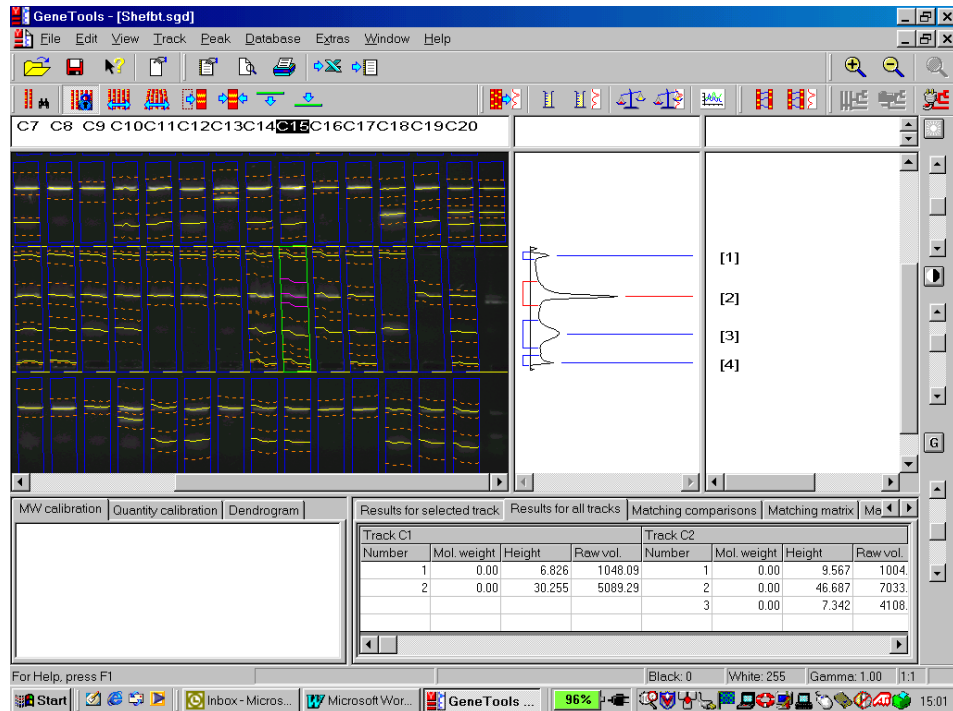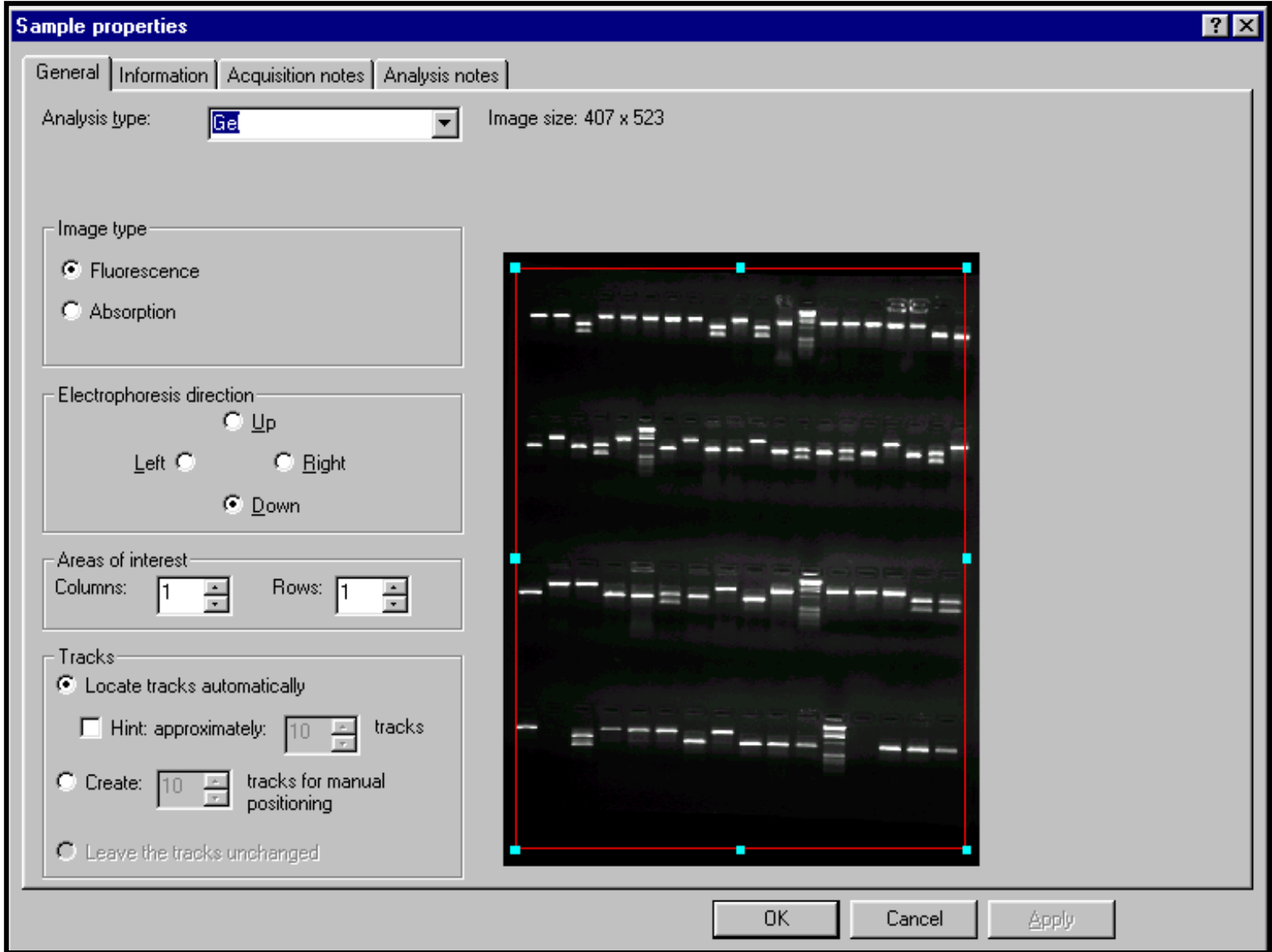# GeneTools Demonstration Notes

