TeloTool User Guide

Dec 2013

Janett Göhring (Max F. Perutz Laboratories, Vienna, Austria) &

Nick Fulcher (Gregor Mendel Institute, Vienna, Austria)

Contents

1. Introduction to TeloTool

TeloTool is designed to perform statistical analysis on telomere length information gathered from Terminal Restriction Fragment analysis (TRF). The TRF assay is a commonly used technique to measure telomere length within a number of organisms and tissues. Because telomere length is not uniform over a whole tissue or organism, telomeric DNA is visualized as a smear on Southern blots when performing TRF analysis. TeloTool is able to extract mean and range information from such telomeric smears in a fast and unbiased way. In addition, TeloTool performs probe intensity corrections to increase the accuracy of extracted data. Data can be easily exported from TeloTool in Microsoft Excel format or as a representative graph.

For a detailed description of formulas and theory behind TeloTool, please see “will be added soon”.

For the source code of TeloTool please visit:
“http://www.mathworks.com/matlabcentral/fileexchange/44573”

For comments, suggestions, or to report problems with TeloTool, please contact Janett Göhring (janett.goehring@univie.ac.at).

2. Using TeloTool – A step by step guide

2.1. Overview

1) Starting TeloTool
2) Loading images
3) Lane recognition
4) Defining marker bands and fit
5) Lane profiles
6) Background correction
7) Results

2.2 Starting TeloTool

TeloTool was developed in Matlab (Mathworks) and runs on a 64bit windows platform which requires installation of the MATLAB Compiler Runtime (MCR) (version 7.17 (R2012a), available at the Mathworks webpage http://www.mathworks.com/products/compiler/mcr/). After installation of the appropriate runtime environment, simply run TeloTool.exe.

Cave! Program may take a minute to load on older machines.

