totallab

life science analysis essentials

TotalLab TL100 – Quick Start

nonlinear

dynamics
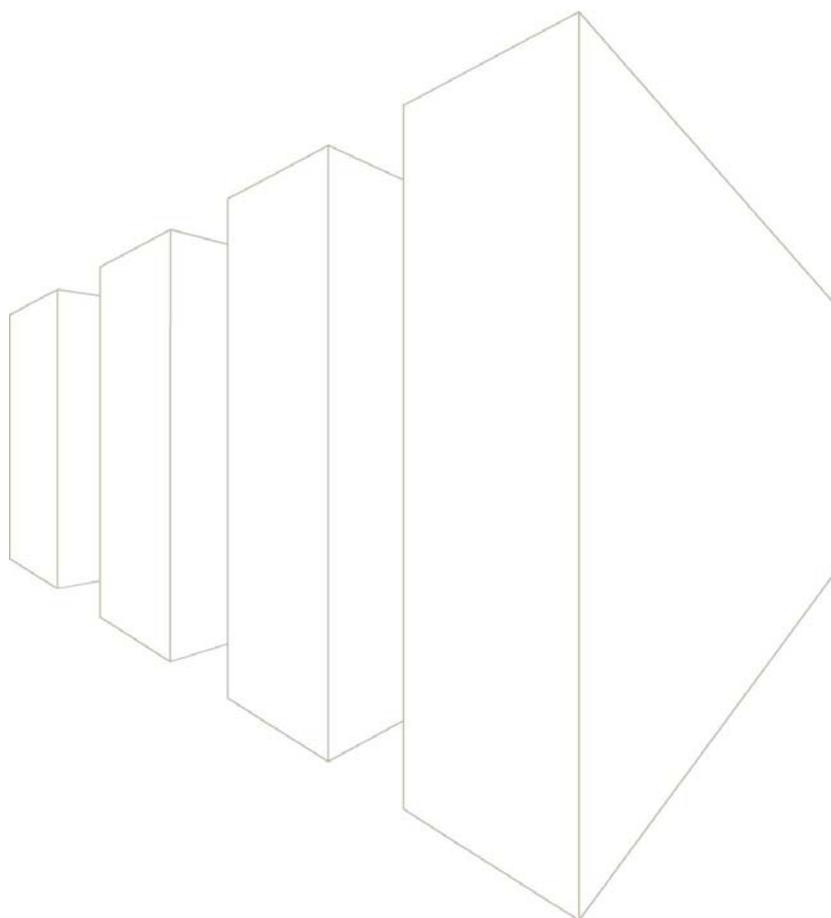
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Introduction

This booklet provides a quick introduction to the TL100 software from the TotalLab range, and guides you through the analysis process in each module. The figures that accompany each section help you work through your analysis and relate to the provided tutorial images.

This Quick Start does not describe all the features in TL100. For more information view the online Help available within the software. If you have any questions or feedback on TL100, please contact Nonlinear Dynamics Technical Support at support@nonlinear.com.

The Control Centre – Getting Started

To start TL100, double-click the TotalLab -TL100 icon on the desktop. Alternatively, select *TotalLab - TL100 v2006* from the Start/Programs menu. The Control Centre window appears.
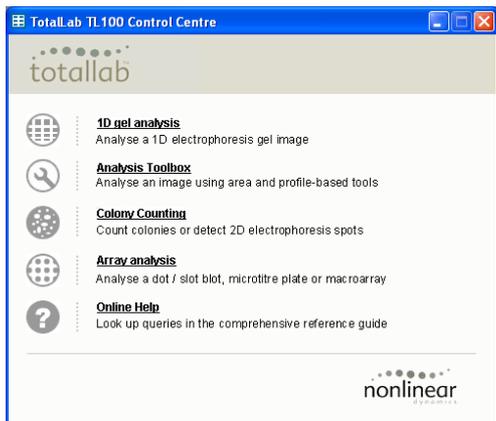

Opening an Image

TL100 can analyse 8- to 16-bit greyscale Tiff images from most types of image capture devices currently available.

