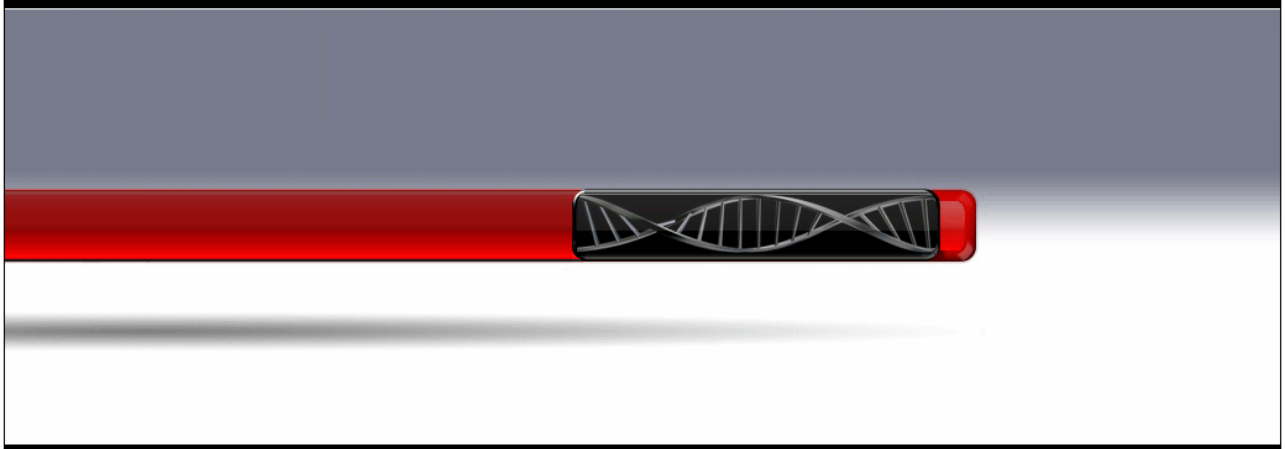
UVITEC

Cambridge

EXPAND YOUR TERRITORIES



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# UVIBAND

## User manual

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## Thank you

Dear Customer,

On behalf of UVItec, we would like to thank you for choosing the UVIband software.

In order to learn the capabilities of your UVIband software, we kindly ask you to read this manual. This manual details how to install and to operate the hardware and the software components.

UVItec is dedicated to your satisfaction and we will be pleased to answer any question you may have. We are also very receptive to your suggestions. Many of the new features and enhancements in this software are a direct result of conversations with our customers. Please do not hesitate to contact us to let us know what you would like to see in the next version of this software.

You can contact us at the following addresses:
⇨ Email: uvi@uvitec.co.uk

⇨ Contact address: UVItec Limited
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Cambridge CB4 1QB
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Fax: +44 (0)1223 306 198

Do not hesitate to visit our website at www.uvitec.co.uk

# UVIBAND
## User manual

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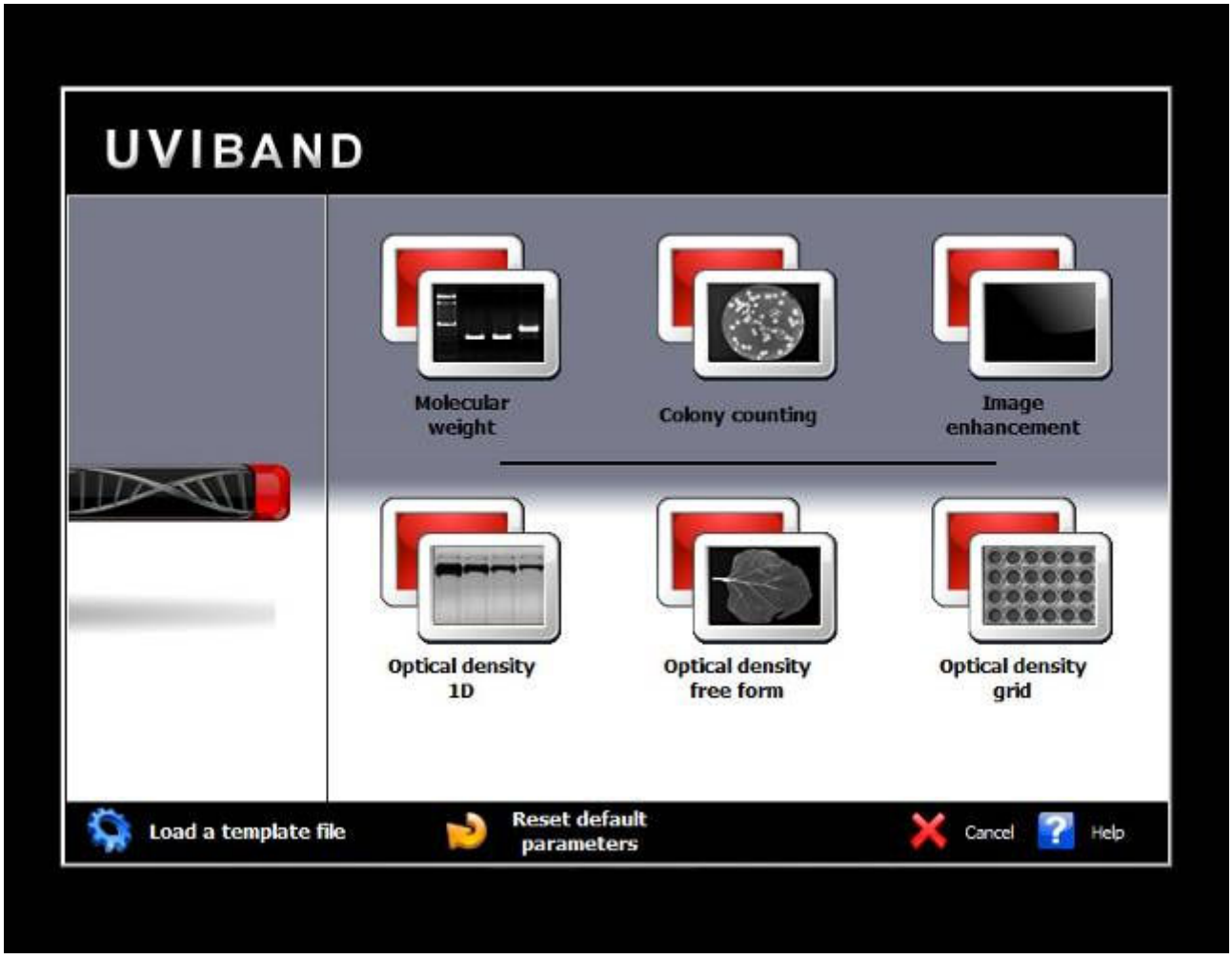


# Introduction

## ➔ UVIband Advanced

# About

UVIband is a sophisticated and intuitive analysis software designed for virtually any fluorescence or chemiluminescence sample.

The software combines the power of a comprehensive set of analytical tools and automatic functions in an incredibly easy to use environment. It includes 8 different analysis modules from molecular weight calculation to volume quantification, through microtitration and GFP quantification.

The analysis can be saved as a template and re-used for further analysis to facilitate routine analysis. All result tables and graphics can be printed or exported in a Windows® compatible format. The image enhancement module prepares your image for publication thanks to a large choice of filters.

## ➔ UVIband Advanced key features


- Transform your 1D gel into quantification data
- Work with different kind of samples such as DNA, RNA, protein, polynucleotide, Petri dish, microtitration plates, plants and in-vivo images
- Ease your analysis by using the same template for the analysis of different images
- Use state of the art analysis algorithms

# End-user license agreement

Please read these instructions before installing and operating
the UVIband Advanced software

Please read carefully the following license agreement. This document is a legal agreement between you, and UVItec Ltd, concerning the use of the enclosed software. This agreement constitutes the complete agreement between you and UVItec Ltd.

By opening the software package or using the CD, you are consenting to be bound by and are becoming a party to this agreement. If you do not agree To all of the terms of this agreement, do not open the package or use the CD. Promptly return the CD, including the written materials, the binder, and all other equipment/containers to the dealer from whom you purchased them.

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We hereby grant you a nonexclusive, limited license to use one copy of the Software on any single computer, provided the Software is in use on only one computer at any time. The Software is "in use" on a computer when it is loaded into temporary memory (RAM) or installed into the permanent memory of a computer—for example, a hard disk, CD-ROM or other storage device.
If the Software is permanently installed on the hard disk or other storage device of a desktop or portable computer (other than a network server) and one person uses that computer more than 80% of the time, then that person may also use the Software on a 2nd computer for the purpose of viewing and post-processing images.

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• This license agreement may be modified only by a written agreement signed by you and us that specifically supersedes this agreement.
• This license agreement is governed by the French laws

# Installation guidelines

## ➔ System requirement

The following sections detail the minimum hardware and software requirements needed for the UVIband Advanced software. These requirements are valid at the time of your purchase and may change with future development of our software products as well as the computers to which they interface.

| | Minimum requirement |
|---|---|
| **Processor** | Pentium, 3.2 GHz, FSB 800 MHz ( bus speed) and upwards |
| **Ram** | 512 Gb and upwards |
| **Hard disk** | 10 Gb and upwards. At least 1Gb free disk space least in order to allow software installation and image storage |
| **Monitor / Video card** | AGP card 1024 x 760 in 16 millions colour mode (24-bit). Upper resolutions supported. Video card with a refresh rate above 70 Hz. |
| **Operating system** | Microsoft Windows 2000 (and upper) - Microsoft Windows XP SP1 (and upper) - Microsoft Windows Vista (32-bit only) |
| **USB Port** | At least one USB port available |

## ➔ Unpacking and installing the UVIband Advanced

Please, open the UVIband Advanced folder carefully and verify the contents:

- Installation manual 1
- UVIband Advanced CD 1
- USB User dongle 1 per license

Remove carefully each component from the folder.
Install the USB user dongle in the USB port of your computer

UVIband Advanced requires Microsoft Windows™ Vista, XP, 2000 or 98.

Microsoft Windows™ must be installed on your computer before any other installation.

The CD disk provided with the manual contains the UVIband Advanced program as well as the pdf manual.

1. Ensure that all other application programs are closed. Windows 2000, XP or Vista users should also ensure that they are logged on with administrator privileges.

2. Insert the disk into your CD drive. The UVIband Advanced InstallShield Wizard automatically runs, and the Setup window appears. If not, double click on the setup.exe file to start the installation of the software

3. The Choose Destination Location window appears. Either click on the Browse button to specify the destination directory, or click on Next to accept the default directory, c:\Programfiles\UVItec\UVIsoft.

4. The Select Program Folder window appears. Click on Next. The Start Copying Files window displays the settings that you selected. Either click on Next to accept the settings, or click on Back to return to the previous screens and revise the settings.

5. When the installation is complete, the system is updated. You should see the UVIband Advanced icon desktop shortcuts. Click twice on the “UVIband Advanced” icon on the Windows Office screen to start the software.

## ➔ Hardware security dongle

The UVIband Advanced software is provided with a hardware security USB dongle.

The UVIband Advanced software is protected using a hardware security key (HSK), which is included in your software package. You must connect the USB dongle to the USB port of your computer before you can run the software.

The USB dongle should be connected to the USB port after the UVIband Advanced CD-Rom installation procedure.

The USB dongle driver is installed during the UVIband Advanced CD-Rom installation procedure.

Users do not have to reboot

The dongle key is your license to use the software. If the dongle key is lost, you will need to purchase a new license.

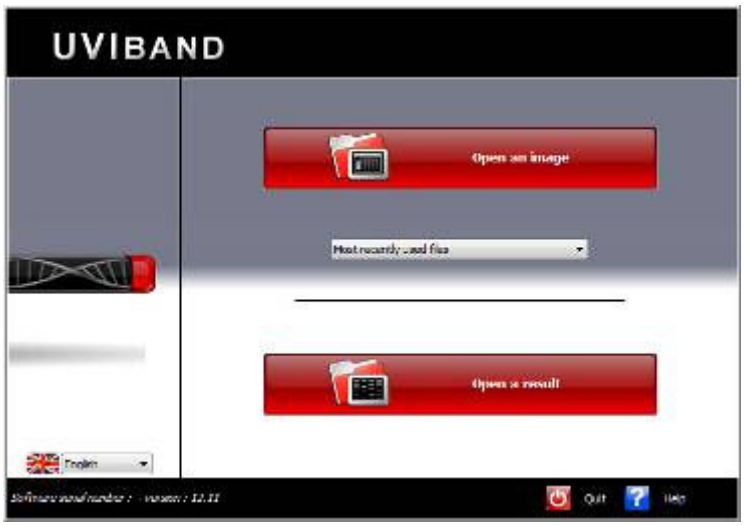
# Quick start

## ➔ Starting the software

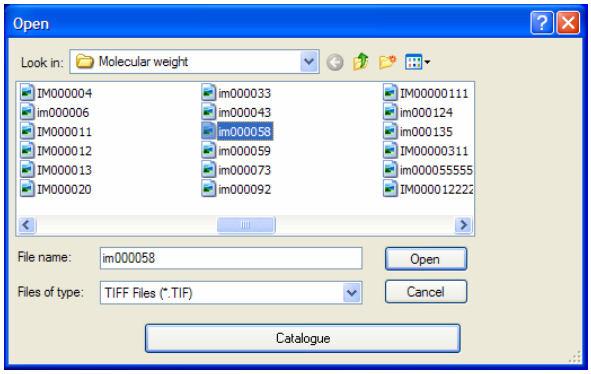
| | |
|---|---|
|  | Click twice on the “UVIband Advanced” icon on the Windows Office screen to start the software. |

## ➔ Open an image or an analysis

The software opens on the following window:

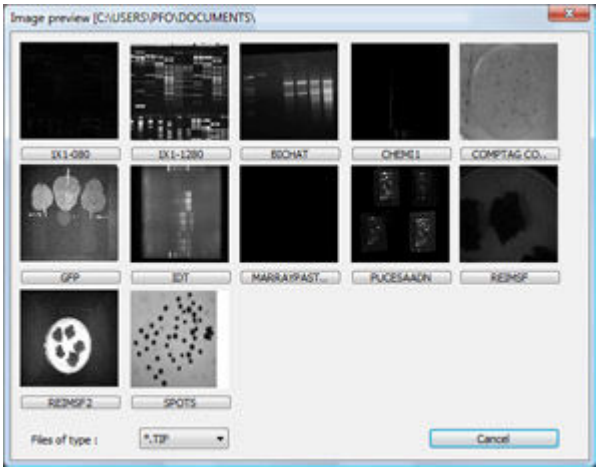



1. Click on the “Open an image” icon to open an image. A pop-up window displays the following menu:



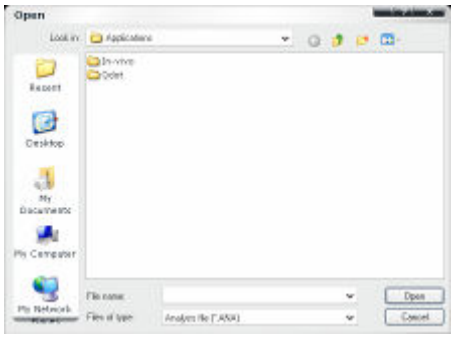
⇨ Browse to specify the image directory
⇨ Double click on the image name you want to load

Note 1: the catalogue function allows a preview of the images loaded in the selected directory. To proceed, click on “Catalogue”.



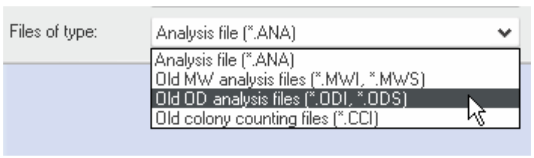

Note 2: the most recently used files allow a fast and easy access to the last 10 images previously open by the UVIband Advanced software.


2. Click on the “Open a result” icon to open a previously saved analysis result. A pop-up window displays the following menu:



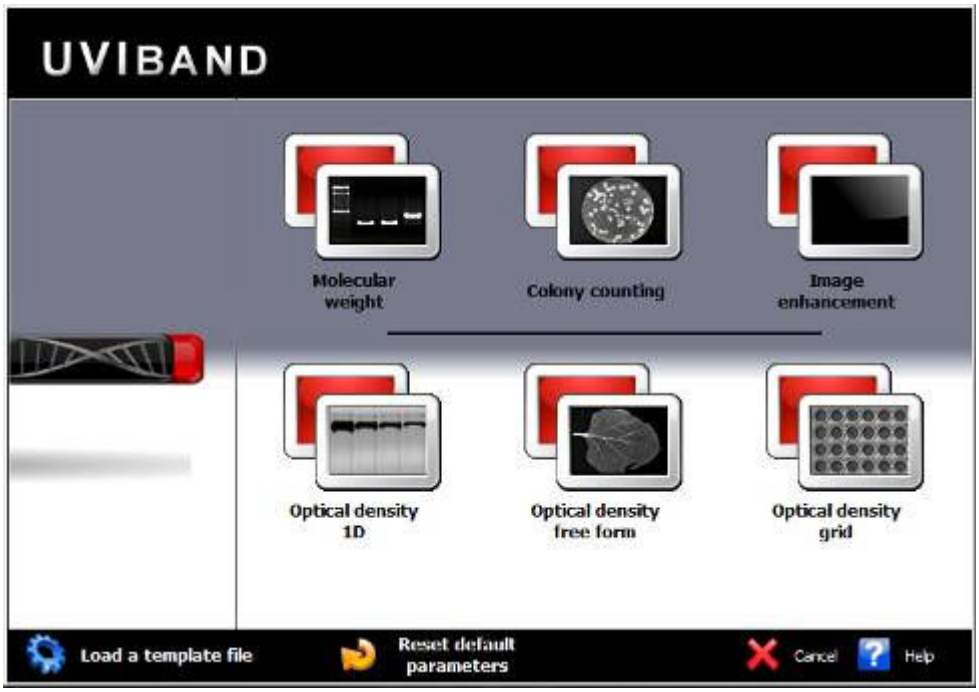

⇨ Browse to specify the analysis directory
⇨ Double click on the analysis name you want to load

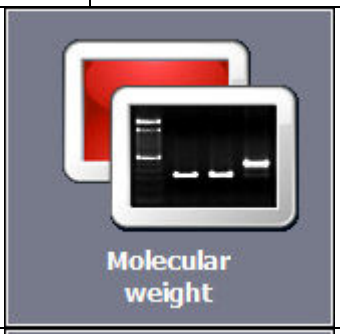
Note 3: Select the appropriate type file to open the analysis file of the former version of UVIband:



## ➔ Access to the analysis modules

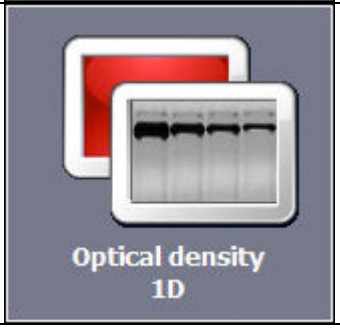
Once an image is opened, a pop-up window displays the software modules menu:



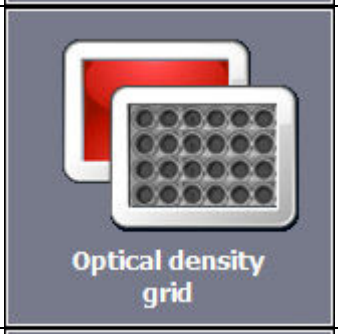




⇨ Select the Molecular weight icon to open the molecular weight analysis (MW) module


⇨ Select the Colony counting icon to open the colony counting (CC) analysis module



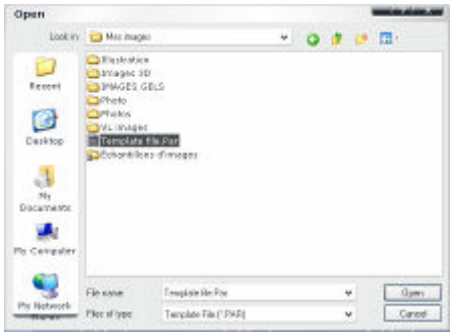

⇨ Select the Optical density - 1D icon to open the optical density (OD) analysis module based on a 1D detection

| | |
|---|---|
|  | ⇨ Select the Optical density - Free form icon to open the optical density (OD) analysis module based on a free form detection |
|  | ⇨ Select the Optical density - Grid icon to open the optical density (OD) analysis module based on a grid detection |
|  | ⇨ Select the Image enhancement icon to open the image enhancement module |

## ➔ Load a template file

Select the “Load a template file” icon to open a template: 


A pop-up window displays the following menu:



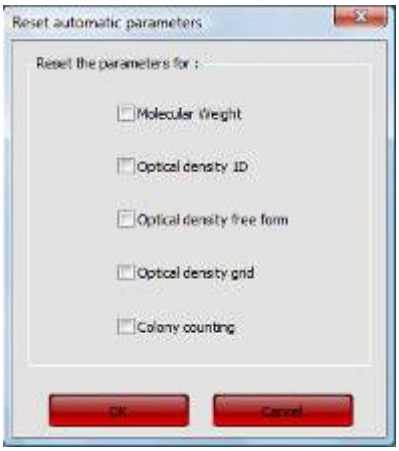

⇨ Browse to specify the template directory
⇨ Double click on the template name you want to load

Note 1: Please refer to the “How to use a template file” part of this manual for more details on the template.

## ➔ Reset to factory setting

Select the “Reset” icon to reset the parameters to the factory setting.

A pop-up window displays the following menu:




⇨ Select the software module for which you want to reset to the factory setting
⇨ Validate by clicking on OK

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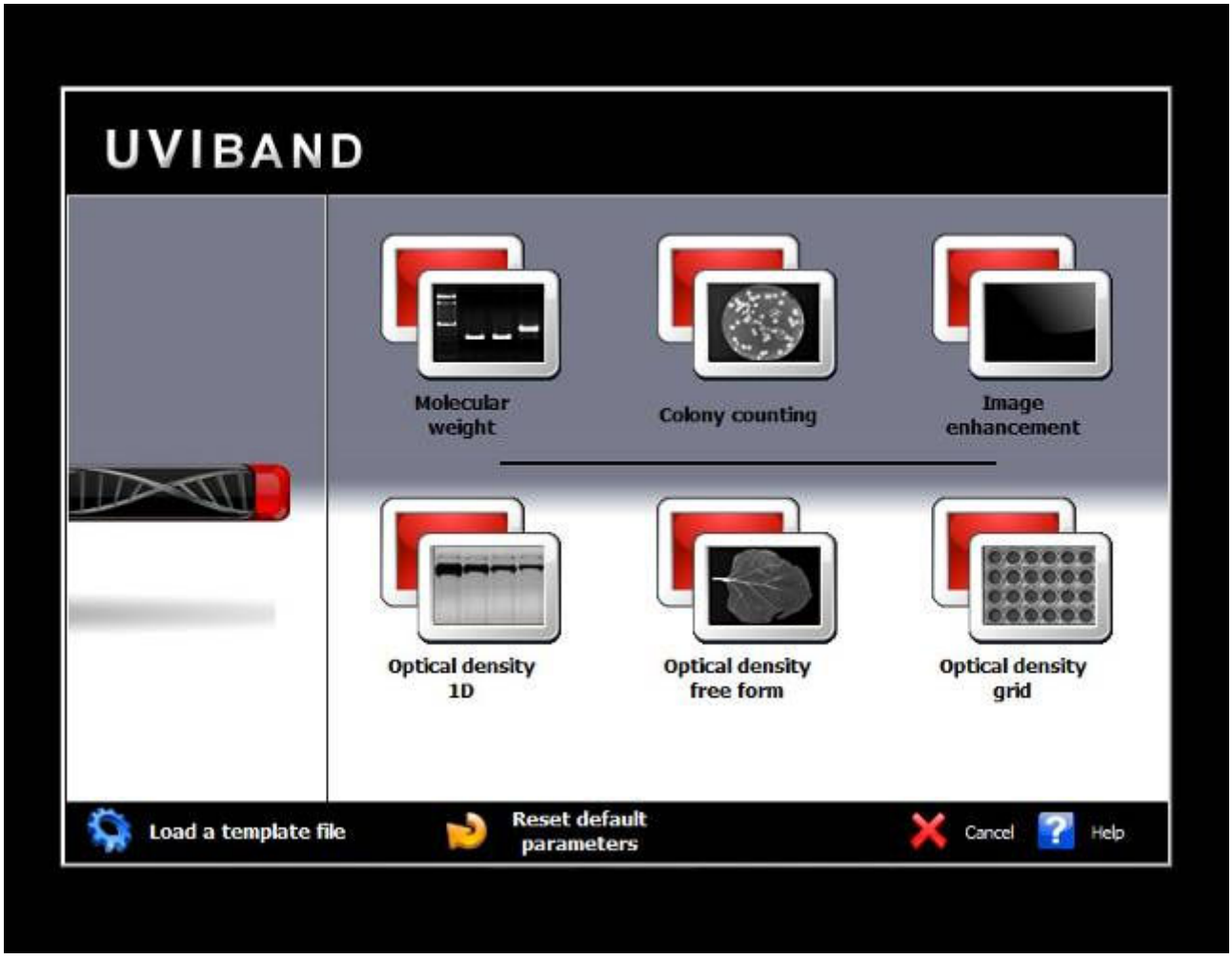



# Molecular weight

## ➔ MW Analysis module

# Molecular weight introduction

## ➔ Objectives and output

The UVIband Advanced Molecular Weight module features the calculation of electrophoretic distances according to markers or standards:

| | |
|---|---|
| In molecular weight | (unit: KiloDalton) |
| In fragment sizes | (unit: Kilobases) |
| In R.F. values | (values between 0 and 1) |
| In isoelectric points | (pH units) |

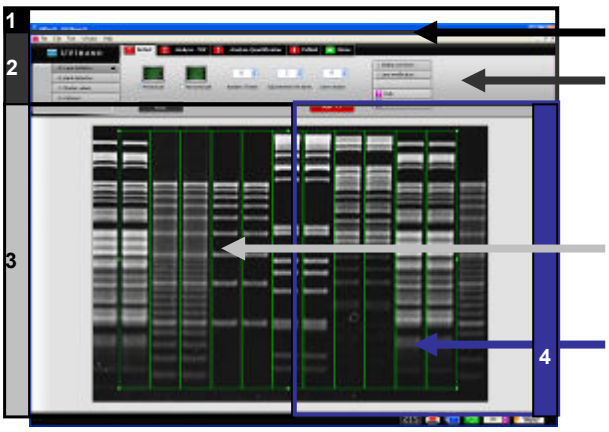
It also features the quantification of spot in volume, percentage or µg.

At the end of the process, you can have the following outputs:
- Molecular weight marker's profile and migration curve
- Sorted and unsorted molecular weight values
- Distance calculation
- Dendrogram in graph or matrix
- Matching with an external master
- Lane's volume and concentration
- 3D profile
- 3D result's graph
- Calibration curve

## ➔ Molecular weight module (MW) operating environment

The MW module opens on the following window:

The UVIband Advanced operating environment is organized into four areas:




1. The menu bar
2. The dash board
3. The image window
4. The working window

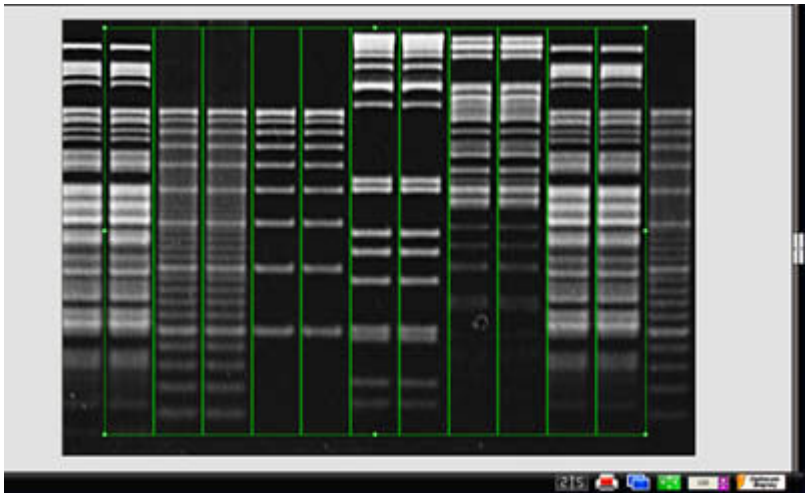
The menu bar contains the following menu:

- ⇨ File
- ⇨ Edit
- ⇨ Windows
- ⇨ Help


The dash board contains four different tabs:

1. Detect
2. Analyse molecular weight (MW)
3. Analyse optical density (OD)
4. Home


The image window displays the active image:



It also contains the image toolbar:


⇨ Display the molecular weight on the image

⇨ Print

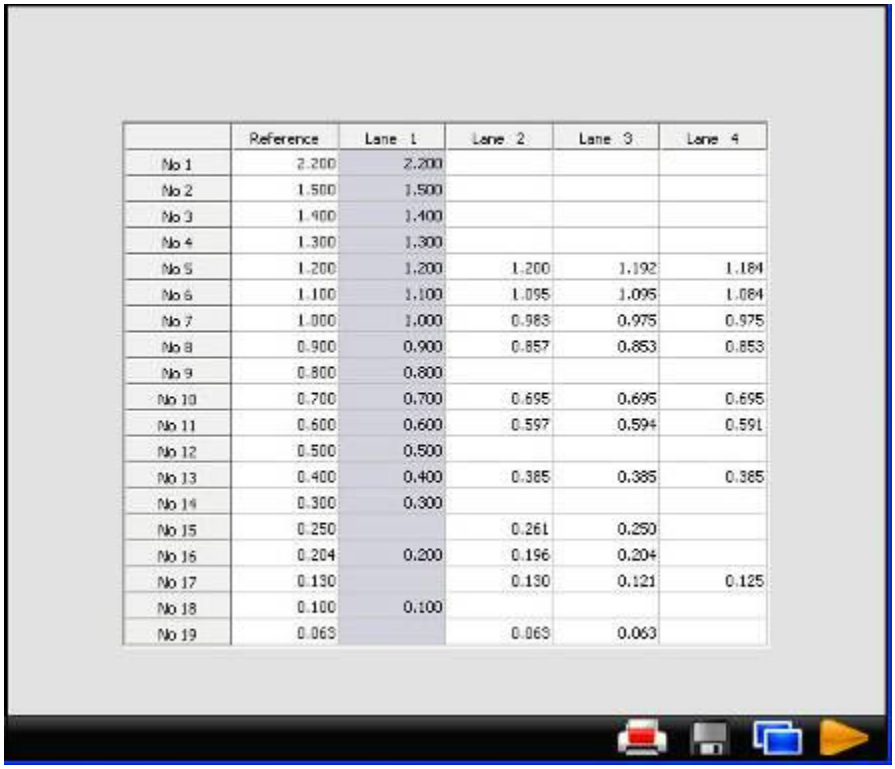
⇨ Copy to clipboard

⇨ Autoscale

⇨ Zoom in or out the image

⇨ Change the optimum display

The working window displays the graphs and tables related to the active analysis:



| | Reference | Lane 1 | Lane 2 | Lane 3 | Lane 4 |
|---|---|---|---|---|---|
| No 1 | 2.200 | 2.200 | | | |
| No 2 | 1.500 | 1.500 | | | |
| No 3 | 1.400 | 1.400 | | | |
| No 4 | 1.300 | 1.300 | | | |
| No 5 | 1.200 | 1.200 | 1.200 | 1.192 | 1.184 |
| No 6 | 1.100 | 1.100 | 1.095 | 1.095 | 1.084 |
| No 7 | 1.000 | 1.000 | 0.983 | 0.975 | 0.975 |
| No 8 | 0.900 | 0.900 | 0.857 | 0.853 | 0.853 |
| No 9 | 0.800 | 0.800 | | | |
| No 10 | 0.700 | 0.700 | 0.695 | 0.695 | 0.695 |
| No 11 | 0.600 | 0.600 | 0.597 | 0.594 | 0.591 |
| No 12 | 0.500 | 0.500 | | | |
| No 13 | 0.400 | 0.400 | 0.385 | 0.385 | 0.385 |
| No 14 | 0.300 | 0.300 | | | |
| No 15 | 0.250 | | 0.261 | 0.250 | |
| No 16 | 0.204 | 0.200 | 0.196 | 0.204 | |
| No 17 | 0.130 | | 0.130 | 0.121 | 0.125 |
| No 18 | 0.100 | 0.100 | | | |
| No 19 | 0.063 | | 0.063 | 0.063 | |

It also contains the working window toolbar:

⇨ Display the molecular weight on the image

⇨ Save the graph or the table

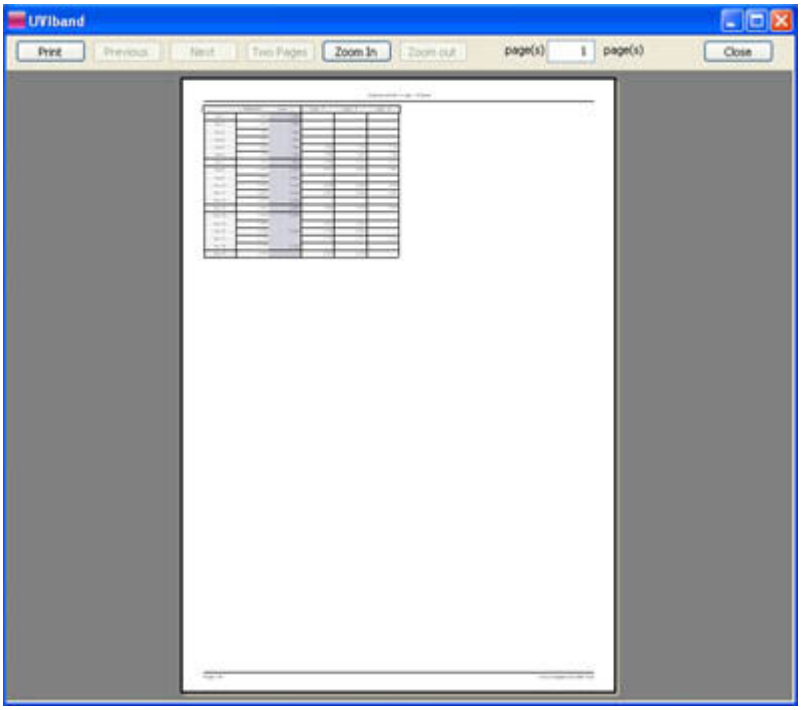
⇨ Copy the graph or the table to clipboard

⇨ Export the table to Excel

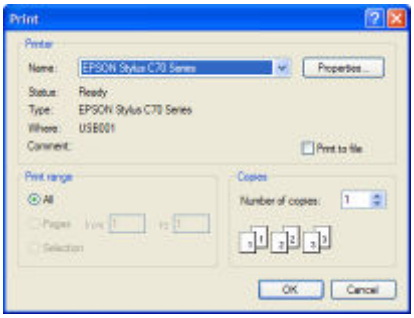
## ➔ Toolbar in details

**Print**

1. Click on the “Print” icon to print the image, the table or the graphs. A pop-up window displays the Print preview: The Print preview displays a preview of the image, as it will be printed.




2. Click on Print to validate the preview. A pop-up window displays the following menu:



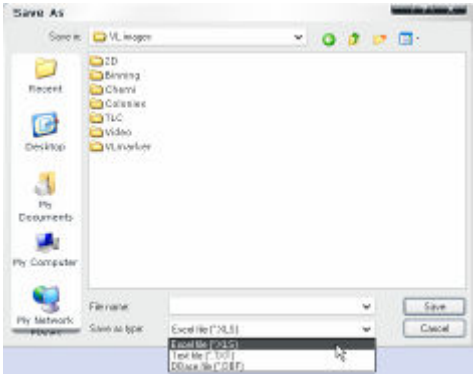

⇨ Select a printer
⇨ Click on Properties to modify the default setting of the printer, if necessary
⇨ Select the number of copies
⇨ Click on OK to validate your options

Note: You can also access the Print menu from the Menu bar (File\Print).

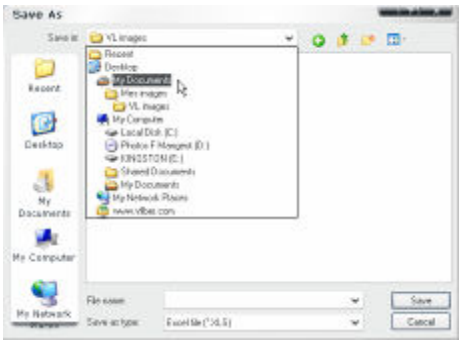
## Save

This function saves a graph or a table. The tables are saved in a Excel™ file format (*.xls). The graphs are saved in a Bitmap format (*.bmp).

1. Click on the “Save” icon.
2. A pop-up window displays the following menu:

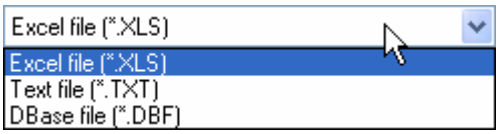



3. Browse to specify the file directory




4. Enter the desired file name, select a file extension and validate

Note: the results could also be saved in a text file format or a Dbase file format:




The graphs can only be saved on a BMP format:


## Copy to clipboard

This function copies an image, a table or a graph onto the clipboard for insertion into another program. This option is identical to the Windows® [Ctrl C] command.

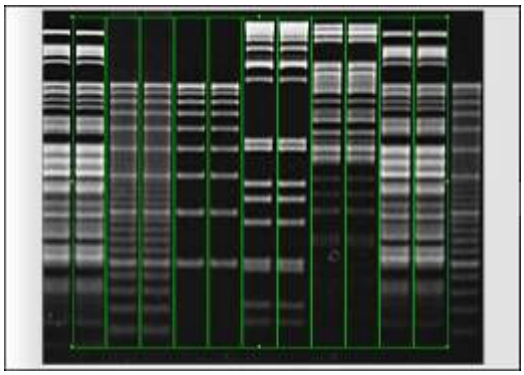
1. To proceed, click on the Copy to clipboard icon. The image, the table or the graph is now ready to be pasted into another application.

2. Open the application that you want to paste the image into, and select from the available pasting options ([Ctrl V] command for Windows® software).

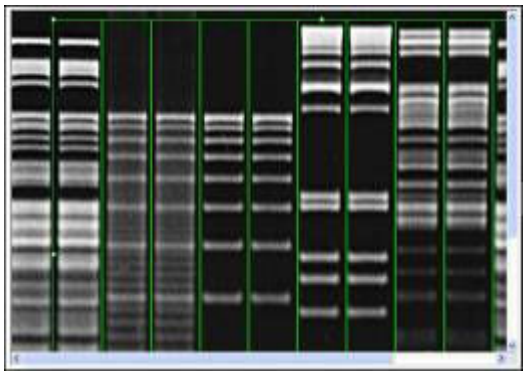
## Auto-scale

1. Click on the “Auto-scale” to resize the image to fit the size of the monitor.

The Auto-scale feature proportions the display of the image to the screen resolution.



Auto-scale (no scroll bar)



No auto-scale (scroll bar)


**Optimum display (for 12, 14 and 16-bit image file)**

The optimum display window is helpful to modify the greyscale selection to enhance the image display: To proceed, click on the “Optimum display” icon. A pop-up window displays the following menu:

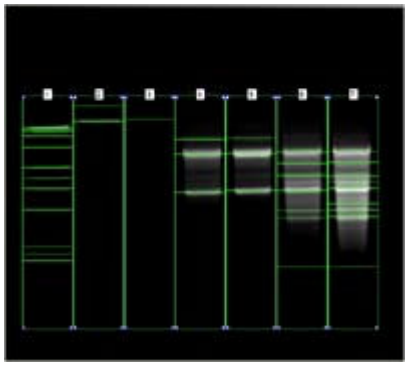
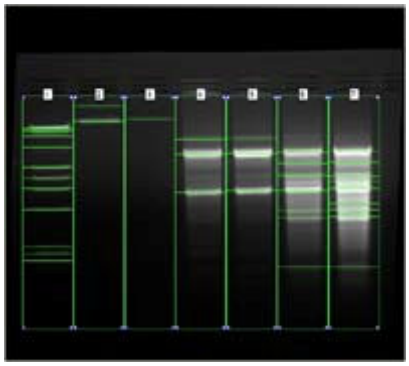
Some images has a 12, 14 or 16-bit format and Windows® can only display 8-bit images (256 grey levels).

Due to this limitation, the UVIband Advanced software handles two images:
⇨ A “memory” image corresponding to the 12, 14 or 16-bit format (4 096, 16 384 or 65 536 grey levels)
⇨ A “display image” corresponding to the image displayed on the screen (256 grey levels)

The easiest way to calculate the “display image” would be to translate the full grey scale each time an image is acquired: the x grey levels values of the “memory” image corresponds to 256 values in the displayed image. In that case, it won’t be possible to visualise faint spots on a dark image.

UVIband Advanced offers the possibility to select the grey level range to translate for the display image calculation. All the grey levels under the “Min value” defined will be converted to 0 (Black) in the displayed image. All the grey levels upper the “Max Value” defined will be set to 255 (White) in the displayed image. The grey levels between those two limits will be converted in an intermediate grey level value following a linear rule.

For both values, you can:
⇨ Edit the value in the corresponding field
⇨ Select the value by dragging and dropping the arrow
⇨ Click on the “optimum display” button: UVIband Advanced will then calculate the ideal values to be selected according to the parameters defined

| | |
|---|---|
|  |  |
| Automatic optimum display | Optimum display enhancement<br>The image appears brighter. The faint bands are more visible. |

Note: The optimum display has no impact on the analysis. Only the display of the image is modified.

**Send to Excel™**

This function transfers the results table to Windows Excel™.

1. To proceed, click on the Send to Excel™ icon. The Excel software is automatically opened by the UVIband Advanced and the table is transferred to Excel™.

# 1- Detect

## ➔ A – Lane definition

1

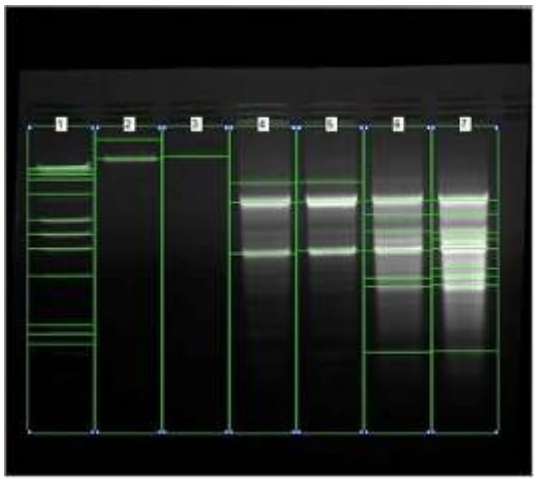
The molecular weight module opens on the Lane definition dashboard of the Detect process:


The dashboard details the lane definition parameters:

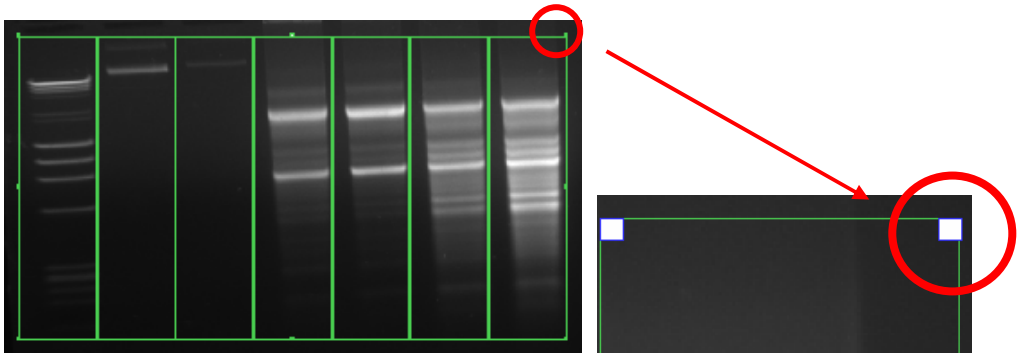

- ⇨ Lanes direction
- ⇨ Number of lanes
- ⇨ Gap between the lanes
- ⇨ Lanes rotation
- ⇨ Smiling correction
- ⇨ Lane modification

**AREA OF INTEREST**

On the image, click and drag to define the analysis area and to overlap the lanes. You can easily adjust the size of the area by clicking on the tags surrounding the area and drag the selected border to the requested size.

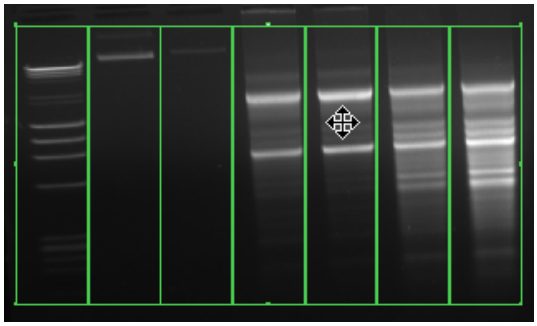


The lanes are defined by green lines, overlaid on the gel image. The gel area is surrounded by square anchors:



To resize the entire lane frame, drag an anchor point in or out. The opposite anchor point will remain fixed while the frame expands or contracts. The frame will expand or contract from the center.

To move the entire frame to a new position, position the mouse on the frame to obtain a cross cursor:



Click and drag the cursor to move the entire frame.

Note: it is not necessary to include the well line in the area of interest window. The calculation of distances does not require this reference line.

**LANE DIRECTION**

Select the direction of the lanes from:

⇨ Horizontal

⇨ Or vertical


The lane direction is automatically modified on the image.

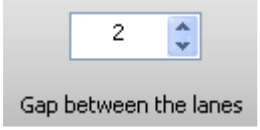
**NUMBER OF LANES**

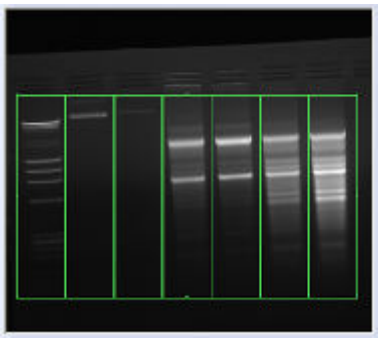
Select the number of lanes:


The number of lane is automatically modified on the image.

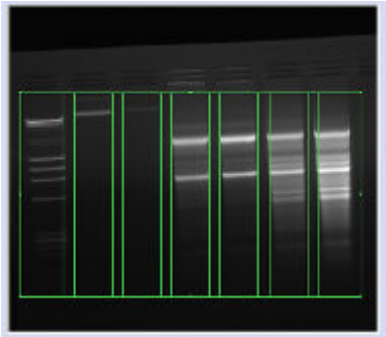
**GAP BETWEEN THE LANES**

Define the gap between the lanes:



**Short gap**

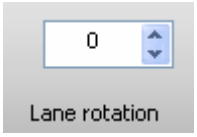


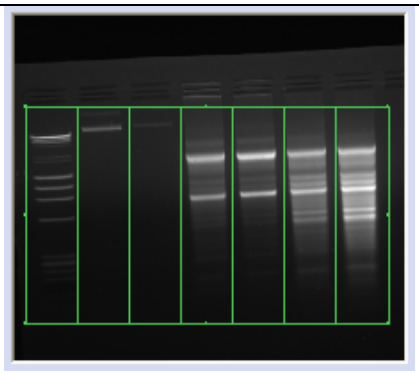
**Large gap**

The gap between the lanes is automatically modified on the image.

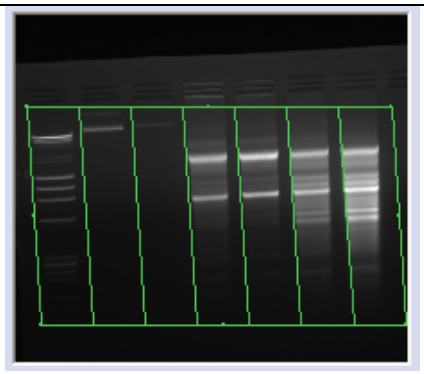
**LANE ROTATION**

Define the lanes rotation to modify the angle of rotation of the lanes' area of interest.





**No lane rotation**



**Lane rotation**

The lane rotation is automatically modified on the image.

Note: Lane rotation could either be positive or negative.

## RESET

The “Reset” button restores the default lane detection parameters.


## NEXT

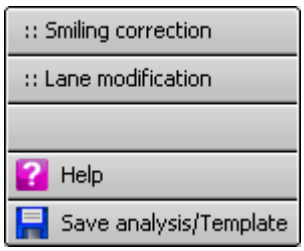
The “Next” button validates your parameter and opens the following analysis step.

| | | |
|---|---|---|
| A- Lane definition | Next >> | B - Band detection |

## OPTION FOLDER

The option folder gathers the following functions:

- ⇨ Smiling correction
- ⇨ Lane modification
- ⇨ Help
- ⇨ Save the analysis or the template

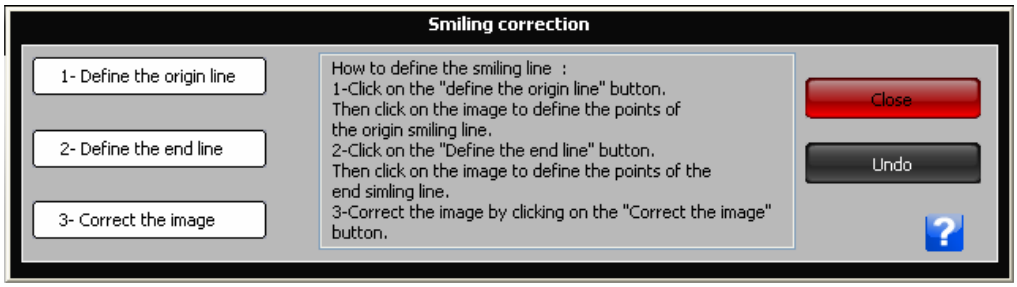



## SMILING CORRECTION

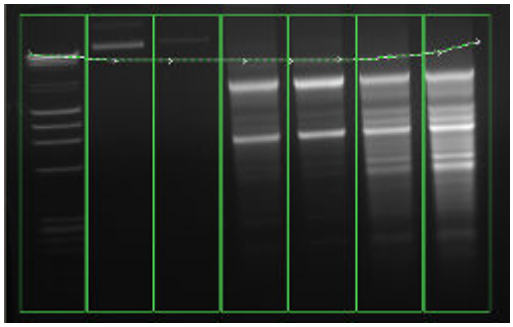
This function allows the treatment of electrophoretic problems such as "smiling" or bent separation front. This correction is based on and requires the definition of internal references in each lane for the recalculation of the electrophoresis distance.

1. Click on the “Smiling correction” button.

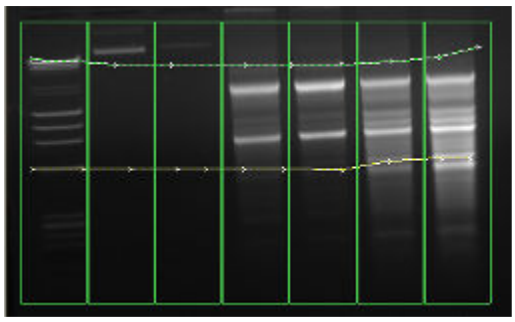

2. A pop-up window displays the following menu:




⇨ Draw first the line of origin by selecting the “Define the origin line button”. Click on the image to define the points corresponding to this line, two at least must be located on the edges of the area of interest.



⇨ Then, draw the front line by selecting the “Define the end line” button. Click on the image to define the points corresponding to this line, two at least must be located on the edges of the area of interest.



3. Click on “Correct the image” to display the migration correction.

Note: You can undo the correction by clicking the “Undo the correction” button.

Note: You need to close the pop-up window by clicking on the “Close” button.

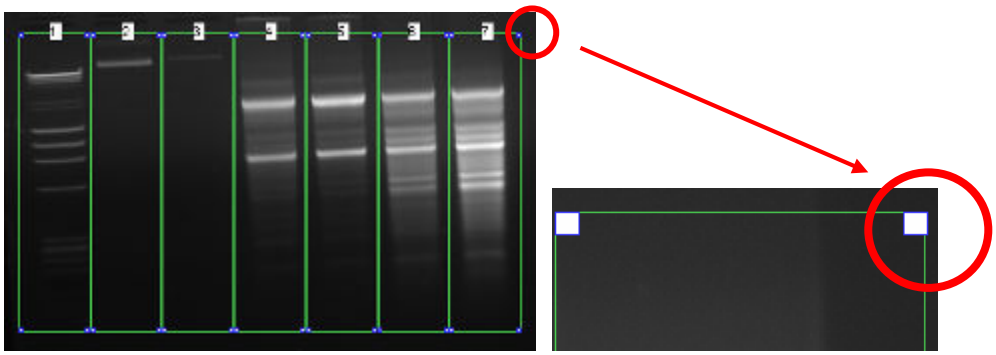
**LANE MODIFICATION**

The lane lines are created in default as part of a lane frame. They can also be modified individually with the Lane modification feature.

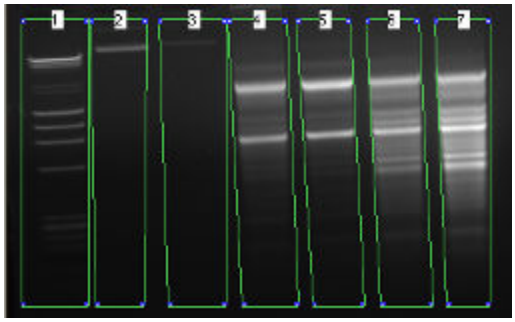
1. Click on the “Lane modification” button.

:: Lane modification

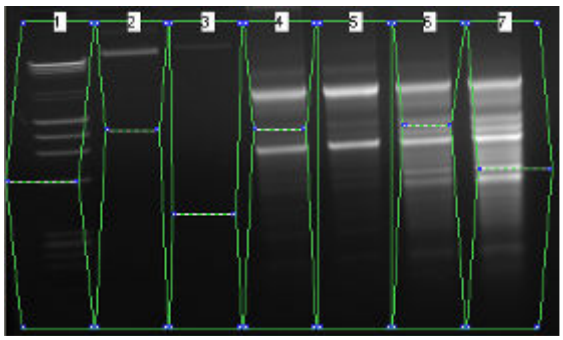
2. A pop-up window displays the each lanes with interactive squares at the corners:



⇨ Click and move these interactive squares to modify the shape of the area of analysis:



⇨ New interactive moving points can be defined by clicking on the longer side of the window:



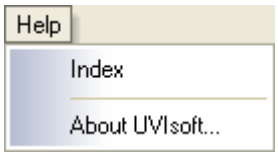
Note: A modification of the area of analysis after a lane modification will cancel this lane modification.

## HELP MENU

Help

Click on the “Help” button. You automatically access the user manual at the chapter corresponding to the function

You can access the help file index through the File\Help from the Menu bar:




## SAVE ANALYSIS / TEMPLATE

This function saves the current analysis. The analysis file will contain the results, the image and all the parameters defined to obtain the results.

The analysis could also be saved as a template for automated analysis routines. Template offers the user the ability to automate many of the repetitive tasks associated with analysis and processing. As a result, you can spend more time evaluating and analyzing results, and less time manipulating set-ups, variables and other settings.

The template automates a task or set of tasks that you perform repeatedly or on a regular basis. It stores all the analysis commands and parameters of an analysis. You can run these parameters with another image whenever you need to perform a new analysis based on the same parameters.

The benefits of the template file are as follows:

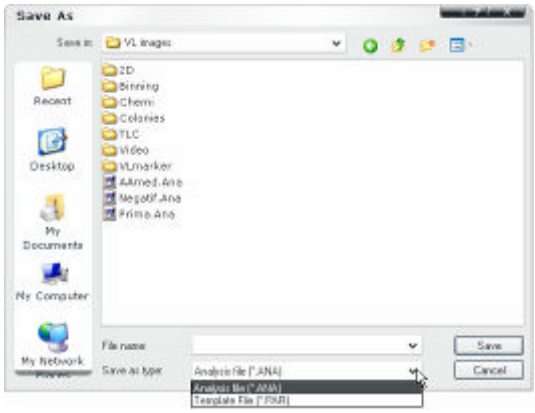
⇨ Time saving

⇨ Reproduction of image analysis parameters

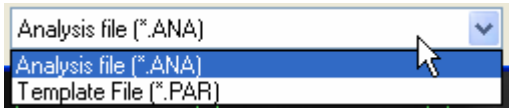
⇨ Templates are modifiable, allowing the user to maintain an original template while modifying it for a slightly different result, with minimal effort

1. Click on the “Save analysis/ Template” button:


2. A pop-up window displays the following menu:

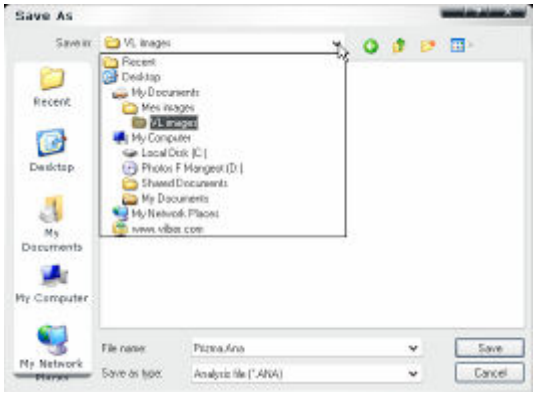



3. Select analysis file or template file:

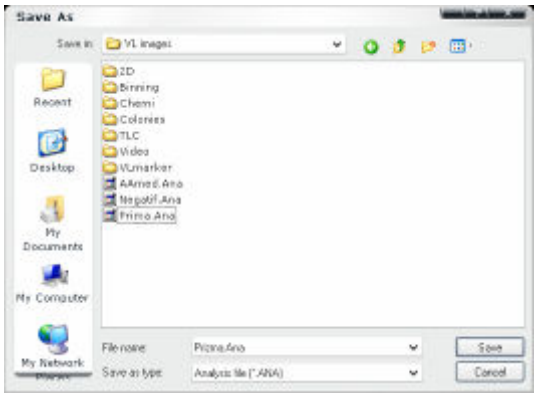



Note: the software proposes Analysis file by default

4. Browse to specify the file directory




5. Enter the desired file name and validate




6. Click on the Save button to create the file.

Note: see “Access to the analysis module” chapter for template or analysis file loading

## ➔ B – Band detection

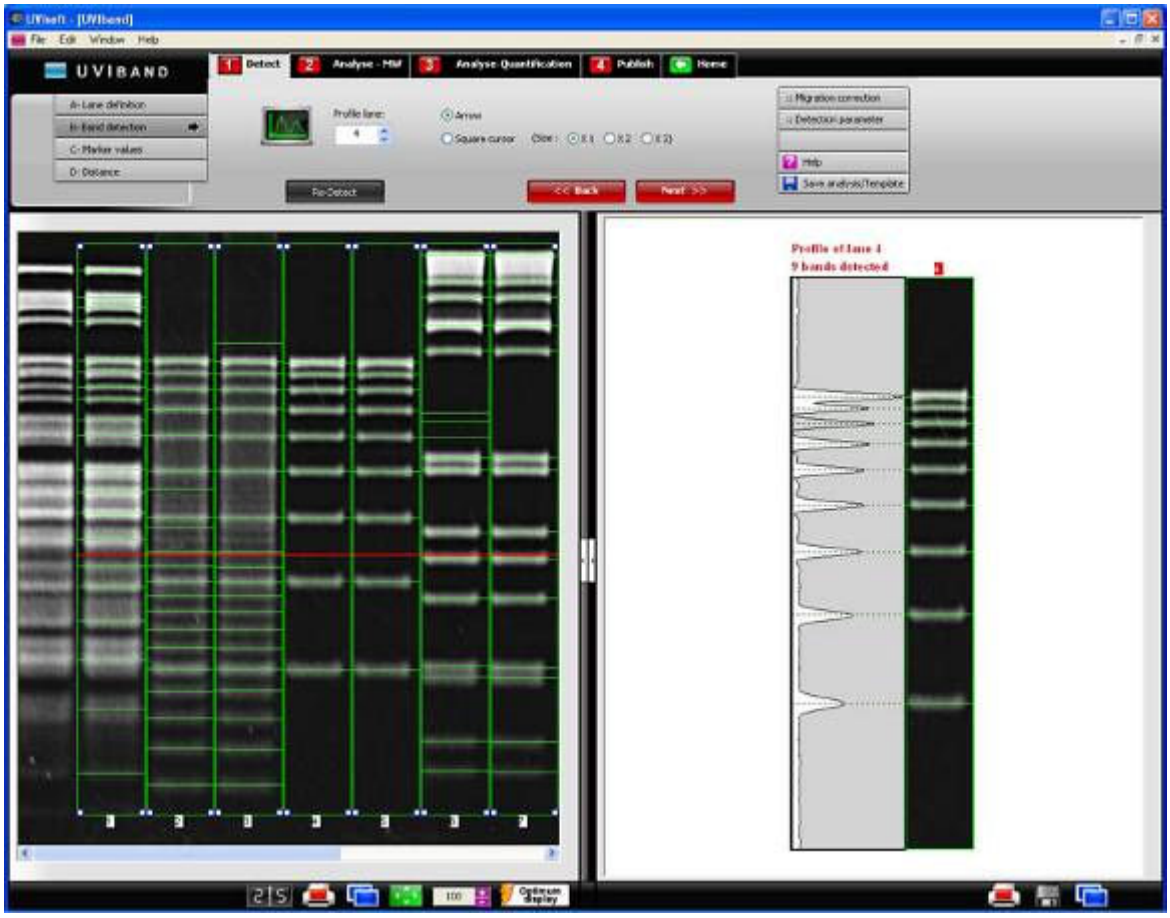
1

The band detection process follows the lane definition:

The band detection process automatically identifies all the bands for the defined lanes. You can also manually mark the bands on the image or on the lane's profile. All bands will be automatically detected when you first access the band detection process, based on default parameters.

The bands are marked by green lines, overlaid on the gel image.

Note: you can either access the lane definition menu by clicking on the next button of the lane definition or directly on the band detection tab.

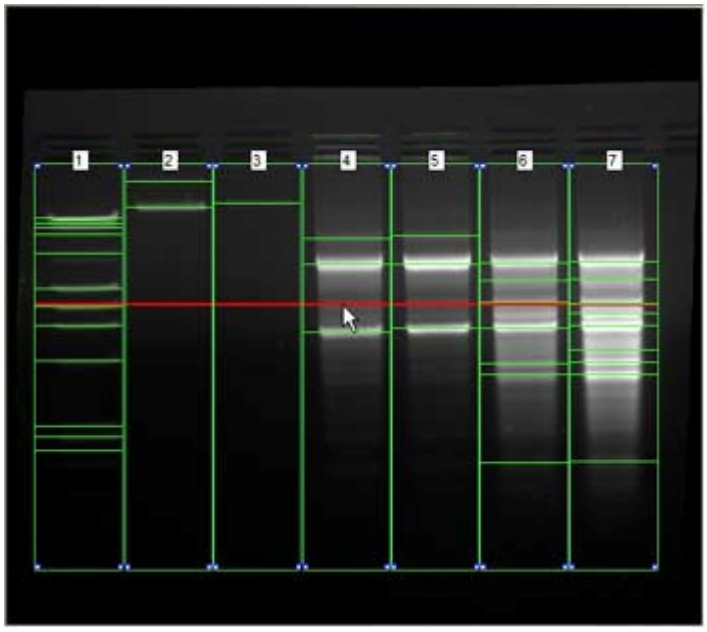



The dashboard details the lane definition parameters:


⇨ Profile lane
⇨ Arrow or square cursor detection

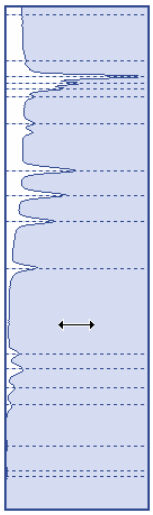
**BAND DETECTION ON THE IMAGE**

You can add or remove bands by clicking directly on the image. Place the cursor at the chosen location and click. The band is immediately added or removed. The red line allows you to check band alignment between lanes.



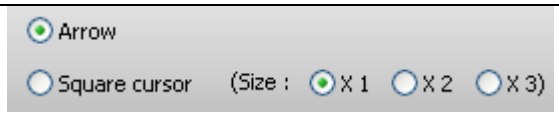

**BAND DETECTION ON THE PROFILE**

The profile is calculated based on the average intensity of each row of pixels across the specified width of the lane. A lane profile provides a visualisation of the intensity of the bands. Bands are represented by peaks.



You can add or remove bands by clicking directly on the profile. To proceed, select your profile lane number:


Select the cursor type:




⇨ The linear cursor has the shape of an arrow ( ⟷ )
⇨ The rectangular cursor has the shape of a square ( □ )

Place the cursor at the chosen profile location and click. The detection line is automatically added or removed.

Note: For arrow cursor, the band is added at the cursor position.
For rectangular cursor, the band is added at the highest position within cursor bounds.

**RE-DETECT**

You can re-detect the bands by clicking the “Re-detect” button. The detection is based on the default parameters.


**NEXT**

The “Next” button validates your parameter and opens the following analysis step.

| | | |
|---|---|---|
| B- Band detection | Next >> | C – Marker values |

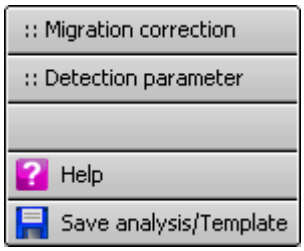
**BACK**

The “Back” button validates your parameter and opens the following analysis step.

| | | |
|---|---|---|
| B- Band detection | << Back | A - Lane definition |

**OPTION FOLDER**

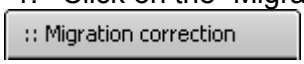
The option folder gathers the following functions:
⇨ Migration correction
⇨ Detection parameter
⇨ Help
⇨ Save the analysis or the template



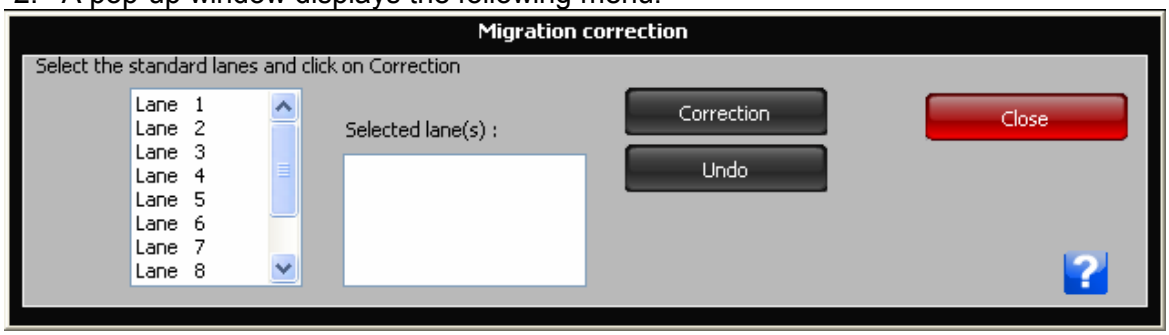

**MIGRATION CORRECTION**

This function corrects the migration distortions by calibrating the image based on the bands of several marker lanes. The band position correction is obtained by realigning lines between each marker’s bands by interpolation.

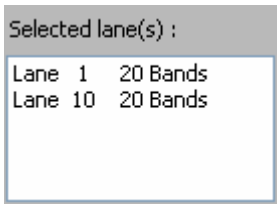
1. Click on the “Migration direction” button.




2. A pop-up window displays the following menu:




Select the marker’s lane:




The menu displays the lane numbers and verifies the number of detected bands is the same for each selected lane.

Note: no realignment is possible if the number of bands between the lanes is different.
Note: you can add or remove detected bands by clicking directly on the image.

3. Click on the “Correction” button. The bands are automatically realigned.

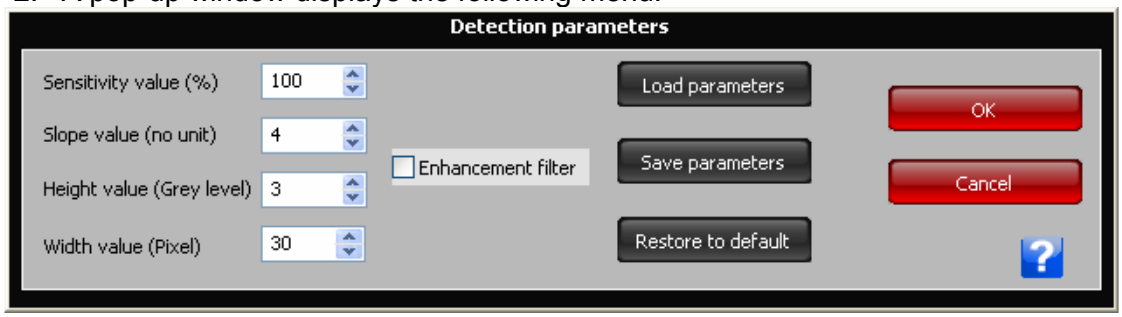

Note: You can undo the migration correction by clicking the “Undo” button.
Note: You need to close the pop-up window by clicking on the “Close” button.

**DETECTION PARAMETER**

You can modify the sensitivity parameters to optimise the band detection. This is particularly useful for instance in the case of several bands not taken into account or if or too much background noise is detected.

1. Click on the “Detection parameter” button.


2. A pop-up window displays the following menu:



- Sensitivity

⇨ Given in %
⇨ It allows distinction between a band in comparison with its surrounding background
⇨ Advantage: an irregular background is taken into account for the band detection

- Slope

⇨ No unit
⇨ The higher the value, the sharper the peaks must be to be detected
⇨ Advantage: a peak can be distinguished from a plateau

- Height:

⇨ In grey level
⇨ It allows detection of a band according to its minimum relative height
⇨ Advantage: a shoulder in a peak can be detected as a second peak

- Width

⇨ In pixel
⇨ Advantage: avoids detection of artefacts

- Filter function

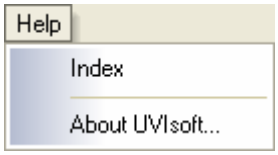
⇨ This function can be activated to avoid detection of superfluous bands due to heavy background.

Note: You can save your detection parameter by clicking on the “Save parameters” button.
Note: You need to close the pop-up window by clicking on the “OK” button.

**HELP MENU**


Click on the “Help” button. You automatically access the user manual at the chapter corresponding to the function

You can access the help file index through the File\Help from the Menu bar:




**SAVE ANALYSIS / TEMPLATE**

This function saves the current analysis. The analysis file will contain the results, the image and all the parameters defined to obtain the results.

The analysis could also be saved as a template for automated analysis routines. Template offers the user the ability to automate many of the repetitive tasks associated with analysis and processing. As a result, you can spend more time evaluating and analysing results, and less time manipulating set-ups, variables and other settings.

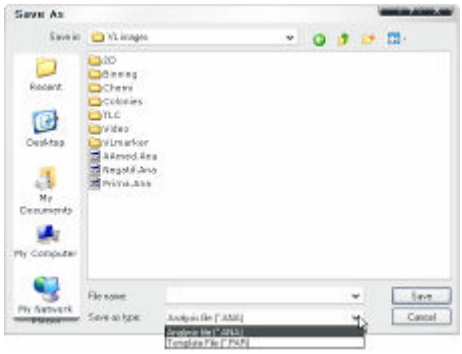
The template automates a task or set of tasks that you perform repeatedly or on a regular basis. It stores all the analysis commands and parameters of an analysis. You can run these parameters with another image whenever you need to perform a new analysis based on the same parameters.

The benefits of the template file are as follows:

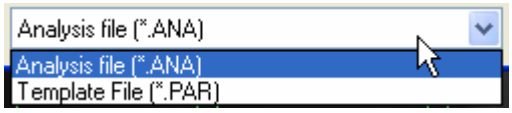
⇨ Time saving
⇨ Reproduction of image analysis parameters
⇨ Templates are modifiable, allowing the user to maintain an original template while modifying it for a slightly different result, with minimal effort

1. Click on the “Save analysis/ Template” button:


2. A pop-up window displays the following menu:

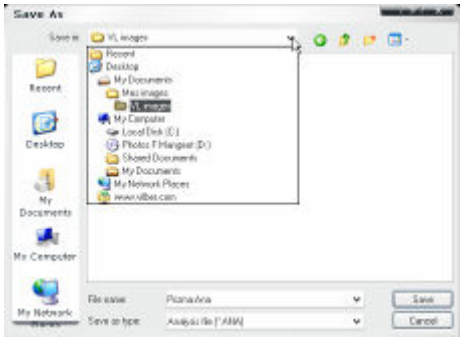


3. Select analysis file or template file:

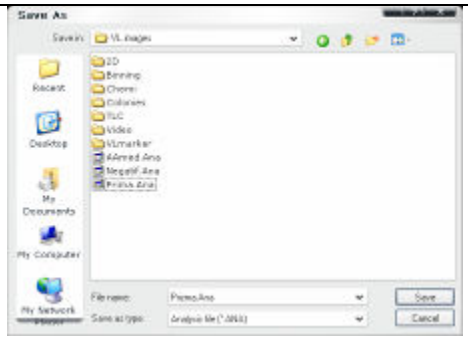



Note: the software proposes Analysis file by default

4. Browse to specify the file directory



5. Enter the desired file name and validate




6. Click on the Save button to create the file.

Note: see “Access to the analysis module” chapter for template or analysis file loading

## ➔ C – Marker values

1

The marker values process follows the band detection. This function allows the assignment of the molecular weight marker's values to the bands of the marker lane.

Note: you can either access marker value menu by clicking on the next button of the band detection or directly on marker value tab.

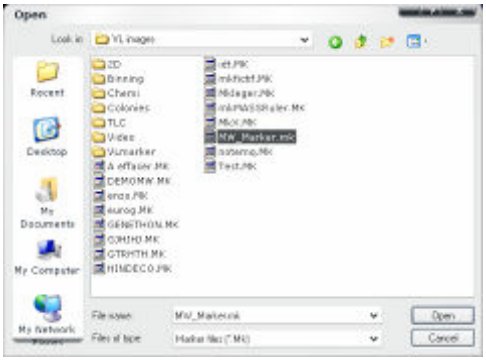

The dashboard details the marker values parameters:


- ⇨ Load a marker
- ⇨ Create a marker
- ⇨ Select the marker's lane
- ⇨ Assign automatically a marker
- ⇨ Assign manually a marker

**LOAD A MARKER**

1. Click on the "Load a marker" button to open a marker value file.

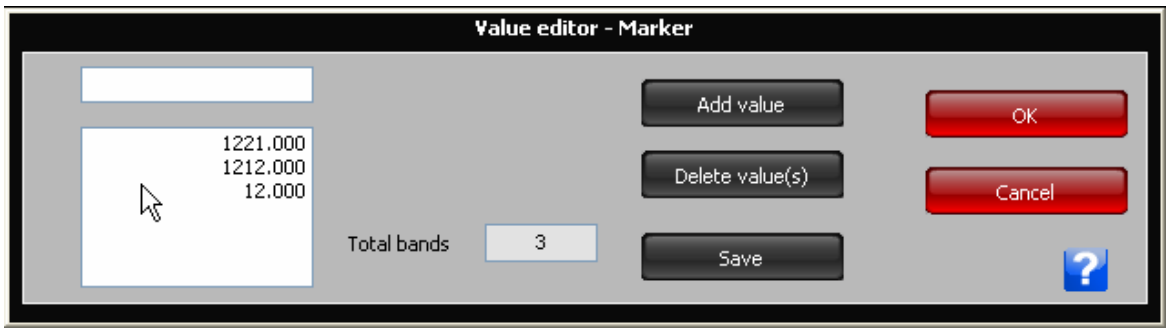
A pop-up window displays the following menu:




⇨ Browse to specify the marker directory
⇨ Double click on the file name you want to load

## CREATE A MARKER

1. Click on the “Create a marker” button to create marker’s values. 

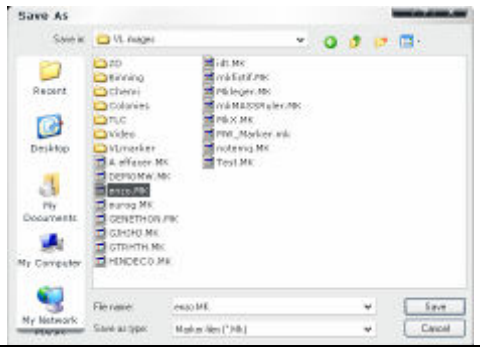




Type your values, band to band, in a descending order. The OK button validates your data.

Note: A minimum of four values is necessary to validate the data.
Note: if an automatic calculation with immediate application of the standard values is carried out, it is not necessary to enter all the bands given by the manufacturer's specifications, but only those which are commonly found on the lanes of the gel.

You can save your molecular weights data and create your own marker library; To proceed, click on the “Save “ button:

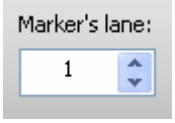

A pop-up window displays the following menu:



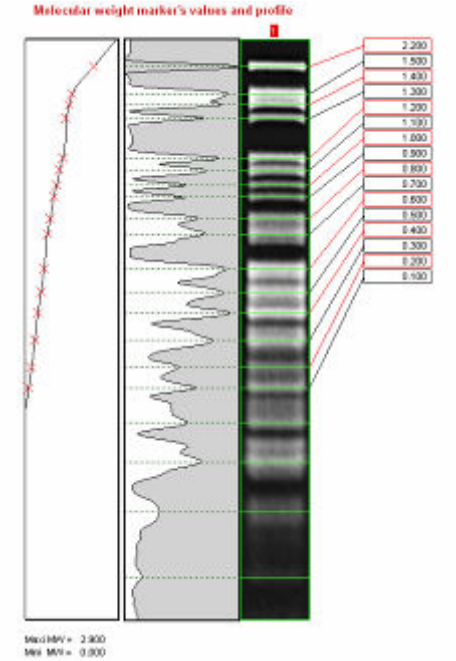
⇨ Browse to specify the directory
⇨ Type the file name and click on Save.

**SELECT THE MARKER'S LANE**

Select the lane corresponding to the molecular weight marker:




The migration curve is automatically displayed next to the lane's profile:

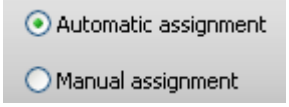



The migration curve allows to check the detection of value application errors, distortion errors, bad separation between the bands, or the quality of the standard itself.

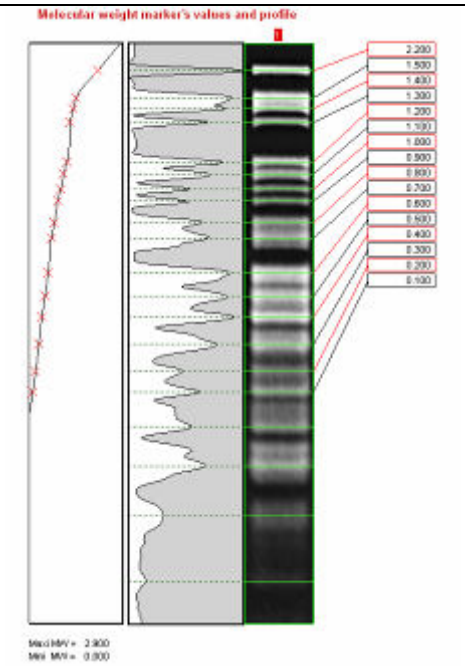
Note: To delete the wrong data, you can either place the cursor arrow on the wrong value itself and click on it, or go for the manual assignment.
Note: The displayed migration curve is of the cubic spline type and must then include a minimum of 4 values.
Note: The minimum MW indicates the minimum molecular weight, which can be calculated, based on the marker's value assignment.
Note: The maximum MW indicates the maximum molecular weight, which can be calculated, based on the marker's value assignment.

**ASSIGN THE MARKER VALUES TO THE BAND**

Assign manually the marker values to the lane by selecting the appropriate option:




For manual assignment, click first on the molecular weight's value to be assigned. The value is highlighted in red. Then, click on the corresponding lane. The value is assigned to the lane:




**NEXT**

The “Next” button validates your parameter and opens the following analysis step.

| | | |
|---|---|---|
| C – Marker values | Next >> | 2- Analyse - MW<br>A- Molecular weight |

**BACK**

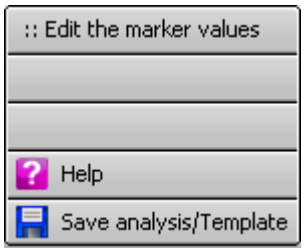
The “Back” button validates your parameter and opens the following analysis step.

| | | |
|---|---|---|
| C – Marker values | << Back | B- Band detection |

**OPTION FOLDER**

The option folder gathers the following functions:

- ⇨ Edit the marker values
- ⇨ Help
- ⇨ Save the analysis or the template



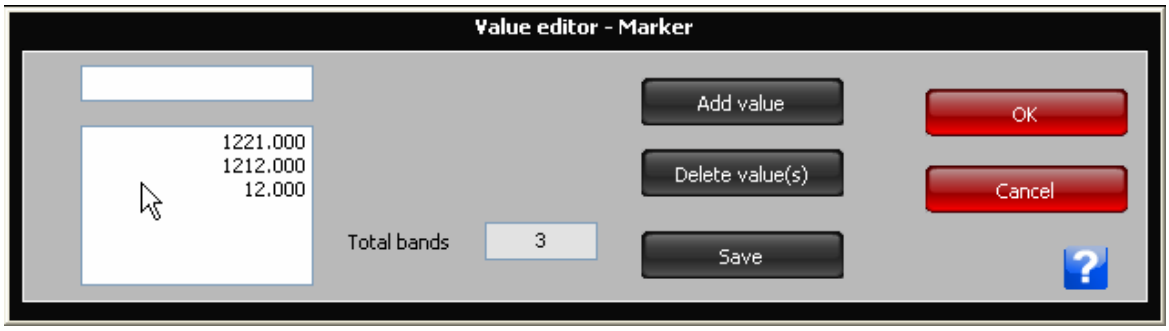

**EDIT THE MARKER VALUES**

This function allows the treatment of electrophoretic problems such as "smiling" or bent separation front. This correction is based on and required the definition of internal references in each lane for the recalculation of the electrophoresis distance.

1. Click on the “Edit the marker values” button.

:: Edit the marker values

2. A pop-up window displays the following menu on which you can modify the marker values:



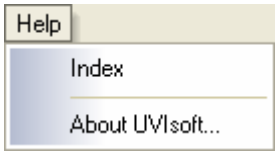

You can add, remove, and save your marker values;

**HELP MENU**

Click on the "Help" button. You automatically access the user manual at the chapter corresponding to the function

Help

You can access the help file index through the File\Help from the Menu bar:




**SAVE ANALYSIS / TEMPLATE**

This function saves the current analysis. The analysis file will contain the results, the image and all the parameters defined to obtain the results.

The analysis could also be saved as a template for automated analysis routines. Template offers the user the ability to automate many of the repetitive tasks associated with analysis and processing. As a result, you can spend more time evaluating and analysing results, and less time manipulating set-ups, variables and other settings.

The template automates a task or set of tasks that you perform repeatedly or on a regular basis. It stores all the analysis commands and parameters of an analysis. You can run these parameters with another image whenever you need to perform a new analysis based on the same parameters.

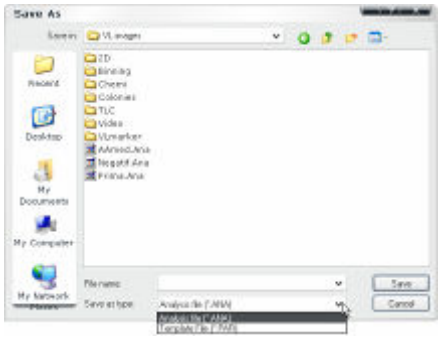
The benefits of the template file are as follows:

⇨ Time savings
⇨ Reproduction of image analysis parameters
⇨ Templates are modifiable, allowing the user to maintain an original template while modifying it for a slightly different result, with minimal effort

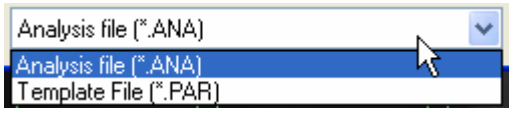
1. Click on the "Save analysis/ Template" button:

Save analysis/Template

2. A pop-up window displays the following menu:

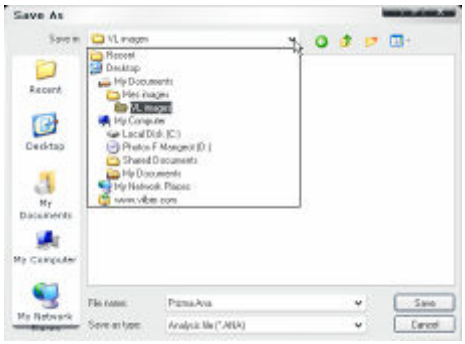


3. Select analysis file or template file:

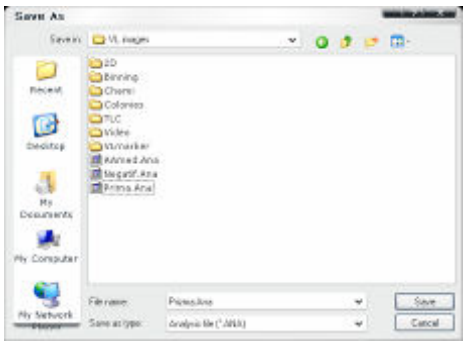



Note: the software proposes Analysis file by default

4. Browse to specify the file directory



5. Enter the desired file name and validate



6. Click on the Save button to create the file.

Note: see “Access to the analysis module” chapter for template or analysis file loading

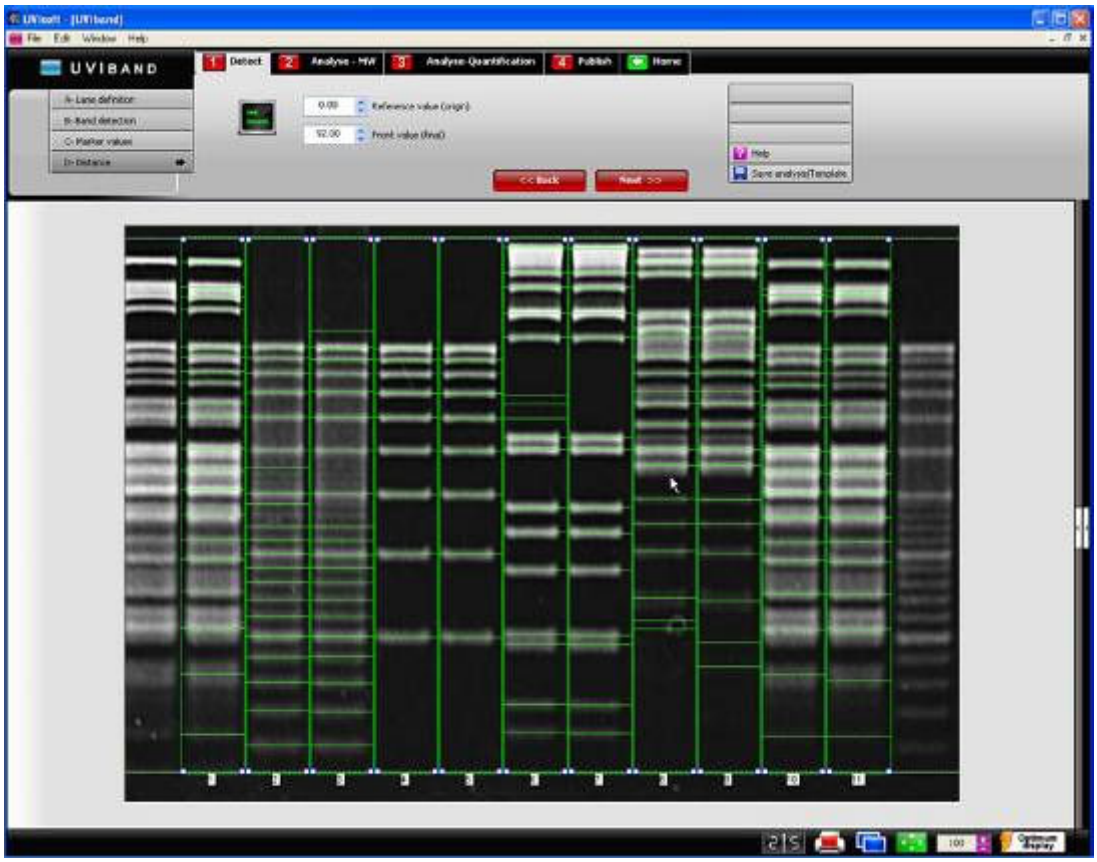
## ➔ D – Distance

1

The distance calculation process follows the band detection:

Note: you can access the distance menu by clicking directly on the distance tab.