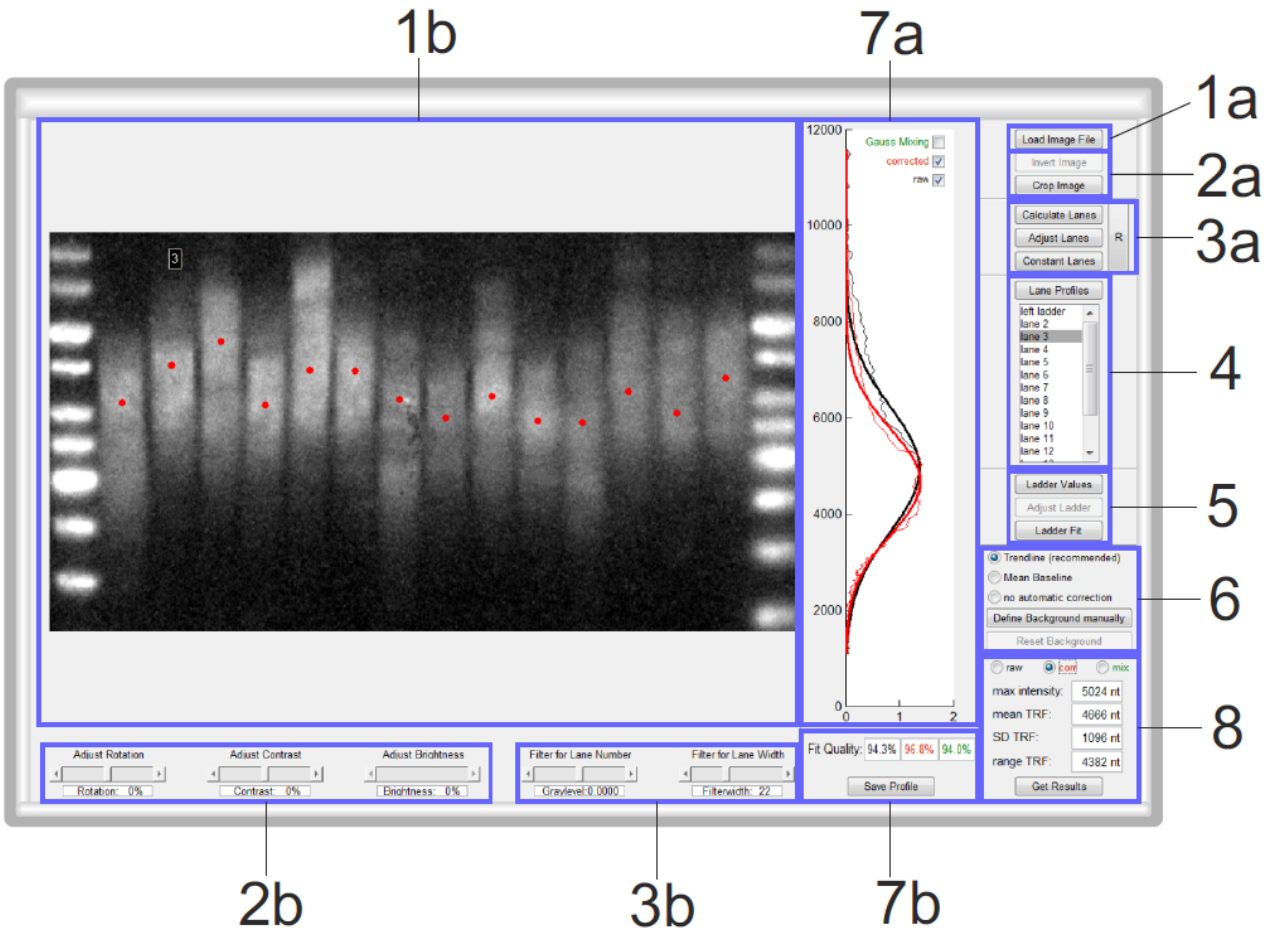

Figure 1. TeloTool user interface

2.3 Loading Images

TeloTool requires 8 or 16 bit TIFF images of TRF blots. Images can be directly loaded into TeloTool via the “Load Image File” button (Fig1-1a) and can be visualized in Fig1-1b. TeloTool contains features for image inversion and cropping (Fig1-2a) along with rotation, brightness, and contrast adjustments (Fig1-2b). Contrast and brightness adjustments only change the visual gel image; analysis is always performed on raw data. The image must always have a black background which can be changed using “Invert Image”. It is also a good idea to examine the integrity of each gel to observe blotches or gel distortions as these will affect the final result. Lanes containing such artifacts should be excluded from analysis.

2.4 Lane Recognition

To detect lanes within the gel, select “Calculate Lanes” (Fig1-3a). Lane number and width can be manually adjusted via “Filter for Lane Number” and “Filter for Lane Width” sliders (Fig 1-3b). When lanes are difficult to define automatically, there is also the option to manually add lanes by selecting “Adjust Lanes” (Fig1-3a). Selecting this will bring up a new interface (Fig 2) where existing lane boundaries can be moved by selecting the appropriate lane from the dropdown box and the “Adjust Selected Lane” button, or new lanes can be added with “Add Lane” button. Each new lane will be added to the right of the selected lane number. The width of each lane can be made constant by selecting “Constant Lanes” which takes the narrowest lane and adjusts each to this width or reset by clicking “R” (Fig1-3a).

