

ImageQuant TL 10.1 Software help

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1 Introduction

This software help describes the features and procedures in ImageQuant[™] TL (IQTL) image analysis software.

The IQTL user interface comes with a number of generic functions and views, and consists of the following modules:

- Gel and Blot Analysis module for analysis of 1D electrophoresis gels and blotting results
- Analysis Toolbox module for area and profile analysis of acquired images
- Array Analysis module for analysis of rectangular arrays
- Colony Counter module for analysis of spots and colonies (primarily for images of microbial colonies)

All modules are opened from a common IQTL hub (startup window). Multiple modules can be run at the same time, but the modules are independent of each other.

• ImageQuant TL GxP module for GxP

ImageQuant TL is a separate software for secure analysis with user access, review of data with electronic signatures, audit traceability, and version control.

Scope of this software help

The scope of this software help is to provide a complete reference for using IQTL software. Click the *Help* button in the software to access the instructions on how to use the IQTL software.

The target users are expected to have the following knowledge:

- Basic knowledge of how to use either the Microsoft[®] Windows[®] or macOS[®] operating system and commands
- · Basic knowledge of image formats and image editing

Target users

This help refers to the following target users:

- User who is working with analysis tools in IQTL
- Administrator who is providing user permissions in ImageQuant TL GxP

Definitions

The following terms and abbreviations are used in this help:

1 Introduction

Term/ Abbreviation	Definition
GxP	A collection of "Good Practice" quality guidelines, where x stands for various fields and work environments, for example, Laboratory or Manufacturing.
Analysis project	The short name for <i>image analysis project file</i> that is used for experiments.
IQTL	The ImageQuant TL software, and the short name is used throughout this help.
MW	Molecular Weight
RF	Retention Factor, or relative mobility. The distance migrated by a band divided by the distance migrated by the dye front. IQTL uses the length of the lane in the calculation.
BG	Background

2 Work with Gel and Blot Analysis

This section contains information about the different views, menus, and options in the IQTL **Gel and Blot Analysis** module. It also contains information on how to use the module to create and edit an analysis, and generate an analysis report.

The module is designed to support fast and accurate analysis of a range of experiment types, including protein- and DNA gels. With IQTL it is possible to do the following:

- Designate lanes
- · Designate background and bands coupled with molecular weight
- Advanced analysis, for example, calibration, lane profile comparison, and normalization of both single- and multi-channel images

Work in general workflow

The Gel and Blot Analysis module has the following general workflow:

- 1. Start in the *Image* view. Views not available are shown as dimmed in the user interface.
- 2. Open an image in the *Image* view.

Result: The Lanes view becomes available.

3. Add lanes to the opened image in the *Lanes* view.

Result: All other views become available except the *Normalize* and *Quantity Calibration* views.

4. Detect bands in the **Bands** view.

Result: The Normalize and Quantity Calibration views become available.

Work with image files

Images up to 1 GB in size can be analyzed using the Analysis Toolbox module, refer to *Chapter 3 Work with Analysis Toolbox, on page 33.* It is also possible to view images in both grayscale and RGB formats, allowing the user to maintain the captured color of the image throughout the analysis.

IQTL supports the following image file formats:

- TIF or TIFF (8- and 16-bit grayscale)
- GEL
- DS (multi-channel files)
- IMG

IMPORTANT

Using JPG, JPEG, and PNG images are possible, but not recommended for quantitative analysis. It is strongly recommended to perform the analysis on the file format according to the device manufacturer. ImageQuant CCD imagers use the TIF files for analysis. For Typhoon scanners, it depends on the application. If the wrong files are analyzed (for example, JPEG files) it could lead to incorrect results.

See the following manuals on the Cytiva website for further guidance:

- Amersham Typhoon Scanner Operating Instructions
- ImageQuant 800 User Manual
- ImageQuant 800 Operating Instructions

Details of image analysis are stored as an **analysis project** file in 1DP file format along with the image file. When opening another image, closing the module, or running a report, you are prompted to save the file even if no changes have been made. The original image file is not changed by the analysis procedures.

Opening an analysis project file automatically opens the associated image file. The same image file can be associated with analysis project files from different modules, but one image file can only be associated with one analysis project file from a given module. Analysis project files are not directly accessible through the IQTL user interface.

Note: The image file name and folder location are stored in the analysis project file, so moving, deleting, or renaming the image file will break the association between the project and the image.

Get started with IQTL Gel and Blot Analysis module

Get started with IQTL Gel and Blot Analysis module as follows:

- 1. Start ImageQuant TL from the Windows start menu, or click on the software icon.
- 2. Click Gel and Blot Analysis in the IQTL hub.



Work with an analysis project

In IQTL you can connect one or several image files to an analysis project.

To access new and existing projects, click the file menu with the following symbol:



In the file menu, select any of the following options:

New Project	Opens a new instance of IQTL in a new window.	
Open Project	Opens an existing saved analysis project. You are prompted to save the ongoing project (if any).	
Open Image	Opens an existing image from the location you want.	
Save Project	Saves the ongoing analysis project.	
Save Project As	Saves the ongoing analysis project with a new name.	
Undo	Undo a change by going back to the previous action (unlimited times).	
Redo	Redo a change by reapplying the action that has been undone (unlimited times).	
lmageQuant TL Hub	Opens the IQTL hub from any view.	
Analysis Report	Generates a project analysis report.	
lmage Information	Shows all metadata of the image in a pop-up window.	
Help	Opens software help. It is partly contextual, meaning that a specific web page shows when clicking help in a specific user interface view.	
About	Shows information about the IQTL software and the version in a pop-up window.	
Quit	Closes the IQTL software. You are prompted to save the ongoing analysis project (if any).	

Work with analysis tools

IQTL is designed with the analysis tools accessible from the left panel, arranged in the most commonly used order. Lane detection, background subtraction, band detection, and molecular weight calibration are considered the basic analysis steps. This is followed by optional analysis steps such as normalization, quantity calibration, and lane profile comparison.

Open IQTL to access the following analysis tools:

Image	Set up image . Click the <i>Image</i> view to open images for analysis as described in <i>Section 2.2 Image view, on page 15</i> .
III Lanes	Define lanes . Click the <i>Lanes</i> view to detect lanes on the image as described in <i>Section 2.3 Lanes view, on page 17</i> .
Δ <u>Λ</u> BG	Define background . Click the <i>BG</i> view to subtract background intensity as described in <i>Section 2.4 Background view, on page 19</i> .
Bands	Define bands . Click the Bands view to create and edit bands on the image as described in Section 2.5 Bands view, on page 22.
mm MW	Define molecular sizes . Click the <i>MW</i> view to add and calibrate standard molecular weight marker curves as described in <i>Section 2.6 Molecular weight calibration view, on page 25</i> .
↔ Compare	Compare lane profile . Click the Compare view to compare lane profiles to a selected reference lane as described in <u>Section 2.7</u> Compare view, on page 27.
% Normalize	Normalize band volume . Click the Normalize view to normalize band volumes as described in Section 2.8 Normalize view, on page 29.
U Quant. Cal.	Quantity Calibration . Click Quant Cal to plot known values against measured volumes and fit a curve to calculate the volumes.
E Results	View analysis results . Click the <i>Results</i> view to view analysis results and generate reports as described in Section 2.10 Results view, on page 31.
S Undo	Undo change . Click Undo to go back to the previous change (unlimited times).
⊘ Redo	Redo change . Click <i>Redo</i> to reapply the change that was set to undone (unlimited times).



Access help. Click Help in any view to access instructions about how to use IQTL.

When using the analysis tools, also the following options are available:

Q Zoom Image	Click Zoom Image to zoom into a selected area. This option is only available for an open image.
F. Fit	Click Fit to restore the view to the whole image. This option is only available for an open image.
😢 Display	Click Display to change image contrast and channel color using a slider or by manually typing values. This option is only available for an open image.
INVERT	Click Invert to invert the pixel intensity. This option is only available for an open image in the Image view.
□Lane 1 ∨	Click <i>Lanes</i> to select lanes to view. Multiple lanes show stacked on the screen.

Tip:

If multiple lanes are selected, a single lane can be selected simply by clicking the lane name.

Click **Channel selector** to select a channel preset for the image from the **Display** window. This option is only available for a multichannel image, see the following example:



Click the **Zoom Window** tab at the bottom right corner of the analysis window to view a small version of the image and a rectangle showing the zoomed in area. In this window you can easily zoom in and out and move the zoomed in rectangle.

Zoom Window



Change contrast and select channel color

With the **Display** option you can change contrast and channel colors for the image.

There are several out-of-the-box **display color presets** with false colors and heatmaps for use. Drag the slider to adjust the contrast. The image in the window updates automatically.



Select one of the display colors for the image from the drop-down menu, then select any of the following options:

- Click Auto to automatically adjust the contrast.
- Click Full to reset the contrast to the maximum settings.
- Click Manual to adjust the contrast range manually.

The following channel presets with false colors and heatmaps are available to apply on the image using the contrast slider:



White.

Background color: Black Bands color: White Mid-point color: Grey



Black.

Background color: White Bands color: Black Mid-point color: Grey



Red.

Background color: Black Bands color: Red Mid-point color: Dark red



Green.

Background color: Black Bands color: Green Mid-point color: Dark green

Blue.

