CLIQS User Guide

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Introduction

Welcome to CLIQS

The CLIQS software provides a complete software package to meet your image analysis requirements. The software consists of the 1D, Array, Colony Counter and Toolbox modules integrated into one package, with each module started from the CLIQS Control Centre.

This software analyses 1D gels, dot and slot blots, microtitre plates, other basic arrays, and colony images captured by the scanner. The Toolbox module analyses an image using area and profile based tools.

The analysis programs are flexible and easy to use.

The User Guide

This user guide has been written with individual sections covering the common interface of the software and its specific modules.

Each module section will guide you through most of the analysis procedures so that you can understand how each module works.

The procedures provide a quick introduction to the analysis modules instead of a comprehensive guide to all the features of this software.

Throughout this guide you will see text distinguished by *two parallel lines*, as illustrated in this small section. This text relates to the relevance of the *tool* or *feature,* when used with a multiplex image.

To help you, pictures are included that show what you should expect to see on the screen.

Note:
You should have basic knowledge of how to use the Microsoft™ Windows operating system. If you are unfamiliar with the Windows environment, see the Microsoft documentation before attempting the step by step procedures.

Getting started

Technical Support

Should any problems arise with the installation or use of CLIQS please contact us at support@totallab.com or Telephone: +44 (0)191 255 8899.

Current subscribers to the Support Subscription package receive technical support as stated in your purchase agreement.

Let's get going!

Click a hyperlink or a button in the Control Centre to start each module.

If you have a dongle version of the software you may need to install dongle drivers. Please click on the link provided

Multiplex Images

Overview

CLIQS allows you to display and analyse multiplex images within the analysis modules, the exception being the Colony Counter module. A multiplex gel contains two or more channels that can be displayed in different viewing modes.

Multiplex image files

CLIQS can analyse multiplex images from 2 formats.

The first format allows the images to be defined using a simple configuration file with the ".ds" extension. These are created by some scanners automatically when doing a multiplex scan. For other scanners/cameras the images for each channel are created separately. In this case we can create the ".ds" file in the software via the Create Multiplex image option in the File menu of the ImageEditor. After using this option the multiplex image file can be used when opening an image later on.

Alternatively some modern systems produce a single colour Tiff image that includes 3 channels of data in one file. In CLIQS these channels are unpacked automatically to produce a ".ds" format as above without you having to worry about converting them.

Displaying Multiplex images

Upon opening a multiplex image its first channel will appear in the Image window, in *Single Channel view* mode. The remaining channel images can be viewed individually in the Second Image View window.

Displaying in single channel view mode

In single viewing mode any one of the channels can be selected and viewed in the main image window.

Displaying in channel overlay view mode

In overlay viewing mode, any combination of channels can be viewed in the main image window

1. Click the *Channel Overlay view* mode button on the main toolbar.
2. On the main toolbar, click on each desired channel image button.

The channels appear as one image, each colour representing a different channel.

Analysing Multiplex images

When analysing multiplex images the parameters you specify are applied to all channels. For some analysis modes you may need to be in Single Channel view mode to apply the parameters e.g. the 1D Molecular Size Calibration parameters.

1D

Introducing 1D

CLIQS 1D is the gel or Western Blot analysis module. The procedures in this section offer basic examples of how to analyse images. You can fine-tune your analysis using the various editing tools.

To help you, pictures are included in the section showing what you should expect to see on the screen.

The software is supplied with two image data files of previously saved data. When following the procedural steps you can restore either of the images used in this section that describes the various features. To restore an image click **Help** | **Restore Tutorial image** or **Help** | **Restore Multiplex image**

The current experiment, lane and channel

Throughout the 1D section you will see references to current experiment and current lane. These are described below.

Current experiment

Current experiment refers to the experiment that is presently loaded. You can only work one experiment at a time.

Current lane

Many of the windows in the software show you data relating to the current lane. Within the Image window the current lane is indicated by a coloured outline – usually green.

All the windows are updated automatically when you change the current lane, and show the data relating to the new selection. You change the current lane selection by clicking another lane number in the Image window.

If you have previously generated a Lane report and then change the current lane, you need to generate a new Lane report.

Current Channel

In *Single Channel viewing* mode, the current channel is the channel displayed in the Image window.

In *Channel Overlay viewing* mode the current channel is the lowest numbered of the selected channels.

Main menu bar

The Main menu bar, located at the top of the main window, provides a number of commands not available through the main toolbar modes.

Import menu

From the Import menu, you can perform the following actions.

Agilent CSV Files

With this command, lane profiles can be imported from other file formats to produce an image in the 1D module, which can then be analysed.

Currently only Agilent CSV (.csv) from a Bioanalyzer can be imported.

When you choose to import Agilent profiles, an **Import Profiles** dialog box is displayed which is a standard multi-file open dialog.

You can select one or more files for import and when you have finished, click the **Open** button. The program will then create an artificial gel, with the lanes corresponding to the imported profiles. The gel is saved as an image file in TIFF format (.tif).

All the files selected for import will be combined, saved as a single image file and a new experiment will automatically be created.

The lanes will have been created automatically and you can then carry on with the analysis in the normal manner.

Print menu

From the Print menu, you can perform the following actions.

Print

There is a print option for each visible window. The **Print** dialog box allows you to select a printer, print range and number of copies for the selected window.

Print Preview

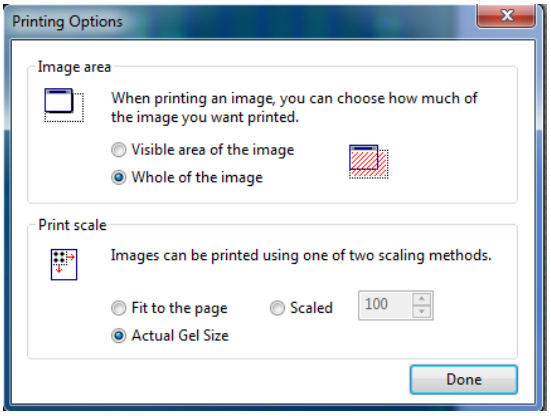
There is a print preview option for each visible window. The **Print Preview** window displays the image or measurements data from the selected window so you can see how it will be printed.

Print Setup

The Print Setup dialog box allows you to select a printer and its properties. You can select a paper size, source and the page orientation.

Printing Options

With these options you can customize the way that the content of windows will be printed, from the commands in this dialog. This includes changing the area of an image that will be printed and setting the scale at which an image will be printed.



Changing the area to be printed from an image

1. Click either Visible area of the image or Whole of the image.
2. Click **Done** to confirm your choice.

You can specify how much of a current image window should be printed and at what scale.

Changing the print range and scale

1. Specify the print range of the window to be printed by clicking either **Visible area of the image** or **Whole of the image**.
2. Select the scale from the **Fit to paper**, **Actual size** or **Scaled** options. If you click Scaled, select or type the percentage in the text box.

   Note:
   The default is set to 100% of the original size.
3. Click **Done** to confirm your choices.

Export menu

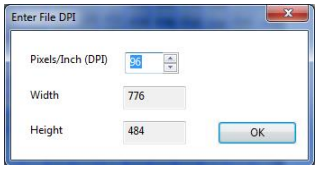
Export to Clipboard

There is an export to clipboard option for each visible window. Click **Export to Clipboard** to copy the data or image displayed in the selected window to the clipboard, so that the data or image can be pasted into other applications. For example the measurements window data can be pasted into a spreadsheet.

Export to File

There is an export to file option for each visible window. Clicking **Export to File** displays a dialog box that allows you to enter a folder and filename.

If you are exporting an image, the default filename is Export.bmp. You are also given the chance to change the DPI of the image saved before naming the file if it is from the Image Window and you are exporting the whole image (see **Export Image Options**)



If you are exporting measurements data the default filename is Export.txt.

The default folder is the CLIQS installation folder.

If you do not want to use the defaults, select the folder in which you want to store the file and type in a name and then press **Save**.

Export to Clip Gallery

This creates a file in exactly the same way as **Export to File** (see above) but instead of saving directly to a folder you select the file is made available via the **Clip Gallery** (see below).

Export Measurements to Excel

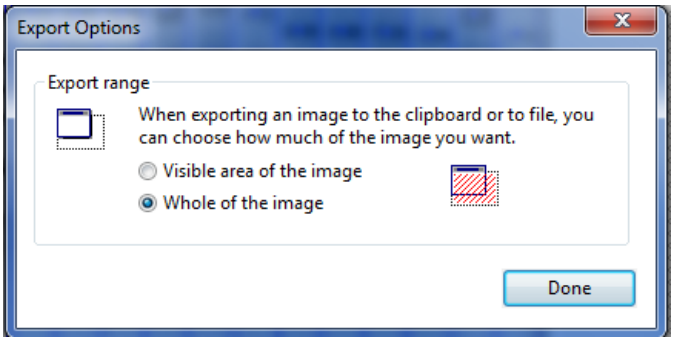
The data in the Measurements window will be copied directly into an Excel spreadsheet.

An Excel workbook automatically opens and the measurement data will be pasted into a new workbook.

If Microsoft Excel is not installed, use the Export to File command.

Image Export Options

With these options you can customize the way that the content of windows will be copied from the commands in this dialog.



Changing the area to be copied from an image

1. Click either Visible area of the image or Whole of the image.
2. Click **Done** to confirm your choice.

You can specify how much of a current image window should be printed and at what scale.

Export Lane Objects

The Export Lane Objects command allows you to save a lane template file, containing information on the current lanes.

1. Click **Export** | **Export Lane Objects** to display the *Save as* dialog box.
2. Select the storage folder and type a filename.
3. Click **Save**.

Import Lane Objects

The Import Lane Objects command allows you to import a lane template file, containing information about analysed lanes.

1. Click **Export** | **Import Lane Objects** to display the Open dialog box.
2. Select the folder and filename.
3. Click **Open.** The Lane information is applied to the current image.

If the current image already has lanes, a message appears advising you that the existing lanes will be deleted before importing the lanes.

Export Lane Profile to Clipboard

1. Click **Export | Export Lane Profile to Clipboard** to export the numerical values of the Current Lane profile, as shown in the Analysis window, to the clipboard.
2. Paste the values into another application.

Export Lane Profile to File

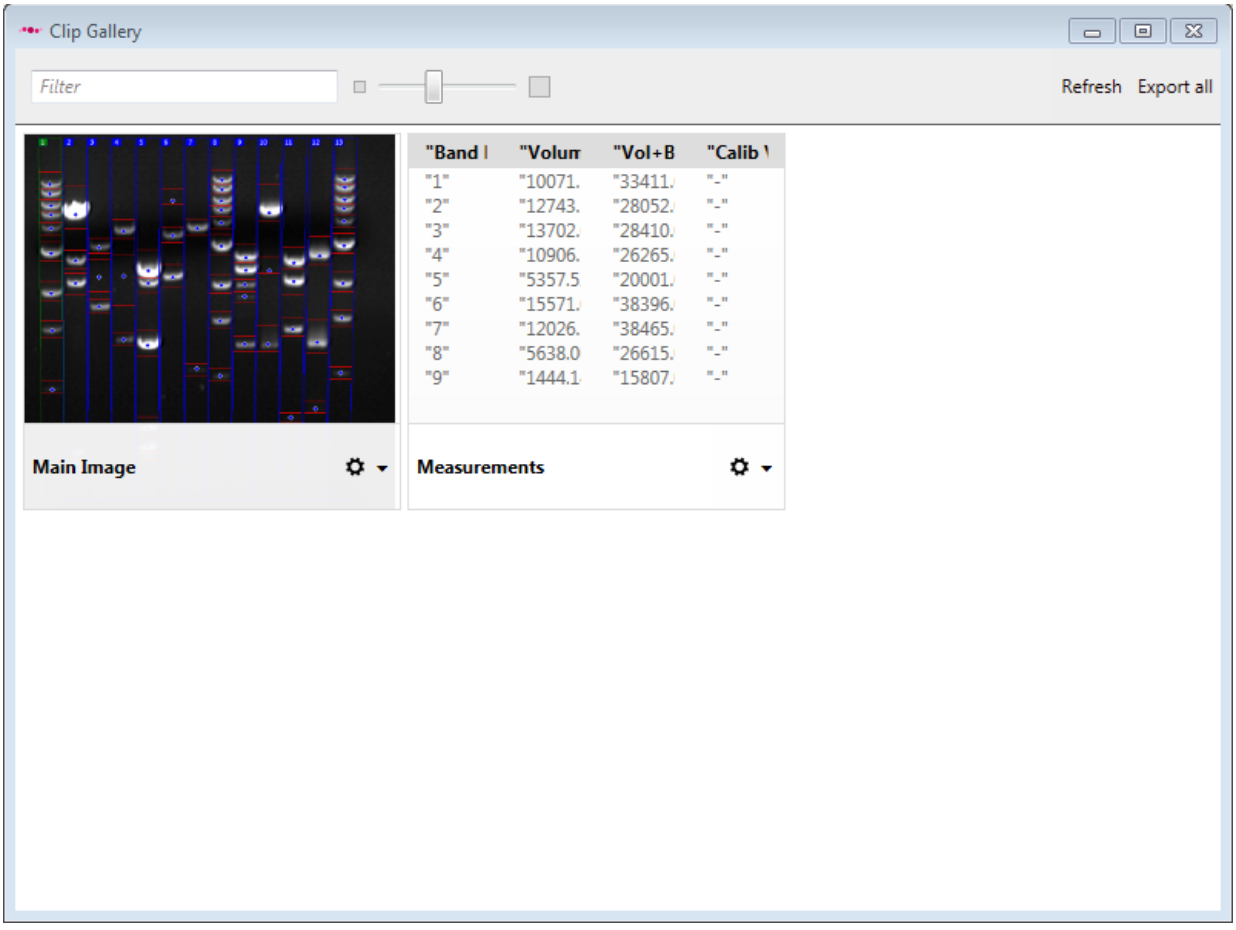
1 Click the **Export** | **Export Lane Profile to File** command to copy the file. The Copy to Text File dialog box displays the default folder as the software installation folder.

2 Type the filename.

3 Click the **Save** button to save the file containing the numerical values of the current lane profile.

Show Clip Gallery

This option shows the Clip Gallery viewer. Here you can see all the images/data you have sent to the clip gallery for this analysed image.



From here you can Copy or Export the information to any program you wish from the drop down menu on each of the items in the gallery

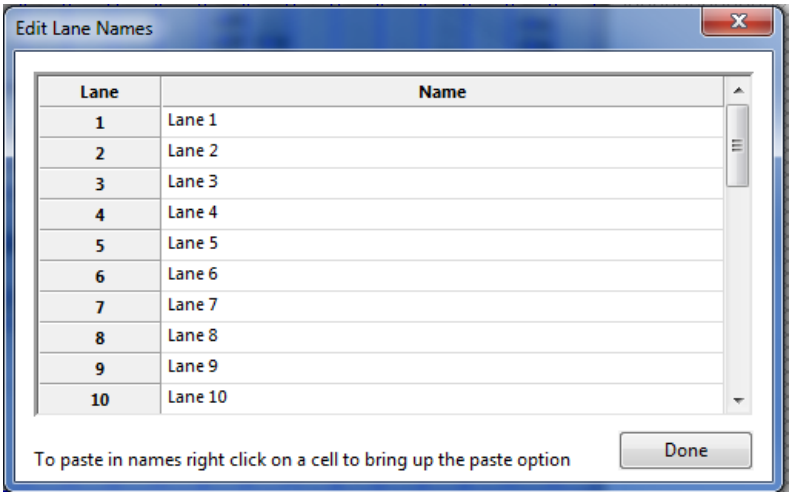
Options menu

Lane Name Entry

From this dialog you can edit lane names easily. Select **Lane Name Entry** to bring up the dialog**.**

This option requires at least one lane to have been created in the experiment. Refer to The Analysis Modes section for Lane Creation.

The dialog box has the appearance and functionality of a simple spreadsheet. Data is displayed within a two dimensional grid, with each row representing a lane and the extra column holding the lane data.



Moving around the dialog

If you are familiar with Excel, you will find that many of the basic cell selection and editing techniques can be used in the dialog box.

This includes copying, cutting and pasting data into the cells. Rectangular regions of cells can be selected to allow the above actions to be applied to multiple cells.

There are also several ways to navigate through the grid:

- **Arrow** keys on your keyboard can be used to step to any cell directly, adjacent to the currently active cell.
- The mouse can be used to select any cell in the grid, by placing the pointer over the cell and left clicking.

Entering lane data

Position the pointer over any of the cells and left click to activate the cell. The cell will be outlined, ready for editing, at which time a data value can be entered by using the keyboard.

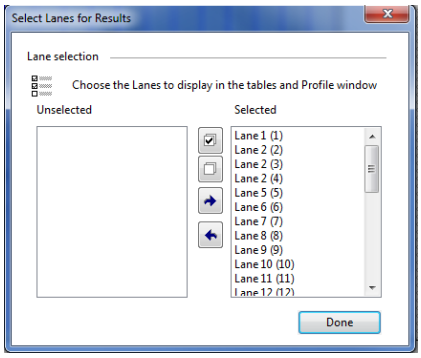
When the name is changed, all windows where the Lane name is displayed will be updated after the dialog box is closed.

You can paste data into the grid using a right click on a cell to bring up a context sensitive menu with a Paste option in it.

Once you have entered the lane data, click **OK.**

Lane Selection

Clicking **Options** | **Lane Selection** displays the Lane Selection dialog box which contains a list of all the lanes in the current gel.



From this list, you can select which lanes will be used as the additional lanes for viewing in the Analysis and Measurements windows. You can also set the order of those lanes by clicking the up and down arrows.

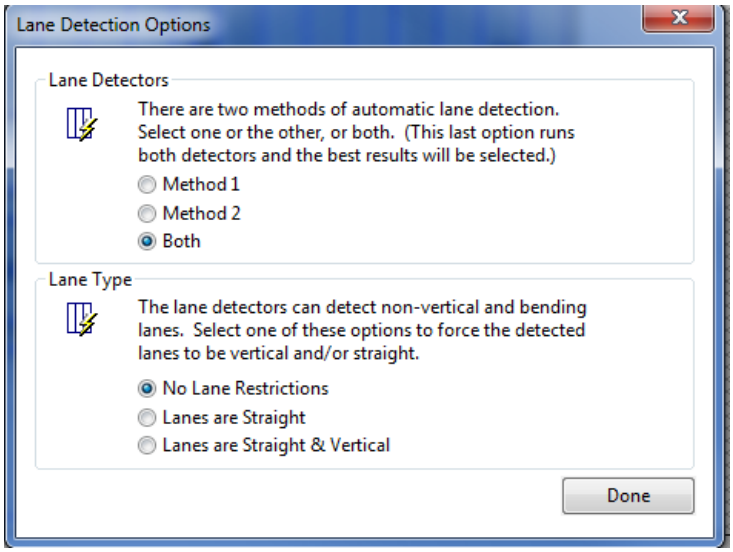
- To select all the detected lanes from the list click the **Select All** button.
- You can select individual lanes by selecting the lane(s) and clicking the Select Lane button.
- You can deselect individual lanes by selecting the lane(s) and clicking the Deselect Lane button.

The order of the lanes only affects the information shown in the Analysis, Measurements and Report windows.

You can clear the selected lanes by clicking the **Deselect all** button.

Lane Detection Options

Clicking **Options** | **Lane Detection Options** displays a dialog box allowing more choice on the Lane Detection Parameters used



Lane Detectors

There are two methods of automatic lane creation and each one can work better on some gels, than on others. You can select either method or choose to use both, in which case the best results will be selected.

Method 1 detects the band edges and attempts to detect the lanes from these.

Method 2 uses the gaps between the lanes to help detect them.

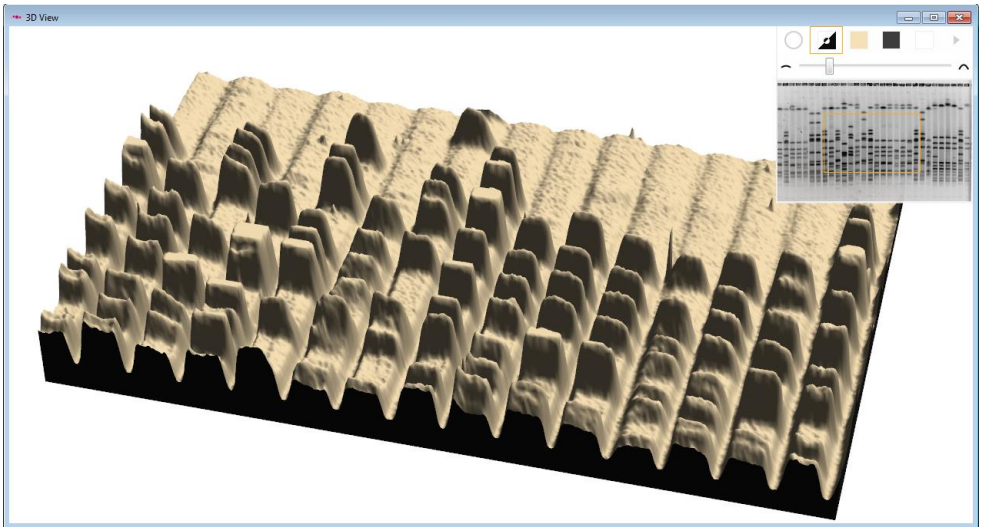
Lane Types

By default, the automatic lane detection will create lanes that are curved, if they need to be. If you wish to make sure they are straight or straight and vertical, choose the relevant option.

3D View menu

Show 3D View

This option allows you to bring up an independent 3D view of the current image being displayed



Each window is separate so you can call up as many as you wish from the same image or different images.

Contrast

The image is created using the current contrast settings not the raw image values so changing the contrast before opening the 3D View will give slightly different results

Options

In the top right corner are the view options. They include

- Continuous rotation
- Invert image
- Change colours for surface, edge and background
- Increase peak sizes
- Change AOI of view

Help menu

Help is provided both as online Help and online Help request links. The commands available from this menu are discussed in the following sections.

Contents

Displays the online Help as a PDF manual.

Restore Tutorial Image

Restores the image used for the tutorial.

Restore Multiplex Image

Restores a default multiplex image so you can see the extra multiple functionality easily.

Example Images

A list of some extra example images to trial with the software.

Start Tutorial

Starts the tutorial within the software and opens the Tutorial Help Pane.

Resume Current Tutorial

Resumes the tutorial within the software from where it last stopped and opens the Tutorial Help Pane.

Tutorial Help Pane

Toggles the display of the Tutorial Help Pane.

About

Displays the name and version number of the software together with the registration information.

The Program Windows

The main window contains three smaller windows. Each of these windows contains a different type of information.

The windows can be moved and resized via the splitter bars between them.

Scrolling windows

Scroll bars are used when the information being displayed is too large to fit on the screen.

The scroll bars are located at the bottom and right side of the image window. They work in the same way as normal scroll bars but allow you to see the range that you are viewing.

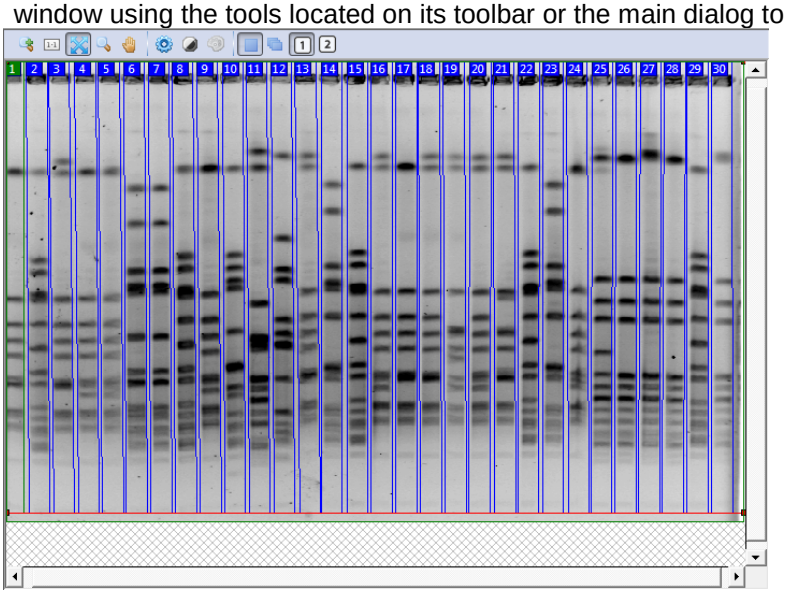
In addition, dragging the scroll box changes the start position of the range you are viewing; you can also resize the scroll box. Since the size of the scroll box reflects the amount of data that is shown in the associated window, resizing the scroll box changes the display magnification. Therefore, if you are viewing an image and make the scroll box smaller, less of the image is used to fill the window and you see the image at an increased magnification.

To resize the scroll box, position the pointer on an edge of the scroll box, the pointer changes to ↔ or ↕. Drag the pointer to the new position.

When the scroll box indicates a display of the total range of the scroll bar, the scroll box becomes transparent. When it indicates that only a portion of the range is visible, the scroll box becomes opaque.

Image window

The Image window is the most important of the windows and displays the image you are currently analysing. The image is overlaid with information determined by the tool you are using and how far through the analysis process you are. Most of your interaction with the software is done within this window using the tools located on its toolbar or the main dialog to the left.



Single viewing mode

When in single channel view mode, this window works in the same way as it does when a non-multiplex image is loaded.

Any one of the channels of a multiplex image can be selected and viewed in this window by clicking the channel button on the image window toolbar.

Overlay viewing mode

In overlay viewing mode, any combination of channels can be viewed in this window.

When in the Overlay viewing mode certain features of the software become unavailable – these are specific to the module and mode. For more information refer to the relevant analysis mode.

The Image window toolbar

The Image window contains a toolbar with the following buttons:

Zooming an image

In this window the button toggles with the Zoom to Fit button undoing any zooming effects.

To zoom the image:

1. Click the zoom button
2. Position the pointer on the area you want to zoom.
3. Drag the pointer over the desired area.

Alternatively, click the zoom button then left click to zoom in and right click to zoom out.

Pressing **Esc** on your keyboard cancels the zoom mode.

Zoom 1:1

Adjusts the magnification of the image to its original size.

Zoom to Fit

Clicking the **Zoom to Fit** button if the image is zoomed, fits the image to the size of the window.

The Zoom to Fit button counteracts any magnification made on an image using the zoom button.

Magnify

Allows you to magnify specific parts of an image.

1. Click the magnify button.
2. Position the pointer over the part of the image you want to magnify. The pointer changes to a small magnifying glass.
3. Hold down the mouse button. The area under the pointer appears in a box.
4. Drag the pointer to view other areas.

Panning

Allows you to move the image around within the Image window when the image is zoomed.

1 Click the Panning button

2 Position the pointer over the image; it changes to a small hand.

3 Drag to change the view.

Options

Allows you to edit the display options for the window. See the **Image Window Options** section for details

Contrast

Click the **Contrast** button to display a dialog box that provides a number of ways to alter the brightness and contrast of an image. For more information, refer to the **Contrast** section

Colour

Click the **Colour** button to display a dialog box that provides a number of ways to alter the base colour of an image or the individual channels. For more information, refer to the **Colour** section

Show Band Boxes

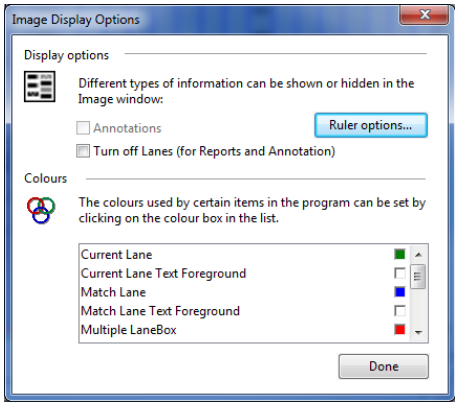
This option displays the lanes and bands with the lane shown as a line through the centre of the lane and the bands shown as red boxes.

Channel buttons

Click the channel buttons to select the channel or channels that display in the main image window. Each channel of a multiplex image enables an extra button

Image Window Options

The parameters shown this dialog relate to the display in the Image window. By selecting the check boxes, you can change the display for the various labels in the image window.



Display options

The display options are:

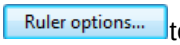
Annotations

This option allows you to show the user-defined annotations created while in Annotation mode.

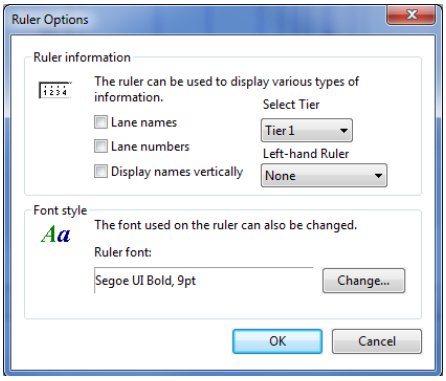
Turn off Lanes (for Reports and Annotations)

This option allows you to toggle the land drawing on or off.
This is useful for printing/reporting an image just with annotations or the ruler for instance

Ruler options button

Click the Ruler options button 

to display the Ruler Options dialog box.



From the Ruler information display options, you will be able to select the following ruler options.

Lane Names

Select the check box if you want to identify the lanes with a name above the image. Clear the check box if you do not want to identify the lanes. Clicking the name on the image and typing the new name in the text box edits the name.

Display Names Vertically

Select the check box to display the lane names vertically. Clear the check box to display the lane names horizontally.

Lane Numbers

Select the check box to display the lane numbers above the image. Clear the check box if you do not want to display the lane numbers above the image.

You can make any lane a current lane by left clicking on the number in the Image window.

Left Hand Ruler
This dropdown list allows you to select the ruler that occupies a gap between the left of the Image window and the left hand side of the gel image. You can select:

None
Not to have a ruler - the gel image is drawn against the left of the window.

Molecular Size
This is only drawn when you have performed Molecular Size calibration, otherwise no ruler is displayed and no space is taken. This will show you the values of Molecular Size for the bands in the leftmost standard lane on the gel.

Band Name
This shows the names of the bands in the current lane. Red names indicate matched bands, which share their name with all other bands matched to the same reference band. This ruler has a maximum size and so some band names may not be completely visible. You can use the Image window to edit the names when they are displayed.

Band PID
This is similar to band name but shows the protein ID field.

Select Tier

From the *Select Tier* list, click the tier to view the various ruler options.

Rulers Font

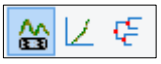
The Font style option displays the font style currently being used by the Image window. To change the style click the **Change** button. The Font dialog box appears allowing you to change the style and size of the fonts.

Colours

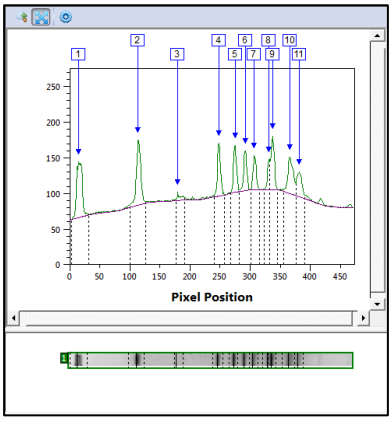
You can change the colours used by the item in the software by clicking the colour wells to the right of the item. The Colour dialog box appears allowing you to select the colour to be used for the item.

Secondary window

On the right hand side of the software is the Secondary window which can display 3 different windows. By default it shows the **Analysis** window but by flipping between the first three buttons you can then display the **Graph** or **Dendrogram** windows



Analysis window



The Analysis window consists of two panes:

Lane Profiles (upper pane)

This pane contains a graph that shows the intensity at each point along the length of the current lane or of additional lanes. Zooming into an area of interest can alter the range of the axes. It is possible to display only the current lane, or the current lane and additional lanes. This option can be selected in the Analysis window Options dialog box.

Note:
The additional lanes will be visible only in the Reports mode.

You can select which additional lanes are displayed on the Lane Selection dialog box from the Options menu.

When you use the *Channel Overlay viewing* mode in a multiplex image only the profiles from the *current lane* of your selected channels are displayed. The relevant coloured line identifies the profile of each channel.

If the *Single Channel viewing* mode is selected, the lane profile of the currently selected channel is displayed.

For information on displaying different lanes refer to the 1D | Experiment Overview section.

Lane Images (lower pane)

This pane shows the images of your lanes. The images displayed here relate to the graphs being shown in the upper pane. The images are displayed as horizontal rectangles so that the features on the image align with the calculated lane profile. If the lane bends on the gel you will see an adjusted image in this pane showing the lane, as it would look if it had not bent.

If additional lanes are displayed in the upper pane, their images will also be displayed in the lower pane.

The lane images are truncated in the lower pane so that they do not extend beyond the start and end of the graph in the upper pane. Where truncation has occurred, the line bordering the image is dotted.

Multiplex

A picture of the chosen lane is displayed for each selected channel when you use the *Channel Overlay viewing* mode in a multiplex image. Each picture has a coloured outline relating to its channel colour.

If the *Single Channel viewing* mode is selected, only the picture of the lane from that channel is displayed.

As well as being able to resize this window, you can adjust the relative size between the panes by dragging the splitter bar  between the two panes. The profile pane has a minimum size.

The Analysis window toolbar

The Image window contains a toolbar with the following buttons:

 **Zooming an image**

In this window the button toggles with the Zoom to Fit button undoing any zooming effects.

To zoom the image:

1. Click the zoom button
2. Position the pointer on the area you want to zoom.
3. Drag the pointer over the desired area.

Alternatively, click the zoom button then left click to zoom in and right click to zoom out.

Pressing **Esc** on your keyboard cancels the zoom mode.

 **Zoom to Fit**

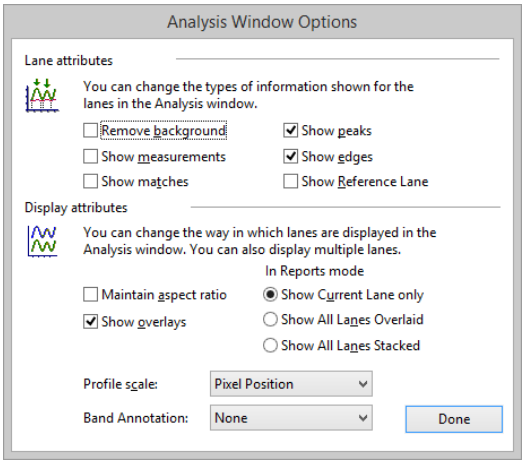
Clicking the **Zoom to Fit** button if the profile is zoomed, fits the profile to the size of the window.

The Zoom to Fit button counteracts any magnification made on an image using the zoom button.

 **Options**

Allows you to edit the display options for the window. See the **Analysis Window Options** section for details

Analysis Window Options



Lane attributes

The Lane attributes contain the following options:

Remove Background

When the software performs background subtraction on the gel you see a purple line under the profile graph indicating the line of background. If you select the Remove background check box, the graph is adjusted so that it shows intensity minus background in its Y-axis.
In addition, the image of the lane is adjusted so that it shows a mock up of how the lane would look if it had no background.

Clear the check box if you do not want to remove the background.

Show peaks

Select this check box to indicate the peaks of bands by a blue arrow over the profile graph and a blue line over the centre of the band in the lane image.

Clear the check box if you do not want to indicate the peaks.

Show measurements

Select the check box to cause the area used in volume measurements to be highlighted by a hatched area between the profile and background line between the edges on the profile graph and by a hatched rectangle between the edges over the lane image. These will not display if Gaussian Volumes have been calculated.

Clear the check box if you do not want to show the measurements.

Show edges

Select this check box to indicate the edges of your bands by vertical dotted lines under the profile graph and over the lane image.

Clear the check box if you do not want to show the edges.

Show Matches

Selecting this option causes dotted lines to be drawn between the matching bands in the lane images, in the lower part of the window.

Show Reference Lane

This option applies in **Reports** mode only and will allow you to display the profile and image of the reference lane in the Profile Window - as is done by default in the **Matching** mode.

Display attributes

The Display attributes contain the following:

Maintain aspect ratio

As you change the horizontal area of interest of the profile graph, the software zooms the lane to align the lane with the profile. When this check box is selected, the lane image is zoomed in both X and Y. However, zooming can make the lane too big or too small to be useful. If you want the lane's height to be fixed at 1:1 clear the check box.

Show overlays

Select the check box to display an overlay on the lane image i.e. the profile band edges and the hatched rectangle between the edges.

Clear the check box if you do not want to display the overlay.

Show Current Lane only

If you want to show the current lane only, click the *Show Current Lane only* button.

Show Additional Lanes in Finish mode

If you want to show the current lane as well as additional lanes, click the *Show Additional Lanes in Finish mode* button. You can select which additional lanes to display in the **Lane Selection dialog** from the Options menu.

Note:
The additional lanes will only be visible in the Reports mode.

Profile scale

This list allows you to select the scale used in the X-axis on the profile graph to indicate position along the lane.

Pixel Position: The basic scale, relating to the number of pixels on the image occupied by the lane.

Inches and mm: If your image file contains information relating to the resolution at which it was captured, these options can be applied.

Rf: Shows the profile graph Retardation factor X-axis coordinates.

Note:
The lane images are warped to line up their features with the Rf and Molecular Weight scales of the profile graph. If the calibration information is not available, the image and profile will not be shown.

Molecular Size: This scale requires that you perform **Molecular Size Calibration** first. The X-axis of the graph is given descending values of Molecular Weight against a logarithmic scale or descending values of pI in a linear scale.

Note:
If changing to Molecular Weight affects your profile or images dramatically, the calibration does not fit very well.

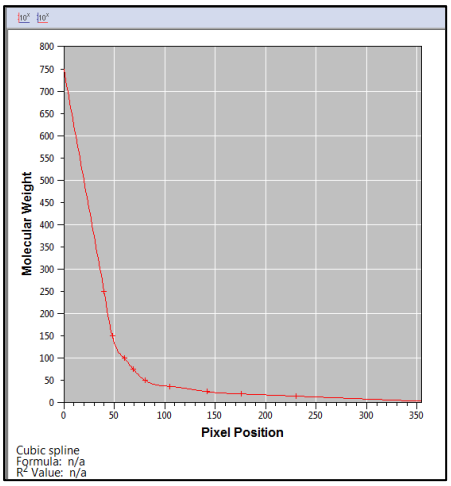
Band Annotations

Choose an annotation type from this dropdown list and value boxes will appear under the lane images in the lower part of this window, showing the band value for that type. Choose **None** to show no band annotations.

Graph window

The graphical information calculated by the **Molecular Size Calibration** and **Quantity Calibration** modes are displayed in in this window. This window appears in place of the Analysis window when you choose the Graph window button on the Second Window toolbar.

The Graph window also shows which curve type was used in the calibration.



The MW curve shows the relationship of the position along the lane (X) to Molecular Weight, pI or Base Pairs (Y).

The Quantity Cal curve shows the relationship of Raw Volume (X) to Calibrated Volume (Y).

