

EXPAND YOUR TERRITORIES





9/2008 index i



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: <u>uvi@uvitec.co.uk</u>

⇒ Contact address: UVItec Limited Avebury House, 36a Union Lane Cambridge CB4 1QB United Kingdom Tel: +44 (0)1223 568 060 Fax: +44 (0)1223 306 198

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

UVIBAND User manual

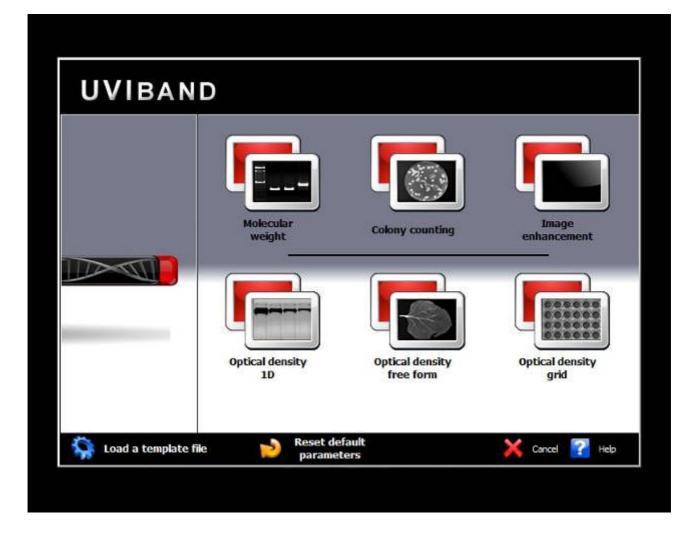
Introduction	7
About	
UVIband Advanced key features	8
End-user license agreement	
Installation guidelines	
System requirement	
Unpacking and installing the UVIband Advanced	11
Hardware security dongle	
Quick start	
Starting the software	
Open an image or an analysis	14
Access to the analysis modules	16
Load a template file	17
Reset to factory setting	
Molecular weight	
Molecular weight introduction	
Objectives and output	20
Molecular weight module (MW) operating environment	
➔ Toolbar in details	
1- Detect	
A – Lane definition	
B – Band detection	
C – Marker values	44
D – Distance	
2- Analyse – Molecular weight	
A – Molecular weight	55
B – Dendrogram	60
C – Matching	
3- Analyse – Quantification	
Principles of quantification	
A – Background subtraction	75
B – Spot separation	
C – Volume of reference	91

➔ D – Calibration	
Publish	
Introduction Return to Home	
➡ Introduction	
Load another image	
Select another function	
➡ Exit the software	
Optical density – 1D	
Optical density / 1D introduction	
Objectives and output	
Optical density / 1D (OD-1D) operating environment	
➔ Toolbar in details	
 1- Window definition → A – Lane definition 	
➡ B – Background subtraction	
C – Spot separation	
2- Analyse – Quantification	
Principles of quantification	
➔ A- Volume of reference	
B- Calibration Publish	
➔ Introduction	
Return to Home	
➔ Introduction	
➔ Load another image	
Select another function	
Exit the software	
Optical density – Free form	
Optical density / Free form introduction	
Objectives and output	
Optical density / Free form (OD-Free form) operating environment	163
➔ Toolbar in details	
1- Window definition	
A – Lane definition	172
B – Background subtraction	177
C – Spot separation	
2- Analyse – Quantification	193
Principles of quantification	193
➔ A – Volume of reference	194
B – Calibration	
Publish	
Introduction	211
Return to Home	A 4 A
➔ Introduction	212
	212 212

Exit the software	213
Optical density - Grid	214
Optical density / Grid introduction	
Objectives and output	215
Optical density / Grid operating environment	215
➔ Toolbar in details	218
1- Window definition	
A – Lane definition	223
B – Background subtraction	228
C – Spot separation	238
2- Analyse – Quantification	245
Principles of quantification	245
➔ A – Volume of reference	246
B – Calibration	255
Publish	
➔ Introduction	
Return to Home	
➔ Introduction	
Load another image	
Select another function	
Exit the software	
Colony counting	
Colony counting introduction	
Kov footuroo	
→ Key features	
 Colony counting module (CC) operating environment 	
 Colony counting module (CC) operating environment Toolbar in details 	268 271
 Colony counting module (CC) operating environment Toolbar in details Automatic counting 	268 271 276
 Colony counting module (CC) operating environment Toolbar in details Automatic counting Detection 	268 271 276 276
 Colony counting module (CC) operating environment Toolbar in details. Automatic counting. Detection Data filtering. 	268 271 276 276
 Colony counting module (CC) operating environment Toolbar in details Automatic counting Detection 	268 271 276 276
 Colony counting module (CC) operating environment Toolbar in details. Automatic counting. Detection Data filtering. Exclusion area Manual counting 	268 271 276 276 281 285 289
 Colony counting module (CC) operating environment Toolbar in details. Automatic counting. Detection Data filtering. Exclusion area Manual counting Publish 	268 271 276 276 281 285 289 293
 Colony counting module (CC) operating environment. Toolbar in details. Automatic counting. Detection Data filtering. Exclusion area Manual counting Publish Introduction 	268 271 276 276 281 285 289 293 293
 Colony counting module (CC) operating environment Toolbar in details. Automatic counting. Detection Data filtering. Exclusion area Manual counting Publish Introduction Return to Home 	268 271 276 276 281 285 289 293 293 294
 Colony counting module (CC) operating environment. Toolbar in details. Automatic counting. Detection Data filtering. Exclusion area Manual counting Publish Introduction Return to Home. Introduction 	268 271 276 281 285 289 293 293 294 294
 Colony counting module (CC) operating environment. Toolbar in details. Automatic counting. Detection	268 271 276 276 281 285 289 293 293 294 294 294
 Colony counting module (CC) operating environment. Toolbar in details. Automatic counting. Detection Data filtering. Exclusion area Manual counting Publish Introduction Return to Home Introduction Load another image Select another function 	268 271 276 276 281 285 289 293 293 294 294 294 295
 Colony counting module (CC) operating environment. Toolbar in details. Automatic counting. Detection Data filtering. Exclusion area Manual counting Publish Introduction Return to Home Introduction Load another image Select another function Exit the software 	268 271 276 276 281 285 289 293 293 294 294 294 295 295
 Colony counting module (CC) operating environment. Toolbar in details. Automatic counting. Detection Data filtering. Exclusion area Manual counting Publish Introduction Return to Home Introduction Load another image Select another function Exit the software 	268 271 276 276 281 285 289 293 293 294 294 294 294 295 295 295
 Colony counting module (CC) operating environment. Toolbar in details Automatic counting Detection Data filtering Exclusion area Manual counting Publish Introduction Return to Home Introduction Exclusion and the rimage Select another function Exit the software Image enhancement 	268 271 276 276 281 285 289 293 293 293 294 294 294 295 295 295 297
 Colony counting module (CC) operating environment. Toolbar in details. Automatic counting. Detection	268 271 276 276 281 285 289 293 293 294 294 294 295 295 295 297 297
 Colony counting module (CC) operating environment. Toolbar in details. Automatic counting. Detection Data filtering. Exclusion area Manual counting Publish Introduction Return to Home. Introduction Load another image Select another function Exit the software Image enhancement Image Enhancement module (IE) operating environment.	268 271 276 276 281 285 289 293 293 293 294 294 294 295 295 295 297 297 297
 Colony counting module (CC) operating environment. Toolbar in details. Automatic counting. Detection Data filtering. Exclusion area Manual counting Publish Introduction Return to Home. Introduction Load another image. Select another function Exit the software Image enhancement Image Enhancement module (IE) operating environment. Toolbar in details. 	268 271 276 276 281 285 289 293 293 293 294 294 294 295 295 295 295 297 297 297 299
 Colony counting module (CC) operating environment. Toolbar in details. Automatic counting. Detection	268 271 276 276 281 285 289 293 293 293 293 294 294 294 295 295 295 297 297 297 299 302
 Colony counting module (CC) operating environment. Toolbar in details Automatic counting Detection Data filtering Exclusion area Manual counting Publish Introduction Return to Home Introduction Load another image Select another function Exit the software Image enhancement Image Enhancement module (IE) operating environment Toolbar in details Image enhancement functions Image enhancement functions Image enhancement functions Introduction 	268 271 276 276 281 285 289 293 293 293 294 294 294 294 295 295 295 297 297 297 297 299 302 302
 Colony counting module (CC) operating environment. Toolbar in details. Automatic counting. Detection	268 271 276 276 281 285 289 293 293 293 293 294 294 294 294 295 295 295 297 297 297 297 297 297 302 303

→ 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 and brightness	
How to use a template file	
Automation with template file	
→ Introduction	
Save template	
➔ Load template	





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.

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➔ 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

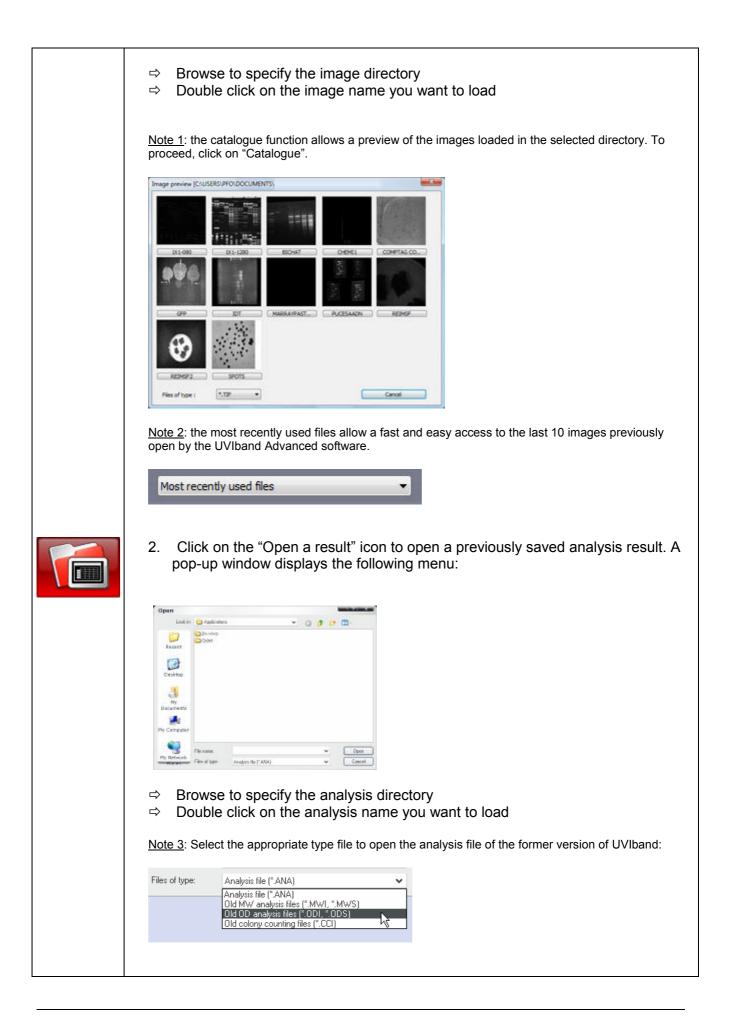
➔ Starting the software

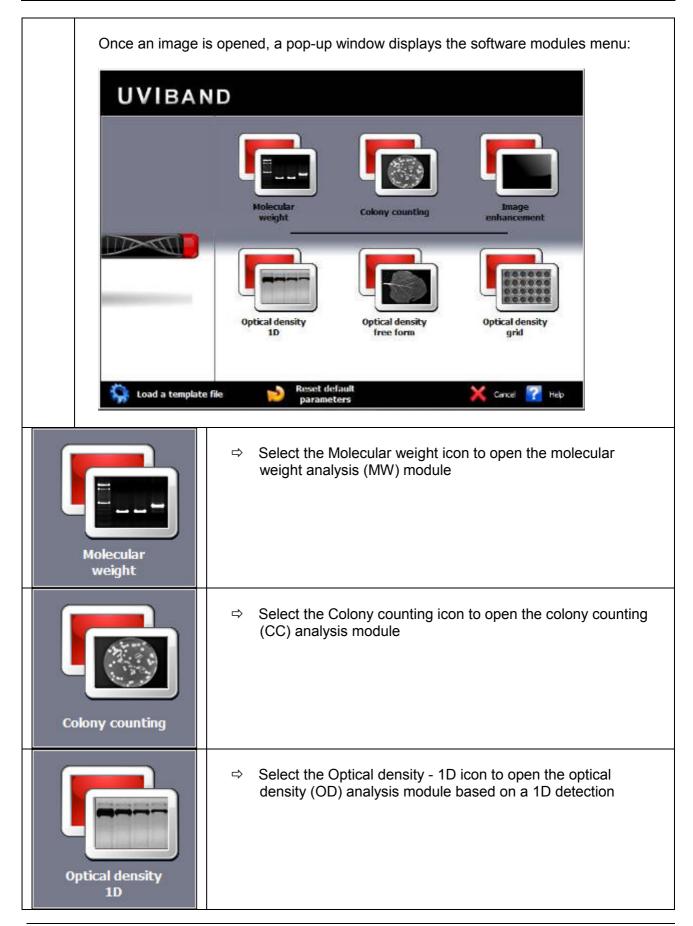


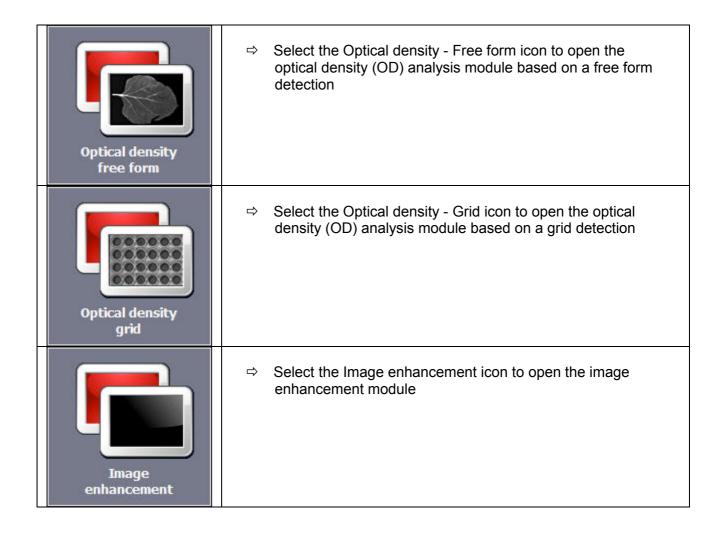
Click twice on the "UVIband Advanced" icon on the Windows Office screen to start the software.

➔ Open an image or an analysis

The software opens on the following window:
UVIBAND Image: Deprive an image: Deprive a
1. Click on the "Open an image" icon to open an image. A pop-up window displays the following menu: Open Image: Image



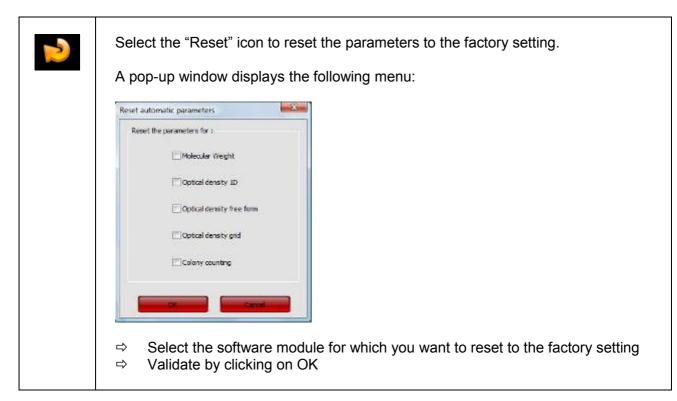




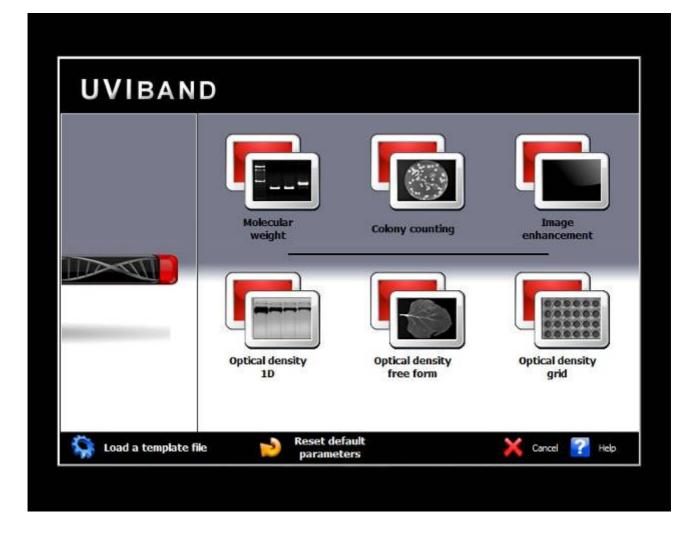
➔ Load a template file

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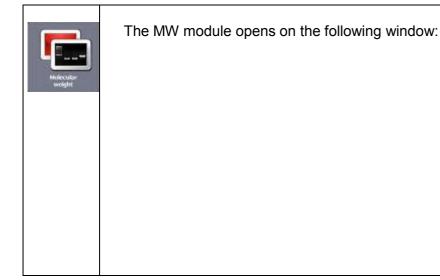
Molecular weight → MW Analysis module

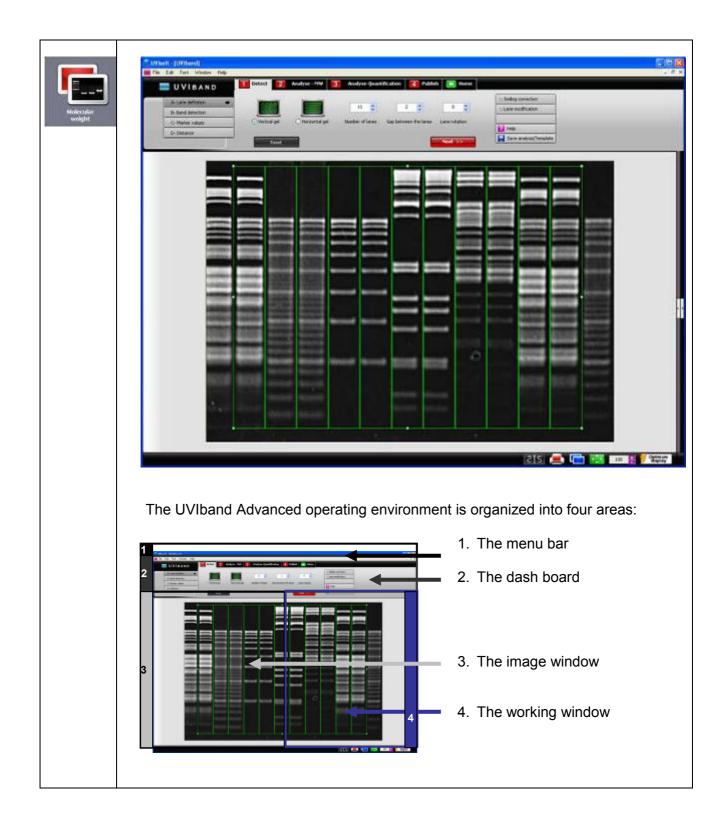
Molecular weight introduction

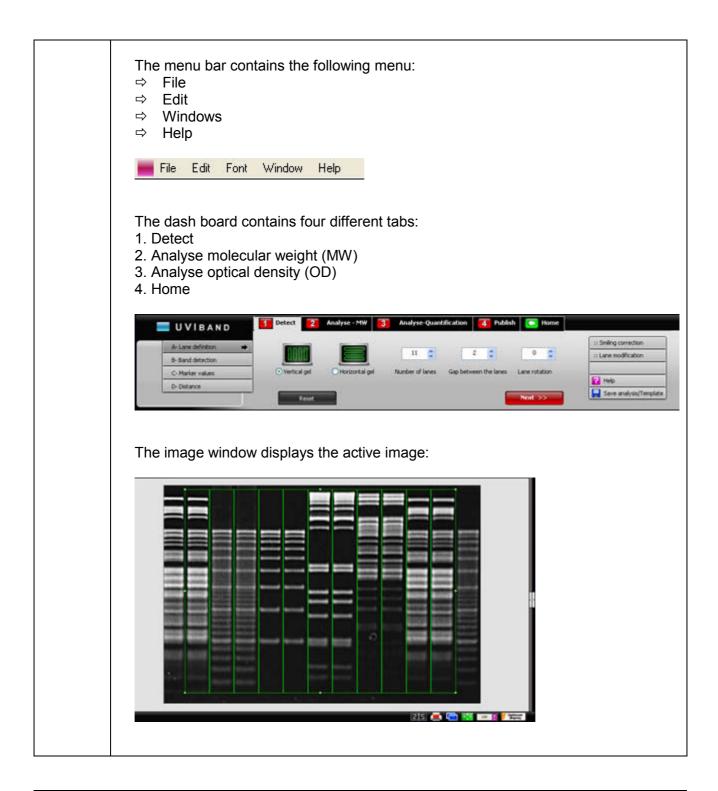
Objectives and output

Andressfor weight	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)
	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

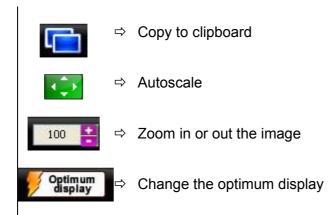
→ Molecular weight module (MW) operating environment







It also cor 2 5	ntains the image toolbar:
25	⇒ Display the molecular weight on the image
	⇔ Print

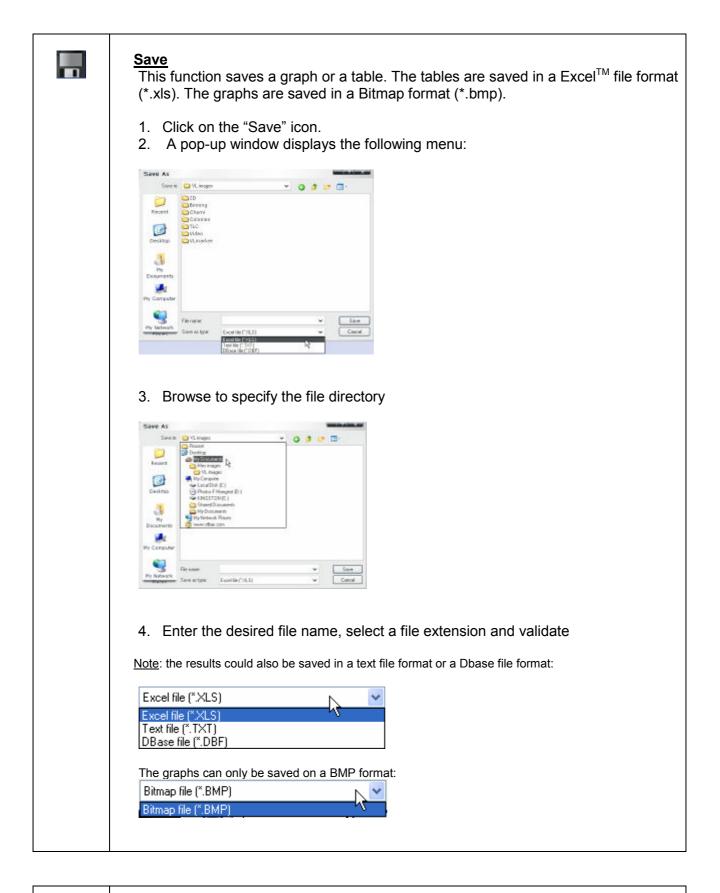


The working window displays the graphs and tables related to the active analysis:

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№ 17 0 130 0.130 0.121 0.125 № 13 0.063 0.063 0.063 0.063 0.063 № 13 0.063 0.063 0.063 0.063 0.063 0.063 Image: Image Image: Image: Image Image: Image: Image Image: Image: Image: Image Image: Imag			0.000	1000	100000	
No 13 0.100 0.100 0.065 0.063 No 19 0.065 0.065 0.063 0.063 Image Image Image Image Image Image Image Image	1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.		0.200		0.0000	0.125
No 19 0.063 0.063 Image: The second seco	1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.		0.100	0.130	0,121	0.125
Contains the working window toolbar: ⇒ Display the molecular weight on the image			0.200			
⇒ Display the molecular weight on the image					-	
\Rightarrow Save the graph or the table						
			-			
\Rightarrow Copy the graph or the table to clipboard	⇔ D	isplay the	molecul	ar weigh		

➔ 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.
Image: A pop-up window displays the
following menu: Image: Select a printer Image: Click on Properties to modify the default setting of the printer, if necessary Image: Select the number of copies Image: Click on OK to validate your options Note: You can also access the Print menu from the Menu bar (File\Print).
Note: You can also access the Print menu from the Menu bar (File\Print).

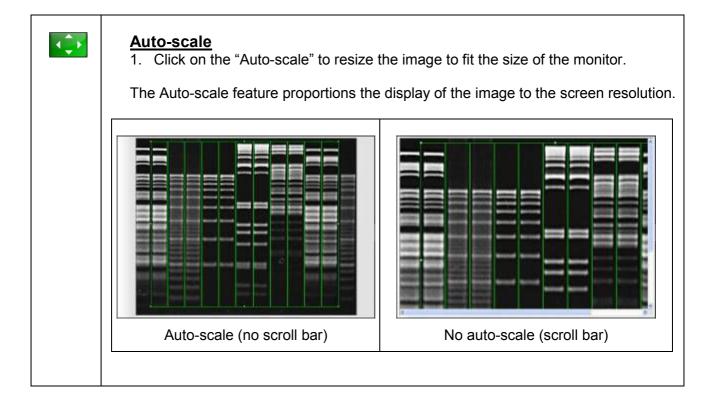


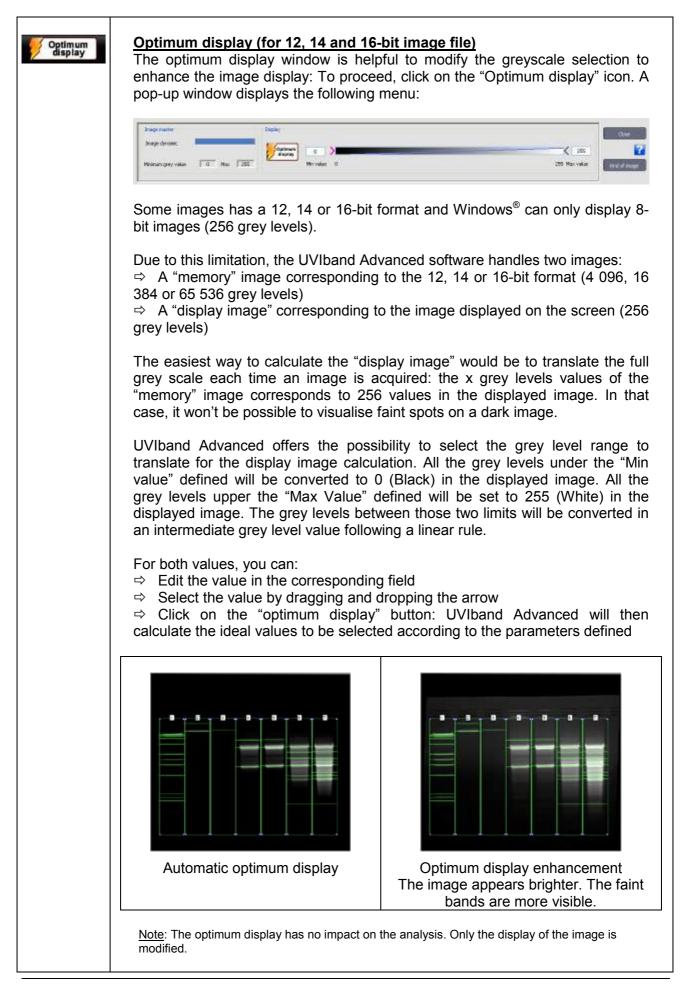


Copy to clipboard

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).







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[™].

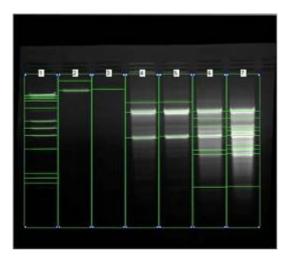
1- Detect

→ A – Lane definition

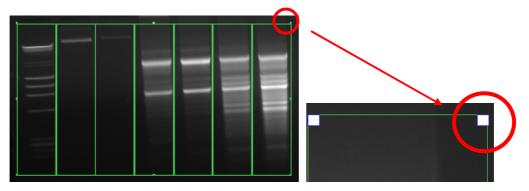
1	The molecular weight module opens on the Lane definition dashboard of the Detect process:
	Vertices Vertice Vertic
	215 🛋 🖬 👀 😫 🖉 98007
	The dashboard details the lane definition parameters:
	UVIBAND Image: Detect integration integratio
	 ⇒ Lanes direction ⇒ Number of lanes ⇒ Gap between the lanes ⇒ 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:



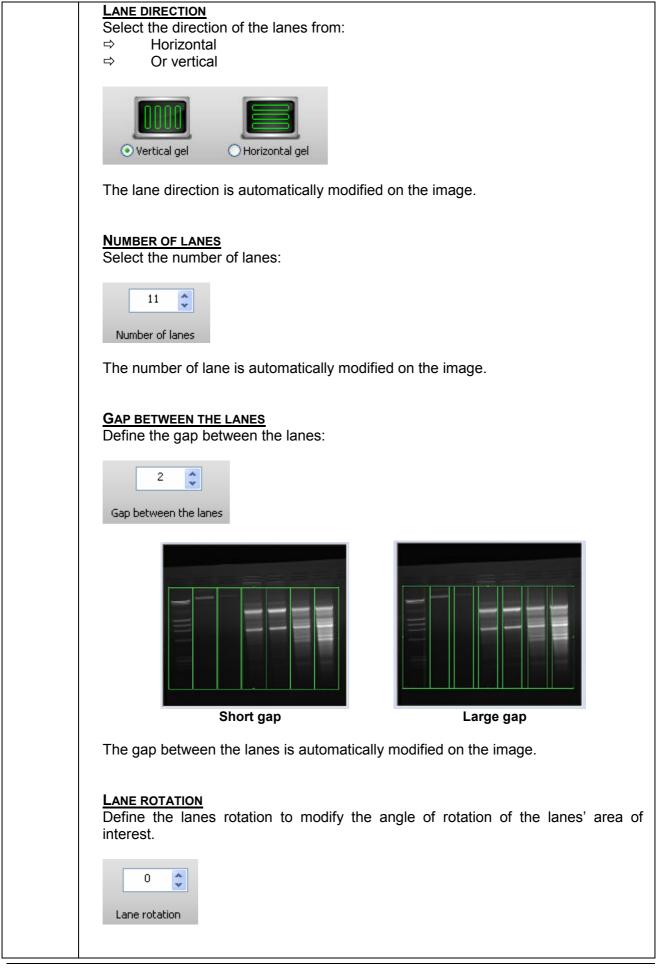
To resize the entire lane frame, drag an anchor point in or out. The opposite anchor point will remain fixed while the frame expands or contracts. The frame will expand or contract from the center.