To open an image in an analysis module click the button in the Control Centre, or if already in the analysis module, select *Open* from the *File* menu. The *Open* window appears.

The demonstration images are located in the *Images* folder of the program group. Select the image and the analysis module you want to use and click on *Open.*

The software automatically saves your analysis throughout the process.
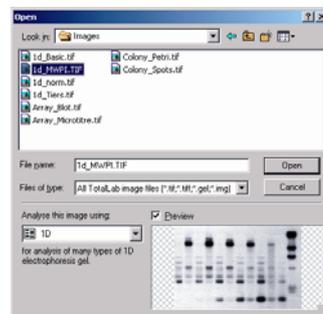
The TL100 Interface

The General Layout

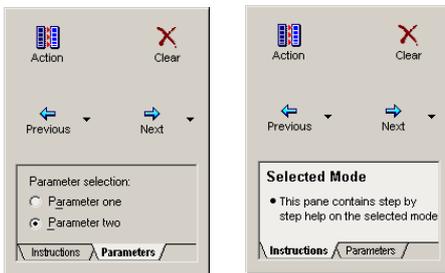The appearance of the program remains the same throughout each analysis module.

A toolbar appears at the top of the window that contains the following buttons.

Additional windows, such as the Image View, Analysis, Measurements Table, MW Curve and Report can be selected from the buttons at the bottom of the Navigator.

The Navigator

The pane at the left of the screen is the Navigator. The Navigator contains the tools required for the current mode of the analysis.

TL100 uses a wizard interface; *Next* and *Previous* buttons allow easy navigation through the analysis modes. The *Restart* button allows you to begin the analysis again

A Help pane is also present that provides information and tips relevant to the current analysis.

Click the *Parameters* tab to view any additional parameters.

Image viewing tools

You access the image viewing tools from the buttons on the Image window.

Zoom: Drag a rectangle on the image window, and then left-click to increase or right-click to decrease the image magnification. Alternatively, you can drag the scroll bar edges.

Zoom 1:1: View the image at a 1:1 ratio.

Fit to window: View the whole image.

Magnify: Allows magnification of a portion of the image.

Panning: The image can be moved around the image window when part of the image is not visible, if for example the zoom tool has been used.

Contrast

The contrast window allows you to improve the way the image is displayed. The values are based on the image calibration during scanning. The original data remains unaltered.

Colour

The colour window allows you to change the colour of the displayed image.

Edit Image

The image manipulation tools are available from the main tool bar and allow you to edit the image. All the edits are logged for a specific image and can be viewed in the Image Properties window.

Options

You can access a variety of options using the button on the main tool bar. These options are specific to the selected analysis module and allow customisation of the Image Window and Measurements Table.

1D Gel Analysis

From the Control Centre, click *1D gel analysis*. Select the image or go to the *Images* folder and select 1D_*MWPI.tif* to follow this example. Click *Open* to launch the 1D analysis module.

Automatic: Performs an automatic analysis using the selected modes. Select or clear the modes in the Navigator. If the analysis requires an area of interest (AOI), you draw the area on the image before clicking *Automatic*.

After an automatic analysis is completed, you can review and edit the data in each analysis mode by selecting the mode button.

You access the option to display multiple profiles in the *Parameters* tab.

Stepwise: Proceed mode-by-mode through the analysis using the *Next* and *Previous* buttons to access the analysis modes.

Lane Creation

To create lanes automatically or manually select the appropriate button in the Navigator.

If you select **Automatic:**

- Click *Create*, and the software automatically detects the lanes. You access the edit mode from the list in the Navigator.

- For automatic analysis within an AOI, drag from the top left to the bottom right on the image and then click *Create*. The software automatically detects the lanes within this area.

- Select multiple tiers in the *Parameters* tab before clicking *Create*.

If you select **Manual:**

- Define the lane template in the *Parameters* tab. Drag from the top left to the bottom right on the image window and TL100 draws the lanes.

