APPLICATION NOTE

A Guide to Background Subtraction Options in GeneTools

Explore the different background subtraction methods available in GeneTools to optimise your analysis

KEY INSIGHTS

- Overview of background subtraction methods in GeneTools
- When and how to use each method

ABOUT

Is image analysis software for quantifying gels, blots, colonies, and microplates with fast, accurate, and automated workflows.

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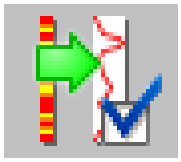
INTRODUCTION

Baseline correction is used to reduce the effect of background noise and emphasise the true signal, helping to improve quantification accuracy and consistency between samples. All automatic background subtraction methods can be applied using the Integration Parameters box, which appears when you click the icon on the GeneTools header bar:



Integratrion Parameters Icon

The automatic background options include:

- 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 were doing it by hand. GeneTools automatically calculates this for you and corrects the background (Figure 1e).

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 (Figure 1b).

The program then chooses the line of lowest slope. This is the red line (Figure 1c) 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.

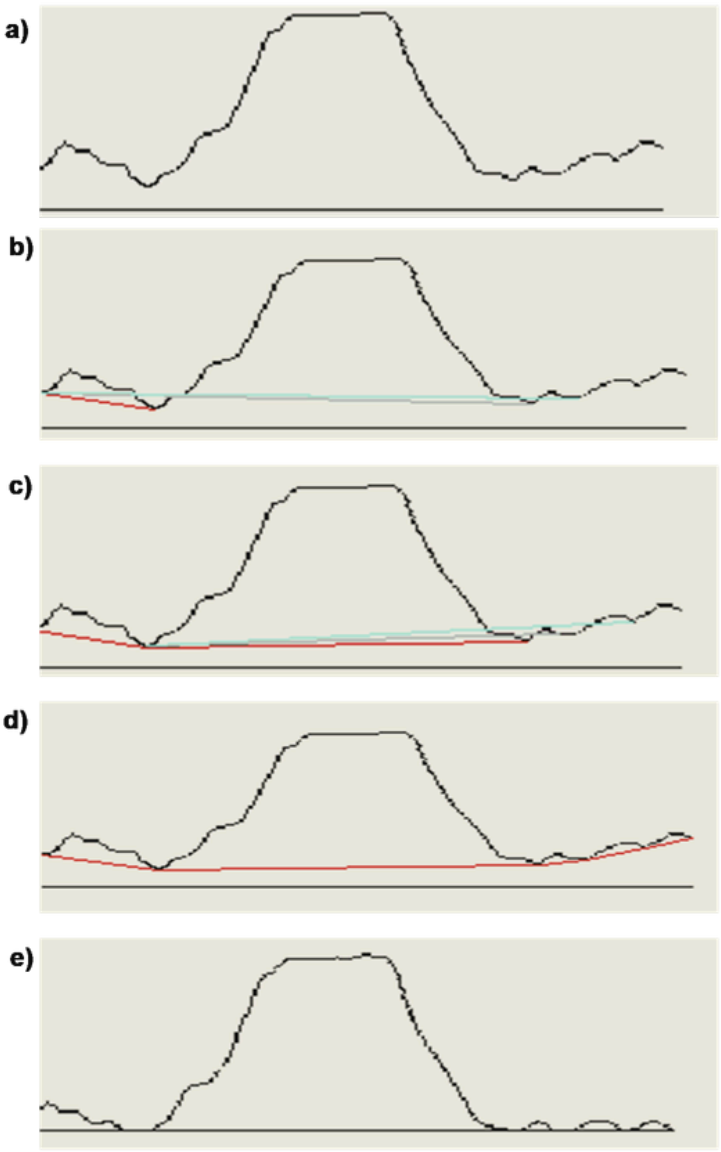


Figure 1- Example of lowest slope background subtraction
In this example only three lines are shown for simplicity.

