GelAnalyzer 19.1 User Manual

1   Installation and startup

GelAnalyzer has native installers for Windows, Linux (Debian), and MacOS. For any other OS a package is available that can be run with Java.

1.1 Windows

Download and extract the GelAnalyzer Windows installer zip file. Run the extracted exe file. A wizard will be shown that guides you through the installation process. Once installation has finished successfully GelAnalyzer should be added to the Start menu and have an icon on the desktop as well.

1.2 MacOS

Download and extract GelAnalyzer MacOS installer zip file. Start the extracted pkg file from Finder by ctrl clicking it and choosing open. You may be notified that the application is of unidentified source. A wizard will be shown that guides you through the installation. Once installation has finished successfully GelAnalyzer will be added to your applications and you should be able to start it from Launchpad.

1.3 Linux

For Linux currently only a Debian (.deb) package is available. It may be installed as any other Debian packages.

1.4 Other OS

1. Make sure you have minimally JDK 13 installed.

2. Download the zip file for the Java version of GelAnalyzer from GelAnalyzer.com.

3. Extract the downloaded zip to any directory you have write access to.

4. Depending on your configuration you may be able to run the gelanalyzer.jar file directly from your file manager. If this does not work then from your OS's shell run:

   java -jar gelanalyzer.jar

2   Previous versions

Note that 19.1 project format is not compatible with any previous versions of GelAnalyzer.

3   Concepts

3.1   Analysis

Analysis is the top-level object in GelAnalyzer and is equivalent to a project in other systems. An analysis is tied to a single image and is self-contained in the sense that once created the image is embedded and thus the analysis file is portable. One running GelAnalyzer instance can operate on one analysis at a time. For information on creating a new analysis refer to section 4.

On top-level an analysis has a single parameter: the **image type**. It may be either *light on dark* or *dark on light* depending on whether the bands in the image are lighter or darker than the background, respectively. Practically this parameter determines whether the intensity values of a lane should be inverted to ensure that bands appear as positive peaks on a lane's profile.

3.2   Lane

A **lane** is a top-down stripe on the image. Its shape is determined by a set of path points and a width paramter. The path points are connected with an interpolating Spline curve. A lane has at least two path points, the start and the end one, in which case its shape is a straight line. During the creation of a new analysis there is an option to rotate the image so that lanes start at the top.

Every lane has a **profile** which is obtained by scanning the image within the lane's area in a top-down manner, at each row averaging the intensities within the width's domain. Since GelAnalyzer currently only supports 8 bit / channel images, the maximal intensity value of a lane's profile is 255. In case of RGB images a pixel's intensity is calculated by converting the RGB color to 8 bit grayscale.

A lane can have a **background** defined on its profile. The background is a set of points that are connected with a straight line forming the **background profile**.

3.3   Band

A **band** is an interval on a lane's profile defined by three attributes: *start index, end index,* and *peak index.* The following restriction applies: *start index <= peak index <= end index.*

A band has the following properties derived:

3.3.1 Raw volume

If there's no background defined on the profile then raw volume is calculated the following way:

$$Raw\,volume = \sum_{i=start\,index}^{end\,index} profile[i]$$

If there is a background defined on the profile then there are two options. If profile values under the background are allowed to be negative then:

$$Raw\,volume = \sum_{i=start\,index}^{end\,index} profile[i] - background\,profile[i]$$

If profile values under the background should be considered zero then:

$$Raw\,volume = \sum_{i=start\,index}^{end\,index} max(\,profile[i] - background\,profile[i], 0)$$

3.3.2 Calibrated volume

A band's calibrated volume may either be set manually, in which case the band is considered as a *quantity calibration band*, or it is calculated by the quantity calibration process (discussed in section 3.5).

3.3.3 Rf

By default a band's Rf value is calculated based on the peak index's position in relation to the profile, i.e.:

$$Rf = peak\,index / profile\,length$$

If Rf calibration is defined on the analysis then band Rf values are calculated as discussed in section 3.4.

3.3.4 MW

A bands molecular weight (MW) may either be set manually, in which case the band is considered as a *MW calibration band*, or it is calculated by the MW calibration process (discussed in section 3.6).