Log scale on Y axis

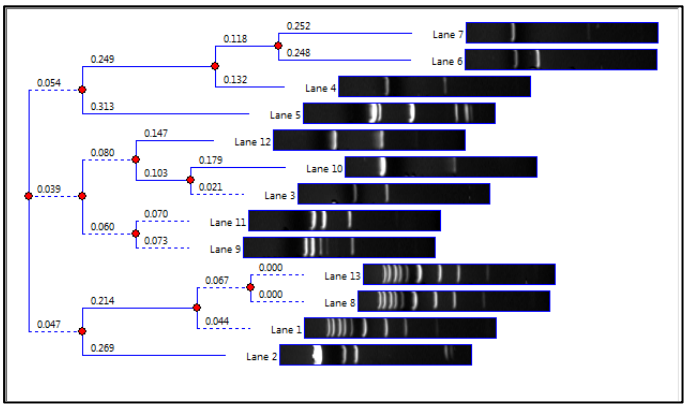
This allows you to have a log scale on the Y axis.

Log scale on X axis

This allows you to have a log scale on the X axis. Can to useful for Molecular Size graphs which may have a linear log relationship.

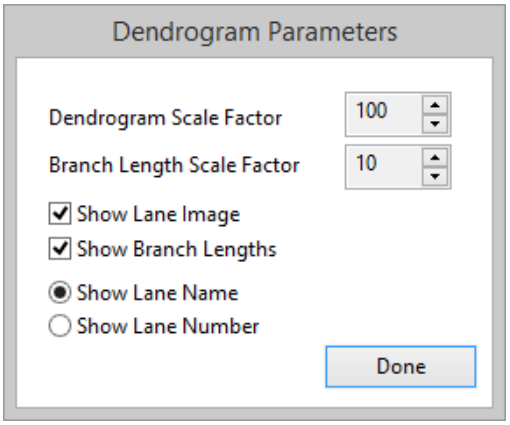
Dendrogram window

This window can be used to build and view dendrograms. The dendrograms show in a hierarchical manner, the similarities of the lanes matched to the current reference lane. This window appears in place of the Analysis window when you choose the Dendrogram window button on the Second Window toolbar.



The display of dendrograms is controlled from the toolbar at the top of this window.

The first button controls display options for the dendrogram and when clicked, will display the following dialog box.



Scale Factor - This value, adjusted by way of the spin buttons, determines how large the dendrogram is drawn.

Branch Length Scale Factor - This determines how the length of the branches is translated into the length of the drawn lines on the dendrogram, when you have Show Branch Lengths selected for Neighbour Joining dendrograms.

Show Lane Image - Select this checkbox to include a picture of the lanes in the dendrogram display.

Show Branch Lengths - The Neighbour Joining dendrogram method represents similarities, using the length of branches. You can turn this option off if you do not want to see this extra information.

Show Lane Name - Select this option button to display the lane name on the dendrogram.

Show Lane Number - Select this option button to display the lane number on the dendrogram.

These other two buttons can be used to generate dendrograms, for display in the main area of this component window. Dendrograms are not updated as you change your analysis and so you must re-build them, using the toolbar buttons.

There are two sorts of dendrogram, which can be built:

Neighbour Joining

See the paper "The Neighbour-joining Method: A New Method for Reconstructing Phylogenetic Trees", Saitou and Nei. Molecular Biology and Evolution (1987) Vol 4 Part 4 p406-425.

UPGMA
(Unweighted Pair-Group Method using Arithmetic Average) See the book "Principles of Numerical Taxonomy", R.R. Sokal and P.H.A. Sneath (section 4.3.3).

Interacting with the Dendrograms

A red circle represents each node of the dendrogram. You can right click on these nodes to display a context menu, which allows you to swap the order of displaying the branches, or hide the branches beneath the node.

Note that the order of displaying the branches does not affect the meaning of the dendrogram.

Neighbour Joining Dendrogram

With this dendrogram, you can choose any node to use as the root of the display - the root is displayed at the extreme left of the dendrogram.

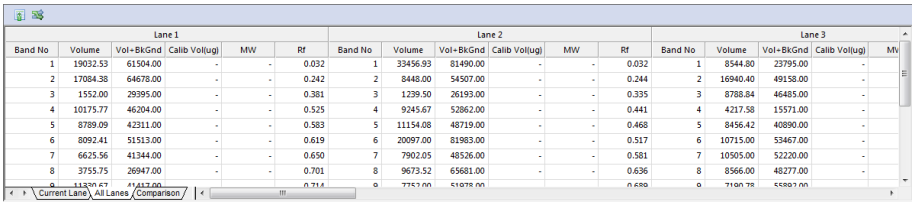
To select a new root, left click on the node you require.

UPGMA Dendrogram

This dendrogram places nodes along a horizontal line of increasing similarity of branches. To see where a node is along the line, left click on it.

A vertical line will extend between the node and the ruler at the bottom of the Dendrogram window to indicate the position.

Measurements window



The Measurements window displays all the analysis results. Four different tables of data are available. Each can be viewed by clicking on its tab at the bottom of the window.

- The ***Selected Lane*** table displays the data fields for all the bands in the selected lane.
- The ***All Lanes*** and ***Comparisons*** tables display the data fields for all the bands in the gel image; however, the *Comparisons* table lines up the bands based on the results of single gel matching..
- ***Matrix -*** A list of the bands in the current reference lane, along with whether each lane has a band matching to the reference band.
- ***Similarity table*****.** This shows the Band Sharing Index between all lanes in the gel and each other.
- The ***Lane Data*** table shows the total volume of the lane and the bands in the lane. It also shows the normalization factor if used for Multiplex Images

From the toolbar select the first button for a list of fields**.** From the list you can select and order the different types of data fields that can be displayed for the bands, in these tables.

Data in the Molecular Size/Base pairs column is shown in three different ways:

Bold shows the specific values assigned by users to standard lanes.

Plain font shows the calculated values.

Italic/red shows the extrapolated values; these values are scientifically unreliable.

 **Data Fields**

Allows you to edit the display options for the window. See the **Measurement Window Options** section for details

Rearrange All Lanes Table

This allows you to display the all lanes table horizontally (by default) or vertically

Show Standard Lanes in Tables

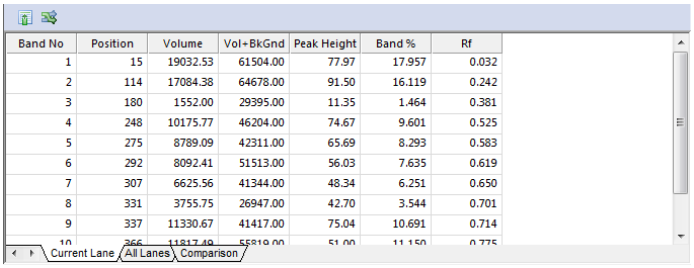
This allows you to toggle if the standard lane data is displayed in the tables

Multiplex

Data for multiplex images in the 1D module displays on a per channel basis. The way your data is displayed in each table is dependent upon which viewing mode you are using.

Single Channel mode:
When you view multiplex images in single channel mode each table displays data as it would for a single gel image.

The *Selected Lane* table displays data from each channel. The title bar of the window shows which lane and channel the data originates from, as shown in the example below.

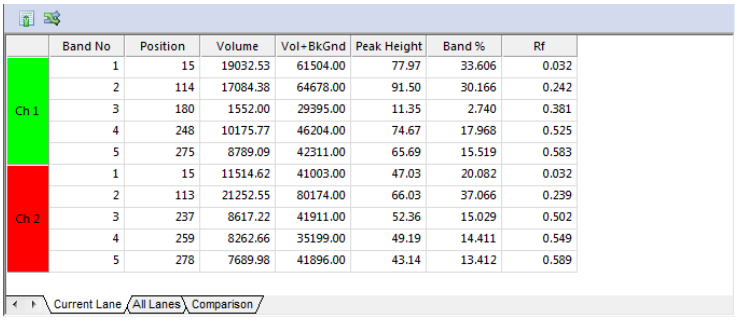


Band No	Position	Volume	Vol+BkGnd	Peak Height	Band %	Rf
1	15	19032.53	61504.00	77.97	17.957	0.032
2	114	17084.38	64678.00	91.50	16.119	0.242
3	180	1552.00	29395.00	11.35	1.464	0.381
4	248	10175.77	46204.00	74.67	9.601	0.525
5	275	8789.09	42311.00	65.69	8.293	0.583
6	292	8092.41	51513.00	56.03	7.635	0.619
7	307	6625.56	41344.00	48.34	6.251	0.650
8	331	3755.75	26947.00	42.70	3.544	0.701
9	337	11330.67	41417.00	75.04	10.691	0.714
10	366	11817.49	55819.00	51.00	11.150	0.775

Current Lane | All Lanes | Comparison

Data displayed in the *All Lanes* and *Comparison* tables relate to each channel independently.

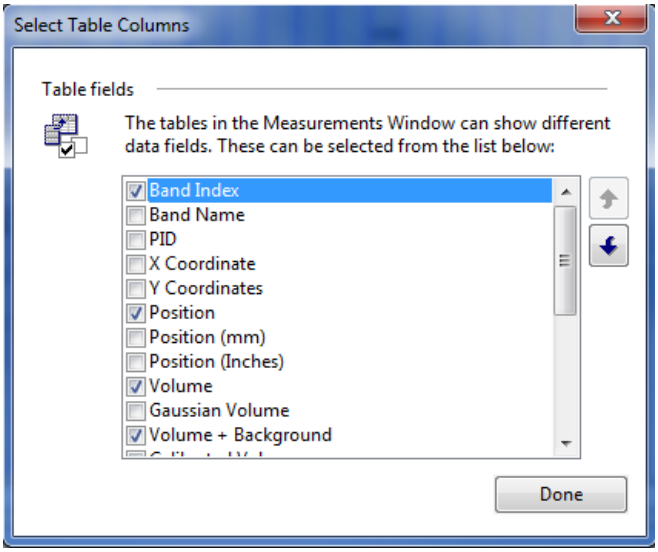
Channel Overlay mode:
The *Selected Lane* table displays data for the chosen lane across all channels. When you use the *Channel Overlay* mode the Measurements window identifies each selected channel with coloured blocks.



	Band No	Position	Volume	Vol+BkGnd	Peak Height	Band %	Rf
Ch 1	1	15	19032.53	61504.00	77.97	33.606	0.032
	2	114	17084.38	64678.00	91.50	30.166	0.242
	3	180	1552.00	29395.00	11.35	2.740	0.381
	4	248	10175.77	46204.00	74.67	17.968	0.525
	5	275	8789.09	42311.00	65.69	15.519	0.583
Ch 2	1	15	11514.62	41003.00	47.03	20.082	0.032
	2	113	21252.55	80174.00	66.03	37.066	0.239
	3	237	8617.22	41911.00	52.36	15.029	0.502
	4	259	8262.66	35199.00	49.19	14.411	0.549
	5	278	7689.98	41896.00	43.14	13.412	0.589

Current Lane | All Lanes | Comparison

Measurement Window Options



The **Selected**, **All Lanes** and **Comparisons** tables available in the Measurements window show the same selected data fields across all tables.

Ensure that you display the desired table in the Measurements window before selecting the check box adjacent to a data field, to display the data.

The check box at the bottom rearranges the **All Lanes** table so that the lanes are shown vertically not horizontally.

Within the software you can select the various data fields that will be displayed in the Measurements window. You can also change the order of these fields by use of the arrows.

To select or deselect a field for display:

Left click the box to the left of the name in the list of fields.

To change the order of the displayed fields:

1 Select the field to move by highlighting the name in the list of fields.

2 Click on the **Move Up** or **Move Dow**n buttons until the field is in the desired position in the list.

Data Fields

This section discusses data field availability. You will find a brief description of each field in the table below.

Data Field name:	**Description**
Area	The area of the Band displaying the number of pixels quantified in the image in order to produce the measurements.
Band Index	The number of the band in a lane.
Band Name	This is the name given to the band by the user
Band PID	This is the protein ID given to the band by the user
Band Percentage	A measure of the band's Volume divided by the Total Volume of all the bands in the lane.
Band Percentage (Calib)	The band's calibrated volume as a proportion to the sum of the calibrated volumes, of all the bands in the lane.
Band Width	The width of the band from one edge to the other in mm
Calib Volume	The Calibrated Volume of the bands.
Coordinates (X and Y)	The Coordinates of the peak in the band.
FWHM	Full Width Half Maximum gives a band width from the points half the peak height to the left and right of the band in mm. This is calculated after **Profile Deconvolution.**

Gaussian Volume	The Gaussian Volume of the bands
Lane Percentage	A measure of the Bands Volume divided by the Volume of the whole Lane.
Molecular Size	Shows a band's molecular size with the units shown in the table column header.
Peak + Background	The maximum value of the Profile of the Band with the Background value added.
Peak Height	The maximum value of the Profile of the Band not including the Background.
Position	The distance, in pixels, of the band's peak from the start of the lane.
Position (mm)	The distance, in mm, of the band's peak from the start of the lane
Position (Inches)	The distance, in Inches, of the band's peak from the start of the lane
Rf	Rf (Retardation Factor) is a measurement of position along the lane, relative to its length. By default, the first position in each lane has an Rf of 0 and the last has an Rf of 1. There is a linear increase in Rf from start to finish.
Volume	The raw volume of the uncalibrated quantity of material in the image feature **after** the background intensity has been removed.
Volume + Background	The uncalibrated quantity of material detected before the background intensity has been removed.

The Band Name for the each band can be edited in these tables.

Status bar

The status bar is the thin bar along the bottom of the main window. It displays information about the current state of the program (e.g. Ready) as well as descriptive messages for a selected command or toolbar button (e.g. Co-ordinates (Pixels): nnn Pixel Value: nnn).

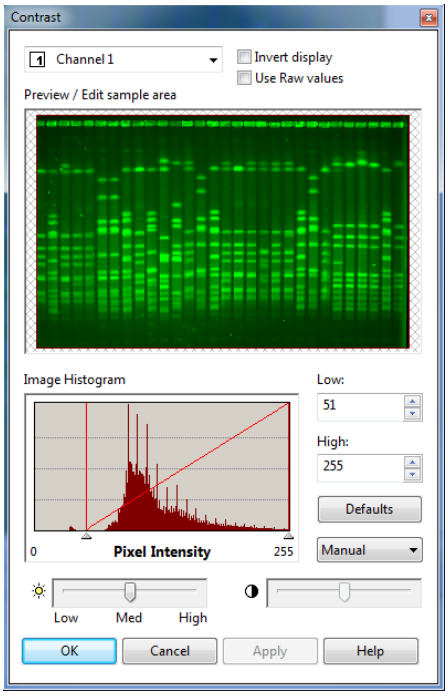
Contrast

The Contrast controls allow you to alter how the selected channels look on screen.

This dialog box is used to change the contrast and brightness of the image.

Multiplex

When you are viewing multiple channels, you can switch to one of the other selected channels from the drop down list. The contrast is edited on a per channel basis.



Preview / Edit sample area

The Preview / Edit sample area displays a thumbnail of the entire image. If the image displays a rectangle on it, known as the area of interest (AOI), the palette is calculated from the AOI area. The default rectangle covers the entire image.

You can change this rectangle by clicking and dragging out a new rectangle; this causes the image to update immediately.

This feature can be very useful when editing very faint parts of the image, as you can set the AOI to a very small area, which produces a very good colour standard in this area.

Image Histogram

The Image Histogram displays the frequency with which each pixel intensity occurs within the area of interest. The peaks on the graph represent the pixel intensities that occur most frequently within the current area of interest.

The left and right red bars on the graph show the range of pixel intensities in the image that will be mapped to different colours in the display image. You can directly modify the brightness and contrast of the display by dragging the bars on the graph.

Move these handles to increase/decrease the contrast.

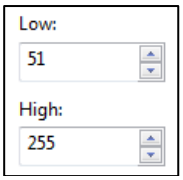
The figures below the image histogram represent the scale of the graph, i.e. the calibration.

You can restore the default contrast at any time by clicking the **Defaults** button.

Low / High edit boxes

The **Low** and **High** edit boxes are synchronised with the two handles below the image histogram.

These edit boxes allow you to enter values to adjust the contrast of your image.



This enhances the contrast display so that for narrow contrast ranges, a sufficient number of grey levels are used giving a higher quality of image display.

You can restore the default contrast at any time by clicking the **Defaults** button.

Brightness

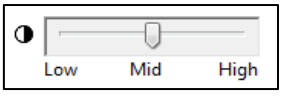
The two sliders at the bottom of the dialog box allow you to adjust the brightness and contrast of the image by moving them between Low, Medium and High.

Drag the slider to change the brightness. High = paler, Low = darker.

Contrast response list

Below the **Defaults** button there is a drop down list. Select the shape of the contrast response curve to help you visualise the features of your image.

Manual - a straight line.
Hi-contrast – a curve calculated from the image to maximise the contrast.
Curve and **Sigmoidal** – the standard shapes of a response curve, adjustable by using the Contrast slider.



The Contrast slider affects the shape of the curve that extends between the contrast limits.

Drag the slider to change the contrast. High = more effect.

Invert display

This check box indicates whether the current colours should be inverted or not; click it to toggle the option on or off. Inverting an image causes it to display like a photographic negative. For example, using the default **Grey Scale** colour scheme white becomes black and vice versa.

Use Raw Values

This check box indicates whether the histogram is calculated after any scanner calibration, the default, or just uses the raw values of the tiff image. Using the raw values will usually increase the contrast.

Auto Default Contrast

By default the software looks at the histogram results and optimizes the default low and high values for contrast stretching. This means you can see more of the important features of the image. If you wish this turned off you can see the image default as stretched from 0 to the maximum in the image. This is a global variable set for all future default display but does not alter the current image unitl you press **Defaults**

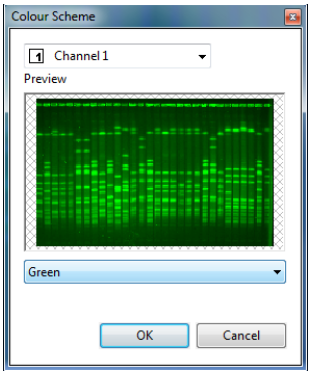
Channel Selector

The drop down at the top of the dialog allows you select which channel you wish to change the contrast for.

Colour

The Colour controls allow you to alter how the selected images look on screen.

The **Preview** shows a thumbnail of the entire image and displays the effects of any changes.



Multiplex

When using multiplex gels you can only launch the Colour Scheme dialog box when in *Multiple Channel viewing* mode. The colour that you choose, from the colour scheme drop down list, is used to change the colour for the channel in *Overlay viewing* mode only.

In *Single Channel viewing* mode the image will be displayed in grey only and cannot be changed.

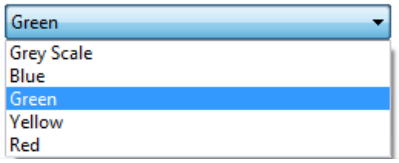
The default choice of colours used to display each channel is dependent upon how many channels there are in the image.
2 Channel images: 1 = green, 2 = red

3 Channel images: 1 = blue, 2 = green, 3 = red

4 Channel images: 1 = blue, 2 = green, 3 = yellow, 4 = red

Colour Scheme List

This is a drop down list box containing the names of all of the installed colour schemes on the system.



Of the various colour schemes that are supplied with the software, two are *false colour* schemes; these can be useful for visualising faint spots or bands, because the human eye is often more sensitive to colour than to shades of grey. To change the colour scheme, select a different scheme from the list.

Reverse colours

This check box indicates whether the current colour scheme should be inverted or not; click it to toggle the option on or off. Inverting an image causes it to display like a photographic negative. For example, using the default **Grey Scale** colour scheme white becomes black and vice versa.

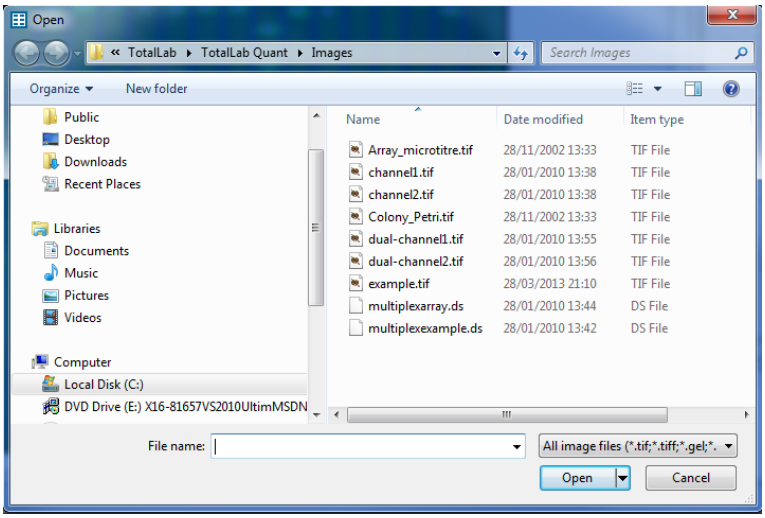
Beginning the Analysis – Open Image

Selecting the **Open Image** command from the main toolbar allows you to start an analysis by Opening an image. It also allows you to Edit an Image, check its properties and Create Multiple Images.

Note:
*If you close the software, open an image or open an existing experiment, the software **automatically saves** the current experiment for you.*

Open Image

You can open an image by clicking the **Open Image** or **Open Multiplex Image** button



To open an image:

1. Select the folder from the *Look in* list.
2. Select the correct file type from the *File of type* list.
3. Click the specific image file from those displayed.
4. Click **Open**. The selected image automatically opens in the Image window allowing you to analyse the image.

Note:
*If you have an **RGB** Colour image it can be opened as a regular image (converted to greyscale) or as a multiplex image (all 3 colours split into 3 channels)*

Edit Image

This option allows a wide range of editing options. Please see the **Image Manipulation** section for more details.

Image Properties

The image properties dialog box has three tabs, File info, Scan info and History. The information in these tabs is read only.

The File info tab displays information about the image, for example the File Name, Size, Image Width and Image Height.

The Scan info tab displays information relating to the scanning of the image, for example the Scanner make, model, date and time of scan and the resolution. It also shows any comments from the scanning.

The History tab displays the name of the image and a scrollable list of any editing made to the image, for example Rotate Clockwise 90.

Start Tutorial

This option allows you to start the included tutorial. A tutorial help pane will appear within the program to help you learn the software

Resume Tutorial

This option allows you to resume the included tutorial and the stage you last looked at. A tutorial help pane will appear within the program to help you learn the software.

Automatic Analysis

Selecting the **Automatic Analysis** option from the toolbar allows you to setup and/or use pre-stored protocols. These protocols record automatic steps of analysis (e.g. band detection) which can then be applied to a new gel..

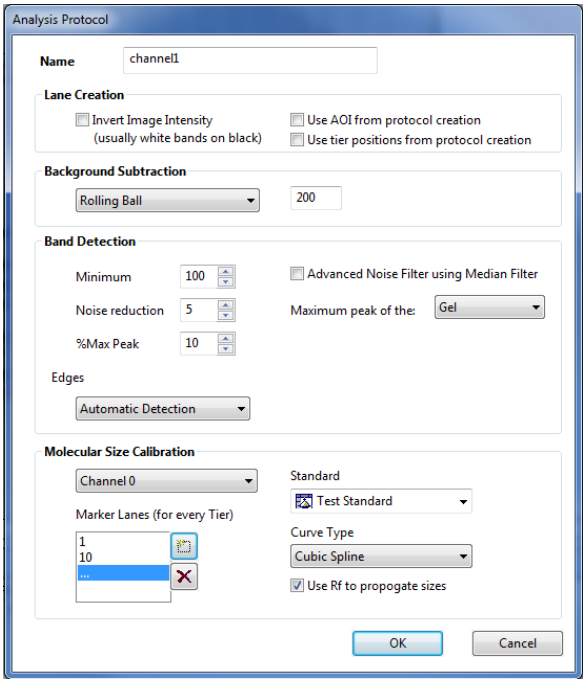
Specifying an area of interest (AOI)

To specify an AOI, move the pointer over the image in the main image window, and drag out an area that you want to analyse. This area is used when analysing your gel. Setting an AOI on noisy gel images can be beneficial when using the automatic analysis mode or when creating lanes.

To clear the AOI, right click on the image.

Creating a Protocol

To create a protocol click on the New button and the protocol creation dialog will appear.



This has many options available so you can automate the analysis process. Here is a summary

Lane creation

Here you can define if you Invert the gel Intensity, use the AOI used with current gel and/or use the tier positions from the current gel.

The last 2 options are useful if you are repeating experiments with same size and structure gel images.

Background Subtraction

Here you can define the automatic background subtraction option to use in the protocol. See the chapter on **Background Subtraction** for more details.

Band Detection

Here you can define the automatic band detection parameters to use in the protocol. See the chapter on **Band Detection** for more details.

Molecular Size Calibration

Here you can define the molecular size parameters to use in the protocol.

You can select the standard to use, edit/create a new standard, curve type and Rf option as in the main calibration mode. The other important decision to make is if to select the channel to use (if multiplex) and to define which lanes will actually be assigned the standard and become markers.

See the chapter on **Molecular Size Calibration** for more details.

Copying a Protocol

Click the copy button to copy the selected protocol. Useful for minor changes to already setup protocols.

Editing a Protocol

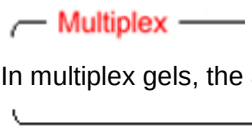
To edit an existing protocol click on this button or double click the selected protocol to bring up the Analysis Protocol dialog.

Deleting a Protocol

Click the delete button to delete the selected protocol.

Running a Protocol

This is where you actually apply the protocol. Select the protocol to use, define the number of tiers if required and then press the Run Protocol button. All the analysis will be done automatically for you to then check the results.



In multiplex gels, the automatic analysis steps selected in the Protocol are applied to every channel.

Lane Creation

You use the **Lane Creation** mode to define the positions and areas occupied by the lanes on the gel image.

Lanes will be created for all channels in a multiplex gel.

After the positions and shapes of these lanes are known, you can perform further operations, such as band detection.

Changing the shape of the existing lanes that already contain bands causes an impact on the data. Therefore you should define them correctly in the early stages. Adding lanes or bands does not cause a loss of data.

Before any analysis can be performed you must locate the lanes on the image then you can use the edit tools to resize or move the boundaries until they match the exact shape of the lanes.

The software automatically numbers each lane when the lane is created. You can name the lanes to identify them by selecting the *Lane names* check box from the Image Windows Options | **Ruler options** dialog box.

Renaming lanes

1 Click the lane name in the Image window ruler that you want to change. An edit box displays.

2 Type the new name, and then click outside of the edit box or press Enter.

You can add more lanes to the gel image at any time. You can also delete lanes if you do not require them.

To define and edit the lanes you should:

Create the lanes automatically or draw the lanes manually.

Optionally, you can:

Set tiers

Create an area of interest (AOI)

Move lanes (in a multibox or individually)

Bend and resize lanes (in a multibox or individually)

Delete lanes

These options are available from the Lane Creation mode, which is started from the **Create Lanes** tool in the main toolbar.

Invert Intensity

Most images have high pixel values (high intensities) in the black areas of the image and low values in the white areas. However, in some images, the spots have a low intensity (and therefore low volume) compared to the background. The "Invert Intensity" command allows you to correct this by inverting all the pixel values before using them to calculate the measurements.

To invert a pixel value, the software subtracts the value from the maximum possible pixel value in the image. For example if the image has a pixel value range from 0 to 255 and the software inverts a pixel that has a value of 50, the inverted value would be (255 - 50) = 205.

To invert the measurements data, click **Invert Intensity** option**,** and then check the data in the measurements window. It can also be required **before** Automatic Lane Creation to improve detection.

Median Profiles

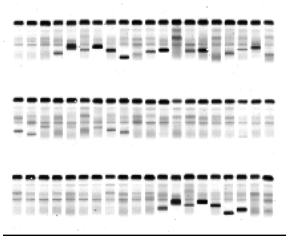
By default the profile of a lane when created uses the average pixel intensity across the width of the lane. This is a perfect representation of the lane for measurement. However sometimes speckles or other image artifacts interfere with this a produce a spike in the profile. This can lead to mistakes in the automatic band detection.

This option allows you to create the profiles using the median value and not the average. This means that speckles etc. will have less chance of altering the profile and lead to better band detection.

This does not alter the band measurement at present so speckles within a band are still measured and no volumes measurements have been altered from previous versions.

Setting tiers

If the gel image has distinct sections you can split the gel image into a number of sections called tiers. Each tier should contain one set of lanes. Ensure that you set the tiers parameter prior to creating the lanes.

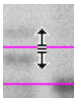


To set tiers on an image:

1 At the bottom of the Mode dialog whilst in **Automatic Lanes AOI** or **Draw New Lanes** sub-modes select the number of tiers required, either by using the spin buttons or typing the number.

Lines defining the tier boundaries appear on the image.

2 Move the lines between the tiers to their desired locations by placing the pointer on the line splitting the tiers and dragging the lane into position. The pointer changes when over the line.



Automatic Lanes (AOI)

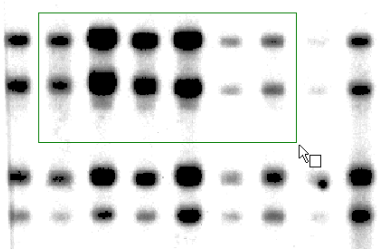
Within the Lane Creation mode you can select an AOI to aid the automatic lane creation.

You select the area from the whole image, across a number of tiers, or within a single tier. The AOI feature can be used to restrict where the software creates the lanes.

Selecting an Area Of Interest

1 Position the pointer on the image at the location of the desired area.

2 Drag out an area on the image, as shown in the example below, and then release the mouse button.



The area of interest can be drawn across distinct sections (tiers); when you click the **Detect Lanes** button the lanes are automatically drawn according to the number of tiers you set.

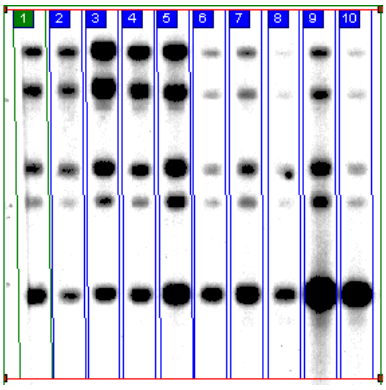
Only one area of interest can be drawn and populated with lanes at any time.

Removing an Area Of Interest

To remove an area of interest, right click anywhere in the image. This cancels the area of interest selection, allowing you to draw another area.

Creating Lanes Automatically

Whether or not the image has distinct sections (tiers), you can automatically create lanes. This is achieved by clicking the **Detect Lanes** button in the mode dialog if the **Automatic Lanes AOI** option is selected. After a few seconds the software detects the lanes in the image and draws the *lane multibox*. If the image has tiers ensure that you set the tiers parameter prior to creating the lanes.



The boundaries of the lanes are shown as rectangles within the multibox area.

Multiplex

Every channel is used during lane detection on multiplex gel.

If you are not satisfied with the multibox, you can edit the lanes in the multibox using the procedures below. After editing the lanes click the **Accept Edit** button at the top of the dialog, this refreshes the data displayed in the program windows.

You see the defined lanes and two red multibox edge lines across the top and bottom of the lanes. These lines are used to move all the lanes simultaneously.
See the section on **Moving Lane Box Edges** for more details.

To move individual lanes, see **Resizing Lanes** and **Moving Lanes**.

The large rectangle that appears is split into small rectangles representing the lanes. If the Lane % width is set to 100, then the lanes use all the available space. The lower the number the less space each lane takes up and the larger the gaps between lanes will be. Lanes are automatically aligned.

If the automatic detection has failed for any reason, click the **Restart** button in the dialog and draw the lanes manually.

If you want to perform densitometry on the bands, ensure that the lane boundary edges you draw closely follow the lane.

Drawing New Lanes

To draw the lanes, you set the parameters in the dialog.

1. If the image displays distinct sections, set the **Number of tiers**.
2. Set the **Number of lanes** you want to draw.
3. Set the **Lane % width**. This specifies how much of the width of each section should be converted into a lane, since it is unlikely that there will not be a gap between them.

You are now ready to draw lanes manually.

4. Drag a rectangle over the gel image in the Image window; the lanes display as you draw. Because the rectangle contains multiple lanes, it is called the lane multibox.

To add extra lanes to a multibox, click the image at the position where you want another lane. If you make a mistake click the **Clear** button and start again. This will clear **all** the lanes.

Editing Lanes

Whether your lanes were created automatically or manually they can be edited by using the Multiple or single lane editing tools located in the dialog for Lane Creation.

Editing within a lane or set of lanes is applied across all channels of a multiplex gel.

Editing lanes in a multiplex gel can be performed in both the single or overlay viewing mode.

Before using the editing tools you must select the edit mode, either **Edit Multiple lanes** or **Edit single lanes**, from the list in the dialog.

Editing multiple lanes

In the *Editing Multiple Lanes* mode there are three main tools.

Bend / Resize Lane Box

Move Lane Box Edges

Add Lanes

After you have added more lanes to a multi lane box you can edit them in either single lane or multiple lane editing mode.

Editing single lanes

In the *Editing Single Lanes* mode there are three main tools.

Bend / Resize Lane

Move Lane

Add Grimaces

Since the lanes might not be straight, you can add and move tie points (small white boxes called handles). You can move a handle inside the lane to bend the lane. By default, the lane has two handles, one at the top and bottom of the lane.

Extra handles along the multibox top and bottom edges might already have been created if the lanes were automatically created.

Bending and Resizing lanes in multiple lane boxes

1. Click the **Bend / Resize Lane Box** button in the dialog.
2. Position the pointer over one of the multibox (red) edge lines. The pointer changes to  .
3. At this point, you can add a handle .

   You can drag a handle to make the edges of the multibox more convex or concave to account for differences in lane length or shape.

Moving the edges of multiple lane boxes

1. Click the Move Lane Box Edges button.
2. Position the pointer over the multibox (red) edge line. The pointer changes to a two-headed arrow, either vertical  or horizontal  .
3. Drag the line to the desired location and release the mouse button.

Adding lanes to multiple lane boxes

1. Click the **Add Lanes** button.
2. Click, within the multibox, where you want an extra lane to appear. A vertical lane appears on the image. This is only possible after Automatic Lane detection.
3. Repeat the previous step as required.

Adding handles

Position the pointer at the location in the lane where you want to add a handle and then **left click**. The pointer changes to  showing where you can add a handle.

Note:
You can drag a handle to a new location at any time.

Deleting handles

To delete a handle, position the pointer over the handle you want to delete (the pointer changes to ) and **right click**. The lane changes shape to reflect the deletion of the handle. Repeat this step to delete all handles not required.

Bending Lanes

1. Click the **Bend / Resize** Lane button in the dialog.
2. To change the angle of a lane, position the pointer over either the top or bottom (white) handles of a lane. The pointer changes to  indicating that the pointer is over a handle.
3. Drag the handle left or right to change the angle of the lane.
4. To bend a lane at any point other than the top or bottom, add (or delete) handles.
5. After you add an additional handle move the handle to the desired location, which causes the lane to bend.

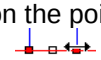
6 Repeat these steps until you have aligned all the lanes.

7 Delete any handles that are not required.

8 If all the lanes are aligned correctly click the **Accept** button in the dialog.

Resizing lanes

1 Click the **Bend /Resize** Lane button in the dialog.

2 Position the pointer over one of the (red) handles. The pointer changes to a double-headed arrow. 

3 Drag the corner handle away from the centre of the lane to make the lane wider, or towards the centre to make the lane narrower. As you move the handle, the lane uniformly resizes about the centre.

4 When you finish the resizing, click the **Accept** button in the dialog. Alternatively, right click in the Image window to accept the changes.

Moving Individual Lanes

1 Click the **Move Lane** button in the dialog.

2 Position the pointer over the lane you want to move. The pointer changes to  showing you that the lane can be moved.

3 Drag the lane to the new position, and then release the mouse button.

4 To move any other lanes, repeat the steps above.

5 After all lanes have been moved to their new positions, click the **Accept** button in the dialog. Alternatively, right click in the Image window to accept the changes.

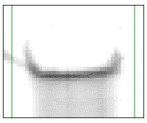
Adding Grimaces

Because lane analysis is performed using the lane profiles, ensure the bands of the lane are straight and perpendicular to the sides of the lane. If the bands of the lane do not meet these criteria, the software allows you to add grimaces to the lanes to compensate for the '*smiling*' effect.

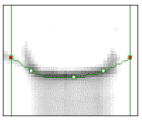
A grimace is effectively a contour line of equal band position across a particular part of a lane. The software calculates the band positions and lane profiles on the assumption that the lane warps uniformly between the horizontal start of the lane and the first grimace, between grimaces and then between the last grimace and the bottom of the lane.

Example of where a grimace is needed

In the section of the gel illustrated below, the band is curved. By putting a grimace over the band, you can compensate for the curvature.



The following illustration shows the gel corrected by adding a Grimace.



Adding Grimaces

1 Click the **Add Grimaces** button in the dialog.

2 Position the pointer at the approximate centre of the band and click; the software adds the grimace to the lane.

You cannot add a grimace outside the lane or too close to another grimace in the lane.

The grimace appears as a horizontal line with red handles to the left and right sides.
You can move these handles to adjust the line to match the angle of the band, and you can add handles to make the line bend to match the curvature of the band.

To add a handle to the grimace.

3 Position the pointer over the grimace line.

The pointer changes to .

4 Click, to add the handle and drag the handle to reposition it.

5 To adjust a grimace handle position the pointer over the handle. The pointer changes to .

6 Drag the handle to reposition it.

To **delete** a grimace, right click on the handle.

The effects of the grimaces can be seen in the Analysis window after you accept the changes. The profile and lane image change according to the new calculations.

Apply Lane Width to Gel

After you draw the lanes, you can resize selected individual lanes or the lanes within a multibox. Afterwards you can then set all the other lanes to the same width as the current lane.

To set the lane widths of all the lanes:

1 Select any of the editing sub-modes in the dialog.

2 Click a lane, to make it the current lane.

3 Click the Apply Lane Width to Gel button.

Deleting Lanes

When you create a lane multibox, you can include lanes, that you do not want to use, or places where you have left a gap on the gel. To avoid these areas being included in the lanes you analyse, you can delete them. You can also delete individual single lanes in the same way.

To delete lanes within the Lane Creation mode:

1 Click a lane to make it the current lane.

2 Click the **Delete Current Lane** button in the dialog when in an editing sub-mode.

Background Subtraction

Background subtraction can be performed on the images to account for the background intensity of the gel material on the image.

Most gel images contain background intensity. In effect, this is the colour of the gel material. This background should be removed from the lanes so that the calculated band volumes represent the volume of the material in the band, rather than the volume of all material including the gel material, at that position.

You start the Background Subtraction mode from the main toolbar.

The various methods of background subtraction can be selected by clicking a button in the dialog.

You choose between five automatic and three manual methods of background subtraction, which are described below.

The chosen method of background subtraction is applied simultaneously to all channels of a multiplex gel.