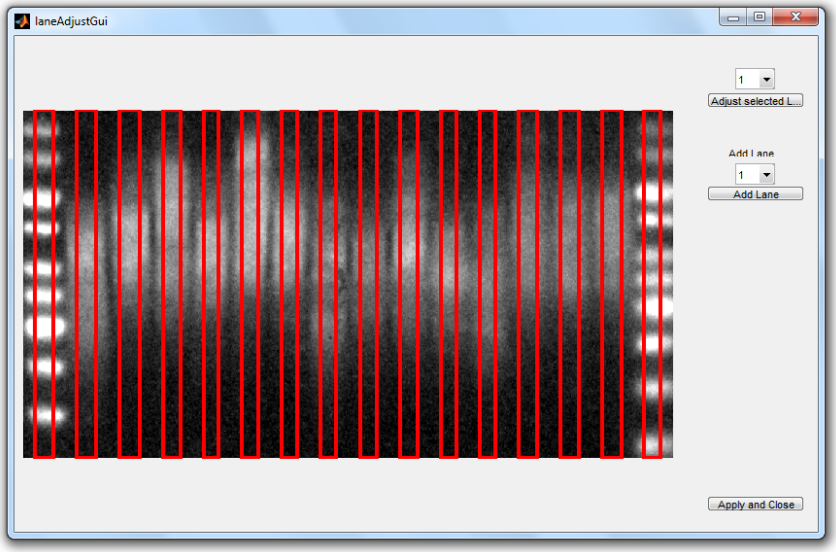

Figure 2. Lane Adjustment Interface

2.5 Defining Ladder Bands and Fit

When the lanes are defined, it is next important to define marker lanes and sizes of ladder bands. TeloTool automatically designates the first and last lanes as size markers; to detect bands within these lanes, select “Lane Profiles” which calculates this over each lane. Red dots which appear in non-marker lanes indicate the maximum signal intensity. To define marker band sizes, first select “Left Ladder” in the lane list (Fig1-4), then “Adjust Ladder” (Fig1-5); this opens a new interface as shown in Fig 4. Peaks of each marker band are automatically recognized, sensitivity of the automatic band recognition can be adjusted with the “Adjust Filter” slider. In the example shown in Fig 3, the final band has run out of the gel on the left side as a consequence of gel distortion, this illustrates the

importance of using flanking marker lanes. In this case, the first nine peaks are designated as true marker values. To remove incorrectly recognized or addition peaks, select “Delete Extremum” and draw a box over the respective red dot. Once marker bands are clearly defined, select “Apply and Close”.

Cave! If the number of elements in the ‘Ladder Values’ does not match with the number of declared ladder peaks, the lane profiles will not be calculated.

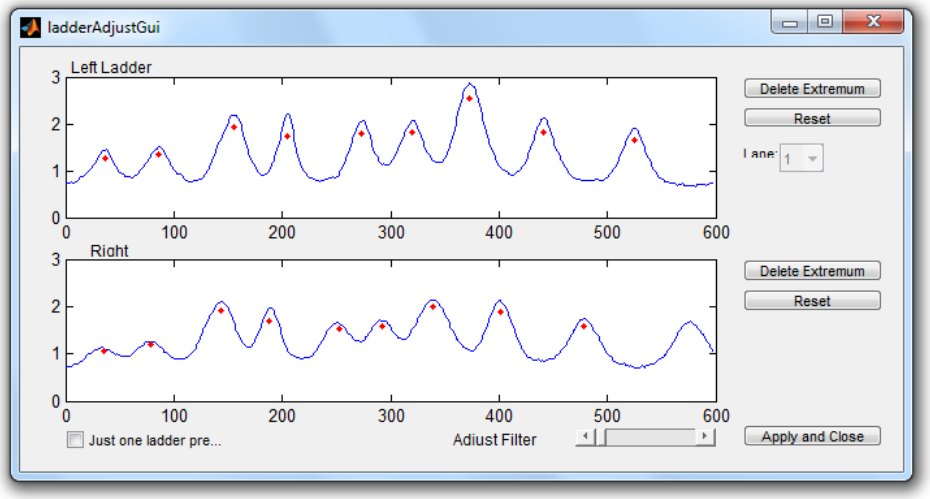

Figure 3. Ladder Adjustment

Next, it’s important to define the size values of each peak. Select “Ladder Values” (Fig1-5) which opens a new interface as shown in Fig. 4. Marker bands can now be entered corresponding to the ladder used; the number of values must be the same as the number of peaks defined in the previous step otherwise an error box will appear. Values entered into this interface can be saved and loaded.

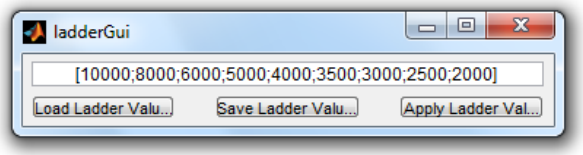

Figure 4. Ladder band size definition

Next, it’s possible to select the fit of the ladder bands. Four possible ladder fitting options are available, to select from these click “Ladder Fit” (Fig1-5) which opens the interface shown in Fig 5. TeloTool will automatically fit a polynomial function of the third order unless defined otherwise. To allow comparison between the different fit options the coefficient of regression (R) and the adjusted R (Adj. R) are provided. The red curve represents the left ladder and the green curve the right ladder.