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

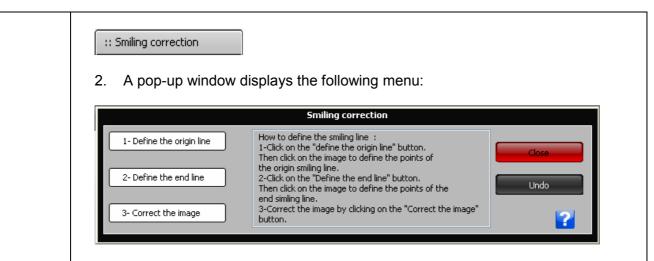
|--|

Click and drag the cursor to move the entire frame.

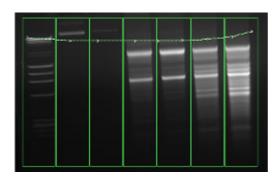
<u>Note</u>: it is not necessary to include the well line in the area of interest window. The calculation of distances does not require this reference line.



No 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.
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 Image: Save the analysis or the template Image: Help Image: Help Image: Save analysis/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.



 \Rightarrow 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.



 \Rightarrow 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.

-	100	_	-	
				Ξ

3. Click on "Correct the image" to display the migration correction.

<u>Note</u>: 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.

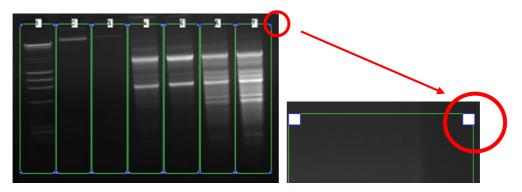
LANE MODIFICATION

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

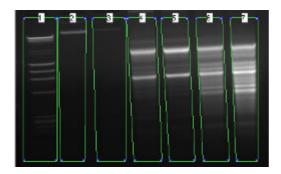
1. Click on the "Lane modification" button.



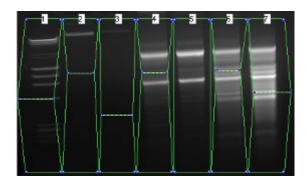
2. A pop-up window displays the each lanes with interactive squares at the corners:



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



 \Rightarrow 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.

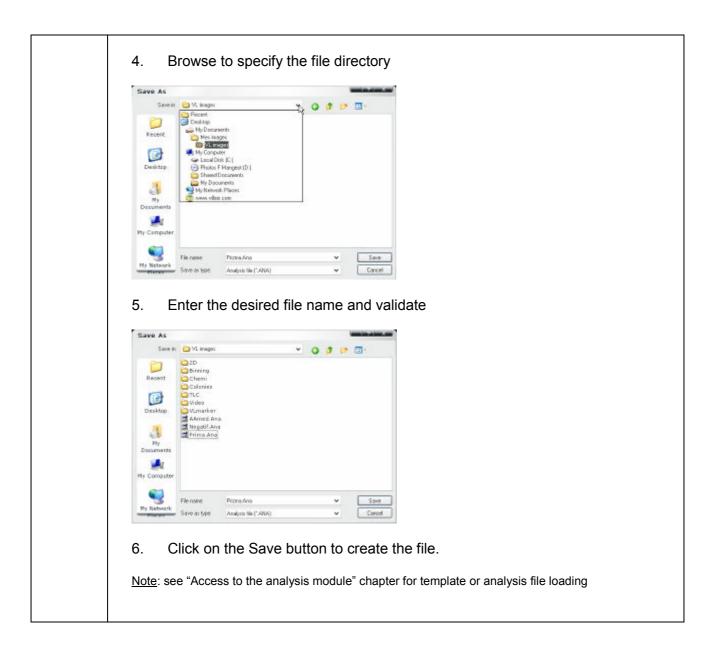
HELP MENU

김 Help

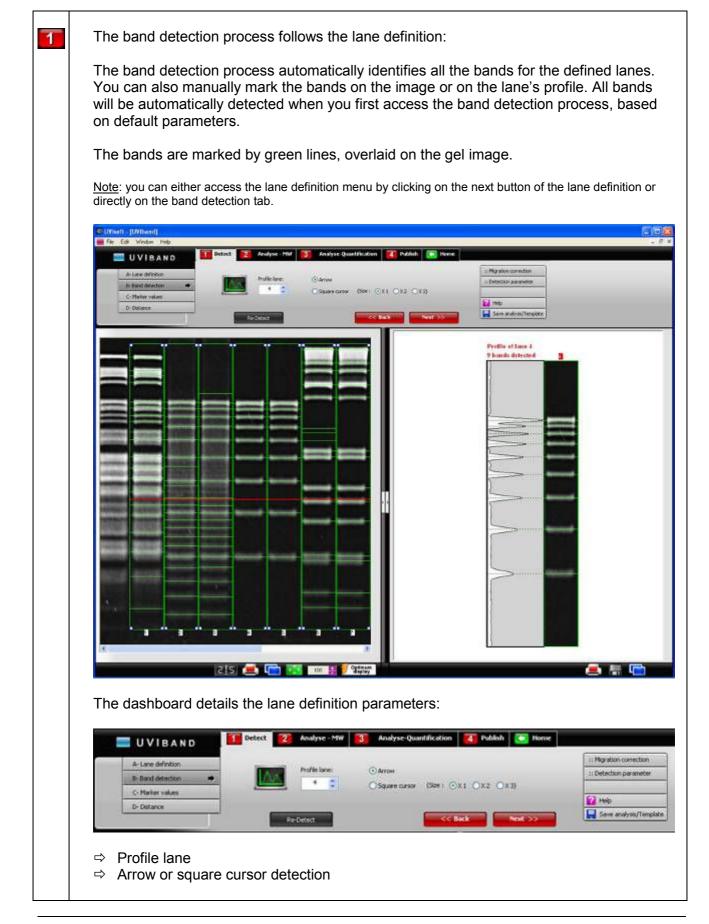
Click on the "Help" button. You automatically access the user manual at the chapter corresponding to the function

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

Help	
	About UVIsoft
_	
<u>Savi</u>	E ANALYSIS / TEMPLATE
	function saves the current analysis. The analysis file will contain the results, mage and all the parameters defined to obtain the results.
Tem asso evalu	analysis could also be saved as a template for automated analysis routines. plate offers the user the ability to automate many of the repetitive tasks iciated with analysis and processing. As a result, you can spend more time uating and analyzing results, and less time manipulating set-ups, variables ar r settings.
regu You	template automates a task or set of tasks that you perform repeatedly or on a lar basis. It stores all the analysis commands and parameters of an analysis. can run these parameters with another image whenever you need to perform w analysis based on the same parameters.
ት ት ት	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 templa e 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:
Save /	
3	een at 🔁 VL anages 🔹 🔍 🥥 🦛 🚍 s
Rece	Colorina Colorina Colorina
Deskt	Division Division Standard
Ny Docum	Megati Ana Prime Ana
Ny Com	
My Reb	Pla name Save
	Save at loss Analysis file (* 244) Cancel Associate Bits (* 2004) Tergolate File (* 2004)
3.	Select analysis file or template file:
A mal	
Anal	ysis file (*.ANA)
Tem	plate File (*.PAR)

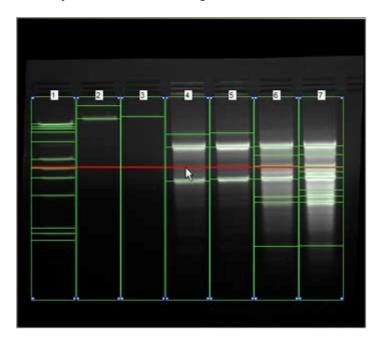


➔ B – Band detection



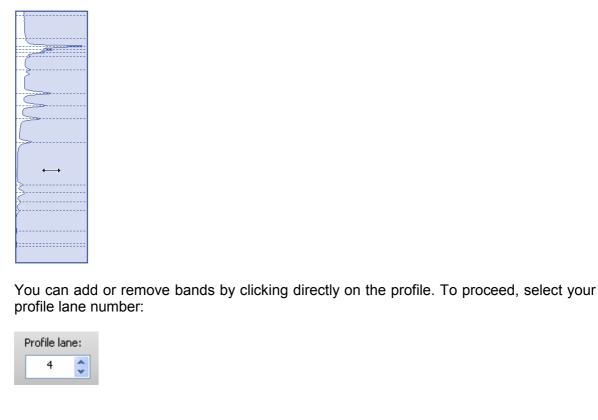
BAND DETECTION ON THE IMAGE

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.



Select the cursor type:

 Arrow 		
-		
○ Square cursor (Size : ⊙)	×1 ○×2 ○×3)	
⇒ The linear cursor has	s the shape of an arrow	v (••)
	sor has the shape of a	
Place the cursor at the	e chosen profile loc:	ation and click. The detection line
automatically added or rer	-	allon and click. The detection line
Note: For arrow cursor, the ban	d is added at the cursor pos	ition.
For rectangular cursor, the band		
_		
<u>Re-DETECT</u> You can re-detect the ban	ds by clicking the "Re-	detect" button. The detection is based o
the default parameters.		
Re-Detect		
<u>NEXT</u> The "Next" button validate	s your parameter and	opens the following analysis step.
		· · · · · · ·
B- Band detection	Next >>	C – Marker values
The "Back" button validate B- Band detection	es your parameter and	opens the following analysis step. A - Lane definition
OPTION FOLDER		
The option folder gathers		
⇒ Migration correctio	n	
Detection paramet		
 ⇒ Detection paramet ⇒ Help 	er	
	er	
⇔ Help	er	
 ⇒ Help ⇒ Save the analysis 	er	
	er	
	er	
 ⇒ Help ⇒ Save the analysis of ∷ Migration correction ∷ Detection parameter 	er	
 ⇒ Help ⇒ Save the analysis of ∷ Migration correction ∷ Detection parameter ☑ Help 	er	
 ⇒ Help ⇒ Save the analysis ∴ Migration correction ∷ Detection parameter ? Help Save analysis/Template 	er	
 ⇒ Help ⇒ Save the analysis ∴ Migration correction ∷ Detection parameter ☑ Help ☑ Save analysis/Template MIGRATION CORRECTION This function corrects the	er or the template e migration distortions	by calibrating the image based on th
 ⇒ Help ⇒ Save the analysis ∴ Migration correction ∷ Detection parameter ☑ Help ☑ Save analysis/Template MIGRATION CORRECTION This function corrects the	er or the template e migration distortions lanes. The band posi	tion correction is obtained by realignin

2. Ap	op-up window displays the following menu: Migration correction
Select t	e standard lanes and click on Correction Lane 1 Lane 2 Lane 3 Lane 4 Lane 5 Lane 6 Lane 7 Lane 8 V
Select t	ne marker's lane:
Selected I Lane 1 Lane 10	ne(s) : 20 Bands 20 Bands
	ealignment is possible if the number of bands between the lanes is different. can add or remove detected bands by clicking directly on the image.
Corr Note: Yo	k on the "Correction" button. The bands are automatically realigned.
Corr Note: Yo	ection
Note: You Note: You Note: You DETECT You can particul	can undo the migration correction by clicking the "Undo" button.
Corr <u>Note</u> : You <u>Note</u> : You <u>DETECT</u> You can particul too muc <u>1. Clic</u>	can undo the migration correction by clicking the "Undo" button. need to close the pop-up window by clicking on the "Close" button. ON PARAMETER modify the sensitivity parameters to optimise the band detection. This is irly useful for instance in the case of several bands not taken into account
Corri Note: You Note: You DETECT You can particul too mud 1. Clid :: Detect	can undo the migration correction by clicking the "Undo" button. need to close the pop-up window by clicking on the "Close" button. ON PARAMETER modify the sensitivity parameters to optimise the band detection. This is irly useful for instance in the case of several bands not taken into account h background noise is detected. k on the "Detection parameter" button.

- 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
- \Rightarrow 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.

<u>Note</u>: You can save your detection parameter by clicking on the "Save parameters" button. <u>Note</u>: You need to close the pop-up window by clicking on the "OK" button.

?	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:

Help	L
	Index
	About UVIsoft

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:

📑 Save analysis/Template

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

Save As						
Save in	🖒 VL integer		*	0	10	11 -
Desktap Overktap My Descurrents	20 Channe Channe Colonies Colonies Colonies Colonies Williarfor Armod Armo Regolf Armo Wilma, Anno					
Ny faziverk	File none: Save at type:	Analysis file (* 1884)				Seve Cancel

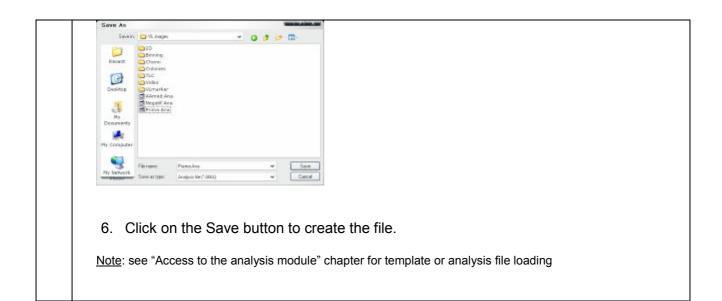
3. Select analysis file or template file:



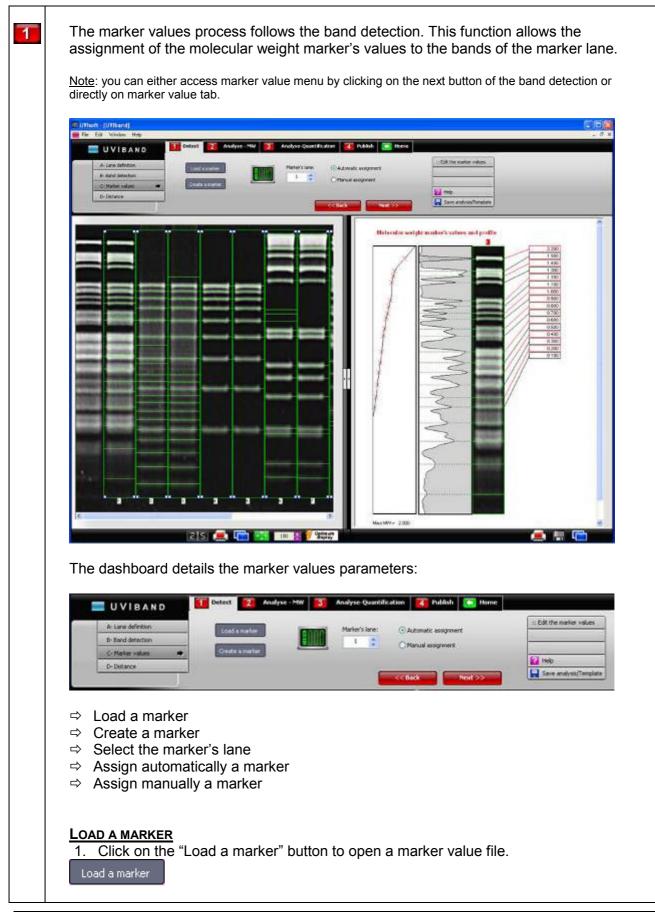
Note: the software proposes Analysis file by default

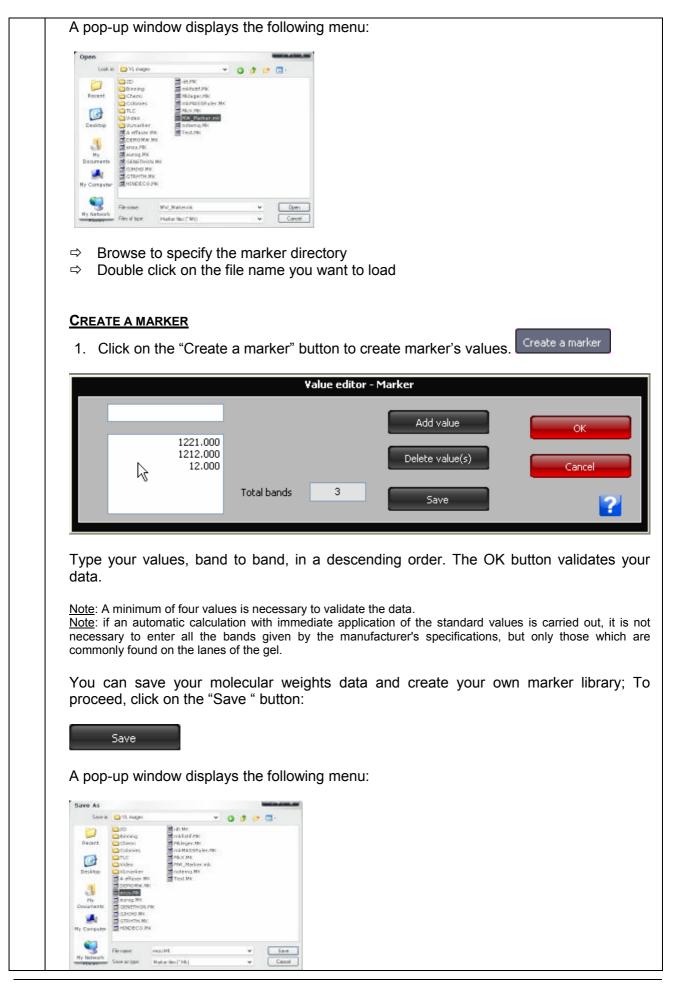
4. Browse to specify the file directory





➔ C – Marker values





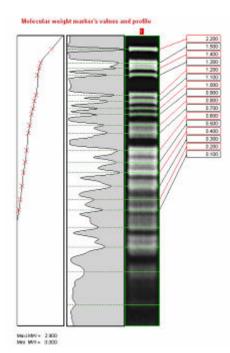
- \Rightarrow Browse to specify the directory
- \Rightarrow Type the file name and click on Save.

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.

<u>Note</u>: 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.

<u>Note</u>: The displayed migration curve is of the cubic spline type and must then include a minimum of 4 values. <u>Note</u>: The minimum MW indicates the minimum molecular weight, which can be calculated, based on the marker's value assignment.

<u>Note</u>: The maximum MW indicates the maximum molecular weight, which can be calculated, based on the marker's value assignment.

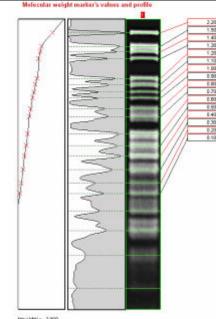
ASSIGN THE MARKER VALUES TO THE BAND

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

Automatic assignment

O Manual assignment

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:



MexiMV+ 2.900 MexiMV+ 0.000

<u>Next</u>

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

C – Marker values	Next >>	2- Analyse - MW 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:

- \Rightarrow Edit the marker values
- ⇔ Help
- ⇒ Save the analysis or the template

:: Edit the marker values		
<mark>?</mark> Help		
📙 Save analysis/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.

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

Value editor - Marker						
		1221.000 1212.000			Add value Delete value(s)	ок
	\mathbb{R}	12.000			Delete value(s)	Cancel
			Total bands	3	Save	2

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:

elp	
	Index
	About UVIsoft

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.

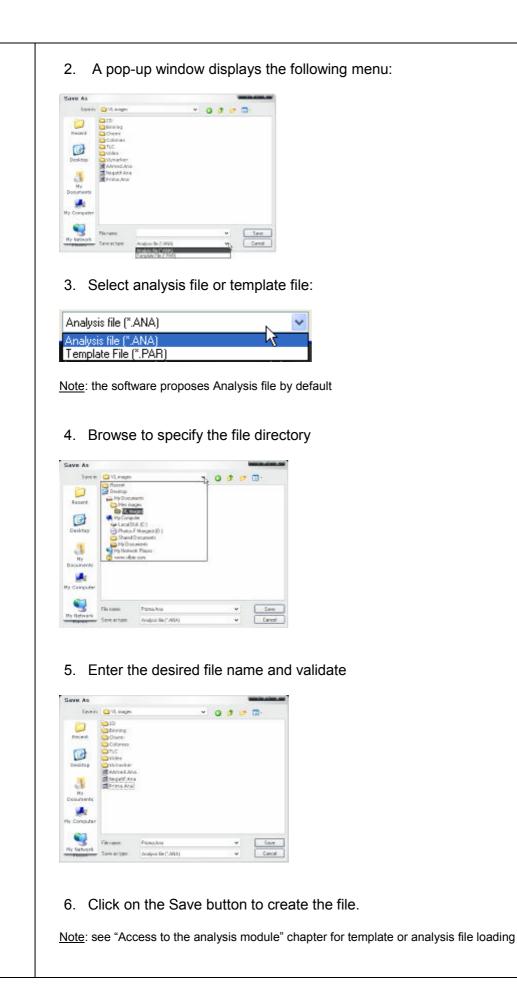
The benefits of the template file are as follows:

- ⇒ Time savings
- ⇒ Reproduction of image analysis parameters

⇒	Templates	are mod	difiable,	allowing	the u	user to	o maintai	n an	original	template	while
modi	fying it for a	a slightly	differen	nt result,	with	minim	al effort				

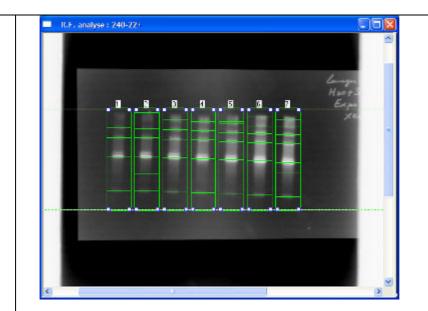
1. Click on the "Save analysis/ Template" button:

Save analysis/Template



➔ 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
	C: Mittert: [Util hand] C: Mittert: [Util hand] C: Mittert: [Util hand] C: Mittert: [Util hand] C: Mitter: Mitter C: Mitter: Mitt
	The dashboard details the marker values parameters:
	UVIBAND Analyse - HW Analyse - WW Analyse - Quantification Publish I home Analyse - Quantification Analyse - Quantification
	 ⇒ 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.

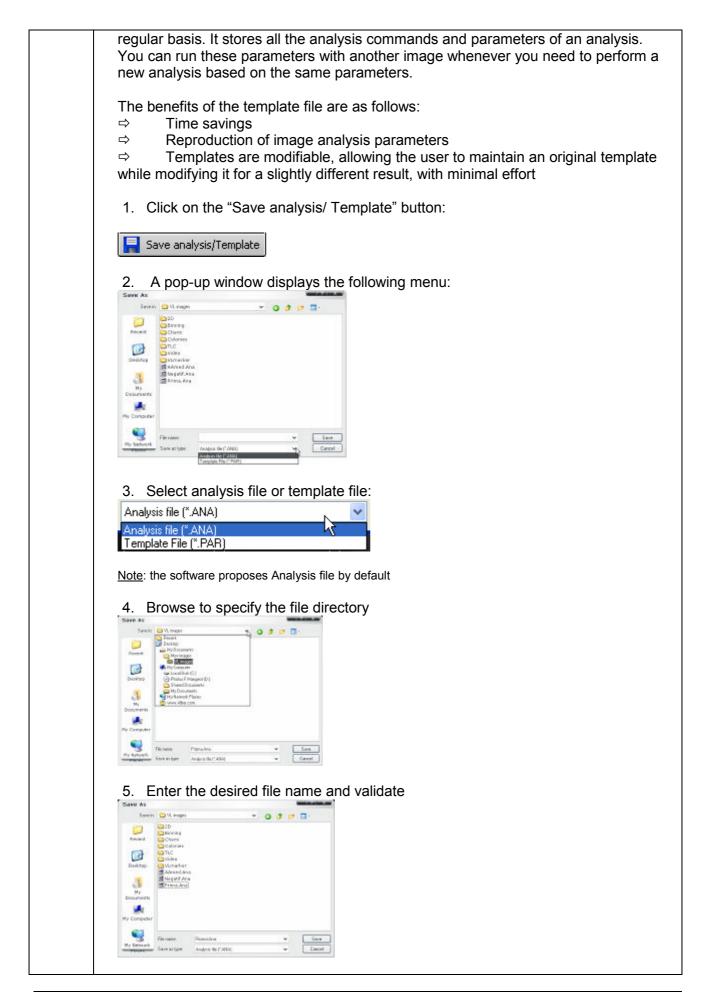
Select the value for the origin and the value the end.



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

	Reference	Lane 1	Lone 2	Lane 3	Lane 4
No 1	2.200	z.200			2
No 2	1.500	1.500			
No 3	1.900	1.400			
No 4	1.300	1.300			
No S	1.200	1.200	1.200	1.192	1.18
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			
	0.063		0.063	0.063	

D – Distance	Next >> 2- Analyse - MW A- Molecular weight
BACK	
	es your parameter and opens the following analysis
D – Distance	<< Back B- Band detection
OPTION FOLDER The option folder gathers	the following functions:
 ⇒ Help ⇒ Save the analysis 	or the template
:: Select the font size	
<table-cell> Help</table-cell>	
📘 Save analysis/Template	
HELP MENU Click on the "Help" buttor	n. You automatically access the user manual at the c
Click on the "Help" buttor corresponding to the fund	n. You automatically access the user manual at the c ction.
Click on the "Help" buttor	
Click on the "Help" buttor corresponding to the func Help You can access the help	
Click on the "Help" buttor corresponding to the func Help You can access the help	ction.
Click on the "Help" buttor corresponding to the func Help You can access the help Help	ction.
Click on the "Help" buttor corresponding to the func Help You can access the help	ction.
Click on the "Help" buttor corresponding to the func Help You can access the help Help Index About UVIsoft	file index through the File\Help from the Menu bar:
Click on the "Help" buttor corresponding to the func Help Vou can access the help Help Index About UVIsoft	file index through the File\Help from the Menu bar: <u>TE</u> urrent analysis. The analysis file will contain the resu
Click on the "Help" buttor corresponding to the func Help You can access the help Help Index About UVIsoft	file index through the File\Help from the Menu bar:
Click on the "Help" buttor corresponding to the func Help Index About UVIsoft SAVE ANALYSIS / TEMPLAT This function saves the c image and all the parame The analysis could also b	tion. file index through the File\Help from the Menu bar: <u>TE</u> urrent analysis. The analysis file will contain the resu eters defined to obtain the results.
Click on the "Help" buttor corresponding to the func Help You can access the help Help Index About UVIsoft Save ANALYSIS / TEMPLAT This function saves the c image and all the parame The analysis could also b Template offers the user	tion. file index through the File\Help from the Menu bar: <u>TE</u> urrent analysis. The analysis file will contain the resu



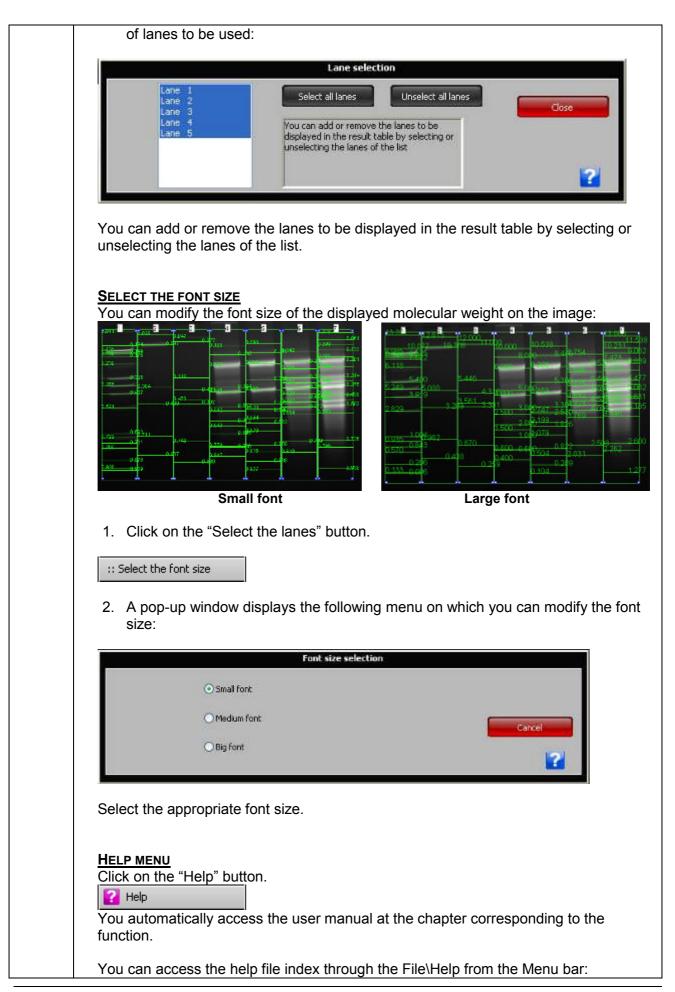
6. Click on the Save button to create the file.
Note: see "Access to the analysis module" chapter for template or analysis file loading