Automatic Background Subtraction Methods in 1D

To use the automatic background subtraction methods select the relevant button from the list in the dialog to perform the subtraction.

To perform automatic background subtraction:

1. Click the button for the method you want to use. The software updates the data automatically. Some options have parameters and altering those and refreshing/clicking again will implement the change.
2. Check the calculations by viewing either table of Selected (current lane) or Comparisons (all lanes) in the Measurements window after band detection has been performed.

The background subtraction methods are:

Rubber Band

This method can be thought of as stretching a rubber band underneath the lane profile. If the values at the ends of the profile are lower than the rest of the profile or the bands are badly separated, do not use this method.

Minimum Profile

This method sets the background level as the lowest value found in the profile. It is not advisable to use this method where the lowest point is found at the extremities of the lane since the result is dependent on where the lane is drawn. This calculation can be hard to repeat between analyses.

Rolling Ball

The Rolling Ball method requires you to enter a value for the size of the rolling ball. This method calculates the background as if a disc, with the radius you have entered, were rolling underneath the lane profile.
The larger the radius of the disc, the less the background rises with the profile.

This method displays both a slider and parameter entry box.

1. Click the **Rolling Ball** button and a parameter entry box appears in the dialog.
2. Enter a specific value in the entry box.

3 Click the **Refresh** button next to the entry box.

4 View the calculations in the tables of the Measurements window.

Lane Edge Subtraction

This method uses the lower of the two values at the edges of the lane length, as the background. If the section of the image used is wider than the bands, then this is a very effective way of removing co-migrating material from your measurements. This method should only be used if the bands are completely enclosed, because when the edges of your lane area intersect any of your bands, band material will be considered as background, severely affecting your results.

Valley to Valley

This method requires that you have performed band detection first. The background is taken as the line between the edges of the bands in the lane. You enter a value of maximum slope in the entry box in the dialog to avoid situations where the edge between overlapping bands, which is not at the background intensity, causes the background to climb too high.

None

Selecting the None method clears any background subtraction calculations.

Manual Background Subtraction Methods

The manual background subtraction methods are:

Image Rectangle

The average intensity within a rectangular area on the image is taken as the background intensity.

To use the Image Rectangle method, you draw a rectangle in the Image window. From this rectangle, the background intensity is applied and calculated for all lanes.

1 Click the Image Rectangle button.

2 Position the pointer over the image window - you will see a small white box attached to the pointer.

3 Draw a rectangle, from the top left to the bottom right.

4 Release the mouse button. The calculations are automatically updated in the Measurements window.

When you use the Image Rectangle method of background subtraction in a multiplex gel, the same rectangular *region* is used across all the channels, although each channel uses its own intensity values within the rectangle.

Image Stripe

A thin vertical rectangle is drawn alongside the lanes, in an area where no band material is present. The average intensity across this rectangle along each of the positions along the lane length is taken as the background for that position across the gel.

1 Click the Image Stripe button.

2 Move the pointer over the image window- you will see a small white box attached to the pointer.

3 Draw a rectangle, from the top left to the bottom right.

4 Release the mouse button. The calculations are automatically updated in the Measurements window.

Multiplex

When you use the Image Stripe method of background subtraction in a multiplex gel, the same stripe *region* is used across all the channels, although each channel uses its own intensity values within the stripe.

Manual Baseline

The Manual baseline method allows you to define the shape of the background line using the profile graph in the Analysis window.

Multiplex

The Manual baseline method is only enabled when you use the single channel viewing mode.

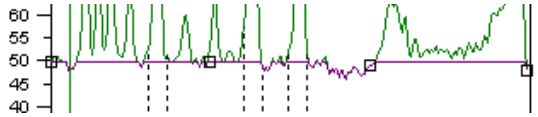
The background for each channel can be edited independently of the other channels, in the Analysis window. Select the channel then follow the steps defined below.

The manual baseline method applies background subtraction to the current lane only. This method recalculates the background every time you complete a mouse interaction in the Analysis window.

Note:
If used incorrectly, the manual baseline method can produce erroneous results. For good laboratory practice you should use an automatic background subtraction method.

Using this method produces a straight line with four movable handles along the bottom of the lane profile graph in the Analysis window. If you had previously used another method to define the background for this lane, the handles fit the current background, which will change when you start editing.

To edit the shape of the background, you must either move existing handles (small white squares) or add additional handles. The background line stretches between the handles using straight lines.



When the pointer is over a central handle, the pointer changes to ; if the handle is at the start or end of the lane, the pointer changes to .

To add a handle:

Position the pointer, over the background line between existing handles and then **click.** The software adds a handle at that position.

To move a handle:

Position the pointer over the handle and drag. As you move the handle, the background line updates to show the effect of the new position. After you release the mouse button the software recalculates the data.

Notice that the background cannot be set above the profile at any given point and the line changes shape where it touches the profile.

To remove a handle:

Position the pointer over the handle and then **right click**. The pointer changes to  . The handle is removed and the background data is recalculated.

You cannot remove the two end handles of the background line.

Band Detection

Band Detection allows the automatic finding of bands and manual editing of bands in the lanes of the image.

After you defined the lanes you are ready to determine the position of the band peaks and edges. The **Detect Bands** mode offers you automatic methods for finding the bands and also allows you to edit the bands manually.

There are two main purposes of band detection: the detection of peaks and the detection of edges.

The peak of a given band is the point in its profile where the image intensity is at its maximum value. This is used to define the band's position in the lane.

The bands are rarely a single pixel in length; therefore, the extents of the band must also be determined so you can measure the band's volume. These edges are found where the software locates the troughs in the lane profile at either side of the band's peak.

To perform band detection, click **Detect Bands** on main toolbar.

With the **Band Detection** mode activated, click the **Detect Bands** button in the dialog.

When you perform band detection or edit bands in multiplex gel you must use the single channel viewing mode. Band detection is performed on a per channel basis. Any parameters set are only applied to the current channel. Therefore, each channel has its own set of band detection settings. This rule also applies when editing bands; any editing is made to the current channel.

Automatic Band Detection

Automatic band detection uses a series of algorithms to find the peaks in the profile to declare as bands and the troughs between them to declare as edges.

Although this method is classed as automatic, some parameters still need to be specified.

1. Enter or change the peak detection parameters at the top of the dialog.
2. Try different values to see which provides the best results. A good starting point is Minimum slope 100, Noise reduction 5, and Percentage maximum peak 3.

When you change the parameters, band detection is automatically performed on all lanes, using the new parameters. This can be useful for instantly seeing the effect of small changes to one of the parameters.

The peak detection parameters are:

Minimum slope

This parameter represents how pronounced the band must be from its surrounding area in the lane. The range for this parameter is 0–999.

A high value means that the transition from the background lane intensity to the band's peak intensity must be sharp. A lower value allows the gradient to be less severe. In general, the lower the minimum slope value, the more bands are detected.

Noise reduction

This parameter 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. Range 0 - 20.

Percentage Maximum Peak

This is a threshold parameter, which discards peaks under a certain size in relation to the highest peak on the gel. The higher the percentage value entered here the fewer the peaks likely to be detected in the profile. The sizes of the peaks are calculated after background subtraction. The range for this parameter is 0 - 100.

In the **Advanced Options** dialog you can set this parameter to work from the highest peak in each lane rather than the whole gel

Edge Detection Parameters

After you are satisfied with the peak detection parameters, that you have entered, you are now ready to enter the parameters for edge detection. By default the edges are detect with the automatic option but it can be changed in the **Advanced Options** dialog.

Single Edge

This option will automatically add a single edge, between the bands at the minimum profile value. It will also stop you having more than one edge between bands, when editing edges.

Automatic detection

Select this parameter if you want the software to automatically detect the band edges. Automatic detection is the preferred method for edge detection. The software identifies an edge as the trough in the profile on either side of the band's peak.

Fixed width

If you want to specify the width of each band (in pixels) thereby determining the position of each edge, select the **Fixed Width** button and enter the required width. The band edges are positioned at an equal distance on either side of the peak.

When you change the edge detection parameters after the bands have been detected, you can see the effects immediately.

The results are shown in the Image and Analysis windows. If the results shown do not agree with what you are expecting, for example, if the software has detected bands that should not be there or if it has not detected enough bands, you can either try other parameters or edit the bands manually.

When you enter different parameters, the new band detection results automatically overwrite the previous bands.

Percentage of Peak

The system reads the intensity at the peak and then moves the edges outwards, until the difference between the intensity at the peak and the intensity at the edge is equal to the specified percentage.

If you change the above values using the spin buttons after bands have been detected, the effects will be seen immediately. Similarly, if you change the method used, the effect will be applied straight away.

Manual Band Editing in the Image window

In **Band Detection** mode, you can add and remove bands in the Image window, you can also move band edges.

Adding bands in the Image window

1. Select the **Add/Delete Bands** option
2. Position the pointer in the Image window where you want to add a band.

   The pointer changes to a drawing tool  indicating that you can add a band at that position. If the pointer displays a different shape you are too near another feature to add a band.
3. Click, to add a band.

If the image is zoomed out, editing the bands and edges might be more difficult. See the section on Zooming in the Image window to find out how to increase the magnification of the image.

A diamond appears centred on the position (or the nearest peak to the position) that you have chosen. Horizontal (red) lines appear above and below the diamond indicating the edges within which the volume measurements are made. The Analysis window also reflects the new band.

Use this method to **add** all required bands.

To **clear** all the bands, click the **Clear** button in the dialog.

To **delete** only selected bands, see the following section on Deleting bands.

Editing band edges in the Image window

The software requires that there be an edge at either side of a band's peak in order to define the total area of the band for measurement. If two bands are near to each other, they might share an edge.
Edges are represented as horizontal lines in the Image window and can be moved into a new position.

To move a band edge:

1. Select the **Move/Delete Edge** option
2. Position the pointer over the band edge you want to move. The pointer changes to ↕.
3. Drag the band edge up or down the lane's length. You will not be able to move the edge past a band or off the lane, or past another band edge.
4. Release the mouse button when the band edge is in the required position. The Analysis window and Measurements tables update to account for the new size of the band.

If you move a shared band edge, a second band edge is created. This band edge can be placed between the upper band and the original band edge or between the original band edge and the lower band. The bands no longer share a single edge.

Deleting band edges in the Image window

If you have two edges between two bands (the bottom edge of one and the top edge of the other), you can remove one of these edges. The bands share the remaining edge.

1. Select the **Move/Delete Edge** option
2. Position the pointer over the band edge you want to remove. The pointer changes to ↕.
3. **Right click,** the software removes the band and updates the Measurements and Analysis windows.

Deleting bands in the Image window

When you delete a band the software removes the band's peak and any unshared edges from the lane, and renumbers the other bands.

1. Select the **Add/Delete Bands** option
2. Position the pointer over the diamond shape of the band you want to remove. The pointer changes to  .
3. **Right click** to delete the band.
   The software removes the band and updates the Analysis window and Measurements tables.

Deleting a group of bands in the Image window

You can delete a group of bands from an area of the gel image. For example, they can be bands that are of no interest or that have been detected in an area of noise by the automatic band detection algorithm.

1. Select the **Add/Delete Bands** option
2. Position the pointer to the top corner, left or right, of the area you want to clear of bands.
3. With the right mouse button depressed, drag the pointer to the opposite corner of the area. The pointer changes to  and an area of red crosshatch appears over the image  while you are dragging the pointer.
4. Click **Yes** to the message 'Do you wish to delete all these bands?'. The bands beneath the drawn area are removed and the software updates the Analysis window and Measurements tables.

Manual Band Editing in the Analysis window

You can add and remove bands in the Analysis window in a similar way to editing in the Image window, but you cannot delete a group of bands. The following instructions describe performing these actions on the profile graph pane in the Analysis window; you can also perform the same interactions with the lower pane.

Adding bands in the Analysis window

1. Select the **Add/Delete Bands** option
2. Position the pointer to the location along the profile, where you want to add a band. The pointer changes to  indicating that you can add a band at that position. The pointer displays a different shape when you are too near another feature to add a band.

   If the profile graph area is small and you are viewing the whole of the profile graph, it might be harder to edit bands and edges. To give a better view, zoom into a desired area. Refer to the Image window | **Zooming an image** section for more information.
3. Click, to add a band. An arrow appears over the profile, directed at the chosen location. Dotted vertical lines appear to the left and right of this location indicating the edges, within which volume measurements are made. The Image window also reflects the new band.

With this method, you can add all the required bands. If you want to clear all the bands, click the **Clear** button in the dialog. To delete some of the bands, see the section below, on deleting bands.

Editing band edges in the Analysis window

Edges are represented as dotted vertical lines in the Analysis window and can be moved.

1. Select the **Move/Delete Edge** option
2. Position the pointer over the edge you want to move. The pointer changes to ⟷.
3. Drag the edge left or right along the lane's length to the desired location. You cannot move the edge past a band or off the lane.
4. Release the mouse button when the edge is in the desired position. The software updates the Image window and Measurements tables accounting for the new band size.

If you move a shared edge, a second edge is created. This edge can be placed between the left band and the original edge or between the original edge and the right band. The bands no longer share a single edge.

Deleting band edges in the Analysis window

If you have two edges between two bands (the right edge of one and the left edge of the other), you can remove one of these edges. The two bands share the remaining edge.

1. Select the **Move/Delete Edge** option
2. Position the pointer over the edge to want to remove. The pointer changes to ⟷.
3. **Right click** the edge. The software removes the edge and updates the Image window and Measurements tables.

Deleting bands in the Analysis window

When you delete a band, the software removes the band's peak and any unshared edges from the lane and renumbers the other bands.

1. Select the **Add/Delete Bands** option
2. Position the pointer over the band you want to remove. The pointer changes to  .
3. **Right click** to delete the band. The software removes the band and updates the Image window and Measurements tables.

Molecular Size Calibration

By using the Molecular Size Calibration mode in a gel image containing molecular weight or pI standard lanes, you can use the software to establish the molecular weight or pI of the bands.

Given the known values for these bands on the standard lanes, the software can interpolate and extrapolate contour lines of known molecular weight/pI horizontally across the gel image. If you have run several standards, these lines can run between the standards, or you can choose one standard as the best example.

After the horizontal contours have been determined, the software uses the selected curve-fitting algorithm to determine the relationship of molecular size to a position along the lane for all the points in all the lanes. From this, you can determine the molecular size of any given band in any of the lanes.

In order to perform molecular size calibration, you must detect the bands in at least the standard lanes, although band detection should be complete by this stage. You can edit the bands of the lanes after calibration without losing the calibration information. If you edit the bands of a standard lane, click the Compute button in the dialog to refresh the curve.

In order to assign the known values to the bands of the standard lane, the software uses mappings lists or standards. Each mapping list contains a set of values for the bands, that separate in a standard lane, and the unit of measurement of those values. There are several mapping lists already defined in the software, but you can define and use your own.

Molecular size calibration is accessed from the **Molecular Size Calibration** button in the main toolbar.

To see the results of your molecular size assignment on the bands, in the Selected and Comparisons tables in the Measurements window ensure that you have the Molecular Size field selected in the Measurements Window **Table Columns** button.

Alternatively, you can set the Analysis window display options by selecting the **Show Molecular Size** check box in the **Analysis window Options** dialog to show the Molecular Size annotations for the bands.

Note:
The annotations are displayed below the Lane image in the Analysis window. If you cannot see the annotations, adjust the size of the lower display box by dragging the edge.

The graph, showing the relationship of molecular weight or pI to the position for the current lane, appears in the Graph window.

Multiplex

When performing Molecular Size Calibration on multiplex gels only one channel can contain MW standards. The standards that you set in the chosen channel are used to generate MW values across all the other channels. You can only set standards on a channel when you use the *Single channel viewing* mode.
To apply standards to a different channel you must:

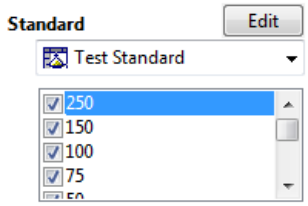
- Ensure that you are in single channel viewing mode.

- Remove the standards from the original channel.

Applying Values To Standard Lanes

To perform **Molecular Size Calibration**, you must first assign the known values to the bands of the standard lane.

1 In the dialog, select the mappings list from the list that represents the values of your standard.



Note:
You can define new Standards or amend existing ones. Refer to Editing Standard Mappings Lists for more information.

2 After you select your standard, the list of its mapping values appears in the list box below the selector. These values can be selected or de-selected by clicking the check boxes at the side of each value.

3 Select the lane that you want to designate as a standard by clicking on it in the Image window. This lane should have all its bands detected by this stage. The gel image should now show the selected standard with its mapping values attached to the bands.

The standard values can be re-positioned on the bands by **holding down** the Left mouse button and **selecting** the band where the **value has been assigned** and dragging it to the required position.

Standard values can be individually added or deleted from the gel image by selecting or deselecting them in the mapping values list. To remove all the assignments from a lane, right click on the lane.

Choosing parameters and performing calibration

Before you can perform the molecular size calculation, you must set the molecular weight parameters in the dialog.

1 Select the Curve type from the list in the dialog.

The curve you select will be used to determine the values between the mapped bands on the standard lanes and between the points of intersection of molecular size contours and the other lanes.

2 Now, select whether to use Rf to propagate. If this option is selected, the molecular weight contours will conform to relative lane position, not absolute.

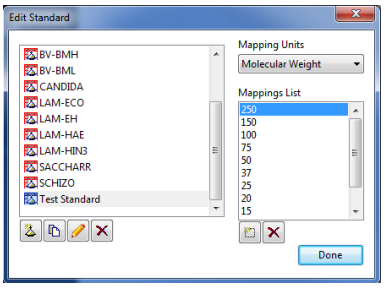
3 To calculate the contours and curves, after you select the Standard, Curve type and Propagation Method, click the **Compute Sizes** Button in the dialog.

The software updates the Analysis window and displays a graph in the Graph window.

To clear the calibration from the gel and all assignments i.e. to restart assigning the mappings, click the **Clear** button.

Editing Standard Mappings Lists

The software provides you with a set of standard mapping lists for common standards. You can use these with the gels or define your own. To enter the editing mode, click the **Edit** button on the dialog. The standards are edited from the **Edit Standard** dialog box that appears.



Adding new standards

1 To create a new standard mappings list, click the **Edit / create Standards** button .

The software creates a new standard with a default name and automatically places you in the renaming mode for that standard.

2 Rename the new standard by typing the new name while the original text is highlighted.

Changing the name of an existing Standard

1 Select the standard you want to rename from the list and then click the **Rename** button below the list.
Alternatively, right click on the selected standard and choose the **Rename** command from its context menu

2 With the original name highlighted, type the new name of the standard mapping list.

To cancel this action either, press ESC to cancel editing without making changes, press ENTER or click outside the entry box to accept the current changes.

3 You can duplicate the currently selected standard by clicking the **Duplicate** button . This creates a copy of the standard and places you in the renaming mode allowing you to type a new standard name.

Note:
You cannot edit the pre-defined standards, but you can duplicate them and then edit the copies to suit your specific requirements.

4 You can delete a standard by selecting it and clicking the **Delete** button .

Editing Standards

You can edit standards that appear in blue  , although you cannot edit standards that appear in red  .

1. If required, change the Mapping Units from the list.
2. Next, add mappings to the mappings list by clicking the **Add new mapping** button  or double click on the last item in the mappings list showing '**…**' and then start editing.
3. Type the value in the text box and press **ENTER** or click the **Add new mapping** button  again.
   The software adds a value to the list, and displays the value in descending order.
4. Repeat the above step to add each value.

   If you want to remove a value, select the value by clicking it in the list and then click the **Delete** button  under the mappings list.
5. After you have finished editing the standards, click the **Done** button on the Edit Standards dialog box.

Gaussian Fitting

Gaussian Fitting is the process of separating a lane profile into a series of individual curves, each representing a single band. The type of curve used to represent each band is known as a Gaussian (Normal) curve. Deconvolution is particularly useful in cases where two or more bands overlap. In such cases, determining the volumes of the individual bands can be difficult.

The **Gaussian Fitting** mode uses a complex curve-fitting algorithm to achieve the best fit possible. However, some profiles will be less suitable for deconvolution than others, particularly those composed of narrow and spiky peaks, which Gaussian curves represent poorly. In addition, if there is a lot of noise within a profile, i.e. small fluctuations that partially obscure the true shape of the profile, then the results of deconvolution may be less accurate.

This option will only become available, once bands have been detected using the **Band Detection** mode. Any background should also be removed before performing deconvolution.

The algorithm relies on an accurate determination of the peak position of each band. It is important to ensure that this has been carried out, as the positioning of detected peak lines within regions that obviously do not contain actual peaks, will degrade the accuracy of the results.

Before applying deconvolution to one or more profiles, you must decide which of the two available algorithms to use – **basic** or **advanced** fitting.

Advanced fitting generally gives accurate results, providing the actual peaks are clearly defined and the profile background has been removed.

Deselecting this option will set the **Basic** fitting algorithm. This is much faster than the Advanced Fitting algorithm, but will usually generate less accurate results. The resulting curves are forced to fit exactly where the bands were originally identified, whereas the Advanced Fitting algorithm can generate curve at any position within the profile. However, it will attempt to fit them at the specified band positions initially. Therefore, the Advanced option has a limited capacity to deal with inaccurately placed band positions.

If the lanes have a large number of detected bands, the Basic fitting algorithm may be preferable, as the Advanced fitting may take an unacceptable amount of time. If the results from the Basic fitting algorithm are unsatisfactory, it is still possible to apply the Advanced Fitting option.

Multiplex

Fitting Gaussians automatically is done on all channels but each channel is treated separately. Editing can only be done in single channel mode just like band editing.

Performing Gaussian Fitting

1. Choose which fitting algorithm you wish to use, by either selecting or deselecting the Advanced Fitting checkbox. The default is deselected.

   Be aware that processing time can be very slow, if there are a large number of peaks present.

2. If you want to apply the deconvolution algorithm to all the lanes in the gel, select the **Apply to all lanes** checkbox. The default is **deselected**.

3. Click the **Fit Gaussians to Peaks** button to perform the deconvolution.

If deconvolution is only being applied to one lane, then during the fitting the pointer will change to an hourglass to indicate that the program is busy. The time taken to finish is proportional to the number of detected bands.

Where deconvolution is being applied to **all** lanes, then a progress bar will be displayed. It is possible to cancel the deconvolution if desired and all lanes that have been processed up until that point will retain the deconvolution information.

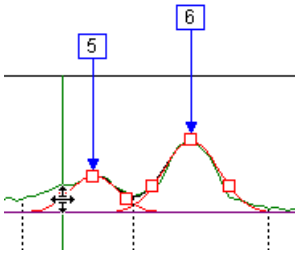
Once the algorithm has finished, a series of red curves will be displayed in the Profile Window, one for each band.

The sum of these curves will approximate the profiles curve. This is shown as a continuous black curve.

The volumes of the Gaussian curves are shown in the Measurement Tables. They are displayed in green once deconvolution has been applied. All other volume-related measurements, such as Lane %, will now use the Gaussian volume and will be displayed in green.

Editing the Results of Deconvolution

It is possible to edit manually the individual curves generated by the deconvolution process. Each curve has three square handles, one at the peak and one either side of the curve. If the pointer is placed over any of these, it changes to a resizing pointer.



- Left click and drag will move and/or resize the curve.
- Dragging the peak handle vertically will change the height of the curve.
- Dragging horizontally will move the whole curve, but will retain the curve shape and size.
- Dragging a side handle horizontally will change the width of the curve. The peak position will remain unchanged and the curve width will change symmetrically, either side of it.

Releasing the left mouse button will end the editing of the current curve and the Gaussian volume in the Measurement tables will be updated accordingly.

Resetting Peaks to Gaussian

You may wish to set the identified peak positions to the peaks of the Gaussian curves.

To do this, click the **Reset Peaks to Gaussian** button in the dialog.

Delete Gaussians

In order to delete the results of deconvolution, click the **Delete Gaussians** button in the dialog.

Quantity Calibration

The purpose of quantity calibration is to relate the band volume in terms of image intensity to real world values. Entering the real-world volumes for bands with known values does this.

There are three different methods of Quantity Calibration, selectable from the dialog. Only one can be used at any one time

When you activate each Quantity Calibration method, new controls appear in the dialog.

The results of band calibrated volume assignment can be viewed in the tables of the Measurements window.

Multiplex

To perform Quantity Calibration on multiplex gels ensure that you are using the *Single channel* viewing mode; the calibration values and colours will not display in the *Channel Overlay* mode.

You must set Quantity Calibration standards for each channel; these settings work independently of the other channels in a multi channel experiment.

Because calibration is performed on a per channel basis you must select the individual channels and then click the **Calibrate** button in the dialog. This rule also applies when you want to *clear* the quantity calibration standards from the channels by clicking the **Clear** button.

Note:
Ensure that you have the Calib Volume and Band Percentage (Calib) table fields selected in the Measurements Window Field dialog box. The tables will then show the Quantity calibrated volume and the Band%(Calib) values, respectively.

Use Calibration Curve

To use this option, select the **Use Calibration Curve** option in the dialog.

By entering a number of known band values the software computes a calibration curve of real-world volume against raw volume. The real-world volumes of the bands with unknown values can then be derived from this curve.

All the bands included in quantity calibration are shown in the Image window with yellow band diamonds; those that are not included have blue diamonds. Yellow and blue are the default colours. The default colours can be changed in the **Image Window Options** dialog box.

You can also view the shape of the calibration curve in the Graph window.

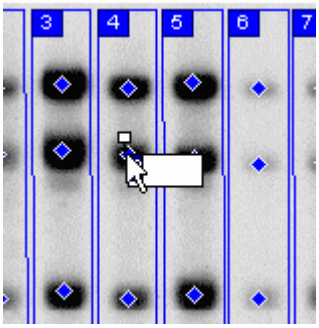
Assigning known volumes

Band volumes are added and edited from the Image window.

The table in the dialog displays a list of the bands you have selected to create the calibration curve together with their assigned calibrated volumes.

1. Ensure that you have the desired table fields selected in order to display your Quantity Calibration results in the Measurements window.
2. Select the required data fields from the Measurement Window Fields dialog box, by selecting the check boxes on the left side.
3. To select a band in the Image window click on the small diamond on the band.

   The pointer changes to when you are over the band. An *in place* edit box appears.



4 Type the band volume in this box and press **Enter** to accept it.

If you have selected the band by mistake, press the Esc key to stop editing. Click OK to the message displayed.

5 The band in the Image window displays the value you have entered. The software also adds the band with the volume, to the table in the dialog.

6 Repeat the steps above to select more bands. Every time a band is added the software recalculates the curve and updates the Graph window.

7 To change a value of a band, click the diamond; the *in place editing* mode highlights the text and allows you to enter a new volume.

8 Press **Enter** to accept or **Esc** to reject the new volume.

Any changes you make are reflected in the tables of the Measurements window, the Quantity Cal curve in the Graph window, and to the table in the parameter tab.

9 Click the **Calibrate** button in the dialog to calibrate all the bands with unknown real-world volume. The software updates the data displayed in the tables of the Measurements window.

Removing bands from the calibration curve

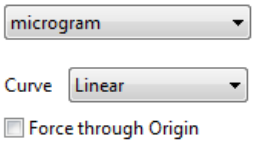
1 Position the pointer over a band you want to remove from the calibration curve.

The pointer changes to when you are over the band.

2 Right click to remove the band. The software updates the quantity calibration curve in the graph window and the measurements tables.

Selecting the curve type and calibration unit

You can select the curve type and calibration unit in the dialog.



1 Click the curve type from the Curve Type list.

2 If you want the curve to pass through the origin, click the **Force through Origin** check box.

3 Click the units for the calibrated values from the Calibration Unit list.

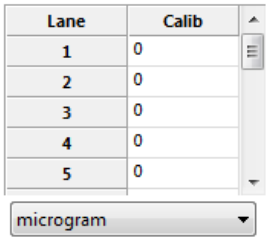
You can change the settings of the curve at any time and the software recalculates the calibration values.

Quantify Individual Lanes

To use this option, select the **Quantify Individual Lanes** option in the dialog.

This tool quantifies bands by assigning a known value to each lane. The bands are then quantified, based upon their relative volume in the respective lane.

The dialog will display a small table.



1. Enter a quantity for each lane into the second column headed **Calib**.
2. Place the pointer over a cell and type in the value.
3. Continue with the above step, until you have a value for all the lanes.
4. Click the units for the calibrated values from the Calibration Unit list.
5. Click the **Calibrate** button to calibrate all the bands with unknown real-world volume. The software updates the data displayed in the tables of the Measurements window.

The table works in a similar way to Excel, allowing you to Copy and Paste not only individual cells into other cells in the table, but also the whole table via the clipboard

Clear all calibrated values

Click the **Clear** button in the dialog to clear the calibration from any of the methods. You can now select a different method if you wish.

Band Normalisation

Normalisation can be used to normalise band volumes by setting the volume of a band or a group of bands to a specific value and then recalculate all the other volumes relative to that value. Normalisation can be useful when comparing the volumes across lanes or gels where the loading might be different.

All the bands used for normalisation are shown in the Image window with yellow band diamonds, those bands that are not included have blue diamonds.

These are the default colours. The default colours can be changed in the Image window Options dialog box.

Choosing Bands and Normalising

Normalisation requires you to select a band or bands in the gel. You will also choose a normalisation value and unit, which is applied to the bands before recalculation, according to the method chosen.

1. Ensure that you have the desired table fields selected to display your results in the Measurements window.
2. Select the required data fields from the Measurements window Field dialog box by clicking the check boxes on the left side.

3 To select a band in the Image window click the small diamond on the band.

Note:

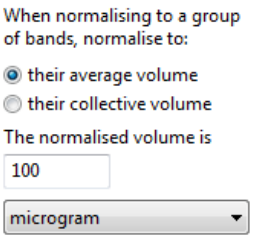
The pointer changes to  *when you are over a selected band.*

4 Repeat the above step as required.

5 Type the Normalised Volume figure in the edit box. The allowed range is 0.0001 to 1000000.

6 Select the unit of measurement from the list.

7 If normalising a group of bands, select either the *group average* or *collective* option by clicking the button in the Parameter tab.



8 Click the **Calibrate** button to calibrate all the bands with unknown real-world volume. The software updates the data displayed in the tables of the Measurements window.

When you click the **Calibrate** button after selecting your bands, the band and lane number will be displayed in the dialog, depending upon how many bands are chosen.

Deselecting bands

To **deselect** a band position the pointer over the band. When the pointer changes to  right click to deselect the band. The software updates the measurements tables.

Deleting values

To delete all the values click the **Clear** button.

Normalisation

The **Normalisation** mode is used to generate the value of normalised volumes for the bands in a gel. This entails:

- Setting the normalised volume for specific band in a lane if a standard gel.
- Selecting a channel in a multiplex gel which contains housekeeping proteins/genes
- Selecting a channel in a multiplex gel which uses total protein in each lane to normalise to.

It then recalculates all normalised volumes for the other bands in the gel using the normalisation factor calculated.

This can be useful when comparing the volumes across lanes and/or gels, where the loading may be different.

When you activate each **Normalisation** method, new controls appear in the dialog.

The results of normalised volume assignment can be viewed in the tables of the Measurements window.

Performing Normalisation for a Standard Gel

To use this option follow these steps.

1 Select a method from the method list

Single Band

All other normalised volumes in the gel are set relative to the selected band.

Matched Bands

This option works by setting the selected band and all other bands to which it is matched to the normalising value you enter.

If a lane has a band matched to the selected band, all the other bands in that lane will have their normalised volumes set, relative to that band. If a lane does not contain a matched band, all volumes in that lane are set to zero.

All Bands in Lane

This option works by setting all volumes in the lane that contains the selected band to the normalising value you enter.

Bands in the other lanes, which are matched to the same reference lane band as the bands in the selected lane, are then set relatively.

If a band in the gel is not matched to the same reference band as one from the selected lane, then the band normalised volume is set to zero.

2 Select a band in the Image window.(or Lane for the All Bands in Lane option) and the selected bands get set to a value of 100 and all other bands calculated based on this value.

This calculation will work on Quantity Calibration values, if they have been calculated for the gel.

Performing Normalisation for a Multiplex Gel

Two normalisation methods can be chosen:

Housekeeping Proteins/Genes

This option is used if you have one channel of your gel assigned with housekeeping proteins or genes.

The idea is that there will be one band per lane in this channel representing the protein/gene and that these should have exactly the same volume. As they will not have exactly the same raw volume a normalisation factor is calculated for each lane. Here is the equation.

1. First find the biggest band in each lane if there is more than one (which there should not be after band editing)
2. Find the biggest band in the whole gel for this channel. This becomes the reference band
3. For each lane calculate the normalisation factor

   NF = volume of band in lane/volume of reference band
4. For each lane calculate the normalised volume of the bands in both channels

   Normalised volume = Raw volume of band/normalisation factor

Total Protein Normalisation

This option is used if you have one channel of your gel assigned with lanes which have a defined total protein.

The idea is that the volume of all the material in each lane(after background subtraction) in this channel should be identical. As they will not have exactly the same raw volume a normalisation factor is calculated for each lane. Here is the equation.

1. Select a lane in the gel to be a reference lane. All other lanes will be normalised relatively to this one.
2. For each lane calculate the normalisation factor

   NF = volume all the material in lane/volume all the material in reference lane
3. For each lane calculate the normalised volume of the bands in both channels

   Normalised volume = Raw volume of band/normalisation factor

Rf Calibration

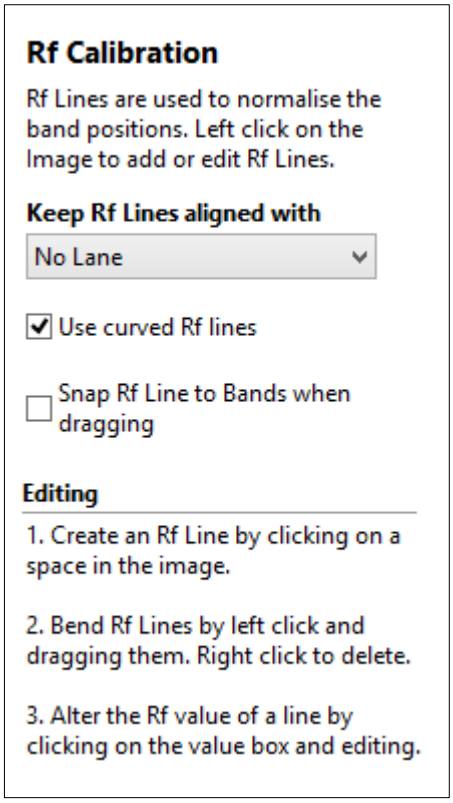
Rf, or **Retardation Factor** is a measurement of position along the lane, relative to the lane length. By default, the first position in each lane has an Rf of 0 and the last has an Rf of 1. There is a linear increase in Rf from start to finish.

If your lanes are uneven, or have spread out as they have gone further down the gel, you may find that the default Rf values are incorrect. The **CLIQS** application allows you to correct the Rf values for your lanes, by adding and moving horizontal lines, which represent points of equal Rf across your gel. The actual Rf value each line represents can be derived from a selected lane, or entered manually.

The relationship of position along the lane to Rf is then calculated for each lane by taking the points where the Rf lines intersect the lanes, and using linear interpolation and extrapolation to find all the Rf values between and outside those points.

Correct Rf calibration can be very useful when assigning molecular weights, or performing band matching.

Enter the mode by selecting the **Rf Calibration** button on the main toolbar.



There are two basic steps to performing Rf:

- Setting the options on the dialog box
- Adding and editing Rf lines

To see the results of your **Rf Calibration** on the bands, examine the tables in the Measurements Table Window and turn on the Rf field. Alternatively, you can use the Analysis Window display options and turn on the **Rf annotations** for the bands and **Rf scale** for the lane. See the topics on the Measurements Table Window and the Analysis Window for more information.

Setting the Rf Options

The **Rf** dialog in the Navigator has four controls:

- Tier selection - this is not displayed, if there is a single tier
- Lane selection to align with
- Use curved Rf lines
- Snap Rf Line to Bands when dragging

You can select your preferences for these settings at any stage in the **Rf** mode, and any existing Rf lines will be updated.

Aligning Rf with a Selected Lane

This is a dropdown list containing lanes. It allows you to determine that the value of Rf for a particular Rf line is derived from the point at which it crosses a given lane.

When an Rf line is added, it is given a default value, depending on the line position, in relation to the other Rf lines. If this alignment option is on, then the value of the Rf line will instead refer to the point at which it crosses the chosen lane. If you bend or move the Rf line, then the value will change according to the new intersection of the line with the given lane - unless you override the value by entering one of your own.

Select the lane to align with or **No Lane** if you want the Rf lines to keep their original values when moved.

If there is more than one tier, you will need to select a tier and a lane per tier, from the dropdown lists in the Navigator.

Use Curved Rf Lines

By default, this option is selected and causes the Rf lines to bend between the tie points, using a cubic spline curve. However, in some gels where you wish to bend the Rf lines drastically, this may not be appropriate and so you should turn this option off to use straight lines to join up the tie points.

Snap Rf Line to Bands When Dragging

When you are bending Rf lines on your image, using handles on those lines, this option can make it easier to move a handle to the exact position of one of the bands on your gel. The option makes the handle "stick" to the location of the band when it is moved, within a few pixels of the position of that band.

Adding and Editing Rf Lines

Rf lines are edited from within the Image window. You must set the alignment and editing options in the Rf dialog in the Navigator.

You can use the pointer to add, bend and start in-place editing for the Rf values of your Rf lines. While editing Rf, you will probably find it most useful to be viewing the whole of your gel and so you are advised to make the Image window zoom the gel image to fit the window. This is done by clicking the **Fit to window** button on the Image window toolbar.

Adding Rf Lines

1 Move the pointer to the place on your gel, where you want to add the line. The pointer should change to  indicating that it is possible to add a new line.

2 Left click and the line will appear across your gel.

At the left hand side of the line, a value box will be shown. This shows the Rf value of that lane. If you have chosen to align with a particular lane, the Rf value will have been calculated from that lane.

At this stage, you have not yet adjusted the Rf values in any of the lanes. To do this you must move the lane, thereby re-mapping the contours of Rf in the gel.

Editing Rf Lines

1 To alter the angle of a line, move the pointer to either end of that line. Since it has not been edited, it does not have any handles. Therefore, you will see the **can-add-handle** pointer  displayed.

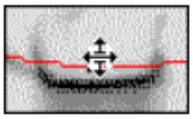
2 Left click and drag the pointer up or down to adjust the angle of this line. As you do so, a white adjustment handle will appear at each end of the line. These handles can be used to adjust the line further.

3 If you have alignment **on**, then you will see that the value of the Rf line changes, when you adjust the angle.

4 If the contour of Rf in your gel is not a straight line, then you can add adjustment handles to the mid-points of your Rf line to make it bend to match the requirements of the gel.