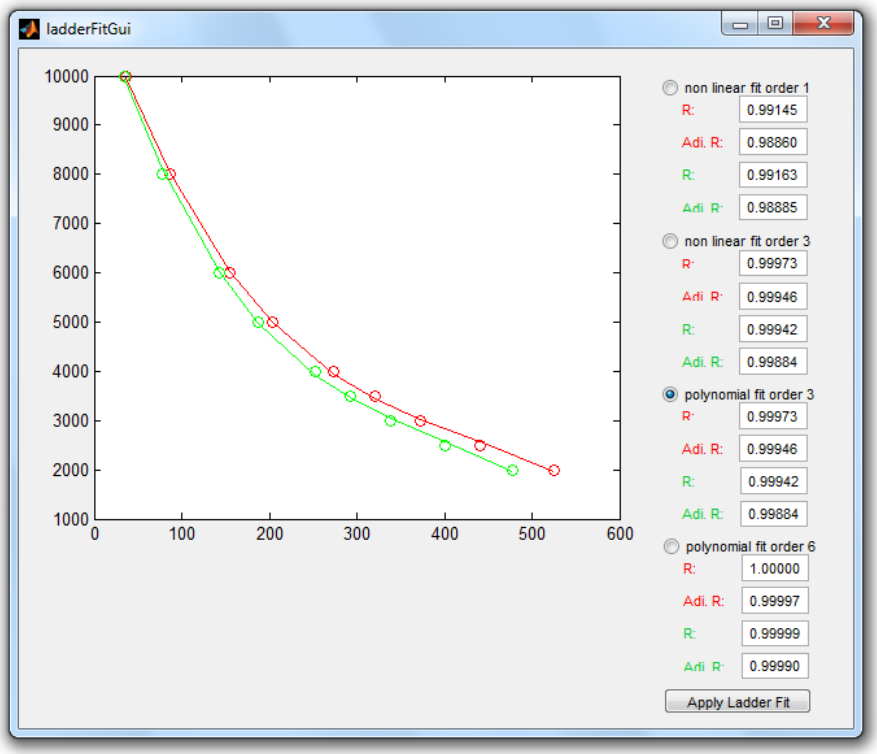

Figure 5. Ladder Fit

2.6 Lane Profiles

After correctly establishing marker values, intensity profiles can now be seen for each lane in panel 7a (Fig 1). Raw, Corrected, and Gauss mixing profiles can be visualized by selecting applicable check boxes (at the top of 7a). Fit quality information can be seen in panel 7b (Fig 1) which indicates percentage fit quality for all available fits. An image of the displayed intensity profile can also be exported through selecting “Save Profile”.

2.7 Background Correction

Three options are available for background subtraction, select from either Trendline, Baseline, or define an area for subtraction (Fig1-6):

- Trendline (Recommended) – Detects the minimum of the first and last 10 pixels of the lane, a line is then calculated between these values and subtracted from raw data.
- Baseline – Takes the average between the first and last 10 pixels of the lane, this is then subtracted from raw data.
- Manual background definition – Area can be manually selected and is subtracted from raw data. This type of correction must be performed before definition of lanes. When it is selected, the user must draw a box in the area chosen for subtraction. New lanes must then be defined; when lanes are already defined, these are removed.

2.8 Results

Once all analysis is complete, data can be displayed for the selected lane in Panel 8 (Fig 1). Clicking “Get Results” in the same panel brings up a new interface as shown in Fig 6. Here you can see the corresponding results table and a graph representing the mean and range of each telomeric smear. Results tables can be exported in Microsoft Excel format; “Save table” saves only the currently

selected table from Raw, Corrected or Gauss mix data whereas “Save All” saves everything within one Excel file. Results graphs can also be exported through “Export Diagram”. It is also possible to exclude specific lanes from the graph. The “Fit Threshold %” box highlights smears in the results graph which lie below a designated threshold.

