in Q-View Software
    a. Enter the software product code (found on the In-Kit Certificate of Analysis) for each plate.
    b. Within **Image Processing**, set the plate overlay.
    c. Perform **Well Assignment**.
    d. Within **Data Analysis**, click **Perform Analysis** to generate charts and reports. Customize and export the results as desired.

Q-View Installation

Software Installation

1. Ensure the computer meets the following minimum specification:
    - Windows® 10 or 11
    Note: The Windows® version of the Q-View Software is required to operate Q-View imagers. The Mac® operating system is not compatible with Q-View imagers.
    - Mac® OS 10.15 or newer
    - 4 processing cores
    - 8 GB RAM
    - USB 2.0 (Type A) or greater for Imager LS and Imager Pro. USB 3.0 (Type A) or greater for Imager Plus. USB C is not supported.
    - Screen resolution of 1280x800 or greater

    For optimal performance:
    - 16+ GB RAM
    - 8 or more processing cores
2. If you do not have a Q-View Software installer, contact a Quansys Biosciences representative by email at sales@quansysbio.com or call 1-888-782-6797.
3. Launch Q-View Software. Click either **Evaluate Q-View** to start a 60 day trial or enter your assigned company name and license key and then click **Continue**. Follow the prompts on the screen to set up user accounts and Q-View imagers. You can change these settings later from the **Settings** menu.

Imager Installation

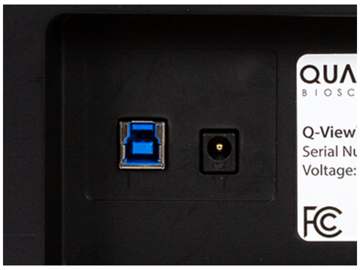


Figure 3. USB and power sockets.

1. Carefully unpack the Q-View imager and place it on a clean work area.
2. Plug the power cord into the back of the imager (Figure 3) and into a surge protector.
3. Plug the USB cord into the back of the imager (Figure 3) and into the computer. For the Q-View Imager Pro and the Q-View Imager Plus, ensure the power switch is in the on position. The Q-View Imager Plus requires USB 3.0 or greater.
4. Clean the tray glass with a lint-free cloth and ethanol, rubbing alcohol, or window cleaner.
5. Launch Q-View Software and open **Settings > Manage Imagers** to discover, name, and focus the imager and to capture a dark field. Dark field capture takes approximately 15 minutes on the Q-View Imager LS and the Q-View Imager Pro, and 35 minutes on the Q-View Imager Plus.

Q-View Imager Pro Notes

- The Q-View Imager Pro requires drivers that are not bundled with the Q-View Software installer. Please contact Quansys Biosciences to obtain and install these additional drivers if needed.
- The button on the front of the Q-View Imager Pro opens and closes the tray. Its color represents the following.
  - **Green**: the imager is on and the tray can be opened
  - **Red**: the tray is open
  - **Yellow**: images are being acquired and the tray is locked
  - **Flashing Red**: the imager is alerting the user to an abnormal condition
- Q-View Software turns the imager on after it is detected and turns it off when the application closes.
- The sensor is cooled to deliver highly reproducible imaging. Cooling will be turned off after one to four hours of inactivity based on your preference. Cooling will be turned back on when image capture begins or sooner if the **Activate Now** link is clicked.
- If the sensor is not at the optimal temperature when the user attempts to capture an image, image capture will be postponed until the temperature target has been reached (generally about one minute).
- Keep the cooling vents on the bottom, sides, and back clear of debris. If the vents are obstructed the imager may overheat. When overheated, the sensor's cooler will be turned off, the open/close button will flash red, and image capture will be prevented.

Q-View Imager Plus Notes

- The Q-View Imager Plus requires drivers that are not bundled with the Q-View Software installer. Please contact Quansys Biosciences to obtain and install these additional drivers if needed.
- The button on the front of the Q-View Imager Plus opens and closes the tray.
- The LED light on the front of the Q-View Imager Plus will change color to indicate the status of the device. The indicator color scheme has been designed to be user friendly for those with normal vision and common forms of color blindness. The table below is the legend for status light indication.

LED Color	Activity	Imager Status
White	Solid	Startup
White	Pulse	Sleep
White	Strobe	Firmware update
Magenta	Solid	Idle, software not connected
Blue-green	Solid	Idle, software connected
Yellow	Solid	Tray in motion or open
Yellow	Strobe	Tray obstruction
Blue	Solid	Plate imaging
Blue	Pulse	Imaging with internal lights
Red	Solid	Fatal error

  Activity Definitions

  - Solid: Constant with no flashing or dimming
  - Pulse: Slow dimming and brightening
  - Strobe: Rapid flashing

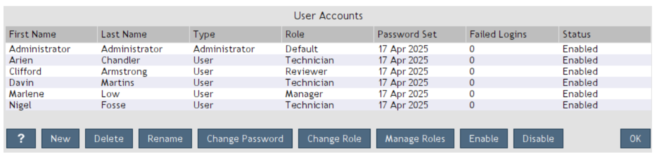
  If 'fatal error' occurs, contact the manufacturer for support.
- The sensors are cooled to deliver highly reproducible imaging. Cooling will be turned off after five minutes of inactivity. Cooling will be turned back on when image capture begins or sooner if the **Activate Now** link is clicked.
- If the sensors are not at the optimal temperature when the user attempts to capture an image, image capture will be postponed until the temperature target has been reached (generally about one minute).
- Keep the cooling vent on the bottom and back clear of debris. If the vents are obstructed the imager may overheat. When overheated, image capture will be prevented.

- The Q-View Imager Plus is designed to operature within an ambient temperature range of 15°C (59°F) to 27°C (80°F). If the ambient temperature falls outside of this range, sensor cooling may be affected and imaging may be unavailable.
- The Q-View Imager Plus is focused during manufacturing and is not user-focusable.
- The Q-View Imager Plus comes equiped with a barcode scanner. Q-View's barcode catalog needs to be kept up-to-date for the scanner to read your plate's product code. See Software and Product Updates for details.
- When importing archived images captured by a Q-View Imager Plus, Q-View needs to be connected to the imager and all four images need to be imported at the same time. This will allow the images to processed and combined into a single image as opposed to importing four distinct, unprocessed images.
- USB 3.0 (Type A) or higher is required. The imager will not connect when plugged into USB 1 or USB 2. USB Type C to Type A conversion is also not supported.

Access Control

User Management

When user accounts are enabled, users are required to log in to use the software. Administrators can manage users during software setup or by opening **Settings > Administration > User Accounts** (Figure 4).



User Accounts

First Name	Last Name	Type	Role	Password Set	Failed Logins	Status
Administrator	Administrator	Administrator	Default	17 Apr 2025	0	Enabled
Arien	Chandler	User	Technician	17 Apr 2025	0	Enabled
Clifford	Armstrong	User	Reviewer	17 Apr 2025	0	Enabled
Davin	Martins	User	Technician	17 Apr 2025	0	Enabled
Marlene	Low	User	Manager	17 Apr 2025	0	Enabled
Nigel	Fosse	User	Technician	17 Apr 2025	0	Enabled

Figure 4. User accounts.