SYNGENE

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GeneTools Demonstration Guide

Upon opening an image or transferring an image from GeneSnap to GeneTools, the first window seen contains the **Sample Properties**.

There are 4 tabs within the Sample Properties window. The first, **General**, contains a **thumbnail** of the image and a number of options which may be selected by the user. These options depend upon the type of analysis selected. Analysis type determines the type of analysis required. There are four options:

Gel should be selected for band detection, molecular weight analysis, peak profiling and densitometry.
Manual Band Quantification is for specific band densitometry.

Spot Blot will automatically detect gridded or non-gridded spot or slot blots or other samples with 'round' data such as ELISA plates. It will perform densitometry, thresholding and area comparisons etc.
Colony (pour plate) is for colony counting and analysis.

The other tabs within Sample Properties are as follows:
Information – lists the user name (at time of capture), capture date, unique ID number, darkroom, camera, lighting and exposure settings.
Acquisition notes and Analysis notes – optional notes which may be added by the user.

Now we will look at the different analysis types and their content.

GEL

When a Gel document is selected, we are looking at tracks of bands upon an electrophoresis gel. Before proceeding to analysis, ensure that all information is correctly entered in the Sample Properties window. Whether the image is **fluorescence** (white bands on black background) or **absorption** (vice versa) is automatically detected – but always check it is correct. The **direction of electrophoresis** is defaulted to Down (this can be changed under individual preferences) so ensure this is checked.
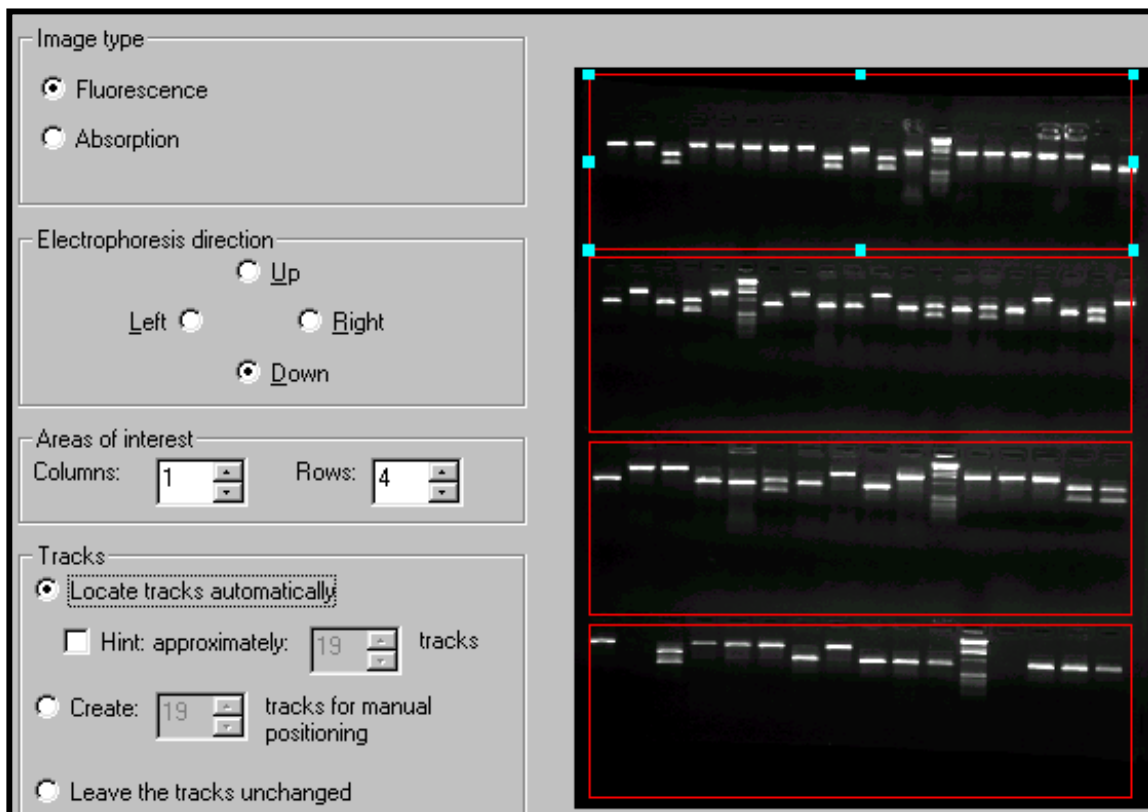
The 'Areas of interest' Columns and Rows boxes need only be altered if analysing a multi-layer gel, or multiple gels within the same image. The red box or boxes determine areas of interest for analysis, and these should be set accordingly. Clicking within each red box enables track detection to be set for that area. The **Tracks** menu determines

how tracks are located. If '**Locate tracks automatically**' is selected, GeneTools will use unique algorithms to determine where tracks are located, even if they are distorted.