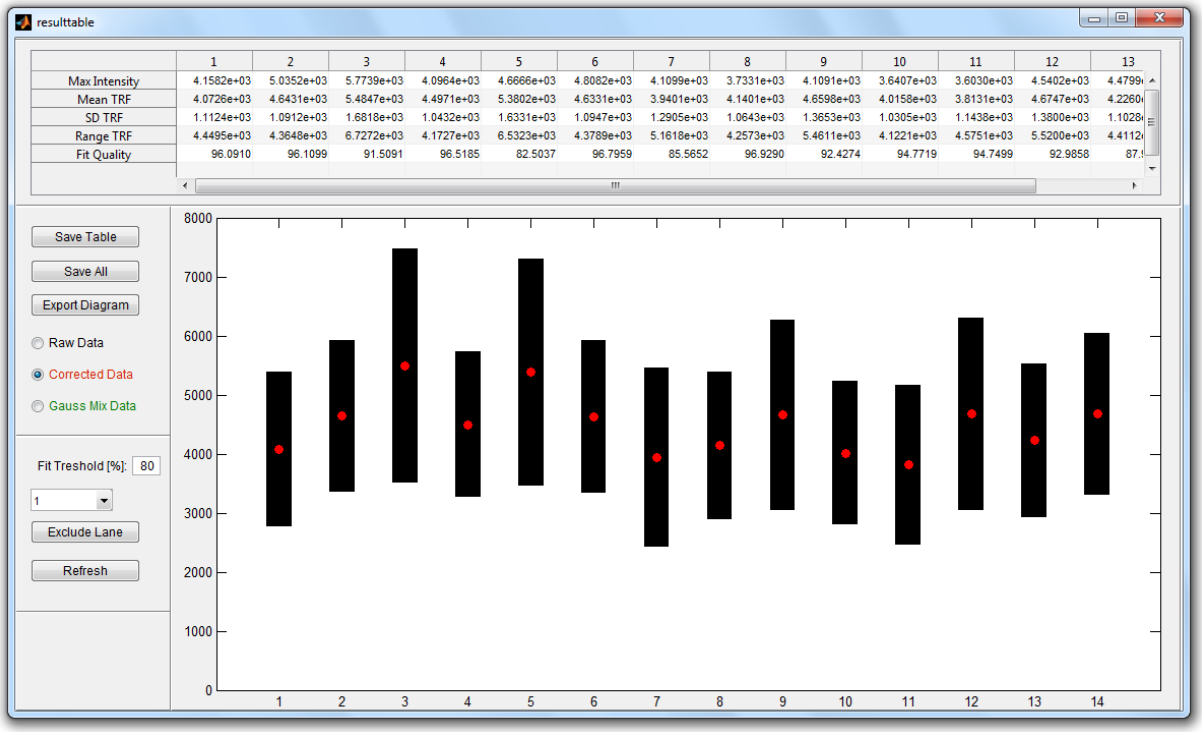

Figure 6. Results

3. Troubleshooting

The following section describes the features labelled in Figure 1 and details for troubleshooting.

#	Description of the feature	Troubleshooting
1a	Press the button 'Load image' to display a gel image in the main axis (1b). The image has to be in TIF format (8- or 16-bit), but is automatically converted into grayscale.	- Error: 'Unknown image format. Please convert into Grayscale manually!' Use any available image manipulation software as 'Gimp' or 'ImageJ' to convert it into a grayscale image. - Error: 'Image format is CMYK. Please convert into Grayscale manually!' Use any available image manipulation software as 'Gimp' or 'ImageJ' to convert it into a grayscale image. - Error: 'Image format has hidden alpha channel. Please remove!' The image was not correctly converted into a grayscale image and probably contains a 4th alpha channel. Use any available image manipulation software as 'Gimp' or 'ImageJ' to convert it into a grayscale image.
1b	**Main axis, which displays the gel at all times.** - For convenience, contrast and brightness changes will be possible, but TRF analysis is based on the raw gel image. - Cropping is performed in this axis. - After the extraction of the lane profiles TeloTool displays red dots within each recognized lane; these represent the position of the maximum intensity of the lane profile.	- Please ensure that the image is not oversaturated. Digital images have to be taken within the linear range of the detector. Adjust exposure time if necessary.
2a	- Press the Button 'Invert image' to yield a gel image with dark background and white TRF signal. The button becomes inactivated as soon as the user continues with the analysis. - Crop the image by pressing 'Crop Image' and click and drag within the main axis (1b). A rectangle of the selection will appear. After the left mouse button is released it will be possible to adjust the dimensions by pressing the blue labels at the edges and corners. Double-click into the rectangle to finalize the selection.	- TeloTool cannot process gel images with a light background. Please press the button to invert the image. If the button is inactivated, please reload the image. - If the image was incorrectly cropped, the image must be reloaded. - Cropping will influence the analysis results. Since TeloTool is based on fitting a Gaussian curve through the intensity profile of each detected lane, it is necessary to analyze the whole TRF smear. If analysis fails or results in bad fit qualities, ensure that the whole smear was used.
2b	- The user can rotate the image by changing the	- If the automatic lane detection fails,