5 Move the pointer over the line you wish to bend. The pointer will change to  indicating that you can add a handle.

6 Left click on the line. An adjustment handle will appear where you clicked. Drag and drop this handle to bend the line.



If you have lane alignment **on**, then the value of the Rf line may change, according to how the bent line intersects the chosen lane. If you have turned **off** the **Curved Line** option, then the line will be formed from straight lines joining the handles.

7 If you have added too many handles to an Rf line, you can right click on the handles that you do not want to delete them.

Editing Rf Line Values

1 If you wish the Rf line to have a specific value, then you can enter the value manually and the Rf line will keep the value, no matter where it is moved.

2 To start editing, move the pointer over the Rf value box of the line.

The pointer will change to  , indicating that you can start editing by clicking on the value box.

3 Click on the value box and an entry box will appear. Type in your desired value. Click the **Esc** key on the keyboard to stop editing and cancel, or click **ENTER** to accept the value that you have entered.

You can also click on the value box of a different Rf line to stop editing the current one, accept the value and start editing the next.

Deleting Rf Lines

1 If you have added too many Rf lines, you can right click on a line you wish to delete. This will remove it from the gel.

2 If you want to set Rf lines back to their default positions at the top and bottom of the gel, then just delete the Rf lines you have added. When only two remain and you delete them, they revert to their default positions.

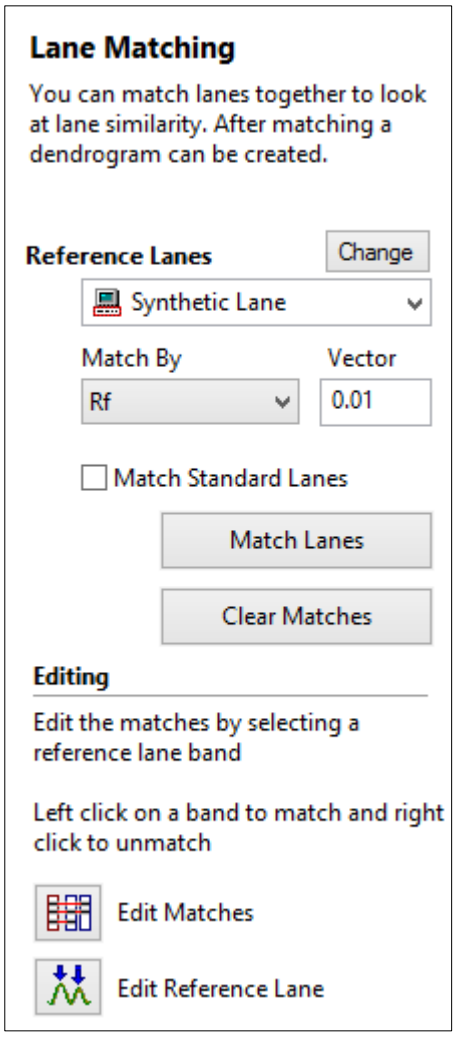
Single Gel Lane Matching

Band matching involves identifying bands in your lanes, which are also found in a common reference lane. The first step in band matching therefore is to choose an appropriate reference lane. Reference lanes should contain all the bands in which you are interested.

You can use more than one reference lane to match to the lanes in your gel and when you do this, the **CLIQS** application will automatically decide which lanes to associate with which reference lanes.

CLIQS can automatically provide you with a synthetic reference lane containing all the bands within your gel, if you do not have a particular sample lane you wish to use. Alternatively, you can select your own reference lanes either from the current gel or from the libraries of reference lanes, which you can create and build up from all your gels.

Enter the mode by selecting the **Single Gel Lane Matching** button on the main toolbar.



To perform all of the functions within Matching, you will need to know how to:

- Add and select reference lanes
- Editing reference lanes
- Set parameters
- Perform automatic matching
- Perform manual matching

The results of your matching can be seen graphically, in the Image and Profile Windows and is represented numerically in the Comparisons, Matrix and Similarity tables in the Measurements Table Window.

In the Image window, matched bands have **red** diamonds over them. Lines are drawn between bands matched to the same reference lane band. If a reference lane band is selected, then the diamond for that band, all the diamonds for the bands matched to it, and the line joining them are drawn in **yellow**.

When you are in **Matching** mode, or displaying the matching overlay for the Image window, a panel appears in the left of this window, showing the current reference lane.

In the Profile Window, **purple** dotted lines are drawn between the bands on the current reference lane and their matched bands in the current lane. If you are in Normal mode and have the comparisons lanes turned on, then dotted lines are drawn between bands in the comparisons lanes, which are matched to the same reference lane band.

Managing Reference Lanes

Reference lanes are used in matching operations with your lanes. In order to use a reference lane with a particular gel, that lane must be added to the list of local reference lanes for that gel. Lanes can be added to the local reference lanes from the normal lanes of the gel.

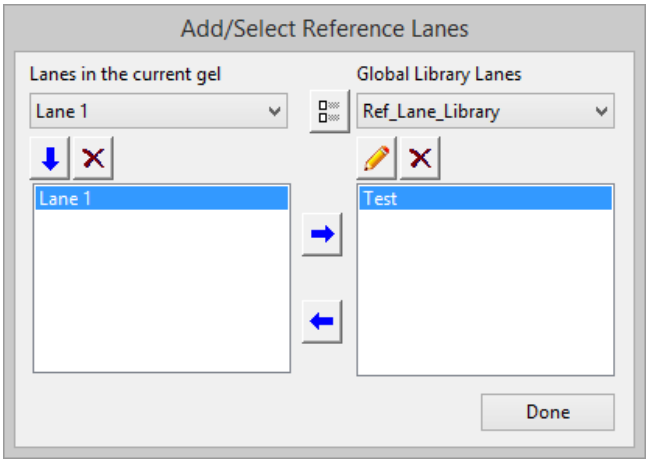
It is also possible to copy reference lanes from the local reference lanes list, into centrally stored libraries of reference lanes, called **global reference lanes libraries**. Lanes can be copied between these libraries and the current gel. By adding the most useful reference lanes to these libraries, you can quickly build up a stock of reference lanes to copy into your gels for analysis.

You can add and remove libraries from the list of global reference lane libraries.

Adding and Selecting Reference Lanes

In order to add or select reference lanes to use with matching, you must use the **Add/Select Reference Lanes** dialog box. This is accessed via the **Change** button on the **Matching** dialog.

Click this button to display the **Add/Select Reference Lanes** dialog box.



This dialog box has three columns of controls.

Controls in the left hand column relate to the current gel and allow you to see which references lanes are currently attached to the gel. You can add to this list from the lanes on the gel. If you select no other lane, then this list defaults to using the synthetic lane. You cannot use normal lanes and synthetic lanes simultaneously.

A synthetic reference lane is generated when you perform automatic matching. The resulting lane will have bands that match to every band on the gel and is generated to fit the matching parameters.

Controls in the right hand column relate to the global libraries of lanes. You can select a library from the dropdown list and see the library lanes underneath.

In the central column, there are buttons for copying the selected global reference lanes from the right to the list on the left and vice versa.

When you copy a lane from your gel into a reference lane list, all the data for that lane is copied. If you were to re-analyse that lane later on, perhaps finding more bands or finding the peaks in a slightly different position, the reference lane data would be unaffected. This could cause an effect where a lane appears not to match, with a reference lane that looks identical to it.

Selecting Reference Lanes

Generally, you will use one of three methods to choose your lanes.

- **See how well the lanes match to a single sample**.
  For this, you will select a single reference lane, either from your gel or perhaps from one of the global reference lanes libraries that you have defined. You will perform matching and find out how well each lane matches to the sample.
- **See what type of sample each lane resembles**.
  For this, you will select a few reference lanes, either from your gel or from one of the global reference lanes libraries. You will perform matching and the software will determine which of the reference lanes best matches each lane.
- **See how all the lanes match to each other**.
  This requires a reference lane that contains most of the different sorts of bands present between the lanes - a synthetic lane is ideal for this purpose, since it contains a band for all the

different bands within the gel. Matching is performed between each lane and the reference lane and you can then see how similar all the lanes are to each other. The All Lanes table in the Matching Tables window is very useful for this purpose.

It is possible to edit reference lanes after they have been added to the reference lanes list for your gel. This allows you to add and remove bands in the reference lane for the purposes of matching.

Since the reference lane is stored separately from the lane it was copied from, the editing will not affect the lane on the gel.

Making a Reference Lane from your Gel

In order to add one of the lanes from the gel to the list of reference lanes for that gel:

1. Select the lane you wish to add from the dropdown list on the **Add/Select Reference Lanes** dialog box.
2. Click the **Add selected lane to list** button to add the lane.

The lane will be added to the reference lane list for the gel. Note that it is not possible to add a lane to this list twice.

3. Repeat the above for all the lanes you wish to add to the list. You can remove these lanes and rename them.

You can perform this operation at any time, using the Experiment Explorer and drag and drop a lane into the reference lanes node.

If your gel is already matched to a synthetic reference lane and you add a lane into the list, the synthetic lane will be deleted and you will lose your matching information.

Renaming a Reference Lane

1. Select the reference lane you wish to rename from the list of reference lanes for your gel.
2. Click on the reference lane when it is selected to start in-place editing. This can also be done by choosing **Rename** from the context menu, which you can display by right clicking on the reference lane in the list.
3. Click the **Esc** key to stop editing the name without accepting your changes. Alternatively, click **ENTER** or click outside the entry box to accept the changes and stop editing. This can also be done within the Experiment Explorer in the Navigator.

Removing a Reference Lane from your Gel

1. Select the reference lane you wish to remove from the list of reference lanes for your gel.
2. Click the **Delete** button.

3. A message will be displayed asking you to confirm this selection - click the **Yes** button to delete or **No** to continue, without making changes.

When you delete the reference lane from your gel, any matching information relating to that lane is lost even if you add it back in immediately.

Copying Reference Lanes between the Global Libraries and your Gel

1. Select the global reference lane library from the dropdown list at the top right of the **Add/Select Reference libraries** list. The lanes within that library will be displayed.

   If you have not defined any libraries, you can do so using the Experiment Explorer. Right click on the Global Reference Lanes node and choose the **Create Reference Library** command.

2. If you wish to copy a lane from this library to the current gel, select the lane you wish to copy and click the …**Global to Local…** button.

   The lane will appear in the list on the left of the dialog box.

3. Alternatively, you can copy any lane from the gel reference lane list to the library - select the required lane and click the …**Local to Global…** button.

   The lane will appear in the global library, with a name determined by the gel name and the lane name within the gel.

No two reference lanes in a library can have the same name and so the name may have a number added to the end to make it unique.

You can copy a synthetic reference lane to the global libraries. However, you must have performed matching, in order to generate the lane before copying.

Editing Reference Lanes

You may wish to add or remove bands from your reference lanes. This is done via the **Edit Reference Lane** tool in the **Matching** mode. This tool is accessed via the button on the dialog that swaps into editing the reference lane not the matching.

You may add or remove bands from the reference lane in the Image window and the Profile Window.

In order to edit the bands of a synthetic reference lane, you must first perform matching to generate the lane. Adding bands to the synthetic lane will have the effect of changing the calculated values of Band Sharing Index for all the matched lanes. Removing bands will have the effect of removing the set of bands matched to them, from the matching process.

In the Image Window

1. Move the pointer over the image in the reference lane pane to the left of the Image window.

2. To add a band, move the pointer to a gap in the reference lane. The pointer will change to  where a reference band can be added.

   Left click to add a band at this point.

3. To remove a band, move the pointer over the diamond for that band.

   The pointer will change to a  shape to indicate that you are over the band. A tool tip will also appear below the pointer, displaying the name and number of the band. Right click to remove this band.

In the Profile Window

You may add and remove bands for the reference lane in this window. This works in exactly the same way as manual band editing. See the section on Band Detection for information on how to edit the bands of the lane manually. Editing the edges of the bands will affect the correlation coefficient, between the reference lane and the lanes to which it is matched.

While you are in reference lane editing mode, the Profile Window shows you the peaks for the reference lane rather than the current lane, as it does the rest of the time.

Automatic Matching

In order to perform automatic matching, you must set the required parameters in the **Matching** dialog and the associated Options dialog in the Navigator. Next, click the **Match Lane** button.

Match Standards

It is unlikely that you would wish to include your molecular weight or pI standard lanes in your matching but if you want to them click this option on

Match By / Vector

Select your required matching method from the dropdown list and enter the required vector in the entry box.

The following options are available:

Position

This uses band position along the lane in pixels. For a band in a lane to match a band in the reference lane, it must be distanced within the number of pixels specified for vector.

Rf

This uses the Rf values of the bands to determine if they are near enough to declare a match. The Rf of a band must be within that limit to be declared a match.

Molecular Size

This uses the logarithm of the molecular size of the bands to determine if they are near enough to declare a match. Using the logarithm ensures that the response to separation along the length of the lane is linear, since molecular weight tends to curve along the lane. If your molecular size calibration is in pI units, then the vector is linear.

If you have used multiple reference lanes, the lanes in the gel, which are best matched to the current reference lane, are highlighted in red. Change the selection of the current reference lane by clicking on the list of reference lanes in the **Matching** dialog. As you change the selection, the highlighting changes to show the lanes matched to the new selection.

Performing Manual Matching

Manual matching can be performed from within either the Image window or the Profile Window. It requires that the **Edit Matches** tool be selected in the dialog.

You can manually edit the matching of a synthetic reference lane, but you will have to perform automatic matching first to cause the synthetic lane to be generated.

Manual Matching in the Image Window

1. You must first select the band on the reference lane, against which you match bands. Moving the pointer over the desired band, will cause the pointer to change to  and a tool tip will appear, giving the band identity. Left click on the band to select it.
2. When the reference lane band is selected, the band diamond is drawn in yellow and so are the diamonds for all bands matched to this reference band. Yellow lines are drawn between bands in the Image window matched to the same reference lane band.
3. To match a band to this reference lane band, you must click on it.

   The pointer will change to  when you are over a band that is unmatched and can be matched, in this way.

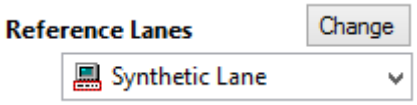
   Only one band in each lane can be matched to a given reference lane band. If you choose to match a second band to the same reference band, the first match will be deleted.
4. To clear the matching of a particular band, right click when the pointer is over that band. The diamond for that band will then turn blue.

   If you are using multiple reference lanes, you can only add matches to lanes that are matched to the currently selected reference lane.
5. Repeat the above steps for all bands to be matched to each reference lane.

Manual Matching in the Profile Window

If you are using multiple reference lanes and the current lane is matched to a different reference lane, an information message will be displayed advising of this. You will be unable to edit matches for the current lane.

1. Select the reference lane you wish to use from the **Matching** dialog in the Navigator.

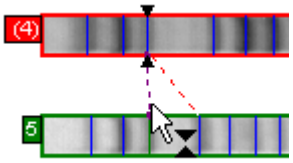


2. Select the current lane you wish to use, either from the Experiment Explorer, or by using the vertical tool bar or keyboard.
3. The reference lane and current lane will be shown, one above the other in the Profile Window. You will see purple dotted lines joining up any matched bands, between the images of the lanes in the lower pane of this window.
4. To add a match, move the pointer over the vertical line representing a band in the image, of either the reference lane or current lane in this window.

   The pointer will change to  to indicate that you can perform a matching operation.

5 To match the band, left click and drag the pointer so that it is over the required band in the other lane.

As you move the pointer, a dotted line between the lane images will show the direction of the matching and a vertical line with arrows either end will show which band would be matched, when you released the mouse button, as in the example below.



6 Release the mouse button, when you are over the required band.

To Delete a Match

Right click over one of the two bands that are matched to each other.

Annotations

Using the Annotations mode, you can add text labels with optional arrows to precisely locate and identify certain areas of an image.

You activate the Annotations mode by clicking the **Annotate** button on the *main toolbar*, or

Create annotations on an image to highlight particular areas of interest. There is no limit to how many annotations you can create in any one image or channel.

Multiplex

Annotations can be created and displayed in multiplex gels regardless of the number of channels or the current view mode. However, it should be noted that you can only create one set of annotations per multiplex gel.

Manual Annotations

To change the annotation style just look through the dialog which contains a variety of options. For example, to change the font click the **Select Font Style** button.

After changing the annotation style, the changes you make are applied to all annotations that are presently selected and any newly created annotations.

Adding and Editing Annotations in the Image window

The following section describes how to use the mouse and the keyboard to Add, Move, Select and Edit the text of annotations in the Image window.

Multiplex

Annotations can be viewed and edited in both single and overlay modes.

We assume that you are using the default settings for annotations when doing the following actions.

Adding an annotation

To add an annotation to the image shown in the image window:

1. Position the pointer over the location in the Image window that you want to annotate. The pointer should be the standard arrow, indicating that you are not over any other items in the window.
2. Left click to add an annotation box. An anchor arrow appears with an attached text entry box, pointing to the location.
3. Type your text into the text entry box.
4. Press **ENTER** to accept the text or press **Esc** to stop editing without accepting any text. You will be able to enter text later.

You have now added an annotation that is indicated as the currently selected annotation by an outline box. You can add more annotations by repeating the above steps.

As an alternative to clicking the image to add a comment, you can position both the arrowhead and the text box of your annotation with a single mouse interaction.

5. Position the pointer at the location where you want the arrowhead, left click and hold down.
6. Move the pointer to the position where you want the text to be centred. As you drag the pointer, the annotation text entry box appears and stretches away from the arrowhead.

7 When you release the button, the text entry box changes to the in-place editing mode. Type in your text and press **Enter**.

Moving annotations

To move an annotation:

1 Position the pointer anywhere on the line between the arrowhead and annotation text box. *You might need to move the text box to see the line.*

2 The pointer changes indicating that you are in position. If this does not happen, you can use the ***zoom in*** function to increase the amount of line available, to pickup the annotation.

3 Drag the annotation to its new location.

Moving part of an annotation

You can also move the position of an annotation anchor arrow or text box.

1 Position the pointer over the anchor arrow or text box you want to move. The pointer changes to, a crosshair for the anchor arrow and the text box becomes outlined.

2 Drag the arrow or text box to the desired location.

Selecting annotations

To select a single annotation you can:

Click on either the arrow, the line between the arrow and the text box, or the text box.

Press the **TAB** key to move between annotations.

Select all

To select **all** the annotations you have created, **right click** anywhere in the image away from any annotation. The context menu appears allowing you to select all the annotations.

Deselect all

To **deselect all** the annotations **right click** anywhere in the image away from any annotation. The context menu appears allowing you to deselect all the selected annotations.

Arbitrary selection

To make an **arbitrary selection** of annotations, **left click** on the first annotation and then **hold down** the **CTRL** key on your keyboard. You can now **click** on each annotation you want to include in your selection. **Right click** in the image for your next action.

To arbitrarily deselect an annotation, **hold down** the **CTRL** key on the keyboard and then **left click** on the annotation.

Editing the text of an annotation

After adding annotations, you can edit the text in the annotation by right clicking the outlined text box and selecting Edit text from the context menu or, left clicking the text box.

While editing annotations, an outline box appears around the selected text box. This outline box does not appear on any image printouts. Annotation boxes are not included on report printouts.

Annotation Fonts

Clicking the Select Font Style button, displays the Font dialog box that allows you to set the font style for your annotations.

Delete selected Annotations

The **Delete Selected** button deletes any currently selected annotation. If you continue to click this button, the annotations will all be deleted in the reverse order that they were added.

Alternatively, position the pointer anywhere over an annotation and right click, then select Delete from the context menu.

There is no **undo** command. If you accidentally delete an annotation, you will need to add it again.

Setting annotation options

The dialog contains the following options.

Text outline style

Select the shape of the border around the text of your annotations from the drop-down list. You can select a rectangle, rounded rectangle or no border.

Fill Colour

Select the colour for the background of the text area of your annotations from the drop-down list. This option is disabled if you have chosen a transparent background.

Line thickness

Select the weight of the lines that is used to draw the anchor arrow of the annotations.

You can choose one of size line thickness or No line.
If you choose No line, your annotation appears as a moveable text box only.

Colour

Select the colour that is used to draw the arrow line and outline of the annotation text box. The default outline colour is green.

Opaque Background

Select the check box if you want the text of the annotation drawn over a coloured background, or clear the check box if you want the text drawn directly onto the image.

Text Orientation

Choose the orientation of the text within the annotation.

Reports

Using the Reports mode you can create PDF reports for the analysis you have just completed.

Analysis Report...

Builds the **Analysis Report** and brings up the PDF report in Acrobat Reader. This report shows the following:

- The picture of the gel as currently shown in the Image window
- A table of lane information including number of bands, band volume and lane volume.
- A Lane Report (see below) of all the lanes in the gel

Quantity Calibration Curve Report...

Builds the **Quantity Calibration** report for the current calibration curve and brings up the PDF report in Acrobat Reader. This report shows:

- The graph of the currently selected quantity calibration curve.
- A table showing each band that has been used to generate the curve. For each of those bands the table shows:
  - the raw volume
  - entered volume
  - volume as derived from the curve
  - difference between 2 and 3 above.

Current Lane Report...

Builds the **Lane Report** and brings up the PDF report in Acrobat Reader. This report shows the following:

- The header information for the gel containing the lane.
- The **Background** and **Band Detection** parameters used to analyse the lane.
- The profile graph for the lane and the image, as displayed in the Profile Window.
- A table, equivalent to the Selected Lane table in the Measurements Table Window, using the fields selected in that window.

Current Lane Molecular Size Report...

Builds the **Molecular Size** report and brings up the PDF report in Acrobat Reader. The report contains the details of **Molecular Size Calibration** for the current lane. This report shows the following:

- The header information for the gel containing the lane.
- The background and band detection parameters used to analyse the lane.
- The values of a series of Additional Lane Data fields.
- The Molecular Weight graph for the lane, as seen in the Graph window.
- A table showing the molecular weights of the bands in the lane.

Report Options

Current Lane Report Notes
To enter lane notes, which display beside the Additional Notes: field on the Lane report, select the required lane from the left hand list and then type the required text in the entry box on the right of the dialog box.

Display Analysis Parameters
Select this checkbox, if you want the Background and Band Detection parameters to appear at the top of the Lane report.

Array

Introducing Array

You use the Array module to analyse microtitre plate images, gridded arrays, and dot and slot blot images.

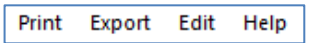
The Array module uses a tool-based approach to analysis. Each tool has a specific task in the analysis process from defining the spots to normalising their measurements and flagging absence and presence.

The analysis process involves a number of different steps that you can perform in any order, repeating or skipping steps when necessary.

The procedures provided in this section are basic examples of how to analyse images. You can fine-tune your analysis using the various editing tools.

The Array module is supplied with example image files. The image used in this section describing the various features can be restored if you want to follow the procedural steps. To restore the image click **Restore Default Image** from the **Help** menu.

Main menu bar



The Main menu bar, located at the top of the main window, provides a number of commands not available through the main toolbar modes.

Print menu

From the Print menu, you can perform the following actions.

Print

There is a print option for each visible window. The **Print** dialog box allows you to select a printer, print range and number of copies for the selected window.

Print Preview

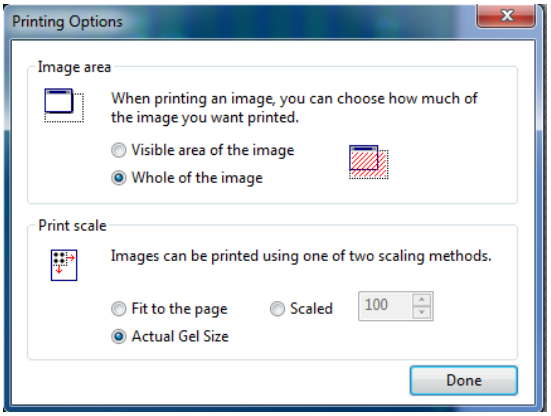
There is a print preview option for each visible window. The **Print Preview** window displays the image or measurements data from the selected window so you can see how it will be printed.

Print Setup

The Print Setup dialog box allows you to select a printer and its properties. You can select a paper size, source and the page orientation.

Printing Options

With these options you can customize the way that the content of windows will be printed, from the commands in this dialog. This includes changing the area of an image that will be printed and setting the scale at which an image will be printed.



Changing the area to be printed from an image

1. Click either Visible area of the image or Whole of the image.
2. Click **Done** to confirm your choice.

You can specify how much of a current image window should be printed and at what scale.

Changing the print range and scale

1. Specify the print range of the window to be printed by clicking either **Visible area of the image** or **Whole of the image**.
2. Select the scale from the **Fit to paper**, **Actual size** or **Scaled** options. If you click Scaled, select or type the percentage in the text box.

Note:
The default is set to 100% of the original size.

3 Click **Done** to confirm your choices.

Export menu

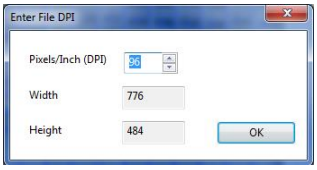
Export to Clipboard

There is an export to clipboard option for each visible window. Click **Export to Clipboard** to copy the data or image displayed in the selected window to the clipboard, so that the data or image can be pasted into other applications. For example the measurements window data can be pasted into a spreadsheet.

Export to File

There is an export to file option for each visible window. Clicking **Export to File** displays a dialog box that allows you to enter a folder and filename.

If you are exporting an image, the default filename is Export.bmp. You are also given the chance to change the DPI of the image saved before naming the file if it is from the Image Window and you are exporting the whole image (see **Export Image Options**)



If you are exporting measurements data the default filename is Export.txt.

The default folder is the CLIQS installation folder.

If you do not want to use the defaults, select the folder in which you want to store the file and type in a name and then press **Save**.

Export to Clip Gallery

This creates a file in exactly the same way as **Export to File** (see above) but instead of saving directly to a folder you select the file is made available via the **Clip Gallery** (see below).

Export Measurements to Excel

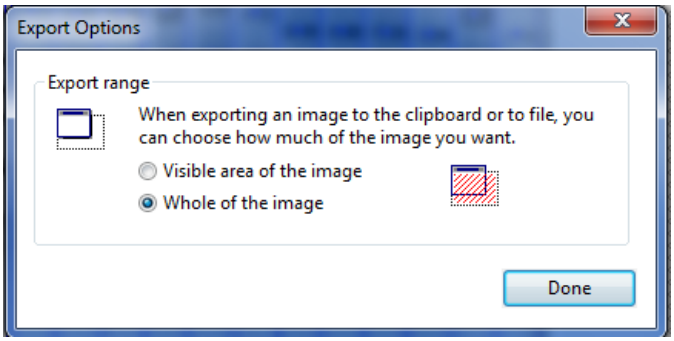
The data in the Measurements window will be copied directly into an Excel spreadsheet.

An Excel workbook automatically opens and the measurement data will be pasted into a new workbook.

If Microsoft Excel is not installed, use the Export to File command.

Image Export Options

With these options you can customize the way that the content of windows will be copied from the commands in this dialog.



Changing the area to be copied from an image

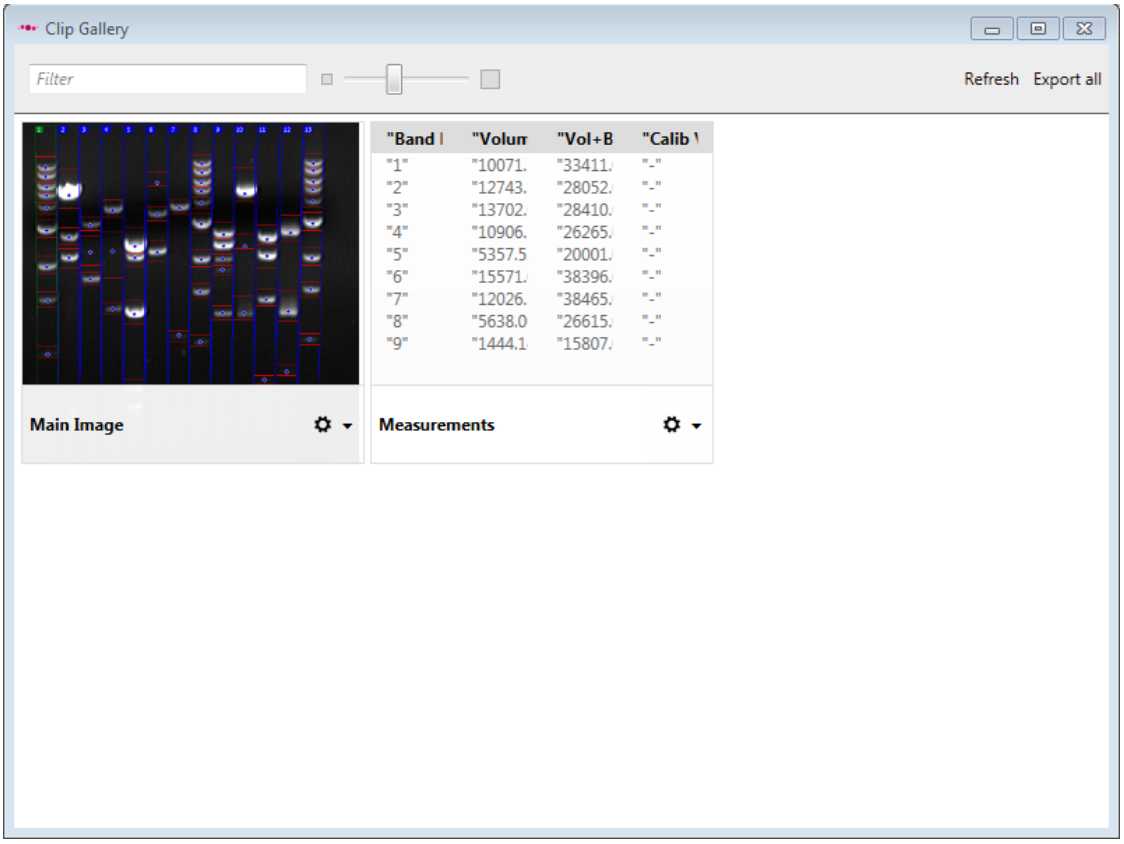
1 Click either Visible area of the image or Whole of the image.

2 Click **Done** to confirm your choice.

You can specify how much of a current image window should be printed and at what scale.

Show Clip Gallery

This option shows the Clip Gallery viewer. Here you can see all the images/data you have sent to the clip gallery for this analysed image.



From here you can Copy or Export the information to any program you wish from the drop down menu on each of the items in the gallery

Edit menu

Copy Grid

This feature allows you to copy a grid from one image and paste it over another image in the same position.

Paste Grid

If the image scales are different, the software resizes the grid. The image that you want to paste the grid over should be of similar size; otherwise, you might see this message - 'Unable to paste the grid from the clipboard because some of the spots are outside of the image'.

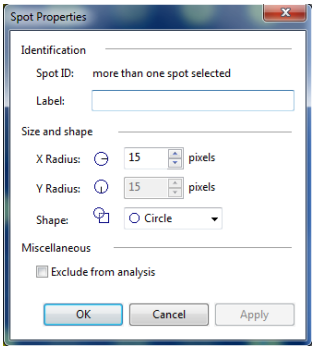
After you paste the grid, you can use the editing tools in the dialog to move or stretch the grid.

Delete Grid

This feature deletes the grid displayed on the current image.

Spot Properties

To display the Spot Properties dialog box right click a spot or a cell in the Measurements window, and then click **Spot Properties** from the context menu.



The various properties of the selected spots can be changed:

Spot label:
Type a label to identify spots in the measurements tables.

X and Y radii:
Select a value in pixels to size the X and Y radius. The Y radius is only available when the spots are all the **Slot** shape.

Shape:
Select a shape from the list. The choices are **Circle**, **Square** or **Slot**.

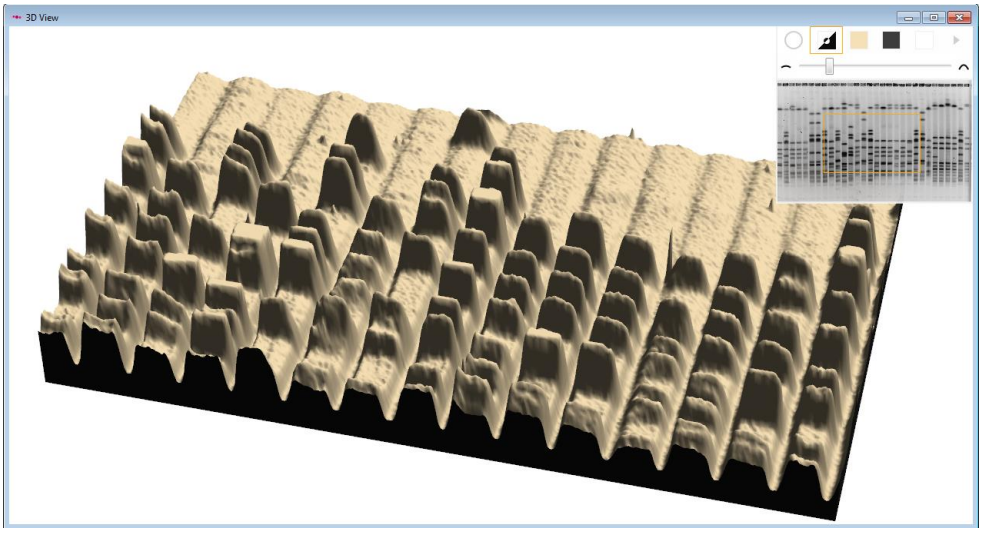
Exclude from analysis:
Select the check box if you do not want the measurements calculated for the selected spots.

For more information, refer to Spot Definition | **Editing spot properties**.

3D View menu

Show 3D View

This option allows you to bring up an independent 3D view of the current image being displayed



Each window is separate so you can call up as many as you wish from the same image or different images.

Contrast

The image is created using the current contrast settings not the raw image values so changing the contrast before opening the 3D View will give slightly different results

Options

In the top right corner are the view options. They include

- Continuous rotation
- Invert image
- Change colours for surface, edge and background
- Increase peak sizes
- Change AOI of view

Help menu

Help is provided both as online Help and online Help request links. The commands available from this menu are discussed in the following sections.

Contents

Displays the online Help as a PDF manual.

Restore Default Image

Restores the image default image used when no other is available.

About

Displays the name and version number of the software together with the registration information.

The Program Windows

The main window contains three smaller windows. Each of these windows contains a different type of information.

The windows can be moved and resized via the splitter bars between them.

Scrolling windows

Scroll bars are used when the information being displayed is too large to fit on the screen.

The scroll bars are located at the bottom and right side of the image window. They work in the same way as normal scroll bars but allow you to see the range that you are viewing.

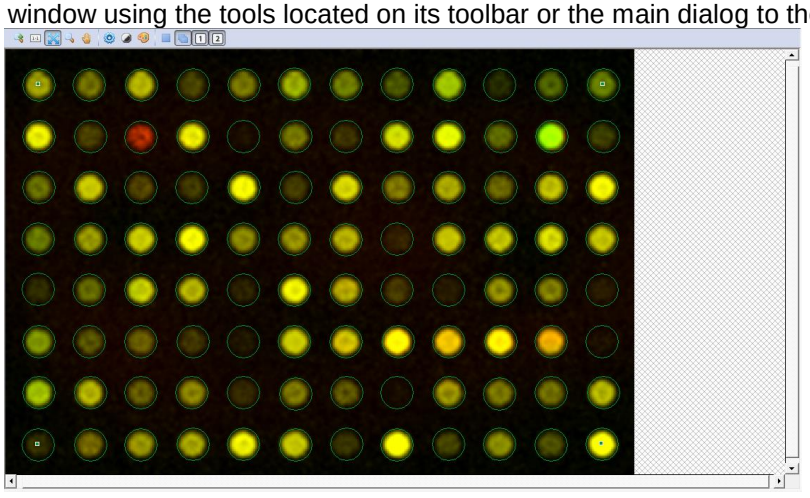
In addition, dragging the scroll box changes the start position of the range you are viewing; you can also resize the scroll box. Since the size of the scroll box reflects the amount of data that is shown in the associated window, resizing the scroll box changes the display magnification. Therefore, if you are viewing an image and make the scroll box smaller, less of the image is used to fill the window and you see the image at an increased magnification.

To resize the scroll box, position the pointer on an edge of the scroll box, the pointer changes to ↔ or ↕. Drag the pointer to the new position.

When the scroll box indicates a display of the total range of the scroll bar, the scroll box becomes transparent. When it indicates that only a portion of the range is visible, the scroll box becomes opaque.

Image window

The Image window is the most important of the windows and displays the image you are currently analysing. The image is overlaid with information determined by the tool you are using and how far through the analysis process you are. Most of your interaction with the software is done within this window using the tools located on its toolbar or the main dialog to the left.



Single viewing mode

When in single channel view mode, this window works in the same way as it does when a non-multiplex image is loaded.

Any one of the channels of a multiplex image can be selected and viewed in this window by clicking the channel button on the image window toolbar.

Overlay viewing mode

In overlay viewing mode, any combination of channels can be viewed in this window.

When in the Overlay viewing mode certain features of the software become unavailable – these are specific to the module and mode. For more information refer to the relevant analysis mode.

The Image window toolbar

The Image window contains a toolbar with the following buttons:

 **Zooming an image**

In this window the button toggles with the Zoom to Fit button undoing any zooming effects.

To zoom the image:

1. Click the zoom button
2. Position the pointer on the area you want to zoom.
3. Drag the pointer over the desired area.

Alternatively, click the zoom button then left click to zoom in and right click to zoom out.

Pressing **Esc** on your keyboard cancels the zoom mode.

 **Zoom 1:1**

Adjusts the magnification of the image to its original size.

 **Zoom to Fit**

Clicking the **Zoom to Fit** button if the image is zoomed, fits the image to the size of the window.

The Zoom to Fit button counteracts any magnification made on an image using the zoom button.

 **Magnify**

Allows you to magnify specific parts of an image.

1. Click the magnify button.
2. Position the pointer over the part of the image you want to magnify. The pointer changes to a small magnifying glass.
3. Hold down the mouse button. The area under the pointer appears in a box.
4. Drag the pointer to view other areas.

Panning

Allows you to move the image around within the Image window when the image is zoomed.

1 Click the Panning button

2 Position the pointer over the image; it changes to a small hand.

3 Drag to change the view.

Options

Allows you to edit the display options for the window. See the **Image Window Options** section for details

Contrast

Click the **Contrast** button to display a dialog box that provides a number of ways to alter the brightness and contrast of an image. For more information, refer to the **Contrast** section

Colour

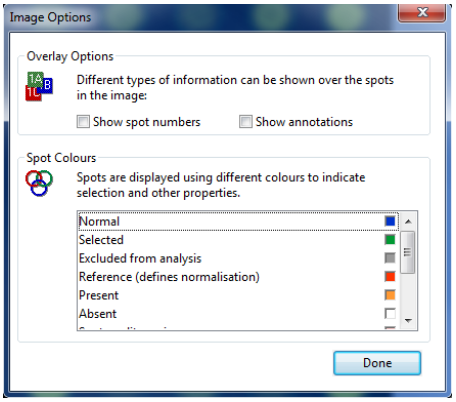
Click the **Colour** button to display a dialog box that provides a number of ways to alter the base colour of an image or the individual channels. For more information, refer to the **Colour** section

Channel buttons

Click the channel buttons to select the channel or channels that display in the main image window. Each channel of a multiplex image enables an extra button

Image Window Options

The parameters shown this dialog relate to the display in the Image window. By selecting the check boxes, you can change the display for the various labels in the image window.



