

GE Healthcare

ImageQuant TL 7.0

Image Analysis Software

Getting Started



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1 Introduction and installation instructions

1.1 Introduction

This booklet provides a quick introduction to ImageQuant™ TL and guides you through the analysis process in each module. The figures that accompany each section help you work through your analysis and relate to the provided tutorial images.

This *Getting Started* does not describe all the features in ImageQuant TL. For more information view the online Help available within the software. If you have any questions or feedback on ImageQuant TL, please contact GE Healthcare Technical Support at igtlsupport@ge.com or your local GE Healthcare representative.

1.2 Installation

Note: Local administrative privileges must be obtained for installation of ImageQuant TL software.

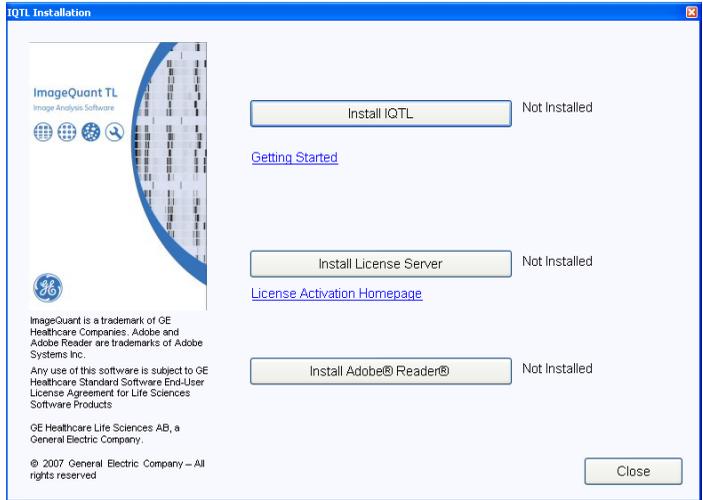
The ImageQuant TL disc contains the installation files for all the items required to run ImageQuant TL software.

- 1 Insert the disc. The Autostart opens the Installation page in your browser. If autostart is disabled, select the DVD/CD in Windows Explorer and launch **setup.exe**.

Note: If the target computer does not have .NET framework installed, an installation of .NET framework will start automatically before the installation dialog appears.

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1.3 E-licensing



- 2 Install each component by clicking the buttons in the installation dialog. Follow the instructions for each installation part.

1.3 E-licensing

ImageQuant TL software requires an electronic license (e-license) to use the program. Once all installations have been performed, ImageQuant TL should be activated with a license file.

1.3.1 E-license types

There are two main types of e-licenses:

Machine (node locked) license

A machine license (also called node locked license) is used for a single computer. This type of license is useful when only one or a few computers are used for working with ImageQuant TL. When using node-locked licenses, one e-license per computer is needed.

Concurrent (floating) license

A concurrent license (also called floating license) is a license that can be used by all computers connected via network to a computer running a license server. This type of license is useful when many users, but not all at the same time, need access to ImageQuant TL. The number of computers that simultaneously can use ImageQuant TL depends on the license, and is administered via the license server. The license server can be installed on any computer in the network.

1.3.2 Activation steps for ImageQuant TL

To activate ImageQuant TL:

- 1 Receive the access code (see *Access code*).
- 2 Find the physical address (Ethernet MAC address of a physical network card) of the computer (see *Finding the physical address of the computer*).
- 3 Collect and place a license file (see Section 1.3.3).
- 4 For a floating license:
Install the e-license server program (see Section 1.3.4).
- 5 Launch ImageQuant TL to test the e-license (see Section 1.4).

Access code

After ordering ImageQuant TL, a letter including an access code (AC) will be sent with a courier to the order's shipment address. Store the access code in a safe place. The access code is necessary for collecting license files (see *Collecting and placing a License file*).

Note: If the access code has not been delivered within 5 days of the order, contact customer service to get the access code via telephone.

Finding the physical address of the computer

Note: It is possible to use Macrovision® LMTOOLS utility for finding the physical address. The LMTOOLS utility is installed when installing the e-license server program, but can also be installed

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separately from the Access Code home page. For instructions see Chapter 1.3.4, Installation of GE Healthcare e-license Server Program.

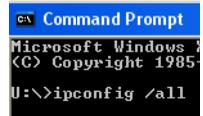
- 1 The physical address of the computer, also called *Ethernet MAC address*, is necessary for identification of the computer when collecting the appropriate e-license file. An example of a computer physical address is: 00-3E-F4-E4-44-76-FE-56. The physical address can only contain digits 0-9 and letters a-f (A-F). Dashes or colons should be removed to be used as the physical address.

*Note: The computer's Internet IP number is **NOT** the physical address of the computer. For questions regarding physical addresses or e-licensing, refer to <http://www.elicensing.amershambiosciences.com/gtlweb/faq.html>.*

- 2 On the computer where the e-license file should be placed, select **Start:All Programs:Accessories:Command Prompt**.

Note: A floating license file should be placed on the License server, a node locked license file should be placed on the computer running ImageQuant TL.

- 3 When the DOS-prompt appears, enter the command `ipconfig /all`, making sure you leave a space between the `g` and the `.`.
- 4 Press Enter.



This will create an output of this type:

```
Host Name . . . . . : hcpbrw-2501
Primary Dns Suffix . . . . . :
Node Type . . . . . : Hybrid
IP Routing Enabled. . . . . : No
WINS Proxy Enabled. . . . . : No
```

Ethernet adapter Local Area Connection:

```
Connection-specific DNS Suffix . :
Description . . . . . : Intel(R) PRO/100+ Alert on LAN*
```

Management Adapter

```
Physical Address. . . . . : 10-A2-B3-07-B0-77
Dhcp Enabled. . . . . : Yes
Autoconfiguration Enabled . . . . : Yes
IP Address. . . . . : 10.255.180.20
Subnet Mask . . . . . : 106.193.0.1
Default Gateway . . . . . : 20.37.0.2
DHCP Server . . . . . : 20.37.2.3
```

- 5 Make a note of the physical address of the computer.

The physical address is the number to the right of **Physical Address** but without any dashes or colons.

In the above case, the physical address for the computer is 10A2B307B077.

Note: If a computer has several physical addresses (there can be one for each Ethernet card in the computer), any physical address not associated with a VPN can be used for identification.