- **New**: Create a new user.
- **Delete**: Delete the selected user(s).
- **Rename**: Rename the selected user.
- **Change Password**: Set the password of the selected user(s).
- **Change Role**: Set the role of the selected user(s).
- **Manage Roles**: Open user role management.
- **Enable**: Enable the selected user(s). Enabled users can log in.
- **Disable**: Disable the selected user(s). Disabled users cannot log in.

Password Policy

Administrators can set their organization's password policy by opening **Settings > Administration > Password Policy** (Figure 5). The password policy defines the following password requirements:

- **Minimum length**: The minimum number of characters.

- **Minimum lower case**: The minimum number of lower case characters.
- **Minimum upper case**: The minimum number of upper case characters.
- **Minimum numeric**: The minimum number of numeric characters.
- **Minimum special**: The minimum number of special characters.

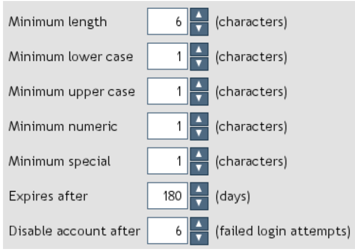


Figure 5. Password policy.

- **Expires after**: The number of days from the day the password was set until the password expires. When a user's password expires, the user will be forced to set a new password at the next successful login.
- **Disable account after**: The number of failed login attempts before the user account is disabled. When a user account is disabled, the user is prevented from logging in until an administrator enables the account. When set to zero, there is no limit to the number of failed login attempts.

User Roles and Permissions

Administrators can manage user roles by opening **Settings > Administration > User Roles** (Figure 6). User roles define permissions which allow or disallow users from accessing various software features. By default, users are assigned the **Default** role which allows all permissions. The default role permissions cannot be modified. Users can view their permissions by opening **Settings > Permissions** (Figure 7).

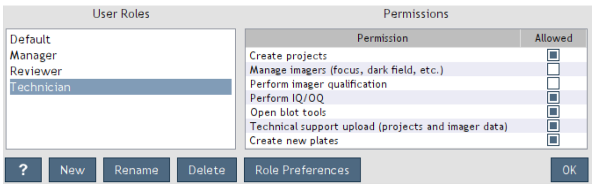


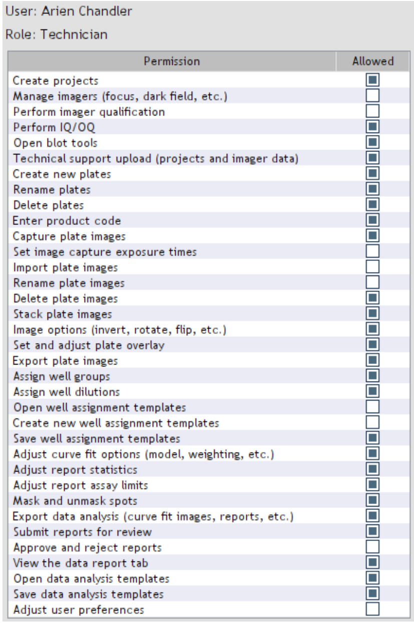
Figure 6. User roles and associated permissions.

- **New**: Create a new user role.
- **Rename**: Change the name of the selected user role.
- **Delete**: Delete the selected user role.
- **Role Preferences**: Set preferences that are applied to the users assigned to the role.

Permissions are granted by selecting the **Allowed** check box adjacent to the permission (Figure 6). Permissions are revoked by deselecting the check box. The permissions are described as follows:

- **Create projects**: Allows users to create new projects.
- **Manage imagers**: Allows users to manage imagers.
- **Perform imager qualification**: Allows users to perform imager qualification.

- **Perform IQ/OQ**: Allows users to perform imager and software IQ/OQ.
- **Open blot tools**: Allows users to open and use blot tools.
- **Technical support upload**: Allows users to upload projects and imager databases for customer support.
- **Create new plates**: Allows users to create new plates.
- **Rename plates**: Allows users to rename existing plates.
- **Delete plates**: Allows users to delete existing plates.
- **Enter product code**: Allows users to enter product codes.
- **Capture plate images**: Allows users to capture plate images.


User: Arien Chandler

Role: Technician

Permission	Allowed
Create projects	☑
Manage imagers (focus, dark field, etc.)	☐
Perform imager qualification	☐
Perform IQ/OQ	☑
Open blot tools	☑
Technical support upload (projects and imager data)	☑
Create new plates	☑
Rename plates	☑
Delete plates	☑
Enter product code	☑
Capture plate images	☑
Set image capture exposure times	☐
Import plate images	☐
Rename plate images	☐
Delete plate images	☑
Stack plate images	☑
Image options (invert, rotate, flip, etc.)	☑
Set and adjust plate overlay	☑
Export plate images	☑
Assign well groups	☑
Assign well dilutions	☑
Open well assignment templates	☐
Create new well assignment templates	☐
Save well assignment templates	☑
Adjust curve fit options (model, weighting, etc.)	☑
Adjust report statistics	☑
Adjust report assay limits	☑
Mask and unmask spots	☑
Export data analysis (curve fit images, reports, etc.)	☑
Submit reports for review	☑
Approve and reject reports	☐
View the data report tab	☑
Open data analysis templates	☑
Save data analysis templates	☑
Adjust user preferences	☐

Figure 7. User permissions.

- **Set image capture exposure times**: Allows users to set image capture exposure times. When this permission is revoked, the recommended exposure time for the connected imager will be used.
- **Import plate images**: Allows users to import plate images.
- **Rename plate images**: Allows users to rename plate images.
- **Delete plate images**: Allows users to delete plate images.
- **Stack plate images**: Allows users to stack plate images. Image stacking is a legacy image processing technique that combines multiple images of varying exposure times into a single image.
- **Image options**: Allows users to access image options which include inverting, rotating and flipping horizontally and vertically.
- **Set and adjust plate overlay**: Allows users to set and adjust plate overlays which include spot and well adjustment, auto-adjust spots and reset spots.
- **Export plate images**: Allows users to export plate images as TIF and JPG files.
- **Assign well groups**: Allows users to assign and modify well groups. Renaming existing wells is always allowed.
- **Assign well dilutions**: Allows users to assign and modify well dilutions.
- **Open well assignment templates**: Allows users to open and apply well assignment templates.
- **Create new well assignment templates**: Allows users to create new well assignment templates.
- **Save well assignment templates**: Allows users to save well assignment templates.