Overlay options

The display options are:

Show Spot Numbers

This option allows you to display the spot number next to the spot in the Image window.

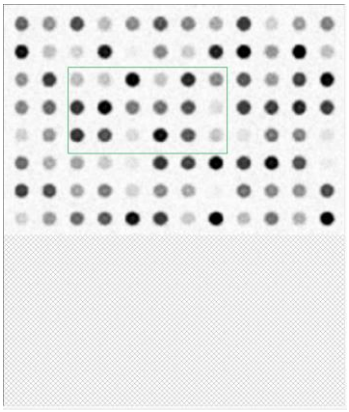
Annotations

This option allows you to show the user-defined annotations created while in Annotation mode.

Spot Colours

You can change the colours used by the spot in the software by clicking the colour wells to the right of the item. The Colour dialog box appears allowing you to select the colour to be used for the item.

Zoom window

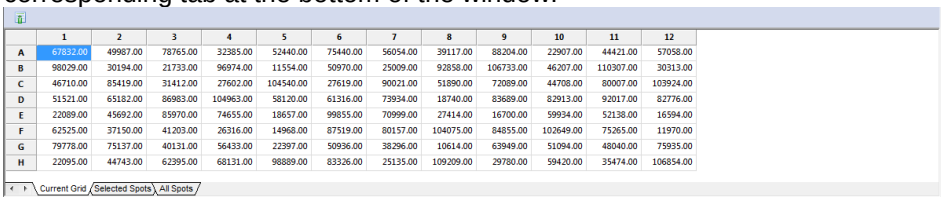


The Zoom window displays the whole image, overlaid with a rectangle representing the area of the image that is currently displayed in the Image window.

Drag the rectangle to a new position to display the corresponding area in the Image window.

Measurements window

The Measurements window displays the analysis results. Three different tables of data are available: Current Grid, Selected Spots and All Spots. Each table can be viewed by clicking the corresponding tab at the bottom of the window.



	1	2	3	4	5	6	7	8	9	10	11	12
A	67832.00	49987.00	78765.00	32385.00	52440.00	75440.00	56054.00	39117.00	88204.00	22907.00	44421.00	57058.00
B	98029.00	30194.00	21733.00	96974.00	11554.00	50970.00	25009.00	92858.00	106733.00	46207.00	110307.00	30313.00
C	46710.00	85419.00	31412.00	27602.00	104540.00	27619.00	90021.00	51890.00	72089.00	44708.00	80007.00	103924.00
D	51521.00	65182.00	86983.00	104963.00	58120.00	61316.00	73934.00	18740.00	83689.00	82913.00	92017.00	82776.00
E	22089.00	45692.00	85970.00	74655.00	18657.00	99855.00	70999.00	27414.00	16700.00	59934.00	52138.00	16594.00
F	62525.00	37150.00	41203.00	26316.00	14968.00	87519.00	80157.00	104075.00	84855.00	102649.00	75265.00	11970.00
G	79778.00	75137.00	40131.00	56433.00	22397.00	50936.00	38296.00	10614.00	63949.00	51094.00	48040.00	75935.00
H	22095.00	44743.00	62395.00	68131.00	98889.00	83326.00	25135.00	109209.00	29780.00	59420.00	35474.00	106854.00

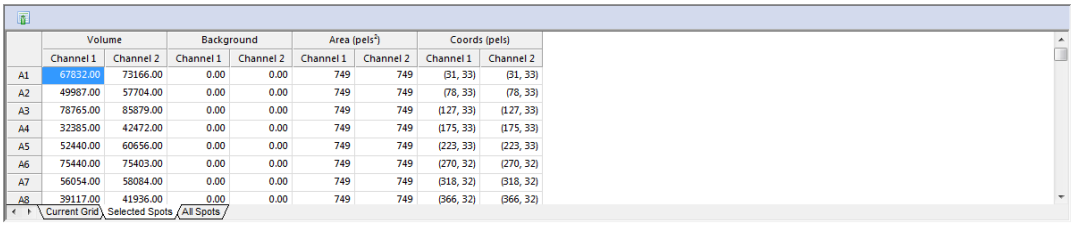
Current Grid | Selected Spots | All Spots

For each table you can select different data fields to display the various properties of the spots. You select and order the data fields using the **Data Fields** button.

Multiplex

You can view data from the current channel of a multiplex image when using the *Single Channel* viewing mode as you would for an individual image. This rule applies to all the tables of the Array module.

To display data for all channels, of a multiplex image select the *Channel Overlay* viewing mode.



	Volume		Background		Area (pels²)		Coords (pels)	
	Channel 1	Channel 2	Channel 1	Channel 2	Channel 1	Channel 2	Channel 1	Channel 2
A1	67832.00	73166.00	0.00	0.00	749	749	(31, 33)	(31, 33)
A2	49987.00	57704.00	0.00	0.00	749	749	(78, 33)	(78, 33)
A3	78765.00	85879.00	0.00	0.00	749	749	(127, 33)	(127, 33)
A4	32385.00	42472.00	0.00	0.00	749	749	(175, 33)	(175, 33)
A5	52440.00	60656.00	0.00	0.00	749	749	(223, 33)	(223, 33)
A6	75440.00	75403.00	0.00	0.00	749	749	(270, 32)	(270, 32)
A7	56054.00	58084.00	0.00	0.00	749	749	(318, 32)	(318, 32)
A8	39117.00	41936.00	0.00	0.00	749	749	(366, 32)	(366, 32)

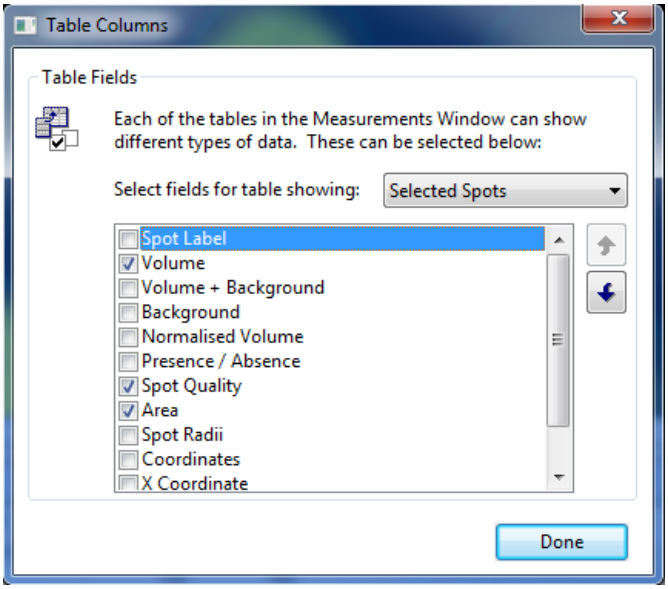
Current Grid | Selected Spots | All Spots

Below each table 'field', you will see a column of data for each spot of the chosen channels.

Data Fields

Allows you to edit the display options for the window. See the **Measurement Window Options** section for details

Measurement Window Options



Using the tables tab you define which data fields you want to display in the different tables.

You can only select one data field for the **Current Grid** table.

The **Selected Spots** and **All Spots** tables available in the Measurements window show the same selected data fields across all tables.

Within the software you can select the various data fields that will be displayed in the Measurements window. You can also change the order of these fields by use of the arrows.

To select or deselect a field for display:

Left click the box to the left of the name in the list of fields.

To change the order of the displayed fields:

1 Select the field to move by highlighting the name in the list of fields.

2 Click on the **Move Up** or **Move Dow**n buttons until the field is in the desired position in the list.

Data Fields

This section discusses data field availability. You will find a brief description of each field in the table below.

Data Field name:	**Description**
Area	The area of the Band, Spot, Colony or other image feature displaying the number of pixels quantified in the image in order to produce the measurements.
Background	The total background for the Band, Spot, Colony or other image feature.
Coordinates (X and Y)	Equivalent to the centre of the detected mass, with the coordinates rounded to the nearest integer.
Normalised Volume	The Normalised Volume of the spots.
Presence / Absence	The Current Grid of Array displays a **1** for spots that contain a sufficient quantity of material to be considered present and **0** for spots not considered as present. The threshold volume can be set in the Presence Flagging dialog box.
Spot Label	Use in reporting and identification of spots in the image. The label for a spot can be edited in the *Spot Properties* dialog box, and in the table when you select the Spot Label field.
Spot Quality	Used to highlight any spots of dubious quality that can affect the measurements results. Spots thought to contain only noise and spots thought to contain a *spike* are highlighted.
Spot Radii	Displays the X and Y radii for the given spot. These will be identical for square and circular spots.
Volume	The raw volume of the uncalibrated quantity of material in the image feature **after** the background intensity has been removed.
Volume + Background	The uncalibrated quantity of material detected before the background intensity has been removed.

Status bar

The status bar is the thin bar along the bottom of the main window. It displays information about the current state of the program (e.g. Ready) as well as descriptive messages for a selected command or toolbar button (e.g. Co-ordinates (Pixels): nnn Pixel Value: nnn).

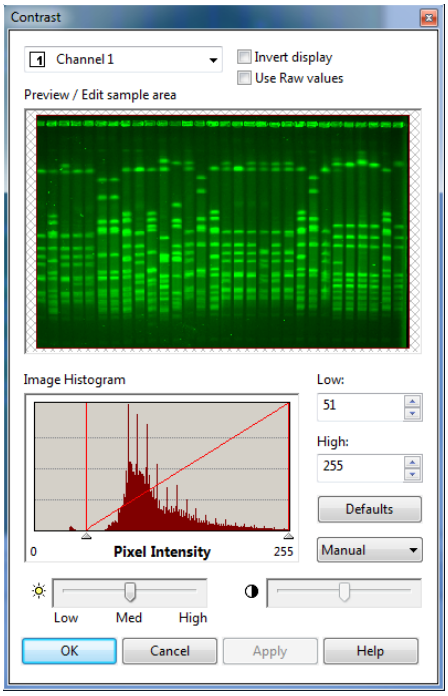
Contrast

The Contrast controls allow you to alter how the selected channels look on screen.

This dialog box is used to change the contrast and brightness of the image.

Multiplex

When you are viewing multiple channels, you can switch to one of the other selected channels from the drop down list. The contrast is edited on a per channel basis.



Preview / Edit sample area

The Preview / Edit sample area displays a thumbnail of the entire image. If the image displays a rectangle on it, known as the area of interest (AOI), the palette is calculated from the AOI area. The default rectangle covers the entire image.

You can change this rectangle by clicking and dragging out a new rectangle; this causes the image to update immediately.

This feature can be very useful when editing very faint parts of the image, as you can set the AOI to a very small area, which produces a very good colour standard in this area.

Image Histogram

The Image Histogram displays the frequency with which each pixel intensity occurs within the area of interest. The peaks on the graph represent the pixel intensities that occur most frequently within the current area of interest.

The left and right red bars on the graph show the range of pixel intensities in the image that will be mapped to different colours in the display image. You can directly modify the brightness and contrast of the display by dragging the bars on the graph.

Move these handles to increase/decrease the contrast.

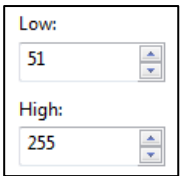
The figures below the image histogram represent the scale of the graph, i.e. the calibration.

You can restore the default contrast at any time by clicking the **Defaults** button.

Low / High edit boxes

The **Low** and **High** edit boxes are synchronised with the two handles below the image histogram.

These edit boxes allow you to enter values to adjust the contrast of your image.



This enhances the contrast display so that for narrow contrast ranges, a sufficient number of grey levels are used giving a higher quality of image display.

You can restore the default contrast at any time by clicking the **Defaults** button.

Brightness

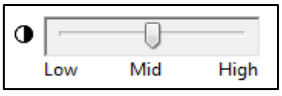
The two sliders at the bottom of the dialog box allow you to adjust the brightness and contrast of the image by moving them between Low, Medium and High.

Drag the slider to change the brightness. High = paler, Low = darker.

Contrast response list

Below the **Defaults** button there is a drop down list. Select the shape of the contrast response curve to help you visualise the features of your image.

Manual - a straight line.
Hi-contrast – a curve calculated from the image to maximise the contrast.
Curve and **Sigmoidal** – the standard shapes of a response curve, adjustable by using the Contrast slider.



The Contrast slider affects the shape of the curve that extends between the contrast limits.

Drag the slider to change the contrast. High = more effect.

Invert display

This check box indicates whether the current colours should be inverted or not; click it to toggle the option on or off. Inverting an image causes it to display like a photographic negative. For example, using the default **Grey Scale** colour scheme white becomes black and vice versa.

Use Raw Values

This check box indicates whether the histogram is calculated after any scanner calibration, the default, or just uses the raw values of the tiff image. Using the raw values will usually increase the contrast.

Auto Default Contrast

By default the software looks at the histogram results and optimizes the default low and high values for contrast stretching. This means you can see more of the important features of the image. If you wish this turned off you can see the image default as stretched from 0 to the maximum in the image. This is a global variable set for all future default display but does not alter the current image unitl you press **Defaults**

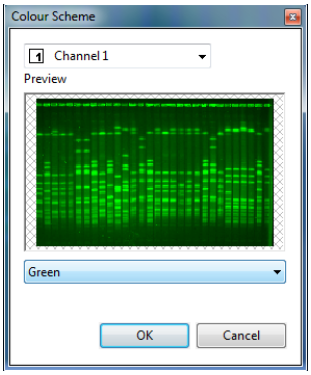
Channel Selector

The drop down at the top of the dialog allows you select which channel you wish to change the contrast for.

Colour

The Colour controls allow you to alter how the selected images look on screen.

The **Preview** shows a thumbnail of the entire image and displays the effects of any changes.



Multiplex

When using multiplex gels you can only launch the Colour Scheme dialog box when in *Multiple Channel viewing* mode. The colour that you choose, from the colour scheme drop down list, is used to change the colour for the channel in *Overlay viewing* mode only.

In *Single Channel viewing* mode the image will be displayed in grey only and cannot be changed.

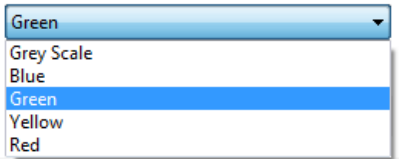
The default choice of colours used to display each channel is dependent upon how many channels there are in the image.
2 Channel images: 1 = green, 2 = red

3 Channel images: 1 = blue, 2 = green, 3 = red

4 Channel images: 1 = blue, 2 = green, 3 = yellow, 4 = red

Colour Scheme List

This is a drop down list box containing the names of all of the installed colour schemes on the system.



Of the various colour schemes that are supplied with the software, two are *false colour* schemes; these can be useful for visualising faint spots or bands, because the human eye is often more sensitive to colour than to shades of grey. To change the colour scheme, select a different scheme from the list.

Reverse colours

This check box indicates whether the current colour scheme should be inverted or not; click it to toggle the option on or off. Inverting an image causes it to display like a photographic negative. For example, using the default **Grey Scale** colour scheme white becomes black and vice versa.

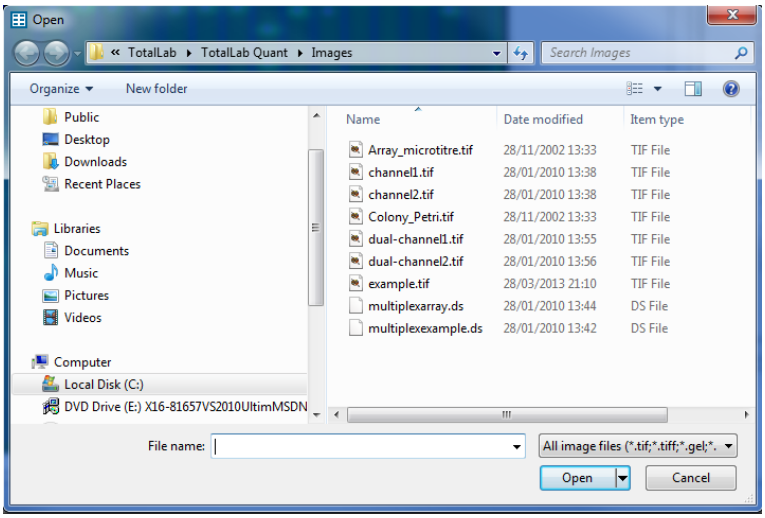
Beginning the Analysis – Open Image

Selecting the **Open Image** command from the main toolbar allows you to start an analysis by Opening an image. It also allows you to Edit an Image, check its properties and Create Multiple Images.

Note:
*If you close the software, open an image or open an existing experiment, the software **automatically saves** the current experiment for you.*

Open Image

You can open an image by clicking the **Open Image** or **Open Multiplex Image** button



To open an image:

1. Select the folder from the *Look in* list.
2. Select the correct file type from the *File of type* list.
3. Click the specific image file from those displayed.
4. Click **Open**. The selected image automatically opens in the Image window allowing you to analyse the image.

Note:
*If you have an **RGB** Colour image it can be opened as a regular image (converted to greyscale) or as a multiplex image (all 3 colours split into 3 channels)*

Edit Image

This option allows a wide range of editing options. Please see the **Image Manipulation** section for more details.

Image Properties

The image properties dialog box has three tabs, File info, Scan info and History. The information in these tabs is read only.

The File info tab displays information about the image, for example the File Name, Size, Image Width and Image Height.

The Scan info tab displays information relating to the scanning of the image, for example the Scanner make, model, date and time of scan and the resolution. It also shows any comments from the scanning.

The History tab displays the name of the image and a scrollable list of any editing made to the image, for example Rotate Clockwise 90.

Spot Definition

Multiplex

In multiplex images, the same individual spot or group of spots is applied across all channels. You can also define the diameter of the spot before creation by setting the X and Y radius for the shape.

You can create, edit or delete spots in both the *Single Channel* and *Channel Overlay* viewing modes.

The basic steps involved in defining spots are:

1 Select a grid type or create a new one.

2 Create the grid either manually or automatically.

In addition you might want to perform any of these optional steps.

Edit the grid properties after creation

Reposition the grid

Adjust the grid to allow for a skewed image

Reposition the individual spots

Resize the individual spots

These steps are discussed below.

Invert Intensity

Most images have high pixel values (high intensities) in the black areas of the image and low values in the white areas. However, in some images, the spots have a low intensity (and therefore low volume) compared to the background. The "Invert Intensity" command allows you to correct this by inverting all the pixel values before using them to calculate the measurements.

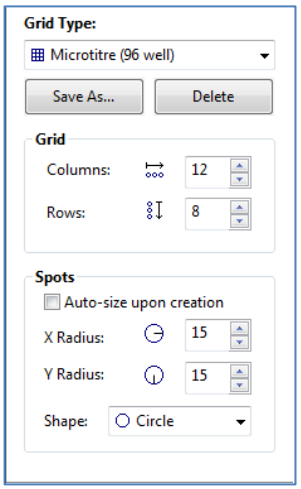
To invert a pixel value, the software subtracts the value from the maximum possible pixel value in the image. For example if the image has a pixel value range from 0 to 255 and the software inverts a pixel that has a value of 50, the inverted value would be (255 - 50) = 205.

To invert the measurements data, click **Invert Intensity** option**,** and then check the data in the measurements window.

Defining the grid layout

The Spot Definition dialog box allows you to accelerate the process of defining the spots on the image. You can store parameters used for creating grids as a grid type that can be quickly selected from the **Grid Type** list at the top of the dialog box.

To begin spot definition, you define the size and location of the spots in the image.



Defining a grid automatically

1. Select a grid type from the list in the dialog.
2. Click the **Detect** button. The grid is automatically drawn in the Image window.

Defining a grid manually

1. Set the number of **columns** and **rows** using the spin buttons in the dialog.
2. Select the **spot shape** from the list.
3. Set the **X** and **Y** radius. Alternatively, click the *Auto-size upon creation* checkbox and let the software detect the optimum radius.
4. Position the pointer over the centre of the top left spot and drag a rectangle over the image to define the grid manually.

The software automatically measures the spots and displays the data in the Measurements window.

If you are not satisfied with the detected grid, you can edit the grid.

Creating a grid type

1. Set values for the dimensions of the grid **columns** and **rows**.
   The maximum grid definition is 48 x 32.
2. Select the **spot shape** from the list.
3. Click the **Save As** button to save the created grid.
4. **Type** a name for the grid and click **OK**. The software adds the new grid to the list.

Deleting a grid type

1. **Select** the grid from the list.
2. Click the **Delete** button below the list.

Repositioning spots

Individual spots or a group of spots can be repositioned in one of the following ways.

Repositioning the whole grid:

1. Click the **Create and Stretch Grid** button in the dialog.
2. Position the pointer over any of the spots other than a corner spot, the pointer changes to  .
3. Drag the grid to the new position.

All the spots must remain within the image for the new grid position to be accepted.

To cancel *repositioning the grids*, right click whilst dragging or press the **Esc** key.

Moving a small group of spots:

1. Click the **Move and Resize Spots** button in the dialog.
2. Position the pointer over the grid and drag, from the top left to bottom right, to highlight the group of spots by drawing a green outline covering the spots.

   Note:
   To select more than one group press the CTRL key when dragging over the additional spots.
3. Position the pointer over one of the selected spots, the pointer changes to  .
4. Drag the group to the new position.

Moving a single spot:

1. Click the **Move and Resize Spots** button in the dialog.
2. Position the pointer over the desired spot, the pointer changes to  .
3. Drag the spot to the new position.

Adjusting a grid to allow for a skewed image

You can uniformly stretch the positions of spots in a grid between the grid's four corners by clicking the **Create and Stretch Grid** mode. This provides a way to quickly adjust the grid to cope with images in which the spots are aligned in a skewed rectangle.

Repositioning the spots by stretching the grid

1. Click the Create and stretch grids button.
2. Position the pointer over the centre of the desired corner spot, the pointer changes to  .
3. Drag one of the grid's square corner handles to a new location. The spot positions are stretched uniformly between the four corner handles of the grid.
4. Repeat steps 2 and 3 until the grid is adjusted.

Resizing spots

Spots can be resized in two ways: by stretching or by defining the spot radius value.

To change the radius by stretching a spot:

1. Click the **Move and Resize** button.
2. Select the spots whose radius you want to change.

3 Position the pointer over the spot edge, at the top, bottom, left or right edge. The pointer changes to either ↕ or ↔.

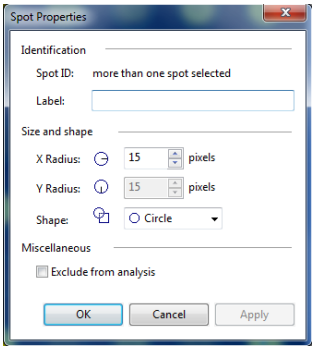
4 Drag the edge to the desired size for all the selected spots.

While the radius of a spot is changing, its value (measured in pixels) is displayed in the status bar.

To **cancel** resizing the spots, right click or press the **Esc** key while dragging.

Editing spot properties

You can edit the properties of the selected spots using the Spot Properties dialog box:



Select a spot by clicking on it or select a group of spots by drawing a rectangle around the desired spots. Then, click the **Edit** | **Spot Properties** command to display the Spot Properties dialog box. Alternatively, right click on an individual spot in the Image window and select **Spot Properties** from the context menu.

You can edit the size and shape of spots as well as their labels (e.g. for sample tracking) and exclude spots from the analysis.

Any spots excluded from analysis will not be measured and will not appear in the Selected Spots or All Spots tables.

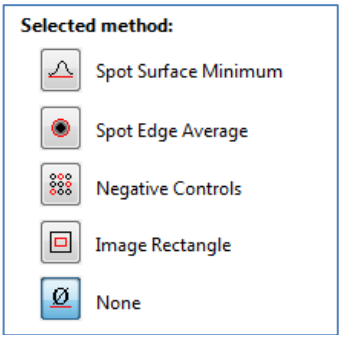
Background Subtraction

Background subtraction is the process by which you remove the part of the image's pixel intensity that is background intensity from the measurements.

Background subtraction is applied across all channels of a multiplex image.

To begin the process select the **Background Subtraction** button in the main toolbar

Click the background subtraction method you want to use from the **Selected method** list in the dialog.



Brief descriptions of the various methods can be found later in this section.

After you select the method and set any required parameters the software automatically calculates the spot volumes and displays the data in the Measurements window.

Providing parameters

Some of the background methods available require you to select extra parameters. For those methods that require parameters, a list of the parameters is shown in the dialog.

Methods that do not require parameters:

Spot Surface Minimum

Spot Edge Average

None

Methods that require extra parameters:

Negative Controls

Image Rectangle

Spot Surface Minimum

The **Spot Surface Minimum** method locates the lowest pixel intensity within the spot and then removes this intensity from the volume for each pixel. You can use this method to ensure that no part of the defined spot is below the background level for each spot.

You should use this method for images with little noise and an even background intensity.

Spot Edge Average

The **Spot Edge Average** method locates the average intensity around the spot's outline, and then applies this as the background for the spot. The background is therefore calculated independently for each spot.

This method is the recommended because it usually provides a good localised background intensity, and is relatively tolerant of noise in the image.

Negative Controls

The **Negative Controls** method averages all the pixels inside the 'flagged' spots in the grids and applies this average as the background intensity. You identify the spots that should be considered as background intensity.

This method can be useful in microtitre plate analysis where certain spots have been set as controls and are therefore known to contain no material.

1. Select the spots you want to change by either clicking on them or drag a selection rectangle around the spots in the Image window. *Notice that the grid over your selection changes to green identifying it as the current selection.*

   To select more than one group press the CTRL key while dragging over the additional groups.

2. Click either **Set negative control** or **Deselect negative control** button in the dialog. *Notice that the software updates the data in the Measurements window automatically.*

Image Rectangle

The Image Rectangle background method requires you to specify a rectangular area of the image. This area is then averaged to calculate the background intensity. This rectangle is initially located at the top left of the image and is drawn in the Image window.

This method can be useful if your image contains a lot of noise and tightly packed spots. However, the Spot Edge Average method might provide better results.

To provide the background rectangle:

1. Position the pointer at the top-left corner of the rectangular background area.
2. Drag the pointer to the bottom right of the rectangular background area. You see the rectangle drawn as you move the pointer.

None

The **None** method allows you to turn off background subtraction altogether. The raw volume calculated for each spot will be the sum of all the pixel intensities in that spot.

Although this method can be useful at times, it is not recommended for good laboratory practice.

Normalisation

The **Normalisation** mode is used to generate the value of normalised volumes for the spots in an array.

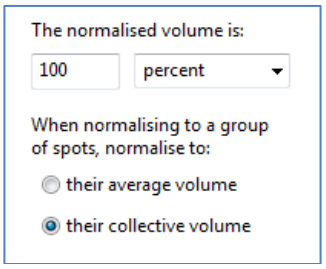
This can be useful when comparing the volumes across spots, where the loading may be different.

The results of normalised volume assignment can be viewed in the tables of the Measurements window.

Performing Normalisation for a Standard Gel

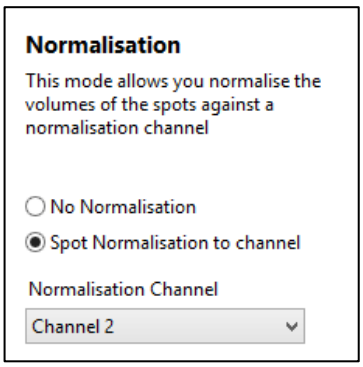
To normalise spot volumes to a selection of spots take the following steps.

1. Select the spots that will be used to normalise the image.
2. Type the normalised volume in the dialog. The range is from **X** (0.0001) to **Y** (1000000).



3. Select the normalised volume unit e.g. nanogram from the list.
4. Click the **average** or **collective** group button.
5. Click the **Normalise** button at the top of the dialog and check the data displayed in the Measurements table.

Performing Normalisation for a Multiplex Gel



This option is used if you have one channel of your array assigned with normalizing spots (such as for in cell westerns).

The idea is that each spot in this normalizing channel should have exactly the same volume. As they will not have exactly the same raw volume a normalisation factor is calculated for each spot. Here is the equation.

1. Find the biggest spot in the whole array for this channel. This becomes the reference spot
2. For each spot calculate the normalisation factor

   NF = volume of spot/volume of reference spot
3. For each spot calculate the normalised volume in both channels

   Normalised volume = Raw volume of spot/normalisation factor

Presence Flagging

Presence flagging allows you to set the level above the background at which the spots should be flagged as having a significant amount of material present. The presence or absence of spots can be seen in the Measurements window after you select the **Presence / Absence** data type.

The basic steps involved in Presence Flagging are:

1 Make an initial estimate of the threshold.

2 Fine-tune the threshold level.

Multiplex

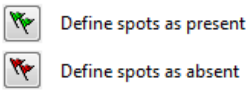
Presence Flagging is applied across all channels of a multiplex image. You can apply presence flagging parameters in both the *Single channel* and *Channel Overlay* viewing mode.

Making an initial estimate

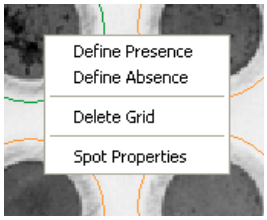
The quickest way to accurately define a threshold for flagging spots as having material present is to make an estimate (automatically or manually), and then adjust the estimate to match what you can see in the image.

To set the initial flagging threshold, either:

Click on a spot, then click either the **Define spots as present** or **Define spots as absent** button in the dialog.



Alternatively, right click a spot in the image and select either the **Define Presence** or **Define Absence** command from the context menu.



Click the **Estimate** button to have the software calculate a threshold for the current image. To do this the software evaluates the volumes of all spots in the image.

Fine-tuning the threshold

After you make an initial estimate for the flagging threshold, you can manually adjust the threshold value so that the presence or absence of the spots (as displayed in the Measurements window) reflects what can be seen in the Image window.

To fine-tune the flagging threshold, either:

Type a value between 0 and 100 in the Flagging threshold text box.

Or,

Drag the slider to the left (towards 0) or the right (towards 100).

Annotations

Using the Annotations mode, you can add text labels with optional arrows to precisely locate and identify certain areas of an image.

You activate the Annotations mode by clicking the **Annotate** button on the *main toolbar*, or

Create annotations on an image to highlight particular areas of interest. There is no limit to how many annotations you can create in any one image or channel.

Multiplex

Annotations can be created and displayed in multiplex gels regardless of the number of channels or the current view mode. However, it should be noted that you can only create one set of annotations per multiplex gel.

Manual Annotations

To change the annotation style just look through the dialog which contains a variety of options. For example, to change the font click the **Select Font Style** button.

After changing the annotation style, the changes you make are applied to all annotations that are presently selected and any newly created annotations.

Adding and Editing Annotations in the Image window

The following section describes how to use the mouse and the keyboard to Add, Move, Select and Edit the text of annotations in the Image window.

Multiplex

Annotations can be viewed and edited in both single and overlay modes.

We assume that you are using the default settings for annotations when doing the following actions.

Adding an annotation

To add an annotation to the image shown in the image window:

1. Position the pointer over the location in the Image window that you want to annotate. The pointer should be the standard arrow, indicating that you are not over any other items in the window.
2. Left click to add an annotation box. An anchor arrow appears with an attached text entry box, pointing to the location.
3. Type your text into the text entry box.
4. Press **ENTER** to accept the text or press **Esc** to stop editing without accepting any text. You will be able to enter text later.

You have now added an annotation that is indicated as the currently selected annotation by an outline box. You can add more annotations by repeating the above steps.

As an alternative to clicking the image to add a comment, you can position both the arrowhead and the text box of your annotation with a single mouse interaction.

1. Position the pointer at the location where you want the arrowhead, left click and hold down.
2. Move the pointer to the position where you want the text to be centred. As you drag the pointer, the annotation text entry box appears and stretches away from the arrowhead.

3 When you release the button, the text entry box changes to the in-place editing mode. Type in your text and press **Enter**.

Moving annotations

To move an annotation:

1 Position the pointer anywhere on the line between the arrowhead and annotation text box. *You might need to move the text box to see the line.*

2 The pointer changes indicating that you are in position. If this does not happen, you can use the ***zoom in*** function to increase the amount of line available, to pickup the annotation.

3 Drag the annotation to its new location.

Moving part of an annotation

You can also move the position of an annotation anchor arrow or text box.

1 Position the pointer over the anchor arrow or text box you want to move. The pointer changes to, a crosshair for the anchor arrow and the text box becomes outlined.

2 Drag the arrow or text box to the desired location.

Selecting annotations

To select a single annotation you can:

Click on either the arrow, the line between the arrow and the text box, or the text box.

Press the **TAB** key to move between annotations.

Select all

To select **all** the annotations you have created, **right click** anywhere in the image away from any annotation. The context menu appears allowing you to select all the annotations.

Deselect all

To **deselect all** the annotations **right click** anywhere in the image away from any annotation. The context menu appears allowing you to deselect all the selected annotations.

Arbitrary selection

To make an **arbitrary selection** of annotations, **left click** on the first annotation and then **hold down** the **CTRL** key on your keyboard. You can now **click** on each annotation you want to include in your selection. **Right click** in the image for your next action.

To arbitrarily deselect an annotation, **hold down** the **CTRL** key on the keyboard and then **left click** on the annotation.

Editing the text of an annotation

After adding annotations, you can edit the text in the annotation by right clicking the outlined text box and selecting Edit text from the context menu or, left clicking the text box.

While editing annotations, an outline box appears around the selected text box. This outline box does not appear on any image printouts. Annotation boxes are not included on report printouts.

Annotation Fonts

Clicking the Select Font Style button, displays the Font dialog box that allows you to set the font style for your annotations.

Delete selected Annotations

The **Delete Selected** button deletes any currently selected annotation. If you continue to click this button, the annotations will all be deleted in the reverse order that they were added.

Alternatively, position the pointer anywhere over an annotation and right click, then select Delete from the context menu.

There is no **undo** command. If you accidentally delete an annotation, you will need to add it again.

Setting annotation options

The dialog contains the following options.

Text outline style

Select the shape of the border around the text of your annotations from the drop-down list. You can select a rectangle, rounded rectangle or no border.

Fill Colour

Select the colour for the background of the text area of your annotations from the drop-down list. This option is disabled if you have chosen a transparent background.

Line thickness

Select the weight of the lines that is used to draw the anchor arrow of the annotations.

You can choose one of size line thickness or No line.
If you choose No line, your annotation appears as a moveable text box only.

Colour

Select the colour that is used to draw the arrow line and outline of the annotation text box. The default outline colour is green.

Opaque Background

Select the check box if you want the text of the annotation drawn over a coloured background, or clear the check box if you want the text drawn directly onto the image.

Text Orientation

Choose the orientation of the text within the annotation.

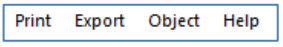
Toolbox

Introducing Toolbox

Toolbox allows you to perform analysis on a wide variety of images. The flexible nature of the software means you are not restricted to a single type of analysis when you use this module.

To help you along the way, where relevant, pictures are included to show what you should expect to see on the screen.

Main menu bar



The Main menu bar, located at the top of the main window, provides a number of commands not available through the main toolbar modes.

Print menu

From the Print menu, you can perform the following actions.

Print

There is a print option for each visible window. The **Print** dialog box allows you to select a printer, print range and number of copies for the selected window.

Print Preview

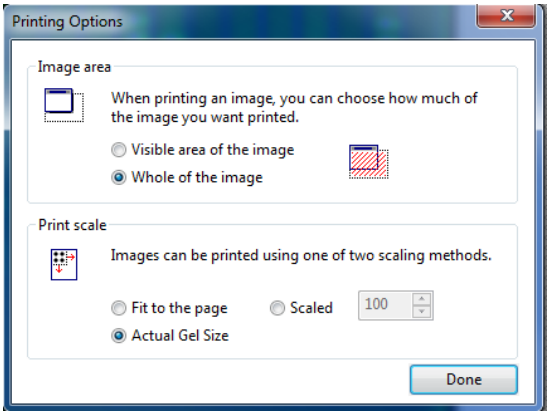
There is a print preview option for each visible window. The **Print Preview** window displays the image or measurements data from the selected window so you can see how it will be printed.

Print Setup

The Print Setup dialog box allows you to select a printer and its properties. You can select a paper size, source and the page orientation.

Printing Options

With these options you can customize the way that the content of windows will be printed, from the commands in this dialog. This includes changing the area of an image that will be printed and setting the scale at which an image will be printed.



Changing the area to be printed from an image

1 Click either Visible area of the image or Whole of the image.

2 Click **Done** to confirm your choice.

You can specify how much of a current image window should be printed and at what scale.

Changing the print range and scale

1 Specify the print range of the window to be printed by clicking either **Visible area of the image** or **Whole of the image**.

2 Select the scale from the **Fit to paper**, **Actual size** or **Scaled** options. If you click Scaled, select or type the percentage in the text box.

Note:
The default is set to 100% of the original size.

3 Click **Done** to confirm your choices.

Export menu

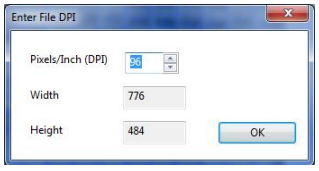
Export to Clipboard

There is an export to clipboard option for each visible window. Click **Export to Clipboard** to copy the data or image displayed in the selected window to the clipboard, so that the data or image can be pasted into other applications. For example the measurements window data can be pasted into a spreadsheet.

Export to File

There is an export to file option for each visible window. Clicking **Export to File** displays a dialog box that allows you to enter a folder and filename.

If you are exporting an image, the default filename is Export.bmp. You are also given the chance to change the DPI of the image saved before naming the file if it is from the Image Window and you are exporting the whole image (see **Export Image Options**)



If you are exporting measurements data the default filename is Export.txt.

The default folder is the CLIQS installation folder.

If you do not want to use the defaults, select the folder in which you want to store the file and type in a name and then press **Save**.

Export to Clip Gallery

This creates a file in exactly the same way as **Export to File** (see above) but instead of saving directly to a folder you select the file is made available via the **Clip Gallery** (see below).

Export Measurements to Excel

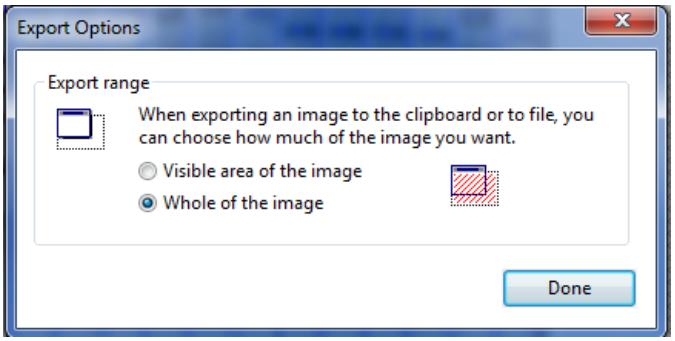
The data in the Measurements window will be copied directly into an Excel spreadsheet.