	position of the 'Adjust Rotation' slider. Please rotate the image to be perpendicular to the window; it will improve the automatic lane detection. The usage of the slider will automatically inactivate all other buttons except 'Crop image'. Please crop the white or black edges to ensure correct trendline and baseline calculations. - The 'Contrast' and 'Brightness' slider can be used to make smears visible; it will help to check correct definition of the lanes. The 'Brightness' slider becomes activated after the 'Contrast' slider has been used.	ensure that the lanes are perpendicular to the analysis window. - If the TRF smears are not seen, or only barely visible, please adjust contrast and brightness. Further analysis is not affected by these adjustments since the original image will be used.
3a	- Press 'Calculate Lanes' to automatically detect the lanes. White rectangles will appear within the main axis, which reach from the top to the bottom of the analysis window. Please note that these are still irregular. If they need to have equal width, press 'Constant Lanes'. - Press 'Adjust Lanes' to open a new GUI, which allows manual adjustments of the lanes. - Press 'Constant Lanes' to equalize the lanes. If the positions sliders in 3b are changed, the lanes will be irregular, so that this button has to be pressed again. - Press 'R' to reset the lane detection to the original parameters. If the slider positions in 3b have been changed, the reset will be using these parameters. Manual adjust via the 'Adjust Lanes' GUI will be lost.	- If no lanes are automatically detected, change the positions of the slider in 3b. - If lanes are incorrectly recognized and the result cannot be improved by using the sliders in 3b, press 'Adjust Lanes' to manually define them.
3b	- The slider 'Filter for Lane Width' and 'Filter for Lane Number adjust the parameters used for the automatic lane detection. Change their position until all lanes are recognized and are wide enough. The filter for the lane number will also affect their width and vice versa. - Please analyze the whole width of the lane. If obvious heterogeneities are present the lanes can manually be adjusted to exclude these artifacts. - If the lanes bend or widen and narrow over the whole length of the smear, manually ('Adjust Lanes') set the lane width to cover only the smear and no blank areas with background. The intensity profiles will be affected otherwise.	- In some instances, the standard ladder is substantially brighter than the TRF smears. This will lead to problems in the automatic lane detection. However, change the position of the slider for 'Lane Width' in small increments until all lanes are detected. - In such cases the filters easily reach their maximum and no lanes can be detected. Start over by pressing 'Calculate Lanes' and reduce the increments even more. - If merged lanes are detected, increase the 'lane number' or adjust the lanes manually by pressing 'Adjust Lanes'.
4	- If the lanes have been set up correctly, press 'Lane Profiles' to obtain the intensity profile of	- If the profile box is not filled with items, no lanes have been set up.