Occasionally the detection algorithm will not locate the tracks accurately. Reasons for this include insufficient resolution and old gels with diffuse bands. (For more information read the track finding document on the GeneTools CD).
In these cases the '**Hint**' facility can be used. The red area of interest box should be positioned as accurately around the tracks as possible (excluding the borders of the gel). Enter the approximate number of tracks in the selection box and select 'OK'.
If the user prefers an exact number of tracks can be manually entered (select the '**Create**' option), this will often require some further manipulation to ensure tracks are precisely placed.

Gels or layers within the same image may have tracks detected automatically or manually. It is possible to return to the Sample Properties window and adjust selections if the tracks are not located to your satisfaction. Simply select the 'Sample Properties' option from the toolbar

If you are analysing a multi-layer gel and wish to change some rows and not others, ensure the 'Leave the tracks unchanged' function is selected for the rows that are satisfactory.

Once tracks have been overlaid automatically or manually they can be manipulated in many ways. Manipulation can be either for a single track or all tracks. **Track width**, **well position, dye front** and **Rf** can all be adjusted. **Individual tracks** can be **bent** and distorted into almost any position. To begin manual manipulation, simply click on the appropriate icon. Once happy with the position of the tracks, they should be **locked** by clicking on the padlock icon. A warning will appear before re-calculating data if appropriate. NB: for descriptions of all the functions of the track adjustment icons see the track menu.
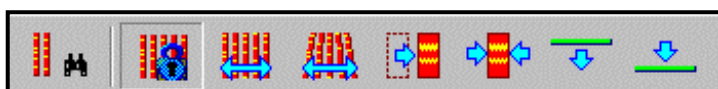
Track manipulation icons

Any tracks that you do not wish to include in analysis can be removed from results tables etc. by selecting '**disable**' from the track menu.
In the lower part of the screen a data table is displayed which can be used to view one or all tracks at once. Individual **peaks** within tracks can be **added**, **deleted** or **adjusted** by clicking in the right hand top pane, which contains the track histogram.
GeneTools enables the user to analyse gels containing both dark and light bands. This is a unique feature that makes the software suitable for the analysis of Zyma gels. To invert a track simply select it and use either a right hand mouse click to reveal the 'invert' option or use the Track menu.

Align banks of tracks
This option appears in the view menu.

Align banks of tracks
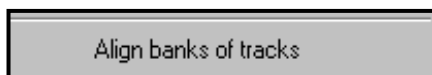
If analysing a gel with tracks running horizontally, this function will rotate them through 90 degrees so they are vertical. This makes the analysis easier to follow on the screen
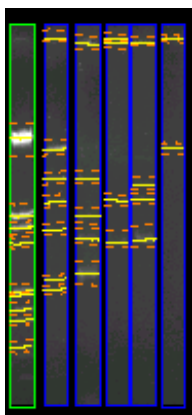
Once all tracks and bands have been detected there are many analysis options. **Molecular weights** can easily be calculated if there are known standards present on the image. Select the lane containing the standards and click on the **Molecular Weight Standard** icon.

Molecular Weight Parameters

Molecular Weight Standard

A window will appear which allows the user to enter values for **Molecular weights** using one of three methods. In the 'manual' tab the user simply selects peaks with known values and assigns the values manually, moving up and down the track as appropriate with the arrow keys. The 'from standard' tab contains a library of pre-entered standards which may be loaded. Simply ensure that the first peak is selected before hitting the assign button. If the appropriate standard is not in the library, simply edit an existing one or create a new standard. Once created, the new standard will enter the library for the next analysis. Any standards assigned can be checked in the curve window before clicking the OK button to accept them. Once entered and assigned, the molecular weight values will appear in red in the appropriate track result box, and all unknown peak values will be calculated from them.
If required the **base pairs** value may be displayed in the track result box. To select this option right click in the results table and all available options will be displayed.

If more than one set of markers is used the results are based on both sets. This can help to correct for smiling. If one selects the **Molecular Weight Parameters**
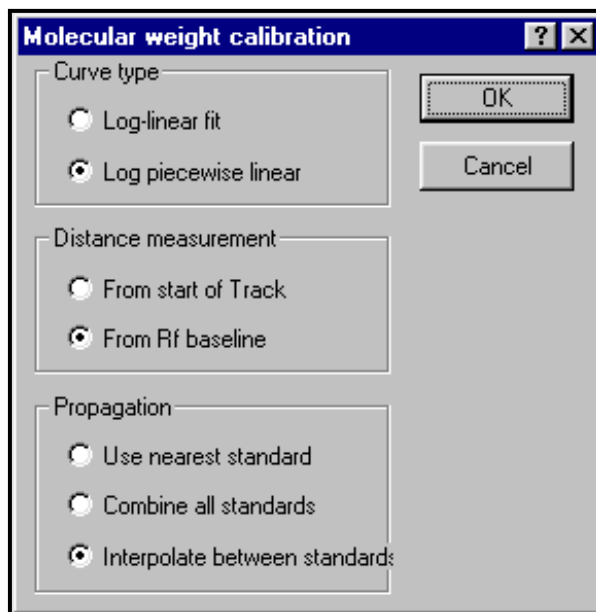

several variables can be applied to data including curve type, distance measurement and propagation.

Propagation controls how the different standards are utilised. Interpolation helps to correct for smiling. Alternatively, use the nearest standard to each track or combine standards if you have both small and large values.

If a **Quantity** is known for one of the bands on the gel, the others can be calculated from it. Click on the known band and then on the **Assign Quantity** icon.