Background color: Black

Bands color: Blue

Mid-point color: Dark blue

Yellow.

Background color: Black

Bands color: Yellow

Mid-point color: Dark yellow

Cyan.

Background color: Black Bands color: Cyan Mid-point color: Dark cyan



Magenta.

Background color: Black Bands color: Magenta Mid-point color: Dark magenta

Silver stain. From very dark to very light in a brown color simulating silver staining.

Coomassie blue. From very dark to very light in a blue color simulating coomassie blue staining.

Heatmap 1.

Background color: From red to blue

Bands color: Green

Mid-point color: Green

Heatmap 2.

Background color: From yellow to cyan

Bands color: Pink

Mid-point color: Dark pink

Heatmap 3.

Background color: From black to blue Bands color: Orange Mid-point color: Dark orange



Threshold 10%. From blue to red, threshold set at 10%.

Threshold 25%. From blue to red, threshold set at 25%.

Threshold 50%. From blue to red, threshold set at 50%.

Threshold 75%. From blue to red, threshold set at 75%.

Threshold 90%. From blue to red, threshold set at 90%.

Start analysis

In the *Image* view, click *Open* to open an image file or an existing analysis project as follows:

Tip: When an image is opened, you have the following options on right-click:

- Copy to Clipboard. Select to take an image snapshot to the clipboard.
- Save to File. Save an image as a PNG file.

By default, the analysis project file is saved in the same folder as the image. But you can save it to any other location if you want.

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2.1 View tutorial images

2.1 View tutorial images

A set of tutorial images is installed with the software to help you to start using ImageQuant TL software. The images are available for each module through **Open Tutorial Image** from the file menu as follows:

2.2 Image view

In IQTL *Image* view, it is possible to set up the image for correct interpretation. It makes sure that the pixel intensities are measured accurately and that the background is set correctly. It is also possible to zoom into the image for closer inspection and review it in 3D.

An open image in IQTL is shown in the following views:

- · Snapshot of 2D intensity view shown at the top left
- Snapshot of 3D intensity view shown at the top right
- Standard image view shown at the bottom half



Click **Toggle Zoom** to enlarge the 3D view. This option is only available for an open image.

Tip:

You can rotate the 3D intensity view image around all axes.

- For Windows, click the left mouse button with and without pressing **Shift**.
- For Mac[®], click either the left or middle mouse buttons while moving the mouse over the image.





For IQTL to measure the bands accurately, the image needs to show cyan bands on a black background (2D intensity view), and as cyan peaks on a black background (3D intensity view). The top of the peaks represent the highest pixel intensity with the background as the lowest. If this is not the case, the image will need to be inverted.

Note: For accurate measurement of bands, always show bands as cyan peaks against a black background.

Saturated pixels are also shown on the 2D intensity view image in orange.

On the right of the image view, the settings panel displays the following:

- Image name and file path, image size, and file size.
- Channels information, where it is possible to rename the channel name by typing into the text box.
- **Open** makes it possible to open a different image.



Integrity test result: Pass Original image from ImageQuant 800 GxP confirmed. **Integrity test result** shows if the image was opened in its original capture state or if the image has been edited. This option is only available for images captured with ImageQuant 800 GxP.

2.3 Lanes view

In *Lanes* view, it is possible to create and edit lanes to continue image analysis. The *Lanes* view includes an image view with an overlay of the Lane Box and individual lanes. The Lane Box and individual lanes can be edited to better fit the image and the number of lanes can be changed.

When creating a lane it defines vertical areas occupied by lanes on the image. Each lane normally represents one sample. Lanes are created manually and can be moved and/or resized once they have been created. It is important to define the lanes correctly at the start of the analysis since changing the lane definition can affect the analysis results.

Define and edit lanes by using the following options on the right panel:



Click **New Box** to draw the area of interest in the image by clicking and dragging the image. You can add several lane boxes to one image.



Click *Edit Box* to edit a lane box.

Resize the box by clicking and dragging the handles at each corner.

Move the box by clicking and dragging the center white diamond shape to the position you want.

Change the shape of each box by clicking and dragging the handles on the sides.



Click *Edit Lanes* to edit an individual lane by clicking and dragging the handles (white diamond shapes) in the center of each lane box.



1 Lane 1

2 Lane 2

LANES

WIDTH

100%

~

Click **Rename** to open individual lanes. Rename the lane by typing the name into the text box.

You can delete an individual lane by clicking the X on an individual lane.

Click *Lanes* to select the number of lanes that you want to create. 10 lanes are selected by default when the lane box is created.

Click *Width* to select the width of the lanes. 100% is selected by default.

2.3 Lanes view



Do the following to create and edit lanes:

- 1. Click **New Box** to draw the area of interest on the image. Multiple areas can also be created.
- 2. Adjust the shape of the lane box by clicking and dragging the white squares on the edges of the box.
- 3. Move the lane box by clicking the center white diamond-shaped handle and drag to the position you want. Sub-divide if more adjustment is needed.
- 4. Change the number of lanes and lane width, if needed, to fit your image.
- Manually adjust the lanes, if needed, to better fit your image by using *Edit Lanes*. Lanes can be moved in any direction and are not connected to the lane box for greater accuracy.
- 6. Adjust individual lane widths by selecting *Individual Lane Widths (px)*. The lane width can be changed manually for a specific lane or by using the arrows. To apply the new lane widths to all lanes, click the double-check sign.



- 7. Rename lanes, if you want.
- 8. Delete lanes that are no longer needed.

2.4 Background view

In **Background** view, it is possible to compare the profiles of the various lanes – it allows users to view one or more lanes simultaneously along with the relevant profiles. It is possible to zoom the profile for a better view of weaker bands. The background can be removed in several ways and the profiles viewed with and without the background displayed.

Background subtraction compensates for the background intensity in the image, so that measured band volumes are a closer representation of the amount of material in the bands. In this view the lane is represented as a profile view. Each point in the profile is generated as an average across the band box.

Background subtraction can be done to make sure that the measured band volumes are more accurate. Background subtraction compensates for the background intensity in the image and can be achieved by using the following methods depending on the experimental data:

- Rolling ball
- Rubber band
- Constant value
- Image rectangle
- None

View lanes by using the following options on the right panel:

~//	Click Select All to select lanes and channels at the top of the list.
	Tip: Click the lane name field to select only that lane to display and clear all other lanes.
Appy to [ALL CHANNELS]	In multiplex images (DS file format), the background subtraction can be adjusted differently to different channels by choosing the background method and apply it to specific channels. To apply the same background method to all channels, select All Channels from the list.
🗸 Table	Click Table to see table data. De-select to enlarge the image.
Сору	Click Copy to copy the table at the bottom of the window, which allows for pasting in Excel [®] , for example.
Export CSV	Click Export CSV to save the table as a CSV file.
Columns	Click Columns to add or remove information in the table.
Zoom Profile	Click Zoom Profile to focus on an area of the profile for closer inspection. Click and drag the area of interest in the profile view.

2 Work with Gel and Blot Analysis

2.4 Background view



Use rolling ball method for background subtraction

The rolling ball method determines the baseline as the highest point on a disc or ball of specified radius as it rolls along under the profile, maintaining contact with the profile at all times. A smaller radius will give a baseline that follows the profile more closely. The radius should be set in relation to the width of the bands. The rolling ball method will create a baseline that follows the profile of diffuse bands more closely than the one of sharp bands.

Background Method			
rolling-ball 🗸			~
Radius (Fraction of L	ane Length	۱)	
6			
0			
-0			
	•	•	•

The radius of the rolling ball is determined as a fraction of the entire lane length. This value can be set in any of the following ways:

- Manually type the value into the Radius box
- Drag the slider to any value between 1/2 and 1/40
- Select any of the following preset options:
 - 1/40
 - 1/10
 - 1/6
 - 1/4
 - 1/2

Use rubber band method

The rubber band method draws a baseline between the lowest points on the profile, as though a rubber band was stretched under the profile. If the ends of the profile are lower than all other points, the baseline will be a straight line between the ends of the profile.

Use constant value method

The constant value method designates a value for the background. This can either be zero, the profile minimum or a value that can be selected on the profile itself by clicking the profile.

Use image rectangle method

Place and adjust the size of the image rectangle in the image window to define the background intensity. The average intensity level within the specified rectangle is used as the background. This value is removed from each pixel in a spot. If the background value is higher than the pixel value, then the value zero is used. The total background can therefore vary between spots.

Adjust the profile Y-axis scale

Right-click the profile, and the following options are available to adjust the scale of the Y-axis:

- Scale Y-axis to here resizes Y-axis to the current y-value of the cursor.
- **Reset Y-axis scale** resets the Y-axis scale to original values. The scale of the Y-axis by default is based on the intensity of the pixels: 0 to 255 for an 8-bit image, and 0 to 65535 for a 16-bit TIF image.
- **Note:** If the image has been previously calibrated on the imager the scale of the Y-axis will reflect the calibrated optical density values instead.

2.5 Bands view

In Bands view, it is possible to detect bands automatically and manually.

Band detection defines bands in the image lanes as follows:

- Band peaks are set at the position of maximum intensity
- Band edges are either detected automatically or set to a fixed number of pixels on either side of the peak

Automatically detected bands can be edited or deleted, and bands can be added manually.