Note: A computer can also have virtual physical addresses, for example, if connected to a VPN software. Make sure that an address related to the physical network card is selected for the installation.

- 6 Close the DOS-prompt window.

1.3.3 Collecting and placing a License file

- 1 Make sure you have the access code and the physical address available.

- 2 On the ImageQuant TL Installation page, click **License Activation Homepage**. The Software Licensing System home page opens.

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1.3 E-licensing

- 3 Collect the License file according to the instructions on the web site. The collection includes the following steps:

- a Enter the **Access Code** and press **Continue**.



Enter Access Code:

- b In the **License Administration Center** page that opens, press **Collect Licences**. The **Select Product and Register Product** page opens:

Collect Licenses

- c Perform step 1 - Select product to collect.



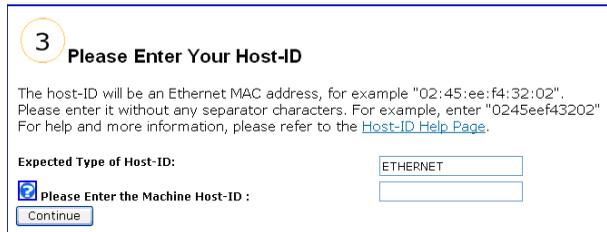
1 Please Select Product to Collect Here

- d Perform step 2 - Register your product.



2 Please Register Your Product Here

- e Perform step 3 - Enter the physical address (**Host-ID**) of the computer:



3 Please Enter Your Host-ID

The host-ID will be an Ethernet MAC address, for example "02:45:ee:f4:32:02". Please enter it without any separator characters. For example, enter "0245eef43202" For help and more information, please refer to the [Host-ID Help Page](#).

Expected Type of Host-ID:

Please Enter the Machine Host-ID :

- f Press **Continue** to proceed to **Collection Confirmation**.



Confirm Collection

Product Selected:- DeCyder MS 1.0 machine license only, beta

Hostid Type: ETHERNET

Host-ID Address Entered: 00d05982592c

Make sure that your e-license is configured correctly and if so, confirm your e-license collection by pressing **Collect license**. Otherwise press **Back** to perform corrections.

- g Save license to file:

Save license to file

We recommend you use the **Save license to file** alternative. A floating license file must be placed on the computer running the e-License server. Place the license file in the folder *C:\Program Files\GE Healthcare\eLicense Server\Licenses* on the License server (see also section Section 1.3.4, step Section 2).

A node locked license file must be placed on the computer running the ImageQuant TL program.

Send by e-mail (not recommended)

If you use the **Send by e-mail** alternative, the license contents are delivered as raw text in the e-mail body. You must copy the license text and paste it as an ASCII text file in, for example, Notepad. Then save the License with the extension *.lic* in your e-license folder.

- 4 For a floating license, continue from section Section 1.3.4.
For a node locked license, continue from section Section 1.4.

1 Introduction and installation instructions

1.3 E-licensing

1.3.4 Installation of GE Healthcare e-license Server Program

The installation in this section should only be performed when using a floating license. If only node-locked licenses are used, continue from Section 1.4.

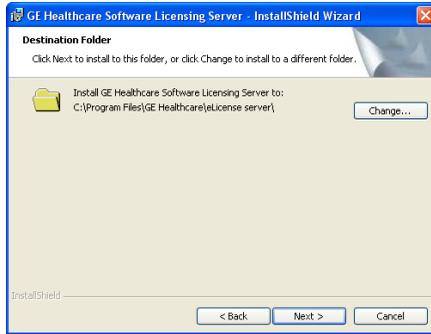
Select the computer on the network where the license server should be installed. All computers running ImageQuant TL must have network access to this computer in order to run.

- 1 On the ImageQuant TL Installation page, click **License Server**. An Install shield wizard dialog is displayed. If not, locate the cd-drive and double click **setup.exe**.



Click **Next**.

- 2 Click **Next** to accept the default destination folder.



- 3 Click **Install** to start the installation.



1 Introduction and installation instructions

1.3 E-licensing

- 4 Click **Finish**.



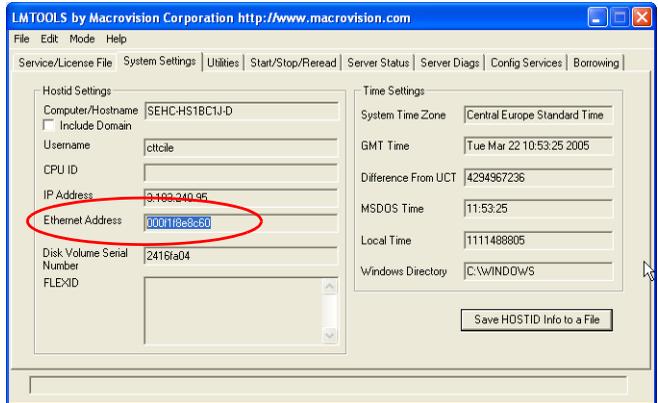
- 5 If the check box **Launch GE Healthcare Software Licensing Server**

Launch GE Healthcare Software Licensing Server

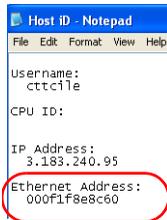
was checked in step Section 4 (it is checked by default), **LTOOLS** a dialog containing information and tools for the e-license server is automatically opened.

To find the physical address of the computer:

- 1 Click the **System Settings** tab.



- 2 The physical address is displayed in the **Ethernet Address** text box.

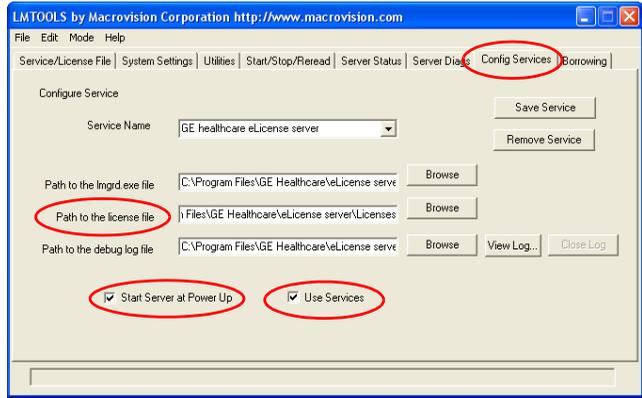


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1.3 E-licensing

Viewing configuration settings

- 1 Click the **Config Services** tab to display the configuration of the e-license server.
- 2 The configuration displayed below should be used for ImageQuant TL.