Lane Editing

Select the edit mode from drop-down menu. If you make changes, click *Accept* to update the lane profile window.

To delete the current lane, highlight the lane you want to delete, and then click on the *Delete* button in the *Parameters* tab.

Edit multiple lanes: Select from the following options in the Navigator.

- *Bend / Resize Lane Box:* For fine control of badly distorted gels, left-click to add a handle, and then drag the handle. Right-click to remove a handle.

- *Move Lane Box Edges:* Drag to move lane box edges on image window.

- *Add Lanes:* Click the location on the image window within the lane box where you need to add the lane.

Edit single lanes: Select from the following options in the Navigator.

- *Bend / Resize:* Left-click to add a handle, and then drag the handle. Right-click to remove a handle.

- *Move:* Select the lanes and then drag the lanes to a new location.

- *Add Grimaces:* Grimaces account for band distortions. Click a band to apply the grimace, and then alter the grimace by adding handles and dragging the handles.

Click *Next* to advance to the Background Subtraction mode, or use the *Next* menu to choose an alternative mode.
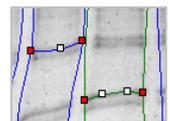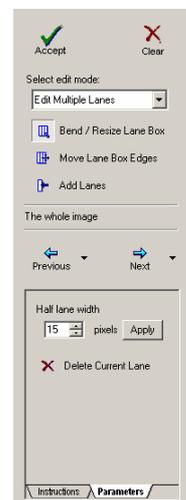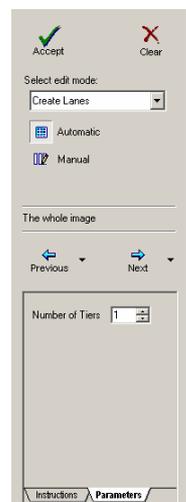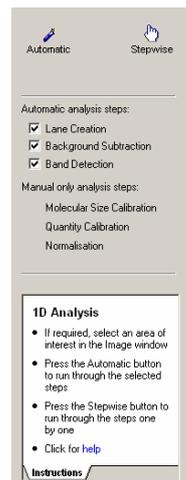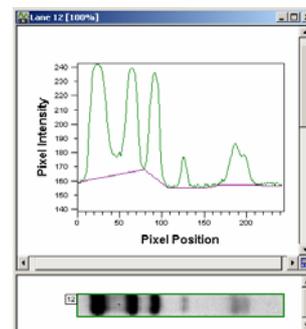
Background Subtraction

The selected background subtraction method is displayed in the Navigator. To choose a different method, select the method from *Parameters* tab.

The following methods are available:

- *Rubber Band:* Select and the software automatically removes the background.

- *Minimum Profile:* Select and the software automatically removes the background.

- *Rolling Ball:* Select and then adjust the radius of the ball in the Navigator, the software automatically removes the background.

- *Image Rectangle:* Select and then draw a rectangle on the part of the image that represents the background.

- *Manual Baseline:* Select and then add or delete the handles in the profile window. Drag a handle to adjust the baseline.

- *None:* Select if you do not want to use background correction.

The lane profile window displays visual confirmation of the background subtraction.

Click *Next* to advance to the Band Detection mode, or use the *Next* menu to choose an alternative mode.

Band Detection

Click the *Detect* button and the software automatically detects the bands and band edges using the currently selected parameters.

Band detection has three parameters:

- **Minimum Slope:** A value that represents how pronounced a band must be from its surrounding area to be recognised. You adjust the value using the slider.

- **Noise Reduction:** Sets the level to determine the difference between noise and band presence. You can adjust the value in the *Parameters* tab.