This function allows the assignment of RF values to the detected bands. To do so, an origin line and a front line must be defined. Usually the origin is set to value 0 and the front to 1, but the UVIband Advanced allows you to set your own values for origin and front lines

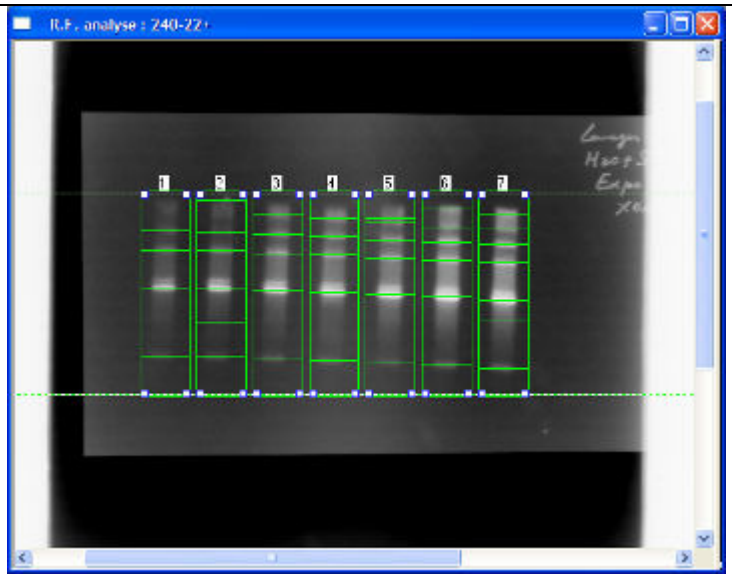



The dashboard details the marker values parameters:


⇨ Define the reference value
⇨ Define the front value

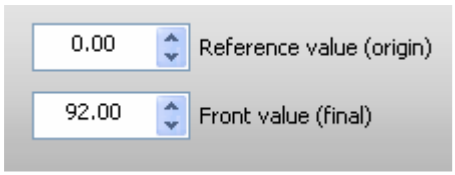
**DEFINE THE REFERENCE AND THE FRONT VALUES**

An origin and a front line are displayed on the image:

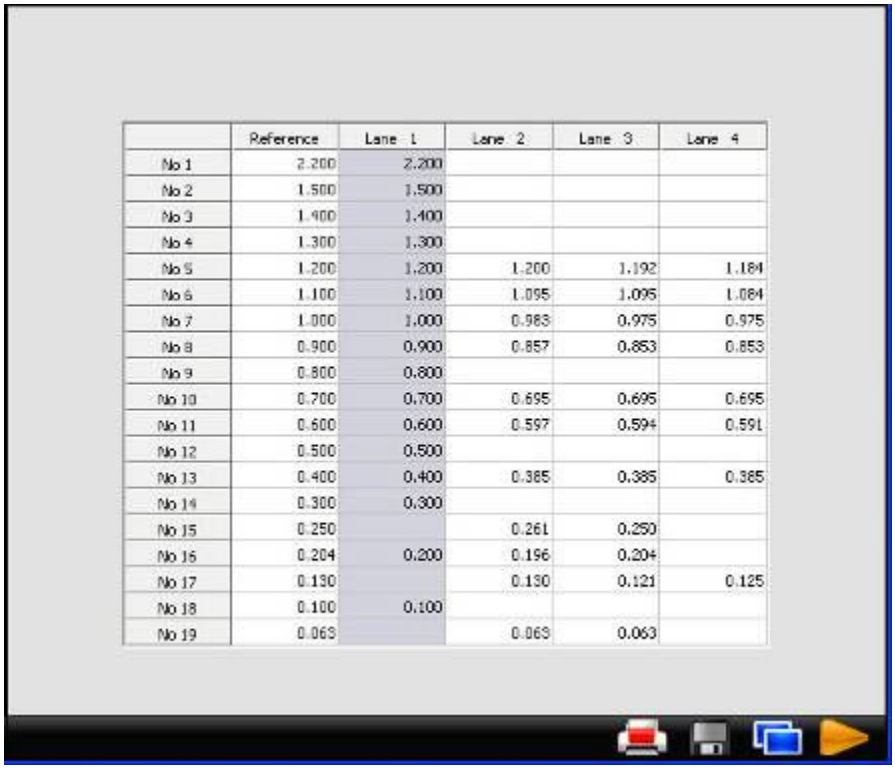



⇨ Click on the first line, keep pressed the left mouse button, and move it to the location for the origin line. Then, release the button.
⇨ Click on the second line and move it to the location for the migration front. Then, release the button. The R.F. values are assigned to the bands.

Select the value for the origin and the value the end.




Then, validate by clicking on Next. The distance table is automatically displayed:



| | Reference | Lane 1 | Lane 2 | Lane 3 | Lane 4 |
|---|---|---|---|---|---|
| No 1 | 2.200 | 2.200 | | | |
| No 2 | 1.500 | 1.500 | | | |
| No 3 | 1.400 | 1.400 | | | |
| No 4 | 1.300 | 1.300 | | | |
| No 5 | 1.200 | 1.200 | 1.200 | 1.192 | 1.184 |
| No 6 | 1.100 | 1.100 | 1.095 | 1.095 | 1.084 |
| No 7 | 1.000 | 1.000 | 0.983 | 0.975 | 0.975 |
| No 8 | 0.900 | 0.900 | 0.857 | 0.853 | 0.853 |
| No 9 | 0.800 | 0.800 | | | |
| No 10 | 0.700 | 0.700 | 0.695 | 0.695 | 0.695 |
| No 11 | 0.600 | 0.600 | 0.597 | 0.594 | 0.591 |
| No 12 | 0.500 | 0.500 | | | |
| No 13 | 0.400 | 0.400 | 0.385 | 0.385 | 0.385 |
| No 14 | 0.300 | 0.300 | | | |
| No 15 | 0.250 | | 0.261 | 0.250 | |
| No 16 | 0.204 | 0.200 | 0.196 | 0.204 | |
| No 17 | 0.130 | | 0.130 | 0.121 | 0.125 |
| No 18 | 0.100 | 0.100 | | | |
| No 19 | 0.063 | | 0.063 | 0.063 | |

## NEXT

The “Next” button validates your parameter and opens the following analysis step.

| | | |
|---|---|---|
| D – Distance |  | 2- Analyse - MW<br>A- Molecular weight |



## BACK

The “Back” button validates your parameter and opens the following analysis step.

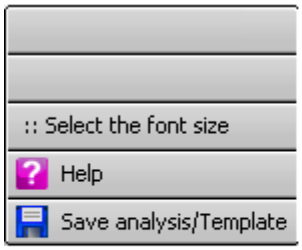
| | | |
|---|---|---|
| D – Distance |  | B- Band detection |



## OPTION FOLDER

The option folder gathers the following functions:

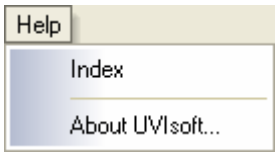
- ⇨ Help
- ⇨ Save the analysis or the template




## HELP MENU

Click on the “Help” button. You automatically access the user manual at the chapter corresponding to the function.


You can access the help file index through the File\Help from the Menu bar:




## SAVE ANALYSIS / TEMPLATE

This function saves the current analysis. The analysis file will contain the results, the image and all the parameters defined to obtain the results.

The analysis could also be saved as a template for automated analysis routines. Template offers the user the ability to automate many of the repetitive tasks associated with analysis and processing. As a result, you can spend more time evaluating and analysing results, and less time manipulating set-ups, variables and other settings.

The template automates a task or set of tasks that you perform repeatedly or on a

regular basis. It stores all the analysis commands and parameters of an analysis. You can run these parameters with another image whenever you need to perform a new analysis based on the same parameters.

The benefits of the template file are as follows:

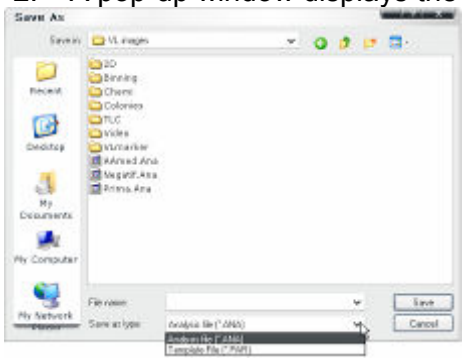
⇨ Time savings

⇨ Reproduction of image analysis parameters

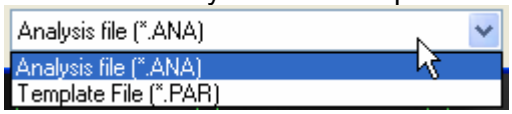
⇨ Templates are modifiable, allowing the user to maintain an original template while modifying it for a slightly different result, with minimal effort

1. Click on the “Save analysis/ Template” button:


2. A pop-up window displays the following menu:

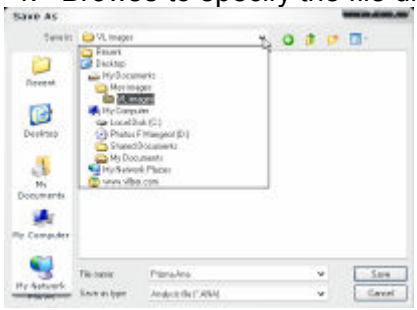


3. Select analysis file or template file:

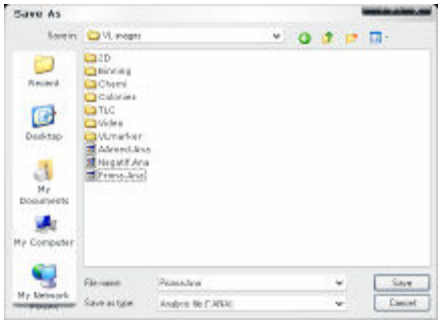



Note: the software proposes Analysis file by default

4. Browse to specify the file directory



5. Enter the desired file name and validate



| | |
|---|---|
| | 6. Click on the Save button to create the file.<br><br>Note: see “Access to the analysis module” chapter for template or analysis file loading |

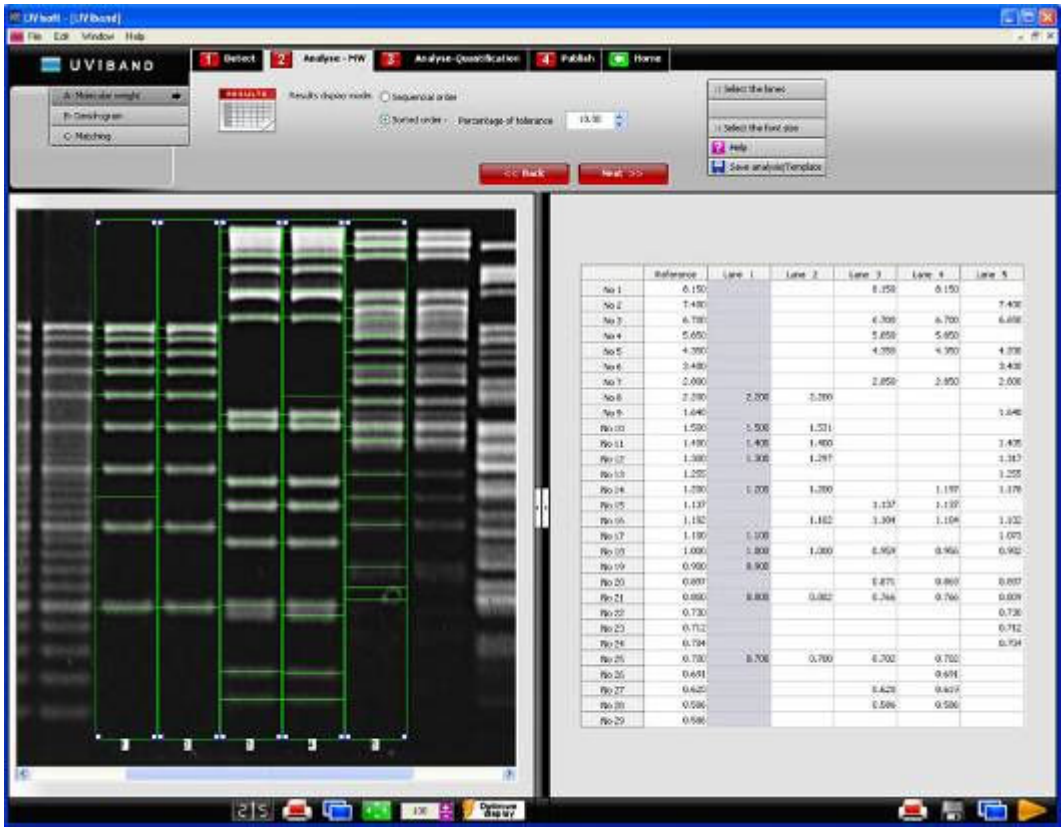
# 2- Analyse – Molecular weight

## ➔ A – Molecular weight

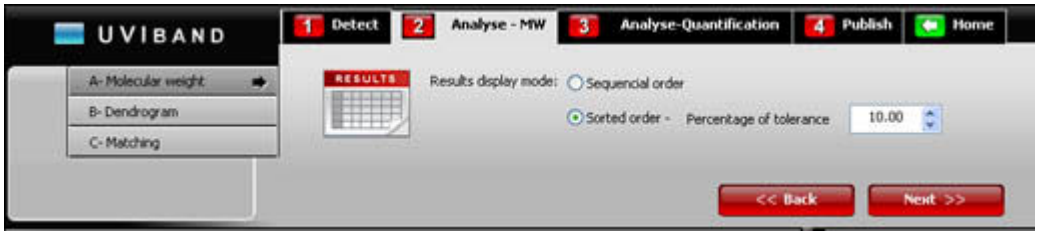
2

The molecular weight results process follows the marker's value assignment.

Note: you can either access the molecular weight results by clicking on the next button of the marker's value assignment or directly by clicking on the Molecular weight tab of the 2-Analyse-MW folder.




The dashboard details the molecular weight results parameters:



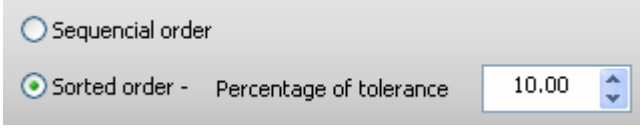

**RESULTS DISPLAY MODE**

There are 2 ways to display the results:

⇨ The sequential order: the calculated molecular weights for each detected band

are displayed in order of appearance

⇨ The sorted order: the results are sorted in order to align on the same line of the array identical values according to a percentage of tolerance. To do so, a reference list of calculated values is created (“Reference” column) and the calculated values are compared to this reference list.




You can defined a percentage of tolerance in order to merge similar molecular weight values on a single line:


The array of results is automatically modified.

**NEXT**

The “Next” button validates your parameter and opens the following analysis step.

| | | |
|---|---|---|
| 2- Analyse - MW<br>A-Molecular weight |  | 3- Analyse - Quantification<br>A- Background subtraction |

**BACK**

The “Back” button validates your parameter and opens the following analysis step.

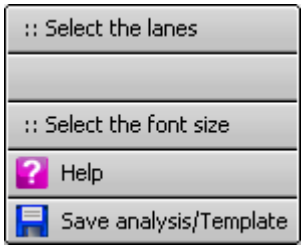
| | | |
|---|---|---|
| 2- Analyse – MW<br>A-Molecular weight |  | 1- Detect<br>C- Marker values |

**OPTION FOLDER**

The option folder gathers the following functions:

⇨ Help

⇨ Save the analysis or the template




**SELECT THE LANES**

You can add or remove the lanes to be displayed in the result table.

1. Click on the “Select the lanes” button.

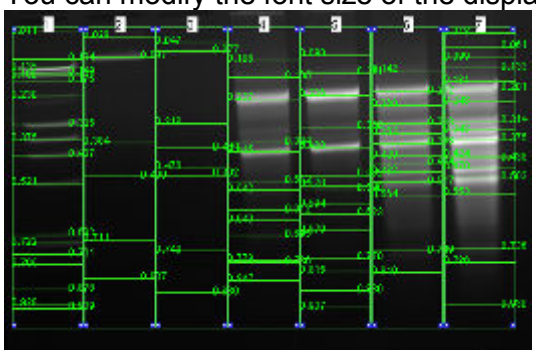

2. A pop-up window displays the following menu on which you can modify the list

of lanes to be used:

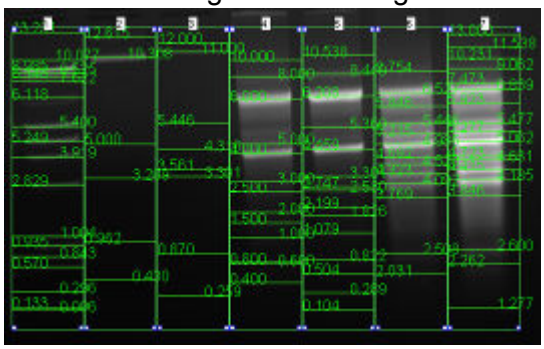

You can add or remove the lanes to be displayed in the result table by selecting or unselecting the lanes of the list.

**SELECT THE FONT SIZE**

You can modify the font size of the displayed molecular weight on the image:



**Small font**



**Large font**

1. Click on the “Select the lanes” button.


2. A pop-up window displays the following menu on which you can modify the font size:

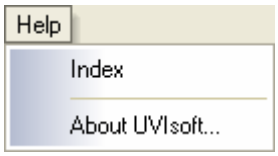

Select the appropriate font size.

**HELP MENU**

Click on the “Help” button.


You automatically access the user manual at the chapter corresponding to the function.

You can access the help file index through the File\Help from the Menu bar:




**SAVE ANALYSIS / TEMPLATE**

This function saves the current analysis. The analysis file will contain the results, the image and all the parameters defined to obtain the results.

The analysis could also be saved as a template for automated analysis routines. Template offers the user the ability to automate many of the repetitive tasks associated with analysis and processing. As a result, you can spend more time evaluating and analysing results, and less time manipulating set-ups, variables and other settings.

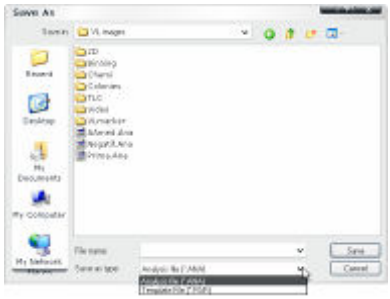
The template automates a task or set of tasks that you perform repeatedly or on a regular basis. It stores all the analysis commands and parameters of an analysis. You can run these parameters with another image whenever you need to perform a new analysis based on the same parameters.

The benefits of the template file are as follows:

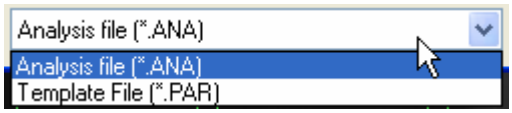
⇨ Time saving
⇨ Reproduction of image analysis parameters
⇨ Templates are modifiable, allowing the user to maintain an original template while modifying it for a slightly different result, with minimal effort

1. Click on the “Save analysis/ Template” button:


2. A pop-up window displays the following menu:

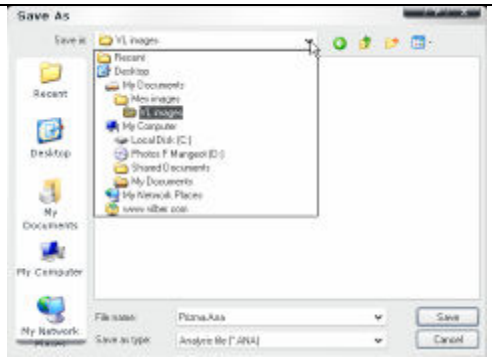


3. Select analysis file or template file:

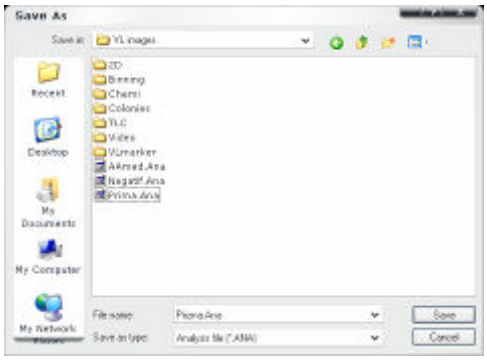



Note: the software proposes Analysis file by default

4. Browse to specify the file directory




5. Enter the desired file name and validate




6. Click on the Save button to create the file.

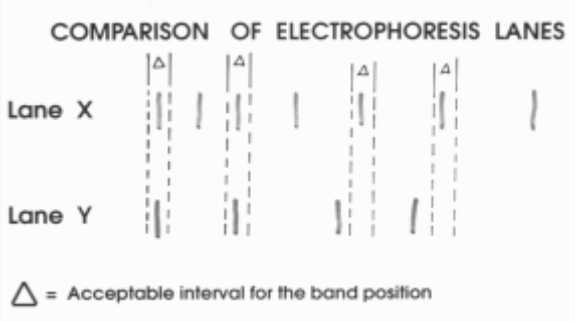
Note: see “Access to the analysis module” chapter for template or analysis file loading

## ➔ B – Dendrogram

2

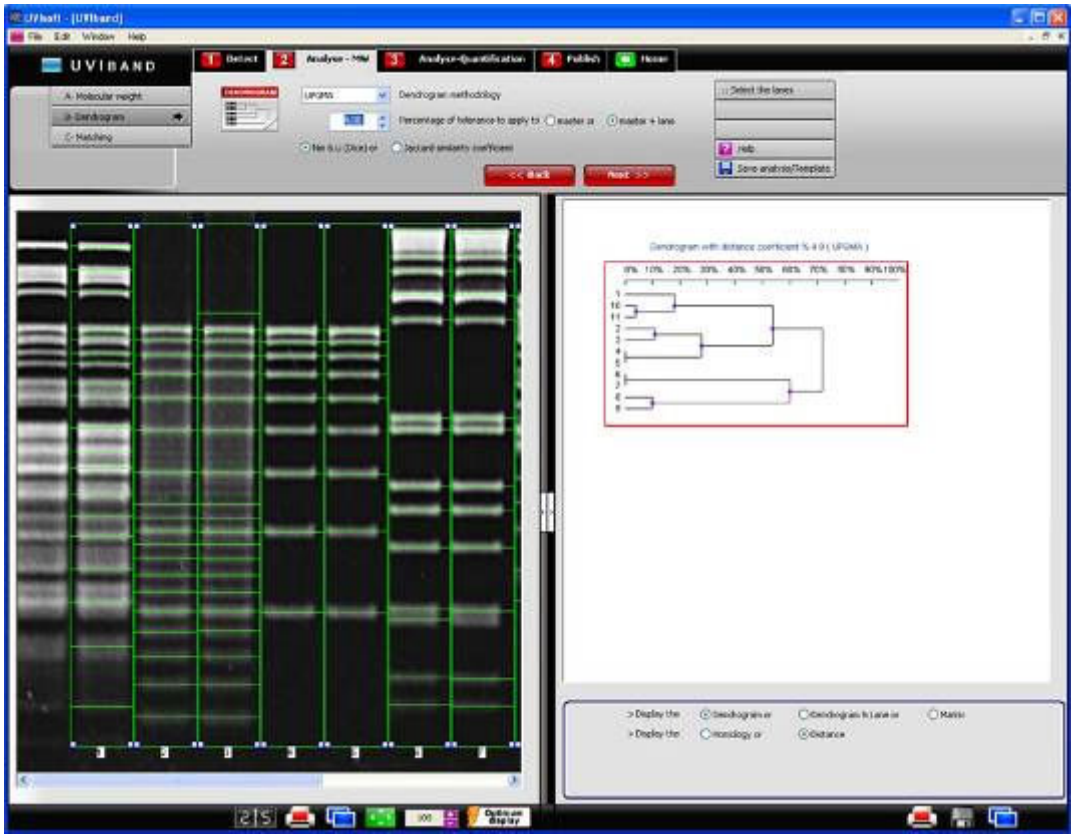
The dendrogram process follows the molecular weight results.

This function allows comparison of bands between lanes of a single image using specific band matching algorithms:




Dendrogram is a schematic representation of sample similarity. The dendrogram tree gathers cluster on a branch length which size varies according to the similarity.

Note: you can only access the Dendrogram menu by clicking on the Dendrogram tab of the 2-Analyse-MW folder.




The dashboard details the dendrogram parameters:


⇨ Select a dendrogram methodology
⇨ Select a similarity coefficient
⇨ Select a percentage of tolerance

**DENDROGRAM METHODOLOGY**

The dendrogram is a way to visualise groups of homology. UVIband Advanced supports several methods of dendrogram calculation:

⇨ Unweighted Pair Group Match Average
⇨ Single Linkage
⇨ Complete Linkage
⇨ Unweigthed Average Linkage
⇨ Average Linkage
⇨ Centroid
⇨ Median
⇨ Ward

For single, complete, unweighted average, average linkage and centroid, median and Ward methods the general formula is as follows:

$$d(R, P+Q) = A*d(R, P) + B*d(R, Q) + E*d(P, Q) + G*|d(R, P) - d(R, Q)|$$

Where:
d(x, y) : distance between 2 lanes x and y ( distance = 1-Homology)
A, B, E, G: are constants specific to the method used (see array)

| | A | B | E | G |
|---|---|---|---|---|
| Single linkage | 0.5 | 0.5 | 0 | -0.5 |
| Complete linkage | 0.5 | 0.5 | 0 | 0.5 |
| Average linkage (unweighted) | 0.5 | 0.5 | 0 | 0 |
| Average linkage | NP/(NP+NQ) | NQ/(NP+NQ) | 0 | 0 |
| Centroid | NP/(NP+NQ) | NQ/(NP+NQ) | (NP*NQ)/(NP+NQ)² | 0 |
| Median | 0.5 | 0.5 | -0.25 | 0 |
| Ward | (NR+NP)/(NR+NP+NQ) | NR+NP+NQ | NR/(NR+NP+NQ) | 0 |

NR: Number of bands in group R
NP: Number of bands in group P
NQ: Number of bands in group Q

For more details on UPGMA and Nei and Li methods, please refer to the following publications:

Timothy J.Beanland and Christopher J. Howe
« The inference of evolutionary trees from molecular data »
Comp. Biochem. Physiol, Vol. 102B, N°4, pp 643-659 1992

Ute Mackenstedt, Kim Luton, Peter R. Baverstock, Alan M. Johnston
« Phylogenetic relationships of Babesia divergens as determined from comparison of small subunit ribosomal RNA gene sequences »
Molecular and biochemical Parasitology, 68 (1994) 161-165

Masatoshi Nei and Wen-Hsiung Li

« Mathematical model for studying genetic variation in terms of restriction endonucleases »
Proc Natl Acad Sci. USA, Vol 76 N°10, pp5269-5273 October 1979

Masatoshi Nei, J. Clairborne Stephens and Naruya Saiton
« Methods for comparing the standard errors of branching points in an evolutionary tree and their application to molecular data from humans and apes »
Mol Biol Evol. 2(1): 66-85 1985

Click on the dendrogram methodology sliding menu to select the dendrogram calculation method:


**SIMILARITY COEFFICIENT**

Click in the heading Similarity coefficient to select either the Nei and Li (Dice) coefficient or the JACCARD coefficient


⇨ Nei and Li coefficient (also called Dice):

Coefficient: a = 2nxy / (nx + ny)

Where nx and ny are the number of bands in the lane "x" and in the lane "y" respectively, and nxy the number of shared bands between the 2 lanes

⇨ Jaccard coefficient:

Coefficient: b = nxy / (nx + ny - nxy)

**PERCENTAGE OF TOLERANCE**

$\Delta$ is a percentage directly read on the drawn curve of the marker:
⇨ The location of the band is then considered with $\pm \Delta$ around its value in Kb, in RF, or in KDa
⇨ The bigger the coefficient, the higher the number of matching bands and conversely

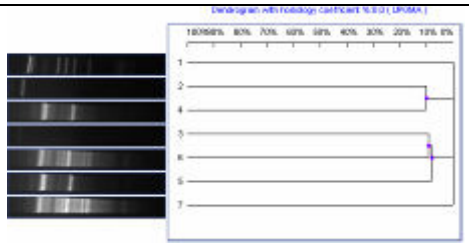

The percentage of tolerance can be applied either:
⇨ Versus the master
⇨ Versus the master and lanes

Versus master:
The coefficient of confidence is only applied to the bands of the reference lane

Versus the master and lanes
The coefficient of confidence is applied to both bands compared

**RESULTS DISPLAY MODE**

Dendrogram can either be displayed by a graphic or a matrix:

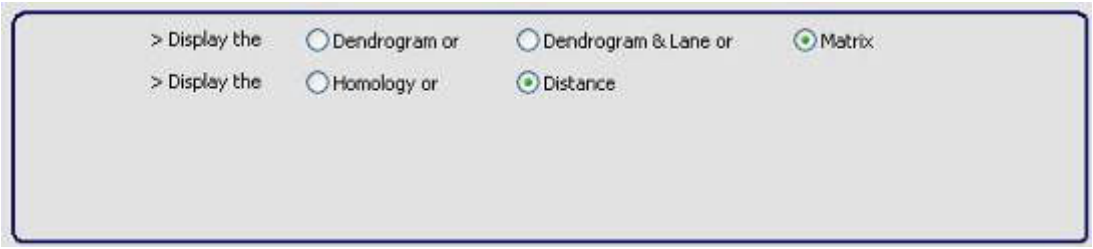


| | Lane 1 | Lane 2 | Lane 3 | Lane 4 | Lane 5 | Lane 6 |
|---|---|---|---|---|---|---|
| Lane 1 | 0 | | | | | |
| Lane 2 | 54 | 0 | | | | |
| Lane 3 | 49 | 11 | 0 | | | |
| Lane 4 | 50 | 28 | 31 | 0 | | |
| Lane 5 | 59 | 28 | 31 | 0 | 0 | |
| Lane 6 | 77 | 92 | 84 | 89 | 89 | 0 |
| Lane 7 | 77 | 92 | 84 | 89 | 89 | 0 |
| Lane 8 | 62 | 67 | 68 | 67 | 67 | 60 |
| Lane 9 | 70 | 67 | 76 | 67 | 67 | 60 |
| Lane 10 | 22 | 49 | 44 | 52 | 52 | 78 |
| Lane 11 | 13 | 54 | 42 | 50 | 50 | 77 |

**Graphical display** **Matrix display**

They can also be expressed in terms of homology or in terms of distance

1. To select your display mode, click on the appropriate selection:




**NEXT**

The “Next” button validates your parameter and opens the following analysis step.

| | | |
|---|---|---|
| B-Dendrogram | Next >> | C- Matching |

**BACK**

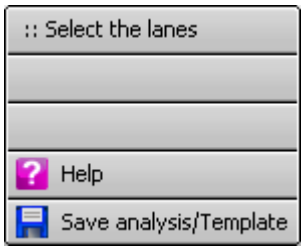
The “Back” button validates your parameter and opens the following analysis step.

| | | |
|---|---|---|
| B-Dendrogram | << Back | A- Molecular weight |

**OPTION FOLDER**

The option folder gathers the following functions:

- ⇨ Select the lanes
- ⇨ Help
- ⇨ Save the analysis or the template




**SELECT THE LANES**

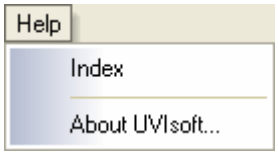
You can add or remove the lanes to be used in dendrogram calculation.

1. Click on the “Select the lanes” button.

2. A pop-up window displays the following menu on which you can modify the list of lanes to be used:


**HELP MENU**


Click on the "Help" button. You automatically access the user manual at the chapter corresponding to the function. You can also access the help file index through the File\Help from the Menu bar:




**SAVE ANALYSIS / TEMPLATE**

This function saves the current analysis. The analysis file will contain the results, the image and all the parameters defined to obtain the results.

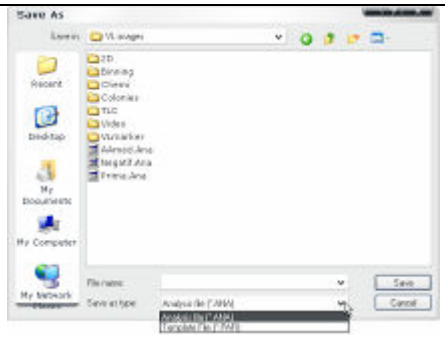
The analysis could also be saved as a template for automated analysis routines. Template offers the user the ability to automate many of the repetitive tasks associated with analysis and processing. As a result, you can spend more time evaluating and analysing results, and less time manipulating set-ups, variables and other settings.

The template automates a task or set of tasks that you perform repeatedly or on a regular basis. It stores all the analysis commands and parameters of an analysis. You can run these parameters with another image whenever you need to perform a new analysis based on the same parameters.

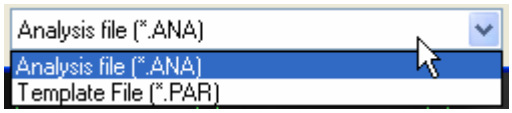
The benefits of the template file are as follows:
⇨ Time saving
⇨ Reproduction of image analysis parameters
⇨ Templates are modifiable, allowing the user to maintain an original template while modifying it for a slightly different result, with minimal effort

1. Click on the "Save analysis/ Template" button:


2. A pop-up window displays the following menu:

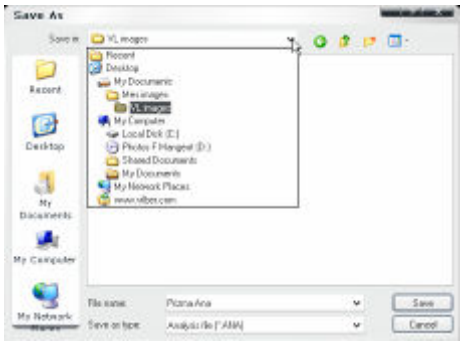


3. Select analysis file or template file:

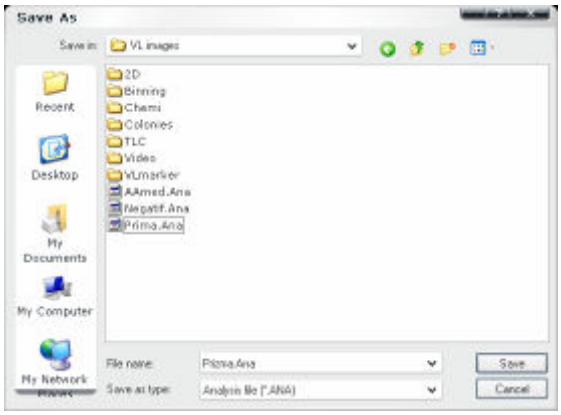



Note: the software proposes “Analysis file” by default

4. Browse to specify the file directory



5. Enter the desired file name and validate



6. Click on the Save button to create the file.

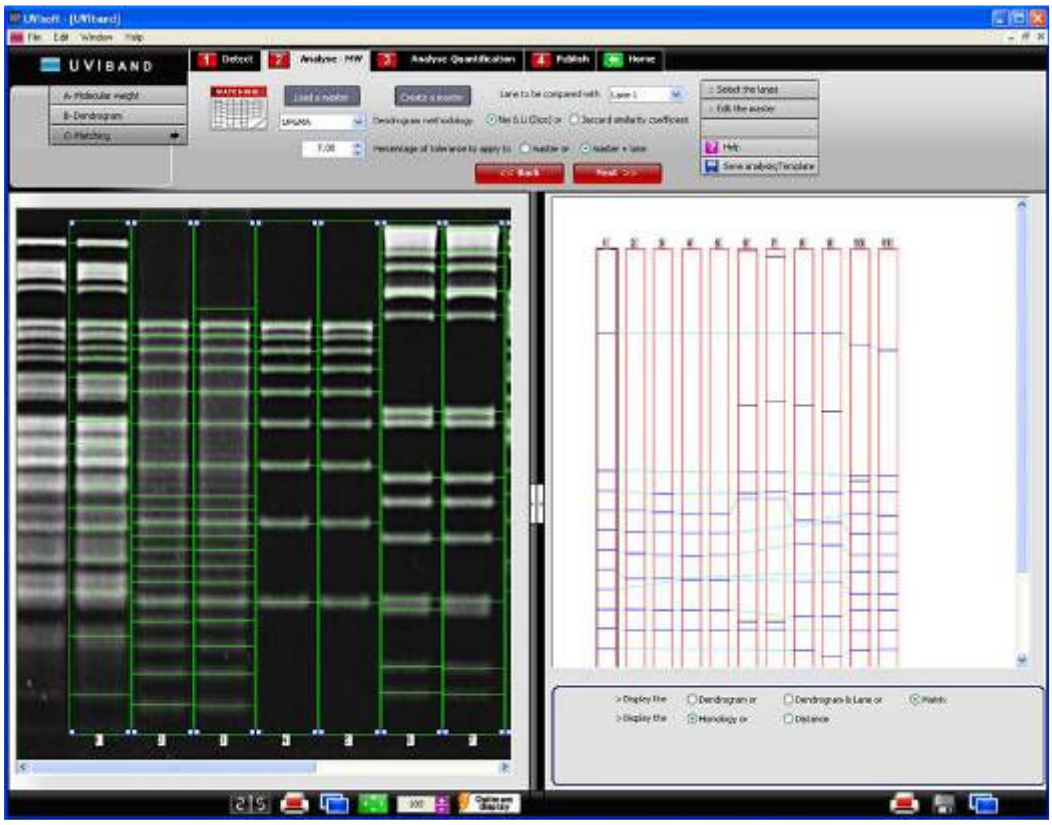
Note: see “Access to the analysis module” chapter for template or analysis file loading

## ➔ C – Matching

2

The matching results process follows the molecular weight results. This function allows to compare a selection of lanes of a gel to a chosen master lane (standard). The master lane can also be one lane of the gel

Note: you can either access matching results by clicking on the next button of the Dendrogram or directly by clicking on the Matching weight tab of the 2-Analyse-MW folder.



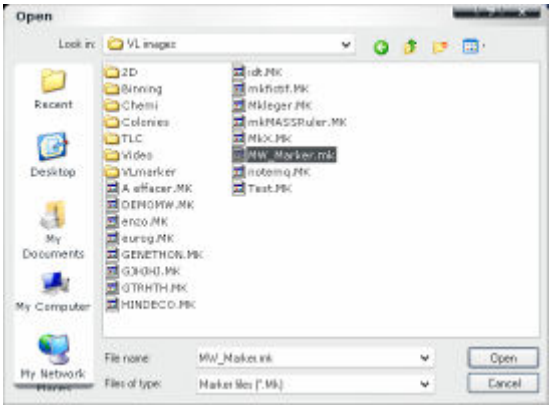

The dashboard details the matching parameters:


- ⇨ Load or create a master
- ⇨ Select the lane to be compared to
- ⇨ Select a dendrogram methodology
- ⇨ Select a similarity coefficient
- ⇨ Select a percentage of tolerance

**LOAD A MASTER**

1. Click on the “Load a master” button to open a master values file.

A pop-up window displays the following menu:




⇨ Browse to specify the marker directory
⇨ Double click on the file name you want to load

**CREATE A MASTER**

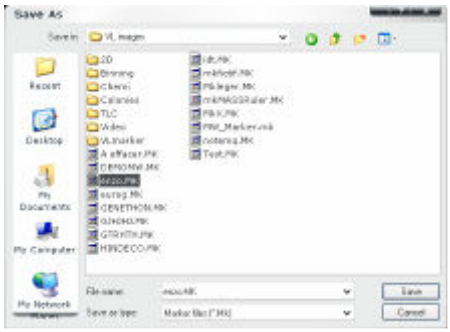
1. Click on the “Create” button to create a new master set of values.



Type your values, band to band, in a descending order. The OK button validates your data.

You can save your molecular weights master data and create your own master’s library; To proceed, click on the “Save “ button:


A pop-up window displays the following menu:



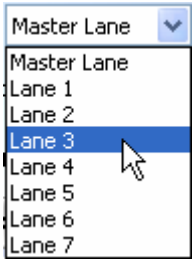

⇨ Browse to specify the directory
⇨ Type the file name and click on Save.

**SELECT THE LANE TO BE COMPARED TO**

Select the lane corresponding to the master.

The master could be:

- ⇨ An external master
- ⇨ Or a lane of the image:


The matching results are automatically displayed.

**DENDROGRAM METHODOLOGY**

The dendrogram is a way to visualise groups of homology. UVIband Advanced supports several methods of dendrogram calculation:

- ⇨ Unweighted Pair Group Match Average
- ⇨ Single Linkage
- ⇨ Complete Linkage
- ⇨ Unweigthed Average Linkage
- ⇨ Average Linkage
- ⇨ Centroid
- ⇨ Median
- ⇨ Ward

For single, complete, unweighted average, average linkages and centroid, median and Ward methods the general formula is as follows:

$$d(R, P+Q) = A*d(R, P) + B*d(R, Q) + E*d(P, Q) + G*|d(R, P) - d(R, Q)|$$

Where:
d(x, y) : distance between 2 lanes x and y ( distance = 1-Homology)
A, B, E, G: are constants specific to the method used (see array)

| | A | B | E | G |
|---|---|---|---|---|
| Single linkage | 0.5 | 0.5 | 0 | -0.5 |
| Complete linkage | 0.5 | 0.5 | 0 | 0.5 |
| Average linkage (unweighted) | 0.5 | 0.5 | 0 | 0 |
| Average linkage | NP/(NP+NQ) | NQ/(NP+NQ) | 0 | 0 |
| Centroid | NP/(NP+NQ) | NQ/(NP+NQ) | $(NP*NQ)/(NP+NQ)^2$ | 0 |
| Median | 0.5 | 0.5 | -0.25 | 0 |
| Ward | (NR+NP)/(NR+NP+NQ) | NR+NP+NQ | NR/(NR+NP+NQ) | 0 |

NR: Number of bands in group R
NP: Number of bands in group P
NQ: Number of bands in group Q

For more details on UPGMA and Nei and Li methods, please refer to the following publications:

Timothy J.Beanland and Christopher J. Howe
« The inference of evolutionary trees from molecular data »
Comp. Biochem. Physiol, Vol. 102B, N°4, pp 643-659 1992

Ute Mackenstedt, Kim Luton, Peter R. Baverstock, Alan M. Johnston
« Phylogenetic relationships of Babesia divergens as determined from comparison of small subunit ribosomal RNA gene sequences »
Molecular and biochemical Parasitology, 68 (1994) 161-165

Masatoshi Nei and Wen-Hsiung Li
« Mathematical model for studying genetic variation in terms of restriction endonucleases »
Proc Natl Acad Sci. USA, Vol 76 N°10, pp5269-5273 October 1979

Masatoshi Nei, J. Clairborne Stephens and Naruya Saiton
« Methods for comparing the standard errors of branching points in an evolutionary tree and their application to molecular data from humans and apes »
Mol Biol Evol. 2(1): 66-85 1985

Click on the dendrogram methodology sliding menu to select the dendrogram calculation method:


**SIMILARITY COEFFICIENT**

Click in the heading Similarity coefficient to select either the Nei and Li (Dice) coefficient or the JACCARD coefficient


⇨ Nei and Li coefficient (also called Dice):
Coefficient: a = 2nxy / (nx + ny)

⇨ Jaccard coefficient:
Coefficient: b = nxy / (nx + ny - nxy)

Where nx and ny are the number of bands in the lane "x" and in the lane "y" respectively, and nxy the number of shared bands between the 2 lanes

**PERCENTAGE OF TOLERANCE**

$\Delta$ is a percentage directly read on the drawn curve of the marker:

⇨ The location of the band is then considered with $\pm \Delta$ around its value in Kb, in RF, or in KDa

⇨ The bigger the coefficient, the higher the number of matching bands and conversely


The percentage of tolerance can be applied either:

⇨ Versus the master

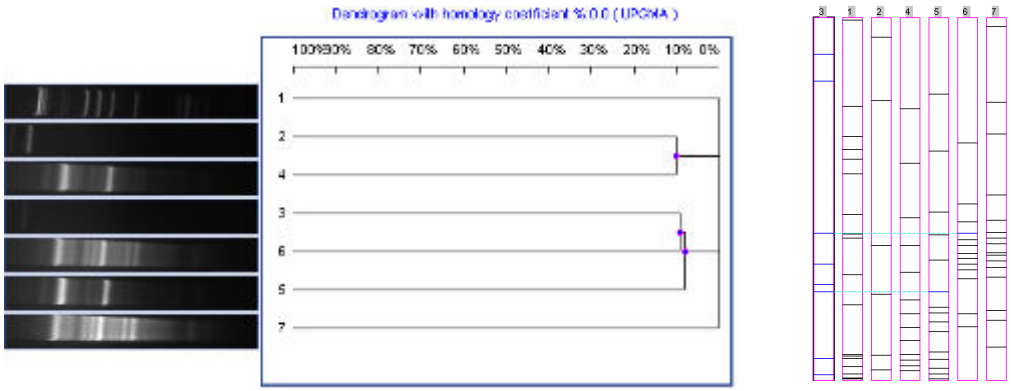
⇨ Versus the master and lanes

Versus master:
The coefficient of confidence is only applied to the bands of the reference lane

Versus the master and lanes
The coefficient of confidence is applied to both bands compared

## RESULTS DISPLAY MODE

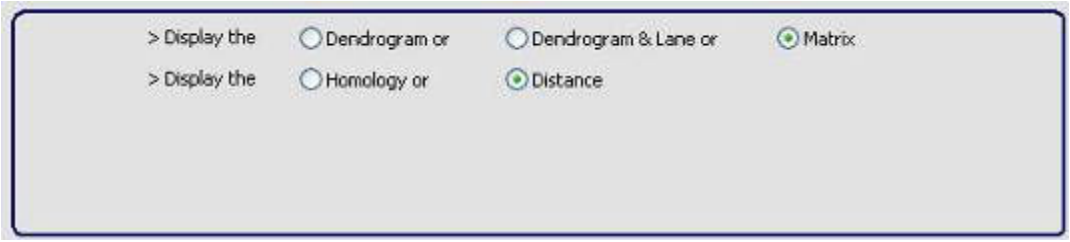
Matching can either be displayed by a graphic or a matrix:




**Graphical display** **Matrix display**

They can also be expressed in terms of homology or in terms of distance

1. To select your display mode, click on the appropriate selection:




## NEXT

The “Next” button validates your parameter and opens the following analysis step.

| | | |
|---|---|---|
| C – Matching | Next >> | 2- Analyse - Quantification<br>A- Background subtraction |

## BACK

The “Back” button validates your parameter and opens the following analysis step.

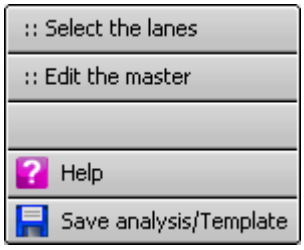
| | | |
|---|---|---|
| C – Matching | << Back | B – Dendrogram |

## OPTIONS FOLDER

The option folders gather the following functions:

⇨ Select the lanes
⇨ Edit the master
⇨ Help

⇨ Save the analysis or the template




**SELECT THE LANES**

You can add or remove the lanes to be used for matching calculation.

1. Click on the “Select the lanes” button.


2. A pop-up window displays the following menu on which you can select the lanes:


You can add or remove the lanes to be used for the matching calculation by selecting or unselecting the lanes of the list.

**EDIT THE MASTER**

1. Click on the “Edit the marker values” button.

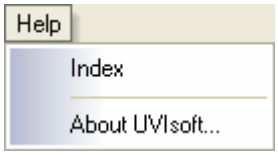

2. A pop-up window displays the following menu on which you can modify the master values:


You can add, remove, and save your master values;

**HELP MENU**

Click on the “Help” button. You automatically access the user manual at the chapter corresponding to the function

You can access the help file index through the File\Help from the Menu bar




**SAVE ANALYSIS / TEMPLATE**

This function saves the current analysis. The analysis file will contain the results, the image and all the parameters defined to obtain the results.

The analysis could also be saved as a template for automated analysis routines. Template offers the user the ability to automate many of the repetitive tasks associated with analysis and processing. As a result, you can spend more time evaluating and analysing results, and less time manipulating set-ups, variables and other settings.

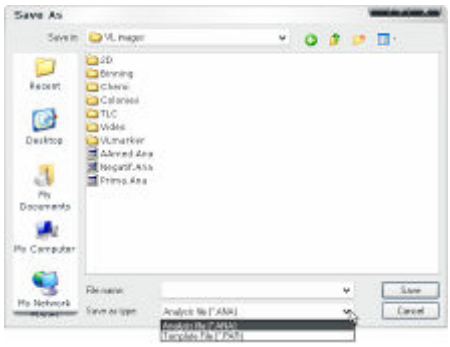
The template automates a task or set of tasks that you perform repeatedly or on a regular basis. It stores all the analysis commands and parameters of an analysis. You can run these parameters with another image whenever you need to perform a new analysis based on the same parameters.

The benefits of the template file are as follows:

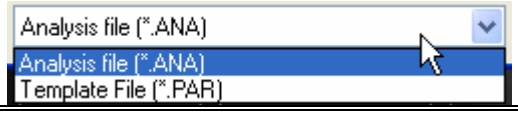
⇨ Time saving
⇨ Reproduction of image analysis parameters
⇨ Templates are modifiable, allowing the user to maintain an original template while modifying it for a slightly different result, with minimal effort

1. Click on the “Save analysis/ Template” button:


2. A pop-up window displays the following menu:

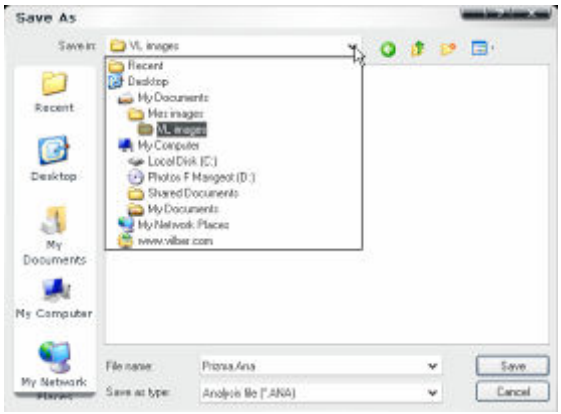


3. Select analysis file or template file:

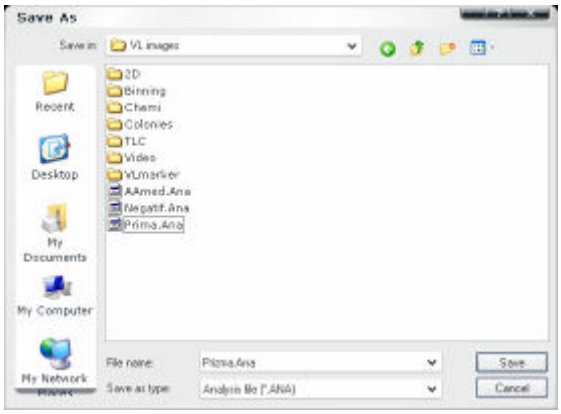


Note: the software proposes Analysis file by default

4. Browse to specify the file directory




5. Enter the desired file name and validate




6. Click on the Save button to create the file.

Note: see “Access to the analysis module” chapter for template or analysis file loading

# 3- Analyse – Quantification

## ➔ Principles of quantification

| | |
|---|---|
| 3 | Volume is the based of the spot quantification process. The volume is the sum of all the intensities included in the defined area (window + separation).<br><br>Quantification is based on the image in pixels whose intensity is coded on a scale.<br>- The scale has 256 grey levels for a 8-bit image<br>- The scale has 4 096 grey levels for a 12-bit image<br>- The scale has 16 384 grey levels for a 14-bit image<br>- The scale has 65 536 grey levels for a 16-bit image<br><br>The quantity (or density) of a spot is calculated from its volume. This is made of the sum of all pixel intensities composing the spot<br><br>In other words, the spot quantity then depends on:<br>- The number of pixels inside the area of the spot<br>- The intensities of these points<br><br>$V = \sum ni\ Ii$<br><br>Image analysis allows comparison in between concentrated intense spots and weaker but more diffused bands.<br><br>Results are given in volumes that may be recalculated according to an OD of reference or a concentration master-curve.<br><br>To measure the amount of a particular spot, you need to define the boundary around the spot and compare the intensity data inside the boundary with the data of other spots or of a standard. |

## ➔ A – Background subtraction

3

The background subtraction process follows the molecular weight results.

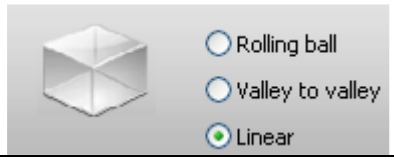
Image background interferes with quantification and data analysis. To this extend, we recommend to perform a background subtraction before any peak volume quantification. The subtraction is automatically done on the analysis area.

Note: As background subtraction permanently changes the image, this is not possible to save the image with a processed background subtraction. However, the process can be saved by saving the complete analysis through the Save analysis process.


The dashboard details the matching parameters:

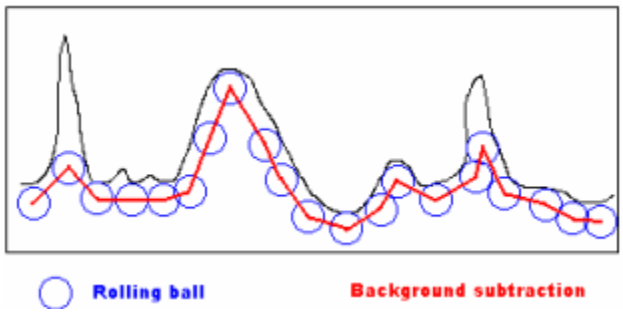

UVIband Advanced has several functions to minimise image background.

⇨ The rolling ball approach
⇨ The valley to valley approach
⇨ The linear approach



**ROLLING BALL**

The rolling ball method is named for a hypothetical ball that rolls along underneath the lane profile, removing different intensity levels along the length of the lane. The ball is rolled under each profile of the image so its movement varies along the image.




The centre of gravity of the ball describes a curve:

⇨ This curve represents the noise to be subtracted.

⇨ The curve depends on the size of the ball and on the size of the peaks.

The size of the ball will affect the position and movements of the centre of gravity and thus it determined the level of background subtraction. A small disk will make a large background subtraction and a large disk the contrary. A disk radius that is too small may subtract almost all image data.

The UVIband Advanced calculates automatically the ideal parameter for background subtraction. This could be manually modified by adjusting the spot size:


To process the rolling ball background subtraction, click on the “Do rolling ball” button:


The changes will be automatically applied to the image.

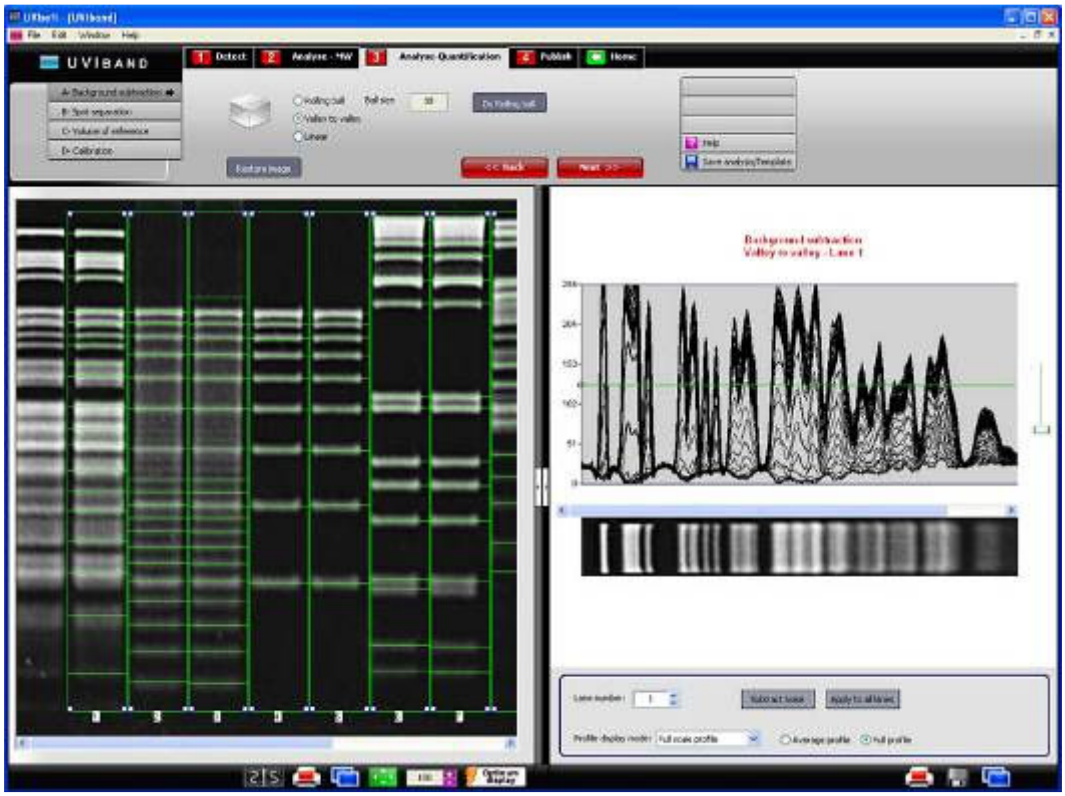
**Note**: few seconds could be necessary to perform the background subtraction.

**VALLEY TO VALLEY**

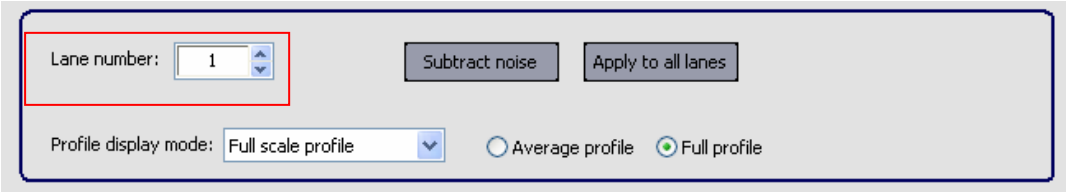
The valley-to valley approach is a lane-based background subtraction. It allows to define manually on the lane profile the level of noise to be subtracted.

1. Click on the “Valley to valley ” button: 

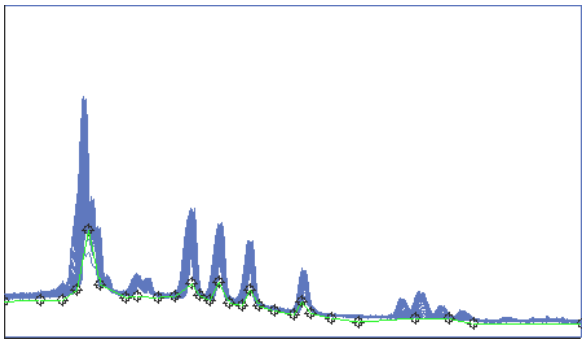
It opens the lane profile window:



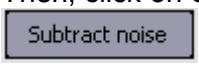
2. In the profile parameters window, select the lane to perform the valley-to-valley approach



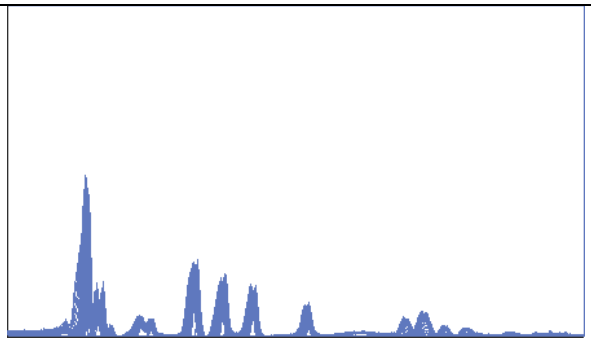

On the profile, click to define the background profile you want to remove:



Then, click on Subtract noise:



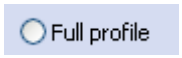
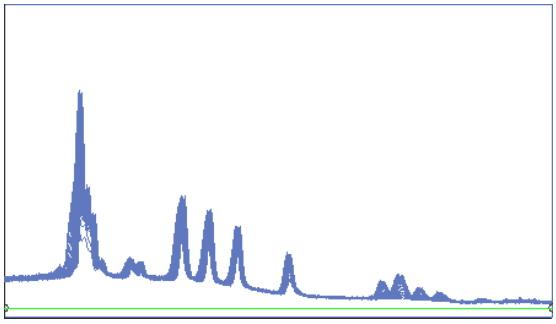
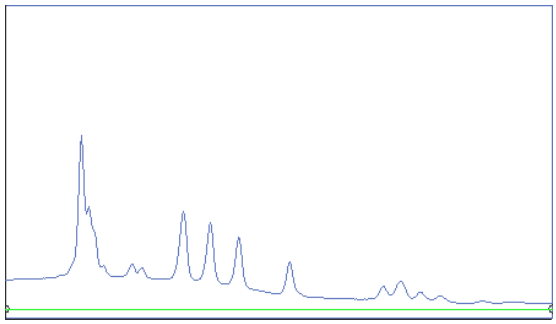
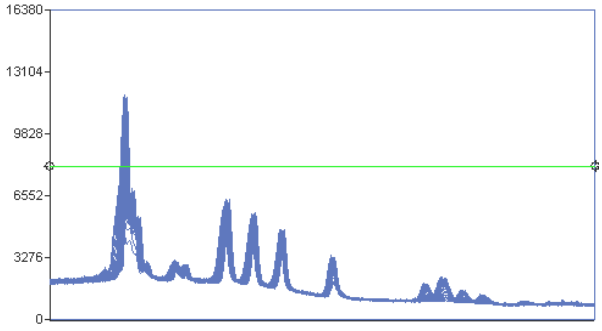

The changes will be automatically applied to the image and to the profile:

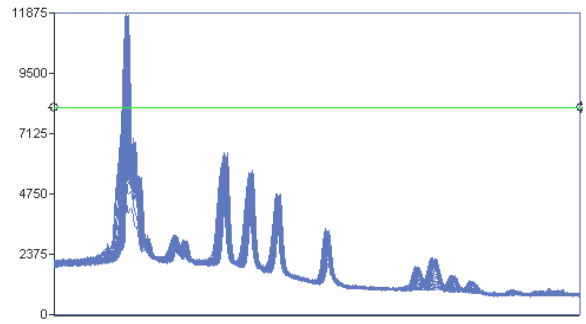
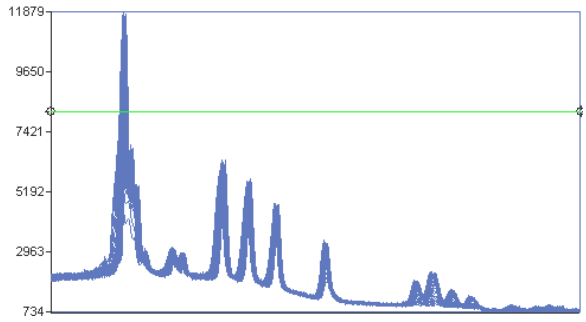


The valley-to-valley approach is a lane-based background subtraction. You can set the same subtraction level for all lanes or specify an individual subtraction level for the selected lane. Any changes you make will be automatically applied to the image.

To apply the same subtraction level for all lanes, click on the “Apply to all lanes” button:


You can easily adjust the profile displays settings as follows:

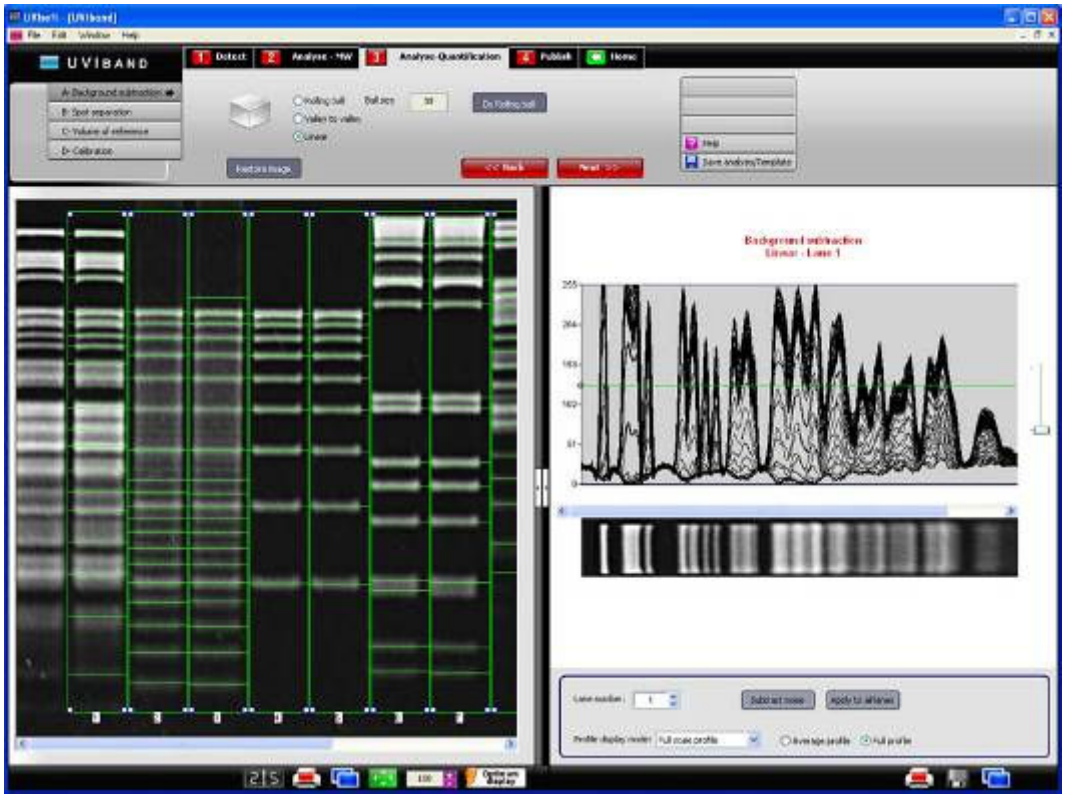
| | |
|---|---|
|   |  |
|   |  |
| Profile display mode:   The profile scale goes from 0 to the image maximum dynamic. |   |

| | |
|---|---|
| Profile display mode:<br>0 to Maximum<br>The profile scale goes from 0 to the lane's maximum intensity; |  |
| Profile display mode:<br>Minimum to Maximum<br>The profile scale goes from the lane's minimum intensity to the lane's maximum intensity; |  |

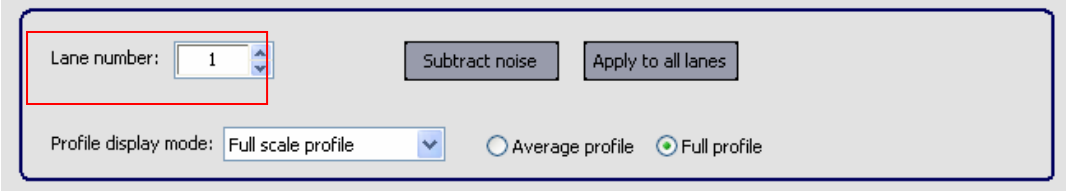
**LINEAR APPROACH**

The linear approach is a lane-based background subtraction. It allows to manually define the level of noise to be subtracted on the lane profile.

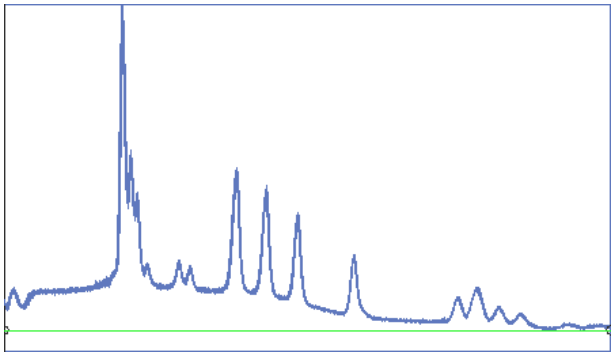
1. Click on the "Linear " button:  . It opens the lane profile window:



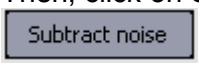

In the profile parameters window, select the lane to perform the linear approach



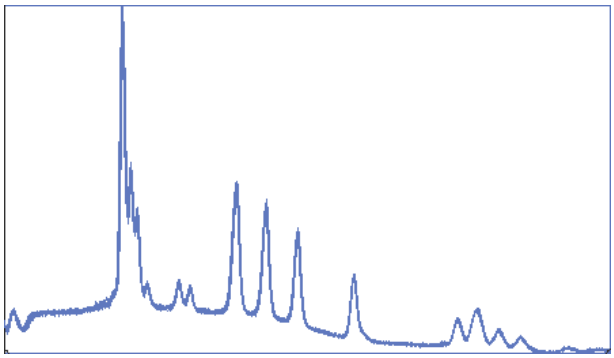

On the profile, click to define the background linear level you want to remove:



Then, click on Subtract noise:

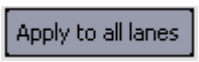



The changes will be automatically applied to the image and to the profile:

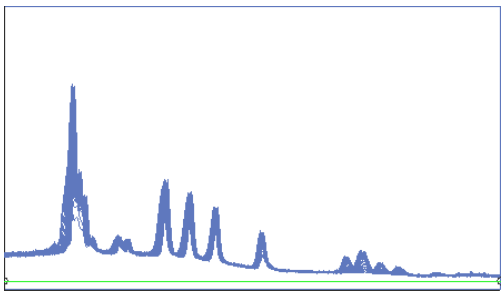
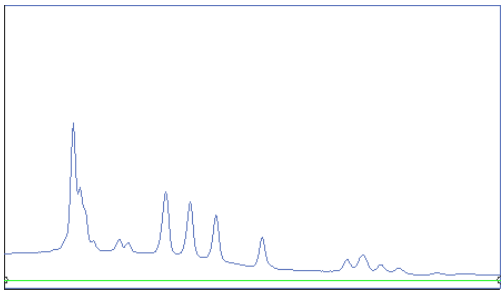
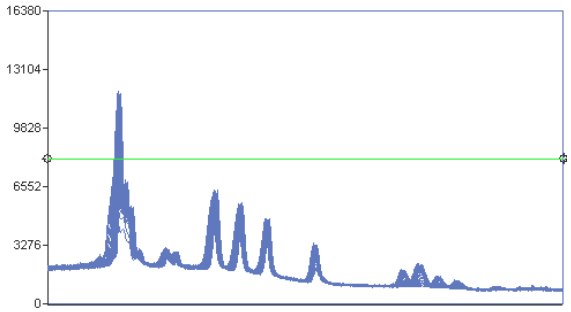
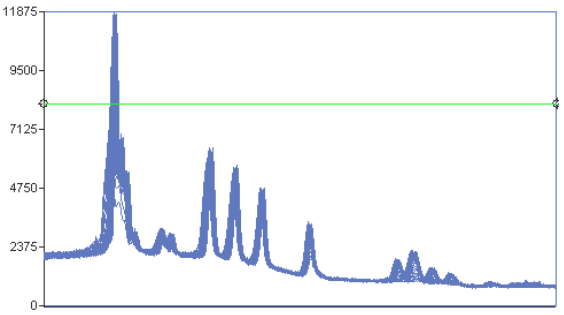
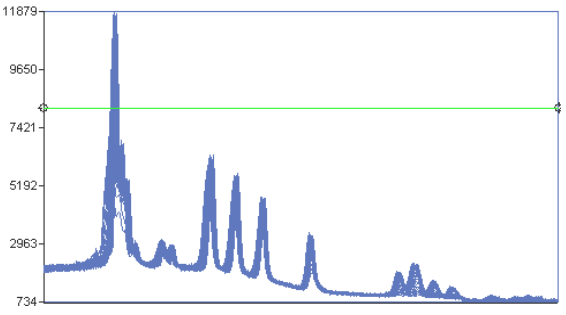


The linear approach is a lane-based background subtraction. You can set the same subtraction level for all lanes or specify an individual subtraction level for the selected lane. Any changes you make will be automatically applied to the image.

To apply the same subtraction level for all lanes, click on the “Apply to all lanes” button:




You can easily adjust the profile displays settings as follows:

| | |
|---|---|
| Full profile |  |
| Average profile |  |
| Profile display mode:<br>Full scale profile<br>The profile scale goes from 0 to the image maximum dynamic. |  |
| Profile display mode:<br>0 to Maximum<br>The profile scale goes from 0 to the lane's maximum intensity; |  |
| Profile display mode:<br>Minimum to Maximum<br>The profile scale goes from the lane's minimum intensity to the lane's maximum intensity; |  |

**NEXT**

The "Next" button validates your parameter and opens the following analysis step.

| | | |
|---|---|---|
| 3 A – Background | Next >> | 3 B- Spot separation |

| | | |
|---|---|---|
| subtraction | | |

**BACK**

The “Back” button validates your parameter and opens the following analysis step.

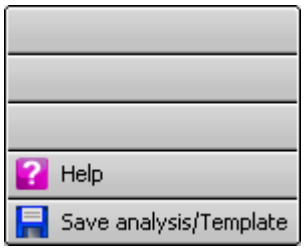
| | | |
|---|---|---|
| 3 A – Background subtraction | << Back | 2 A – Molecular weight |

**OPTION FOLDER**

The option folder gathers the following functions:

⇨ Help

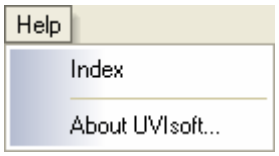
⇨ Save the analysis or the template




**HELP MENU**

Click on the “Help” button. You automatically access the user manual at the chapter corresponding to the function


You can access the help file index through the File\Help from the Menu bar




**SAVE ANALYSIS / TEMPLATE**

This function saves the current analysis. The analysis file will contain the results, the image and all the parameters defined to obtain the results.

The analysis could also be saved as a template for automated analysis routines. Template offers the user the ability to automate many of the repetitive tasks associated with analysis and processing. As a result, you can spend more time evaluating and analysing results, and less time manipulating set-ups, variables and other settings.

The template automates a task or set of tasks that you perform repeatedly or on a regular basis. It stores all the analysis commands and parameters of an analysis. You can run these parameters with another image whenever you need to perform a new analysis based on the same parameters.

The benefits of the template file are as follows:

⇨ Time saving

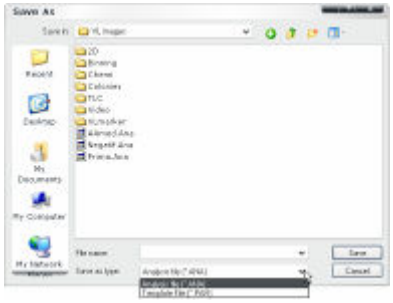
⇨ Reproduction of image analysis parameters

⇨ Templates are modifiable, allowing the user to maintain an original

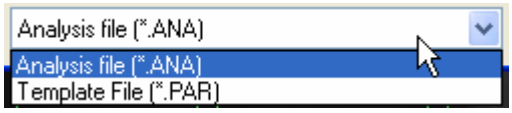
template while modifying it for a slightly different result, with minimal effort

1. Click on the “Save analysis/ Template” button:


2. A pop-up window displays the following menu:

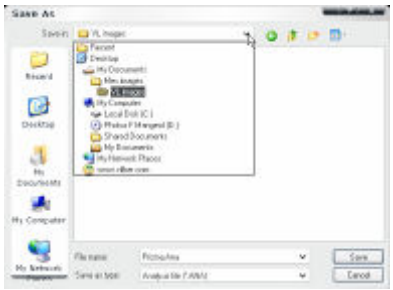


3. Select analysis file or template file:

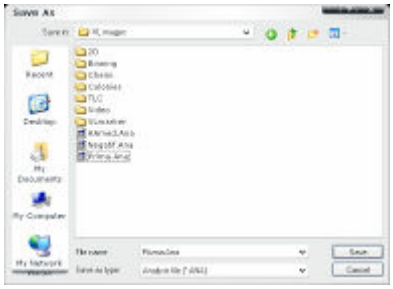



Note: the software proposes Analysis file by default

4. Browse to specify the file directory



5. Enter the desired file name and validate



6. Click on the Save button to create the file.

Note: see “Access to the analysis module” chapter for template or analysis file loading

## ➔ B – Spot separation

3

In order to measure the volume of a particular spot, you need:

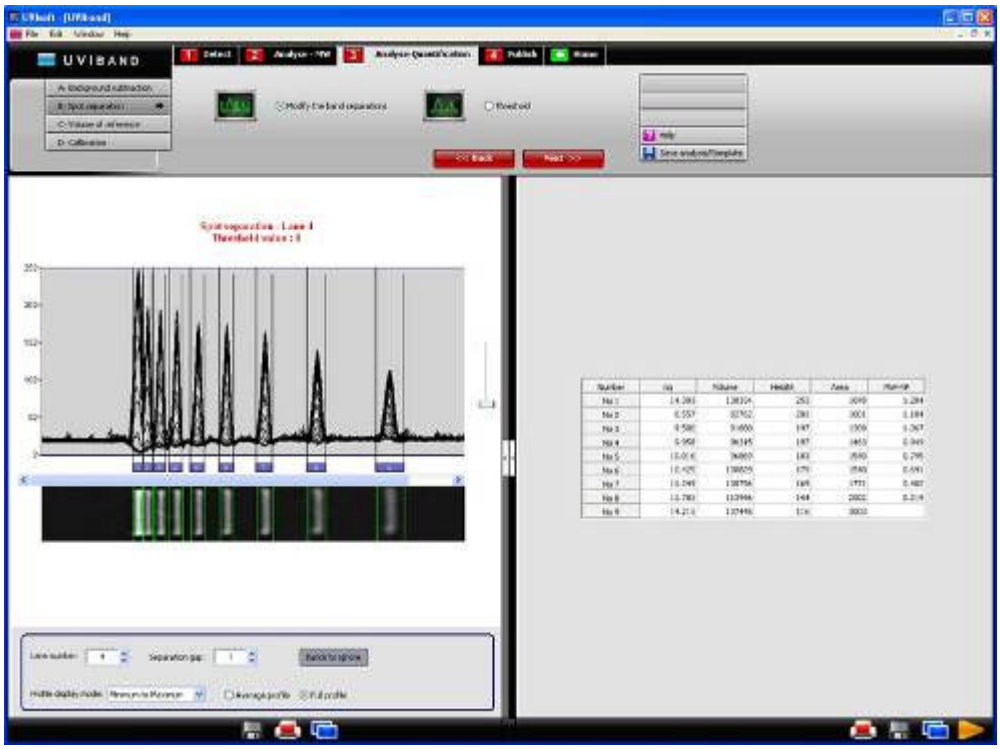
⇨ To define the boundary around the spot;

⇨ To compare the intensity data inside the boundary with the data of other spots or of a standard.

A volume is the sum of the pixel intensity inside a defined boundary. The purpose of the spot separation is to define this boundary.

The spot separation process follows the background subtraction.

Note: you can either access the spot separation function by clicking on the next button of the background subtraction or directly by clicking on the spot separation of the Analyse-Quantification folder.




The dashboard details the spot separation parameters:


⇨ Modify the spot separation

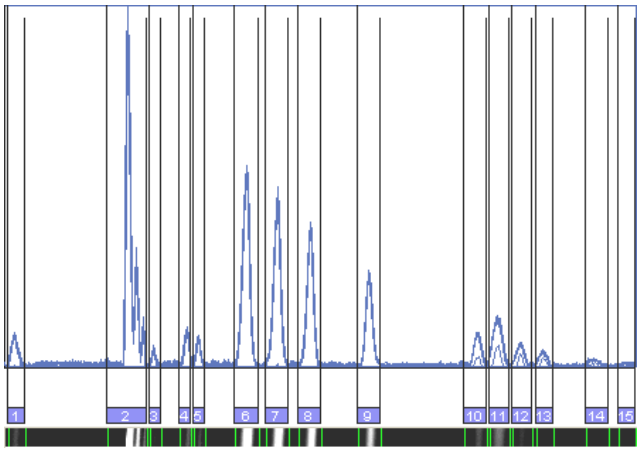
⇨ Standard threshold

⇨ Extended threshold

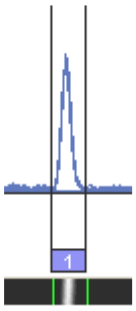
**MODIFY THE SPOT SEPARATION**

UVIband Advance proposes by default an automatic predefined spot separation based on the band detection. You can modify the default spot separation by selecting the “Modify the spot separation” option.

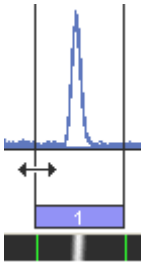

The default separation is illustrated on the lane's profile:




The brackets illustrate the bands boundaries:



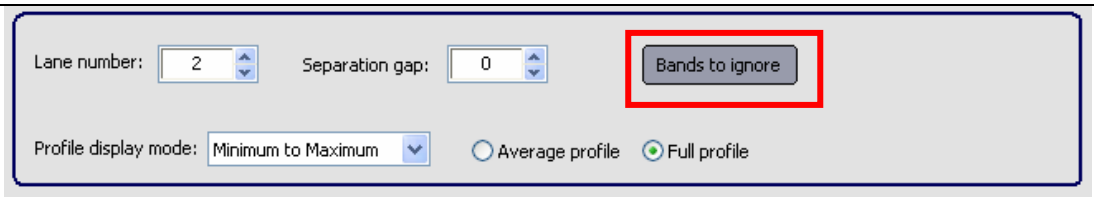

You can easily reposition a band's boundaries. In order to do so, click on the bracket and drag the cursor:



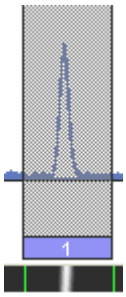

Drag the cursor until the area of the band that you want to define has been completely enclosed.

**Note**: When you release the mouse button, the band's volume is automatically recalculated to take into account the new area of interest.

To ignore a band, select "Bands to ignore" from the profile's parameter menu:



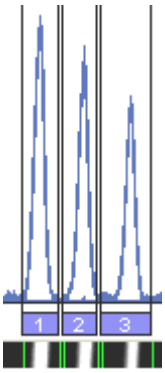

Then, click on the band you want to ignore:



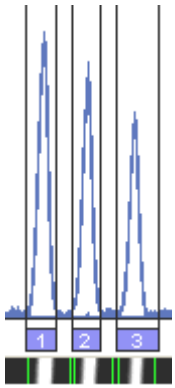

The band is then highlighted in grey and discarded from the result table:

Note: you can ignore more than one band at a time.
Note: to stop the process, click again on the “Bands to ignore” button.

To increase the gap in between the lane, select the “Separation gap” option from the profile’s parameter menu:




**Limited separation gap**

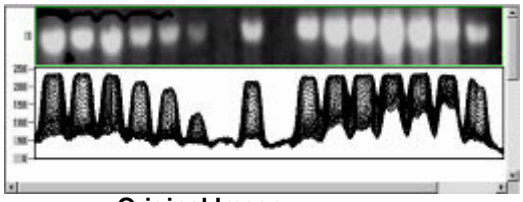



**Extended separation gap**

**DEFINE A THRESHOLD**

The threshold defines the detection level to take into account for the volume quantification. It allows to distinguish between bands and smears on the lane.

**Case when you should use detection level** (Threshold):
There is still a strong background even after the background subtraction



**Original Image**

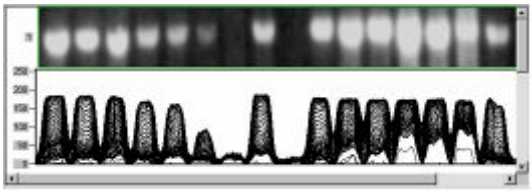


**Image with subtracted background**

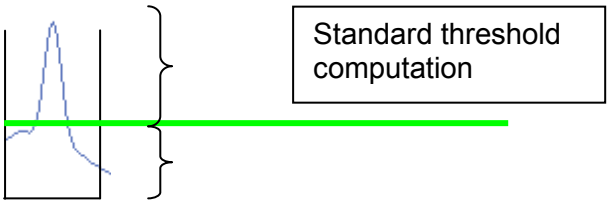
The spot contours must be isolated more precisely from the smears where they are located

The threshold method calculates the volume which is above the threshold:

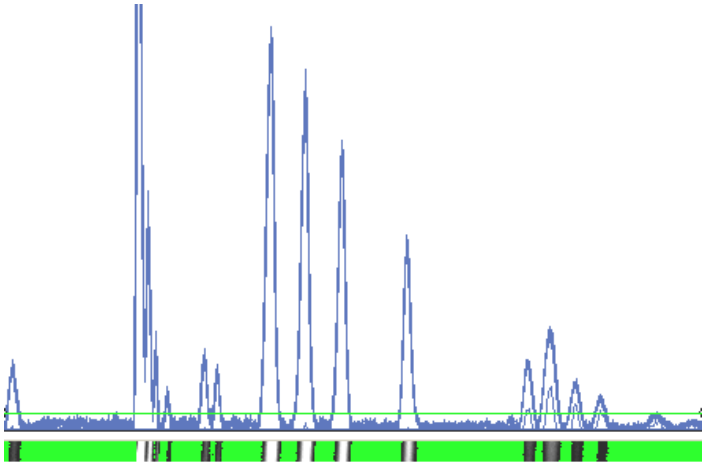
Volume= ∑ (Pixels intensities)

⇨ Pixel intensities=0 if Pixel<Threshold

⇨ Pixel intensities= (Pixel intensities) if Pixel>Threshold




Move upwards or downward the horizontal line appearing on the profile:



This displays a green contour that encloses pixels whose intensity is equal to or greater than that of the pixel at the cursor. If the contour does not encircle the band, reposition the cursor and click again. A new contour will be drawn in place of the old one.

The green area under the profile represents the range of values discarded to calculate the volume. The contour should completely surround the data you want to quantify.

The defined threshold is automatically applied to the selected lane. The results are recalculated taking into account the threshold:

| Number | // | Volume | Height | Area | MW-RF |
|---|---|---|---|---|---|
| No 1 | | 224452 | 2173 | 126 | 31.714 |
| No 2 | | 561516 | 2110 | 294 | 24.000 |
| No 3 | | 1216111 | 2429 | 574 | 13.143 |
| No 4 | | 1072687 | 11699 | 210 | 8.812 |
| No 5 | | 388143 | 6775 | 70 | 7.391 |
| No 6 | | 373495 | 5360 | 98 | 6.459 |
| No 7 | | 531756 | 2988 | 224 | 5.767 |
| No 8 | | 1140205 | 3070 | 490 | 4.945 |
| No 9 | | 1144095 | 6172 | 392 | 3.847 |
| No 10 | | 943922 | 5602 | 350 | 2.930 |
| No 11 | | 1138471 | 4700 | 602 | 2.453 |
| No 12 | | 1235282 | 3269 | 966 | 1.987 |
| No 13 | | 385044 | 1870 | 294 | 1.417 |
| No 14 | | 401562 | 2191 | 252 | 0.973 |
| No 15 | | 243847 | 1541 | 195 | 0.774 |
| No 16 | | 213134 | 1311 | 191 | 0.573 |
| No 17 | | 4827 | 973 | 5 | 0.389 |
| No 18 | | 0 | 0 | 0 | 0.267 |

⇨ The volume is the sum of intensities included in the spot area of analysis.
⇨ The height is the maximum spot intensity, in grey levels.
⇨ The area is the zone defined for each spot area of analysis.

The threshold approach is on a lane-based basis. You can set the same threshold for all lanes or specify an individual threshold for the selected lane. Any changes you make will be automatically applied to the image.

To apply the same subtraction level for all lanes, click on the “Apply to all lanes” button:

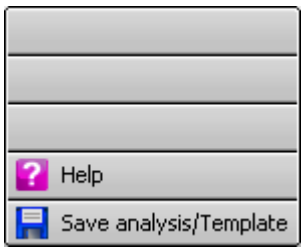

**NEXT**
The “Next” button validates your parameter and opens the following analysis step.

| | | |
|---|---|---|
| 3 B- Spot separation | Next >> | 3 C – Volume of reference |

**BACK**
The “Back” button validates your parameter and opens the following analysis step.

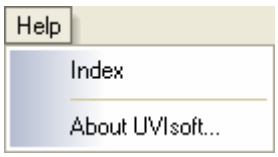
| | | |
|---|---|---|
| 3 B- Spot separation | << Back | 1 C – Background subtraction |

**OPTION FOLDER**
The option folder gathers the following functions:
⇨ Help
⇨ Save the analysis or the template




**HELP MENU**
Click on the “Help” button. You automatically access the user manual at the chapter corresponding to the function


You can access the help file index through the File\Help from the Menu bar



## SAVE ANALYSIS / TEMPLATE

This function saves the current analysis. The analysis file will contain the results, the image and all the parameters defined to obtain the results.

The analysis could also be saved as a template for automated analysis routines. Template offers the user the ability to automate many of the repetitive tasks associated with analysis and processing. As a result, you can spend more time evaluating and analysing results, and less time manipulating set-ups, variables and other settings.

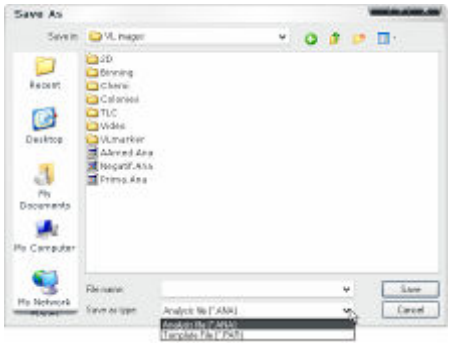
The template automates a task or set of tasks that you perform repeatedly or on a regular basis. It stores all the analysis commands and parameters of an analysis. You can run these parameters with another image whenever you need to perform a new analysis based on the same parameters.