	each lane. This action will fill the list box underneath it with 'left ladder', 'lane i' to 'right ladder'. Click on a list box item to see the respective intensity profile for each lane within the profile axis (7a). Initially, only the ladders can be viewed since they have to be set up correctly before the intensity profiles of the TRF smears can be converted to their molecular weight. - If the ladders are correctly set up, every item can be selected and viewed in 7a. Orientation is facilitated by displaying a number above the selected lane in the main axis (1b).	- Error: 'Please adjust the ladders first! Both ladders and the ladder values must have the same number of elements.' Before the lanes containing the TRF smears can be displayed according to their molecular weight, the standard ladders have to be set up first. Select the 'left ladder' and press 'Adjust Ladder' (refer to section 2.5).
5	- Select the left or right ladder within the list box and press 'Adjust Ladder' to open a new GUI (for more information refer to the 'Adjust Ladder' GUI chapter). The button is only active if a ladder is selected. - Press 'Ladder Values' to open a new GUI. Type in the molecular weight of the standard ladder from highest to lowest value. Please add the brackets "[and]" before and after the vector. Separate them by ";" The ladder values used in the last session are automatically saved and loaded. Save and Load options are implemented. To finalize the chosen vector press 'Apply Ladder Values'. -If the number of visible extrema and the number of ladder values matches, the button 'Ladder Fit' will open a new GUI. Here, the fit for the ladder can be chosen (for more information refer to the 'Ladder Fit' GUI chapter).	- The ladder will be displayed within the profile axis (7a) and the automatically detected extrema are displayed as red dots. If any extreme is recognized wrongly, press 'Adjust Ladder' and delete the respective extreme. - Please ensure that the number of ladder elements is the same as the number of ladder bands visible in the gel image. If a band 'ran out' of the gel, delete that value from the list. - If the number of visible bands in the two ladders differs, delete the surplus extreme via the 'Adjust Ladder' GUI - In rare instances the ladder fit is suboptimal. The user may choose between different fit options which may lead to improved results.
6	**Background correction** The user may choose between different background subtraction modes - The 'Trendline' calculates a linear vector between the background value of the top and the bottom of the lane and subtracts it over the whole range from the intensity profile - The 'Baseline' calculates the mean between the background intensity at the top and the bottom of the lane and subtracts this constant factor from the intensity profile. - The option 'no automatic correction' disables background correction.	- Since results can be substantially influenced by the manual background correction, it is recommended to use the more accurate trend- or baseline option, which calculates the background per lane instead of the whole gel. - When selecting manual definition of background correction, this option will lead to the reset of the lanes and profiles. If manual Background correction is necessary, consider performing it before the lane detection.

	- The button 'Define Background manually' enables the user to draw a rectangle within the main axis (1b) to define an area of background. The average intensity in this area is used to be subtracted from the intensity profile of all lanes. - 'Reset Background' deletes the manually defined background and restores the automatic background subtraction.	
7a	**Profile axis, which displays the intensity profiles and respective Gaussian fits of each lane.** - If a ladder is selected red dots are displayed indicating the recognized extrema (bands). - If a lane is selected the intensity profile of the TRF smear is displayed, according to its molecular weight (y-axis). The red dot indicates the position of the maximum intensity of the profile. There are 3 different options for probe correction(all of them can be displayed at once) - The black check box 'raw' displays the raw data and its superpositioned Gaussian fit. The profile and the fit will be displayed in black color. - The red check box 'corrected' displays the probe corrected data and its superpositioned Gaussian fit. The profile and the fit will be displayed in red. This refers to method 1* of the probe correction. - The green check box 'Gauss mixing' displays the probe corrected data and its superpositioned Gaussian fit. The profile and the fit will be displayed in green color. This refers to method 2* of the probe correction. * For more details on the probe intensity correction, see the publication Göhring et. al 2013.	- Please select from Raw, Corrected, or Gauss mixing to display respective profiles. If unselected, only the maximum intensity of the profiles will be displayed as a red dot. - If only the intensity profile is visible, the calculation of the Gaussian fit failed. This indicates that the data is either not following a normal distribution, or a poor quality image is being used. Gel artifacts can lead to such disruptions.
7b	**Display of the fit quality of the Gaussian Fit for the raw data, the corrected data and the Gaussian mixing.** - The numbers have the same color code as described in 7a (black for the raw data, red for the corrected data and green for the Gaussian mixing). - The user may save the visible profile as .tif, .jpg, .pdf. or .eps by pressing the button 'Save Profile'.	- Negative Values or NaN Values indicate that the Gaussian fitting failed. - For conversion to .pdf, the open-source program pdftops has to be downloaded and the path declared. TeloTool will ask for the declaration of the respective path to the program the first time a profile is saved. - For conversion to .eps, the open-source program ghostscript has to be downloaded

		and the path declared. TeloTool will ask for the declaration of the respective path to the program the first time a profile is saved.
8	**Display of the results for the selected lane.** - The user may choose between 'raw' (for raw data, in black), 'corr' (for corrected data, in red) and 'mix' (for the Gauss-mixing, in green). The text fields underneath the ratio buttons display the maximum intensity in nt (which is the same for all options), the mean TRF, the standard deviation and the range. - The button 'Get Results' opens a new GUI, which displays the results for all (for more information refer to the 'Results' GUI chapter)	- Negative Values or NaN values indicate that the Gaussian fitting failed.