- **Percentage Maximum Peak:** Measures the relationship between each band in comparison to the tallest band and determines at what level to include a faint band. You can adjust the value in the *Parameters* tab.
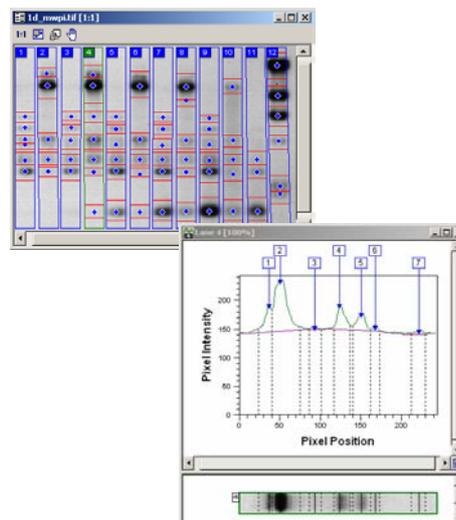
Band edges can be determined automatically or all the edges can be set to a fixed width. You can edit the bands and edges either on the image or lane profile window. The pointer changes according to its proximity to the bands and edges.

- To remove a band/edge, right-click.

- To add a band/edge, left-click.

- To move an edge, position the pointer over an existing edge and drag the pointer. The image and profile windows can be viewed in greater detail by zooming in.

The Measurements Table window contains the current results. You can alter the fields displayed in the table using the *Options* from the main toolbar

Click *Next* to open the Molecular Size Calibration mode, or use the *Next* menu to choose an alternative mode.

Molecular Size Calibration

Before you can perform molecular size calibration, you must set up the molecular size reference lane in the *Parameters* tab.

- Select the standard from the library or create a new standard.

- To create a new or edit an existing standard, click *Edit.*

    Edit standard: Create a duplicate of the existing standard before editing the values.

    Create new standard: Click the button, select the mapping units and add the standard values.

- Select the curve type that best fits the data, in the example you see a linear log curve.

- Click within a lane to assign standards, right-click to deselect.

- If there are distortions in the gel that were compensated for when creating the lanes, then *Use Rf to propagate.*

- Deselect any values in the *Parameters* tab that you do not want to use. The software retains these settings until you change them.

- Click *Compute* and the calibrated figures appear in the Measurements Table.

If all the bands are nicely separated, selecting a lane should result in perfect automatic assignment from top down. If there is a problem, you can move the assigned ladder by dragging the yellow line in the image window.

For the demo image *1D_MWPI.TIF*, the standard is *Lambda-Hind III* and the standard lane is lane 12.

Click *Next* to open the Quantity Calibration mode, or use the *Next* menu to choose an alternative mode.
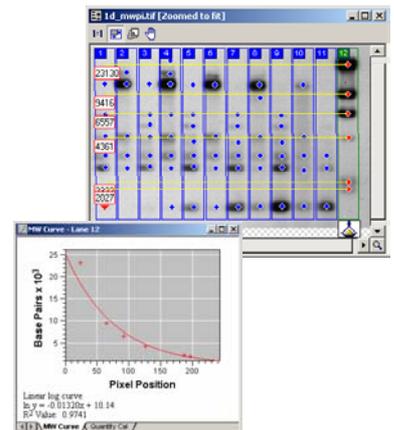
Quantity Calibration

This mode allows calibration of bands on a gel to known volumes, also accounting for nonlinear staining effects. A standard curve is produced and the software automatically calculates values for the unknowns.

- Click the bands where the quantities are known from the experimental set up.

- Add the known volume in the box that appears. You select the units in the *Parameters* tab.

- Select a curve method and click *Calibrate.* The volumes appear in the Measurements Table.

Click *Next* to open the Normalisation mode, or use the *Next* menu to choose an alternative mode.

Normalisation

The normalisation protocol allows the use of known values, or the bands can be expressed as a percentage or a proportion of one or more selected bands. If actual volumes are not known, the band volume can be set to 100 (ignore units), and the unknown bands expressed as a percentage of the known band(s).

- Select one or several known band(s) (right-click to deselect).

- Select the required units in the *Parameters* tab.

- Type the amount to be associated with the known bands.

- Select either their average volume or collective volume.

- Click *Normalise*.