2- Analyse – Molecular weight

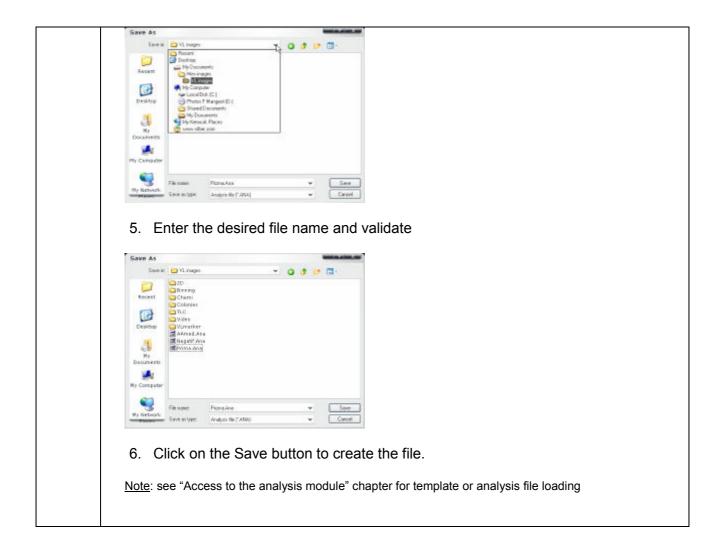
➔ A – Molecular weight

C Modrey.	T Delet 2 Andys	e - MW 3 Andree Q oversels () Segmental anter () Noted onder - De		ah 💽 tian X = 👙		i select the larves i Select the face i Select the face i Select analysis	and the second			<u>E</u> E
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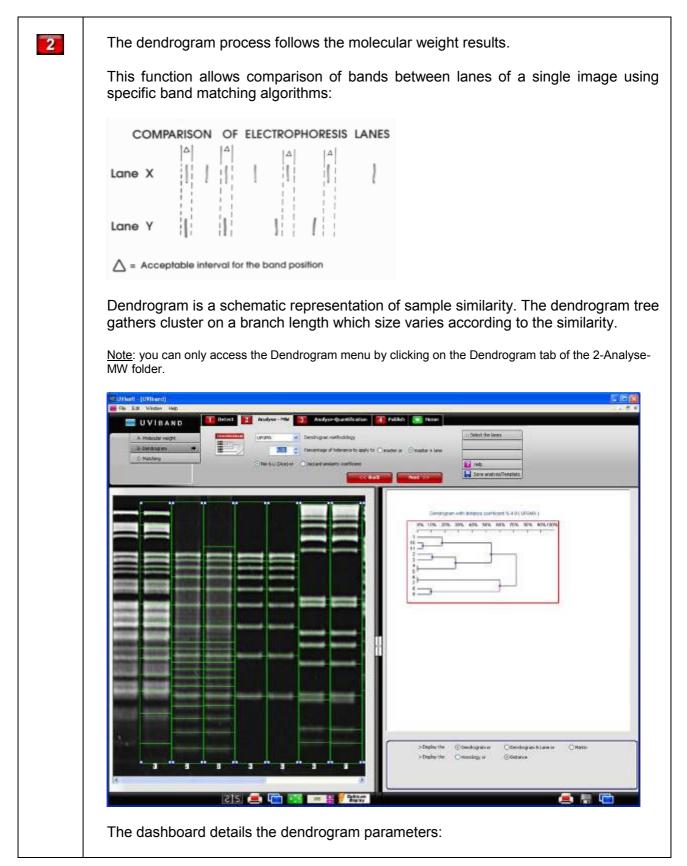
are displayed in order of appearance	
The sorted order: the results are sorted in orde array identical values according to a percentage of st of calculated values is created ("Reference" co are compared to this reference list.	f tolerance. To do so, a reference
◯ Sequencial order	
Sorted order - Percentage of tolerance 10.00 ♀	
You can defined a percentage of tolerance in o veight values on a single line:	order to merge similar molecular
Percentage of tolerance 5.00	
he array of results is automatically modified.	
<u>IEXT</u> ⁻he "Next" button validates your parameter and ope	ens the following analysis step.
	3- Analyse - Quantification A- Background subtraction
S S DOCK	ens the following analysis step. 1- Detect C- Marker values
DPTION FOLDER The option folder gathers the following functions: ⇒ Help ⇒ Save the analysis or the template	
:: Select the lanes	
:: Select the font size	
Help Save analysis/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 c	on which you can modify the list



The template automates a task or set of tasks that you perform repeatedly or regular basis. It stores all the analysis commands and parameters of an anal You can run these parameters with another image whenever you need to pe new analysis based on the same parameters. The benefits of the template file are as follows: ⇒ Time saving ⇒ Reproduction of image analysis parameters		
About UVIsoft SACE ANAL YSIS / TEMPLATE This function saves the current analysis. The analysis file will contain the ress image and all the parameters defined to obtain the results. The analysis could also be saved as a template for automated analysis routin 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 tir evaluating and analysing results, and less time manipulating set-ups, variable other settings. The template automates a task or set of tasks that you perform repeatedly or regular basis. It stores all the analysis commands and parameters of an anal You can run these parameters with another image whenever you need to per the set of the template file are as follows:		
SAVE ANALYSIS / TEMPLATE This function saves the current analysis. The analysis file will contain the restinage and all the parameters defined to obtain the results. The analysis could also be saved as a template for automated analysis routin 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 tir evaluating and analysing results, and less time manipulating set-ups, variable other settings. The template automates a task or set of tasks that you perform repeatedly or regular basis. It stores all the analysis commands and parameters of an anal You can run these parameters with another image whenever you need to perform run these parameters with another image whenever you need to perform run these parameters with another image whenever you need to perform a saving 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: Image saving Save analysis/Template 2. A pop-up window displays the following menu: Image save analysis file or template file: A pop-up window displays the following menu: Image save file ("ANA) Image she ("ANA) Image she ("ANA)	Index	
 This function saves the current analysis. The analysis file will contain the resimage and all the parameters defined to obtain the results. The analysis could also be saved as a template for automated analysis routin 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 tir evaluating and analysing results, and less time manipulating set-ups, variable other settings. The template automates a task or set of tasks that you perform repeatedly or regular basis. It stores all the analysis commands and parameters of an analyou can run these parameters with another image whenever you need to penew 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 tewhile modifying it for a slightly different result, with minimal effort 1. Click on the "Save analysis/Template" button: Save analysis/Template 3. Select analysis file or template file: Analysis file ("ANA) Analysis file ("ANA) Analysis file ("ANA) Analysis file ("ANA) 	About UVIsoft	
 This function saves the current analysis. The analysis file will contain the resimage and all the parameters defined to obtain the results. The analysis could also be saved as a template for automated analysis routin 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 tir evaluating and analysing results, and less time manipulating set-ups, variable other settings. The template automates a task or set of tasks that you perform repeatedly or regular basis. It stores all the analysis commands and parameters of an analyou can run these parameters with another image whenever you need to penew 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 tewhile modifying it for a slightly different result, with minimal effort 1. Click on the "Save analysis/Template" button: Save analysis/Template 3. Select analysis file or template file: Analysis file ("ANA) Analysis file ("ANA) Analysis file ("ANA) Analysis file ("ANA) 		
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 the evaluating and analysing results, and less time manipulating set-ups, variable other settings. The template automates a task or set of tasks that you perform repeatedly or regular basis. It stores all the analysis commands and parameters of an anal You can run these parameters with another image whenever you need to pe new analysis based on the same parameters. The benefits of the template file are as follows:	This function saves t	he current analysis. The analysis file will contain the resul
regular basis. It stores all the analysis commands and parameters of an anal You can run these parameters with another image whenever you need to penew 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 tew while modifying it for a slightly different result, with minimal effort Click on the "Save analysis/Template" button: Save analysis/Template A pop-up window displays the following menu: Time saving Salect analysis file or template file: Analysis file ("ANA) Analysis file ("ANA) Analysis file ("ANA) Template File ("PAR)	Template offers the u associated with analy	user the ability to automate many of the repetitive tasks ysis and processing. As a result, you can spend more time
 Time saving Reproduction of image analysis parameters Templates are modifiable, allowing the user to maintain an original te 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: Templates are used to the series of the se	regular basis. It store You can run these pa	es all the analysis commands and parameters of an analys arameters with another image whenever you need to perfo
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Save analysis/Template 2. A pop-up window displays the following menu: Image: Save analysis for the following menu: Image: Save analysis file or template file: Analysis file (*.ANA) Image: Save analysis file or template file:	 ⇒ Reproduction ⇒ Templates ar 	e modifiable, allowing the user to maintain an original tem
2. A pop-up window displays the following menu: Image: Comparison of the system Image: Comparison of the system 3. Select analysis file or template file: Image: Comparison of the system	1. Click on the "Sa	ve analysis/ Template" button:
3. Select analysis file or template file: Analysis file (*.ANA) Analysis file (*.ANA) Analysis file (*.ANA)	📘 Save analysis/Templat	te
Analysis file (*.ANA) Analysis file (*.ANA)	2. A pop-up windo	w displays the following menu:
Analysis file (*.ANA) Analysis file (*.ANA) Template File (*.PAR)	Tanan Vingen v Vingen	× Sra
Analysis file (*.ANA) Template File (*.PAR)	3. Select analysis f	ïle or template file:
	Analysis file (*.ANA)	
· · · · · · · · · · · · · · · · ·		ses Analysis file by default
4. Browse to specify the file directory		



➔ B – Dendrogram



	Detect 2 Analyse - MW	Analyse Quantification	Publish 🔛 Home		
A-Molecular weight B-Dendrogram	UPONA OP	chogram methodislogy centage of tolerance to apply to 🔘 n	naster or 🕐 naster + lane	dect the larves HMp Save analyse/Template	
⇒ Select a simila	rogram methodology arity coefficient antage of tolerance				
supports several n	is a way to visualise nethods of dendrogram air Group Match Averag age verage Linkage	calculation:	mology. UVIbanc	Advanced	
and Ward method d (R, P+Q) = A*d(Where:	lete, unweighted avera s the general formula is R, P) + B*d(R, Q) + E*c petween 2 lanes x and y	s as follows: d(P, Q) + G* de	(R, P) - d(R, Q)	bid, median	I
	nstants specific to the n				
		В		G	
Single linkage	A 0.5	0.5	Е 0	-0.5	
Complete linkage	0.5	0.5	0	0.5	
Average linkage	0.5	0.5	0	0	
(unweighted)					
Average linkage	NP/(NP+NQ)	NQ/(NP+NQ)	0	0	
Centroid	NP/(NP+NQ)	NQ/(NP+NQ)	(NP*NQ)/(NP+NQ) ²	0	
Median			-0.25 NR/(NR+NP+NQ)	0	
Ward	(NR+NP)/(NR+NP+NQ)	NR+NP+NQ	NR/(NR+NP+NQ)	0	
publications: Timothy J.Beanland a « The inference of evo	nds in group P ands in group Q on UPGMA and Nei ar nd Christopher J. Howe olutionary trees from molecul	lar data »	please refer to th	ne following	I
Ute Mackenstedt, Kim	siol, Vol. 102B, N°4, pp 643- Luton, Peter R. Baverstock Inships of Babesia diverger	, Alan M. Johnstor		small subunit	:

« 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:
UPGMA Dendrogram methodology
<u>SIMILARITY COEFFICIENT</u> Click in the heading Similarity coefficient to select either the Nei and Li (Dice) coefficient or the JACCARD coefficient
• Nei & Li (Dice) or • • • • • • • • • • • • • • • • • •
⇒ 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 △ is a percentage directly read on the drawn curve of the marker: ⇒ The location of the band is then considered with ± △ around its value in Kb, in RF, or in KDa ⇒ The bigger the coefficient, the higher the number of matching bands and conversely
4.00 Percentage of tolerance
 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:

Image: Second	1. To se	also be exp elect your dis > Display the	ressed in te splay mode,	rms of hor click on th	nology ne appro	or in ter	Matrix d ms of diselection	istance	
Image:	1. To se	also be exp elect your dis > Display the	ressed in te splay mode,	rms of hor click on th	nology ne appro	or in ter	Matrix d ms of diselection	er e	
Image:	I. To se	also be exp elect your dis > Display the	ressed in te splay mode,	rms of hor click on th	nology ne appro	or in ter	Matrix d ms of di selectior	istance n:	
Graphical display Matrix display hey can also be expressed in terms of homology or in terms of distance . To select your display mode, click on the appropriate selection: > Display the Opendrogram or Opendrogram & Lane or OMatrix > Display the Opendrogram or Opendrogram & Lane or OMatrix > Display the Opendrogram or Opendrogram & Lane or Omatrix > Display the Opendrogram or Opendrogram & Compared the following analysis B-Dendrogram Next >> AcK he "Back" button validates your parameter and opens the following analysis B-Dendrogram C- Matching AcK he "Back" button validates your parameter and opens the following analysis B-Dendrogram C- Matching PTION FOLDER A- Molecular weight he option folder gathers the following functions: Select the lanes Help Help	To se	also be exp elect your dis > Display the	ressed in te splay mode,	rms of hor click on th	nology ne appro	or in ter opriate s	Matrix d ms of di selectior	so so lisplay istance n:	
hey can also be expressed in terms of homology or in terms of distance . To select your display mode, click on the appropriate selection: > Display the Opendrogram or Opendrogram & Lane or OMatrix > Display the Homology or Obstance EXT he "Next" button validates your parameter and opens the following analysi B-Dendrogram C- Matching ACK he "Back" button validates your parameter and opens the following analysi B-Dendrogram << Back	. To se	also be exp elect your dis > Display the	ressed in te splay mode,	click on th	ne appro	or in ter opriate s	ms of di selectior	istance n:	
> Display the Opendrogram or Opendrogram & Lane or OMetrix > Display the Homology or Obstance MEXT The "Next" button validates your parameter and opens the following analysi B-Dendrogram Next >> B-Dendrogram C- Matching BACK The "Back" button validates your parameter and opens the following analysi B-Dendrogram Next >> C- Matching BACK The "Back" button validates your parameter and opens the following analysi B-Dendrogram << Back A- Molecular weight OPTION FOLDER The option folder gathers the following functions: Select the lanes Help		> Display the	ODendrogram	n or O De	ndrogram (-			
Next The "Next" button validates your parameter and opens the following analysi B-Dendrogram Next >> B-Dendrogram C- Matching BACK The "Back" button validates your parameter and opens the following analysi B-Dendrogram << Back	NEXT	> Display the	⊖ Homology o	r 💿 Dis	stance				
The "Next" button validates your parameter and opens the following analysi B-Dendrogram Next >> C- Matching BACK C- Matching The "Back" button validates your parameter and opens the following analysi B-Dendrogram << Back A- Molecular weight OPTION FOLDER The option folder gathers the following functions: ⇒ Select the lanes ⇒ Help	NFXT								
B-Dendrogram Next >> C- Matching B-ACK The "Back" button validates your parameter and opens the following analys B-Dendrogram << Back A- Molecular weight OPTION FOLDER The option folder gathers the following functions: ⇒ Select the lanes ⇒ Help Help Help Help		t" button vali	dates vour i	parameter	and on	ens the	followin	ia analv	sis
BACK The "Back" button validates your parameter and opens the following analys B-Dendrogram << Back									
The "Back" button validates your parameter and opens the following analys B-Dendrogram << Back	B-De	endrogram		Next >>		C- Ma	atching		
The option folder gathers the following functions: ⇒ Select the lanes ⇒ Help			idates your		and op				sis
			oro the falls	owing func	tions:				
Select the lanes	 ⇒ Sele ⇒ Help ⇒ Save 	ect the lanes b e the analys		nplate					

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

Lane 1 Lane 2	Select all lanes Unselect all lanes	-
Lane 3		Close
Lane 4 Lane 5	You can add or remove the lanes to be displayed in the result table by selecting or	
	unselecting the lanes of the list	

HELP MENU

🕜 Help	

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:

Help	
	Index
	About UVIsoft

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:

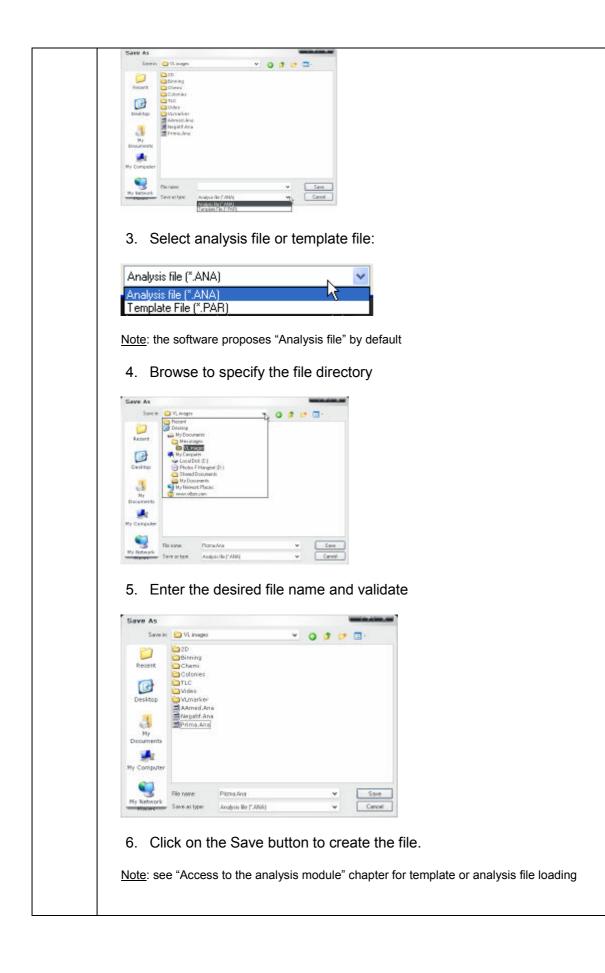
- ⇒ Time saving
- ⇒ Reproduction of image analysis parameters

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

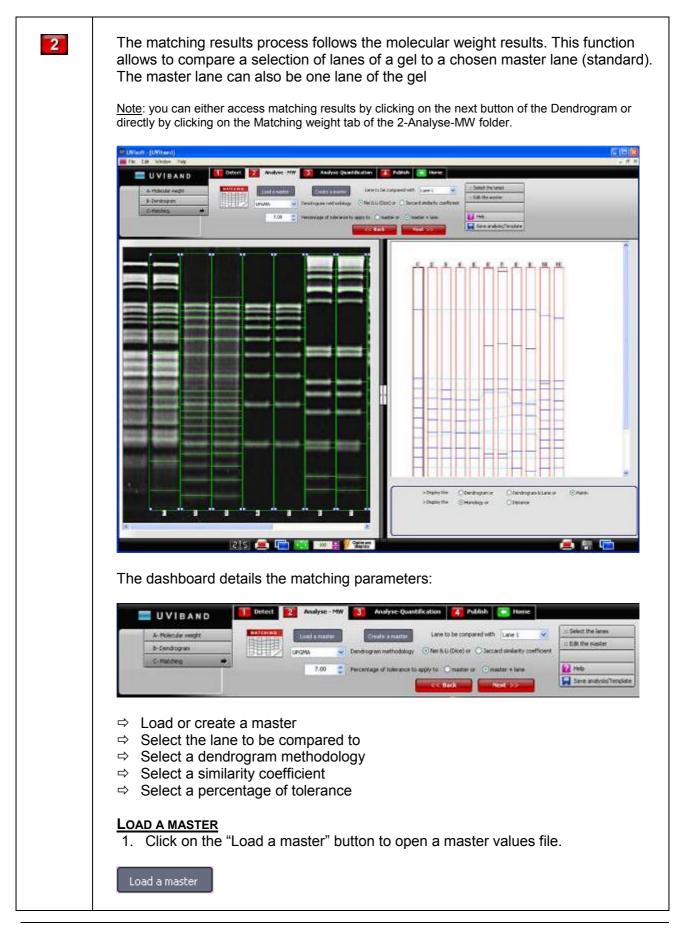
1. Click on the "Save analysis/ Template" button:

📑 Save analysis/Template

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



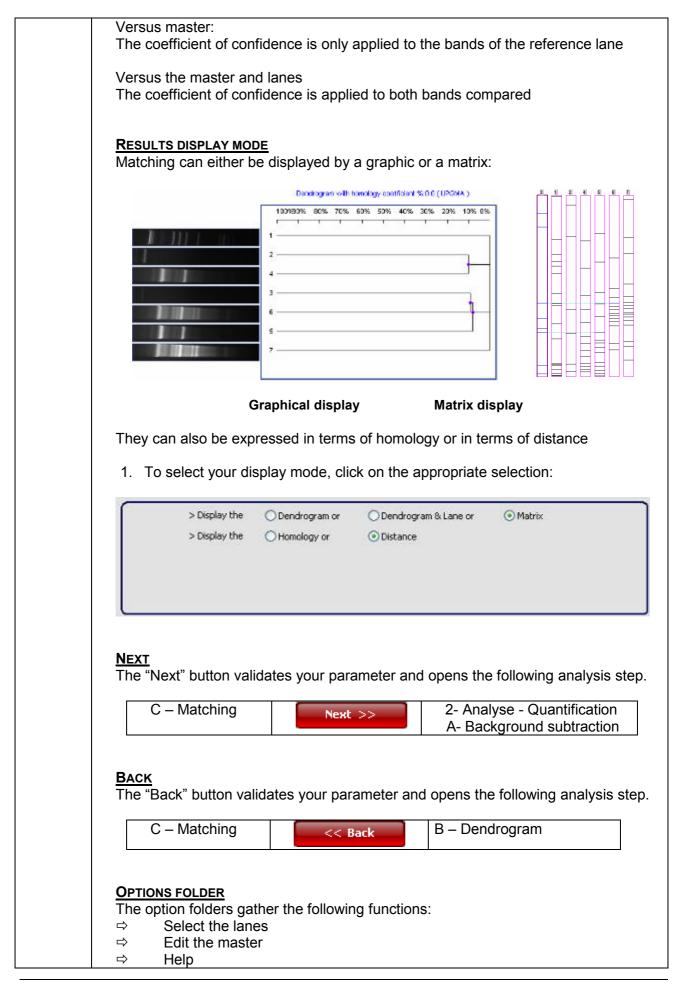
→ C – Matching



A pop-up window displays the following menu:	
Open Look in: O VL images V O S (*)	
Moning Initial Mike Recard Chemia Initial Mike Colonies Initial Mikes Initial Mikes Image: Colonies Image: Colonies Initial Mikes Image: Colonies Image: Colonies Initial Mikes Image: Colonies Image: Colonies Image: Colonies Image: Colonies Image: Colonies Image: Colonies	
With Network File name MW_Makes with Copen Hy Network Files of type: Markes Bes ("Mk) Cancel	
 ⇒ Browse to specify the marker directory ⇒ Double click on the file name you want to load 	
 <u>CREATE A MASTER</u> 1. Click on the "Create" button to create a new master set of values. 	
Create a master	
Value editor - Master Add value OK Delete value(s) Total bands	
Type your values, band to band, in a descending order. The OK button validate your data.	S
You can save your molecular weights master data and create your own master library; To proceed, click on the "Save " button:	S
Save	
A pop-up window displays the following menu:	
State State Some In VL magn V Some In VL magn V Some In Some In Induction In	
 ⇒ Browse to specify the directory ⇒ Type the file name and click on Save. 	

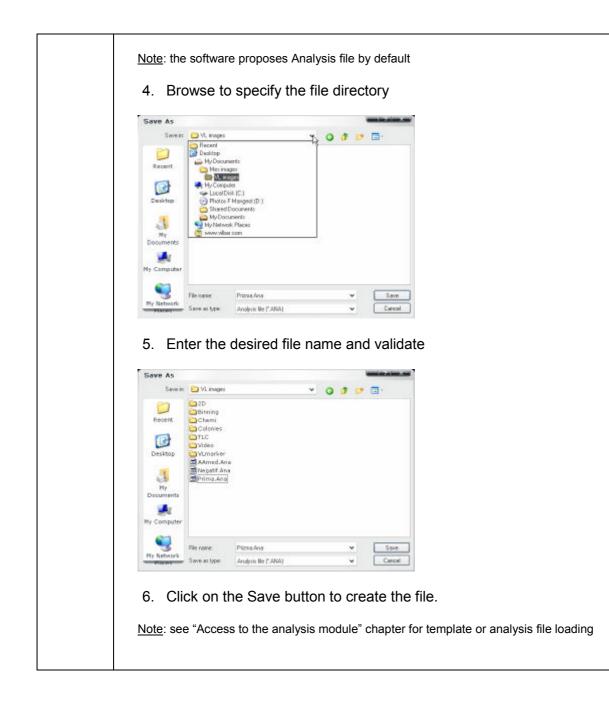
The master could				
⇒ An external r				
\Rightarrow Or a lane of t	ine image:			
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Lane 1				
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Lane 5				
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supports soveral	mothode of dondroaran			
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 ⇒ Unweighted F ⇒ Single Linkag ⇒ Complete Lin ⇒ Unweigthed A ⇒ Average Linkag ⇒ Average Linkag ⇒ Centroid ⇒ Median ⇒ Ward For single, complete the second of the sec	Pair Group Match Avera e kage Average Linkage age lete, unweighted average ds the general formula i (R, P) + B*d(R, Q) + E*	ge, average linka s as follows: d(P, Q) + G* d(F y (distance = 1-	R, P) - d(R, Q) Homology)	id, n
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⇒ Unweighted F ⇒ Single Linkag ⇒ Complete Lin ⇒ Unweigthed A ⇒ Average Linkag ⇒ Average Linkag ⇒ Median ⇒ Ward For single, comp and Ward method d (R, P+Q) = A*d Where: d(x, y) : distance A, B, E, G: are co Single linkage Complete linkage Average linkage (unweighted) Average linkage	Pair Group Match Avera e kage Average Linkage age lete, unweighted average ds the general formula i (R, P) + B*d(R, Q) + E* between 2 lanes x and onstants specific to the particular <u>A</u> 0.5 0.5 0.5 0.5	ge, average linka s as follows: d(P, Q) + G* d(I y (distance = 1- method used (se <u>B</u> 0.5 0.5 0.5 0.5 NQ/(NP+NQ)	R, P) - d(R, Q) Homology) ee array) Ee array) 0 0 0	-((

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:
UPGMA Vendrogram methodology
<u>SIMILARITY COEFFICIENT</u> Click in the heading Similarity coefficient to select either the Nei and Li (Dice) coefficient or the JACCARD coefficient
⊙ Nei & Li (Dice) or ○ Jaccard similarity 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 △ is a percentage directly read on the drawn curve of the marker: ⇒ The location of the band is then considered with ± △ around its value in Kb, in RF, or in KDa ⇒ The bigger the coefficient, the higher the number of matching bands and conversely
7.00 Percentage of tolerance
 The percentage of tolerance can be applied either: ⇒ Versus the master ⇒ Versus the master and lanes



⇒ Save the analysis or the template
:: Select the lanes
:: Edit the master
Save analysis/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.
:: Select the lanes
 A pop-up window displays the following menu on which you can select the lanes:
Lane selection
Lane 1 Lane 2 Lane 3 Lane 4 Lane 5 Vou can add or remove the lanes to be displayed in the result table by selecting or unselecting the lanes of the lst
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.
:: Edit the master
A pop-up window displays the following menu on which you can modify the master values:
Value editor - Master
Add value
Valuace add remove, and as a your master values:
You can add, remove, and save your master values;
HELP MENU
Pelp Help

	ick on the "Help" button. You automatically access the user manual at the apter corresponding to the function
Yo	ou can access the help file index through the File\Help from the Menu bar
н	elp Index About UVIsoft
Th	AVE ANALYSIS / TEMPLATE his function saves the current analysis. The analysis file will contain the results, e image and all the parameters defined to obtain the results.
Te as ev	he analysis could also be saved as a template for automated analysis routines. Emplate offers the user the ability to automate many of the repetitive tasks asociated with analysis and processing. As a result, you can spend more time raluating and analysing results, and less time manipulating set-ups, variables and her settings.
ree Yo	the template automates a task or set of tasks that you perform repeatedly or on a gular basis. It stores all the analysis commands and parameters of an analysis. Bu can run these parameters with another image whenever you need to perform new analysis based on the same parameters.
Th	ne benefits of the template file are as follows:
⇔ ⇔ ¢ wr	Time saving Reproduction of image analysis parameters Templates are modifiable, allowing the user to maintain an original template nile 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:
a D D	As As Constants Server V. Insper V O B O D - Server V. Subserver V O B O D - Server V. Subserver V. Subser
-	Poin number V Lune Reference® Analysis No. (* AAA) Careed Reference® Reference® Careed
3.	. Select analysis file or template file:
	nalysis file (*.ANA)
	nalysis file (*.ANA) K

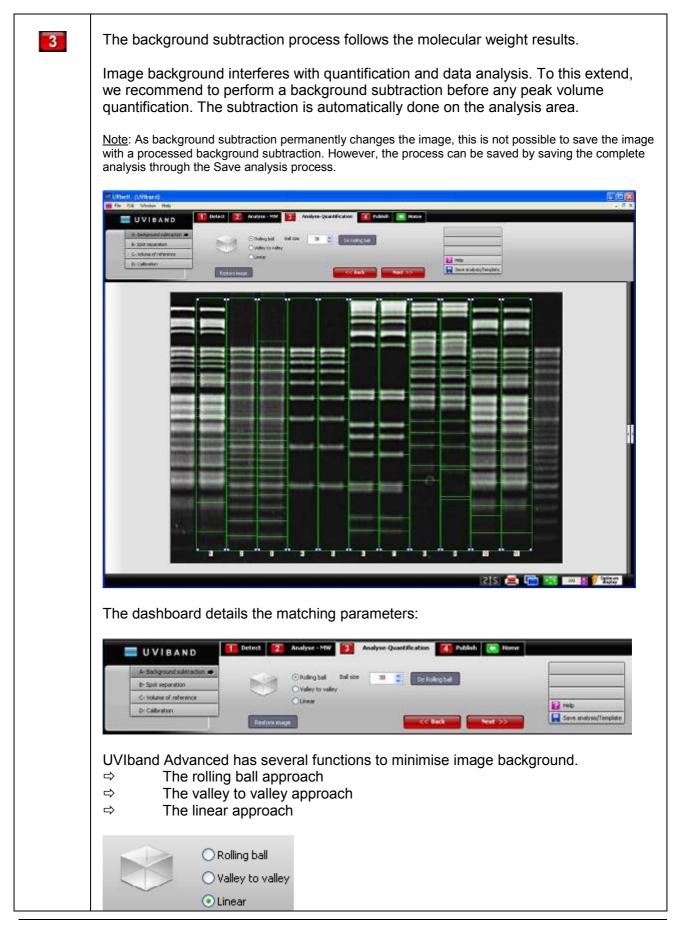


3- Analyse – Quantification

➔ 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). 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 = ∑ ni li 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 – Background subtraction



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.

\mathcal{X} $\wedge \mathcal{M}$		S
	AB	

The centre of gravity of the ball describes a curve:

 \Rightarrow This curve represents the noise to be subtracted.

 \Rightarrow 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:

🔾 Rolling ball	Ball size	38

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

Do Rolling ball

The changes will be automatically applied to the image.