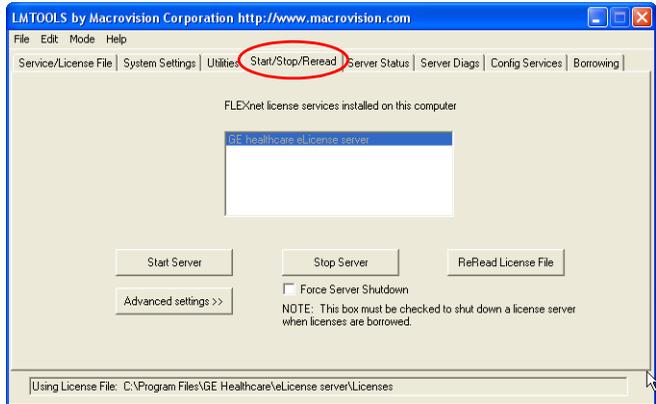


The field **Path to the license file** is the directory where the floating license file should be placed. The default directory for floating license files is *C:\Program Files\GE Healthcare\License Server\Licenses*.

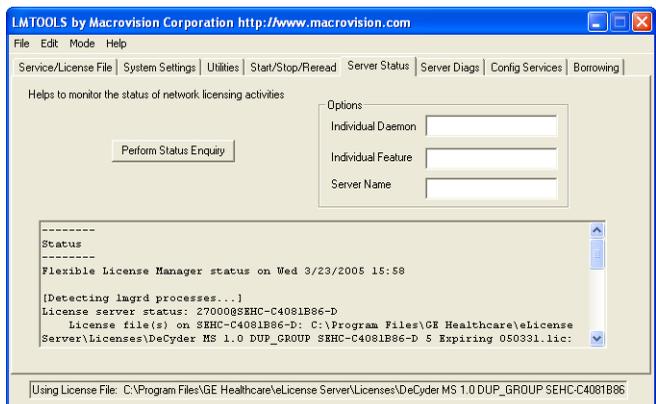
Do not change the default path. The floating license file which has been collected from the Web (in section Section 1.3.3) must be placed in the directory that is set here.

- 3 We recommend that you select **Use Services** to run the license server as a Windows® Service.
- 4 We also recommend that you select **Start Service at Power Up** to automatically start the license server when the machine boots.
- 5 Click **Save Service** even if you made no changes in steps 3-4.

- 6 Select the **Start/Stop/Reread** tab to control the license manager. Information about the license file is displayed at the bottom of the dialog:



- 7 Select the **Start Server** button to start the license server.
- 8 To confirm that the License Server is operating with the selected license, select the **Server Status** tab and press the **Perform Status Enquiry** button. Then scroll to the bottom of the text box. The information given here will confirm that the License has been accepted and the License Server is operable.



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1.4 Testing e-License

9 Select **File:Exit** and continue with section Section 1.4.

Note: Using LMTOOLS to control the floating license server is covered in detail in the Macrovision Licensing End User Manual.

1.4 Testing e-License

To be able to start ImageQuant TL, the correct license file must be found. ImageQuant TL will, at every login, check that the license file is available.

1.4.1 Test procedure

After installation of ImageQuant TL software, downloading and placing the license file, start ImageQuant TL to test that the license file can be found. Test ImageQuant TL software on all computers where the program is installed.

Note: The previous sections must have been successfully completed to start ImageQuant TL.

- 1 Start ImageQuant TL.
- 2 If a valid license file is available, ImageQuant TL will start, displaying the license agreement.

Note: ImageQuant TL will use the last used license if it is still available and valid.

If you want it to use another license, disable (make sure the program cannot find) the license type(s) that should not be used by, for example, changing the name for a node locked license or disconnecting the network for a floating (concurrent) license, during log in.

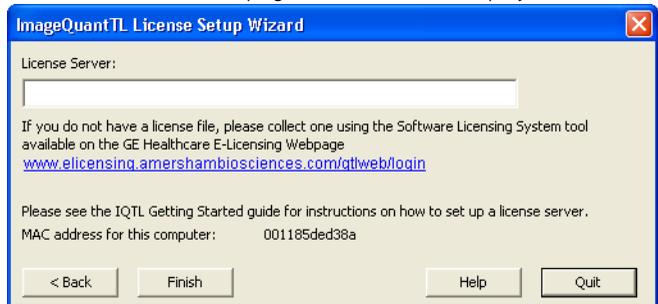
License failure

- 1 If one or more of the requested licenses are not available, the **ImageQuant TL License Setup Wizard** dialog is displayed.



- 2 If using a floating (concurrent) license:

- Select the ***I am using a Floating E-license*** radio button.
- Click ***Next***. The next page in the wizard is displayed

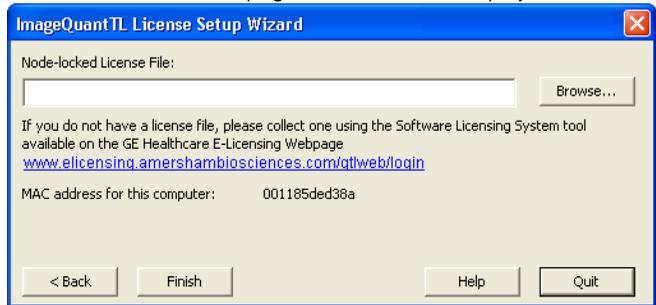


- Enter the network name of the machine that is running the ***GE Healthcare Software Licensing Server***.
- Click ***Finish***.
Once the license file is found, ImageQuant TL will start, displaying the license agreement.

1 Introduction and installation instructions

1.5 Finishing the installation

- 3 If using a node locked (machine) license:
 - Select the ***I am using a Node Locked E-license*** radio button.
 - Click **Next**. The next page in the wizard is displayed.



- Enter or browse for the location where you have stored the license file on the machine.
- Click **Finish**.
Once the license file is found, ImageQuant TL will start, displaying the license agreement.