Detect bands

Click Detect Bands to detect bands automatically with default settings.

Tip: Holding the pointer above an area in the lane profile will show a green line that corresponds to a yellow line in the image view.

Alternatively, do the following to detect bands automatically by changing the default settings:

- Change one or several of the parameter's settings, then click **Detect Bands** to apply changes and detect bands, or drag the slider to get the settings you want.
- Revert to default settings by clicking **Defaults** \rightarrow **Detect bands**.

The band detection parameter settings are as follows:

Min-Slope	Sets a minimum value for the slope of the band, defined as the slope of a line from the start or end of the band (the first or last pixel above background) to the peak intensity. Setting a high value will detect only sharp bands. The start and end of the band for slope calculation do not necessarily coincide with the band edges.	
	Default: 100 within range 0 – 999.	
Noise	Represents the degree to which small local peaks should be ignored on the profile and is designed to eliminate noise in the image. Noise reduction has no effect on the profile itself, only the number of peaks detected. In general, the higher the Noise Reduction value, the fewer peaks detected.	
	Default: 4 within range 0 – 20.	
% Max-Pe	ak Detects only bands with a peak intensity (after background subtraction) equal to or higher than this value.	
	Default: 3 within range 0 – 100 %.	
Edge Mod Single Edg		
Edge Mod Automatic	5	
Edge Mod Fixed Widt	Sets the band width to the specified number of pixels, with the peak in the center of the band. If bands are too close together for the fixed width to be applied, half the width value will be applied on either side of the peak, as far as possible. All bands are set to the same width.	
Edge Mod Percentag of Peak		
	or multi-channel images, different automated band detection parameters an be selected for each channel.	

Bands are detected by analysis of the original image, regardless of any changes on contrast applied in the image window.

Use manual band detection and editing

Create bands manually using the profile of each lane or by clicking directly in the image.

2.5 Bandsview



Click on the image or lane profile to create new bands and right-click on bands to delete them. The band edges can be adjusted in the lane profile view.



To adjust the Y-axis scale of the bands, see Adjust the profile Y-axis scale, on page 21.

2.6 Molecular weight calibration view

In *Molecular Weight Calibration* view, it is possible to use the lane box (created in the previous steps) to accurately determine the molecular weight of all the bands across the whole image. Markers can be selected from presets or added manually.

Molecular size calibration uses the band position in lanes with standards to calculate the properties of the bands in other samples.

Note: If more than one lane box has been drawn, a different molecular weight marker can be applied to each box.

Although the function is referred to as molecular size calibration, it can be used equally to determine pl values on isoelectric focusing gels or migration properties on TLC plates.

Apply and calculate molecular sizes

Do the following to apply and calculate molecular sizes:

- Select the marker lane from the image view by clicking on the name visible on the top. One or several selected marker lanes will now be visible on the right setting panel. To delete marker lanes, click **X** in the bottom right corner in the right-side settings panel.
- To apply a standard, click **Change** in the curve settings window and select any of the available preset marker and calibration curve.

The following calibration curve options are available for each molecular weight marker for best fit:

- Log
- Cubic Spline
- First Order Lagrange
- Exponential
- Exponential (offset)
- Power
- Power (regression)
- Straight Line
- Click to delete incorrect bands from the marker lanes if needed.
- If you make a mistake and want to start again, click **Undo**.
- To use a different molecular weight marker, click **Change** and select any one of the others available.

It is also possible to choose which channel to use for a multiplex image to determine molecular weight. Selected lanes to be used for molecular weight calibration are listed and can also be deleted.

2.6 Molecular weight calibration view

Add and edit molecular weight templates

Do the following to add and edit molecular weight templates:

1. Click *Edit MW Standard Templates*. A pop-up window opens.



2. Create or edit an existing template and change units for protein and DNA gels, or use percentage for RF.

The following molecular weight marker presets are available for use:

- Amersham ECL Rainbow Full Range
- Amersham ECL Rainbow High Range
- Amersham ECL Rainbow Low Range
- Amersham ECL DualVue
- Amersham ECL Plex
- 3. Save your changes.

2.7 Compare view

In **Compare** view, it is possible to perform an advanced analysis for lane comparisons. IQTL automatically performs a pair-wise correlation calculation for all possible lane pairs. The pairwise correlation results are displayed in the **Similarity table**. The similarity score is a dimensionless number ranging from –1 (anti-correlation) to 0 (no correlation) to 1 (total positive correlation). Thus, a score of 1 means the lane profiles are identical.

The similarity scores are also used for grouping all lanes in a **Dendrogram**. The **Dendrogram** tree diagram can be copied and saved to a file.

Select a reference lane to view more details in the **Scatter Plot** display of relative lane volume (y-axis) and similarity scores (x-axis). This pane can also be exported. This plot is used to compare chemical similarity based on separation profile (similarity score) with amount of total signal in the lane.

Note: The lanes that are compared are always in the same channel image.

Typical samples include the following:

- Complex samples, for example, cell lysates
- · Samples with overlapping bands
- Samples with shifted bands, for example, gel shift assays and DNA fragment analysis.
- Protein purification samples, for example, fractions from an ÄKTA™ Bioprocess system
- **Note:** Dilution series are not suitable samples to compare because weak bands often appear shifted to slightly lower Rf positions and the similarity score is very sensitive to peak shifts.
- **Tip:** If the migration is not uniform, for example if lanes are curved or bent, adjust the lane box to reflect these differences. Try to minimize any "close neighbor effects", i.e. the tendency that lanes close to the reference lane have higher similarity score than lanes farther away from the reference lane. The effect is caused by nonuniform migration and can be adjusted for by setting the lane box correctly and include reference samples in multiple lanes.

Do the following to compare lanes:

- Select Reference Lane from the settings panel (reference lane can also be selected by clicking the lane numbers on the left side of the Similarity Table or on the Dendrogram).
- 2. Select the *Alignment* method.
- 3. View similar lanes together by clicking the nodes of the **Dendrogram**.
- 4. Select a group of points on the Scatter Plot, then click and drag to make a box.

Set parameter settings for comparing lanes



The following parameter settings are available for comparing lanes:

Reference Lane	Select reference lane from the drop down of available lanes. This is used to calculate the values on the Y-axis of the Scatter Plot .
Alignment	Select any of the following alignment methods:
	• MW . If present, this uses molecular weight calibration for lane alignment (recommended).

• Auto RF. Calculates RF values using lane length.

The **Compare** view shows the analyzed data in different view formats that can be selected. The different displays are interactive. For example, selecting a point or group of points on the scatter plot are reflected on the other views.

The following view formats are available:

Image	Shows image view including lanes. The color codes on the lanes match other display views in the window.
Similarity Table	Shows similarity scores for all lane pairs.
Profiles	Shows overlaid profiles of selected lanes and are color coded accordingly.

2.8 Normalize view

In **Normalize** view, it is possible to normalize the calculated band volumes. Normalization calculates band volumes relative to a defined value for one or more reference bands. Normalization is useful in comparing relative band volumes between different lanes or different images where total sample loading can be different.

The following normalization methods are available:

In-Channel Band (single	Uses the volume of a single band to calculate the normalization factor.
band only)	 Select the reference band manually on the image display, or select the largest band automatically by clicking Use Largest.
	 Select a <i>Ref. Value</i> scale factor for the calculation (possible values are 1, 10, 100, 1000, 100000, 1000000). The normalized band area is calculated using <i>Ref. Value</i> x (volume/ref. volume).
Multi- channel Band	First select a Reference Channel . The largest band by volume (as measured in the Reference Channel) is found for each lane and marked as the housekeeping (HK) band. Each lane is then assigned a Normalization Factor (NF) by dividing the largest overall HK volume by the HK band volume for that lane in the Reference Channel . Bands are assigned normalized values by multiplying their measured volume by the NF for the lane.
Total Protein	First select a Reference Channel . For each lane, a normalization factor (NF) is calculated by dividing the total volume of the most abundant lane by its own total volume. All calculations of NF factors are based on the Reference Channel volumes. Then select the Normalization Factor Denominator , either the whole lane volume or the sum of all band volumes for the lane. Bands in all channels are assigned normalized values by multiplying their measured volume by the NF for the lane. Select Common y-axis scale to scale the y-axis in graphs for
	easy comparison.

2.9 Quantity calibration view

In **Quantity Calibration** view, bands with known amounts are plotted against measured volumes (total sum of pixel intensities), the user selects a curve-type, and a curve is fitted to the data. Calibrated band volumes are then calculated using the curve. Also, the bands with known volumes will get calibrated volumes based on the curve. It is normal that band volumes follow a linear dose-response curve.



Click on bands in the image and click **ADD**, followed by entering the known values of each band. Fit the curve by selecting the best option under **Fit Type**.



2.10 Results view

In **Results** view, it is possible to review and export the data from the analysis. Data can be viewed by lane and by channel and includes a variety of different options. Data can also be exported to a CSV file for further analysis with detailed appendices, if required. The following result options are available for use:

Review data selection

Lanes	Select which lanes are displayed in the results table.
Channels	Select which channels are displayed in the results table. Select all by checking the box for All Lanes.

