Amersham™ ImageQuant™ TL analysis software

IMAGING SYSTEMS, SOFTWARE, AND ACCESSORIES

ImageQuant™ TL software is an advanced analysis software designed specifically for images of 1D gels, Western blots, multiwell plates, and colony plates. In addition, the analysis toolbox module has several generic tools for quantitative analysis of a wide range of sample types.

Why ImageQuant TL analysis software?

This software encompasses a comprehensive suite of tools and modules designed for various types of image and data analysis. It includes capabilities for analyzing 1D gels and blots with features such as band detection, background subtraction, molecular weight calibration, multi-channel normalization, and quantity calibration. Users can compare samples using similarity scores and dendrograms. Additionally, the software provides a general image analysis toolbox for defining and analyzing areas and line profiles of interest, an array analysis module for multi-well plates, a colony counter module for bacterial colony detection, and an image editor for basic image modifications like rotation and cropping.

ImageQuant TL software is the software of choice for research labs to make sure their image data does not get modified. When using its analysis modules, no matter the operations the user performs, the image raw data remains intact.

The software supports a wide range of image file formats including TIF, IMG, JPG, and GEL, and is compatible with both Windows and Apple Mac systems. For regulated environments, a GxP version ensures compliance with 21 CFR Part 11, featuring data integrity and traceability, including electronic signatures.

Top 10 features

- Multi-image view: facilitates navigation across various analysis windows, empowering users
- 2. Alignment tool: Enables alignment of images from different acquisition methods, ensuring easy comparison and confidence in your results.
- 3. ImageQuant TL analysis software is the only fully validated software compatible with the Amersham™ range of ImageQuant 800 CCD imagers and Typhoon™ laser scanners enabling you to work efficiently and achieve the best results from your image data.

Gel and blot

- 4. Chromatogram import with fractions and overlay charts: correlates gel bands and chromatogram fractions and visualize multiple curves with ease - just as you do with UNICORN™ software.
- Normalization: Correct sample application differences effortlessly and leverage the total signal in the reference channel, like Cy™5 Quickstain, for precise, normalized values. Plus, view both normalized and non-normalized values for full transparency.
- 6. Dendrograms similarity score: Our advanced tools offer an easy zoom and expand view, enabling quick, unbiased comparisons of different samples across various applications. Perfect for DNA gels, Southern blots, and protein electrophoresis, our solution simplifies the way you measure sample similarity, without the need for specialized software. See how your samples stack up to the reference with a clear similarity dendrogram tree. Combine it with the alignment tool to create image overlays with different acquisition methods (e.g. to overlay fluorescence and phosphor images).

Tissue imaging

7. Automatic quantification of shapes: simplifies your research with the ability to create numerous non-regular shapes easily, reducing user bias and saving time.

Colony counting

8. Transformation/transfection efficiency protocols: the power to fine-tune transformation sensitivity with auto-detection of colonies and easy setting of transformation threshold levels, ensuring every colony counts.

Array analysis

- Multi-channel fluorescence: reduces complexity in the analysis of multi-channel images, making your assays more efficient.
- 10. Positive or negative: swiftly categorize wells as positive when signals surpass the threshold.



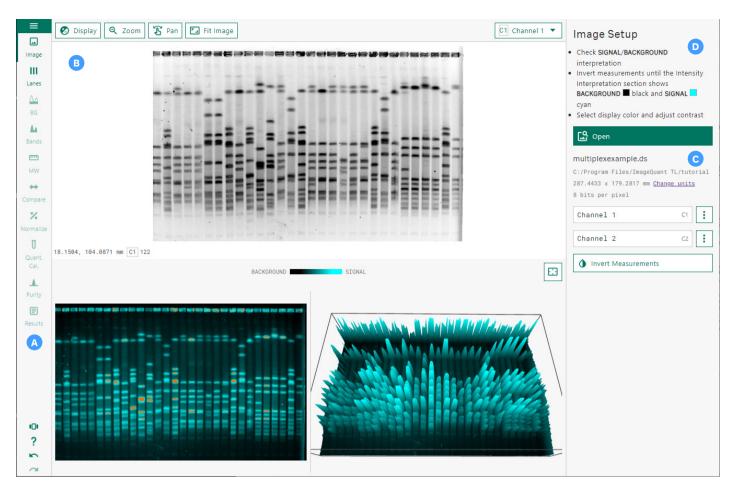


Fig 1. Streamlined ImageQuant TL software user interface with four sections. (A) a navigation panel with quick access to image analysis steps, (B) a large central view of your analyzed image, along with other analysis-dependent data like 3D views, lane profiles, and more, (C) a settings panel where you can easily adjust analysis steps, and (D) an instructions panel with information on each step.