- **Adjust curve fit options**: Allows users to adjust curve fit options which include regression models, weighting and negative well subtraction.
- **Adjust report statistics**: Allows users to adjust report statistics which include pixel intensity, concentration, percent backfit and assay statistics.
- **Adjust report assay limits**: Allows users to adjust report assay limits which include assay and lot LLD, LLOQ and ULOQ values.
- **Mask and unmask spots**: Allows users to mask and unmask spots individually or by well.
- **Export data analysis**: Allows users to export data analysis comprising charts and reports.
- **Submit reports for review**: Allows users to submit reports for review. This permission only applies when the Require reviewer signature option is selected.
- **Approve and reject reports**: Allows users to approve or reject reports that have been submitted for review. This permission only applies when the Require reviewer signature option is selected.
- **View the data report tab**: Allows users to view the data tab. This is the report that shows either pixel intensities or concentrations.
- **Open data analysis templates**: Allows users to open and apply data analysis templates.
- **Save data analysis templates**: Allows users to save data analysis templates.
- **Adjust user preferences**: Allows users to adjust their preferences. Default preferences can be defined in User Roles.

Electronic Signatures

When documents are saved, electronic signatures (Figure 8) are required if user accounts are enabled. The name of the signee, a reason for the signature, and a timestamp are appended to the document. Electronic signatures apply to the following:

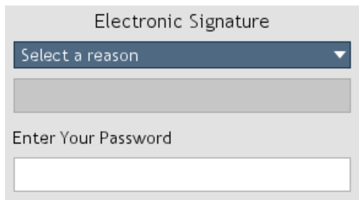


Figure 8. Electronic signature.

- **Well assignment templates**: When a well assignment template is saved, an electronic signature is required. The template file is encrypted to prevent modification.
- **Data analysis templates**: When a data analysis template is saved, an electronic signature is required. The template file is encrypted to prevent modification.
- **Report review submission**: When a report is submitted for review, an electronic signature is required.
- **Report approval**: When a report is approved, an electronic signature is required.

- **Report rejection**: When a report is rejected, an electronic signature is required.
- **Report exporting**: When a report is exported, an electronic signature is required. The signatures are found at the bottom of the exported CSV file.
- **Application settings**: When settings are exported, an electronic signature is required. The settings file is encrypted to prevent modification.

Electronic signature settings (Figure 9) can be accessed by opening **Settings > Administration > Electronic Signature Settings**. You can create or delete signature reasons for specific categories. The order of the reasons can by modified by moving them up or down in the list.

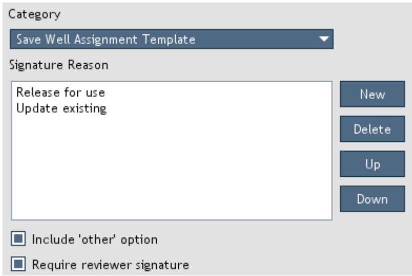


Figure 9. Electronic signature settings.

When the **Include 'other' option** is selected, an 'other' option will be included in the reason drop-down list (Figure 8). A free-form text field will be enabled allowing the user to enter a custom reason. When the **Require reviewer signature** option is selected, exporting reports requires that they first be submitted for review by users with the **Submit reports for review** permission and subsequently approved by users with the **Approve and reject reports** permission.

Image Processing

Image Acquisition

1. Place the plate in the Q-View imager and close the lid/tray. Select the desired imager and enter the exposure time(s) and image name(s) (Figure 10). If the desired imager is not listed, select **Refresh** from the drop-down to scan for imagers.

   The recommended exposure settings for Q-Plex kits are:
   - Q-View Imager LS: 270 seconds
   - Q-View Imager Pro: 300 seconds
   - Q-View Imager Plus: 300 seconds

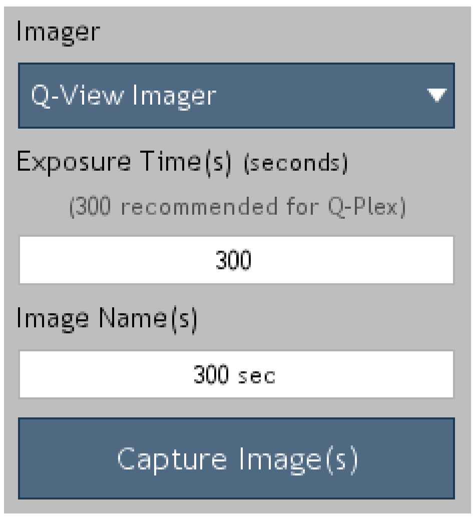


Figure 10. Image acquisition options.

2. Capture the image(s) by clicking **Capture Image(s)**. You can continue to **Well Assignment** while images are being captured.
3. Add a plate tab for each physical plate to be imaged by clicking the **+** tab (Figure 11). Custom plate names can be set by double clicking the plate name.

Figure 11. Plates organized by tabs.

Note: The **Enable Internal Illumination** option is available for Q-View Imager Pro models when activated in **Settings > Preferences**. When enabled, the imager's internal focus lights are turned on during image acquisition. This may be useful for quality control but should not be enabled when capturing Q-Plex images.

Import Images

Before importing an image, ensure it is grayscale and has a pixel depth of at least 12 bits. Q-View Software can open TIFF, CR2, BMP, PNG, and JPEG files. Low bit depth and lossy images (BMP, PNG, and JPEG) are insufficient for analysis and should be imported for display purposes only.

To import an image, click **Image Processing > Import Image** or drag and drop image files onto the list of images.

Manage Images

Images can be renamed, deleted, or stacked by right-clicking an image name (Figure 12). Image details can be viewed by selecting properties. Click **Export** to save an image as a lossless TIFF or compressed JPEG. JPEG files are suitable for display purposes only and should not be used for analysis.

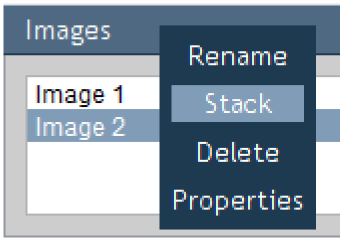


Figure 12. Image pop-up menu.

Align Plate Overlay

1. Enter the **Software Product Code** from the In-Kit Certificate of Analysis into the **Product Code** field.
2. Use **Image Options** to adjust image gamma, zoom, and orientation as desired. Images with black spots on a white background need to be inverted by clicking **Image Options > Invert Image**.
3. If you are using the **Auto-Set Plate Overlay** feature, the initial overlay position will be set automatically. Otherwise, click **Overlay Options > Set Plate Overlay**, and click and drag the mouse cursor from the center of the top-left well to the center of the bottom-right

well. Use the following **Overlay Options** (Figure 13), if needed, to manually adjust the overlay:

- **Set Plate Overlay**: Set the plate overlay by dragging from the center of the top-left well to the center of the bottom-right well.
- **Adjust Plate Overlay**: Pivot or expand/contract the entire overlay via clicking and dragging the corner wells highlighted in blue.
- **Adjust Spot** and **Adjust Well**: Click and drag to move either an individual spot in the overlay, or all spots in a well. Use the keyboard arrow keys for more precise adjustments.
- **Auto-Adjust Spots**: Automatically adjusts all spots to improve the alignment of the overlay.
- **Reset Spots**: Reset all spots in the overlay to their default locations.
- **Hide Plate Overlay**: View the image without the plate overlay.