Review band table display

Band ID	Shows the global band ID across the gel. Each band will have a unique ID.
Channel	Shows the channel number the band is part of.
Lane #	Shows the name of the lane.
Band #	Shows the band number within the given lane.
Position	Shows the position of the band in the lane.
Dist. Unit	Shows the length of the unit.
Туре	Shows the band that has been detected, manually added, or edited.
Peak	Shows the calculated peak intensity (no background).
Peak - Raw	Shows the peak intensity including background.
Volume	Shows the summarized volume of pixel intensities in the band box (no background).
Volume - Raw	Shows the sum of the pixel intensity for all pixels in a band.
Volume - Norm.	Shows the normalized volume (if normalization applied).
Volume - Calib.	Shows the calibrated volume (if quantity calibration applied).
Band %	Shows the volume of a band expressed as a percentage of total volume of all bands in the lane.
Lane %	Shows the volume of a band expressed as a percentage of the volume of the whole lane.
Extents (px)	Shows the extent of the band in pixels.
Length (px)	Shows the length of a band in pixels.

Band Area	Shows the area of the band, that is, the number of pixels.
(px)	
Vol. Unit	Shows the unit of volume.

Review lane table display

ID	Shows the lane ID.
Name	Shows the lane name.
Width (mm)	Shows the lane width in mm.
Length (mm)	Shows the lane length in mm.
Width (px)	Shows the lane width in pixels.
Length (px)	Shows the lane length in pixels.
Volume	Shows the volume, that is, the sum of pixels, of the lane (no background).
Vol. Raw	Shows the volume of the lane with background included.
Bands	Shows the number of bands in lane.
Band Vol.	Shows the sum of band volumes (no background).
Band Vol. Raw	Shows the sum of band volumes with background.
Volume Unit	Shows unit of volume.

Export results to CSV file

Export the analysis results to a CSV file as follows:

- 1. Select **Detailed Appendices** to add all data for profiles, raw profiles, background, and volumes at each pixel from the analyzed image.
- 2. Click *Export CSV* to export the added data to a CSV file.

Generate analysis report

Generate an analysis report as follows:

- 1. Select *Analysis Report* from the file menu.
- 2. Browse file location, type the file name, and save the analysis report as PDF file.

3 Work with Analysis Toolbox

The *Analysis Toolbox* module is a general-purpose tool to allow analysis of images from a wide range of experiments.

The user can define different shapes (rectangles, ellipses, or polygons) and a number of parameters are calculated for each object and displayed in table form. It is also possible to draw line objects and display the corresponding intensity profiles. All data can then be exported by using **Copy** and **Paste**.

For information about the file menu, working with the analysis view features, changing contrast and display channel color, refer to *Chapter 2 Work with Gel and Blot Analysis, on page 5*.

Work with Analysis Toolbox

L Image	<i>Image Setup</i> . Click <i>Open</i> to set up an image for analysis.
🗖 Area	Area Analysis . Click to draw and analyze objects such as rectangles, ellipses, and polygons.
≁ Profile	Profile Analysis . Click to draw and analyze lines, polylines, and curve profiles.

The following editing tools are available for use:



Edit. Click to move or resize drawn objects in the image. Objects can also be nudged using the arrow keys.

Windows:

Click and drag to make an area that covers the objects that you want to select. Press **Ctrl** and **A** to select all objects in an image. To select specific objects, press **Ctrl** or **Shift** before clicking on one or several objects and click again to clear them.

Mac:

Click and drag to make an area that covers the objects that you want to select. Press **Control** and **A** to select all objects in an image. To select specific objects, press **Control** before clicking on one or several objects to select or clear them.



The following tools are available in the area view for use:



BG shape. Select shape to define as background.

Note:

Objects drawn outside the image area are given the value zero.

The following tools are available in the profile view for use:



Zoom Profile. Click and drag the magnifying glass from left to right to focus on an area of the profile for a close inspection.

|--|

Reset Zoom. Click to reset the view to the whole length of the profile.

To adjust the Y-axis scale of the bands, see Adjust the profile Y-axis scale, on page 21.

View measurements table

The following information is shown in the measurements table:

ID	Shows the original ID number of each shape.
Name	Shows the name of lines and shapes. You can also rename them by clicking the Info button and type the new name.
Width	Shows the width of the shape in mm.
Height	Shows the height of the shape in mm.
Area (px)	Shows the area of the shape in pixels.
Volume	Shows the sum of pixel intensities (with background subtracted, if turned on).
Raw Volume	Shows the sum of pixel intensities (without background subtracted, if turned on).
	Note: If a shape under Background Shape is not selected, then both Volume and Raw Volume have the same value.
BG	Note: If a shape under Background Shape is not selected, then both
BG BG level	Note: If a shape under Background Shape is not selected, then both Volume and Raw Volume have the same value.
	Note: If a shape under Background Shape is not selected, then both Volume and Raw Volume have the same value. Select to use the shape as the background.
BG level	Note: If a shape under Background Shape is not selected, then both Volume and Raw Volume have the same value. Select to use the shape as the background. Shows background level.
BG level Mean	Note: If a shape under Background Shape is not selected, then both Volume and Raw Volume have the same value. Select to use the shape as the background. Shows background level. Shows mean pixel intensity.

Work with Array Analysis 4

The Array Analysis module is designed for analysis of images of rectangular arrays, such as multi-well plates, dot-blot, and slot-blot images. Array objects in the image are analyzed for volume (total sum of pixel intensities) and may be flagged as present or absent based on a user-defined threshold.

Work in general workflow

The *Array Analysis* module is designed for the following general workflow:

- 1. Start in the *Image* view.
- 2. Open an image in the *Image* view, then the *Grid* view is available.
- 3. When you have added a grid in the Grid view, then all other views are available (Background, Normalize, Quantity Calibration, and Prescence).

Get started using the Array Analysis module

Get started with the IQTL Array Analysis module as follows:

- 1. Start ImageQuant TL from the Windows start menu.
- 2. Click Array Analysis in the IQTL hub to open the Array Analysis module.

For information about the file menu, analysis view features, changing contrast, and display channel color, refer to Chapter 2 Work with Gel and Blot Analysis, on page 5.

Work with analysis tools

The following analysis tools are available for use:



Image Setup. Click Open to set up an image for analysis.

Define Grid. Click to define and edit the grid and the wells. Multiple grids can be analyzed in the same image.



Subtract Background. Click to subtract background intensity.


Normalize. Click to normalize well volumes with reference to a selected array object.



Quantity Calibration. Click to plot known values against measured volumes and fit a curve to calculate calibrated volumes.



Presence Flagging. Click to flag wells as **Present** or **Absent** on the basis of array object volume (total pixel intensity) above or below a threshold.

Image view

The *Image* view contains settings for the image window display. The image can be viewed in both 2D and 3D and by zooming in and out. Saturated pixels are shown in the 2D intensity image in orange. The 3D view can be enlarged and rotated for better viewing.

For more information, refer to Chapter 2 Work with Gel and Blot Analysis, on page 5.

Grid view

The first step in analyzing an array is to define the grid and allow adjustments of the whole grid as well as individual array objects as required by the image.

New	B Grid	Edit	Grid	E	0 dit Wells
cols			rows		
12			8		‡
Drag or	the imag	je to crea	96 ate a 12 x		384
Grid	Dimen	sions	Well Si	ze	
G1	12	x 8	80%	\sim	×
➔ Import Grid					
∋	Export (Grid			

In the grid view you have the following options:

Define the grid layout	Under New Grid the layout of the grid is determined, for example, the number of columns and rows. Two predefined grid layouts, 96 and 384 well arrays, can be selected as templates.		
Make a grid	To make a grid, click and drag in the image. To make a second grid, if there is more than one array in the image that you want to analyze, click and drag again. Remove grids by clicking the X next to the grid in the grid table.		
	Note: For multiplex images, the values in the table will only be displayed for one specific selected channel at a time.		
Adjust the grid	To adjust the grid, select Edit Grid . To move, distort, or resize the grid, drag the handles in the center of the corner spots. The array object size can be adjusted by changing the percentage under Well Size .		
Adjust individual	To adjust individual array sizes, select Edit Wells . Click an array object to move it or resize it as follows:		
wells	• Move wells . To move an array object, drag with the pointer inside one of the selected array objects.		
	• Resize wells . To resize an array object, drag the white diamond that appears outside of the spot after selecting it. This will affect only the currently selected array object. Alternatively change all the array object's radii under Well Size in the grid table.		

To change the shape of array objects, select a new shape using **Well Shape** under **Edit Wells**. This will affect all array objects. You cannot mix different shapes in the same array.

Save grid layouts by using *Export Grid* and reused grids by using *Import Grid*.

Background view

Background subtraction compensates for the background intensity in the image, so that measured volumes are a closer representation of the amount of material in the array objects.

For multi-channel images, the same background subtraction method is applied to all channels, but each channel uses separate baseline calculations.

Select any of the following methods for background subtraction from *Background method* panel:

SurfaceSelect to use the lowest pixel intensity in the array object asminimumbackground. The background is determined separately for each
array object.