The benefits of the template file are as follows:

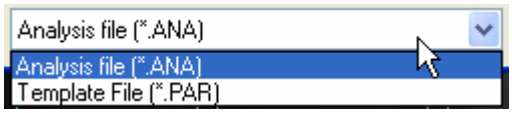
⇨ Time saving
⇨ Reproduction of image analysis parameters
⇨ Templates are modifiable, allowing the user to maintain an original template while modifying it for a slightly different result, with minimal effort

1. Click on the “Save analysis/ Template” button:


2. A pop-up window displays the following menu:

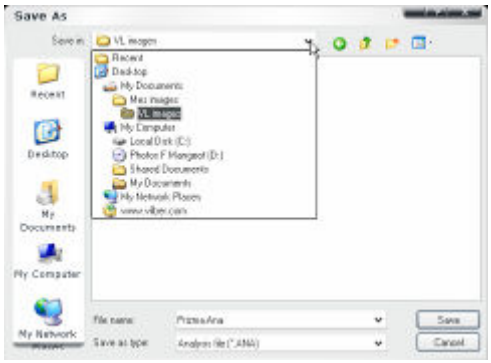


3. Select analysis file or template file:

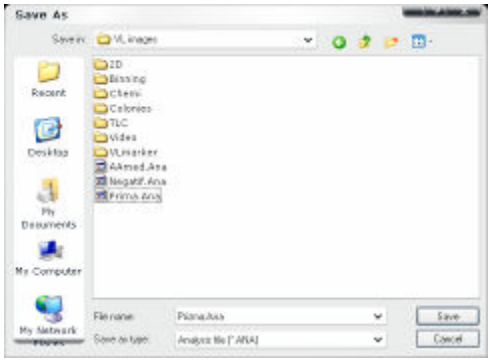



Note: the software proposes Analysis file by default

4. Browse to specify the file directory




5. Enter the desired file name and validate




6. Click on the Save button to create the file.

Note: see “Access to the analysis module” chapter for template or analysis file loading

## ➔ C – Volume of reference

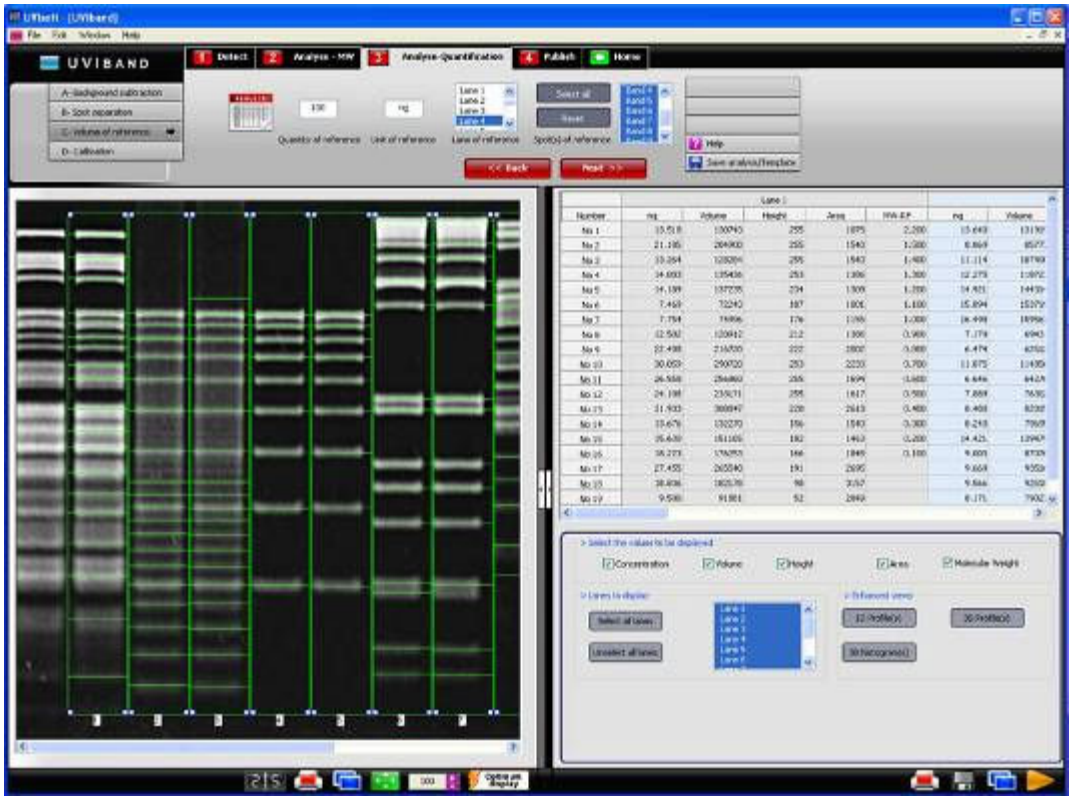
3

A volume is the total signal intensity inside a defined boundary drawn on a lane.

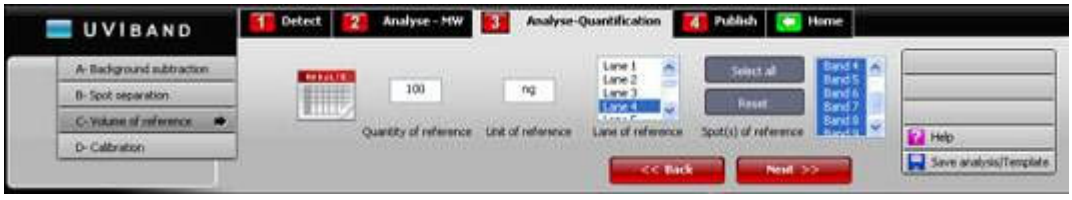
The purpose of the volume of reference is to use volumes of known concentration to calculate the unknown concentrations.

The volume of reference process follows the spot separation.

Note: you can either access the volume of reference function by clicking on the next button of the background subtraction or directly by clicking on the volume of reference of the 3-Analyse-Quantification folder.




The dashboard details the volume of reference parameters:




⇨ The quantity of reference
⇨ The unit of reference
⇨ The lane of reference
⇨ The spot(s) of reference

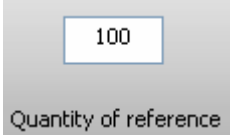
**QUANTITY OF REFERENCE**

The calculation of the unknown concentrations is based:

⇨ On the calculated volumes

⇨ On the known concentration. The known concentration is the quantity of reference. The quantity of reference could correspond to one or several spots.
The purpose of the quantity of reference is to define the known concentration:

In the “Quantity of reference” edit field, type the quantity of known concentration you want to have as a reference:




**UNIT OF REFERENCE**

The unit of reference is the header unit of the concentration. You can define your own header such as % or µg.

In the “Unit of reference” edit field, type the unit you want to be displayed in the results table:


Unit of reference
**Percentage as unit of reference**


Unit of reference
**µg as unit of reference**

**LANE OF REFERENCE**

The lane of reference defines the lane of the known concentration.
Select the lane of reference from the list:


Lane of reference

If a single lane is selected, only the volumes of this reference lane will be used to calculate the relationship between the volume and the quantity. The other concentrations are calculated based on the concentration/volume relationship of this specific lane.

| | Lane 1 | | Lane 3 | | Lane 4 | |
|---|---|---|---|---|---|---|
| Number | % | Volume | % | Volume | % | Volume |
| No 1 | 44.708 | 6635518 | 178.291 | 26461728 | 49.658 | 7370205 |
| No 2 | 25.475 | 3780895 | 64.424 | 9561786 | 47.652 | 7072517 |
| No 3 | 14.264 | 2117062 | 9.885 | 1467075 | | 0 |
| No 4 | 9.304 | 1380926 | | | | 0 |
| No 5 | 3.574 | 530507 | | | | 0 |
| No 6 | 1.840 | 273100 | | | | 0 |
| No 7 | 0.835 | 123860 | | | | |
| No 8 | | 0 | | | | |
| No 9 | | 0 | | | | |

Illustration 1: 100% / lane 1 / all bands. Total concentration lane 1= 100%

If “All lanes” is selected, for each lane a new relationship between volume and quantity will be recalculated, according to the band’s lane selected. For instance, the defined

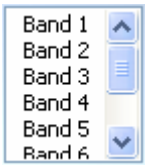
parameters are 100% for all band all lanes; the results table could be as follows. Lane by lane, the total band concentration is 100%:

| | Lane 1 | | Lane 3 | | Lane 4 | |
|---|---|---|---|---|---|---|
| Number | % | Volume | % | Volume | % | Volume |
| No 1 | 44.708 | 6635518 | 70.582 | 26461728 | 51.031 | 7370205 |
| No 2 | 25.475 | 3780895 | 25.504 | 9561786 | 48.969 | 7072517 |
| No 3 | 14.264 | 2117062 | 3.913 | 1467075 | | 0 |
| No 4 | 9.304 | 1380926 | | | | 0 |
| No 5 | 3.574 | 530507 | | | | 0 |
| No 6 | 1.840 | 273100 | | | | 0 |
| No 7 | 0.835 | 123860 | | | | |
| No 8 | | 0 | | | | |
| No 9 | | 0 | | | | |

Illustration 2: 100% / all lanes / all bands. Total concentration all lanes= 100%

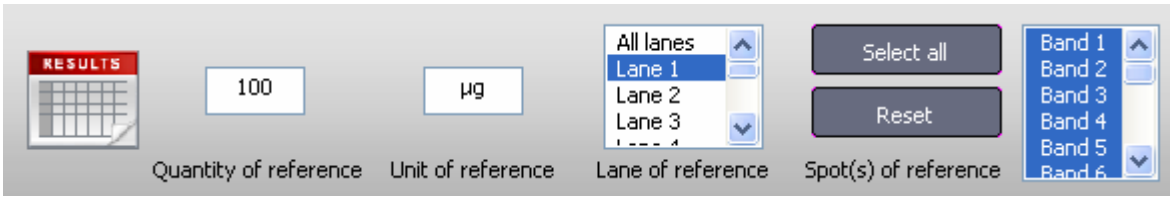
**SPOT(S) OF REFERENCE**

The quantity of reference could correspond to one or several spots of the selected lane. Select one or several spots of the lane of reference from the list:




**EXAMPLE 1**

Let's consider the known concentration is 3µg contains in the first spot of lane 3. The settings should then be as follows:

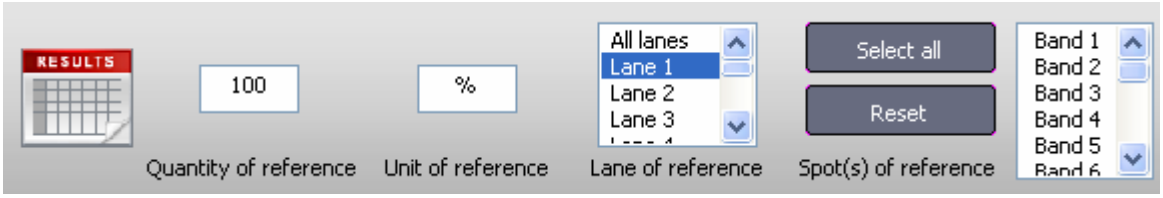



The results table indicates the following for lane 3:

| Number | µg | Volume | Height | Area | MW-RF |
|---|---|---|---|---|---|
| No 1 | 3.000 | 4285313 | 4071 | 1775 | 10.000 |
| No 2 | 9.267 | 13237182 | 3438 | 5396 | 8.000 |
| No 3 | 0.942 | 1345357 | 2740 | 568 | 6.000 |
| No 4 | 0.467 | 667689 | 2692 | 284 | 5.000 |
| No 5 | 12.560 | 17940927 | 2651 | 10224 | 4.000 |
| No 6 | 0.358 | 511654 | 1305 | 426 | 3.000 |
| No 7 | 3.885 | 5549237 | 1275 | 5112 | 2.500 |
| No 8 | 1.626 | 2322765 | 1176 | 2414 | 2.000 |
| No 9 | 0.465 | 664510 | 1000 | 710 | 1.500 |

**EXAMPLE 2**

Let's consider the known concentration is 100% contains in all the spots of lane 1. The settings should then be as follows:




The results table indicates the following for lane 1:

| Number | % | Volume | Height | Area | MW-RF |
|---|---|---|---|---|---|
| No 1 | 3.978 | 1715709 | 2744 | 781 | 9.896 |
| No 2 | 15.367 | 6627687 | 4310 | 2769 | 7.998 |
| No 3 | 11.431 | 4930041 | 4642 | 2130 | 7.710 |
| No 4 | 12.333 | 5319454 | 2612 | 2414 | 4.561 |
| No 5 | 2.112 | 911077 | 2323 | 426 | 4.000 |
| No 6 | 35.571 | 15341999 | 2191 | 10508 | 2.678 |
| No 7 | 19.207 | 8284193 | 1270 | 8591 | 1.872 |

## NEXT

The “Next” button validates your parameter and opens the following analysis step.

| | | |
|---|---|---|
| 3 C – Volume of reference | Next >> | 3 D – Calibration |

## BACK

The “Back” button validates your parameter and opens the following analysis step.

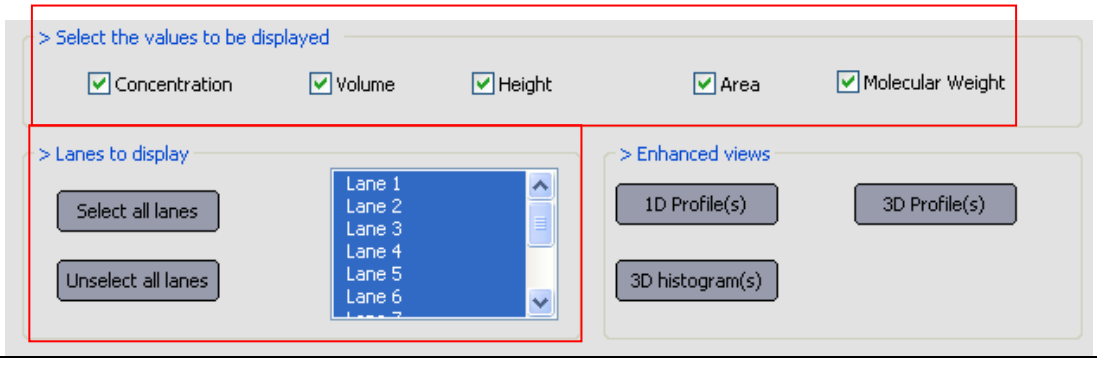
| | | |
|---|---|---|
| 3 C – Volume of reference | << Back | 3 B- Spot separation |

## RESULT TABLE

In the result parameter window, you can select the lanes and the values to be displayed in the results tables:

- ⇨ Concentration
- ⇨ Volume
- ⇨ The maximum intensity
- ⇨ The area
- ⇨ The molecular weight

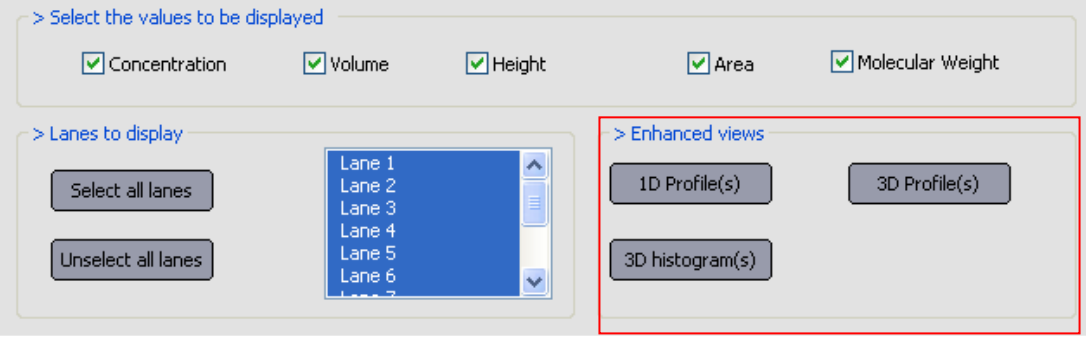
1. To select your display mode, click on the appropriate selection:



## GRAPHICAL VIEW

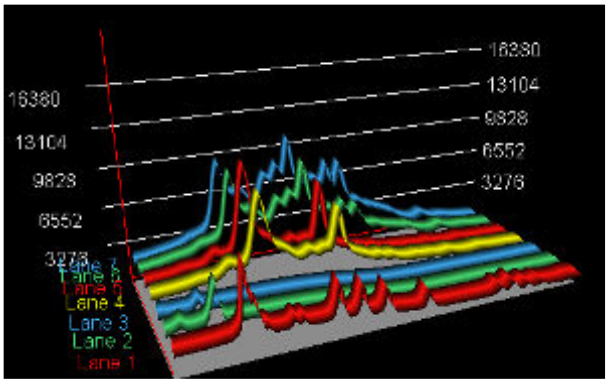
In the results parameter window, you can select the graphical results tables:

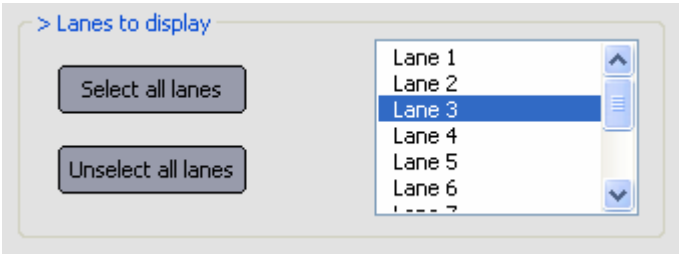
- ⇨ 1D profile
- ⇨ 3D profile
- ⇨ 3D histogram




Note: For all enhanced views, you can modify the angle of vision of the 3D view: Move the mouse cursor on the 3D area, click and drag the view in the direction you want to rotate. Release the mouse when satisfactory.

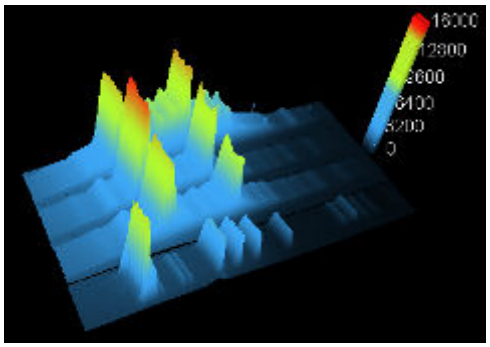
The 1D profile allows you to superimpose the intensity profiles of any number of selected lanes.
To proceed, click on the 1D Profile and select the lanes to be superimposed:

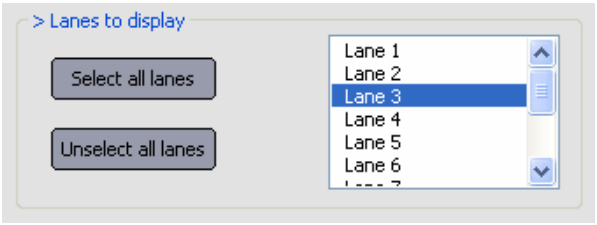




Note: To reposition the 1D profile window, position your cursor at the top of the box. The cursor appearance will change to a multidirectional arrow symbol. You can then drag the box to a new position.
Note: To increase the size of the 1D profile window, position your cursor at the edge of the window and click and drag to extend its size.
Note: Click on Print to print the 1D profile window
Note: Click on Send to clipboard to save the graph in the Windows ® clipboard (Past/copy Windows ® feature).

The 3D profile displays the three-dimensional rendering of any selected lanes.
To proceed, click on the 3D Profile button and select the lanes to be displayed:

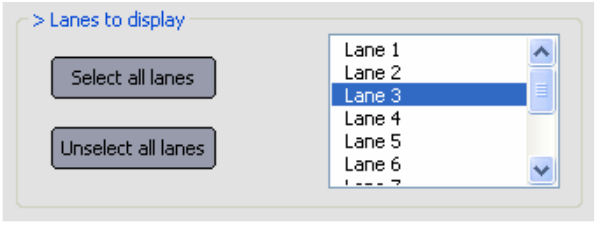


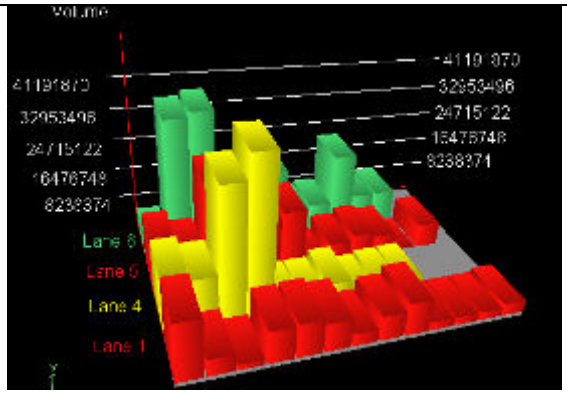

Note: To reposition the 1D profile window, position your cursor at the top of the box. The cursor appearance will change to a multidirectional arrow symbol. You can then drag the box to a new position.
Note: To increase the size of the 1D profile window, position your cursor at the edge of the window and click and drag to extend its size.
Note: Click on Print to print the 1D profile window
Note: Click on Send to clipboard to save the graph in the Windows ® clipboard (Past/copy Windows ® feature).

The 3D histogram displays the three-dimensional histogram of selected results:

- ⇨ Volume
- ⇨ Calculated quantities
- ⇨ Maximum intensities

To proceed, click on the 3D Histogram button and select the lanes to be displayed:

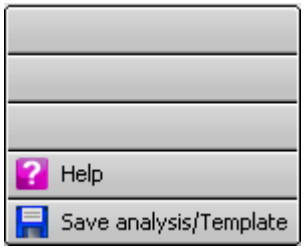



Note: To reposition the 1D profile window, position your cursor at the top of the box. The cursor appearance will change to a multidirectional arrow symbol. You can then drag the box to a new position.
Note: To increase the size of the 1D profile window, position your cursor at the edge of the window and click and drag to extend its size.
Note: Click on Print to print the 1D profile window
Note: Click on Send to clipboard to save the graph in the Windows ® clipboard (Past/copy Windows ® feature).

## OPTION FOLDER

The option folder gathers the following functions:

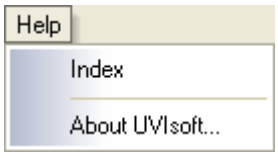
⇨ Help
⇨ Save the analysis or the template




## HELP

Click on the “Help” button. You automatically access the user manual at the chapter corresponding to the function


You can access the help file index through the File\Help from the Menu bar




## SAVE ANALYSIS / TEMPLATE

This function saves the current analysis. The analysis file will contain the results, the image and all the parameters defined to obtain the results.

The analysis could also be saved as a template for automated analysis routines. Template offers the user the ability to automate many of the repetitive tasks associated with analysis and processing. As a result, you can spend more time evaluating and analysing results, and less time manipulating set-ups, variables and other settings.

The template automates a task or set of tasks that you perform repeatedly or on a regular basis. It stores all the analysis commands and parameters of an analysis. You can run these parameters with another image whenever you need to perform a new analysis based on the same parameters.

The benefits of the template file are as follows:

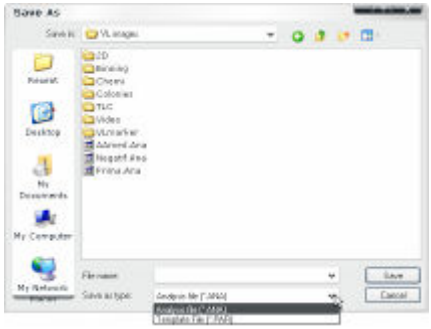
⇨ Time saving

⇨ Reproduction of image analysis parameters

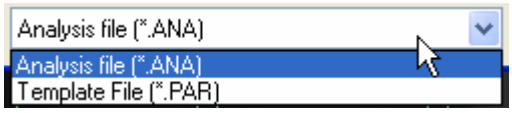
⇨ Templates are modifiable, allowing the user to maintain an original template while modifying it for a slightly different result, with minimal effort

1. Click on the “Save analysis/ Template” button: 


2. A pop-up window displays the following menu:

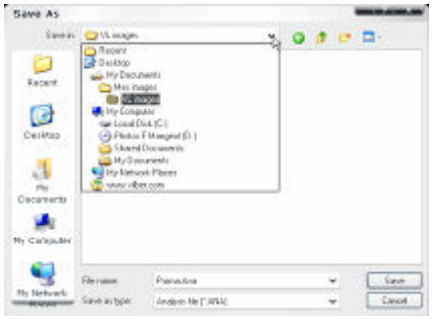


3. Select analysis file or template file:

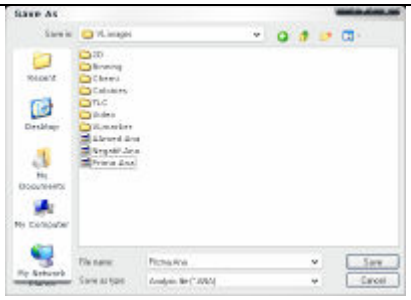



Note: the software proposes Analysis file by default

4. Browse to specify the file directory



5. Enter the desired file name and validate



6. Click on the Save button to create the file.

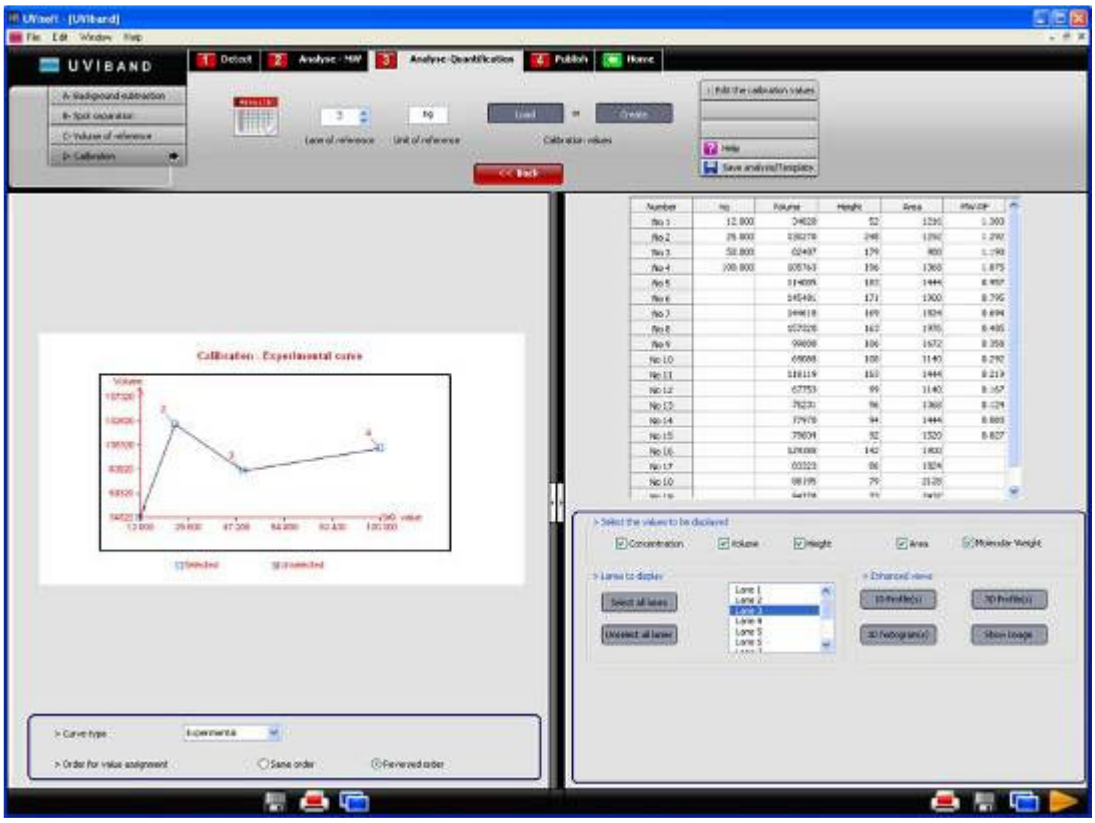
Note: see “Access to the analysis module” chapter for template or analysis file loading

## ➔ D – Calibration

3

The calibration process follows Volume of reference. The calibration is the calculation of the concentration based on a concentration master or on a calibration curve on which you can select all or few points.

Note: you can either access the calibration function by clicking on the next button of the Volume of reference or directly by clicking on the Calibration of the 3-Analyse-Quantification folder.




The dashboard details the volume of reference parameters:


- ⇨ The lane of reference
- ⇨ The unit of reference
- ⇨ The calibration values

**LANE OF REFERENCE**

The lane of reference defines the lane of the known concentration.
Select the lane of reference from the list:


Lane of reference

**UNIT OF REFERENCE**

The unit of reference is the header unit of the concentration. You can define your own header such as % or µg.

In the “Unit of reference” edit field, type the unit you want to be displayed in the results table:


Unit of reference
**Percentage as unit of reference**


Unit of reference
**µg as unit of reference**

**THE CALIBRATION VALUES**

1. Click on the “Load” or “Create” button to enter calibration’s values.

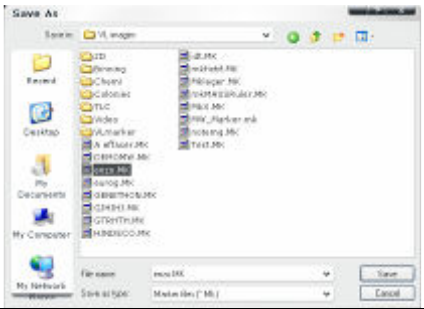

For “Create”, a pop-up window displays the following menu:


Type your values, band to band, in a descending order. The OK button validates your data.

Note: if an automatic calculation with immediate application of the standard values is carried out, it is not necessary to enter all the bands given by the manufacturer's specifications, but only those which are commonly found on the lanes of the gel.

You can save your calibration data and create your own calibration library; To proceed, click on the “Save “ button:

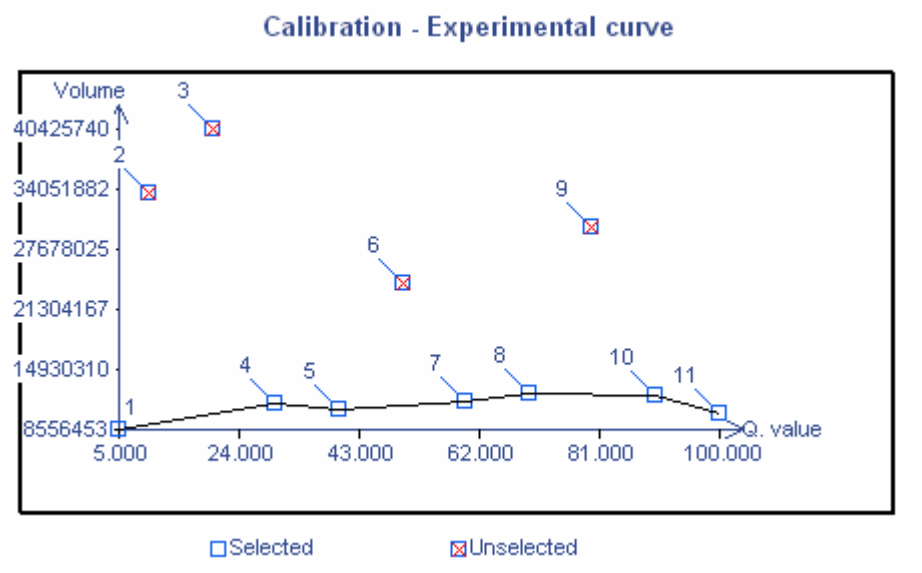

A pop-up window displays the following menu:



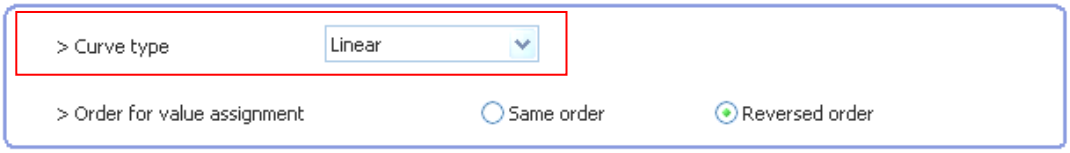
⇨ Browse to specify the directory
⇨ Type the file name and click on Save.

**MASTER CURVE**

After the values of the master-curve are defined, the calibration curve is displayed. You can unselect wrong values or points out of the curve by directly clicking on them

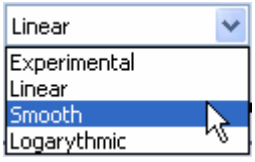



In the profile parameters window, select the curve type:

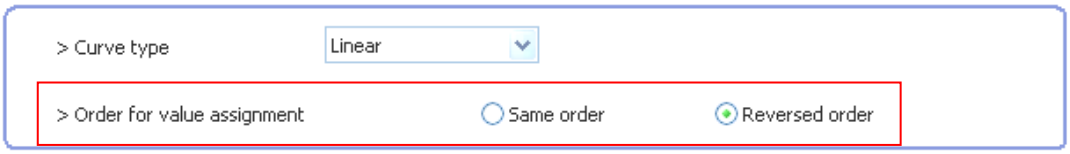



Four mathematical models can be used:
⇨ Experimental: the curve simply links the values (point to point), without any mathematical model,
⇨ Linear curve: displays a model with linear regression
⇨ Smoothed: displays a smoothed curve (polynomial spline, at least 4 points must be entered)

⇨ Logarithmic curve: displays a model with logarithmic regression




You can also select the order for the spot display:
⇨ - Same order as the values of the master-curve
⇨ - Reversed order (depending on the order of the defined values)

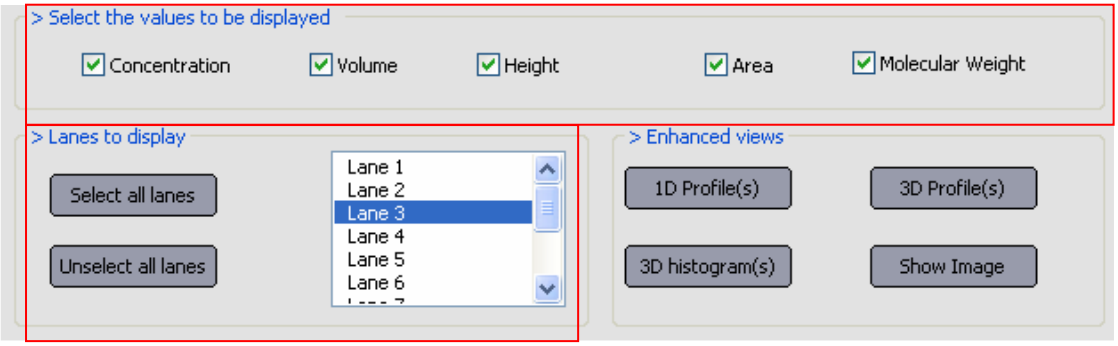



## RESULT TABLE

In the result parameter window, you can select the lanes and the values to be displayed in the results tables:

- ⇨ Concentration
- ⇨ Volume
- ⇨ The maximum intensity
- ⇨ The area
- ⇨ The molecular weight

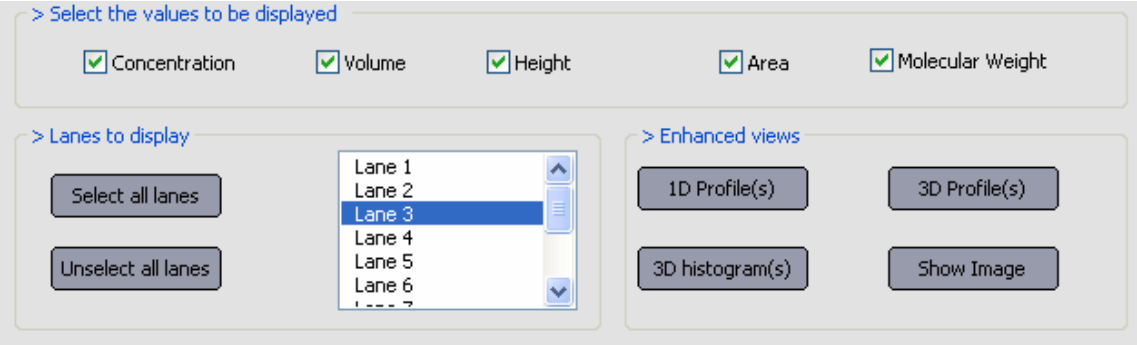
To select your display mode, click on the appropriate selection:




## GRAPHICAL VIEW

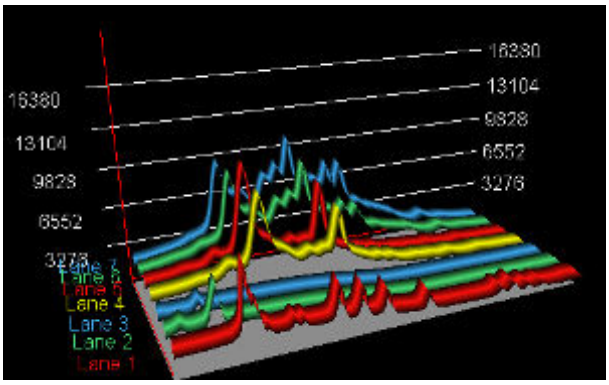
In the results parameter window, you can select the graphical results tables:

- ⇨ 1D profile
- ⇨ 3D profile
- ⇨ 3D histogram



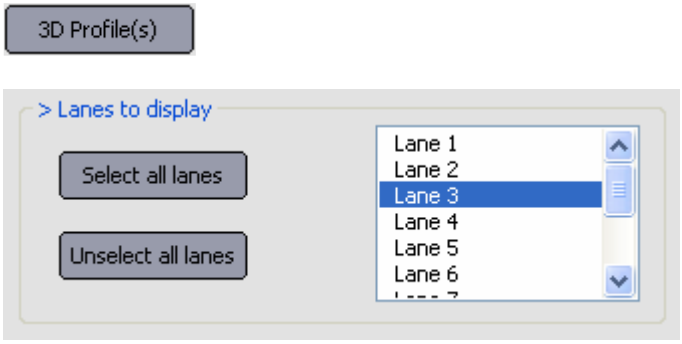

Note: For all enhanced views, you can modify the angle of vision of the 3D view : Move the mouse cursor on the 3D area, click and drag the view in the direction you want to rotate. Release the mouse when satisfactory.

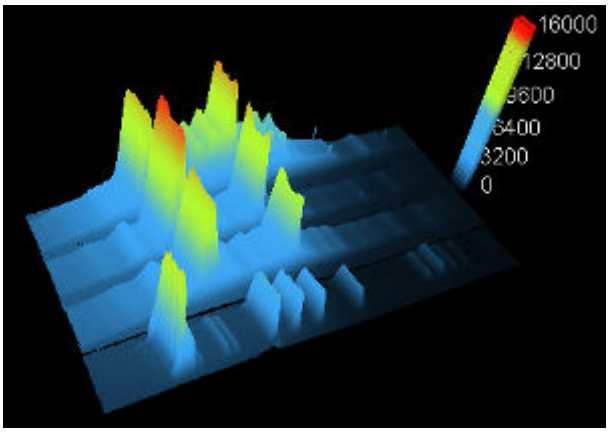
The 1D profile allows you to superimpose the intensity profiles of any number of selected lanes.
To proceed, click on the 1D Profile and select the lanes to be superimposed:




Note: To reposition the 1D profile window, position your cursor at the top of the box. The cursor appearance will change to a multidirectional arrow symbol. You can then drag the box to a new position.
Note: To increase the size of the 1D profile window, position your cursor at the edge of the window and click and drag to extend its size.
Note: Click on Print to print the 1D profile window
Note: Click on Send to clipboard to save the graph in the Windows ® clipboard (Past/copy Windows ® feature).

The 3D profile displays the three-dimensional rendering of any selected lanes.
To proceed, click on the 3D Profile button and select the lanes to be displayed:



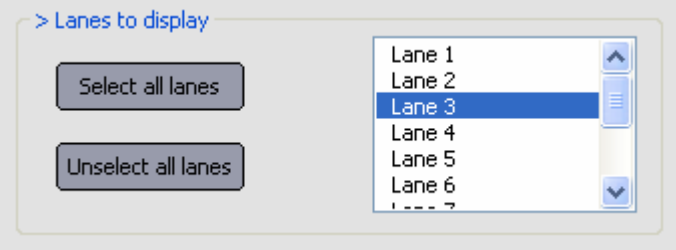



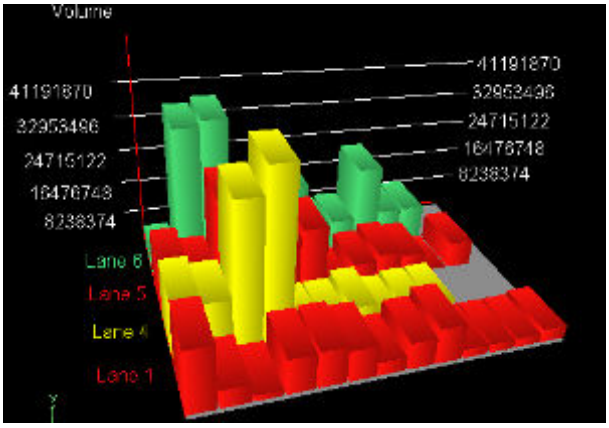

Note: To reposition the 1D profile window, position your cursor at the top of the box. The cursor appearance will change to a multidirectional arrow symbol. You can then drag the box to a new position.
Note: To increase the size of the 1D profile window, position your cursor at the edge of the window and click and drag to extend its size.
Note: Click on Print to print the 1D profile window
Note: Click on Send to clipboard to save the graph in the Windows ® clipboard (Past/copy Windows ® feature).

The 3D histogram displays the three-dimensional histogram of selected results:

- ⇨ Volume
- ⇨ Calculated quantities
- ⇨ Maximum intensities

To proceed, click on the 3D Histogram button and select the lanes to be displayed:

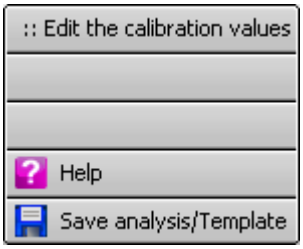




Note: To reposition the 1D profile window, position your cursor at the top of the box. The cursor appearance will change to a multidirectional arrow symbol. You can then drag the box to a new position.
Note: To increase the size of the 1D profile window, position your cursor at the edge of the window and click and drag to extend its size.
Note: Click on Print to print the 1D profile window
Note: Click on Send to clipboard to save the graph in the Windows ® clipboard (Past/copy Windows ® feature).

## OPTION FOLDER

The option folder gathers the following functions:

- ⇨ Edit the calibration values
- ⇨ Help
- ⇨ Save the analysis or the template




## EDIT THE CALIBRATION VALUES

Click on the “Edit the calibration values” button.

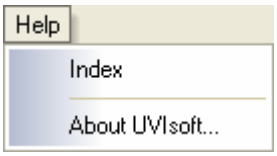

A pop-up window displays the following menu on which you can modify the calibration values:


You can add, remove, and save your marker’s value;

**HELP MENU**

Click on the “Help” button. You automatically access the user manual at the chapter corresponding to the function


You can access the help file index through the File\Help from the Menu bar




**SAVE ANALYSIS / TEMPLATE**

This function saves the current analysis. The analysis file will contain the results, the image and all the parameters defined to obtain the results.

The analysis could also be saved as a template for automated analysis routines. Template offers the user the ability to automate many of the repetitive tasks associated with analysis and processing. As a result, you can spend more time evaluating and analysing results, and less time manipulating set-ups, variables and other settings.

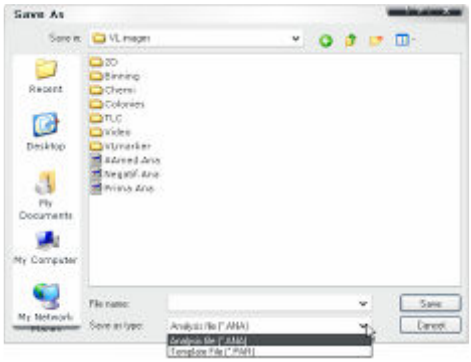
The template automates a task or set of tasks that you perform repeatedly or on a regular basis. It stores all the analysis commands and parameters of an analysis. You can run these parameters with another image whenever you need to perform a new analysis based on the same parameters.

The benefits of the template file are as follows:

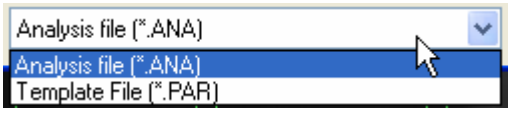
⇨ Time saving
⇨ Reproduction of image analysis parameters
⇨ Templates are modifiable, allowing the user to maintain an original template while modifying it for a slightly different result, with minimal effort

Click on the “Save analysis/ Template” button:

A pop-up window displays the following menu:

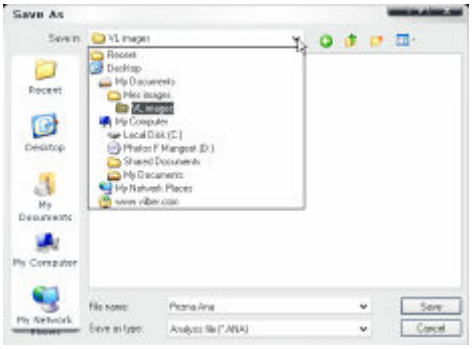


Select analysis file or template file:

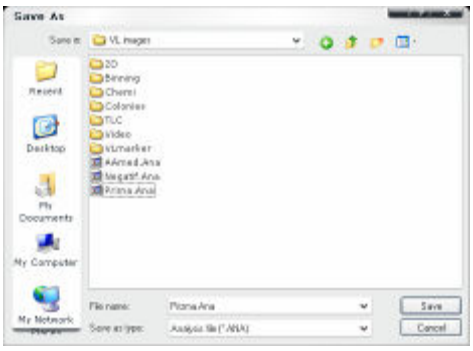



Note: the software proposes Analysis file by default

Browse to specify the file directory



Enter the desired file name and validate



Click on the Save button to create the file.

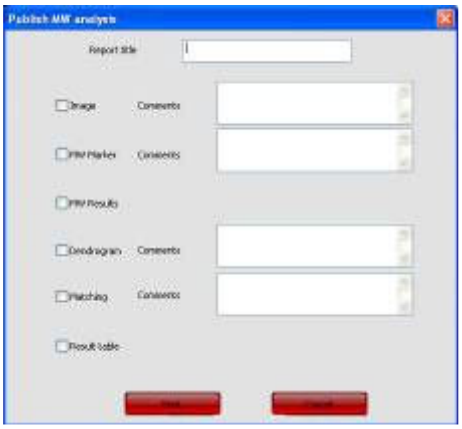
Note: see “Access to the analysis module” chapter for template or analysis file loading

# Publish

## ➔ Introduction

The purpose of the Publish function is to prepare a printed report of your results. You can easily organise your report with titles and comments and your own selection of data to be published among the following:
- Sample image
- Molecular weight marker's data and migration curve
- Molecular weight result table
- Dendrogram results
- Lane matching results
- Quantification result table

To proceed, select the Publish tab. A pop-up window displays the following menu:



⇨ Enter a report title if any
⇨ Select the options to be printed
⇨ Add comments or not per option

Click on the "Print" button. A pop-up window displays the following menu

⇨ Select a printer
⇨ If necessary, click on Properties to modify the default setting of the printer,
⇨ Select the number of copies
⇨ Click on OK to validate your options

# Return to Home

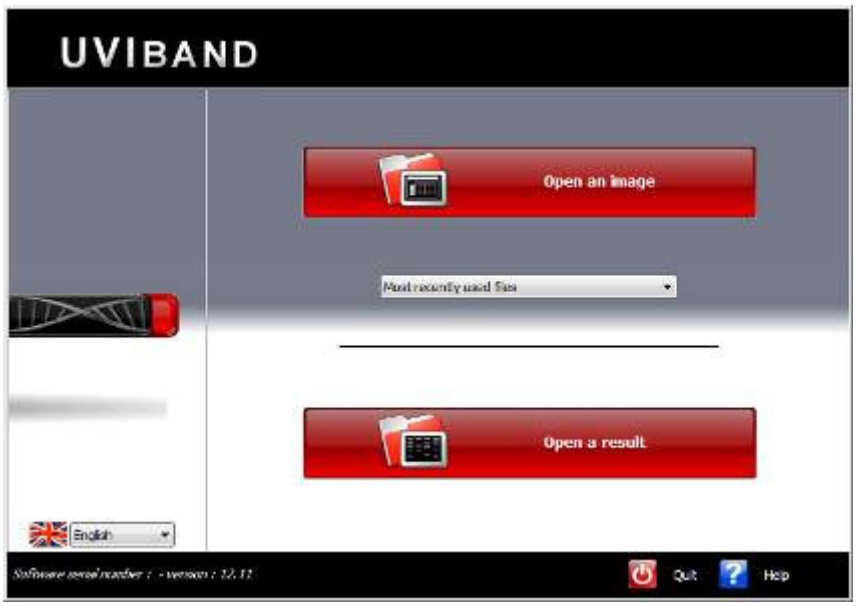
## ➔ Introduction

The home dashboard is the hub to other functions of the software:

- ⇨ Open another image file or another result file
- ⇨ Select another analysis module
- ⇨ Exit the software


## ➔ Load another image

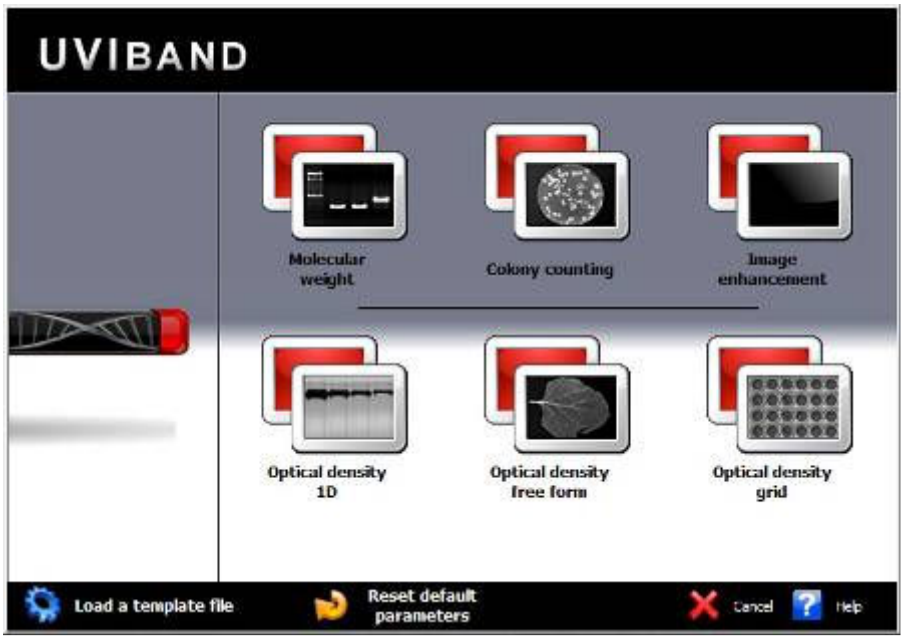
To return to the main menu, click on the home icon. A new menu appears with the main menu task bar functions:




- ⇨ Click on the “Open an image” icon to open an image
- ⇨ Click on the “Open a result” icon to open a previously saved analysis result

## ➔ Select another function

To return to the analysis menu, click on the analysis icon. A new menu appears with the analysis module task bar functions:




Click on the appropriate icon to select an analysis module.

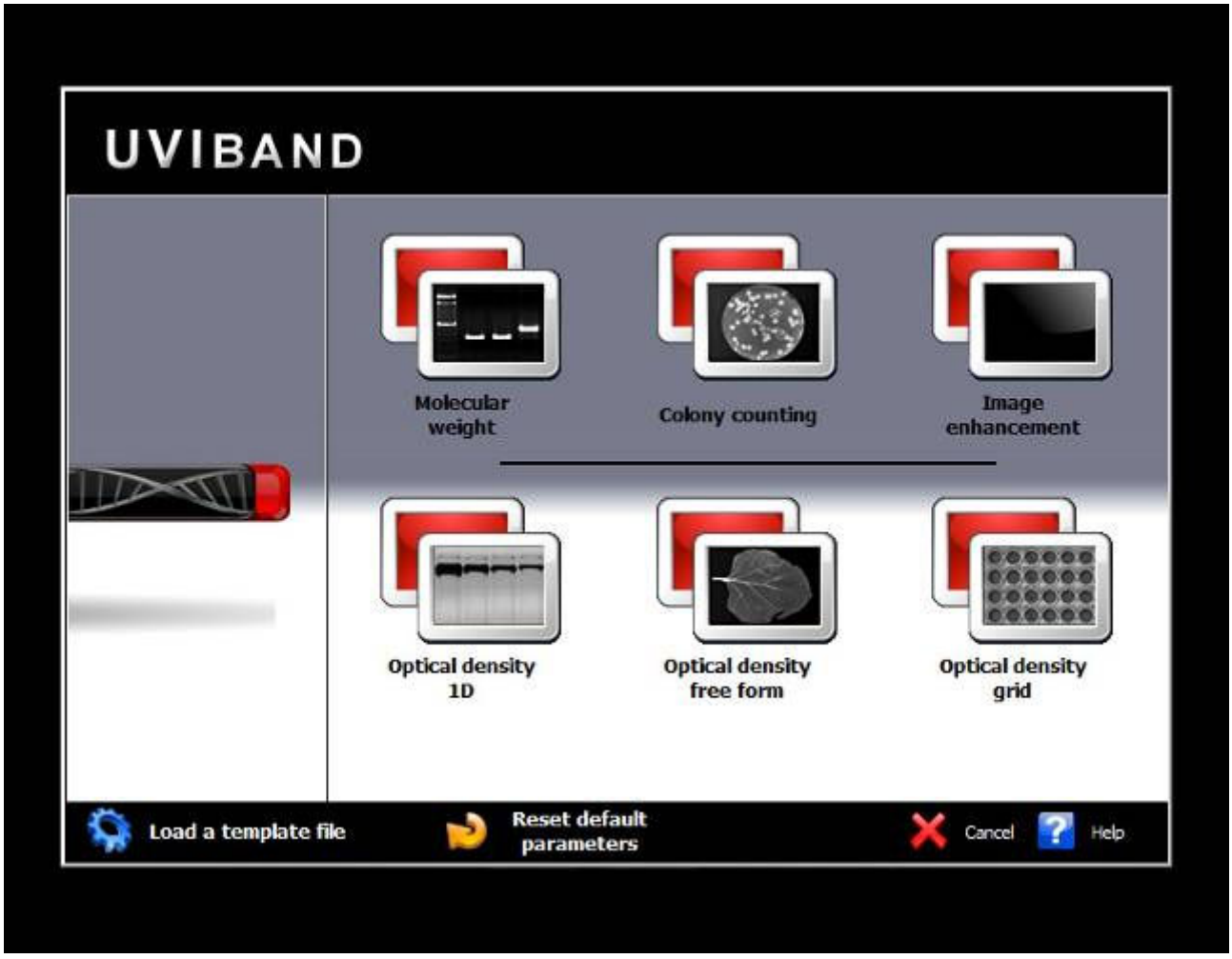
- ⇨ Select the Molecular weight icon to open the molecular weight analysis (MW) module
- ⇨ Select the Colony counting icon to open the colony counting (CC) analysis module
- ⇨ Select the Optical density - 1D icon to open the optical density (OD) analysis module based on a 1D detection
- ⇨ Select the Optical density - Free form icon to open the optical density (OD) analysis module based on a free form detection
- ⇨ Select the Optical density - Grid icon to open the optical density (OD) analysis module based on a grid detection
- ⇨ Select the Image enhancement icon to open the image enhancement module

## ➔ Exit the software

To close UVIband Advanced, select Exit from the File menu.

You will be prompted to save your analysis.

UVITEC
Cambridge




# Optical density – 1D
## ➔ OD-1D Analysis module

# Optical density / 1D introduction

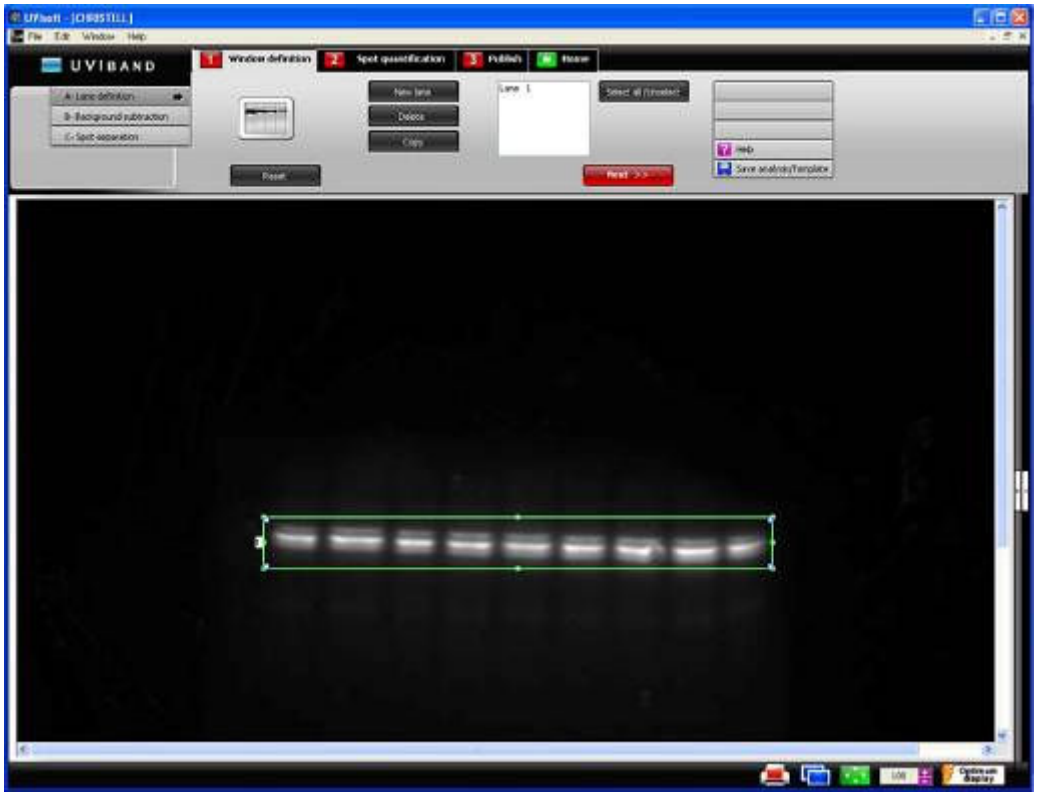
## ➔ Objectives and output

The UVIband Advanced Optical density /1D module features the quantification of spot in volume, percentage or µg.

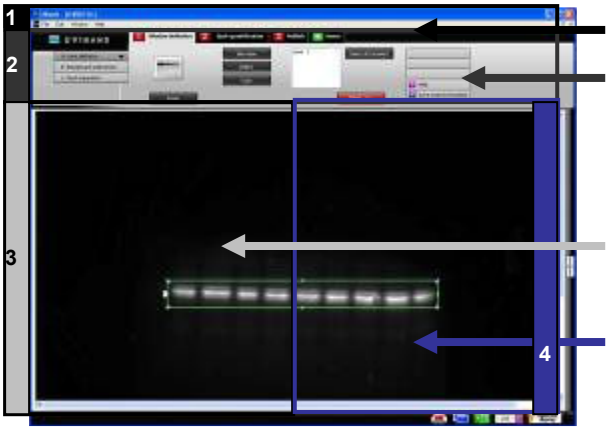
At the end of the process, you can have the following outputs:
- Lane's volume and concentration
- 3D profile
- 3D result's graph
- Calibration curve

## ➔ Optical density / 1D (OD-1D) operating environment

The OD-1D module opens on the following window:




The UVIband Advanced operating environment is organised into four areas:




1. The menu bar
2. The dash board
3. The image window
4. The working window

The menu bar contains the following menu:

- ⇨ File
- ⇨ Edit
- ⇨ Windows
- ⇨ Help


The dash board contains three different tabs:

1. Window definition
2. Spot quantification
3. Publish
4. Home


The image window displays the active image:

It also contains the image toolbar:


 ⇨ Save a screen capture of the view

 ⇨ Print

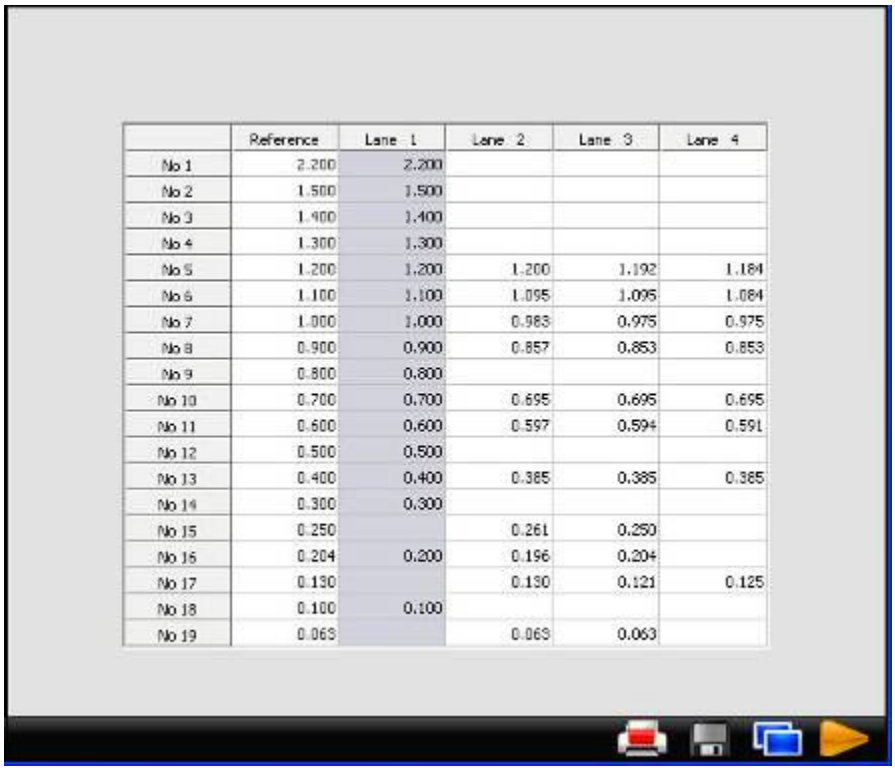
 ⇨ Copy to clipboard

 ⇨ Autoscale

⇨ Zoom in or out the image

⇨ Change the optimum display

The working window displays the graphs and tables related to the active analysis:



| | Reference | Lane 1 | Lane 2 | Lane 3 | Lane 4 |
|---|---|---|---|---|---|
| No 1 | 2.200 | 2.200 | | | |
| No 2 | 1.500 | 1.500 | | | |
| No 3 | 1.400 | 1.400 | | | |
| No 4 | 1.300 | 1.300 | | | |
| No 5 | 1.200 | 1.200 | 1.200 | 1.192 | 1.184 |
| No 6 | 1.100 | 1.100 | 1.095 | 1.095 | 1.084 |
| No 7 | 1.000 | 1.000 | 0.983 | 0.975 | 0.975 |
| No 8 | 0.900 | 0.900 | 0.857 | 0.853 | 0.853 |
| No 9 | 0.800 | 0.800 | | | |
| No 10 | 0.700 | 0.700 | 0.695 | 0.695 | 0.695 |
| No 11 | 0.600 | 0.600 | 0.597 | 0.594 | 0.591 |
| No 12 | 0.500 | 0.500 | | | |
| No 13 | 0.400 | 0.400 | 0.385 | 0.385 | 0.385 |
| No 14 | 0.300 | 0.300 | | | |
| No 15 | 0.250 | | 0.261 | 0.250 | |
| No 16 | 0.204 | 0.200 | 0.196 | 0.204 | |
| No 17 | 0.130 | | 0.130 | 0.121 | 0.125 |
| No 18 | 0.100 | 0.100 | | | |
| No 19 | 0.063 | | 0.063 | 0.063 | |

It also contains the working window toolbar:

⇨ Display the molecular weight on the image

⇨ Save the graph or the table

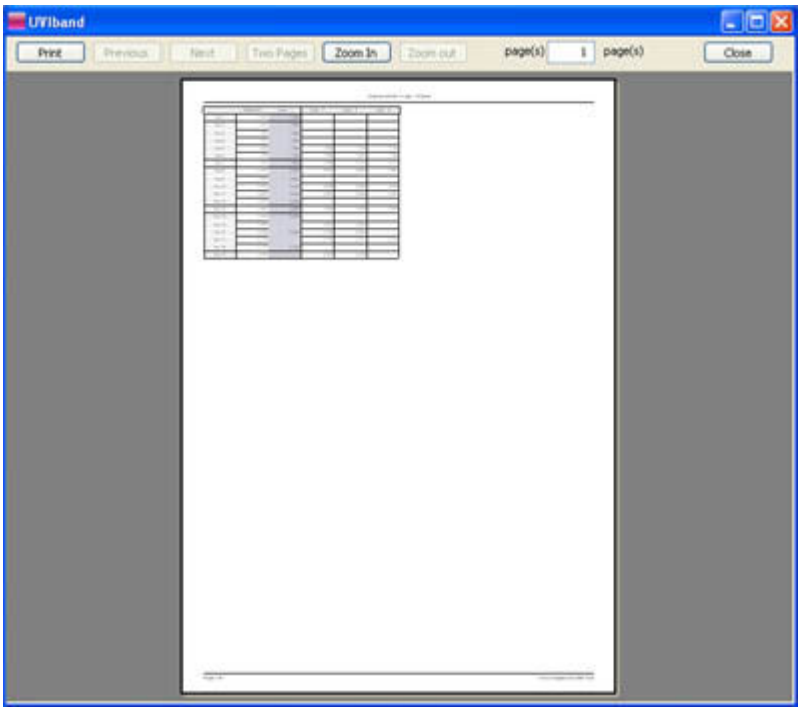
⇨ Copy the graph or the table to clipboard

⇨ Export the table to Excel

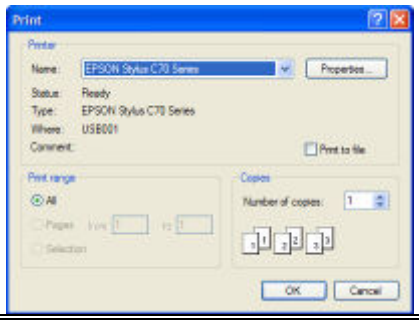
## ➔ Toolbar in details

**Print**

1. Click on the “Print” icon to print the image, the table or the graphs. A pop-up window displays the Print preview: The Print preview displays a preview of the image, as it will be printed.




2. Click on Print to validate the preview. A pop-up window displays the following menu:



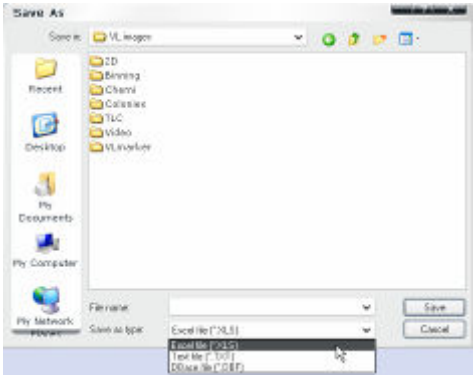
⇨ Select a printer
⇨ Click on Properties to modify the default setting of the printer, if necessary
⇨ Select the number of copies
⇨ Click on OK to validate your options

Note: You can also access the Print menu from the Menu bar (File\Print).

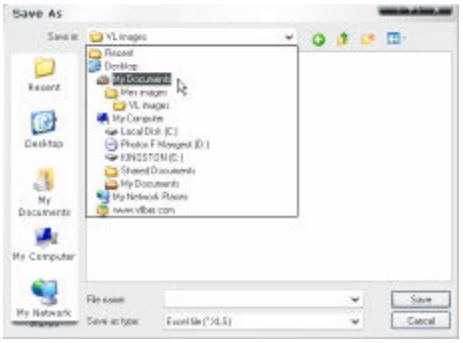
**Save**

This function saves a graph or a table. The tables are saved in a Excel™ file format (*.xls). The graphs are saved in a Bitmap format (*.bmp).

1. Click on the “Save” icon.
2. A pop-up window displays the following menu:



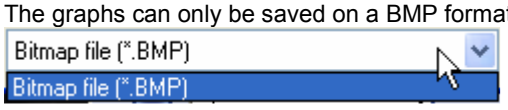

3. Browse to specify the file directory




4. Enter the desired file name, select a file extension and validate

Note: the results could also be saved in a text file format or a Dbase file format:


The graphs can only be saved on a BMP format:



| | |
|---|---|

## Copy to clipboard

This function copies an image, a table or a graph onto the clipboard for insertion into another program. This option is identical to the Windows® [Ctrl C] command.

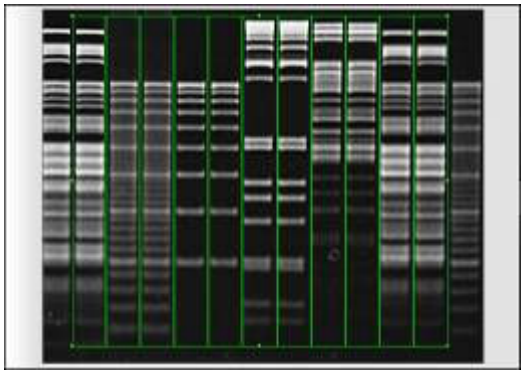
1. To proceed, click on the Copy to clipboard icon. The image, the table or the graph is now ready to be pasted into another application.

2. Open the application that you want to paste the image into, and select from the available pasting options ([Ctrl V] command for Windows® software).

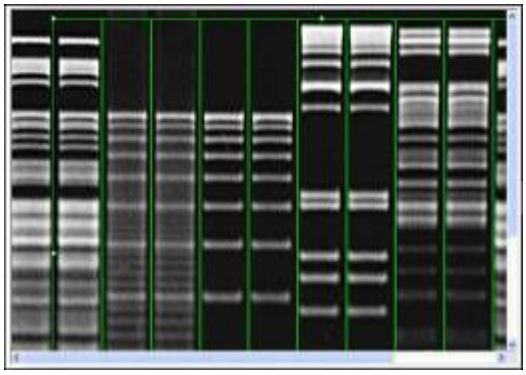
## Auto-scale

1. Click on the “Auto-scale” to resize the image to fit the size of the monitor.

The Auto-scale feature proportions the display of the image to the screen resolution.



Auto-scale (no scroll bar)



No auto-scale (scroll bar)


**Optimum display (for 12, 14 and 16-bit image file)**

The optimum display window is helpful to modify the greyscale selection to enhance the image display: To proceed, click on the “Optimum display” icon. A pop-up window displays the following menu:

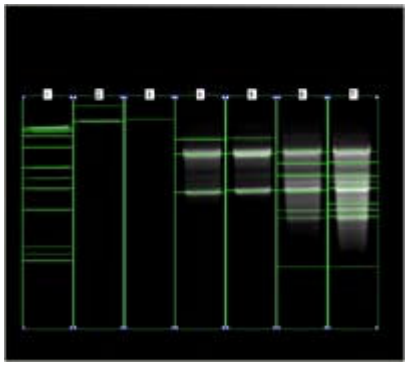
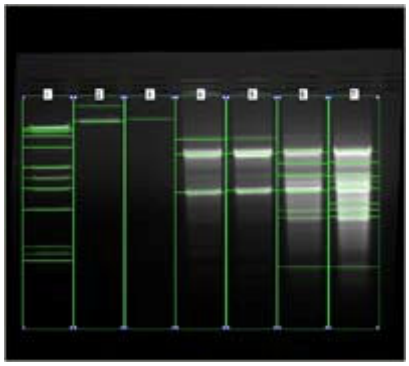
Some images has a 12, 14 or 16-bit format and Windows® can only display 8-bit images (256 grey levels).

Due to this limitation, the UVIband Advanced software handles two images:
⇨ A “memory” image corresponding to the 12, 14 or 16-bit format (4 096, 16 384 or 65 536 grey levels)
⇨ A “display image” corresponding to the image displayed on the screen (256 grey levels)

The easiest way to calculate the “display image” would be to translate the full grey scale each time an image is acquired: the x grey levels values of the “memory” image corresponds to 256 values in the displayed image. In that case, it won’t be possible to visualise faint spots on a dark image.

UVIband Advanced offers the possibility to select the grey level range to translate for the display image calculation. All the grey levels under the “Min value” defined will be converted to 0 (Black) in the displayed image. All the grey levels upper the “Max Value” defined will be set to 255 (White) in the displayed image. The grey levels between those two limits will be converted in an intermediate grey level value following a linear rule.

For both values, you can:
⇨ Edit the value in the corresponding field
⇨ Select the value by dragging and dropping the arrow
⇨ Click on the “optimum display” button: UVIband Advanced will then calculate the ideal values to be selected according to the parameters defined

| | |
|---|---|
|  |  |
| Automatic optimum display | Optimum display enhancement<br>The image appears brighter. The faint bands are more visible. |

Note: The optimum display has no impact on the analysis. Only the display of the image is modified.

**Send to Excel™**

This function transfers the results table to Windows Excel™.

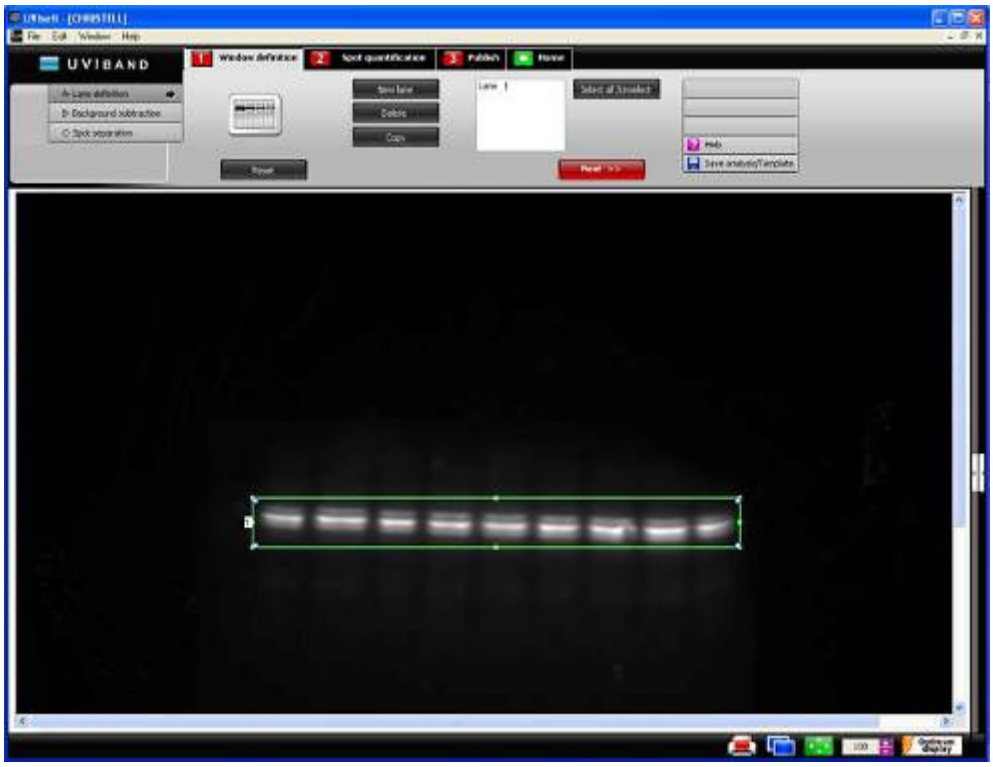
To proceed, click on the Send to Excel™ icon. The Excel software is automatically opened by the UVIband Advanced and the table is transferred to Excel™.

# 1- Window definition

## ➔ A – Lane definition

1

The Optical Density / 1D module opens on the Lane definition dashboard of the Window definition process:

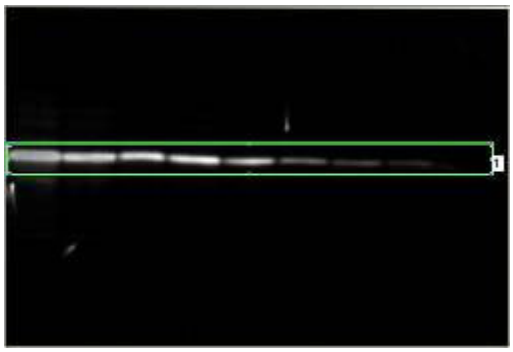



The dashboard details the lane definition parameters:

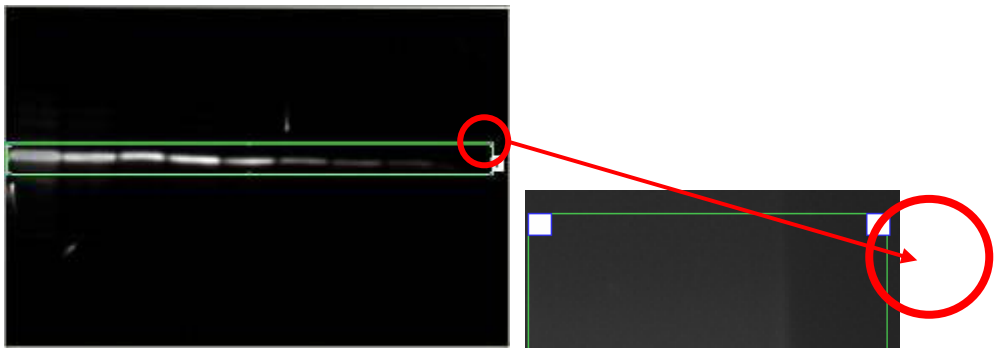

- ⇨ New lane
- ⇨ Delete
- ⇨ Copy
- ⇨ Select / unselect all lanes

**DEFINE A NEW LANE**

On the image, click on the top left corner of the lane, then drag to define the size of the analysis area. You can easily adjust the size of the area by clicking on the tags surrounding the area and drag the selected border to the requested size.

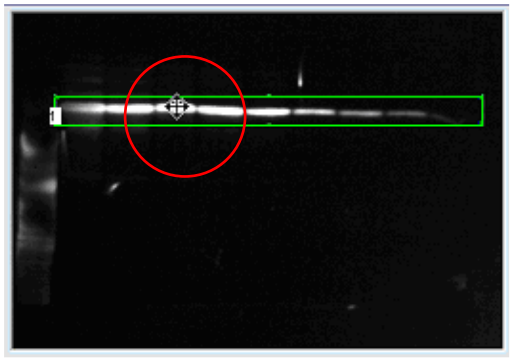


The lane is defined by green lines, overlaid on the image. The area is surrounded by square anchors:



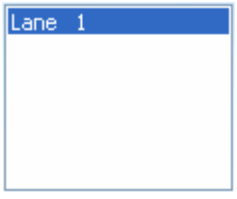
To resize the entire lane frame, drag an anchor point in or out. The opposite anchor point will remain fixed while the frame expands or contracts. The frame will expand or contract from the centre.

To move the entire frame to a new position, position the mouse on the frame to obtain a cross cursor: Click and drag the cursor to move the entire frame.

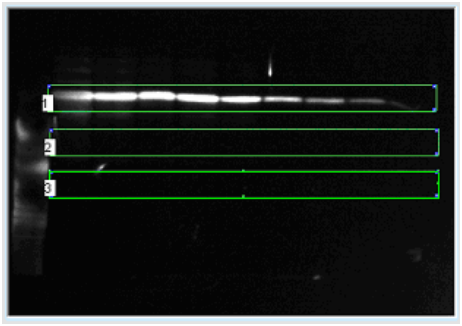


**COPY A LANE**

To copy a lane, select the lane in the lane list:



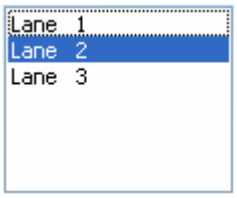

Click on the Copy button. The lane is then duplicated:



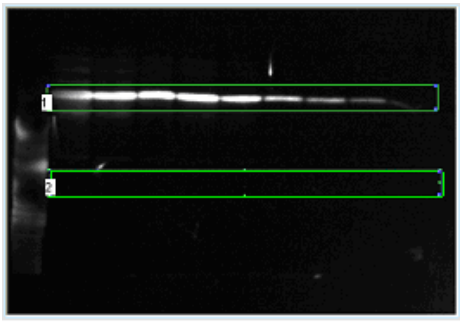
The number of lanes is automatically modified in the lane list.
You can move the lane frame to a new position. In order to do so, position the mouse on the. Click and drag the cursor to move the frame.

**DELETE A LANE**

To copy a lane, select the lane in the lane list:




Click on the Delete button. The lane is then deleted.



The number of lanes is automatically modified in the lane list.
You can move the lane frame to a new position. In order to do so, position the mouse on the. Click and drag the cursor to move the frame.

**NEXT**

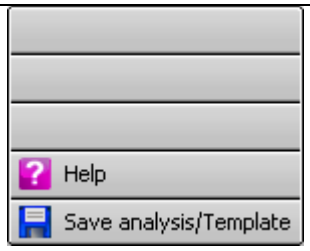
The “Next” button validates your parameter and opens the following analysis step.

| | | |
|---|---|---|
| 1 A – Lane definition | Next >> | 1 B – Background subtraction |

**OPTION FOLDER**

The option folder gathers the following functions:

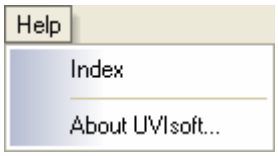
⇨ Help
⇨ Save the analysis or the template




**HELP MENU**

Click on the “Help” button. You automatically access the user manual at the chapter corresponding to the function


You can access the help file index through the File\Help from the Menu bar:




**SAVE ANALYSIS / TEMPLATE**

This function saves the current analysis. The analysis file will contain the results, the image and all the parameters defined to obtain the results.

The analysis could also be saved as a template for automated analysis routines. Template offers the user the ability to automate many of the repetitive tasks associated with analysis and processing. As a result, you can spend more time evaluating and analysing results, and less time manipulating set-ups, variables and other settings.

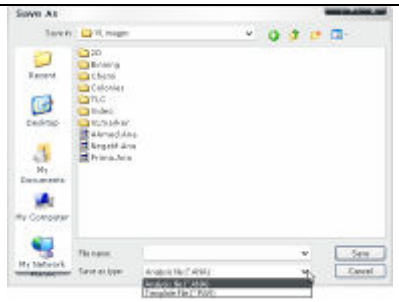
The template automates a task or set of tasks that you perform repeatedly or on a regular basis. It stores all the analysis commands and parameters of an analysis. You can run these parameters with another image whenever you need to perform a new analysis based on the same parameters.

The benefits of the template file are as follows:

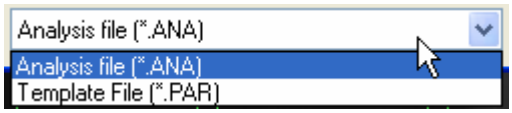
⇨ Time saving
⇨ Reproduction of image analysis parameters
⇨ Templates are modifiable, allowing the user to maintain an original template while modifying it for a slightly different result, with minimal effort

1. Click on the “Save analysis/ Template” button:


2. A pop-up window displays the following menu:

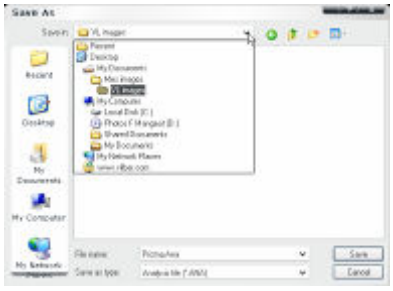


3. Select analysis file or template file:

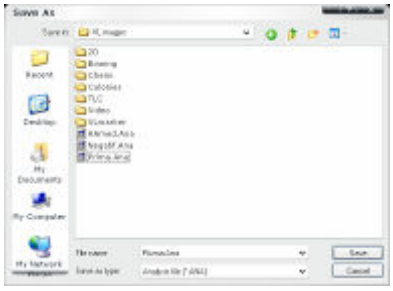



Note: the software proposes Analysis file by default

4. Browse to specify the file directory



5. Enter the desired file name and validate



6. Click on the Save button to create the file.

Note: see “Access to the analysis module” chapter for template or analysis file loading

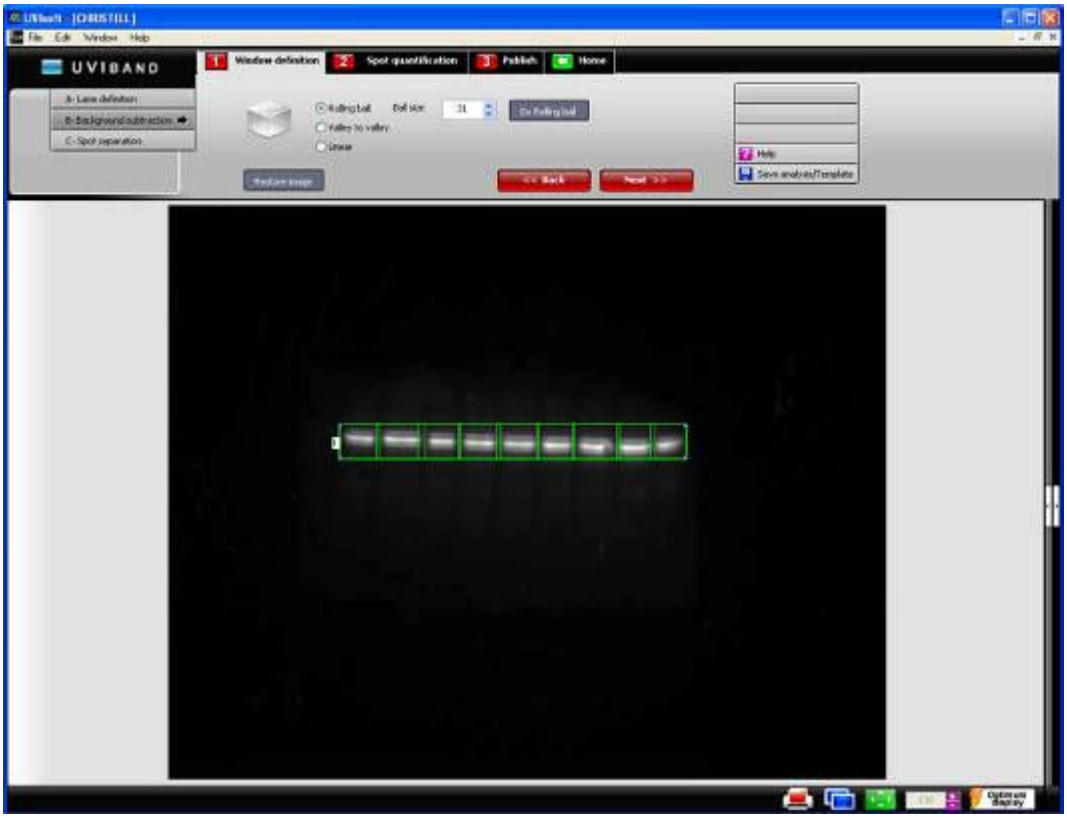
## ➔ B – Background subtraction

1

The background subtraction process follows lane definition.

Image background interferes with quantification and data analysis. To this extend, we recommend to perform a background subtraction before any peak volume quantification. The subtraction is automatically done on the analysis area.

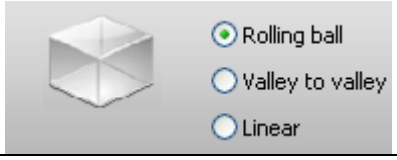
Note: As background subtraction permanently changes the image, this is not possible to save the image with a processed background subtraction. However, the process can be saved by saving the complete analysis through the Save analysis process.



The dashboard details the matching parameters:


UVIband Advanced has several functions to minimise image background.

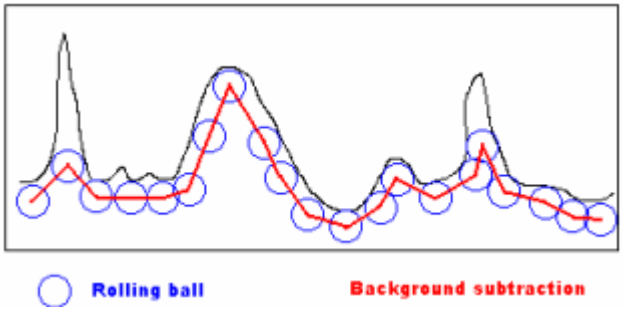
⇨ The rolling ball approach
⇨ The valley to valley approach
⇨ The linear approach



**ROLLING BALL**

The rolling ball method is named for a hypothetical ball that rolls along underneath the lane profile, removing different intensity levels along the length of the lane.

The ball is rolled under each profile of the image so its movement varies along the image.




The centre of gravity of the ball describes a curve:

⇨ This curve represents the noise to be subtracted.

⇨ The curve depends on the size of the ball and on the size of the peaks.

The size of the ball will affect the position and movements of the centre of gravity and thus it determined the level of background subtraction. A small disk will make a large background subtraction and a large disk the contrary. A disk radius that is too small may subtract almost all image data.

The UVIband Advanced calculates automatically the ideal parameter for background subtraction. This could be manually modified by adjusting the spot size:


To process the rolling ball background subtraction, click on the “Do rolling ball” button:


The changes will be automatically applied to the image.