Quantity Calibration Parameters
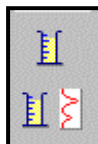
Assign Quantity

 Enter the value for the band in the window. The value will appear in red under a 'quantity' column in the data window, and all unknown values are calculated relative to it. If the **Quantity Calibration Parameters** icon is selected one can alter the curve type to enter a number of known standards (increases accuracy) or to enter some units for the quantity (e.g.:ng).
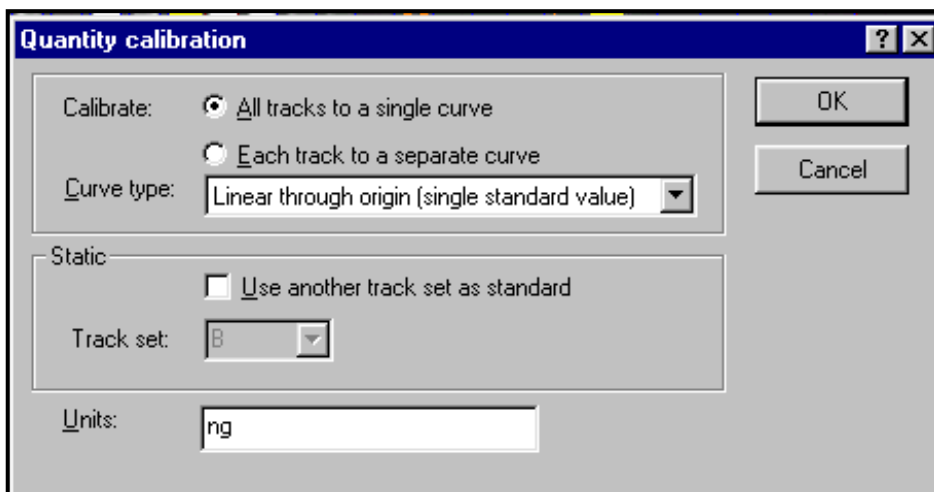
Quantity calculations may be made using one calibration curve for the whole image, or treating each track as a separate sub-image with it's own standard curve. Select the appropriate setting for your sample in 'quantity calibration parameters'. If choosing the latter method, it is possible to propagate a quantity across each track, using either peak number or peak position (Rf) to achieve this. Select your chosen method of propagation in 'assign quantity'.

Within Multi-layer gels it is possible to use a quantity calibration standard from one set of tracks for another set of tracks or indeed another gel. Simply select as appropriate. If the 'use another track as standard' box is ticked, the desired track set may then be selected from the drop down menu.

Band Matching is another component of GeneTools.   This function allows a selected band to be matched against other bands in other tracks on the gel.   Click on the **Band Matching Parameters** icon to select whether matching should be by Position, Rf or MW. Tolerance % may also be set within this menu.
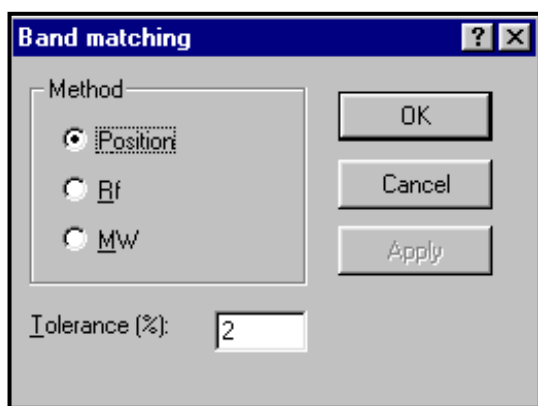
Once happy, select the track of interest and click on the **Band Matching** icon.
The bands within the selected track will be joined to any matching ones within the set detection limits.   A **Dendogram** will also be produced which can be maximised by using the right hand mouse button. The dendrograms in GeneTools are based on the 'neighbour-joining method'.   For more information on this process please consult

GeneTools online help under the heading 'Dendrograms'.   For further information concerning matching view Matching **Comparisons**, Matching **Matrix** and Matching **Coefficients** in the data table.
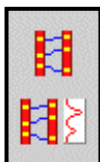
Band Matching Parameters

Set Matching Standard

To view one or more of the tracks on the same graph, click on the **Profile Comparison** icon.

This feature can be used to compare tracks on the same or different gels. This is an incredibly useful feature and represents a major advantage over most competitor software. Just ensure that all gels of interest have an image window open within GeneTools before clicking on profile comparison. Expand the gel icon on the RHS of the screen by clicking on +.   Then double left click on tracks as required adding or removing them from the graph. Any track may be selected as the reference by selecting it and clicking on right hand mouse button. The reference track will automatically be added to the graph but it can be removed although it will remain the reference until unselected or another track is selected. The track properties may also be selected by clicking the right hand mouse button.

To alter the scale of the graph click on the zoom in button. To view the required section of graph when in zoom use the scroll bar. If wished, up to twelve different colours can be assigned to tracks under the Extras/ Configuration menu.

The Profile comparison may be exported to Word™ or saved to clipboard as required (see later for more details on reporting functions).