3.4   Rf calibration

Rf calibration serves the purpose of making Rf values of bands in different lanes comparable. It can also be used to compensate for gel run distortions to a certain extent. Rf calibration is achieved by defining a number of fixed Rf curves on the image. A **Rf curve** is the same kind of Spline curve that forms the path of lanes. Opposed to lanes, Rf curves are horizontal, i.e., their start and end points are always at the left and right side of the image, respectively. Each Rf curve has a value assigned to it.

If Rf calibration is defined on the analysis then a band's Rf value gets calculated in relation to the Rf curve above and under it using linear interpolation. If a band happens to lie above the 0.0 or under the 1.0 curve then it's Rf value will be 0.0 or 1.0, respectively. It is up to the user to pay attention that bands lie within the bounds of Rf curves.

3.5   Quantity calibration

The quantity calibration process consists of fitting a function on the known {*raw volume, calibrated volume*} pairs coming from quantity calibration bands and then calculating the *calibrated volume* of every band in the analysis using the fitted function.

GelAnalyzer currently supports four calibration functions:

- Linear: $y = a * x + b$

- Quadratic: $y = a * x^2 + b * x + c$

- Linear log: $\ln(y) = a*x + b$

- Log: $y = a*\exp(b*x) + c$

3.6 MW calibration

MW calibration is the same as quantity calibration with the difference that it's based on the {Rf, MW} pairs coming from MW calibration bands and the goal is to calculate the MW of every band in the analysis by from their Rf values. The possible functions to be fitted are the same as in the case of quantity calibration.

3.6.1 MW standard

GelAnalyzer supports MW standards as a list of MW values in decreasing order. MW standards can be loaded on a lane or MW calibration bands of a lane can be stored as an MW standard.

4 Creating a new analysis

New Analysis

Once the program has been started there are two options available: creating a new analysis or opening an existing one. To create a new analysis, choose "New analysis" from the "File" menu or click the corresponding button in the toolbar. A file open dialog will be shown to select the gel image. Only PNG, JPEG, and GIF image formats are supported.

After the image has been opened the New analysis wizard is shown. To step forward click the check button or press ENTER. To step back use the back arrow or BACKSPACE. The steps in the wizard are the following:

1. Selecting the image type. The image type is either "light on dark" or "dark on light" based on whether the bands are lighter or darker than the background, respectively. The image type may be changed later on in the Analysis menu.

2. <u>Rotate the image so that lanes are vertical and lanes start at the top of the image!</u> It is not possible to rotate the image after the analysis has been created!

3. Select a crop region if necessary. Keep the left mouse button pressed to select the region. The region may be resized by dragging its edges. Right click to delete the region and thus keep the whole image. Only this cropped image will be stored in the analysis so pay attention that all relevant information are within the crop region!

5 Graphical User Interface

5.1 Main window

5.1.1 Main window menus

5.1.1.1 File menu

- Create, save, load Analysis.

- Export data
- Copy data to the clipboard.

5.1.1.2   Analysis menu

- Open the MW standard manager.
- Set the image type.
- Set the Quantity and MW calibration functions.
- Set weather profile values of a lane under the baseline should be considered zero or are allowed to be negative.

5.1.1.3   Windows menu

- Rearrange internal windows.
- Move a given window to the front.
- Turn on/off showing hints in the internal windows.

5.1.2 Main window toolbar

The Main window's toolbar is divided into two groups by default. Buttons in the first group are shortcuts for creating loading and saving Analysis. The second group is the program mode selector where each button corresponds to a mode discussed in section 6.

In certain program modes there are additional action buttons that appear in a third group.

5.2   Image window

This is where the gel image is displayed and where operations related to lanes, Rf-, quantity-, and MW calibration take place. A drop down box to select a zoom scale is also shown here. The zoom scale has no effect on the measurements.

5.3   Profile window

The profile window displays the intensity profile chart of the selected lane. Operations related to bands and background definition/subtraction also take place here. The horizontal axis of the chart represents the distance from the lane's start position in pixels, and the vertical axis represents the intensity values. Since all input images are converted to 8 bit grayscale format internally, the maximal intensity value of any profile is 255. The lane image is displayed below the chart.

A zoom selection drop-down is available whenever a lane is selected to control the horizontal resolution of the chart. There are three modes available:

- Fixed zoom values (100%, 200%, and 300%). The length of the profile is scaled with the chosen value. Use the horizontal scroll bar to scroll the profile.
- Fit profile mode: The length of the profile is fit to the chart's width. Scrolling is not possible in this case.