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

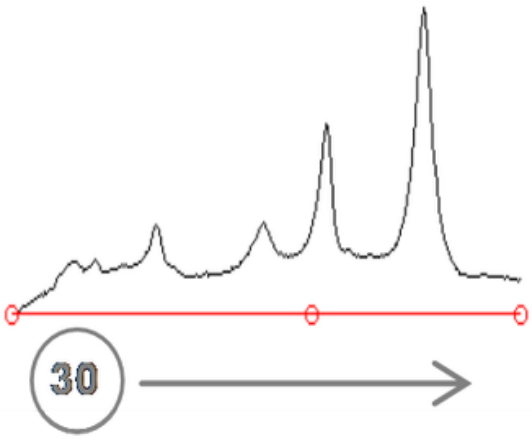


Figure 2-Background subtraction using the rolling disk method

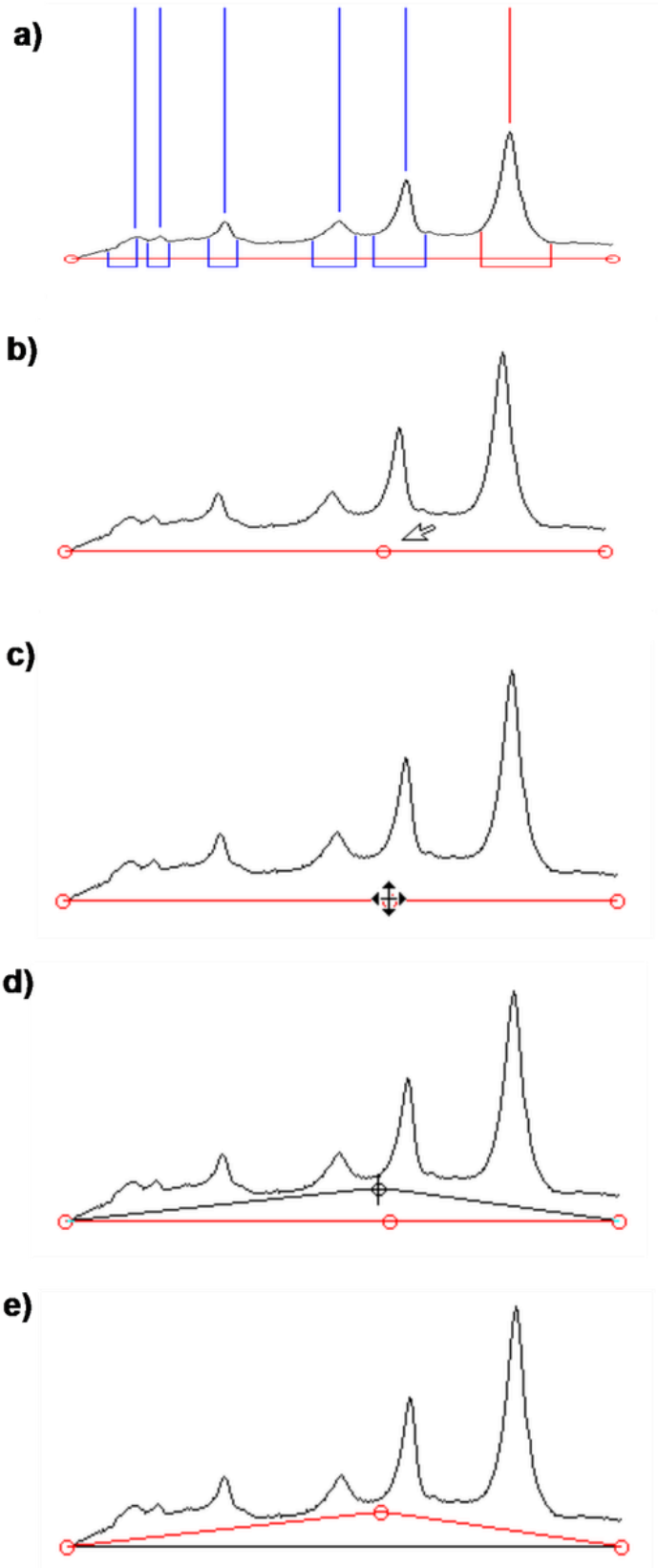
Manual Baseline Corrections

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 colour selected for the manual baseline (Figure 3a)
3. If required, double-click on the baseline to add additional drag handles so that you can bend the line. N.B. Any peak markers and boundaries will be removed from the profile (Figure 3b).
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 (Figure 3c). Drag the handle to reshape the baseline (Figure 3d) then drop the handle in its new position (Figure 3e).

N.B. 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.



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