- **Edge average** Select to use the average intensity on the array object edge. The background is determined separately for each array object.
- Negative
controlSelect to use the average intensity of an array object as the
background for all array objects. Select an array object and click
Set negative control to define a negative control array object.
To restore a negative control array object to normal status, select
the array object and click Clear negative control. This method
uses the same background value for all array objects in the grid. If
the background value is higher than the pixel value, then the
value zero is used.
- ImageSelect to use the average intensity level within a specifiedrectanglerectangle on the image. Move the image window and adjust the
size to define the rectangle for background intensity. The image
rectangle will produce a single value for background. This is
removed from each pixel in an array object. If the background
value is higher than the pixel value, then the value zero is used.

Note:

Only one negative control or image rectangle can be chosen or placed within an image even when there are more than one grid.

None Select to use no background subtraction.

Normalization view

Normalization calculates the array object's normalized volumes relative to a defined value for one or more reference array objects. If multiple reference array objects are selected, the defined value may be set as the average or sum of the selected array object volumes. Normalization is useful in comparing relative array object volumes between different images where total loading may be different.

Do normalization as follows:

- Set one or several reference wells by clicking on the array objects or dragging a box over the chosen area to highlight all array objects in that area, in the image view. The selected array objects will have a different color.
- Press Set Reference Well to select reference array objects or Clear Reference Well to remove selected array objects. Selected array objects can be seen listed under Selected Wells.
- 3. Select a *Scale Factor*: 1, 10, 100, or 1000.

Quantity Calibration view

Quantity Calibration plots known amounts of array objects against measured volumes and fit a curve. The calibrated volumes of the array objects, which also include the array objects with known volumes, are then calculated from the curve.

Click ADD.



For more information about how to add known values, refer to Section 2.9 Quantity calibration view, on page 30.

Presence Flagging view

Array objects are flagged as present or absent based on volume (total sum of pixel intensities) above or below a threshold. The software automatically estimates a threshold value based on the array object volume and the background. You can adjust the threshold to suit your analysis.

Adjust thresholds for presence flagging as follows:

- To adjust the threshold so that all array objects with a volume higher than a given array object are flagged as present, select the array object and click **Selected is Present**. Conversely, to adjust the threshold so that all array objects with a volume lower than a given array object are flagged as absent, select the array object and click **Selected is Absent**.
- You can also adjust the threshold by dragging the *Flagging threshold* slider or entering a numerical value (range 1–100) for the threshold. *YES*, indicates array objects that contain a sufficient quantity of material to be considered present, and *NO* indicates array objects not considered as present.

The numerical threshold value does not correspond directly to any array object volume.

5 Work with Colony Counter

The **Colony Counter** module makes it possible to analyze spots that are not in a regular array and is designed primarily for use with images of microbial colonies on, for example, agar plates. Colony analysis provides data on number, size (area and volume), average intensity, and circularity of spots. The module can also be used for analysis of 2D gel images.

Work in general workflow

The **Colony Counter** module is designed for the following general workflow:

- 1. Start in the *Image* view.
- 2. Open an image then all other views are available (Detect, Edit, and Background).

Get started using the Colony Counter module

Get started with the IQTL Colony Counter module as follows:

- 1. Start ImageQuant TL from the Windows start menu.
- 2. Click Colony Counter in the IQTL hub to open the Colony Counter module.

For information about file menu, analysis view features, changing contrast, and display channel color, refer to *Chapter 2 Work with Gel and Blot Analysis, on page 5*.

Work with analysis tools

The following analysis tools are available for use:



Image view

The *Image* view contains settings for the image window display. The image can be viewed in both 2D and 3D and by zooming in and out. Saturated pixels are shown in the 2D intensity image in orange. The 3D view can be enlarged and rotated. For more details, see *Chapter 2 Work with Gel and Blot Analysis, on page 5*.

Detect view

To analyze an image in the colony counter module, set an area of interest (AOI) to define the image area that will be analyzed, then adjust the detection parameters as required.



In the colony detect view you have the following options:

Set an area of	Set an area of interest as follows:		
interest	1.	Click and drag in the image or press + next to Samples to add an AOI.	
	2.	Choose whether the AOI should be circular or rectangular.	
	3.	Adjust the AOI by moving the whole area by pressing in the white diamond shape in the middle or by moving the corners with the mouse.	
	4.	You can switch between circular and rectangular AOI without redrawing the area.	
	-		

5. Add more AOIs by repeating the same procedure.

Set detection Control colony detection by the following parameters:

- parameters Sensitivity
 - Operator Size
 - Noise Factor
 - Background
 - Auto Splitting

To save specific detection parameters, click *i* and select *Export Detection Parameters*.

To open saved detection parameters, click *i* and select *Import Detection Parameters*.

Initialize Sensitivity
Defaults
Import Detection Parameters
Export Detection Parameters
Reset AOI
Delete Sample

All values, for the detection parameters, can be changed by adjusting the sliders. Detect the colonies in the AOI by clicking **Detect**.

- **Sensitivity**. Controls the sensitivity of the detection algorithm (range 1–10 000). Higher values will detect fainter colonies. To obtain a default value for the sensitivity based on the selected area of interest, click *Initialize Sensitivity*.
- Noise factor. Represents the width of the edge and center filters of the detection operator. The higher this value, the greater the compensation for noise in the image and hence the greater the accuracy. However, increasing this will also slow detection considerably, as you are greatly increasing the amount of processing required per pixel. The noise factor has a minimum value of 1. The maximum value depends on the selected operator size. It is equal to the odd number that is nearest to half the current operator size.
- **Operator size**. Represents the width of the detection operator, proportional to the size of the spots you are trying to detect. Higher values will tend to detect only the larger colonies and miss the smaller ones.
- **Background**. Represents a bias value added to all pixel values before any calculations are performed. Increasing the background value decreases the detected area of colonies.

Note:

This value is not related to the value used in background subtraction in the other modules.

• Automatic splitting. Controls automatic splitting of overlapping colonies. Drag the slider to higher values to increase the number of overlapping colonies that are split automatically. You can also split overlapping colonies manually in *Edit* view.

Note:

The total number of detected colonies are displayed for each **Sample** (selected area of interest) at the top-right of the window.

Note:

The color that circles the detected colonies can be changed by clicking **Color** at the top right of the window.

ColumnsThe data displayed in the table can be selected by clickingColumns...and ticking the boxes next to the chosen parameters,for example, Area, Circularity, and Volume.

Note:

The results table can be exported either by exporting a CSV file or by copying and pasting to suitable software.

Edit view

Editing includes the following functions:

- Drawing and erasing colonies
- Deleting colonies
- Splitting overlapping colonies

In the colony edit view you have the following options:



Select. Select a colony by clicking on it in the image view, which then highlights the colony in the table and vice versa. Several colonies can also be selected by dragging the pointer across the image view. It is also possible to select colonies with low circularity by pressing the *Low Circularity* button. The selected colony/colonies can be deleted by clicking *Delete Selected Colonies*.

Draw. Select the appropriate colony radius under **Draw** and click on the chosen colonies to detect them. Colonies can also be drawn by clicking the pointer in the middle of the colony and dragging outwards until the desired size is reached.



Delete. To delete colonies, select **Delete** and either click on the colony or press and drag to choose a larger area to delete. Colonies can also be deleted by clicking **Select** \rightarrow **Delete Selected Colonies**.

Split. To split the detected area for overlapping colonies into separate colonies, select the option and then drag it where you want to split the area. To restore split colonies to a single detection area, use the **Undo** function.

Background view

Background subtraction compensates for the background intensity in the image, so that measured colony volumes are a closer representation of the amount of material in the colonies. There are three options available to select from the **Background Methods** menu.

To subtract background intensity, select the subtraction method and click **Recalculate Measurements**. To cancel background subtraction, select **None** as a background method and click **Recalculate Measurements**.

In the colony background view you have the following options for subtraction methods:

lmage rectangle	Uses the average intensity level within a specified rectangle on the image. Position the image window to define the rectangle for background intensity. The image rectangle will produce a single value for background. This is removed from each pixel in a spot. If the background value is higher than pixel value, then the value zero is used.
Mode non- spot	Uses the average pixel intensity in a rectangular border enclosing the colony, with a width specified as the margin value. Any colonies in the border area are ignored.
None	Uses no background subtraction.

Note:

Only one negative control or image rectangle can be chosen or placed within an image even when there is more than one grid.

6 Create a multiplex image

The **Create Multiplex** module makes it possible to create multiplex images that can then be used for further analysis in the IQTL software.

Using multiplex images allows for a more detailed analysis of your images, particularly where you may require normalization of results. Imaging devices generate each channel of a multiplex image as a separate image file. Using the Create Multiplex module, you can create DS files using these multi-channel images that can then be analyzed further in other modules as a single multiplex image.

Create a multiplex image

Create a multiplex image as a DS file as follows:

- 1. Click Browse.
- 2. Browse and select images from the various channels you want (TIF or GEL file format).
- 3. Click Write DS File to create and save the multiplex image.
- 4. Quit and open the preferred module to continue analysis of the DS multichannel image file.
- **Note:** To open a DS file it must be in the same folder as the images it refers to.

7 Edit an image using the editor

The *Image Editor* makes it possible to edit images before any specific analysis. It is possible to edit TIF and GEL multiplex images with the Image Editor.