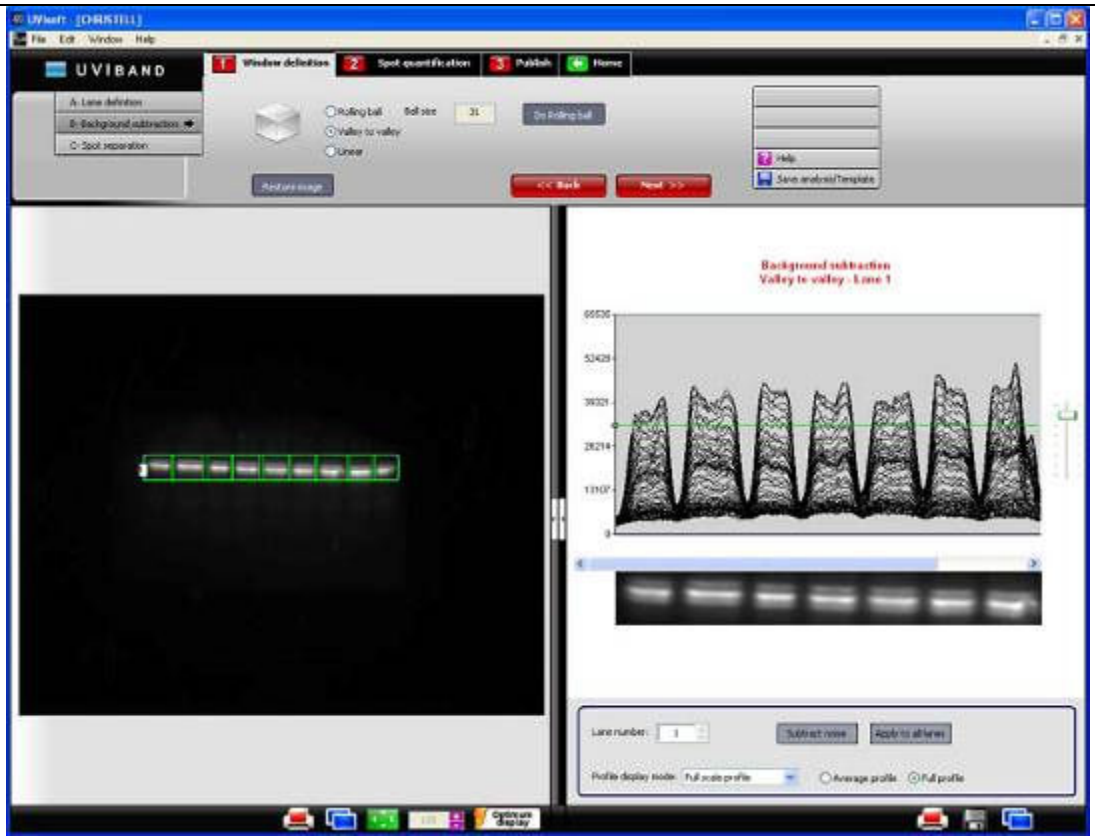
**Note**: few seconds could be necessary to perform the background subtraction.

**VALLEY TO VALLEY**

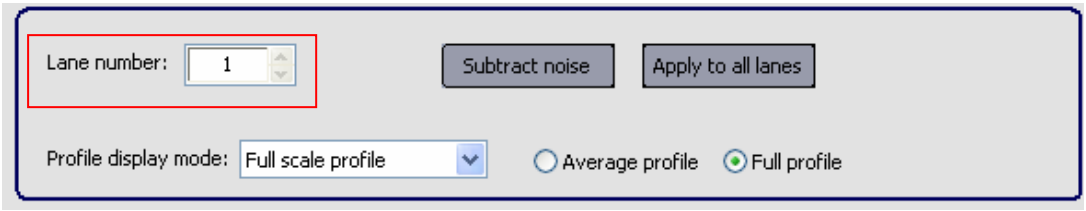
The valley-to valley approach is a lane-based background subtraction. It allows to manually define on the lane profile the level of noise to be subtracted.

1. Click on the “Valley to valley ” button:

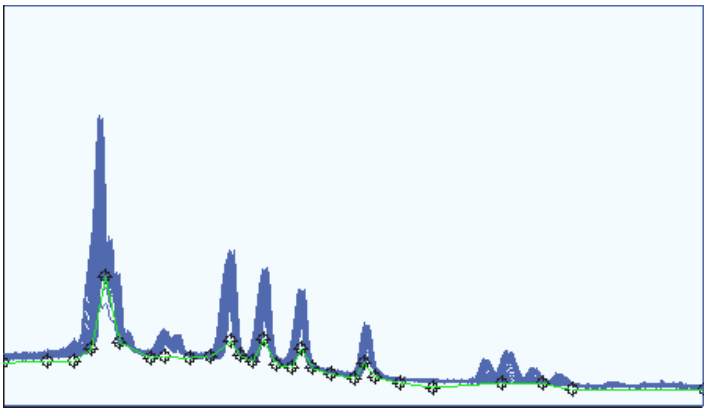

It opens the lane profile window:




2. In the profile parameters window, select the lane to perform the valley-to-valley approach

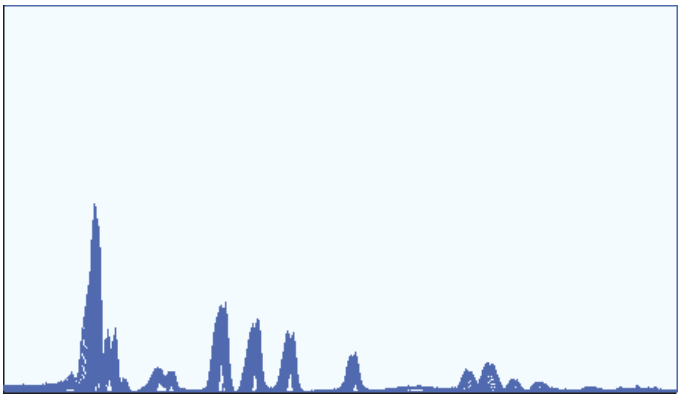



On the profile, click to define the background profile you want to remove:



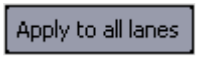
Then, click on Subtract noise:


The changes will be automatically applied to the image and to the profile:

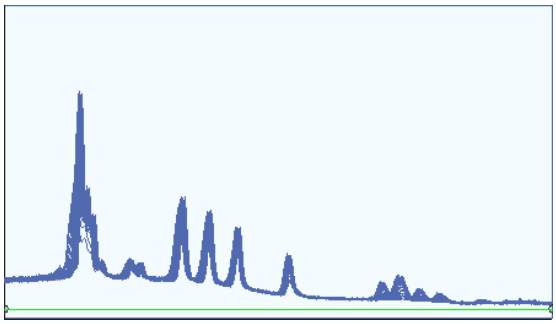
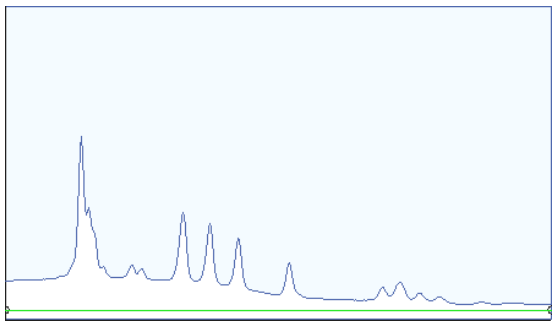
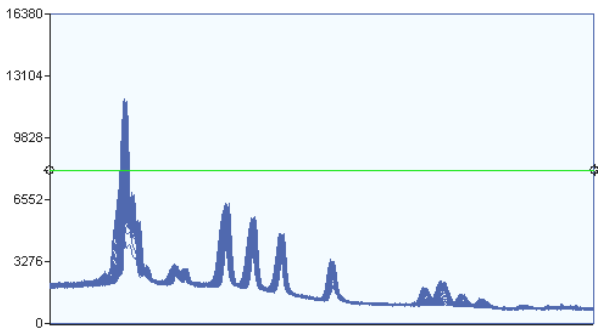


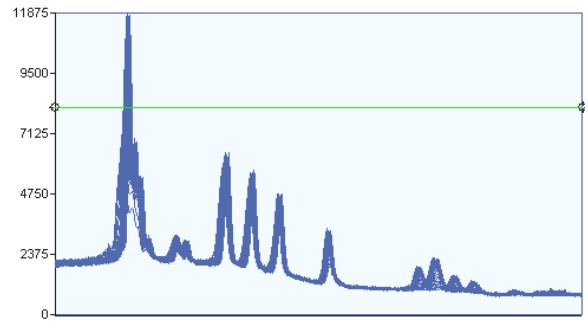
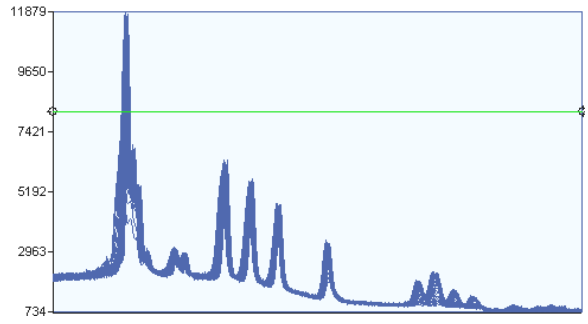
The valley-to-valley approach is a lane-based background subtraction. You can set the same subtraction level for all lanes or specify an individual subtraction level for the selected lane. Any changes you make will be automatically applied to the image.

To apply the same subtraction level for all lanes, click on the “Apply to all lanes” button:




You can easily adjust the profile displays settings as follows:

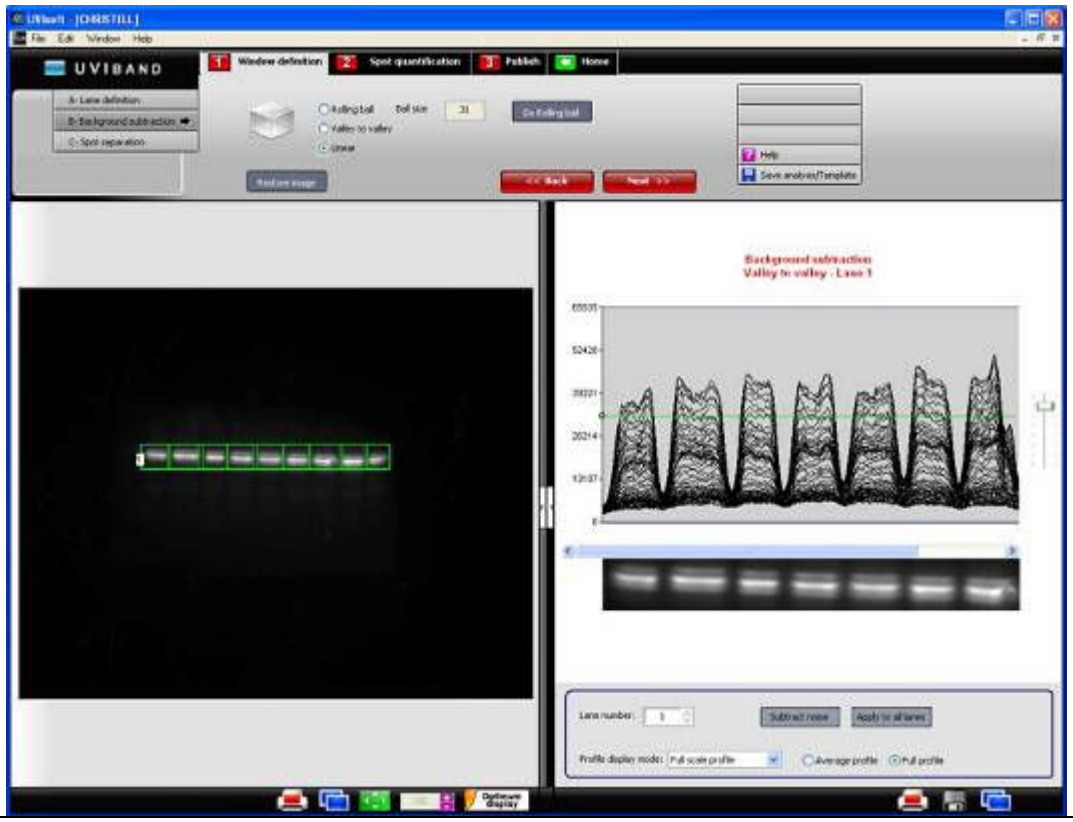
| | |
|---|---|
|  |  |
|  |  |
|  <br>  <br>The profile scale goes from 0 to the image maximum dynamic. |  |

| | |
|---|---|
| Profile display mode:<br>0 to Maximum<br><br>The profile scale goes from 0 to the lane's maximum intensity; |  |
| Profile display mode:<br>Minimum to Maximum<br><br>The profile scale goes from the lane's minimum intensity to the lane's maximum intensity; |  |

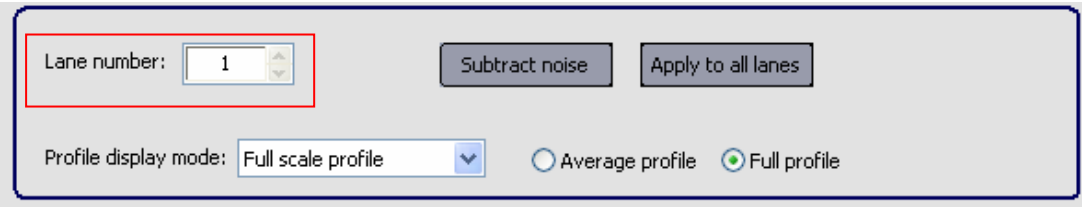
**LINEAR APPROACH**

The linear approach is a lane-based background subtraction. It allows to manually define the level of noise to be subtracted on the lane profile.
To proceed, click on the "Linear " button:

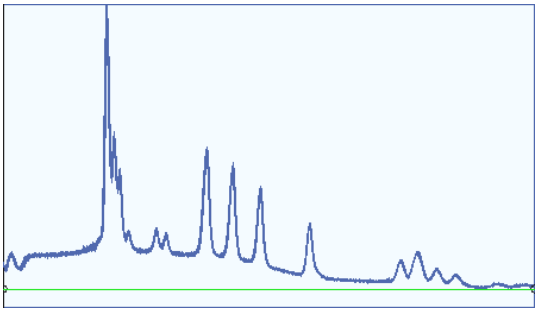

It opens the lane profile window:



In the profile parameters window, select the lane to perform the linear approach

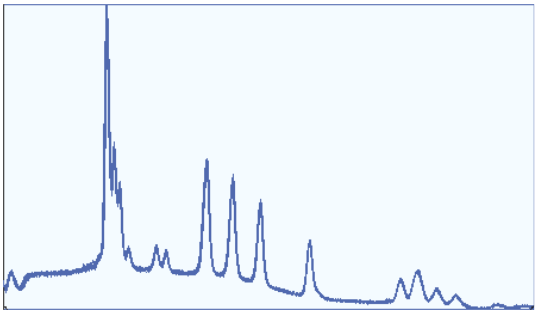



On the profile, click to define the background linear level you want to remove:



Then, click on Subtract noise: 

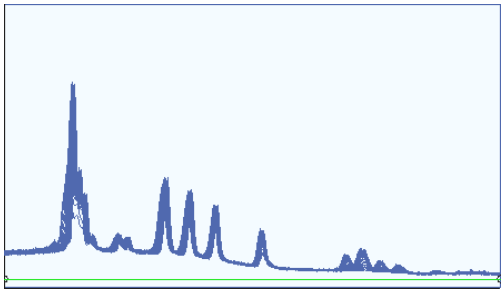
The changes will be automatically applied to the image and to the profile:

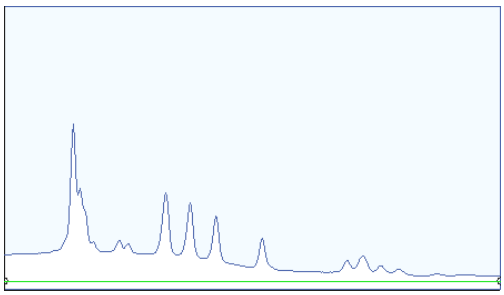
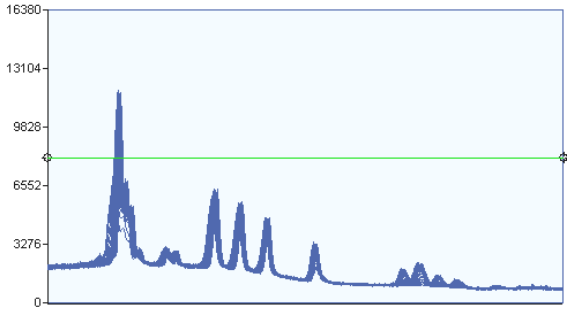
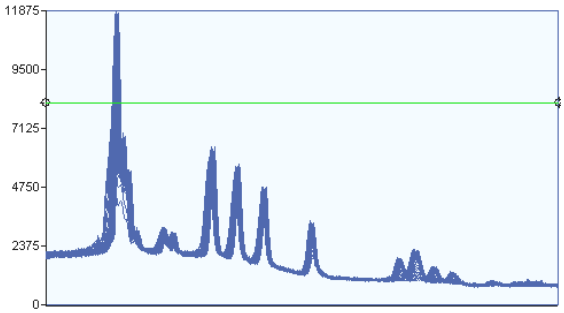
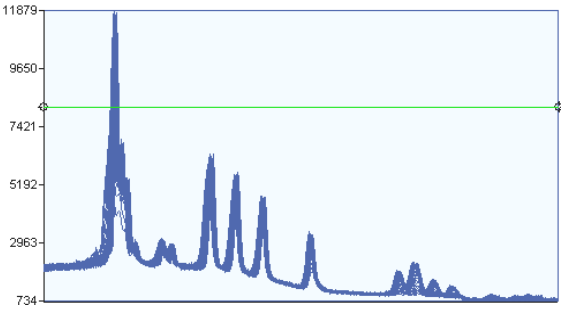


The linear approach is a lane-based background subtraction. You can set the same subtraction level for all lanes or specify an individual subtraction level for the selected lane. Any changes you make will be automatically applied to the image.

To apply the same subtraction level for all lanes, click on the “Apply to all lanes” button:


You can easily adjust the profile displays settings as follows:

| | |
|---|---|
|  |  |

| | |
|---|---|
| Average profile |  |
| Profile display mode:<br>Full scale profile<br>The profile scale goes from 0 to the image maximum dynamic. |  |
| Profile display mode:<br>0 to Maximum<br>The profile scale goes from 0 to the lane's maximum intensity; |  |
| Profile display mode:<br>Minimum to Maximum<br>The profile scale goes from the lane's minimum intensity to the lane's maximum intensity; |  |

**NEXT**

The "Next" button validates your parameter and opens the following analysis step.

| | | |
|---|---|---|
| 1 B – Background subtraction | Next >> | 1 C- Spot separation |

**BACK**

The "Back" button validates your parameter and opens the following analysis step.

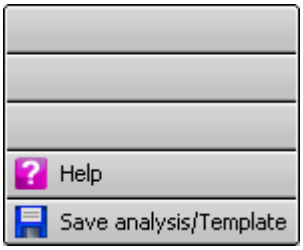
| | | |
|---|---|---|
| 1 B – Background subtraction | << Back | 1 A – Lane definition |

**OPTION FOLDER**

The option folder gathers the following functions:

⇨ Help

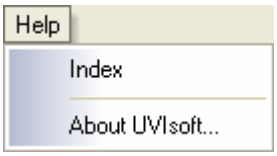
⇨ Save the analysis or the template




**HELP MENU**

Click on the “Help” button. You automatically access the user manual at the chapter corresponding to the function


You can access the help file index through the File\Help from the Menu bar




**SAVE ANALYSIS / TEMPLATE**

This function saves the current analysis. The analysis file will contain the results, the image and all the parameters defined to obtain the results.

The analysis could also be saved as a template for automated analysis routines. Template offers the user the ability to automate many of the repetitive tasks associated with analysis and processing. As a result, you can spend more time evaluating and analysing results, and less time manipulating set-ups, variables and other settings.

The template automates a task or set of tasks that you perform repeatedly or on a regular basis. It stores all the analysis commands and parameters of an analysis. You can run these parameters with another image whenever you need to perform a new analysis based on the same parameters.

The benefits of the template file are as follows:

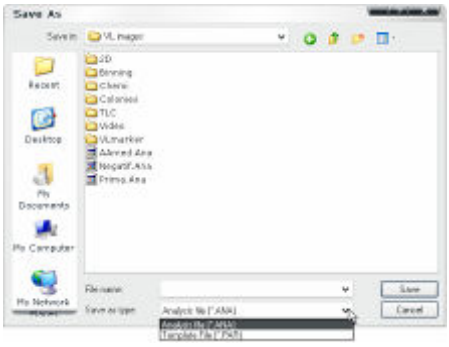
⇨ Time saving

⇨ Reproduction of image analysis parameters

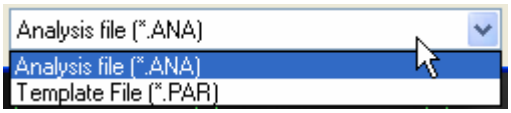
⇨ Templates are modifiable, allowing the user to maintain an original template while modifying it for a slightly different result, with minimal effort

1. Click on the “Save analysis/ Template” button:

2. A pop-up window displays the following menu:

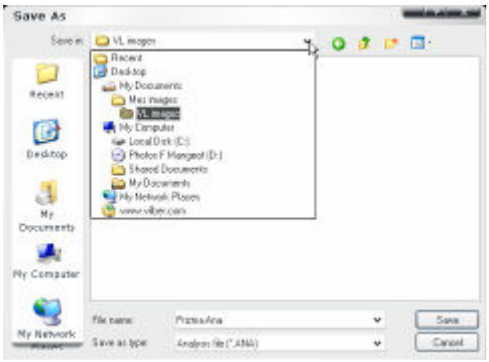



3. Select analysis file or template file:

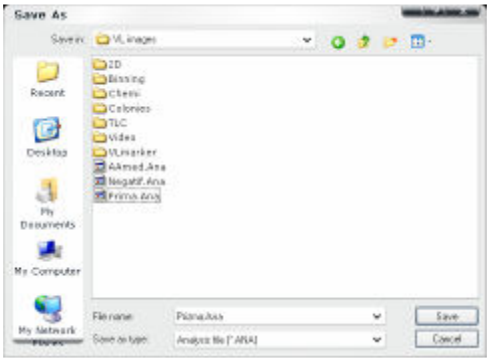



Note: the software proposes Analysis file by default

4. Browse to specify the file directory




5. Enter the desired file name and validate




6. Click on the Save button to create the file.

Note: see “Access to the analysis module” chapter for template or analysis file loading

## ➔ C – Spot separation

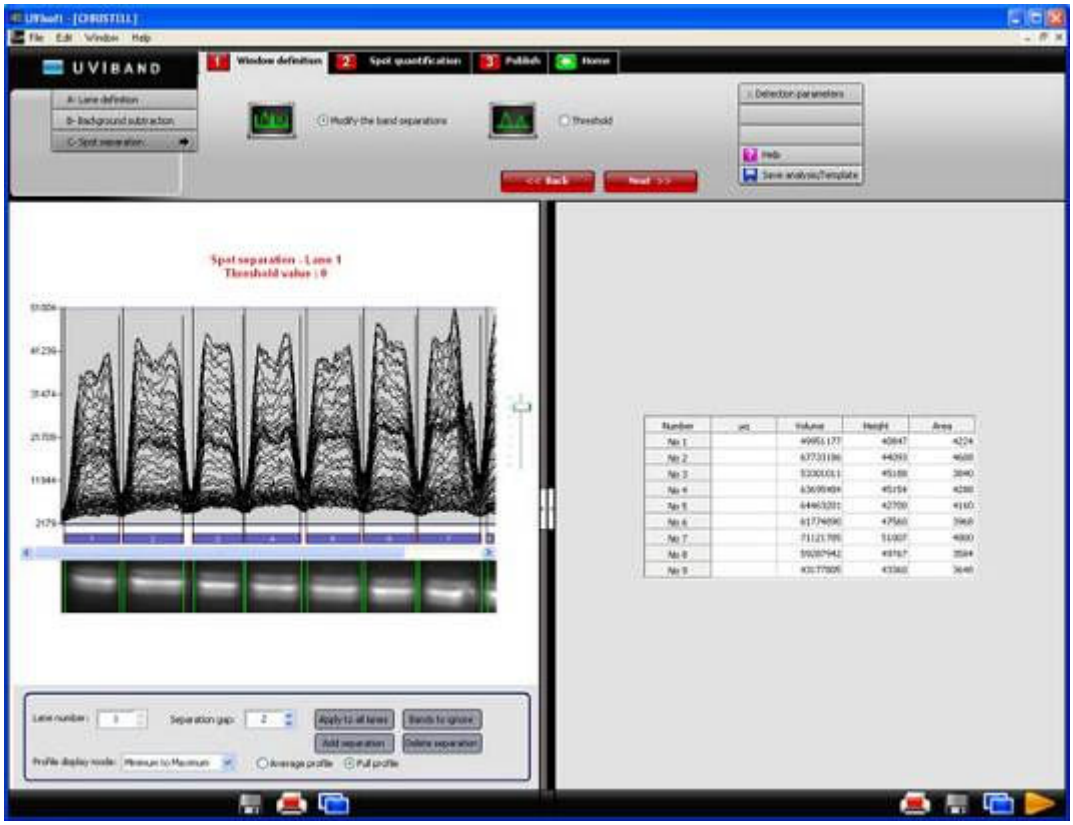
1

In order to measure the volume of a particular spot, you need:
⇨ To define the boundary around the spot;
⇨ To compare the intensity data inside the boundary with the data of other spots or of a standard.

A volume is the sum of the pixel intensity inside a defined boundary. The purpose of the spot separation is to define this boundary.

The spot separation process follows the background subtraction.

Note: you can either access the spot separation function by clicking on the next button of the background subtraction or directly by clicking on the spot separation of the Window definition folder.




The dashboard details the spot separation parameters:

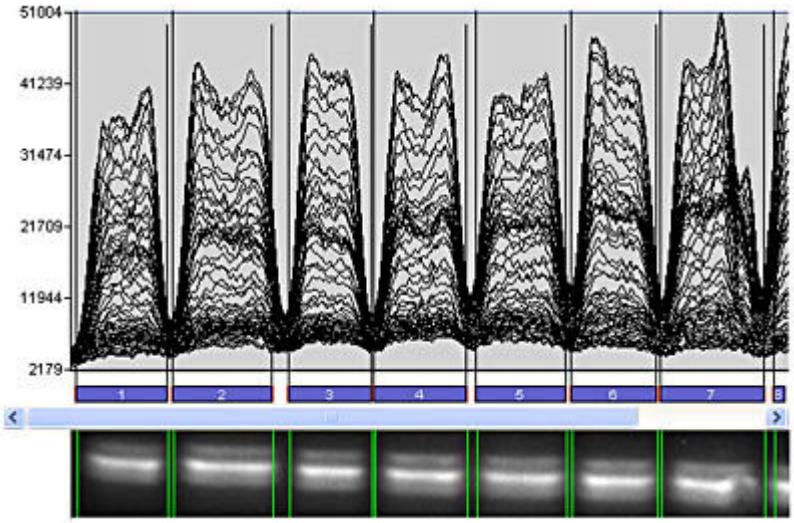

⇨ Modify the spot separation
⇨ threshold

**MODIFY THE SPOT SEPARATION**

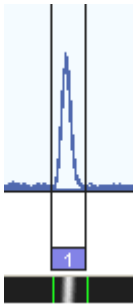
UVIband Advance proposes by default an automatic predefined spot separation based on the

band detection. You can modify the default spot separation by selecting the “Modify the spot separation” option.

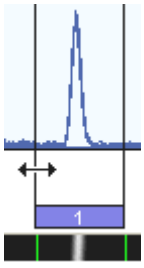

The default separation is illustrated on the lane’s profile:




The brackets illustrate the bands boundaries:



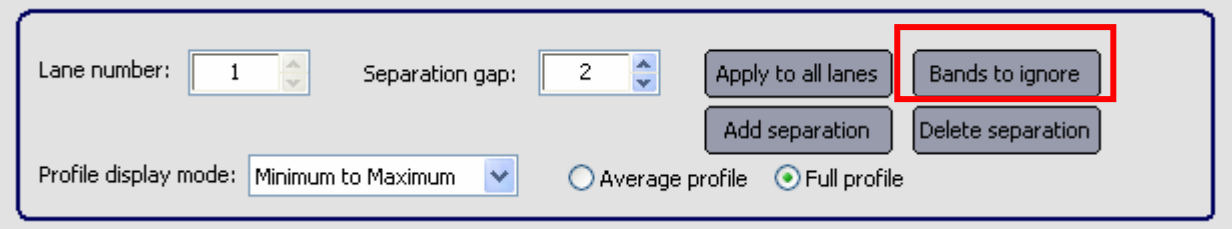

You can easily reposition a band’s boundaries. In order to do so, click on the bracket and drag the cursor:



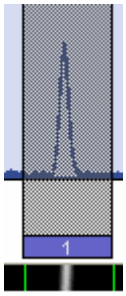

Drag the cursor until the area of the band that you want to define has been completely enclosed.

**Note**: When you release the mouse button, the band’s volume is automatically recalculated to take into account the new area of interest.

To ignore a band, select “Bands to ignore” from the profile’s parameter menu:



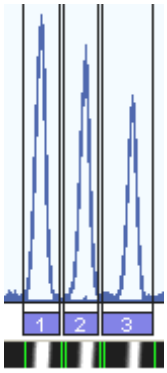

Then, click on the band you want to ignore:



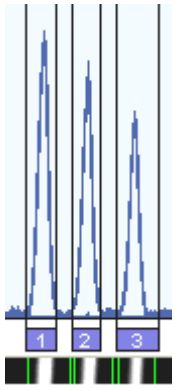

The band is then highlighted in grey and discarded from the result table:

Note: you can ignore more than one band at a time.
Note: to stop the process, click again on the “Bands to ignore” button.

To increase the gap in between the lane, select the “Separation gap” option from the profile’s parameter menu:




**Limited separation gap**



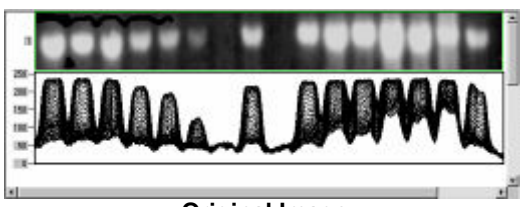

**Extended separation gap**

**DEFINE A THRESHOLD**

The threshold defines the detection level to take into account for the volume quantification. It allows to distinguish between bands and smears on the lane.

**Case when you should use detection level (Threshold)**

There is still a strong background even after the background subtraction



**Original Image**

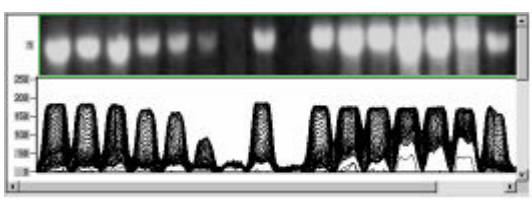


**Image with subtracted background**

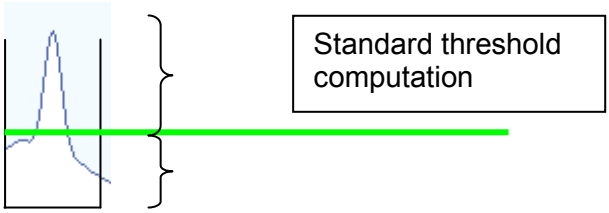
The spot contours must be isolated more precisely from the smears where they are located. The threshold calculates the volume which is above the threshold:

Threshold:

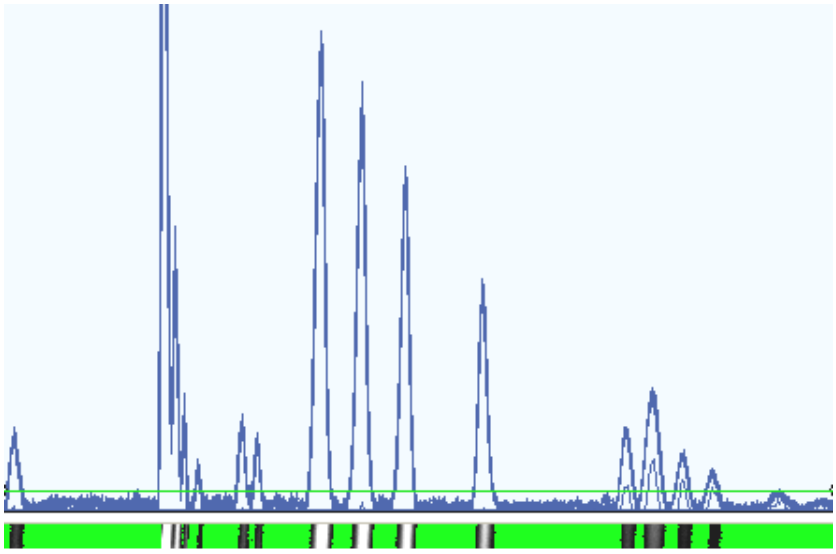
Volume= ∑ (Pixels intensities)

⇨ Pixel intensities=0 if Pixel<Threshold

⇨ Pixel intensities= (Pixel intensities) if Pixel>Threshold




Move upwards or downward the horizontal line appearing on the profile:



This displays a green contour that encloses pixels whose intensity is equal to or greater than that of the pixel at the cursor. If the contour does not encircle the band, reposition the cursor and click again. A new contour will be drawn in place of the old one.

The green area under the profile represents the range of values discarded to calculate the volume. The contour should completely surround the data you want to quantify.

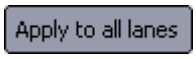
The defined threshold is automatically applied to the selected lane. The results are recalculated taking into account the threshold:

| Number | // | Volume | Height | Area | MW-RF |
|---|---|---|---|---|---|
| No 1 | | 224452 | 2173 | 126 | 31.714 |
| No 2 | | 561516 | 2110 | 294 | 24.000 |
| No 3 | | 1216111 | 2429 | 574 | 13.143 |
| No 4 | | 1072687 | 11699 | 210 | 8.812 |
| No 5 | | 388143 | 6775 | 70 | 7.391 |
| No 6 | | 373495 | 5360 | 98 | 6.459 |
| No 7 | | 531756 | 2988 | 224 | 5.767 |
| No 8 | | 1140205 | 3070 | 490 | 4.945 |
| No 9 | | 1144095 | 6172 | 392 | 3.847 |
| No 10 | | 943922 | 5602 | 350 | 2.930 |
| No 11 | | 1138471 | 4700 | 602 | 2.453 |
| No 12 | | 1235282 | 3269 | 966 | 1.987 |
| No 13 | | 385044 | 1870 | 294 | 1.417 |
| No 14 | | 401562 | 2191 | 252 | 0.973 |
| No 15 | | 243847 | 1541 | 195 | 0.774 |
| No 16 | | 213134 | 1311 | 191 | 0.573 |
| No 17 | | 4827 | 973 | 5 | 0.389 |
| No 18 | | 0 | 0 | 0 | 0.267 |

⇨ The volume is the sum of intensities included in the spot area of analysis.
⇨ The height is the maximum spot intensity, in grey levels.
⇨ The area is the zone defined for each spot area of analysis.

The threshold approach is on a lane-based basis. You can set the same threshold for all lanes or specify an individual threshold for the selected lane. Any changes you make will be automatically applied to the image.

To apply the same subtraction level for all lanes, click on the “Apply to all lanes” button:



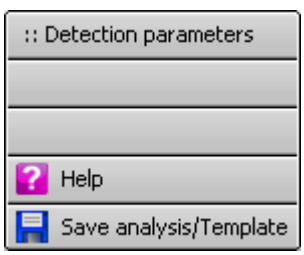

**NEXT**
The “Next” button validates your parameter and opens the following analysis step.

| | | |
|---|---|---|
| 1 C- Spot separation | Next >> | 2 A – Volume of reference |

**BACK**
The “Back” button validates your parameter and opens the following analysis step.

| | | |
|---|---|---|
| 1 C- Spot separation | << Back | 1 B – Background subtraction |

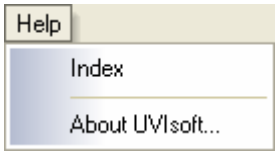
**OPTION FOLDER**
The option folder gathers the following functions:
⇨ Help
⇨ Save the analysis or the template



## HELP MENU

Click on the “Help” button. You automatically access the user manual at the chapter corresponding to the function


You can also access the help file index through the File\Help from the Menu bar




## SAVE ANALYSIS / TEMPLATE

This function saves the current analysis. The analysis file will contain the results, the image and all the parameters defined to obtain the results.

The analysis could also be saved as a template for automated analysis routines. Template offers the user the ability to automate many of the repetitive tasks associated with analysis and processing. As a result, you can spend more time evaluating and analysing results, and less time manipulating set-ups, variables and other settings.

The template automates a task or set of tasks that you perform repeatedly or on a regular basis. It stores all the analysis commands and parameters of an analysis. You can run these parameters with another image whenever you need to perform a new analysis based on the same parameters.

The benefits of the template file are as follows:

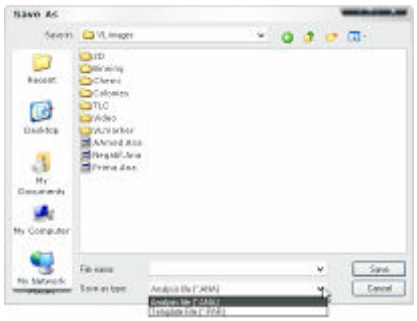
⇨ Time saving

⇨ Reproduction of image analysis parameters

⇨ Templates are modifiable, allowing the user to maintain an original template while modifying it for a slightly different result, with minimal effort

1. Click on the “Save analysis/ Template” button:

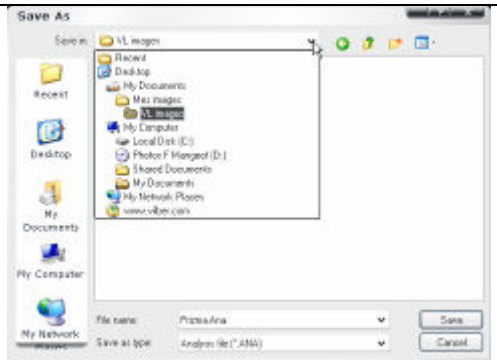

2. A pop-up window displays the following menu:



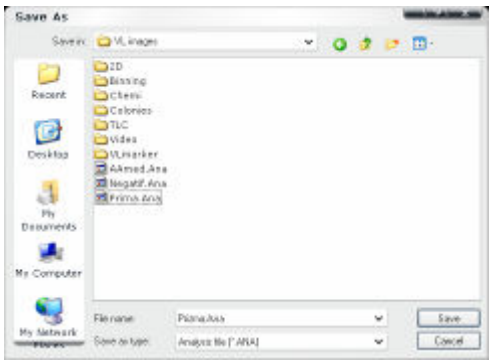
3. Select analysis file or template file:

Note: the software proposes Analysis file by default

4. Browse to specify the file directory




5. Enter the desired file name and validate




6. Click on the Save button to create the file.

Note: see “Access to the analysis module” chapter for template or analysis file loading

# 2- Analyse – Quantification

## ➔ Principles of quantification

| | |
|---|---|
| 2 | Volume is the based of the spot quantification process. The volume is the sum of all the intensities included in the defined area (window + separation).<br><br>Quantification is based on the image in pixels whose intensity is coded on a scale.<br>- The scale has 256 grey levels for a 8-bit image<br>- The scale has 4 096 grey levels for a 12-bit image<br>- The scale has 16 384 grey levels for a 14-bit image<br>- The scale has 65 536 grey levels for a 16-bit image<br><br>The quantity (or density) of a spot is calculated from its volume. This is made of the sum of all pixel intensities composing the spot<br><br>In other words, the spot quantity then depends on:<br>- The number of pixels inside the area of the spot<br>- The intensities of these points<br><br>$V = \sum ni\ Ii$<br><br>Image analysis allows comparison in between concentrated intense spots and weaker but more diffused bands.<br><br>Results are given in volumes that may be recalculated according to an OD of reference or a concentration master-curve.<br><br>To measure the amount of a particular spot, you need to define the boundary around the spot and compare the intensity data inside the boundary with the data of other spots or of a standard. |

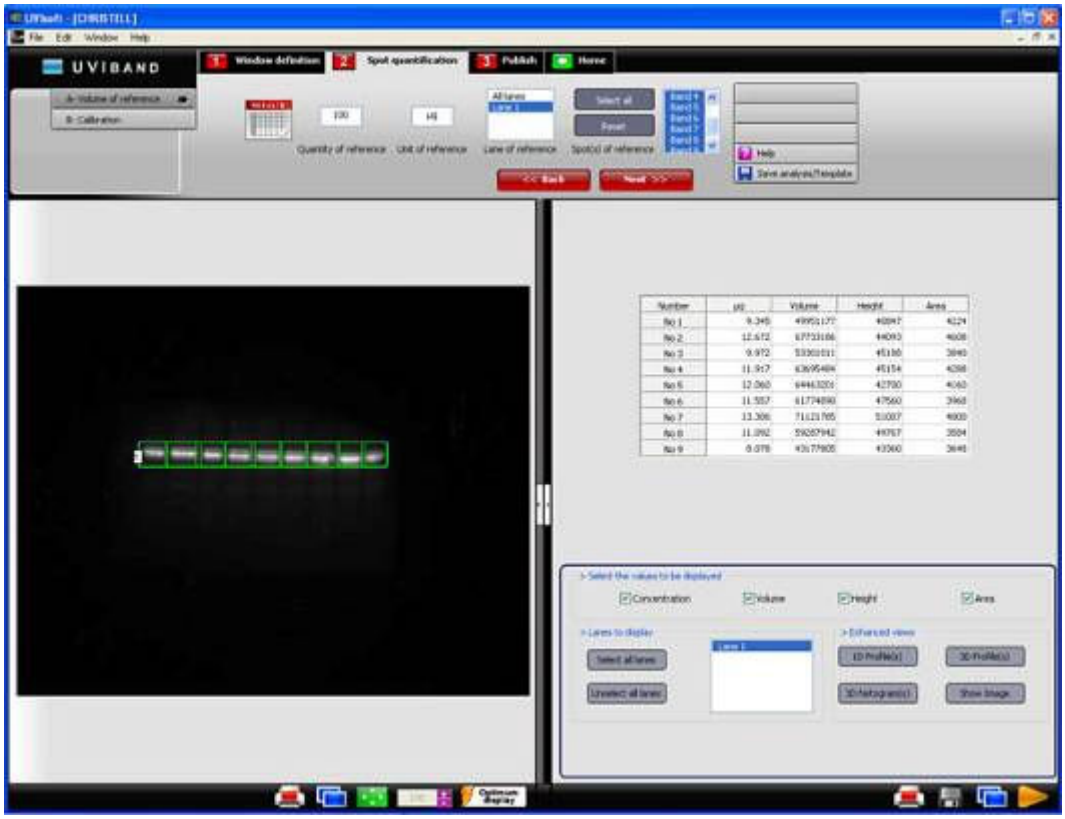
## ➔ A- Volume of reference

2

A volume is the total signal intensity inside a defined boundary drawn on a lane.

The purpose of the volume of reference is to use volumes of known concentration to calculate the unknown concentrations. The volume of reference process follows the spot separation.

Note: you can either access the volume of reference function by clicking on the next button of the background subtraction or directly by clicking on the volume of reference of the 2-Spot Quantification folder.




The dashboard details the volume of reference parameters:

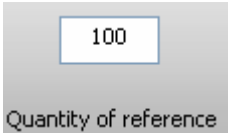

⇨ The quantity of reference
⇨ The unit of reference
⇨ The lane of reference
⇨ The spot(s) of reference

**QUANTITY OF REFERENCE**

The calculation of the unknown concentrations is based:
⇨ On the calculated volumes
⇨ On the known concentration. The known concentration is the quantity of

reference.

The quantity of reference could correspond to one or several spots.
The purpose of the quantity of reference is to define the known concentration. In the "Quantity of reference" edit field, type the quantity of known concentration you want to have as a reference:




**UNIT OF REFERENCE**

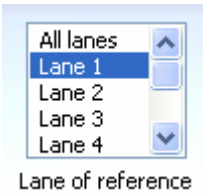
The unit of reference is the header unit of the concentration. You can define your own header such as % or µg.
In the "Unit of reference" edit field, type the unit you want to be displayed in the results table:


Unit of reference
**Percentage as unit of reference**


Unit of reference
**µg as unit of reference**

**LANE OF REFERENCE**

The lane of reference defines the lane of the known concentration.
Select the lane of reference from the list:




If a single lane is selected, only the volumes of this reference lane will be used to calculate the relationship between the volume and the quantity. The other concentrations are calculated based on the concentration/volume relationship of this specific lane.

| | Lane 1 | | Lane 3 | | Lane 4 | |
|---|---|---|---|---|---|---|
| Number | % | Volume | % | Volume | % | Volume |
| No 1 | 44.708 | 6635518 | 178.291 | 26461728 | 49.658 | 7370205 |
| No 2 | 25.475 | 3780895 | 64.424 | 9561786 | 47.652 | 7072517 |
| No 3 | 14.264 | 2117062 | 9.885 | 1467075 | | 0 |
| No 4 | 9.304 | 1380926 | | | | 0 |
| No 5 | 3.574 | 530507 | | | | 0 |
| No 6 | 1.840 | 273100 | | | | 0 |
| No 7 | 0.835 | 123860 | | | | |
| No 8 | | 0 | | | | |
| No 9 | | 0 | | | | |

Illustration 1: 100% / lane 1 / all bands. Total concentration lane 1= 100%

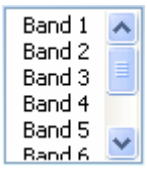
If "All lanes" is selected, for each lane a new relationship between volume and quantity will be recalculated, according to the band's lane selected. For instance, the defined parameters are 100% for all band all lanes; the results table could be as follows. Lane by lane, the total band concentration is 100%:

| | Lane 1 | | Lane 3 | | Lane 4 | |
|---|---|---|---|---|---|---|
| Number | % | Volume | % | Volume | % | Volume |
| No 1 | 44.708 | 6635518 | 70.582 | 26461728 | 51.031 | 7370205 |
| No 2 | 25.475 | 3780895 | 25.504 | 9561786 | 48.969 | 7072517 |
| No 3 | 14.264 | 2117062 | 3.913 | 1467075 | | 0 |
| No 4 | 9.304 | 1380926 | | | | 0 |
| No 5 | 3.574 | 530507 | | | | 0 |
| No 6 | 1.840 | 273100 | | | | 0 |
| No 7 | 0.835 | 123860 | | | | |
| No 8 | | 0 | | | | |
| No 9 | | 0 | | | | |

Illustration 2: 100% / all lanes / all bands. Total concentration all lanes= 100%

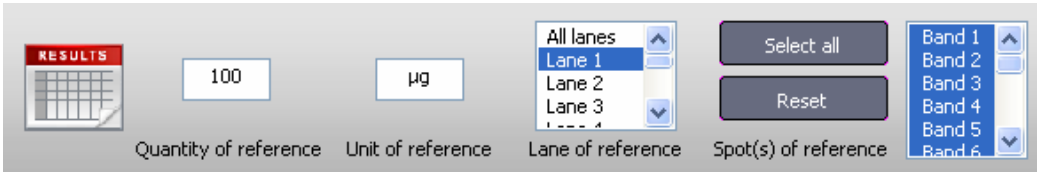
## SPOT(S) OF REFERENCE

The quantity of reference could correspond to one or several spots of the selected lane. Select one or several spots of the lane of reference from the list:




## EXAMPLE 1

Let's consider the known concentration is 3µg contains in the first spot of lane 3. The settings should then be as follows:

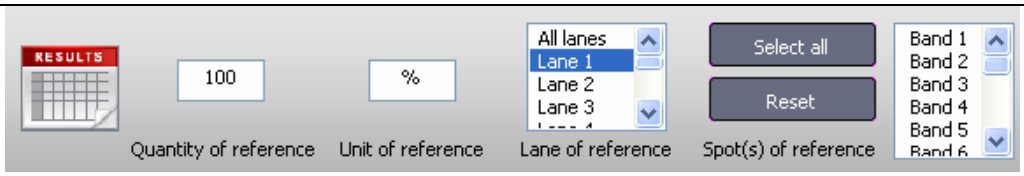



The results table indicates the following for lane 3:

| Number | µg | Volume | Height | Area | MW-RF |
|---|---|---|---|---|---|
| No 1 | 3.000 | 4285313 | 4071 | 1775 | 10.000 |
| No 2 | 9.267 | 13237182 | 3438 | 5396 | 8.000 |
| No 3 | 0.942 | 1345357 | 2740 | 568 | 6.000 |
| No 4 | 0.467 | 667689 | 2692 | 284 | 5.000 |
| No 5 | 12.560 | 17940927 | 2651 | 10224 | 4.000 |
| No 6 | 0.358 | 511654 | 1305 | 426 | 3.000 |
| No 7 | 3.885 | 5549237 | 1275 | 5112 | 2.500 |
| No 8 | 1.626 | 2322765 | 1176 | 2414 | 2.000 |
| No 9 | 0.465 | 664510 | 1000 | 710 | 1.500 |

## EXAMPLE 2

Let's consider the known concentration is 100% contains in all the spots of lane 1. The settings should then be as follows:




The results table indicates the following for lane 1:

| Number | % | Volume | Height | Area | MW-RF |
|---|---|---|---|---|---|
| No 1 | 3.978 | 1715709 | 2744 | 781 | 9.896 |
| No 2 | 15.367 | 6627687 | 4310 | 2769 | 7.998 |
| No 3 | 11.431 | 4930041 | 4642 | 2130 | 7.710 |
| No 4 | 12.333 | 5319454 | 2612 | 2414 | 4.561 |
| No 5 | 2.112 | 911077 | 2323 | 426 | 4.000 |
| No 6 | 35.571 | 15341999 | 2191 | 10508 | 2.678 |
| No 7 | 19.207 | 8284193 | 1270 | 8591 | 1.872 |

**NEXT**

The "Next" button validates your parameter and opens the following analysis step.

| | | |
|---|---|---|
| 2 A – Volume of reference | Next >> | 2 B – Calibration |

**BACK**

The "Back" button validates your parameter and opens the following analysis step.

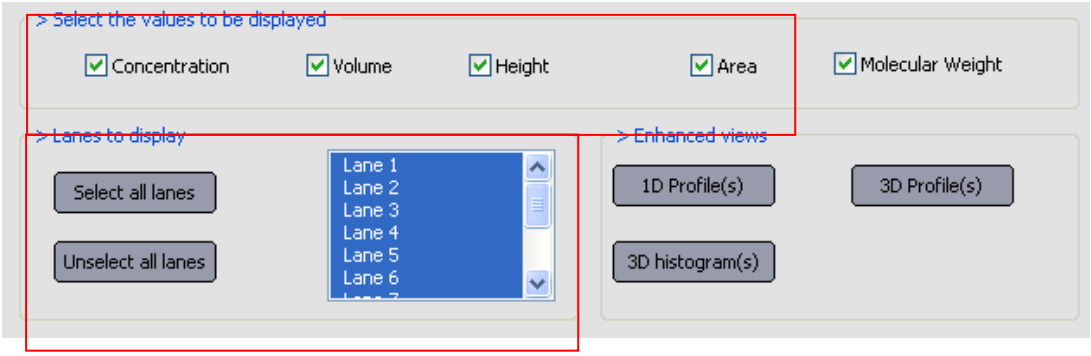
| | | |
|---|---|---|
| 2 A – Volume of reference | << Back | 1 C- Spot separation |

**RESULT TABLE**

In the result parameter window, you can select the lanes and the values to be displayed in the results tables:

- ⇨ Concentration
- ⇨ Volume
- ⇨ The maximum intensity
- ⇨ The area

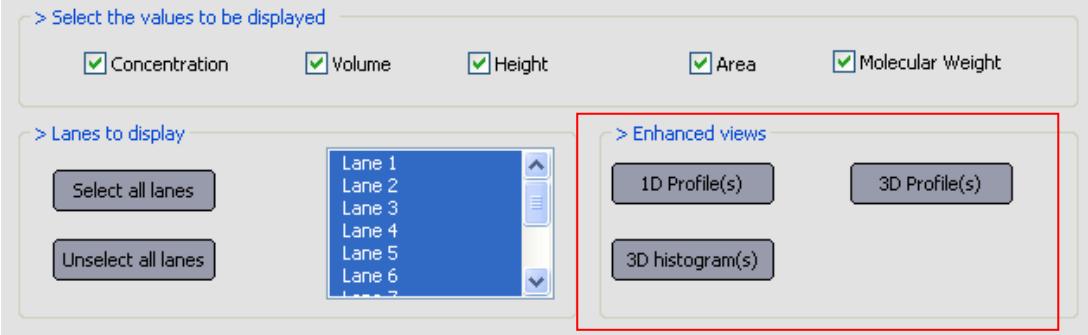
To select your display mode, click on the appropriate selection:




**GRAPHICAL VIEW**

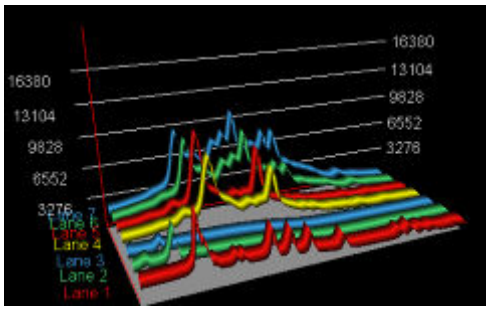
In the results parameter window, you can select the graphical results tables:

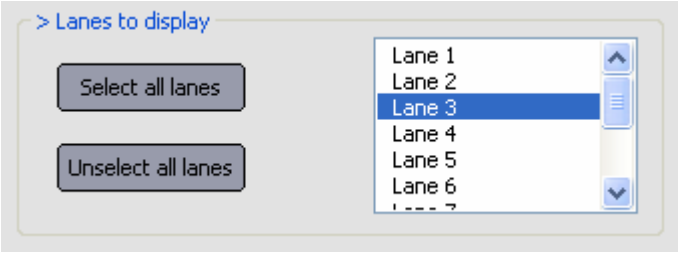
⇨ 1D profile
⇨ 3D profile
⇨ 3D histogram




Note: For all enhanced views, you can modify the angle of vision of the 3D view: Move the mouse cursor on the 3D area, click and drag the view in the direction you want to rotate. Release the mouse when satisfactory.

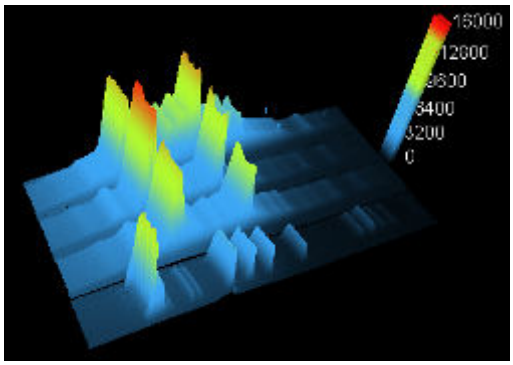
The 1D profile allows you to superimpose the intensity profiles of any number of selected lanes.
To proceed, click on the 1D Profile and select the lanes to be superimposed:

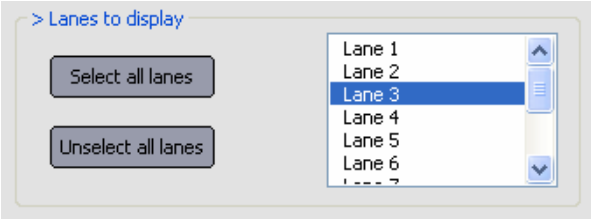




Note: To reposition the 1D profile window, position your cursor at the top of the box. The cursor appearance will change to a multidirectional arrow symbol. You can then drag the box to a new position.
Note: To increase the size of the 1D profile window, position your cursor at the edge of the window and click and drag to extend its size.
Note: Click on Print to print the 1D profile window
Note: Click on Send to clipboard to save the graph in the Windows ® clipboard (Past/copy Windows ® feature).

The 3D profile displays the three-dimensional rendering of any selected lanes.
To proceed, click on the 3D Profile button and select the lanes to be displayed:

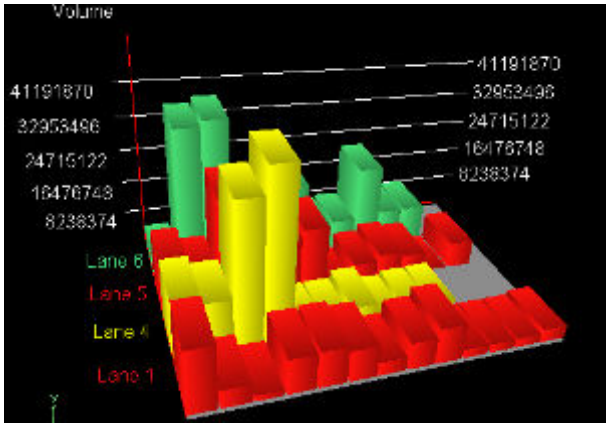



Note: To reposition the 1D profile window, position your cursor at the top of the box. The cursor appearance will change to a multidirectional arrow symbol. You can then drag the box to a new position.
Note: To increase the size of the 1D profile window, position your cursor at the edge of the window and click and drag to extend its size.
Note: Click on Print to print the 1D profile window
Note: Click on Send to clipboard to save the graph in the Windows ® clipboard (Past/copy Windows ® feature).

The 3D histogram displays the three-dimensional histogram of selected results:
- ⇨ Volume
- ⇨ Calculated quantities
- ⇨ Maximum intensities

To proceed, click on the 3D Histogram button and select the lanes to be displayed:


Note: To reposition the 1D profile window, position your cursor at the top of the box. The cursor appearance will change to a multidirectional arrow symbol. You can then drag the box to a new position.
Note: To increase the size of the 1D profile window, position your cursor at the edge of the window and click and drag to extend its size.
Note: Click on Print to print the 1D profile window

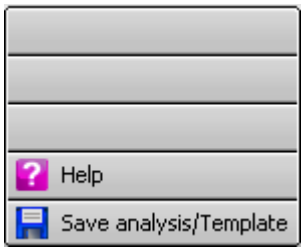
Note: Click on Send to clipboard to save the graph in the Windows ® clipboard (Past/copy Windows ® feature).

## OPTION FOLDER

The option folder gathers the following functions:

⇨ Help

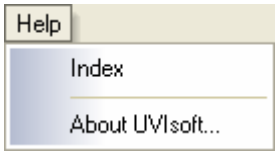
⇨ Save the analysis or the template




## HELP

Click on the “Help” button. You automatically access the user manual at the chapter corresponding to the function


You can access the help file index through the File\Help from the Menu bar




## SAVE ANALYSIS / TEMPLATE

This function saves the current analysis. The analysis file will contain the results, the image and all the parameters defined to obtain the results.

The analysis could also be saved as a template for automated analysis routines. Template offers the user the ability to automate many of the repetitive tasks associated with analysis and processing. As a result, you can spend more time evaluating and analysing results, and less time manipulating set-ups, variables and other settings.

The template automates a task or set of tasks that you perform repeatedly or on a regular basis. It stores all the analysis commands and parameters of an analysis. You can run these parameters with another image whenever you need to perform a new analysis based on the same parameters.

The benefits of the template file are as follows:

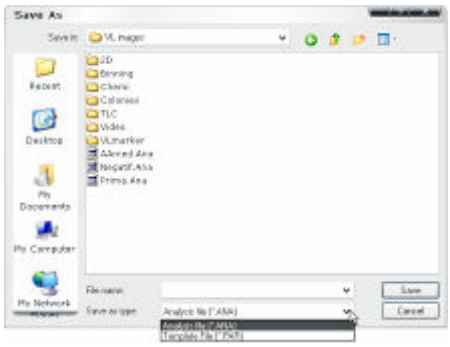
⇨ Time saving

⇨ Reproduction of image analysis parameters

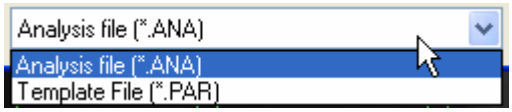
⇨ Templates are modifiable, allowing the user to maintain an original template while modifying it for a slightly different result, with minimal effort

1. Click on the “Save analysis/ Template” button:


2. A pop-up window displays the following menu:

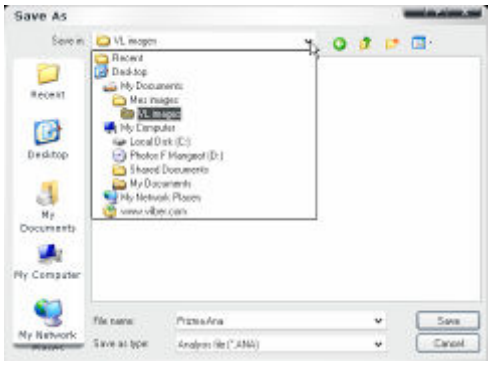



3. Select analysis file or template file:

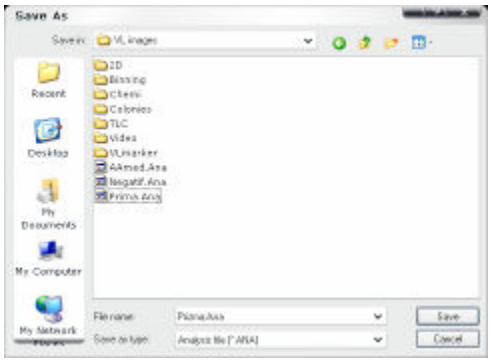



Note: the software proposes Analysis file by default

4. Browse to specify the file directory




5. Enter the desired file name and validate




6. Click on the Save button to create the file.

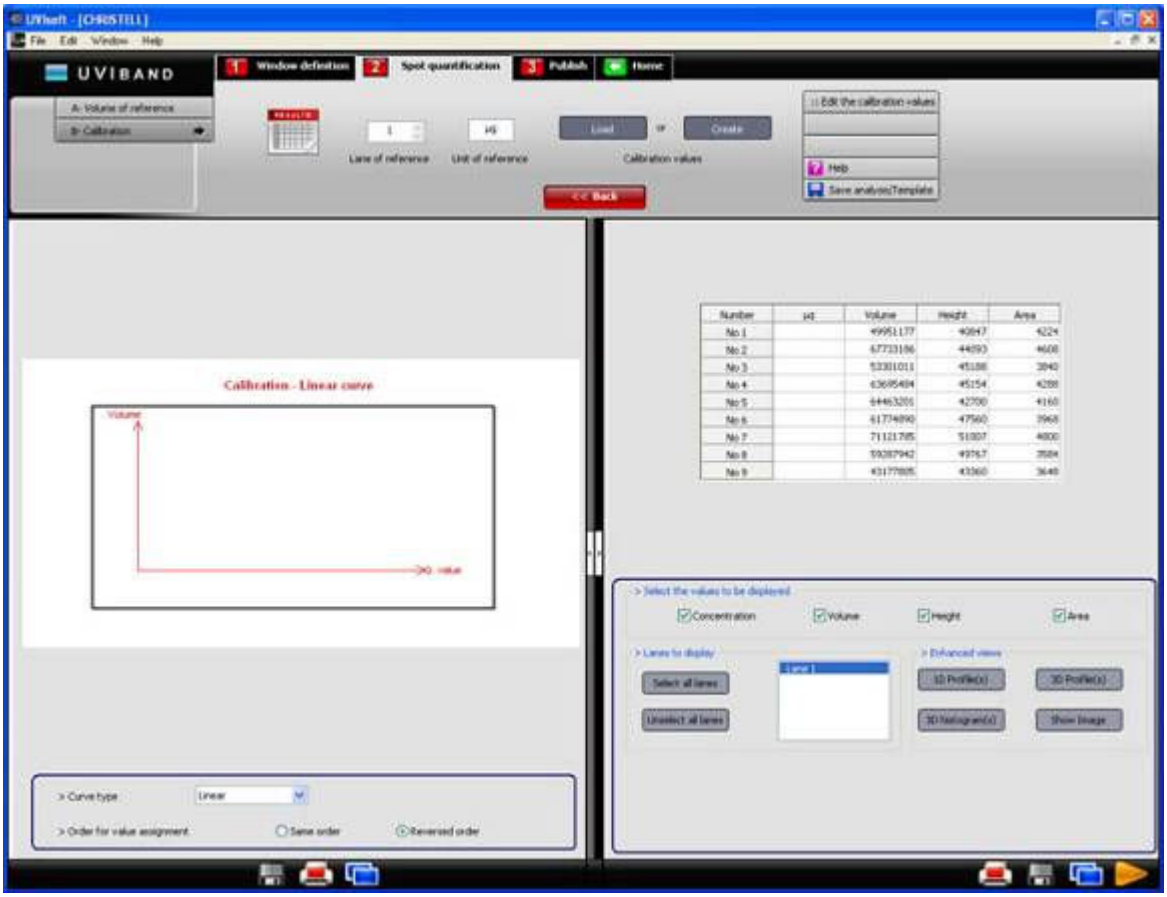
Note: see “Access to the analysis module” chapter for template or analysis file loading

## ➔ B- Calibration

2

The calibration process follows the Volume of reference function. The calibration is the calculation of the concentration based on a concentration master or on a calibration curve on which you can select all or few points.

Note: you can either access the calibration function by clicking on the next button of the Volume of reference or directly by clicking on the Calibration of the 2-Spot quantification folder.

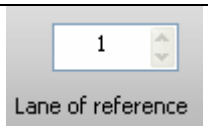



The dashboard details the volume of reference parameters:


- ⇨ The lane of reference
- ⇨ The unit of reference
- ⇨ The calibration values

**LANE OF REFERENCE**

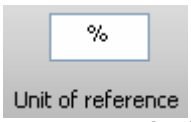
The lane of reference defines the lane of the known concentration.
Select the lane of reference from the list:



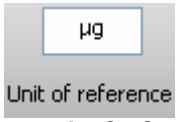

**UNIT OF REFERENCE**

The unit of reference is the header unit of the concentration. You can define your own header such as % or µg.

In the "Unit of reference" edit field, type the unit you want to be displayed in the results table:




**Percentage as unit of reference**




**µg as unit of reference**

**THE CALIBRATION VALUES**

Click on the "Load" or "Create" button to enter calibration's values.

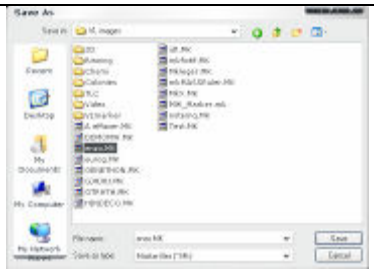

For "Create", a pop-up window displays the following menu:


Type your values, band to band, in a descending order. The OK button validates your data.

Note: if an automatic calculation with immediate application of the standard values is carried out, it is not necessary to enter all the bands given by the manufacturer's specifications, but only those which are commonly found on the lanes of the gel.

You can save your calibration data and create your own calibration library; To proceed, click on the "Save " button:

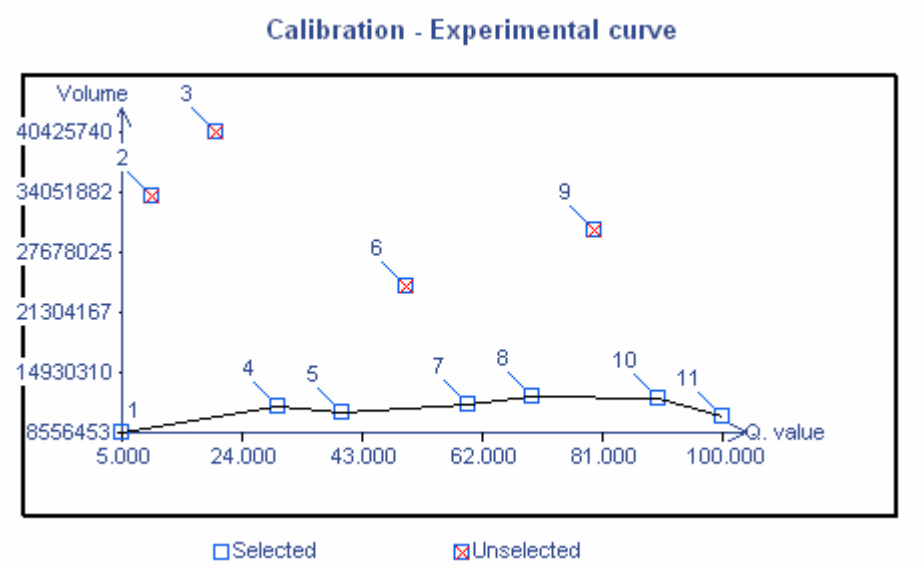

A pop-up window displays the following menu:



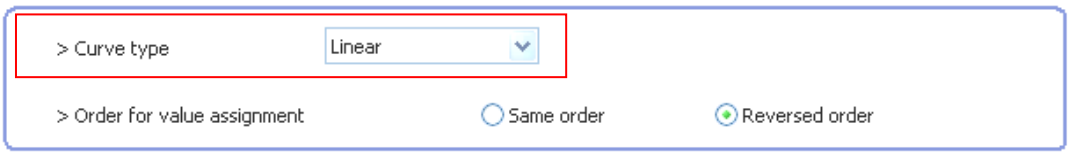
⇨ Browse to specify the directory
⇨ Type the file name and click on Save.

**MASTER CURVE**

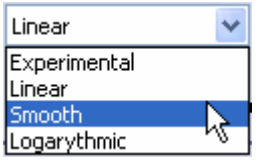
After the values of the master-curve are defined, the calibration curve is displayed. You can unselect wrong values or points out of the curve by directly clicking on them



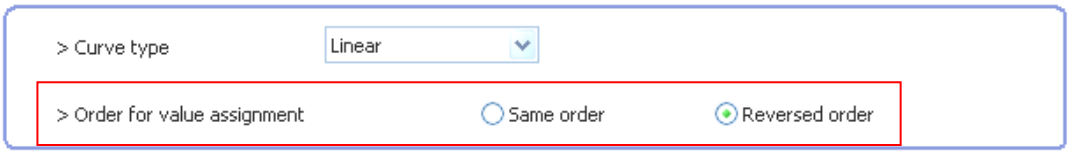

In the profile parameters window, select the curve type:




Four mathematical models can be used:
⇨ Experimental: the curve simply links the values (point to point), without any mathematical model,
⇨ Linear curve: displays a model with linear regression
⇨ Smoothed: displays a smoothed curve (polynomial spline, at least 4 points must be entered)
⇨ Logarithmic curve: displays a model with logarithmic regression



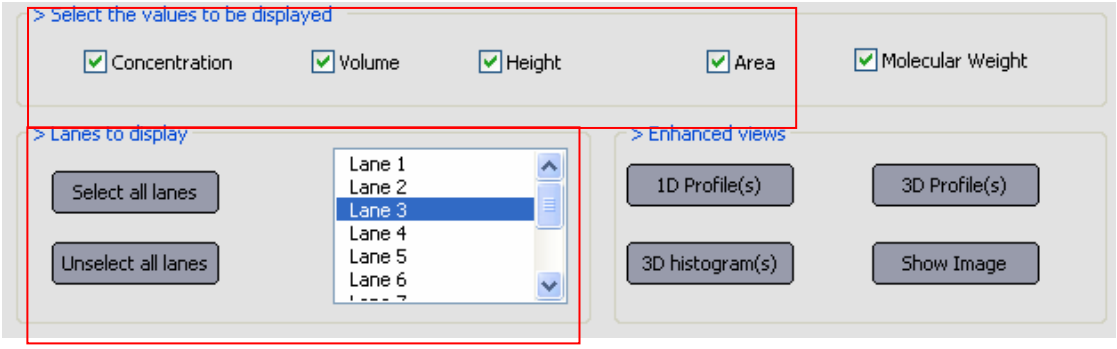
You can also select the order for the spot display:
⇨ - Same order as the values of the master-curve
⇨ - Reversed order (depending on the order of the defined values)




**RESULT TABLE**

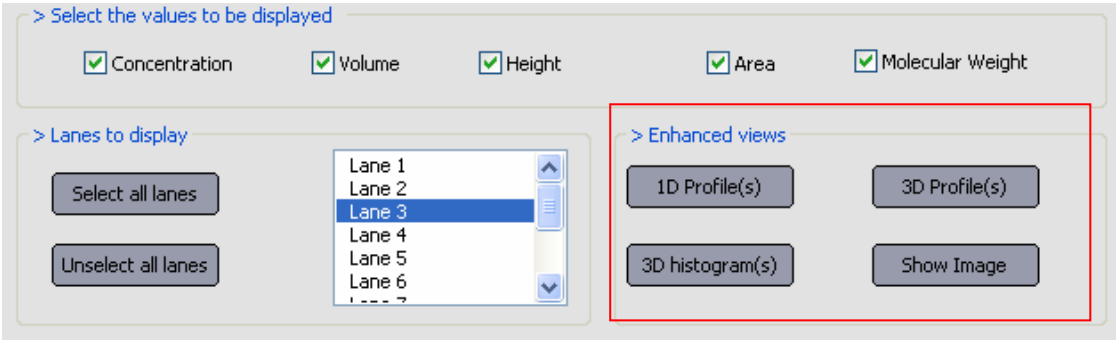
In the result parameter window, you can select the lanes and the values to be displayed in the results tables:
⇨ Concentration
⇨ Volume
⇨ The maximum intensity
⇨ The area

To select your display mode, click on the appropriate selection:



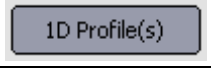

**GRAPHICAL VIEW**

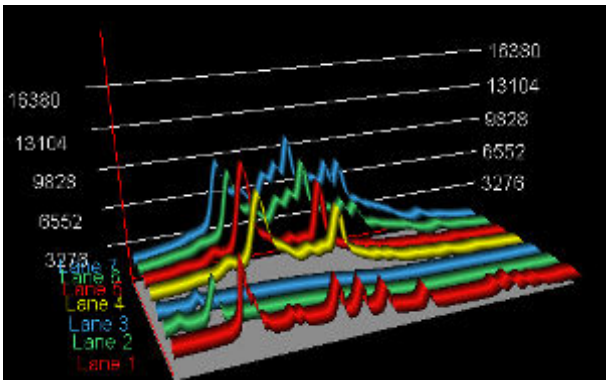
In the results parameter window, you can select the graphical results tables:
⇨ 1D profile
⇨ 3D profile
⇨ 3D histogram

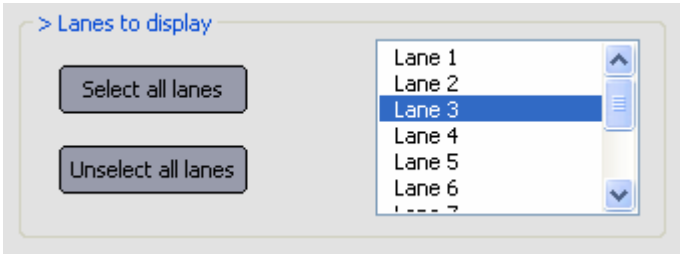



Note: For all enhanced views, you can modify the angle of vision of the 3D view : Move the mouse cursor on the 3D area, click and drag the view in the direction you want to rotate. Release the mouse when satisfactory.

The 1D profile allows you to superimpose the intensity profiles of any number of selected lanes.

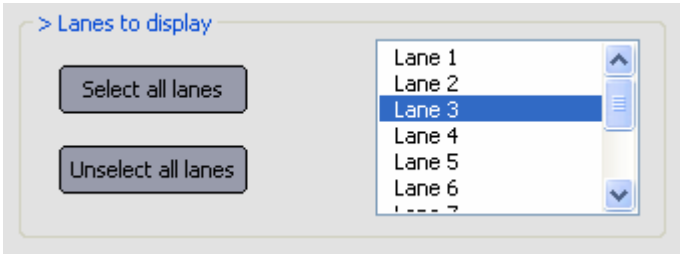
To proceed, click on the 1D Profile and select the lanes: 

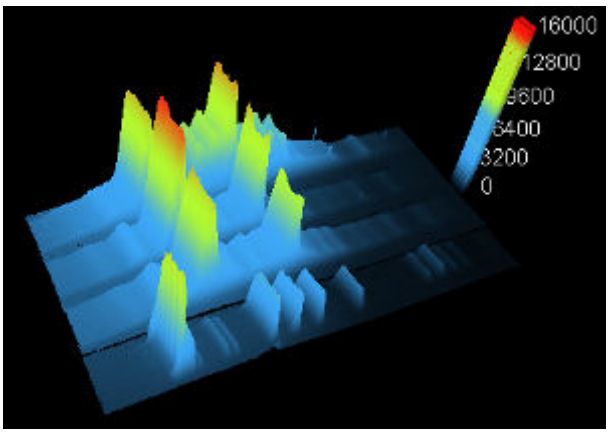



Note: To reposition the 1D profile window, position your cursor at the top of the box. The cursor appearance will change to a multidirectional arrow symbol. You can then drag the box to a new position.
Note: To increase the size of the 1D profile window, position your cursor at the edge of the window and click and drag to extend its size.
Note: Click on Print to print the 1D profile window
Note: Click on Send to clipboard to save the graph in the Windows ® clipboard (Past/copy Windows ® feature).

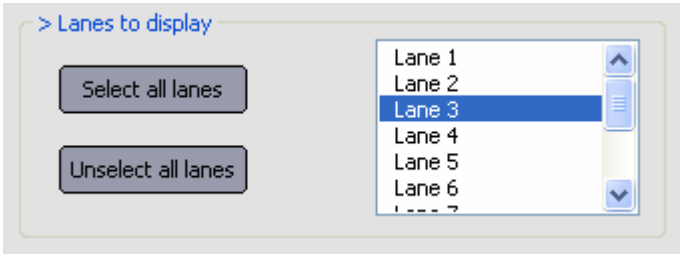
The 3D profile displays the three-dimensional rendering of any selected lanes.
To proceed, click on the 3D Profile button and select the lanes to be displayed:

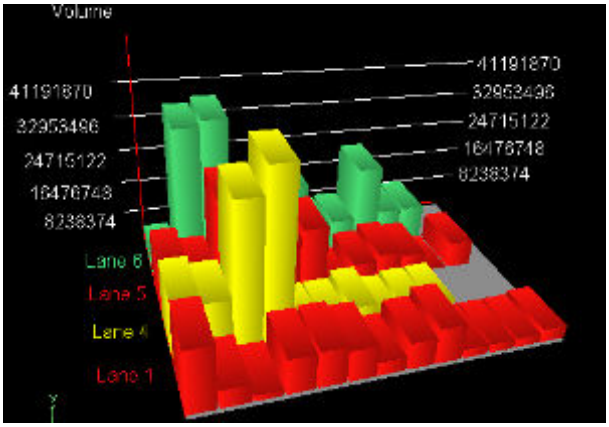



Note: To reposition the 1D profile window, position your cursor at the top of the box. The cursor appearance will change to a multidirectional arrow symbol. You can then drag the box to a new position.
Note: To increase the size of the 1D profile window, position your cursor at the edge of the window and click and drag to extend its size.
Note: Click on Print to print the 1D profile window
Note: Click on Send to clipboard to save the graph in the Windows ® clipboard (Past/copy Windows ® feature).

The 3D histogram displays the three-dimensional histogram of selected results:
⇨ Volume
⇨ Calculated quantities
⇨ Maximum intensities
To proceed, click on the 3D Histogram button and select the lanes to be displayed:

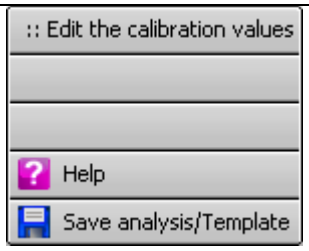




Note: To reposition the 1D profile window, position your cursor at the top of the box. The cursor appearance will change to a multidirectional arrow symbol. You can then drag the box to a new position.
Note: To increase the size of the 1D profile window, position your cursor at the edge of the window and click and drag to extend its size.
Note: Click on Print to print the 1D profile window
Note: Click on Send to clipboard to save the graph in the Windows ® clipboard (Past/copy Windows ® feature).

## OPTION FOLDER

The option folder gathers the following functions:
⇨ Edit the calibration values
⇨ Help
⇨ Save the analysis or the template




**EDIT THE CALIBRATION VALUES**

1. Click on the “Edit the calibration values” button.

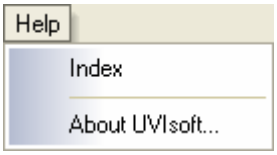

2. A pop-up window displays the following menu on which you can modify the calibration values:


You can add, remove, and save your marker’s value;

**HELP MENU**

Click on the “Help” button. You automatically access the user manual at the chapter corresponding to the function


You can access the help file index through the File\Help from the Menu bar




**SAVE ANALYSIS / TEMPLATE**

This function saves the current analysis. The analysis file will contain the results, the image and all the parameters defined to obtain the results.

The analysis could also be saved as a template for automated analysis routines. Template offers the user the ability to automate many of the repetitive tasks associated with analysis and processing. As a result, you can spend more time evaluating and analysing results, and less time manipulating set-ups, variables and other settings.

The template automates a task or set of tasks that you perform repeatedly or on a regular basis. It stores all the analysis commands and parameters of an analysis. You can run

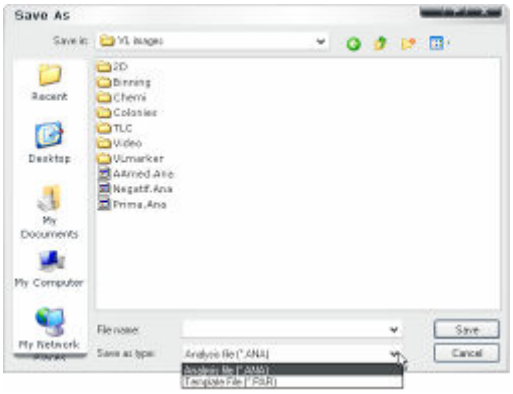
these parameters with another image whenever you need to perform a new analysis based on the same parameters.

The benefits of the template file are as follows:

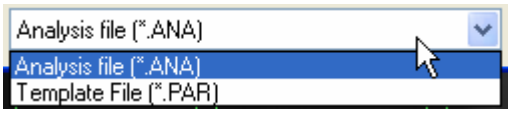
⇨ Time saving
⇨ Reproduction of image analysis parameters
⇨ Templates are modifiable, allowing the user to maintain an original template while modifying it for a slightly different result, with minimal effort

1. Click on the “Save analysis/ Template” button:


2. A pop-up window displays the following menu:

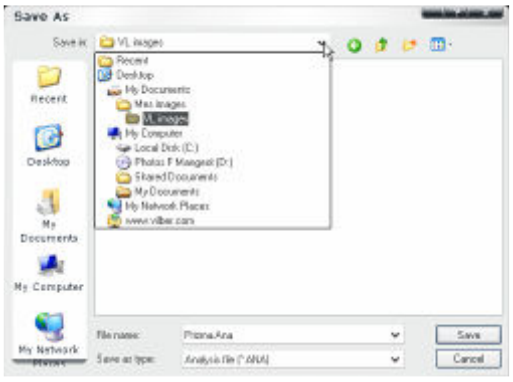



3. Select analysis file or template file:

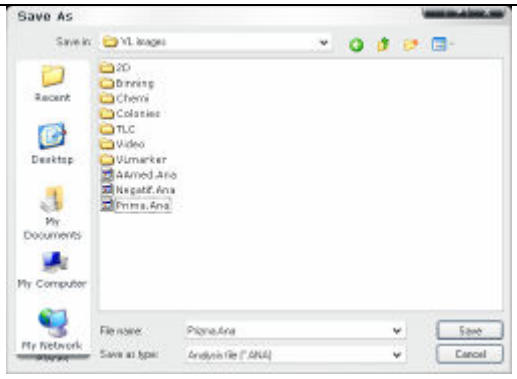



Note: the software proposes Analysis file by default

4. Browse to specify the file directory




5. Enter the desired file name and validate




6. Click on the Save button to create the file.

Note: see “Access to the analysis module” chapter for template or analysis file loading

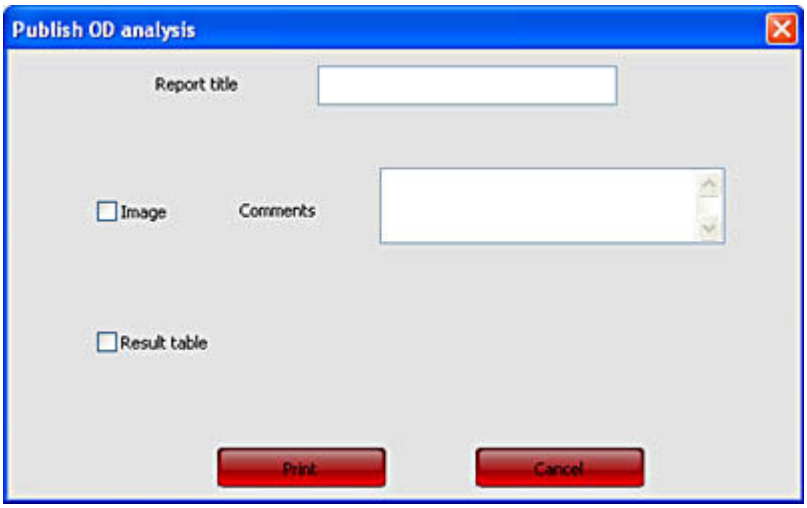
# Publish

## ➔ Introduction

3

The purpose of the Publish function is to prepare a printed report of your results. You can easily organise your report with titles and comments and your own selection of data to be published among the following:
- Sample image
- Quantification result table

To proceed, select the Publish tab. A pop-up window displays the following menu:




⇨ Enter a report title if any
⇨ Select the options to be printed
⇨ Add comments or not per option

Click on the “Print” button. A pop-up window displays the following menu


⇨ Select a printer
⇨ If necessary, click on Properties to modify the default setting of the printer,
⇨ Select the number of copies
⇨ Click on OK to validate your options

# Return to Home

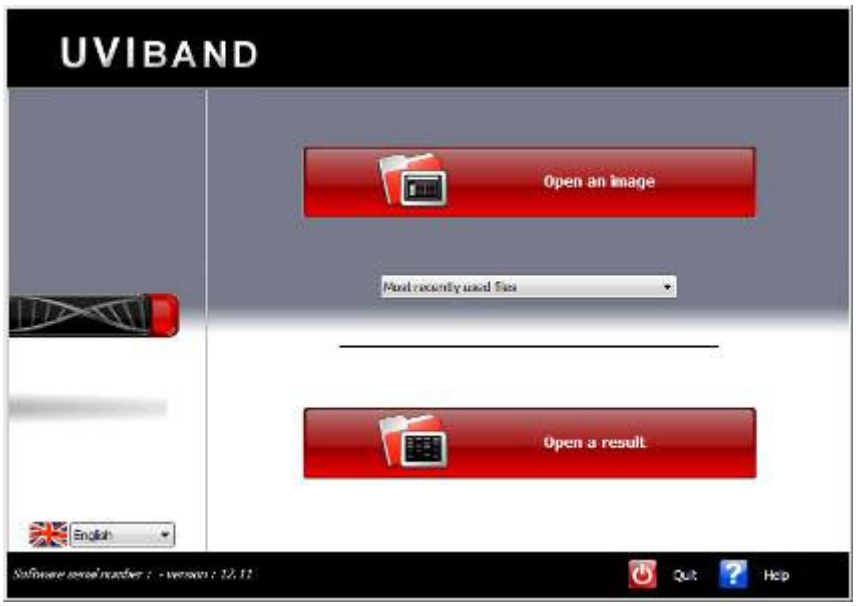
## ➔ Introduction

The home dashboard is the hub to other functions of the software:

- ⇨ Open another image file or another result file
- ⇨ Select another analysis module
- ⇨ Exit the software


## ➔ Load another image

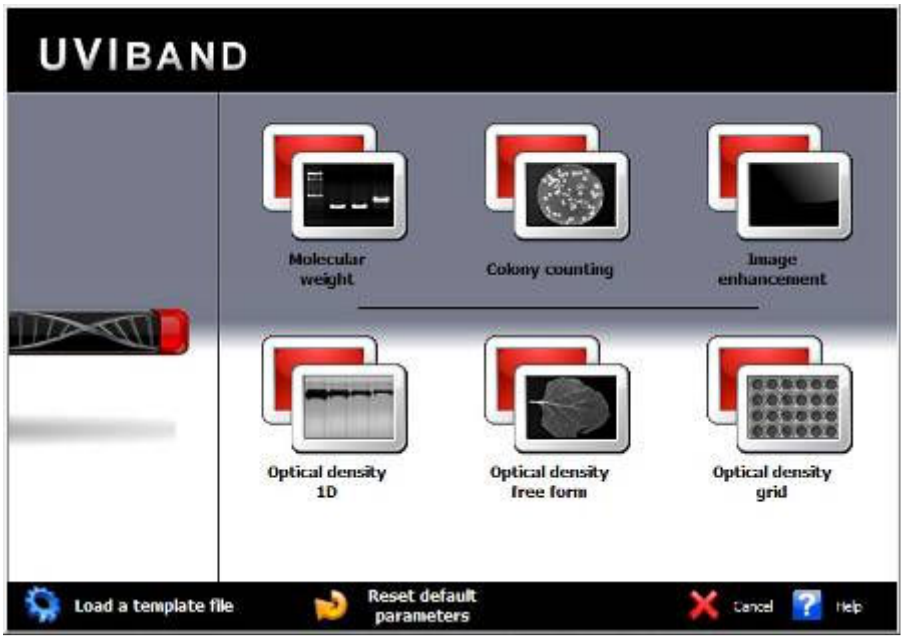
To return to the main menu, click on the home icon. A new menu appears with the main menu task bar functions:




- ⇨ Click on the “Open an image” icon to open an image
- ⇨ Click on the “Open a result” icon to open a previously saved analysis result

## ➔ Select another function

To return to the analysis menu, click on the analysis icon. A new menu appears with the analysis module task bar functions:




Click on the appropriate icon to select an analysis module.