The **Integration Parameters** menu also relates to gel analysis.
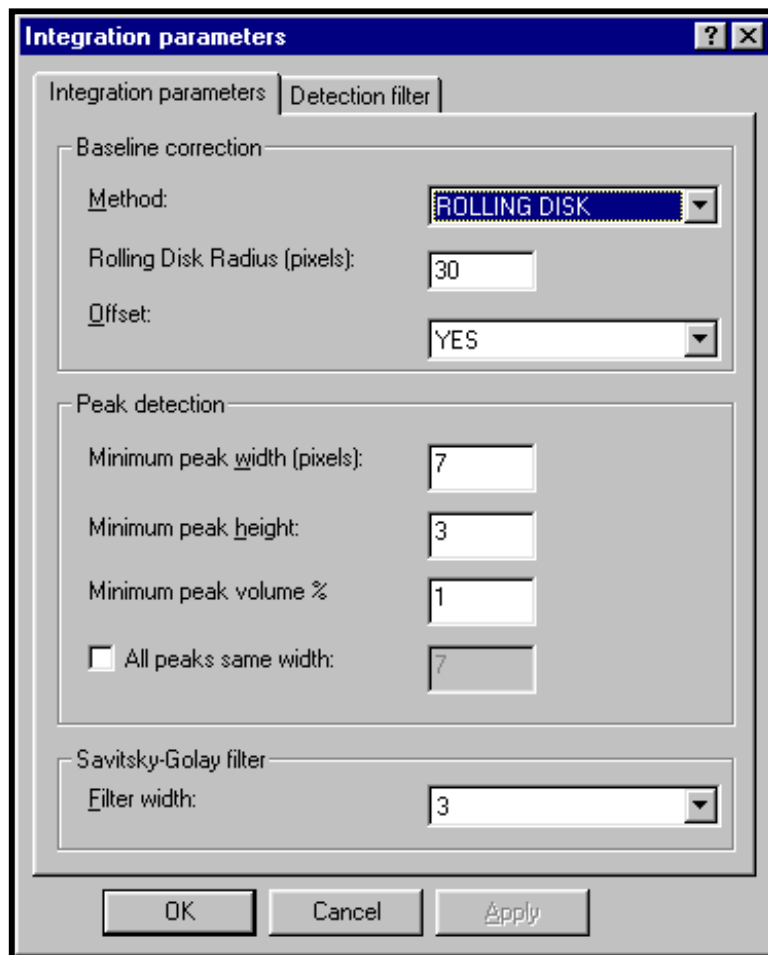
In this menu various parameters can be adjusted. **Baseline correction** is defaulted to '**Lowest slope'** (this method uses a line that connects all the points on the profile, using the lowest possible slope) but several options are provided:

The **'Rolling disk'** option is especially popular in the analysis of ethidium bromide stained gels. These gels have an intrinsically high background and results are improved dramatically by applying a correction. For this method the program first calculates the position of the line formed by the centre of a disk with the set radius rolled along below the profile.
The baseline is then one radius length above this line, and the corrected signal is measured as the height above this baseline.
'**Track borders'** uses the border of the track as a background.
'**Track borders and slope'** is a combination method. The signal is corrected for the track borders, then the lowest slope method is used.
'**None**' will not apply a background correction.

Please note that selecting 'edit manual baseline' from the **Track** menu enables the user to set a manual baseline.

Peak detection parameters may be altered to modify the sensitivity of automatic location. Lower the default values to increase sensitivity and vice versa.

The **Savitsky-Golay** filter is used in integrating the image. The greater the set width the greater the smoothing effect of the filter.

Detection filters are for use when working with coloured images. Selecting a particular filter enables analysis of only that colour within the image. To analyse the whole image, use no filter.

Using **Save Sample Defaults** under the Extras menu will save all the settings within **Integration Parameters**.
To switch back to a saved set of defaults select **Load Sample Defaults** from the Extras menu.   If necessary GeneTools will automatically re-locate tracks and extract data.
Please note that only one set of sample defaults per analysis type may be saved under a user name at one time.


MANUAL BAND QUANTIFICATION

This document type should be selected if the user wishes to carry out densitometry analysis on a typical electrophoresis gel. The function will automatically open with the rectangular drawing tool selected.

Click and drag on left mouse button to draw around bands of interest. A double left mouse click will produce a box identical to the last drawn.
Boxes are automatically numbered in order of drawing and will appear, complete with Raw volume figures, in a data box at the base of the screen (to display various other properties in this table, click the right-hand mouse button and select options as required). If you require all sample boxes to be of identical size, select the **Spots same size** icon. The boxes will be the same size when the icon is depressed. In manual band quantification, the default setting is for different sized bands.
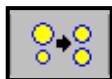
If bands are not rectangular, use the 'freehand drawing' tool

Click and hold the left-hand mouse button to draw around the band. Once drawn the shape can be altered by clicking and dragging on the blue drag handles.
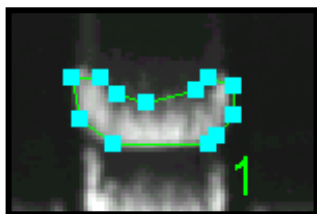
When all required bands have been drawn around click on the **Lock Position** icon to prevent accidental changes.

If you require repositioning after locking, you will need to select the **Position any spot** icon.

Background correction is set by clicking the icon:

This will produce a window with a drop down menu. The default setting is '**None**'.
For the most accurate analysis it is best to apply a background correction. There are two choices:
 If you select '**Automatic**', an individual background measurement will be calculated from the four pixels at the corners of each sample box. This is suitable for most gel samples whose background is uneven across the image.

If '**Manua**l' is selected, the user can now draw any number of background boxes upon the image. The background measurement for each data box will now be taken from the **nearest** manual background box.
NB: When using this option please ensure that you draw at least one background box on the sample, do not just select 'manual' then click 'OK'.