Common functions

Export presentation image with dpi options

After opening an image, the user usually needs to adjust the contrast, especially for multi-channel images. The next step in the analysis is to create different objects, for example lanes, bands, rectangles, circles, lines, and spots. With the function export presentation image, the displayed image can be exported at any stage during the analysis, with or without an overlay of the analysis objects. It is possible to save the exported image in BMG, JPEG, or PNG file formats. These exported images can be used for making presentations or be part of figures for publication.

Note that the contrast setting and the image export do not affect the original raw image data. Most scientific journals require that authors also provide the original images supporting all blot and gel results reported in an article's figures. These are the original images used for analysis, in TIF, GEL and IMG file formats.

The image detector determines the number of pixels in the original image and the magnification of the imaging system decides the pixel size. The export presentation image allows the user to increase the number of pixels in the exported image. This changes the dpi (dots per inches) and the pixel size of the exported image. When submitting figures for scientific publications, 300 dpi is a generally accepted resolution.

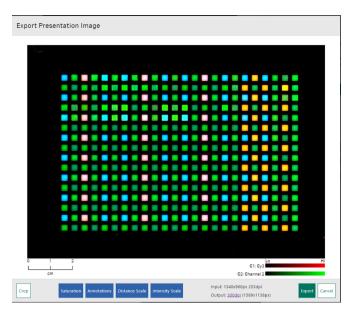
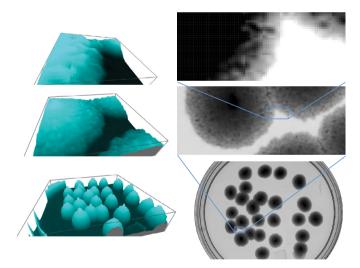


Fig 2. Multichannel fluorescence images from a Amersham Typhoon scanner. Export presentation image function allows the user to export high resolution images of color and grayscale images during the analysis steps. Highlighted saturated pixels and overlays can be included in the exported image. It is also possible to crop the image, and to select the output dpi (number of pixels) of the exported image to meet any publishing requirements.

Deep zoom-in to view single pixels

ImageQuant TL software has a zoom tool which allows the user to easily zoom in on individual pixels. For samples that have items of interest in close proximity, it is advantageous to have a powerful zoom-in tool to view fine image details.



 $\textbf{Fig 3.} \ \ \text{Deep zoom-in tool allows resolving the finest image details and easily check the intensity of individual pixels.}$

Gel and blot analysis module

In the gel and blot analysis modules, there are multiple steps. Figures 4, 5 and 6 illustrate examples at different stages of the analysis.



Fig 4. Lane detection, along with annotation and manual lane editing.

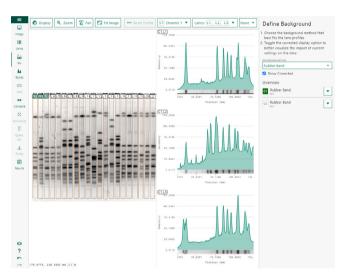


Fig 5. In the background view, users can select between different background subtraction methods, including rolling ball, rubber band and constant value.



Fig 6. Automatic band detection with options to edit bands manually as needed.

Lane profile comparison

Scientists often compare samples visually across different lanes, but this can be difficult if samples have many bands. Visual examination is also time-consuming and may be biased. The ImageQuant TL software lane profile comparison tool analyzes lanes and determines similarity. This analysis tool will give you an overview of all samples, automatically compare all lanes to a selected reference lane, and calculate a dendrogram similarity tree that groups all samples based on the calculated similarity score. The lane profile comparison tool plots a similarity score against relative volume compared to a reference lane. This correlation calculation helps you to quickly determine how similar the samples are without any bias. The resulting similarity scatter plot provides an overview of all lanes, both in terms of chemical similarity (the separation profile) and amount. Based on all possible pair-wise comparisons, it is possible to construct a dendrogram of all the samples in the lanes. This grouping of samples is based on lane profile similarity and corresponds to how similar samples are chemically in the electrophoresis steps. The software displays this dendrogram and the results table it is based on for easy export and reporting

You can use lane profile comparison to:

- Evaluate purified samples during manufacturing of biopharmaceuticals.
- · Determine sample purity for batch release of drugs.
- Select a specific fraction from a chromatography run by comparing lanes of different fractions.
- · Quickly identify artifacts or odd spikes in lanes.
- · Analyze complex samples like cell lysates (Fig 8).
- Speed-up gel shift assays and DNA fragment analysis.