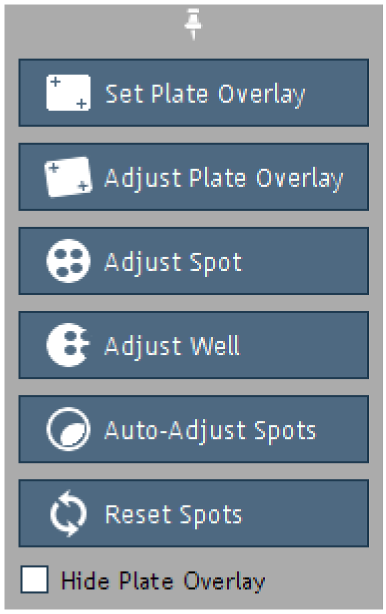


Figure 13. Overlay Options.

Auto-Set Plate Overlay

When using a Q-View imager, the **Auto-Set Plate Overlay** feature, found in **Settings > Preferences**, can be enabled to cause the software to provide the initial plate overlay position. This feature is activated after manually setting a plate overlay for the first time. Once activated, the **Auto-Set Plate Overlay** feature will automatically set the overlay onto all captured images as soon as the product code is entered. Be sure to review the accuracy of the overlay placement for each captured image.

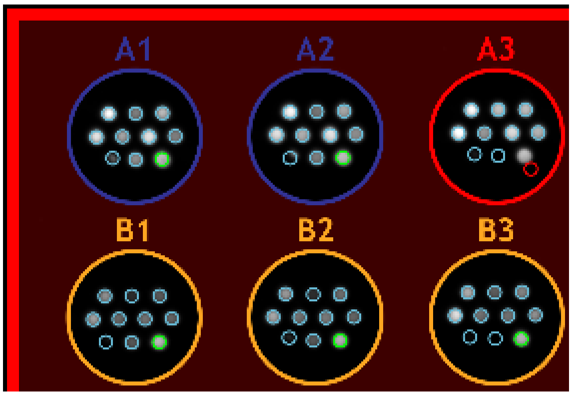


Figure 14. Plate overlay.

Auto Spot Finding

When using a **Q-View Imager LS**, **Q-View Imager Pro**, or **Q-View Imager Plus** with **Q-Plex** plates, **Auto Spot Finding** will be active and will make it easier to accurately set the plate overlay either manually or via the **Auto-Set Plate Overlay** feature.

Assay Controls

For products with defined assay controls, the assay control spots that are in range are outlined in green. Spot(s) and associated well(s) that are out of range are outlined in red (Figure 14).

Well Assignment

Assign well types, names, and dilution factors in either Q-View Software or a spreadsheet application such as Microsoft Excel. Use **Well Assignment Templates** to quickly assign repeat layouts.

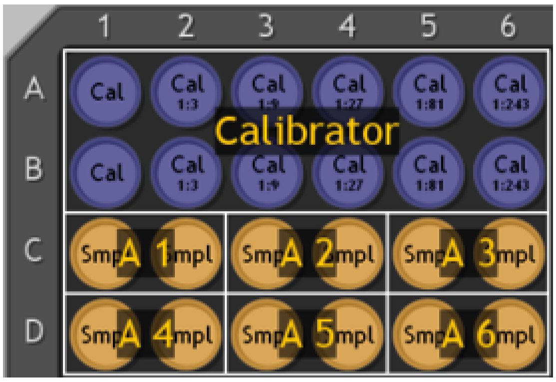


Figure 15. Well assignment.

Assigning Wells in Q-View Software

To assign well types and names, select the desired wells by clicking and dragging the mouse over the plate image (Figure 15). Hold the control key to select discontiguous groups of wells. Click **Groups** (Figure 16) and enter custom names if desired. Select one of the well types defined below to register the assignment.

Figure 16. Group tools.

- **Calibrator**: The kit calibrator (antigen standard).
- **Negative**: The calibrator curve negative (blank) where only sample diluent was loaded.
- **Sample**: The biological sample (unknown) being tested.
- **Control**: User positive control samples. The known concentrations of these samples may be predefined by Quansys Biosciences with an acceptable range. If an analyte in a predefined control fails to meet the criteria, an error code will be included in the report tab of **Data Analysis**.
- **Rename**: Rename well groups without modifying well type.

Calibrators may be assigned dilution factors or grouped by predefined concentrations for each analyte. Predefined concentrations are created by Quansys Biosciences and assigned by the user by selecting a well group, clicking the **Calibrator** button (Figure 16) and then choosing from a list of group names in a subsequent pop-up window.

Use **Sequential Naming** to automatically name groups of wells with sequentially increasing suffixes.

To assign dilution factors (Figure 17), select the desired wells and click the **Dilutions** button. Enter the dilution factor of the initial dilution in the **Initial Dilution** field. For example, enter the number "2" for a 1:2 (50%) dilution. If a dilution series was continued from that initial dilution, enter the series dilution factor. For example, to indicate a 1:10 dilution series from the 1:2 (e.g. 1:20, 1:200, 1:2000), enter "10" in the **Dilution Factor** field. Press any of the directional buttons, e.g. **Top to Bottom**, to register the assignment.

Figure 17. Dilution tools.

Assigning Wells in a Spreadsheet

To assign well types, names, and dilutions in spreadsheet software such as Microsoft Excel, click **Templates > New Template**, then name and save the newly created template file. Once saved, a blank well assignment template will open in the default spreadsheet software. Fill this out and save your changes. Apply the template as described below.

Well Assignment Templates

To save your current well assignment as a template, click **Templates > Save Template**. To apply a previously saved template to a plate, either click **Templates > Open Template**, or drag and drop the template file onto the plate diagram.

Data Analysis

Once you have completed **Image Processing** and **Well Assignment**, select **Data Analysis**. Click **Perform Analysis** to generate charts, concentrations, and statistics based on optimal regression settings provided by the product definition. Click the **Curve Fit Options** button to customize regression models, weighting, and negative well subtraction settings.

Curve Fit Options

The default 4PL or 5PL regression models are generally recommended for most Q-Plex data, as these are widely considered to provide the best overall fit for immunoassay curves. If your data is abnormal or you are trying to optimize the fit for a particular range, one of the other regression models, along with masking, may better fit your needs. You can choose from the following **Curve Fit Options** (Figure 18):

Regression Models

- **5 Parameter Logistic (5PL)**: This non-linear regression model is generally considered the best for fitting sigmoidal immunoassay calibrator curves and is recommended for Q-Plex kits.

Regression					
Analyte	5PL	4PL	Point To Point	Qualitative	Auto Select
Ferritin	☐	☐	☐	☐	☒
AGP	☐	☐	☐	☐	☒
Tg	☐	☐	☐	☐	☒
sTfR	☐	☐	☐	☐	☒
CRP	☐	☐	☐	☐	☒
HRP2	☐	☐	☐	☐	☒
RBP4	☐	☐	☐	☐	☒

Figure 18. Curve fit regression options.

- **4 Parameter Logistic (4PL)**: This non-linear regression model, which is slightly less complex than 5PL, is also considered an acceptable model for analyzing ELISA calibrator curves, particularly if the calibrator curve is symmetric.
- **Point to Point**: Also known as spline analysis, unknowns are determined by straight lines between each calibrator point in this regression model.

