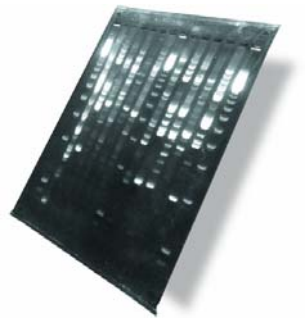
nonlinear dynamics

Quick Start Manual

Phoretix 1D

version 2003



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Introduction

Welcome to the Phoretix 1D Quick Start Manual.

This document has been constructed to provide a valuable starting point when first using the software. By running through the procedures described, you will become proficient in using and demonstrating this product within a very short period of time.

Should you want to discuss any aspects of the software or if you experience any difficulties, please do not hesitate to contact us. Our **Coverwise Support Team** has immediate access to the development, sales and applications teams to ensure the most knowledgeable answer, quickly delivered.

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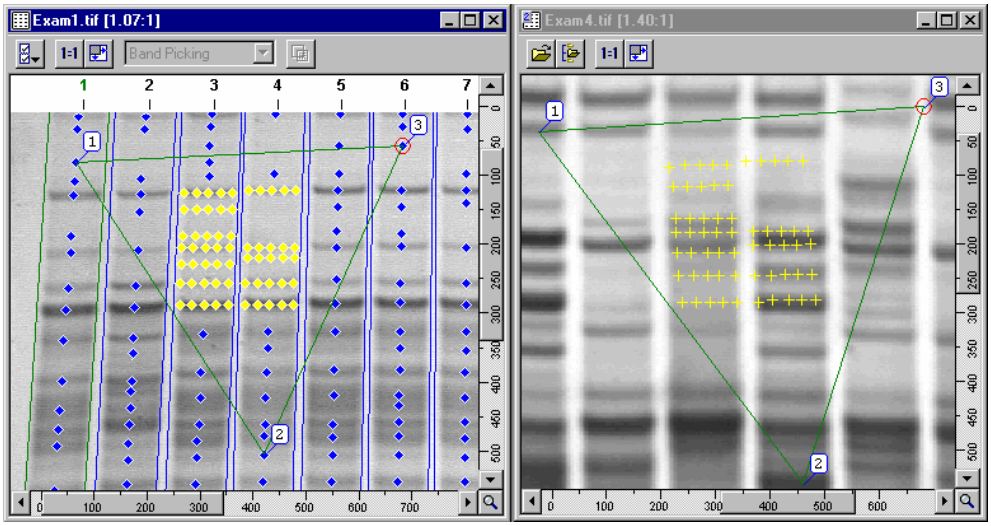
When contacting us, please include as much information about yourself and the problems you are encountering as possible to ensure a speedy resolution.

Key product points: Phoretix 1D version 2003

- Provides automatic detection of lanes and bands.
- High throughput screening is facilitated with Analysis Protocols.
- Automatic analysis of multi-tiered gels.
- Complex band pattern matching, easily handles intra- and inter-gel variation.
- MADGE, MegaBACE™ and ALF™ gel compatible.
- Distorted gels analysed accurately by accounting for the distortions using flexible lanes, grimaced bands and flexible Rf lines.
- Comprehensive gel analysis features include: band quantitation, molecular size determination, pI calculation, various normalisation and calibration techniques and a range of band matching facilities.
- Quantitative analysis, highly accurate MW determination and densitometry.
- Band pattern matching and lane relationship studies: Automatic band matching to an automatically produced synthetic reference lane that may be edited.
- Comprehensive data management tools, to organise lane data and create libraries.
- Extensive display options, annotated image and lane profiles, tables, graphs, dendrograms, reports, matrices and histograms.
- Powerful band picking tool for the coordinate mapping of selected bands in a current gel, to coordinates relating to locations on a separate robot device. Numerous robot types supported.



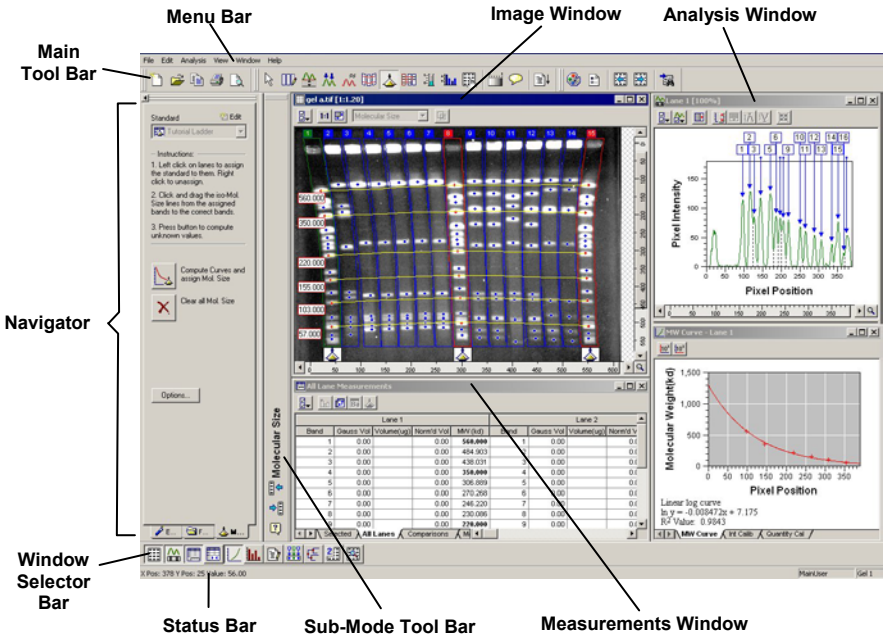
Program Layout

Overview

When ***Phoretix 1D*** is opened, the program window is displayed, with the **Menu Bar** and **Main Tool Bar** at the top of the main window.

Below this is the **Navigator** on the left side of the window. It is the control panel from which most of the analysis is controlled.

The main part of the program window consists of the various component windows, such as the **Image**, **Analysis** and **Measurements** windows.



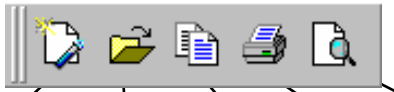
These component windows can be activated or minimised by pressing the appropriate button on the **Window Selector Bar**, at the bottom of the main program window, beneath the Navigator.

Finally, at the base of the program window is the Status Bar, providing image coordinate data and program status information.

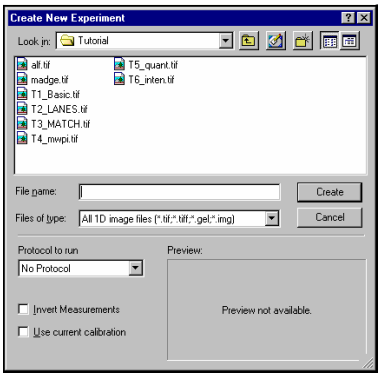
Main Tool Bar

The main toolbar is located just below the main menu bar and provides quick access to the most frequently used modes and commands in the program. Each command or mode can also be accessed using the main menu bar.

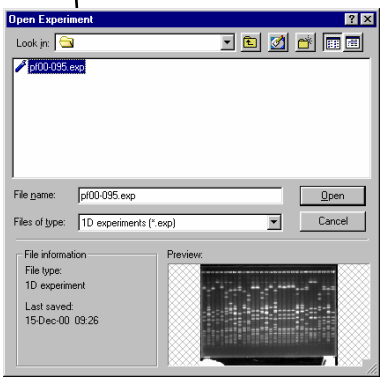
Viewing the toolbar from the left side, the **first section** consists of standard windows options:



Create New Experiment



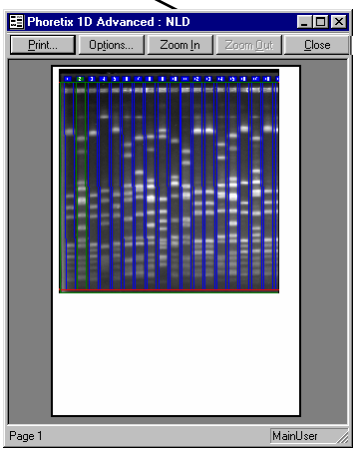
Open an Experiment



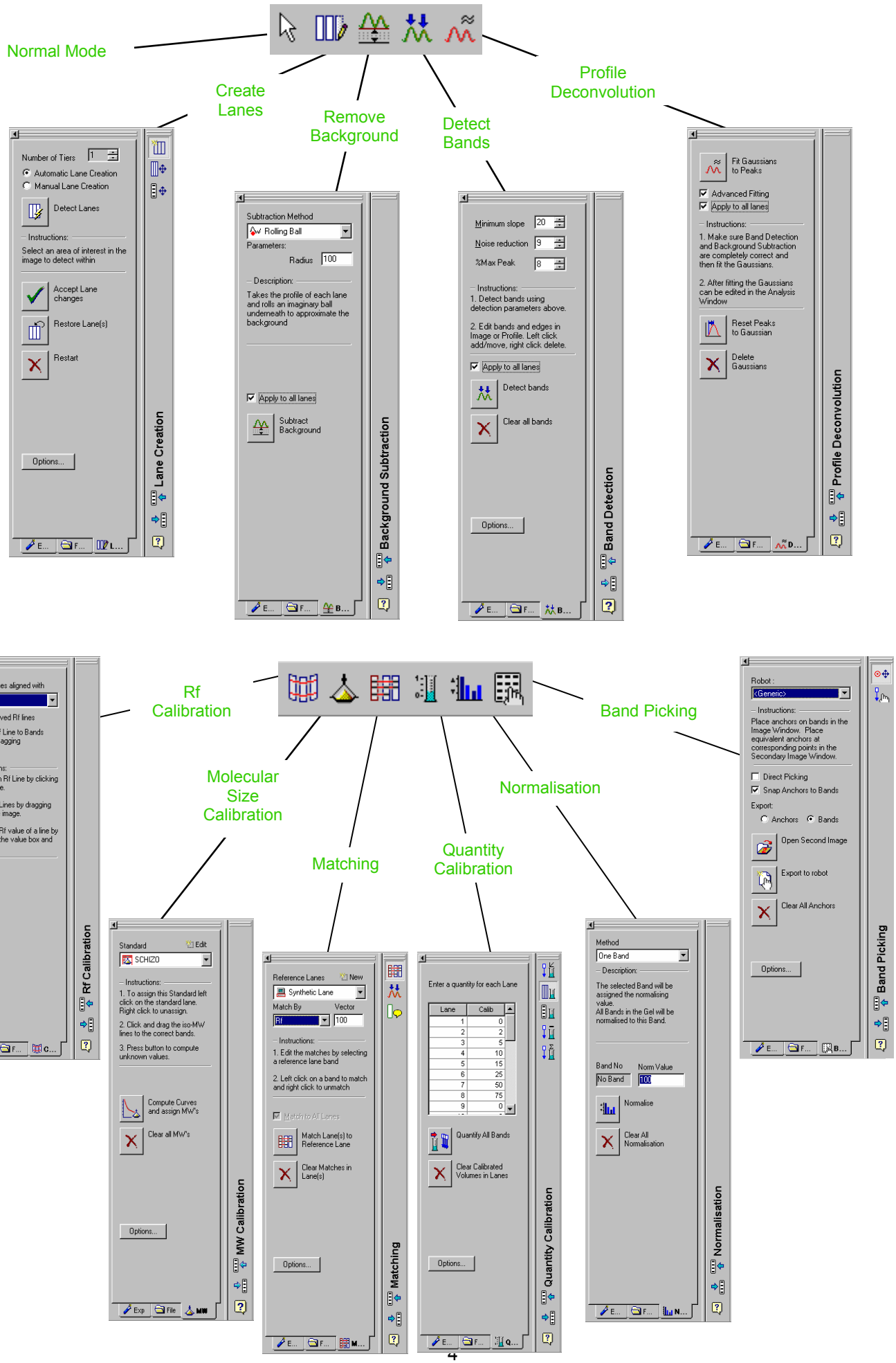
Copy to Clipboard

Print

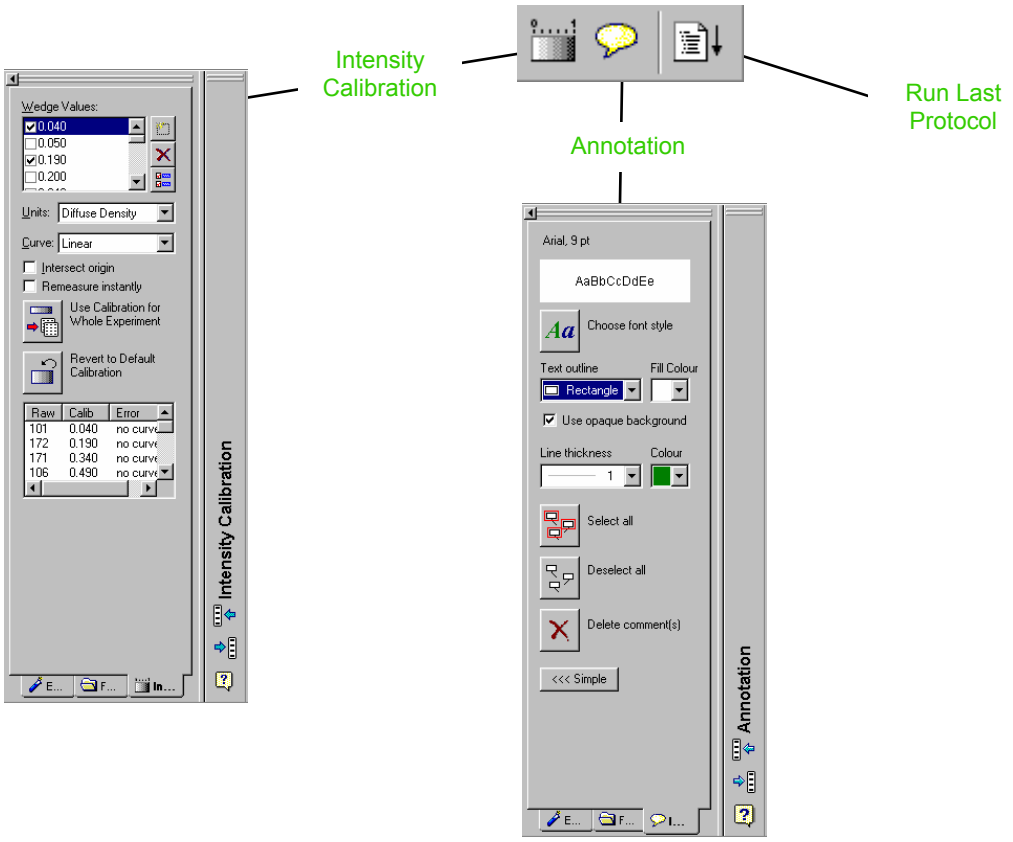
Print Preview



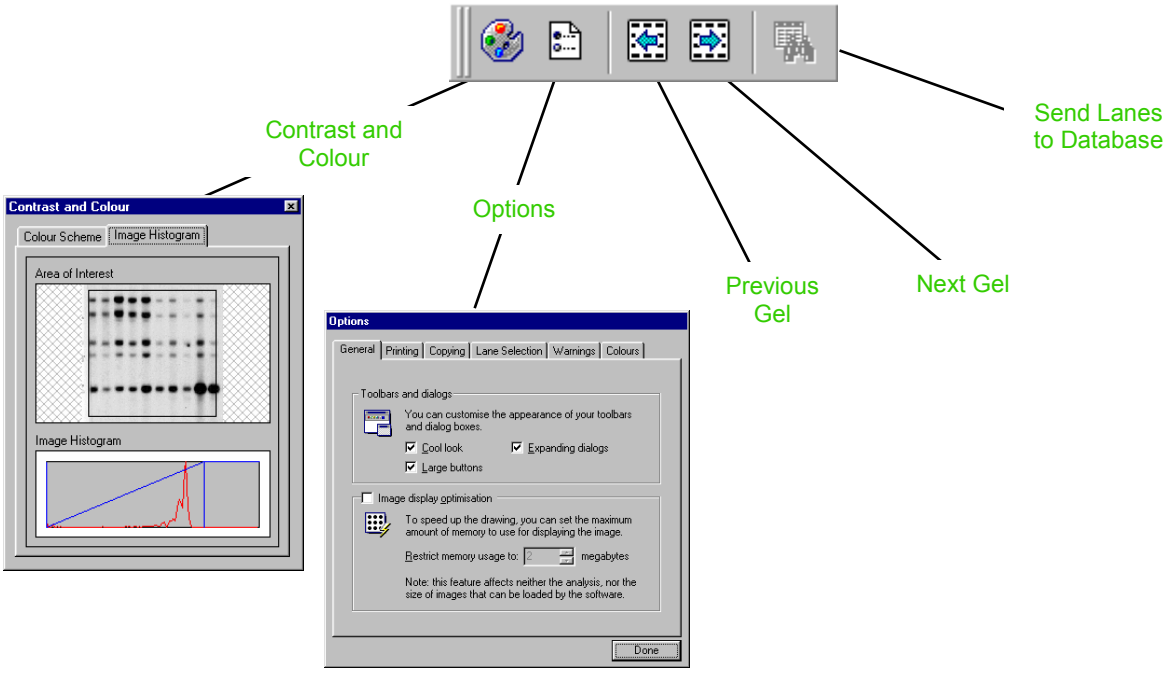
The **second section** of the toolbar consists of the **main processes for analysis**.