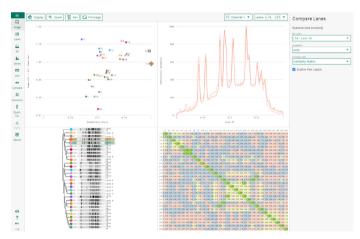


Fig 7. The lane profile comparison tool automatically performs a full quantitative comparison of different lane profiles based on profile shape and peak volumes. These parameters reflect chemical similarity and amount of sample in the lane. The analysis is based on correlation of lane profiles and comparing lane volumes. The user can select to display: The image or all pair-wise profile correlations in the similarity table. The scatter plot (top left) shows the calculated similarity score for a selected reference lane. Users can select the reference lanes in the table, or in the dropdown list. Lane alignment can either be based on the lane area or on the molecular weight (MW) calibration (if available). The software calculates and presents lane grouping in dendrogram form (bottom left).

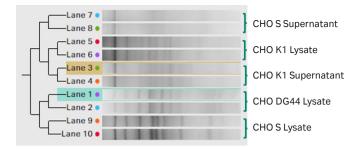


Fig 8. The comparison tool, grouped lysate samples and revealed similarities between sample groups. For example, the K1 cell lysate and supernatant lysate were similar and closely related.

Normalization function

The normalization function provides you with several ways to normalize the calculated values of band volumes. You can normalize to a single band or lane and to total protein and housekeeping proteins for multiplex images.

- Selected or largest band: Normalize band volumes based on selected or largest bands
- Housekeeping: Lane-wise normalization based on housekeeping (largest) bands in each lane
- Total protein method: Uses the total lane volume, or the sum
 of all bands in a lane, for the user-selected reference channel
 for lane-wise normalization of all bands in all channels. Figure 9
 shows total protein normalization for multiplex images.

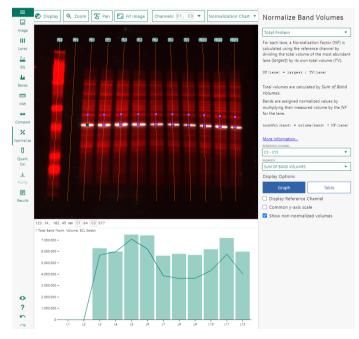


Fig 9. Total protein normalization is easily done for multichannel images in ImageQuant TL software. The total sum of band volumes for each lane of a selected reference channel are used for normalization. In this example, the lysate samples were labeled with Cy5 and the target protein was Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) detected with ECL™ reagent in a Western blot experiment. The line in the graph represents the non-normalized values.

Quantity calibration function

Quantity calibration allows you to calculate the amount of material in all bands in the image from the band volume (total sum of pixel intensities), calculated from values entered for bands with known amounts. This function is often used to check purity and amount of sample.

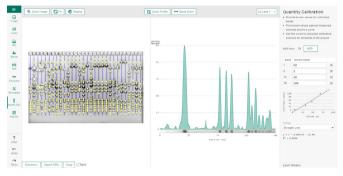


Fig 10. Bands with known values are used to plot and calculate all band volumes using quantity calibration. After choosing the best fit for the curve, the equation can be exported.

Useful features, include:

- · Choose from a wide variety of curve fits.
- Detect changes in real time as bands are entered for curve fitting.
- Copy and paste the equation for the plotted curve for documentation.

ÄKTA™ chromatogram import function

The use of SDS-PAGE to analyze protein samples from an ÄKTA™ system is often the analytical method of choice to measure purity, confirm molecular weight, and to detect protein modifications such as glycosylation, hydrolysis, and degradation. The new ImageQuant TL 11 software purity step allows import and annotation of chromatogram curves from ÄKTA systems to connect peaks in chromatograms to samples run on SDS-PAGE gels. This software function allows users to quickly link the SDS-PAGE purity analysis with the ÄKTA chromatogram, and report all results together in a single pdf file.