- Manual zoom: With this tool a portion of the profile can be selected to be shown. After choosing this option the cursor turns into a crosshair and selection is available by pressing left mouse button and selecting the area while keeping the mouse pressed. The maximal allowed magnification is 1000% this way.

Without background

The Profile window toolbar has a special toggle button which is active whenever a background is defined on the selected lane. With the "Without background" button it's possible to toggle on/off the visualization of subtracting the defined background from the lane's profile.

5.4   Analysis info window

This window displays a table whose rows represent a band of the analysis. There is a single menu item "Show" where you may select whether to show bands of all lanes, the selected lane, or only the MW / Quantity calibration ones. Whenever you select a lane in the image window this table will switch to showing the bands of that lane.

Those calibrated volume and MW values that has been assigned to a band by the user are shown in the table with bold font.

5.5   Calibration curves window

This window shows the fitted quantity and molecular-weight calibration curves along with their equations and the coefficient of determination (R^2) of the fit. The calibration bands are marked with a red square and hovering the mouse over them will display all details of the given band in the bottom of the window.

5.6   MW standard manager

The MW standard manager can be opened from the Analysis menu. The left panel shows a list of the standards and the right panel shows the selected one's values. Value can be added and removed using the two buttons under the right panel. Standards can be created, renamed and deleted from the menu.

6   Program modes

GelAnalyzer always operates in a given mode which determines what kind of operations are possible. There are 6 modes altogether: Lanes, Bands, Background subtraction, Rf calibration, Quantity calibration, MW calibration. To switch between modes use the buttons in the main window's toolbar. There is no fixed workflow and it is possible to switch between modes and do modifications without losing consistency in the analysis data.

6.1   Lanes mode

Lanes mode

This is the default mode when a new analysis is created or one is opened. Action buttons corresponding to Lanes mode are in the Image window's toolbar. In lanes mode it is possible to detect, add, modify, and delete lanes. All these operations take

place in the Image window. It is possible to select a lane by left-clicking in its region. The mouse pointer will change to a hand when it's within a lane's region. There are four operations possible in Lanes mode: detecting lanes automatically, adding lanes manually, modifying existing lanes, and deleting lanes.

6.1.1 Detect lanes

Detect lanes

GelAnalyzer has a built in method to detect lanes automatically. This is a convenience feature and it is not guaranteed that its outcome will be satisfactory for the further analysis. It is possible to manually modify any lanes including the ones detected automatically.

After clicking the "Detect lanes" button a window will be shown where the region of the lane detection can be defined. It works the same way as in the case of the crop region selection during the new analysis creation. If no region is defined the whole image will be used. To approve click the check button in the toolbar or press ENTER. Close the window or press ESCAPE to cancel.

6.1.2 Add a new lane manually

Add lane

To add a new lane manually first click the "Add lane" button. After this the mouse pointer will change into a cross and defining the new lane takes three left-clicks on the image:

1. First select the new lane's start position (the upper one).

2. Then select the lane's end position (the lower one).

3. Finally, set the new lane's width.

Neither the start nor the end point of the new lane can be defined within the region of an existing lane.

6.1.3 Modify existing lanes

Modify lanes

Lanes are defined by their control points and their width. The control points determine the lane's path. Minimally there are two control points in which case the path is a straight line. By adding new control points to a lane its shape can be bent and thus make it follow better lanes that are not straight on the image.

After activating the lane modification sub-mode the central path and control points of the lanes will be shown. To quit from lane modification mode click the button again or press ESCAPE. Whenever a lane is modified its profile will be refreshed and consequently all the bands that are defined on it are deleted. The following modifications are possible:

Add control point to a lane: Left-click the central path of a lane where the mouse pointer has a shape of a cross. By keeping the mouse button pressed it's possible to move the newly added point and thus changing the shape of the lane.

Move control points of a lane: Move the mouse over the control points where the mouse pointer changes to a hand shape. Grab the point by pressing the left mouse button and move it while keeping the button pressed.

Change the width of a lane: Move the mouse to the edge of the lane where the mouse pointer will change into a horizontal resize shape. Grab and move the edge of the lane with the left mouse button.

Delete control point of a lane: Move the mouse over the control points where the pointer changes to a hand shape. Right-click to delete the control point. The start and end control points cannot be deleted.
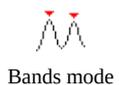
6.1.4 Delete lanes

Delete
selected lane

To delete the selected lane click the corresponding button in the Image window's toolbar or press DELETE.