The third section of the main toolbar contains **additional processes for analysis:**

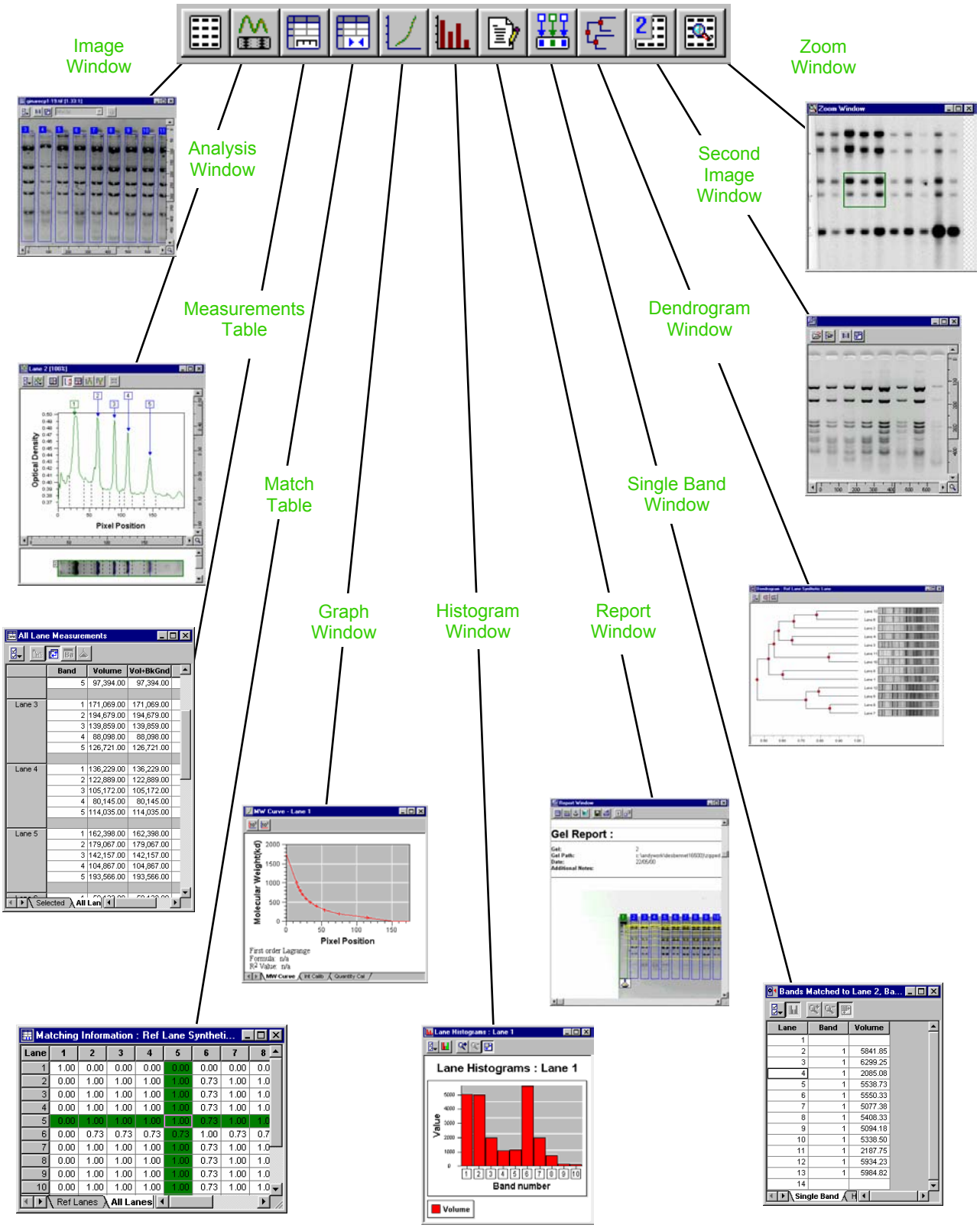


Finally, the **fourth section** contains the **additional facilities for analysis:**



Window Selector Bar

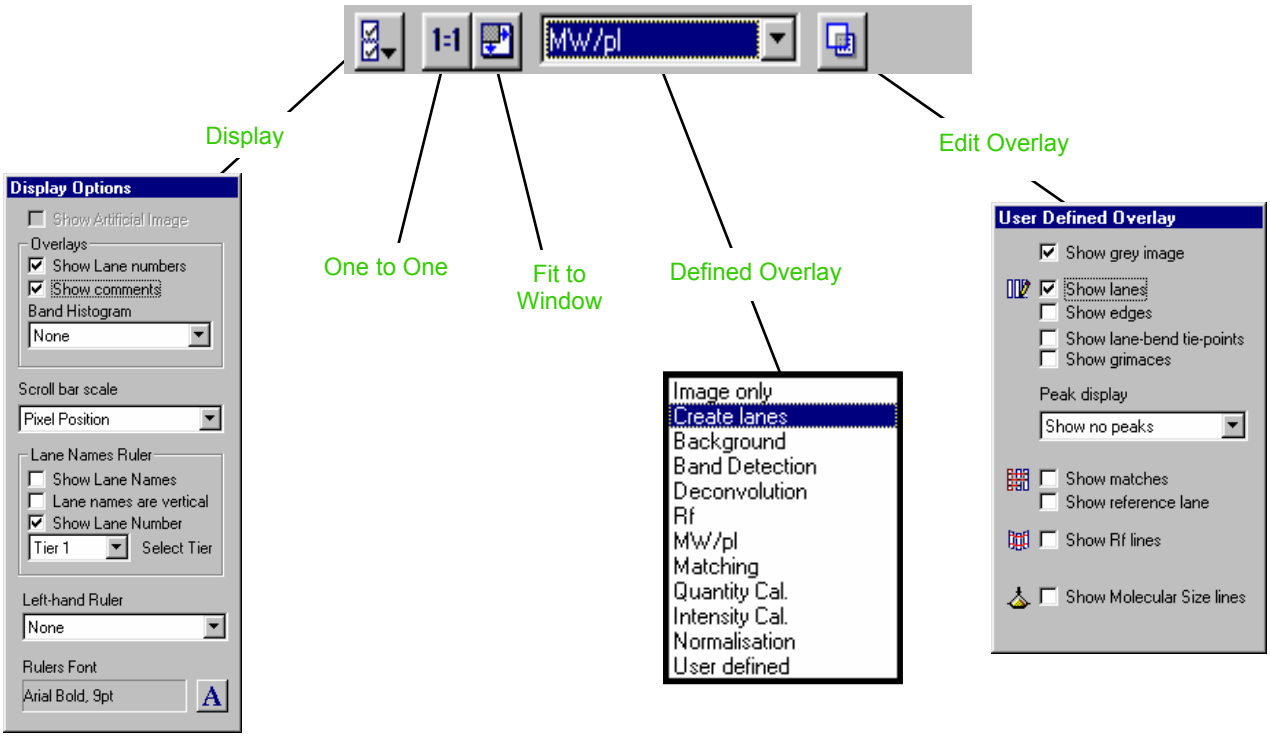
At the bottom of the program window is the **Window Selector Bar**, consisting of the eleven **Child Window** icons.



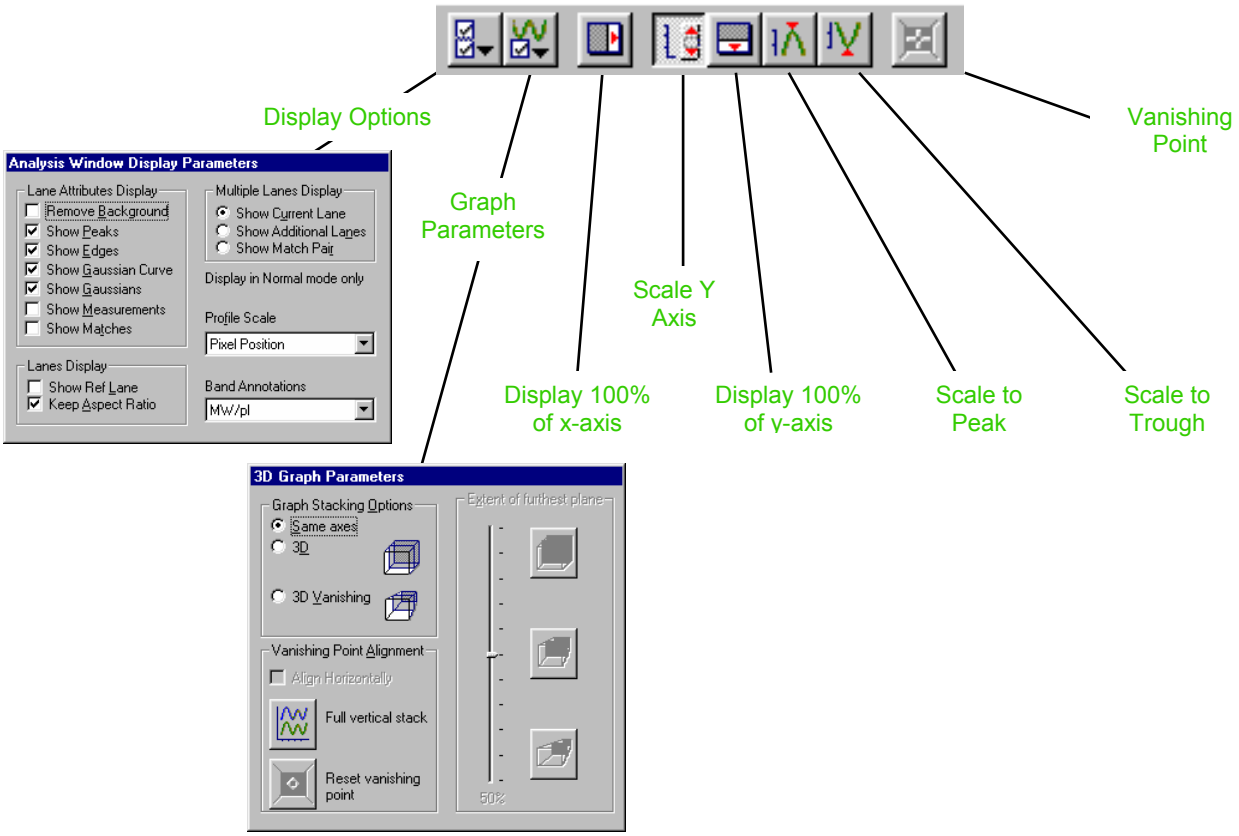
Toolbars on Child Windows

The two main Child Windows for performing analysis - the Image Window and the Analysis Window, also have toolbars. Icons on these toolbars launch popup dialogs that allow you to modify the parameters for that window.

For the **Image Window** they are:



For the **Analysis Window** they are:



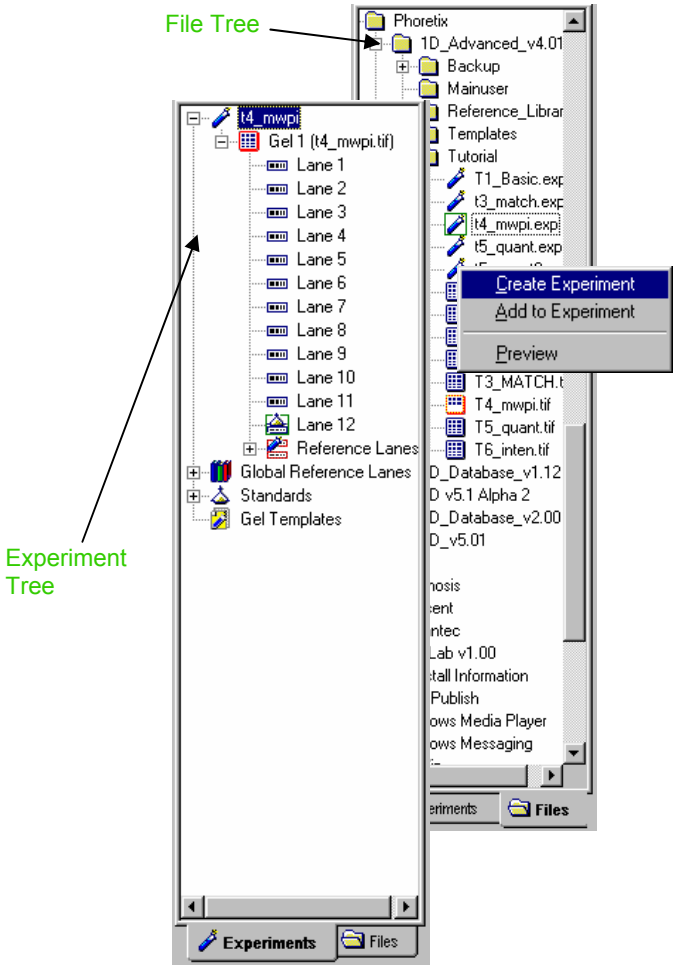
Navigator

At the left hand side of the screen is the Navigator. It is the integrated control panel for the software. Navigator appearance is specific for with each analysis mode - see previous pages.

When you first run the program the Navigator has two tabs at the bottom. The first shows a **File Tree** - this is similar to Explorer but **only** shows files related to the 1D software. It is possible to perform all **File** operations using this tree.

The other tab in the Navigator is the **Experiment Tree**. This contains the layout of the current experiment and also allows you to select the current gel that is shown in the Image Window, simply by double-clicking on the gel name.

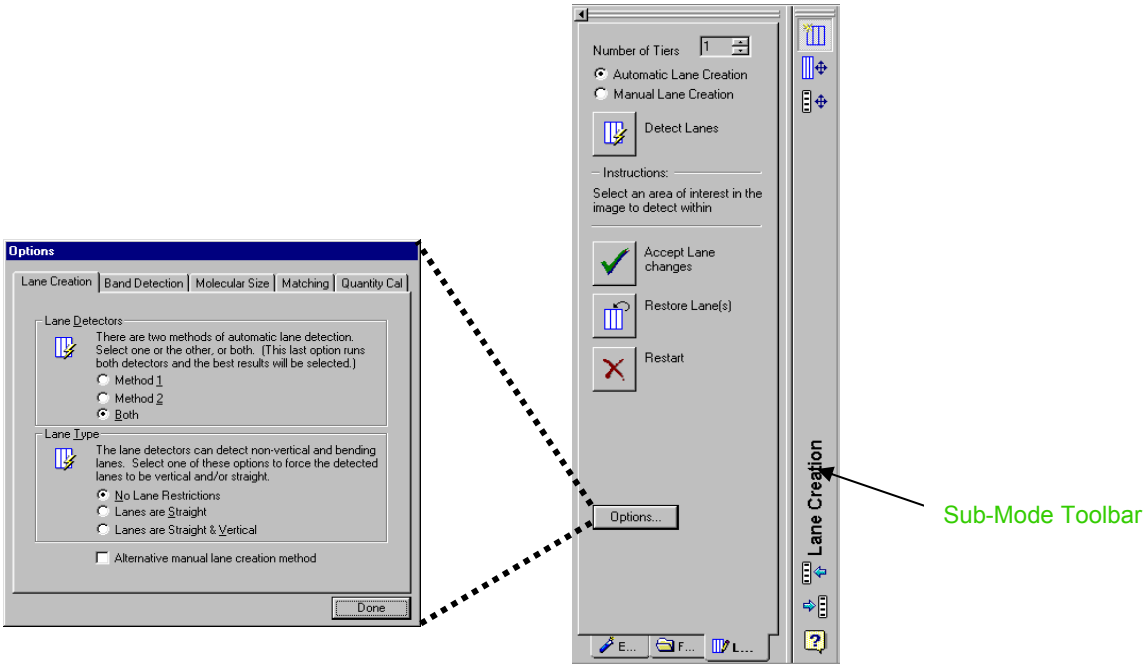
Other operations can be performed on these gels. These are available using a **right-click context menu**, or from the various menus at the top of the program window.