Array Analysis

From the Control Centre, click *Array analysis* and select an image or go to the *Images* directory and select either *Array_Blot.tif* or *Array_Microtitre.tif*. Click *Open* to launch the Array analysis module.

Define Spots

Create and Stretch Grids:

- Select the grid type, spot shape and spot size from the *Parameters* tab and then click *Detect* to automatically define the spots. To auto-size spots select the checkbox.

- Alternatively, select the grid type, spot shape and size, and then define the grid by positioning the pointer in the centre of the top left well. Drag the pointer to the centre of the bottom right well.

Adjustments

- Drag to move the entire grid.

- Reshape the grid by dragging a handle.

Move and Resize Spots:

- To move individual spots, click a spot (Ctrl-click each spot or drag out an area to choose multiple spots). Then reposition the spot(s) exactly over the well.

- Clear the Show Spot Numbers checkbox in the Options window.

- To alter the diameter of a spot, change the spot radius in the Parameters tab (make sure you clear the auto-size checkbox). Alternatively, right-click the spot and open the Spot Properties window and change the size and shape using the pixel values. In addition you can add the Spot Label in this field.

- Alternatively, click the spot and drag the perimeter to the required size as shown in the figure.
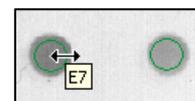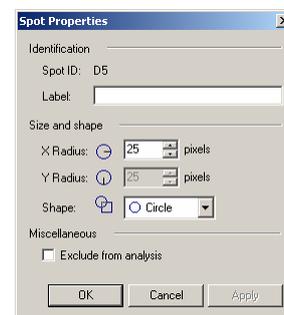
You should use a smaller diameter with microtitre plates, not necessarily including the perimeter of each well's contents. With a blot, you should use a wider diameter.

Click *Next* to advance to the Background Subtraction mode, or use the *Next* menu to choose an alternative mode.

Background Subtraction

Select a method from the *Parameters* tab. You can choose from the following five methods.

- Spot Surface Minimum: Select and the software automatically removes the background.

- Spot Edge Average: Select and the software automatically removes the background.

- Negative Controls: Select, and then define the negative control(s) in the image window.

- Image Rectangle: Select, and then draw a rectangle on the part of the image that represents the background.

- None: Select if you do not want to use background subtraction.

The values appear in the Measurements Table. You can change the fields using the *Options* window.

Click *Next* to advance to Normalisation mode, or use the *Next* menu to choose an alternative mode.

Normalisation

The Normalisation protocol allows the use of known values, or the spots can be expressed as a percentage or a proportion of one or more selected spots. If the actual volumes are not known, the spot volume can be set to 100 percent and the software calculates the unknown spots in relation to that spot.

- Select one or more known spots on the image window.

- Select the required units in the *Parameters* tab and type the amount associated with the known spot(s).

- Select average volume or collective volume.

- Click *Normalise.*

Click *Next* to advance to the Presence Flagging mode, or use the *Next* menu to choose an alternative mode.

Presence Flagging

This mode sets limits above which a spot is deemed to be present on the image, which is indicated in a table as a spot presence (1) or absence (0).

Detection is automatic if you select *Estimate*, or you can manually adjust the value using the slider. Alternatively, you can define a spot as absent or present using the buttons in the Navigator.
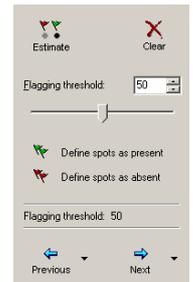
Colony Counter Analysis

From the Control Centre select *Colony Counting,* and then select an image or go to the *Images* directory and select *Colony_Petri.tif*. Click *Open* to launch the Colony Counter analysis module.

Feature Detection

You select the area of interest (AOI) shape in the *Parameters* tab, and then draw an AOI on the image window. The information about the detected features automatically appears in the Measurements Table.

You can change the following parameters:

- **Detection parameters:** Modify using the sliders for sensitivity and operator size. Selecting Initialize Sensitivity automatically determines the sensitivity for a given operator size.