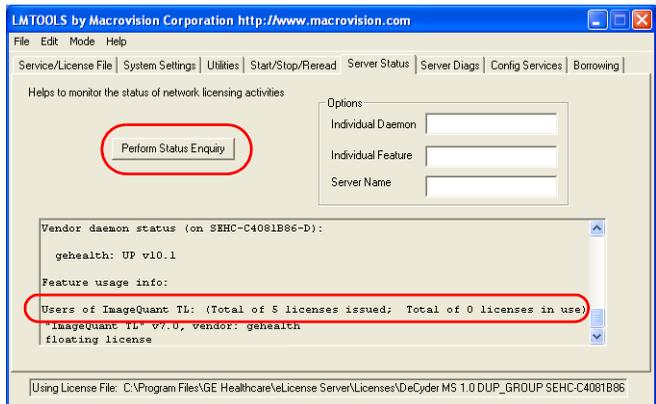
1.5 ***Finishing the installation***

- 1 Eject the DVD.
- 2 Restart the computer.

1.6 Changing license configuration

If the set of licenses needs to be changed on an e-license server that is already installed and in operation, perform the following steps:

- 1 Open the **LMTTOOLS** utility by locating the folder where the license server installation is stored, and double-click **lmttools.exe**.  **LMTTOOLS** opens.
- 2 Make sure that all licenses offered by the e-license server are checked in. All e-license server users must have returned their license to the server, that is, closed their program windows. Control this by opening the **Server Status** tab. Press the **Perform Status Enquiry** button.  Then scroll to the bottom of the text box. The information given here will confirm that no license is in use.



- 3 Click the **Start/Stop/Reread** tab and stop the e-license server by pressing the **Stop Server** button. 
- 4 Update the set of licenses that should be used by the e-license server, for example, if a new license shall be added, copy it to the folder where the current set of e-licenses resides, by default `C:\Program Files\GE Healthcare\eLicense Server\Licenses`. Please

1 Introduction and installation instructions

1.6 Changing license configuration

observe that all licenses in this folder must be valid and have the same vendor name, in our case, **gehealth**.

```
"ImageQuant TL" v7.0, vendor: gehealth
```

- 5 In the **LMTOOLS Start/Stop/Reread** tab, press the **Start server** button to restart the e-license server.

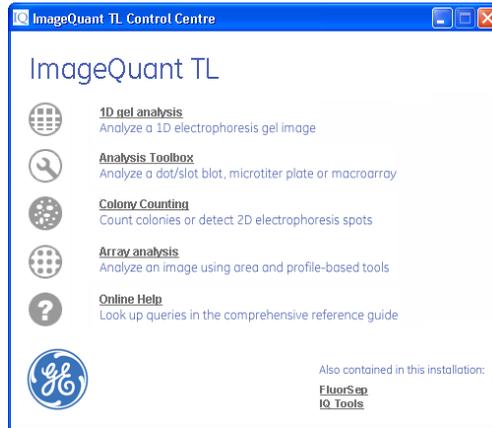


- 6 To confirm that the new set of licenses has been accepted and are available as expected, select the **Server Status** tab and press the **Perform Status Enquiry** button. Then scroll to the bottom of the text box. The information given here will confirm that the License has been accepted and the License Server is operable, see the screen dump in step 2.
- 7 Select **File:Exit**.



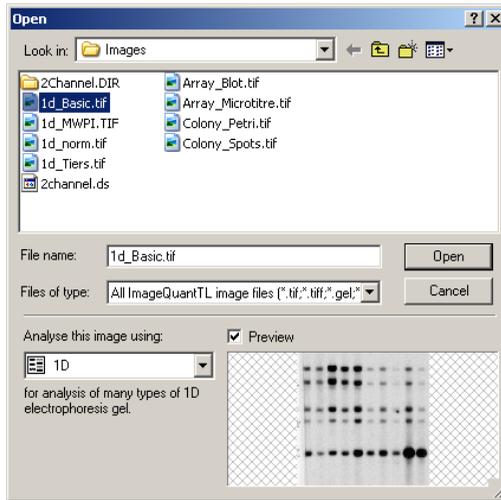
2 Getting started

To start ImageQuant TL, double-click the **ImageQuant TL** icon on the desktop. Alternatively, select ImageQuant TL v7.0 from the **Start/Programs** menu. The **Control Centre** window appears.



2.1 Opening an image

ImageQuant TL can analyse 8- to 16-bit grey scale **Tiff** images, **.gel** files, and **.ds** files from most types of image capture devices currently available.



To open an image in an analysis module click the button in the **Control Centre**, or if already in the analysis module, select **Open** from the **File** menu. The **Open Image** window appears.

The demonstration images are located in the **Images** folder of the ImageQuant TL program group. Select the image and the analysis module you want to use and click on **Open**.

The software automatically saves your analysis throughout the process.

ImageQuant TL can perform a full analysis of multichannel images (**.ds format**). When a multichannel image is loaded, the interface changes to allow access to the multichannel analysis features.

Note: *Colony counter module cannot load multichannel images.*

2 Getting started

2.1 Opening an image

The **Overlay** and **Single** buttons on the main tool bar allow you to select the **image** view. If you select **Overlay**, you choose the channels to be viewed using the channel selector buttons. This determines which channel data appears in the main image window, the lane profile window and the measurements table.

To assist with the analysis, you can access a second image window using the buttons under the Navigator. The second image window allows you to visually compare the channels you select using the buttons in the window. If you want to edit the image, you use the main image window. The software updates the second image window automatically.

Refer to the **Help** pane throughout the analysis process, or refer to the footnotes throughout this document that describe how an analysis would be carried out on a multichannel image.

3 ImageQuant TL interface

3.1 *The general layout*

The appearance of the program remains the same throughout each analysis module.

A toolbar appears at the top of the window that contains the following buttons.



Additional windows, such as the Image View, Analysis, Measurements Table, MW Curve, Report and Second Image Window (to be used when analysing multichannel images) can be selected from the buttons at the bottom of the Navigator.