Analysis Modes

Having opened or created a new experiment, the Navigator displays a **third tab**, which takes on the appearance of the current analysis mode, as in the example below - Lane Creation. The Navigator displays the relevant tools for the current analysis step. Several of the Analysis modes have additional options, accessed using the **Options** button.

Running down the right hand side of the Navigator is the **Sub-Mode Toolbar**. This vertical toolbar displays the current mode and offers extra tools depending upon the mode selected.

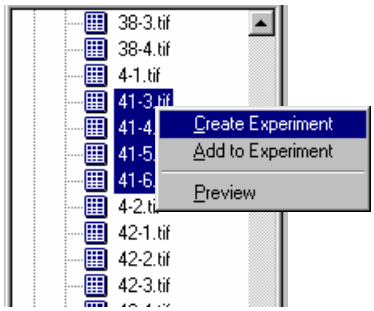


Analysis Protocol

Stage 1: Managing Files and Experiments

To set up an Experiment the files must be in the same folder.

Selecting files: Select **files tab** at the bottom of the **Navigator.** Move to the folder containing the image files (in greyscale tiff format). Expand the folder by clicking on the plus node and select the files to be included in the experiment, by holding down the **Ctrl** button as you select the files.

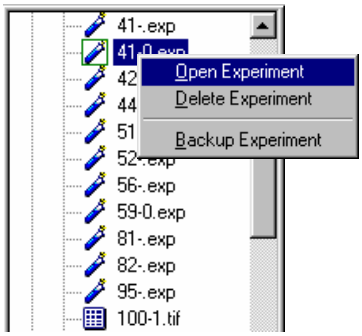


Creating

Creating an Experiment: Right click on the selected files and choose **Create Experiment**. The images are loaded and the first image in the series is loaded into the Image Window. The Navigator will now automatically switch to **Lane Creation**, the first step in the analysis procedure.

Notice: the Navigator now has three tabs.

Opening an Existing Experiment: All existing experiments in the file tree of the Navigator are identified with an icon that resembles a test-tube. When the experiment is highlighted, the gel image files analysed in this experiment will flash in the file tree. To **open** the experiment either double click on the test-tube icon, or right click and select **Open Experiment**.



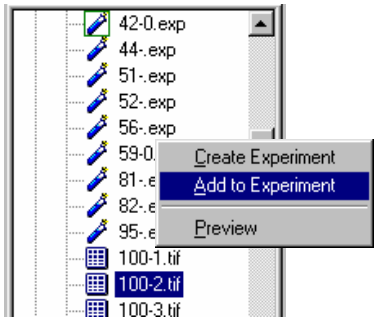
Backing up the current experiment: can be done here by right clicking on the experiment file (with the exception of the current experiment).

Deleting existing experiments: can also be performed using this context menu.

Opening

Adding Gel Images: Additional images that are present in the **same folder** can be added to existing experiments by right clicking on them when in file view.

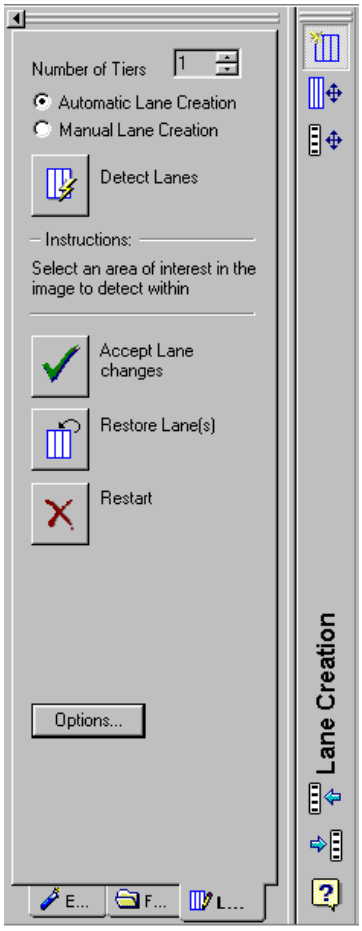
Note: You can **preview** the images being added to the experiment by selecting the preview option in this context menu.



Adding

Multiple Experiments: During analysis the software does **not** change the raw data of the image files. Therefore unlimited experiments can be created from the **same image files**, by repeating the above process.

Stage 2: Lane Creation



Automatic Lane Definition

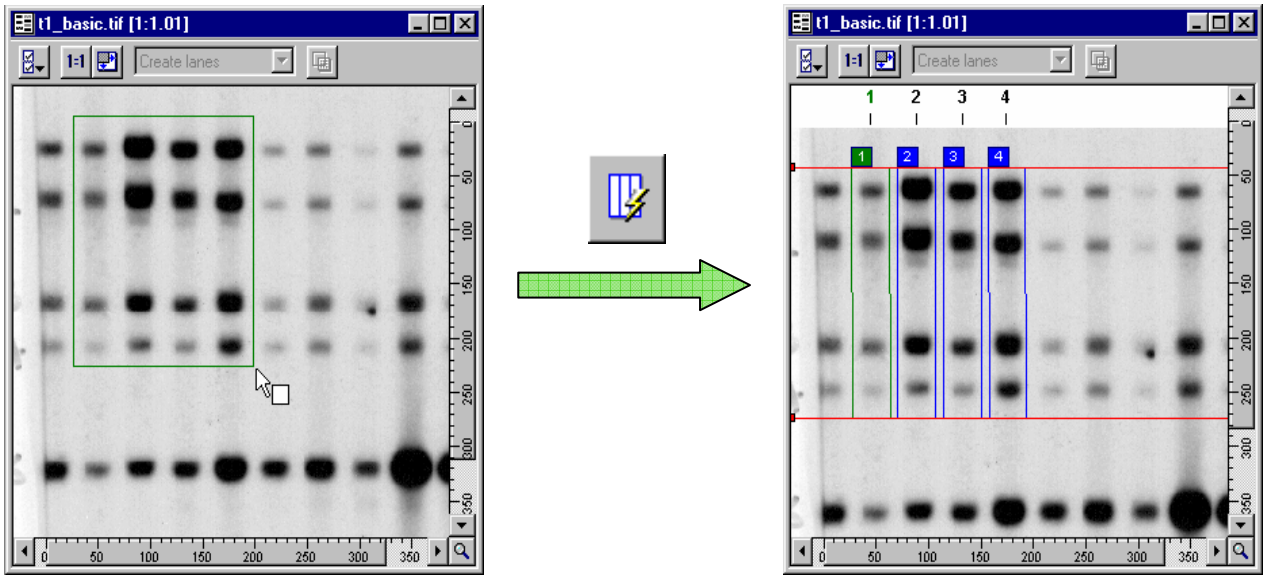
Automatic and Manual Lane creation allows the definition of one or more areas called **Multi Boxes,** in which the position, width and number of lanes on your gel image is defined.

To detect lanes on an image first select the **Create Lanes** icon from the **Lane Creation Sub Mode Toolbar**.

Automatic lane creation can operate on **all of the image** or a user defined **Area of Interest** on the image.

To Detect:

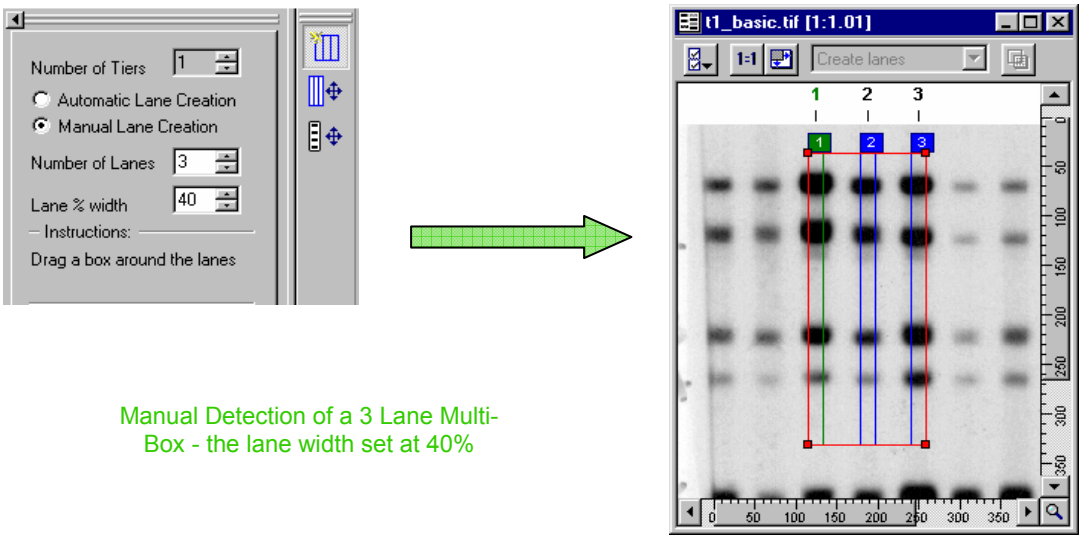
- **all of the image** - click on the **Detect Lanes** icon - this will create a **Multi-Box**.
- an **Area of Interest** on the image - first define the area by dragging out a rectangle and then press the **Detect Lanes** icon.
- when complete the sub-mode will change to **Move Multiple Lanes**.



Define and detect lanes in an Area of Interest

Manual Lane Definition

When **Manual Lane Creation** is selected, you can define both the number and width of lane overlays that will appear in the **Multi-Box**, when it is created in the Image Window.



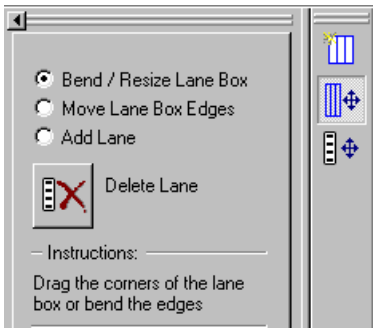
Manual Detection of a 3 Lane Multi-Box - the lane width set at 40%

For a range of reasons: sample content, electrophoresis conditions, gel quality etc. lanes can exhibit a variety of distortions that have to be accommodated in the **Lane Creation** facility.

Accurate band detection and volume measurement is dependent on the quality of lane creation. Hence we have included a number of tools that enable the user to rapidly edit the position, size, width and linearity of the lane overlays after automatic or manual creation.

There are tools provided to operate at the level of:

- **the multi-box** 
- **the lane** 



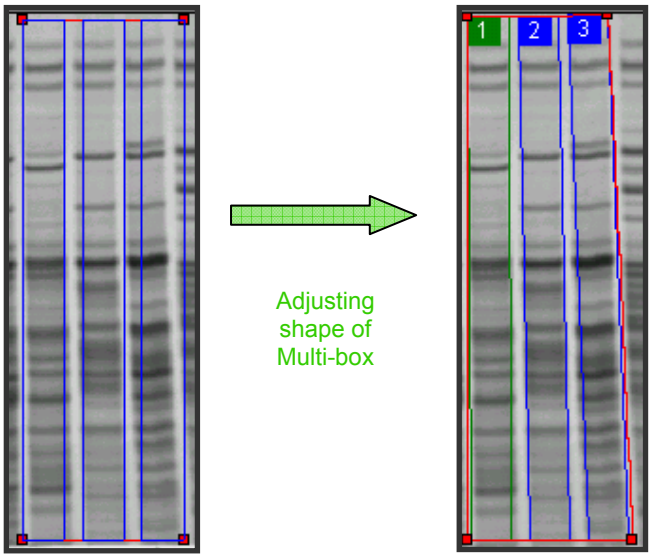
Multi-Box Adjustment

Editing the Multi-box

For the purposes of this example we are only interested in detecting the bands in three of the lanes on a gel image. In the Navigator panel (set for manual lane creation as shown above) set the number of lanes to exactly 3 and an initial lane width of 80%.

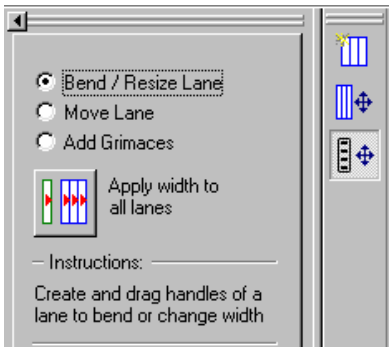
Select the first icon on the **Sub-Mode Toolbar**, then holding down the left mouse button drag out the box over the three lanes.

The box will now look like the first image on the right. To adjust the Multi-box, select the second icon on the **Sub-Mode Toolbar.** By dragging the **red boxes** at each corner the box can be reshaped to adjust for some of the lane distortion.



Adjusting shape of Multi-box

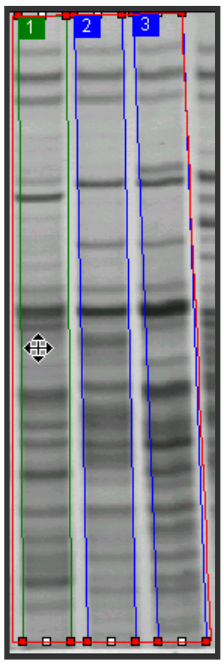
Editing Individual Lanes



Each lane within the Multi-Box can be individually moved, resized, have tie points added to allow reshaping of the lane and the addition of **Grimaces**, to account for poorly-shaped bands.

Select lane: Either click on the lane overlay in the image, the lane number, ruler or use the lane navigation icons at the bottom of the **Sub-Mode Toolbar**. The current lane overlay will now be coloured green.

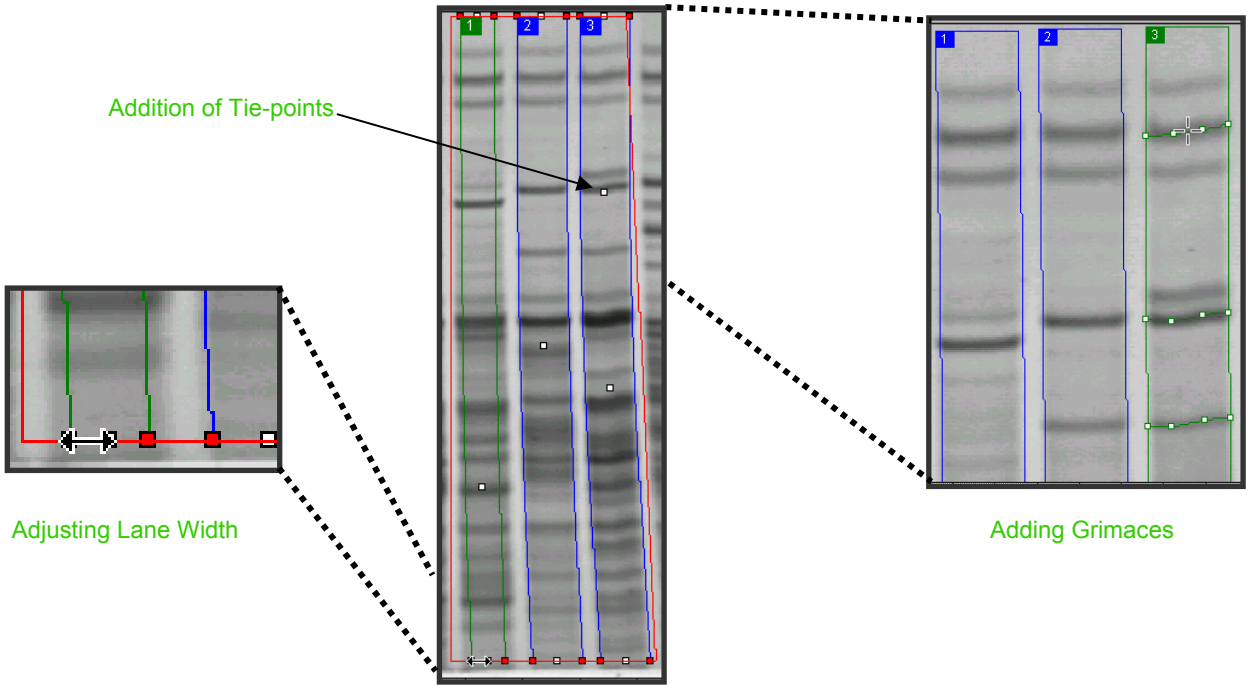
Move Lane: Select **Move Lane** in the Navigator. The pointer now changes to a **four way arrow** when it is located over a lane. Holding down the left mouse button allows you to move the lane overlay.



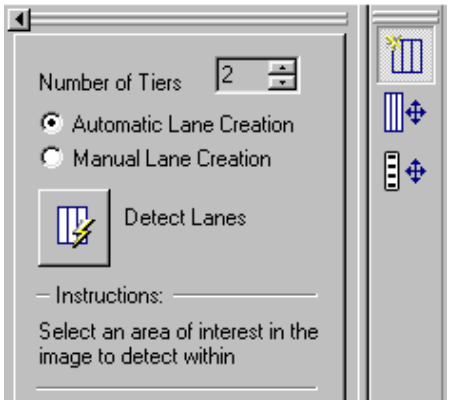
Resize Lane: Select the **Bend/resize lane** tools. Now when you move the pointer up and down the lane it appears as a **cross hair**. Each time you left click you add a tie point (small white marker). Each tie point can be repositioned by dragging it left or right. This includes the default tie points at the top and bottom of the lane. The middle graphic in the diagram below shows the reshaping of the lanes around added tie points. A tie point can be removed by right clicking on it.