An Excel workbook automatically opens and the measurement data will be pasted into a new workbook.

If Microsoft Excel is not installed, use the Export to File command.

Image Export Options

With these options you can customize the way that the content of windows will be copied from the commands in this dialog.



Changing the area to be copied from an image

1. Click either Visible area of the image or Whole of the image.
2. Click **Done** to confirm your choice.

You can specify how much of a current image window should be printed and at what scale.

Export Lines to Clipboard

1. Click **Export | Export Lines to Clipboard** to export the numerical values of the profile lines, as shown in the Line window, to the clipboard.
2. Paste the values into another application.

Export Lines to File

1. Click **Export | Export Lines to File** to export the numerical values of the profile lines, as shown in the Line window, to a file.
2. Type the filename.
3. Click the **Save** button to save the file containing the numerical values of the current lane profile.

Export Shapes

Having created a specific object you might want to apply it to another image. Before applying an object to an image you must save it by 'exporting' it.

1. Click **Export** | **Export shapes** to display the **Save As** dialog box.
2. Select a **folder** or create a new folder, **type** a filename and click **Save**. The export file is saved as a Shape Object file with an .obs extension.

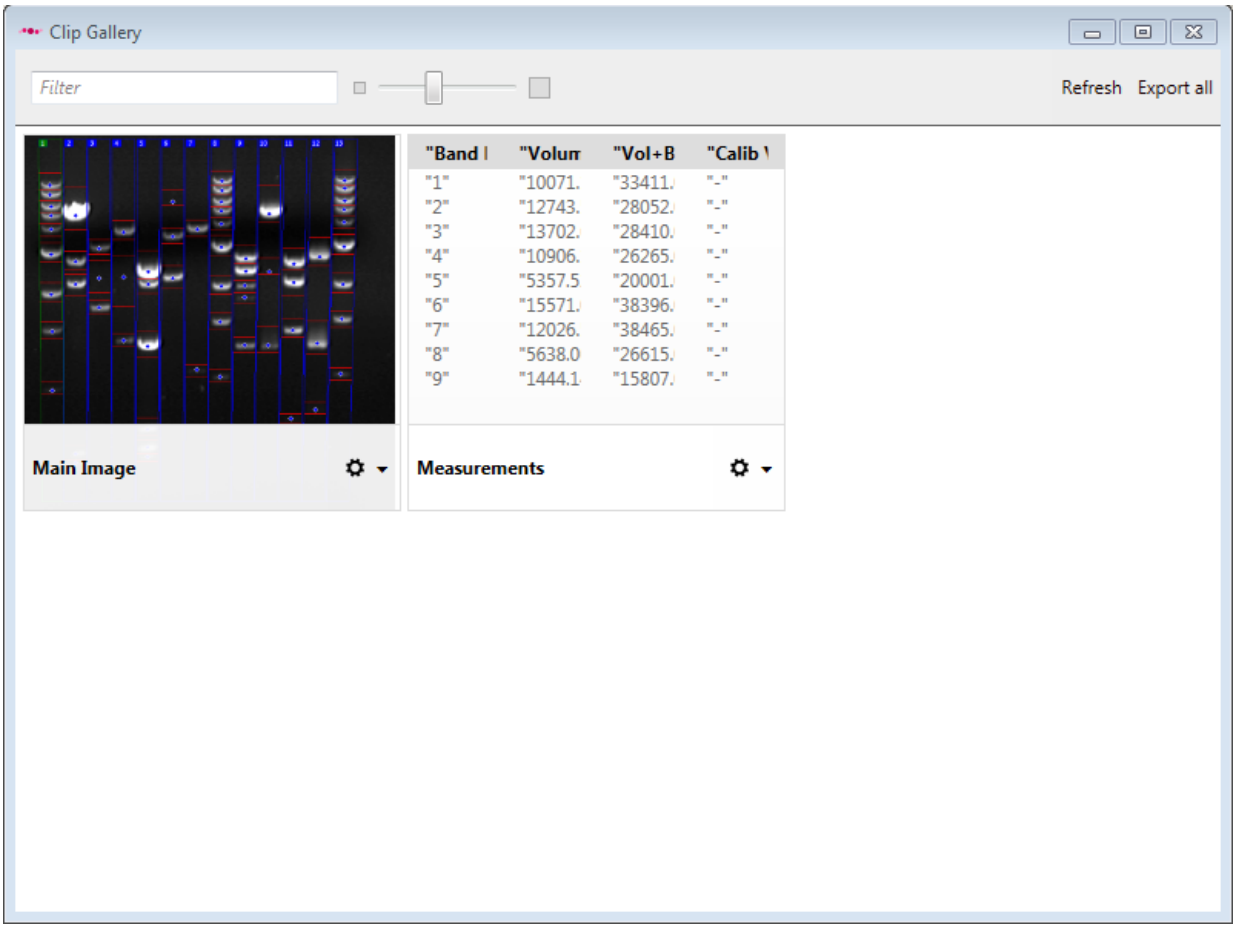
Import Shapes

Click **Export** | **Import Shapes** to display the **Open** dialog box.

Select the **folder** where the Shape object file is stored, and then select the **Shape object file**. Click **Open**. The object appears on the current image and can be moved if required.

Show Clip Gallery

This option shows the Clip Gallery viewer. Here you can see all the images/data you have sent to the clip gallery for this analysed image.



From here you can Copy or Export the information to any program you wish from the drop down menu on each of the items in the gallery

Object menu

Multiplex

The **Undo** and **Redo** commands are applied to all channels of a multiplex image.

Undo

Use this command to undo the last action taken in sequence; the action can be Create, Delete, Move, Edit, or Properties.

Redo

Use this command to redo the last previously undone action taken in sequence; the action can be Create, Delete, Move, Edit, or Properties.

Cut

Use this command to cut a line or shape from the active Image window.

Copy

Use this command to **copy** the line or shape selected in the active Image window to the clipboard. The copied line or shape can be pasted into another position on the image or onto another image.

Paste

Use this command to **paste** the cut or copied line or shape into another position on the image or onto another image in the same position. The software resizes the line or shape if the scale of the image is different.

Duplicate

After you draw an object on the image, for example a Polygon, you can duplicate the object and place a copy in a new location.

You can reposition the object in a new location on the image, and the software calculates that area.

Repositioning an object

1. Position the pointer over the outline of the object. The pointer changes to  showing that you are in the correct position.
2. Left click to display the handles of the object.
3. Drag the object to the new location, and then release the mouse button.

Delete

Use this command to **delete** a selected line or shape in the Image window.

Select All

This command is used to select all the objects that appear on the image.

Invert selection

Use this command to reverse objects that are selected and those that are not. For example, if you have three objects drawn on the image and one of them is selected, applying this feature selects the remaining two objects and deselects the originally selected object.

You can then, for example, move the object(s) to a different area or change the properties of the selected object(s).

Shape Properties

Use this command to define the properties for the current object. For more information, refer to the Shape Definition | Object Editing | **Shape Properties** section.

Show/Hide

Click **Object** | **Show/Hide** to display the Show/Hide objects dialog box. From the list, you can select any object and apply the show or hide command. This feature affects the data shown in the Area window.

Note:
If you hide an object the software does not delete the object.

Group Selection

If you have multiple *area* or *line* objects drawn in the Image window, you can group them. Grouped object properties can be changed in the same way as single objects. For more information on changing properties, refer to the Shape Definition | Object Editing | **Shape Properties** section.

To group objects, press the **Ctrl** key and click each object in the Image window that you want to include in a group. When selected, each object displays handles (small squares). With all desired objects selected, click **Group Selection** from the Object menu. A dotted rectangle appears around the grouped objects.

Ungroup

Use this command to change a group of objects into individual objects.

1. Position the pointer over an object, the pointer changes to  .
2. If you click an object that is part of a group, a dotted rectangle appears, identifying a group.
3. Click **Ungroup** from the Object menu.

Alignment

Use this command to change the object's format in the following ways: Left, Right, Top, Bottom, Top/Bottom Centre and Left/Right Centre.

You can space a group of three or more objects equidistantly using either the Space Across or Space Down commands. You must select at least 3 objects for this feature to work. Objects cannot overlap. If the objects overlap a message appears and the software cancels the action.

The formats are:

Space Across - All selected objects are spaced out evenly between the leftmost and rightmost objects.

Space Down - All selected objects are spaced out evenly between the top and bottom objects.

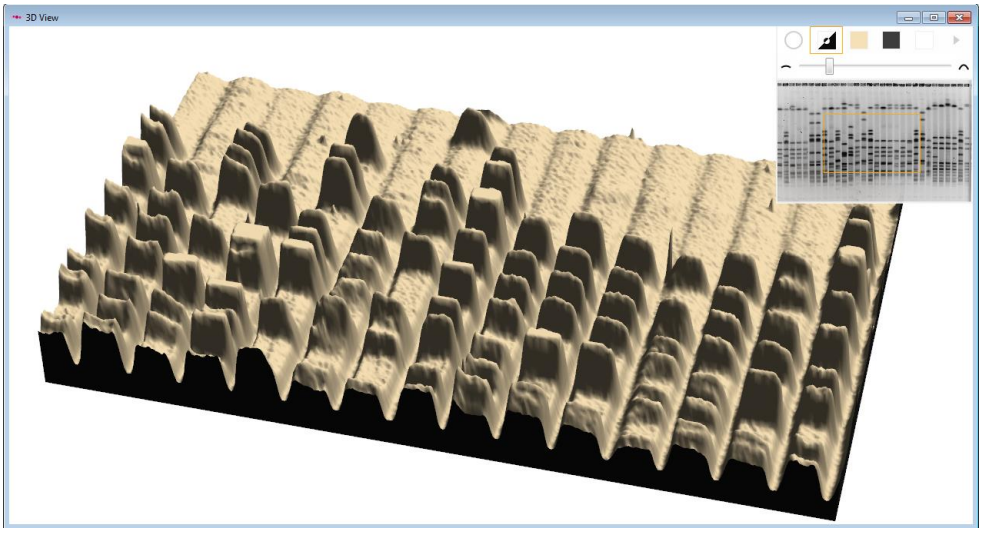
Renumber Selection

This option renumbers the selected objects from left to right

3D View menu

Show 3D View

This option allows you to bring up an independent 3D view of the current image being displayed



Each window is separate so you can call up as many as you wish from the same image or different images.

Contrast

The image is created using the current contrast settings not the raw image values so changing the contrast before opening the 3D View will give slightly different results

Options

In the top right corner are the view options. They include

- Continuous rotation
- Invert image
- Change colours for surface, edge and background
- Increase peak sizes
- Change AOI of view

Help menu

Help is provided both as online Help and online Help request links. The commands available from this menu are discussed in the following sections.

Contents

Displays the online Help as a PDF manual.

Restore Default Image

Restores the default image used by the module when no others are available.

About

Displays the name and version number of the software together with the registration information.

The Program Windows

The main window contains three smaller windows. Each of these windows contains a different type of information.

The windows can be moved and resized via the splitter bars between them.

Scrolling windows

Scroll bars are used when the information being displayed is too large to fit on the screen.

The scroll bars are located at the bottom and right side of the image window. They work in the same way as normal scroll bars but allow you to see the range that you are viewing.

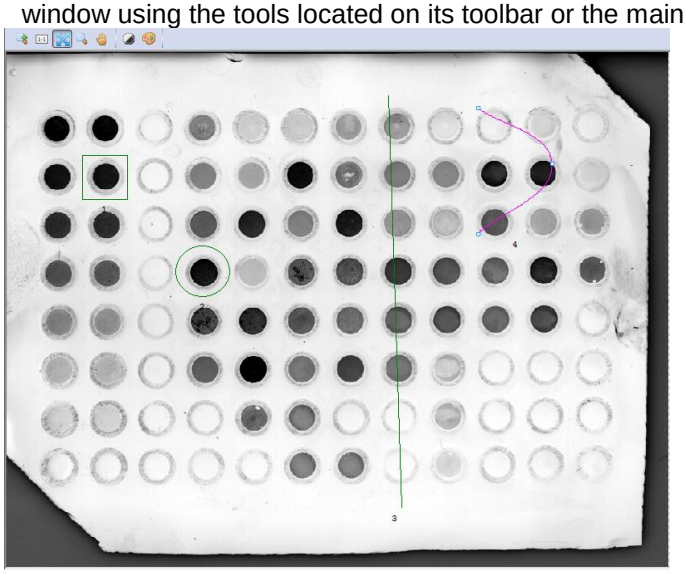
In addition, dragging the scroll box changes the start position of the range you are viewing; you can also resize the scroll box. Since the size of the scroll box reflects the amount of data that is shown in the associated window, resizing the scroll box changes the display magnification. Therefore, if you are viewing an image and make the scroll box smaller, less of the image is used to fill the window and you see the image at an increased magnification.

To resize the scroll box, position the pointer on an edge of the scroll box, the pointer changes to ↔ or ↕. Drag the pointer to the new position.

When the scroll box indicates a display of the total range of the scroll bar, the scroll box becomes transparent. When it indicates that only a portion of the range is visible, the scroll box becomes opaque.

Image window

The Image window is the most important of the windows and displays the image you are currently analysing. The image is overlaid with information determined by the tool you are using and how far through the analysis process you are. Most of your interaction with the software is done within this window using the tools located on its toolbar or the main dialog to the left.



Multiplex

Background shapes are not visible on multiplex images when the channels are displayed in the *Channel Overlay view* mode. Therefore, you cannot Create, Edit or Delete background shapes in this viewing mode.

As with all of the modules, most of the interaction with the software is done within this window using the tools located in the dialog.

Context menu

Right clicking a shape or line within the Image window accesses the context menu. For information on the *Cut*, *Copy*, *Paste*, *Duplicate* and *Delete* commands, refer to the Toolbox | **Edit menu** section. For information on *Properties,* refer to the Shape Definition | Object Editing | **Shape Properties** section.

Single viewing mode

When in single channel view mode, this window works in the same way as it does when a non-multiplex image is loaded.

Any one of the channels of a multiplex image can be selected and viewed in this window by clicking the channel button on the image window toolbar.

Overlay viewing mode

In overlay viewing mode, any combination of channels can be viewed in this window.

When in the Overlay viewing mode certain features of the software become unavailable – these are specific to the module and mode. For more information refer to the relevant analysis mode.

The Image window toolbar

The Image window contains a toolbar with the following buttons:

 **Zooming an image**

In this window the button toggles with the Zoom to Fit button undoing any zooming effects.

To zoom the image:

1. Click the zoom button
2. Position the pointer on the area you want to zoom.
3. Drag the pointer over the desired area.

Alternatively, click the zoom button then left click to zoom in and right click to zoom out.

Pressing **Esc** on your keyboard cancels the zoom mode.

 **Zoom 1:1**

Adjusts the magnification of the image to its original size.

 **Zoom to Fit**

Clicking the **Zoom to Fit** button if the image is zoomed, fits the image to the size of the window.

The Zoom to Fit button counteracts any magnification made on an image using the zoom button.

 **Magnify**

Allows you to magnify specific parts of an image.

1. Click the magnify button.
2. Position the pointer over the part of the image you want to magnify. The pointer changes to a small magnifying glass.

3 Hold down the mouse button. The area under the pointer appears in a box.

4 Drag the pointer to view other areas.

 **Panning**

Allows you to move the image around within the Image window when the image is zoomed.

1 Click the Panning button

2 Position the pointer over the image; it changes to a small hand  .

3 Drag to change the view.

 **Contrast**

Click the **Contrast** button to display a dialog box that provides a number of ways to alter the brightness and contrast of an image. For more information, refer to the **Contrast** section

 **Colour**

Click the **Colour** button to display a dialog box that provides a number of ways to alter the base colour of an image or the individual channels. For more information, refer to the **Colour** section

 **Channel buttons**

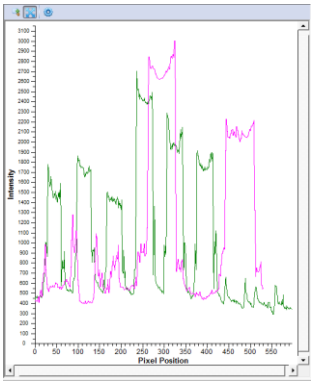
Click the channel buttons to select the channel or channels that display in the main image window. Each channel of a multiplex image enables an extra button

Line window

This window displays the analysis of line objects.

To display data for the current channel, of a multiplex image select *Single Channel view* mode.

Each plot on the graph has the same colour as the line it represents in the Image window. To change a line colour, refer to the Shape Definition | Object Editing | **Shape Properties** section.



Position the pointer over the graph in the Line window. The pixel position on the graph is highlighted in the Image window.

The Line window toolbar

The Image window contains a toolbar with the following buttons:

Zooming an image

In this window the button toggles with the Zoom to Fit button undoing any zooming effects.

To zoom the image:

1. Click the zoom button
2. Position the pointer on the area you want to zoom.
3. Drag the pointer over the desired area.

Alternatively, click the zoom button then left click to zoom in and right click to zoom out.

Pressing **Esc** on your keyboard cancels the zoom mode.

Zoom to Fit

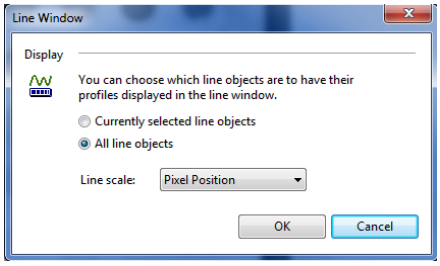
Clicking the **Zoom to Fit** button if the profile is zoomed, fits the profile to the size of the window.

The Zoom to Fit button counteracts any magnification made on an image using the zoom button.

Options

Allows you to edit the display options for the window. See the **Line Window Options** section for details

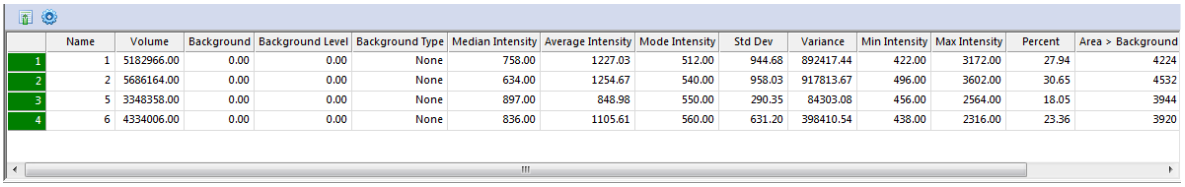
Line Window Options



This dialog allows you to choose which line objects have their profiles displayed in the Line window. Click either **Currently selected line objects** or **All line objects**.

It also allows you to choose a scale for the line window.

Measurements window



	Name	Volume	Background	Background Level	Background Type	Median Intensity	Average Intensity	Mode Intensity	Std Dev	Variance	Min Intensity	Max Intensity	Percent	Area > Background
1	1	5182966.00	0.00	0.00	None	758.00	1227.03	512.00	944.68	892417.44	422.00	3172.00	27.94	4224
2	2	5686164.00	0.00	0.00	None	634.00	1254.67	540.00	958.03	917813.67	496.00	3602.00	30.65	4532
3	5	3348358.00	0.00	0.00	None	897.00	848.98	550.00	290.35	84303.08	456.00	2564.00	18.05	3944
4	6	4334006.00	0.00	0.00	None	836.00	1105.61	560.00	631.20	398410.54	438.00	2316.00	23.36	3920

This window displays the measurements for the area objects

The Name and Comment of an area object can be edited using the Properties dialog box.

Multiplex

To display data for the current channel, of a multiplex image select *Single Channel view* mode.

Data Fields

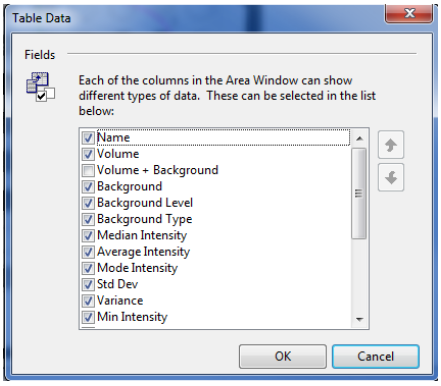
Allows you to edit the displayed fields for the window. See the **Measurement Window Fields** section for details

Options

Allows you to edit some display options for the window. See the **Measurement Window Options** section for details.

Measurement Window Fields

This dialog allows you to select which fields you want to display in the Area window, and the order in which you want the fields displayed. To display any of the fields select its check box. Click the up and down arrows to change the order of the selected fields.



Within the software you can select the various data fields that will be displayed in the Measurements window. You can also change the order of these fields by use of the arrows.

To select or deselect a field for display:

Left click the box to the left of the name in the list of fields.

To change the order of the displayed fields:

1 Select the field to move by highlighting the name in the list of fields.

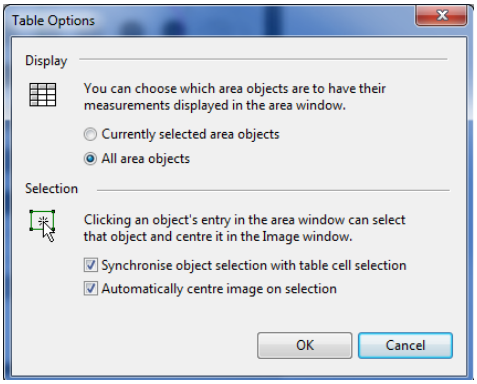
2 Click on the **Move Up** or **Move Down** buttons until the field is in the desired position in the list.

Data Fields

This section discusses data field availability. You will find a brief description of each field in the table below.

Data Field name:	Description
Area	The area of the Band, Spot, Colony or other image feature displaying the number of pixels quantified in the image in order to produce the measurements.
Area > Background	Area > Background is the number of pixels which have an intensity greater than the background level.
Average Intensity	The volume of the image feature divided by its area.
Background	The total background for the Band, Spot, Colony or other image feature.
Background Level	The average background intensity in the Image Rectangle used in the background subtraction method of the Toolbox module.
Background Type	The method of background subtraction used.
Centre (X and Y)	The X, Y position of the centre of a given shape, relative to the top-left of the image (position 0,0).
Comment	Displays the text you typed into the data field.
Height	Shows the height in pixels of the bounding rectangle of the shape.
Max Intensity	The maximum pixel intensity in a given area.
Median Intensity	The median pixel intensity in a given area.
Min Intensity	The minimum pixel intensity in a given area.
Mode Intensity	The most common pixel intensity for a given area.
Name	The name of the shape object. Note: *The name displayed in the table can be changed using in-place editing or the Properties dialog box.*
Percent	The percentage of total volume for all area objects represented by a given object.
Std Dev	The standard deviation of the pixel intensities within a given area.
Variance	The variance of the pixel intensities within a given area. Population variance as opposed to the Sample variance.
Volume	The raw volume of the uncalibrated quantity of material in the image feature **after** the background intensity has been removed.
Volume + Background	The uncalibrated quantity of material detected before the background intensity has been removed.
Width	The width in pixels of the bounding rectangle of the shape.

Measurement Window Options



This dialog allows you to choose which area objects to display in the Area window. Click either **Currently selected area objects** or **All area object**s. You can also synchronise the object selection with the table cell or automatically centre an area object in the Area window.

Status bar

The status bar is the thin bar along the bottom of the main window. It displays information about the current state of the program (e.g. Ready) as well as descriptive messages for a selected command or toolbar button (e.g. Co-ordinates (Pixels): nnn Pixel Value: nnn).

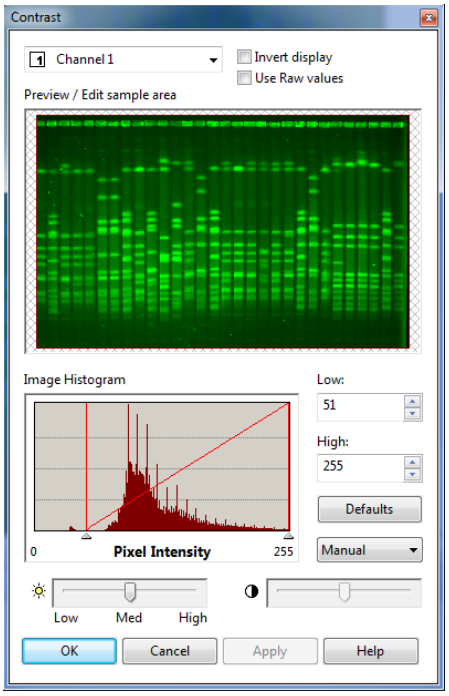
Contrast

The Contrast controls allow you to alter how the selected channels look on screen.

This dialog box is used to change the contrast and brightness of the image.

Multiplex

When you are viewing multiple channels, you can switch to one of the other selected channels from the drop down list. The contrast is edited on a per channel basis.



Preview / Edit sample area

The Preview / Edit sample area displays a thumbnail of the entire image. If the image displays a rectangle on it, known as the area of interest (AOI), the palette is calculated from the AOI area. The default rectangle covers the entire image.

You can change this rectangle by clicking and dragging out a new rectangle; this causes the image to update immediately.

This feature can be very useful when editing very faint parts of the image, as you can set the AOI to a very small area, which produces a very good colour standard in this area.

Image Histogram

The Image Histogram displays the frequency with which each pixel intensity occurs within the area of interest. The peaks on the graph represent the pixel intensities that occur most frequently within the current area of interest.

The left and right red bars on the graph show the range of pixel intensities in the image that will be mapped to different colours in the display image. You can directly modify the brightness and contrast of the display by dragging the bars on the graph.

Move these handles to increase/decrease the contrast.

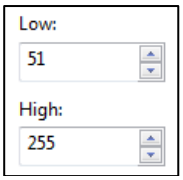
The figures below the image histogram represent the scale of the graph, i.e. the calibration.

You can restore the default contrast at any time by clicking the **Defaults** button.

Low / High edit boxes

The **Low** and **High** edit boxes are synchronised with the two handles below the image histogram.

These edit boxes allow you to enter values to adjust the contrast of your image.



This enhances the contrast display so that for narrow contrast ranges, a sufficient number of grey levels are used giving a higher quality of image display.

You can restore the default contrast at any time by clicking the **Defaults** button.

Brightness

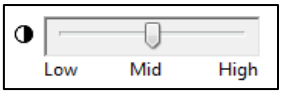
The two sliders at the bottom of the dialog box allow you to adjust the brightness and contrast of the image by moving them between Low, Medium and High.

Drag the slider to change the brightness. High = paler, Low = darker.

Contrast response list

Below the **Defaults** button there is a drop down list. Select the shape of the contrast response curve to help you visualise the features of your image.

Manual - a straight line.
Hi-contrast – a curve calculated from the image to maximise the contrast.
Curve and **Sigmoidal** – the standard shapes of a response curve, adjustable by using the Contrast slider.



The Contrast slider affects the shape of the curve that extends between the contrast limits.

Drag the slider to change the contrast. High = more effect.

Invert display

This check box indicates whether the current colours should be inverted or not; click it to toggle the option on or off. Inverting an image causes it to display like a photographic negative. For example, using the default **Grey Scale** colour scheme white becomes black and vice versa.

Use Raw Values

This check box indicates whether the histogram is calculated after any scanner calibration, the default, or just uses the raw values of the tiff image. Using the raw values will usually increase the contrast.

Auto Default Contrast

By default the software looks at the histogram results and optimizes the default low and high values for contrast stretching. This means you can see more of the important features of the image. If you wish this turned off you can see the image default as stretched from 0 to the maximum in the image. This is a global variable set for all future default display but does not alter the current image unitl you press **Defaults**

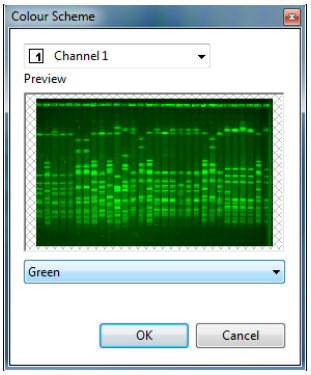
Channel Selector

The drop down at the top of the dialog allows you select which channel you wish to change the contrast for.

Colour

The Colour controls allow you to alter how the selected images look on screen.

The **Preview** shows a thumbnail of the entire image and displays the effects of any changes.



Multiplex

When using multiplex gels you can only launch the Colour Scheme dialog box when in *Multiple Channel viewing* mode. The colour that you choose, from the colour scheme drop down list, is used to change the colour for the channel in *Overlay viewing* mode only.

In *Single Channel viewing* mode the image will be displayed in grey only and cannot be changed.

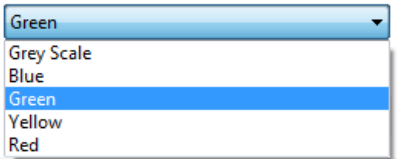
The default choice of colours used to display each channel is dependent upon how many channels there are in the image.
2 Channel images: 1 = green, 2 = red

3 Channel images: 1 = blue, 2 = green, 3 = red

4 Channel images: 1 = blue, 2 = green, 3 = yellow, 4 = red

Colour Scheme List

This is a drop down list box containing the names of all of the installed colour schemes on the system.



Of the various colour schemes that are supplied with the software, two are *false colour* schemes; these can be useful for visualising faint spots or bands, because the human eye is often more sensitive to colour than to shades of grey. To change the colour scheme, select a different scheme from the list.

Reverse colours

This check box indicates whether the current colour scheme should be inverted or not; click it to toggle the option on or off. Inverting an image causes it to display like a photographic negative. For example, using the default **Grey Scale** colour scheme white becomes black and vice versa.

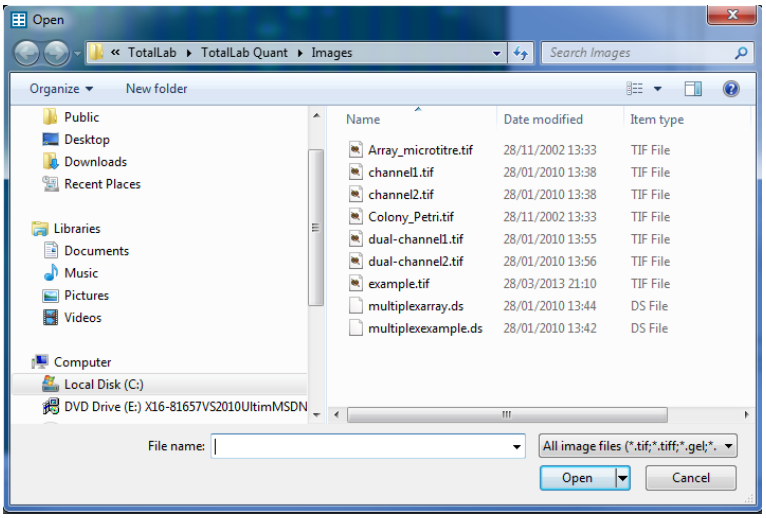
Beginning the Analysis – Open Image

Selecting the **Open Image** command from the main toolbar allows you to start an analysis by Opening an image. It also allows you to Edit an Image, check its properties and Create Multiple Images.

Note:
*If you close the software, open an image or open an existing experiment, the software **automatically saves** the current experiment for you.*

Open Image

You can open an image by clicking the **Open Image** or **Open Multiplex Image** button



To open an image:

1. Select the folder from the *Look in* list.
2. Select the correct file type from the *File of type* list.
3. Click the specific image file from those displayed.
4. Click **Open**. The selected image automatically opens in the Image window allowing you to analyse the image.

Note:
*If you have an **RGB** Colour image it can be opened as a regular image (converted to greyscale) or as a multiplex image (all 3 colours split into 3 channels)*

Edit Image

This option allows a wide range of editing options. Please see the **Image Manipulation** section for more details.

Image Properties

The image properties dialog box has three tabs, File info, Scan info and History. The information in these tabs is read only.

The File info tab displays information about the image, for example the File Name, Size, Image Width and Image Height.

The Scan info tab displays information relating to the scanning of the image, for example the Scanner make, model, date and time of scan and the resolution. It also shows any comments from the scanning.

The History tab displays the name of the image and a scrollable list of any editing made to the image, for example Rotate Clockwise 90.

Shape Definition

In this mode, the dialog displays the objects in two categories, areas and lines. It also displays the Selector and Autotrace tools.

While creating, moving, or resizing any of the objects, you can press the **Esc** key to cancel the action.

Multiplex

Each channel of a multiplex image has an independent set of shapes. Shapes can only be created, edited or deleted in *Single Channel view* mode – these functions are not available in Channel Overlay View mode. When you delete all the objects using the **Clear** button in the dialog, only the objects on the current channel are deleted.

Invert Intensity

Most images have high pixel values (high intensities) in the black areas of the image and low values in the white areas. However, in some images, the spots have a low intensity (and therefore low volume) compared to the background. The "Invert Intensity" command allows you to correct this by inverting all the pixel values before using them to calculate the measurements.

To invert a pixel value, the software subtracts the value from the maximum possible pixel value in the image. For example if the image has a pixel value range from 0 to 255 and the software inverts a pixel that has a value of 50, the inverted value would be (255 - 50) = 205.

To invert the measurements data, click **Invert Intensity** option**,** and then check the data in the measurements window.

Areas

The measurements for *area* shapes are displayed in the Area window. The Areas category has five specific shapes.

Rectangle

The rectangle shape allows you to measure any rectangular area on an image. Click the **Rectangle** button  and drag to create a rectangle.

Polygon

The polygon shape allows you to measure any polygonal area on an image. Click the **Polygon** button  ; position the pointer where you want to start the first side of the polygon and click. Continue dragging and clicking to add new points to the polygon. When you have completed the polygon, right click to add the final point, which connects the line back to the first point, closing the polygon.

Ellipse

The ellipse shape allows you to measure any elliptical area on an image. Click the **Ellipse** button  and drag out an area.

Closed Spline

To create a closed spline click the **Closed spline** button , and then use the same method as for creating a polygon – the only difference is that the points are connected by a smooth curve instead of a series of straight lines.

Grid

Click the **Grid** button to display the Grid Dimensions Rows and Columns Entry boxes.

Type the number of rows and columns for the grid, and then click **OK**.

Draw out a grid over the image. Each cell is measured independently and displayed in the Area window.

Lines

The measurements for lines appear in the Line window. You can choose among four types of line.

Line

Click the **Line** button and then drag to draw a straight line between two points.

Polyline

To create a connected line between two or more points use the polyline feature. Click the **Polyline** button and drag to create the first point of the polyline. Continue clicking and dragging to add new points to the polyline. When you have completed the polyline, right click to add the final point.

Spline

To create a smooth curve between three or more points on the image use the spline feature. To create a spline, click the **Spline** button and then use the same method as for a polyline.

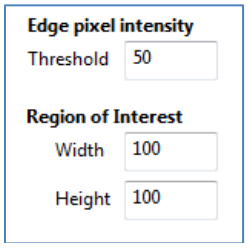
Freehand

The freehand feature allows you to create a single line of any shape.

Click the **Freehand** button and drag a line on the image. The freehand line follows any movement you make with the pointer in the Image window.

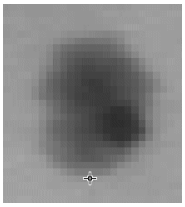
Autotrace

Click the **Autotrace** button , and then the autotrace parameters appear

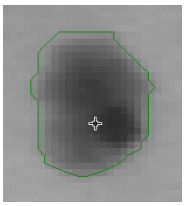


1 *Zoom* into an area of the image using the zooming tool .

2 To set the Edge pixel intensity threshold **right click** the outer area of the feature, as in the example below or type the threshold directly in the threshold text box.



3 Click the centre of the feature.



The software automatically draws the autotrace shape.

If the Autotrace object is not drawn as expected, try a different threshold.

Selector

The *Selector* feature is an editing sub-mode. To activate this feature click the **Selecto**r button  in the dialog. The *Selector* feature remains active until you select another feature.

To select a single object or many objects use either of the following methods:

Click on a single object.

Press the **Ctrl** key and click on the outlines of several objects.

Drag out an area surrounding all the objects you want to select.

With the object or group of objects selected you can then perform any of the following editing commands:

Change the properties of the objects

Reposition the objects as a group

Use any of the editing commands: *cut*, *copy, paste, duplicate* or *delete*

To resize an object you must select the object individually.

To **deselect** an object or group of objects, press the **Ctrl** key and click any object or group not required.

Object Editing

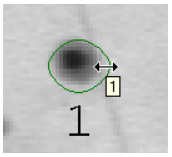
After you have created an object on your image, you can resize and move the object or change the properties using the Selector tool.For more information, refer to the following sections.

Resizing and moving objects

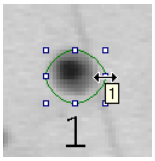
Click the **Selector** button  in the dialog to activate the editing sub-mode.

Resizing an object

1 Position the pointer over the edge of the object you want to resize. Depending upon what shape of object you are editing the pointer changes to either ↕ or ↔ when you are over a movable handle.



2 Click and drag the handle to a new position and release the mouse button.

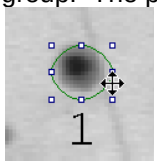


At any time while dragging, you can press the Esc key to cancel the action and restore the object back to its original location.

Handles of freehand lines or in multiple selections cannot be moved.

Moving an object or group of objects

1 Click the boundary anywhere other than a movable handle, of an individual object or object group. The pointer changes to ✥.



2 Drag the object or group to the new location on the image.

Moving multiple objects

1 Press the **Ctrl** key and click each object.

2 Drag the selection of objects to the new location on the image.

Undo

Use the **Edit** | **Undo** command to undo any object editing (for example name, size, colour or delete). This feature allows you to safely experiment with the various methods of editing objects.

Redo

The **Edit** | **Redo** command allows you to redo any operation that you have previously undone.

Clipboard Commands

While creating shape objects, you might want to use the same object several times. There are two ways to create a copy of the selected objects.

Use the **Edit** | **Copy** command. This places a copy of the selected objects into the clipboard. Use the **Edit** | **Paste** command to paste the selection into another image or back onto the same image.

Or, create a copy of the selected objects for use on the same image, by using the **Edit** | **Duplicate** command. This places a copy of the object on top of the original, that can be moved.

Shape Properties

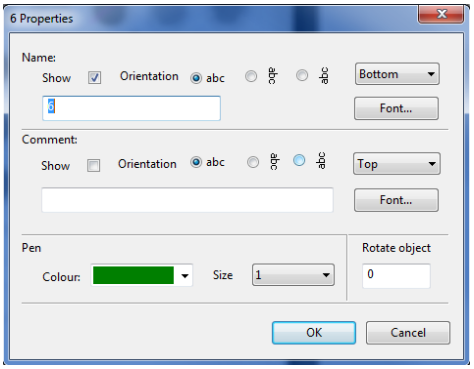
You can use the Properties dialog box to:

Edit the objects name, enter a comment, and choose to show the text or not.