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

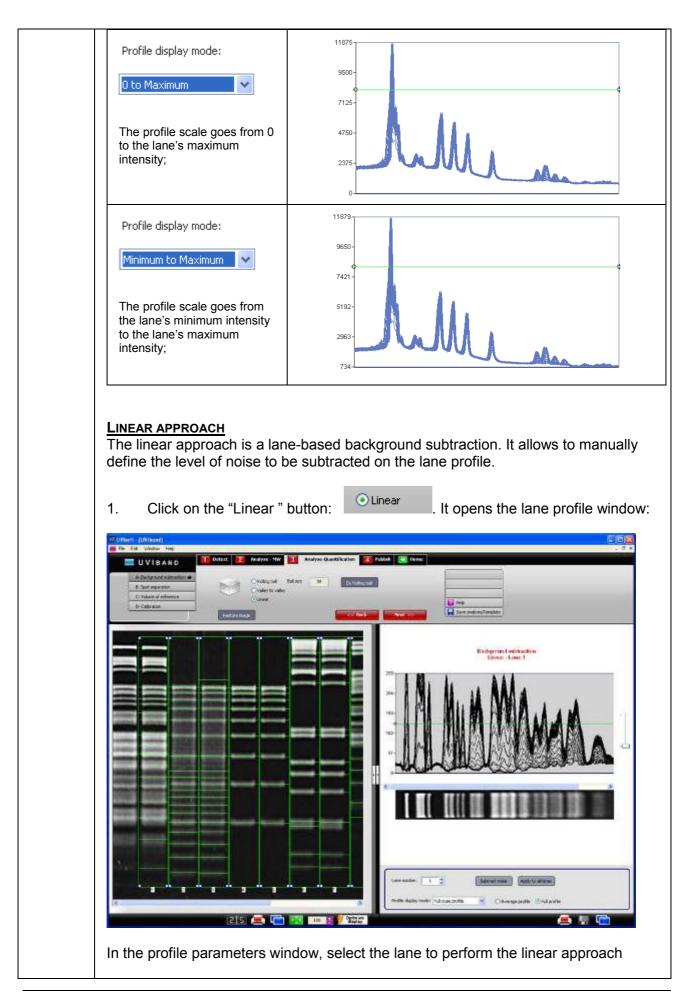
VALLEY TO VALLEY

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

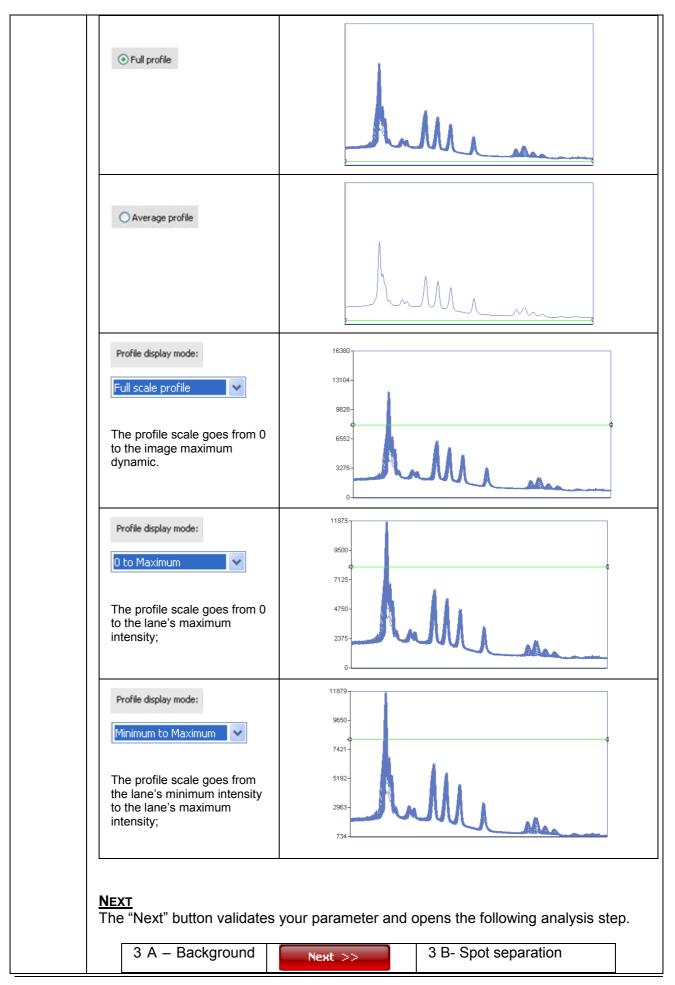
1. Click on the "Valley to valley " button: Valley to valley It opens the lane profile window:

Utherit UNitanel Par Ed: Webar Heis Order, 14 Order, 14
Contract noise in the second profile in t
On the profile, click to define the background profile you want to remove: $\int \int \int \int \int \int \int \int \partial f d d d d d d d d d d d d d d d d d d$
Then, click on Subtract noise: Subtract noise The changes will be automatically applied to the image and to the profile:

the same subtraction level for	h is a lane-based background subtraction. You can set or all lanes or specify an individual subtraction level for ges you make will be automatically applied to the image.
To apply the same subtractic button:	on level for all lanes, click on the "Apply to all lanes"
Apply to all lanes	
You can easily adjust the pro	ofile displays settings as follows:
O Full profile	Lulli
O Average profile	<u> </u>
Profile display mode: Full scale profile	16380 13104- 9828-
The profile scale goes from 0 to the image maximum dynamic.	



	ane number: 1 Subtract noise Apply to all lanes rofile display mode: Full scale profile Average profile Full profile
Then	he profile, click to define the background linear level you want to remove:
	tract noise
~	Julli
subtr	linear approach is a lane-based background subtraction. You can set the same raction level for all lanes or specify an individual subtraction level for the selected Any changes you make will be automatically applied to the image.
butto	
	can easily adjust the profile displays settings as follows:



	subtraction			
Bac The		es your parameter and c	opens the following analysis st	ep.
	3 A – Background subtraction	<< Back	2 A – Molecular weight]
	Help	the following functions: is or the template		
	Help Save analysis/Template			
Clic	<u>P MENU</u> k on the "Help" button. responding to the funct Help		ess the user manual at the cha	apter
	-	ile index through the Fil	e\Help from the Menu bar	
This			lysis file will contain the result e results.	s, the
Ten ass eva	nplate offers the user t ociated with analysis a	he ability to automate m ind processing. As a res	or automated analysis routines nany of the repetitive tasks sult, you can spend more time anipulating set-ups, variables	
regi You	ular basis. It stores all	the analysis commands eters with another image	it you perform repeatedly or or and parameters of an analysi whenever you need to perfo	S.
The ⇒ ⇒ ⇒	-	image analysis parame	eters 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:
Savet Savet Savet
3. Select analysis file or template file:
Analysis file (*.ANA) Analysis file (*.ANA) Template File (*.PAR)
Note: the software proposes Analysis file by default
4. Browse to specify the file directory
Bases At Soften Soften Compare Soften Soften
5. Enter the desired file name and validate
Sover AL Soveral Soveral Soveral Basers Soveral Basers Soveral Codesis Soveral Basers Soveral Codesis Soveral Basers Soveral Codesis Soveral Soveral Soveral Sov
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

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

 \Rightarrow To define the boundary around the spot;

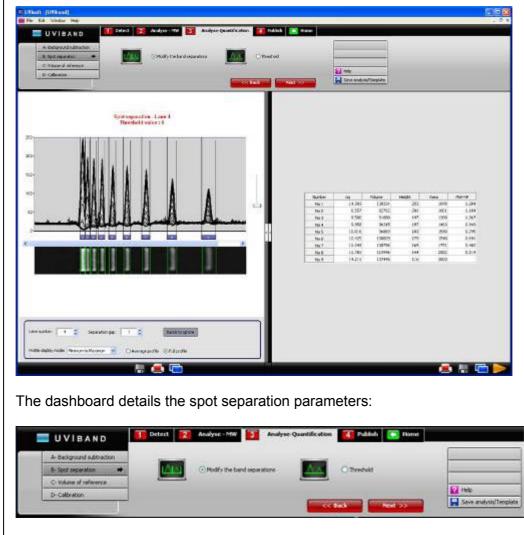
3

 \Rightarrow 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.

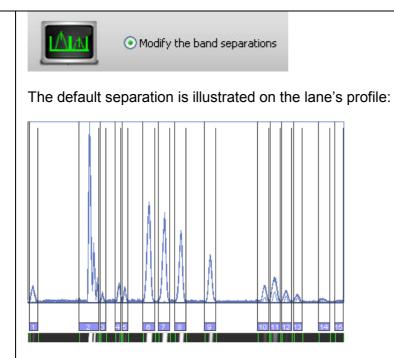
<u>Note</u>: you can either access the spot separation function by clicking on the next button of the background subtraction or directly by clicking on the spot separation of the Analyse-Quantification folder.



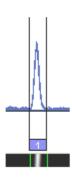
- → Modify the spot separation
- ⇒ Standard threshold
- ⇒ Extended threshold

MODIFY THE SPOT SEPARATION

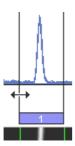
UVIband Advance proposes by default an automatic predefined spot separation based on the band detection. You can modify the default spot separation by selecting the "Modify the spot separation" option.



The 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:

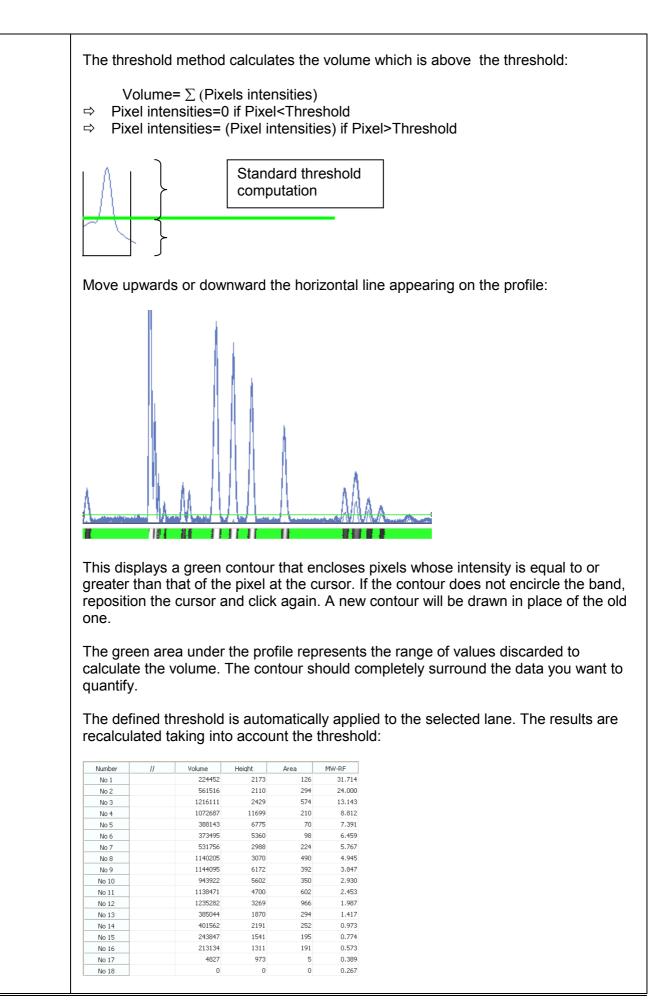


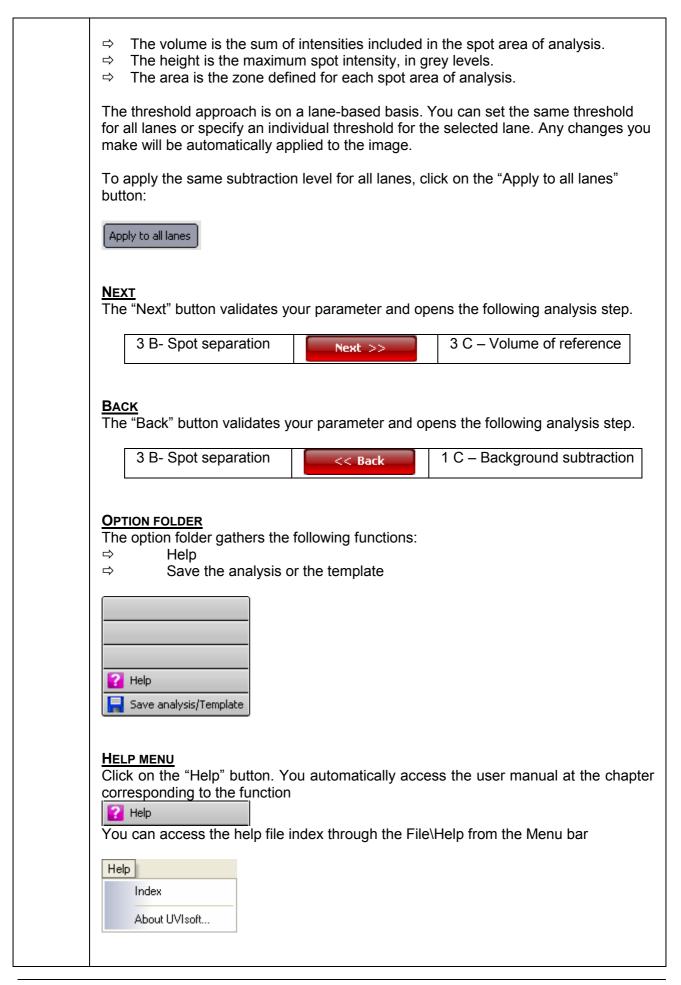
Drag the cursor until the area of the band that you want to define has been completely enclosed.

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:

Lane number: 2 Separation gap: 0 Profile display mode: Minimum to Maximum	Bands to ignore
Then, click on the band you want to ignore:	
The band is then highlighted in grey and dis	carded from the result table:
<u>Note</u> : you can ignore more than one band at a time. <u>Note</u> : to stop the process, click again on the "Bands to	o ignore" button.
To increase the gap in between the lane, se profile's parameter menu:	lect the "Separation gap" option from the
1 2 3	
Limited separation gap	Extended separation gap
DEFINE A THRESHOLD The threshold defines the detection level to quantification. It allows to distinguish between Case when you should use detection level	en bands and smears on the lane.
There is still a strong background even after	
AAAAAAA AAAAAAAA	AAAAAA A AAAAAA
Original Image	Image with subtracted background
The spot contours must be isolated more pro located	ecisely from the smears where they are





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:

 \Rightarrow 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:

Save analysis/Template

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

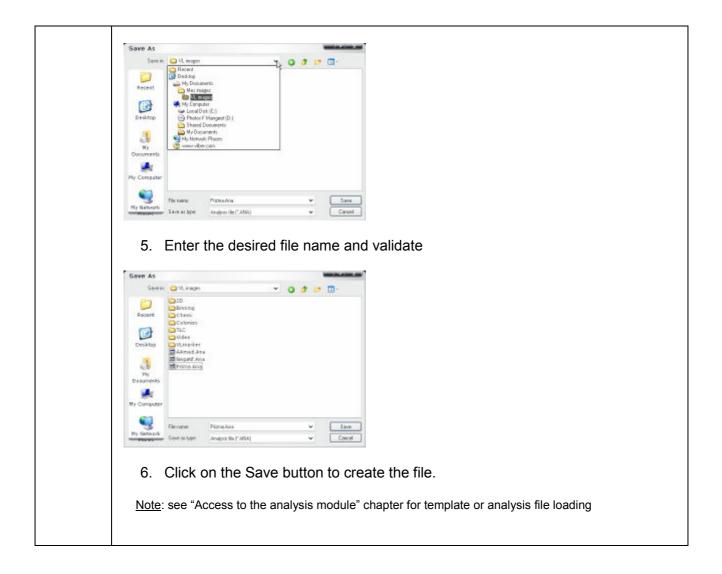
Hecent Desktop	VL Integer 2D Celoraing Calorated TLC Webe ULmarker Alavael Are		v	0	đ 1		10
Recent Desktop	Ethening Chenii Calonieil TUC Videa Vutnatker						
Ph Decements	Rogert Ann Frithe Ann						
in Careadar							
	De name				¥	E	Saw
Ha Network	inve outupe	Analysis for I	_	_	2		Casel

3. Select analysis file or template file:

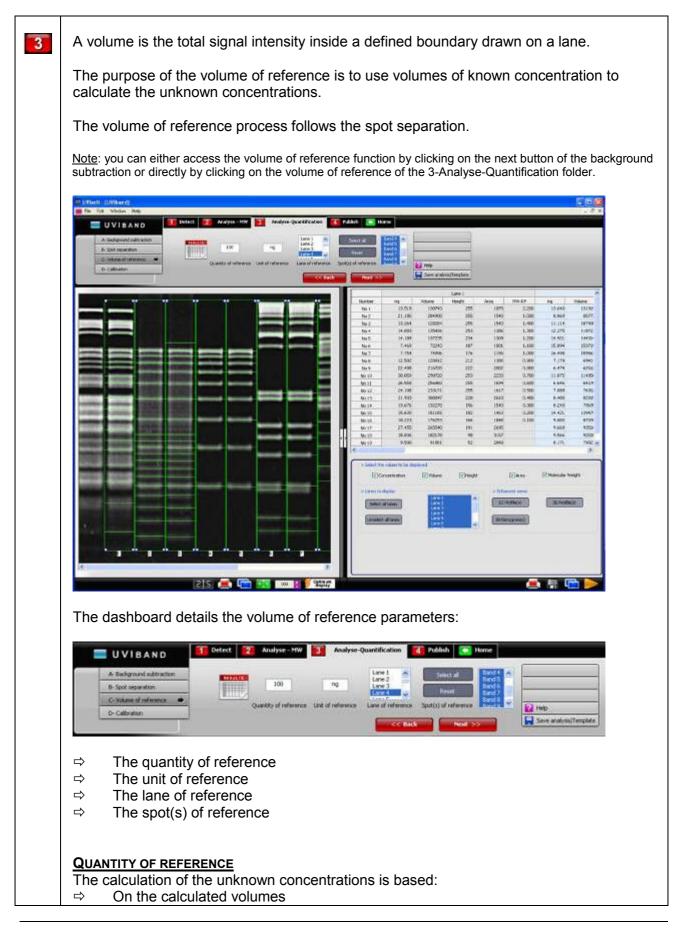
Analysis file (*.ANA)	N V
Analysis file (*.ANA)	1
Template File (*.PAR)	

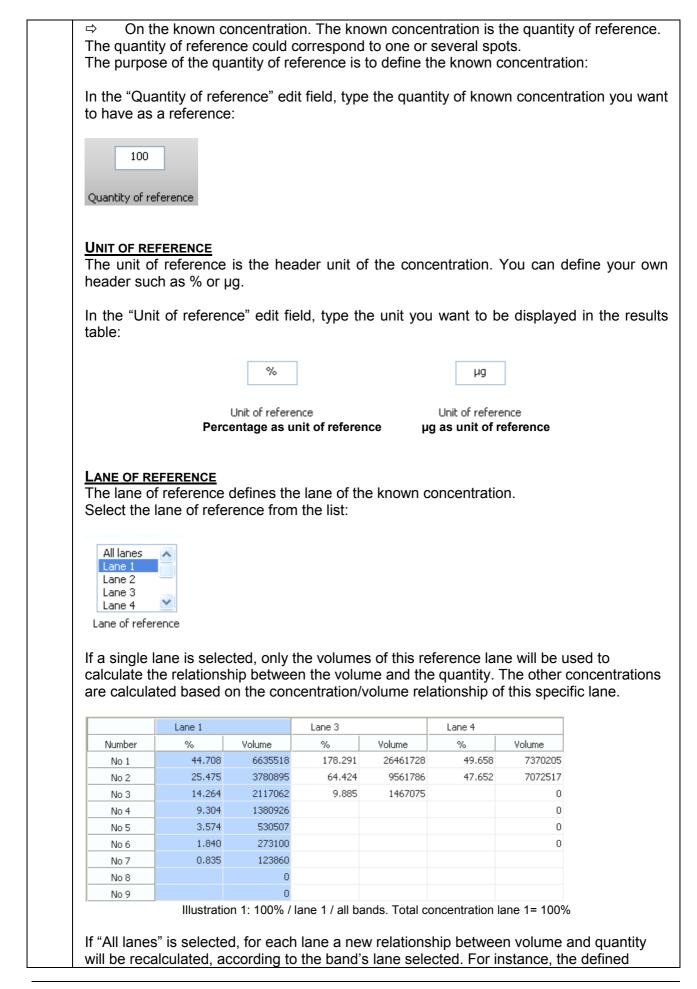
Note: the software proposes Analysis file by default

4. Browse to specify the file directory



➔ C – Volume of reference





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:

Band 1	~
Band 2	
Band 3	
Band 4	_
Band 5	
Band 6	<u> </u>

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:

RESULTS		All lanes	Select all	Band 1 🔺 Band 2
100	μg	Lane 2 Lane 3 👽	Reset	Band 3 Band 4 Band 5
Quantity of reference	Unit of reference	Lane of reference	Spot(s) of reference	Band 6

Number	μq	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

The results table indicates the following for lane 3:

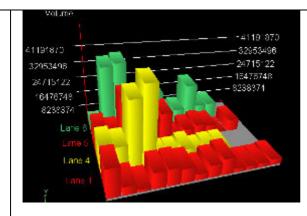
EXAMPLE 2

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

RESULTS	100	%	All la Lane Lane	2	Select all Reset	Bar Bar Bar Bar
	uantity of referen				5pot(s) of referenc	e Bar
	able indicates					
Number	3.978	Volume 1715709	Height 2744	Area 781	MW-RF 9.896	
No 1 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	
BACK	/olume of refe		Next >>		- Calibration	
BACK The "Back" I		es your para		pens the foll	 Calibration lowing analysi Spot separati 	
BACK The "Back" b 3 C - \ 3 C - \ A RESULT TAB In the result in the result in the result ⇒ Concent ⇒ Concent ⇒ Volume ⇒ The max ⇒ The area	outton validate /olume of refe parameter wi s tables: ration	es your para erence	meter and op << Back	pens the foll	lowing analysi	is step
BACK The "Back" B 3 C - V RESULT TAB In the result in the result \Rightarrow Concent \Rightarrow Concent \Rightarrow Volume \Rightarrow The max \Rightarrow The area \Rightarrow The mole	outton validate /olume of refe parameter wi s tables: ration imum intensi	es your para erence ndow, you ca	meter and o << Back	bens the foll	lowing analysi Spot separati	is step
BACK The "Back" to 3 C - N 3 C - N Concent \Rightarrow The results \Rightarrow Concent \Rightarrow Concent \Rightarrow Concent \Rightarrow The max \Rightarrow The max \Rightarrow The mole 1. To sel	button validate /olume of refe parameter wis tables: ration timum intensit ecular weight ect your disp	es your para erence ndow, you ca ty lay mode, cli	meter and o << Back	bens the foll	lowing analysi Spot separati	ion be disp

	> Select the values to be d	isplayed			
	Concentration	Volume	🗹 Height	🗹 Area	Molecular Weight
;	> Lanes to display Select all lanes Unselect all lanes	Lane 1 Lane 2 Lane 3 Lane 4 Lane 5 Lane 6		> Enhanced views 1D Profile(s) 3D histogram(s)	3D Profile(s)
					view: Move the mouse cur ease the mouse when satis
_	proceed, click or				superimposed.
9	80 104 9828 5552	-	18280 13104 9828 6552 3276		
	3273 Faire 2 Lane 3 Lane 3 Lane 2 Lane 1				
	> Lanes to display Select all lanes Unselect all lanes	Lan Lan Lan Lan Lan	e2 e3 e4		

3D Profile(s)
18000 12900 8000 8100 900
Select all lanes Lane 1 Lane 2 Lane 2 Lane 3 Lane 4 Lane 5 Lane 6
<u>Note</u> : 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. <u>Note</u> : 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. <u>Note</u> : Click on Print to print the 1D profile window <u>Note</u> : 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:
3D histogram(s)
Select all lanes Unselect all lanes Lane 4 Lane 5 Lane 6



<u>Note</u>: 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. <u>Note</u>: 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

🛜 Help

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

Help	
	Index
	About UVIsoft

SAVE ANALYSIS / TEMPLATE

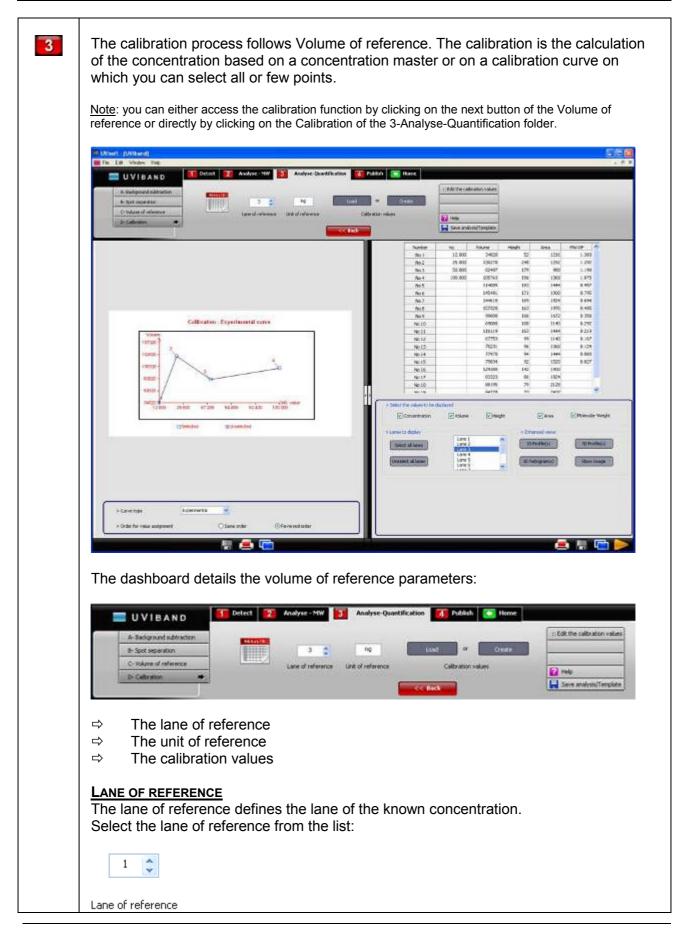
This function saves the current analysis. The analysis file will contain the results, the image and all the parameters defined to obtain the results.

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.

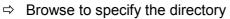
The template automates a task or set of tasks that you perform repeatedly or on a regular basis. It stores all the analysis commands and parameters of an analysis. You can run these parameters with another image whenever you need to perform a new analysis based on the same parameters.
The benefits of 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:
Save As Save 🕲 Ourigen - 🗢 🕒 😰
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Votes Lexing Water Lexing Water Wate
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Ny Computer Ny Norman Computer (1992) Ny Ny Norman Computer (1992) Ny Ny N
Temporar Fair 2000
3. Select analysis file or template file:
Analysis file (*.ANA)
Analysis file (*.ANA)
Note: the software proposes Analysis file by default
4. Browse to specify the file directory
Save As Save O'Longe State Control Con
Recent Contraction
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The carrier and the com
Statework Far runne Parmation Same The formula Parmation Cancel Cancel
5. Enter the desired file name and validate

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Ne Arbury	Do name:	Pichailea Andre Br(*2001	Constitutions?	
	- Save at tipe	Analysis Br (* 2005)	Earosi	to create the file.

➔ D – Calibration



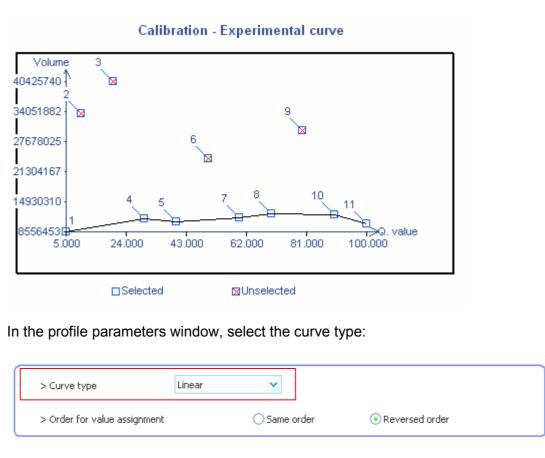
<u>UNIT OF REFERENCE</u> The unit of reference is the header unit of the concentration. You can define your own header such as % or μ g.
In the "Unit of reference" edit field, type the unit you want to be displayed in the results table:
γ μg
Unit of referenceUnit of referencePercentage as unit of referenceµg as unit of reference
THE CALIBRATION VALUES 1. Click on the "Load" or "Create" button to enter calibration's values.
Load or Create
For "Create", a pop-up window displays the following menu:
Value editor - Master Value editor - Master Add value Coccentration Add value Cancel Cancel Total bands Save Cancel Cancel Coccentration Cancel Coccentration
Type your values, band to band, in a descending order. The OK button validates your data.
<u>Note</u> : 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:
Save
A pop-up window displays the following menu:
Sava As Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Income Incom



⇒ 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



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)

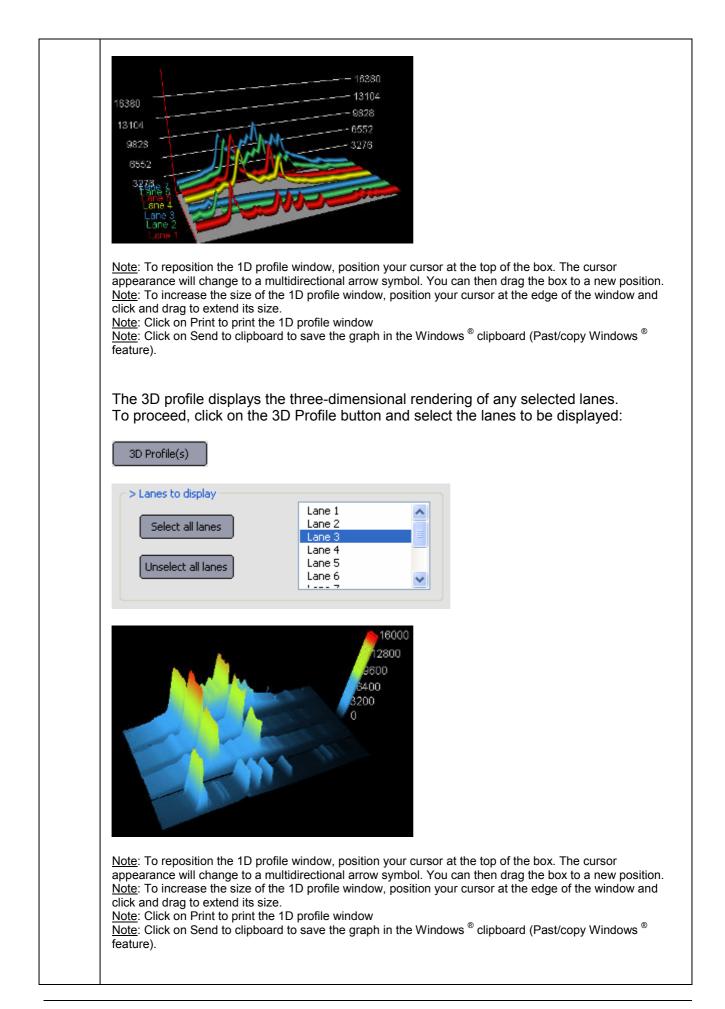
⇒ Logarithmic curve: displays a model with logarithmic regression

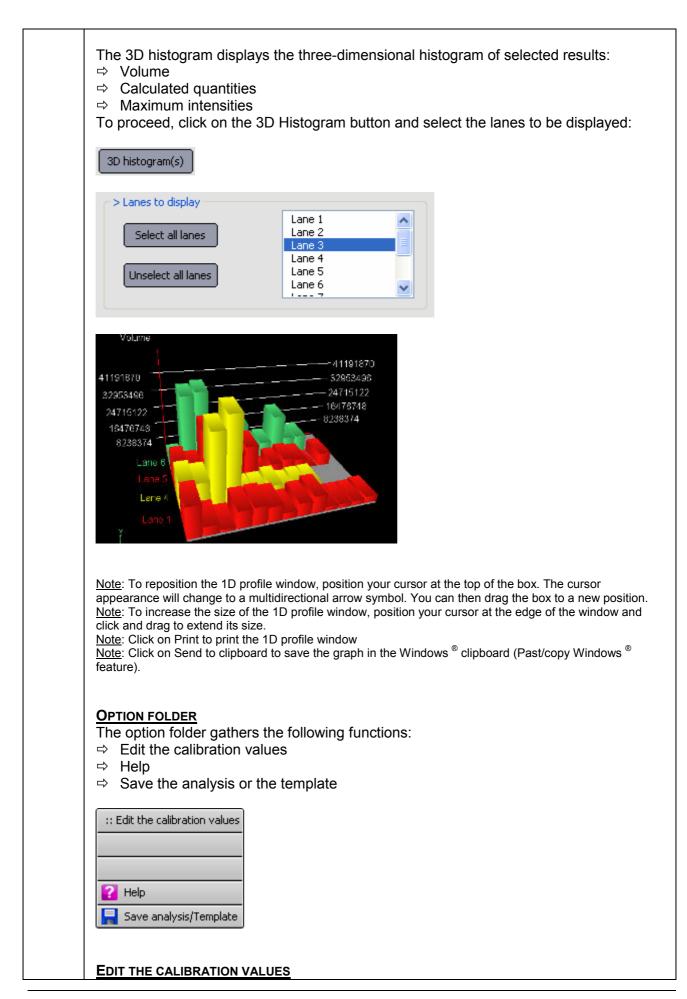
Linear	*
Experimental	
Linear	
Smooth	
Logarythmic	~

You can also select the order for the spot display:

- ⇒ Same order as the values of the master-curve
- \Rightarrow Reversed order (depending on the order of the defined values)

R	ESULT TABLE				
In	the result parameters the result parameters and the result splayed in the result splayed		ou can select the	lanes and the	e values to be
⇒	Concentration				
合合	The maximum inte	ensity			
合合		eiaht			
		-	al on the energy	riata aclastia	
	To select your disp	•			1.
ſ	> Select the values to be disp Concentration	Volume	🔽 Height	🗸 Area	Molecular Weight
		Volume			
ſ	> Lanes to display	Lane 1		nhanced views	
	Select all lanes	Lane 2 Lane 3 Lane 4		1D Profile(s)	3D Profile(s)
		Lane 4			
	Unselect all lanes	Lane 5 Lane 6	3) histogram(s)	Show Image
	Unselect all lanes) histogram(s)	Show Image
	RAPHICAL VIEW the results parame 1D profile 3D profile	Lane 6			
In 分 分	RAPHICAL VIEW the results parame 1D profile 3D profile	ter window, y			
In 分 分	RAPHICAL VIEW the results parame 1D profile 3D profile 3D histogram	ter window, y			
In 分 分	RAPHICAL VIEW the results parame 1D profile 3D profile 3D histogram	ter window, y	vou can select the	e graphical re	sults tables:
In 分 分	RAPHICAL VIEW the results parame 1D profile 3D profile 3D histogram > Select the values to be dis	Lane 6 ter window, y played Volume Lane 1 Lane 2	vou can select the	e graphical res	sults tables:
In 分 分	RAPHICAL VIEW the results parame 1D profile 3D profile 3D histogram > Select the values to be dis Concentration > Lanes to display Select all lanes	Lane 6 Lane 7 ter window, y volume Volume Lane 1 Lane 2 Lane 4 Lane 5	vou can select the	e graphical res	sults tables:
In 分 分	RAPHICAL VIEW the results parame 1D profile 3D profile 3D histogram > Select the values to be dis Concentration > Lanes to display	Lane 6 ter window, y played Volume Lane 1 Lane 2 Lane 3 Lane 4	vou can select the	e graphical res	sults tables:





Click on the "Edit the calibration values" buttor

:: Edit the calibration values

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

547W		Value editor	r - Master	
K-			Add value	OK
100.000 90.000				-
80.000			Delete value(s)	Cancel
70.000		н —		
60.000	Total bands	6		
50.000		1	Save	2

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

Index

About UVIsoft...