The following image editing tools are available for use:

SAVE AS	Save as. Click to save an edited image.
Revert	Revert . Click to restore the original image.
Q Zoom Image	Click Zoom Image to zoom into a selected area. This option is only available for an open image.
F. Fit	Click Fit to restore the view to the whole image. This option is only available for an open image.
😢 Display	Click Display to change image contrast and channel color using a slider or by manually typing values. This option is only available for an open image.
[] Horizontal Flip	Horizontal Flip. Click to flip the image horizontally.
····· Vertical Flip	Vertical Flip. Click to flip the image vertically.
다. Crop	Crop . Drag the edges of the rectangle using the white squares to choose the area to crop, then click Apply .
Rotate 90° Left	Rotate 90° left. Click to rotate the image 90 degrees to the left.
Rotate 90° Right	<i>Rotate 90° right</i> . Click to rotate the image 90 degrees to the right.
♣ Free Rotate	<i>Free Rotate</i> . Click to rotate the image by free hand, or using the +/- 0.1 and 5 degree buttons, then click <i>Apply</i> .

Use the Image Editor

Use the image editor as follows:

- Select the image to edit when opening the Image Editor module.
- Save the edited image with a new name to the folder you want.

Note: Save the image file you have edited with a new name to be able to refer to the unedited original image, if needed.

Note: Edited multiplex images will be saved as a single, multichannel file of the same format as the original images, i.e., .TIF or .GEL.

8 Using GxP functions

IQTL GxP is a separate software package to use for analysis of experiments in a secure data environment and supports the need for traceability and control of data.

IQTL GxP provides the following functionality for regulated applications:

- User access control (security includes personal usernames and passwords, and electronic signatures)
- Version control
- Experimental audit trail

Note: ImageQuant TL GxP is currently only available for Windows.

IQTL GxP is a true client/server tool. It is designed to scale from a single PC all the way up to a server with many connected network clients.

The IQTL GxP needs to be configured by Setup Secure Folder and GxP Admin Tool (also called Server) and Setup IQTL GxP application (also called Client).

- Secure folder stores images and experiment data. It also contains the database file which stores the user data and respective roles/privileges. The admin user responsible for the IQTL GxP Admin tool creates and maintains this Secure storage.
- IQTL GxP Admin Tool maintains and configures the Secure Folder, GxP Users, and user data. It also controls the connectivity (e.g. HTTP and HTTPS) between IQTL GxP Admin(Server) and IQTL GxP Application(Client).
- **IQTL GxP Application** is the module where users perform image analysis in a safe, authentication-based GxP environment. All the important events of analysis steps are logged and audited by the software. The application never operates directly on the Secure folder maintained by the IQTL GxP Admin tool. Only authenticated users added by the IQTL GxP Admin tool can perform analysis on this IQTL GxP Application.
- **Note:** It is recommended that an admin user configures and operates IQTL GxP Admin tool in a secured IT controlled system. Therefore, the IQTL GxP Admin tool is a standalone application separate from the IQTL GxP application.

Work with the ImageQuant TL GxP application

The IQTL GxP application is used for management of versions and approval of experiments. It is used to check out and check in image and experiment files to the secure folder and to approve analysis results. The IQTL GxP tool is to be used along with the standard IQTL analysis software to secure and manage image and analysis project files.

Note: The analysis itself is carried out in the IQTL module which does not provide any security features.

The following functions are available in IQTL GxP client for use:

Filters	 Filter Search Results applies several filters to the projects in the project list.
	• Last 7 days shows all projects created in the last 7 days.
	• Last 30 days shows all projects created in the last 30 days.
	 Last 90 days shows all projects created in the last 90 days.
	Active shows all active projects.
	 Awaiting QC shows all projects awaiting QC
	Checked out shows all checked out projects (locked)
	 Checked out (by me) shows all projects checked out by me (locked)
	• Awaiting signoff shows all projects awaiting final sign off.
	• Signed off shows all projects that are complete.
	• Signed off (by me) shows all projects signed off by me.
	• All shows all projects.
	• Custom filter lists all custom filters created in Advanced Find/ Filter tool.
Projects	Lists names and statuses of all projects in Secure Folder
	Click a project to access it
Refresh Projects List	 Updates the Projects List to include new projects and to update project states
	Only applicable if more than one Client is accessing a single Server and Secure Folder
Advanced Find/Filter	Project name searches project name keywords.
rina/riiter	• Last user searches all projects by last user access.
	 Project state searches all projects by a list of all possible project states such as Awaiting QC, Active etc.
	Data range searches all projects by selected dates.
	 Save as filter combines multiple search options and saves it as a filter for future use.

Tip:

Name the custom filter with descriptive names.

Advanced

Options

- **Cancel checkout** cancels checkout, and does not perform any check-in. The working folder is cleared but does not record any data in the secure folder.
- **Working folder** opens working folder for review when the project is checked out. Additional documents can be added to the working folder when the project is checked out.
- *Inspect image* allows the following visual inspection of the checked-out image:
 - Image name
 - Image resolution
 - Image dimensions
 - Image calibration (if present)
 - Image view
 - Image histogram

The following is a mandatory step if QC is required before analysis selected in the Admin Tool. It requires that the user has QC permission to visually inspect image when the project is in **Awaiting QC** state, including the following options:

- QC Pass image can now be analyzed
- **QC Fail** image cannot be analyzed
- *Edit image* opens **Image Editor** tool for Windows that allows image editing. See *Chapter 7 Edit an image using the editor, on page 47* for functions available on this tool.
- Audit Report Generates a PDF file of the full project history.
- Analysis
Report• Once an analysis has been performed, it generates a PDF file
of the analysis report, which includes a full report of the
analysis for the project and indicates whether the project has
been signed off, including details of who signed it off and when
it was signed off.

Work with the ImageQuant TL GxP workflow

Different users need to have access to the different steps in the workflow, which can be defined by the Admin when users are created. Refer to *Administer users and permissions, on page 53*.

Use the following workflow for the IQTL GxP client:

- 1. Log in . Log in using username and password.
- 2. Add project. Browse and add files including image to be analyzed to the project. A name and notes can also be added at this stage. The project files are stored in a secure payload file in the secure folder and protected from untracked modification by a strong cryptographic hashing function.

- Check out. Check out to copy the image stored in the secure folder to the working folder for analysis. Once the image is checked out by a user, the project is locked to prevent other users from making changes. The working folder is created in the Temp folder of the specific user currently logged onto the PC.
- 4. **Analyze**. Automatically open the ImageQuant TL software for analysis. The image file can now be analyzed. Once analysis is completed, close the analysis view, and save when prompted. A copy of the analysis data (.1dp) and an audit trail of the full analysis care captured in the working folder with the image.
- 5. **Check in**. Check in to copy the data stored in the working folder to the secure folder, which now includes the completed analysis. This action also deletes the data stored in the working folder.
- 6. **Request sign-off**. Mark the project as complete, which changes the status to *Awaiting Sign off*.
- 7. **Check out (R/O)**. Open the analysis in read-only format so that the reviewer can review the analysis. Once the analysis review is complete, close the analysis window. This action copies the image stored in secure folder to the working folder in a read-only format for the reviewer.
- 8. **Sign-off**. Reject or approve the analysis. The user must type password to complete this step. Rejecting returns the project to active state. Signing off the analysis places it in the complete state. Undo approve option is available.
- 9. **Cancel sign-off**: Cancel the sign-off request and make the project again active for further analysis.

Prevent image file tampering feature

For additional security, the Amersham IQTL 800 GxP captures images with a special fingerprint (security tag), which can be read during analysis. This function makes sure that image files are not tampered with after imaging and before analysis.

The integrity of the image is verified by the IQTL software and a **Pass** result is displayed as follows:



Integrity test result: Pass Original image from ImageQuant 800 GxP confirmed.

Work with IQTL GxP secure folder

The Secure Folder is the "vault" in which the analysis data, user information, and server setup information is stored. IQTL GxP allows the secure folder to be set up locally or remotely with very stringent permissions. The image and analysis data are potentially captured and saved here at several points throughout the project for additional security.

The experiments can only be checked out from the secure folder for further analysis by one person at a time. Moreover, all actions are recorded, enabling traceability and repeatability. Refer to *IQTL GxP regulatory requirements, on page 57*.

The secure folder contains the following:

- Payload folder includes packaged, fingerprinted versions of the work users check into the system.
- Database file includes the user information and access logs.
- Server Config file includes server setup information.

Location

- Ideally, the secure folder should be located on a network drive, not on the PC where the client is installed.
- If the Secure Folder is also installed on the local PC, it is critical to make sure that the user permissions are set up correctly.

Permissions

- Due to the design of the software, the secure folder can be set up with very strict permissions.
- Only Administrators (Sys-admin) need access to the secure folder, which is automatically created on software installation.
- Set up permissions as follows:
 - Administrators can read, write, and change the secure folder, but cannot delete any content.
 - Other users cannot access the secure folder (to secure data integrity, and prevent malicious or accidental modification to the data).

Work with database setup

IQTL GxP has been developed to support a range of different laboratories from small companies with a local PC and one staff member to much larger facilities with a hierarchy of multiple users using clients on several PCs but connected to a single secure folder. This flexibility means that the setup is very important, which can be done either locally or remotely.

Set up local database

The simplest installation architecture supported by IQTL GxP is the fully local setup. This is where both the server and client are on the same PC.

The reason to use this setup is simplicity. If your needs are limited to a single PC, it is possible to largely ignore the client/server nature of the TLVC application and just get on with the business of analyzing images and recording work.