Lane Width: To alter the **width** of any lane (using the resize lane tool) move the pointer slowly over the red tie points at the lane corners. The pointer will now change into a **two-way arrow**. Dragging this left or right adjusts the lane width - see the left graphic in the diagram below.

Grimacing: Sometimes the shapes of the bands in a lane look misshapen. To ensure that they are detected accurately one can added **grimaces.** These are user-defined flexible overlays, containing multiple tie-points that reflect the contours of the bands. This allows the software to automatically correct for such lane distortion during the **Band Detection** stage of analysis.



Detecting Lanes on Multi-tier Images

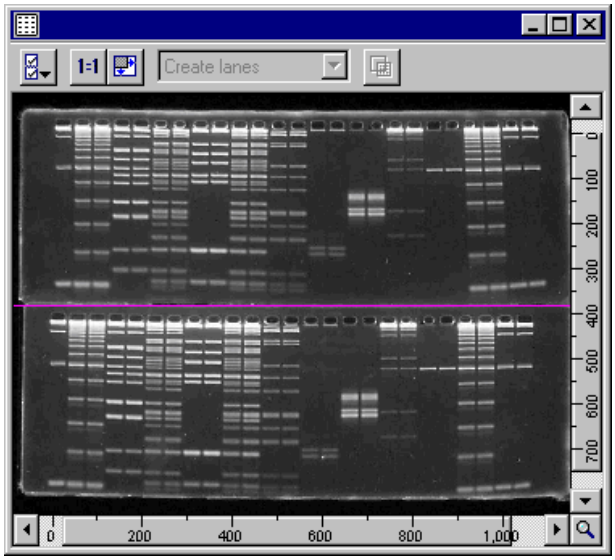


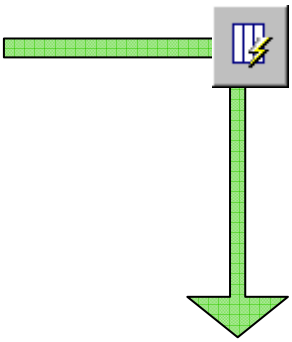
Multi-Box Adjustment

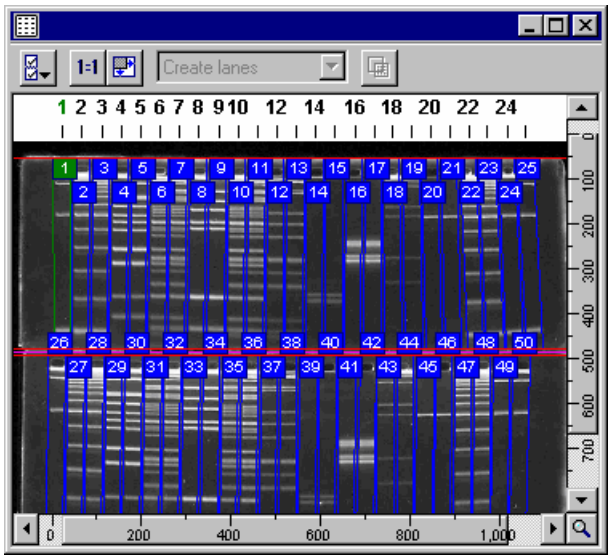
To detect multiple tiers on an image, select the required number of tiers by entering a value in the box at the top of the Lane Creation Navigator.

If two tiers are required (as shown in the example below) then you will see a purple line appear on the defining the end of the first tier and the start of the second one.

The user can adjust the position of the red line by dragging it to the appropriate location on the Image Window.

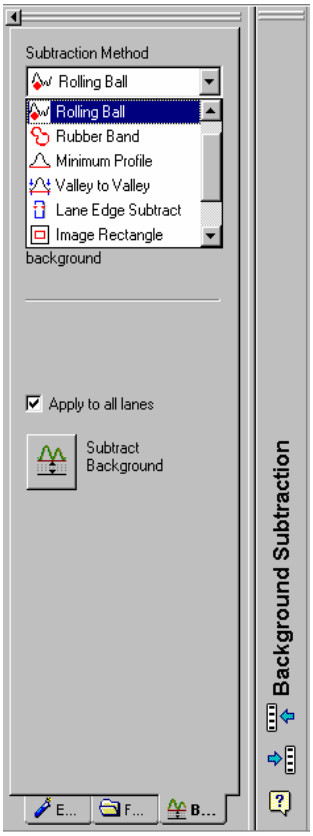






Lanes are detected by clicking on the **Detect Lanes** icon on the Lane Creation Navigator.
Once the detection of the lanes in the tiers is completed satisfactorily, the rest of the analysis proceeds as for one tier.

Stage 3: Background Subtraction



Background Subtraction

The **Background Subtraction** icon follows lane creation on the main toolbar.

Subtraction Methods

There are several different algorithms available for determining the background values. Some commonly used ones are highlighted below. To perform a subtraction method select one from the menu on the Navigator, as shown on the right. Now click on the **Subtract Background** icon to execute the algorithm. The **Measurements Table** is immediately updated to show the changes in measurements. By default, all lanes in the gel are immediately analysed using the same algorithm, although you can disable this using the **Apply to all lanes** checkbox on the Navigator dialog.

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

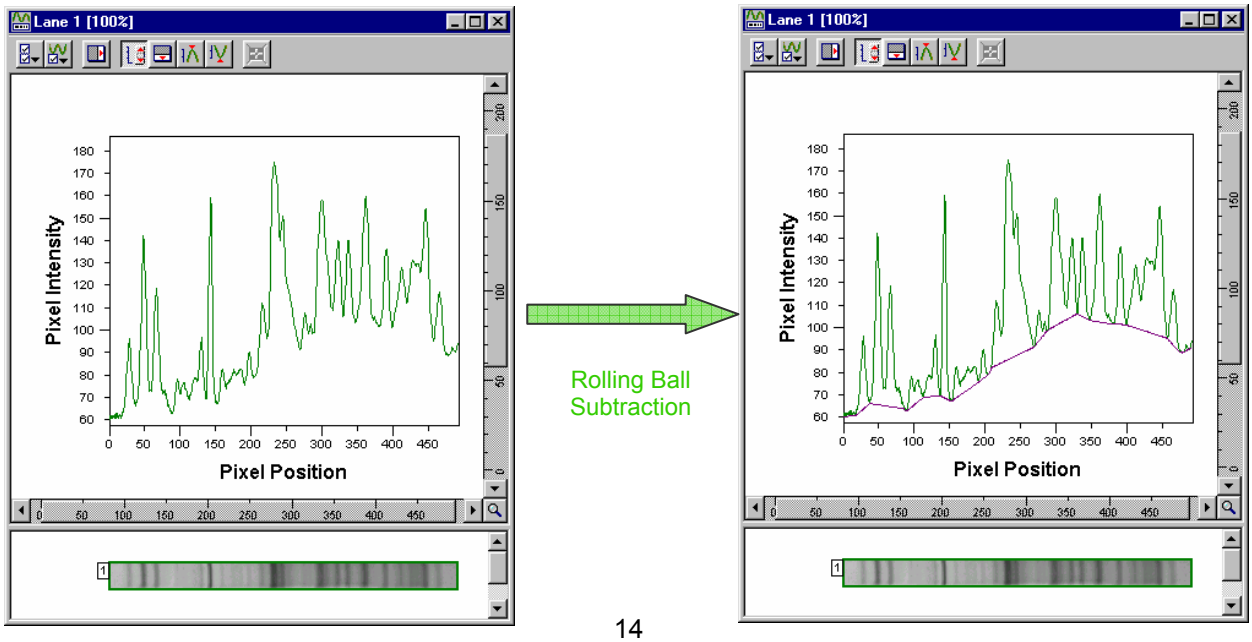
Rubber Band: - This method can be thought of as stretching a rubber band underneath the lane profile. It is not recommended for poorly-separated bands.

Minimum Profile: - This method takes the lowest value on the profile of each lane as the background for that lane.

Manual Methods: are also available in the Navigator selection box.

Viewing Background Subtraction:

To examine the effect of the background subtraction method being used, ensure that the **Analysis Window** is highlighted. The diagrams below compare the **Rolling Ball** method (Radius 200) with the **No Background** situation.



Stage 4: Band Detection

Detect Bands

The **Detect Bands** icon follows background subtraction on the main toolbar.

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.

To detect bands in all the lanes, select the **Apply to all lanes** check box.

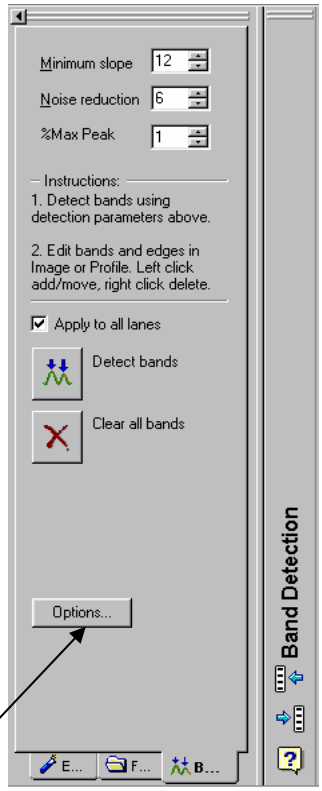
There are three key parameters controlling band detection:

Minimum slope: This figure represents how pronounced the band must be, with respect to the surrounding area in the lane. In general, the lower the minimum slope value, the more bands are detected.

Noise reduction: The figure represents the degree to which small local peaks should be ignored on the profile. It is designed to eliminate noise in the image. In general, the higher the Noise Reduction value, the fewer peaks detected.

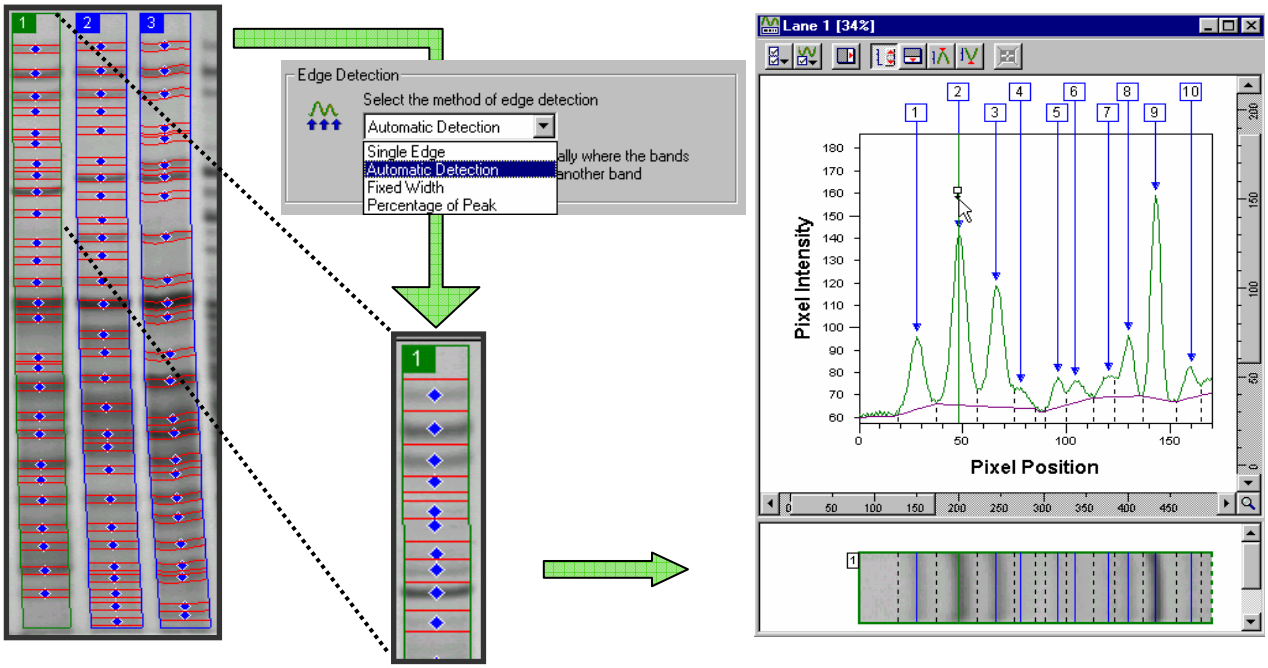
Percentage Maximum Peak: This is a threshold parameter that discards peaks that are less than a certain size, in relation to the highest peak on the gel/lane. The higher the percentage value entered here, the fewer the peaks likely to be detected in the profile.

Detection of the three lanes described in analysis stages **2** and **3** were detected, using the parameters shown in the Navigator above. The difference between automatic and fixed width edge detection is shown on the diagram below. The dialog to change the edge detection method is found by clicking on **Options**.



Detect Bands

Using the **Analysis** or Image Windows you can easily add or remove bands manually, using left click to add and right click to remove band overlays.



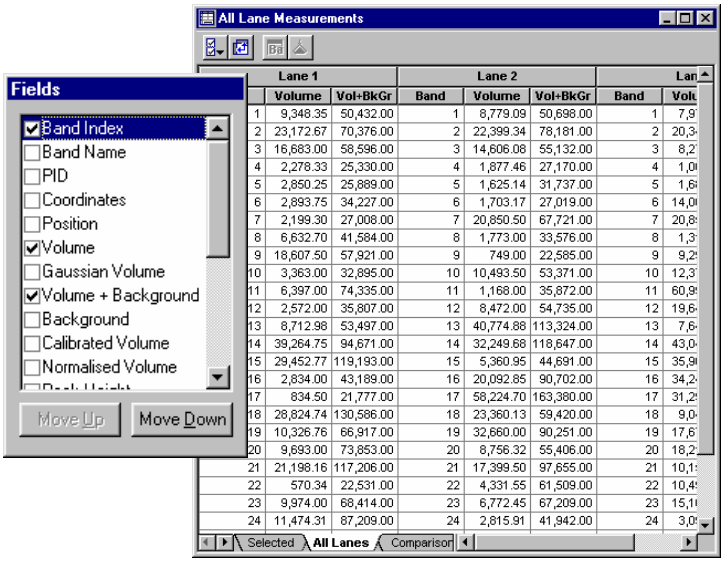
Remember, if you redetect the bands automatically any manual editing will be lost!

Band Measurement

Automatic Calculation: Band volume measurement occurs automatically when the **Detect Bands** icon is pressed.

Editing on the Fly: As each change in the band detection parameters or manual edit is made, re-measurement of the bands occurs automatically thus making the editing process both faster and more efficient.

Displaying Measurements: The Measurements Window, which is visible by default, now shows a list of all the bands that have been detected and their **volumes**. Other information about the bands in this table can be displayed by clicking the **select fields** button in the Measurements Window. The pop-out menu contains a list of check-boxes for the available fields.



Measurements Table

Stage 5: Profile Deconvolution

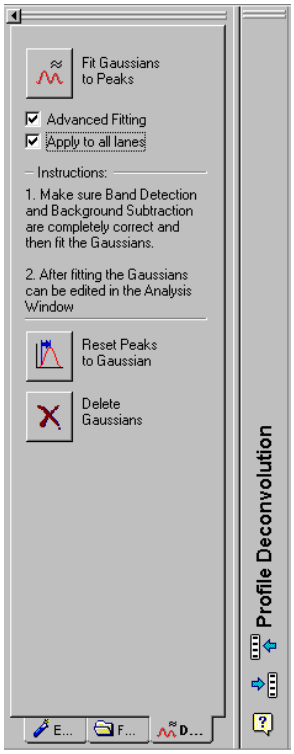
The **Profile Deconvolution** icon follows band detection on the main toolbar.