3.2 *The navigator*

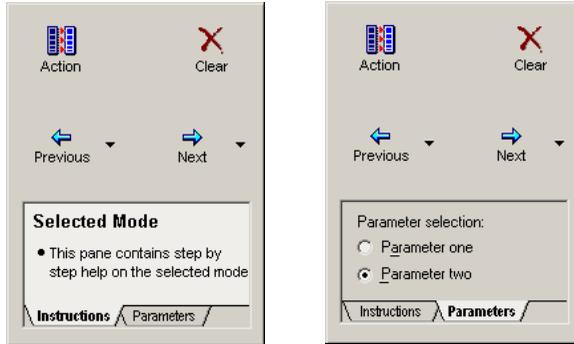
The pane at the left of the screen is the Navigator. The Navigator contains the tools required for the current mode of the analysis.

ImageQuant TL uses a wizard interface; **Next** and **Previous** buttons allow easy navigation through the analysis modes. The **Restart** button allows you to begin the analysis again

3 ImageQuant TL interface

3.3 Image viewing tools

A **Help** pane is also present that provides information and tips relevant to the current analysis.



- Click the **Parameters** tab to view any additional parameters.

3.3 Image viewing tools

You access the image viewing tools from the buttons on the Image window.



- **Zoom:** Drag a rectangle on the image window, and then left-click to increase or right-click to decrease the image magnification. Alternatively, you can drag the scroll bar edges.



- **Zoom 1:1:** View the image at a 1:1 ratio.



- **Fit to window:** View the whole image.



- **Magnify:** Allows magnification of a portion of the image.



- **Panning:** The image can be moved around the image window when part of the image is not visible, if for example the zoom tool has been used.

3.4 **Contrast**

The contrast window allows you to improve the way the image is displayed. The values are based on the image calibration during scanning. The contrast can be altered in either the **Single** or **Overlay** view. When analysing in **Overlay** you select the channels for contrast adjustment from the drop-down menu. The original data remains unaltered.

3.5 **Colour**

The colour window allows you to change the colour of the displayed image. You cannot make alterations to the colour display in the **Overlay** view.

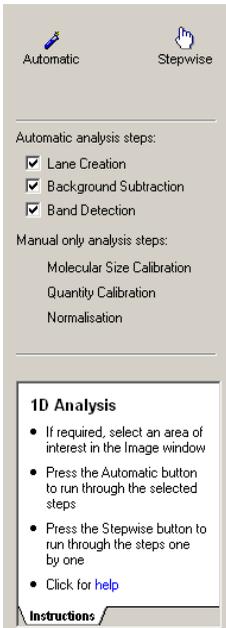
3.6 **Edit Image**

The image manipulation tools are available from the main tool bar and allow you to edit the image. All the edits are logged for a specific image and can be viewed in the **Image Properties** window. Editing can be performed in either the **Single** or **Overlay** view. All the edits appear in all the channels of a multichannel image.

3.7 **Options**

You can access a variety of options using the button on the main tool bar. These options are specific to the selected analysis module and allow customisation of the **Image Window and Measurements** Table.

4 1D gel analysis



From the **Control Centre**, click **1D gel analysis**. Select the image or go to the **Images** folder and select **1D_MWPI.tif** to follow this example. Click **Open** to launch the 1D analysis module.

Automatic

Performs an automatic analysis using the selected modes. Select or clear the modes in the Navigator. If the analysis requires an area of interest (AOI), you draw the area on the image before clicking **Automatic**.

After an automatic analysis is completed, you can review and edit the data in each analysis mode by selecting the mode button.

You access the option to display multiple profiles in the **Parameters** tab.

Note: *Automatic analyses all channels simultaneously. You can edit the image in the individual modes. To draw an AOI on a multichannel image prior to **Automatic** analysis, use the **Overlay** view so that all the lanes are analysed.*

Stepwise

Proceed mode-by-mode through the analysis using the **Next** and **Previous** buttons to access the analysis modes.

4.1 Lane creation

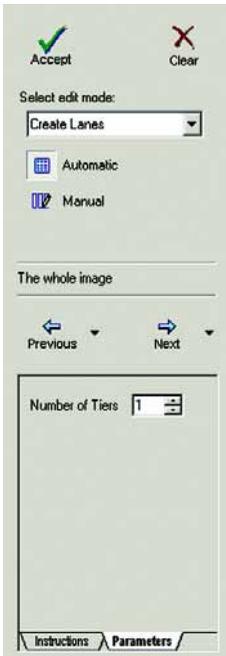
To create lanes automatically or manually select the appropriate button in the Navigator.

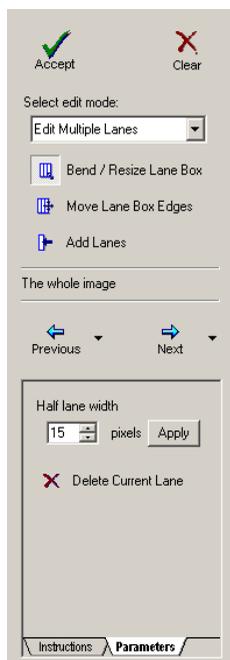
If you select **Automatic**:

- Click **Create**, and the software automatically detects the lanes. You access the edit mode from the list in the Navigator.
- For automatic analysis within an AOI, drag from the top left to the bottom right on the image and then click **Create**. The software automatically detects the lanes within this area.
- Select multiple tiers in the **Parameters** tab before clicking **Create**.

If you select **Manual**:

- Define the lane template in the **Parameters** tab. Drag from the top left to the bottom right on the image window and ImageQuant TL draws the lanes.





4.2 Lane Editing

Select the edit mode from drop-down menu. If you make changes, click **Accept** to update the lane profile window.

To delete the current lane, highlight the lane you want to delete, and then click on the **Delete** button in the **Parameters** tab.

Edit multiple lanes

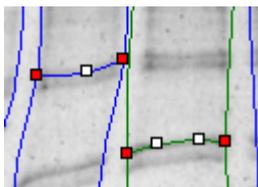
Select from the following options in the Navigator:

- **Bend / Resize Lane Box:** For fine control of badly distorted gels, left-click to add a handle, and then drag the handle. Right-click to remove a handle.
- **Move Lane Box Edges:** Drag to move lane box edges on image window.
- **Add Lanes:** Click the location on the image window within the lane box where you need to add the lane.

Edit single lanes

Select from the following options in the Navigator:

- **Bend / Resize:** Left-click to add a handle, and then drag the handle. Right-click to remove a handle.
- **Move:** Select the lanes and then drag the lanes to a new location.
- **Add Grimaces:** Grimaces account for band distortions. Click a band to apply the grimace, and then alter the grimace by adding handles and dragging the handles.