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:

Save analysis/Template

A pop-up window displays the following menu:
Same A
Mr Bernerki Sove an Upper Proceeding of the Context Sove an Upper Proceeding of the Context Sove and Upper Comptone Prior P
Select analysis file or template file:
Analysis file (*.ANA)
Note: the software proposes Analysis file by default
Browse to specify the file directory
Save Ar Sever A Sever A Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Protect Pro
Phy Consuder Presentations Consent Phy Consuder States Inter States Consent Phy Consent States Inter States
Enter the desired file name and validate
Same A Mathematical A Same A Mathematical A Same A Mathematical A Same A Same
We have: Picroscie Picroscie Same Encode Mr Notwork Some strippe Anagoss lis (*Akk) Concel
Click on the Save button to create the file.
Note: see "Access to the analysis module" chapter for template or analysis file loading

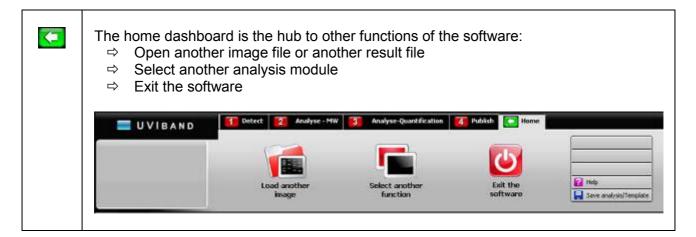
Publish

➔ Introduction

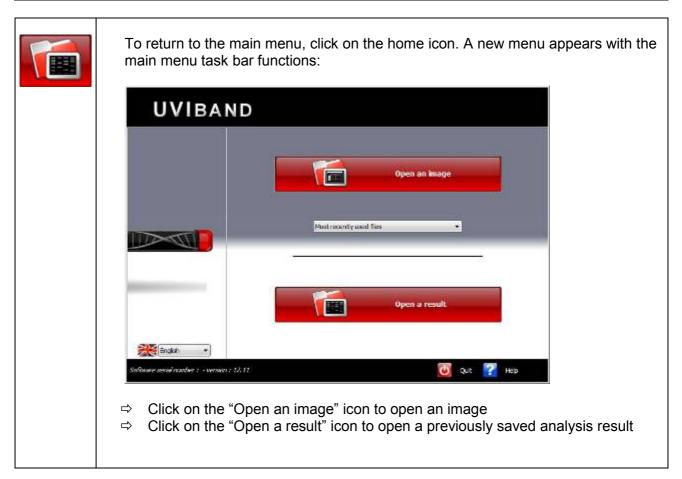
4 The purpose of the Publish function is to prepare a printed report of your results. You can easily organise your report with titles and comments and your own selection of data to be published among the following: - Sample image - Molecular weight marker's data and migration curve - Molecular weight result table - Dendrogram results - Lane matching results - Quantification result table To proceed, select the Publish tab. A pop-up window displays the following menu: Bub MM as Report Side opiation. 2 Cittle Crimines Ke Depásoran Druthing 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 Proveties ERSON Dates ("This loss [Prints 06 Canoel ⇒ 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

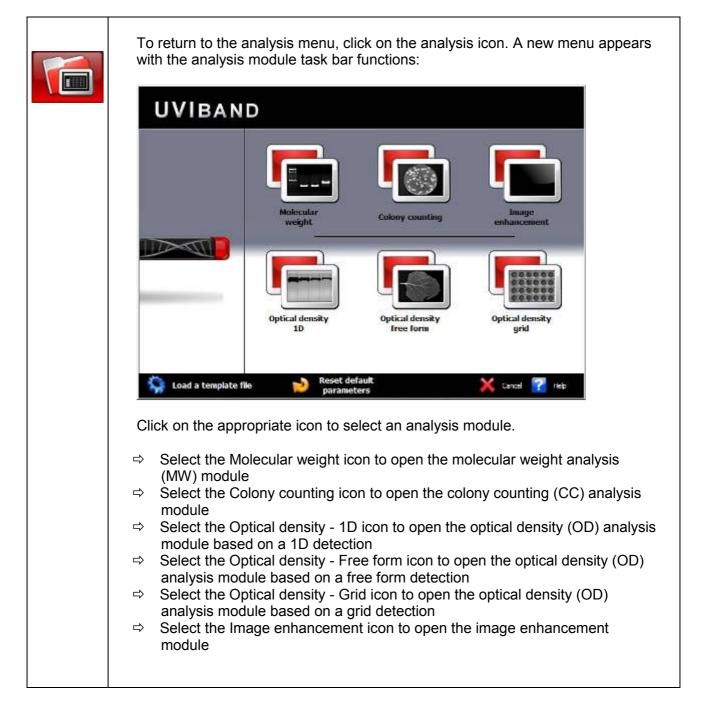
➔ Introduction



➔ Load another image



Select another function

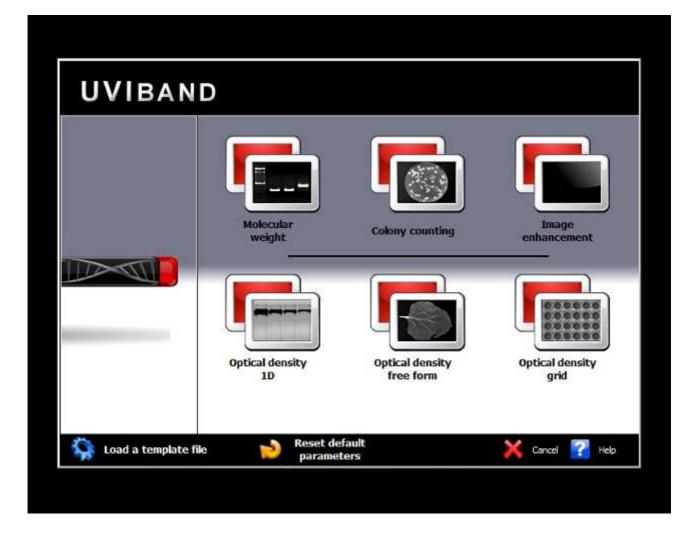


➔ Exit the software

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

You will be prompted to save your analysis.

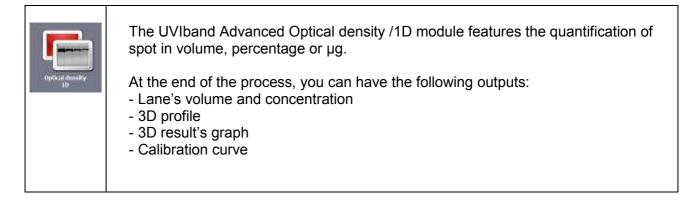




Optical density – 1D → OD-1D Analysis module

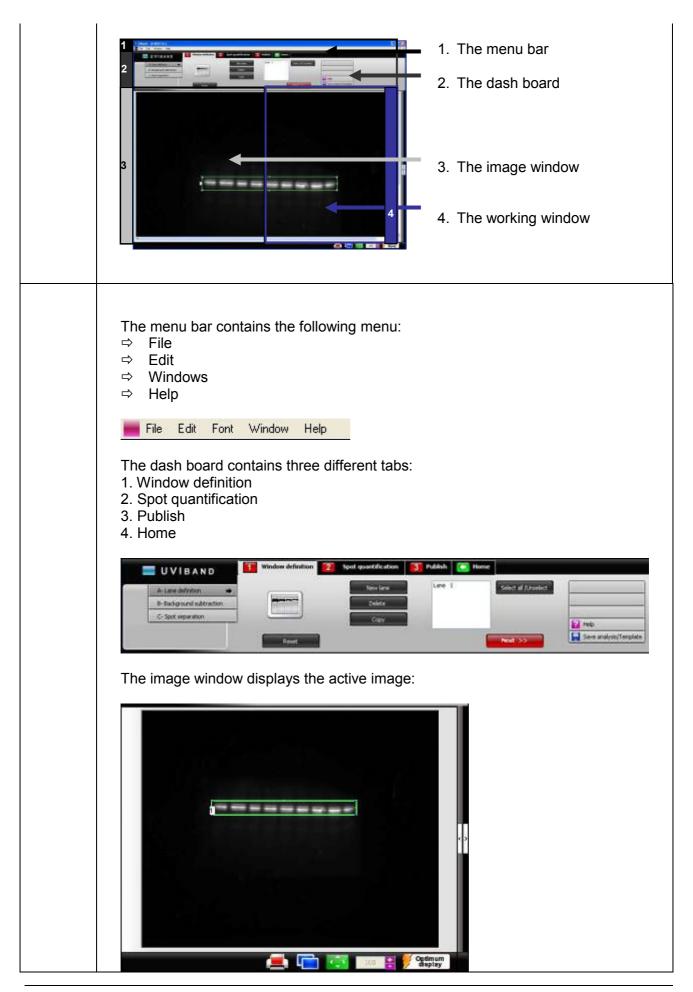
Optical density / 1D introduction

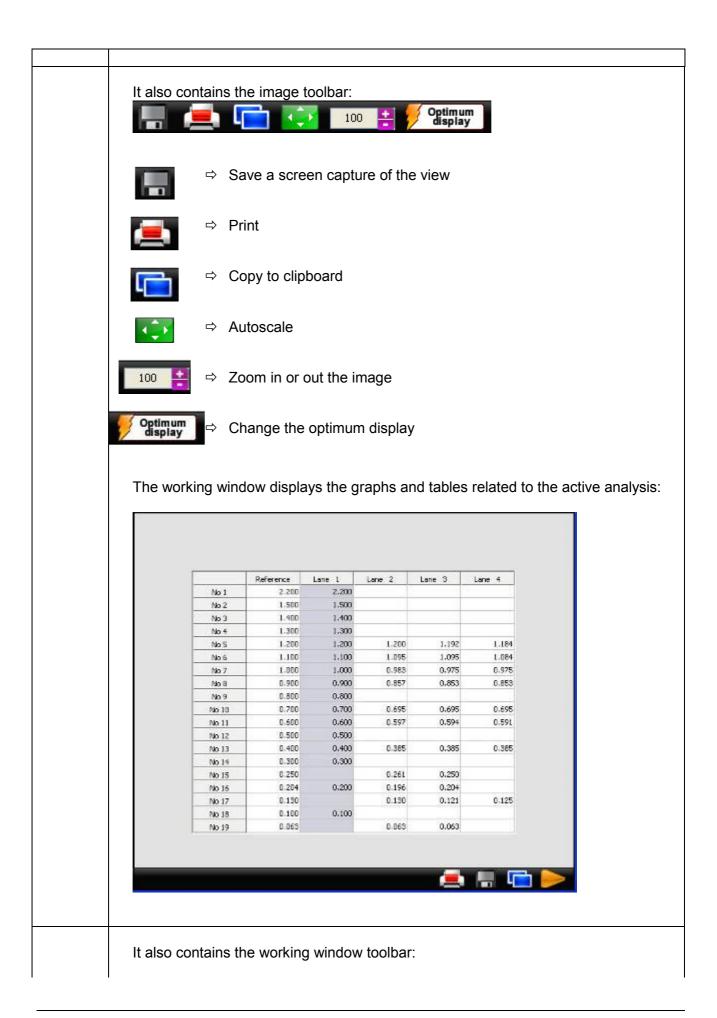
Objectives and output

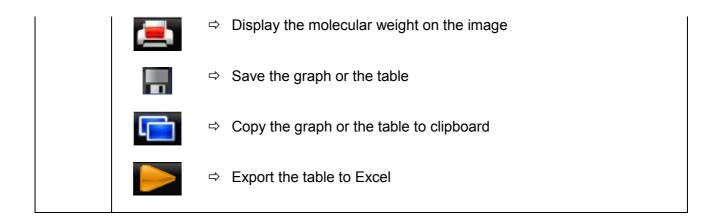


➔ Optical density / 1D (OD-1D) operating environment

	The OD-1D module opens on the following window:
Optical formity	C Urfuett - (C SSSTEL) Image: Comparison of the state of the st
	The UVIband Advanced operating environment is organised into four areas:







➔ Toolbar in details

T

 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. 						
Prize Previous Next Tree Project Dage(s) Dege(s) Close						
Click on Print to validate the preview. A pop-up window displays the following menu:						
Print Print Name EFSON Sylue C10 Server Subur Ready Type EPSON Sylue C10 Server Where USB01 Connext Prest to the Print range Copies OK Cancel						

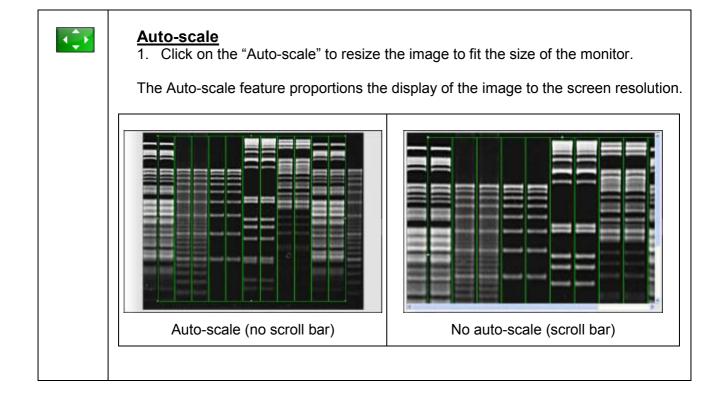
 ⇒ Select a printer ⇒ Click on Properties to modify the default setting of the printer, if necessary ⇒ Select the number of copies ⇒ Click on OK to validate your options
Note: You can also access the Print menu from the Menu bar (File\Print).

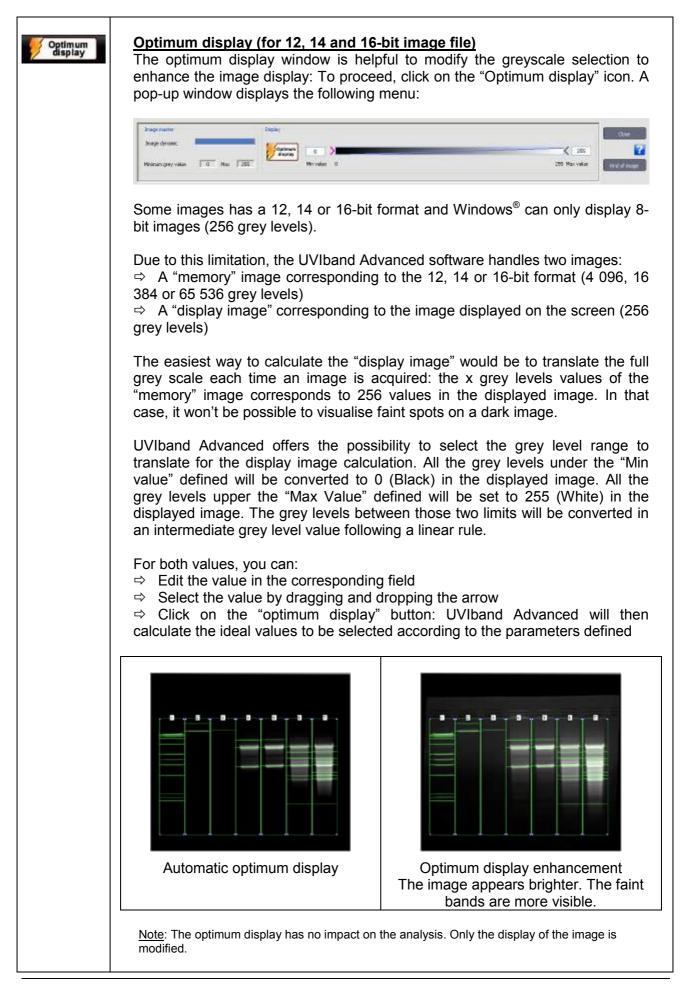
Г

Τ

<u>Save</u> 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:
Same II. Millingen Some II. 2D Brootet Columbus Columbus Columbus Columbus Columbus Observedte Columbus Prootet Columbus
Pro Computer Pro Matroork Pro Matroork Case as type Excerting (201) Text for (201) Text for (201) Text for (201) Text for (201)
3. Browse to specify the file directory
He Computer He Computer He Retwork He Retwork Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service Service
4. Enter the desired file name, select a file extension and validate <u>Note</u> : the results could also be saved in a text file format or a Dbase file format:
Excel file (*.XLS) Excel file (*.XLS) Text file (*.TXT) DBase file (*.DBF)
The graphs can only be saved on a BMP format: Bitmap file (*.BMP) Bitmap file (*.BMP)

<u>Copy to clipboard</u> 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).
available pasting options ([Ctrl V] command for Windows [®] software).



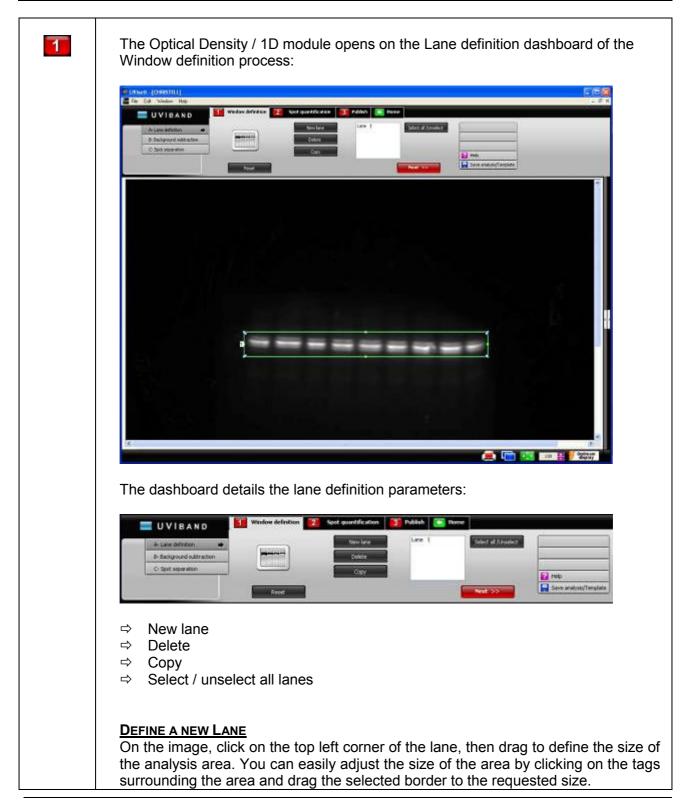


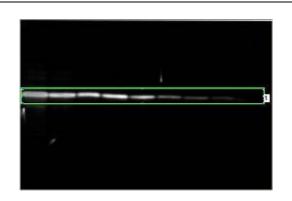


Send to Excel[™] This function transfers the results table to Windows Excel[™].

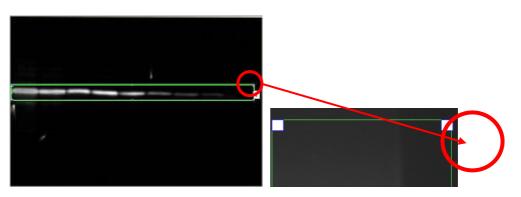
To proceed, click on the Send to Excel^{TM} icon. The Excel software is automatically opened by the UVIband Advanced and the table is transferred to Excel^{TM} .

➔ A – Lane definition



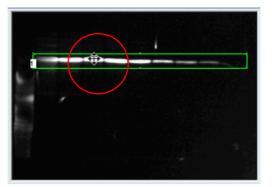


The lane is defined by green lines, overlaid on the image. The area is surrounded by square anchors:



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.

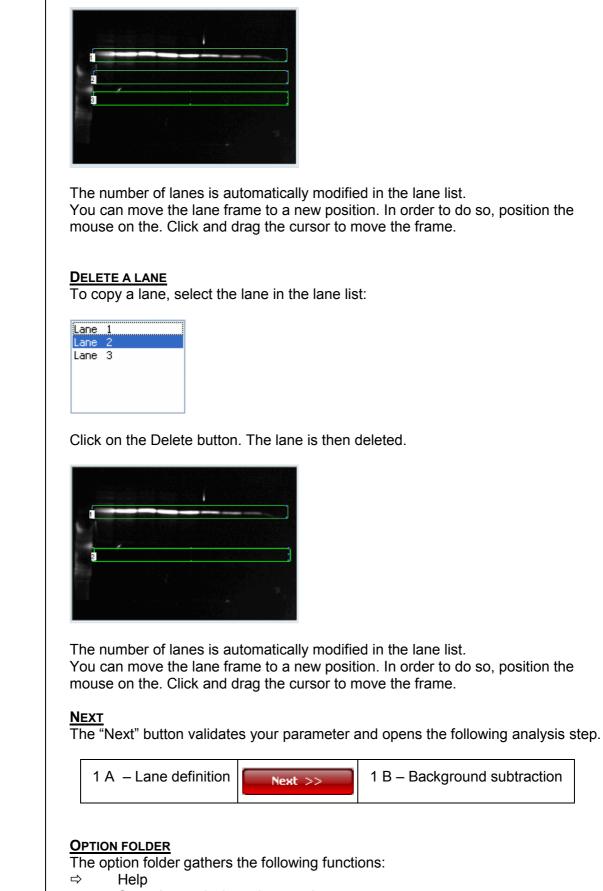
To move the entire frame to a new position, position the mouse on the frame to obtain a cross cursor: Click and drag the cursor to move the entire frame.



<u>COPY A LANE</u> To copy a lane, select the lane in the lane list:

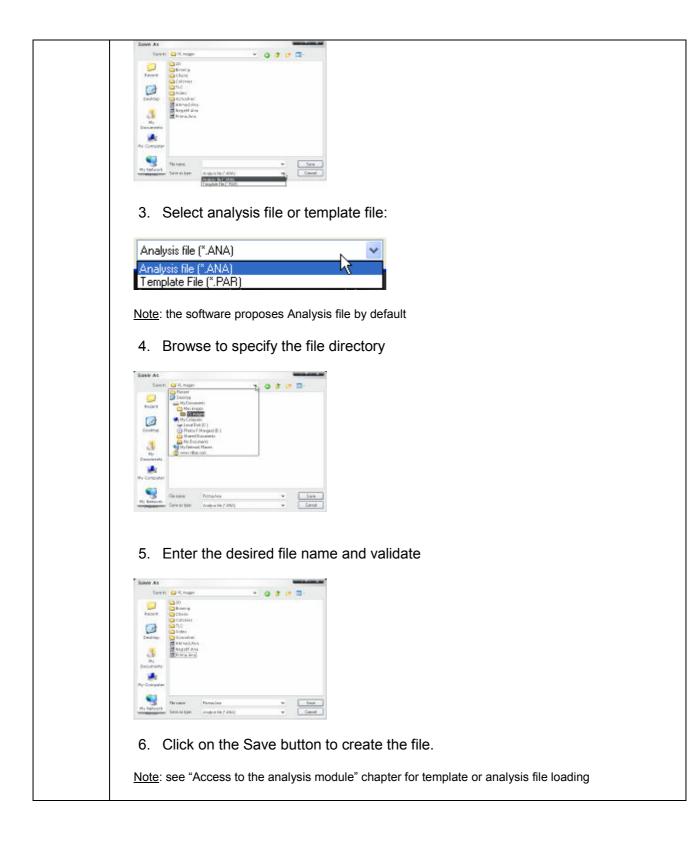
Lane 1

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

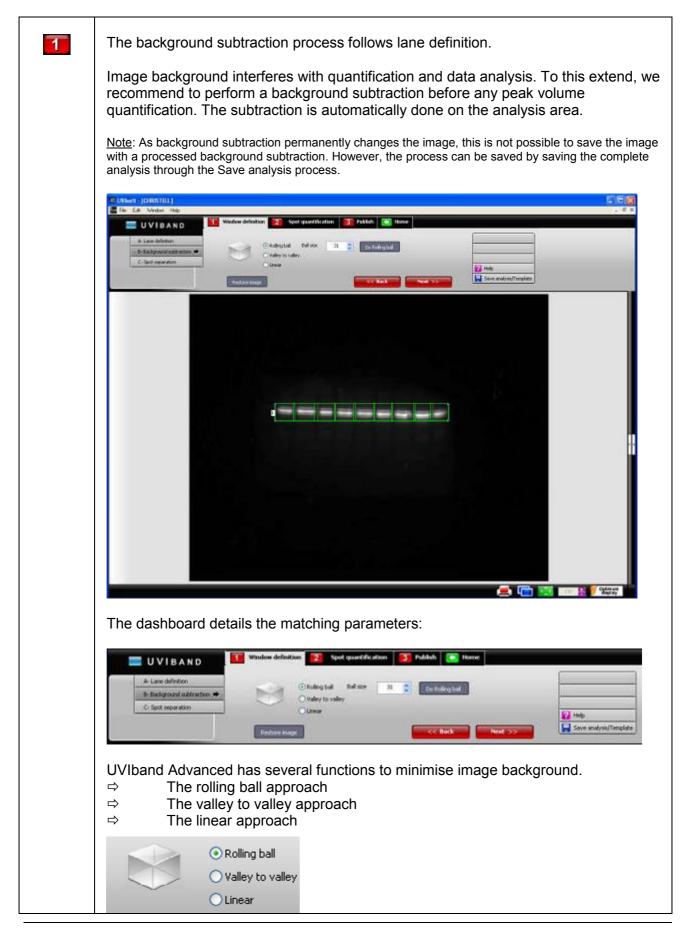


 \Rightarrow Save the analysis or the template

Help
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:
Help Index About UVIsoft
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:
Save analysis/Template
2. A pop-up window displays the following menu:



➔ B – Background subtraction



ROLLING BALL

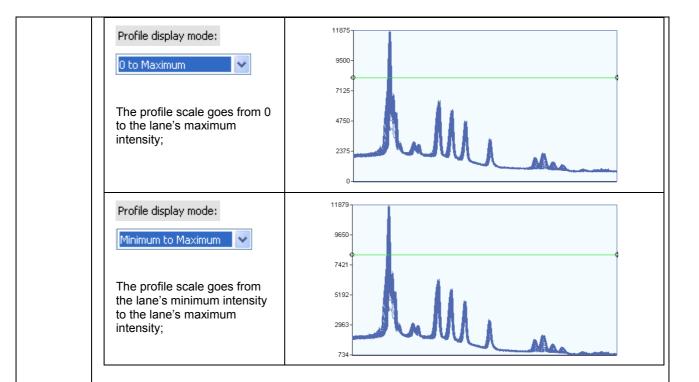
The rolling ball method is named for a hypothetical ball that rolls along underneath the lane profile, removing different intensity levels along the length of the lane.