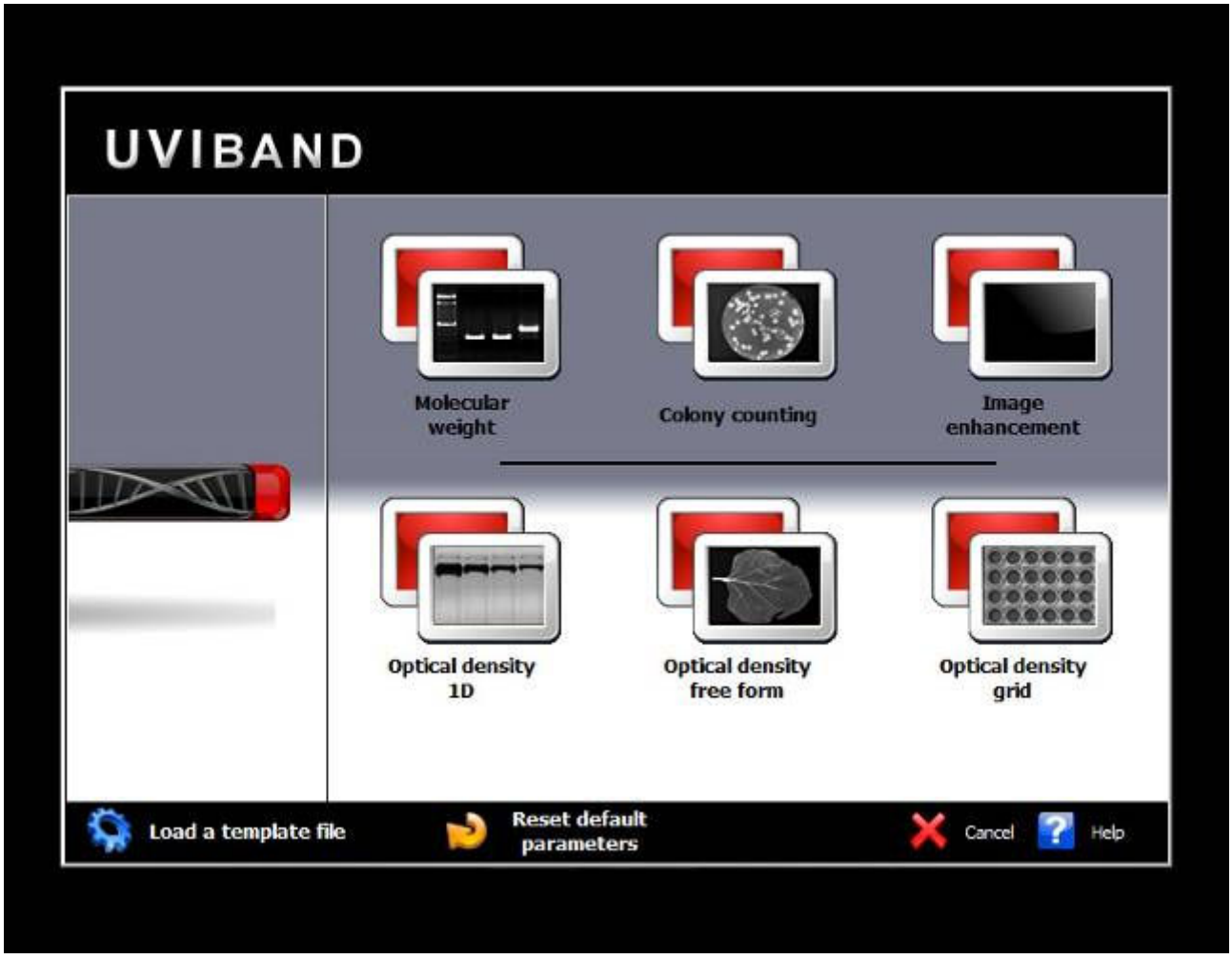
- ⇨ Select the Molecular weight icon to open the molecular weight analysis (MW) module
- ⇨ Select the Colony counting icon to open the colony counting (CC) analysis module
- ⇨ Select the Optical density - 1D icon to open the optical density (OD) analysis module based on a 1D detection
- ⇨ Select the Optical density - Free form icon to open the optical density (OD) analysis module based on a free form detection
- ⇨ Select the Optical density - Grid icon to open the optical density (OD) analysis module based on a grid detection
- ⇨ Select the Image enhancement icon to open the image enhancement module

## ➔ Exit the software

To close UVIband Advanced, select Exit from the File menu.

You will be prompted to save your analysis.

UVITEC
Cambridge




## Optical density – Free form

## ➔ OD-Free form Analysis module

# Optical density / Free form introduction

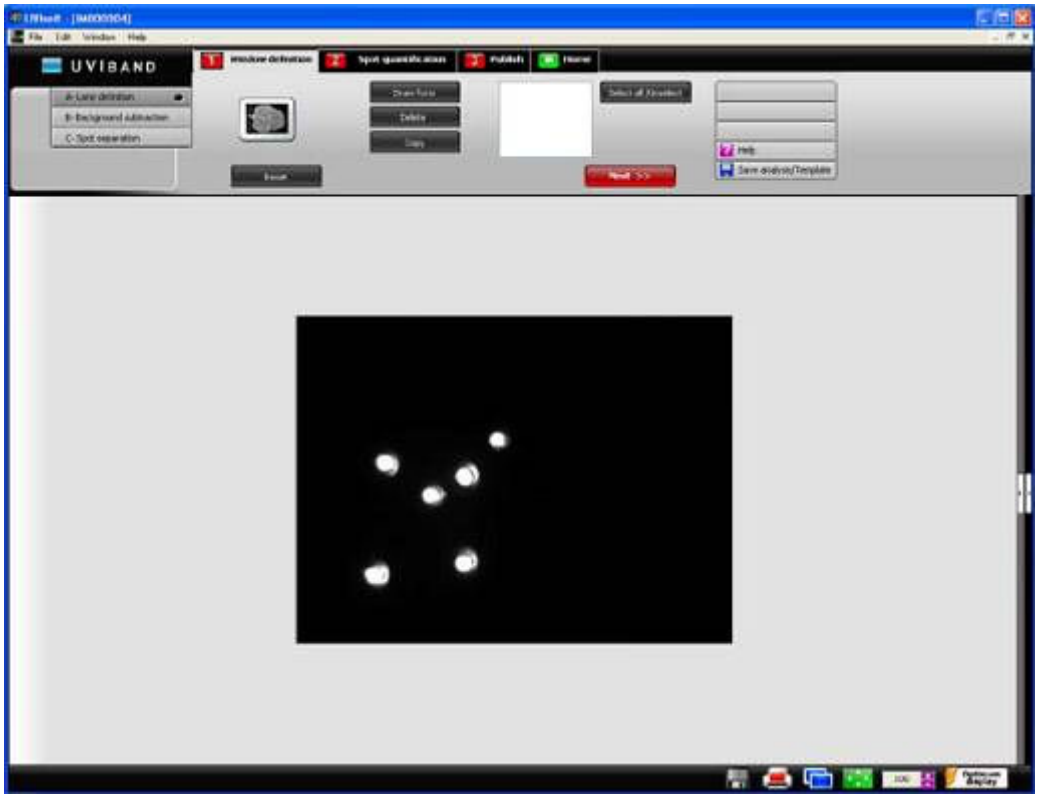
## ➔ Objectives and output

The UVIband Advanced Optical density /Free form module features the quantification of spot in volume, percentage or µg.

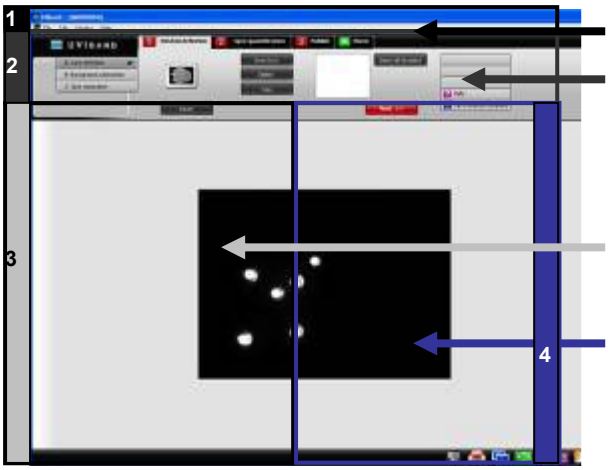
At the end of the process, you can have the following outputs:
- Lane's volume and concentration
- 3D profile
- 3D result's graph
- Calibration curve

## ➔ Optical density / Free form (OD-Free form) operating environment

The OD-Free form module opens on the following window:



The UVIband Advanced operating environment is organised into four areas:




1. The menu bar
2. The dash board
3. The image window
4. The working window

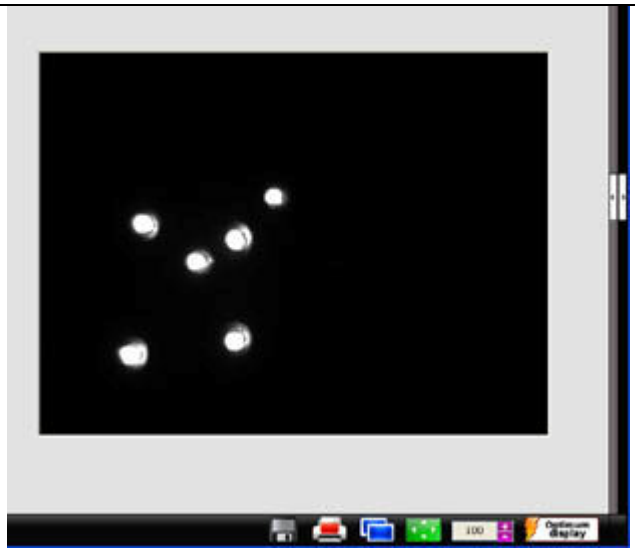
The menu bar contains the following menu:

- ⇨ File
- ⇨ Edit
- ⇨ Windows
- ⇨ Help


The dash board contains four different tabs:

1. Detect
2. Analyse molecular weight (MW)
3. Analyse optical density (OD)
4. Home


The image window displays the active image:



It also contains the image toolbar:


 ⇨ Save the screen capture of the view

 ⇨ Print

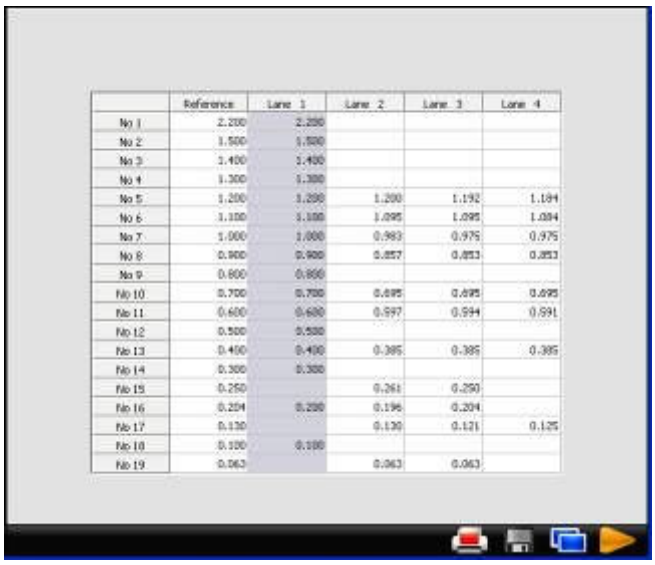
 ⇨ Copy to clipboard

 ⇨ Autoscale

⇨ Zoom in or out the image

⇨ Change the optimum display

The working window displays the graphs and tables related to the active analysis:



| | Reference | Lane 1 | Lane 2 | Lane 3 | Lane 4 |
|---|---|---|---|---|---|
| No 1 | 2.200 | 2.200 | | | |
| No 2 | 1.500 | 1.500 | | | |
| No 3 | 1.400 | 1.400 | | | |
| No 4 | 1.300 | 1.300 | | | |
| No 5 | 1.200 | 1.200 | 1.200 | 1.192 | 1.184 |
| No 6 | 1.100 | 1.100 | 1.095 | 1.095 | 1.084 |
| No 7 | 1.000 | 1.000 | 0.983 | 0.975 | 0.975 |
| No 8 | 0.900 | 0.900 | 0.857 | 0.853 | 0.853 |
| No 9 | 0.800 | 0.800 | | | |
| No 10 | 0.700 | 0.700 | 0.695 | 0.695 | 0.695 |
| No 11 | 0.600 | 0.600 | 0.597 | 0.594 | 0.591 |
| No 12 | 0.500 | 0.500 | | | |
| No 13 | 0.400 | 0.400 | 0.385 | 0.385 | 0.385 |
| No 14 | 0.300 | 0.300 | | | |
| No 15 | 0.250 | | 0.261 | 0.250 | |
| No 16 | 0.204 | 0.200 | 0.196 | 0.204 | |
| No 17 | 0.130 | | 0.130 | 0.121 | 0.125 |
| No 18 | 0.100 | 0.100 | | | |
| No 19 | 0.063 | | 0.063 | 0.063 | |

It also contains the working window toolbar:

 ⇨ Display the molecular weight on the image

 ⇨ Save the graph or the table

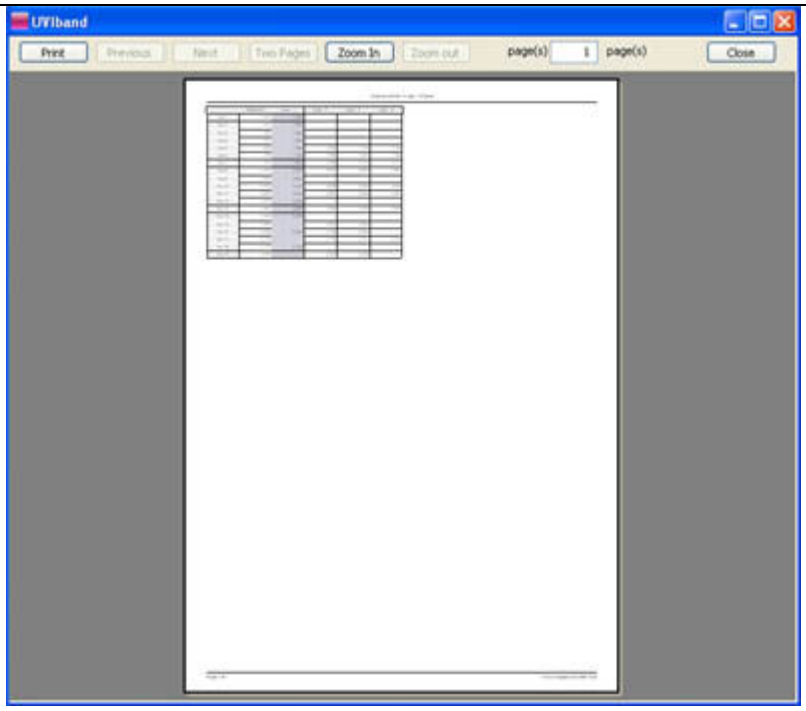
 ⇨ Copy the graph or the table to clipboard

 ⇨ Export the table to Excel

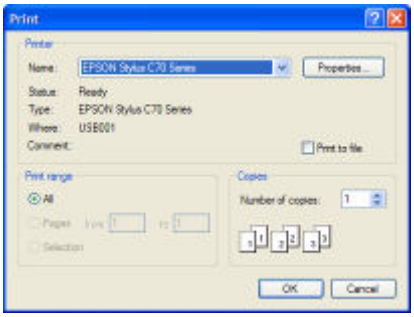
## ➔ Toolbar in details

**Print**

Click on the “Print” icon to print the image, the table or the graphs. A pop-up window displays the Print preview: The Print preview displays a preview of the image, as it will be printed.




Click on Print to validate the preview. A pop-up window displays the following menu:



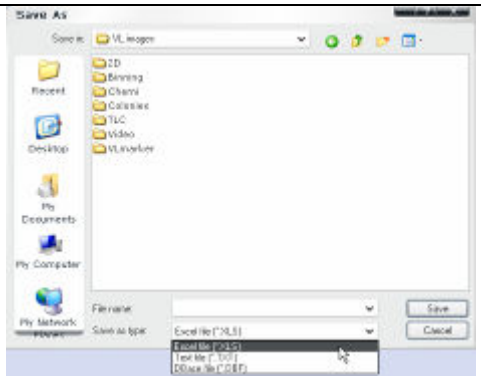

- ⇨ Select a printer
- ⇨ Click on Properties to modify the default setting of the printer, if necessary
- ⇨ Select the number of copies
- ⇨ Click on OK to validate your options

Note: You can also access the Print menu from the Menu bar (File\Print).

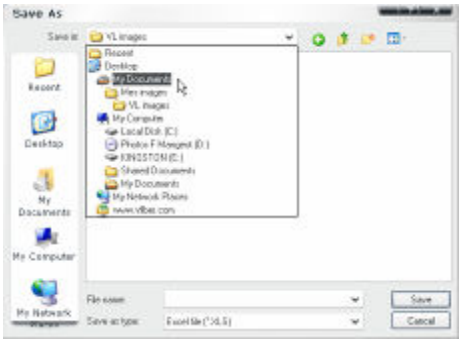
**Save**

This function saves a graph or a table. The tables are saved in a Excel™ file format (*.xls). The graphs are saved in a Bitmap format (*.bmp).

Click on the “Save” icon.
A pop-up window displays the following menu:

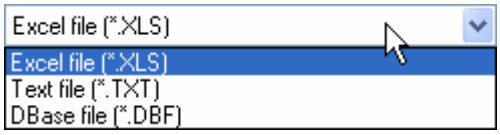


Browse to specify the file directory

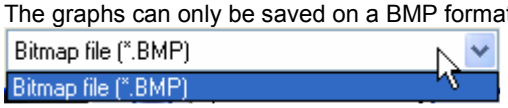


Enter the desired file name, select a file extension and validate

Note: the results could also be saved in a text file format or a Dbase file format:




The graphs can only be saved on a BMP format:




## Copy to clipboard

This function copies an image, a table or a graph onto the clipboard for insertion into another program. This option is identical to the Windows® [Ctrl C] command.

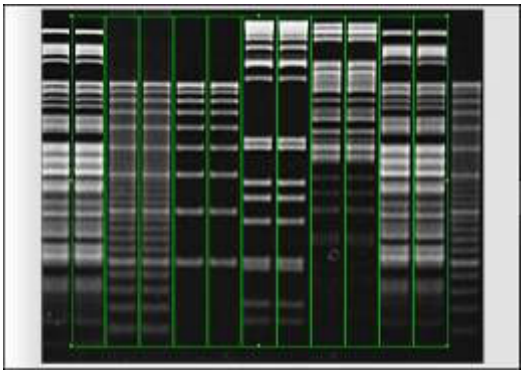
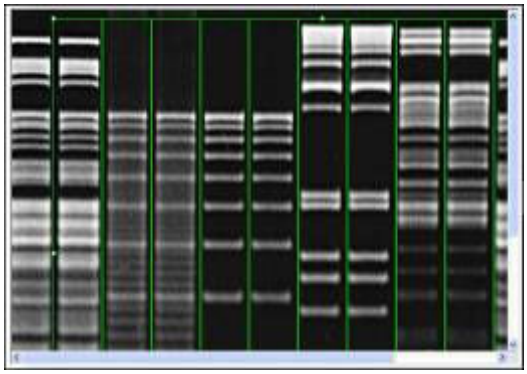
To proceed, click on the Copy to clipboard icon. The image, the table or the graph is now ready to be pasted into another application.

Open the application that you want to paste the image into, and select from the available pasting options ([Ctrl V] command for Windows® software).

**Auto-scale**

Click on the “Auto-scale” to resize the image to fit the size of the monitor.

The Auto-scale feature proportions the display of the image to the screen resolution.

| | |
|---|---|
|  |  |
| Auto-scale (no scroll bar) | No auto-scale (scroll bar) |


**Optimum display (for 12, 14 and 16-bit image file)**

The optimum display window is helpful to modify the greyscale selection to enhance the image display: To proceed, click on the “Optimum display” icon. A pop-up window displays the following menu:

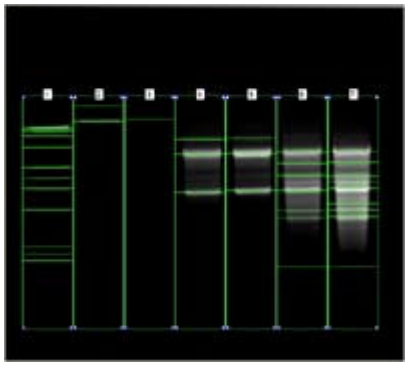
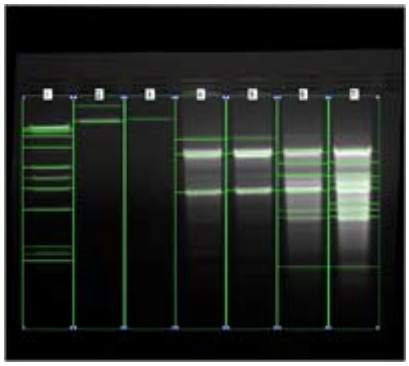
Some images has a 12, 14 or 16-bit format and Windows® can only display 8-bit images (256 grey levels).

Due to this limitation, the UVIband Advanced software handles two images:
⇨ A “memory” image corresponding to the 12, 14 or 16-bit format (4 096, 16 384 or 65 536 grey levels)
⇨ A “display image” corresponding to the image displayed on the screen (256 grey levels)

The easiest way to calculate the “display image” would be to translate the full grey scale each time an image is acquired: the x grey levels values of the “memory” image corresponds to 256 values in the displayed image. In that case, it won’t be possible to visualise faint spots on a dark image.

UVIband Advanced offers the possibility to select the grey level range to translate for the display image calculation. All the grey levels under the “Min value” defined will be converted to 0 (Black) in the displayed image. All the grey levels upper the “Max Value” defined will be set to 255 (White) in the displayed image. The grey levels between those two limits will be converted in an intermediate grey level value following a linear rule.

For both values, you can:
⇨ Edit the value in the corresponding field
⇨ Select the value by dragging and dropping the arrow
⇨ Click on the “optimum display” button: UVIband Advanced will then calculate the ideal values to be selected according to the parameters defined

| | |
|---|---|
|  |  |
| Automatic optimum display | Optimum display enhancement<br>The image appears brighter. The faint bands are more visible. |

Note: The optimum display has no impact on the analysis. Only the display of the image is modified.

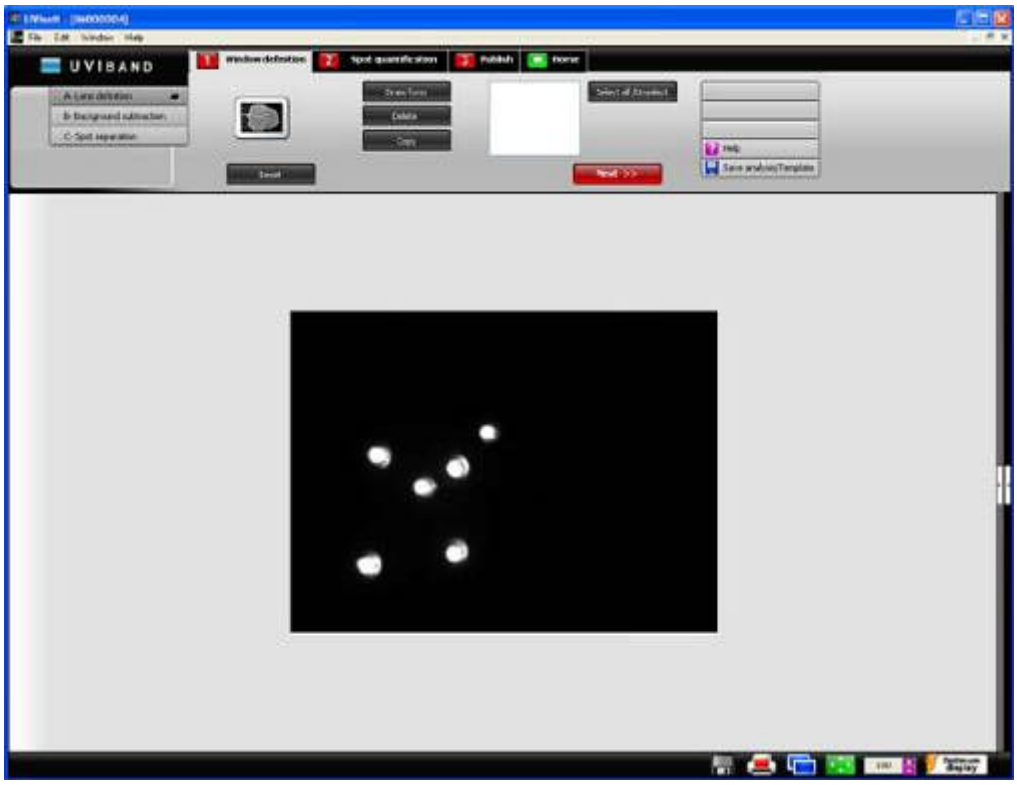
| | |
|---|---|
|  | **Send to Excel™**<br>This function transfers the results table to Windows Excel™.<br><br>To proceed, click on the Send to Excel™ icon. The Excel software is automatically opened by the UVIband Advanced and the table is transferred to Excel™. |

# 1- Window definition

## ➔ A – Lane definition

1

The Optical Density / Free form module opens on the Lane definition dashboard of the Window definition process:



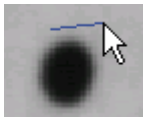

The dashboard details the lane definition parameters:


- ⇨ New lane
- ⇨ Delete
- ⇨ Copy
- ⇨ Select / unselect all lanes

**DEFINE A NEW LANE**

On the image, click on the Draw from button:

⇨ Click on the image to define the first point



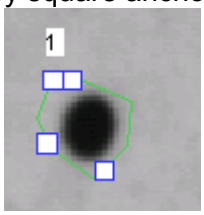
⇨ Change the mouse position to define on edge of the area. Click to validate this edge.

⇨ Repeat these steps as necessary to define the free form area

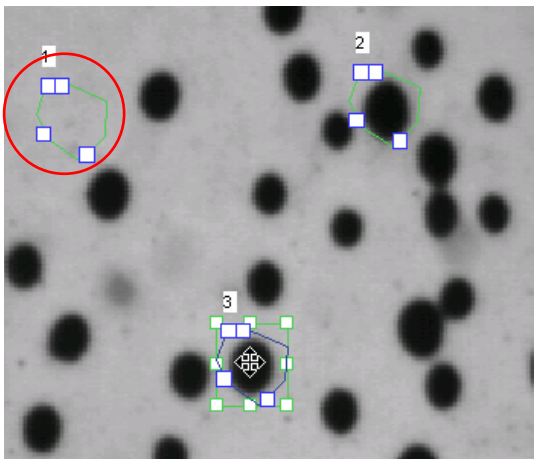
⇨ Click on the Validate button to define the area


⇨ Click on the Abort button to cancel the definition


The lane is defined by green lines, overlaid on the image. The area is surrounded by square anchors:

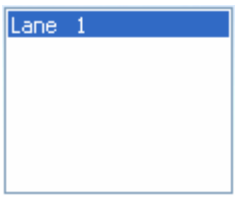



To move the entire frame to a new position,
⇨ Select the frame
⇨ Position the mouse on the frame to obtain a cross cursor
⇨ Click and drag the cursor to move the entire frame

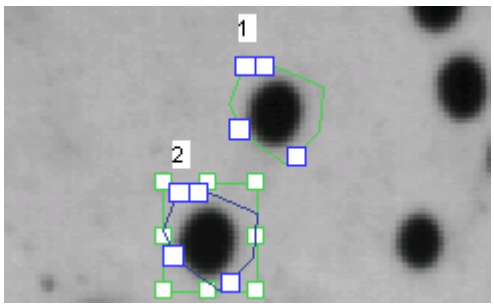



**COPY A LANE**

To copy a lane, select the lane in the lane list:



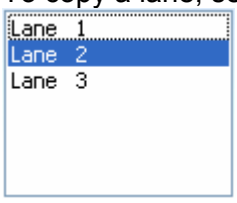

Click on the Copy button. The lane is then duplicated:




The number of lanes is automatically modified in the lane list.
You can move the lane frame to a new position. In order to do so, position the mouse on the. Click and drag the cursor to move the frame.

**DELETE A LANE**

To copy a lane, select the lane in the lane list:




Click on the Delete button. The lane is then deleted.

The number of lanes is automatically modified in the lane list.
You can move the lane frame to a new position. In order to do so, position the mouse on the. Click and drag the cursor to move the frame.

**NEXT**

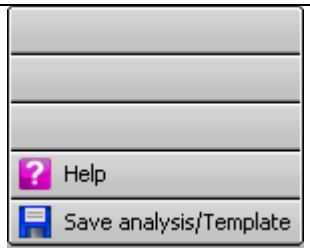
The “Next” button validates your parameter and opens the following analysis step.

| | | |
|---|---|---|
| 1 A – Lane definition | Next >> | 1 B – Background subtraction |

**OPTION FOLDER**

The option folder gathers the following functions:

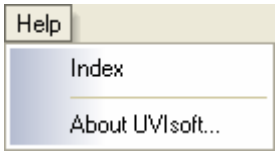
- ⇨ Help
- ⇨ Save the analysis or the template




**HELP MENU**

Click on the “Help” button. You automatically access the user manual at the chapter corresponding to the function


You can access the help file index through the File\Help from the Menu bar:




**SAVE ANALYSIS / TEMPLATE**

This function saves the current analysis. The analysis file will contain the results, the image and all the parameters defined to obtain the results.

The analysis could also be saved as a template for automated analysis routines. Template offers the user the ability to automate many of the repetitive tasks associated with analysis and processing. As a result, you can spend more time evaluating and analysing results, and less time manipulating set-ups, variables and other settings.

The template automates a task or set of tasks that you perform repeatedly or on a regular basis. It stores all the analysis commands and parameters of an analysis. You can run these parameters with another image whenever you need to perform a new analysis based on the same parameters.

The benefits of the template file are as follows:

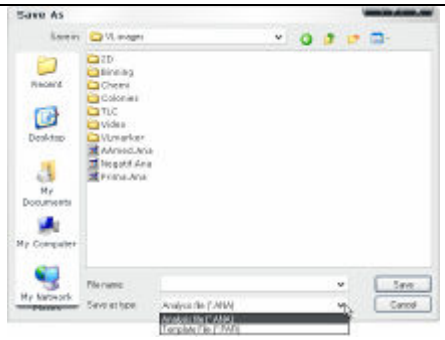
⇨ Time saving

⇨ Reproduction of image analysis parameters

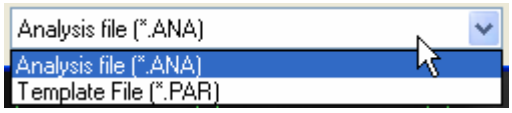
⇨ Templates are modifiable, allowing the user to maintain an original template while modifying it for a slightly different result, with minimal effort

1. Click on the “Save analysis/ Template” button:


2. A pop-up window displays the following menu:

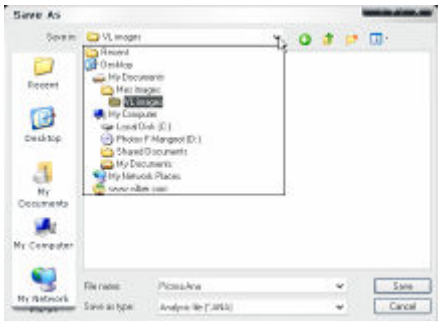


3. Select analysis file or template file:

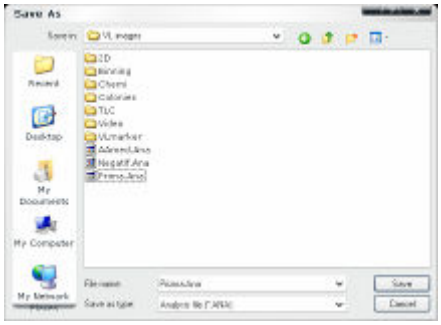



Note: the software proposes Analysis file by default

4. Browse to specify the file directory



5. Enter the desired file name and validate



6. Click on the Save button to create the file.

Note: see “Access to the analysis module” chapter for template or analysis file loading

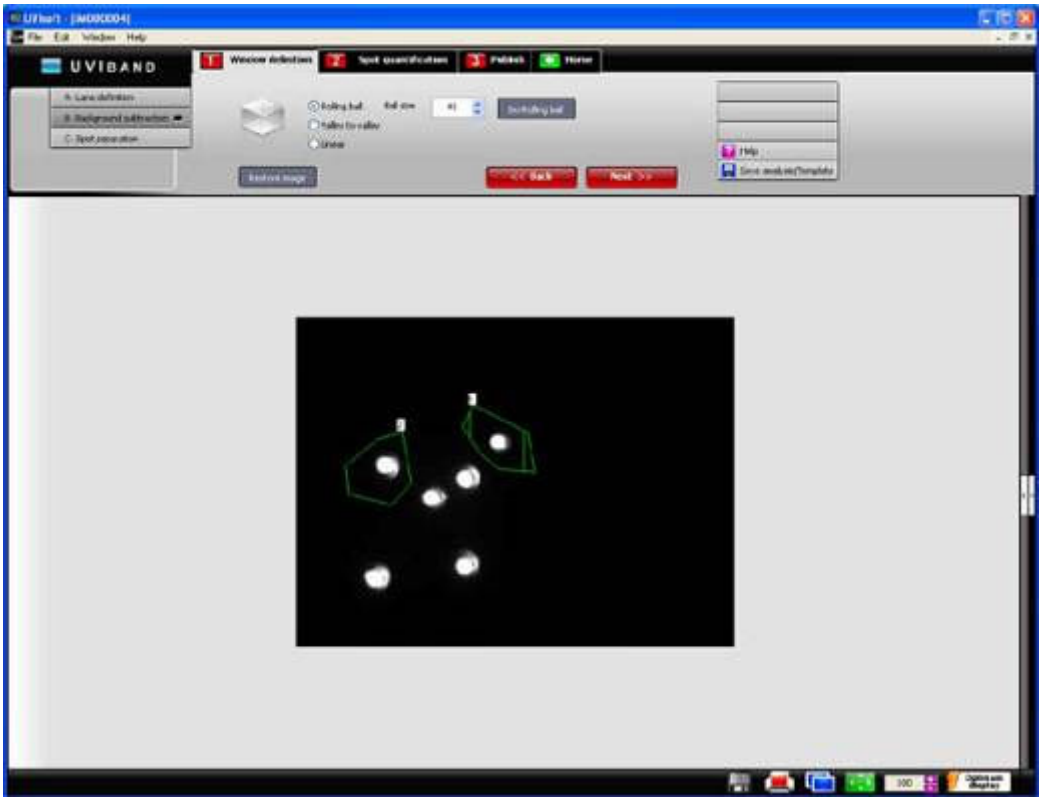
## ➔ B – Background subtraction

1

The background subtraction process follows lane definition.

Image background interferes with quantification and data analysis. To this extend, we recommend to perform a background subtraction before any peak volume quantification. The subtraction is automatically done on the analysis area.

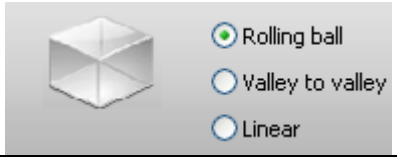
Note: As background subtraction permanently changes the image, this is not possible to save the image with a processed background subtraction. However, the process can be saved by saving the complete analysis through the Save analysis process.




The dashboard details the matching parameters:

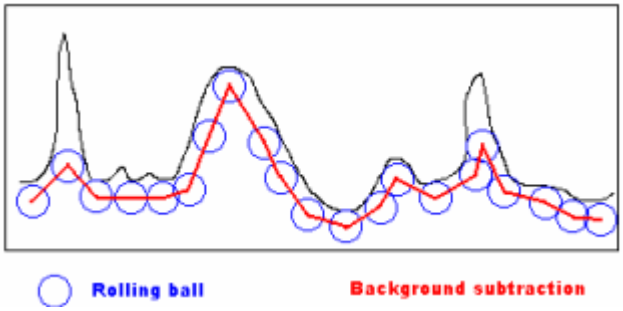

UVIband Advanced has several functions to minimise image background.

⇨ The rolling ball approach
⇨ The valley to valley approach
⇨ The linear approach



**ROLLING BALL**

The rolling ball method is named for a hypothetical ball that rolls along underneath the lane profile, removing different intensity levels along the length of the lane.
The ball is rolled under each profile of the image so its movement varies along the image.




The centre of gravity of the ball describes a curve:

⇨ This curve represents the noise to be subtracted.

⇨ The curve depends on the size of the ball and on the size of the peaks.

The size of the ball will affect the position and movements of the centre of gravity and thus it determined the level of background subtraction. A small disk will make a large background subtraction and a large disk the contrary. A disk radius that is too small may subtract almost all image data.

The UVIband Advanced calculates automatically the ideal parameter for background subtraction. This could be manually modified by adjusting the spot size:


To process the rolling ball background subtraction, click on “Do rolling ball”:


The changes will be automatically applied to the image.

**Note**: few seconds could be necessary to perform the background subtraction.

**VALLEY TO VALLEY**

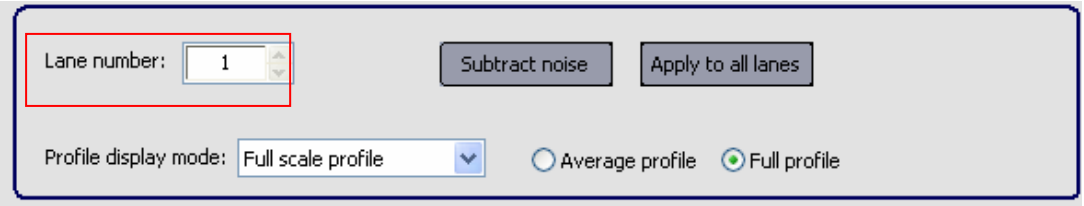
The valley-to valley approach is a lane-based background subtraction. It allows to manually define on the lane profile the level of noise to be subtracted.

1. Click on the “Valley to valley ” button: 

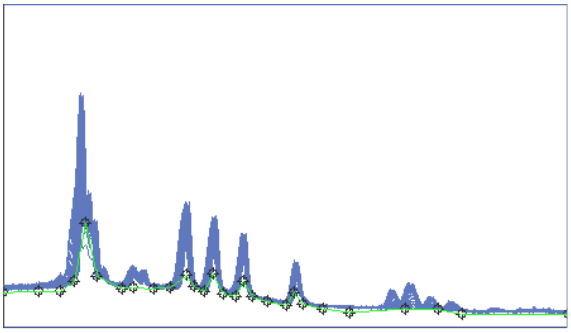

It opens the lane profile window:


2. In the profile parameters window, select the lane to perform the valley-to-valley

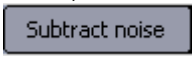
approach



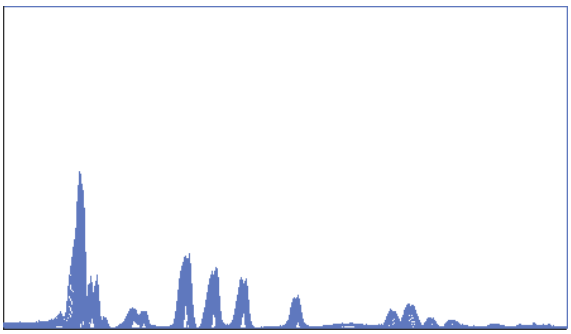

On the profile, click to define the background profile you want to remove:



Then, click on Subtract noise:

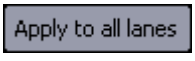



The changes will be automatically applied to the image and to the profile:

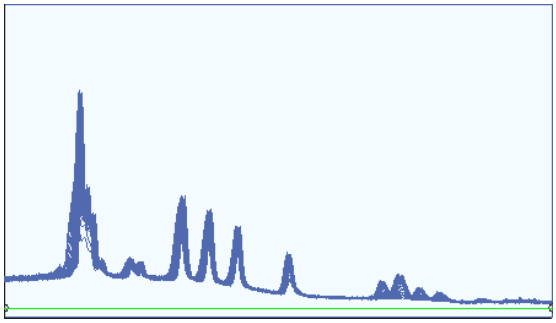
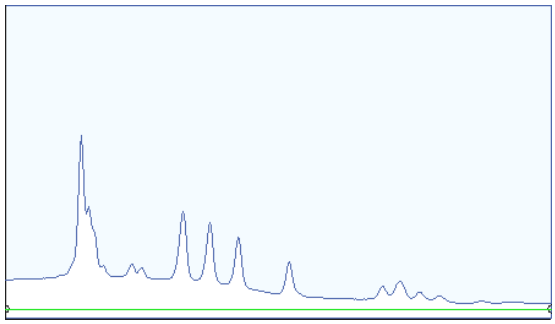
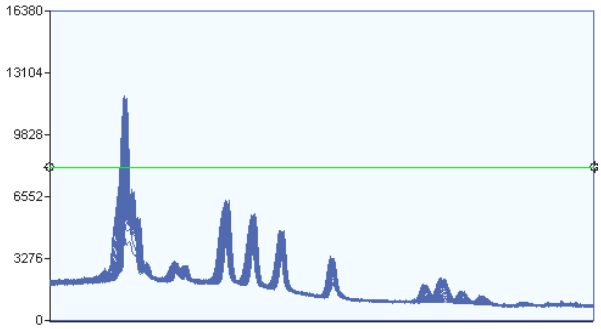
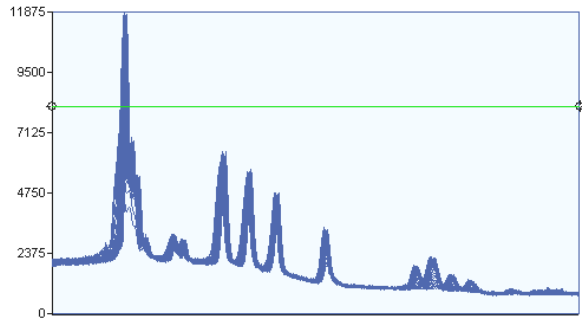
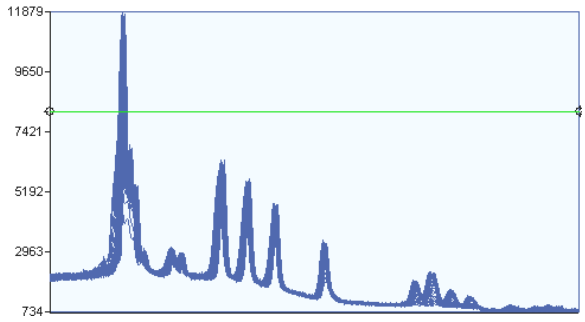


The valley-to-valley approach is a lane-based background subtraction. You can set the same subtraction level for all lanes or specify an individual subtraction level for the selected lane. Any changes you make will be automatically applied to the image.

To apply the same subtraction level for all lanes, click on the “Apply to all lanes” button:




You can easily adjust the profile displays settings as follows:

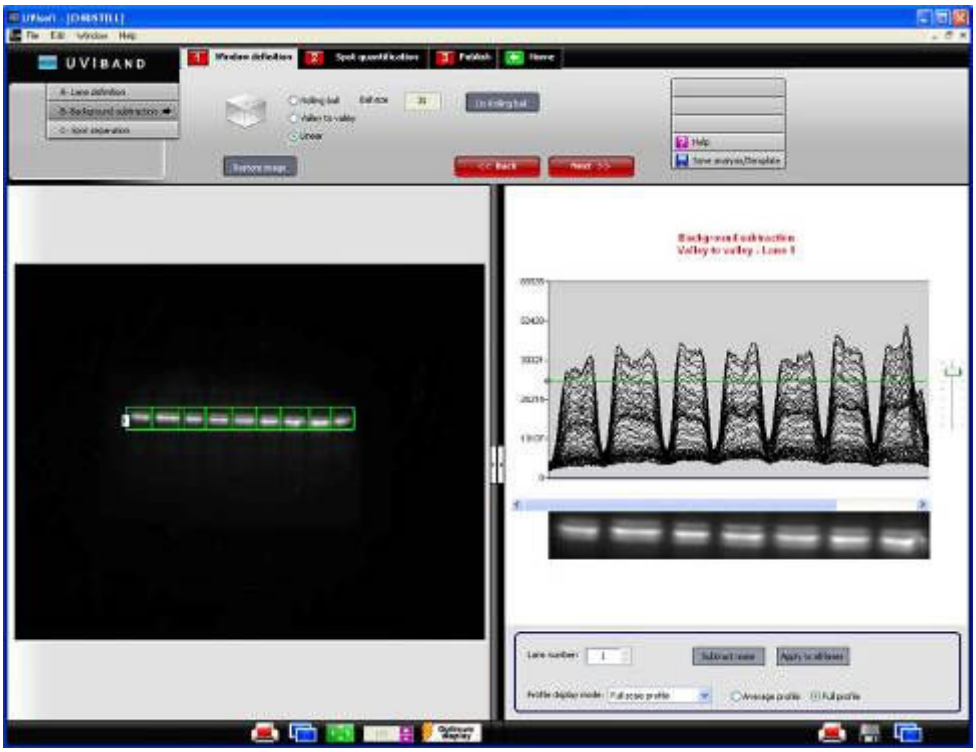
| | |
|---|---|
| Full profile |  |
| Average profile |  |
| Profile display mode:<br>Full scale profile<br>The profile scale goes from 0 to the image maximum dynamic. |  |
| Profile display mode:<br>0 to Maximum<br>The profile scale goes from 0 to the lane's maximum intensity; |  |
| Profile display mode:<br>Minimum to Maximum<br>The profile scale goes from the lane's minimum intensity to the lane's maximum intensity; |  |

**LINEAR APPROACH**

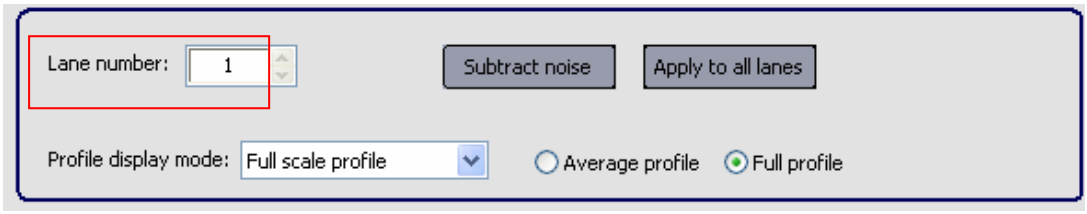
The linear approach is a lane-based background subtraction. It allows to manually define the level of noise to be subtracted on the lane profile.

1. Click on the “Linear ” button: 

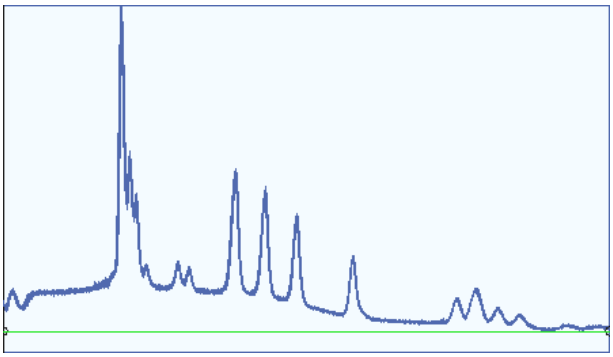

It opens the lane profile window:



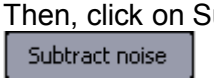

In the profile parameters window, select the lane to perform the linear approach



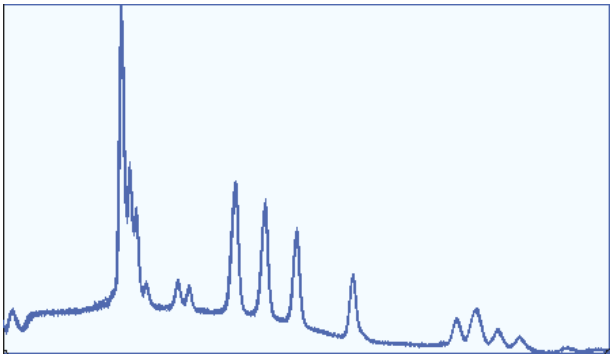

On the profile, click to define the background linear level you want to remove:



Then, click on Subtract noise:

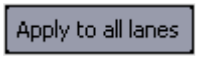


The changes will be automatically applied to the image and to the profile:

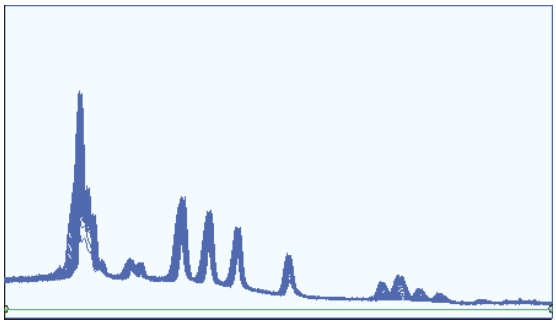
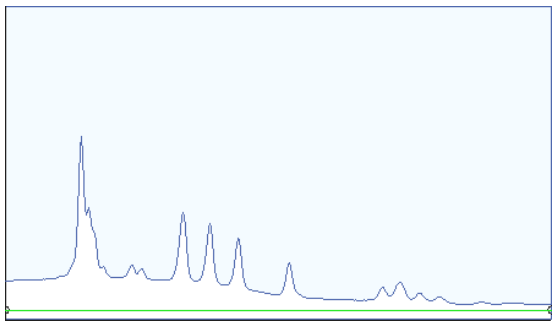


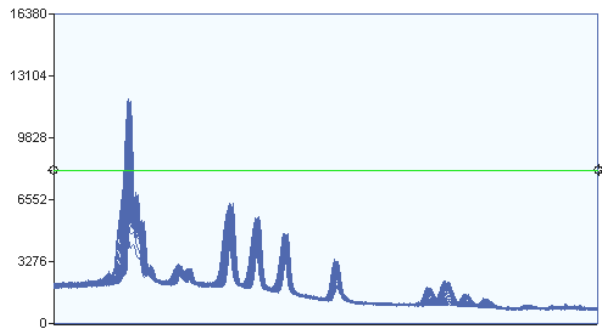
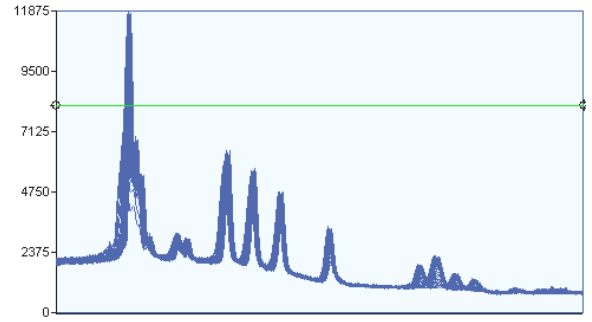
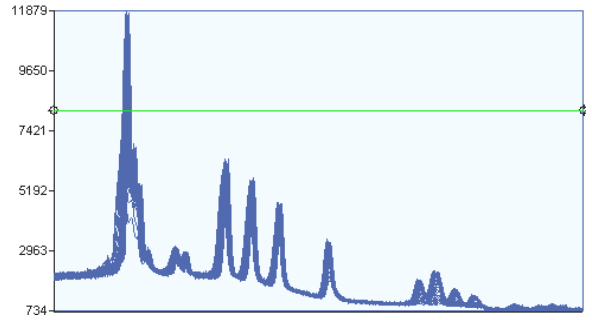
The linear approach is a lane-based background subtraction. You can set the same subtraction level for all lanes or specify an individual subtraction level for the selected lane. Any changes you make will be automatically applied to the image.

To apply the same subtraction level for all lanes, click on the “Apply to all lanes” button:




You can easily adjust the profile displays settings as follows:

| | |
|---|---|
|   |  |
|   |  |

| | |
|---|---|
| Profile display mode:<br>Full scale profile<br>The profile scale goes from 0 to the image maximum dynamic. |  |
| Profile display mode:<br>0 to Maximum<br>The profile scale goes from 0 to the lane's maximum intensity; |  |
| Profile display mode:<br>Minimum to Maximum<br>The profile scale goes from the lane's minimum intensity to the lane's maximum intensity; |  |

**NEXT**

The "Next" button validates your parameter and opens the following analysis step.

| | | |
|---|---|---|
| 1 B – Background subtraction | Next >> | 1 C- Spot separation |

**BACK**

The "Back" button validates your parameter and opens the following analysis step.

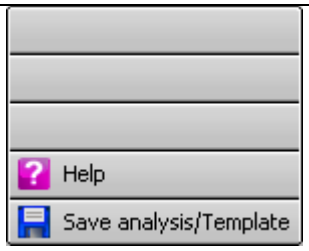
| | | |
|---|---|---|
| 1 B – Background subtraction | << Back | 1 A – Lane definition |

**OPTION FOLDER**

The option folder gathers the following functions:

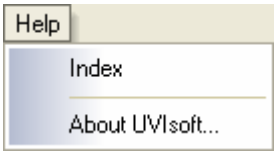
⇨ Help

⇨ Save the analysis or the template




**HELP MENU**


Click on the “Help” button. You automatically access the user manual at the chapter corresponding to the function. You can access the help file index through the File\Help from the Menu bar




**SAVE ANALYSIS / TEMPLATE**

This function saves the current analysis. The analysis file will contain the results, the image and all the parameters defined to obtain the results.

The analysis could also be saved as a template for automated analysis routines. Template offers the user the ability to automate many of the repetitive tasks associated with analysis and processing. As a result, you can spend more time evaluating and analysing results, and less time manipulating set-ups, variables and other settings.

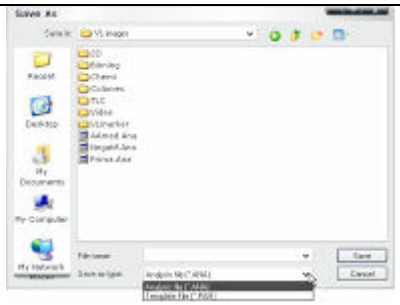
The template automates a task or set of tasks that you perform repeatedly or on a regular basis. It stores all the analysis commands and parameters of an analysis. You can run these parameters with another image whenever you need to perform a new analysis based on the same parameters.

The benefits of the template file are as follows:

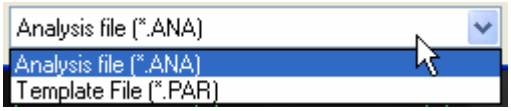
⇨ Time saving
⇨ Reproduction of image analysis parameters
⇨ Templates are modifiable, allowing the user to maintain an original template while modifying it for a slightly different result, with minimal effort

1. Click on the “Save analysis/ Template” button:


2. A pop-up window displays the following menu:

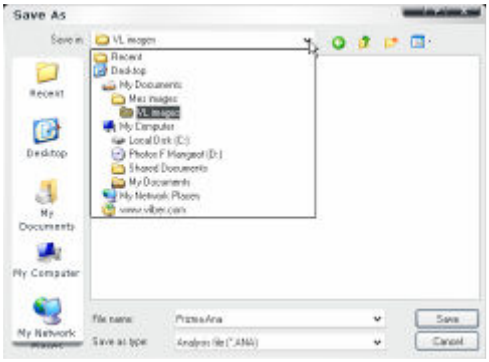


3. Select analysis file or template file:

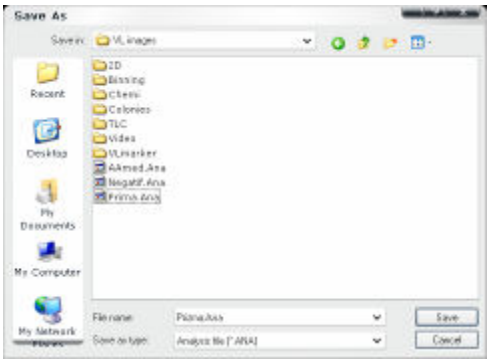



Note: the software proposes Analysis file by default

4. Browse to specify the file directory




5. Enter the desired file name and validate




6. Click on the Save button to create the file.

Note: see “Access to the analysis module” chapter for template or analysis file loading

## ➔ C – Spot separation

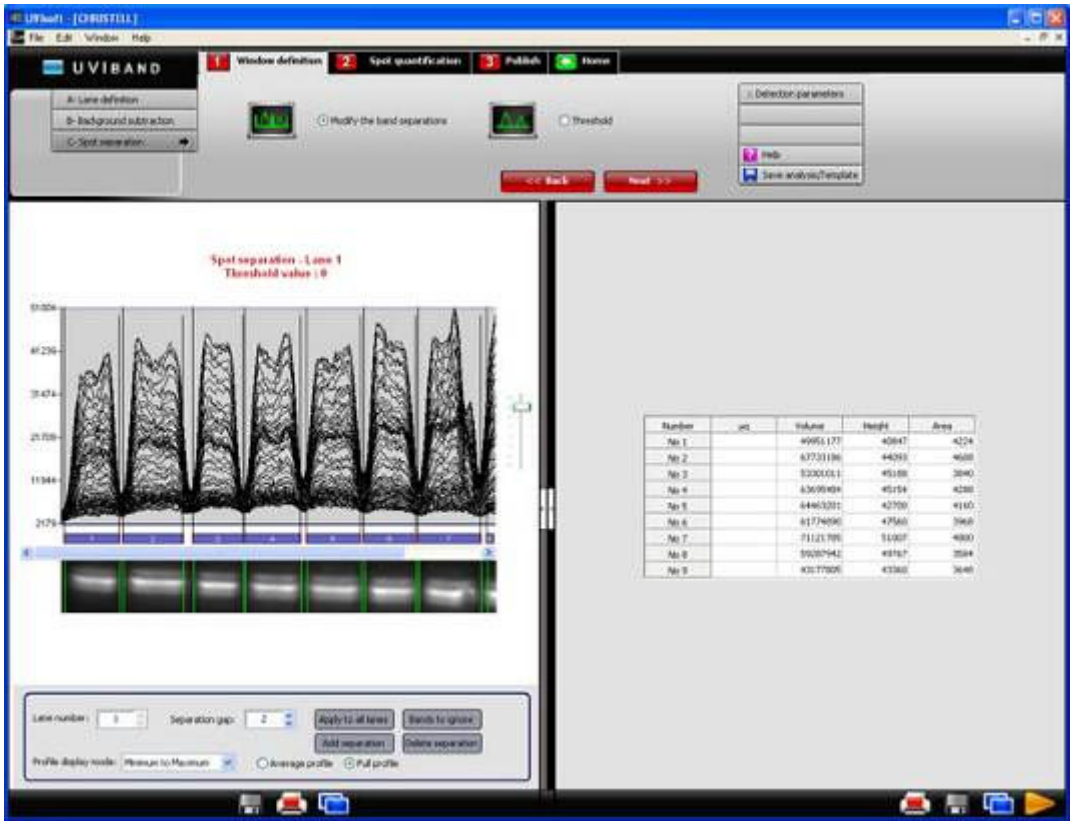
1

In order to measure the volume of a particular spot, you need:
⇨ To define the boundary around the spot;
⇨ To compare the intensity data inside the boundary with the data of other spots or of a standard.

A volume is the sum of the pixel intensity inside a defined boundary. The purpose of the spot separation is to define this boundary.

The spot separation process follows the background subtraction.

Note: you can either access the spot separation function by clicking on the next button of the background subtraction or directly by clicking on the spot separation of the Window definition folder.




The dashboard details the spot separation parameters:

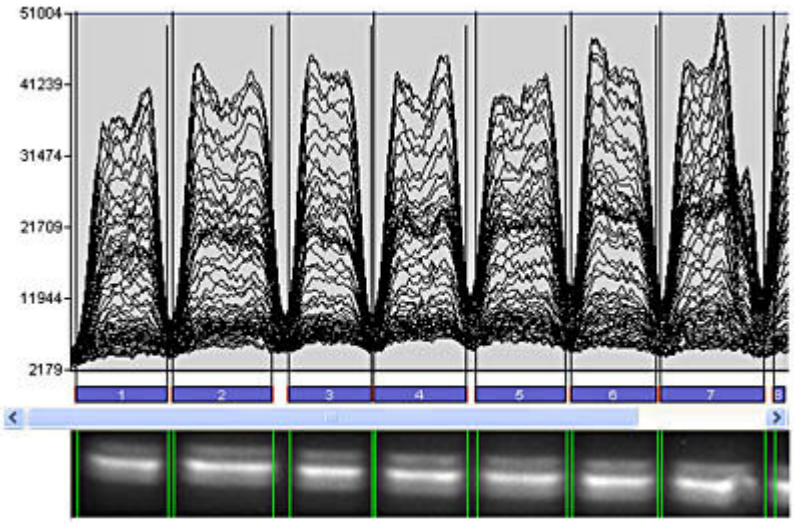

⇨ Modify the spot separation
⇨ Standard threshold
⇨ Extended threshold

**MODIFY THE SPOT SEPARATION**

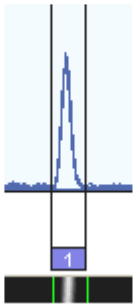
UVIband Advance proposes by default an automatic predefined spot separation based on the

band detection. You can modify the default spot separation by selecting the “Modify the spot separation” option.

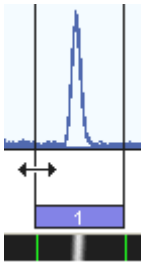

The default separation is illustrated on the lane’s profile:




The brackets illustrate the bands boundaries:



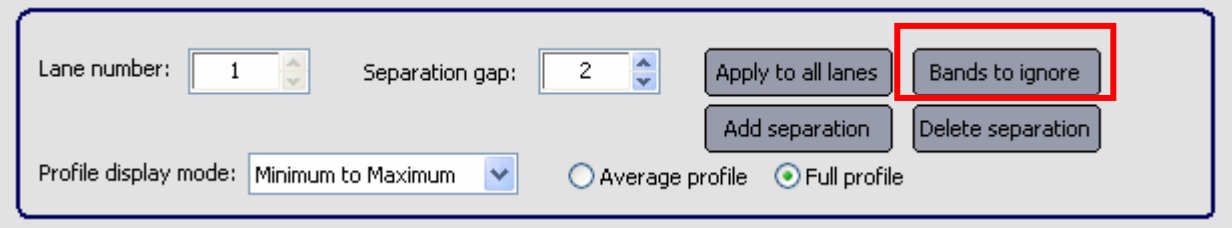

You can easily reposition a band’s boundaries. In order to do so, click on the bracket and drag the cursor:



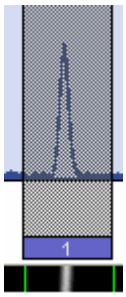

Drag the cursor until the area of the band that you want to define has been completely enclosed.

**Note**: When you release the mouse button, the band’s volume is automatically recalculated to take into account the new area of interest.

To ignore a band, select “Bands to ignore” from the profile’s parameter menu:



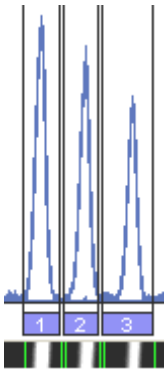

Then, click on the band you want to ignore:



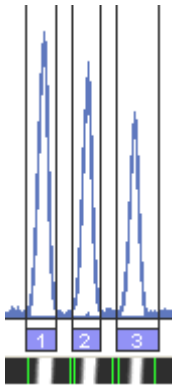

The band is then highlighted in grey and discarded from the result table:

Note: you can ignore more than one band at a time.
Note: to stop the process, click again on the “Bands to ignore” button.

To increase the gap in between the lane, select the “Separation gap” option from the profile’s parameter menu:




**Limited separation gap**

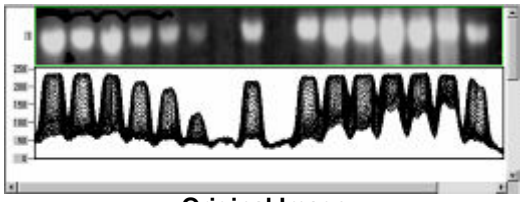



**Extended separation gap**

## DEFINE A THRESHOLD

The threshold defines the detection level to take into account for the volume quantification. It allows to distinguish between bands and smears on the lane.

Case when you should use detection level (Threshold)
There is still a strong background even after the background subtraction



**Original Image**

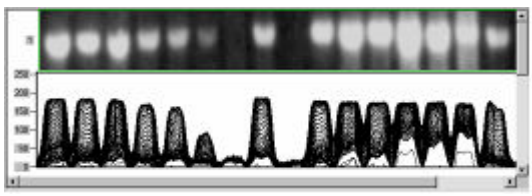


**Image with subtracted background**

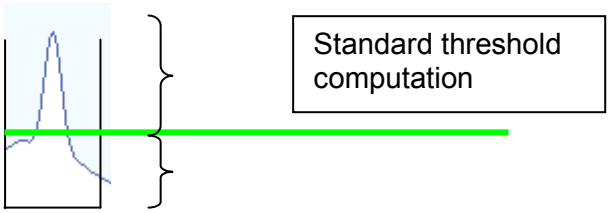
The spot contours must be isolated more precisely from the smears where they are located

The threshold calculates the volume which is above the threshold:
Standard threshold:

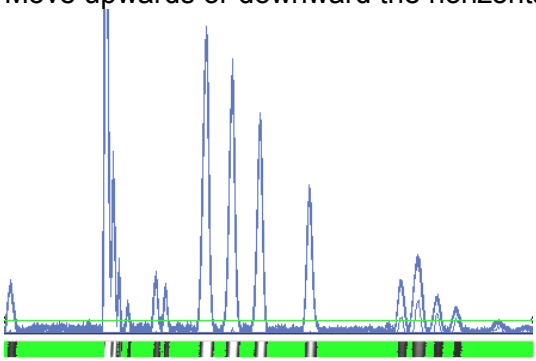
Volume= ∑ (Pixels intensities)

⇨ Pixel intensities=0 if Pixel<Threshold

⇨ Pixel intensities= (Pixel intensities) if Pixel>Threshold




Move upwards or downward the horizontal line appearing on the profile:



This displays a green contour that encloses pixels whose intensity is equal to or greater than that of the pixel at the cursor. If the contour does not encircle the band, reposition the cursor and click again. A new contour will be drawn in place of the old one.

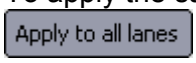
The green area under the profile represents the range of values discarded to calculate the volume. The contour should completely surround the data you want to quantify.
The defined threshold is automatically applied to the selected lane. The results are recalculated taking into account the threshold:

| Number | // | Volume | Height | Area | MW-RF |
|---|---|---|---|---|---|
| No 1 | | 224452 | 2173 | 126 | 31.714 |
| No 2 | | 561516 | 2110 | 294 | 24.000 |
| No 3 | | 1216111 | 2429 | 574 | 13.143 |
| No 4 | | 1072687 | 11699 | 210 | 8.812 |
| No 5 | | 388143 | 6775 | 70 | 7.391 |
| No 6 | | 373495 | 5360 | 98 | 6.459 |
| No 7 | | 531756 | 2988 | 224 | 5.767 |
| No 8 | | 1140205 | 3070 | 490 | 4.945 |
| No 9 | | 1144095 | 6172 | 392 | 3.847 |
| No 10 | | 943922 | 5602 | 350 | 2.930 |
| No 11 | | 1138471 | 4700 | 602 | 2.453 |
| No 12 | | 1235282 | 3269 | 966 | 1.987 |
| No 13 | | 385044 | 1870 | 294 | 1.417 |
| No 14 | | 401562 | 2191 | 252 | 0.973 |
| No 15 | | 243847 | 1541 | 195 | 0.774 |
| No 16 | | 213134 | 1311 | 191 | 0.573 |
| No 17 | | 4827 | 973 | 5 | 0.389 |
| No 18 | | 0 | 0 | 0 | 0.267 |

⇨ The volume is the sum of intensities included in the spot area of analysis.
⇨ The height is the maximum spot intensity, in gray levels.
⇨ The area is the zone defined for each spot area of analysis.

The threshold approach is on a lane-based basis. You can set the same threshold for all lanes or specify an individual threshold for the selected lane. Any changes you make will be automatically applied to the image.

To apply the same subtraction level for all lanes, click on the “Apply to all lanes” button:




**NEXT**

The “Next” button validates your parameter and opens the following analysis step.

| | | |
|---|---|---|
| 1 C- Spot separation | Next >> | 2 A – Volume of reference |

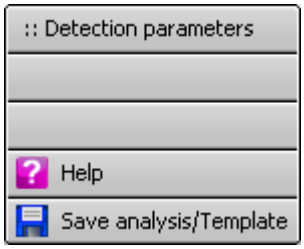
**BACK**

The “Back” button validates your parameter and opens the following analysis step.

| | | |
|---|---|---|
| 1 C- Spot separation | << Back | 1 B – Background subtraction |

**OPTION FOLDER**

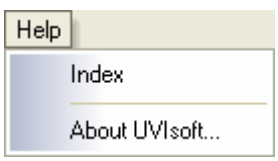
The option folder gathers the following functions:
⇨ Help
⇨ Save the analysis or the template




**HELP MENU**

Click on the “Help” button. You automatically access the user manual at the chapter corresponding to the function


You can also access the help file index through the File\Help from the Menu bar




**SAVE ANALYSIS / TEMPLATE**

This function saves the current analysis. The analysis file will contain the results, the image and all the parameters defined to obtain the results.

The analysis could also be saved as a template for automated analysis routines.

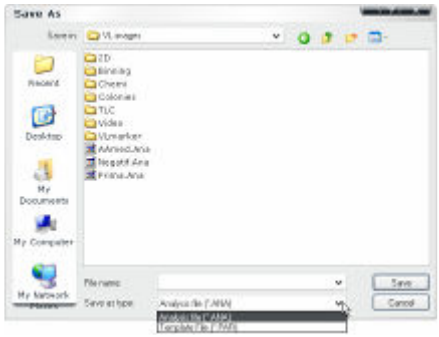
Template offers the user the ability to automate many of the repetitive tasks associated with analysis and processing. As a result, you can spend more time evaluating and analysing results, and less time manipulating set-ups, variables and other settings.

The template automates a task or set of tasks that you perform repeatedly or on a regular basis. It stores all the analysis commands and parameters of an analysis. You can run these parameters with another image whenever you need to perform a new analysis based on the same parameters.

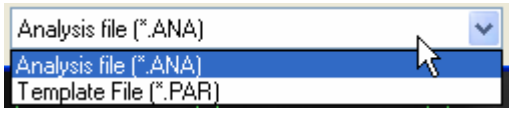
The benefits of the template file are as follows:
⇨ Time saving
⇨ Reproduction of image analysis parameters
⇨ Templates are modifiable, allowing the user to maintain an original template while modifying it for a slightly different result, with minimal effort

1. Click on the “Save analysis/ Template” button:


2. A pop-up window displays the following menu:

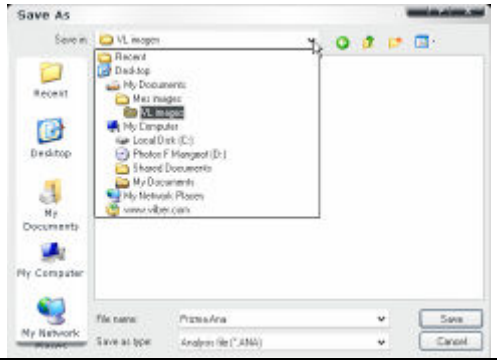



3. Select analysis file or template file:

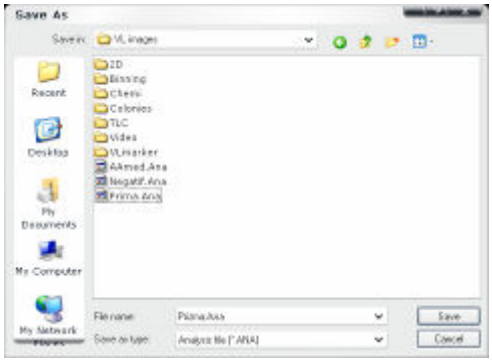



Note: the software proposes Analysis file by default

4. Browse to specify the file directory



5. Enter the desired file name and validate




6. Click on the Save button to create the file.

Note: see “Access to the analysis module” chapter for template or analysis file loading

# 2- Analyse – Quantification

## ➔ Principles of quantification

2

Volume is the based of the spot quantification process. The volume is the sum of all the intensities included in the defined area (window + separation).

Quantification is based on the image in pixels whose intensity is coded on a scale.
- The scale has 256 grey levels for a 8-bit image
- The scale has 4 096 grey levels for a 12-bit image
- The scale has 16 384 grey levels for a 14-bit image
- The scale has 65 536 grey levels for a 16-bit image

The quantity (or density) of a spot is calculated from its volume. This is made of the sum of all pixel intensities composing the spot

In other words, the spot quantity then depends on:
- The number of pixels inside the area of the spot
- The intensities of these points

$$V = \sum ni\ Ii$$

Image analysis allows comparison in between concentrated intense spots and weaker but more diffused bands.

Results are given in volumes that may be recalculated according to an OD of reference or a concentration master-curve.

To measure the amount of a particular spot, you need to define the boundary around the spot and compare the intensity data inside the boundary with the data of other spots or of a standard.

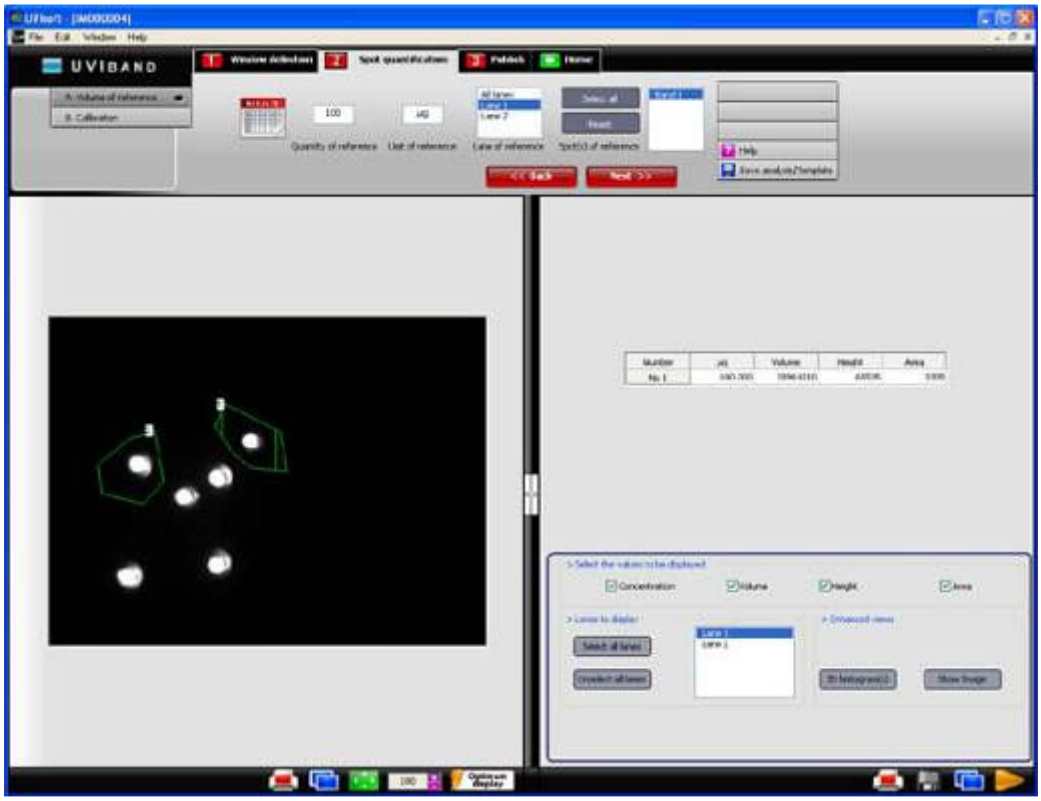
## ➔ A – Volume of reference

2

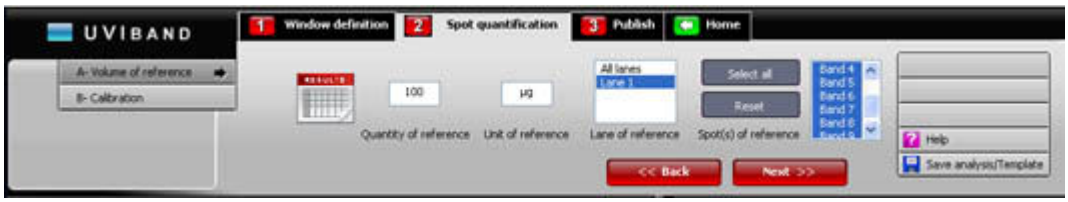
A volume is the total signal intensity inside a defined boundary drawn on a lane.

The purpose of the volume of reference is to use volumes of known concentration to calculate the unknown concentrations. The volume of reference process follows the spot separation.

Note: you can either access the volume of reference function by clicking on the next button of the background subtraction or directly by clicking on the volume of reference of the 2-Spot Quantification folder.




The dashboard details the volume of reference parameters:




- ⇨ The quantity of reference
- ⇨ The unit of reference
- ⇨ The lane of reference
- ⇨ The spot(s) of reference

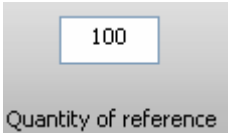
**QUANTITY OF REFERENCE**

The calculation of the unknown concentrations is based:

- ⇨ On the calculated volumes
- ⇨ On the known concentration. The known concentration is the quantity of

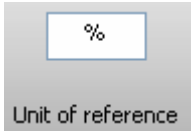
reference.

The quantity of reference could correspond to one or several spots.
The purpose of the quantity of reference is to define the known concentration:
In the “Quantity of reference” edit field, type the quantity of known concentration you want to have as a reference:

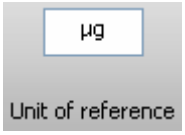



**UNIT OF REFERENCE**

The unit of reference is the header unit of the concentration. You can define your own header such as % or µg.
In the “Unit of reference” edit field, type the unit you want to be displayed in the results table:



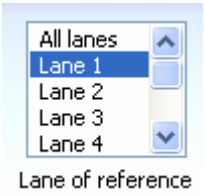

**Percentage as unit of reference**




**µg as unit of reference**

**LANE OF REFERENCE**

The lane of reference defines the lane of the known concentration.
Select the lane of reference from the list:




If a single lane is selected, only the volumes of this reference lane will be used to calculate the relationship between the volume and the quantity. The other concentrations are calculated based on the concentration/volume relationship of this specific lane.

| | Lane 1 | | Lane 3 | | Lane 4 | |
|---|---|---|---|---|---|---|
| Number | % | Volume | % | Volume | % | Volume |
| No 1 | 44.708 | 6635518 | 178.291 | 26461728 | 49.658 | 7370205 |
| No 2 | 25.475 | 3780895 | 64.424 | 9561786 | 47.652 | 7072517 |
| No 3 | 14.264 | 2117062 | 9.885 | 1467075 | | 0 |
| No 4 | 9.304 | 1380926 | | | | 0 |
| No 5 | 3.574 | 530507 | | | | 0 |
| No 6 | 1.840 | 273100 | | | | 0 |
| No 7 | 0.835 | 123860 | | | | |
| No 8 | | 0 | | | | |
| No 9 | | 0 | | | | |

Illustration 1: 100% / lane 1 / all bands. Total concentration lane 1= 100%

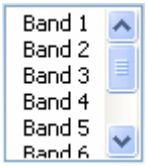
If “All lanes” is selected, for each lane a new relationship between volume and quantity will be recalculated, according to the band’s lane selected. For instance, the defined parameters are 100% for all band all lanes; the results table could be as follows. Lane by lane, the total band concentration is 100%:

| | Lane 1 | | Lane 3 | | Lane 4 | |
|---|---|---|---|---|---|---|
| Number | % | Volume | % | Volume | % | Volume |
| No 1 | 44.708 | 6635518 | 70.582 | 26461728 | 51.031 | 7370205 |
| No 2 | 25.475 | 3780895 | 25.504 | 9561786 | 48.969 | 7072517 |
| No 3 | 14.264 | 2117062 | 3.913 | 1467075 | | 0 |
| No 4 | 9.304 | 1380926 | | | | 0 |
| No 5 | 3.574 | 530507 | | | | 0 |
| No 6 | 1.840 | 273100 | | | | 0 |
| No 7 | 0.835 | 123860 | | | | |
| No 8 | | 0 | | | | |
| No 9 | | 0 | | | | |

Illustration 2: 100% / all lanes / all bands. Total concentration all lanes= 100%

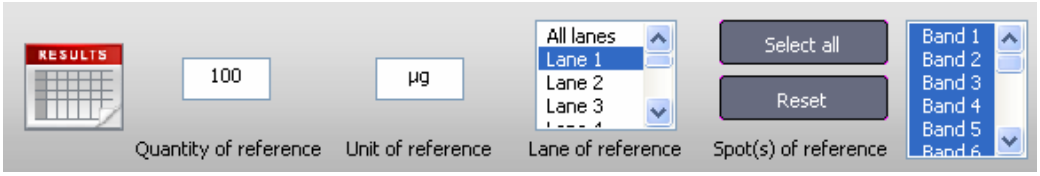
## SPOT(S) OF REFERENCE

The quantity of reference could correspond to one or several spots of the selected lane.
Select one or several spots of the lane of reference from the list:




## EXAMPLE 1

Let's consider the known concentration is 3µg contains in the first spot of lane 3. The settings should then be as follows:

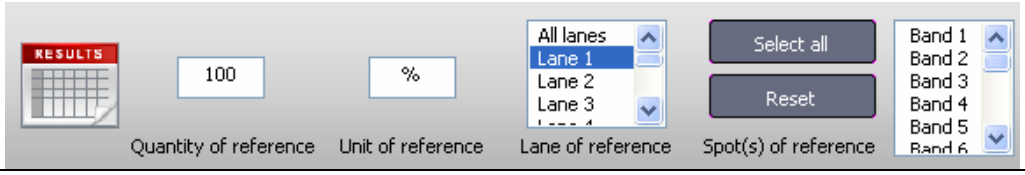



The results table indicates the following for lane 3:

| Number | µg | Volume | Height | Area |
|---|---|---|---|---|
| No 1 | 3.000 | 4285313 | 4071 | 1775 |
| No 2 | 9.267 | 13237182 | 3438 | 5396 |
| No 3 | 0.942 | 1345357 | 2740 | 568 |
| No 4 | 0.467 | 667689 | 2692 | 284 |
| No 5 | 12.560 | 17940927 | 2651 | 10224 |
| No 6 | 0.358 | 511654 | 1305 | 426 |
| No 7 | 3.885 | 5549237 | 1275 | 5112 |
| No 8 | 1.626 | 2322765 | 1176 | 2414 |
| No 9 | 0.465 | 664510 | 1000 | 710 |

## EXAMPLE 2

Let's consider the known concentration is 100% contains in all the spots of lane 1. The settings should then be as follows:



The results table indicates the following for lane 1:

| Number | % | Volume | Height | Area |
|---|---|---|---|---|
| No 1 | 3.978 | 1715709 | 2744 | 781 |
| No 2 | 15.367 | 6627687 | 4310 | 2769 |
| No 3 | 11.431 | 4930041 | 4642 | 2130 |
| No 4 | 12.333 | 5319454 | 2612 | 2414 |
| No 5 | 2.112 | 911077 | 2323 | 426 |
| No 6 | 35.571 | 15341999 | 2191 | 10508 |
| No 7 | 19.207 | 8284193 | 1270 | 8591 |

**NEXT**

The “Next” button validates your parameter and opens the following analysis step.

| | | |
|---|---|---|
| 2 A – Volume of reference | Next >> | 2 B – Calibration |

**BACK**

The “Back” button validates your parameter and opens the following analysis step.

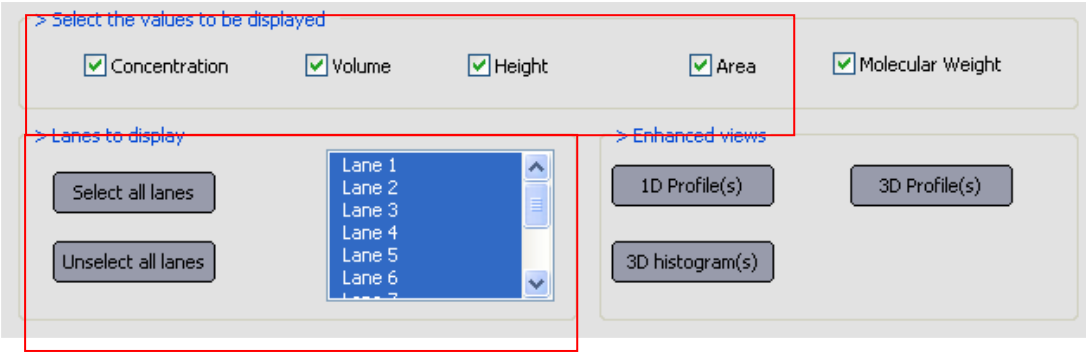
| | | |
|---|---|---|
| 2 A – Volume of reference | << Back | 1 C- Spot separation |

**RESULT TABLE**

In the result parameter window, you can select the lanes and the values to be displayed in the results tables:

- ⇨ Concentration
- ⇨ Volume
- ⇨ The maximum intensity
- ⇨ The area

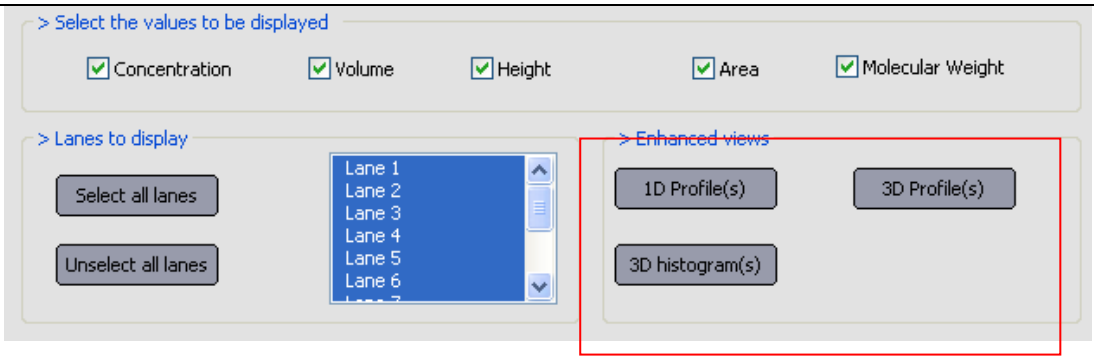
1. To select your display mode, click on the appropriate selection:




**GRAPHICAL VIEW**

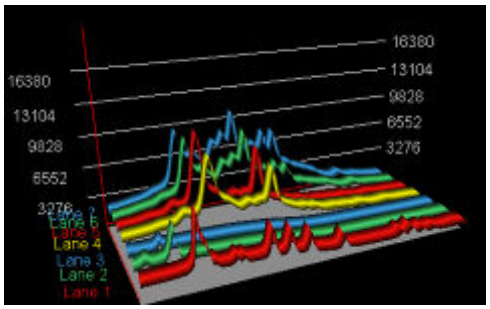
In the results parameter window, you can select the graphical results tables:

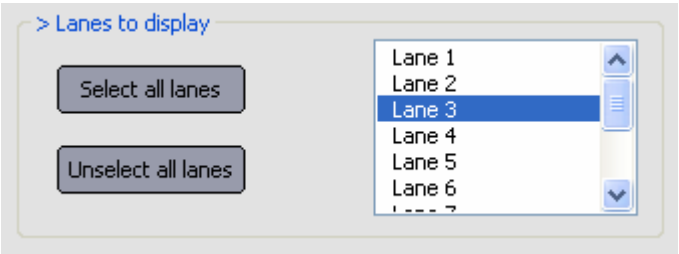
- ⇨ 1D profile
- ⇨ 3D profile
- ⇨ 3D histogram




Note: For all enhanced views, you can modify the angle of vision of the 3D view: Move the mouse cursor on the 3D area, click and drag the view in the direction you want to rotate. Release the mouse when satisfactory.