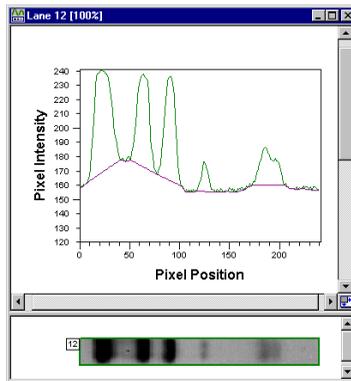


Click **Next** to advance to the **Background Subtraction** mode, or use the **Next** menu to choose an alternative mode.

Note: Lane creation is carried out simultaneously across all the channels of a multichannel image and can be performed in **Single** or **Overlay** view. Any edits are applied to all the channels. When editing lanes or drawing the AOI, the **Overlay** view should be selected so that all the lanes are visible.

4.3 Background Subtraction

The selected background subtraction method is displayed in the Navigator. To choose a different method, select the method from **Parameters** tab.



The following methods are available:

- **Rubber Band:** Select and the software automatically removes the background.
- **Minimum Profile:** Select and the software automatically removes the background.
- **Rolling Ball:** Select and then adjust the radius of the ball in the Navigator, the software automatically removes the background.

4 1D gel analysis

4.3 Background Subtraction

- **Image Rectangle:** Select and then draw a rectangle on the part of the image that represents the background.
- **Manual Baseline:** Select and then add or delete the handles in the profile window. Drag a handle to adjust the baseline.
- **None:** Select if you do not want to use background correction.

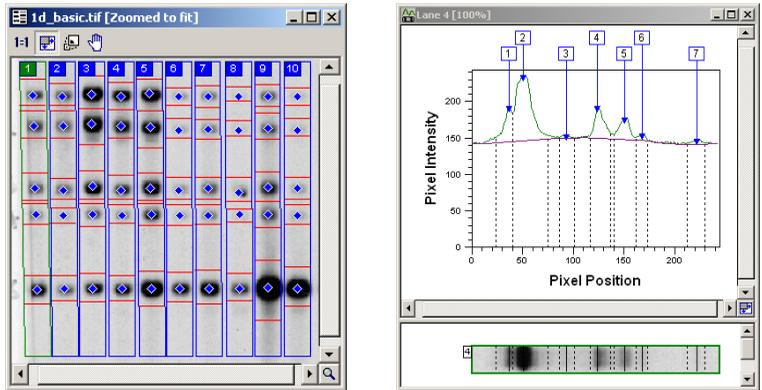
The lane profile window displays visual confirmation of the background subtraction.

Click **Next** to advance to the **Band Detection** mode, or use the **Next** menu to choose an alternative mode.

Note: *Background subtraction is performed simultaneously across all the channels of a multichannel image and can be performed in either the **Single** or **Overlay** view. If the **Manual Baseline** method is selected, you change each individual channel in **Single** view, but you view all channels in **Overlay** view. The manual baselines cannot be edited in **Overlay** view.*

4.4 Band Detection

Click the **Detect** button and the software automatically detects the bands and band edges using the currently selected parameters.



Band detection has three parameters:

- **Minimum Slope:** A value that represents how pronounced a band must be from its surrounding area to be recognised. You adjust the value using the slider.
- **Noise Reduction:** Sets the level to determine the difference between noise and band presence. You can adjust the value in the *Parameters* tab.
- **Percentage Maximum Peak:** Measures the relationship between each band in comparison to the tallest band and determines at what level to include a faint band. You can adjust the value in the *Parameters* tab.

Band edges can be determined automatically or all the edges can be set to a fixed width. You can edit the bands and edges either on the image or lane profile window. The pointer changes according to its proximity to the bands and edges.

- To remove a band/edge, right-click.

4 1D gel analysis

4.4 Band Detection

- To add a band/edge, left-click.
- To move an edge, position the pointer over an existing edge and drag the pointer. The image and profile windows can be viewed in greater detail by zooming in.

The **Measurements Table** window contains the current results. You can alter the fields displayed in the table using the **Options** from the main toolbar

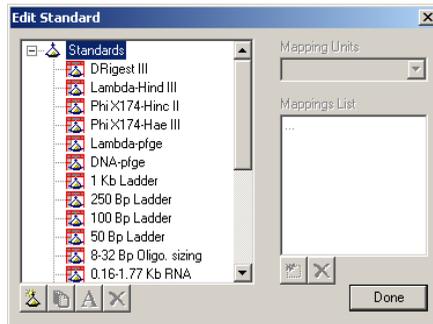
Click **Next** to open the **Molecular Size Calibration** mode, or use the **Next** menu to choose an alternative mode.

Note: *Band detection must be performed on each channel individually in the **Single** view. Edits can be performed on the main image window and lane profile window. The second image window can be used for visual comparisons between the channels. When band detection is complete, all the channels can be viewed in **Overlay** mode.*

4.5 Molecular Size Calibration

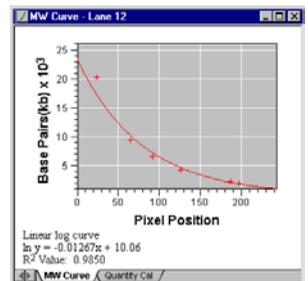
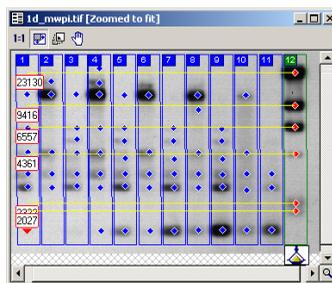
Before you can perform molecular size calibration, you must set up the molecular size reference lane in the **Parameters** tab.

- 1 Select the standard from the library or create a new standard.
- 2 To create a new or edit an existing standard, click **Edit** .



Edit standard: Create a duplicate of the existing standard before editing the values.

Create new standard: Click the button, select the mapping units and add the standard values.



- 3 Select the curve type that best fits the data, in the example you see a linear log curve.
- 4 Click within a lane to assign standards, right-click to deselect.