The drawback is security and integrity. Having the secure folder on a PC that users have file-system access to will always come with a possibility of accidental or deliberate data integrity compromise. For this reason, we recommend that the server and client always be located on different PCs.

Use the following workflow for the IQTL GxP local database setup:

- 1. Create the secure folder. This step is performed outside of the application. Choose a location that is accessible to the Windows user that will be using the client tool on a daily basis.
- 2. Install and run the server.

- Provide the path to the secure folder you created and click Connect. You may see a "No Data Found" message – this is expected given it is a new, empty folder. Click "Initialize Folder" and you will see the main Server administration interface.
- 4. The most important option to configure for a local setup is the "Allow remote clients" setting. If this option is disabled (the default value), the server can be launched without requiring any administrator level permissions and can therefore be launched on demand by the client application. For this setup, no further server configuration is required, and you can now move on to the user setup section towards the end of this document.

Set up remote database

Remote database setup is the recommended option where the server and secure folder are located on different PCs to one or several clients. This separation is necessary to provide a security layer between users and controlled data.

Our recommended approach is to co-locate the server and the secure folder on the same PC, but there is no requirement to do so. The only hard requirement is that the User Account you will use to run the server application have read and write access to the secure folder via Windows folder permissions. Delete permission is not necessary.

As in the local setup, you are responsible for creating the Secure Folder and making sure its permissions are set such that clients have no access to it.

Use the following workflow for the IQTL GxP remote database setup:

- 1. Create the Secure Folder. This step is performed outside of the application. Choose a location that is accessible to the Windows user that will be using the client tool on a daily basis.
- 2. Launch the server application and provide the path in the box provided and follow the instructions to complete initialization until reaching the main server interface.
- 3. Given that clients will be running on remote PCs, configure the server to accept remote connections. Do this by turning on the "Allow remote clients" option in the "SERVER" section of the main interface. Once this option is selected, click "(Re)Start Server" to apply the changes.
- **Note:** To configure the Server to use HTTPS instead of (or in addition to) HTTP, enable the required methods in the Admin tool and restart the Server. For clients to connect successfully to an HTTPS instance on the server, you must configure Windows to attach a digital certificate to the HTTPS port that is used (HTTP and HTTPS must use different ports). This workflow is out of the scope of this help but is strongly recommended if you plan to allow access to clients outside of networks in direct control.

Administer users and permissions

The IQTL GxP Admin Tool controls the setup of user security. It is used to create and assign analysis permissions to users and to control access to the Secure Folder.

The following functions are available in IQTL GxP Admin Tool for use:

Secure Folder	Click to show the location of the Secure Folder with the following options:		
	• View the Access Log allows the review of the Access Log.		
	• Add Archive Volume extends the capacity of the Secure Folder across one or more additional folders/drives.		
Server	Click <i>Allow remote clients</i> with the following options:		
	- Clear this option to run as a local service accessible only to clients on local PC with no external security risks.		
	 Select this option to run as a remotely accessible service visible to clients on networked PC. 		
	 Select <i>HTTP</i> when access to remote clients is not allowed. Note that it is less secure access between client and server than HTTPS. Port can be changed. (Default is 17080). 		
	• Select <i>HTTPS</i> when access to remote clients is allowed. Port can be changed. (Default is 17443).		
Startup	Name the Windows Service with any of the following options:		
	Removed (Remove)		
	Queried (Query)		
	Stopped (Stop)		
	Started (Start)		
Client Options	 Select Mandatory approval comment to require that a comment to be added at any approval (QC/Sign off). 		
	• Select Require QC before analysis to require a user with the QC permission to review and approve image quality prior to analysis.		
Password Restrictions	For internal users, set any of the following password restriction options:		
	• Expire . Enter password expiry from 0 (never) to 10 years.		
	• <i>Minimum length</i> . Enter password minimum length from 0 to 32 characters.		
	• Require mix of upper/lower case characters . Toggle mix of upper and lower case characters for the password on or off.		
	• Require non-alphanumeric characters . Toggle non- alphanumeric characters (special characters, such as +, %, etc.) for the password on or off.		
	 Prevent repeats. Toggle to prevent reuse of old passwords on or off. 		

Users	List all users created of the software, including permissions.
	Active users are always listed. Inactive user can be listed by
	selecting the Show Inactive Users option. Click to select any of
	the following options:

- Edit User to edit user permissions.
- Set Password to set new user password.
- **Expire Password** to expire current password and force the user to enter a new password on next login.

Note:

This will have no effect if the No Password Expiry rule is set.

- **De-activate User** to set an active user account to inactive.
- **Re-activate User** to set an inactive user account to active again.

New User Create new user with one of the following options:

- Internal User. Create user using IQTL GxP with a unique username and password governed by the password restrictions.
- Windows User. Assign user using username and password that exists in the Windows Active Directory® on the relevant domain. Password is controlled outside Admin Tool. Once the domain and username is specified, click Check Names to verify that the user exists.

The following permission options are available for use to each created user:

- Add Project. Select to add image to Secure Folder.
- Check out. Select to copy image from Secure Folder to Working Folder.
- Check out R/O. Select to copy image from Secure Folder to Working Folder for read-only view.
- *Emergency Login*. Select so that two users can log in as a third user in case of emergency.
- **QC**. Select to approve or reject an image if QC is required before analysis.
- Approve. Select to approve analyses from other users.
- Self-Approve. Select to approve own analyses.

QC Workflow

The QC Workflow is a set of steps to enforce a policy where analysis cannot proceed until the input data (images) have passed a QC check.

The QC Workflow is a global setting, controlled by the IQTL GxP Admin tool. Once enabled, when a project is created, it is placed in an "Awaiting QC" state rather than the usual "Ready" state.

While "Awaiting QC", the actions available to perform on the project are limited to QC functions.

Once the project has been "QC Passed", it is placed in the "Ready" state and analysis can begin.

Follow the below steps to perform a QC-Check analysis:

- 1. Enable QC in Client Option of IQTL GxP Admin tool.
- 2. Add a user with QC privileges enabled.
- 3. Log in to the IQTL GxP application as a non-QC user and create a project. The project state will be QC-Awaited. It will not be possible to check-out and analyze unless the project is QC passed.
- 4. To proceed for further analysis, please log in again as a QC user. Check-out R/O, Inspect, and make a QC pass or QC fail.
- 5. Once the project is QC passed, then the project is ready for analysis by the earlier requested user.

Emergency Login Workflow

Emergency login provides the ability to sign in to the GxP application posing as any normal user, without needing to know their password. This is helpful if a user has checked-out projects and then becomes unavailable for whatever reason.

To achieve this, two provisioned users (i.e. users with the "Emergency Login" permission) must supply *their* credentials.

Follow the steps below to create and login as an Emergency user:

- 1. Add two users with Emergency permission enabled. These two users shall not be mixed with another normal user.
- 2. Run the IQTL GxP application. At the login screen, enter the username of the absent user you want to sign in as, but leave the password blank, and click on *Emergency Sign In*.
- 3. Once the *Emergency Sign In* window opens, log in with two emergency sign-in users.
- 4. The IQTL GxP application is now logged in as an absent user.
- **Note:** During this session, all the events are tracked in audit trails as performed by an absent user. There will be an entry in the access log recording information about emergency users logged in as absent user.

IQTL GxP regulatory requirements

Regulatory authorities require pharmaceutical development and manufacturing companies to use quality-assured equipment and to follow detailed and carefully documented analytical and manufacturing procedures. The purpose of this control effort is to ensure consistent and reliable quality in pharmaceutical products that reach the market.

GxP regulations describes requirements for companies and laboratories and include guidelines on how standard protocols can be used for safe electronic record keeping.

IQTL GxP has functions that supports compliance to the **21 CFR Part 11** regulation by the Food & Drug Administration (FDA) and **EU GMP Annex 11**.

The following functions support IQTL GxP 21 CFR Part 11:

• Audit trails (21 CFR 11.10 (e))

- Project audit history complete log of user activity
- Analysis log detailed application history
- Access log full list of all successful/failed login attempts

Limiting system access (21 CFR 11.10 (d))

- Robust password access
- Ability to use Windows User Access Control for authentication
- Fine grained feature access based on permissions
- Client/server approach prevents user access to the secure folder
- Electronic signatures (21 CFR 11, Subpart B)
 - Approval functionality limited to configured users
- Password restrictions (21 CFR 11, 11.200 (a))
 - Full compliance with passwords complexity requirements
- Emergency logins (21 CFR 11, 11.200 (3))
 - Two users (with appropriate permissions) can provide credentials to pose as another for emergency situations
- Version History for change control (21 CFR 11, 11.10 (k))
 - Resilient data integrity enforced throughout the lifetime of a project
 - Detailed log of all operations performed on a project
- **Note:** IQTL GxP is intended to address GxP requirements in compliance with 21 CFR part 11 and EU GMP Annex 11. It is the user organization's responsibility to make sure that the software is appropriately validated within the context of the user's particular environment, and to make sure that the other Part 11 or EU GMP Annex 11 compliance elements are implemented appropriately.