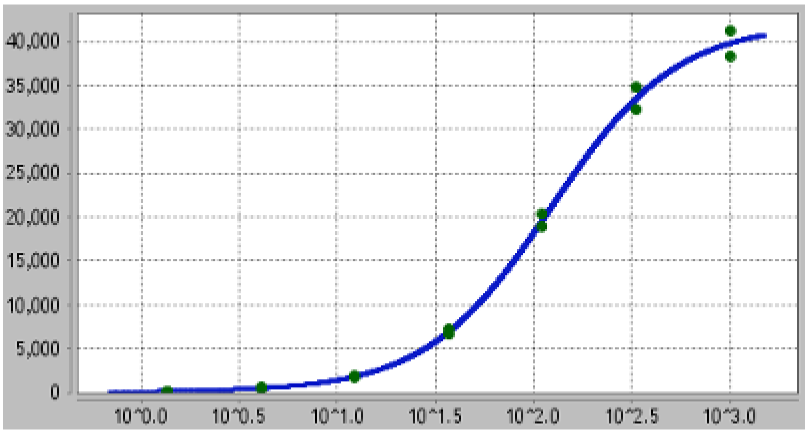


Figure 19. Example 5 Parameter Logistic chart.

- **Qualitative**: This option is for kits that do not come with a calibrator; no concentrations are calculated. Bar charts show the average pixel intensity for each well group and dilution.
- **Auto Select**: This option selects the best regression model and weighting for each assay by fitting and comparing all regression models.

In products with spot replicates, replicates within each well are averaged and treated as single data points. Calibrator curves for assays with spot replicates are fit to the replicate average using the specified regression model.

Calibrator curves for our High Dynamic Range Singleplex assays are automatically fit using the HDR algorithm irrespective of the chosen curve fit option.

Weighting

Signal	Weighting								
Negative Subtraction	None	1/y^.9	1/y^1.5	1/y^2	1/r^.25	1/r^.5	Auto Select	Auto Select Favor Low	Auto Select Favor High
☐	☐	☐	☐	☐	☐	☐	☒	☐	☐
☐	☐	☐	☐	☐	☐	☐	☒	☐	☐
☐	☐	☐	☐	☐	☐	☐	☒	☐	☐
☐	☐	☐	☐	☐	☐	☐	☒	☐	☐
☐	☐	☐	☐	☐	☐	☐	☒	☐	☐
☐	☐	☐	☐	☐	☐	☐	☒	☐	☐
☐	☐	☐	☐	☐	☐	☐	☒	☐	☐

Weighting options are available after selecting **Show Advanced Options**. Weighting is used to reduce the impact of outliers

Figure 20. Curve fit negative subtraction and weighting options.

and offset heteroscedasticity. In ELISA calibrator curves, the residuals (variability) tend to increase as the concentration of the calibrator (y) increases. To improve curve fit, various weighting methods can be applied. General weighting (y) can be applied to normalize the residuals across the curve based on the signal of each calibrator point. Weighting based on residuals (r), reduces the impact of outliers on the curve fit. The larger the number associated with either weighting method, the more variation there will be between the impact of the calibrator points on the final curve fit.

Curve fit can be evaluated by looking at the percent backfit value in the report. When samples fall at the low or high end of the curve, goodness of fit should be prioritized at that end of the curve. Selecting **Favor Low** or **Favor High** evaluates the percent backfit across the curve and will automatically select the weighting method that results in the best fit for either the low or high end of the curve.

Negative Well Subtraction

Negative well subtraction (Figure 20) automatically deducts the average signal (pixel intensity) of the negative wells from the signal of all other wells for each analyte. Curve fit and calculated concentrations are not affected by negative well subtraction. The default state, on or off, can be changed in **Settings > Preferences**.

Viewing and Customizing the Data

Concentrations, pixel intensities, and statistics are displayed based on the selected **Data Analysis** tab (Figure 21), spot masking, and settings in the **Statistics** and **Assay Limits** menus.

Results are organized into three tabs for each plate:

Figure 21. Data Analysis tabs.

- **Charts Tab**: Displays the regression model charts for quantitative assays, or bar graphs for qualitative assays. The selected regression model and weighting are included above each chart when enabled (**Statistics > Assay > Show Regression Model**). Hover the mouse over a point to view (x, y) values. Right-click to access masking options and chart properties. Click and drag from top-left to bottom-right to zoom in. Click and drag from bottom-right to top-left to zoom out.
- **Report Tab**: Displays concentrations and other calculations based on settings in the **Statistics** and **Assay Limits** menus.

- **Data Tab**: Displays concentrations or pixel intensity values based on settings in the **Statistics** and **Assay Limits** menus.

Other values that may appear in the **Report** and **Data** tabs are:

- **Incalculable (High/Low)**: The concentration could not be calculated typically because it fell either above or below the parameters of the curve. The reported value can be customized in **Settings > Preferences**.
- **NA**: Not Applicable. Note that certain statistics cannot be calculated if there is only one replicate present or there are no negative wells assigned.
- **No Fit**: The curve did not fit using the selected curve fit settings.
- **Empty**: No well assignment was provided by the user for the well.

Mask Outliers

Select and right-click (Figure 22) data points within the **Chart**, **Report** or **Data** tab to mask (exclude from all calculations) or unmask spots and wells.

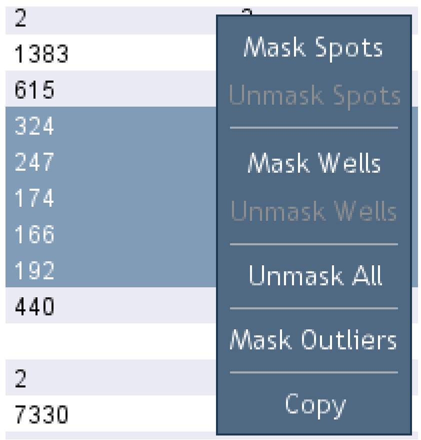


Figure 22. Masking spots.

Q-View Software can automatically identify and mask outliers in calibrator curve data. To enable this feature, open **Settings > Preferences** and select **mask calibrator outliers by default**. When you unmasked an auto-masked outlier, Q-View Software will not auto-mask the point again when fitting curves until you right click a chart or report and select **Mask Outliers** (Figure 22) from the drop-down menu.

Statistics

Click **Statistics** (Figure 23) to select which calculations are shown.

If multiple dilutions of a sample were present, select **Auto-Select Dilution** to view only the data for the dilution that fell on the most linear part of the calibrator curve, instead of data for every dilution of the sample.

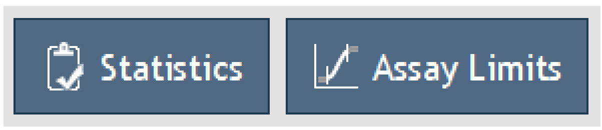


Figure 23. Statistics and Assay Limits.