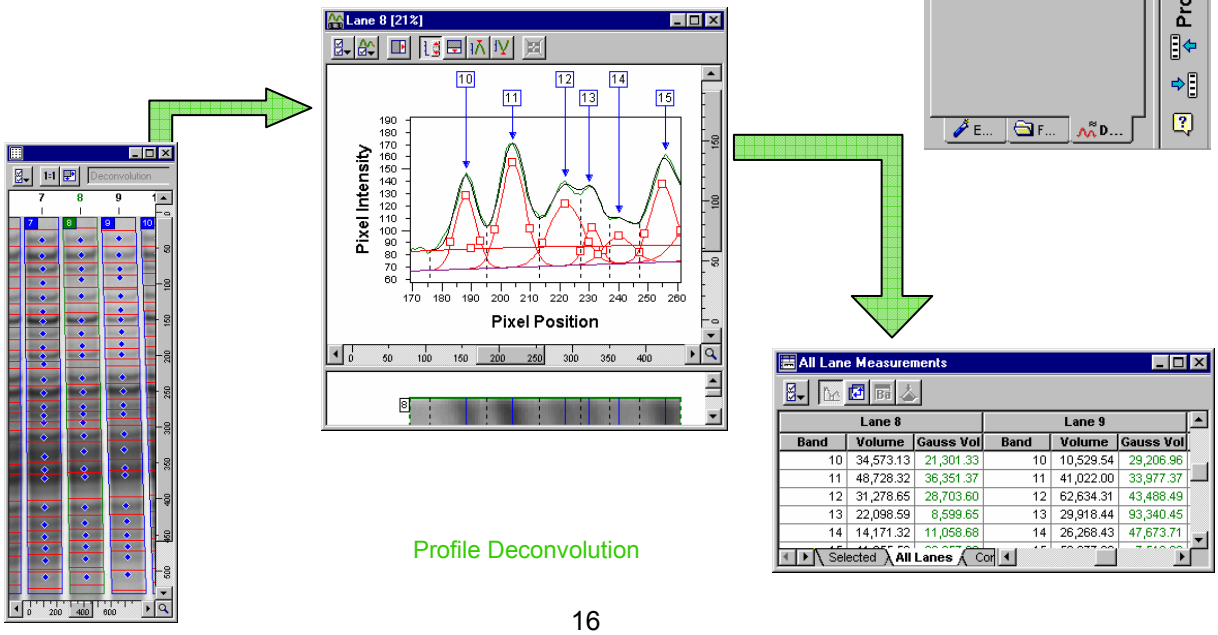
Profile Deconvolution is the process of separating a lane profile into a series of individual curves, each representing a single band. The software will automatically recalculate band volumes for merged or overlapping peaks and for bands displaying saturation.

When Profile Deconvolution is implemented the **Analysis Window** will display the Gaussian fit in **red** overlaying the original **green** profile.

Manual handles are provided to allow you to visually adjust the computed fit if required.

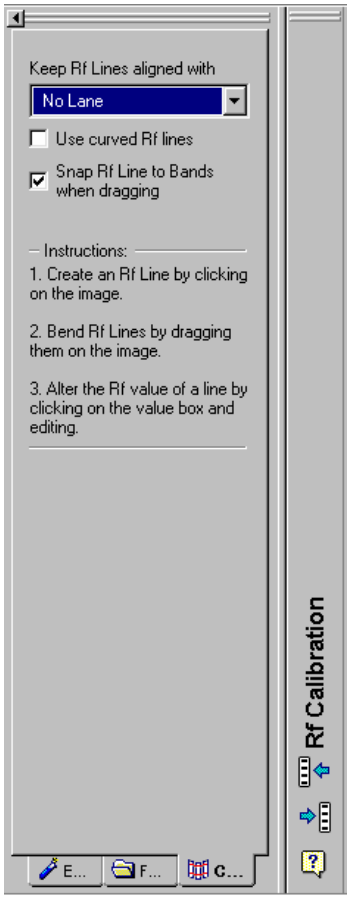
The **Measurements Table** now has an additional column displaying the **Gaussian Volume**.





Profile Deconvolution

Stage 6: Rf Calibration



Rf Calibration

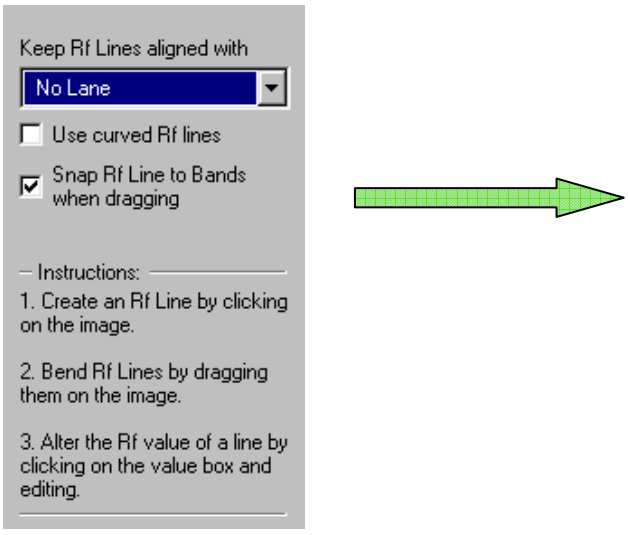
The **Rf Calibration** icon follows Profile Deconvolution on the main toolbar.

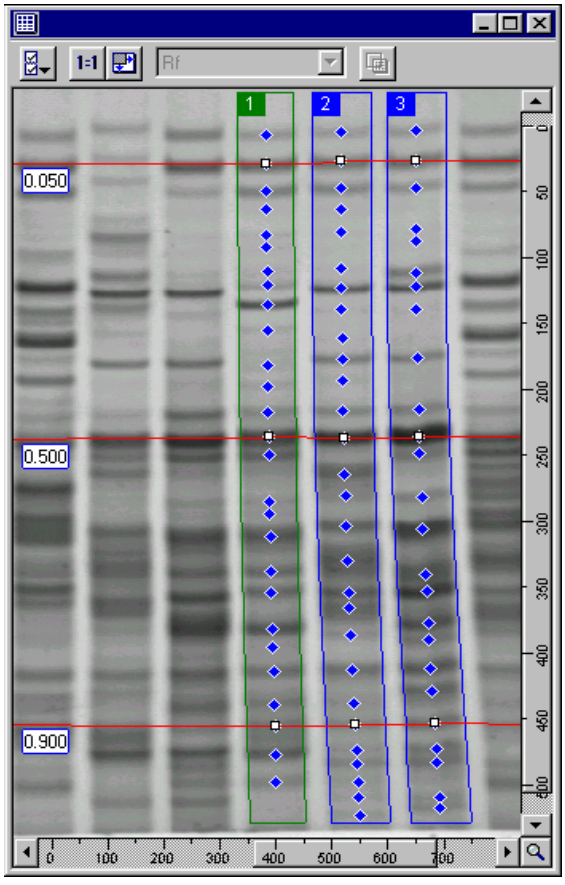
Rf or **Retardation Factor** is a measurement of position along the lane, relative to lane length. By default, the first position in each lane has an Rf of **0** and the last position has an Rf of **1**. There is a linear increase in Rf from start to finish.

To Add/Remove an Rf line: move the pointer over the Image Window and left click to add a new line. Use right click on an existing Rf line, to remove it.

Bending Rf lines: you will probably find it most useful to be viewing the whole of your gel. Move the mouse over the line you wish to bend. Left click to add a tie point or right click to remove one. Tie points can be dragged to the required position or automatically **snapped to bands** of interest, using the facility in the Navigator (as shown below).

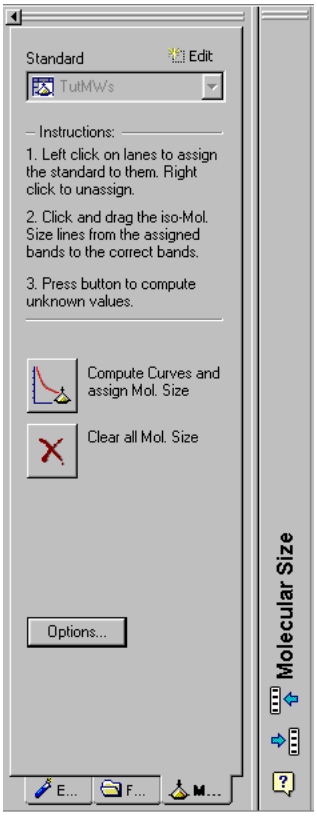
Reassigning Values to Rfs: To change the Rf value assigned to a line, first select the box containing the numerical value by left clicking on it. Now over-type the value with the new value. The box will now have a blue outline signifying a user-defined value for the Rf line. When comparing data across gels, it is important to assign similar bands with the same Rf.





Placement of Rf Lines

Stage 7: Molecular Size Calibration



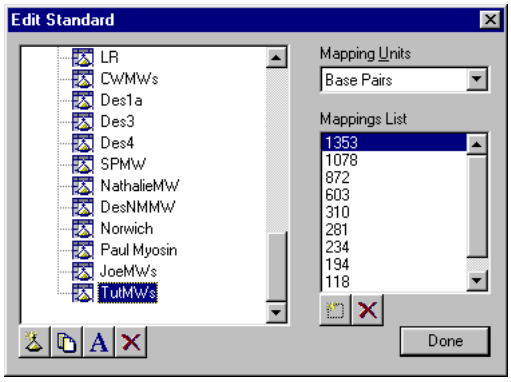
MW Calibration

The **Molecular Size Calibration** icon follows Rf calibration on the main toolbar.

To estimate the **molecular weight** or **pI** of the bands in your lanes, you will have run a standard lane (or several) alongside the normal lanes on your gel. The standard lane will have bands with known values of Molecular Weight, Base Pairs or pI.

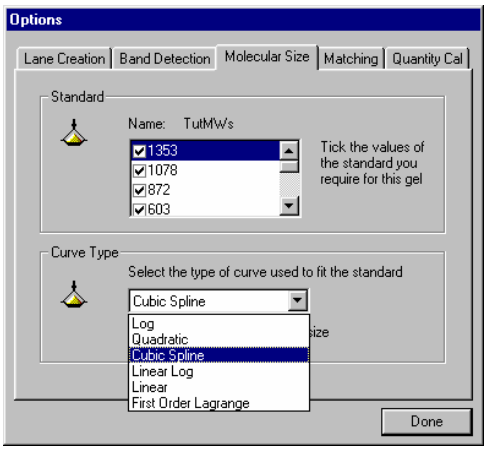
Using these known values, the program creates contours of known Molecular Size horizontally across the gel. If you have run several standards, these lines can run **between the standards**, or you can simply choose one standard as the best example and propagate the MW **using Rf** or the **position on the gel**.

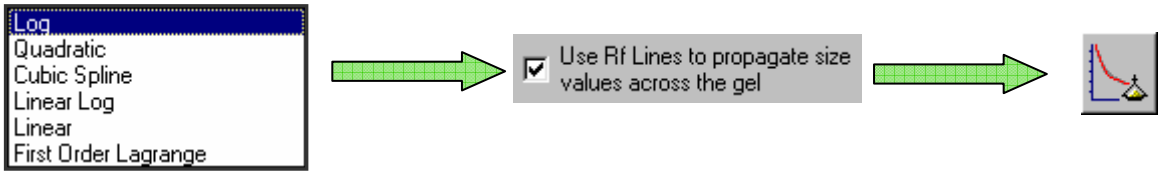
Step 1: Enter known values for standards lane(s) using the **Edit Standard** dialog, which is accessed by pressing the **Edit** icon at the top of the Navigator.



Step 2: Select a Standard Lane by clicking on it and assign the standard values either singly or all together, using the tools provided in the top panel of the Navigator.

Step 3: Now choose the **Curve Fitting Algorithm** and the method of propagation from the **Options** dialog, accessed with the **Options** button on the Navigator.



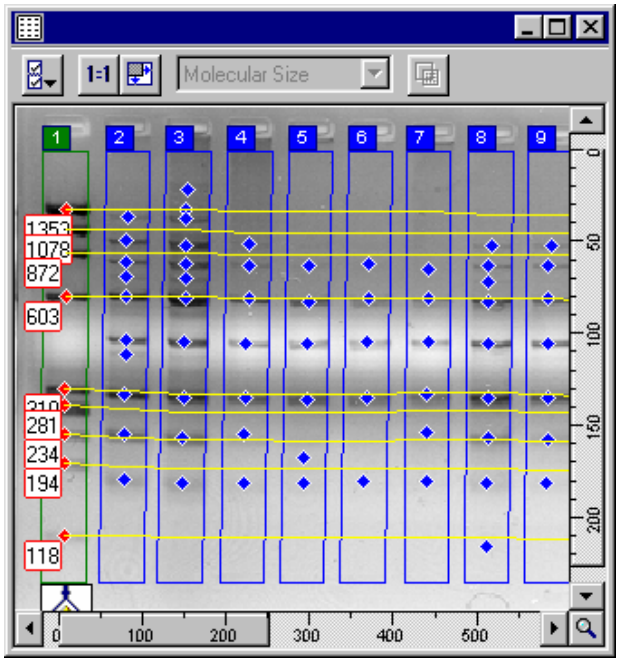


Curve Fitting Algorithm

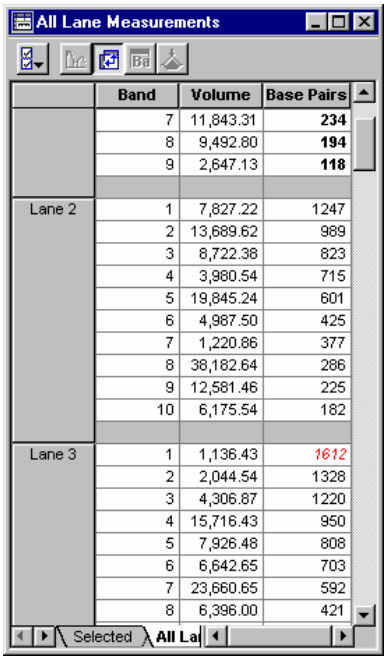
Propagation Method

Compute and Assign MW

Note: You can edit the bands of the lanes after MW calibration without losing the calibration information. However, if you edit the bands of a standard lane, then it ceases to be a standard lane until you re-assign the known values to its bands, at which point their MW values will automatically appear in an updated table.



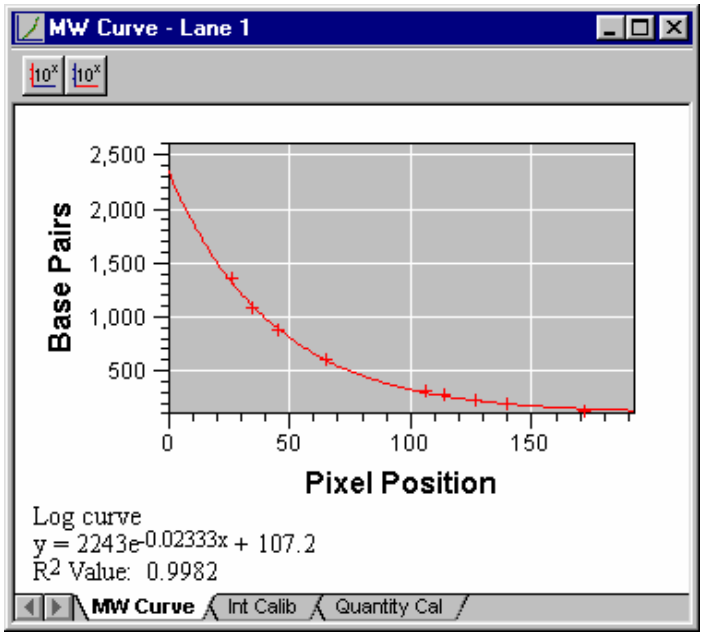
Interpolation of MW values using Rf lines



Measurements Table

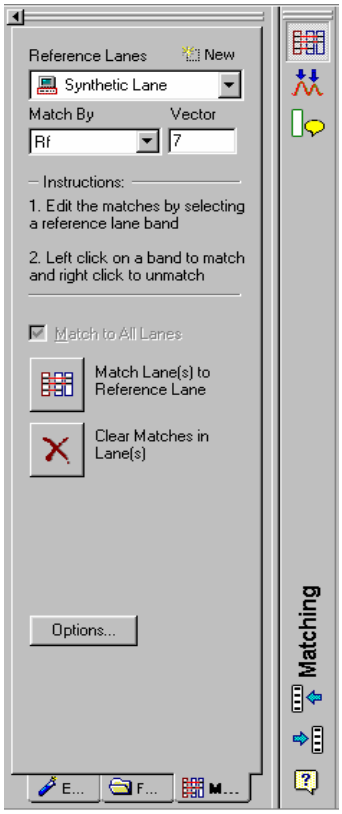
This is the advantage of being able to **edit on the fly**.

Note: Correct choice of Curve Fitting Algorithm ensures that the interpolation of unknown values generates reliable estimates for molecular weight. In the case shown below, the **best fit** to the standards data was achieved using a **log curve fitting algorithm.**



Beware: if you choose to adjust the auto detect parameters and redetect bands then all assignments of MW will be lost.

Stage 8: Matching



Matching

The **Matching** icon follows molecular size calibration on the main toolbar.