- **Background, noise factor and spot splitting:** Can be adjusted in the Parameters tab.
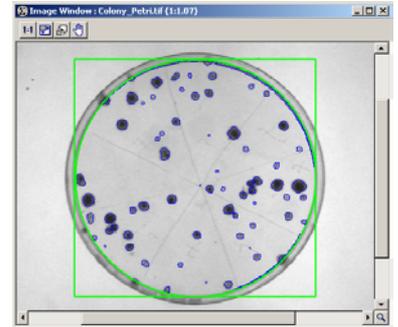
You can use the zoom tool to determine if editing is necessary.

Click *Next* to open the Edit mode, or use the *Next* menu to choose an alternative mode.

Feature Editing

The Navigator contains the following controls:

- *Draw or Erase Features:* Left-click and drag to draw a feature using the selected pen size. Right-click and drag to erase part of a feature.

- *Delete Features:* Click a feature if you want to delete.

- *Split Features:* Manually split a feature by dragging a line at the required position. Use *Draw* or *Erase* to make minor changes.

After editing, the Measurements Table can be updated by clicking *Renumber Spots*.

Click *Next* to open the Background Selection mode, or use the *Next* menu to choose an alternative mode.
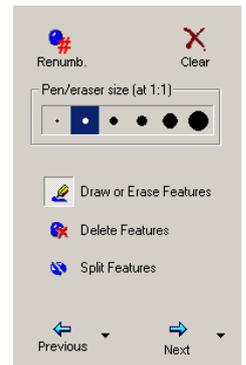
Background Subtraction

Select a method from the *Parameters* tab. The methods are:

- *Mode of Non-Spot:* Calculates the background based on the most common pixel value in the region around the feature. You can adjust the margin in the Navigator.

- *Image Rectangle:* Requires you to draw a rectangle on the part of the image that represents the background.

The results are displayed in the Measurements Table.

Toolbox

From the Control Centre click *Analysis Toolbox*, and then select an image. Click *Open* to launch the Toolbox analysis module.

Shape Definition

- Select a drawing tool from the choice of *Areas*, *Lines,* or *Autotrace* in the Navigator.

- **Areas and Lines:** Drag to create the object on the image window. Some objects require multiple clicks, right-click to complete the object.

- **Grid:** Type the grid dimensions in the box that appears. Drag the grid on the image window.

- **Autotrace:** Right-click the outer edge of the feature to determine the *Edge pixel intensity* (the value appears in the *Parameters* tab), and then left-click the centre of the feature to detect and draw the object.

- **Selector:** Allows you to select objects for editing. Drag through an area to select multiple objects.
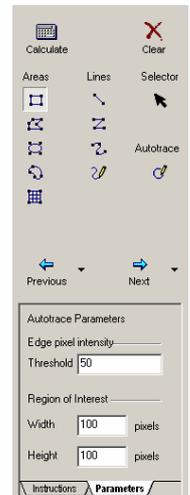
Shape Editing

- Click the *Selector* button, and then click the object, (or CTRL-click for multiple objects) that you want to edit.

- Drag a handle to resize the object.

- Drag an object to a new location.

- Right-click to copy and paste objects.

Click *Next* to open the Background Subtraction mode.

Background subtraction

Select the method from the *Parameters* tab. You can use different methods on different features on the same image.

- *Local Average:* Select and the software automatically performs the background subtraction on the selected feature.

- *Local Median:* Select and the software automatically performs the background subtraction on the selected feature.

- *Histogram Peak:* Select and the software automatically performs the background subtraction on the selected feature.

- *Image Rectangle / Ellipse:* Select and draw an object on the part of the image window that represents the background. If multiple objects are drawn, select the object to apply from the drop-down menu in the *Parameters* tab and the software automatically subtracts the background.

In *Selector* mode, click the feature that requires background subtraction. Choose a method from the *Parameters* tab and the software automatically subtracts the background.

To simultaneously subtract the background from multiple objects, click the *Selector* button and drag an area. Choose a method from the *Parameters* tab and the software automatically subtracts the background.