9 System requirements

To be able to use ImageQuant TL, the following system requirements need to be met:

- Operating systems: Windows 10 (64 bit), or Mac® OS X® 10.14, or higher.
- Processor: 1.2 GHz and better.
- Memory: Minimum 2 Gb, recommended 8 Gb installed RAM, or higher.
- Free hard disk space: Recommended 1 Gb.
- Microsoft .NET® 5.0: See instructions below.

Minimum and recommended specifications are important in order to provide good software performance and reduce installation and operational issues.

Note: You need to have administrator rights for the installation. To use the software you do not need administrator rights. IQTL GxP is only available for Windows.

Workflow

Install ImageQuant TL according to the following workflow:

- Download the software installation package from Cytiva.com, or run the DVD installation disk.
- Collect your eLicense using the activation ID you have received via e-mail.
- Download and install Microsoft .NET 5.0.
- Install the ImageQuant TL software.
- Configure the license.

Install Microsoft.NET 5.0

The Microsoft .NET package is not provided with the ImageQuant TL or IQTL GxP software package. Microsoft .NET is needed to run ImageQuant TL and IQTL GxP software.

Install.NET 5.0 as follows:

- Download Microsoft.NET 5.0 (.NET Runtime [Version]) from https:// dotnet.microsoft.com/download/dotnet/5.0.
- 2. Double-click the Windows desktop runtime file to run the installation.

10 Administer electronic license

The ImageQuant TL software requires an electronic license (eLicense) to operate. The license file is collected from the Cytiva software licensing web page using an activation ID.

The activation ID is delivered through e-mail after ordering ImageQuant TL. Store the activation ID in a safe place. For details on how to collect the eLicense using this activation ID, refer to Section 10.1 Collect license using activation ID, on page 63. Once the eLicense has been downloaded onto your computer, proceed with the following steps to activate and begin using the software.

Note: If the access code has not been delivered within 7 days of the order, contact technical support.

Choose license type

You can administer one of the following license types:

- Node-locked license is generated for a specific computer and does not require a network connection after installation.
- Floating license is handled by a license server to grant access for running ImageQuant TL.

To collect your electronic license using this activation ID, refer to Section 10.1 Collect license using activation ID, on page 63.

Node-locked license

Follow the steps below when using a Node-locked license:

- 1. Download and save the Node-locked license in any folder on your computer.
- 2. Start the ImageQuant TL software.
- 3. When the ImageQuant TL control hub opens, click **Configure license**.
- 4. Select Node locked as license type.
- 5. Browse to the folder where you saved the Node-locked license and click *Apply*.
- 6. Now the software is ready to use and you can return to the hub and use the ImageQuant TL software.

Floating license

To use the software with a floating license follow the steps below:

A. Install the eLicense server.

- **B.** Set up the eLicense server.
- **C.** Configure the floating license in IQTL software.

A. Install eLicense server

To install the eLicense server on the computer where the license will be located:

- 1. Browse the downloaded software installation package for **Cytiva Software** *Licensing Server Setup*.
- 2. Double-click the file Cytiva Software Licensing Server Setup.
- 3. Click **Next**.
- 4. Accept the EULA terms and click **Next**.
- 5. Accept the default destination folder (recommended) and click Next.
- 6. Click Install to start the installation.
- 7. Click *Finish* to complete the installation of the license server.
- 8. B. Set up eLicense Server.

B. Set up eLicense Server

The eLicense server needs to be set up after being installed using the Admin Tool.

1. Set up the eLicense server

The eLicense server must be set up correctly to use the floating electronic license file. Do the following to set up the eLicense server:

- 1. Download the floating license file from the license website at *http://www.cytiva.com/eDelivery*.
- 2. Save the file in the license file path used by the eLicense server. The default folder in Windows 10 is C:\Program Files\Cytiva\eLicense server\licenses.
- 3. Restart the computer to initialize the eLicense server.

2. Set up eLicense server administrator

Use any of the following ways to set up a floating license:

- eLicense Configuration app (simplest way):
 - 1. Open the e-Licence Configuration app from the Windows start menu.

• License Configuration	×
Cytiva Configure e-License	
An e-License is required to run the software. It is used to authorize your computer with the e-License server. These are the steps required to configure an e-License: 1. Select and copy your computers Ethemet address (also know as MAC address or physical address), which is require	red in
step 2, using the button below. The address will then be copied to the clipboard. 482AE3982265, (Ethernet, Intel(R) Ethernet Connection (7) I219-LM) Copy to Clip 2 Use the link before the connection (7) I219-LM	
 Use the link below to access the Cytiva e-license web. Follow the instructions on the web page to retrieve an e-lice Note: Paste the Ethernet address when invited to do so. This is required to generate an e-license. Also remember t press the activation button and to save the e-license on a file. www.cytiva.com/eDelivery 	
3. Browse to select the retrieved e-License file. Thereafter, press "Configure e-License". Browse	
	ose

- 2. Browse to the downloaded floating license file, then click **Configure e-License** and **Close**.
- License Server Admin Tool app (advanced, containing information and tools for the eLicense server program):
 - Open License Server Admin Tool from the Windows start menu, or open LMTOOLS (lmtools.exe) from the folder where the server installation is stored. For details about how to use LMTOOLS is described in Flexera Licensing End User Manual.
 - 2. Look up the host ID of the computer, meaning Ethernet MAC address, which identifies the computer and is used to activate the eLicense.
 - 3. Click the **System Settings** tab in the **LMTOOLS** app and enter the MAC address in the **Ethernet** box.
 - 4. Check that the **Path to the license file** contains the file path for the floating license. The default directory for floating license files is C:\Program Files(x86)\Cytiva\eLicense Server\Licenses. Do not change the default path.

By default, Use Services is selected, to run the license server as a Windows Service.

By default, **Start Service** at power up is also selected, to automatically start the license server when the computer boots.

Click **Save Service** even if you did not change anything in this step.

C. Configure the floating license in IQTL software

After the floating license is configured in License Server, all the systems connecting to License Server can follow the steps below to use the floating license.

- 1. When the ImageQuant TL hub opens, click **Configure license**.
- Click Back and select License type as Floating. After this, select Remote Server option and fill in the IP address of the computer where the license server is installed.

Note: In case of local system where the server is installed, kindly use Local PC option instead of Remote Server.

- 3. Click the *Apply* button.
- 4. Now the software is ready to use and you can return to the hub and use the ImageQuant TL software.

In this chapter

Section		See page
10.1	Collect license using activation ID	63

10.1 Collect license using activation ID

Do the following to collect and activate the electronic license:

- 1. Go to *http://www.cytiva.com/eDelivery* and log on by selecting *Activation ID* and typing in the activation ID that you received by e-mail during your purchase.
- 2. Select Activation & Entitlements → List Entitlements.

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© See all Recent Files Description The accounts you are connently assigned to do not have any erithments. Please context your system administrator.	Announcements GE Healthcare Life Sciences in now Cytiva. Get reacquainted with Cytiva.	

3. Select the entitlement in the list for IQTL, and activate it by clicking **Action** \rightarrow **Activate**.

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4. Type your personal details and click **Next**.

Home Activation Entitleme					
Activate Licenses	5				
Please add or select at	least one host and c	lick Next to config	jure counts.		
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Sold to:	IQTL demo license		Activation ID	Product	Expiration date
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Ship-to address:					
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Organization *					
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SoftwareUpdates		~			
Cancel					

5. Under **Configure Hosts**, click **±** to add host.

10.1 Collect license using activation ID

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Configure Hosts					
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Cancel					

 In Create License Host, enter the Host ID (also called MAC address) for the computer where you have installed ImageQuant TL. Then click OK to generate the license file. For details on how to find the Host ID, refer to Find Host ID (MAC address), on page 64.

Create License Host Please select a license host type and enter an appropriate value.	\otimes
Nodelocked hosts:: ETHERNET V = XXXXXXXXXXXX	
OK Cancel	

7. Under *License Details*, select *View* → *Save to File* to save the license file to any folder you want on your computer. The license file is ready to be used.

III List Entitlements - FlesNet Opera 🗴 🕂											- 0
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Complete											

Find Host ID (MAC address)

Note:	The following step is applicable only for node-locked licenses.

Windows

Do the following to find the *Host ID* for Windows:

- 1. In the Windows search bar, type cmd and press Enter on your keyboard.
- 2. Type ipconfig /all and press Enter.

- 3. Check for a Network adapter for the current system.
 - **Note:** For systems connected to a network via an Ethernet cable, choose **Ethernet Adapter Ethernet**.

Or, if the system is connected to the network via a Wireless network adapter, choose **Wireless LAN adapter Wi-Fi**.

Kindly choose the appropriate network adapter based on the connectivity of the system.

4. Write a note of the string numbers and letters shown under **Physical Address**.

Note: Enter this **Host ID** without the hyphens – when collecting your electronic license on the eDelivery website.

Мас

Do the following to find the *Host ID* for macOS:

- 1. Open the Apple menu and click the Apple icon.
- 2. Scroll to System Preferences.
- 3. Select *Network* → *Wi-Fi* → *Advanced*.
- 4. Copy the MAC address shown under the *Wi-Fi Address* or *Airport Address*.

11 Contact technical support

If there are any problems with the installation or use of ImageQuant TL, please contact your local sales support office, or use the contact form found at *https://www.cytiva.com/contact*.

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cytiva.com/iqtl

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