Change the font and orientation of the text.

Change the pen size and colour used to draw the shape object.

Rotate the object around its centre of gravity by changing the number of degrees by which it is rotated.



To access the **Properties** dialog box you can either:

Click on the object and then click **Shape Properties** from the Object menu

Click on an object and press **Alt**+**Enter**

Select **Properties** from the context menu after right clicking an object

Double click on an object, when over an object boundary.

Background Subtraction

Background subtraction is the process by which you remove the part of the image's pixel intensity that is background from the measurements.

To begin the process click the **Background Subtraction** button from the main toolbar.

You can choose to assign the same background method to all shapes or just the selected ones. For just the selected ones

1. Make sure the option to **Apply to**: is set to Selected Shapes
2. Click the **Selector** button  in the dialog.
3. Click an individual object in the Image window. Alternatively, you can select multiple objects by drawing an area around a group of objects.
4. Click a background subtraction method option in the dialog.
5. View the data in the Measurements window.

For assigned to all Shapes

1. Make sure the option to **Apply to**: is set to All Shapes
2. Click a background subtraction method option in the dialog.
3. View the data in the Measurements window.

Multiplex

Each channel of a multiplex image has an independent set of objects that are used when subtracting the background of the objects.

You can only create, edit or delete objects when the *Single Channel view* mode is selected. You will notice that existing background objects are not visible in the *Channel Overlay view* mode.

Only the objects on the current channel are deleted when you click the **Clear** button in the dialog.

Select each channel individually, and then click **Subtract** to remove the background of the objects from the current channel.

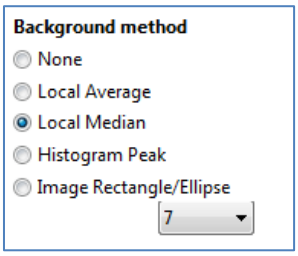
Allow Background Intensity higher than object intensity

By default when you subtract the background level from each pixel in an object you simply take the result, positive or negative, and add them together to get the final volume. So for example if the object has 2 pixels of value 10 and 15 with a background level of 11 then the resulting volume is 10-11 = -1 and 15-11=4 added together = 3.

However this may not be a valid option for you so you can instead force the lowest value, after background removed, to be 0. So in the previous example the first value would not be -1 it would be 0 and the result would be 4.

Background subtraction methods

The dialog contains the following background subtraction methods.



Note:
You can apply different background subtraction methods to different objects. The measurement tables reflect each type.

None

Use the **None** method to turn off background subtraction. The calculated raw volume will be the sum of all pixel intensities in that area.

Although this method can be useful, it is not recommended for good laboratory practice.

Local Average

The local average method determines the mean of all the pixel values in the object outline and uses this value for the background.

Local Median

The local median method determines the median of all the pixel values in the object outline and uses this value for the background.

Histogram Peak

The histogram peak method determines the background of an area by selecting a strip, from top to bottom of the image, equal to the object width and calculating the modal value of the pixels. The value that occurs most often within this area is used as the background value.

Image Rectangle/Ellipse

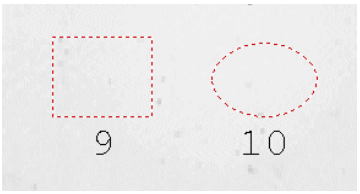
The Image Rectangle/Ellipse method requires you to specify a rectangular or elliptical area that best represents the background intensity of the image.
The mean intensity of the pixels of the area is used to calculate the background intensity.

Specifying a Rectangle or Ellipse background area

1 Click either the **Rectangle**  or **Ellipse**  button in the dialog.

2 Position the cursor  on the background area of the image.

3 Drag an area in the image, the cursor changes to  . A dotted line identifies the area, with the drawing object sequence number below it.



4 Click the Selector button in the dialog.

5 Click an individual object in the Image window. Alternatively, you can select multiple objects by drawing an area around a group of objects.

6 If you have more than one rectangle or ellipse shape, select a shape from the pull down list below the Rectangle/Ellipse method.

7 Click the Image Rectangle/Ellipse button in the dialog. The data in the Measurements window automatically updates.

Resizing a Rectangle or Ellipse background area

1 Click the **Selector** button in the dialog.

2 Position the pointer over the *shape* boundary, click to select the shape. The pointer changes to a two-headed arrow ↔ when positioned over a handle.

3 Drag the handle to a new position.

At any time while dragging, press the **Esc** key to cancel the action and restore the area back to its original location.

Moving a Rectangle or Ellipse background area

1 Click the **Selector** button in the dialog.

2 Click a *Rectangle* or *Ellipse* boundary anywhere other than on a handle. The pointer changes to ✥.

3 Drag the *Rectangle* or *Ellipse* to a new location.

Annotations

Using the Annotations mode, you can add text labels with optional arrows to precisely locate and identify certain areas of an image.

You activate the Annotations mode by clicking the **Annotate** button on the *main toolbar*, or

Create annotations on an image to highlight particular areas of interest. There is no limit to how many annotations you can create in any one image or channel.

Multiplex

Annotations can be created and displayed in multiplex gels regardless of the number of channels or the current view mode. However, it should be noted that you can only create one set of annotations per multiplex gel.

Manual Annotations

To change the annotation style just look through the dialog which contains a variety of options. For example, to change the font click the **Select Font Style** button.

After changing the annotation style, the changes you make are applied to all annotations that are presently selected and any newly created annotations.

Adding and Editing Annotations in the Image window

The following section describes how to use the mouse and the keyboard to Add, Move, Select and Edit the text of annotations in the Image window.

Multiplex

Annotations can be viewed and edited in both single and overlay modes.

We assume that you are using the default settings for annotations when doing the following actions.

Adding an annotation

To add an annotation to the image shown in the image window:

1. Position the pointer over the location in the Image window that you want to annotate. The pointer should be the standard arrow, indicating that you are not over any other items in the window.
2. Left click to add an annotation box. An anchor arrow appears with an attached text entry box, pointing to the location.
3. Type your text into the text entry box.
4. Press **ENTER** to accept the text or press **Esc** to stop editing without accepting any text. You will be able to enter text later.

You have now added an annotation that is indicated as the currently selected annotation by an outline box. You can add more annotations by repeating the above steps.

As an alternative to clicking the image to add a comment, you can position both the arrowhead and the text box of your annotation with a single mouse interaction.

1. Position the pointer at the location where you want the arrowhead, left click and hold down.
2. Move the pointer to the position where you want the text to be centred. As you drag the pointer, the annotation text entry box appears and stretches away from the arrowhead.

3 When you release the button, the text entry box changes to the in-place editing mode. Type in your text and press **Enter**.

Moving annotations

To move an annotation:

1 Position the pointer anywhere on the line between the arrowhead and annotation text box. *You might need to move the text box to see the line.*

2 The pointer changes indicating that you are in position. If this does not happen, you can use the ***zoom in*** function to increase the amount of line available, to pickup the annotation.

3 Drag the annotation to its new location.

Moving part of an annotation

You can also move the position of an annotation anchor arrow or text box.

1 Position the pointer over the anchor arrow or text box you want to move. The pointer changes to, a crosshair for the anchor arrow and the text box becomes outlined.

2 Drag the arrow or text box to the desired location.

Selecting annotations

To select a single annotation you can:

Click on either the arrow, the line between the arrow and the text box, or the text box.

Press the **TAB** key to move between annotations.

Select all

To select **all** the annotations you have created, **right click** anywhere in the image away from any annotation. The context menu appears allowing you to select all the annotations.

Deselect all

To **deselect all** the annotations **right click** anywhere in the image away from any annotation. The context menu appears allowing you to deselect all the selected annotations.

Arbitrary selection

To make an **arbitrary selection** of annotations, **left click** on the first annotation and then **hold down** the **CTRL** key on your keyboard. You can now **click** on each annotation you want to include in your selection. **Right click** in the image for your next action.

To arbitrarily deselect an annotation, **hold down** the **CTRL** key on the keyboard and then **left click** on the annotation.

Editing the text of an annotation

After adding annotations, you can edit the text in the annotation by right clicking the outlined text box and selecting Edit text from the context menu or, left clicking the text box.

While editing annotations, an outline box appears around the selected text box. This outline box does not appear on any image printouts. Annotation boxes are not included on report printouts.

Annotation Fonts

Clicking the Select Font Style button, displays the Font dialog box that allows you to set the font style for your annotations.

Delete selected Annotations

The **Delete Selected** button deletes any currently selected annotation. If you continue to click this button, the annotations will all be deleted in the reverse order that they were added.

Alternatively, position the pointer anywhere over an annotation and right click, then select Delete from the context menu.

There is no **undo** command. If you accidentally delete an annotation, you will need to add it again.

Setting annotation options

The dialog contains the following options.

Text outline style

Select the shape of the border around the text of your annotations from the drop-down list. You can select a rectangle, rounded rectangle or no border.

Fill Colour

Select the colour for the background of the text area of your annotations from the drop-down list. This option is disabled if you have chosen a transparent background.

Line thickness

Select the weight of the lines that is used to draw the anchor arrow of the annotations.

You can choose one of size line thickness or No line.
If you choose No line, your annotation appears as a moveable text box only.

Colour

Select the colour that is used to draw the arrow line and outline of the annotation text box. The default outline colour is green.

Opaque Background

Select the check box if you want the text of the annotation drawn over a coloured background, or clear the check box if you want the text drawn directly onto the image.

Text Orientation

Choose the orientation of the text within the annotation.

Colony Counter

Introducing Colony Counter

You use the Colony Counter module to analyse images by detecting and measuring image features, 2D spots, or colonies. You can display and print measurement data from the detected colonies.

To help you along the way, where relevant, pictures are included in the section showing what you should expect to see on the screen.

The procedures provided in this section are basic examples of how to analyse colonies. You can fine-tune your analysis using the various editing tools.

The Colony Counter module is supplied with two image data files of previously saved data stored in the TIFF file format. The image, used in this section to describe the various features, can be restored if you want to follow the procedural steps. To restore the image click **Restore Default Image** from the **Help** menu.

Note:
The Colony Counter module does not have the capability of opening multiplex images.

Main menu bar

The Main menu bar, located at the top of the main window, provides the majority of commands and options available in the software.

File menu

From the File menu, you can perform the following actions.

Note:
If you close the module, open an image or open an existing experiment, the software ***automatically saves*** *the current experiment for you.*

Open Image

The **Open Image** command displays a dialog box that allows you to locate and open an image file. The same dialog box appears if you click the **Open** button on the toolbar. For more information refer to the section about **Opening an image**.

Invert Measurements

Most images have high pixel values (high intensities) in the black areas of the image and low values in the white areas. However, in some images, the spots have a low intensity (and therefore low volume) compared to the background. The "Invert Measurements" command allows you to correct this by inverting all the pixel values before using them to calculate the measurements.

To invert a pixel value, the software subtracts the value from the maximum possible pixel value in the image. For example if the image has a pixel value range from 0 to 255 and the software inverts a pixel that has a value of 50, the inverted value would be (255 - 50) = 205.

To invert the measurements data, click **File | Invert Measurements,** and then check the data in the measurements window.

Image Properties

The image properties dialog box has three tabs, File info, Scan info and History. The information in these tabs is read only.

The File info tab displays information about the image, for example the File Name, Size, Image Width and Image Height.

The Scan info tab displays information relating to the scanning of the image, for example the Scanner make, model, date and time of scan and the resolution. It also shows any comments from the scanning.

The History tab displays the name of the image and a scrollable list of any editing made to the image, for example Rotate Clockwise 90.

Print

The Print dialog box allows you to select a printer, print range and number of copies.

To print the image and/or the measurements data, you need to activate the window.

Print Setup

The Print Setup dialog box allows you to select a printer and its properties. You can select a paper size, source and the page orientation.

Print Preview

The **Print Preview** window displays the image or measurements data from the selected window so you can see how it will be printed.

Clicking the Preview button on the main toolbar also displays the Print Preview window.

Printing Options

The **Printing Options** command displays the Options, Reporting tab that allows you to amend the print range and scale. For more information refer to View menu | Options | **Reporting Tab**.

The type of dialog box that will be displayed depends on whether a window with an image or with tabular data has been selected for printing.

Alternatively, clicking the **Options** button in the print preview window displays the Options, Reporting tab.

Exit

Click **Exit** to close the active analysis module, and return to the Control Centre window.

Edit menu

The Edit menu contains commands that are common to all modules. Some modules have additional menu items, which are described in the Edit Menu section for that module.

The following sections discuss the common commands.

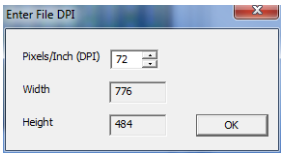
Export to Clipboard

Click **Export to Clipboard** to copy the data or image displayed in the active window to the clipboard, so that the data or image can be pasted into other applications. For example the measurements window data can be pasted into a spreadsheet. Alternatively, clicking the **Export** button on the toolbar copies the data or image.

Export to File

Clicking **Export to File** displays a dialog box that allows you to enter a folder and filename.

If you are exporting an image, the default filename is Export.bmp. You are also given the chance to change the DPI of the image saved before naming the file



If you are exporting measurements data the default filename is Export.txt.

The default folder is the CLIQS installation folder.

If you do not want to use the defaults, select the folder in which you want to store the file and type in a name and then press **Save**.

Export to Excel

Export to Excel is only available when the Image or Measurements window is active and if Microsoft Excel is installed on your computer. The data in the Measurements window will be copied directly into an Excel spreadsheet.

Image data (calibrated values of pixel intensities) is also exported directly to Excel. Each cell in the table represents one pixel. Data is exported on a per channel basis.

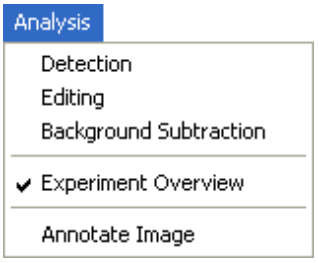
An Excel workbook automatically opens and the measurement data will be pasted into a new workbook.

If Microsoft Excel is not installed, use the Export to File command.

Edit Image

This feature allows you to manipulate the image. For more information refer to the **Image Manipulation** section.

Analysis menu



These tools are discussed in the following sections.

Detection

Editing

Background Subtraction

Annotations

View menu

The View menu contains the following commands.

Zoom to Fit

The Zoom to Fit command can be switched on or off by clicking this command. When switched on, the image will scale up or down to fit the window whenever the image window is re-sized.

Contrast

Clicking the Contrast command displays the contrast dialog box. Alternatively, you can click the Contrast button on the toolbar.

For more information refer to the **Contrast** section.

Invert Colour

Clicking the Invert Colour command simply inverts the colour of the image. Alternatively, you can click the Invert Colour button on the toolbar.

For more information refer to the **Colour** section.

Colour

Clicking the Colour command displays the colour dialog box.

For more information refer to the **Colour** section.

Options

Clicking **Options** displays a dialog box containing various tabs. Each tab allows you to select settings relevant to the active analysis module.

For more information refer to The Common Interface | **Options** section.

Window menu

The Window menu contains two commands. The **Arrange windows** command allows you to open and move the windows into a pre-defined layout. The **Close all Windows** command allows you to close all open windows, but does not close the experiment or the software.

The Window menu also displays the list of the open windows in the module. A tick mark denotes the active window.

Help menu

Help is provided both as online Help and online Help request links. The commands available from this menu are discussed in the following sections.

Contents

Displays the online Help. Alternatively, you can press F1 to access the Help topics.

Restore default Image

Restores the default image relevant to each analysis module.

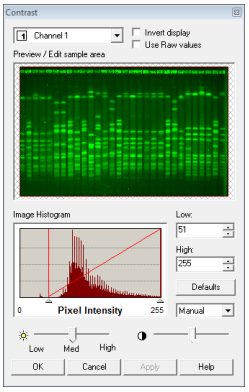
About

Displays the name and version number of the software together with the registration information.

Contrast

Making changes to the Contrast controls only affects the current analysis module. The Contrast controls allow you to alter how the selected channels look on screen.

This dialog box is used to change the contrast and brightness of the image.



Preview / Edit sample area

The Preview / Edit sample area displays a thumbnail of the entire image. If the image displays a rectangle on it, known as the area of interest (AOI), the palette is calculated from the AOI area. The default rectangle covers the entire image.

You can change this rectangle by clicking and dragging out a new rectangle; this causes the image to update immediately.

This feature can be very useful when editing very faint parts of the image, as you can set the AOI to a very small area, which produces a very good colour standard in this area.

Image Histogram

The Image Histogram displays the frequency with which each pixel intensity occurs within the area of interest. The peaks on the graph represent the pixel intensities that occur most frequently within the current area of interest.

The left and right red bars on the graph show the range of pixel intensities in the image that will be mapped to different colours in the display image. You can directly modify the brightness and contrast of the display by dragging the bars on the graph.

Move these handles to increase/decrease the contrast.

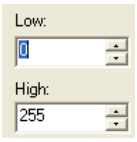
The figures below the image histogram represent the scale of the graph, i.e. the calibration.

You can restore the default contrast at any time by clicking the **Defaults** button.

Low / High edit boxes

The **Low** and **High** edit boxes are synchronised with the two handles below the image histogram.

These edit boxes allow you to enter values to adjust the contrast of your image.



This enhances the contrast display so that for narrow contrast ranges, a sufficient number of grey levels are used giving a higher quality of image display.

You can restore the default contrast at any time by clicking the **Defaults** button.

Brightness

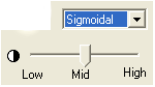
The two sliders at the bottom of the dialog box allow you to adjust the brightness and contrast of the image by moving them between Low, Medium and High.

Drag the slider to change the brightness. High = paler, Low = darker.

Contrast response list

Below the **Defaults** button there is a drop down list. Select the shape of the contrast response curve to help you visualise the features of your image.

Manual - a straight line.
Hi-contrast – a curve calculated from the image to maximise the contrast.
Curve and **Sigmoidal** – the standard shapes of a response curve, adjustable by using the Contrast slider.



The Contrast slider affects the shape of the curve that extends between the contrast limits.

Drag the slider to change the contrast. High = more effect.

Invert display

This check box indicates whether the current colours should be inverted or not; click it to toggle the option on or off. Inverting an image causes it to display like a photographic negative. For example, using the default **Grey Scale** colour scheme white becomes black and vice versa.

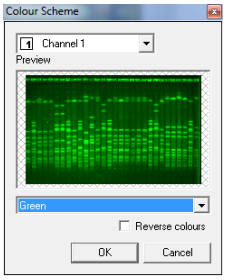
Use Raw Values

This check box indicates whether the histogram is calculated after any scanner calibration, the default, or just uses the raw values of the tiff image. Using the raw values will usually increase the contrast.

Colour

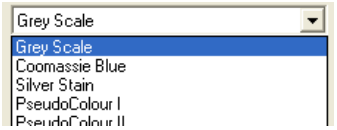
Making changes to the Colour controls only affects the current analysis module. The Colour controls allow you to alter how the selected images look on screen.

The **Preview** shows a thumbnail of the entire image and displays the effects of any changes.



Colour Scheme List

This is a drop down list box containing the names of all of the installed colour schemes on the system.



Of the various colour schemes that are supplied with the software, two are *false colour* schemes; these can be useful for visualising faint spots or bands, because the human eye is often more sensitive to colour than to shades of grey. To change the colour scheme, select a different scheme from the list.

Reverse colours

This check box indicates whether the current colour scheme should be inverted or not; click it to toggle the option on or off. Inverting an image causes it to display like a photographic negative. For example, using the default **Grey Scale** colour scheme white becomes black and vice versa.

Options

The **Options** dialog box displays various tabs; each tab allows you to select settings relevant to the individual analysis modules. To display the Options dialog box click **View** | **Options**. Alternatively, click the **Options** button on the toolbar.

Image tab

The Image tab allows you to change the various display settings in the Colony Counter module.

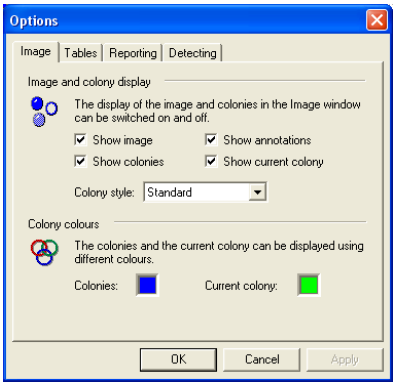


Image and Colony display

By clearing the **Show Image** check box, the gel image of the experiment will only be visible in the Zoom window, and the Image window will display only the detected colonies, after the detection has been performed.

You can hide the detected colonies in the Image window by clearing the **Show Colonies** or **Show Current Colony** check boxes.

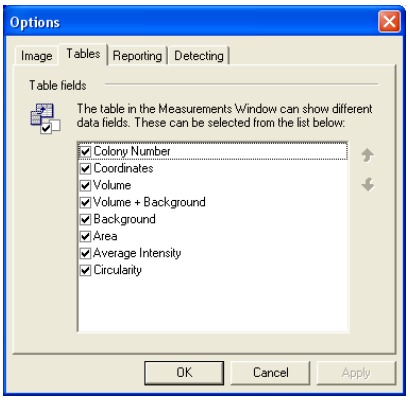
The four choices on the **Colony Style** list are: Standard, Outline, Crosshairs and Bullets.

Colony colours

To change colours, **click** the coloured box next to the item to launch the Color dialog box. Select a new colour and click **OK**.

Tables tab

You use the tables tab to define which data fields you want to display in the Measurements table. You can also change the order of these fields by use of the arrows.



To select or deselect a field for display:

Left click the box to the left of the name in the list of fields.

To change the order of the displayed fields:

1 Select the field to move by highlighting the name in the list of fields.

2 Click on the **Move Up** or **Move Dow**n buttons until the field is in the desired position in the list.

In the Array module each table in the Measurements window can display different sets of data. Only a single field can be selected for the Current Grid table. Therefore the **Move Up** and **Move Down** buttons are not enabled for the Current Grid table. The selected field is displayed on the Measurements table title bar.

Data Fields

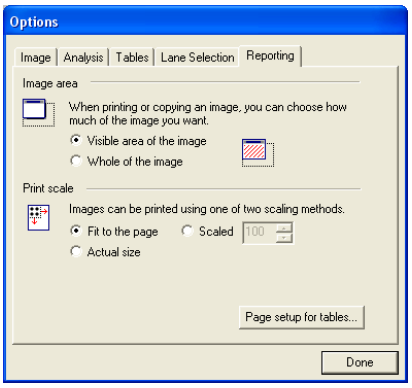
This section discusses data field availability for each module. You will find a brief description of each field in the table below.

Not all data fields are available within the various tables displayed in the Measurements window or Area window of the individual modules. The following table shows the data fields available for each analysis module:

Data Field name:	Description
Area	The area of the Band, Spot, Colony or other image feature displaying the number of pixels quantified in the image in order to produce the measurements.
Average Intensity	The volume of the image feature divided by its area.
Background	The total background for the Band, Spot, Colony or other image feature.
Circularity	A measurement, indicating the roundness of a colony (0-1.0). The closer the value is to 1.0 the rounder the colony is. The main use is to detect a colony that might consist of more than one colony.
Colony Number	The unique number given to a detected colony. Colonies are numbered from top left to bottom right across the selected area. If a detected colony is deleted or partially deleted during the analysis, the colony number is eliminated. In the case of a partially deleted colony, the remainder of the colony is issued with a new identification number at the bottom of the list. To identify any detected colony, position the pointer over the colony and wait a second or two for the number to appear.
Coordinates (X and Y)	Equivalent to the centre of the detected mass, with the coordinates rounded to the nearest integer.
Volume	The raw volume of the uncalibrated quantity of material in the image feature **after** the background intensity has been removed.
Volume + Background	The uncalibrated quantity of material detected before the background intensity has been removed.

Reporting tab

You can customize the way that the content of windows will be copied and printed, from the commands in this tab. This includes changing the area of an image that will be copied or printed, setting the scale at which an image will be printed, and changing the way that tables will be printed.



The settings on this tab affect the Copy to Clipboard and Copy to File commands from the Edit menu.

Changing the area to be copied from an image

1. Click either Visible area of the image or Whole of the image.
2. Click **Done** to confirm your choice.

You can specify how much of a current image window should be printed and at what scale.

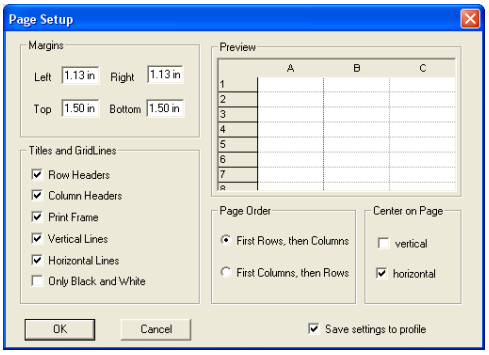
Changing the print range and scale

1. Specify the print range of the window to be printed by clicking either **Visible area of the image** or **Whole of the image**.
2. Select the scale from the **Fit to paper**, **Actual size** or **Scaled** options. If you click Scaled, select or type the percentage in the text box.

   Note:
   The default is set to 100% of the original size.
3. Click **Done** to confirm your choices.

Page setup for tables

Clicking the **Page setup for tables** button displays the following dialog box.



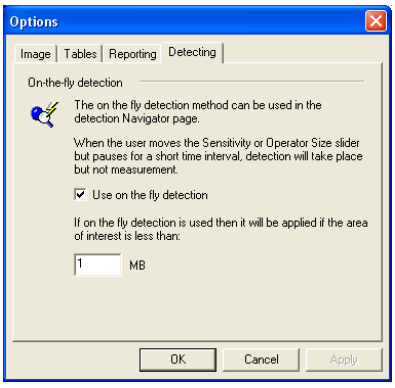
From this dialog box you can dictate how the measurements table will be printed. You can also specify the order in which the grid cells should be printed.

Changing the page setup for tables

1. Set the margins or accept the defaults.
2. Select or clear the Titles and Gridline options.
3. Set the page order and orientation. A preview of how the table will appear when printed can be seen at the top right of the dialog box.
4. Click on **OK** to confirm your choices.

Note:
The margins you set are added to those used by your printer.

Detecting tab



On the fly detection

When you move the Sensitivity or Operator size sliders in the Navigator and pause for a few seconds detection takes place, this is called *On the fly detection*. By default this feature is selected; clear the check box to switch it off.

AOI size

If the *Use on the fly detection* feature is selected, it will only be applied if the area of interest is less than the size displayed in the text box. The default size is 1 Mb.

Toolbar

This section describes the toolbar buttons that are common to all the modules. In addition, each module displays an additional set of buttons, which are located in the Navigator. These are discussed in each of the relevant module sections.

The main toolbar, located just below the main menu bar, provides quick access to the software's most frequently used tools and commands.

Open button

Clicking the **Open** button 

displays the Open dialog box and allows you to open an existing experiment as described in the **Opening an image** section.

Export button

Click the **Export** button 

to copy the data or image that is displayed in the active window to the clipboard.

Print button

Click the **Print** button 

to send the data from the active window to the default printer.

Preview button

Before printing, you can preview the desired image or data. By clicking the **Preview** button 

the active window changes into a print preview window. Refer to the File Menu | **Print Preview** section for more information.

Annotate button

Click the **Annotate** button 

to activate the annotation mode, which allows you to highlight features of interest on your images or to add text to the image.

Refer to The Common Interface| **Annotations** section for more information.

Contrast button

Click the **Contrast** button 

to display a dialog box that provides a number of ways to alter the brightness and contrast of an image. For more information, refer to the **Contrast** section.

Invert Colour button

Click the **Invert Colour** button 

to reverse the colour of the displayed image. For more information, refer to the **Colour** section.

Options button

Click the **Options** button 

to display the Options dialog box, which contains various tabs that allow you to select settings for the analysis modules. Alternatively, select **View menu | Options**. For more information refer to The Common Interface | **Options** section.

Edit Image button

Click the **Edit Image** button 

to open the Image Editor dialog box that allows you to edit the displayed image in various ways. For more information refer to the **Image Manipulation** section.

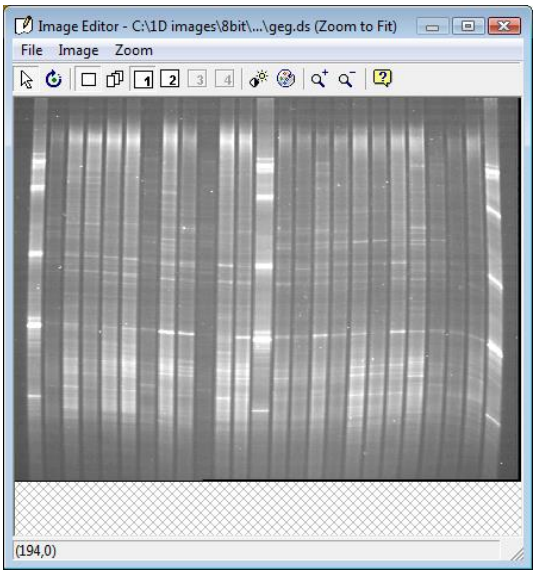
Image Manipulation

You should perform all image manipulation before analysing the data in this software. This includes any rotation, cropping or alignment of the images that may be necessary.

However, the software includes a set of tools that allow you to make basic modifications to the images. Care should be taken when using these tools, if the images have already been analysed in an experiment, because manipulating the image could result in the loss of data.

Clicking the **Edit Image** button 

opens the Image Editor. Alternatively, from the **Edit menu**, click **Edit Image**.



The Image Editor provides tools to Remove Noise, Crop, Rotate and Flip the image.

Image Editor Window

The Image Editor window has its own toolbar

From this toolbar you can set markers, rotate the image, select a channel and choose how to view the channels.

This window also has a menu bar. The features on these menus are discussed in later sections.

Opening images for editing

From the Image Editor | File menu, click **Open…** to display the *Load a Gel Image* dialog box. Select an image and start editing.

Saving the edited image

When you have performed image editing you can save the edited image by clicking **File** | **Save as…**. If you choose to save over an existing file, you will be asked for confirmation.

Note:
You cannot save an edited image using the same filename if the image is currently open in the Image window.

Editing cannot be undone after saving the image. Refer to **Reverting an image** for more information.

Reverting an image

If you are not satisfied with your editing you can restore the image to the original state when you last loaded or saved the image.

From the **File** menu click **Revert** to undo all editing in the current session to an image or channels.

Editing cannot be undone after saving the image.

Cropping images

To reduce disk usage and speed up analysis of your image, you can cut the image down to contain only the area in which you are interested. This is known as cropping.

Setting the crop area

Before using the cropping tool, you must select an area on the sample image by following these steps.

1. Click and drag out a rectangle on the image in the Image Editor. The cropping area is indicated by green dotted lines.

   Right clicking anywhere in the image cancels the selected area.

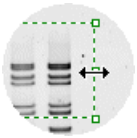
2. Next, select **Image** | **Crop to area**. Everything outside the selected area will be removed, leaving the cropped area in the Image Editor.

To cancel this area once cropped, select **File** | **Revert**.

Moving the crop area

To move the existing cropping rectangle, place the pointer over one of the rectangle edges and then drag the rectangle to the new location. Notice that the pointer changes to  when over a line.

To change the shape or area, drag the handles *(small boxes)* of the rectangle.



Notice the pointer changes to a two-headed arrow line when over a handle.

You must be in Selection mode  to move or resize a cropping area.

For more information on moving a cropping rectangle refer to **Moving a marker**.

Saving cropping rectangles

Cropping rectangles can be saved along with any marker. For more information refer to **Saving marker configurations**.

Rotating images

An image can be rotated if it has been scanned the wrong way around.

Click either **Image** | **Rotate clockwise** or **Rotate anticlockwise** to rotate the image 90° in the selected direction.

Freeform Rotating

Selecting **Freeform rotate** places a grid over the image. To change the orientation of the image take the following steps:

1. Place the pointer anywhere in the Image Editor; the pointer changes to .
2. Drag the grid into the new position.

   The image rotates upon releasing the mouse button.
3. Repeat as necessary.

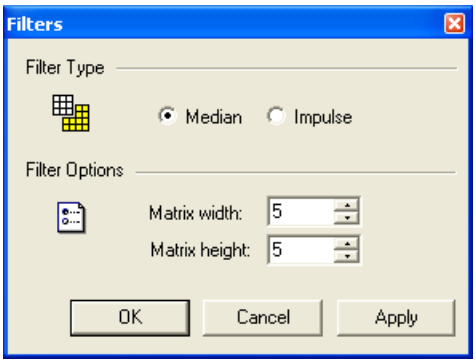
Flipping an image

Clicking either **Image** | **Flip horizontal** or **Flip vertical** flips the image around the horizontal and vertical axis.

Filtering the image

Applying a filter to an image removes noise from the image. You should not remove noise unless it is necessary.

1. Clicking **Image** | **Filter…** displays the Filters dialog box that allows you to set the filter parameters.



2. Select the Filter type, either **Median** or **Impulse**.
3. Set the **Matrix Width** and **Height** by using the spin buttons or type the parameters in the text box.
4. Click **Apply** to apply the filters to the image. The *apply* command displays the changes and leaves the dialog box open allowing you to change the filter if desired. However, if you click **OK** the filter is applied to the whole image and the dialog box closes.
5. Filters are applied to the whole image or all channels of a multiplex image.

Changing the Contrast and Colour

You can change the contrast and colour in the Image Editor. For more information refer to The Common Interface sections discussing Contrast and Colour.

Zooming an image

Using the following commands on the Zoom menu can change the magnification of an image or channel.

Zoom in/Zoom out – Zooming in, increases the level of magnification and zooming out decreases the level of magnification.

Zoom 1 to 1 – Reverts the image to its original size.

Zoom to fit – Scales the image up or down to fit the window whenever the Image Editor window is re-sized.

Navigator

The Navigator occupies the left side of the module main window. It displays the tools relating to each analysis mode of the active module.

The features of the Navigator follow the same basic layout; therefore, learning to use each of the integrated modules is relatively easy.

The features common to all modules are discussed in the following sections.

Refer to the module section for the individual analysis features of that module.

Clear button

Clicking the **Clear** button 

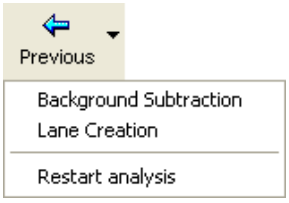
clears only the analysis effects of the current mode on the image.

Previous button

Clicking the **Previous** button 

displays the last analysis tool in the Navigator that you used.

Clicking the down arrow to the right of the button, displays a list of analysis modes. The list is presented in the suggested order of analysis. You can select any mode displayed.

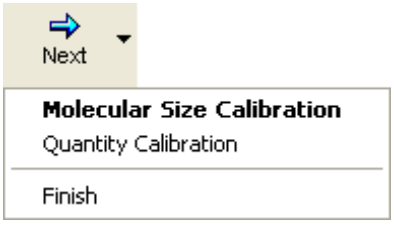


Next button

Clicking the **Next** button 

displays the next set of analysis tools in the Navigator.

Clicking the down arrow to the right of the button, displays a list of analysis modes. The list is presented in the suggested order of analysis. You can select any mode displayed.



Restart button

After you complete your analysis using the modes or you click **Next** | **Finish**, the Navigator displays the Experiment Overview page, and the **Restart** button.

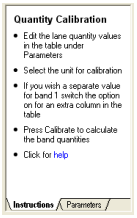
Clicking the **Restart** button 

displays a message telling you that you are about to restart the analysis and asks whether or not you want to continue. If you click **Yes**, the current image is cleared of any analysis performed.

When you select a mode, the relevant analysis tools are displayed in the Navigator allowing you to refine your analysis. Modes can be selected in any order.

Help pane

A Help pane similar to the one below is displayed at the bottom of the Navigator.



This area displays basic guidance relevant to the active module's analysis mode.

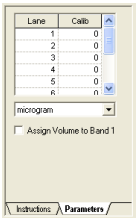
The text in red is relevant to multiplex images.

Help hyperlink

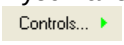
The 'Click for help' hyperlink in the Navigator Help pane displays the online Help.

Parameters pane

The Parameters pane displays the parameters for the current analysis mode of the active module. It allows you to set parameters and start the analysis operations.



If an unacceptable value is entered in a text box, the text box will change colour.

If you have the software resized to a smaller region the Navigator displays a **Controls** button 

. Clicking this button produces a popup window showing the Help and Parameter panes that contain the analysis tools.

The Program Windows

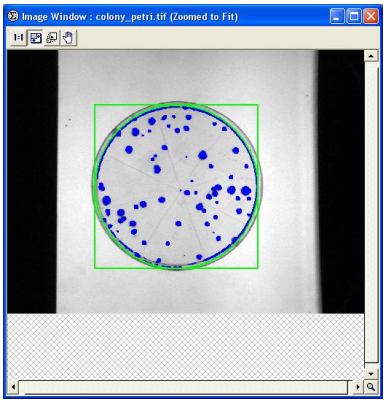
The main window contains a number of smaller windows. Each of these windows contains a different type of information. The windows can be activated using the buttons on the Window Selector bar below the Navigator.

The windows can be moved and resized; they can also be hidden. If you are working with a single window, it can be maximised, giving the largest possible viewing space.

Discussed below are the windows common to all modules of this software

Image window

The Image window is the most important of the smaller windows and displays the image you are currently analysing. The image is overlaid with information determined by the tool you are using and how far through the analysis process you are. Most of your interaction with the software is done within this window using the tools located on the main toolbar or Navigator.



The Image window toolbar

The Image window contains a toolbar with the following buttons:

Zooming an image

You will also find the zoom button in the Analysis and Line windows of the various modules

In these windows the button toggles with the Zoom to Fit button undoing any zooming effects.

To zoom the image:

1. Click the zoom button
2. Position the pointer on the area you want to zoom.
3. Drag the pointer over the desired area.

Alternatively, click the zoom button then left click to zoom in and right click to zoom out.

Pressing **Esc** on your keyboard cancels the zoom mode.

Zoom 1:1

Adjusts the magnification of the image to its original size.

Zoom to Fit

Clicking the **Zoom to Fit** button if the image is zoomed, fits the image to the size of the window.

The Zoom to Fit button counteracts any magnification made on an image using the zoom button .

Magnify

Allows you to magnify specific parts of an image.

1 Click the magnify button.

2 Position the pointer over the part of the image you want to magnify. The pointer changes to a small magnifying glass.

3 Hold down the mouse button. The area under the pointer appears in a box.

4 Drag the pointer to view other areas.

Panning

Allows you to move the image around within the Image window when the image is zoomed.