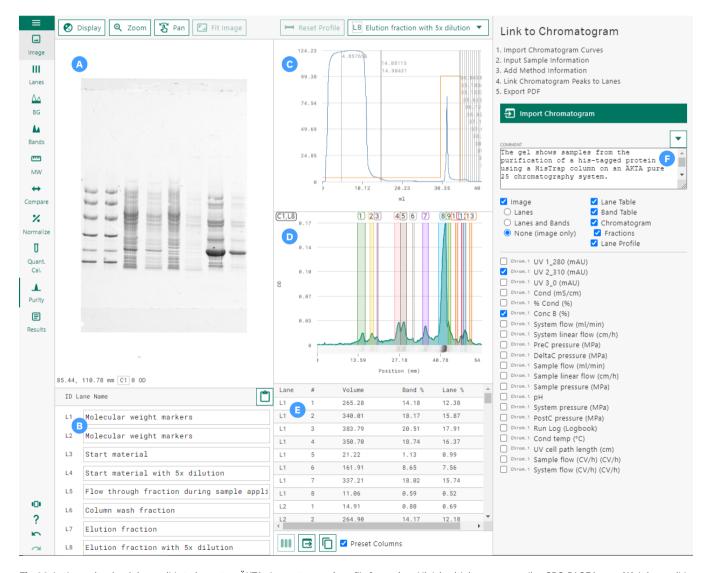


Fig 11. In the purity view it is possible to import an ÄKTA chromatogram (.asc file format) and link it with its corresponding SDS-PAGE image (A). It is possible to copy and paste directly all sample names into the lane name table (B), for example from an Excel table, and display the chromatogram curves contained in the .asc file (C). The selected lane profile is shown (D) and the corresponding band purity results of the highlighted lane are displayed in the band table (E). If there is injection/fraction data in the .asc file, this information can be imported in the user comments field (F).

Analysis toolbox module

The analysis toolbox module provides tools for analyzing images that are not specific to the other modules. It is also useful to check certain parameters, such as the profile of a line of pixels, or calculating signal-to-noise ratios. This module also analyses images with samples of non-regular shapes, like tissue sections. You can use the different drawing tools to generate the area of interest (line, polyline, curve, auto-trace shape, rectangle, ellipse, polygon) as well as to define features in the image. The profile window displays intensity profiles along the lines and shapes you define. The software calculates area and volume (i.e., number of pixels and total pixel intensity), with or without subtraction of background intensity.

Example of applications are:

- Quantify target molecules in tissue sections
- Quickly check the signal-to-noise ratio in an image
- Compare profiles of two similar shapes (e.g. different lanes) in a rapid manner
- · Quantify samples of different contours.

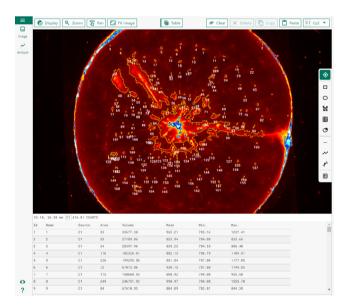


Fig 12. Multi-object detection in images of tissue sections is easy with **detect objects** tool in analysis toolbox module.

Array analysis module

The array analysis module is designed for analysis of images consisting of rectangular arrays, such as microplates, dot blot, and slot blot images. The module is helpful when determining the volume and presence within each well, such as target protein.

Key benefits include:

- Choose grids from common templates such as 96- or 384-well plates, select shapes of the grid, such as circle or squares, and customize the size of individual wells.
- · Detect presence or absence of samples in wells.
- Multiple grids for images with more than one array. Each grid can be modified to fit each individual array.
- Easily normalize your assay by setting reference wells and plot a curve from known well volumes using quantity calibration.



Fig 13. The well volumes of a dilution series in a 96-well plate are normalized and analyzed using the normalize option in the array analysis module.

Colony counter module

The colony counter module provides functions for analysis of spots that are not in a regular array — primarily for use with images of microbial colonies. It can also be used for basic analysis of proteins separated on a 2D gel.

Key benefits include:

- Transformation/transfection efficiency protocol: report number of transformed/transfected colonies versus total number of colonies.
- · Automatic detection, count, and volumes of colonies.
- Add, delete, or split colonies easily.
- Select multiple areas of interest for analysis of images with more than one colony.

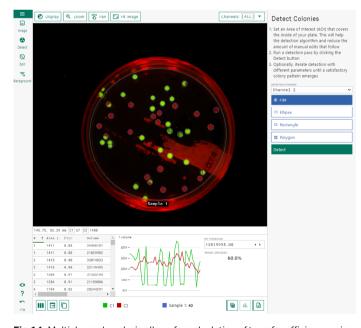


Fig 14. Multichannel analysis allows for calculation of transfer efficiency using fluorescent proteins as reporters. Images captured by Dr. Annemarie Kuipers and Dr. Albert van Dijk, University of Utrecht, using an Amersham ImageQuant 800 system.

Image editor module

The image editor module allows you to edit images before analysis.