4 1D gel analysis

4.5 Molecular Size Calibration

- 5 If there are distortions in the gel that were compensated for when creating the lanes, then use **Rf** to propagate.
- 6 Deselect any values in the **Parameters** tab that you do not want to use. The software retains these settings until you change them.
- 7 Click **Compute** and the calibrated figures appear in the **Measurements** Table.

If all the bands are nicely separated, selecting a lane should result in perfect automatic assignment from top down. If there is a problem, you can move the assigned ladder by dragging the yellow line in the image window.

For the demo image **1D_MWPI.TIF**, the standard is *Lambda-Hind III* and the standard lane is lane 12.

Click **Next** to open the Quantity Calibration mode, or use the **Next** menu to choose an alternative mode.

Note: *Molecular weight calibration must be performed in the **Single channel** view. When the standard is applied to a lane, click **Compute** and ImageQuant TL calculates the molecular weight across all the channels. The **Measurements** Table shows the data relating to the channel selected in the main image window.*

4.6 Quantity Calibration

This mode allows calibration of bands on a gel to known volumes, also accounting for nonlinear staining effects. A standard curve is produced and the software automatically calculates values for the unknowns.

- 1 Click the bands where the quantities are known from the experimental set up.
- 2 Add the known volume in the box that appears. You select the units in the **Parameters** tab.
- 3 Select a curve method and click **Calibrate**. The volumes appear in the **Measurements** Table.
- 4 Click **Next** to open the **Normalisation** mode, or use the **Next** menu to choose an alternative mode.

Note: *Quantity Calibration must be performed in the **Single channel** view. You can assign known quantities to the bands on a channel, click **Calibrate** and the volumes appear in the **Measurements** Table for that channel. Quantity calibration data can be calculated for each channel individually.*

4.7 Normalisation

The normalisation protocol allows the use of known values, or the bands can be expressed as a percentage or a proportion of one or more selected bands. If actual volumes are not known, the band volume can be set to 100 (ignore units), and the unknown bands expressed as a percentage of the known band(s).

- 1 Select one or several known band(s) (right-click to deselect).
- 2 Select the required units in the **Parameters** tab.
- 3 Type the amount to be associated with the known bands.
- 4 Select either their average volume or collective volume.
- 5 Click **Normalise**.

Note: *Normalisation must be performed in the **Single channel** view. Normalised values can be calculated for each channel individually.*

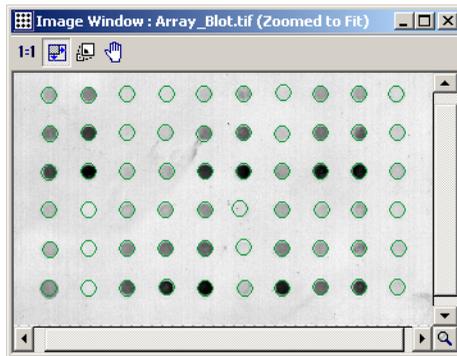
5 Array analysis

From the **Control Centre**, click **Array analysis** and select an image or go to the **Images** directory and select either **Array_Blot.tif** or **Array_Microtitre.tif**. Click **Open** to launch the **Array analysis** module.

Array analysis can be performed in either the **Single** or **Overlay** view. Each stage of the analysis is performed on all the channels.

5.1 Define Spots

5.1.1 Create and Stretch Grids

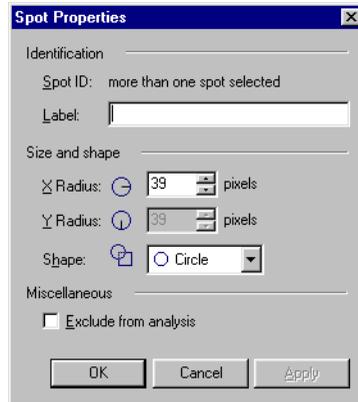


- Select the grid type, spot shape and spot size from the **Parameters** tab and then click **Detect** to automatically define the spots. To auto-size spots select the checkbox.
- Alternatively, select the grid type, spot shape and size, and then define the grid by positioning the pointer in the centre of the top left well. Drag the pointer to the centre of the bottom right well.

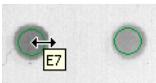
5.1.2 Adjustments

- Drag to move the entire grid.
- Reshape the grid by dragging a handle.

5.1.3 Move and Resize Spots



- To move individual spots, click a spot (Ctrl-click each spot or drag out an area to choose multiple spots). Then reposition the spot(s) exactly over the well.
- Clear the **Show Spot Numbers** check box in the **Options** window.
- To alter the diameter of a spot, change the spot radius in the **Parameters** tab (make sure you clear the auto-size check box). Alternatively, right-click the spot and open the **Spot Properties** window and change the size and shape using the pixel values. In addition you can add the **Spot Label** in this field.
- Alternatively, click the spot and drag the perimeter to the required size as shown in the figure.



You should use a smaller diameter with microtitre plates, not necessarily including the perimeter of each well's contents. With a blot, you should use a wider diameter.

Click **Next** to advance to the Background Subtraction mode, or use the **Next** menu to choose an alternative mode.

5.2 Background subtraction

Select a method from the **Parameters** tab. You can choose from the following five methods.

- **Spot Surface Minimum:** Select and the software automatically removes the background.
- **Spot Edge Average:** Select and the software automatically removes the background.
- **Negative Controls:** Select, and then define the negative control(s) in the image window.
- **Image Rectangle:** Select, and then draw a rectangle on the part of the image that represents the background.
- **None:** Select if you do not want to use background subtraction.

The values appear in the **Measurements** Table. You can change the fields using the **Options** window.

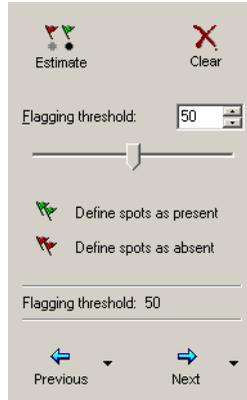
Click **Next** to advance to **Normalisation** mode, or use the **Next** menu to choose an alternative mode.

5.3 Normalisation

The Normalisation protocol allows the use of known values, or the spots can be expressed as a percentage or a proportion of one or more selected spots. If the actual volumes are not known, the spot volume can be set to 100 percent and the software calculates the unknown spots in relation to that spot.