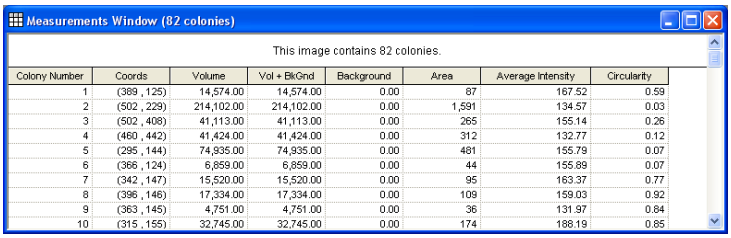
1 Click the Panning button

2 Position the pointer over the image; it changes to a small hand.

3 Drag to change the view.

Measurements window

The Measurement window displays the data in a table format that consists of various calculations from the detected colonies. You select the fields to display in the window from the Options | **Tables tab**.

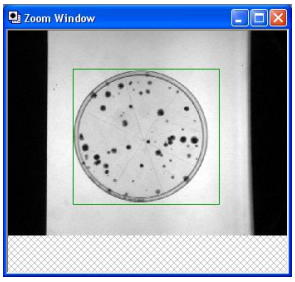


Colony Number	Coords	Volume	Vol + BkGnd	Background	Area	Average Intensity	Circularity
1	(389 , 125)	14,574.00	14,574.00	0.00	87	167.52	0.59
2	(502 , 229)	214,102.00	214,102.00	0.00	1,591	134.57	0.03
3	(502 , 408)	41,113.00	41,113.00	0.00	265	155.14	0.26
4	(460 , 442)	41,424.00	41,424.00	0.00	312	132.77	0.12
5	(295 , 144)	74,935.00	74,935.00	0.00	481	155.79	0.07
6	(366 , 124)	6,859.00	6,859.00	0.00	44	155.89	0.07
7	(342 , 147)	15,520.00	15,520.00	0.00	95	163.37	0.77
8	(396 , 146)	17,334.00	17,334.00	0.00	109	159.03	0.92
9	(363 , 145)	4,751.00	4,751.00	0.00	36	131.97	0.84
10	(315 , 155)	32,745.00	32,745.00	0.00	174	188.19	0.85

Preferred data fields for each analysis module can be selected/cleared using the Options | Tables tab for each module. Refer to the Options | Tables tab | **Data Fields** for more information.

Zoom window

You see an overall view of the image and a rectangle containing the zoomed area, of the gel in the Image window.



Scrolling windows

Scroll bars are used when the information being displayed is too large to fit on the screen.

The scroll bars are located at the bottom and right side of the image window. They work in the same way as normal scroll bars but allow you to see the range that you are viewing.

In addition, dragging the scroll box changes the start position of the range you are viewing; you can also resize the scroll box. Since the size of the scroll box reflects the amount of data that is shown in the associated window, resizing the scroll box changes the display magnification. Therefore, if you are viewing an image and make the scroll box smaller, less of the image is used to fill the window and you see the image at an increased magnification.

To resize the scroll box, position the pointer on an edge of the scroll box, the pointer changes to ↔ or ↕. Drag the pointer to the new position.

When the scroll box indicates a display of the total range of the scroll bar, the scroll box becomes transparent. When it indicates that only a portion of the range is visible, the scroll box becomes opaque.

Window Selector bar

The window selector bar located at the bottom of the Navigator contains three buttons for activating the Image, Zoom and Measurements windows used in the Colony Counter module.

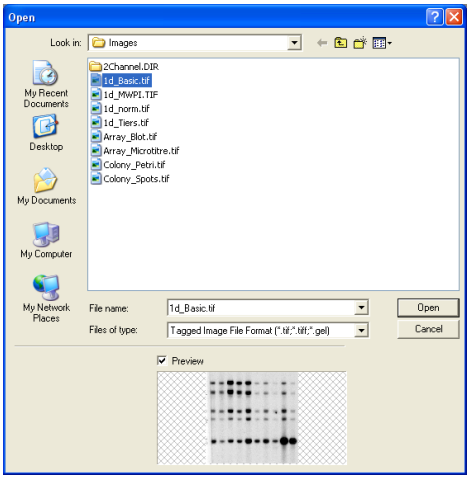
Clicking a button displays or closes the window.

Status bar

The status bar is the thin bar along the bottom of the main window. It displays information about the current state of the program (e.g. Ready) as well as descriptive messages for a selected command or toolbar button (e.g. Co-ordinates (Pixels): nnn Pixel Value: nnn).

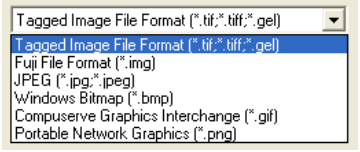
Opening an image

You can open an image by clicking **File** | **Open** image or by clicking the **Open** button on the main toolbar



To open an image:

1. Select the folder from the *Look in* list.
2. Select the correct file type from the *File of type* list.



3. Click the specific image file from those displayed.
4. Select the desired analysis module from the *Analyse this image using* list.
5. Click **Open**. The selected image automatically opens in the relevant modules Image window allowing you to analyse the image.

If you select the **Preview** check box a preview of the file displays in the small pane.

If the image does not display in the Image window after the module has opened, click the Image window button on the window selector bar, below the Navigator.

Keyboard shortcuts

Each menu is activated by clicking the Alt key and the appropriate underlined letter e.g. File Menu **Alt**+**F**.

Below is a list of common menu commands and their shortcut keys

File | Open Image	**Ctrl+O**
File | Print	**Ctrl+P**
Edit | Export to Clipboard	**Ctrl+E**
Help | Contents	**F1**

Detection

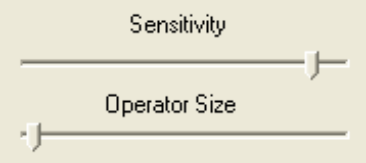
This procedure describes the basic steps to detect colonies and display measurement data relevant to the detected colonies of the image.

Detection usually takes place automatically after you change a parameter. The results appear in the Measurements window. Because of this feature, you rarely have to click the Detect button in this mode.

The detect colonies process is controlled by a number of parameters both in the upper Navigator and in the Parameters tab.

Basic Detection method

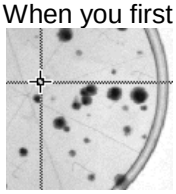
The upper Navigator contains two parameters, Sensitivity and Operator size.



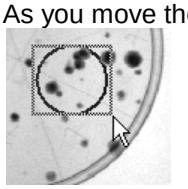
These sliders control the same parameter in the Parameters tab. To detect colonies using these parameters:

Drag to select an area of interest in the Image window.

When you first position the pointer on the image the pointer displays as a cross hair.



As you move the pointer it changes to a circle (AOI) enclosed by a box.



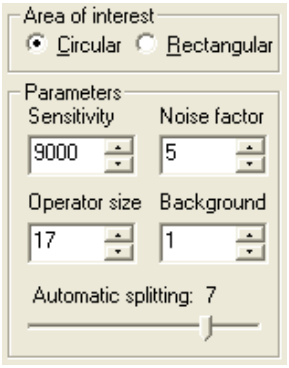
Change the parameters by moving the Sensitivity and Operator size sliders. When you release the slider the software performs automatic detection.

If the Measurements window displays the following message "This image contains no colonies", reset the sensitivity slider or re-select the area to be measured. Clicking the **Initialise Sensitivit**y button  allows the software to find an initial value.

If you briefly pause when moving the sliders, the *On the fly detection* feature allows you to observe the potential colonies. This feature is selected by default and can be switched off using the Options | Detection dialog box.

The Parameters tab includes the basic parameters described above and a number of advanced parameters. These parameters can be changed by entering numerical values.

Setting the parameters



To set the parameters for:

Area of interest

Click either the **Circular** or **Rectangular** button. Detection takes place automatically.

Initialising sensitivity

After you enter a numeric value or move the **Initialise Sensitivit**y slider in the upper Navigator, the software provides an initial value for the sensitivity based on the selected area of interest.

The sensitivity range is 1 – 10000.

Operator size

This is the width of the detection operator and is proportional to the size of the spots you are trying to detect. The operator size range is an *odd* number between 5 – 699.

Noise factor

This represents the width of the edge and centre 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 range is an *odd* number with the 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.

Background

This is a bias value added to all pixel values before any calculations are performed and therefore allowing detection of images that have a very low intensity background.

The background range is 0 – 14999.

Change the value by clicking the up or down spin buttons to the right of the text box. When you release the spin button detection takes place automatically. Alternatively, you can change the parameter by typing the value in the text box. Click the **Detect** button to redetect the colonies.

Automatic splitting

Automatic splitting is the process when the software attempts to split partially overlapping colonies.

The automatic splitting range is 0 – 9.

If the parameter is set to **0** the software does not attempt to split colonies; increase the value to activate this feature.

When you have finished editing the colonies click the **Next** button in the Navigator.

Editing Detected Colonies

After detecting the colonies three basic tools allow you to edit the colonies. The tools are: *Draw or Erase Features, Delete Features, and Split Features*. There are additional commands to *Re-number and Clear detected colonies*. Click the various buttons in the Navigator to activate the tools.

This section describes the basic steps of editing colonies. Your actual editing depends upon the parameters that you initially set in the Navigator for detecting the colonies.

When you draw undetected colonies in the Image window, the coordinates are displayed on the status bar at the bottom of the screen when the pointer is held over the edited colony.

After you have edited a colony, the software automatically re-measures the colonies and updates the Measurements table.

Drawing Undetected Colonies

1. Set the Pen/eraser size in the upper Navigator.
2. Click the **Draw or Erase Features** button  to activate the Draw command. The pointer changes to a pen when you move it over the image.
3. Position the pointer over the desired drawing area, and click or drag the pointer. Alternatively, if you want to extend an existing colony, position the pointer on the desired colony and click or drag the pointer. This extends the colony pixel by pixel.

If you connect the finish point to the start point of the area that you have drawn, the software fills the area.

You remain in the drawing/erasing mode until you select another mode.

Erasing Colonies

Erasing colonies differs from deleting colonies in that, the erasing mode erases a colony pixel by pixel and the Deleting colony mode removes the entire colony.

To erase an entire colony or part of a colony:

Set the Pen/eraser size in the upper Navigator.

Activate the Erase option by clicking the **Draw or Erase Features** button  . The pointer changes to a pen when you move it over the image.

Position the pointer over the desired area for deletion. Either right click an individual colony or drag the pointer across many colonies. Alternatively, with the pen set to a smaller size, select a point in a detected colony and right click. This reduces or deletes the colony pixel by pixel.

Unlike the drawing command, if you connect the finish point to the start point of the area you want to erase, the software does **not** erase the entire area.

Deleting Colonies

To delete a whole colony as opposed to part of a colony:

1. Click the **Delete Features** button  in the upper Navigator.
2. Position the pointer over the image.
3. Select the colony to be deleted and click. A red cross appears to the right of the pointer when it is over a colony. The software removes the detected colony from the image window, but not from the original image file.

You remain in deletion mode until you select another mode.

Splitting Colonies

This option allows you to split larger detected colonies.

1. Click the **Split Features** button  in the upper Navigator.
2. Position the pointer over the colony that you want to split. The pointer changes to a scalpel. 
3. Click and drag the pointer in any direction i.e. vertically, horizontally or diagonally, to split the colony.

If the colony is too small it might be deleted rather than split.

Renumbering Detected Colonies

After any editing, you should **re-number** the detected colonies by clicking the **Renumb** button 

in the Navigator.

By selecting a row of colony data from the Measurements table, you can identify the newly re-numbered colonies.

Clearing All Colonies

Clicking on the **Clear** button 

in the Navigator allows you to clear **all** previously detected colonies.

To clear individual colonies select the **Delete Features** button  .

A message box asking for confirmation will prompt you before all colonies are deleted.

When you have finished editing colonies click the **Next** button in the Navigator.

Background Subtraction

The software allows you to define the background volume across the images. This background can appear in the image because of excess staining of the gel and other gel properties, or the background can occur because of the process in which the image was captured.

Background subtraction is the process by which you remove from the measurements the part of the image's pixel intensity that is background intensity.

The various methods of background subtraction are selected by clicking a button in the Parameters tab of the Navigator.

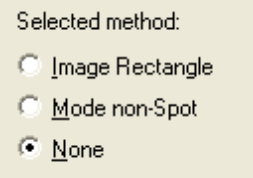


Image Rectangle method

To use the image rectangle background subtraction method:

1. Click the Image rectangle button in the Parameters tab of the Navigator.
2. Drag a rectangle in the image window that represents the background.

When you release the button, the software calculates the background level as the average intensity in the rectangle. For each colony, its area is multiplied with the background level, which gives the colony's *background*. The background value is subtracted from the colony's *volume* as measured without background subtraction. The software updates the measurements table automatically.

Clearing an image rectangle

You can clear the image rectangle by right clicking inside the rectangle. This also removes the background values from the Measurements table.

Repositioning an image rectangle

You can reposition a rectangle at any time by re-drawing the rectangle as many times as desired. The measurements are re-calculated automatically when you release the button.

Mode of non-spot method

The **Mode of none spot** method works by first finding the rectangle that completely encloses each colony. This rectangle is then expanded by a set number of pixels to give an area for examination (the default is 45 pixels in every direction).

To use the Mode of none spot background subtraction method:

1. Click the Mode non-Spot button in the Parameters tab of the Navigator.
2. In the **Margin** parameter text box, in the upper Navigator, type the number of pixels that you want the rectangle to be expanded by and click the **Subtract** button.

   Alternatively, you can change this value by using the up and down spin buttons on the side of the text box. When you release the button the data updates automatically in the measurements table.

The pixels in this area that are not part of any colony are then examined, and the most frequently occurring pixel intensity is deemed to be the background value for that colony.

In 12 and 16 bit images, the number of possible different pixel intensities means that using the most frequent pixel value is unlikely to produce good results. When this method is used on such an image, each pixel intensity is assigned to one or more overlapping groups of intensities. The background value is then calculated as the average of the most heavily occupied group.

None

This method clears any background subtraction calculations. It has the same effect as clicking the **Clear** button in the Navigator.

Clearing background values

If you have used any background subtraction method, automatic or manual, clicking the **Clear** button in the Navigator clears the background values from the Measurements table.

Annotations

Using the Annotations mode, you can add text labels with optional arrows to precisely locate and identify certain areas of an image.

You activate the Annotations mode -

by clicking the **Annotate** button 

on the *main toolbar*, or

by clicking the **Annotate Image** command on the *Analysis menu*.

After you click the Annotate button the Navigator changes to show the font and parameter options.

Create annotations on an image to highlight particular areas of interest. There is no limit to how many annotations you can create in any one image or channel.

Manual Annotations

To change the annotation style, click the **Parameters** tab in the Navigator, which contains a variety of options. For example, to change the font click the **Font** button 

near the top of the Navigator.

After changing the annotation style in the Parameters tab, the changes you make are applied to all annotations that are presently selected and any newly created annotations.

Adding and Editing Annotations in the Image window

The following section describes how to use the mouse and the keyboard to Add, Move, Select and Edit the text of annotations in the Image window.

We assume that you are using the default settings for annotations when doing the following actions.

Adding an annotation

To add an annotation to the image shown in the image window:

1. Position the pointer over the location in the Image window that you want to annotate. The pointer should be the standard arrow, indicating that you are not over any other items in the window.
2. Left click to add an annotation box. An anchor arrow appears with an attached text entry box, pointing to the location.
3. Type your text into the text entry box.
4. Press **ENTER** to accept the text or press **Esc** to stop editing without accepting any text. You will be able to enter text later.

You have now added an annotation that is indicated as the currently selected annotation by an outline box. You can add more annotations by repeating the above steps.

As an alternative to clicking the image to add a comment, you can position both the arrowhead and the text box of your annotation with a single mouse interaction.

1. Position the pointer at the location where you want the arrowhead, left click and hold down.
2. Move the pointer to the position where you want the text to be centred. As you drag the pointer, the annotation text entry box appears and stretches away from the arrowhead.
3. When you release the button, the text entry box changes to the in-place editing mode. Type in your text and press **Enter**.

Moving annotations

To move an annotation:

1. Position the pointer anywhere on the line between the arrowhead and annotation text box. *You might need to move the text box to see the line.*
2. The pointer changes indicating that you are in position. If this does not happen, you can use the ***zoom in*** function to increase the amount of line available, to pickup the annotation.
3. Drag the annotation to its new location.

Moving part of an annotation

You can also move the position of an annotation anchor arrow or text box.

1. Position the pointer over the anchor arrow or text box you want to move. The pointer changes to, a crosshair for the anchor arrow and the text box becomes outlined.
2. Drag the arrow or text box to the desired location.

Selecting annotations

To select a single annotation you can:

Click on either the arrow, the line between the arrow and the text box, or the text box.

Press the **TAB** key to move between annotations.

Select all

To select **all** the annotations you have created, **right click** anywhere in the image away from any annotation. The context menu appears allowing you to select all the annotations.

Deselect all

To **deselect all** the annotations **right click** anywhere in the image away from any annotation. The context menu appears allowing you to deselect all the selected annotations.

Arbitrary selection

To make an **arbitrary selection** of annotations, **left click** on the first annotation and then **hold down** the **CTRL** key on your keyboard. You can now **click** on each annotation you want to include in your selection. **Right click** in the image for your next action.

To arbitrarily deselect an annotation, **hold down** the **CTRL** key on the keyboard and then **left click** on the annotation.

Editing the text of an annotation

After adding annotations, you can edit the text in the annotation by right clicking the outlined text box and selecting Edit text from the context menu or, left clicking the text box.

While editing annotations, an outline box appears around the selected text box. This outline box does not appear on any image printouts. Annotation boxes are not included on report printouts.

Annotation Fonts

Clicking the Select Font Style button, displays the Font dialog box that allows you to set the font style for your annotations.

Delete selected Annotations

The  button deletes any currently selected annotation. If you continue to click this button, the annotations will all be deleted in the reverse order that they were added.

Alternatively, position the pointer anywhere over an annotation and right click, then select Delete from the context menu.

There is no **undo** command. If you accidentally delete an annotation, you will need to add it again.

Setting annotation options

Located at the bottom of the Navigator is the Annotation parameter tab. This tab contains the following options.

Text outline style

Select the shape of the border around the text of your annotations from the drop-down list. You can select a rectangle, rounded rectangle or no border.

Fill Colour

Select the colour for the background of the text area of your annotations from the drop-down list. This option is disabled if you have chosen a transparent background.

Line thickness

Select the weight of the lines that is used to draw the anchor arrow of the annotations.

You can choose one of size line thickness or No line.
If you choose No line, your annotation appears as a moveable text box only.

Colour

Select the colour that is used to draw the arrow line and outline of the annotation text box. The default outline colour is green.

Opaque Background

Select the check box if you want the text of the annotation drawn over a coloured background, or clear the check box if you want the text drawn directly onto the image.

Text Orientation

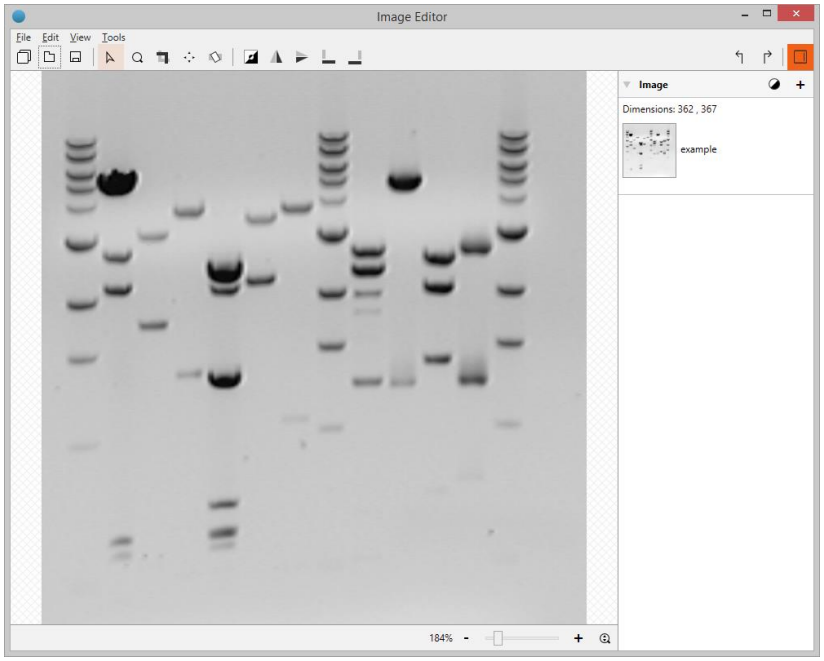
Choose the orientation of the text within the annotation.

Image Editor and Create Multiple Images

You should perform all image editing before analysing the data in this software. This includes any rotation, cropping or alignment of the images that may be necessary.

However, the software includes a set of tools that allow you to make basic modifications to the images. Care should be taken when using these tools, if the images have already been analysed in an experiment, because manipulating the image could result in the loss of data.

Go to the **Image Editor** link in the **Control Centre**.



The Image Editor provides tools to Crop, Rotate, Translate and Flip the image.

Image Editor Window

The Image Editor window has its own toolbar

From this toolbar you can select the mode to edit the image.

This window also has a menu bar. The features on these menus are discussed in later sections.

Opening images for editing

From the Image Editor | File menu, click **Open or use the Open button** to display the *Open file* dialog box. Select an image and start editing.

Saving the edited image

When you have performed image editing you can save the edited image by clicking **File** | **Save or the Save button**. If you choose to save over an existing file, you will be asked for confirmation.

Editing cannot be undone after saving the image.

Undo and Redo

If you are not satisfied with your editing you can undo or redo the edits you have made in this session using the Undo and Redo buttons at the top left of the editor.

Editing cannot be undone after saving the image.

Zooming an image

You can change the zoom on the image in a number of ways.

- The Zoom button on the toolbar allows you to left/right click on the image to zoom in or out. Alternatively you can drag out an area to zoom into
- If you go to the bottom of the image you can use the clide to zoom in or out. Also there is a fit to window button so you can see the entire image

Guide Image

If you wish to crop, rotate or translate and image and would like to compare the image to an independent image you can open a **Guide Image.** This image will appear behind the main image so you compare them easily.

Cropping images

To reduce disk usage and speed up analysis of your image, you can cut the image down to contain only the area in which you are interested. This is known as cropping. Click on the **Crop** button to start cropping

Once in the cropping mode you can see the crop rectangle displayed over the image. Drag/move the rectangle over the image until you get the correct position and then press the **Apply** button.

Translating images

If you wish to simply move the image left/right, up/down then go to the Translate option

You can use the arrow keys to nudge the image by the number of pixels you have entered. This is more useful for multiplex images (see section on them later).

Freeform Rotating images

An image can be rotated if it has been scanned at an angle using the Rotate option.

A grid will appear on the image. Use it to line up with the image features by dragging on the image or using the nudging buttons in the mode bar.

Invert an image

Click on the Invert button to swap white and black levels of the image.

Flipping an image

Click one of the two flipping buttons to simply flip the image vertically or horizontally.

Rotating an image 90º

Click one of the two rotating buttons to simply rotate the image 90 degrees

Changing the Contrast

You can change the contrast of the images in the Image Editor. Click on the black and white circle to open the contrast tab. By default you get a range of values to click on

If you select the … button you can get an advanced option showing the image histogram. You can auto set the contrast using the circle button or drag the vertical bars on the histogram

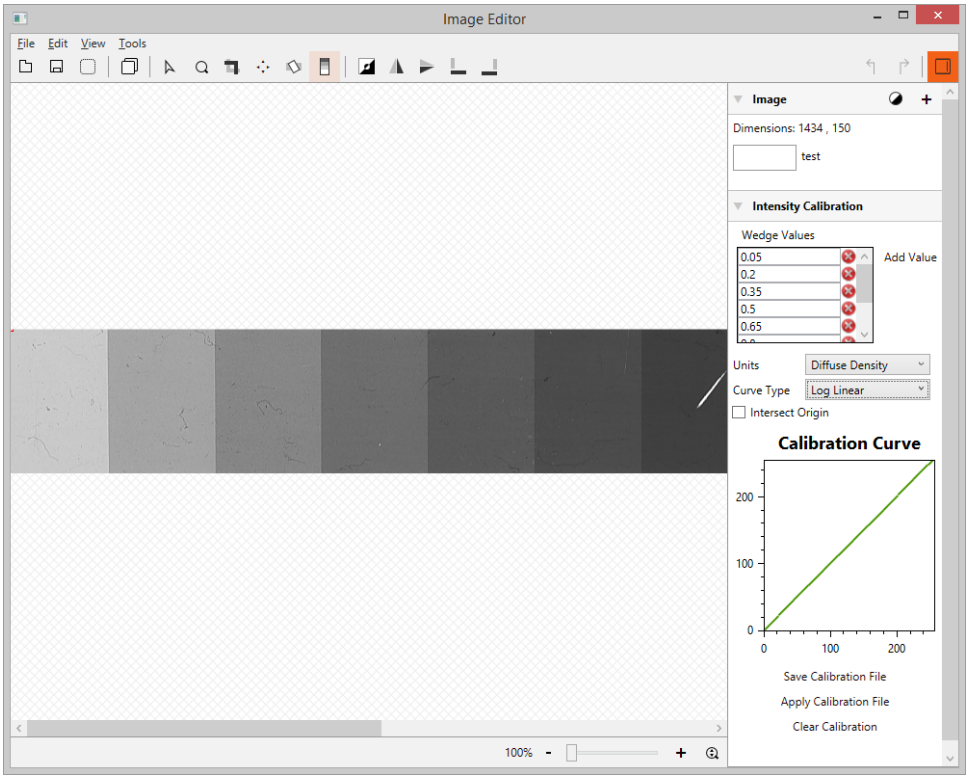
Intensity Calibration

If you wish to increase the accuracy of your imaging and want to account for any non-linearity in your scanner or camera capture system you can use Step Wedge calibration.

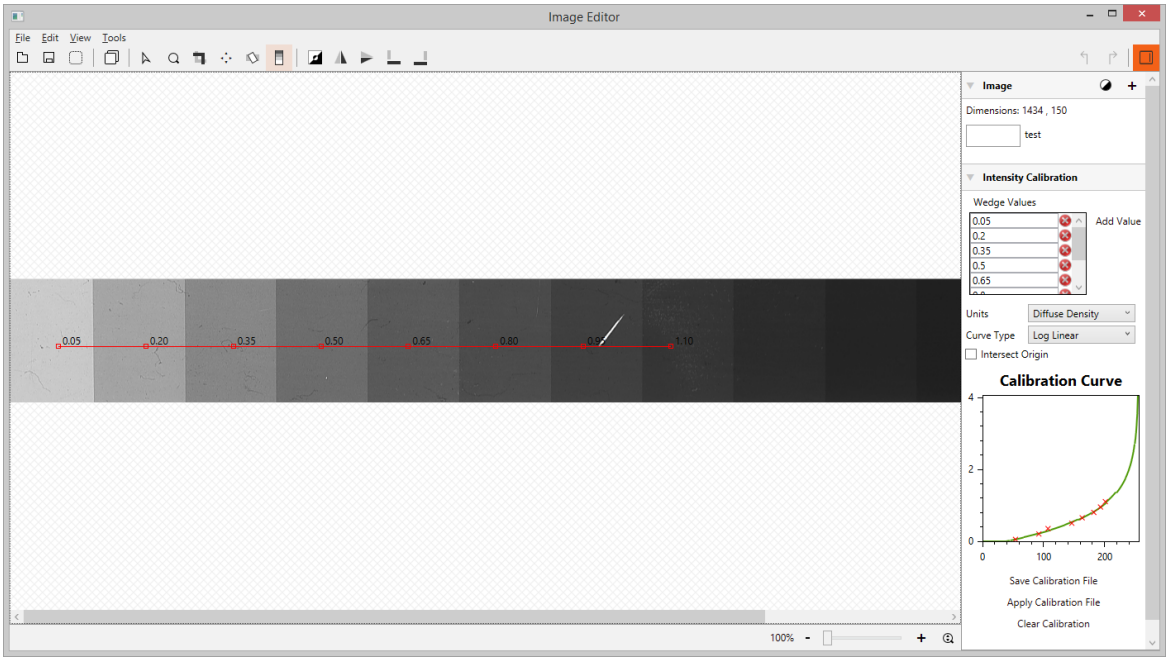
First you use a picture of a step wedge to create a calibration curve. Then you load in images scanned at exactly the same scanner parameters and then apply the calibration result to the image. This data is then stored in the image and will be used in the analysis software.

Create Calibration Curve

Open a step wedge image you have scanned in and go to the calibration mode.



1. First make sure the Wedge Values are correct. These will be published with the step wedge itself. You can Add Values using the button on the right. You can delete them using the X buttons next to the value and you can edit the value itself by clicking on the value itself and typing.
2. Then go to the other options and make sure they are also correct. Units are normally in Diffuse Density but you need to check the step wedge. The curve type and intersect origin options will determine how the curve is calibrated.
3. To now create the curve you need to drag out the values on the image so that each rectangle is centered on a wedge.



4. If you are happy with the curve results you can Save the Calibration File out so that it can be used on other images.
5. When you close the image you can also save the data in the image so it will be kept with the step wedge image.

Apply Calibration Curve

Open a gel/blot image you have scanned in and go to the calibration mode.

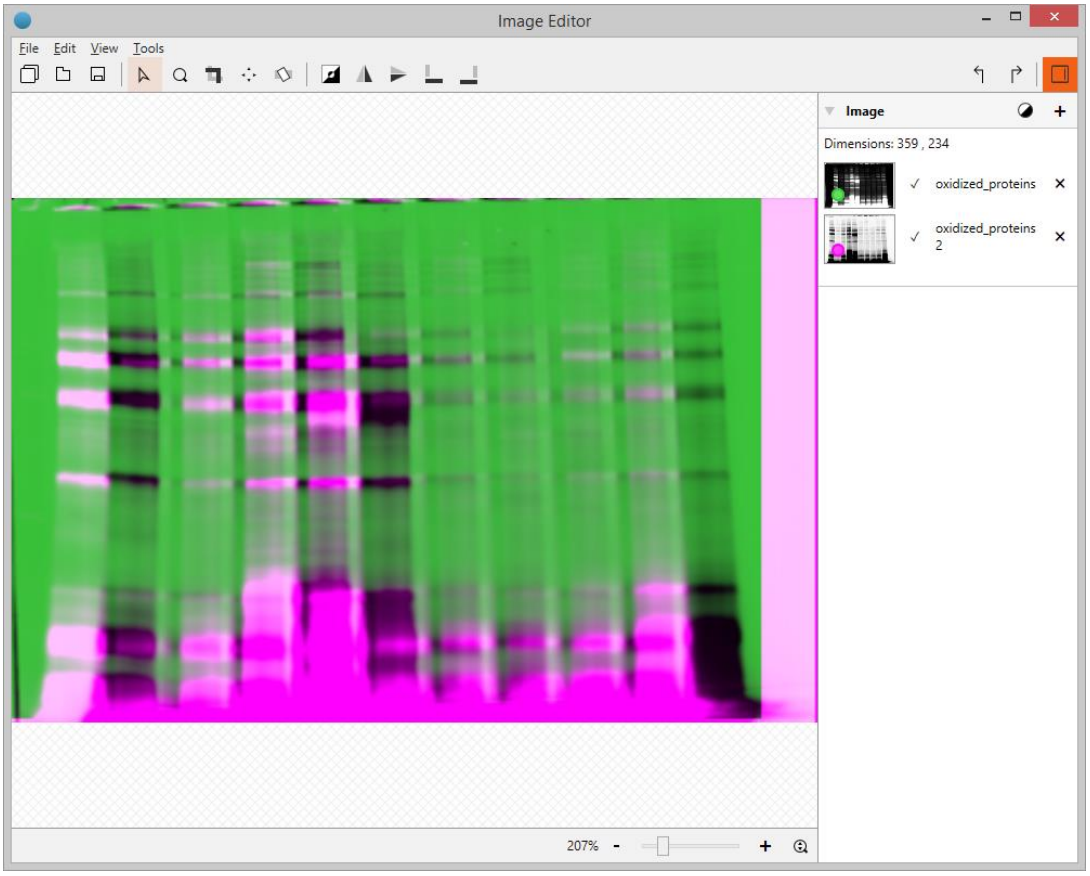
1. Click on Apply Calibration Curve and choose the correct calibration file created previously.
2. You can see the results of the wedge and curve in the bar on the side and then when you close the image you should save the image to store the calibration.

Multiplex Images

Multiple images can be edited or created in the Image Editor.

Create Multiplex Image

A multiplex image consists of 2 or more (usually 3) separate grey scale images that are overlaid on one another. A small information file (.ds) keeps the information on the image names used in the same folder as the original images. To create a new multiples image just use the File menu or the first toolbar button and select the images you need in the combined image. This is how they will appear



Open Multiplex Image

Open a multiplex image with the normal open image option but remember to choose the .ds file to open.

Add Channel to Multiplex Image

Use the '+' button in the top right to add another channel image to the multiplex.

Remove Channel from Multiplex Image

Use the 'x' buttons next to a channel image on the image list to remove a channel.

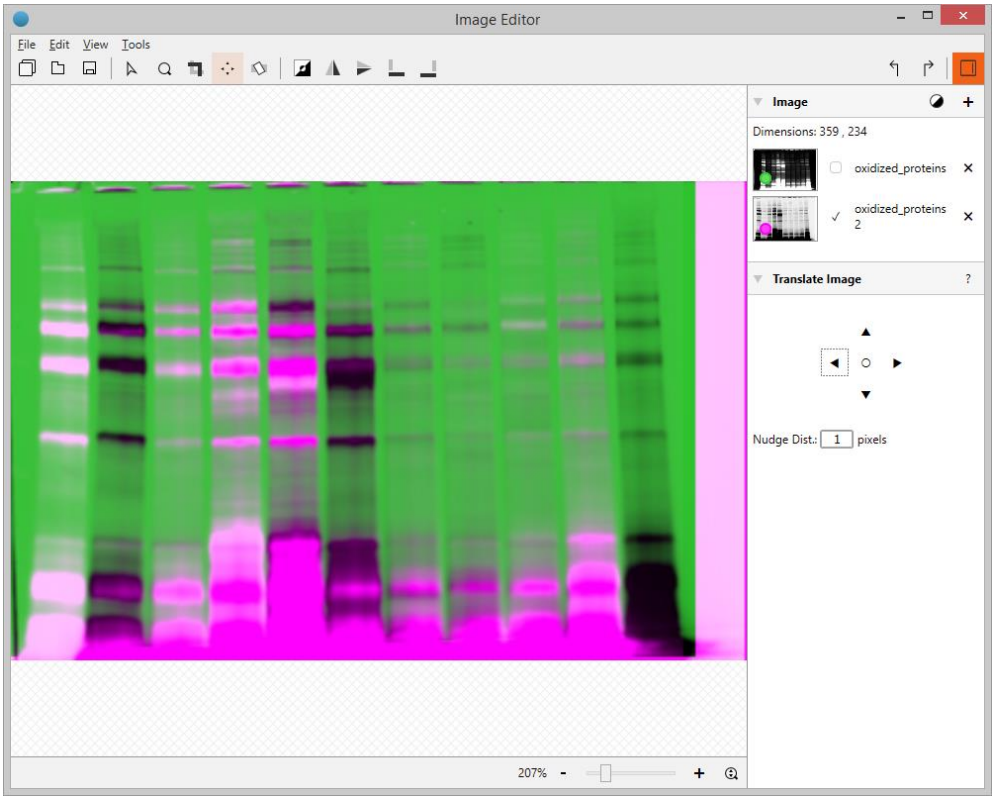
Save Multiplex Image

Use the standard save option on the toolbar or in the File menu.

Editing on one channel only

This very useful option allows you to edit on only the selected channels. This is best used on rotate or translate issues when you wish to line up the images correctly.

To do this look at the image list and choose click on the '✓' button next the images that you do NOT wish to edit. This can be toggled back later. This means you can, for instance, translate one channel image so it now overlays the other channel correctly as in this example where the image capture had been offset slightly



Glossary of Terms

Background subtraction

Background subtraction is the name given to the removal of the background intensity that runs throughout your images.

Check box

A square box that is selected or cleared to turn on or off an option. More than one check box can be selected.

Context menu

A context menu is a small popup menu allowing you to select commands pertaining to the current feature.

Current window

This is the window that has the highlighted title bar and is currently active.

Data file

The storage of analytical information about the gel on the computer hard disk or network drive.

Dialog box

A window containing buttons and controls allowing you to set options or perform operations.

Handles

A small rectangle over a feature on the image, which allows you to interact with that feature usually to re-size it.

Image Rectangle

The method of background subtraction, which uses the average intensity in an area on the image as the background intensity.

Mode

The current set of interactions possible to perform analysis in the program.

Modules

The modules consist of 1D, Array, Colony Counter and Toolbox, which are the tools used for analysing 1D gels, dot and slot blots, microtitre plates and colony images captured by a scanner.

Molecular Size Calibration

A mode, which is used to relate the position along a lane to the Molecular Size of material that would migrate there.

Object

This can be a Line or Shape used to define an area or subtract background values in the Toolbox module.

On the Fly

The software automatically re-measures data or re-plots a graph when any detection changes are applied to images.

Palette

A table of colours that allows you to change the colours used to display an image. Rather than specifying particular colours directly, images can be drawn by referring to particular entries in this table. Therefore if the palette is changed, the image colours changes.
This often gives the impression that the image itself has changed when this is actually not the case. The software adjusts the colours to give you different displays of image contrast without changing the image.

Pixels

A pixel (an abbreviation of Picture Element) is the smallest part of a digitised image and is the unit of quantification. A digital image is simply a two dimensional array of pixels. In a greyscale image, each pixel contains a measurement of the image at that point, whereas in a Binary (Monochrome) Image the pixel is an on/off switch that indicates whether the system believes that there is a Band at that position. (There will be many pixels to an individual band).

Rf

Rf (Retardation Factor) is a measurement of the position along the lane, relative to its length. By default, the first position in each lane has an Rf of 0 and the last has an Rf of 1. There is a linear increase in Rf from start to finish.

Rolling ball

This is an automatic background subtraction method. The method calculates the background as if a ball, with the radius you specify, were rolling underneath the profile.

Rubber band

This is an automatic background subtraction method.
It can be thought of as stretching a rubber band underneath the lane profile.

Single view mode

Displays an individual multiplex image in the Image window.

Spike

Spike is the term used for a small dark artifact within a spot. This can result from, for example dust or errant bits of labeled target.

Spin buttons

These are small buttons at the side of a text box that allow you to increment or decrement a parameter by a set value.

Standard Lanes

A lane with known values of Molecular Weight or pI for its bands.

Step wedge

A translucent film divided into steps of known diffuse density. Step wedges are used in Intensity Calibration to provide areas of an image that can be used to calibrate all pixel intensities.

Tab

A control that allows you to switch between multiple views, for example, the different panes in the lower Navigator, or the various settings of the Options dialog box.

Text box

Text boxes allow you to enter numeric values for parameters. The text box will turn yellow if an unacceptable value is entered.

Toggle

To change an item's state between two possible choices, normally On or Off.