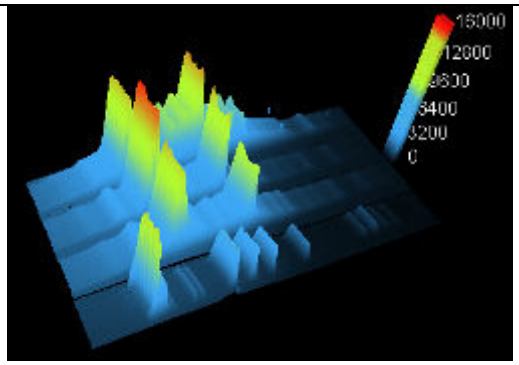
The 1D profile allows you to superimpose the intensity profiles of any number of selected lanes.
To proceed, click on the 1D Profile and select the lanes to be superimposed:

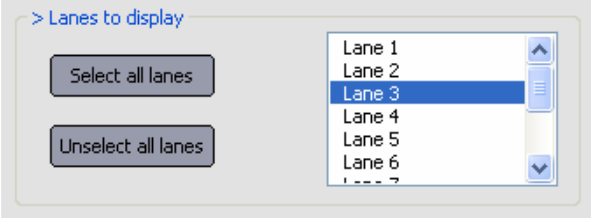




Note: To reposition the 1D profile window, position your cursor at the top of the box. The cursor appearance will change to a multidirectional arrow symbol. You can then drag the box to a new position.
Note: To increase the size of the 1D profile window, position your cursor at the edge of the window and click and drag to extend its size.
Note: Click on Print to print the 1D profile window
Note: Click on Send to clipboard to save the graph in the Windows ® clipboard (Past/copy Windows ® feature).

The 3D profile displays the three-dimensional rendering of any selected lanes.
To proceed, click on the 3D Profile button and select the lanes to be displayed:



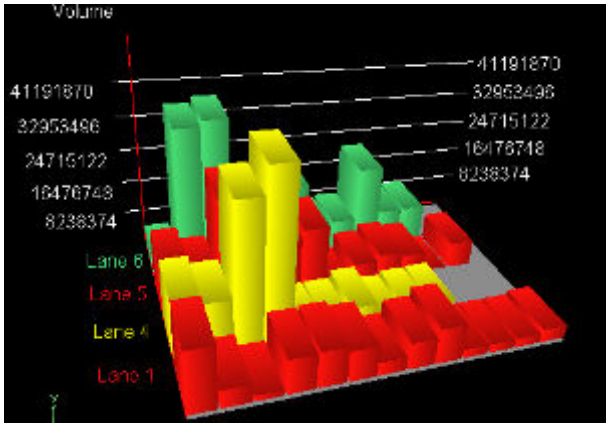




Note: To reposition the 1D profile window, position your cursor at the top of the box. The cursor appearance will change to a multidirectional arrow symbol. You can then drag the box to a new position.
Note: To increase the size of the 1D profile window, position your cursor at the edge of the window and click and drag to extend its size.
Note: Click on Print to print the 1D profile window
Note: Click on Send to clipboard to save the graph in the Windows ® clipboard (Past/copy Windows ® feature).

The 3D histogram displays the three-dimensional histogram of selected results:

- ⇨ Volume
- ⇨ Calculated quantities
- ⇨ Maximum intensities

To proceed, click on the 3D Histogram button and select the lanes to be displayed:


Note: To reposition the 1D profile window, position your cursor at the top of the box. The cursor appearance will change to a multidirectional arrow symbol. You can then drag the box to a new position.
Note: To increase the size of the 1D profile window, position your cursor at the edge of the window and click and drag to extend its size.
Note: Click on Print to print the 1D profile window

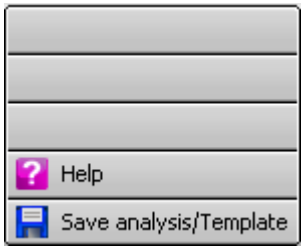
Note: Click on Send to clipboard to save the graph in the Windows ® clipboard (Past/copy Windows ® feature).

**OPTION FOLDER**

The option folder gathers the following functions:

⇨ Help

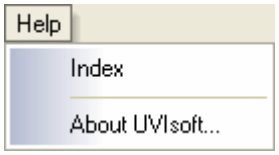
⇨ Save the analysis or the template




**HELP**

Click on the “Help” button. You automatically access the user manual at the chapter corresponding to the function


You can access the help file index through the File\Help from the Menu bar




**SAVE ANALYSIS / TEMPLATE**

This function saves the current analysis. The analysis file will contain the results, the image and all the parameters defined to obtain the results.

The analysis could also be saved as a template for automated analysis routines. Template offers the user the ability to automate many of the repetitive tasks associated with analysis and processing. As a result, you can spend more time evaluating and analysing results, and less time manipulating set-ups, variables and other settings.

The template automates a task or set of tasks that you perform repeatedly or on a regular basis. It stores all the analysis commands and parameters of an analysis. You can run these parameters with another image whenever you need to perform a new analysis based on the same parameters.

The benefits of the template file are as follows:

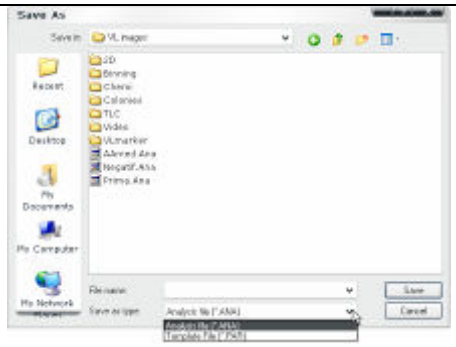
⇨ Time saving

⇨ Reproduction of image analysis parameters

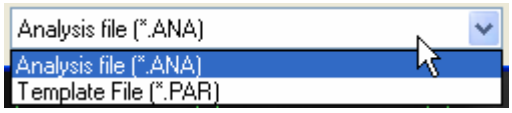
⇨ Templates are modifiable, allowing the user to maintain an original template while modifying it for a slightly different result, with minimal effort

1. Click on the “Save analysis/ Template” button:


2. A pop-up window displays the following menu:

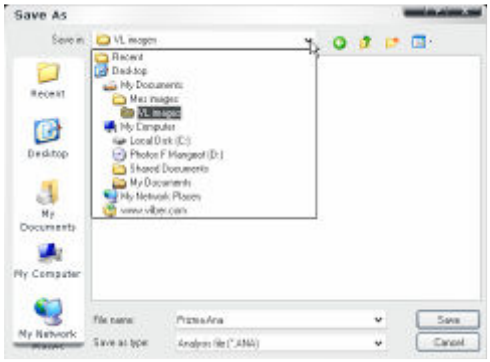


3. Select analysis file or template file:

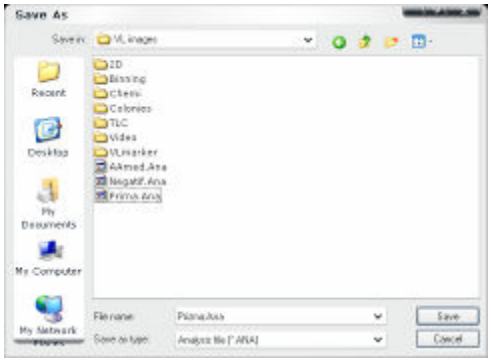



Note: the software proposes Analysis file by default

4. Browse to specify the file directory



5. Enter the desired file name and validate



6. Click on the Save button to create the file.

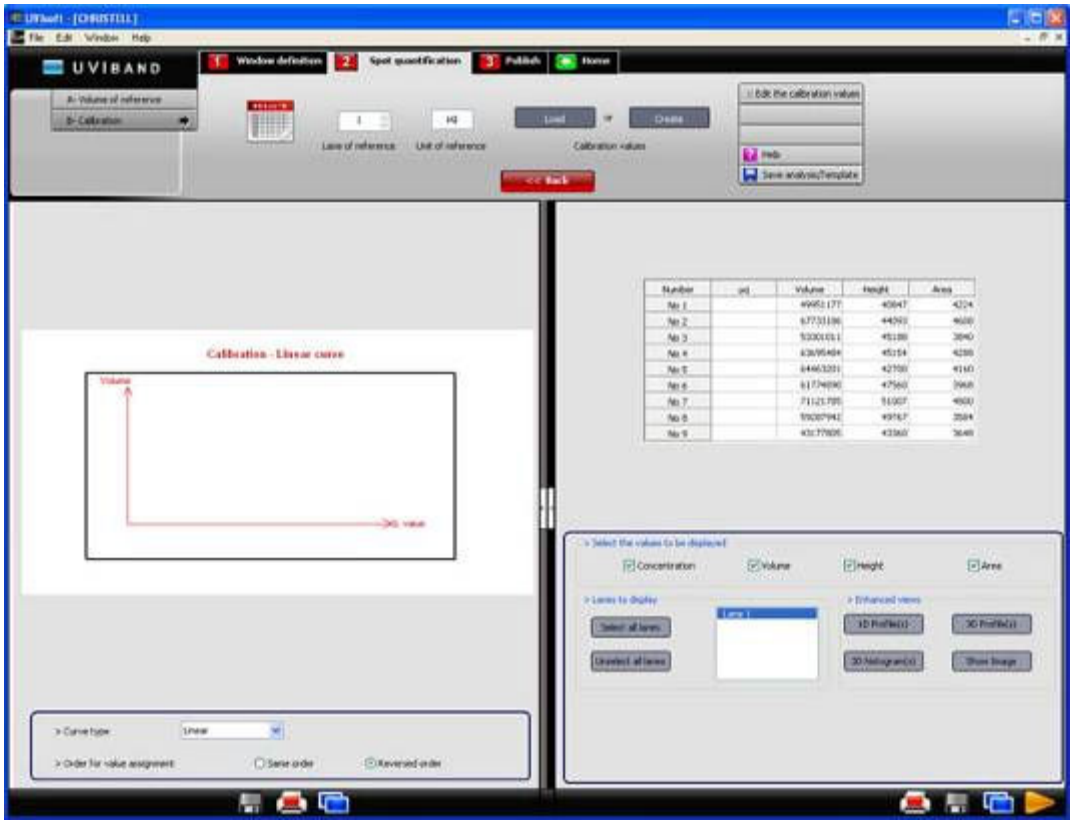
Note: see “Access to the analysis module” chapter for template or analysis file loading

## ➔ B – Calibration

2

The calibration process follows Volume of reference. The calibration is the calculation of the concentration based on a concentration master or on a calibration curve on which you can select all or few points.

Note: you can either access the calibration function by clicking on the next button of the Volume of reference or directly by clicking on the Calibration of the 2-Spot quantification folder.




The dashboard details the volume of reference parameters:


⇨ The lane of reference
⇨ The unit of reference
⇨ The calibration values

**LANE OF REFERENCE**

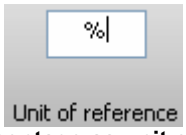
The lane of reference defines the lane of the known concentration.
Select the lane of reference from the list:


Lane of reference

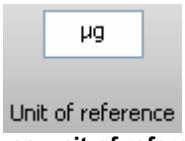
**UNIT OF REFERENCE**

The unit of reference is the header unit of the concentration. You can define your own header such as % or µg.

In the “Unit of reference” edit field, type the unit you want to be displayed in the results table:




**Percentage as unit of reference**




**µg as unit of reference**

**THE CALIBRATION VALUES**

Click on the “Load” or “Create” button to enter calibration’s values.

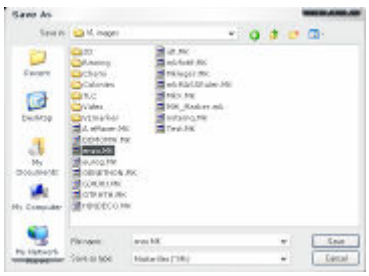

For “Create”, a pop-up window displays the following menu:


Type your values, band to band, in a descending order. The OK button validates your data.

Note: if an automatic calculation with immediate application of the standard values is carried out, it is not necessary to enter all the bands given by the manufacturer's specifications, but only those which are commonly found on the lanes of the gel.

You can save your calibration data and create your own calibration library; To proceed, click on the “Save “ button:


A pop-up window displays the following menu:

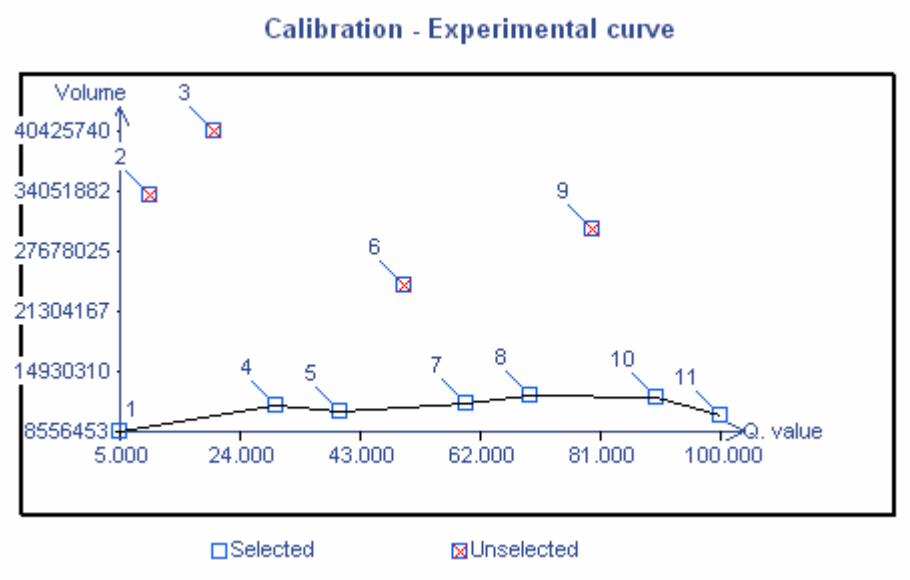


⇨ Browse to specify the directory

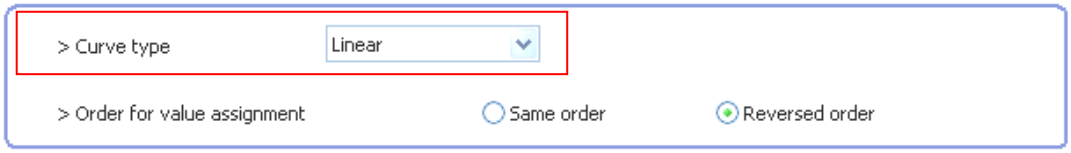
⇨ Type the file name and click on Save.

**MASTER CURVE**

After the values of the master-curve are defined, the calibration curve is displayed. You can unselect wrong values or points out of the curve by directly clicking on them




In the profile parameters window, select the curve type:



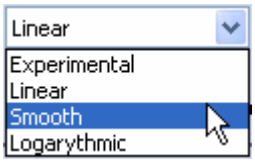

Four mathematical models can be used:

⇨ Experimental: the curve simply links the values (point to point), without any mathematical model,

⇨ Linear curve: displays a model with linear regression

⇨ Smoothed: displays a smoothed curve (polynomial spline, at least 4 points must be entered)

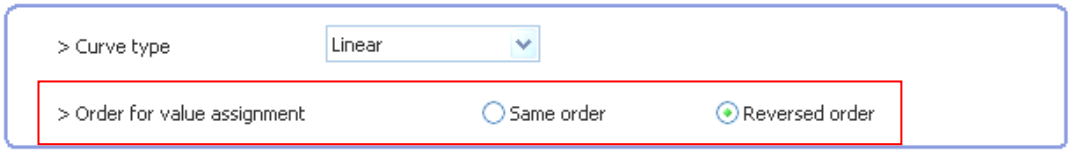
⇨ Logarithmic curve: displays a model with logarithmic regression




You can also select the order for the spot display:

⇨ - Same order as the values of the master-curve

⇨ - Reversed order (depending on the order of the defined values)

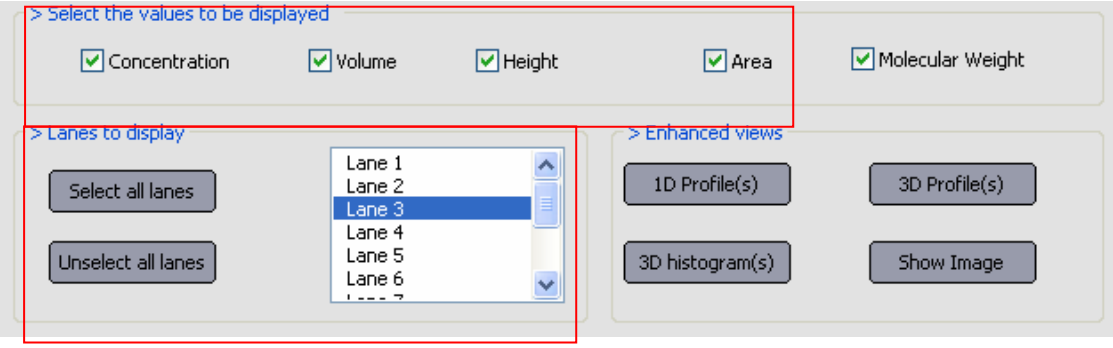


**RESULT TABLE**

In the result parameter window, you can select the lanes and the values to be displayed in the results tables:

- ⇨ Concentration
- ⇨ Volume
- ⇨ The maximum intensity
- ⇨ The area

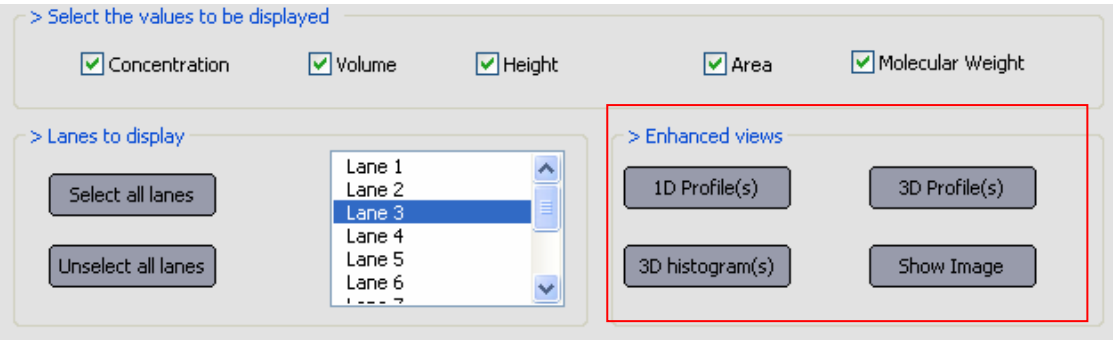
To select your display mode, click on the appropriate selection:




**GRAPHICAL VIEW**

In the results parameter window, you can select the graphical results tables:

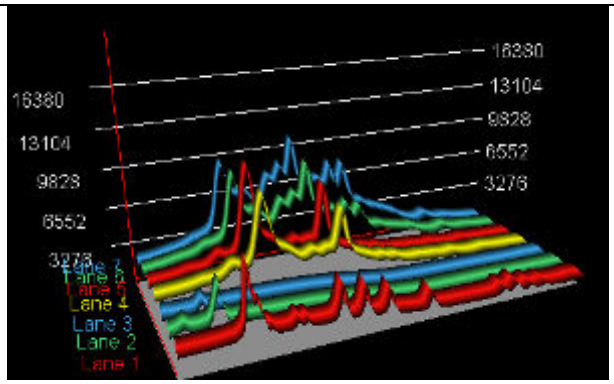
- ⇨ 1D profile
- ⇨ 3D profile
- ⇨ 3D histogram

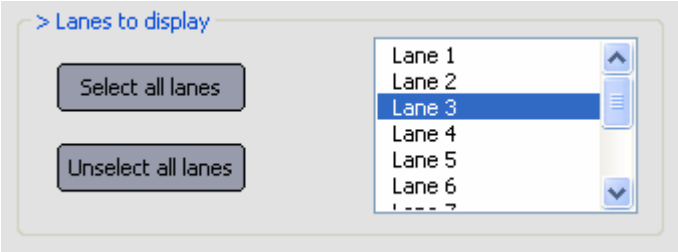



Note: For all enhanced views, you can modify the angle of vision of the 3D view : Move the mouse cursor on the 3D area, click and drag the view in the direction you want to rotate. Release the mouse when satisfactory.

The 1D profile allows you to superimpose the intensity profiles of any number of selected lanes.

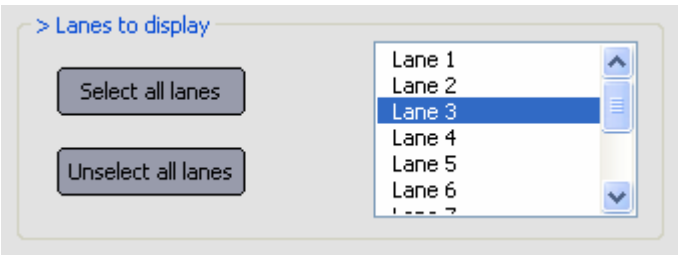
To proceed, click on the 1D Profile and select the lanes: 

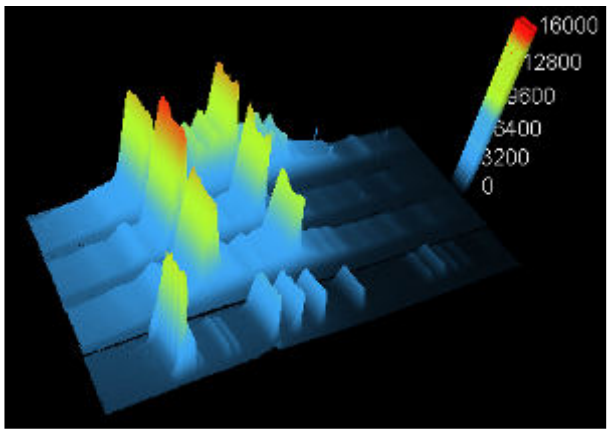



Note: To reposition the 1D profile window, position your cursor at the top of the box. The cursor appearance will change to a multidirectional arrow symbol. You can then drag the box to a new position.
Note: To increase the size of the 1D profile window, position your cursor at the edge of the window and click and drag to extend its size.
Note: Click on Print to print the 1D profile window
Note: Click on Send to clipboard to save the graph in the Windows ® clipboard (Past/copy Windows ® feature).

The 3D profile displays the three-dimensional rendering of any selected lanes.
To proceed, click on the 3D Profile button and select the lanes to be displayed:

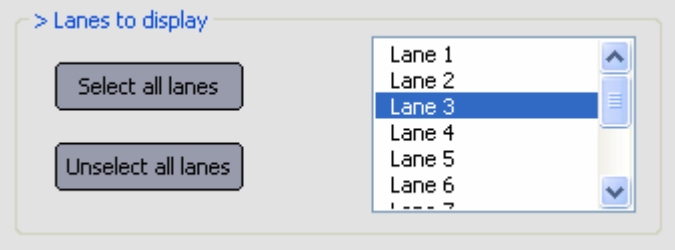



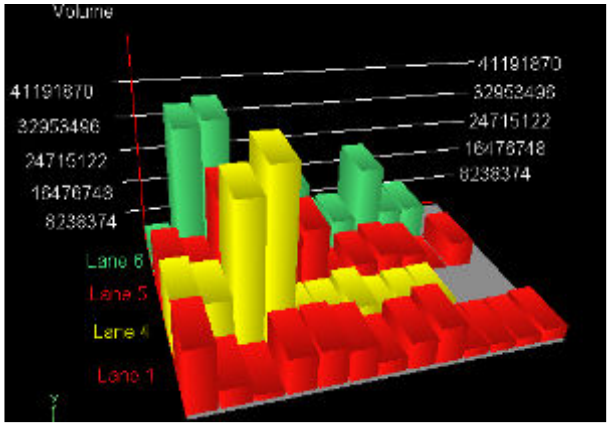
Note: To reposition the 1D profile window, position your cursor at the top of the box. The cursor appearance will change to a multidirectional arrow symbol. You can then drag the box to a new position.
Note: To increase the size of the 1D profile window, position your cursor at the edge of the window and click and drag to extend its size.
Note: Click on Print to print the 1D profile window
Note: Click on Send to clipboard to save the graph in the Windows ® clipboard (Past/copy Windows ® feature).

The 3D histogram displays the three-dimensional histogram of selected results:

- ⇨ Volume
- ⇨ Calculated quantities
- ⇨ Maximum intensities

To proceed, click on the 3D Histogram button and select the lanes to be displayed:

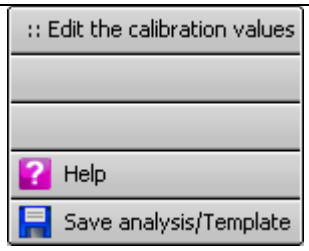




Note: To reposition the 1D profile window, position your cursor at the top of the box. The cursor appearance will change to a multidirectional arrow symbol. You can then drag the box to a new position.
Note: To increase the size of the 1D profile window, position your cursor at the edge of the window and click and drag to extend its size.
Note: Click on Print to print the 1D profile window
Note: Click on Send to clipboard to save the graph in the Windows ® clipboard (Past/copy Windows ® feature).

## OPTION FOLDER

The option folder gathers the following functions:

- ⇨ Edit the calibration values
- ⇨ Help
- ⇨ Save the analysis or the template




**EDIT THE CALIBRATION VALUES**

Click on the “Edit the calibration values” button.

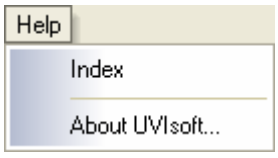

A pop-up window displays the following menu on which you can modify the calibration values:


You can add, remove, and save your marker’s value;

**HELP MENU**


Click on the “Help” button. You automatically access the user manual at the chapter corresponding to the function

You can access the help file index through the File\Help from the Menu bar




**SAVE ANALYSIS / TEMPLATE**

This function saves the current analysis. The analysis file will contain the results, the image and all the parameters defined to obtain the results.

The analysis could also be saved as a template for automated analysis routines. Template offers the user the ability to automate many of the repetitive tasks

associated with analysis and processing. As a result, you can spend more time evaluating and analysing results, and less time manipulating set-ups, variables and other settings.

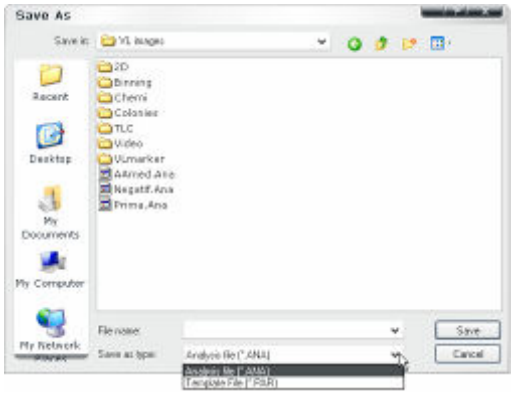
The template automates a task or set of tasks that you perform repeatedly or on a regular basis. It stores all the analysis commands and parameters of an analysis. You can run these parameters with another image whenever you need to perform a new analysis based on the same parameters.

The benefits of the template file are as follows:

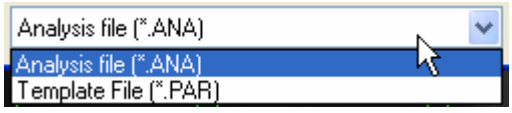
⇨ Time saving
⇨ Reproduction of image analysis parameters
⇨ Templates are modifiable, allowing the user to maintain an original template while modifying it for a slightly different result, with minimal effort

1. Click on the “Save analysis/ Template” button:


2. A pop-up window displays the following menu:

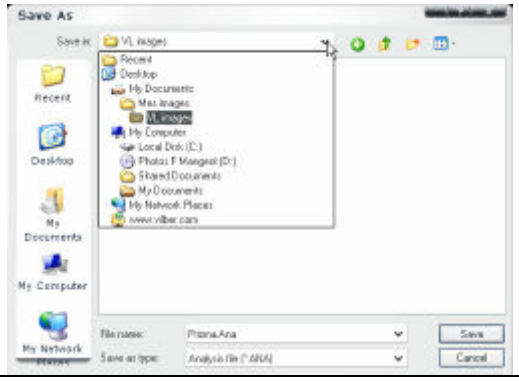



3. Select analysis file or template file:

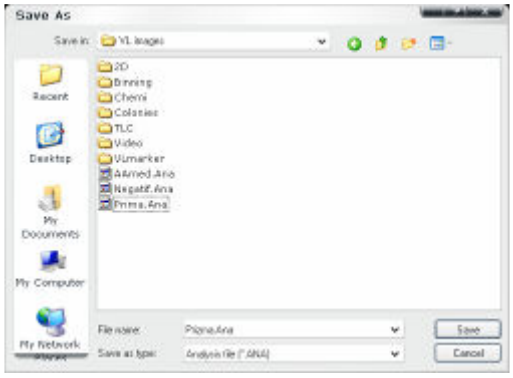



Note: the software proposes Analysis file by default

4. Browse to specify the file directory



5. Enter the desired file name and validate




6. Click on the Save button to create the file.

Note: see “Access to the analysis module” chapter for template or analysis file loading

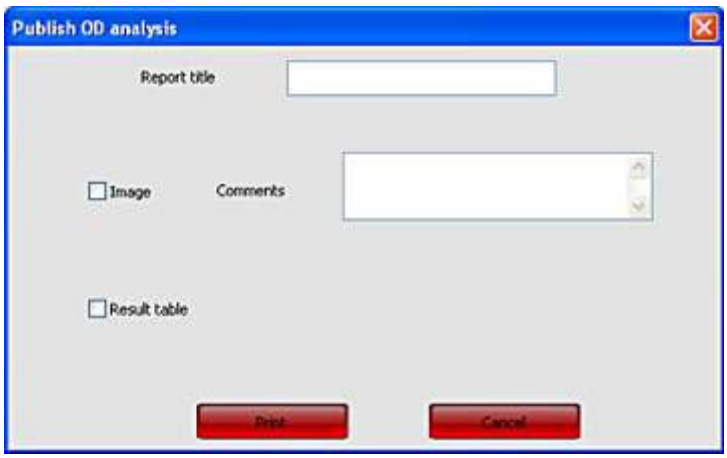
# Publish

## ➔ Introduction

3

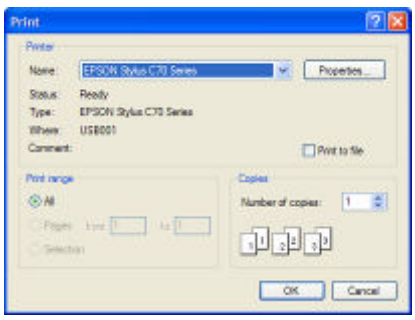
The purpose of the Publish function is to prepare a printed report of your results. You can easily organise your report with titles and comments and your own selection of data to be published among the following:
- Sample image
- Quantification result table

1. To proceed, select the Publish tab. A pop-up window displays the following menu:




⇨ Enter a report title if any
⇨ Select the options to be printed
⇨ Add comments or not per option

2. Click on the “Print” button. A pop-up window displays the following menu




⇨ Select a printer
⇨ If necessary, click on Properties to modify the default setting of the printer,
⇨ Select the number of copies
⇨ Click on OK to validate your options

# Return to Home

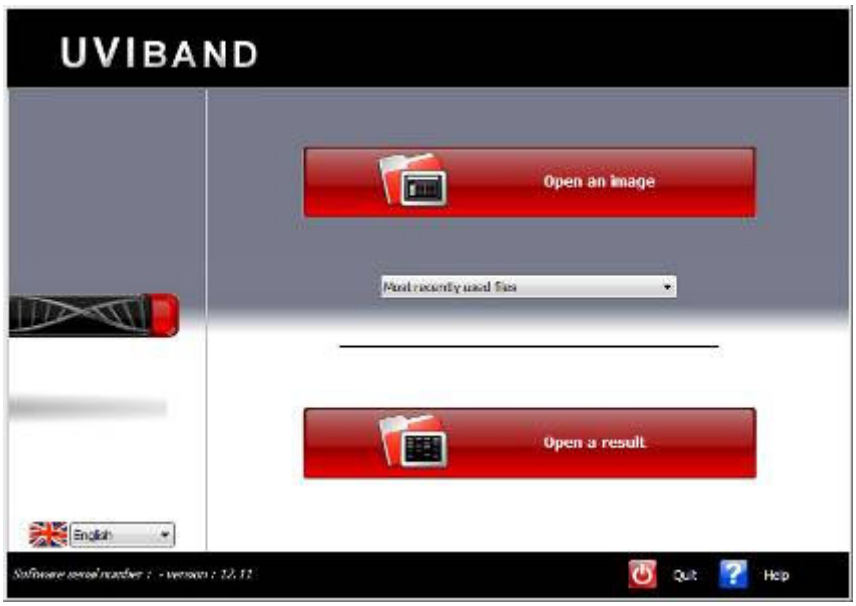
## ➔ Introduction

The home dashboard is the hub to other functions of the software:

- ⇨ Open another image file or another result file
- ⇨ Select another analysis module
- ⇨ Exit the software


## ➔ Load another image

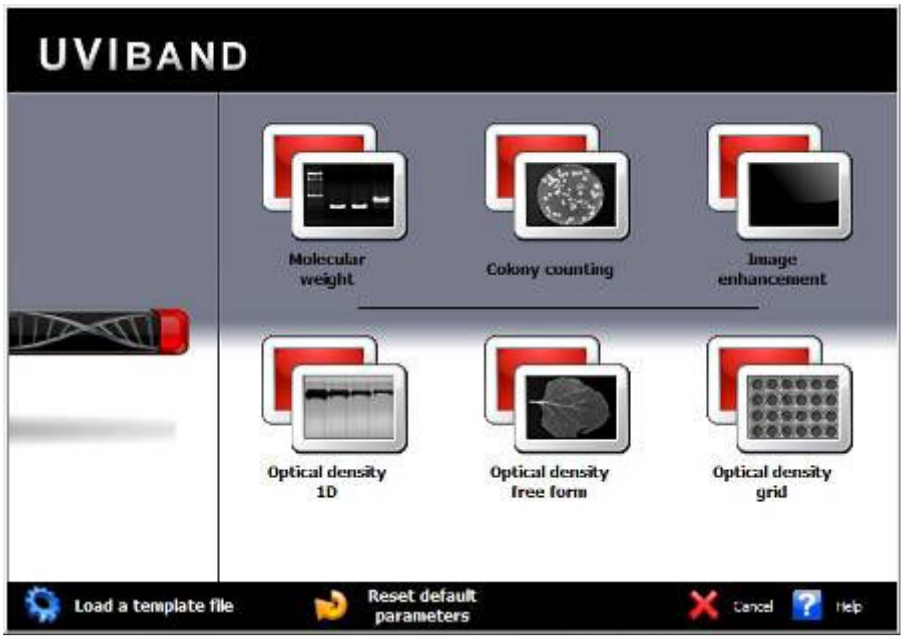
To return to the main menu, click on the home icon. A new menu appears with the main menu task bar functions:




- ⇨ Click on the “Open an image” icon to open an image
- ⇨ Click on the “Open a result” icon to open a previously saved analysis result

## ➔ Select another function

To return to the analysis menu, click on the analysis icon. A new menu appears with the analysis module task bar functions:




Click on the appropriate icon to select an analysis module.

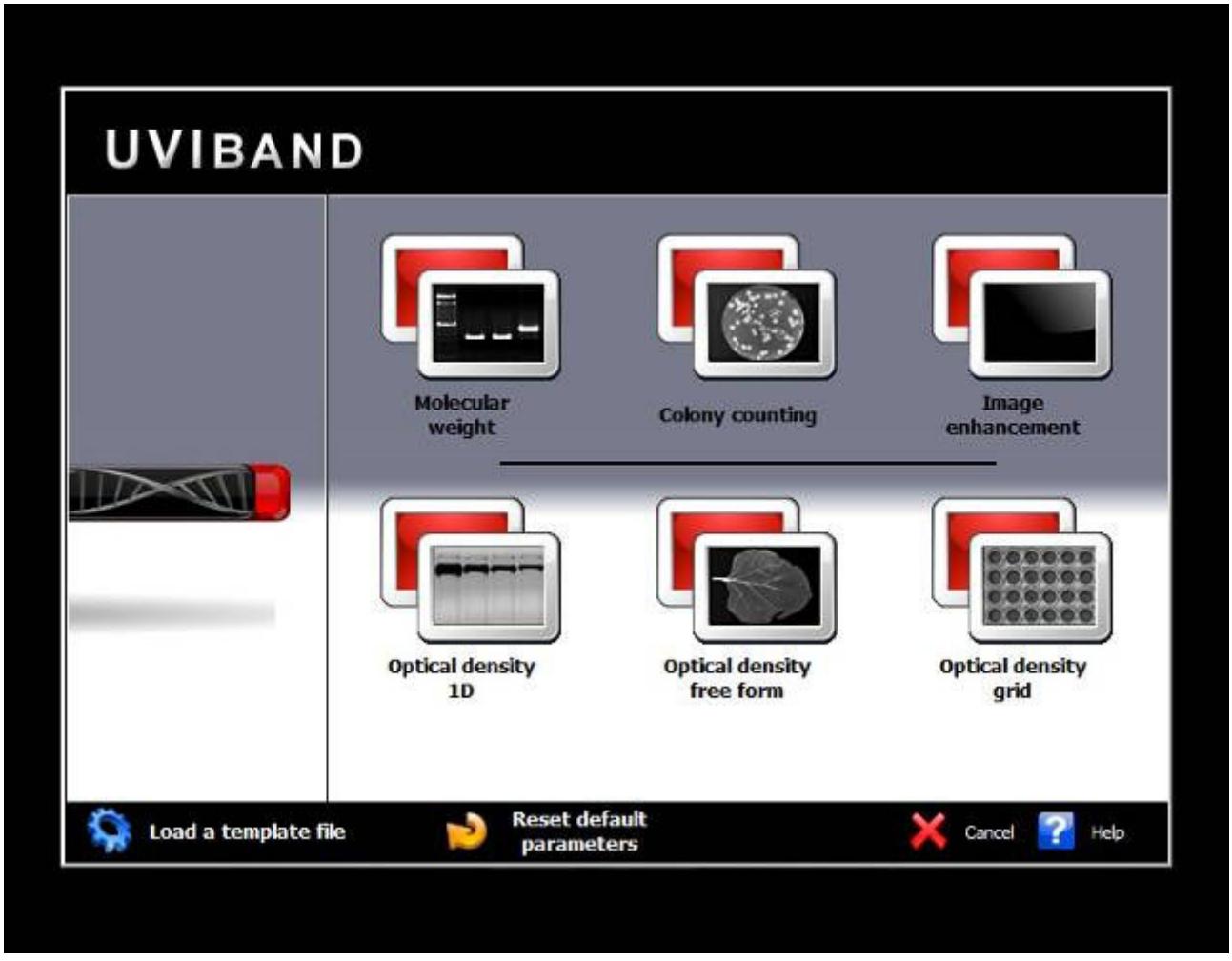
- ⇨ Select the Molecular weight icon to open the molecular weight analysis (MW) module
- ⇨ Select the Colony counting icon to open the colony counting (CC) analysis module
- ⇨ Select the Optical density - 1D icon to open the optical density (OD) analysis module based on a 1D detection
- ⇨ Select the Optical density - Free form icon to open the optical density (OD) analysis module based on a free form detection
- ⇨ Select the Optical density - Grid icon to open the optical density (OD) analysis module based on a grid detection
- ⇨ Select the Image enhancement icon to open the image enhancement module

## ➔ Exit the software

To close UVIband Advanced, select Exit from the File menu.

You will be prompted to save your analysis.

UVITEC
Cambridge




# Optical density - Grid
# ➔ OD-Grid Analysis module

# Optical density / Grid introduction

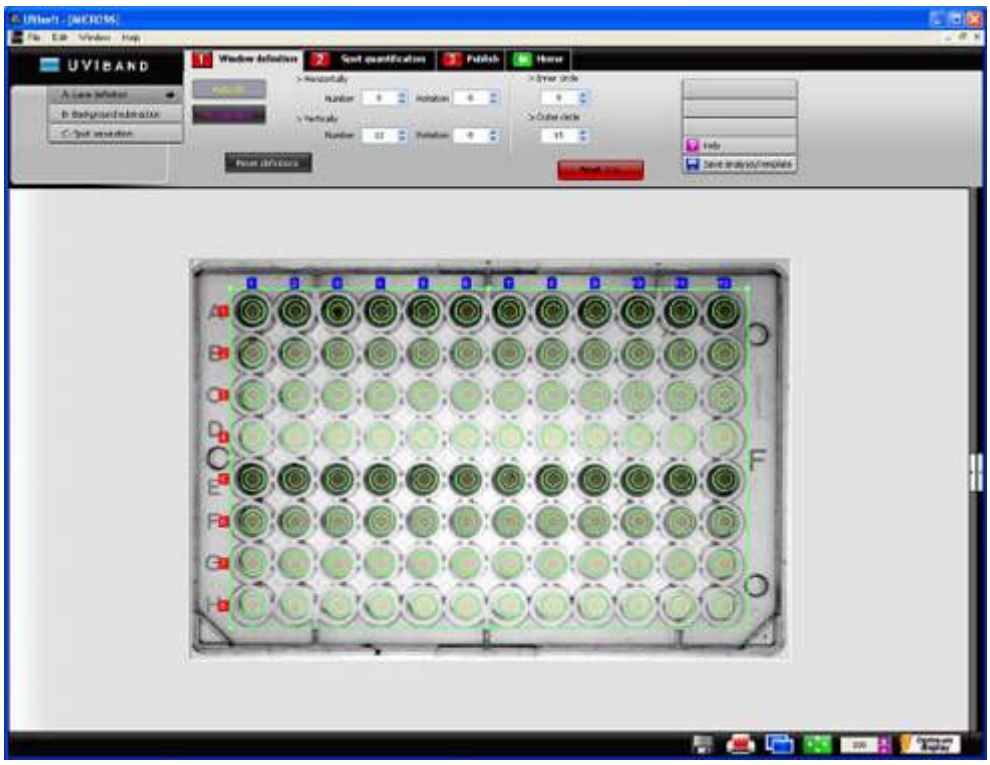
## ➔ Objectives and output

The UVIband Advanced Optical density /Free form module features the quantification of spot in volume, percentage or µg.

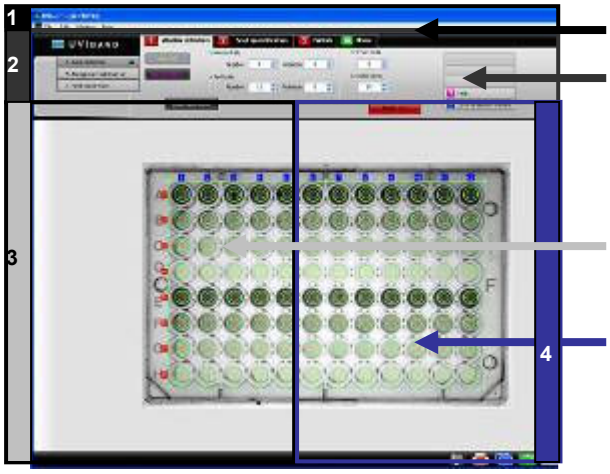
At the end of the process, you can have the following outputs:
- Lane's volume and concentration
- 3D profile
- 3D result's graph
- Calibration curve

## ➔ Optical density / Grid operating environment

The OD-Grid module opens on the following window:




The UVIband Advanced operating environment is organised into four areas:




1. The menu bar
2. The dash board
3. The image window
4. The working window

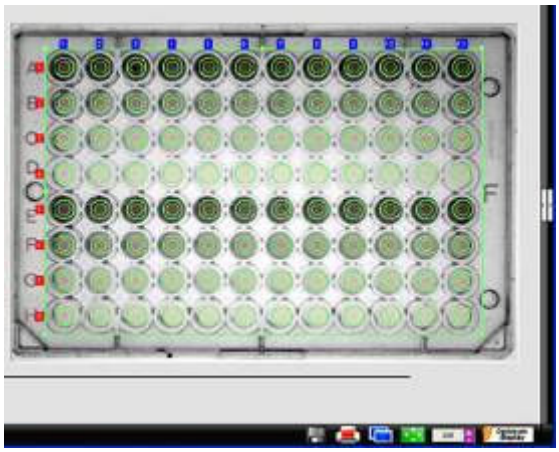
The menu bar contains the following menu:

- ⇨ File
- ⇨ Edit
- ⇨ Windows
- ⇨ Help

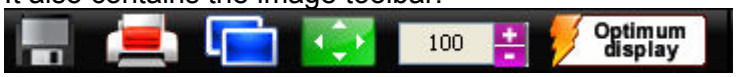

The dash board contains four different tabs:

1. Window definition
2. Spot quantification
3. Home


The image window displays the active image:



It also contains the image toolbar:




 ⇨ Save a screen capture of the view

 ⇨ Print

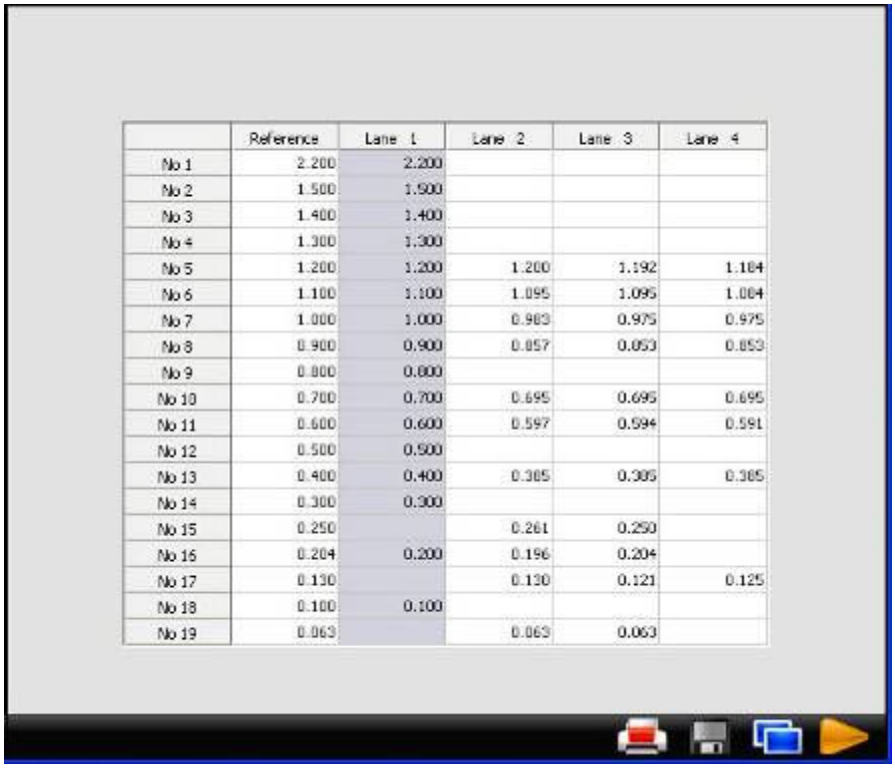
 ⇨ Copy to clipboard

 ⇨ Autoscale

⇨ Zoom in or out the image

⇨ Change the optimum display

The working window displays the graphs and tables related to the active analysis:



| | Reference | Lane 1 | Lane 2 | Lane 3 | Lane 4 |
|---|---|---|---|---|---|
| No 1 | 2.200 | 2.200 | | | |
| No 2 | 1.500 | 1.500 | | | |
| No 3 | 1.400 | 1.400 | | | |
| No 4 | 1.300 | 1.300 | | | |
| No 5 | 1.200 | 1.200 | 1.200 | 1.192 | 1.184 |
| No 6 | 1.100 | 1.100 | 1.095 | 1.095 | 1.084 |
| No 7 | 1.000 | 1.000 | 0.983 | 0.975 | 0.975 |
| No 8 | 0.900 | 0.900 | 0.857 | 0.853 | 0.853 |
| No 9 | 0.800 | 0.800 | | | |
| No 10 | 0.700 | 0.700 | 0.695 | 0.695 | 0.695 |
| No 11 | 0.600 | 0.600 | 0.597 | 0.594 | 0.591 |
| No 12 | 0.500 | 0.500 | | | |
| No 13 | 0.400 | 0.400 | 0.385 | 0.385 | 0.385 |
| No 14 | 0.300 | 0.300 | | | |
| No 15 | 0.250 | | 0.261 | 0.250 | |
| No 16 | 0.204 | 0.200 | 0.196 | 0.204 | |
| No 17 | 0.130 | | 0.130 | 0.121 | 0.125 |
| No 18 | 0.100 | 0.100 | | | |
| No 19 | 0.063 | | 0.063 | 0.063 | |

It also contains the working window toolbar:

 ⇨ Display the molecular weight on the image

 ⇨ Save the graph or the table

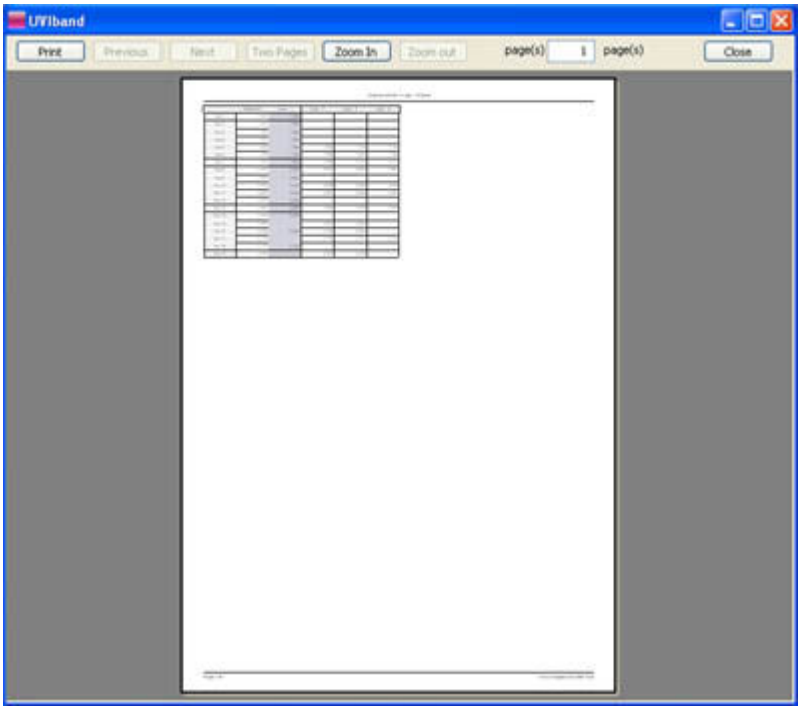
 ⇨ Copy the graph or the table to clipboard

 ⇨ Export the table to Excel

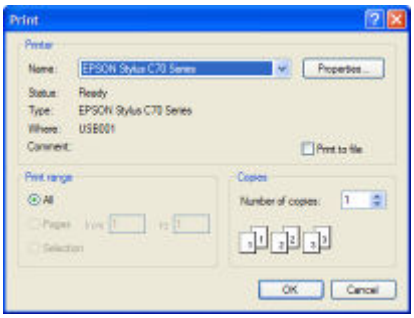
## ➔ Toolbar in details

**Print**

Click on the “Print” icon to print the image, the table or the graphs. A pop-up window displays the Print preview: The Print preview displays a preview of the image, as it will be printed.




Click on Print to validate the preview. A pop-up window displays the following menu:




⇨ Select a printer
⇨ Click on Properties to modify the default setting of the printer, if necessary

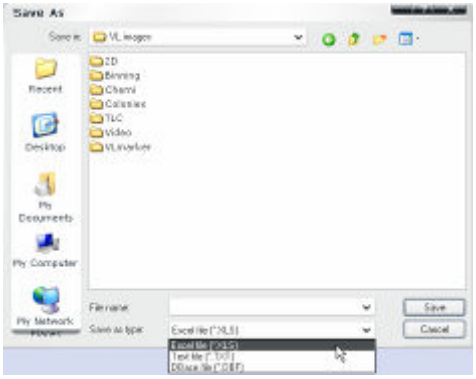
⇨ Select the number of copies
⇨ Click on OK to validate your options

Note: You can also access the Print menu from the Menu bar (File\Print).

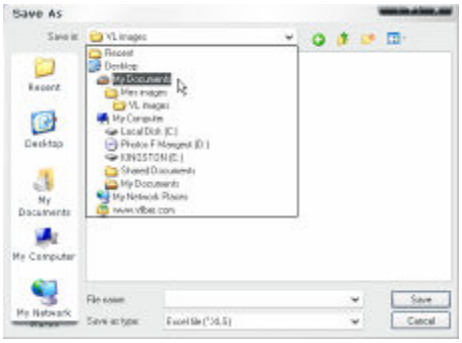
**Save**

This function saves a graph or a table. The tables are saved in a Excel™ file format (*.xls). The graphs are saved in a Bitmap format (*.bmp).

Click on the “Save” icon.
A pop-up window displays the following menu:

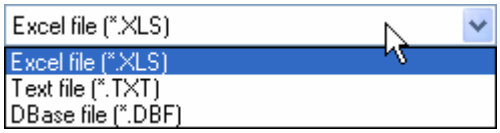



Browse to specify the file directory




Enter the desired file name, select a file extension and validate

Note: the results could also be saved in a text file format or a Dbase file format:




The graphs can only be saved on a BMP format:

## Copy to clipboard

This function copies an image, a table or a graph onto the clipboard for insertion into another program. This option is identical to the Windows® [Ctrl C] command.

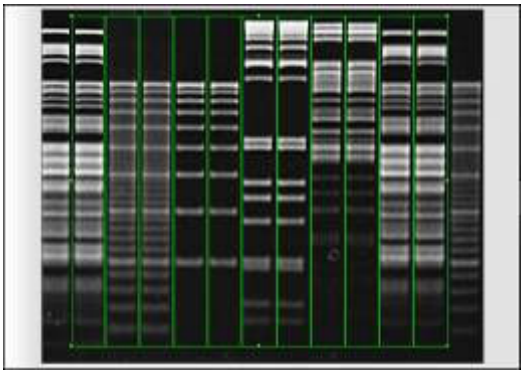
To proceed, click on the Copy to clipboard icon. The image, the table or the graph is now ready to be pasted into another application.

Open the application that you want to paste the image into, and select from the available pasting options ([Ctrl V] command for Windows® software).

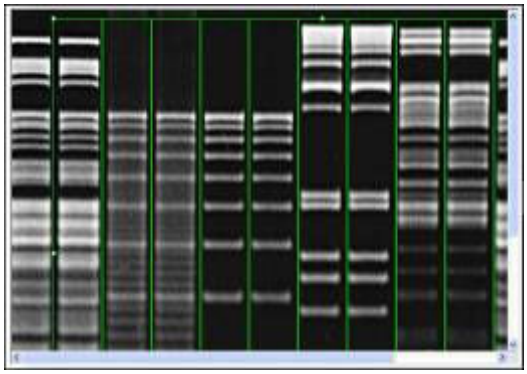
## Auto-scale

Click on the “Auto-scale” to resize the image to fit the size of the monitor.

The Auto-scale feature proportions the display of the image to the screen resolution.



Auto-scale (no scroll bar)



No auto-scale (scroll bar)


**Optimum display (for 12, 14 and 16-bit image file)**

The optimum display window is helpful to modify the greyscale selection to enhance the image display: To proceed, click on the “Optimum display” icon. A pop-up window displays the following menu:

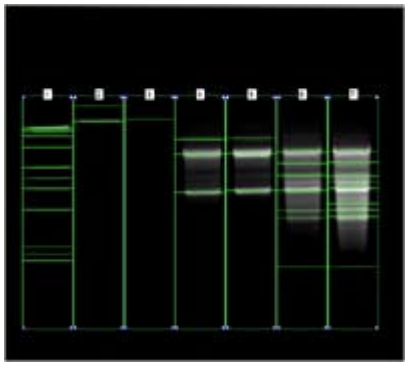
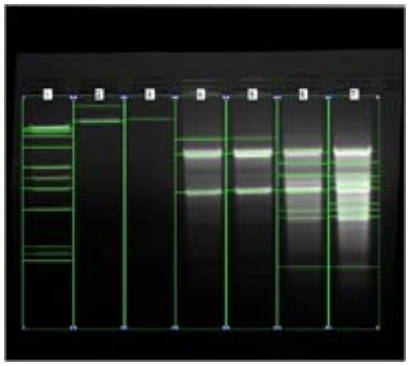
Some images has a 12, 14 or 16-bit format and Windows® can only display 8-bit images (256 grey levels).

Due to this limitation, the UVIband Advanced software handles two images:
⇨ A “memory” image corresponding to the 12, 14 or 16-bit format (4 096, 16 384 or 65 536 grey levels)
⇨ A “display image” corresponding to the image displayed on the screen (256 grey levels)

The easiest way to calculate the “display image” would be to translate the full grey scale each time an image is acquired: the x grey levels values of the “memory” image corresponds to 256 values in the displayed image. In that case, it won’t be possible to visualise faint spots on a dark image.

UVIband Advanced offers the possibility to select the grey level range to translate for the display image calculation. All the grey levels under the “Min value” defined will be converted to 0 (Black) in the displayed image. All the grey levels upper the “Max Value” defined will be set to 255 (White) in the displayed image. The grey levels between those two limits will be converted in an intermediate grey level value following a linear rule.

For both values, you can:
⇨ Edit the value in the corresponding field
⇨ Select the value by dragging and dropping the arrow
⇨ Click on the “optimum display” button: UVIband Advanced will then calculate the ideal values to be selected according to the parameters defined

| | |
|---|---|
|  |  |
| Automatic optimum display | Optimum display enhancement<br>The image appears brighter. The faint bands are more visible. |

Note: The optimum display has no impact on the analysis. Only the display of the image is modified.

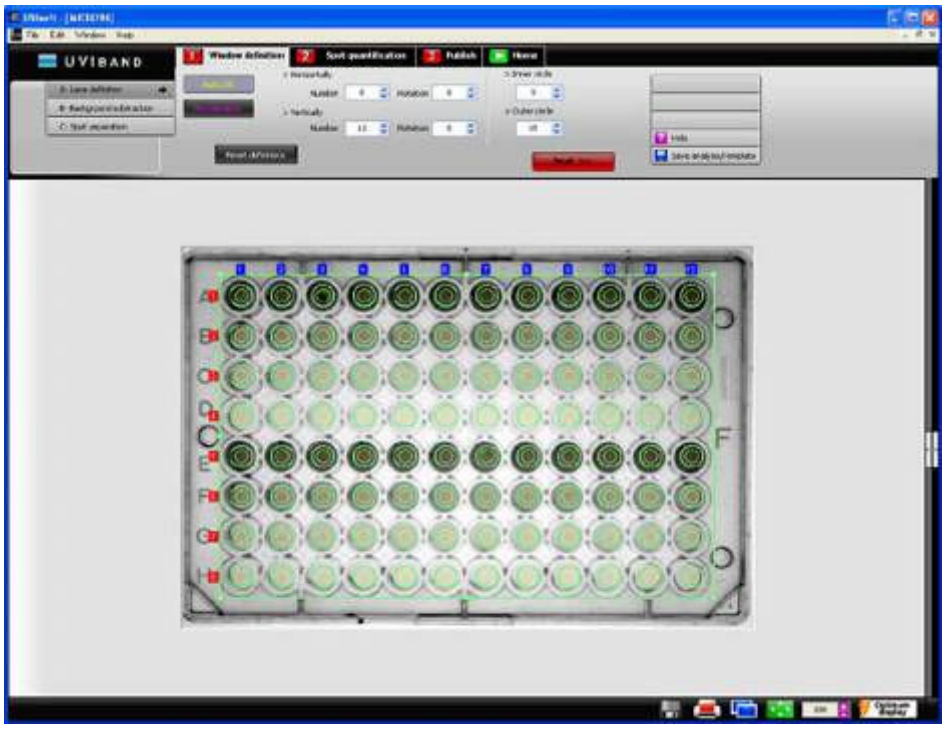
| | |
|---|---|
|  | **Send to Excel™**<br>This function transfers the results table to Windows Excel™.<br><br>To proceed, click on the Send to Excel™ icon. The Excel software is automatically opened by the UVIband Advanced and the table is transferred to Excel™. |

# 1- Window definition

## ➔ A – Lane definition

1

The Optical Density / Grid module opens on the Lane definition dashboard of the Window definition process:




The dashboard details the lane definition parameters:


⇨ Area of analysis
⇨ Automatic optical density
⇨ Microtitration

**AREA OF ANALYSIS**

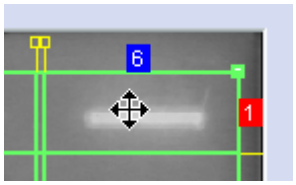
The following is valid for both microtitration and automatic optical density functions.

On the image, click and drag to define the analysis area and to overlap the lanes. You can easily adjust the size of the area by clicking on the tags surrounding the area and drag the selected border to the requested size.

The lanes are defined by green lines, overlaid on the gel image. The gel area is surrounded by square anchors:

To resize the entire lane frame, drag an anchor point in or out. The opposite anchor point will remain fixed while the frame expands or contracts. The frame will expand or contract from the centre.

To move the entire frame to a new position, position the mouse on the frame to obtain a cross cursor:




Click and drag the cursor to move the entire frame.

## AUTOMATIC OPTICAL DENSITY

On the image, click on the Automatic OD button: 


The dashboard details the Automatic Optical density parameters:

Select the following parameters:

- ⇨ Number of horizontal lines
- ⇨ Rotation of the horizontal lines
- ⇨ Gap between the horizontal lines
- ⇨ Number of vertical rows
- ⇨ Rotation of the vertical rows
- ⇨ Gap between the vertical rows

The area of analysis is automatically modified on the image.

## AUTOMATIC OPTICAL DENSITY

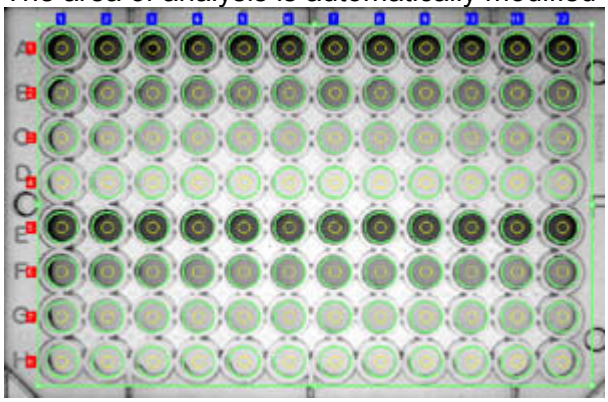
On the image, click on the Microtitration button: 


The dashboard details the Microtitration parameters:


Select the following parameters:

- ⇨ Number of horizontal lines
- ⇨ Rotation of the horizontal lines
- ⇨ Number of vertical rows
- ⇨ Rotation of the vertical rows
- ⇨ Size of the inner circle
- ⇨ Size of the outer circle

The area of analysis is automatically modified on the image:



## RESET

The “Reset” button restores the default lane detection parameters.


## NEXT

The “Next” button validates your parameter and opens the following analysis step.

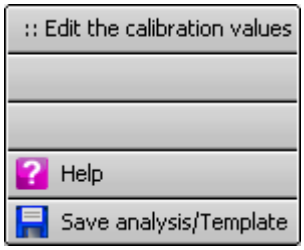
| | | |
|---|---|---|
| 1 A – Lane definition | Next >> | 1 B – Background subtraction |

**OPTION FOLDER**

The option folder gathers the following functions:

⇨ Help

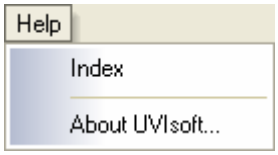
⇨ Save the analysis or the template




**HELP MENU**

Click on the “Help” button. You automatically access the user manual at the chapter corresponding to the function


You can access the help file index through the File\Help from the Menu bar




**SAVE ANALYSIS / TEMPLATE**

This function saves the current analysis. The analysis file will contain the results, the image and all the parameters defined to obtain the results.

The analysis could also be saved as a template for automated analysis routines. Template offers the user the ability to automate many of the repetitive tasks associated with analysis and processing. As a result, you can spend more time evaluating and analysing results, and less time manipulating set-ups, variables and other settings.

The template automates a task or set of tasks that you perform repeatedly or on a regular basis. It stores all the analysis commands and parameters of an analysis. You can run these parameters with another image whenever you need to perform a new analysis based on the same parameters.

The benefits of the template file are as follows:

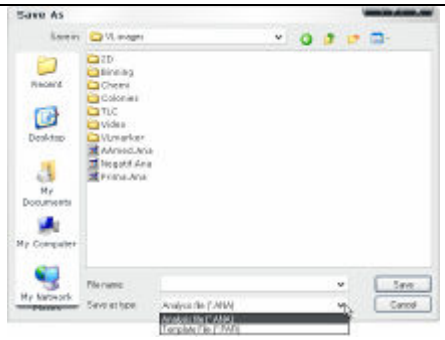
⇨ Time saving

⇨ Reproduction of image analysis parameters

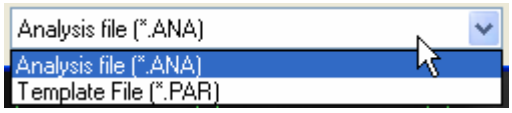
⇨ Templates are modifiable, allowing the user to maintain an original template while modifying it for a slightly different result, with minimal effort

1. Click on the “Save analysis/ Template” button:


2. A pop-up window displays the following menu:

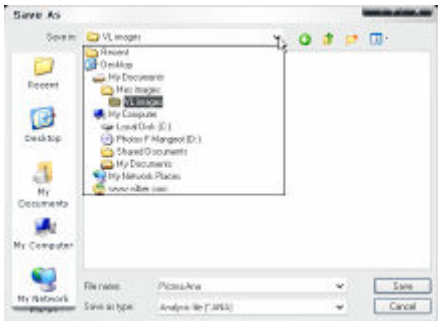


3. Select analysis file or template file:

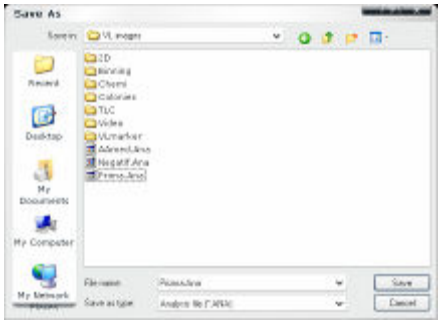



Note: the software proposes Analysis file by default

4. Browse to specify the file directory



5. Enter the desired file name and validate



6. Click on the Save button to create the file.

Note: see “Access to the analysis module” chapter for template or analysis file loading

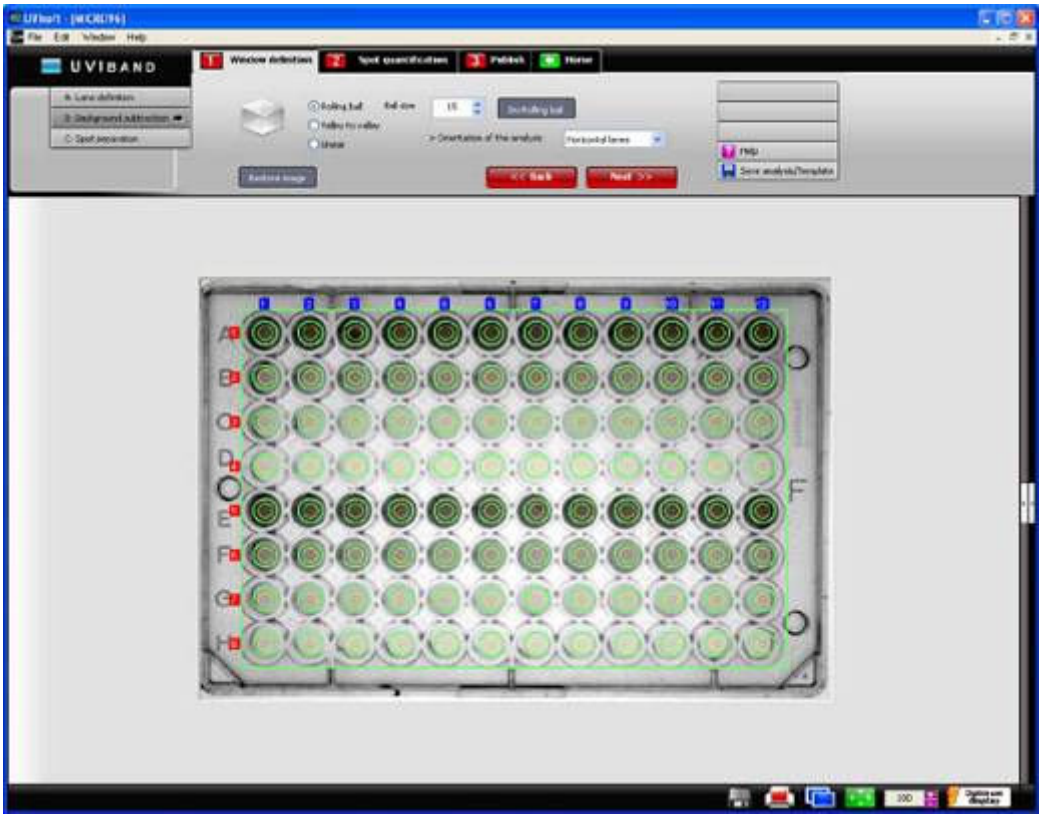
## ➔ B – Background subtraction

1

The background subtraction process follows lane definition.

Image background interferes with quantification and data analysis. To this extend, we recommend to perform a background subtraction before any peak volume quantification. The subtraction is automatically done on the analysis area.

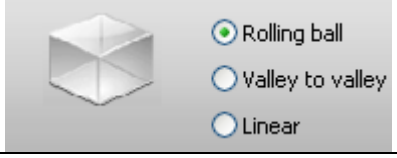
Note: As background subtraction permanently changes the image, this is not possible to save the image with a processed background subtraction. However, the process can be saved by saving the complete analysis through the Save analysis process.



The dashboard details the matching parameters:

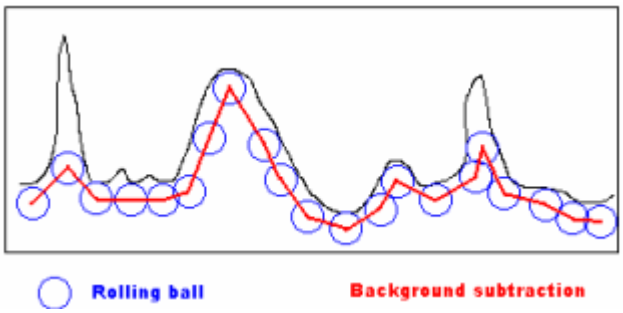

UVIband Advanced has several functions to minimise image background.

⇨ The rolling ball approach
⇨ The valley to valley approach
⇨ The linear approach



**ROLLING BALL**

The rolling ball method is named for a hypothetical ball that rolls along underneath the lane profile, removing different intensity levels along the length of the lane.
The ball is rolled under each profile of the image so its movement varies along the image.




The centre of gravity of the ball describes a curve:

⇨ This curve represents the noise to be subtracted.

⇨ The curve depends on the size of the ball and on the size of the peaks.

The size of the ball will affect the position and movements of the centre of gravity and thus it determined the level of background subtraction. A small disk will make a large background subtraction and a large disk the contrary. A disk radius that is too small may subtract almost all image data.

The UVIband Advanced calculates automatically the ideal parameter for background subtraction. This could be manually modified by adjusting the spot size:


To process the rolling ball background subtraction, click on the “Do rolling ball” button:


The changes will be automatically applied to the image.

**Note**: few seconds could be necessary to perform the background subtraction.

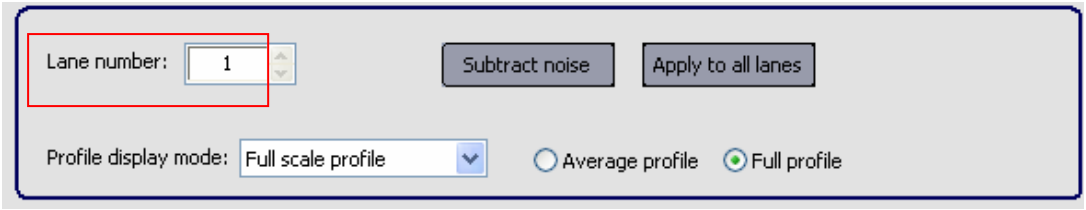
**VALLEY TO VALLEY**

The valley-to valley approach is a lane-based background subtraction. It allows to manually define on the lane profile the level of noise to be subtracted.

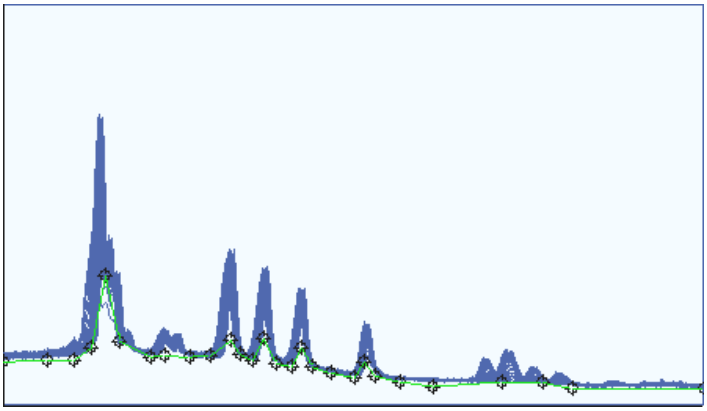
Click on the “Valley to valley ” button: 


It opens the lane profile window:

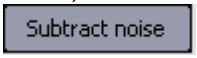

In the profile parameters window, select the lane to perform the valley-to-valley approach



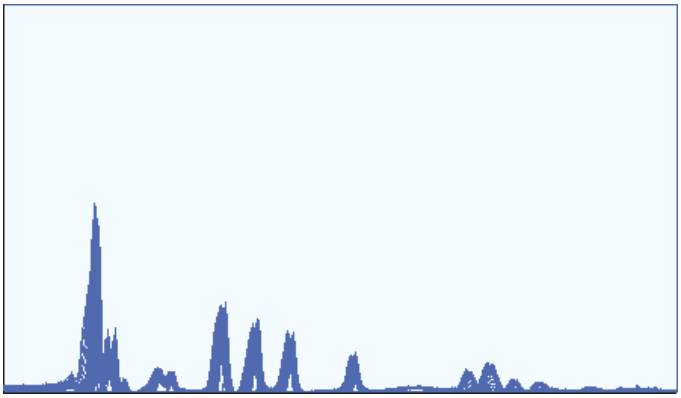

On the profile, click to define the background profile you want to remove:



Then, click on Subtract noise:

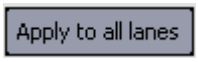



The changes will be automatically applied to the image and to the profile:

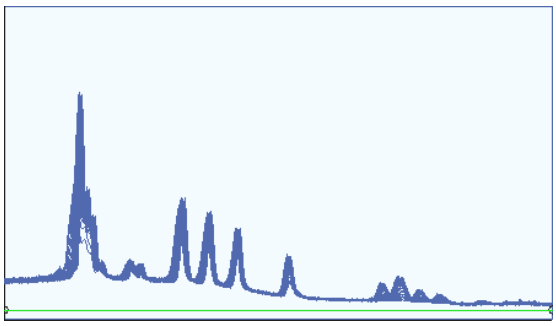
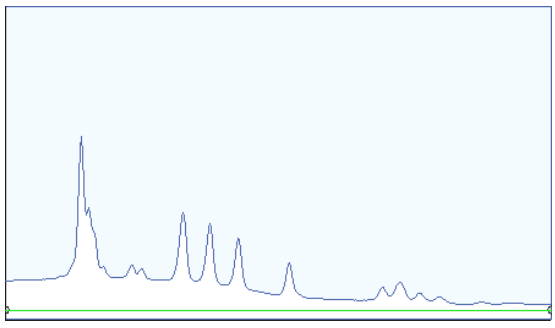


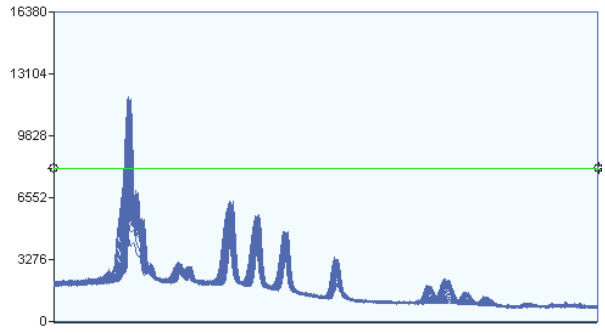
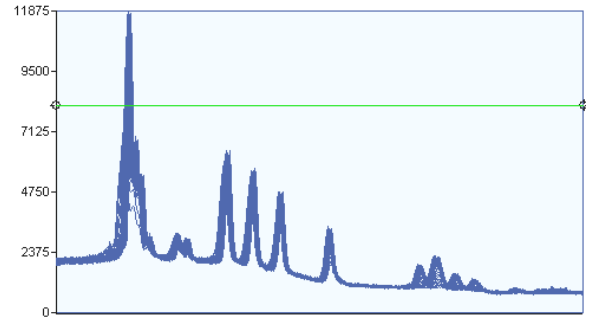
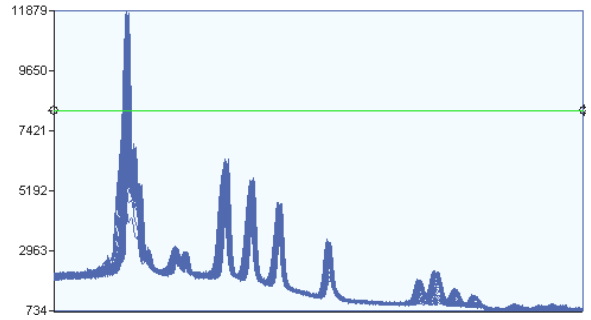
The valley-to-valley approach is a lane-based background subtraction. You can set the same subtraction level for all lanes or specify an individual subtraction level for the selected lane. Any changes you make will be automatically applied to the image.

To apply the same subtraction level for all lanes, click on the “Apply to all lanes” button:




You can easily adjust the profile displays settings as follows:

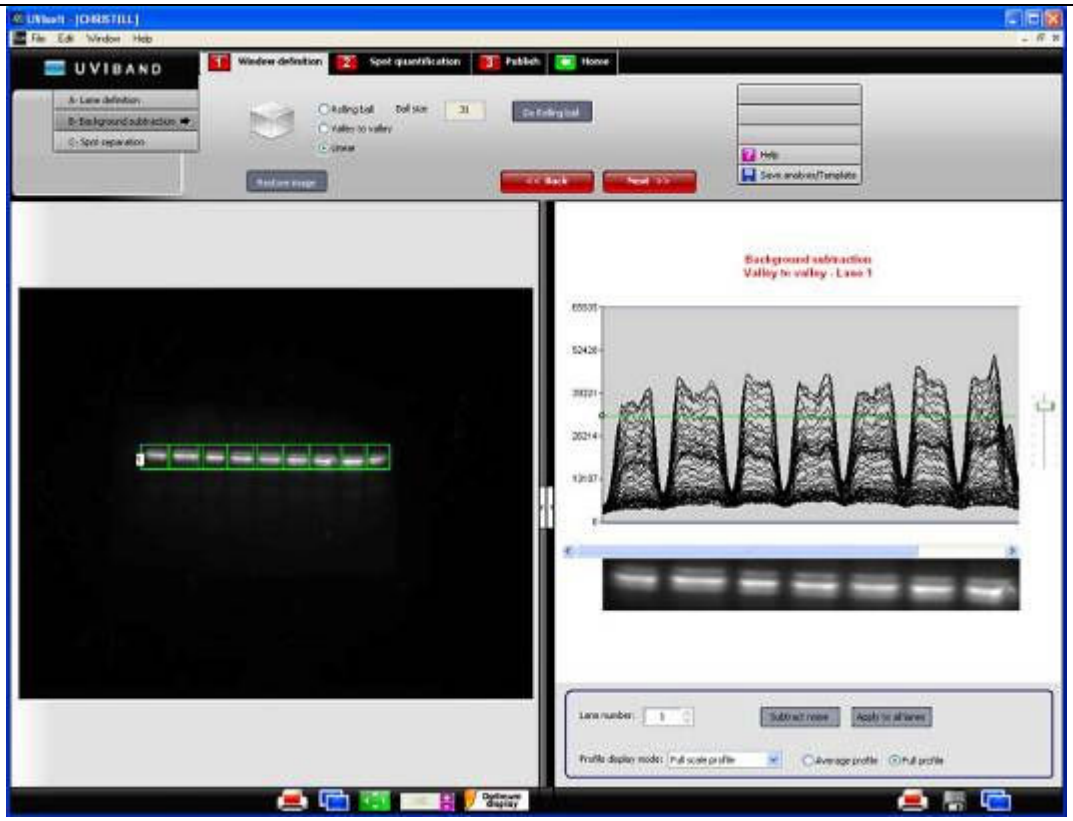
| | |
|---|---|
|   |  |
|   |  |

| | |
|---|---|
| Profile display mode:<br>Full scale profile<br>The profile scale goes from 0 to the image maximum dynamic. |  |
| Profile display mode:<br>0 to Maximum<br>The profile scale goes from 0 to the lane's maximum intensity; |  |
| Profile display mode:<br>Minimum to Maximum<br>The profile scale goes from the lane's minimum intensity to the lane's maximum intensity; |  |

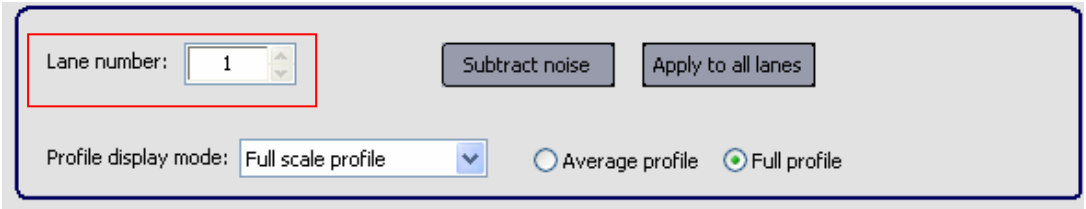
**LINEAR APPROACH**

The linear approach is a lane-based background subtraction. It allows to manually define the level of noise to be subtracted on the lane profile.
To proceed, click on the "Linear " button:

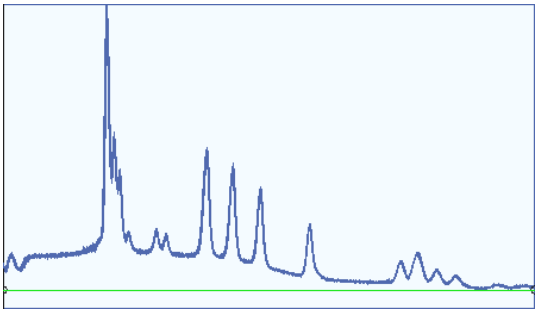

It opens the lane profile window:




In the profile parameters window, select the lane to perform the linear approach

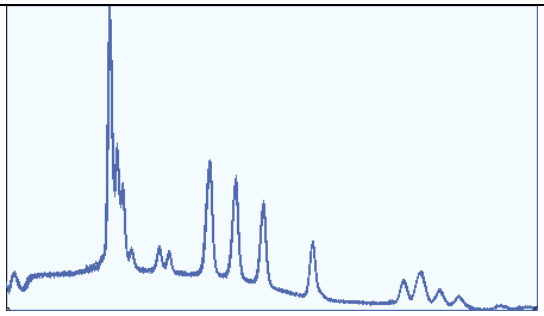



On the profile, click to define the background linear level you want to remove:



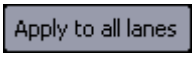
Then, click on Subtract noise: 

The changes will be automatically applied to the image and to the profile:

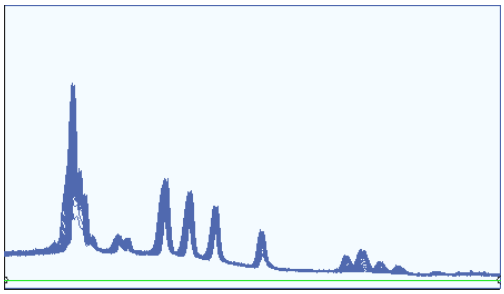
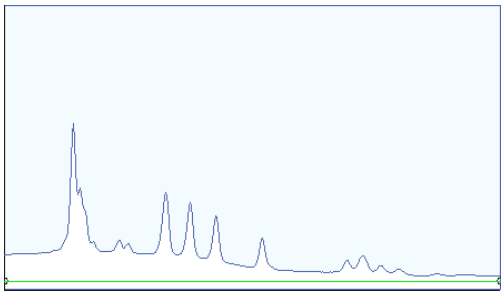
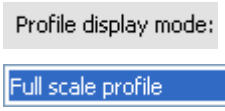
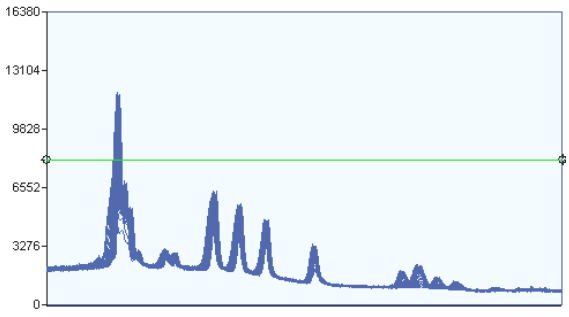


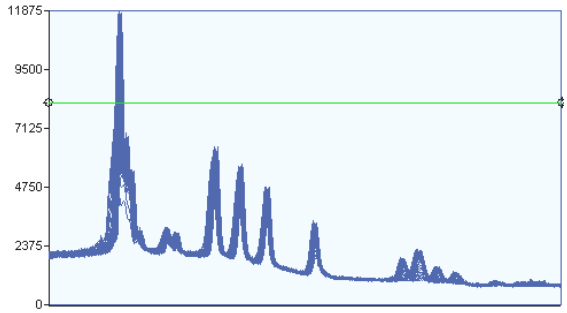
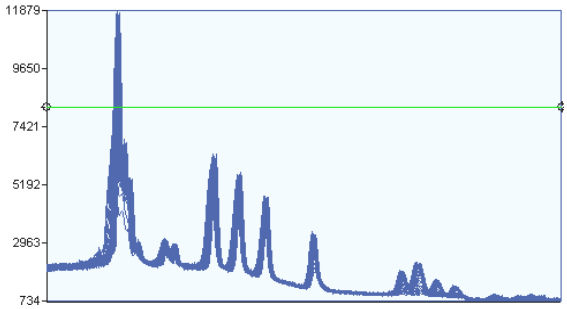
The linear approach is a lane-based background subtraction. You can set the same subtraction level for all lanes or specify an individual subtraction level for the selected lane. Any changes you make will be automatically applied to the image.

To apply the same subtraction level for all lanes, click on the “Apply to all lanes” button:




You can easily adjust the profile displays settings as follows:

| | |
|---|---|
|  |  |
|  |  |
|  <br>The profile scale goes from 0 to the image maximum dynamic. |  |

| | |
|---|---|
| Profile display mode:<br>0 to Maximum<br><br>The profile scale goes from 0 to the lane's maximum intensity; |  |
| Profile display mode:<br>Minimum to Maximum<br><br>The profile scale goes from the lane's minimum intensity to the lane's maximum intensity; |  |

**NEXT**

The "Next" button validates your parameter and opens the following analysis step.

| | | |
|---|---|---|
| 1 B – Background subtraction | Next >> | 1 C- Spot separation |

**BACK**

The "Back" button validates your parameter and opens the following analysis step.