Delete all
lanes

It is possible to delete all lanes of the analysis at once. To do this click the corresponding button in the Image window's toolbar.

6.2   Bands mode

Bands mode

In bands mode it is possible to detect, manually add, modify, and delete bands on lanes. Action buttons that affect all lanes are shown in the main window's toolbar and those affecting the selected lane only are shown in the Profile window's toolbar.

6.2.1 Detect bands

Detect bands

GelAnalyzer has a built-in function to detect bands automatically on the selected or on all lanes simultaneously. To detect bands on all lanes use the corresponding button in the main window's toolbar. Use the same button in the Profile window's toolbar to detect bands on the selected lane only. Be aware that this operation will delete previously defined bands on the lane it's applied to.

The method has three parameters that can be fine tuned every time it's applied. The peak threshold and minimal height parameters control the sensitivity of the method and the peak maximum width determines how wide the individual bands can be in percentage to the length of the lane.

6.2.2 Add band manually

Add band

Adding band to a lane takes place in the Profile window, thus, first the targeted lane has to be selected. After clicking the "Add band" button it takes two left-clicks on the profile region to define a new band:

1.   Select the start position.

2.   Select the end position. The end position must be to the right of the start position.

It is not possible to define a band that overlaps with an existing band. To cancel the band adding process right-click anywhere in the profile region or press ESCAPE.

6.2.3 Deleting bands

To delete a single band on the selected lane right-click within its region in the Profile window.

It is also possible to delete all bands of the selected lane by clicking the delete button in the Profile window's toolbar. It is also possible to delete all bands on all lanes at once by clicking the delete button in the main window's toolbar.

6.2.4 Modify existing bands

Start, end, and peak markers of existing bands may be modified in the Profile window. To do so grab the markers with left mouse button pressed where the mouse pointer has the shape of a hand and drag them to the desired position. Whenever the start or end of a band is modified the peak marker will automatically point to the position where the profile has the maximal value within the band's boundaries.

Modifications done to a band are reflected on the state of the analysis as well. For example, if a band is modified then its volume is recalculated. If the band was used as a quantity calibration band then the quantity calibration curve will be refitted and the calibrated volume of all bands in the analysis will be recalculated.

6.2.5 Delete bands

There are three ways to delete bands: delete a single band of the selected lane, delete all bands of the selected lane, and delete all bands of all lanes in the analysis.

In order to delete a single band of the selected lane right-clicking within its region.

In order to delete all bands of the selected lane click the delete button in the Profile window's toolbar.

In order to delete all bands of all lanes click the delete button in the main window's toolbar.

Deleting bands are instantly reflected on the state of the analysis.

6.3 Background definition mode

Background definition mode
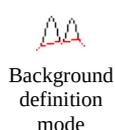
Background definition mode makes it possible to correct the uneven illumination of the image by defining a background profile on a lane which will then be subtracted when calculating band volumes.

There are three built-in methods for auto-detecting background to a lane and it's possible to define and modify the background definition manually as well.

6.3.1 Auto detect background

Auto detect background

It is possible to detect background on the selected lane or on all lanes of the analysis. To detect background click the corresponding button in the Profile window's toolbar. To detect background on all lanes use the button in the main window's toolbar.

There are three methods for background detection:

The **Rolling ball** method has one parameter which is the maximal peak width in percentage of the profile length. The method itself may be imagined as if a ball of the given size is rolled over the inverted profile and the points where it touches the profile will consist the detected background.

The **Morphological** method is very similar to Rolling ball and takes the same parameter only that it operates on a different principle.

The **Valley to valley** method differs from the other two in that it can only be applied if there's at least one band defined on the targeted lane. The resulting background points are composed of the band start/end profile values.

6.3.2 Define/modify a lane's background manually

To manually define background on a lane first click anywhere on the profile. A constant background will be added with two points at the two ends of the profile. Background points can be moved by dragging them while keeping the left mouse button pressed (mouse pointer has the shape of a hand when it's over a background point). To insert a point into the background click at the desired location. To delete background points right-click on them (first and last background points cannot be deleted).

Anytime a lane's background gets modified its band's volumes are refreshed instantly. If any of the bands are used in quantity calibration then that also gets refreshed and applied to every band of the analysis.