To assign a quantity to a known band, click on the **Quantity** icon.
A window will appear and the calibrated quantity can be entered. Quantities of the other bands are now automatically calculated. For greater accuracy more than one known value may be added. To enable this, click on the **Quantity Calibration Parameters** icon. A window will appear and the curve type may be altered to allow multiple standard values. A unit type e.g.: nanograms may also be entered at this stage. To view the quantity calibration graph click on the tab in the lower half of the screen.
NB: Quantities may also be assigned by using a right hand mouse button click.
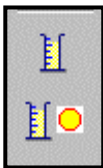
Quantity Calibration Parameters
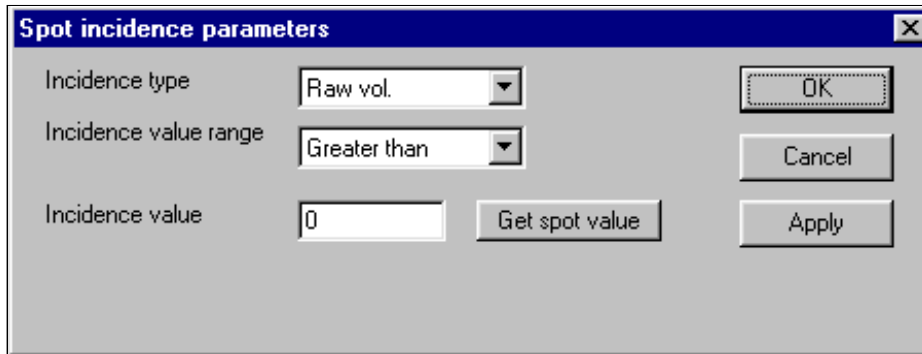
Assign Quantity

Incidence Parameters
Within Manual Band quantification it is possibly to calculate the incidence of bands based upon upper and lower limits of various parameters. To use this function, click on the icon:

A window will appear :

Incidence type can be set according to Raw volume, % Total Raw Volume, Pixel Area or Quantity. The **Incidence value range** may have an upper and lower cut-off (select **two-value range**) or may be greater than or less than an input value. To obtain values to define incidence, simply click on the appropriate sample box and click **'Get spot value'**. To display the selected bands, select the **Incidence** tab in the data window.

SPOT BLOT
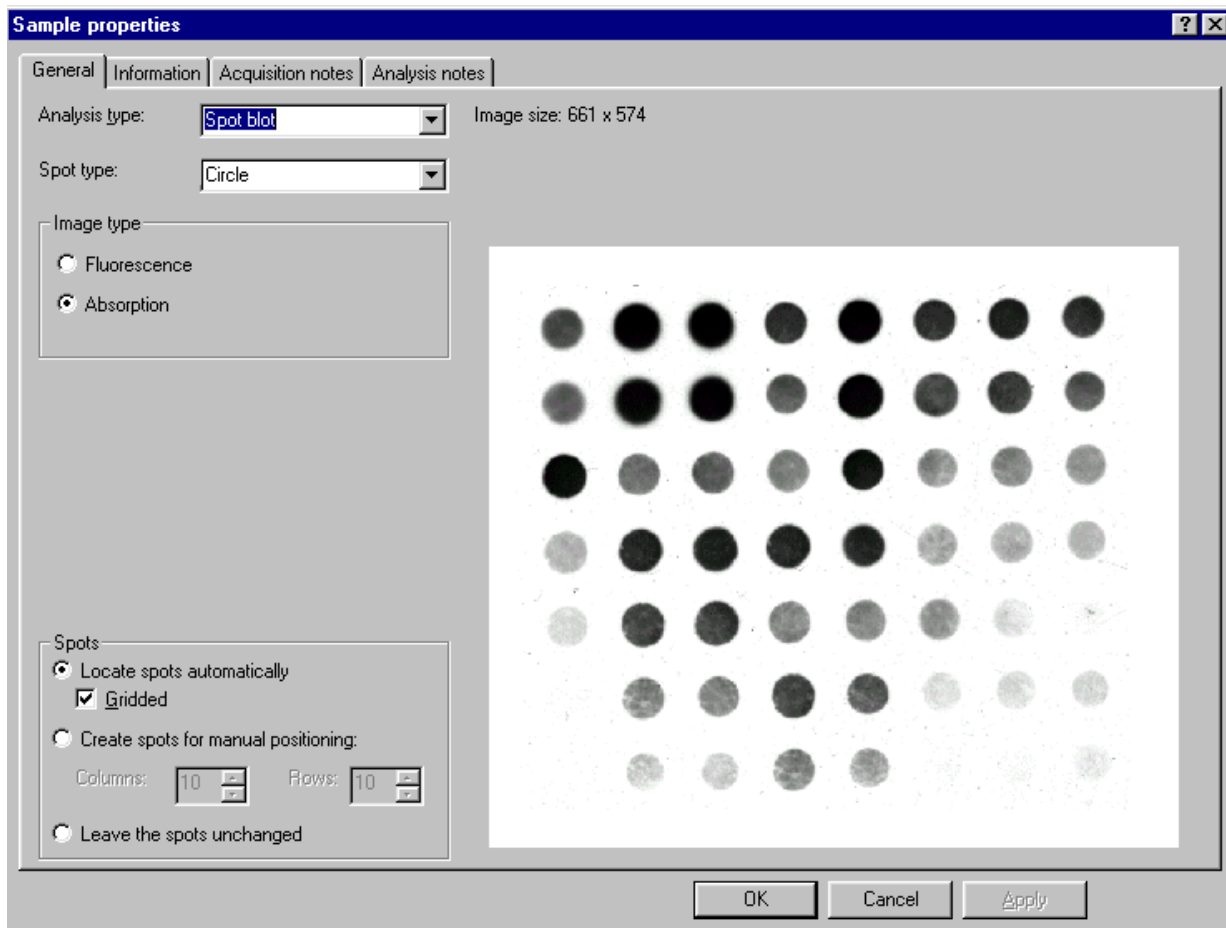
Select **Spot Blot** as document type. A Sample Properties window will appear:

Firstly, select whether the spot type is circle (most usual), or rectangular (slot blot). This software option will automatically detect spots whether they are in a gridded or random formation. If your spots are in a grid then select this setting. Please note however, that you will be unable to add or delete spots from the grid without returning to the Sample Properties box to amend row and column numbers. If the boxes for numbers of rows and columns are left blank, GeneTools will search for all spots within the image. On a poor image or in cases when only part of the array is of interest, enter numbers for columns and rows to obtain a template that may be adjusted as required.