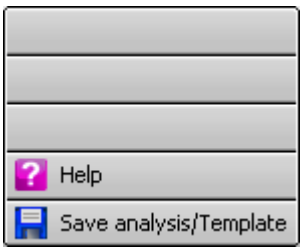
| | | |
|---|---|---|
| 1 B – Background subtraction | << Back | 1 A – Lane definition |

**OPTION FOLDER**

The option folder gathers the following functions:

⇨ Help

⇨ Save the analysis or the template




**HELP MENU**

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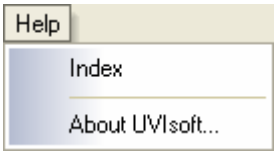


**SAVE ANALYSIS / TEMPLATE**

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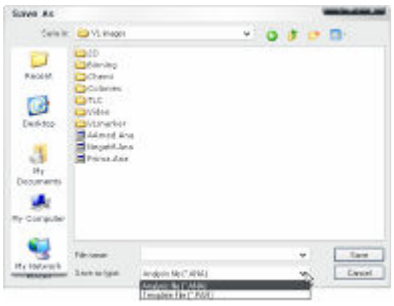
The benefits of the template file are as follows:

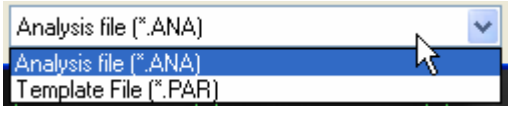
⇨ Time saving
⇨ Reproduction of image analysis parameters
⇨ Templates are modifiable, allowing the user to maintain an original template while modifying it for a slightly different result, with minimal effort

1. Click on the “Save analysis/ Template” button:


2. A pop-up window displays the following menu:

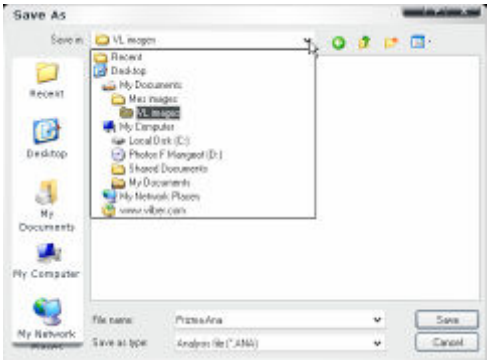


3. Select analysis file or template file:

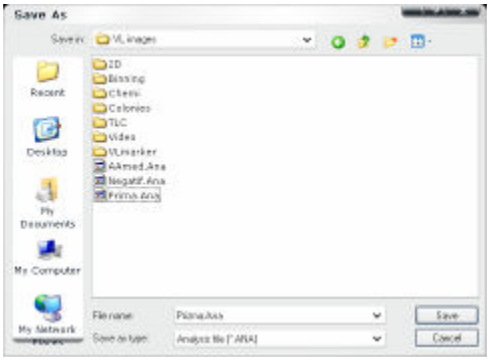



Note: the software proposes Analysis file by default

4. Browse to specify the file directory




5. Enter the desired file name and validate




6. Click on the Save button to create the file.

Note: see “Access to the analysis module” chapter for template or analysis file loading

## ➔ C – Spot separation

1

In order to measure the volume of a particular spot, you need:

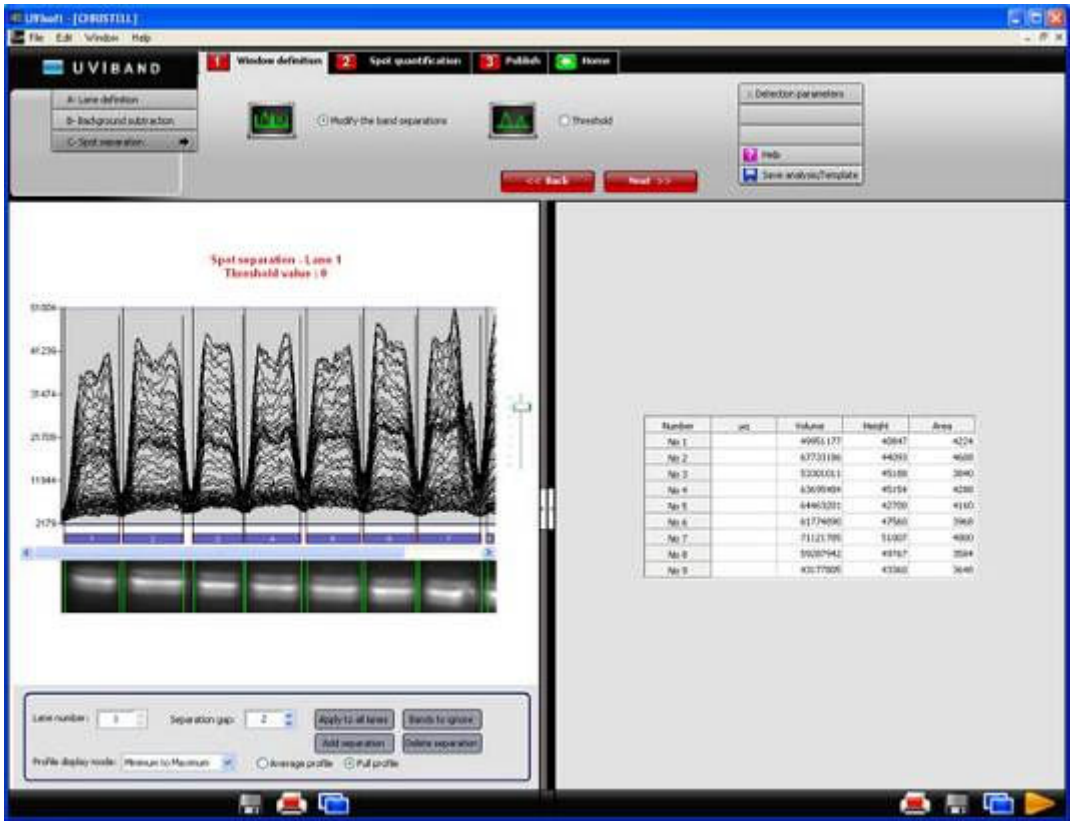
⇨ To define the boundary around the spot;

⇨ To compare the intensity data inside the boundary with the data of other spots or of a standard.

A volume is the sum of the pixel intensity inside a defined boundary. The purpose of the spot separation is to define this boundary.

The spot separation process follows the background subtraction.

Note: you can either access the spot separation function by clicking on the next button of the background subtraction or directly by clicking on the spot separation of the Window definition folder.




The dashboard details the spot separation parameters:


⇨ Modify the spot separation

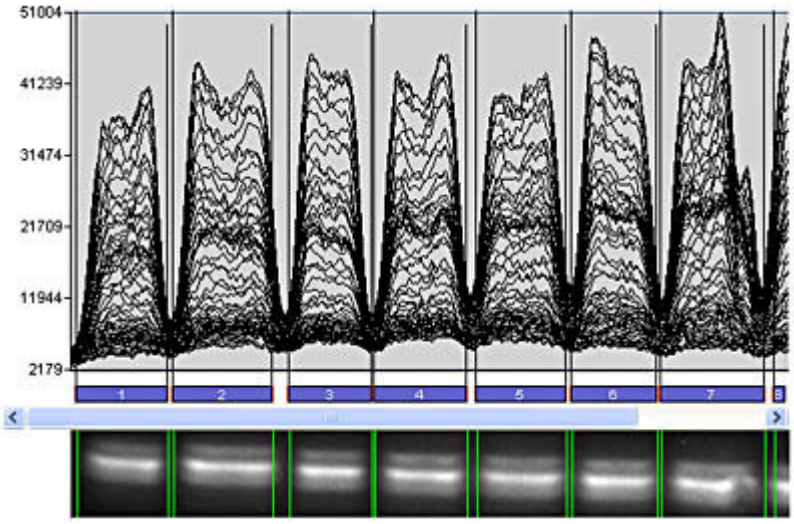
⇨ Standard threshold

⇨ Extended threshold

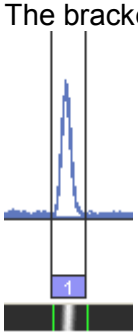
**MODIFY THE SPOT SEPARATION**

UVIband Advance proposes by default an automatic predefined spot separation based on the band detection. You can modify the default spot separation by selecting the “Modify the spot separation” option.

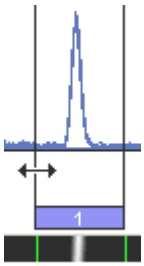

The default separation is illustrated on the lane’s profile:




The brackets illustrate the bands boundaries:



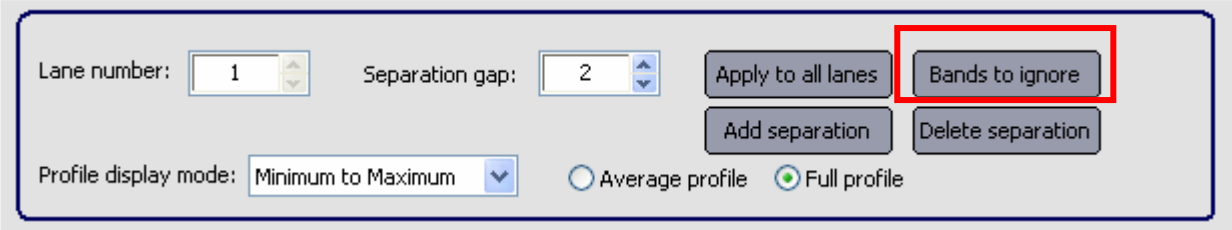

You can easily reposition a band’s boundaries. In order to do so, click on the bracket and drag the cursor:



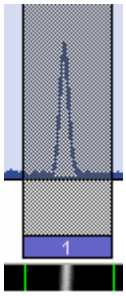

Drag the cursor until the area of the band that you want to define has been completely enclosed.

**Note**: When you release the mouse button, the band’s volume is automatically recalculated to take into account the new area of interest.

To ignore a band, select “Bands to ignore” from the profile’s parameter menu:



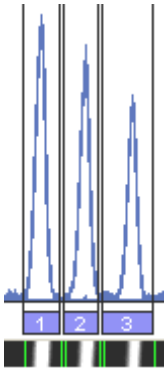

Then, click on the band you want to ignore:



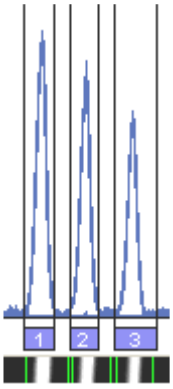

The band is then highlighted in grey and discarded from the result table:

Note: you can ignore more than one band at a time.
Note: to stop the process, click again on the “Bands to ignore” button.

To increase the gap in between the lane, select the “Separation gap” option from the profile’s parameter menu:




**Limited separation gap**



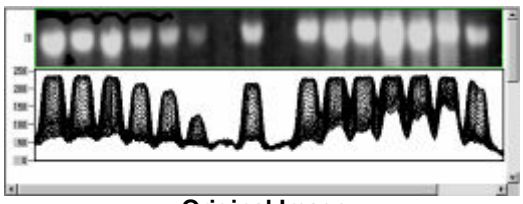

**Extended separation gap**

**DEFINE A THRESHOLD**

The threshold defines the detection level to take into account for the volume quantification. It allows to distinguish between bands and smears on the lane.

**Case when you should use detection level (Threshold)**

There is still a strong background even after the background subtraction



**Original Image**

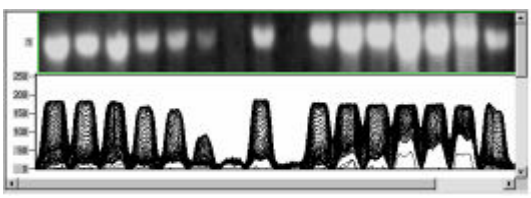


**Image with subtracted background**

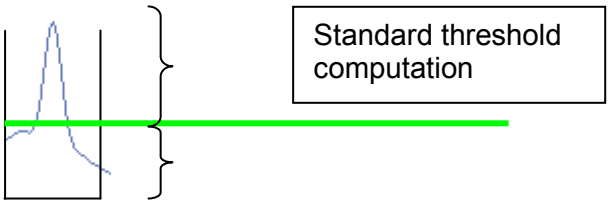
The spot contours must be isolated more precisely from the smears where they are located. The threshold calculates the volume which is above the threshold:

Threshold:

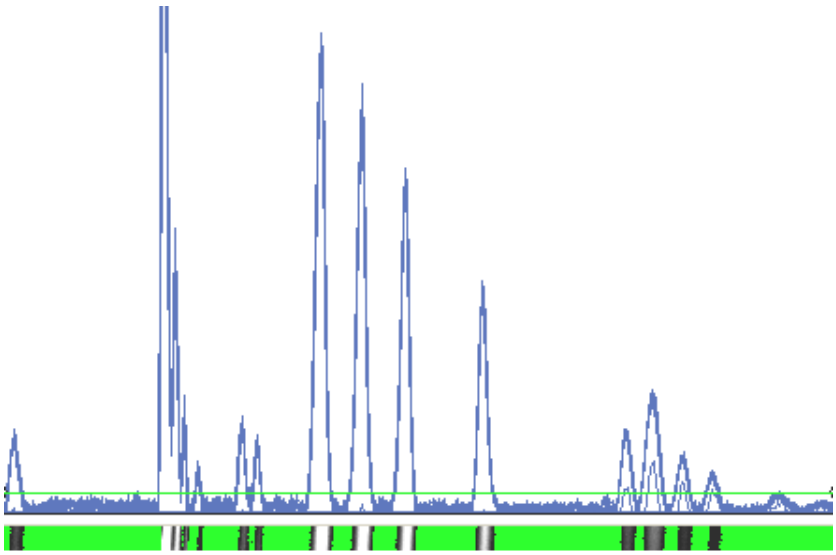
Volume= ∑ (Pixels intensities)

⇨ Pixel intensities=0 if Pixel<Threshold

⇨ Pixel intensities= (Pixel intensities) if Pixel>Threshold




Move upwards or downward the horizontal line appearing on the profile:



This displays a green contour that encloses pixels whose intensity is equal to or greater than that of the pixel at the cursor. If the contour does not encircle the band, reposition the cursor and click again. A new contour will be drawn in place of the old one.

The green area under the profile represents the range of values discarded to calculate the volume. The contour should completely surround the data you want to quantify.

The defined threshold is automatically applied to the selected lane. The results are recalculated taking into account the threshold:

| Number | // | Volume | Height | Area | MW-RF |
|---|---|---|---|---|---|
| No 1 | | 224452 | 2173 | 126 | 31.714 |
| No 2 | | 561516 | 2110 | 294 | 24.000 |
| No 3 | | 1216111 | 2429 | 574 | 13.143 |
| No 4 | | 1072687 | 11699 | 210 | 8.812 |
| No 5 | | 388143 | 6775 | 70 | 7.391 |
| No 6 | | 373495 | 5360 | 98 | 6.459 |
| No 7 | | 531756 | 2988 | 224 | 5.767 |
| No 8 | | 1140205 | 3070 | 490 | 4.945 |
| No 9 | | 1144095 | 6172 | 392 | 3.847 |
| No 10 | | 943922 | 5602 | 350 | 2.930 |
| No 11 | | 1138471 | 4700 | 602 | 2.453 |
| No 12 | | 1235282 | 3269 | 966 | 1.987 |
| No 13 | | 385044 | 1870 | 294 | 1.417 |
| No 14 | | 401562 | 2191 | 252 | 0.973 |
| No 15 | | 243847 | 1541 | 195 | 0.774 |
| No 16 | | 213134 | 1311 | 191 | 0.573 |
| No 17 | | 4827 | 973 | 5 | 0.389 |
| No 18 | | 0 | 0 | 0 | 0.267 |

⇨ The volume is the sum of intensities included in the spot area of analysis.
⇨ The height is the maximum spot intensity, in grey levels.
⇨ The area is the zone defined for each spot area of analysis.

The threshold approach is on a lane-based basis. You can set the same threshold for all lanes or specify an individual threshold for the selected lane. Any changes you make will be automatically applied to the image.

To apply the same subtraction level for all lanes, click on the “Apply to all lanes” button:

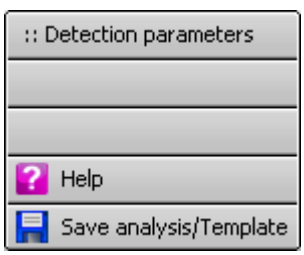

**NEXT**
The “Next” button validates your parameter and opens the following analysis step.

| | | |
|---|---|---|
| 1 C- Spot separation | Next >> | 2 A – Volume of reference |

**BACK**
The “Back” button validates your parameter and opens the following analysis step.

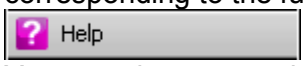
| | | |
|---|---|---|
| 1 C- Spot separation | << Back | 1 B – Background subtraction |

**OPTION FOLDER**
The option folder gathers the following functions:
⇨ Help
⇨ Save the analysis or the template

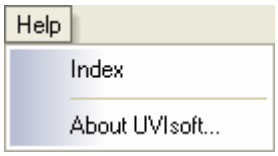


## HELP MENU

Click on the “Help” button. You automatically access the user manual at the chapter corresponding to the function




You can also access the help file index through the File\Help from the Menu bar




## SAVE ANALYSIS / TEMPLATE

This function saves the current analysis. The analysis file will contain the results, the image and all the parameters defined to obtain the results.

The analysis could also be saved as a template for automated analysis routines.

Template offers the user the ability to automate many of the repetitive tasks associated with analysis and processing. As a result, you can spend more time evaluating and analysing results, and less time manipulating set-ups, variables and other settings.

The template automates a task or set of tasks that you perform repeatedly or on a regular basis. It stores all the analysis commands and parameters of an analysis. You can run these parameters with another image whenever you need to perform a new analysis based on the same parameters.

The benefits of the template file are as follows:

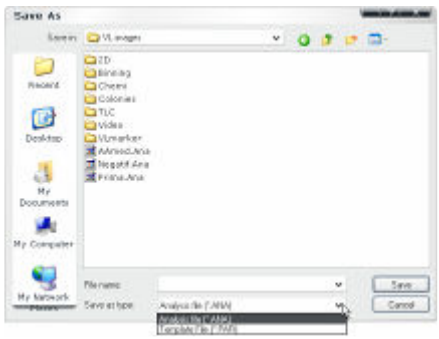
⇨ Time saving

⇨ Reproduction of image analysis parameters

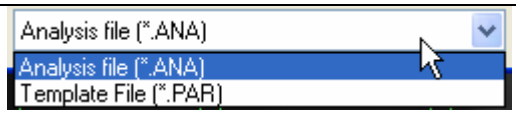
⇨ Templates are modifiable, allowing the user to maintain an original template while modifying it for a slightly different result, with minimal effort

1. Click on the “Save analysis/ Template” button:


2. A pop-up window displays the following menu:

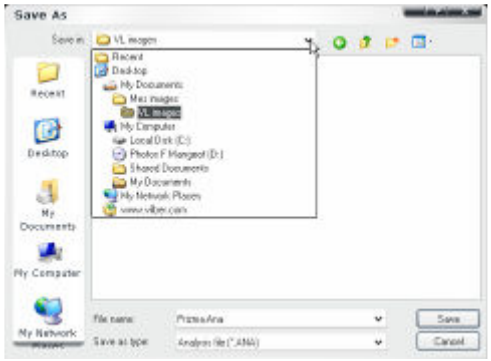



3. Select analysis file or template file:

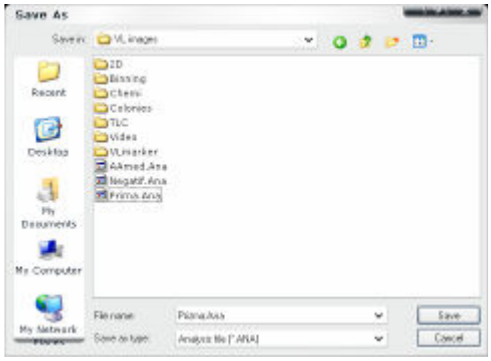



Note: the software proposes Analysis file by default

4. Browse to specify the file directory




5. Enter the desired file name and validate




6. Click on the Save button to create the file.

Note: see “Access to the analysis module” chapter for template or analysis file loading

# 2- Analyse – Quantification

## ➔ Principles of quantification

2

Volume is the based of the spot quantification process. The volume is the sum of all the intensities included in the defined area (window + separation).

Quantification is based on the image in pixels whose intensity is coded on a scale.
- The scale has 256 grey levels for a 8-bit image
- The scale has 4 096 grey levels for a 12-bit image
- The scale has 16 384 grey levels for a 14-bit image
- The scale has 65 536 grey levels for a 16-bit image

The quantity (or density) of a spot is calculated from its volume. This is made of the sum of all pixel intensities composing the spot

In other words, the spot quantity then depends on:
- The number of pixels inside the area of the spot
- The intensities of these points

$$V = \sum ni\ Ii$$

Image analysis allows comparison in between concentrated intense spots and weaker but more diffused bands.

Results are given in volumes that may be recalculated according to an OD of reference or a concentration master-curve.

To measure the amount of a particular spot, you need to define the boundary around the spot and compare the intensity data inside the boundary with the data of other spots or of a standard.

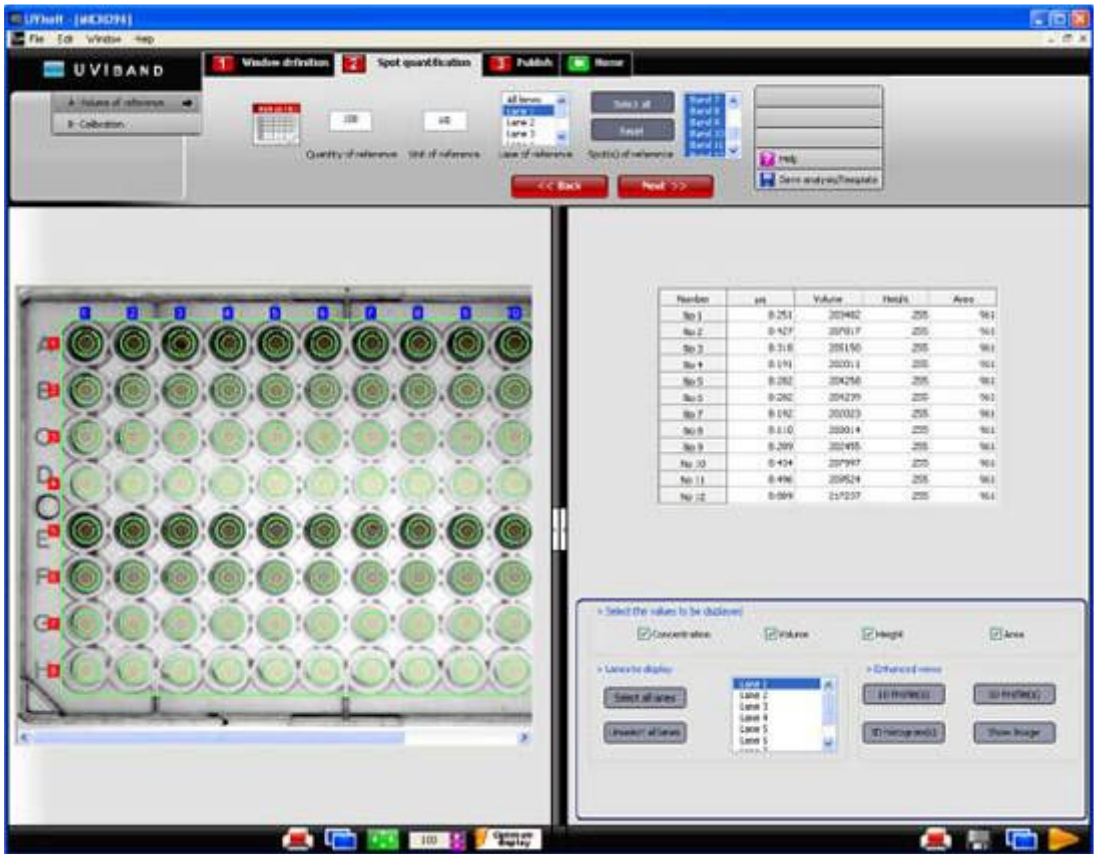
## ➔ A – Volume of reference

2

A volume is the total signal intensity inside a defined boundary drawn on a lane.

The purpose of the volume of reference is to use volumes of known concentration to calculate the unknown concentrations. The volume of reference process follows the spot separation.

Note: you can either access the volume of reference function by clicking on the next button of the background subtraction or directly by clicking on the volume of reference of the 2-Spot Quantification folder.




The dashboard details the volume of reference parameters:


⇨ The quantity of reference
⇨ The unit of reference
⇨ The lane of reference
⇨ The spot(s) of reference

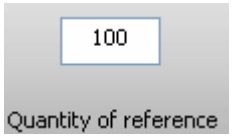
**QUANTITY OF REFERENCE**

The calculation of the unknown concentrations is based:

⇨ On the calculated volumes
⇨ On the known concentration. The known concentration is the quantity of reference.

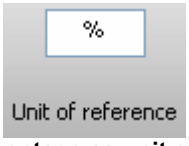
The quantity of reference could correspond to one or several spots.
The purpose of the quantity of reference is to define the known concentration:

In the “Quantity of reference” edit field, type the quantity of known concentration you want to have as a reference:

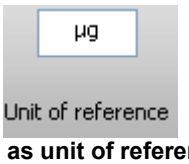



**UNIT OF REFERENCE**
The unit of reference is the header unit of the concentration. You can define your own header such as % or µg.

In the “Unit of reference” edit field, type the unit you want to be displayed in the results table:




**Percentage as unit of reference**




**µg as unit of reference**

**LANE OF REFERENCE**
The lane of reference defines the lane of the known concentration.
Select the lane of reference from the list:


If a single lane is selected, only the volumes of this reference lane will be used to calculate the relationship between the volume and the quantity. The other concentrations are calculated based on the concentration/volume relationship of this specific lane.

| | Lane 1 | | Lane 3 | | Lane 4 | |
|---|---|---|---|---|---|---|
| Number | % | Volume | % | Volume | % | Volume |
| No 1 | 44.708 | 6635518 | 178.291 | 26461728 | 49.658 | 7370205 |
| No 2 | 25.475 | 3780895 | 64.424 | 9561786 | 47.652 | 7072517 |
| No 3 | 14.264 | 2117062 | 9.885 | 1467075 | | 0 |
| No 4 | 9.304 | 1380926 | | | | 0 |
| No 5 | 3.574 | 530507 | | | | 0 |
| No 6 | 1.840 | 273100 | | | | 0 |
| No 7 | 0.835 | 123860 | | | | |
| No 8 | | 0 | | | | |
| No 9 | | 0 | | | | |

Illustration 1: 100% / lane 1 / all bands. Total concentration lane 1= 100%

If “All lanes” is selected, for each lane a new relationship between volume and

quantity will be recalculated, according to the band's lane selected. For instance, the defined parameters are 100% for all band all lanes; the results table could be as follows. Lane by lane, the total band concentration is 100%:

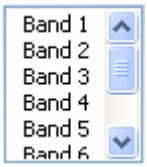
| | Lane 1 | | Lane 3 | | Lane 4 | |
|---|---|---|---|---|---|---|
| Number | % | Volume | % | Volume | % | Volume |
| No 1 | 44.708 | 6635518 | 70.582 | 26461728 | 51.031 | 7370205 |
| No 2 | 25.475 | 3780895 | 25.504 | 9561786 | 48.969 | 7072517 |
| No 3 | 14.264 | 2117062 | 3.913 | 1467075 | | 0 |
| No 4 | 9.304 | 1380926 | | | | 0 |
| No 5 | 3.574 | 530507 | | | | 0 |
| No 6 | 1.840 | 273100 | | | | 0 |
| No 7 | 0.835 | 123860 | | | | |
| No 8 | | 0 | | | | |
| No 9 | | 0 | | | | |

Illustration 2: 100% / all lanes / all bands. Total concentration all lanes= 100%

**SPOT(S) OF REFERENCE**

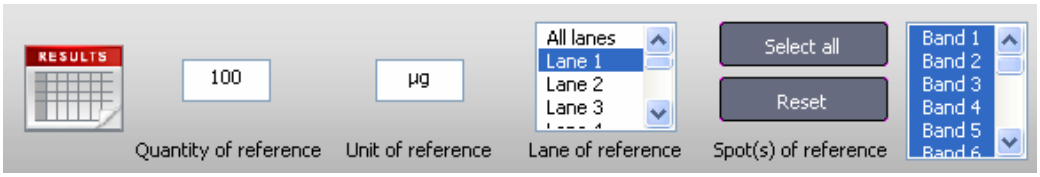
The quantity of reference could correspond to one or several spots of the selected lane.

Select one or several spots of the lane of reference from the list:




**EXAMPLE 1**

Let's consider the known concentration is 3µg contains in the first spot of lane 3. The settings should then be as follows:

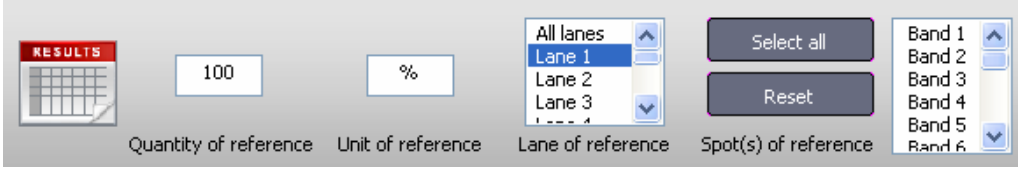



The results table indicates the following for lane 3:

| Number | µg | Volume | Height | Area | MW-RF |
|---|---|---|---|---|---|
| No 1 | 3.000 | 4285313 | 4071 | 1775 | 10.000 |
| No 2 | 9.267 | 13237182 | 3438 | 5396 | 8.000 |
| No 3 | 0.942 | 1345357 | 2740 | 568 | 6.000 |
| No 4 | 0.467 | 667689 | 2692 | 284 | 5.000 |
| No 5 | 12.560 | 17940927 | 2651 | 10224 | 4.000 |
| No 6 | 0.358 | 511654 | 1305 | 426 | 3.000 |
| No 7 | 3.885 | 5549237 | 1275 | 5112 | 2.500 |
| No 8 | 1.626 | 2322765 | 1176 | 2414 | 2.000 |
| No 9 | 0.465 | 664510 | 1000 | 710 | 1.500 |

**EXAMPLE 2**

Let's consider the known concentration is 100% contains in all the spots of lane 1. The settings should then be as follows:




The results table indicates the following for lane 1:

| Number | % | Volume | Height | Area | MW-RF |
|---|---|---|---|---|---|
| No 1 | 3.978 | 1715709 | 2744 | 781 | 9.896 |
| No 2 | 15.367 | 6627687 | 4310 | 2769 | 7.998 |
| No 3 | 11.431 | 4930041 | 4642 | 2130 | 7.710 |
| No 4 | 12.333 | 5319454 | 2612 | 2414 | 4.561 |
| No 5 | 2.112 | 911077 | 2323 | 426 | 4.000 |
| No 6 | 35.571 | 15341999 | 2191 | 10508 | 2.678 |
| No 7 | 19.207 | 8284193 | 1270 | 8591 | 1.872 |

**NEXT**

The "Next" button validates your parameter and opens the following analysis step.

| | | |
|---|---|---|
| 2 A – Volume of reference | Next >> | 2 B – Calibration |

**BACK**

The "Back" button validates your parameter and opens the following analysis step.

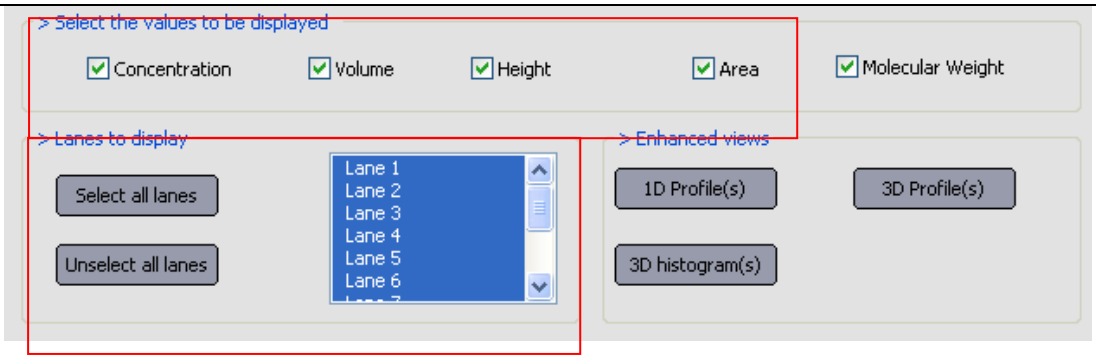
| | | |
|---|---|---|
| 2 A – Volume of reference | << Back | 1 C- Spot separation |

**RESULT TABLE**

In the result parameter window, you can select the lanes and the values to be displayed in the results tables:

- ⇨ Concentration
- ⇨ Volume
- ⇨ The maximum intensity
- ⇨ The area

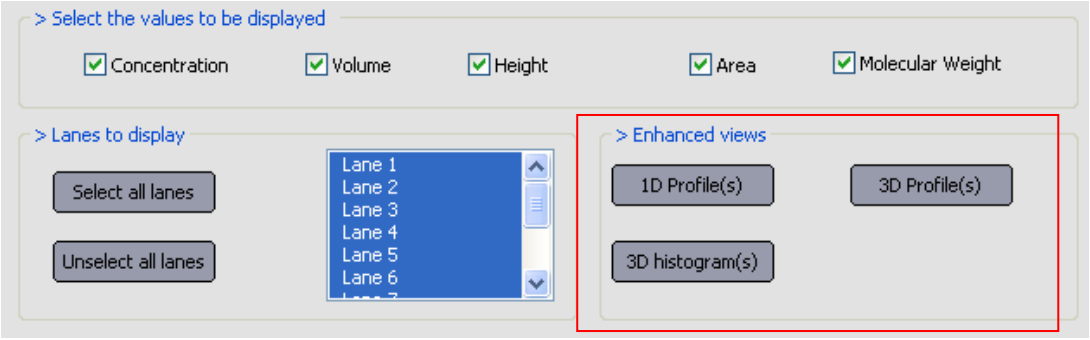
1. To select your display mode, click on the appropriate selection:




## GRAPHICAL VIEW

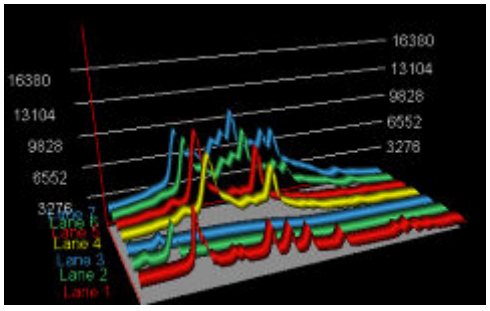
In the results parameter window, you can select the graphical results tables:

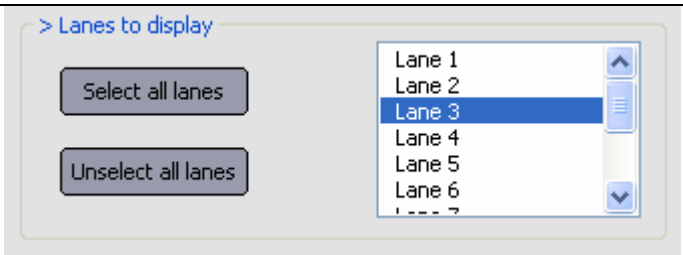
- ⇨ 1D profile
- ⇨ 3D profile
- ⇨ 3D histogram




Note: For all enhanced views, you can modify the angle of vision of the 3D view: Move the mouse cursor on the 3D area, click and drag the view in the direction you want to rotate. Release the mouse when satisfactory.

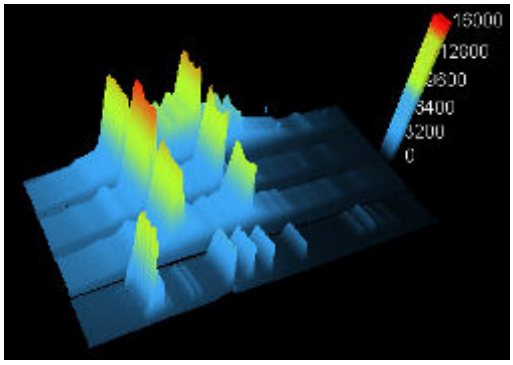
The 1D profile allows you to superimpose the intensity profiles of any number of selected lanes.
To proceed, click on the 1D Profile and select the lanes to be superimposed:

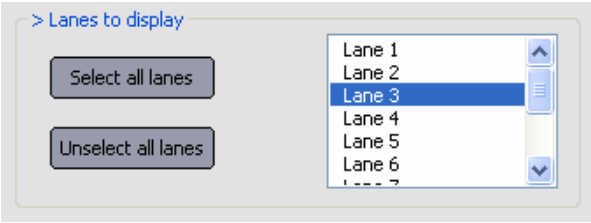






Note: To reposition the 1D profile window, position your cursor at the top of the box. The cursor appearance will change to a multidirectional arrow symbol. You can then drag the box to a new position.
Note: To increase the size of the 1D profile window, position your cursor at the edge of the window and click and drag to extend its size.
Note: Click on Print to print the 1D profile window
Note: Click on Send to clipboard to save the graph in the Windows ® clipboard (Past/copy Windows ® feature).

The 3D profile displays the three-dimensional rendering of any selected lanes.
To proceed, click on the 3D Profile button and select the lanes to be displayed:

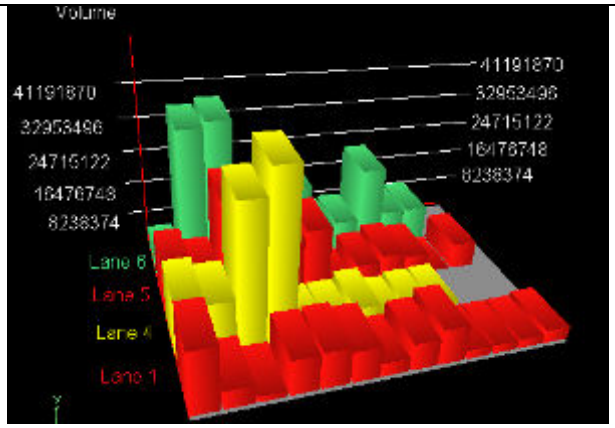




Note: To reposition the 1D profile window, position your cursor at the top of the box. The cursor appearance will change to a multidirectional arrow symbol. You can then drag the box to a new position.
Note: To increase the size of the 1D profile window, position your cursor at the edge of the window and click and drag to extend its size.
Note: Click on Print to print the 1D profile window
Note: Click on Send to clipboard to save the graph in the Windows ® clipboard (Past/copy Windows ® feature).

The 3D histogram displays the three-dimensional histogram of selected results:
- ⇨ Volume
- ⇨ Calculated quantities
- ⇨ Maximum intensities

To proceed, click on the 3D Histogram button and select the lanes to be displayed:



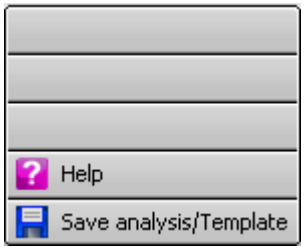

Note: To reposition the 1D profile window, position your cursor at the top of the box. The cursor appearance will change to a multidirectional arrow symbol. You can then drag the box to a new position.
Note: To increase the size of the 1D profile window, position your cursor at the edge of the window and click and drag to extend its size.
Note: Click on Print to print the 1D profile window

Note: Click on Send to clipboard to save the graph in the Windows ® clipboard (Past/copy Windows ® feature).

**OPTION FOLDER**

The option folder gathers the following functions:

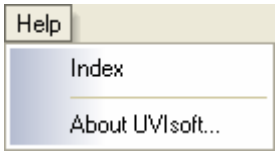
⇨ Help
⇨ Save the analysis or the template




**HELP**

Click on the “Help” button. You automatically access the user manual at the chapter corresponding to the function


You can access the help file index through the File\Help from the Menu bar




**SAVE ANALYSIS / TEMPLATE**

This function saves the current analysis. The analysis file will contain the results, the image and all the parameters defined to obtain the results.

The analysis could also be saved as a template for automated analysis routines. Template offers the user the ability to automate many of the repetitive tasks associated with analysis and processing. As a result, you can spend more time evaluating and analysing results, and less time manipulating set-ups, variables and

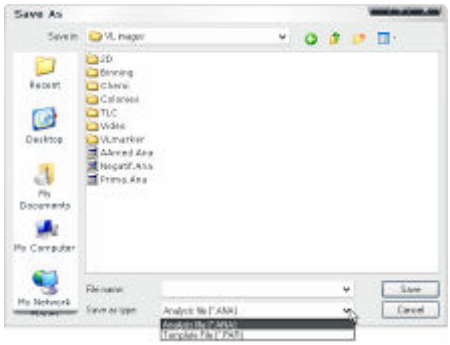
other settings.

The template automates a task or set of tasks that you perform repeatedly or on a regular basis. It stores all the analysis commands and parameters of an analysis. You can run these parameters with another image whenever you need to perform a new analysis based on the same parameters.

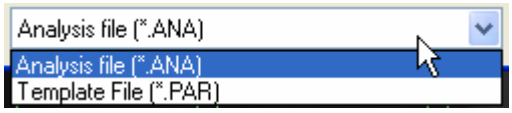
The benefits of the template file are as follows:
⇨ Time saving
⇨ Reproduction of image analysis parameters
⇨ Templates are modifiable, allowing the user to maintain an original template while modifying it for a slightly different result, with minimal effort

1. Click on the “Save analysis/ Template” button:


2. A pop-up window displays the following menu:

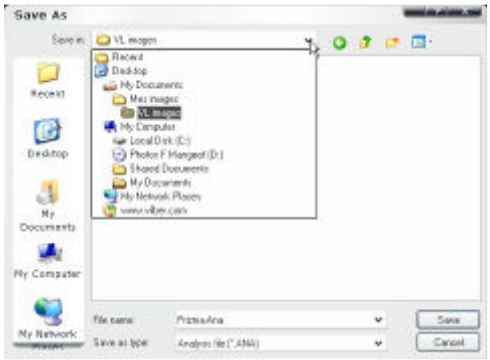



3. Select analysis file or template file:

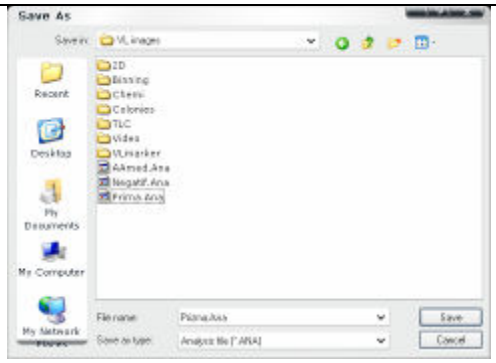



Note: the software proposes Analysis file by default

4. Browse to specify the file directory




5. Enter the desired file name and validate




6. Click on the Save button to create the file.

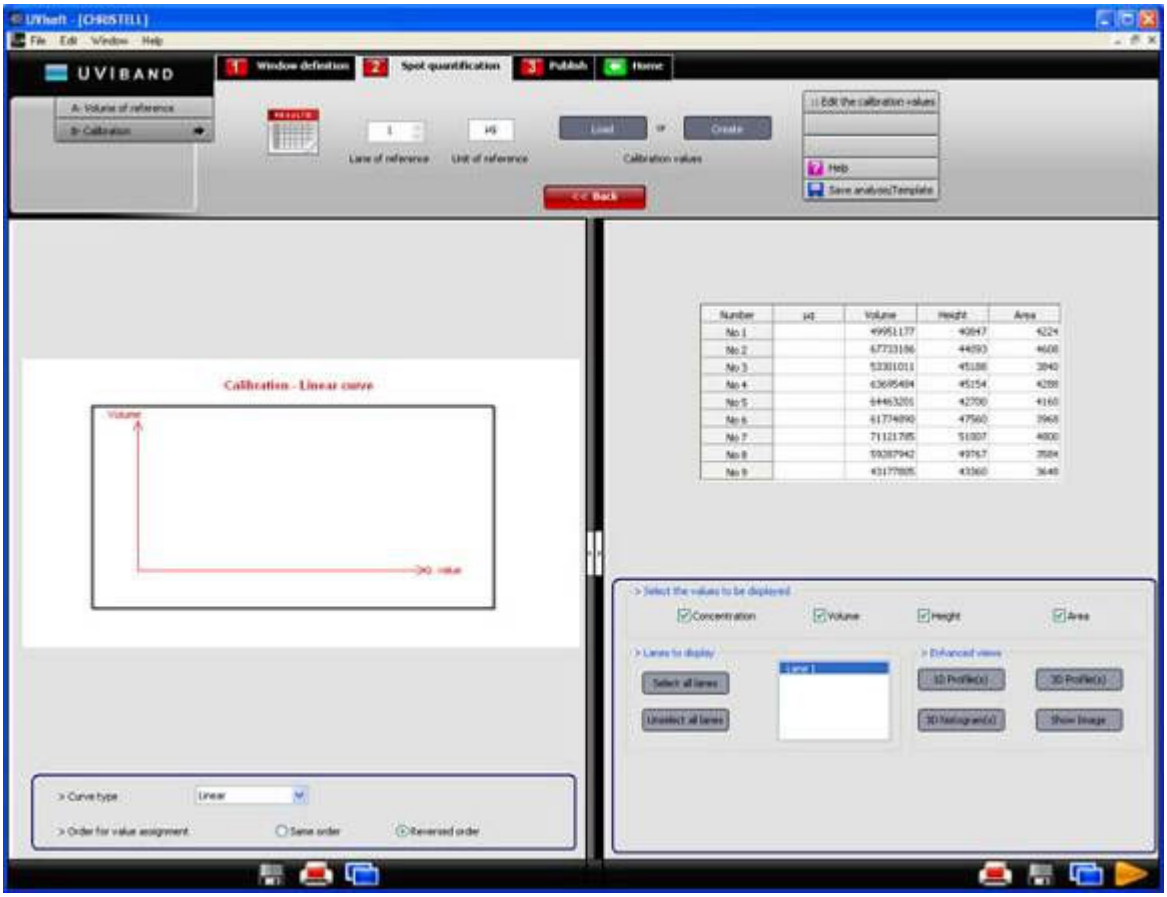
Note: see “Access to the analysis module” chapter for template or analysis file loading

## ➔ B – Calibration

2

The calibration process follows the Volume of reference function. The calibration is the calculation of the concentration based on a concentration master or on a calibration curve on which you can select all or few points.

Note: you can either access the calibration function by clicking on the next button of the Volume of reference or directly by clicking on the Calibration of the 2-Spot quantification folder.

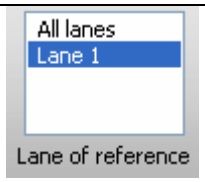



The dashboard details the volume of reference parameters:


- ⇨ The lane of reference
- ⇨ The unit of reference
- ⇨ The calibration values

**LANE OF REFERENCE**

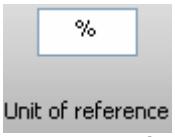
The lane of reference defines the lane of the known concentration.
Select the lane of reference from the list:



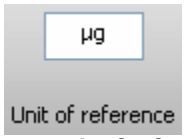

**UNIT OF REFERENCE**

The unit of reference is the header unit of the concentration. You can define your own header such as % or µg.

In the "Unit of reference" edit field, type the unit you want to be displayed in the results table:




**Percentage as unit of reference**




**µg as unit of reference**

**THE CALIBRATION VALUES**

Click on the "Load" or "Create" button to enter calibration's values.

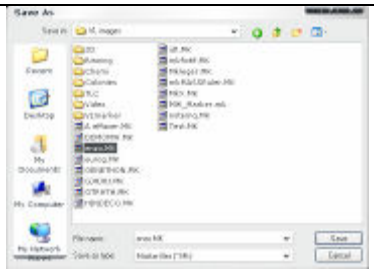

For "Create", a pop-up window displays the following menu:


Type your values, band to band, in a descending order. The OK button validates your data.

Note: if an automatic calculation with immediate application of the standard values is carried out, it is not necessary to enter all the bands given by the manufacturer's specifications, but only those which are commonly found on the lanes of the gel.

You can save your calibration data and create your own calibration library; To proceed, click on the "Save " button:

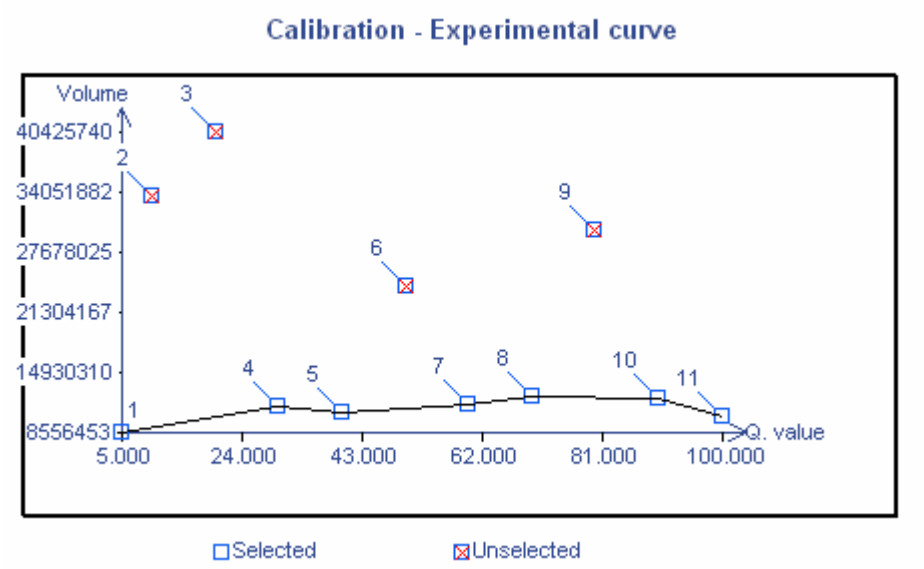

A pop-up window displays the following menu:



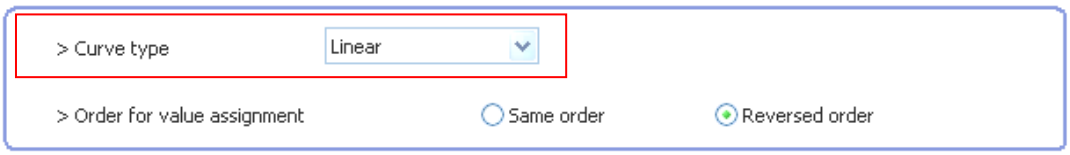
⇨ Browse to specify the directory
⇨ Type the file name and click on Save.

**MASTER CURVE**

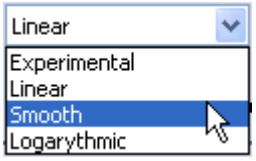
After the values of the master-curve are defined, the calibration curve is displayed. You can unselect wrong values or points out of the curve by directly clicking on them



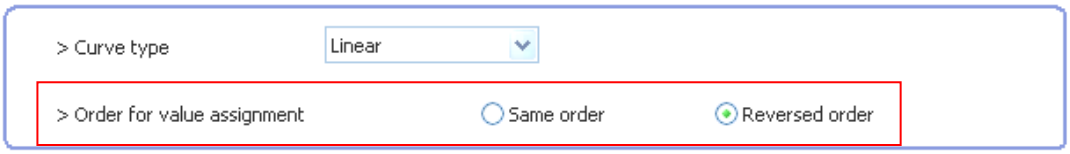

In the profile parameters window, select the curve type:




Four mathematical models can be used:
⇨ Experimental: the curve simply links the values (point to point), without any mathematical model,
⇨ Linear curve: displays a model with linear regression
⇨ Smoothed: displays a smoothed curve (polynomial spline, at least 4 points must be entered)
⇨ Logarithmic curve: displays a model with logarithmic regression



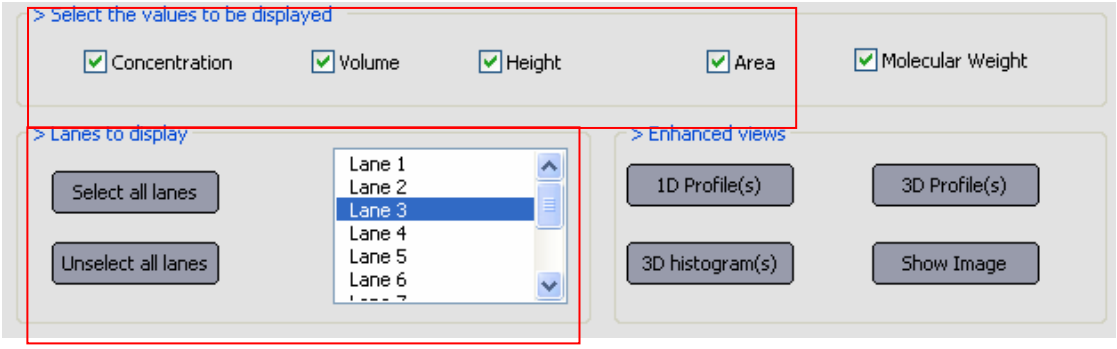
You can also select the order for the spot display:
⇨ - Same order as the values of the master-curve
⇨ - Reversed order (depending on the order of the defined values)




**RESULT TABLE**

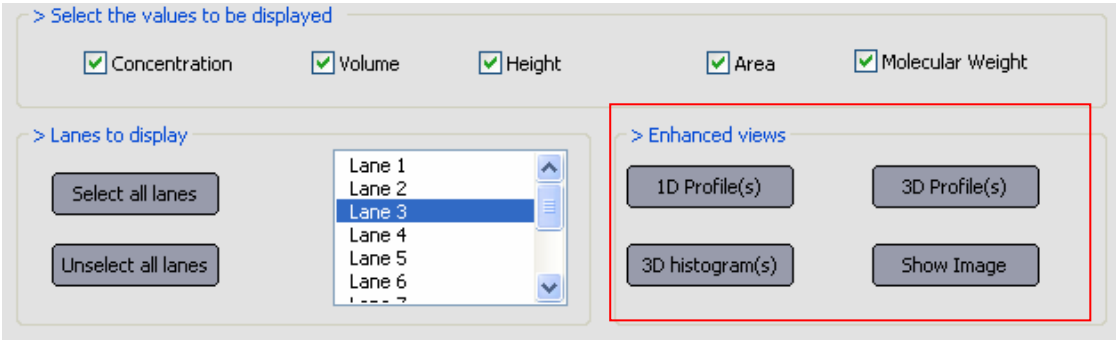
In the result parameter window, you can select the lanes and the values to be displayed in the results tables:
⇨ Concentration
⇨ Volume
⇨ The maximum intensity
⇨ The area

To select your display mode, click on the appropriate selection:



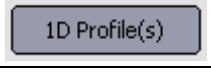

**GRAPHICAL VIEW**

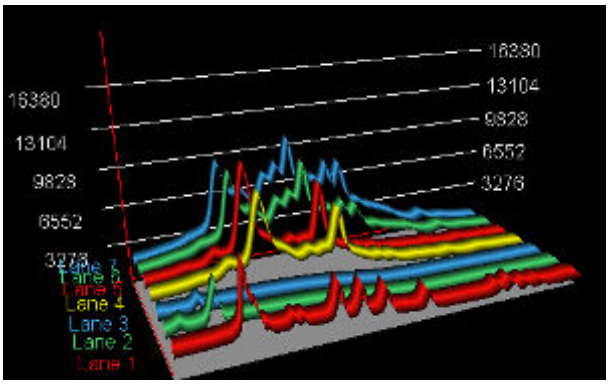
In the results parameter window, you can select the graphical results tables:
⇨ 1D profile
⇨ 3D profile
⇨ 3D histogram

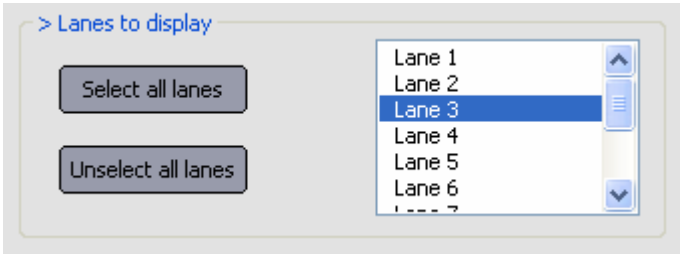



Note: For all enhanced views, you can modify the angle of vision of the 3D view : Move the mouse cursor on the 3D area, click and drag the view in the direction you want to rotate. Release the mouse when satisfactory.

The 1D profile allows you to superimpose the intensity profiles of any number of selected lanes.

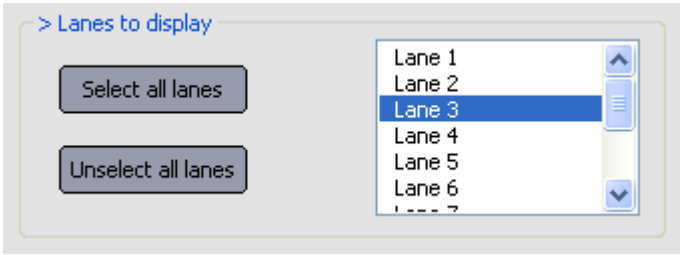
To proceed, click on the 1D Profile and select the lanes: 

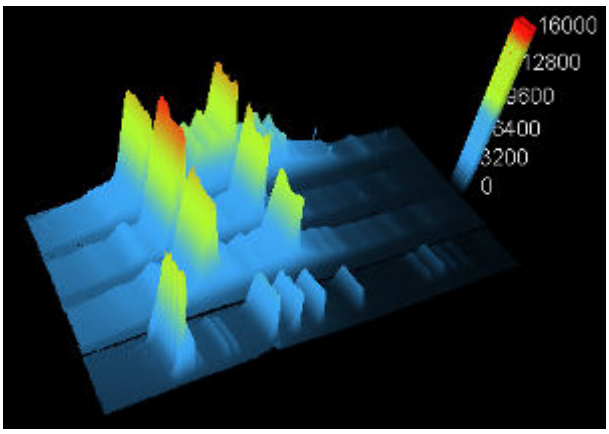



Note: To reposition the 1D profile window, position your cursor at the top of the box. The cursor appearance will change to a multidirectional arrow symbol. You can then drag the box to a new position.
Note: To increase the size of the 1D profile window, position your cursor at the edge of the window and click and drag to extend its size.
Note: Click on Print to print the 1D profile window
Note: Click on Send to clipboard to save the graph in the Windows ® clipboard (Past/copy Windows ® feature).

The 3D profile displays the three-dimensional rendering of any selected lanes.
To proceed, click on the 3D Profile button and select the lanes to be displayed:

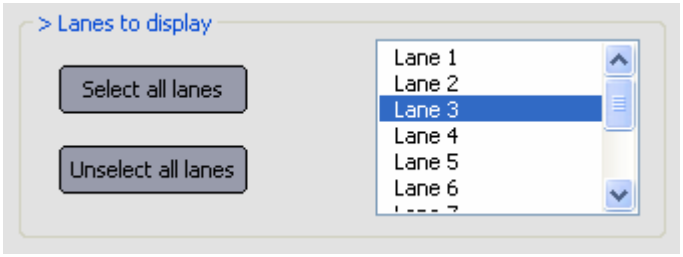



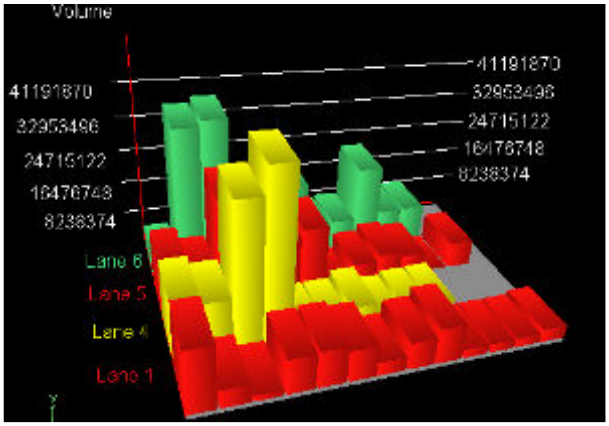



Note: To reposition the 1D profile window, position your cursor at the top of the box. The cursor appearance will change to a multidirectional arrow symbol. You can then drag the box to a new position.
Note: To increase the size of the 1D profile window, position your cursor at the edge of the window and click and drag to extend its size.
Note: Click on Print to print the 1D profile window
Note: Click on Send to clipboard to save the graph in the Windows ® clipboard (Past/copy Windows ® feature).

The 3D histogram displays the three-dimensional histogram of selected results:
- ⇨ Volume
- ⇨ Calculated quantities
- ⇨ Maximum intensities

To proceed, click on the 3D Histogram button and select the lanes to be displayed:

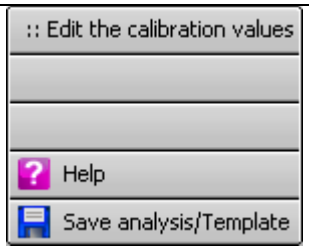




Note: To reposition the 1D profile window, position your cursor at the top of the box. The cursor appearance will change to a multidirectional arrow symbol. You can then drag the box to a new position.
Note: To increase the size of the 1D profile window, position your cursor at the edge of the window and click and drag to extend its size.
Note: Click on Print to print the 1D profile window
Note: Click on Send to clipboard to save the graph in the Windows ® clipboard (Past/copy Windows ® feature).

## OPTION FOLDER

The option folder gathers the following functions:
- ⇨ Edit the calibration values
- ⇨ Help
- ⇨ Save the analysis or the template




**EDIT THE CALIBRATION VALUES**

1. Click on the “Edit the calibration values” button.

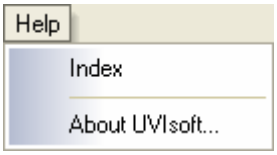

2. A pop-up window displays the following menu on which you can modify the calibration values:


You can add, remove, and save your marker’s value;

**HELP MENU**

Click on the “Help” button. You automatically access the user manual at the chapter corresponding to the function


You can access the help file index through the File\Help from the Menu bar




**SAVE ANALYSIS / TEMPLATE**

This function saves the current analysis. The analysis file will contain the results, the image and all the parameters defined to obtain the results.

The analysis could also be saved as a template for automated analysis routines. Template offers the user the ability to automate many of the repetitive tasks associated with analysis and processing. As a result, you can spend more time evaluating and analysing results, and less time manipulating set-ups, variables and other settings.

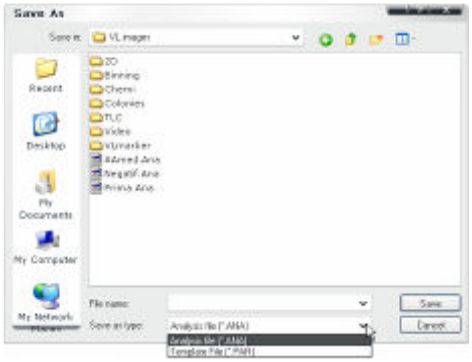
The template automates a task or set of tasks that you perform repeatedly or on a regular basis. It stores all the analysis commands and parameters of an analysis. You can run these parameters with another image whenever you need to perform a new analysis based on the same parameters.

The benefits of the template file are as follows:

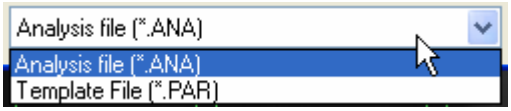
⇨ Time saving
⇨ Reproduction of image analysis parameters
⇨ Templates are modifiable, allowing the user to maintain an original template while modifying it for a slightly different result, with minimal effort

1. Click on the “Save analysis/ Template” button:


2. A pop-up window displays the following menu:

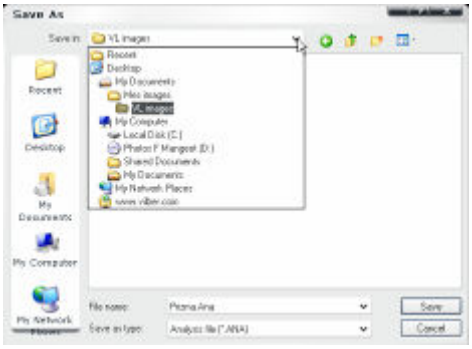


3. Select analysis file or template file:

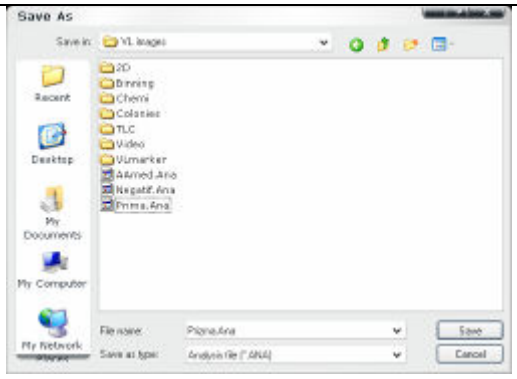



Note: the software proposes Analysis file by default

4. Browse to specify the file directory



5. Enter the desired file name and validate




6. Click on the Save button to create the file.

Note: see “Access to the analysis module” chapter for template or analysis file loading

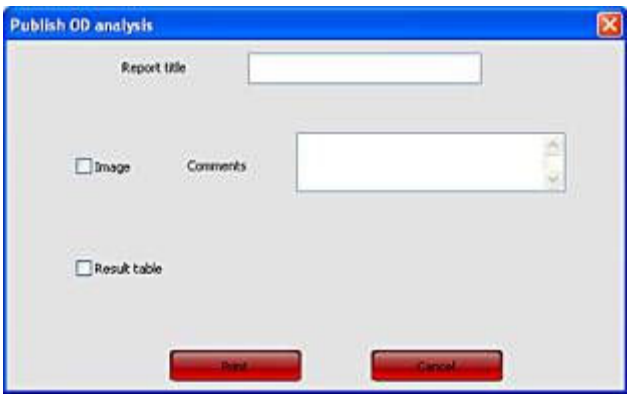
# Publish

## ➔ Introduction

3

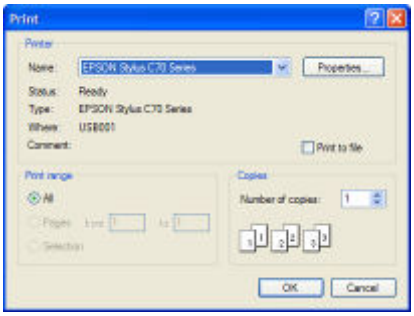
The purpose of the Publish function is to prepare a printed report of your results. You can easily organise your report with titles and comments and your own selection of data to be published among the following:
- Sample image
- Quantification result table

1. To proceed, select the Publish tab. A pop-up window displays the following menu:




⇨ Enter a report title if any
⇨ Select the options to be printed
⇨ Add comments or not per option

2. Click on the “Print” button. A pop-up window displays the following menu




⇨ Select a printer
⇨ If necessary, click on Properties to modify the default setting of the printer,
⇨ Select the number of copies
⇨ Click on OK to validate your options

# Return to Home

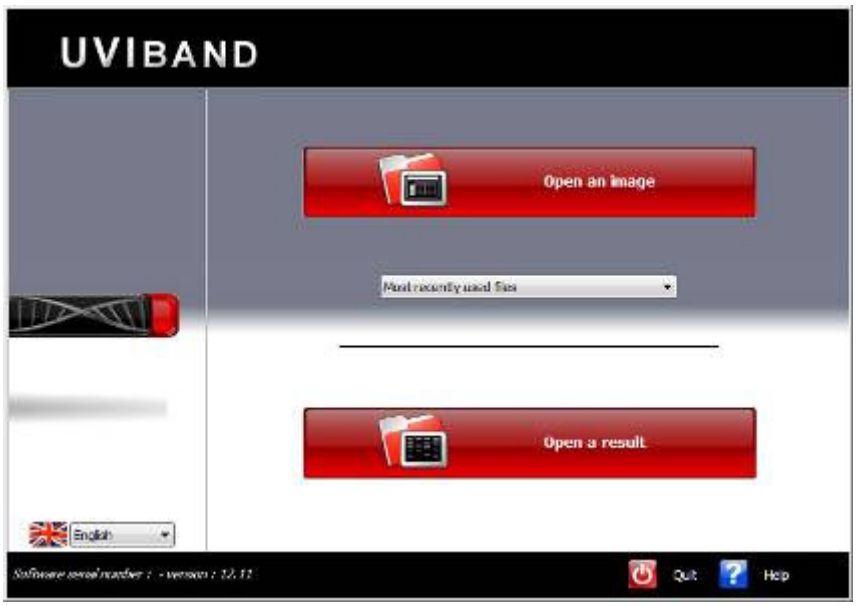
## ➔ Introduction

The home dashboard is the hub to other functions of the software:

- ⇨ Open another image file or another result file
- ⇨ Select another analysis module
- ⇨ Exit the software


## ➔ Load another image

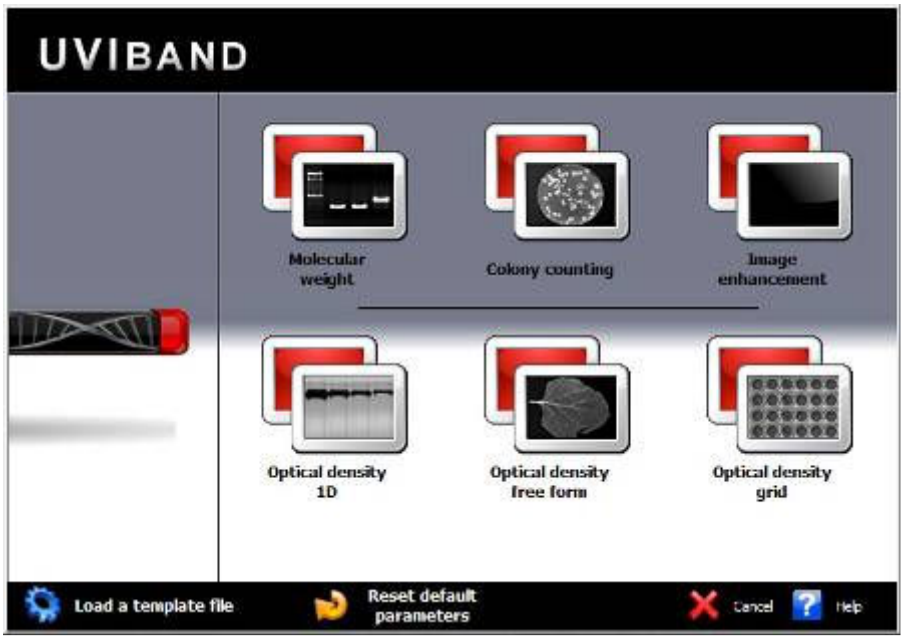
To return to the main menu, click on the home icon. A new menu appears with the main menu task bar functions:




- ⇨ Click on the “Open an image” icon to open an image
- ⇨ Click on the “Open a result” icon to open a previously saved analysis result

## ➔ Select another function

To return to the analysis menu, click on the analysis icon. A new menu appears with the analysis module task bar functions:




Click on the appropriate icon to select an analysis module.

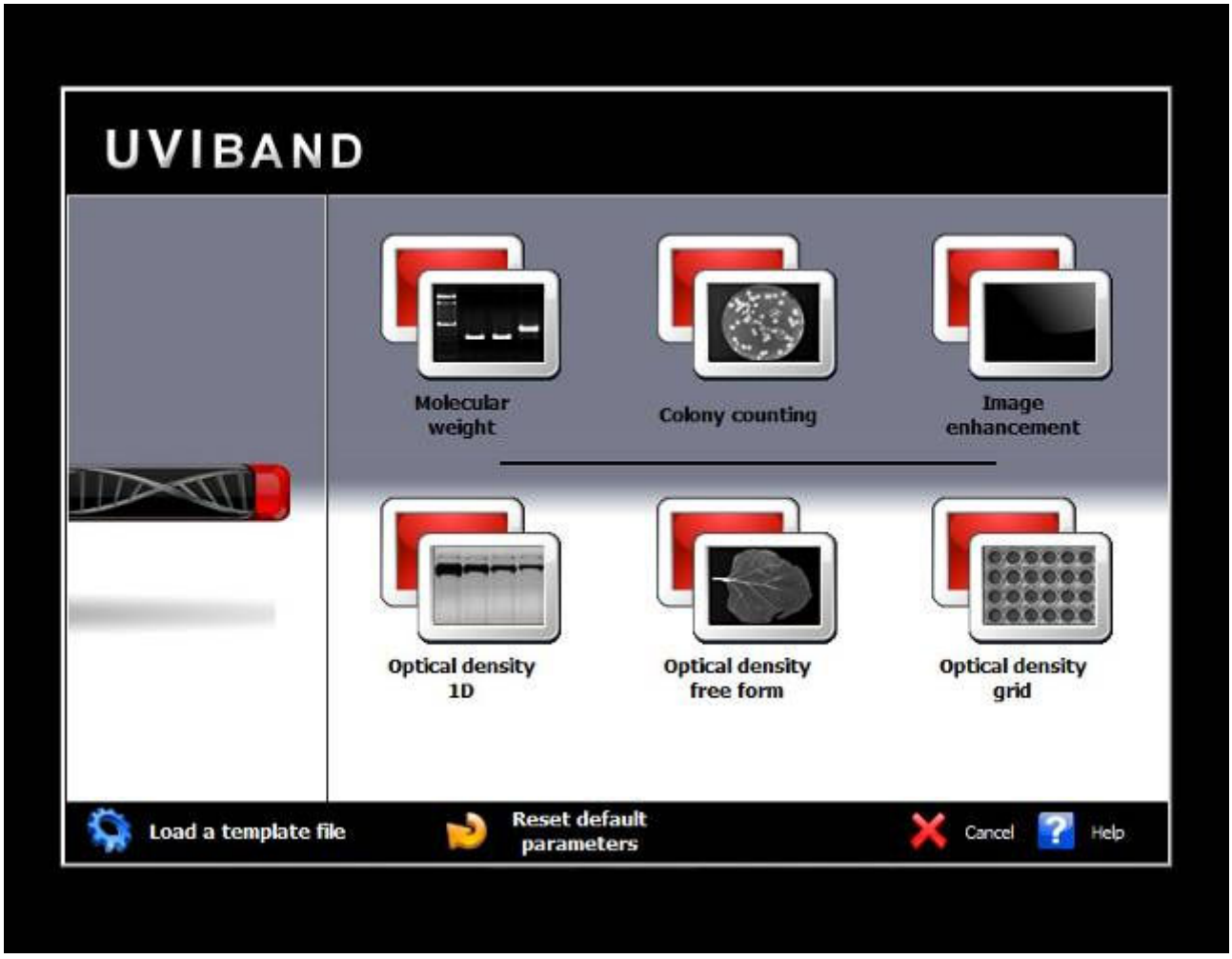
- ⇨ Select the Molecular weight icon to open the molecular weight analysis (MW) module
- ⇨ Select the Colony counting icon to open the colony counting (CC) analysis module
- ⇨ Select the Optical density - 1D icon to open the optical density (OD) analysis module based on a 1D detection
- ⇨ Select the Optical density - Free form icon to open the optical density (OD) analysis module based on a free form detection
- ⇨ Select the Optical density - Grid icon to open the optical density (OD) analysis module based on a grid detection
- ⇨ Select the Image enhancement icon to open the image enhancement module

## ➔ Exit the software

To close UVIband Advanced, select Exit from the File menu.

You will be prompted to save your analysis.

UVITEC
Cambridge




# Colony counting
# ➔ CC Analysis module

# Colony counting introduction

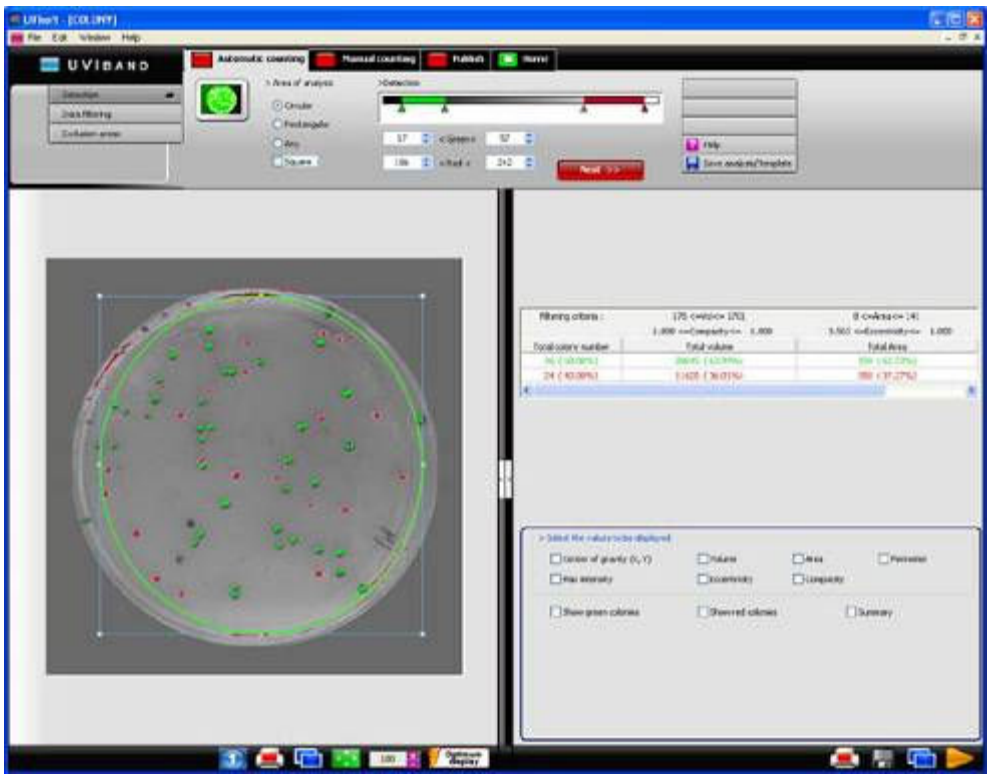
## ➔ Key features


The UVIband Advanced Colony counting module features the calculation of the number of colonies and their characteristics (Automatic colony counting) or the manual counting of colonies with the mouse (Manual colony counting).

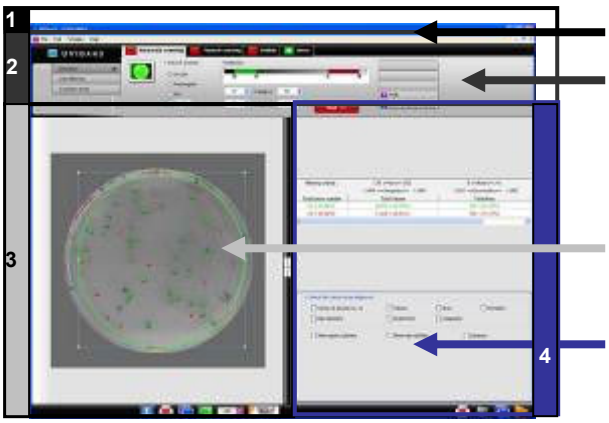
At the end of the process, you can have the following outputs:
- Number of colonies of type I (detected as green) and II (detected as red)
- The gravity, the compacity, the volume, the area, the perimeter and the eccentricity of each detected colony.

## ➔ Colony counting module (CC) operating environment


The Colony counting module opens on the following window:



The UVIband Advanced operating environment is organised into four areas:




5. The menu bar
6. The dash board
7. The image window
8. The working window

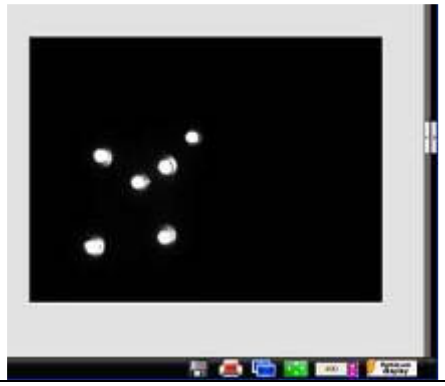
The menu bar contains the following menu:

- ⇨ File
- ⇨ Edit
- ⇨ Windows
- ⇨ Help

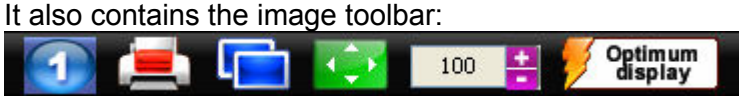

The dash board contains 3 different tabs:

1. Automatic counting
2. Manual counting
3. Publish
4. Home


The image window displays the active image:



It also contains the image toolbar:




⇨ Display the colony numbers on the image

⇨ Print

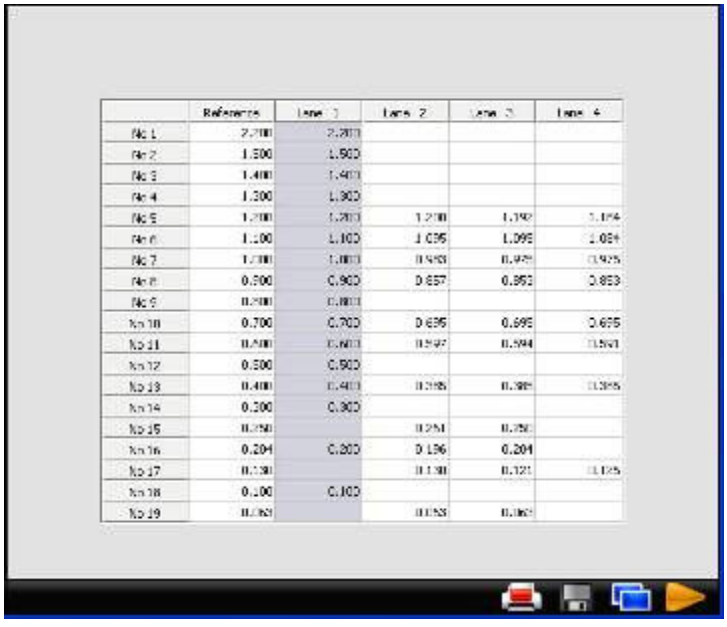
⇨ Copy to clipboard

⇨ Autoscale


⇨ Zoom in or out the image


⇨ Change the optimum display

The working window displays the graphs and tables related to the active analysis:



It also contains the working window toolbar:

⇨ Print the results

⇨ Save the graph or the table

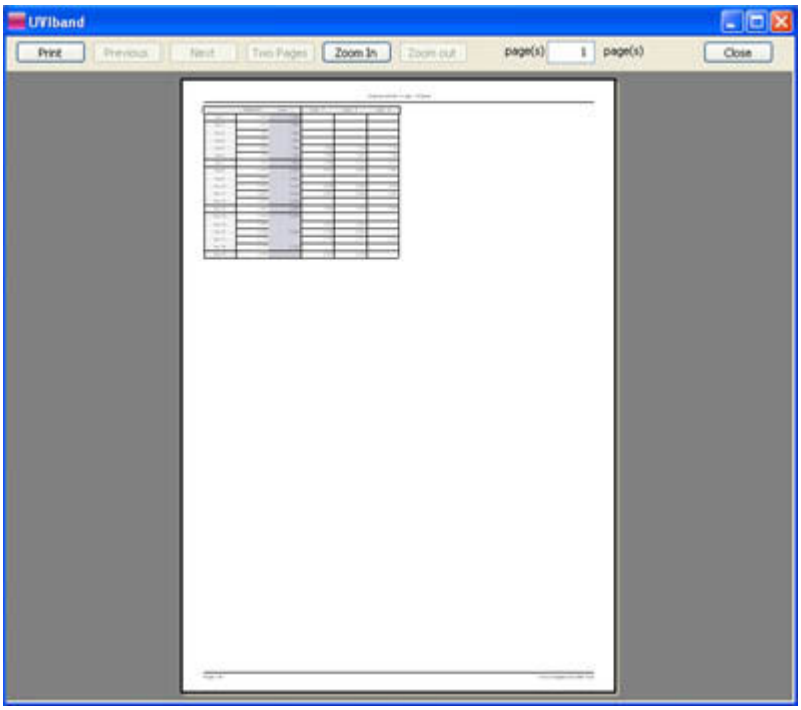
⇨ Copy the graph or the table to clipboard

⇨ Export the table to Excel

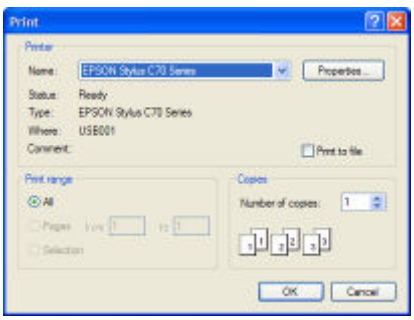
## ➔ Toolbar in details

**Print**

Click on the “Print” icon to print the image, the table or the graphs. A pop-up window displays the Print preview: The Print preview displays a preview of the image, as it will be printed.




Click on Print to validate the preview. A pop-up window displays the following menu:



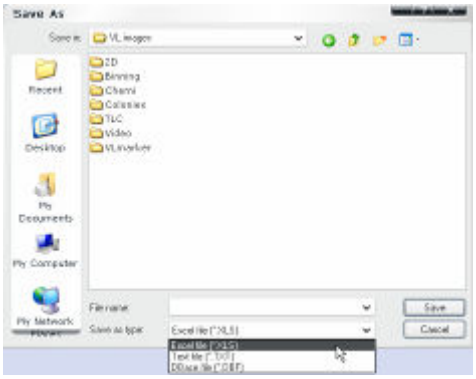

⇨ Select a printer
⇨ Click on Properties to modify the default setting of the printer, if necessary
⇨ Select the number of copies
⇨ Click on OK to validate your options

Note: You can also access the Print menu from the Menu bar (File\Print).

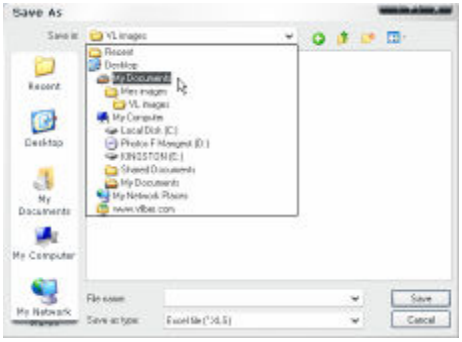
**Save**

This function saves a graph or a table. The tables are saved in a Excel™ file format (*.xls). The graphs are saved in a Bitmap format (*.bmp).

Click on the “Save” icon.
A pop-up window displays the following menu:

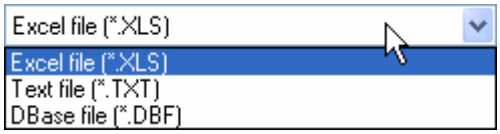



Browse to specify the file directory

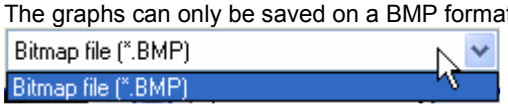



Enter the desired file name, select a file extension and validate

Note: the results could also be saved in a text file format or a Dbase file format:




The graphs can only be saved on a BMP format:



## Copy to clipboard

This function copies an image, a table or a graph onto the clipboard for insertion into another program. This option is identical to the Windows® [Ctrl C] command.

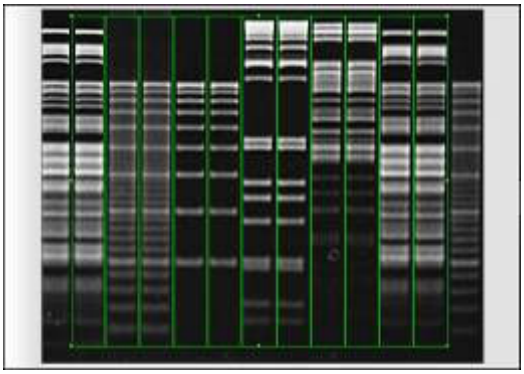
To proceed, click on the Copy to clipboard icon. The image, the table or the graph is now ready to be pasted into another application.

Open the application that you want to paste the image into, and select from the available pasting options ([Ctrl V] command for Windows® software).

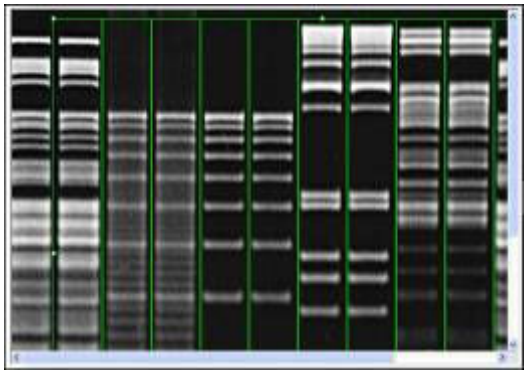
## Auto-scale

Click on the “Auto-scale” to resize the image to fit the size of the monitor.

The Auto-scale feature proportions the display of the image to the screen resolution.



Auto-scale (no scroll bar)



No auto-scale (scroll bar)


**Optimum display (for 12, 14 and 16-bit image file)**

The optimum display window is helpful to modify the greyscale selection to enhance the image display: To proceed, click on the “Optimum display” icon. A pop-up window displays the following menu:

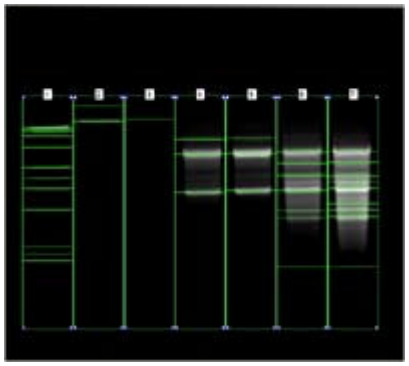
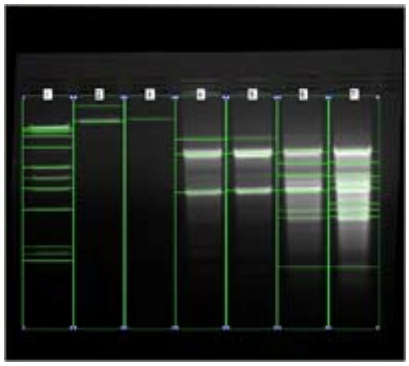
Some images has a 12, 14 or 16-bit format and Windows® can only display 8-bit images (256 grey levels).

Due to this limitation, the UVIband Advanced software handles two images:
⇨ A “memory” image corresponding to the 12, 14 or 16-bit format (4 096, 16 384 or 65 536 grey levels)
⇨ A “display image” corresponding to the image displayed on the screen (256 grey levels)

The easiest way to calculate the “display image” would be to translate the full grey scale each time an image is acquired: the x grey levels values of the “memory” image corresponds to 256 values in the displayed image. In that case, it won’t be possible to visualise faint spots on a dark image.

UVIband Advanced offers the possibility to select the grey level range to translate for the display image calculation. All the grey levels under the “Min value” defined will be converted to 0 (Black) in the displayed image. All the grey levels upper the “Max Value” defined will be set to 255 (White) in the displayed image. The grey levels between those two limits will be converted in an intermediate grey level value following a linear rule.