Key benefits include:

- Free rotate or manual rotation (with 0.1 degree of accuracy)
- Crop
- Flip
- Save edited images as a new file to preserve the original image file

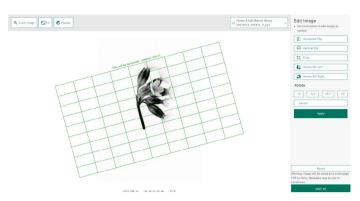


Fig 15. A cowslip, imaged using Cy5 filter settings in the ImageQuant 800 software, is rotated in image editor. The free rotation option can be used both by rotating the grid using the computer mouse or by clicking on the rotate buttons for finer adjustments.

Image integrity

Cytiva's imaging instruments are the first to generates images with encrypted digital fingerprints. This fingerprint is inspected in the image integrity checker stand-alone software, generating a pass result and certificate of conformity if the image has not been modified in any way. This compatibility with image integrity checker is useful for both researchers and journal editors to validate the authenticity of data, submitted electronically by the scientific community for peer reviewed articles. See cytiva.com/imageintegrity for more information.

ImageQuant TL GxP module for full data integrity

Quality guidelines and regulations check whether pharmaceutical products are safe for their intended use, and that the manufacturing, control, storage, and distribution processes adhere to documented quality processes. GxP is an abbreviation for good practice — "x" can stand for manufacturing or laboratory. GxP regulations describe requirements and guidelines on how you can use standard protocols for safe electronic record keeping.

GxP focuses on traceability, accountability, and data integrity. The ImageQuant TL GxP module along with the Amersham ImageQuant 800 GxP CCD imager help support compliance with FDA 21 CFR Part 11 and EU GMP Annex 11. The ImageQuant TL GxP module provides the data security you need in a regulated environment. Achieve full control of 1D gel electrophoresis and Western blot data with these features:

- Save protocol feature to speed up analysis and remove user bias
- Robust password control which can be configured to follow the required password policy,
- User groups with different access levels based on role,
- Audit trails with complete user activity, login attempt, and detailed analysis logs,
- Client-server based data protection for safe archiving of data in a secure folder (Fig 17),
- Electronic signatures for approvers with check-in and checkout process for data archiving and approval,
- · Emergency login capability, and
- Images generated by ImageQuant 800 GxP module check image authenticity automatically before analysis.



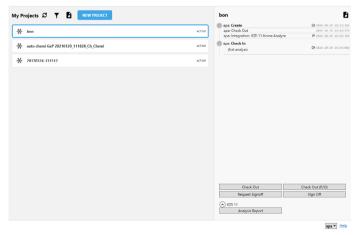


Fig 16. ImageQuant TL GxP module organizes image files by project. Users can then see images waiting for approval and authorized users can sign off.



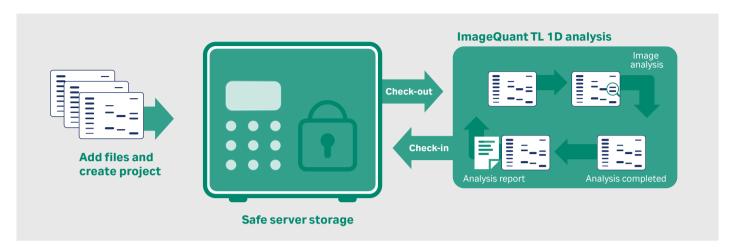


Fig 17. Client-server data archiving process in ImageQuant TL GxP version.

System requirements

ImageQuant TL software version	Operating system
11	Windows 11 Pro
	Windows 11 Enterprise
	Mac OS Catalina 10.15, or higher
GxP	Windows 11 Pro
	Windows 11 Enterprise

Ordering information

New user license codes

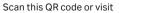
Description	Product code
ImageQuant TL software 11 node locked license	29800680
ImageQuant TL software 11 node locked license (5 pack)	29800681
ImageQuant TL software 11 node locked license (20 pack)	29800682
ImageQuant TL software 11 floating license	29800683
ImageQuant TL software 11 floating license (5 pack)	29800684
ImageQuant TL software 11 floating license (20 pack)	29800685
ImageQuant TL software 11 GxP node locked license	29800688
ImageQuant TL 11 GxP node locked license (5 pack)	29803812

Description	Product code
ImageQuant TL 11 GxP floating license	29803813
ImageQuant TL 11 GxP floating license (5 pack)	29803814
ImageQuant TL software 11 Mac OS node locked license	29800686
ImageQuant TL software 11 Mac OS node locked license (5 pack)	29800687

Upgrade license codes

Description	Product code
ImageQuant 11 software upgrade to Pro node locked license	29803817
ImageQuant 11 software upgrade to Pro floating license	29803818
ImageQuant 11 software upgrade to Pro Mac as node locked	29803819
ImageQuant 11 software upgrade Pro floating license (5 pack)	29803820
ImageQuant 11 software upgrade Pro node license (5 pack)	29803821

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