When happy, click OK and wait for GeneTools to locate spots. Once located, you must firstly decide whether or not all spots are the same size. Set this by clicking on the icon **Spots same size.** When the icon is faded, this is the same-size setting.

The default settings are same-size for gridded arrays, and not for non-gridded arrays.

For a grid, any adjustment in positioning is best made by moving the control point spots. These spots are shown in red. These will default to be the corner spots. If these are not visible, click on the appropriate icon:

To alter the control points, double-click on the spot no longer required. The spot will turn green. To designate another spot as a control point, simply double click. Only three control points may be used at any one time.
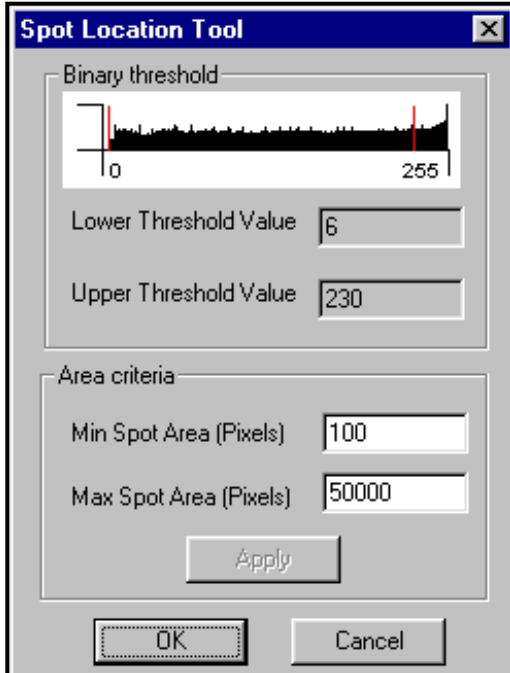To position individual spots, select the **Position any spot** icon.

 When analysing a non-gridded spot blot, spots can be added by double clicking, and deleted by right hand mouse button click.

To locate spots based on an intensity threshold, select the **Locate spots** icon

A window will be displayed:

Using the mouse, move the red lines on the Binary threshold to determine which spots are included in the analysis. Please note that this tool is most useful when analysing non-gridded samples. Spots which are not coloured blue will not be included in the analysis. For gridded samples, the spot locator is useful if spots are of variable size. Spots that are part of a grid will not be removed from the results table even If they are not coloured blue. Spots can also be selected/rejected by altering the minimum/maximum pixel areas.

Background correction is set by clicking the icon:
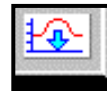
This will produce a window with a drop down menu. The default setting is '**None**'.
For the most accurate analysis it is best to apply a background correction. There are two choices:
 If you select '**Automatic**', an individual background measurement will be calculated from the four corner pixels of each spot. This is suitable for most gel samples whose background is uneven across the image.

If '**Manua**l' is selected, the user can now draw any number of background boxes upon the image. The background measurement for each spot will now be taken from the nearest manual background box.
NB: When using this option please ensure that you draw at least one background box on the sample, do not just select 'manual' then click 'OK'.

To assign a quantity to a known spot, click on the **Assign Quantity** icon.
A window will appear and the calibrated quantity can be entered. Quantities of the other spots are now automatically calculated. For greater accuracy more than one known value may be added. To enable this, click on the **Quantity Calibration Parameters** icon. A window will appear and the curve type may be altered to allow multiple standard values. A unit type e.g.: nanograms may also be entered at this stage. To view the quantity calibration graph click on the tab in the lower half of the screen.
NB: Quantities may also be assigned by using a right hand mouse button click.
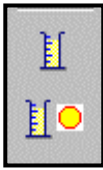
Quantity Calibration Parameters

Assign Quantity

Spot Incidence Parameters

Within Manual Band quantification it is possibly to calculate the incidence of spots based upon upper and lower limits of various parameters. To use this function, click on the icon:

A window will appear :

Spot incidence parameters			
Incidence type	Raw vol.		OK
Incidence value range	Greater than		Cancel
Incidence value	0	Get spot value	Apply

Incidence type can be set according to Raw volume, % Total Raw Volume, Pixel Area or Quantity. The **Incidence value range** may have an upper and lower cut-off (select **two-value range**) or may be greater than or less than an input value. To obtain values to define incidence, simply click on the appropriate spot and click **'Get spot value'.**
To display the selected spots, select the **Incidence** tab in the data window.

<u>**COLONY COUNTING**</u>

Select **Colony** option (pour plate) under Sample Properties. Before proceeding to analysis, select whether you wish to count dark colonies, light colonies or both together. (A dark colony is determined as one appearing darker than the background media. Remember that when using transillumination, colonies normally considered light, usually appear dark against the background agar).

Upon entering colony counting, your image will appear on the right hand side of the screen, with all analysis functions to the left. The first task is to define the frame.