For both values, you can:
⇨ Edit the value in the corresponding field
⇨ Select the value by dragging and dropping the arrow
⇨ Click on the “optimum display” button: UVIband Advanced will then calculate the ideal values to be selected according to the parameters defined

| | |
|---|---|
|  |  |
| Automatic optimum display | Optimum display enhancement<br>The image appears brighter. The faint bands are more visible. |

Note: The optimum display has no impact on the analysis. Only the display of the image is modified.

| | |
|---|---|
|  | **Send to Excel™**<br>This function transfers the results table to Windows Excel™.<br><br>To proceed, click on the Send to Excel™ icon. The Excel software is automatically opened by the UVIband Advanced and the table is transferred to Excel™. |

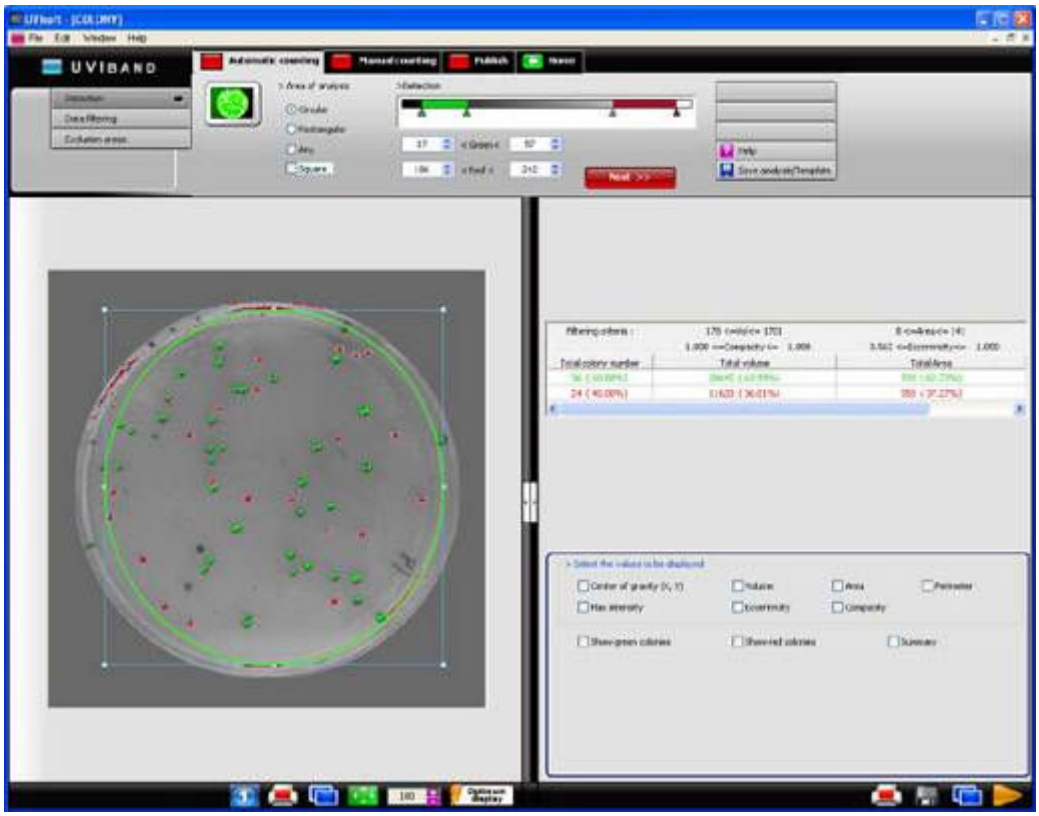
# Automatic counting

## ➔ Detection

The detection process automatically identifies all the colonies for a defined area of analysis lanes.
The colonies will be automatically detected when you first access the band detection process, based on default parameters.

Two types of colonies can be detected:

- Type A called green type, overlaid on green on the image.
- Type B, called red type, overlaid on red on the image.

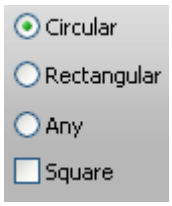



The dashboard details the lane definition parameters:


⇨ Define the area of analysis
⇨ Adjust the detection parameters

**DEFINE THE AREA OF ANALYSIS**

You could define a circular, a rectangular or a free form area of analysis:




To define a free form, click on the first point of the area on the image. Move the cursor to define one edge of the area. Validate this edge by clicking once. Then, repeats this procedure as many times as necessary.

Select the “Square“ option to obtain a circle instead of an ellipse or a square instead of a rectangle.

**ADJUST THE DETECTION PARAMETERS**

The detection parameters are summarised in the following bar graph:

The green and red areas represent the grey level range used to determine both kinds of colonies.

Click on the coloured triangle and drag them to a new place to modify the detection range. A preview of the detection is displayed on the image.

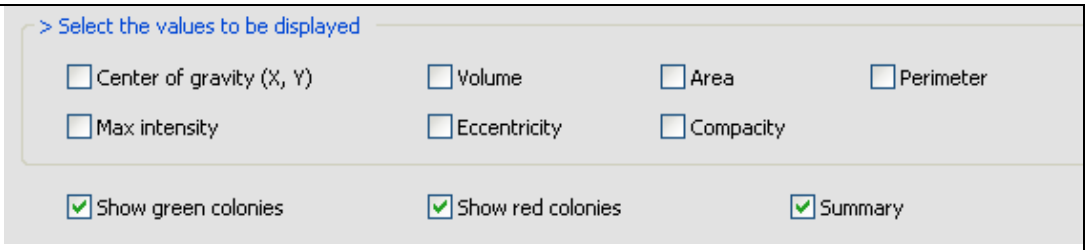
Note: you could also type the grey level range in the detailed green and red parameters:


**RESULT TABLE**

In the result parameter window, you can select the lanes and the values to be displayed in the results tables:

- ⇨ Centre of gravity
- ⇨ Volume
- ⇨ Area
- ⇨ Perimeter
- ⇨ Maximum intensity
- ⇨ Eccentricity
- ⇨ Compacity

To select your display mode, click on the appropriate selection:




- Centre of gravity

⇨ Co-ordinates of the centre of gravity of the detected colony

- Volume

⇨ The volume is the sum of intensities included in the colony area

- Area

⇨ The area is number of pixels which defined the colony

- Perimeter

⇨ The perimeter is the circumference of the colony

- Maximum intensity

⇨ The maximum intensity is the grey level height of the spot.

- Eccentricity

⇨ Smears and artefacts can be eliminated with this coefficient which calculates how stretched is the colony

⇨ Eccentricity = $\frac{\text{Minimum width}}{\text{Maximum length}}$ 0 <eccentricity <1

- Compacity

⇨ The compacity indicates how concentrate is the colony

⇨ Compacity= $\frac{4\,\pi\,\text{Area}}{\text{Perimeter}^2}$ 0 <compacity <1

**NEXT**

The "Next" button validates your parameter and opens the following analysis step.

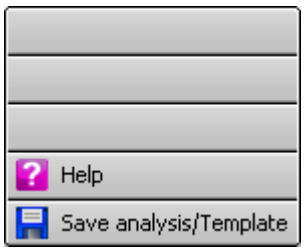
| | | |
|---|---|---|
| Detection | Next >> | Data filtering |

**OPTION FOLDER**

The option folder gathers the following functions:

⇨ Help

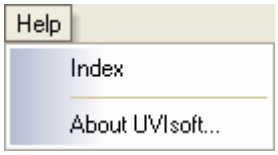
⇨ Save the analysis or the template



## HELP MENU

Click on the “Help” button. You automatically access the user manual at the chapter corresponding to the function


You can access the help file index through the File\Help from the Menu bar




## SAVE ANALYSIS / TEMPLATE

This function saves the current analysis. The analysis file will contain the results, the image and all the parameters defined to obtain the results.

The analysis could also be saved as a template for automated analysis routines. Template offers the user the ability to automate many of the repetitive tasks associated with analysis and processing. As a result, you can spend more time evaluating and analysing results, and less time manipulating set-ups, variables and other settings.

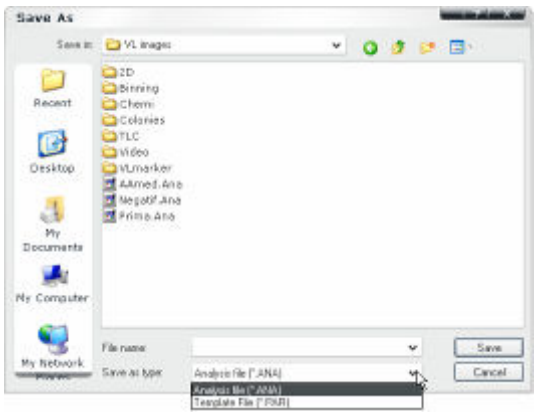
The template automates a task or set of tasks that you perform repeatedly or on a regular basis. It stores all the analysis commands and parameters of an analysis. You can run these parameters with another image whenever you need to perform a new analysis based on the same parameters.

The benefits of the template file are as follows:

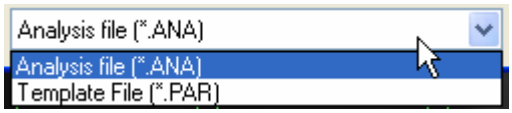
⇨ Time saving
⇨ Reproduction of image analysis parameters
⇨ Templates are modifiable, allowing the user to maintain an original template while modifying it for a slightly different result, with minimal effort

1. Click on the “Save analysis/ Template” button:


2. A pop-up window displays the following menu:

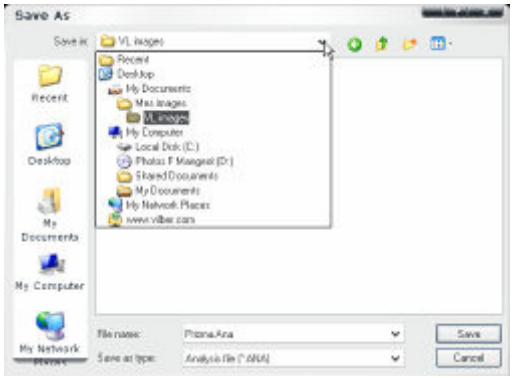


3. Select analysis file or template file:

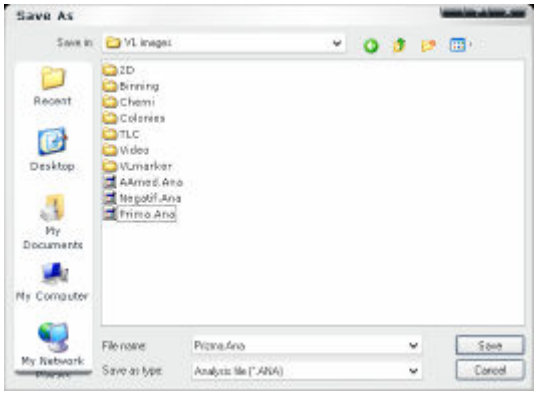



Note: the software proposes Analysis file by default

4. Browse to specify the file directory




5. Enter the desired file name and validate




6. Click on the Save button to create the file.

Note: see “Access to the analysis module” chapter for template or analysis file loading

## ➔ Data filtering

The data filtering summaries the colony detection parameters:


The dashboard details the data filtering parameters:

- ⇨ Volume
- ⇨ Perimeter
- ⇨ Area
- ⇨ Eccentricity
- ⇨ Compacity

**DATA FILTERING PARAMETERS**

You can modify the sensitivity parameters to optimise the band detection. This is particularly useful for instance in the case of several bands not taken into account or if or too much background noise is detected.

- Volume

⇨ The volume is the sum of intensities included in the colony area

- Perimeter

⇨ The perimeter is the circumference of the colony

- Area

⇨ The area is number of pixels which defined the colony

- Eccentricity

⇨ Smears and artefacts can be eliminated with this coefficient which calculates how stretched is the colony

⇨ Eccentricity= $\frac{\text{Minimum width}}{\text{Maximum length}}$ 0 <eccentricity <1

- Compacity

⇨ The compacity indicates how concentrate is the colony

⇨ Compacity= $\frac{4\,\pi\,\text{Area}}{\text{Perimeter}^2}$ 0 <compacity <1

**RESET FILTERING**

The “Reset” button restores the default data filtering parameters.


**NEXT**

The “Next” button validates your parameter and opens the following analysis step.

| | | |
|---|---|---|
| Data filtering | Next >> | Exclusion area |

**BACK**

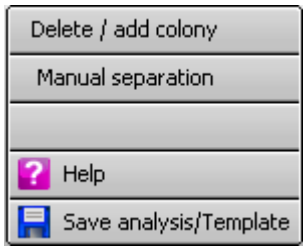
The "Back" button validates your parameter and opens the following analysis step.

| | | |
|---|---|---|
| Data filtering | << Back | Detection |

**OPTION FOLDER**

The option folder gathers the following functions:

- ⇨ Delete / Add colonies
- ⇨ Manual separation
- ⇨ Help
- ⇨ Save the analysis or the template




**DELETE / ADD COLONIES**

This function allows you to discard a specific colony or to recall it.

Note: You can not add a non detected colony

1. Click on the "Delete / Add colony" button.


2. Move the mouse cursor on the image and click on the colony you want to delete. To delete or recall a colony, click on the colony centre. You can delete or recall as many spot as necessary

Note: Click one more time on the "Delete / Add colony" button to exit this function.

**MANUAL SEPARATION**

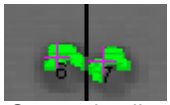
You can separate several colonies that have been detected as a single one.

1. Click on the "Manual separation" button.


2. Move the mouse cursor on the image and click with the left mouse button to define the origin of the separation line. Move the mouse cursor to the end point of the separation line and click with the left button to validate the position.

The number of colonies is then recalculated taking account this separation line.


No separation

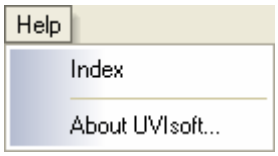

Separation line

Note: Click one more time on the “Manual separation” button to exit this function.

## HELP MENU

Click on the “Help” button. You automatically access the user manual at the chapter corresponding to the function


You can access the help file index through the File\Help from the Menu bar:




## SAVE ANALYSIS / TEMPLATE

This function saves the current analysis. The analysis file will contain the results, the image and all the parameters defined to obtain the results.

The analysis could also be saved as a template for automated analysis routines. Template offers the user the ability to automate many of the repetitive tasks associated with analysis and processing. As a result, you can spend more time evaluating and analysing results, and less time manipulating set-ups, variables and other settings.

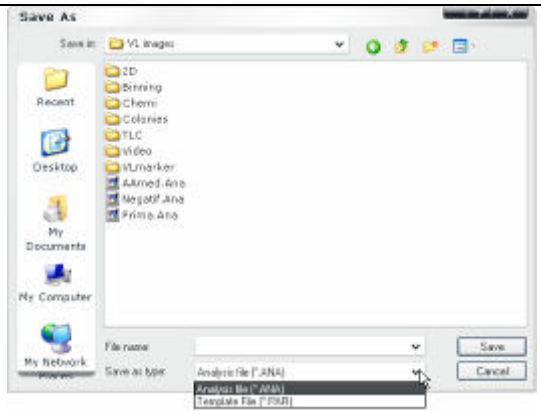
The template automates a task or set of tasks that you perform repeatedly or on a regular basis. It stores all the analysis commands and parameters of an analysis. You can run these parameters with another image whenever you need to perform a new analysis based on the same parameters.

The benefits of the template file are as follows:

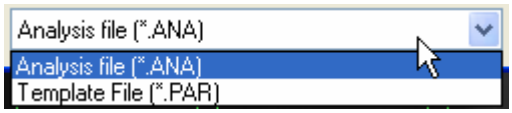
⇨ Time saving
⇨ Reproduction of image analysis parameters
⇨ Templates are modifiable, allowing the user to maintain an original template while modifying it for a slightly different result, with minimal effort

1. Click on the “Save analysis/ Template” button:


2. A pop-up window displays the following menu:

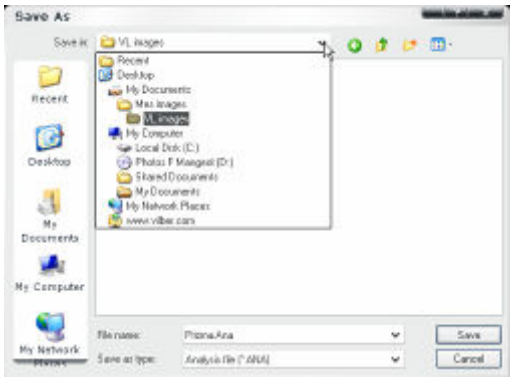



3. Select analysis file or template file:

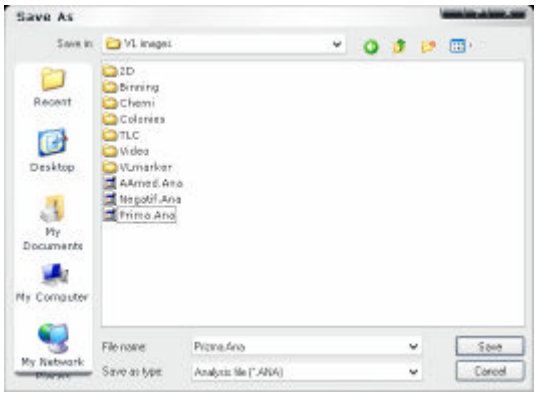



Note: the software proposes Analysis file by default

4. Browse to specify the file directory




5. Enter the desired file name and validate




6. Click on the Save button to create the file.

Note: see “Access to the analysis module” chapter for template or analysis file loading

## ➔ Exclusion area

The exclusion area feature allows you to define contaminated areas to be excluded from the counting. No colony will be counted in these defined areas.


The dashboard details the lane definition parameters:

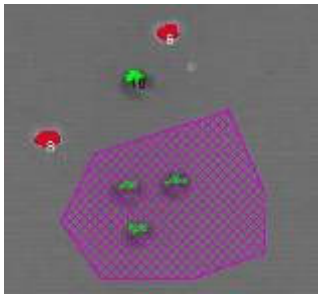
- ⇨ Add an exclusion area
- ⇨ Delete an exclusion area
- ⇨ Reset the exclusion areas

**ADD AN EXCLUSION AREA**

Select the Add exclusion function:

Add exclusion area

To define an exclusion area, click on the first point of the area on the image. Move the cursor to define one edge of the area. Validate this edge by clicking once. Then, repeats this procedure as many times as necessary.



Click on the “Validate” button to define the area or on “Abort” to cancel the definition.

Validate definition

The colonies number is automatically recalculated.

Note: you can define as many exclusion areas as necessary.

**DELETE AN EXCLUSION AREA**

The “Delete area” button cancels the defined exclusion area. To proceed, click on the exclusion area you want to delete.

Delete area

**RESET THE EXCLUSION AREA**

The “Reset area(s)” button restores the original area of analysis and cancels all the defined exclusion area.


**BACK**

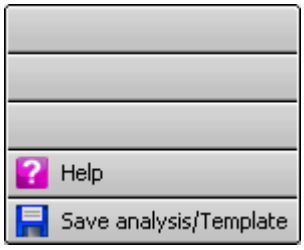
The “Back” button validates your parameter and opens the following analysis step.

| | | |
|---|---|---|
| Exclusion area | << Back | Data filtering |

**OPTION FOLDER**

The option folder gathers the following functions:

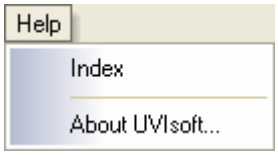
- ⇨ Help
- ⇨ Save the analysis or the template




**HELP MENU**

Click on the “Help” button. You automatically access the user manual at the chapter corresponding to the function:


You can access the help file index through the File\Help from the Menu bar:




**SAVE ANALYSIS / TEMPLATE**

This function saves the current analysis. The analysis file will contain the results, the image and all the parameters defined to obtain the results.

The analysis could also be saved as a template for automated analysis routines. Template offers the user the ability to automate many of the repetitive tasks associated with analysis and processing. As a result, you can spend more time evaluating and analysing results, and less time manipulating set-ups, variables and other settings.

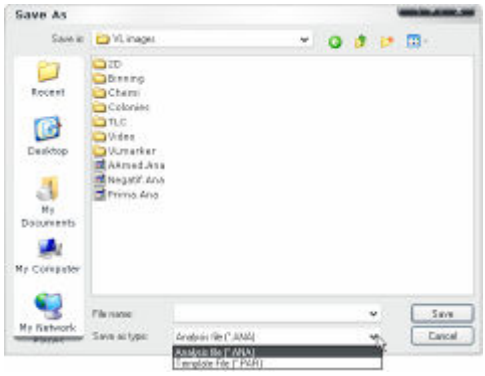
The template automates a task or set of tasks that you perform repeatedly or on a regular basis. It stores all the analysis commands and parameters of an analysis. You can run these parameters with another image whenever you need to perform a new analysis based on the same parameters.

The benefits of the template file are as follows:

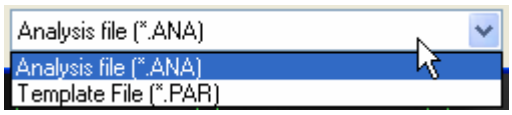
⇨ Time saving
⇨ Reproduction of image analysis parameters
⇨ Templates are modifiable, allowing the user to maintain an original template while modifying it for a slightly different result, with minimal effort

1. Click on the “Save analysis/ Template” button:


2. A pop-up window displays the following menu:

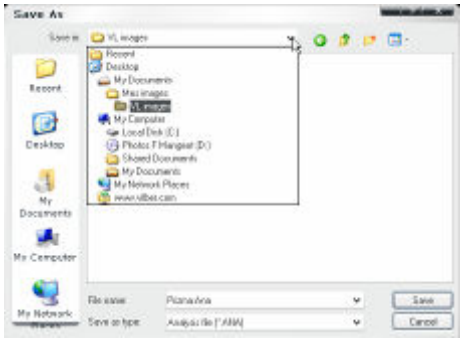



3. Select analysis file or template file:

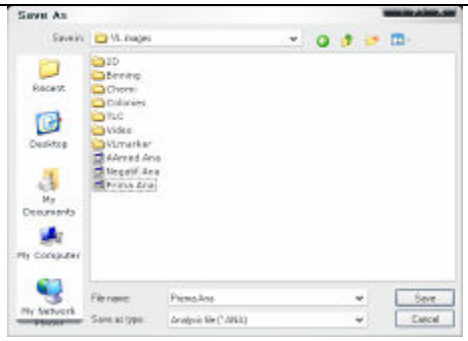



Note: the software proposes Analysis file by default

4. Browse to specify the file directory




5. Enter the desired file name and validate



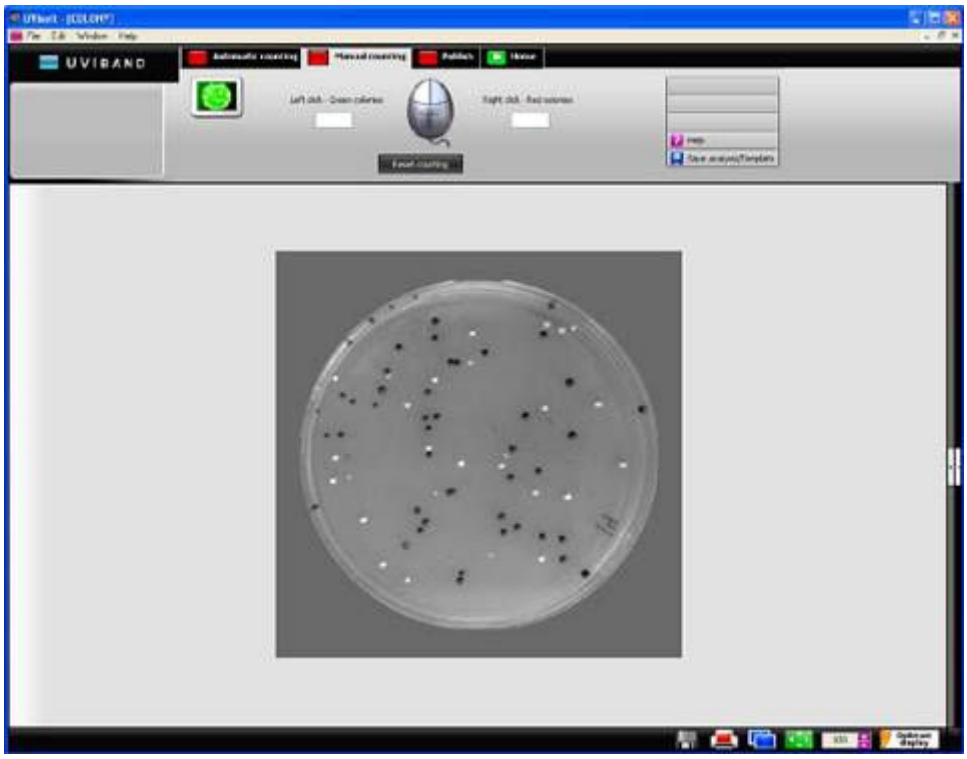

6. Click on the Save button to create the file.

Note: see “Access to the analysis module” chapter for template or analysis file loading

# Manual counting

## ➔ Manual counting

The manual Colony counting opens on this screen:




The dashboard details the manual counting parameters:


⇨ Left click for green type colonies
⇨ Right click for red type colonies
⇨ Reset counting

**LEFT CLICK FOR GREEN TYPE COLONIES**

Click on the left mouse button to count a green type colony. The colony is then added to the green type counter:

47

Note: you can cancel a counted colony by clicking once again with the left mouse button.

**RIGHT CLICK FOR RED TYPE COLONIES**

Click on the right mouse button to count a red type colony. The colony is then added to the red type counter:


Note: you can cancel a counted colony by clicking once again with the right mouse button.

**RESET COUNTING**

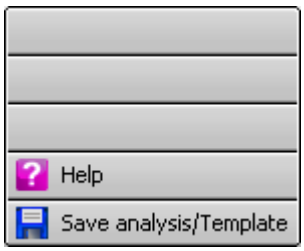
The “Reset” button restores the counters to zero.


**OPTION FOLDER**

The option folder gathers the following functions:

⇨ Help

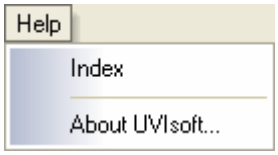
⇨ Save the analysis or the template




**HELP MENU**

Click on the “Help” button. You automatically access the user manual at the chapter corresponding to the function


You can access the help file index through the File\Help from the Menu bar:




**SAVE ANALYSIS / TEMPLATE**

This function saves the current analysis. The analysis file will contain the results, the image and all the parameters defined to obtain the results.

The analysis could also be saved as a template for automated analysis routines. Template offers the user the ability to automate many of the repetitive tasks associated with analysis and processing. As a result, you can spend more time evaluating and analysing results, and less time manipulating set-ups, variables and other settings.

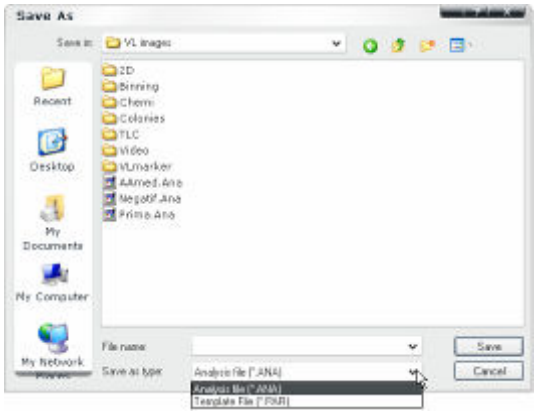
The template automates a task or set of tasks that you perform repeatedly or on a regular basis. It stores all the analysis commands and parameters of an analysis. You can run these parameters with another image whenever you need to perform a new analysis based on the same parameters.

The benefits of the template file are as follows:

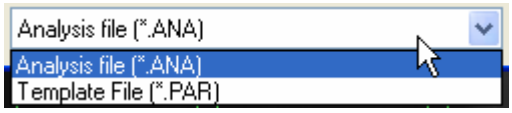
⇨ Time saving
⇨ Reproduction of image analysis parameters
⇨ Templates are modifiable, allowing the user to maintain an original template while modifying it for a slightly different result, with minimal effort

1. Click on the “Save analysis/ Template” button:


2. A pop-up window displays the following menu:

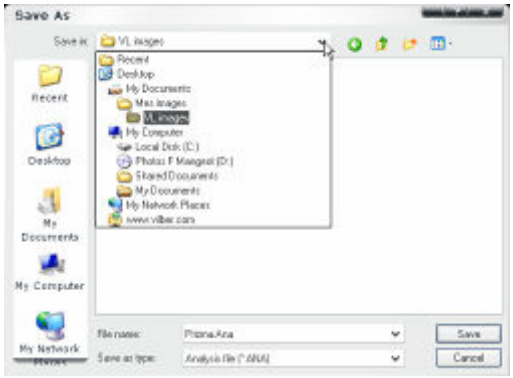



3. Select analysis file or template file:

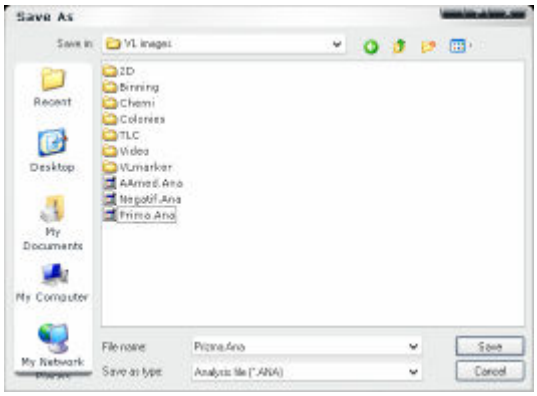



Note: the software proposes Analysis file by default

4. Browse to specify the file directory




5. Enter the desired file name and validate




6. Click on the Save button to create the file.

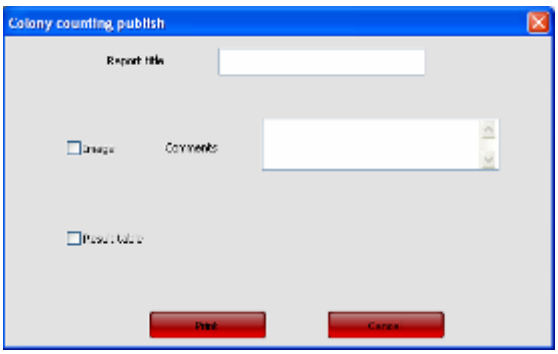
Note: see “Access to the analysis module” chapter for template or analysis file loading

# Publish

## ➔ Introduction

The purpose of the Publish function is to prepare a printed report of your results. You can easily organise your report with titles and comments and your own selection of data to be published among the following:
- Sample image
- Quantification result table

1. To proceed, select the Publish tab. A pop-up window displays the following menu:




⇨ Enter a report title if any
⇨ Select the options to be printed
⇨ Add comments or not per option

2. Click on the “Print” button. A pop-up window displays the following menu


⇨ Select a printer
⇨ If necessary, click on Properties to modify the default setting of the printer,
⇨ Select the number of copies
⇨ Click on OK to validate your options

# Return to Home

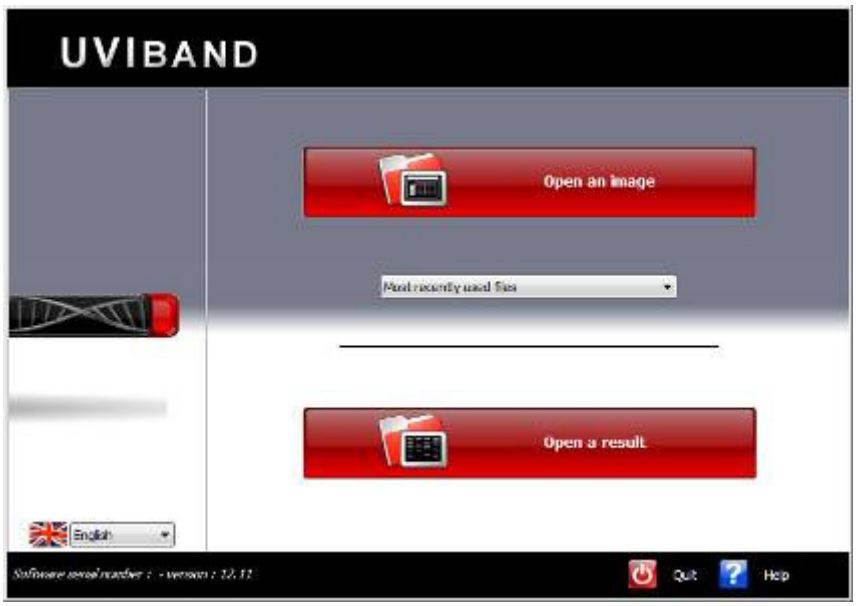
## ➔ Introduction

The home dashboard is the hub to other functions of the software:

- ⇨ Open another image file or another result file
- ⇨ Select another analysis module
- ⇨ Exit the software


## ➔ Load another image

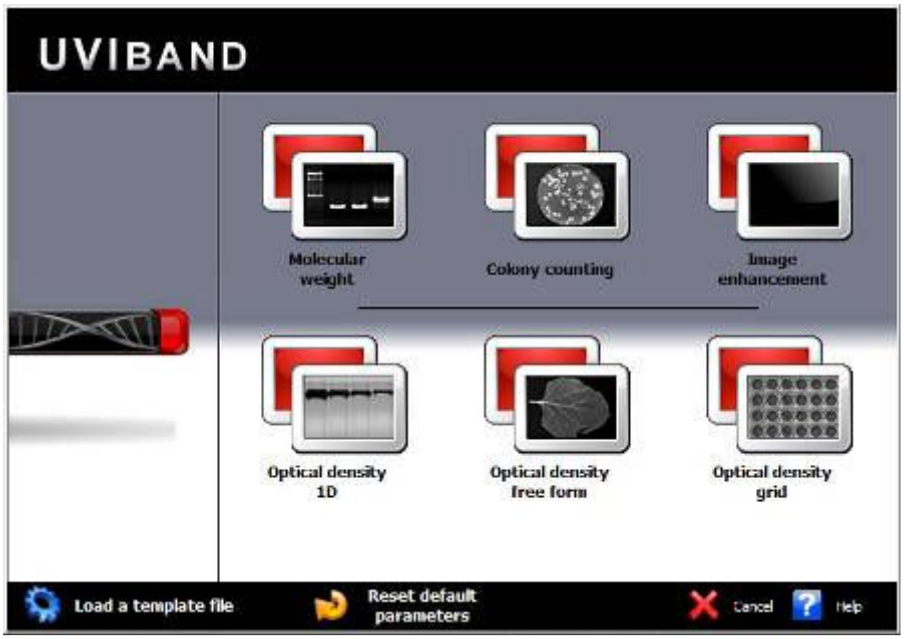
To return to the main menu, click on the home icon. A new menu appears with the main menu task bar functions:




- ⇨ Click on the “Open an image” icon to open an image
- ⇨ Click on the “Open a result” icon to open a previously saved analysis result

## ➔ Select another function

To return to the analysis menu, click on the analysis icon. A new menu appears with the analysis module task bar functions:



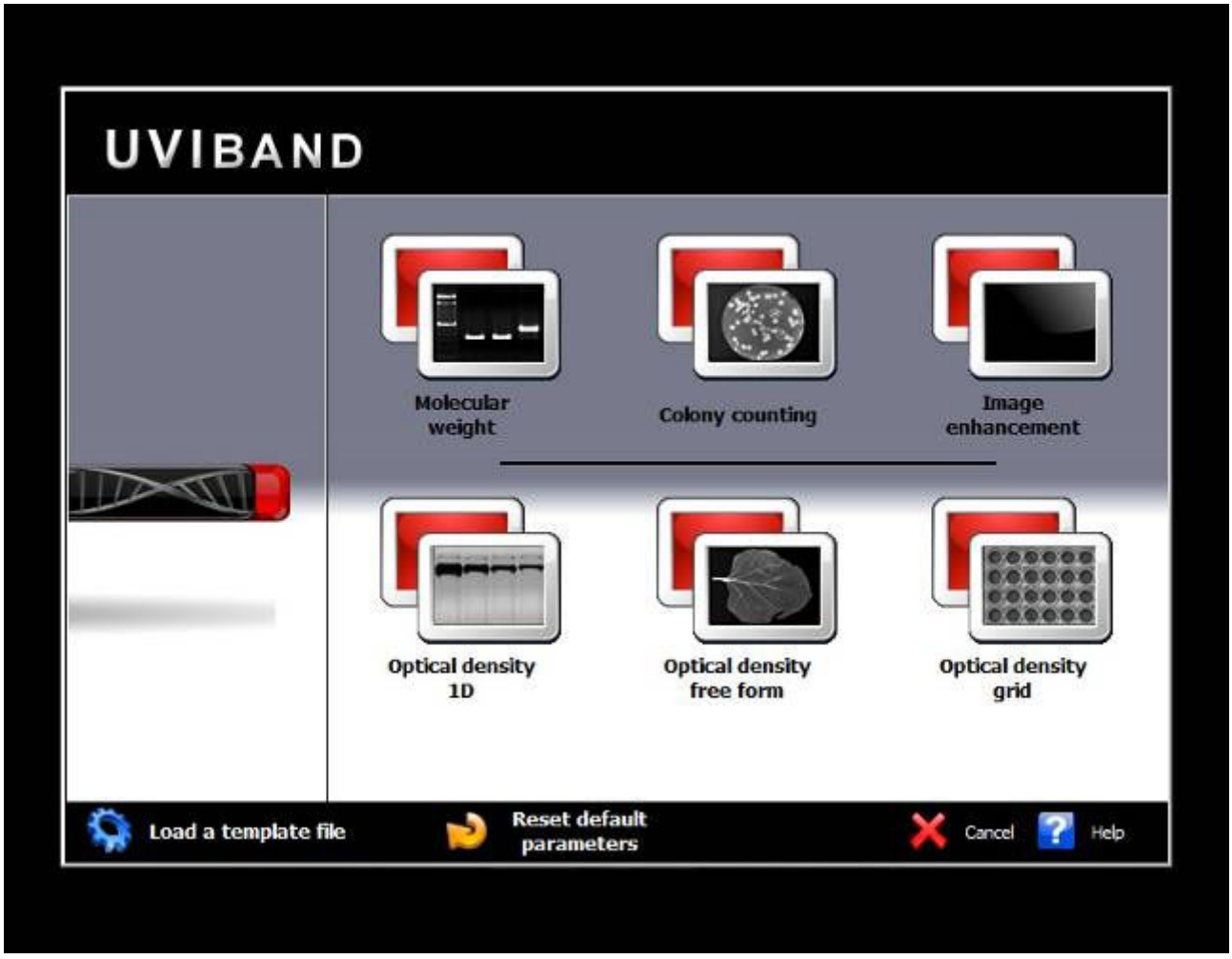

Click on the appropriate icon to select an analysis module.

- ⇨ Select the Molecular weight icon to open the molecular weight analysis (MW) module
- ⇨ Select the Colony counting icon to open the colony counting (CC) analysis module
- ⇨ Select the Optical density - 1D icon to open the optical density (OD) analysis module based on a 1D detection
- ⇨ Select the Optical density - Free form icon to open the optical density (OD) analysis module based on a free form detection
- ⇨ Select the Optical density - Grid icon to open the optical density (OD) analysis module based on a grid detection
- ⇨ Select the Image enhancement icon to open the image enhancement module

## ➔ Exit the software

To close UVIband Advanced, select Exit from the File menu.

You will be prompted to save your analysis.




# Image enhancement
## ➔ Image enhancement module

# Image enhancement introduction

## ➔ Objectives and output

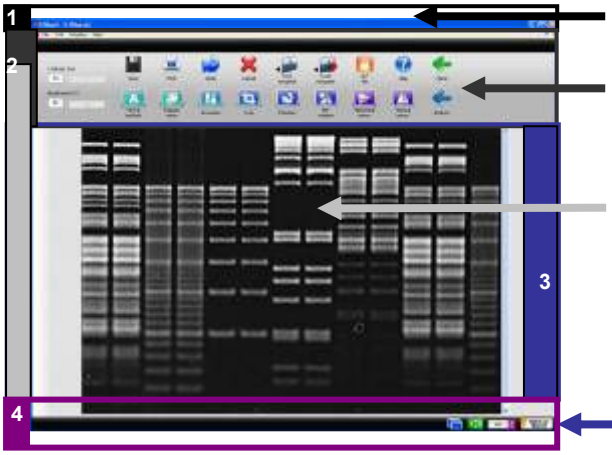

The UVIband Advanced Image Enhancement module includes features to enable editing of comments, inversion, contrast, brightest adjustments and colorimetric.

The image enhancement features are as follows:
- Text & symbols editing
- Pseudo colours imaging
- Image inversion
- Image cropping
- Rotation
- Mirroring
- Contrast/ Brightness adjustment
- Good Laboratory Practice management
- Image enhancement template management

## ➔ Image Enhancement module (IE) operating environment

The image enhancement module opens on the following window:

The UVIband Advanced Image Enhancement operating environment is organised into four areas:




1. The menu bar
2. The dash board
3. The image window
4. The toolbar

The menu bar contains the following menu:

- ⇨ File
- ⇨ Edit
- ⇨ Font
- ⇨ Windows
- ⇨ Help


The dash board contains the image enhancement features:

The image window displays the active image:

It also contains the image toolbar: 


 ⇨ Copy to clipboard

 ⇨ Autoscale

⇨ Zoom in or out the image

⇨ Change the optimum display

## ➔ Toolbar in details

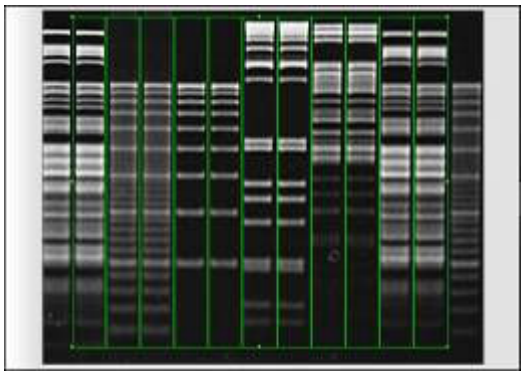
**Copy to clipboard**
This function copies an image, a table or a graph onto the clipboard for insertion into another program. This option is identical to the Windows® [Ctrl C] command.

To proceed, click on the Copy to clipboard icon. The image, the table or the graph is now ready to be pasted into another application.

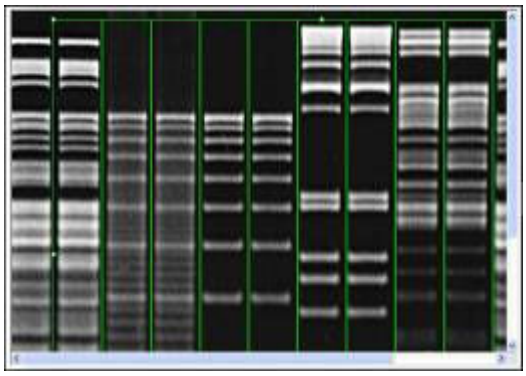
Open the application that you want to paste the image into, and select from the available pasting options ([Ctrl V] command for Windows® software).

**Auto-scale**
Click on the “Auto-scale” to resize the image to fit the size of the monitor.

The Auto-scale feature proportions the display of the image to the screen resolution.



Auto-scale (no scroll bar)



No auto-scale (scroll bar)


**Optimum display (for 12, 14 and 16-bit image file)**

The optimum display window is helpful to modify the greyscale selection to enhance the image display: To proceed, click on the “Optimum display” icon. A pop-up window displays the following menu:

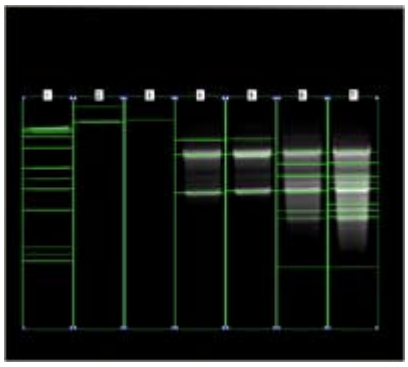
Some images has a 12, 14 or 16-bit format and Windows® can only display 8-bit images (256 grey levels).

Due to this limitation, the UVIband Advanced software handles two images:
⇨ A “memory” image corresponding to the 12, 14 or 16-bit format (4 096, 16 384 or 65 536 grey levels)
⇨ A “display image” corresponding to the image displayed on the screen (256 grey levels)

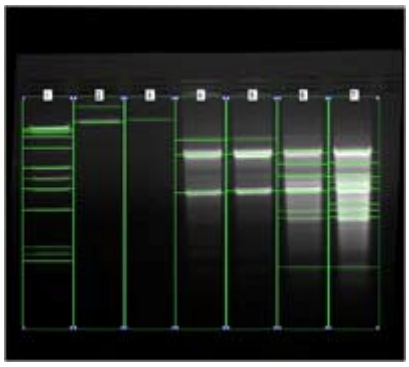
The easiest way to calculate the “display image” would be to translate the full grey scale each time an image is acquired: the x grey levels values of the “memory” image corresponds to 256 values in the displayed image. In that case, it won’t be possible to visualise faint spots on a dark image.

UVIband Advanced offers the possibility to select the grey level range to translate for the display image calculation. All the grey levels under the “Min value” defined will be converted to 0 (Black) in the displayed image. All the grey levels upper the “Max Value” defined will be set to 255 (White) in the displayed image. The grey levels between those two limits will be converted in an intermediate grey level value following a linear rule.

For both values, you can:
⇨ Edit the value in the corresponding field
⇨ Select the value by dragging and dropping the arrow
⇨ Click on the “optimum display” button: UVIband Advanced will then calculate the ideal values to be selected according to the parameters defined



Automatic optimum display



Optimum display enhancement
The image appears brighter. The faint bands are more visible.

Note: The optimum display has no impact on the analysis. Only the display of the image is modified.

# Image enhancement functions

## ➔ Introduction

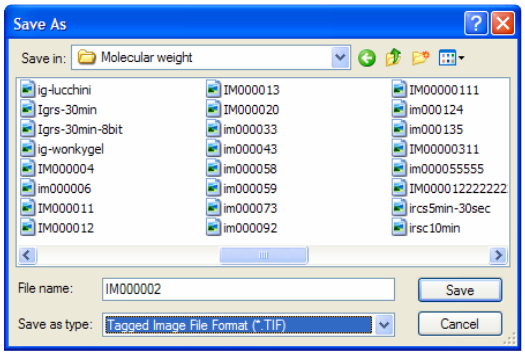
The dashboard contains all the image enhancement functions:

- ⇨ Save
- ⇨ Print
- ⇨ Undo
- ⇨ Cancel
- ⇨ Save template
- ⇨ Load template
- ⇨ GLP file
- ⇨ Help
- ⇨ Home
- ⇨ Text and symbols
- ⇨ Pseudo colours
- ⇨ Inversion
- ⇨ Crop
- ⇨ Rotation
- ⇨ 90° rotation
- ⇨ Horizontal mirror
- ⇨ Vertical mirror
- ⇨ Analyse
- ⇨ Contrast & brightness

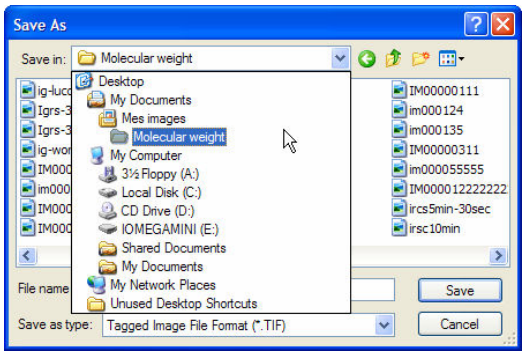
## ➔ Save

This function saves a previously unsaved image to a new file, or update the changes to an existing image file, or save an image to a new file or file location. You can either save the displayed image (8-bit format) or the complete ( 8, 12, 14, 16-bit image).

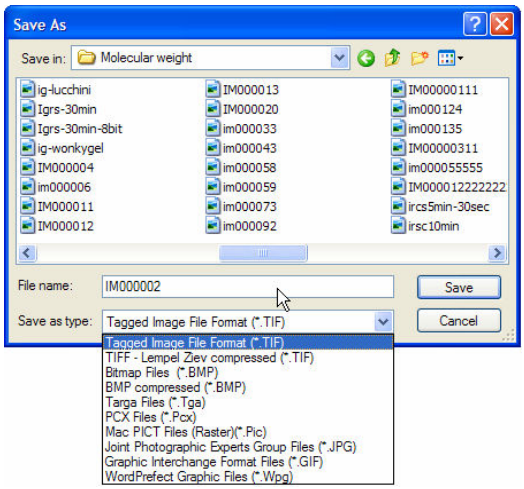
1. Click on the “Save” icon. A pop-up window displays the following menu:




2. Browse to specify the image directory



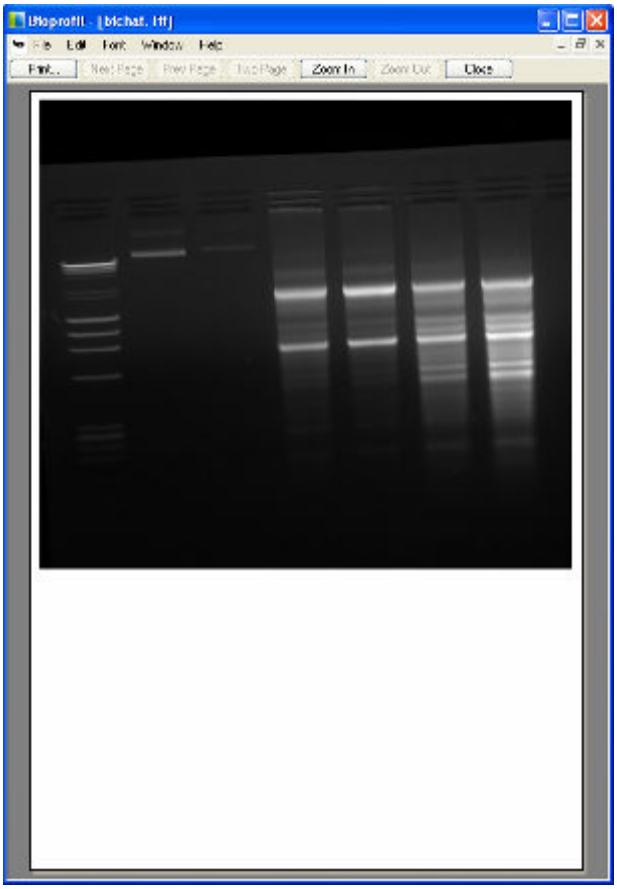

3. Enter the desired file name, select a file extension and validate



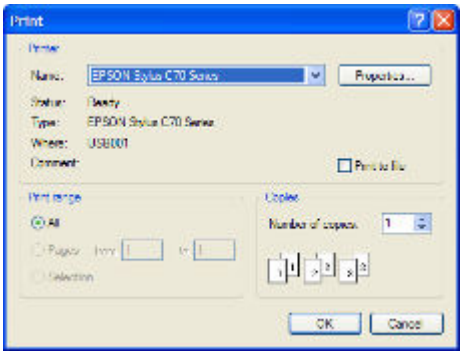
## ➔ Print

This function prints a previously opened image as it appears in the image window.

1. Click on the “Print” icon. The Print preview displays a preview of the image as it will be printed:

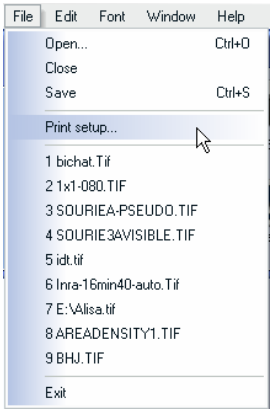



2. Click on the “Print” button. A pop-up window displays the following menu:

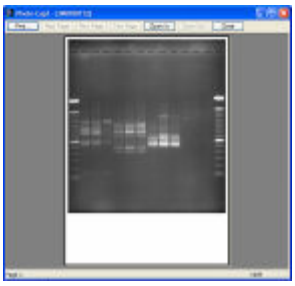



⇨ Select a printer
⇨ If necessary, click on Properties to modify the default setting of the printer,
⇨ Select the number of copies
⇨ Click on OK to validate your options

Note: You can also access the Print menu from the Menu bar (File\Print). To proceed, select File\Print Preview from the Menu bar:

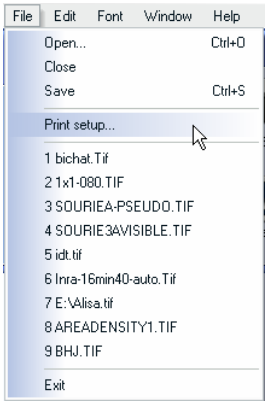



A pop-up window displays the print preview:

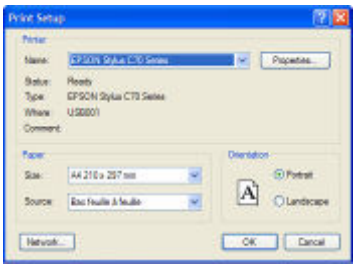


⇨ Click on Print to print as previewed
⇨ Click on Close to close the Print preview and to go back to the main menu

3. The Print Setup allows you to choose a printer and to configure the printing. To proceed, select File\Print Setup from the Menu bar:




A pop-up window displays the print setup menu:



| | |
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| | ⇨ Select a printer<br>⇨ Click on Properties to modify the default setting of the printer, if necessary<br>⇨ Select the paper size and source; select the orientation<br>⇨ Click on OK to validate your options<br><br>Note: After you have installed and setup your printer, the procedure for setting up and configuring a printer is the same as in other Windows program. |

## ➔ Undo

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|  | The undo function undoes the last image enhancement modification you made. The Undo option allows several levels of edits. In other words, you can undo step by step the preceding edit.<br><br>To Undo an action, click on the undo icon. The list of the last editing actions is displayed:<br><br>  <br><br><br>Select from this list, the actions you want to undo. The Undo applies automatically on the image. |

## ➔ Cancel

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|  | Click on the “Cancel image change(s)” icon to undo all previous image treatments. The original image is then displayed without all the further modifications. |

## ➔ Save template

The template automates a task or set of tasks that you perform repeatedly or on a regular basis. It stores all the image enhancement parameters as well as the analysis set-up. You can run these parameters with another image whenever you need to perform a new analysis based on the same parameters.

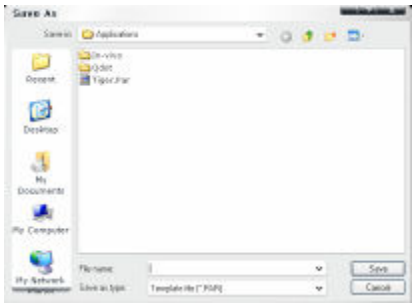
The benefits of the template file are as follows:

⇨ Time saving

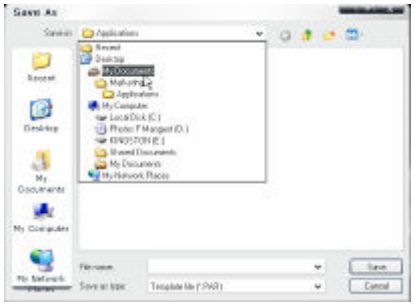
⇨ Reproduction of image analysis parameters

⇨ Templates are modifiable, allowing the user to maintain an original template while modifying it for a slightly different result, with minimal effort

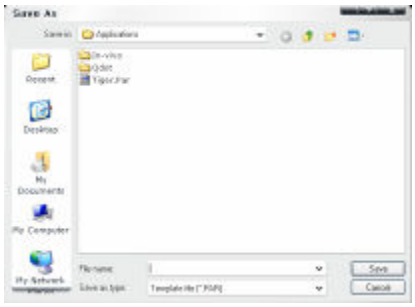
1. Click on the “Save template” icon. A pop-up window displays the following menu:



2. Browse to specify the file directory



3. Enter the desired file name and validate

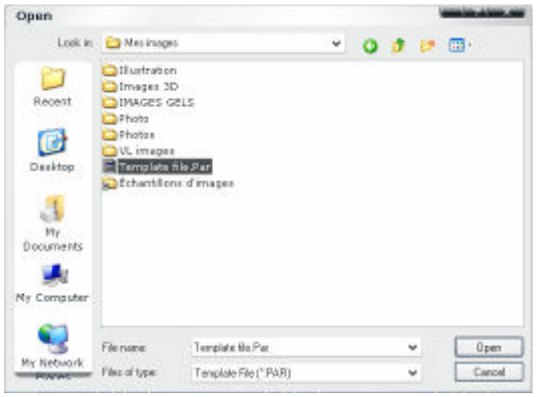


4. Click on the Save button to create the file.

## ➔ Load template

To load a template, select the “Load a template file” icon:

A pop-up window displays the following menu:

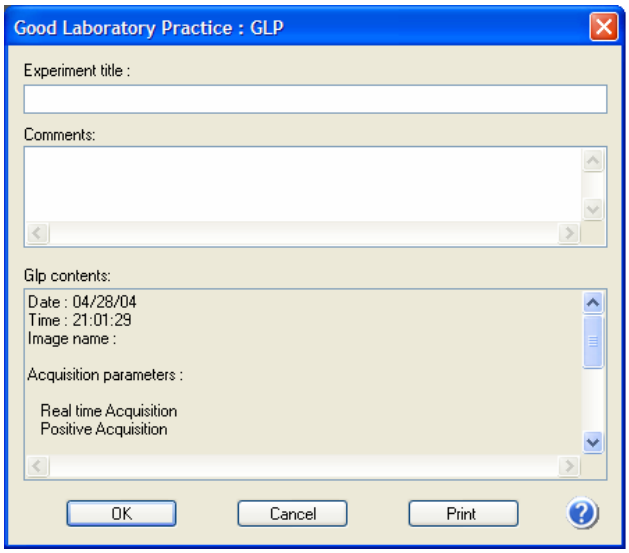



⇨ Browse to specify the template directory
⇨ Double click on the template name you want to load

Note 1: Please refer to the “How to use a template file” part of this manual for more details on the template.

## ➔ GLP file

The Good Laboratory Practice (GLP) file is made to track all the image treatments performed with the software. Click on the “GLP” icon. A pop-up window displays the following menu:



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| | ⇨ Enter the experiment title and comments.<br>⇨ All other image acquisition parameters (Real time acquisition or integration time, positive or negative image) are recorded.<br>⇨ Treatments on frozen images (contrast, brightness, inversion) are also recorded. |

## ➔ Help

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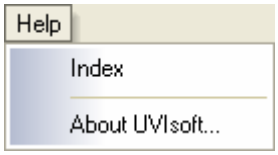
|  | The Help menu contains information about the UVIband Advanced software and enables you to access the on-line help system.<br><br>  <br><br><br>Click on the “Help” button. You automatically access the user manual at the chapter corresponding to the function<br><br>You can access the help file index through the File\Help from the Menu bar:<br><br>  <br> |

## ➔ Home

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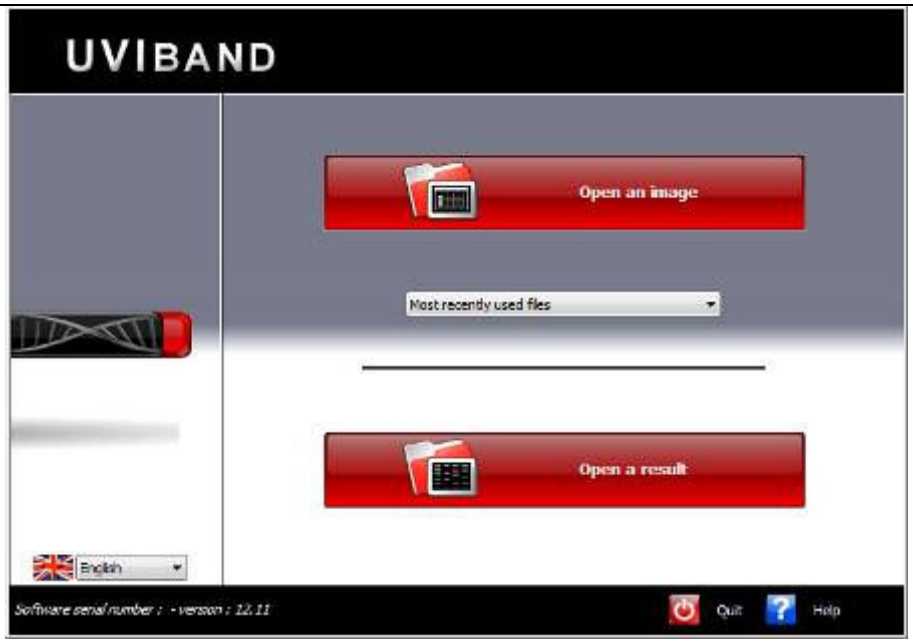
|  | To return to the main menu, click on the home icon. A new menu appears with the main menu task bar functions: |



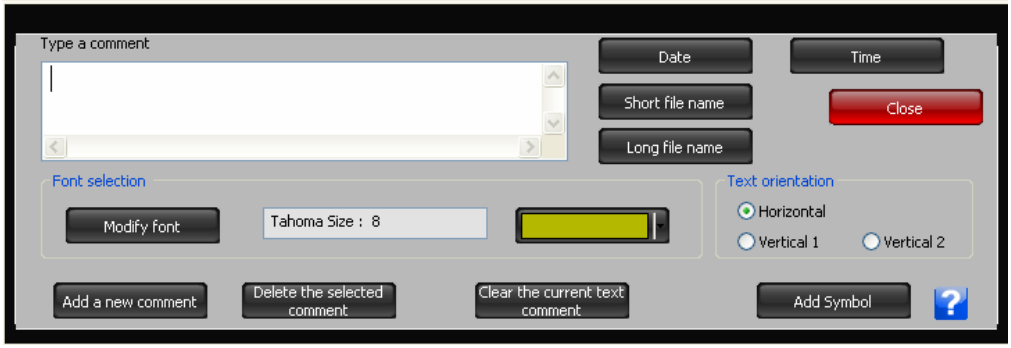

## ➔ Text and symbols

Image annotation is useful when you want to add information directly on your image or when you want to prepare formal presentation. With the UVIband Advanced software, you can:

⇨ Add text to an image.

⇨ Use symbols such as an arrow, line, rectangle or any symbols defined in a font, to emphasise a particular area in an image.

1. To annotate an image, click on the “Text and symbols” icon. A pop-up window displays the following menu:




2. Enter the text in the text editor window

⇨ Select the font

⇨ Select the font size

⇨ Select the font colour

⇨ Click on OK to validate

You can insert symbol by clicking on the Symbol button. You can also add the following items to the image:

⇨ Date. Add the current date to the image. This date defaults to the date set on

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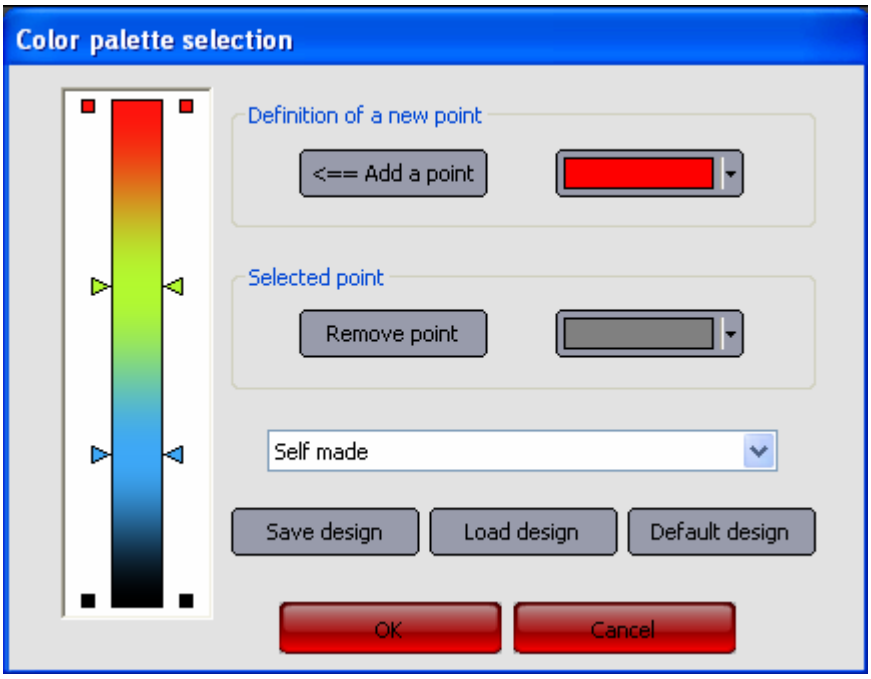
| | the computer you are using.<br>⇨ Time. Add the current time to the image. This time defaults to the time set on the computer you are using.<br>⇨ Full image name. Add the image title to the image. The title defaults to the file name and location of the opened image.<br>⇨ Short image name. Add the image title to the image. The title defaults to the file name of the opened image. |

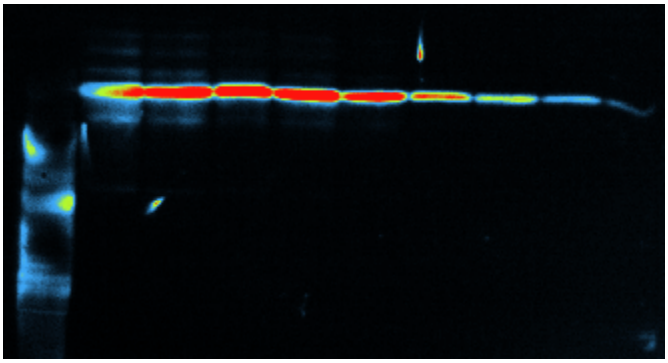
## ➔ Pseudo colours

The pseudo colours can display different types or levels of fluorescence in an image. It replaces the original grey levels of the image by another palette colour. To proceed, click on the Pseudo-colour icon. A pop-up window displays the following menu in which you can adjust the colours of the image:

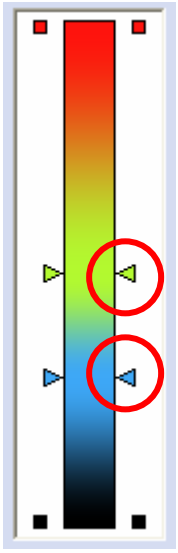



The image is then displayed with the default pseudo-colours settings.

For instance, the image could be as followed:



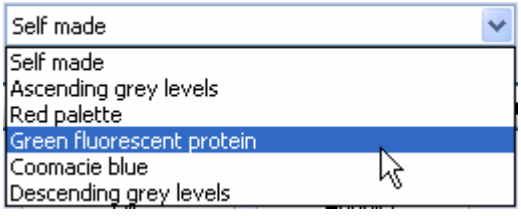
**Colours adjustments**
For the bicolour selection, click on the arrow to define the value of the colour you want to modify. While keeping the mouse button pressed, move the arrow to its new value. Release the mouse button when value is satisfactory, the image is automatically updated. You can repeat these operations as many time as necessary for all pseudo colours.



**Add or remove a colour**
Click on Add a point to add a colour on the pseudo colours list. Select the colour from the Add a point palette:


Select the point to remove and click on Remove point to remove a colour from the pseudo colours list.

**Default, predefined and user defined palette design**
The UVIband Advanced software has several predefined palette designs. Select your palette design from the design list:

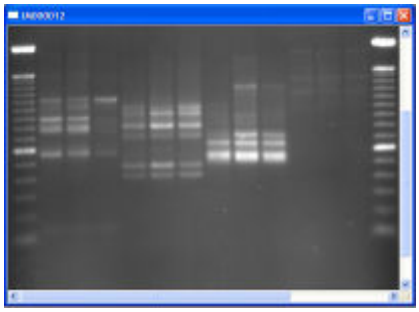


You can also save and load your own palette design. Define the set of colours you want to apply and click on Save to save the palette design. Click on Load to open your palette design.

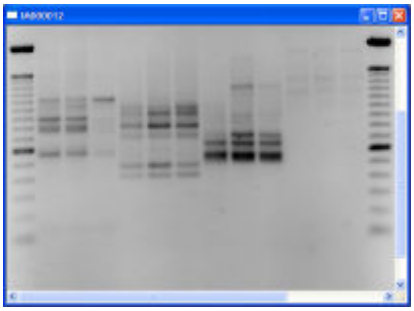

To come back to the default design, click on the default design button:


## ➔ Inversion

Click on the “Inversion” to inverse the grey level of the image. This makes a negative image.



**Before**

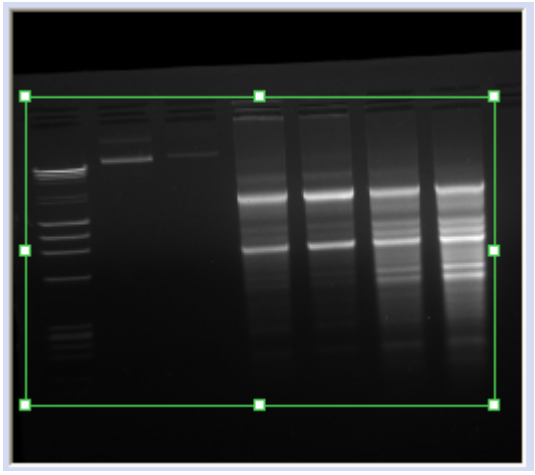


**After**

## ➔ Crop

The Crop function cuts out a portion of an image. You can crop an image to better reflect the area of interest. After cropping, only the selected area is part of the image. The other part is discarded from the image.

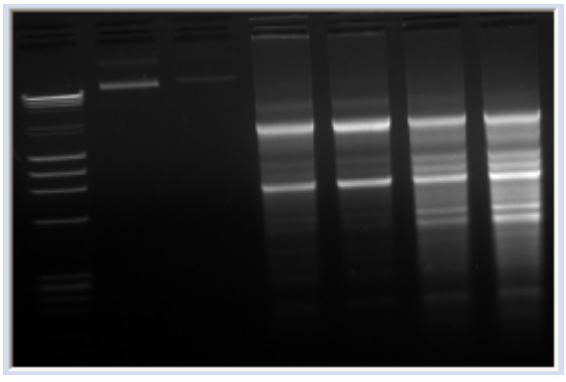
To crop an image, click on the Crop icon. A green window overlays the image:



Press and hold the left mouse button on the corner square. Drag the cursor over the image until the dotted rectangle covers the area of the image that you want to crop.

Click on the Crop button to crop according to your area of interest:


A new image is opened with the cropped area:

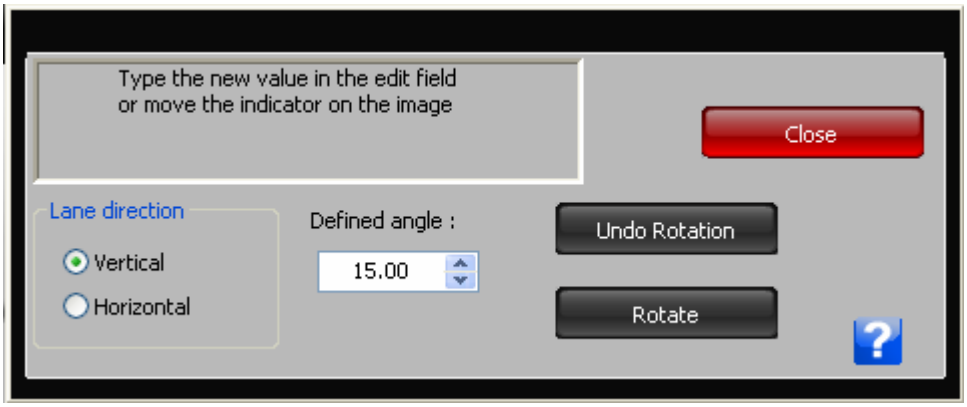


Note: this image is a new unsaved image. You need to save this image.

Note: The former image is still opened.

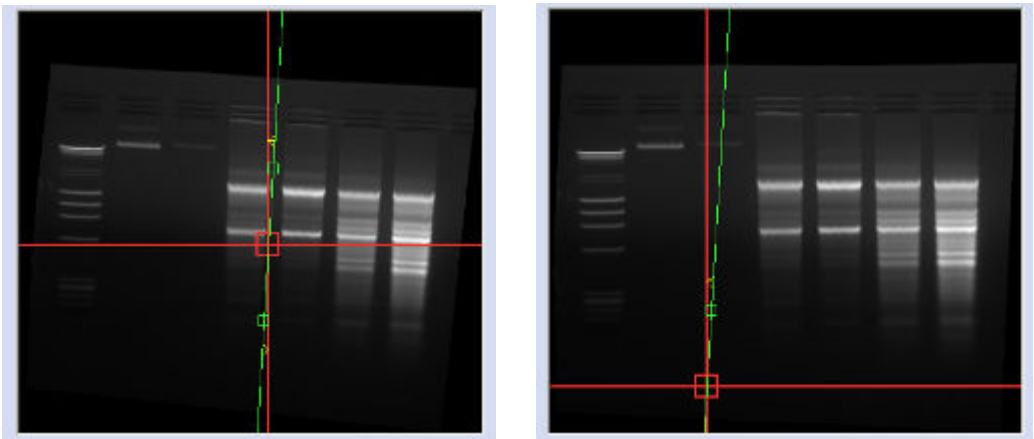
## ➔ Rotation

Use the Rotation function to rotate the image in increments other than 90°. Click on the rotation icon. A pop-up window display the following menu:




You can also define the angle of rotation in degrees. To complete the rotation, click on the Rotate button. Click on the Undo button to come back as previously.

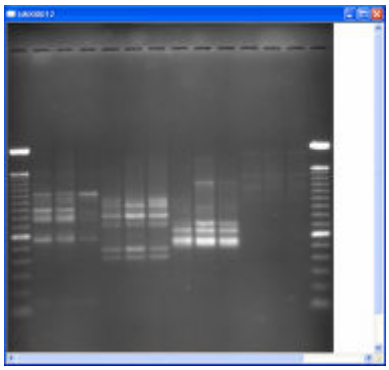
Thanks to the axis, which appears on the image, you can also directly rotate the image using this overlay. To perform the rotation, position the cursor on the green square and drag in the yellow arrow direction. As you drag, the arrow will rotate and the angle in the box will change. Adjust the green lane to be as parallel as possible as the lanes. To complete the rotation, click on the Rotate button.



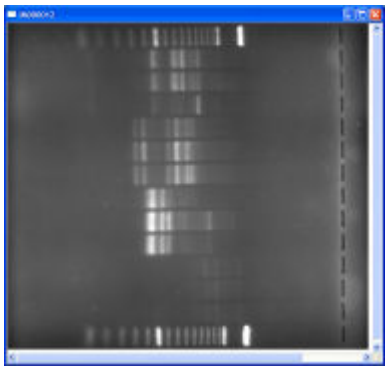
**Adjust the green line to be as parallel as possible from the lane. Then, clock on Rotate.**

## ➔ 90° rotation

Click on the “90° Rotation” icon to rotate right the image. The image is rotated clockwise in 90° increments.



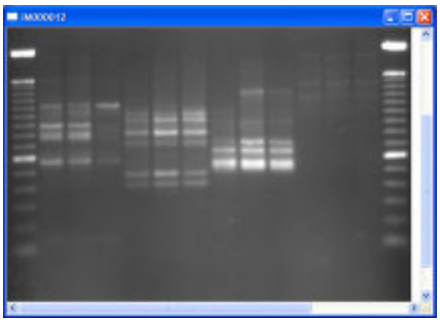
**Before**



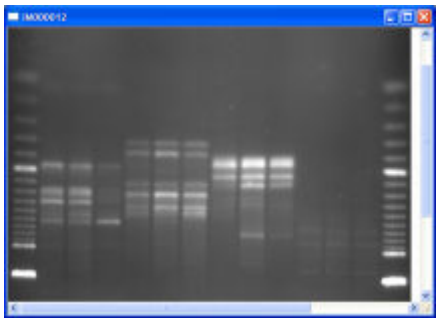
**After**

## ➔ Horizontal mirror

Click on the “Horizontal mirror” icon to flip the image from top to bottom.



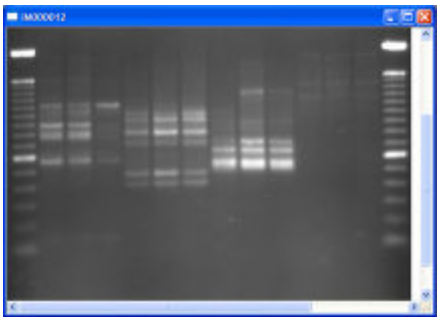
**Before**



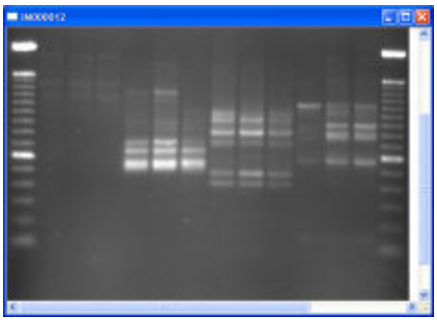
**After**

## ➔ Vertical mirror

Click on the “Vertical mirror” icon to flip the image from right to left.



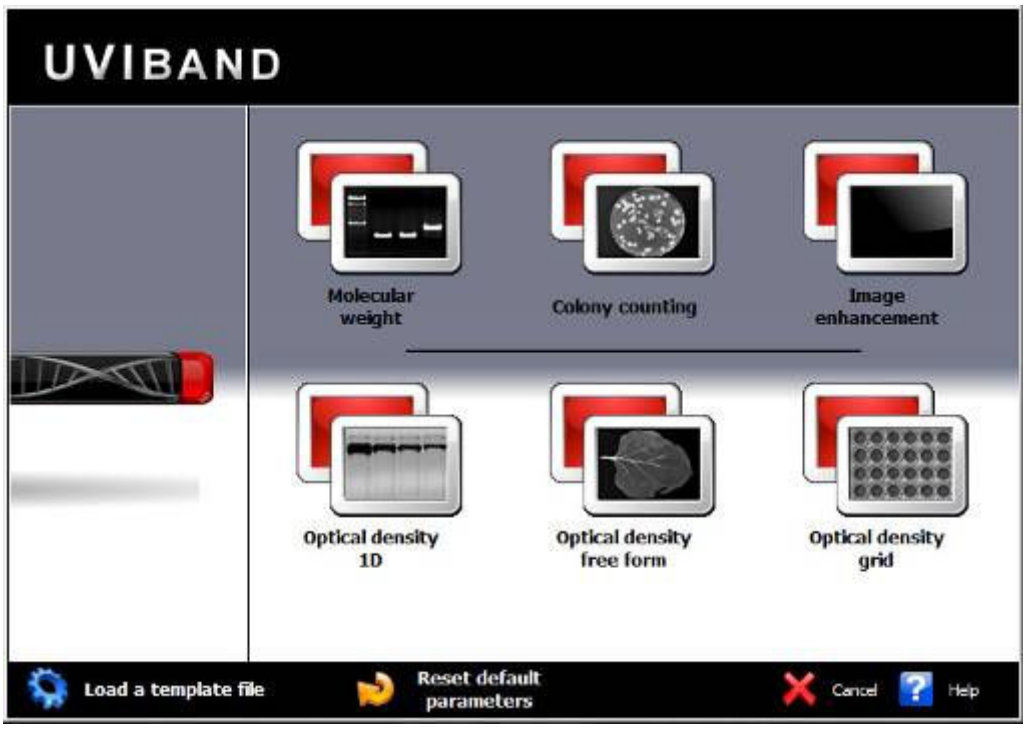
Before



After

## ➔ Analyse

To return to the analysis menu, click on the analysis icon. A new menu appears with the analysis module task bar functions:

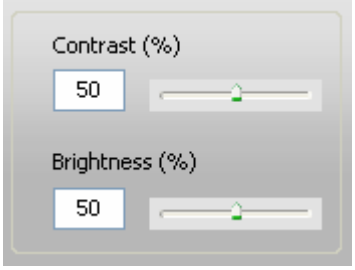


## ➔ Contrast and brightness

You can adjust the contrast or the brightness.

⇨ The contrast exaggerates or subdues the difference between the bright and the dark area of an entire image;

⇨ The brightness lightens or darkens an entire image.

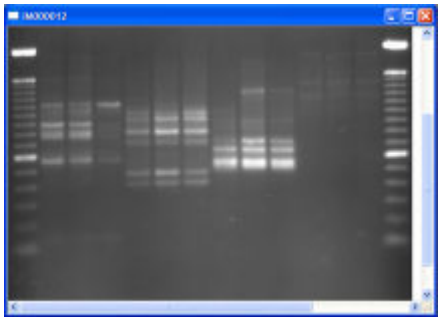
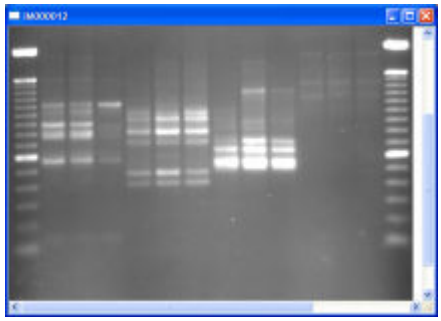
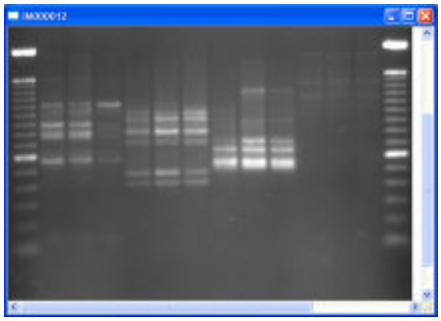
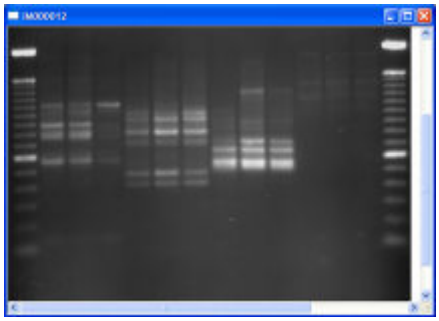



1. Specify the percentage to add or remove from the image.

⇨ Increase the brightness or the contrast by defining a value above 50%

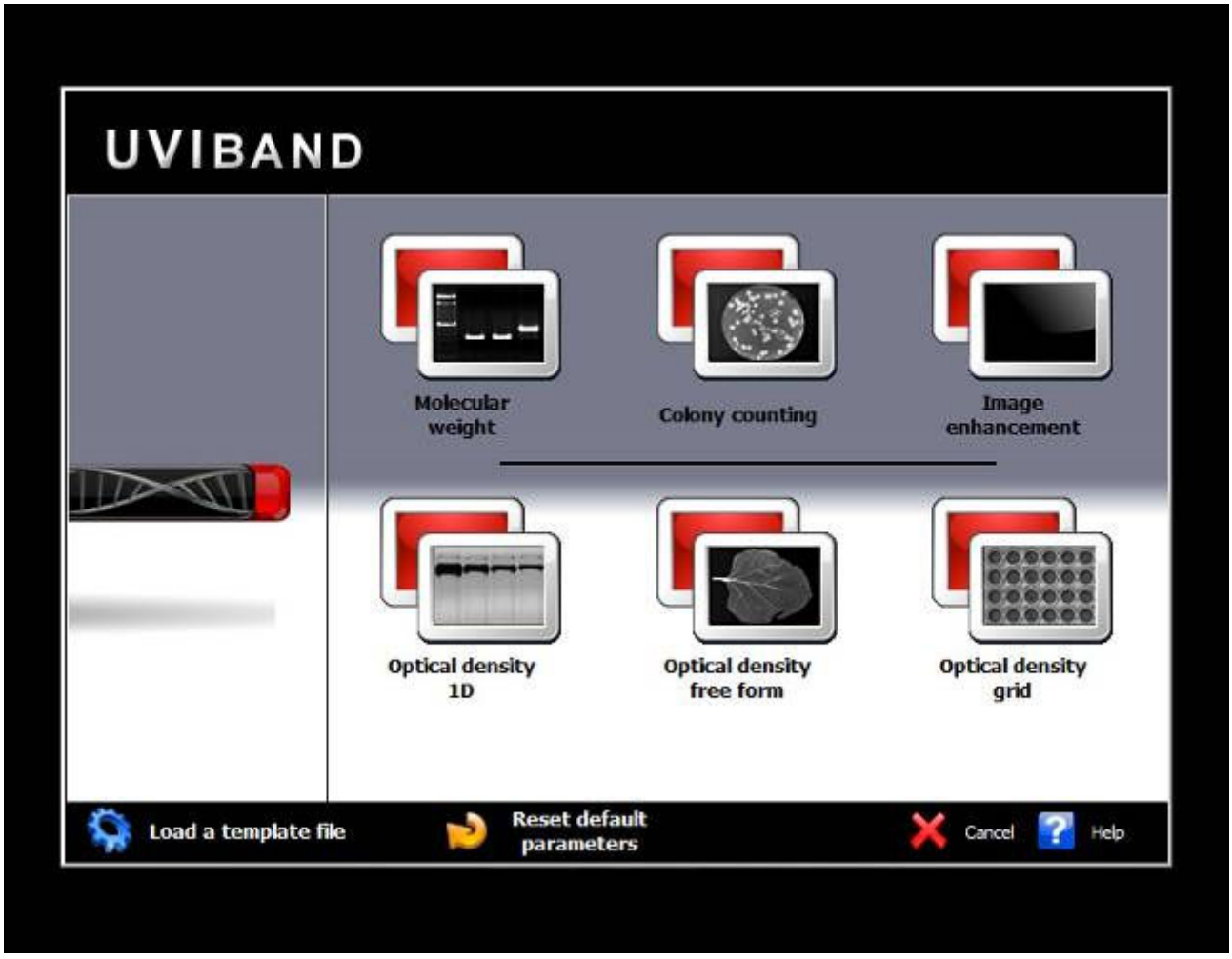
⇨ Decrease the brightness or the contrast by defining a value inferior to 50%

⇨ The image is automatically updated

| | |
|---|---|
|  **Contrast adjustment Before** |  **Contrast adjustment After (i.e. contrast 70%)** |
|  **Brightness adjustment Before** |  **Brightness adjustment After (i.e. brightness 40%)** |

UVITEC

Cambridge




# How to use a template file
# ➔ UVIband Advanced

# Automation with template file

## ➔ Introduction

The analysis or the image enhancement pattern could be saved as a template for automated analysis routines. Template is a tool for automated analysis routine. It offers the user the ability to automate many of the repetitive tasks associated with analysis and processing. As a result, you can spend more time evaluating and analysing results, and less time manipulating set-ups, variables and other settings.

The template automates a task or set of tasks that you perform repeatedly or on a regular basis. It stores all the analysis commands and parameters of an analysis. You can run these parameters with another image whenever you need to perform a new analysis based on the same parameters.

The benefits of the template file are as follows:

⇨ Time saving
⇨ Reproduction of image analysis parameters
⇨ Templates are modifiable, allowing the user to maintain an original template while modifying it for a slightly different result, with minimal effort

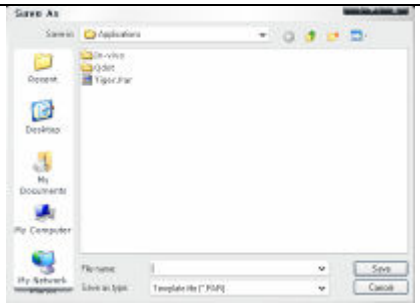
The template allows you to save objects such as lanes, band detection parameters, molecular weight markers, volume calibration standard, text and line overlays in a template file. The template file can then be automatically applied to an image just by loading the image and applying the template file.

The template file is automatically created during the analysis process. You decide to save or not this file to keep it for further analysis.

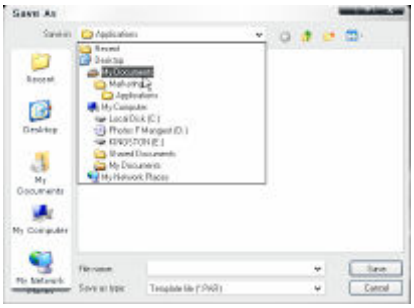
## ➔ Save template

or

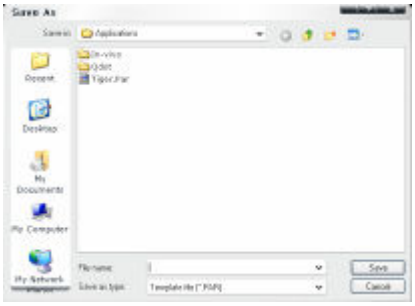

1. Click on the “Save template” icon. A pop-up window displays the following menu:



2. Browse to specify the file directory



3. Enter the desired file name and validate



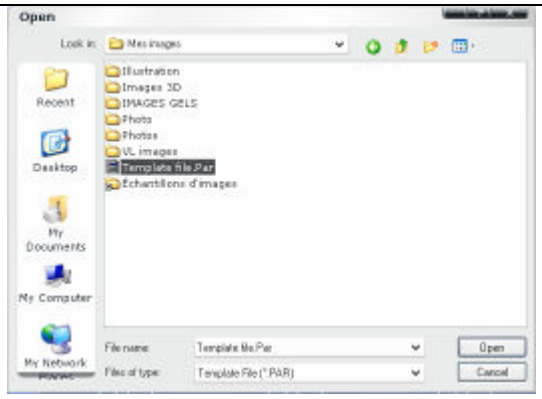
4. Click on the Save button to create the file.

## ➔ Load template

or


To load a template, select the “Load a template file” icon:

A pop-up window displays the following menu:




⇨ Browse to specify the template directory
⇨ Double click on the template name you want to load

Note 1: You need first to load an image before to load a template.