Assay Limits

Click **Assay Limits** (Figure 23) to modify how extrapolated data is displayed:

- **ULOQ and LLOQ – Upper and Lower Limits of Quantification**: These are the average of the highest/lowest calibrator points with a %backfit of 120%-80%, a %CV of <30%, and a positive mean pixel intensity difference between the calibrator point and the negative control. These are the recommended upper and lower range limits, allowing concentrations only for data within the quantifiable range of the calibrator curve (interpolated) to be displayed.

- **LLD – Lower Limit of Detection**: The lower limit can alternately be set to the LLD to view concentrations down to this value. LLD is the lowest concentration of analyte that can be reliably distinguished from background noise. Note that values below the LLOQ are extrapolated.
- **LOB - Limit of Blank**: The lower limit can alternately be set to the LOB to view concentrations down to this value. LOB is the expected signal when measuring samples that contain no analyte. Note that values below the LLOQ are extrapolated.
- **LOD - Limit of Detection**: The lowest concentration of analyte that can be reliably distinguished from the LOB with a defined level of statistical confidence.
- **Lot LLD, LLOQ, or ULOQ**: The Lot limits, only available for certain products, are the assigned Lot LLD, LLOQ and ULOQ values determined by Quansys Biosciences.
- **No Limit**: Q-View Software will display concentrations for all points that can be calculated with the chosen regression model, including those extrapolated below the LLOQ and the LLD.

Evaluating the Quality of the Curve Fit

Backfitting of the calibrator is a practical method for evaluating the quality of the curve fit. This is done by comparing the calculated concentration of each calibrator to its known (theoretical) concentration using the equation: calculated concentration / theoretical concentration x 100%. Q-View Software displays this result in the report as percent backfit. The preferred result for each calibrator point is a backfit value of 80%-120%, although 70%-130% may be acceptable.

Data Analysis Templates

Custom curve fit settings, statistics and assay limits can be saved in the **Data Analysis Templates** section (Figure 24). To save your current settings as a template, click **Save Template**. To apply a saved template, click **Open Template** or drag and drop a template file onto the data analysis section.

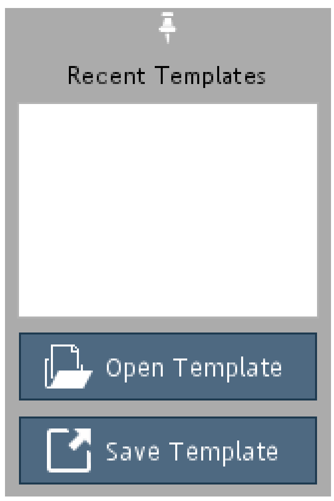


Figure 24. Data analysis templates.

Error Codes

The error code column in the report alerts you to potential problems with your assay. Error codes are defined at the bottom of the report and include:

- **Assay Controls (AC)**: Within a product, analytes may be designated as assay controls and assigned an acceptable range of pixel intensity values. This error is raised when an analyte's measured pixel intensity falls outside its defined range.
- **Process Controls (PC)**: When a product includes a process control, users must select a process control during well assignment. The selected control determines the well group name and specifies acceptable concentration ranges for associated analytes. This error is raised when an analyte's calculated concentration is outside the defined range.
- **Spot Overlay Out of Bounds (SB)**: This error is raised if any wells in the plate overlay overlap one another, or if any individual spots fall outside the defined boundaries of their respective wells.
- **Pixel Intensity Coefficient of Variation (CV)**: This error is raised when the coefficient of variation (CV) between a sample and its corresponding control exceeds 30%.
- **Masked Outliers (MO)**: This error is raised when one or more spots are automatically identified as statistical outliers and subsequently masked during analysis.

Submit Report For Review

When **User Accounts** are enabled and the **Require reviewer signature** option is selected, reports are required to be submitted for review (Figure 25) and subsequently approved before they can be exported. Users are required to have the **Submit reports for review** permission to perform this action. If a report changes after it has been submitted for review, resubmission for review is required. This does not apply if the user who modifies the report has the **Approve and reject reports** permission.

Figure 25. Submit for review.

Approve/Reject Report

Users with the **Approve and reject reports** permission can approve or reject reports (Figure 26) submitted for review. Rejected reports must be resubmitted for review and approved before they can be exported.
Approved reports can be exported by users who have the **Export data analysis** permission.

Figure 26. Review report.

Export

Click **Export** (Figure 27) to save charts as images, or the currently displayed values in the **Report** or **Data** tabs as a spreadsheet (CSV).

Figure 27. Export.

Q-View Imager Qualification

To qualify imagers, light is measured at constant intensities across the dynamic range of the imager and compared to expected values. Quansys Biosciences supplies two qualification plates; one for Q-View Imager LS and Q-View Imager Pro and another for Q-View Imager Plus. Performing qualification on a regular basis ensures that the imager consistently meets performance specifications.

Settings

Q-View Software imager qualification settings (Figure 29) can be accessed from the **Qualification > Q-View Imager Qualification > Settings** menu (Figure 28). The settings are accessible to only administrators.

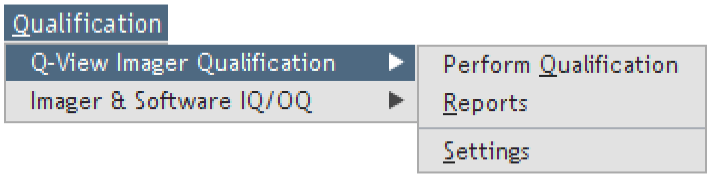


Figure 28. Q-View Imager Qualification menu.

- **Enable Q-View Imager Qualification**: When enabled, image acquisition is restricted based on the qualification settings and status.
- **Qualification Expiration**: Defines the amount of time that an imager is considered qualified.
- **Disallow image acquisition for non-admin users when qualification expires**: When selected, non-administrator users are not allowed to capture images when qualification expires (Figure 30).

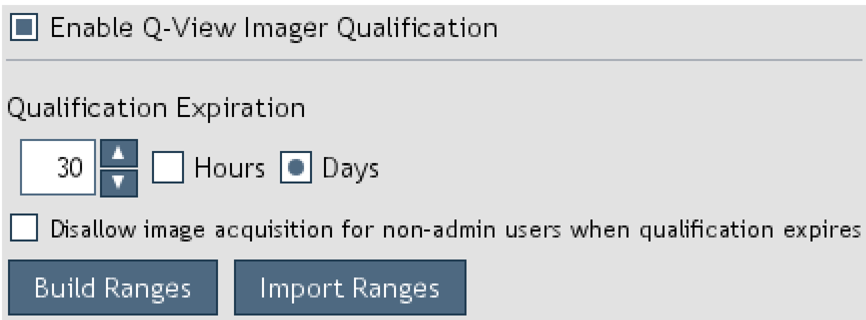


Figure 29. Q-View Imager Qualification settings.

- **Build Ranges**: Builds qualification range parameters by processing a series of qualification plate images.
- **Import Ranges**: Imports predefined ranges provided by Quansys Biosciences.

Perform Qualification

For Q-View Imager LS and Q-View Imager Pro, power on the **Q-View Qualification Plate** and place it in the imager face down in the lower right corner of plate deck. For Q-View Imager Plus, place the **Q-View Qualification Plate** in the imager with the barcode facing the rear.