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

The Automatic Default for Matching uses a **Synthetic Reference Lane**, generated by the program that contains all the bands within the gel.

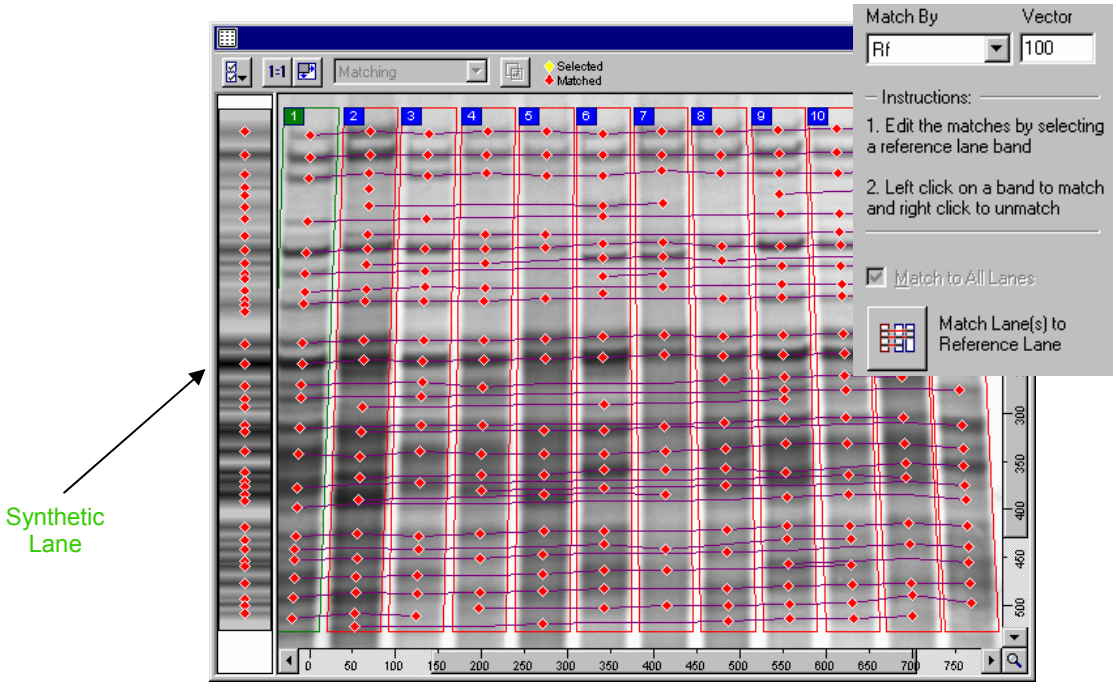
Alternatively you can select your own reference lanes either from the current gel or from the libraries of reference lanes, which you can create and build up from all your gels.

To Match: using the default **synthetic lane** simply click on the **Match lane(s) to the Reference Lane** button on the Navigator.

There are **Three Matching Methods:**

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 by the vector.

Rf: Uses the Rf values of the bands to determine if they are near enough to declare a match. The vector is in 10,000ths and a band's Rf must be within that limit to be declared a match.



Matching based on Rf

Molecular Size: This uses the logarithm of the molecular weight of the bands to determine if they are near enough to declare a match.

Each **Matching Method** uses a **Matching Vector**. This value controls the threshold for assigning a match in the three methods described above. By increasing the value you increase the chance of declaring a match between two bands.

Dendrogram Generation

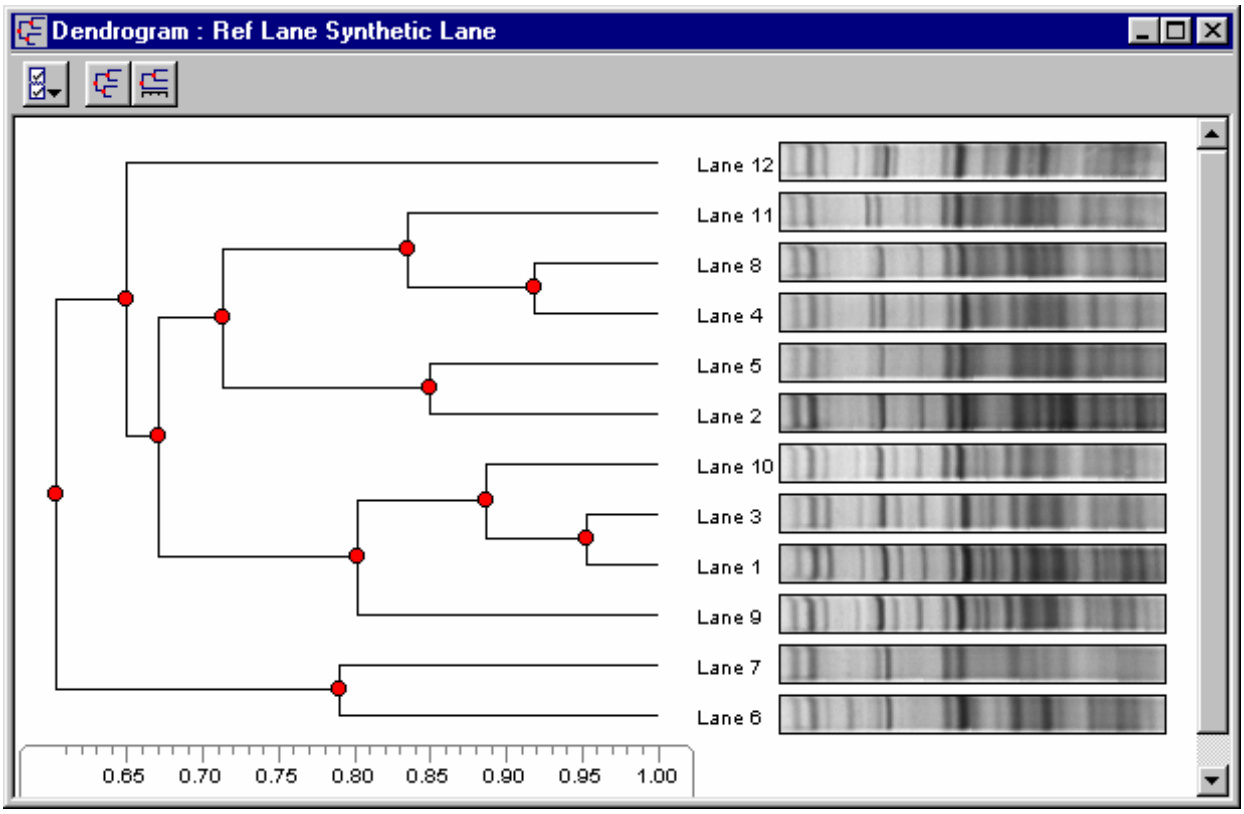
The **Dendrogram window** can be used to build and view dendrograms. The dendrograms display in a hierarchical manner, the similarities of the lanes matched to the current reference lane.

The display of dendrograms is controlled from the tool bar at the top of the Dendrogram window. Having performed matching on your lanes, select one of the following methods.

Neighbour Joining: The Neighbour Joining method clusters the sequences in a pairwise fashion.

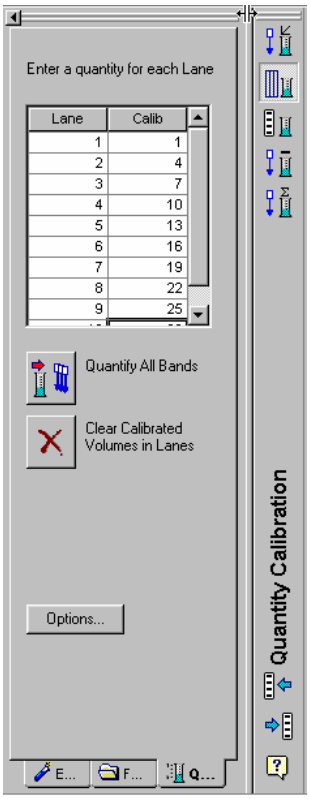
UPGMA: (Unweighted Pair-Group Method using Arithmetic Average) The UPGMA option constructs a tree by successive clustering using an average-linkage method of clustering.

The diagram below shows the UPGMA Dendrogram for the data matched in the previous section.



UPGMA Dendrogram

Stage 9: Quantity Calibration



Quantity Calibration

The purpose of quantity calibration is to relate band volume in terms of image intensity to **known** values of bands or lanes. This is performed by entering **known** volumes for bands against the corresponding detected bands. Then other bands volumes can be derived from the curve.

The **Quantity Calibration** mode is started from the button on the main tool bar.

To enter **known** values for the standard curve just click on the band in the Image Window and enter the appropriate value.

Then choose the **Calibration Unit** and **Curve Type**.

There are **five** Quantity Calibration Modes:

Use curve to quantify selected bands. This is performed by entering known volumes for bands against the corresponding detected bands. Then other bands volumes can be derived from the curve.

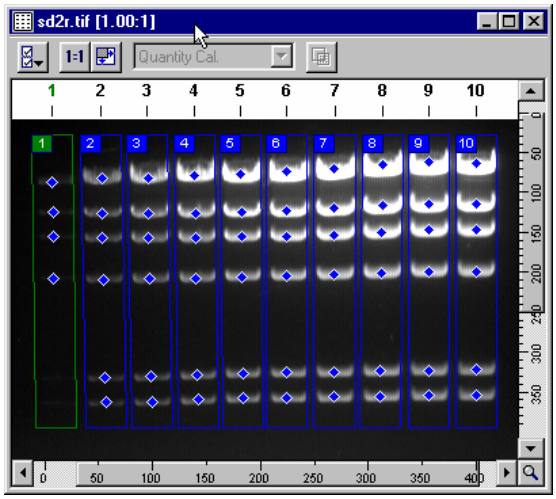
Quantify Individual Bands. Enter a value for total lane loading to compute individual band quantity in lane.

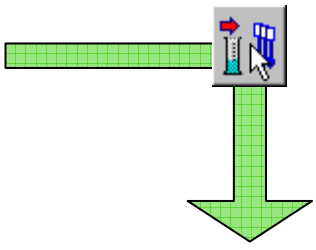
Quantify Single Lane. Entering a total quantity for a defined lane.

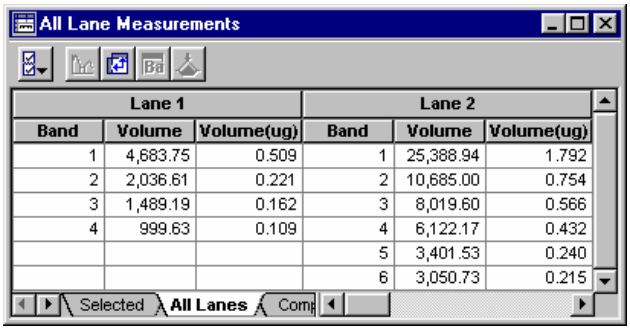
Quantify Average of Selected Bands. Select a group of bands and assign a value to average volume.

Quantify Total of Selected Bands. Select a group of bands and assign a value to total volume.

To assign all bands using the curve just click on the above icon in the Navigator. Interpolated values will now appear in the **calibrated volume field** in the **Measurements Table** as shown below.







Stage 10: Normalisation

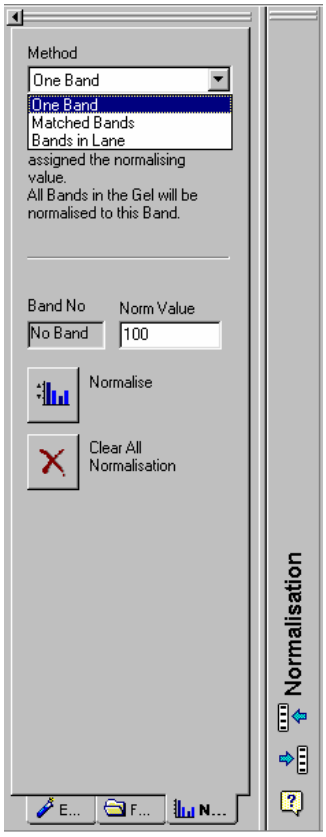
The **Normalisation** icon is to the right of the Quantity Calibration icon on the main toolbar.

Normalisation Mode is used to generate the value of normalised volumes for the bands in a gel. There are three methods available:

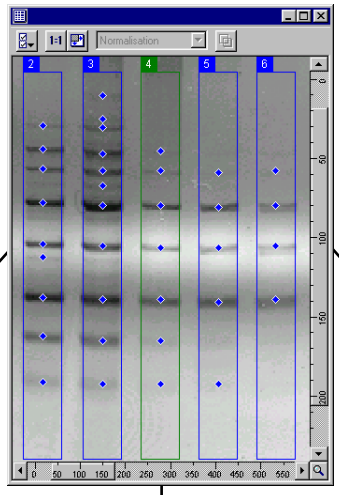
1. Normalise to one specific band in a lane.
2. Normalise a set of all bands matched to the same reference lane band.
3. Normalise to all the bands of a specific lane.

Normalisation is useful when comparing the volumes across lanes and/or gels where the loading may be different.

Note: methods **2** and **3** are only active if Band Matching has been performed.



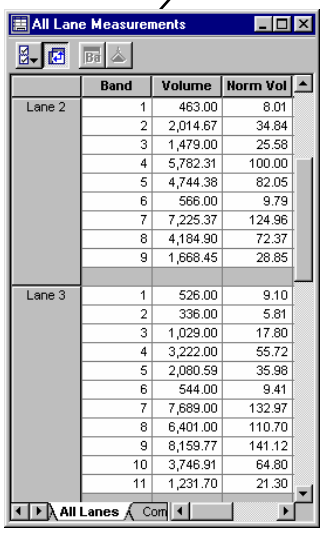
Normalisation



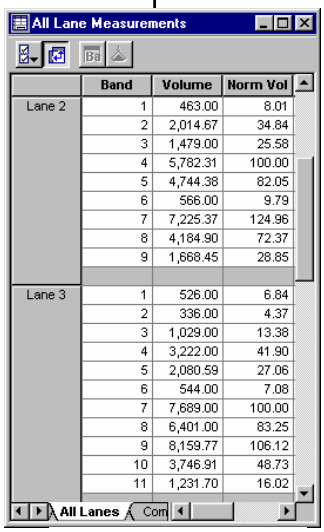
One Band

Bands in Lane

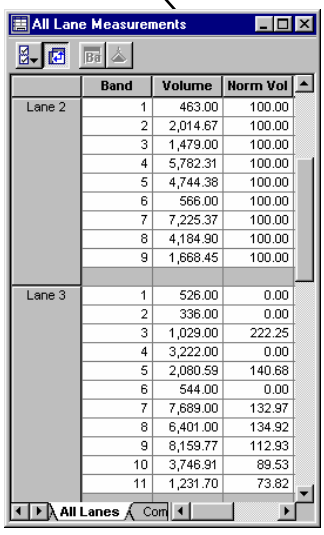
Matched Bands



Normalise to Band 4 in Lane 2



Normalise the set of bands matched to Band 4 in Lane 2



Normalise to all bands in Lane 2

Stage 11: Band Picking

To enter the Band Picking mode, select the **Band Picking** icon to the right of the Quantity Calibration icon on the main toolbar.

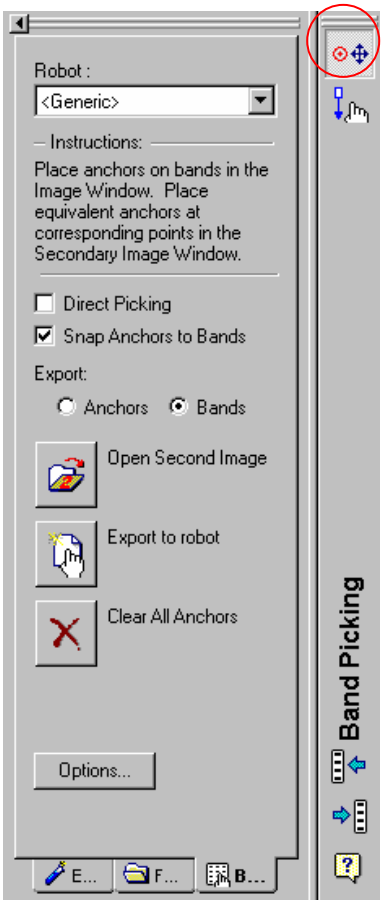
The purpose of the Band Picking mode is to map the coordinates of selected bands in the current gel, to coordinates relating to the location of the equivalent band on other gels, as viewed on a separate robot band picking device.