- 1 Select one or more known spots on the image window.
- 2 Select the required units in the **Parameters** tab and type the amount associated with the known spot(s).
- 3 Select average volume or collective volume.
- 4 Click **Normalise**.
- 5 Click **Next** to advance to the **Presence Flagging** mode, or use the **Next** menu to choose an alternative mode.

5.4 Presence flagging



This mode sets limits above which a spot is deemed to be present on the image, which is indicated in a table as a spot presence (1) or absence (0).

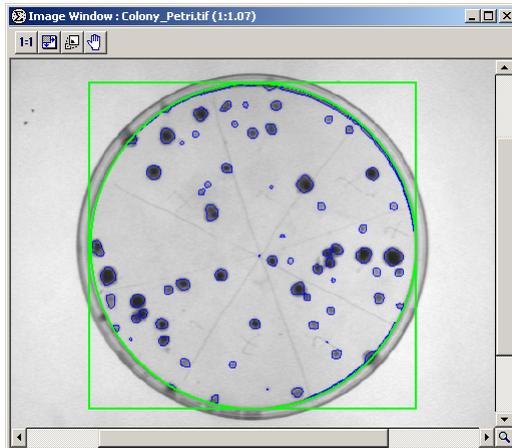
Detection is automatic if you select **Estimate**, or you can manually adjust the value using the slider. Alternatively, you can define a spot as absent or present using the buttons in the Navigator.

6 Colony counter analysis

From the **Control Centre** select **Colony Counting**, and then select an image or go to the **Images** directory and select **Colony_Petri.tif**. Click **Open** to launch the **Colony Counter Analysis** module.

Note: *Multichannel images cannot be analysed using this module.*

6.1 Feature Detection



You select the area of interest (AOI) shape in the **Parameters** tab, and then draw an AOI on the image window. The information about the detected features automatically appears in the **Measurements** Table.

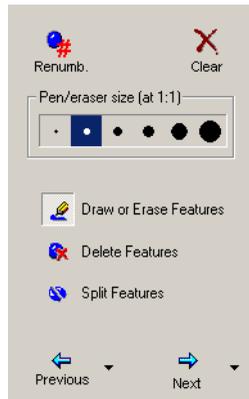
You can change the following parameters:

- **Detection parameters:** Modify using the sliders for sensitivity and operator size. Selecting **Initialize Sensitivity** automatically determines the sensitivity for a given operator size.
- **Background, noise factor and spot splitting:** Can be adjusted in the **Parameters** tab.

You can use the zoom tool to determine if editing is necessary.

Click **Next** to open the **Edit** mode, or use the **Next** menu to choose an alternative mode.

6.2 Feature Editing



The Navigator contains the following controls:

- **Draw or Erase Features:** Left-click and drag to draw a feature using the selected pen size. Right-click and drag to erase part of a feature.
- **Delete Features:** Click a feature if you want to delete.
- **Split Features:** Manually split a feature by dragging a line at the required position. Use *Draw* or *Erase* to make minor changes.

After editing, the **Measurements** Table can be updated by clicking **Renumber Spots**.

Click **Next** to open the **Background Selection** mode, or use the **Next** menu to choose an alternative mode.

6.3 Background subtraction

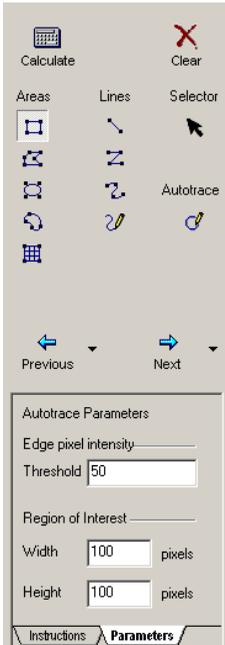
Select a method from the **Parameters** tab. The methods are:

- **Mode of Non-Spot:** Calculates the background based on the most common pixel value in the region around the feature. You can adjust the margin in the Navigator.
- **Image Rectangle:** Requires you to draw a rectangle on the part of the image that represents the background.

The results are displayed in the **Measurements** Table.

7 Toolbox

From the **Control Centre** click **Analysis Toolbox**, and then select an image. Click **Open** to launch the **Toolbox analysis** module.



7.1 Shape definition

- Select a drawing tool from the choice of **Areas**, **Lines**, or **Autotrace** in the Navigator.
- **Areas and Lines:** Drag to create the object on the image window. Some objects require multiple clicks, right-click to complete the object.
- **Grid:** Type the grid dimensions in the box that appears. Drag the grid on the image window.
- **Autotrace:** Right-click the outer edge of the feature to determine the **Edge pixel intensity** (the value appears in the **Parameters** tab), and then left-click the centre of the feature to detect and draw the object.
- **Selector:** Allows you to select objects for editing. Drag through an area to select multiple objects.

7.2 Shape Editing

- Click the **Selector** button, and then click the object, (or CTRL-click for multiple objects) that you want to edit.
- Drag a handle to resize the object.
- Drag an object to a new location.
- Right-click to copy and paste objects.

Click **Next** to open the **Background Subtraction** mode.

Note: *Shape definition must be performed on each channel individually in the **Single** view. You can edit the image in the main image window and compare the channels in the second image window. When shape definition is complete, all the channels can be viewed in the **Overlay** mode.*

7.3 Background subtraction

Select the method from the **Parameters** tab. You can use different methods on different features on the same image.

- **Local Average:** Select and the software automatically performs the background subtraction on the selected feature.
- **Local Median:** Select and the software automatically performs the background subtraction on the selected feature.
- **Histogram Peak:** Select and the software automatically performs the background subtraction on the selected feature.
- **Image Rectangle / Ellipse:** Select and draw an object on the part of the image window that represents the background. If multiple objects are drawn, select the object to apply from the drop-down menu in the **Parameters** tab and the software automatically subtracts the background.

In **Selector** mode, click the feature that requires background subtraction. Choose a method from the **Parameters** tab and the software automatically subtracts the background.

To simultaneously subtract the background from multiple objects, click the **Selector** button and drag an area. Choose a method from the **Parameters** tab and the software automatically subtracts the background.

Background subtraction must be performed on each channel individually in the **Single** view. When background subtraction is complete all the channels can be viewed in **Overlay** mode.

7 Toolbox

7.3 Background subtraction

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