Figure 30. Image capture is restricted.

Select **Qualification > Q-View Imager Qualification > Perform Qualification** from the menu (Figure 28) or click **Q-View Imager Qualification has expired** (Figure 30) from the **Image Acquisition** screen. Confirm that the serial number on the qualification plate matches the serial number in the window. The process takes about 5 minutes to complete.

Reports

To view and export qualification reports, select **Qualification > Q-View Imager Qualification > Reports** (Figure 28) from the menu.

Recertification

For Q-View Imager LS and Q-View Imager Pro, qualification plate certification expires annually. The expiration date is shown on the back of the plate. Once certification has expired, please contact Quansys Biosciences to arrange to have your plate recertified. Once your plate has been recertified and returned to you, it is necessary to rebuild the qualification ranges.

Build Ranges

To build qualification ranges, open **Qualification > Q-View Imager Qualification > Settings > Build Ranges** and follow the instructions provided by the software.

Imager & Software IQ/OQ

IQ/OQ is an add-on module that ensures the installation and operational functions of the imager and software are performing correctly. These tests can be used upon initial evaluation and subsequent evaluations to ensure consistent operation of the Q-View imager and software. Following the IQ/OQ testing, a report is generated that may be used for regulatory compliance and equipment control.

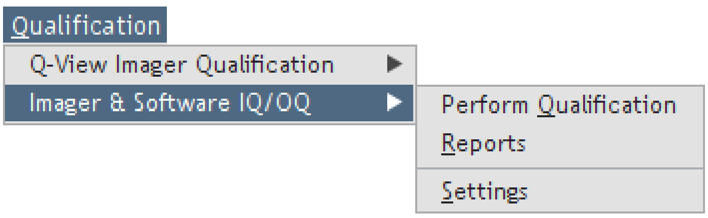


Figure 31. Imager & Software IQ/OQ menu.

Settings

Imager & Software IQ/OQ settings (Figure 32) can be accessed from the **Qualification > Imager & Software IQ/OQ > Settings** menu (Figure 31). The settings are accessible to only administrators.

- **Enable Imager & Software IQ/OQ**: When enabled, image acquisition is restricted based on the qualification settings and status.

- **IQ/OQ Expiration**: Defines the amount of time qualification is considered valid.

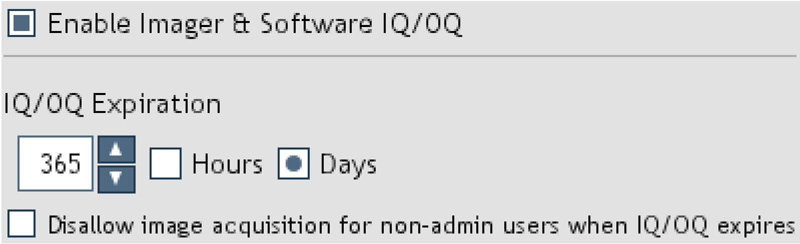


Figure 32. Imager & Software IQ/OQ settings.

- **Disallow image acquisition for non-admin users when IQ/OQ expires**: When selected, non-administrator users are not allowed to capture images when IQ/OQ expires (Figure 33).

Perform Qualification

In the Q-View Software, select **Qualification > Imager & Software IQ/OQ > Perform Qualification** from the menu (Figure 31) or click **Imager & Software IQ/OQ has expired** (Figure 33) from the **Image Acquisition** screen. The process takes about 15 minutes to complete.

Reports

To view and export qualification reports, select **Qualification > Imager & Software IQ/OQ > Reports** (Figure 31) from the menu.

Figure 33. Image capture is restricted.

Blot Tools

Click **Support > Legacy Blot Tools** to make custom selections for western blots, northern blots, southern blots, dot blots, and other chemiluminescent images. Click **Close** to return to the previous **Image Processing** screen.

Selection Options

Click **Selection Options** and choose one of the following tools to make a custom selection (Figure 34).

Auto Select: Automatically select a group of similar, contiguous pixels by clicking on the center of the grouping. The number of shades included in the selection correlates to the sensitivity value (0-100) entered in the **Sensitivity** field.

The selection is indicated by a bright red border and filled with translucent red while unselected areas are indicated by a translucent gray fill. Make sure the intended pixel grouping is selected, rather than just a ring around the grouping (Figure 35).

Rectangle and Ellipse: Create a rectangular or circular selection by

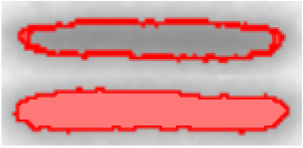


Figure 35. Auto select tool. On the top, only the outer edge is selected, whereas on the bottom, the entire area is selected. The light red fill indicates the selection boundaries.

clicking and dragging the location where the selection is to be placed.

Polygon: Draw a custom, straight-edged selection by clicking on the image where each vertex is desired. Right-click to close the polygon.

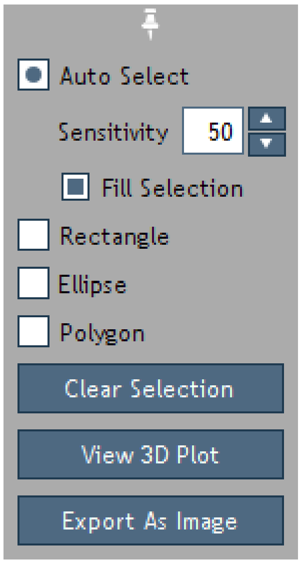


Figure 34. Blot tools.

Selections can be moved by dragging with the mouse, cleared with **Clear Selection**, and exported as a JPEG image by clicking **Export As Image**.

Manipulate the selection boundaries by clicking and dragging, or use the following keyboard commands.

Tool	Arrow keys move selection	Alt + arrow keys to lengthen/shorten shape	+/- keys to resize
Auto Select	√	–	–
Rectangle	√	√	√
Ellipse	√	√	√
Polygon	√	–	–

Note: Hold shift to amplify actions.

Illuminance Plot

To create a plot of the illuminance of a selected area (Figure 36), click **View 3D Plot**. The 3D plot will appear in a new window. The plot can be viewed at any angle by clicking and dragging. Hold control while dragging to move the plot. Roll the mouse wheel to zoom in and out. Various plotting options are available on the right side of the window.

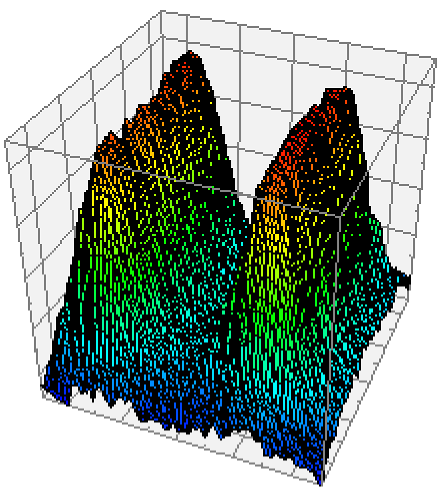


Figure 36. 3D illuminance plot.