In general terms, the procedure for Band Picking consists of:

1. Choosing a robot from a dropdown list on the Band Picking mode Navigator.
2. Decide on how to map the coordinates of selected bands. Usually, this will be performed by using triangulation, or the alternative Ettan robot procedure. The user may prefer to use Direct Picking of bands and so avoid mapping altogether.
3. Choosing the bands to export. In some cases, the user may choose to export anchors only.
4. Export the bands or anchors and create the coordinates file for the robot. Where no robot is selected, the coordinate values are calculated and presented in a Measurements table.

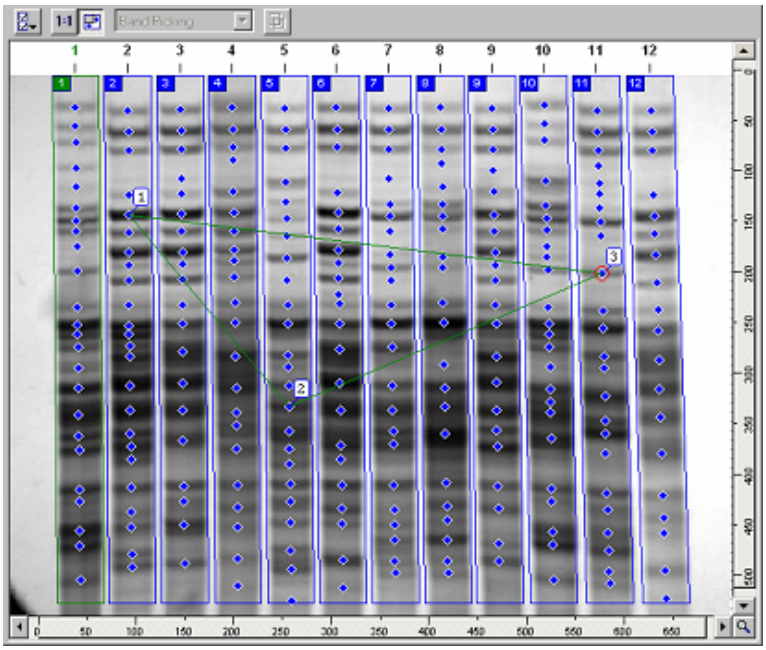
Before entering the mode, you should have performed the required image analysis procedures: lane detection, background subtraction and band detection. In addition, you should open a second image by pressing the **Open Second Image** button in the Navigator dialog. In the dialog that then opens, select the image to which you wish to map the coordinates of the bands of the current gel. Press the **Open** button to display the image.

In the event that the Second Image window is hidden by other child windows, double-click the **Secondary Image** window icon on the Window selector bar.



Having pressed the **Edit Anchors** button, select at least **3** anchor points on images in both the main and secondary image windows.

Use the **Snap Anchors to Bands** option for accurate placement. As more anchor points are added, non-overlapping green triangles are drawn. Use the right-hand mouse button to deselect an anchor if required.

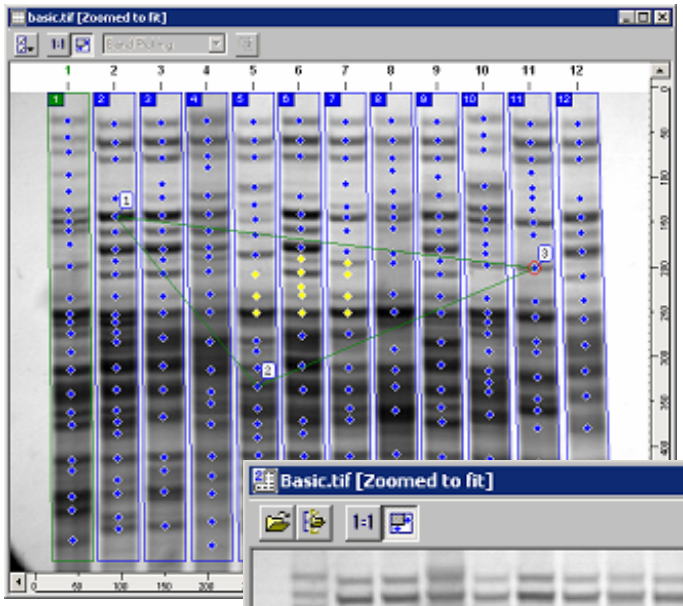


Clicking the **Direct Picking** checkbox will remove all anchors.

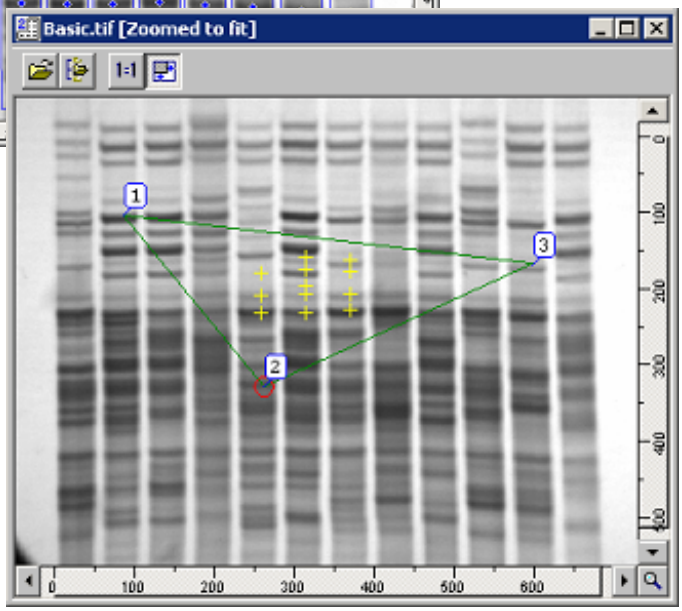
By use of left click or left click and drag, selected spots can be directly picked from the primary image and their coordinates can be exported to a text file for the robot or directory.

The **Pick Bands** button is then used to select those bands to be picked. A single **left click** will select the chosen band, a double click will select all the bands, while **left click + Ctrl** will allow an area to be defined, within which the bands will be selected.

Main Image Window

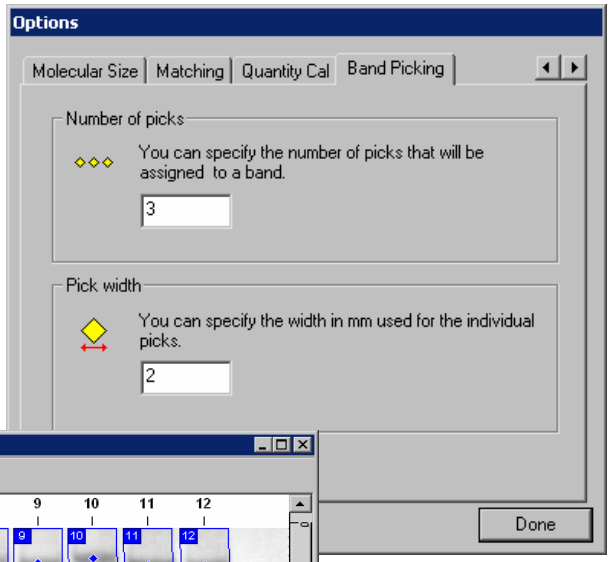


Second Image Window

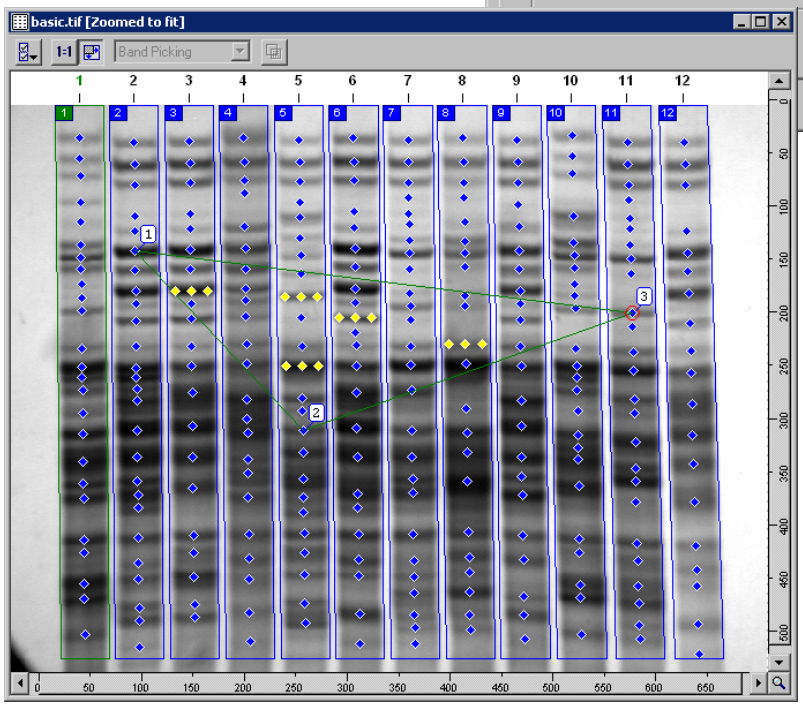


The corresponding points on the second image that lie within the anchor triangle will be selected.

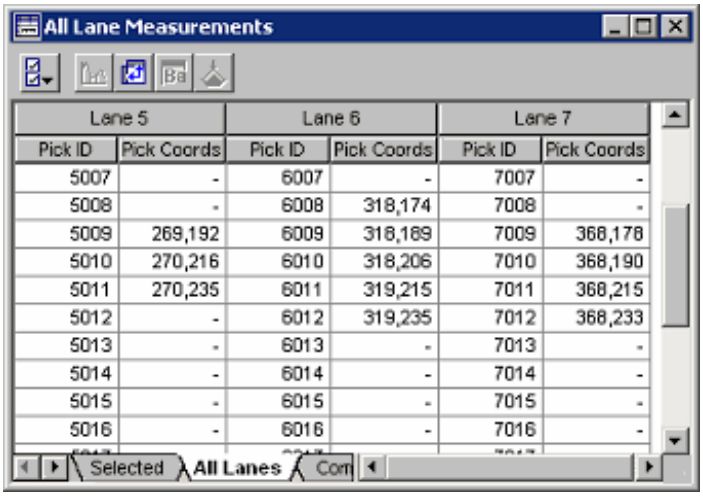
The criteria for multiple picks can be defined using the specific **Band Picking Options** dialog, accessed by selecting the **Options** button on the Band Picking **Navigator**.



The bands to picked can then be selected as outlined previously.



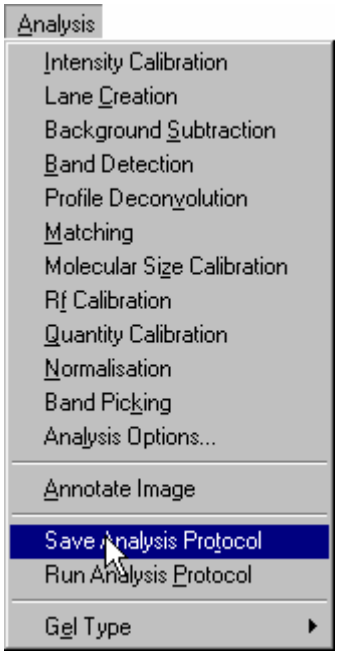
Finally, the **Export Coordinates** button is used to transfer the band coordinates as a **pick list file** to the robot. The information can then be displayed in the measurements table.



Lane 5		Lane 6		Lane 7	
Pick ID	Pick Coords	Pick ID	Pick Coords	Pick ID	Pick Coords
5007	-	6007	-	7007	-
5008	-	6008	318,174	7008	-
5009	269,192	6009	318,189	7009	368,178
5010	270,216	6010	318,206	7010	368,190
5011	270,235	6011	319,215	7011	368,215
5012	-	6012	319,235	7012	368,233
5013	-	6013	-	7013	-
5014	-	6014	-	7014	-
5015	-	6015	-	7015	-
5016	-	6016	-	7016	-

1. Analysis Protocols

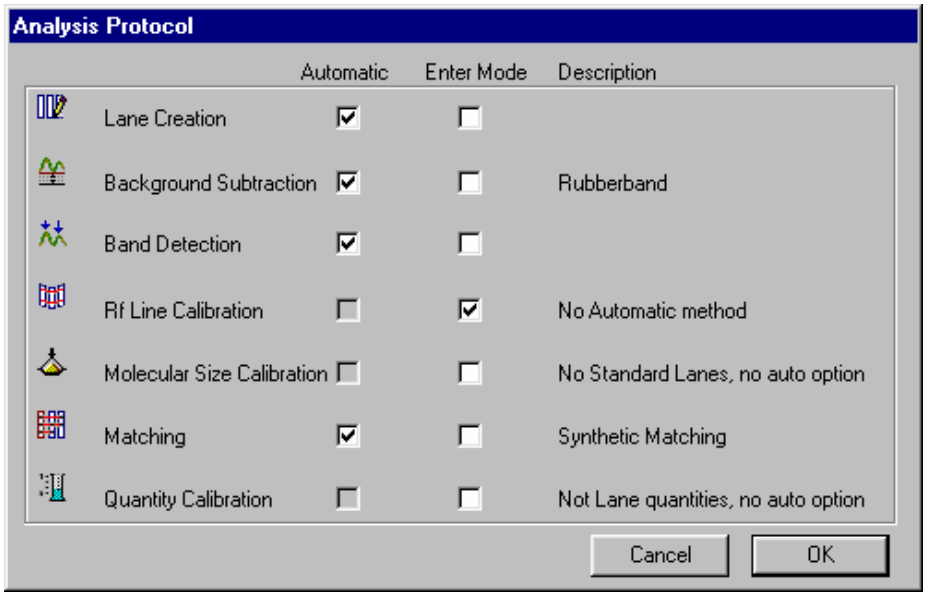
If you are analysing more than one gel in an experiment or are continually analysing similar types of gels, then analysis protocols can be employed to automate many of the analysis steps. The only step that is not possible to automate is the placement of Rfs.



Having analysed the first gel in an experiment, the **Analysis Protocol** can be saved and then automatically used to analyse the next gel.

To save a new **Analysis Protocol** at the end of analysis select the **Save Analysis Protocol** option from the analysis menu, as shown to the right. This will open the dialog illustrated below.

Tick the steps you wish to automate. If you wish to manually enter parameters for any of the steps within the protocol then tick the **Enter Mode** box for the appropriate step in the analysis.



To run a stored analysis protocol, first select the protocol to run from the **Run Analysis Protocol** dialog.

If analysis has already been performed on the current gel, then when you run a protocol you will be warned that the existing analysis data will be overwritten.

To **run the last used protocol**, click on the icon on the main toolbar.

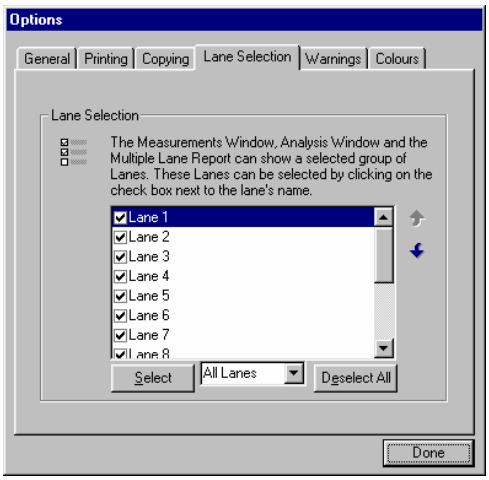
2. Displaying Multi-Lane Information

Once a gel has been analysed there are many ways to examine the data, especially if the lanes have been matched.

Lane Selection

First select the **Normal Mode** and then select the lanes to be compared from the **View Menu | Options | Lane Selections** dialog. Tick the required lanes.

Note: If the current lane is to be included in the selection and you are only comparing them in the **Analysis Window**, then do not tick it as required.



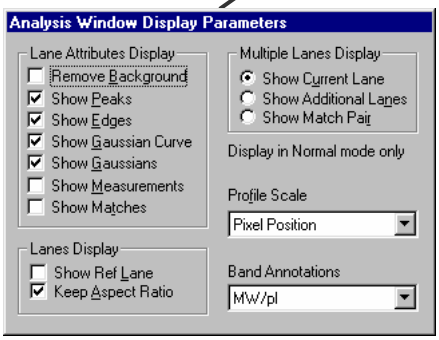
Lane Selections

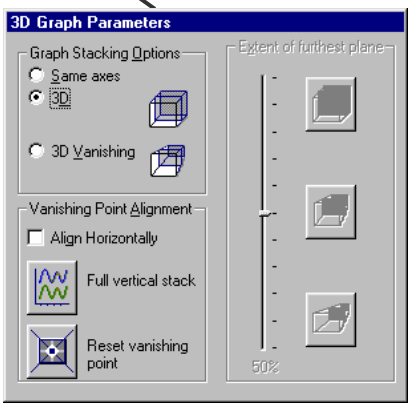
Setting up the Analysis Window Display:

The first two icons in the Analysis Window activate two display dialogs.