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

Andrada
Rolling ball Background subtraction
 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:
O Rolling ball size 31 S1 S S1 S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S
To process the rolling ball background subtraction, click on the "Do rolling ball" button:
Do Rolling ball
The changes will be automatically applied to the image.
Note: few seconds could be necessary to perform the background subtraction.
<u>VALLEY TO VALLEY</u> 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:
O Valley to valley
It opens the lane profile window:

© Uywan: (DBISHII) ■ Fis. tot: / Wrdos: Help
UVERAND Senderschelles Zept quantification A Lass similars Consequence of lations Consequence of lations
Background subtraction Valley to valley - 8 zme 1
 In the profile parameters window, select the lane to perform the valley-to-valley approach
Lane number: 1 Subtract noise Apply to all lanes Profile display mode: Full scale profile Image: Average profile Image: Full scale profile
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 subtracti button:	on level for all lanes, click on the "Apply to all lanes"				
Apply to all lanes					
You can easily adjust the pr	ofile displays settings as follows:				
⊙ Full profile	Lulle man				
O Average profile	Anton				
Profile display mode: Full scale profile	16380				
The profile scale goes from 0 to the image maximum dynamic.	6552- 3276-				

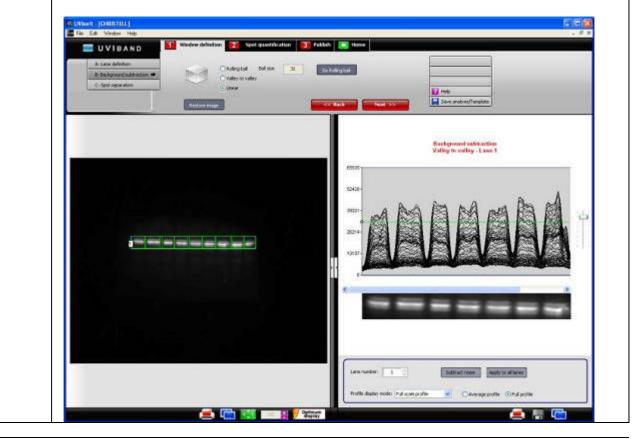


LINEAR APPROACH

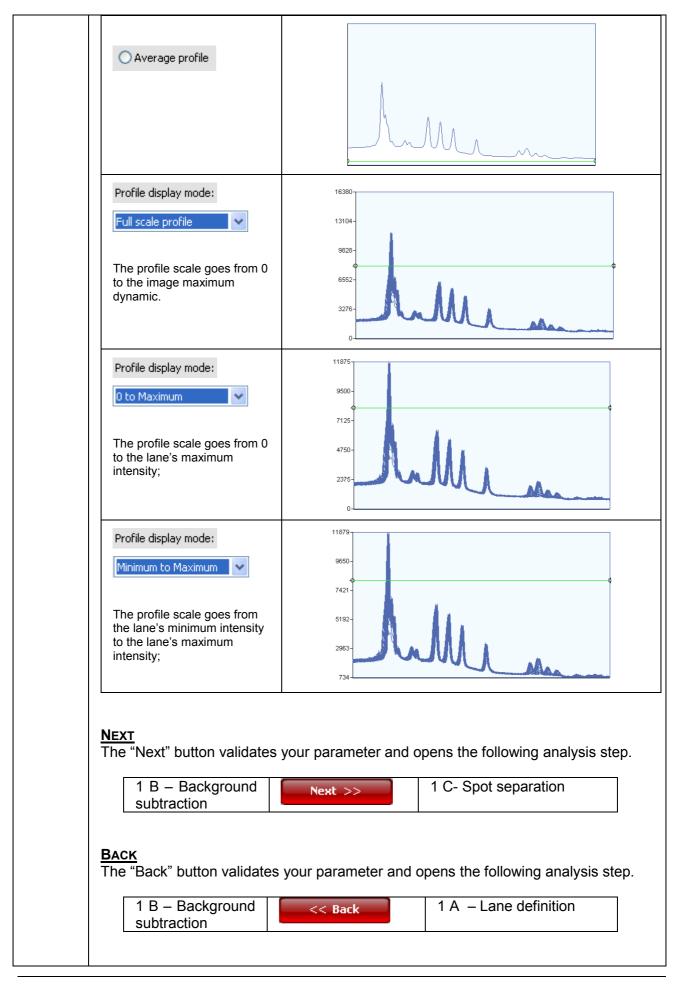
The linear approach is a lane-based background subtraction. It allows to manually define the level of noise to be subtracted on the lane profile. To proceed, click on the "Linear" button:

OLinear

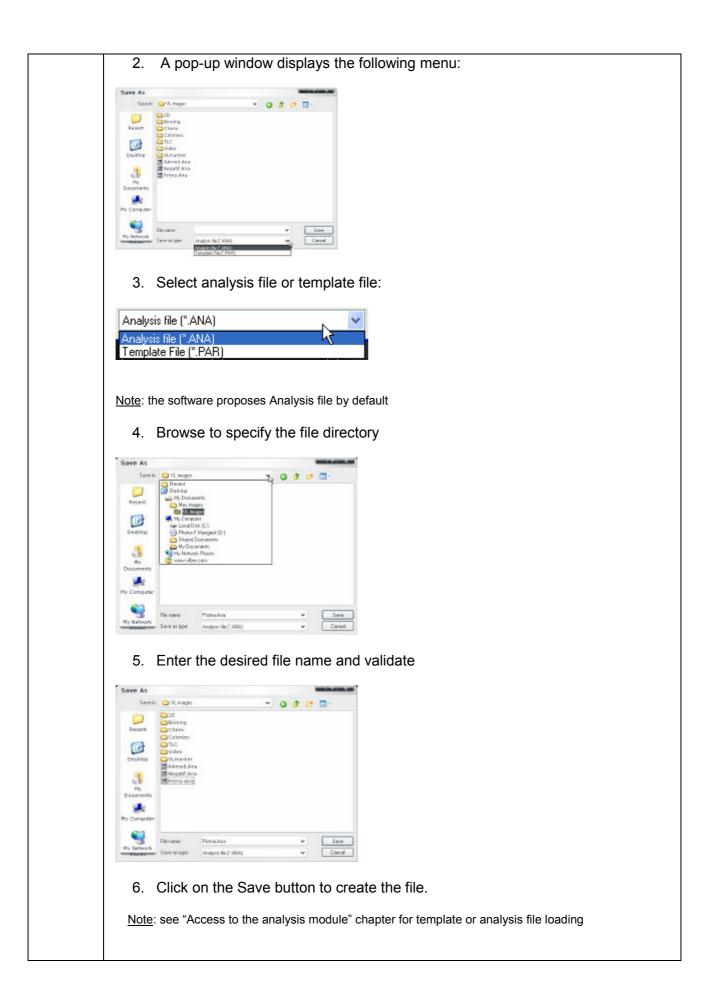
It opens the lane profile window:



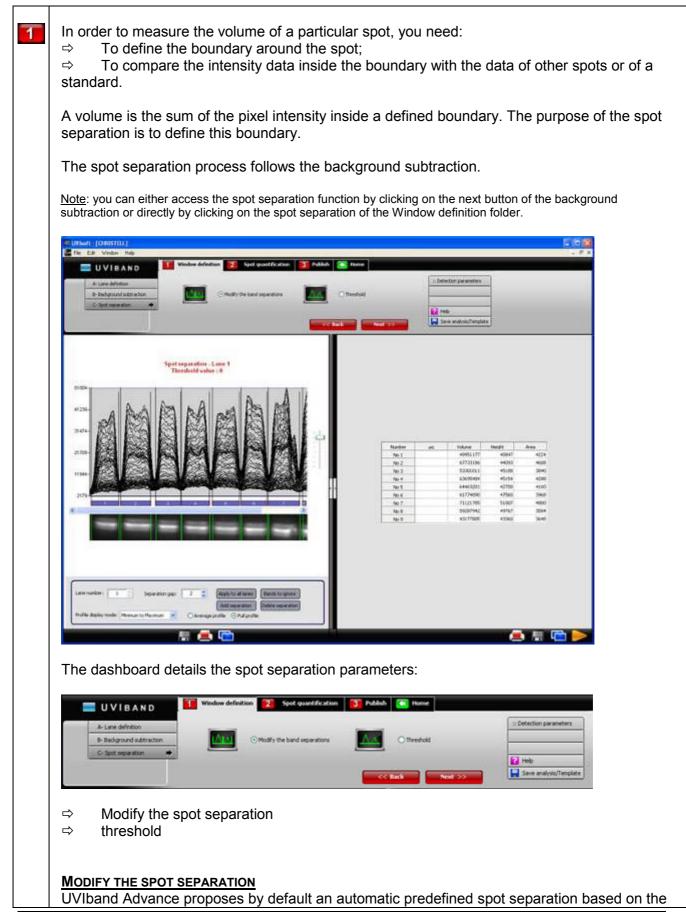
In the profile parameters window, select the lane to perform the linear approach					
Lane number: 1 Subtract noise Apply to all lanes Profile display mode: Full scale profile Image: Average profile Image: Full scale profile					
On the profile, click to define the background linear level you want to remove:					
Mum					
Then, click on Subtract noise: Subtract noise The changes will be automatically applied to the image and to the profile:					
MMM					
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:					
Apply to all lanes					
You can easily adjust the profile displays settings as follows:					
Sell profile					

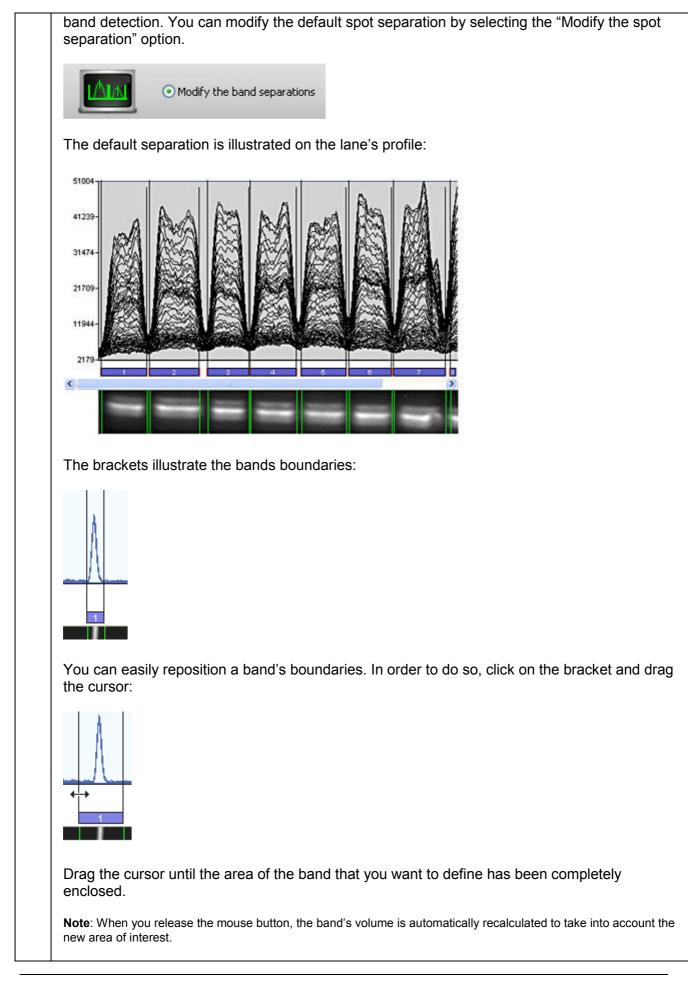


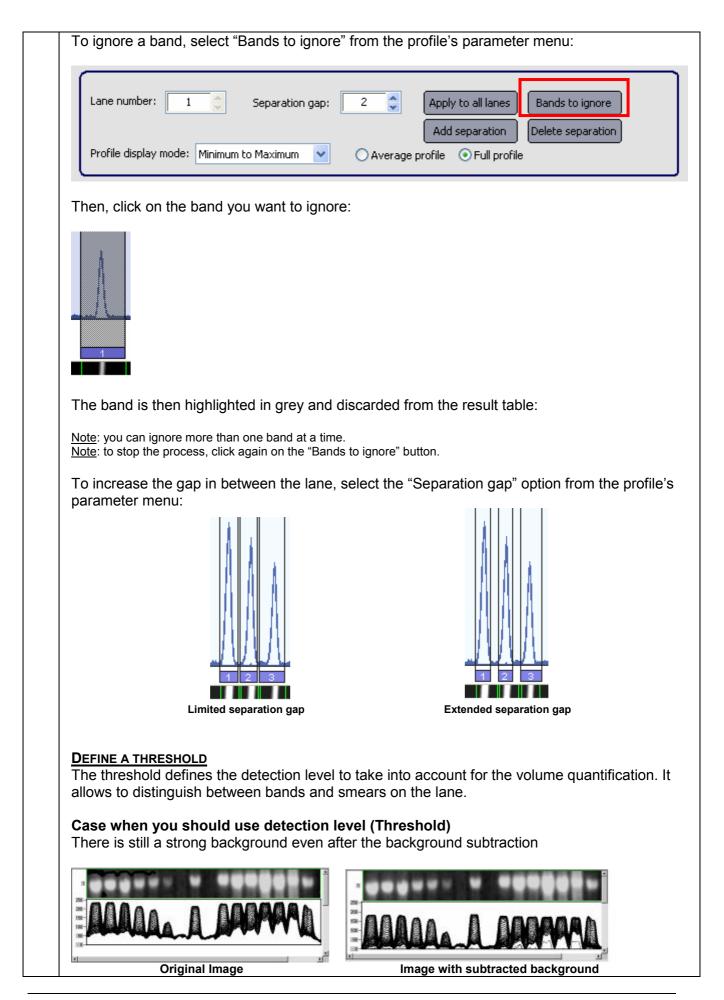
OPTION FOLDER The option folder gathers the following functions: ⇒ Help ⇒ Save the analysis or the template
Help
HELP MENU Click on the "Help" button. You automatically access the user manual at the chapter corresponding to the function Help
You can access the help file index through the File\Help from the Menu bar
Help Index About UVIsoft
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:
Reversion analysis/Template

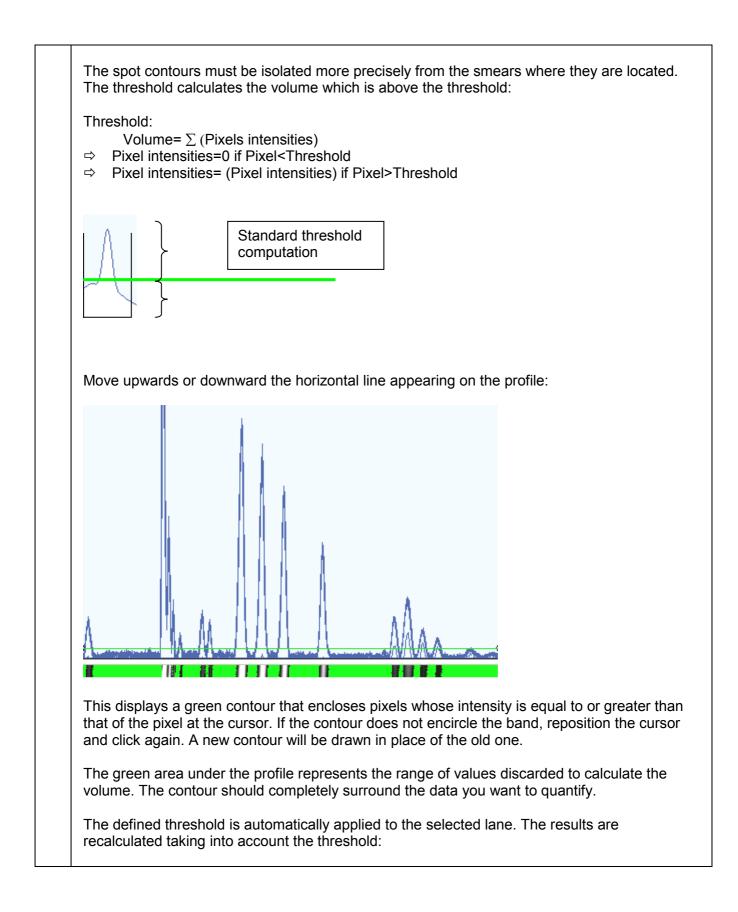


➔ C – Spot separation









Number	11	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

 \Rightarrow The volume is the sum of intensities included in the spot area of analysis.

 \Rightarrow The height is the maximum spot intensity, in grey levels.

 \Rightarrow 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:

Apply to all lanes

<u>Next</u>

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

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

<u>Васк</u>

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

1 C- Spot separation	<< Back	1 B – Background subtraction
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OPTION FOLDER

The option folder gathers the following functions:

⇒

김 Help

⇒

Save the analysis or the template

:: Detection parameters

Help

Save analysis/Template

HELP MENU

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

김 Help

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

Help

Index

About UVIsoft...

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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:

📑 Save analysis/Template

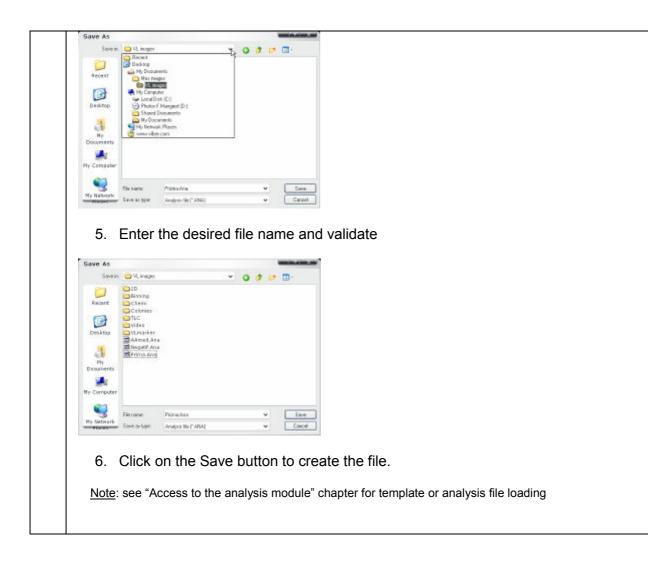
2. A pop-up window displays the following menu:

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3. Select analysis file or template file:

Note: the software proposes Analysis file by default

4. Browse to specify the file directory

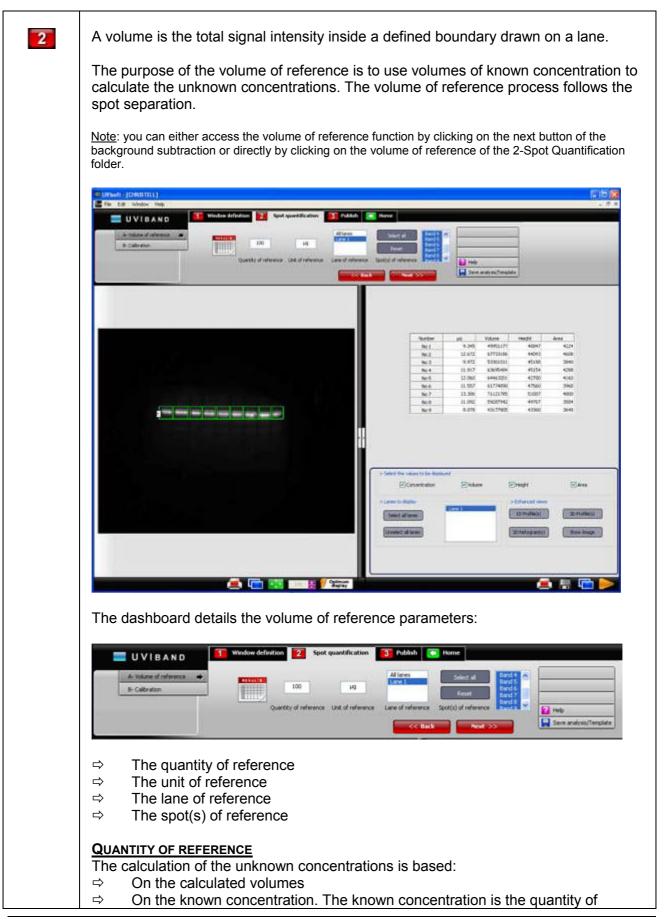


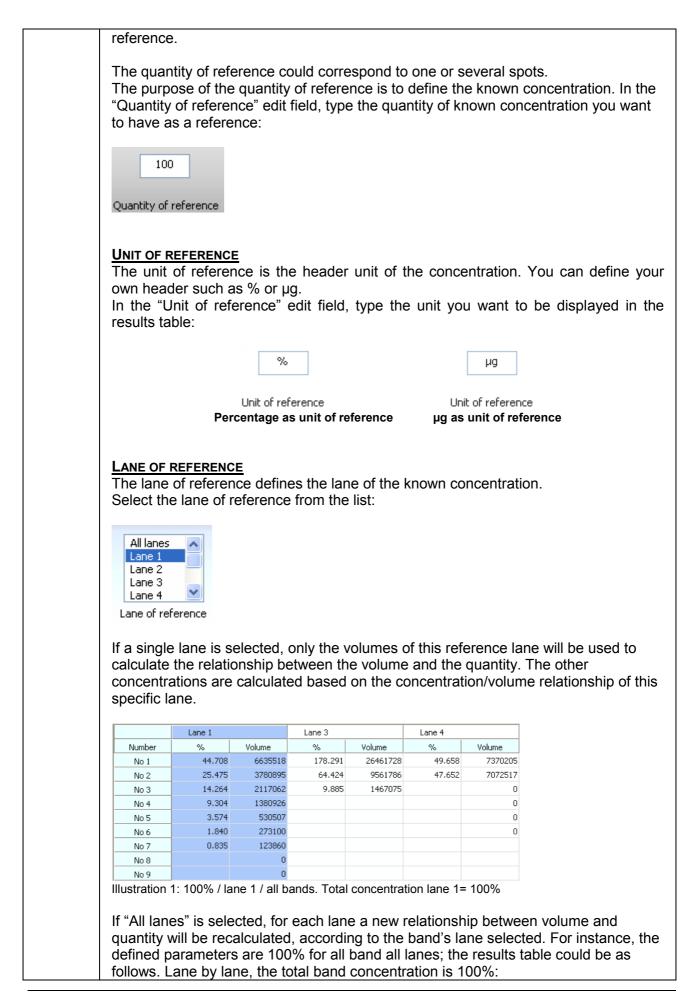
2- Analyse – Quantification

➔ 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 = Σ ni li
	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





	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:

Band 1	~
Band 2	
Band 3	
Band 4	_
Band 5	
Band 6	×.

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:

RESULTS		All lanes	Select all	Band 1 🔺 Band 2
100	μg	Lane 2 Lane 3	Reset	Band 3 Band 4 Band 5
Quantity of reference	Unit of reference	Lane of reference	Spot(s) of reference	Band 6

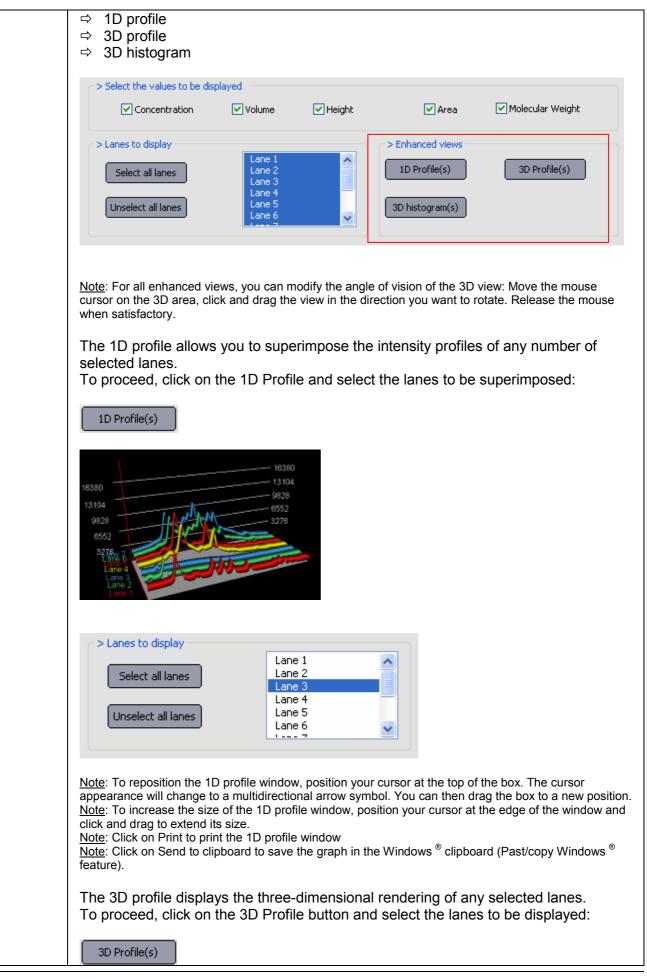
The results table indicates the following for lane 3:

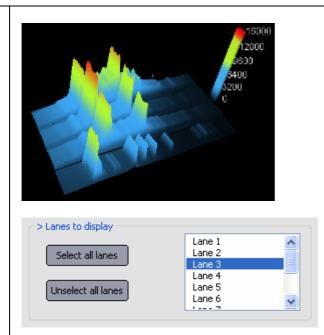
Number	ρų	Volume	Height	Area	MW-RF
No 1	3.000	4285313	4071	1775	10.000
No 2	9.267	13237182	3438	5396	8.000
No 3	0.942	1345357	2740	568	6.000
No 4	0.467	667689	2692	284	5.000
No 5	12.560	17940927	2651	10224	4.000
No 6	0.358	511654	1305	426	3.000
No 7	3.885	5549237	1275	5112	2.500
No 8	1.626	2322765	1176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

Oua	ntity of reference	Unit of reference	Lane 2 Lane 3	e Spot(s) of r	Band 5		
	able indicate						
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		
The "Back" k			meter and op	ens the fol	lowing analys		
2 A – V	/olume of refe		meter and op << Back		lowing analys - Spot separat		
The "Back" to 2 A – V RESULT TABI In the result lisplayed in Concent Concent Official The max The max The area Select you	Volume of refe	erence indow, you c ables: ty de, click on th	<< Back	lanes and	- Spot separat		
The "Back" to 2 A – V RESULT TABI n the result displayed in ⇒ Concent ⇒ Concent ⇒ Volume ⇒ The max ⇒ The area o select you	Volume of reference parameter with the results tar ration timum intension r display mod	erence indow, you c ables: ty de, click on th	<< Back	lanes and	- Spot separat		





<u>Note</u>: 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. <u>Note</u>: 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.

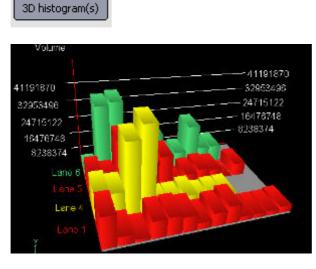
Note: Click on Print to print the 1D profile window

<u>Note</u>: 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:



<u>Note</u>: 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. <u>Note</u>: 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

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

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The option folder gathers the following functions:

- ⇒ Help
- ⇒ Save the analysis or the template

김 Help
📘 Save analysis/Template

Help

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

<table-cell> Help</table-cell>

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

Help	
	Index
	About UVIsoft

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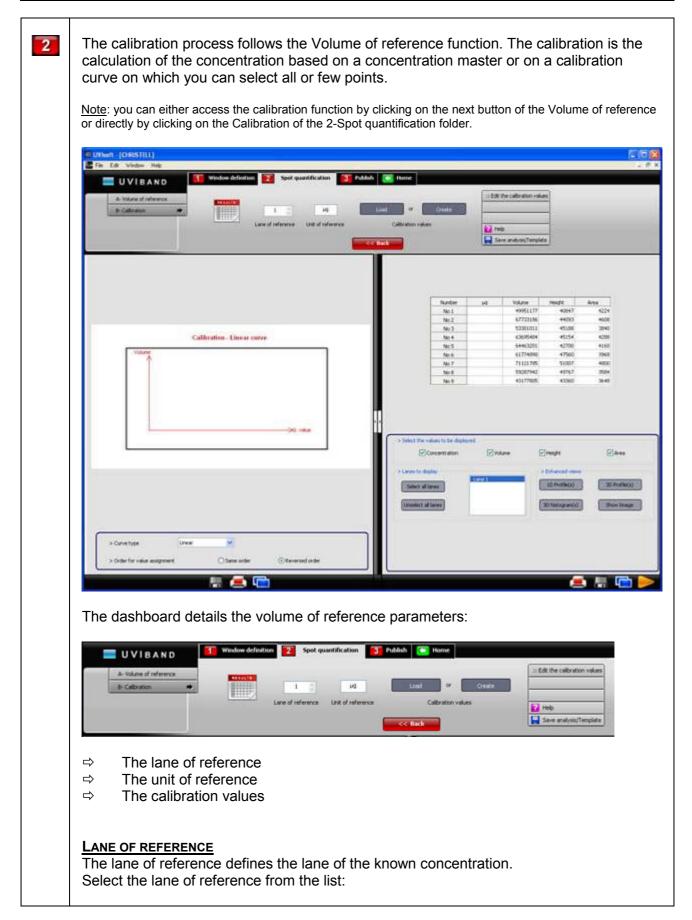
1. Click on the "Save analysis/ Template" button:

📕 Save analysis/Template

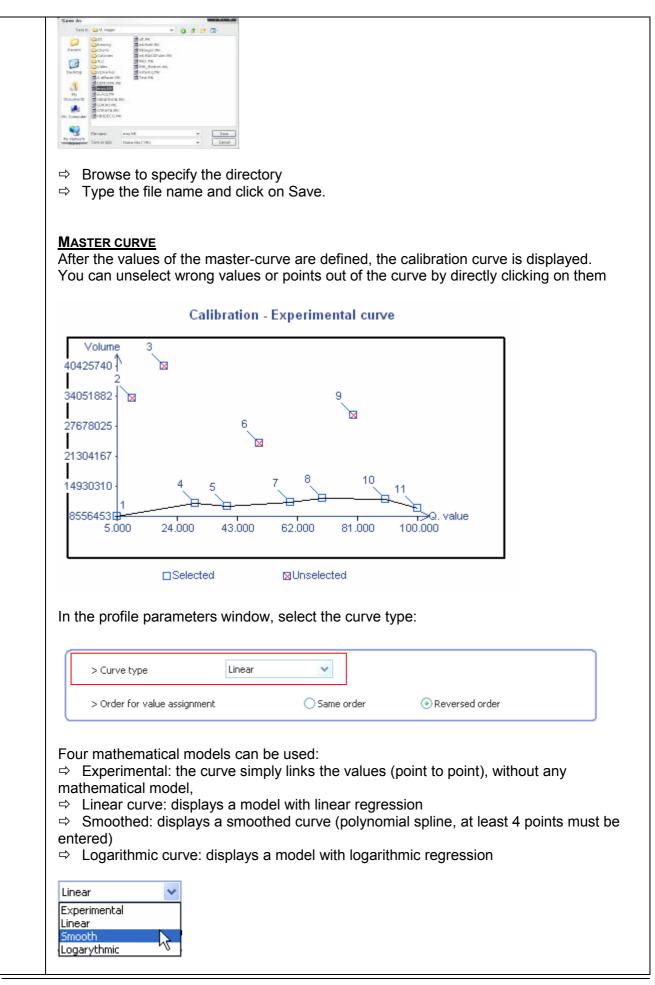
2. A pop-up window displays the following menu:



➔ B- Calibration

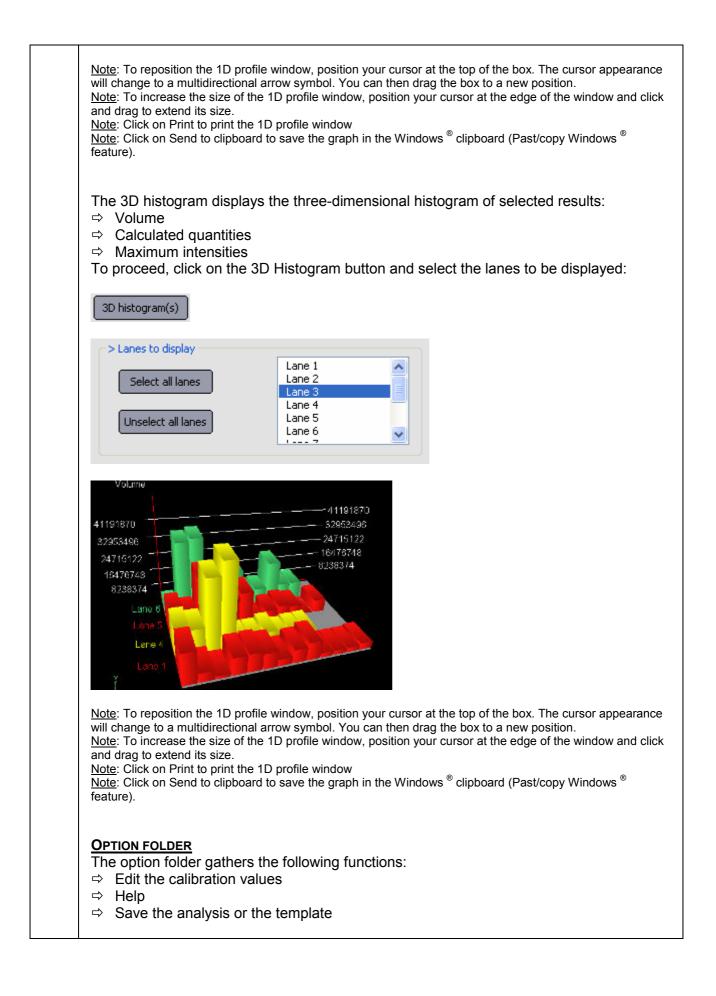


1	
header such as %	ence is the header unit of the concentration. You can define your ow
	% µg Unit of reference Unit of reference Percentage as unit of reference µg as unit of reference
THE CALIBRATION Click on the "Loa	<u>VALUES</u> Id" or "Create" button to enter calibration's values.
Load	or Create
For "Create", a p	op-up window displays the following menu:
	Value editor - Master Add value Add value C Cancel Total bands C Cancel Cancel
Type your value data.	s, band to band, in a descending order. The OK button validates yo
	c calculation with immediate application of the standard values is carried out, it is r all the bands given by the manufacturer's specifications, but only those which a he lanes of the gel.
You can save yo click on the "Save	ur calibration data and create your own calibration library; To procee e " button:
Save	

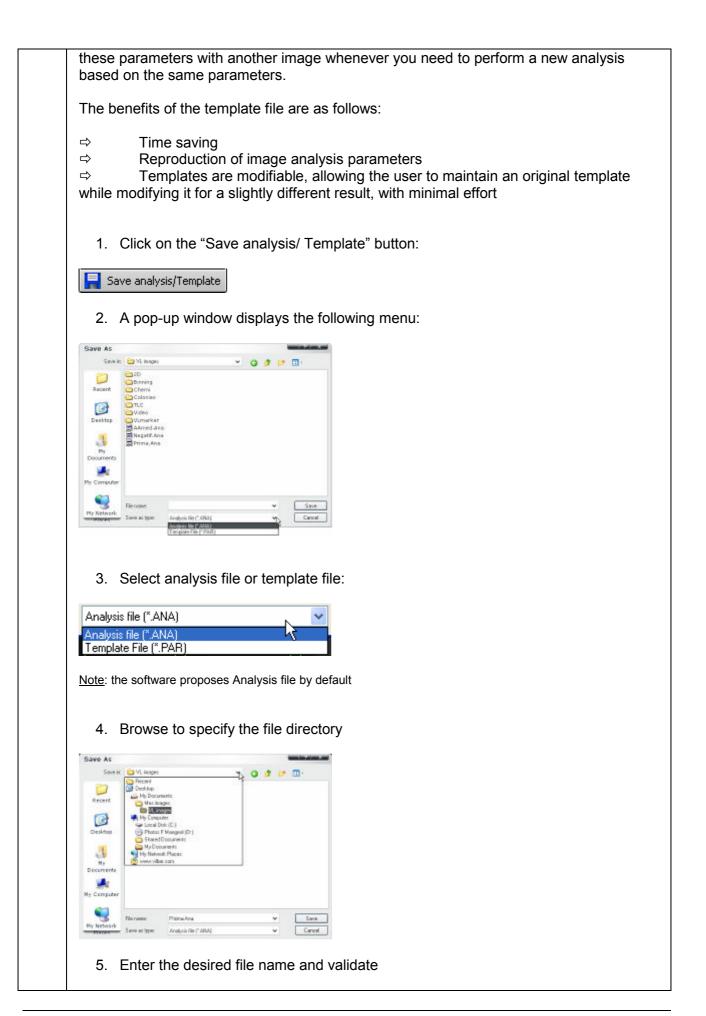


	Linear	*		
> Order for value assi	gnment	◯ Same order	 Reverse 	ed order
 the results tables Concentration Volume The maximum in The area 	ntensity			ne values to be disp
o select your displ	•	on the approp	oriate selection:	
Concentration	Volume	🗹 Height	🗹 Area	Molecular Weight
> Lanes to display		h (> Enhanced views	
Select all lanes	Lane 1 Lane 2		1D Profile(s)	3D Profile(s)
Unselect all lanes	Lane 3 Lane 4 Lane 5 Lane 6		3D histogram(s)	Show Image
the results param 1D profile 3D profile		you can select ♥ Height	✔ Area	esults tables: ✓ Molecular Weight
 the results param 1D profile 3D profile 3D histogram Select the values to be only and the values to be values	displayed	✓ Height	Area Enhanced views	Molecular Weight
 the results param 1D profile 3D profile 3D histogram Select the values to be of Concentration 	Volume Volume Lane 1 Lane 2 Lane 3		✔ Area	
 3D profile 3D histogram Select the values to be of Concentration Lanes to display 	displayed Volume Lane 1 Lane 2	✓ Height	Area Enhanced views	Molecular Weight

16380 13104 9828 6552 3278 6552 3278 5662 3278 5662 200 200 200 200 200 200 200 200 200	
> Lanes to display Select all lanes Unselect all lanes	Lane 1 Lane 2 Lane 3 Lane 4 Lane 5 Lane 6 Lane 7
will change to a multidirectio <u>Note</u> : To increase the size of and drag to extend its size. <u>Note</u> : Click on Print to print to <u>Note</u> : Click on Send to clipbo feature). The 3D profile displays	orofile window, position your cursor at the top of the box. The cursor appearance onal arrow symbol. You can then drag the box to a new position. If the 1D profile window, position your cursor at the edge of the window and click the 1D profile window oard to save the graph in the Windows [®] clipboard (Past/copy Windows [®] the three-dimensional rendering of any selected lanes. e 3D Profile button and select the lanes to be displayed:
Select all lanes	Lane 1 Lane 2 Lane 3 Lane 4 Lane 5 Lane 6



- 10 A	
:: Edit the c	calibration values
🛜 Help	
	nalysis/Template
EDIT THE C	CALIBRATION VALUES
1. Clic	ck on the "Edit the calibration values" button.
:: Edit the c	calibration values
2 A n	op-up window displays the following menu on which you can modify the
	bration values:
	Value editor - Master
N	
13.	Add value CK
	90.000 Delete value(s) Cancel
	70.000 60.000 Total bands 6
	50.000 Save
You can a	add, remove, and save your marker's value;
HELP MEN	
	he "Help" button. You automatically access the user manual at the chapter iding to the function
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You can a	access the help file index through the File\Help from the Menu bar
Help	
Index	
	UVIsoft
	UVIsoft
About	
About	UVIsoft LYSIS / TEMPLATE tion saves the current analysis. The analysis file will contain the results, the
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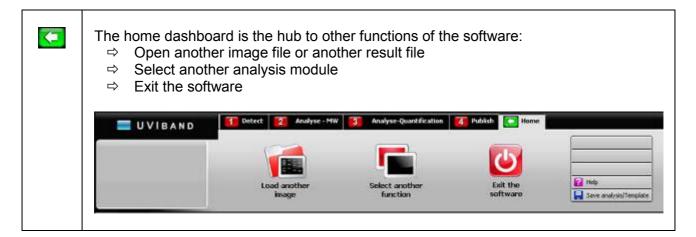
Publish

➔ Introduction

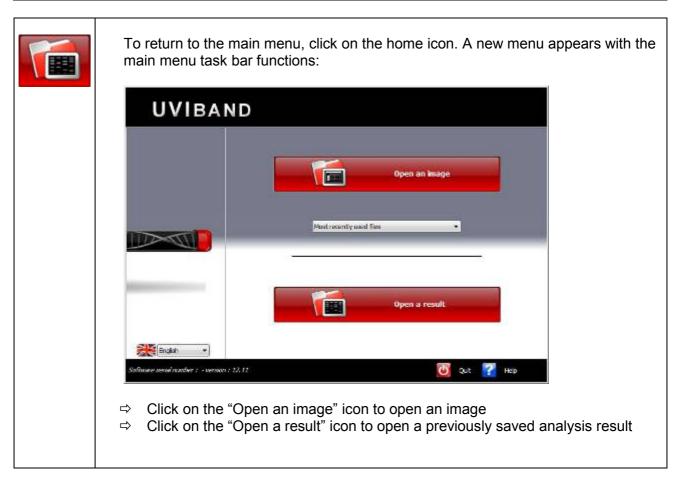
3	The purpose of the Publish function is to prepare a printed report of your results. You can easily organise your report with titles and comments and your own selection of data to be published among the following: - Sample image - Quantification result table To proceed, select the Publish tab. A pop-up window displays the following menu:
	Publish OD analysis
	Report title
	Image Comments
	Result table
	Print Cancel
	 ⇒ 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
	Print Perter None Stons Reduk Type USB001 Comment: Point ruge State Print State Image State State Image State Image State Image State Image Image </th
	 ⇒ 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

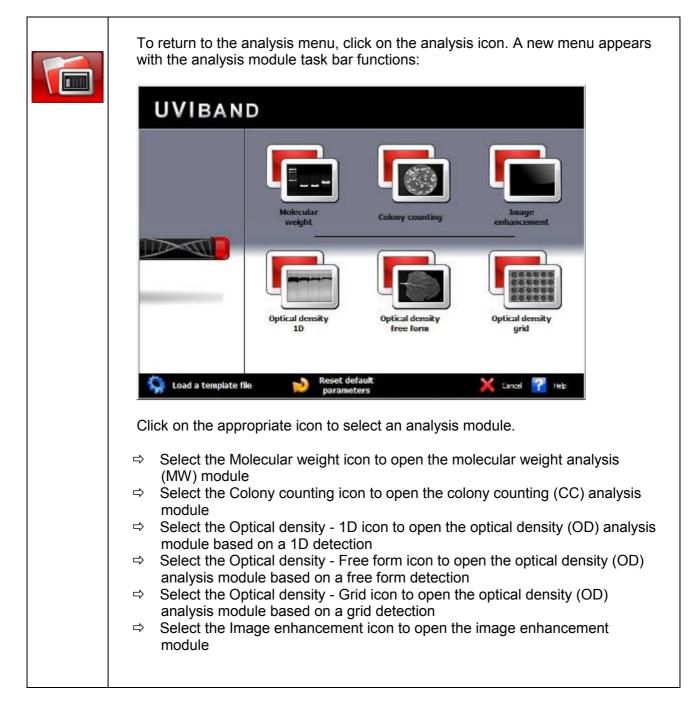
➔ Introduction



➔ Load another image



Select another function

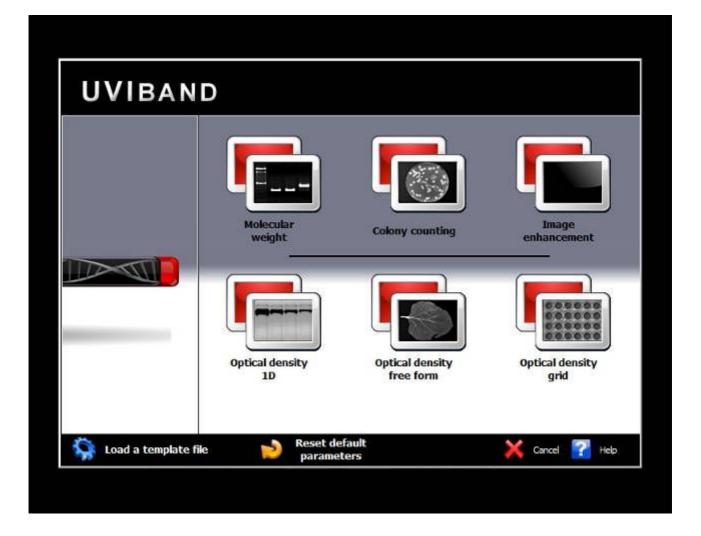


➔ Exit the software

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

You will be prompted to save your analysis.





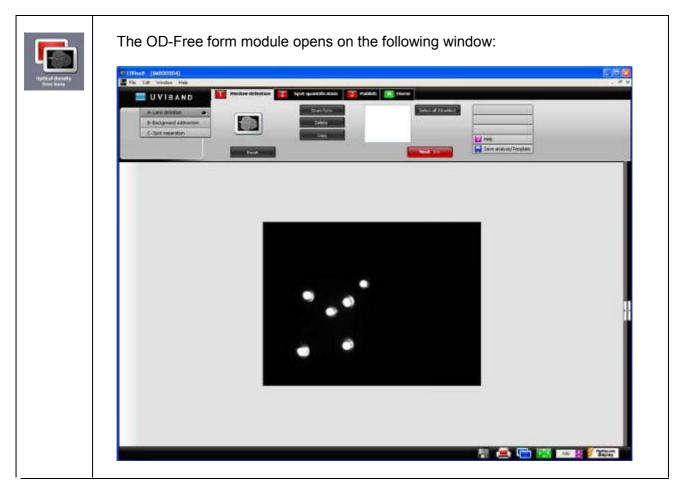
Optical density – Free form → OD-Free form Analysis module

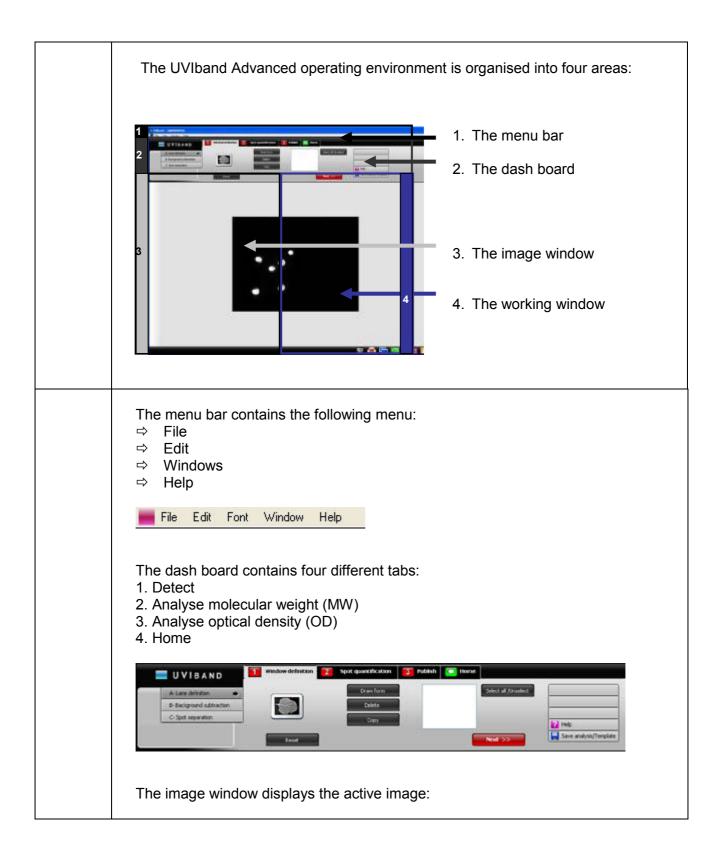
Optical density / Free form introduction

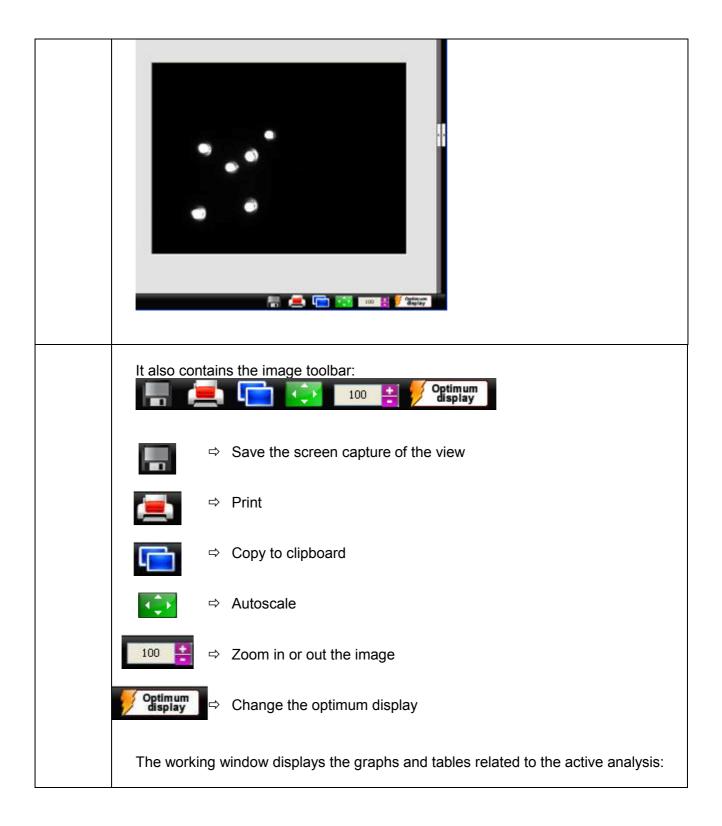
➔ Objectives and output

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







-	Reference	Lane 1	Lare 2	Large 3	Lone 4
No i	2.200	2.290			
No 2	1.500	1,500			
No 3 No 4	1.400	1.300			
No 5	1.200	1.290	1.200	1.192	1.184
No 6	1.100	3.300	1.095	1.095	1.004
No 7	1.900	1,000	0.983	0.975	0.975
No 8	0.900	9.900	6.857	0,053	0.853
No P	0.600	0,890			
No 10	0.700	0.700	0.695	0,695	0.695
No 11	0,600	0.600	0.997	0.994	0,991
No.12	0.500	0.530			
No L1	0.400	0.400	0.095	0.385	0.385
No 14	0.300	0.300	2000		
No 15	0.250		0.261	0.250	
No 16	0.204	8,290	0.196	0.204	0.125
No 17 No 10	0.500	0.100	916.09	9.645	0.044
No 19	0.063		0.063	0.063	
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also conta			-		oolbar
	> Disp	lay the	e mole	dow to	oolbar weigh
	DispSave	lay the	e mole graph o	dow to cular y	oolbar weigh table

➔ Toolbar in details



<u>Print</u>

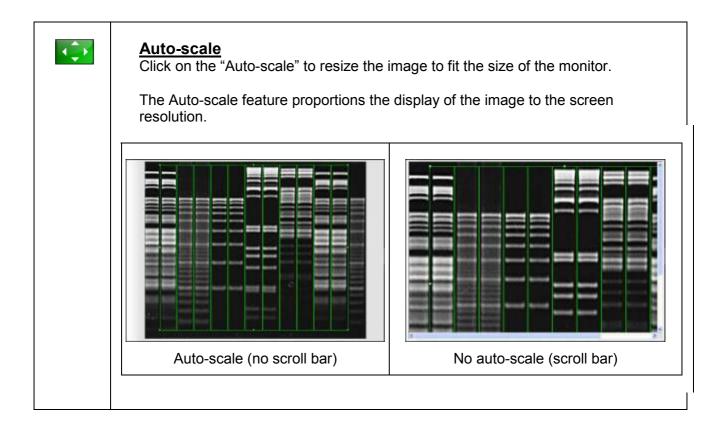
Click on the "Print" icon to print the image, the table or the graphs. A pop-up window displays the Print preview: The Print preview displays a preview of the image, as it will be printed.

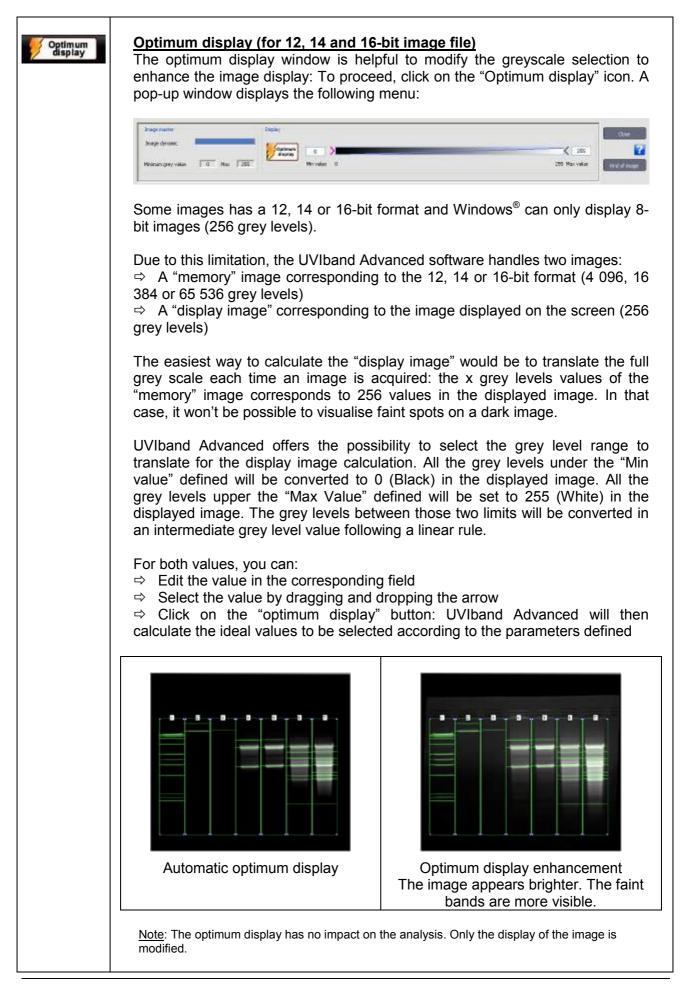
E Wilband	
Prote Previous. Filmit Tres Fegers Zoom In Zoom cut Page(s) 1 page(s) Close	
December of the	
Click on Print to validate the preview. A pop-up window displays the following	
menu:	
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Type: EP90W Stylus C70 Series Where: USE001	
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\rightarrow Coloct a printer	
 ⇒ Select a printer ⇒ Click on Properties to modify the default setting of the printer, if necessar 	c) /
 ⇒ Click on Properties to modify the default setting of the printer, if necessar ⇒ Select the number of copies 	y
 ⇒ Click on OK to validate your options 	
Note: You can also access the Print menu from the Menu bar (File\Print).	

<u>Save</u> 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:

	Save As
	Sere is 🔐 VL leager 🔹 💽 🥵 📳
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	Desurrets
	my Computer
	Provide Server Server
	Encode (see (2015)
	The Mr (100) by Downing (200)
	Browse to specify the file directory
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	Me Conjune
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	The matching Service S
	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:
	Excel file (*.XLS) 💦 🔽
	Excel file (*.XLS)
	Text file (*.TXT)
	DBase file (*.DBF)
	The graphs can only be saved on a BMP format:
	Bitmap file (*.BMP)
	Bitmap file (*.BMP)
1	

<u>Copy to clipboard</u> 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).



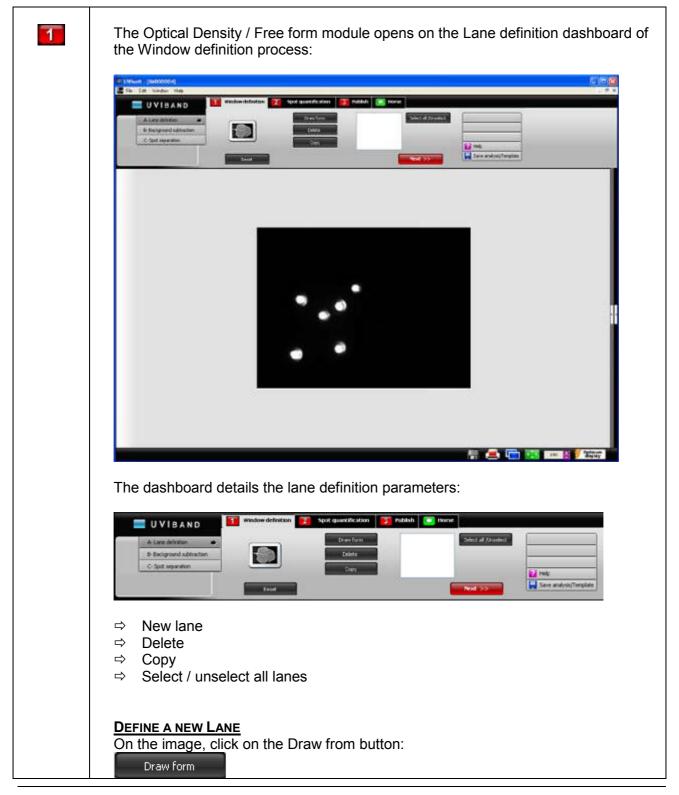




Send to Excel[™] This function transfers the results table to Windows Excel[™].

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

➔ A – Lane definition



⇒ 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.



 \Rightarrow Repeat these steps as necessary to define the free form area

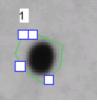


⇒ Click on the Validate button to define the area
Validate definition

⇒ Click on the Abort button to cancel the definition

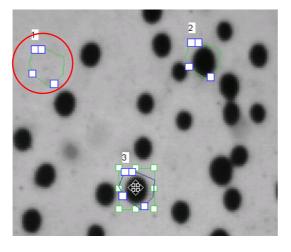


The lane is defined by green lines, overlaid on the image. The area is surrounded by square anchors:



To move the entire frame to a new position,

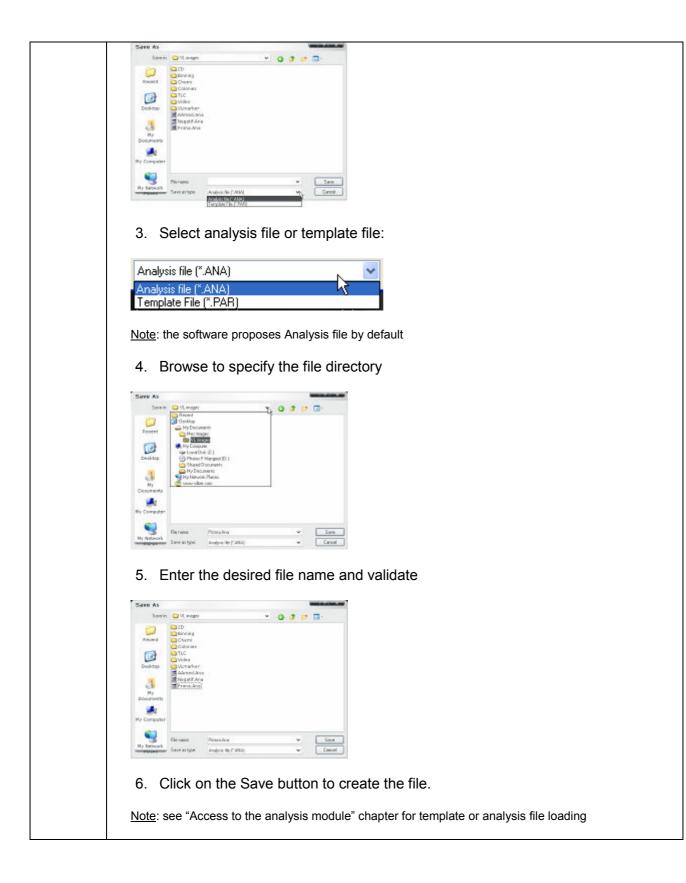
- ⇒ Select the frame
- \Rightarrow Position the mouse on the frame to obtain a cross cursor
- \Rightarrow Click and drag the cursor to move the entire frame



<u>COPY A LANE</u> To copy a lane, select the lane in the lane list:

Lane 1
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: Lane 1 Lane 2 Lane 3
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.
<u>Next</u> 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
HELP MENUClick on the "Help" button. You automatically access the user manual at the chapter corresponding to the functionHelpYou can access the help file index through the File\Help from the Menu bar:
Help Index About UVIsoft
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: Save analysis/Template
2. A pop-up window displays the following menu:



➔ 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:
	A Lare defeator B Background mit/sation C Spot represtion O Rules bill B Background mit/sation C Spot represtion Restore satisfies Restore satisfies Restore satisfies
	 UVIband Advanced has several functions to minimise image background. ⇒ The rolling ball approach ⇒ The valley to valley approach ⇒ The linear approach
	Rolling ball Valley to valley Linear

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.

A	Lada
Rolling ball	Background subtraction

The centre of gravity of the ball describes a curve:

- \Rightarrow This curve represents the noise to be subtracted.
- \Rightarrow The curve depends on the size of the ball and on the size of the peaks.

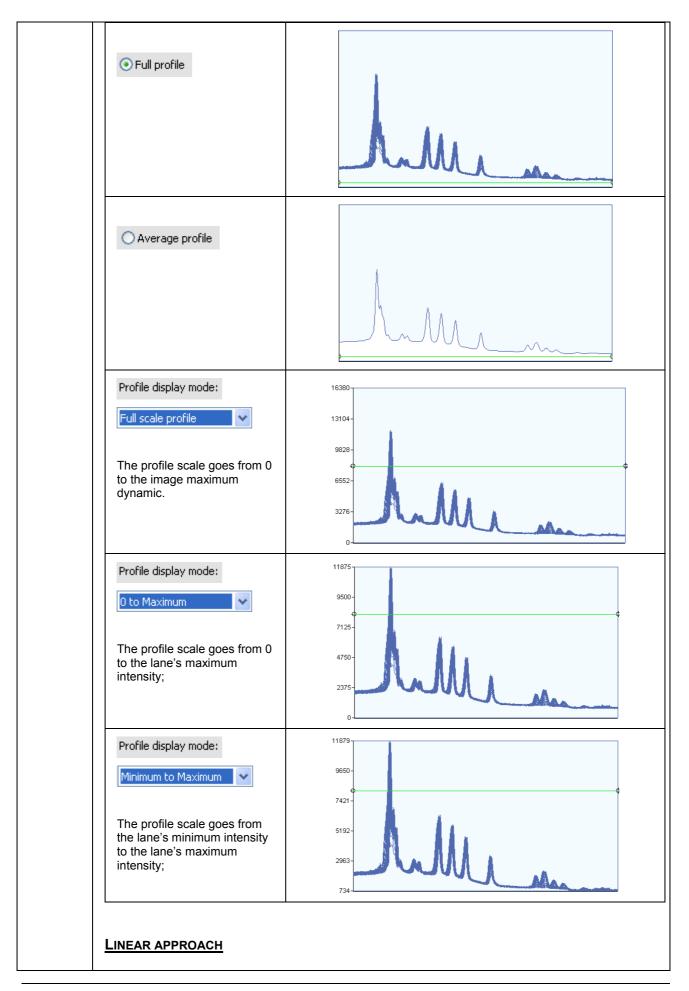
The size of the ball will affect the position and movements of the centre of gravity and thus it determined the level of background subtraction. A small disk will make a large background subtraction and a large disk the contrary. A disk radius that is too small may subtract almost all image data.