Define frame
- ⦿ Adjust frame
- ○ Draw exclude region
- ○ Adjust exclude region(s)
- ○ Add/ remove colonies

Use the mouse to position the circular frame over the colony plate. Avoid taking the frame right up to the edges, where shadowing may be mistaken for colonies.
If there are regions within the plate that you do not wish to include, such as areas of contamination or pen markings, these can be excluded. Select '**Draw exclude region**' and then use to mouse to draw as accurately as possible around the unwanted region. This will no longer be included in the analysis. Exclude regions may be adjusted after drawing if required.
Next, adjust the sensitivity of detection to include all the colonies of interest.

Select ☐ Show colony shapes

to determine what is being picked up as colonies and to help avoid picking up shadows or undissolved agar.

Detection
Sensitivity   Medium
☐ Separate colonies
Set area limits
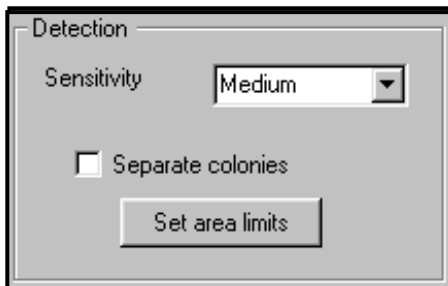
Choose from a range of settings between **Very Low** and **Very High**.

Once happy with sensitivity, click on **Separate Colonies** to apply the separation algorithm.

In order to be more specific about which colonies are detected, select **Set area limits** tab. Here it is possible to input maximum and minimum colony sizes. It may take some experimentation to obtain the correct set of parameters for your colony, but once done these settings can be saved as **Sample Defaults** (see later)

Results
Class Split:

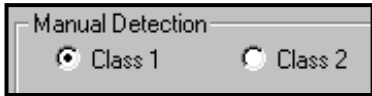|	Class 1	Class 2
Count :	136	0
% Area :	3.5	0.0
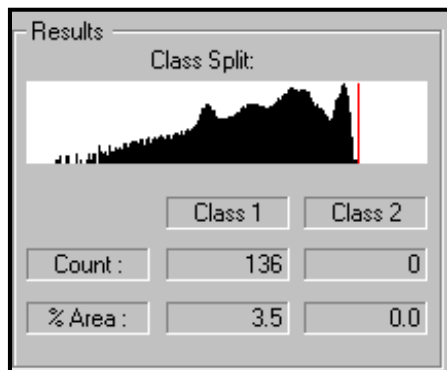
The Class Split window is used when looking at two shades of dark or light colonies. Use the mouse to adjust the red line and split the colonies into two class types.

Manual Detection
To use the manual detection function, select **Add/remove colonies** option

◉ Add/ remove colonies

This will enable the manual detection option

For a single colour colony plate have Class 1 selected. Add or delete colonies by double clicking the left-hand mouse button. Double-clicking an existing colony will delete it, double clicking elsewhere will add a colony. Class 2 will be required if two colour plates are being counted.

When printing to the thermal printer within the colony counting package, the image is printed along with colony counts and % area readings. All of the usual capture settings are also displayed.

SAMPLE PROPERTIES

If you change your mind about the type of analysis being carried out at any time, simply click on the Sample Properties icon to return to this window.

REPORT GENERATION

GeneTools is capable of producing a fully GLP compliant report with details of all analysis carried out. By clicking on **Report Setup** icon

the required features can be selected or removed. Headings for report setup are changed under GeneTools configuration in the Extras menu. Default settings from a previous report may also be imported using this menu.

Review the report prior to printing using the **Print Preview** icon.

Be aware that to print out your report you must have an A4 printer attached, and this should be selected prior to viewing the Print preview.

ONE BUTTON WORD™ TRANSFER

Simply by clicking on the appropriate icon, the entire GeneTools report, including graphs, will be transferred to Microsoft Word™. This is a very powerful function enabling the end user to have complete flexibility concerning report production and further analysis.

ONE BUTTON EXCEL™ TRANSFER

Any data generated in a report table during a GeneTools analysis can be transferred to Excel™ with a single mouseclick.   Simply select the table of interest if appropriate i.e.: in molecular weight analysis you may want all tracks or a single track or even matching

comparisons. Click on the Excel™ icon and the displayed data will instantly be dropped into a spreadsheet, along with capture information to identify the data source.
NB: Obviously, Excel™ software must be loaded onto the PC you are using! Some older versions of Excel™ will not accept data directly. In these instances, firstly save the data as a CSV file (see below).

SAVING DATA AS CSV (TEXT) FILES

By clicking the appropriate icon it is possible to save data as a CSV (comma separated variable) file. This can be opened in most software packages.

SAVE TO CLIPBOARD

This icon, when selected in the main GeneTools analysis, will place the image onto clipboard. This can then be pasted into Word™ or alternative applications. By right clicking within the dendrogram, quantity calibration or molecular weight curve windows, this information may also be placed onto clipboard. This feature adds to the reporting flexibility of GeneTools.

CONFIGURATION WINDOW

This window, found within the 'Extras' drop down menu allows various adjustments to be made and stored under user preferences.   There are four tabs within the menu:
General – for setting of document type, font, auto-location of tracks etc.
Import – for importation of defaults from previous analyses e.g. mol weight, sample track and report settings.
Colours – to alter the colours of various lines and plots appearing within the analysis program to suit personal preferences.
Molecular Weight – to import libraries and set the graph default to log or linear.

USER PREFERENCES

These are stored under your username so when returning to GeneTools it is not necessary to reset various parameters from Sample properties to molecular weight libraries. To change username select the function from the Extras menu. This function makes GeneTools the most flexible and user specific analysis package on the market today.