Profile Display Parameters

Profile Display Alignment

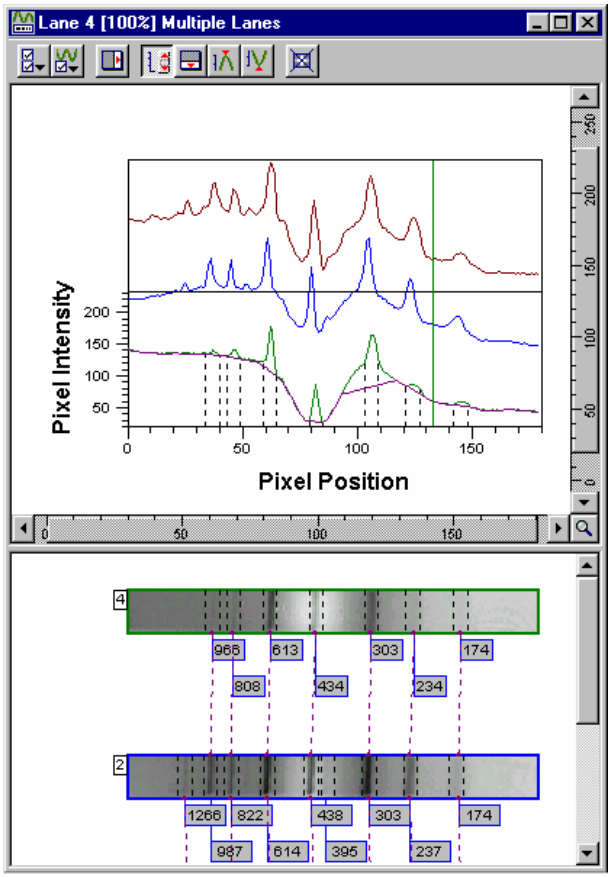




Using the display dialogs described on the previous page, the Analysis Window has been set up to display the **Current** lane profile - **lane 4** - and two additional profiles for **lanes 2 and 3**.

Note: The profile colour corresponds to the colour surrounding the bitmap representation of the lane shown in the lower panel.

The lower panel also shows which bands are matched and in this case the **Band Annotations** is set to displaying the **Molecular Size.**

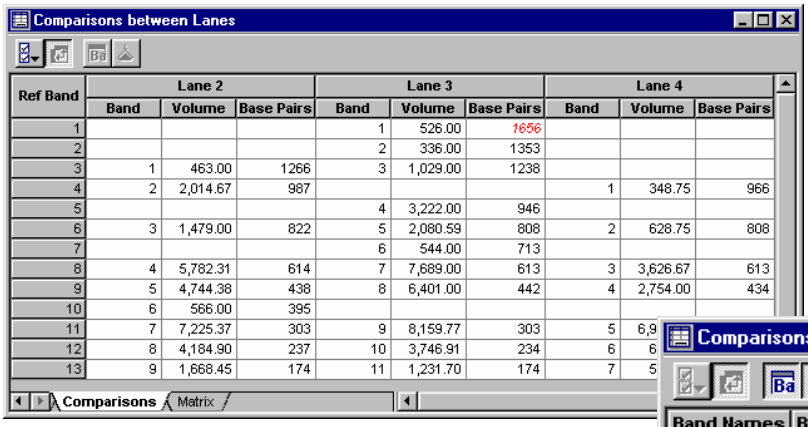


Multi-Lanes in Analysis Window

Displaying details of Selected Lanes:

In the lane selections dialog shown on the previous page make sure that lanes **2**, **3** and **4** are ticked.

In the **Measurements Table** click on the **Comparisons** tab at the bottom right of the table, this will display details of the three lanes aligning the matched bands (see below).

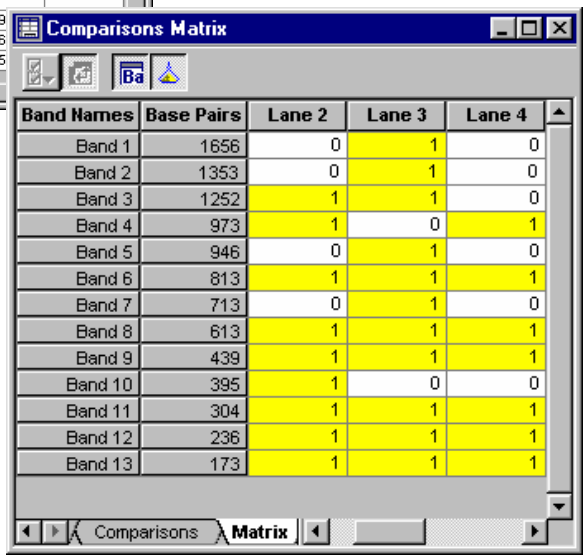


Ref Band	Lane 2			Lane 3			Lane 4		
	Band	Volume	Base Pairs	Band	Volume	Base Pairs	Band	Volume	Base Pairs
1				1	526.00	1656			
2				2	336.00	1353			
3	1	463.00	1266	3	1,029.00	1238			
4	2	2,014.67	987				1	348.75	966
5				4	3,222.00	946			
6	3	1,479.00	822	5	2,080.59	808	2	628.75	808
7				6	544.00	713			
8	4	5,782.31	614	7	7,689.00	613	3	3,626.67	613
9	5	4,744.38	438	8	6,401.00	442	4	2,754.00	434
10	6	566.00	395						
11	7	7,225.37	303	9	8,159.77	303	5	6,9	
12	8	4,184.90	237	10	3,746.91	234	6	6	
13	9	1,668.45	174	11	1,231.70	174	7	5	

Multi-Lanes Comparisons

A comparisons matrix can also be displayed in the Measurements Table. The designation **1** indicates a match and the designation **0** indicates no match.

Note: the Base Pair value is the average value of the matched bands.

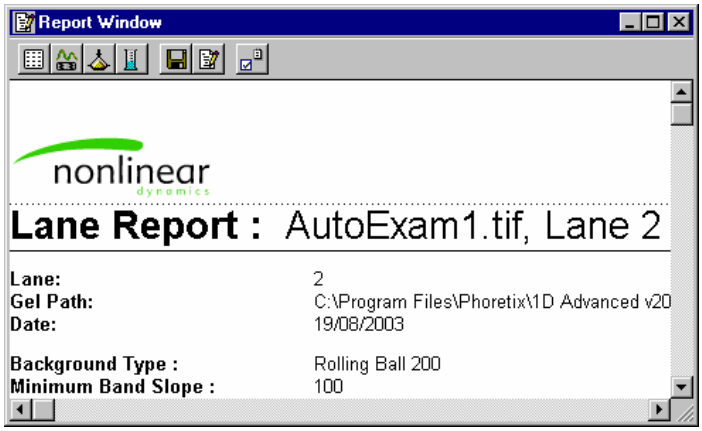


Band Names	Base Pairs	Lane 2	Lane 3	Lane 4
Band 1	1656	0	1	0
Band 2	1353	0	1	0
Band 3	1252	1	1	0
Band 4	973	1	0	1
Band 5	946	0	1	0
Band 6	813	1	1	1
Band 7	713	0	1	0
Band 8	613	1	1	1
Band 9	439	1	1	1
Band 10	395	1	0	0
Band 11	304	1	1	1
Band 12	236	1	1	1
Band 13	173	1	1	1

Multi-Lanes Matrix

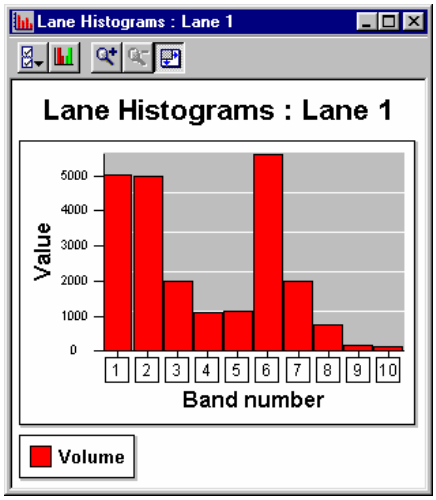
3. Reports

The program features an **Automatic Report Generator** and this will ensure GLP reporting or provide an easy way of keeping records for a laboratory notebook. Gel, Lane, Molecular Weight Calibration and Quantity Calibration reports can all be generated.



4. Histograms

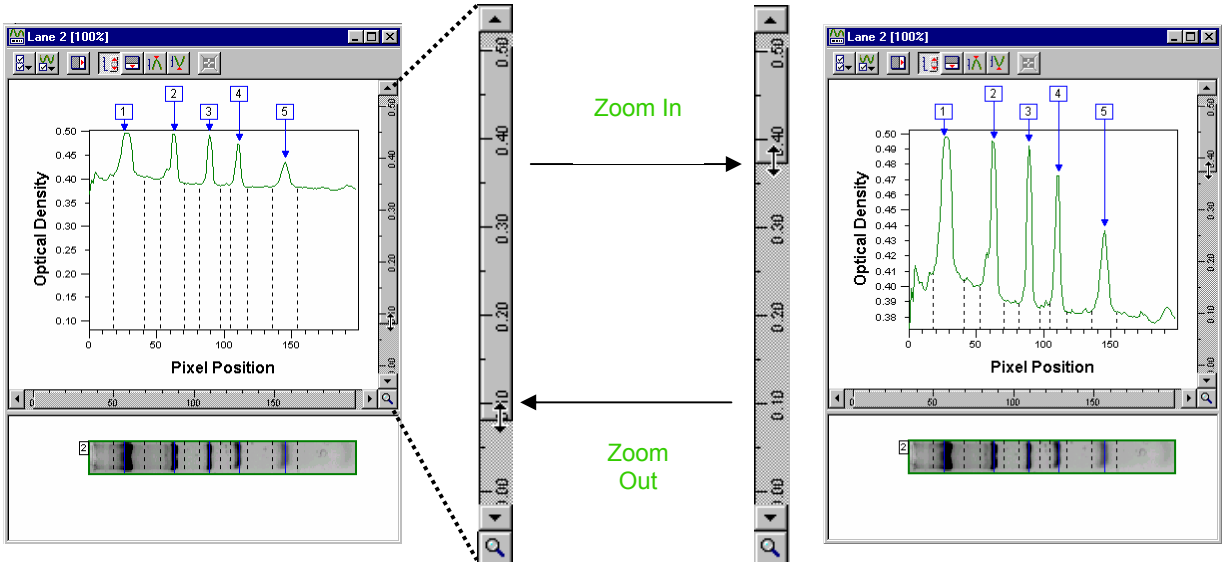
The **Histograms** window allows the user considerable control over the graphical layout, with the user able to select the colours of the bars in the histogram, their order and what fields are displayed from the window toolbar.



Histograms

5. Zoom Function on Windows

The Zoom Function offers infinite flexibility for viewing the images. One can zoom in and out using the dedicated zoom feature in the bottom right of the Image Window (magnifying glass), or use the resizable scroll bars as shown below by dragging the ends of the **Thumb** (double headed pointer arrow).



Zooming In and Out of the Image using the Resizable Scroll Bars

6. Band Names, Lane Names and Annotations

Annotations can be user defined and placed as the user desires using the Annotation Facility. The **Display Options** dialog accessed by clicking on the first icon in the **Image Window**, allows you to switch Band and Lane Names on or off.

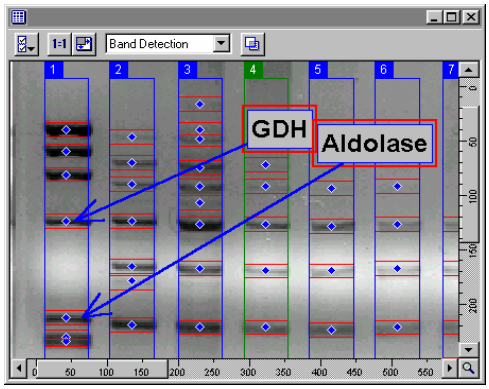
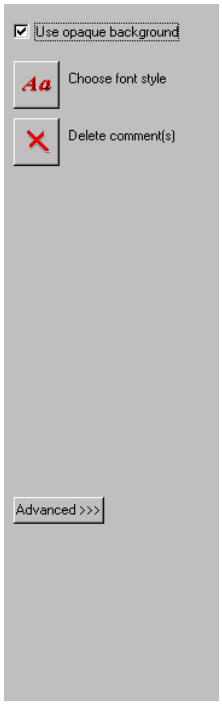
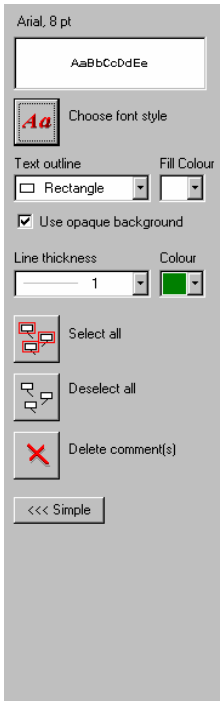
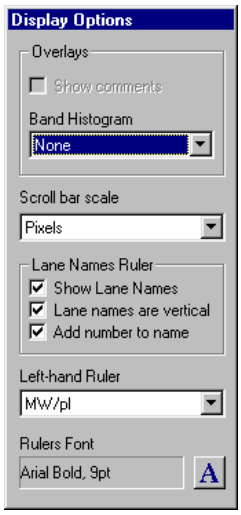


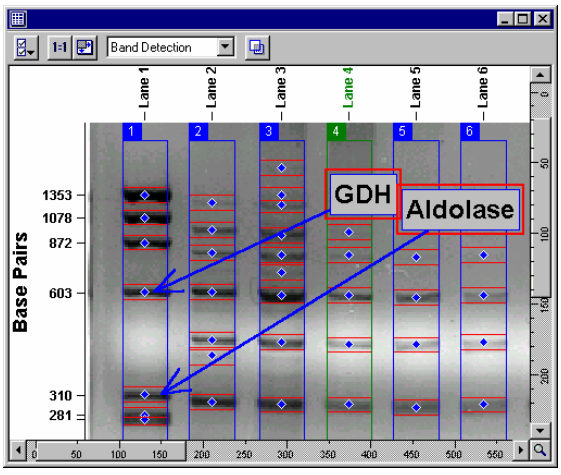
Image Annotation



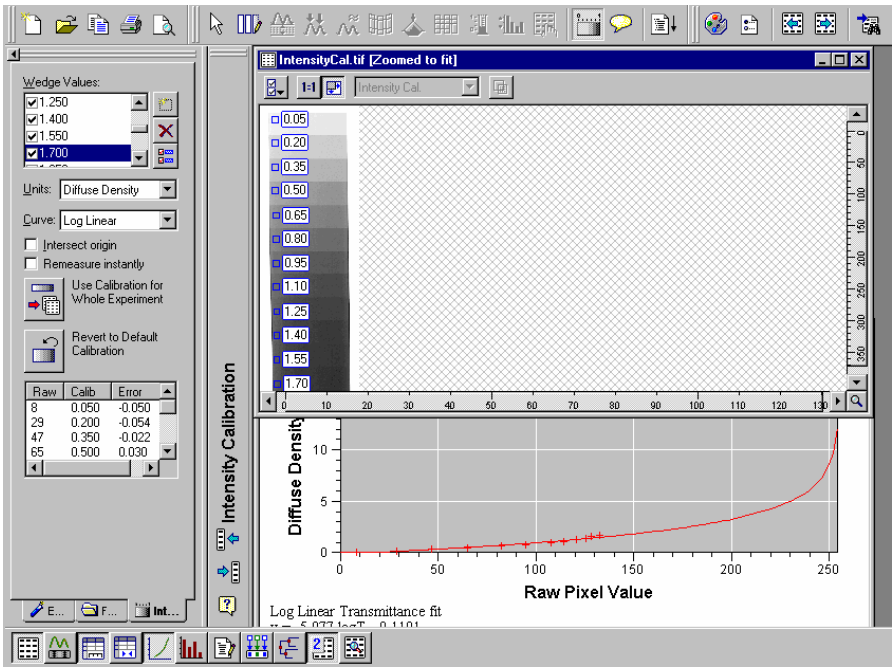




Changing Display Options



7. Intensity Calibration



The image to the right shows the calibration of the image capture device, using intensity calibration based on the capture of a calibrated **Step Wedge**.

This step wedge has known intensity values assigned to it by the manufacturer.

These are placed correctly over the image and the calibration is fitted using a curve of choice and using **diffuse or optical densities** or **counts** as the unit. This calibration can then be passed to a whole series of images captured at the same time.

The step wedge can be captured in the same image as the gel image or as a separate image at the same time and settings.