6.4   Rf calibration mode

Rf calibration
mode

In Rf calibration mode its possible define Rf curves on the image and modify their shape and Rf value. All these operations take place in the Image window.

6.4.1 Add default start and end Rf curves

Add default
start and end
Rf curves
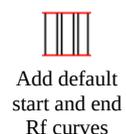
In case if lanes have different start or end levels its advisable to set absolute start and end Rf curves even if no gel run distortion is present on the image. This can be done by clicking the corresponding button in the Image window's toolbar. If there are lanes on the image the start (0.0) and end (1.0) Rf curves will be set to the highest lane start and the lowest lane end, respectively. If no lanes are yet defined then the start and end curves will be added at 10% and 90% of the image's height, respectively.

6.4.2 Add Rf curve

Add Rf curve
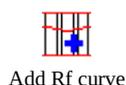
In order to add a new Rf curve between the 0.0 and 1.0 curves click the corresponding action button in the Image window's toolbar then click at the desired location for the new Rf curve on the image. It is possible use this method even if no 0.0 and 1.0 curves are defined in which case these will be added automatically as if the "Add default start and end Rf curves" method was used.

6.4.3 Change the value of Rf curves

To set the value of an Rf curve (except for the first and last one) left-click on the text area with current value in it. The area will become active and then its possible edit the value. Press ENTER to confirm or press ESCAPE to cancel.

Changing the value of an Rf curve is instantly reflected on the state of the entire analysis.

6.4.4 Modify an Rf curve's shape

Modifying an Rf curve's shape is done the same way as in the case of lanes. That is, by left-clicking anywhere on the curve will add a new curve point. Right-clicking on a curve point (except of the first and last one) deletes it. Changing the shape of the curve is done through dragging the curve's points.

6.4.5 Deleting an Rf curve

An Rf curve can be deleted by right-clicking anywhere on the curve where there's no curve point. The 0.0 and 1.0 curves cannot be deleted this way, for that use the "Delete Rf calibration" button in the Image window's toolbar.

6.5   Quantity calibration mode

Quantity calibration is based on assigning known volume value to bands in the analysis. A band that has a calibrated volume value assigned to it is referred to as a quantity calibration band. Once the required number of such calibration bands are available the calibration curve is calculated and all band in the analysis have their calibrated volumes calculated. The number of required calibration bands depends on the type of curve to fit.

Quantity
calibration
mode

The process of assigning calibrated volume values to given bands takes place in the Image window.

6.5.1 Assign calibrated volume to a band

Each band is marked with a white circle on the image. To assign calibrated volume value to a band left-click on its marker. A text field will be shown where the value can be entered. Press ENTER to confirm or ESCAPE to cancel. The value must be greater than or equal to 0.

6.5.2 Modify the calibrated volume of a calibration band

By left-clicking the band marker the value becomes editable. Press ENTER to confirm or ESCAPE to cancel.

6.5.3 Remove a band from quantity calibration

To remove a band from quantity calibration right-click on its marker.

6.5.4 Delete quantity calibration

It is possible to remove all bands of the analysis from the calibration and thus remove quantity calibration from the analysis in one step. To do this click the delete button in the Main window's toolbar.

6.5.5 Set the calibration function

The quantity calibration function can be set in the Analysis menu in the Main window's menu bar.

6.6 Molecular weight calibration mode

Molecular weight calibration mode

The operations in MW calibration mode are the same as in quantity calibration mode. For MW values only integers greater than 0 are allowed.

In MW mode its also possible to load an MW standard onto the selected lane or save its bands' MW values as a MW standard.

6.6.1 Load MW standard on selected lane

Load MW standard on selected lane

To load an MW standard to the selected lane's bands click the button in the Main window's toolbar then select the MW standard from the dialog shown afterwards.

6.6.2 Save selected lane as MW standard

Save selected lane as MW standard

To create an MW standard from the selected lane's bands' MW values click the button in the Main window's toolbar then enter the name for the new Standard in the shown dialog.

7 Copy/Export data

To copy the contents of the individual windows go to the Main window's File menu → Copy submenu options. In case of the Image, Profile, and Calibration curve windows the content is an image. In case of the Analysis info window the content is textual data that can be pasted into any spreadsheet program.

For the mage, Profile, and Calibration curve windows it's also possible to save their content to an image file. For this option go to the Main window's File menu → Export submenu options.