Blot Report

The **Blot Report** is an exportable table that includes custom names, number of pixels selected, mean pixel intensities, and standard deviations. To add a selection to the table, click **Add to**

Report. You can add as many entries as you would like. The information for the current selection is shown at the bottom of the drop-down. The table can be viewed and edited at any time by clicking **View Report**. To view the pixel coordinates and intensities for a selection, click the blue **# Pixels** label.

Administrative

Software and Product Updates

Software, product definitions, barcode catalog, and imager firmware updates can be obtained automatically if set to do so in **Settings > Administration > Q-View Updates** (Figure 37). If updates are not occurring, your network settings may need to be modified in **Settings > Administration > Network Settings**.

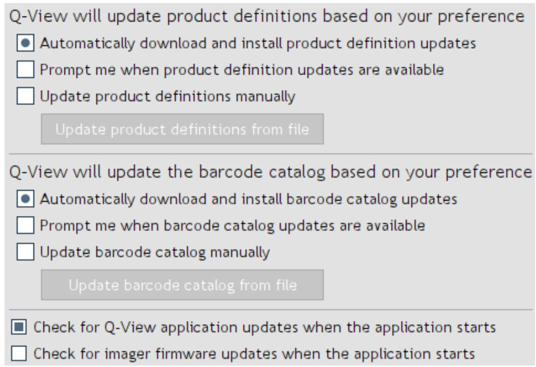


Figure 37. Update options.

If your computer does not have Internet access, contact Quansys Biosciences at sales@quansysbio.com or 1-888-782-6797 to arrange for regular software updates to be sent to you.

To apply product catalog updates manually, go to **Settings > Administration > Q-View Updates** and select **Update product definitions manually**, then click the **Update product definitions from file** button. A file selection window will appear. Navigate to the product definitions file you received from Quansys Biosciences and select **Open**.

To apply barcode catalog updates manually, go to **Settings > Administration > Q-View Updates** and select **Update barcode catalog manually**, then click the **Update barcode catalog from file** button. A file selection window will appear. Navigate to the barcode catalog file you received from Quansys Biosciences and select **Open**.

Settings

Use the options under the **Settings** menu as follows (Figure 38):

- **Change Password**: Change the password of the currently logged in user.
- **Permissions**: View the permissions of the currently logged in user.
- **Preferences**: Set preferences for auto-set plate overlay, negative well subtraction, reporting, Q-View Imager Pro temperature regulation, image stacking, notifications and internal illumination.

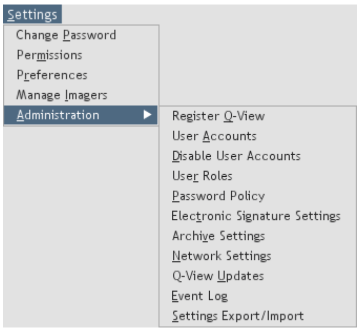


Figure 38. Administrative options.

- **Manage Imagers**: Discover, name, focus, capture dark fields and update firmware for Q-View imagers.
- **Administration**: When logged in as an administrator, you have access to the following settings:
    - **Register Q-View**: Enter your company name and assigned license key to unlock all of the software's features.
    - **User Accounts**: Create, delete, or edit user accounts.
    - **Disable User Accounts**: User login will no longer be required to use Q-View Software.
    - **User Roles**: Manage user roles and permissions.
    - **Password Policy**: Manage your organization's password policy.
    - **Electronic Signature Settings**: Manage electronic signature settings.
    - **Archive Settings**: Enable or disable automatic image archiving and set the retention period.
    - **Network Settings**: Specify settings for Internet access for software updates. If the computer running Q-View Software has multiple network devices, you may need to manually select the device that has access to the Internet in order to receive updates. To select a network device, deselect the **Auto-select** check box and choose the network interface and IP address from which Internet traffic will originate. If access goes through a proxy, click the **Internet connection requires proxy** check box and enter the IP address or host name and associated port number. If your proxy requires authentication, enter the user name and password.
    - **Q-View Updates**: Set both software and product definition update preferences.
    - **Event Log**: View logged application events.
    - **Settings Export/Import**: To ease the administrative burden of managing multiple installations of Q-View Software, settings from one installation of Q-View Software can be exported as a configuration file and imported into other installations of Q-View Software. This feature is only available when **User Accounts** are enabled. The applicable settings are user roles, user accounts and preferences, password policy, electronic signature settings, archive settings, Q-View update settings, network settings, Q-View imager qualification settings, and imager & software IQ/OQ settings.

Event Log

A variety of events and setting modifications are recorded in the event log. The event log (Figure 39) can be accessed under **Settings >**

Administration > Event Log. Events can be filtered by type, user, and date. The log can be exported by clicking the **Export** button.

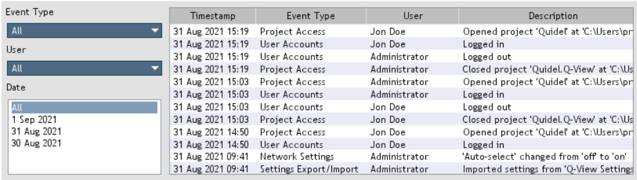


Figure 39. Event log.

Project History

Whenever a project is closed or a report is exported, the full report is recorded if any changes exist. Project history (Figure 40) can be accessed by opening **Project > Project History**. By selecting two entries in the history list, you can compare reports to identify differences. Differences are highlighted in yellow. Reports can exported by clicking the associated **Export** button.

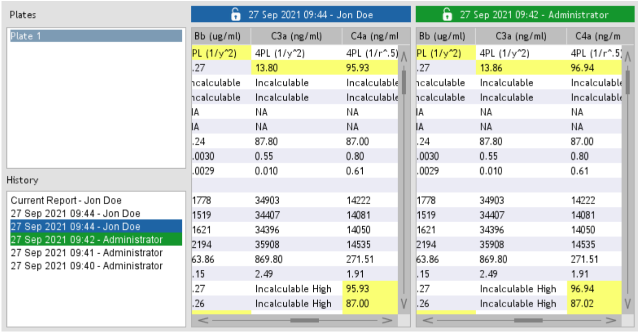


Figure 40. Project history.

Uninstalling Q-View Software

Q-View Software can be uninstalled through your computer's settings or by opening the Q-View Software installer and clicking **Remove**.

Support

This manual is available in the software by clicking the help button (Figure 41), under **Support > Q-View Software Manual**, and online at www.quansysbio.com/manuals.

Figure 41. Help button.

Technical/Troubleshooting

Tips for many aspects of using multiplex ELISA's are available online at www.quansysbio.com/tech-tips.

Technical support data such as projects and imager databases can be sent to Quansys Biosciences directly from Q-View Software by opening **Support > Technical Support Upload** and selecting either **Upload Project** or **Upload Imager Database**. If your computer is not connected to the Internet, the upload options will prepare the data for manual upload which is accomplished by moving the produced file(s) to a computer with Internet access and visiting support.quansysbio.com.

Contact Quansys Biosciences

To contact Quansys Biosciences, please:
Call us direct at 1-435-752-0531
or Toll Free within the US 1-888-QUANSYS (888-782-6797)