



Handy Helper

Application Note 20

What is Background Subtraction?

Baseline correction is used to reduce the effect of background and to emphasise the signal. All automatic background subtraction can be carried out in the integration parameters box, which appears when you click on the icon found on the GeneTools header bar:



Integration parameters

Integration parameters | Detection filter

Baseline correction

Method: ROLLING DISK

Rolling Disk Radius (pixels): 30

Offset: YES

Peak detection

Minimum peak width (pixels): 7

Minimum peak height: 3

Minimum peak volume %: 1

All peaks same width: 7

Savitsky-Golay filter

Filter width: 3

OK Cancel Apply

The automatic background options include:

- None
- Track Borders
- Lowest Slope
- Track Borders & Slope
- Rolling Disk

ROLLING DISK

NONE

TRACK BORDERS

LOWEST SLOPE

TRACK BORDERS & SLOPE

ROLLING DISK

None

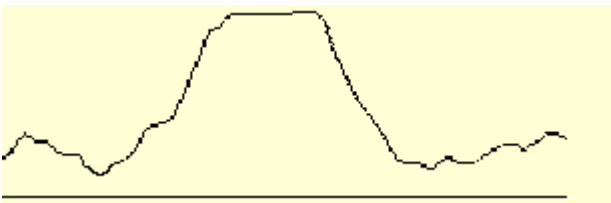
No background corrections are made.

Track Borders

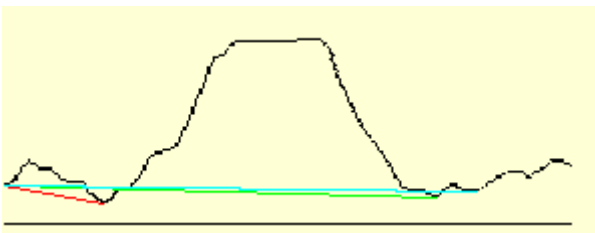
The signal is calculated relative to the points at the track border. This means that the first pixel outside the track is used for background correction of the other pixels inside. The outside value is probably an average of the first pixels either side of the track.

Lowest Slope

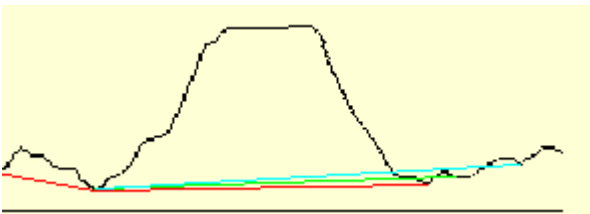
To understand how lowest slope works, consider the following example, which goes through the process as if you had to do it by hand. Naturally all this is done automatically by GeneTools and all you would see is the profile in the first picture change into the profile in the last picture;



To find the first segment of the baseline, the program starts at one end of the profile and draws imaginary lines from the start point to all other points on the profile. The following example just shows three of these lines:

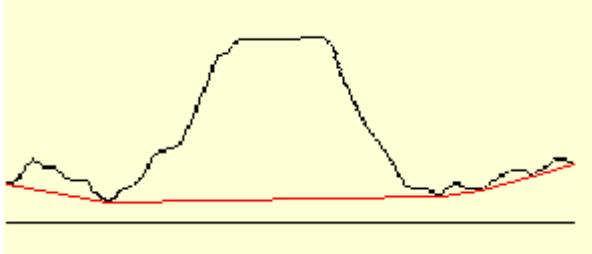


The program then chooses the line of lowest slope. This is the red line in this example as the green and blue lines have larger (less negative) slopes (lines to all other points on the profile have even larger slopes). To find the second segment of the baseline, the program moves to the end of the first segment and again draws imaginary lines from here to the remaining points on the profile. Again the following example only draws three of these lines:

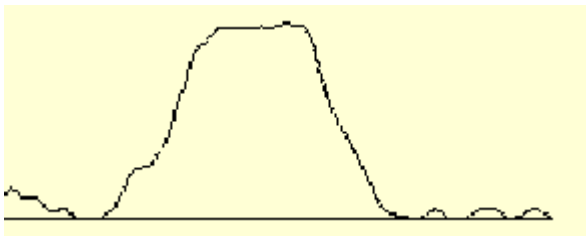


The program again chooses the line of lowest slope (the red line again in this example) and repeats this process until it gets to the other end of the profile:

/More....



The corrected signal is then measured as the height above this baseline:



Track Borders & Slope

A combination of Track borders and lowest slope whereby the signal is corrected for the track borders, and then the lowest slope correction is made.

Rolling Disk

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. The larger the radius of the disc, the less the background rises with the profile.

Manual Baseline Corrections

You can use the Integration parameters dialog box to set the type of automatic baseline correction to be used for a Sample, but you can also set a manual baseline.

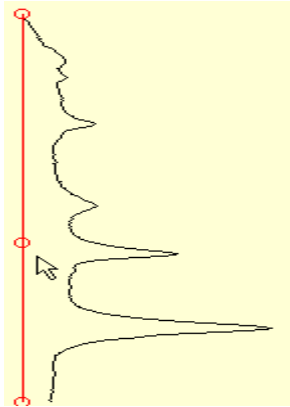
To set a manual baseline for a track:

- 1) Click on the track in the Image pane to select it.
 - 2) Choose Edit manual baseline from the Track menu or the context menu displayed when you right-click in the Image pane or Profile pane. The baseline will be displayed with circular drag handles at its ends and in the color selected for the manual baseline - press for how to set the color of components in GeneTools.
 - 3) If required, double-click on the baseline to add additional drag handles so that you can bend the line.
- Any peak markers and boundaries will be removed from the profile.

/More....

4) To re-shape the baseline:

- Position the pointer over the drag handle you want to move. The pointer will change to a four-way arrow.
- Drag the handle to reshape the baseline.
- Drop the handle in its new position.



Note: You cannot drag a handle above the profile line. While you are dragging a handle, the new baseline may be drawn above the profile, but when you release the mouse button it will be reshaped so that it always lies below it.