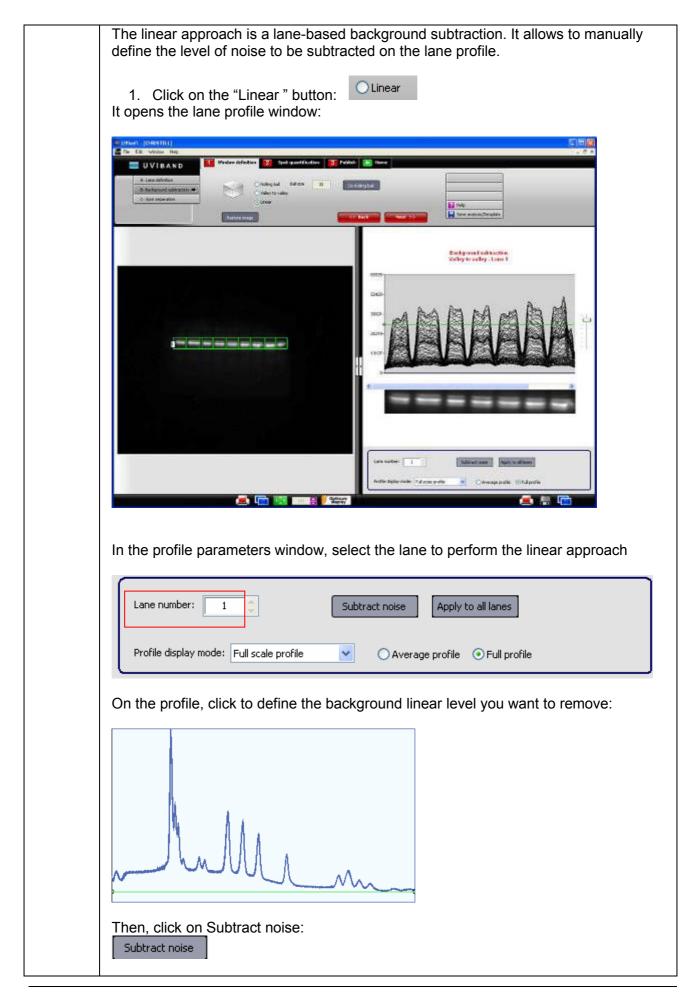
The UVIband Advanced calculates automatically the ideal parameter for background subtraction. This could be manually modified by adjusting the spot size:

O Rolling ball Ball size 31 S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S
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.
 <u>VALLEY TO VALLEY</u> 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: Valley to valley It opens the lane profile window:
A-Leve defention Image: Spot quantification Image: Publish (image: Home) A-Leve defention Image: Publish (image: Home) Image: Publish (image: Home) A-Leve defention Image: Publish (image: Home) Image: Publish (image: Home) A-Leve defention Image: Publish (image: Home) Image: Publish (image: Home) A-Leve defention Image: Publish (image: Home) Image: Publish (image: Home) A-Leve defention Image: Publish (image: Home) Image: Publish (image: Home) A-Leve defention Image: Publish (image: Home) Image: Publish (image: Home) A-Leve defention Image: Publish (image: Home) Image: Publish (image: Home) A-Leve defention Image: Publish (image: Home) Image: Publish (image: Home) A-Leve defention Image: Publish (image: Home) Image: Publish (image: Home) A-Leve defention Image: Publish (image: Home) Image: Publish (image: Home) Image: Publish (image: Home) Image: Publish (image: Publish (image: Home) Image: Publish (image: Publish (

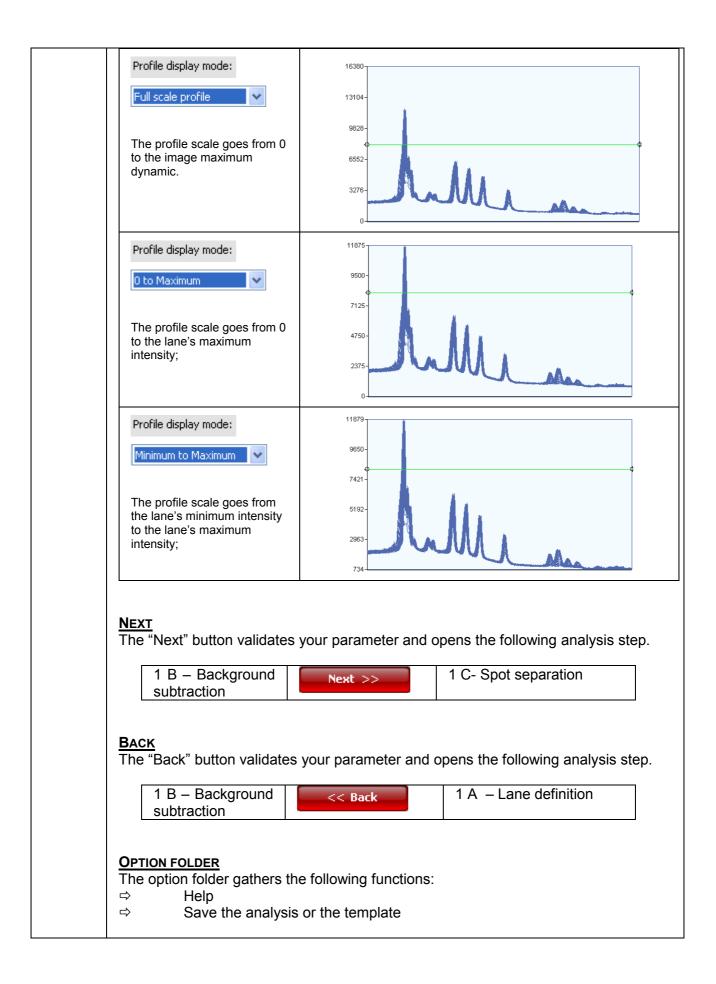
2. In the profile parameters window, select the lane to perform the valley-to-valley

approach
Lane number: 1 Subtract noise Apply to all lanes Profile display mode: Full scale profile Image of the scale profile Image of the scale profile
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: Apply to all lanes
You can easily adjust the profile displays settings as follows:

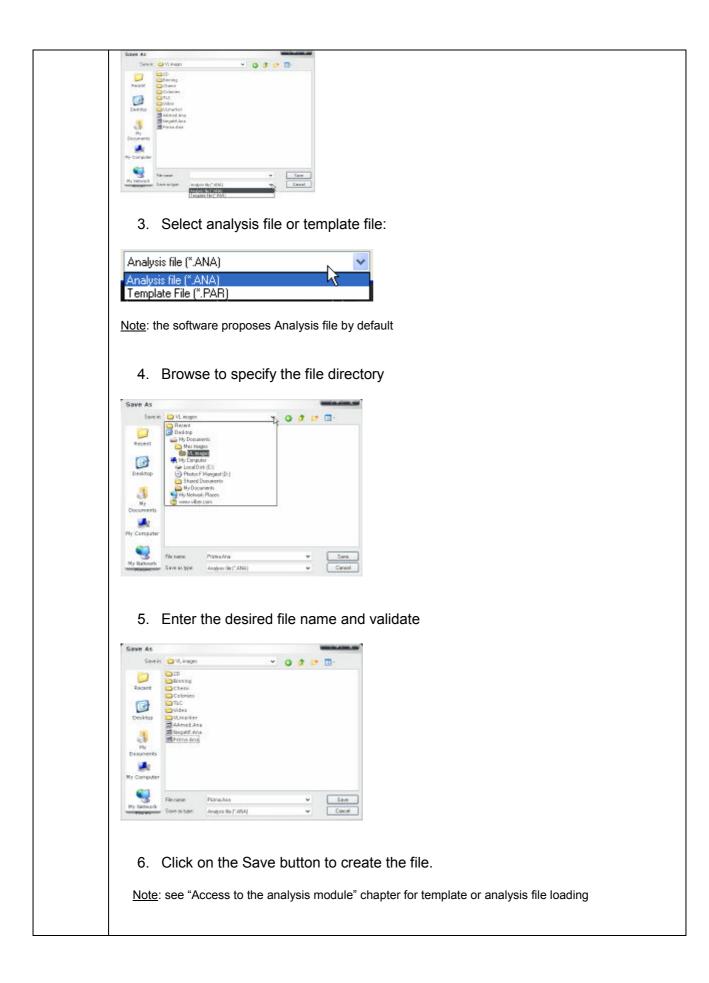




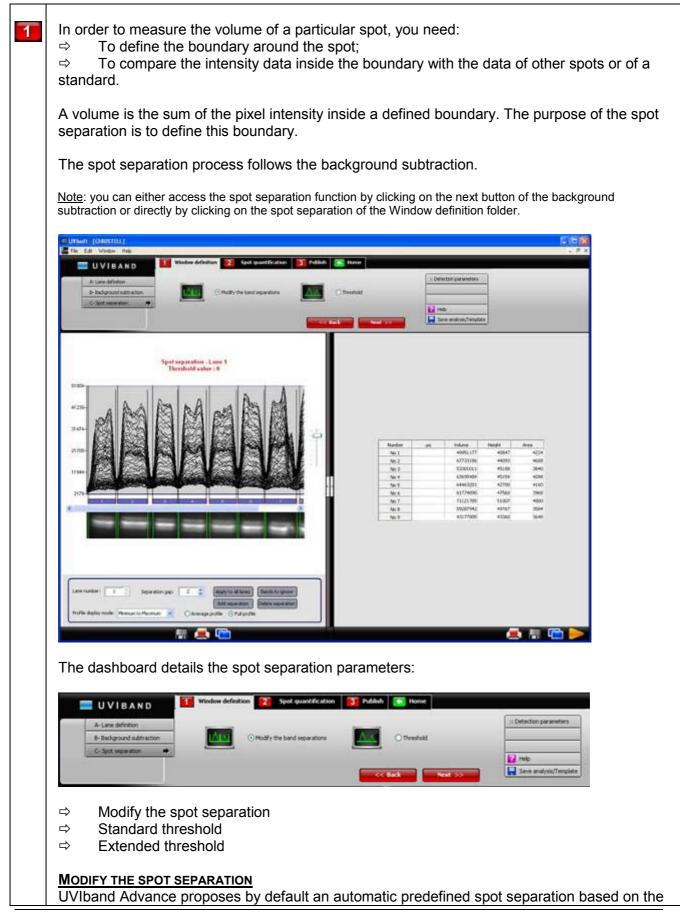
The changes will be automatically applied to the image and to the profile:
Mum
The linear approach is a lane-based background subtraction. You can set the same subtraction level for all lanes or specify an individual subtraction level for the selected lane. Any changes you make will be automatically applied to the image.
To apply the same subtraction level for all lanes, click on the "Apply to all lanes" button: Apply to all lanes
You can easily adjust the profile displays settings as follows:
Sell profile
Average profile

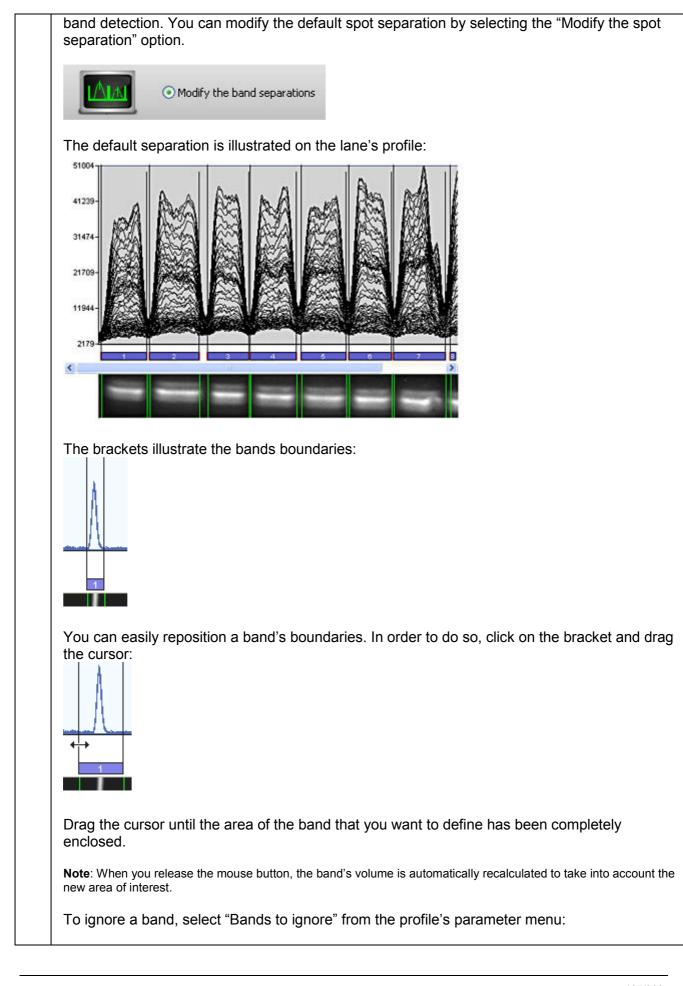


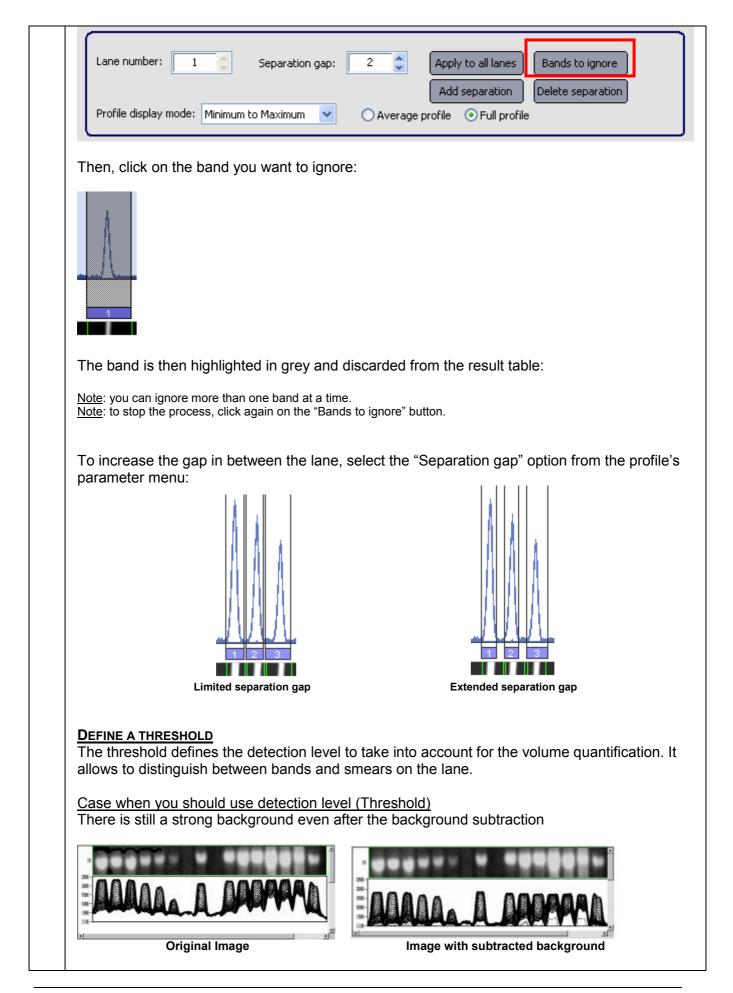
	elp ave analysis/Template
Help	MENU
<table-cell> He</table-cell>	elp
corres	on the "Help" button. You automatically access the user manual at the chapter sponding to the function. You can access the help file index through the elp from the Menu bar
Help	
I	ndex
А	about UVIsoft
Templ assoc evalua	nalysis could also be saved as a template for automated analysis routines. late offers the user the ability to automate many of the repetitive tasks iated with analysis and processing. As a result, you can spend more time ating and analysing results, and less time manipulating set-ups, variables and settings.
regula You c	emplate automates a task or set of tasks that you perform repeatedly or on a ar basis. It stores all the analysis commands and parameters of an analysis. an run these parameters with another image whenever you need to perform a nalysis based on the same parameters.
	enefits of the template file are as follows:
⇔ ⇔ ⇔ templa	Time saving Reproduction of image analysis parameters Templates are modifiable, allowing the user to maintain an original ate while modifying it for a slightly different result, with minimal effort
1.	Click on the "Save analysis/ Template" button:
📙 Sa	ave analysis/Template
2.	A pop-up window displays the following menu:
	рар ар станование и станование и При при при при при при при при при при п

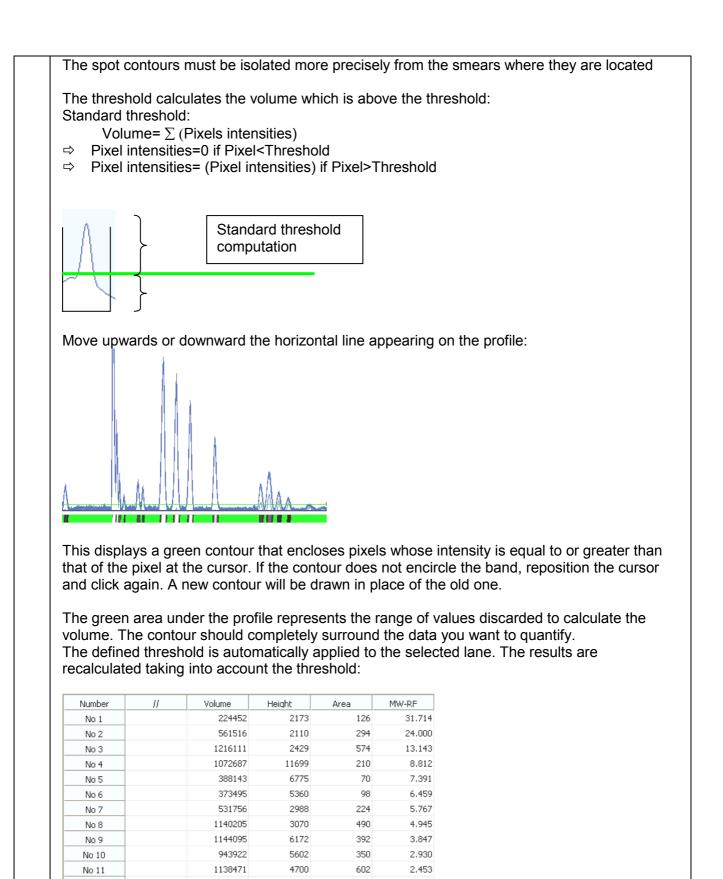


➔ C – Spot separation









1235282

385044

401562

243847

213134

4827

0

No 12

No 13

No 14

No 15

No 16

No 17

No 18

3269

1870

2191

1541

1311

973

0

966

294

252

195

191

5

0

1.987

1.417

0.973

0.774

0.573

0.389

0.267

		eshold for the selected	. You can set the same threshold for all lane. Any changes you make will be
To a	• • • •		click on the "Apply to all lanes" button:
Apply	to all lanes		
NEX			
The		your parameter and c	opens the following analysis step.
	1 C- Spot separation	Next >>	2 A – Volume of reference
Bacı The		s your parameter and	opens the following analysis step.
гс	1 C- Spot separation	· ·	1 B – Background subtraction
		<< Back	
:: De	etection parameters		
_			
?	Help		
	Save analysis/Template		
	•		ess the user manual at the chapter
Click		on	
Click corre	esponding to the function		
Click corre	Help	elp file index through the through the theory of theory of the theory of the theory of the theory of the theory of theory of theory of theory of the theory of the theory of the	he File\Help from the Menu bar
Click corre	Help can also access the he	elp file index through the state of the stat	he File\Help from the Menu bar
Click corre	Help can also access the he	elp file index through th	he File\Help from the Menu bar

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:

📙 Save analysis/Template

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

Save As							
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9	Ple neme						See
Hy Natavork	Severet hore	Anijsa (k. (ANI)			- 6	m.	Canod

3. Select analysis file or template file:

Analysis file (*.ANA)	N 🔽
Analysis file (*.ANA)	2
Template File (*.PAR)	

Note: the software proposes Analysis file by default

4. Browse to specify the file directory



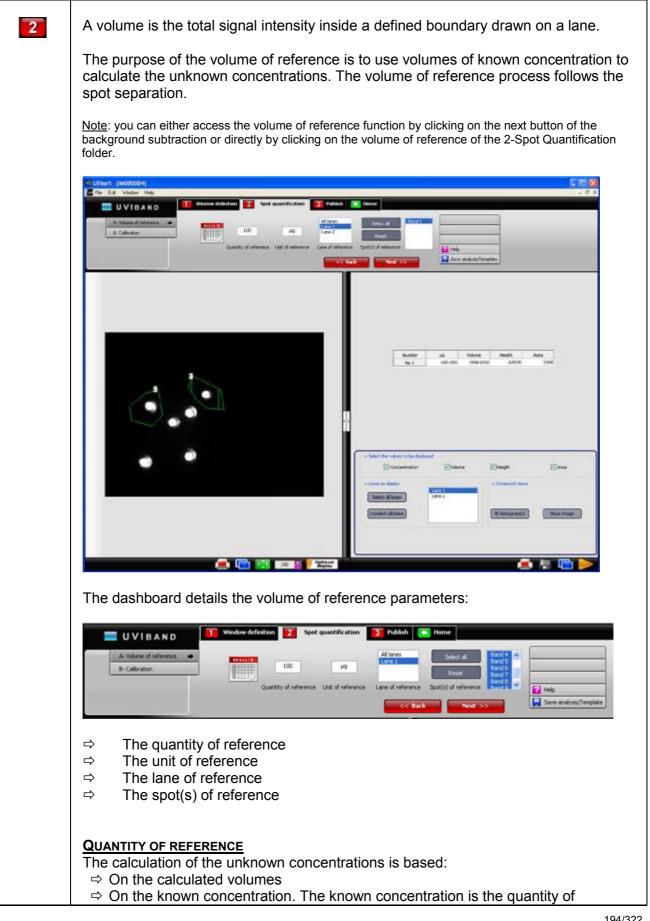
Savern	D VI, inages		· • • •	• 🔟 •			
Recent	Dissing Octem						
0	Colonies TLC Vides						
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3	Regatif. Ana						
Phy Desuments							
My Computer							
Ny Metwork	Fienate	Piana.kus	v	Save			
	Sove as type.	Anagest No (* ARA)	*	Cancel			
6.	Click c	on the Sa	ve butto	n to cre	ate the file.		

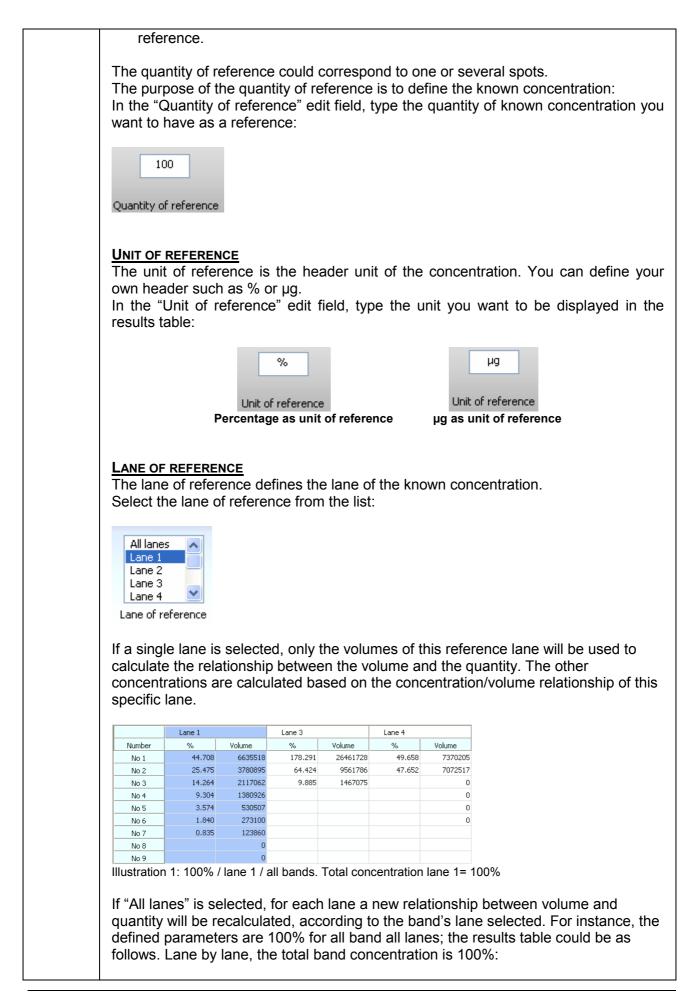
2- Analyse – Quantification

➔ 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
	$V = \sum ni li$
	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





	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:

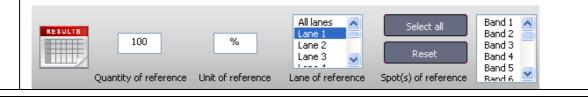
RESULTS 100		All lanes	Select all	Band 1 A
	μg	Lane 2 Lane 3 🗸	Reset	Band 3 Band 4 Band 5
Quantity of reference	Unit of reference	Lane of reference	Spot(s) of reference	Band 6

The results table indicates the following for lane 3:

Number	μq	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:



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

The results table indicates the following for lane 1:

<u>Next</u>

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

T		1
2 A – Volume of reference	Next >>	2 B – Calibration

Васк

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

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

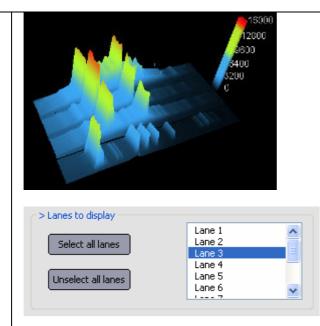
RESULT TABLE

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
- \Rightarrow The area
 - 1. To select your display mode, click on the appropriate selection:

Concentration	Volume	🗹 Height	🗹 Area	Molecular Weight
Lanes to display Select all lanes Unselect all lanes	Lane 1 Lane 2 Lane 3 Lane 4 Lane 5 Lane 6		> Enhanced views 1D Profile(s) 3D histogram(s)	3D Profile(s)
APHICAL VIEW			t the graphical	results tables:

> Select the values to be dis	played			
Concentration	Volume	🗹 Height	🗹 Area	Molecular Weight
> Lanes to display Select all lanes Unselect all lanes	Lane 1 Lane 2 Lane 3 Lane 4 Lane 5 Lane 6		> Enhanced views 1D Profile(s) 3D histogram(s)	3D Profile(s)
<u>Note</u> : For all enhanced vic cursor on the 3D area, cli when satisfactory.				
The 1D profile allows selected lanes. To proceed, click on		-	• •	-
1D Profile(s)				
16380 13104 9828 6552 3276 / Lane 4 Lane 3 Lane 2 Lane 1				
> Lanes to display Select all lanes Unselect all lanes	Lane Lane Lane Lane Lane Lane	2 3 4 5		
	o a multidirectio e of the 1D profi is size. nt the 1D profile	nal arrow sym le window, pos window	bol. You can then dra sition your cursor at t	ag the box to a new position. the edge of the window and
The 3D profile displa To proceed, click on				
3D Profile(s)				



<u>Note</u>: 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. <u>Note</u>: 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).

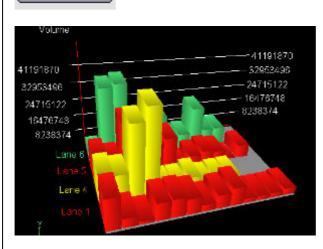
The 3D histogram displays the three-dimensional histogram of selected results:

⇔ Volume

3D histogram(s)

- ⇒ Calculated quantities
- ⇒ Maximum intensities

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

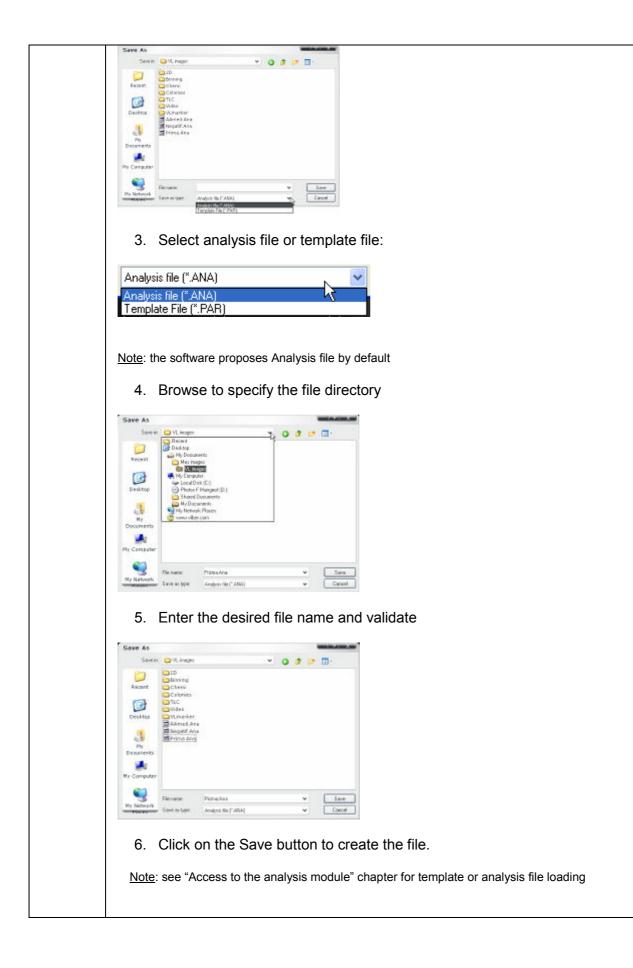


<u>Note</u>: To reposition the 1D profile window, position your cursor at the top of the box. The cursor appearance will change to a multidirectional arrow symbol. You can then drag the box to a new position. <u>Note</u>: To increase the size of the 1D profile window, position your cursor at the edge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

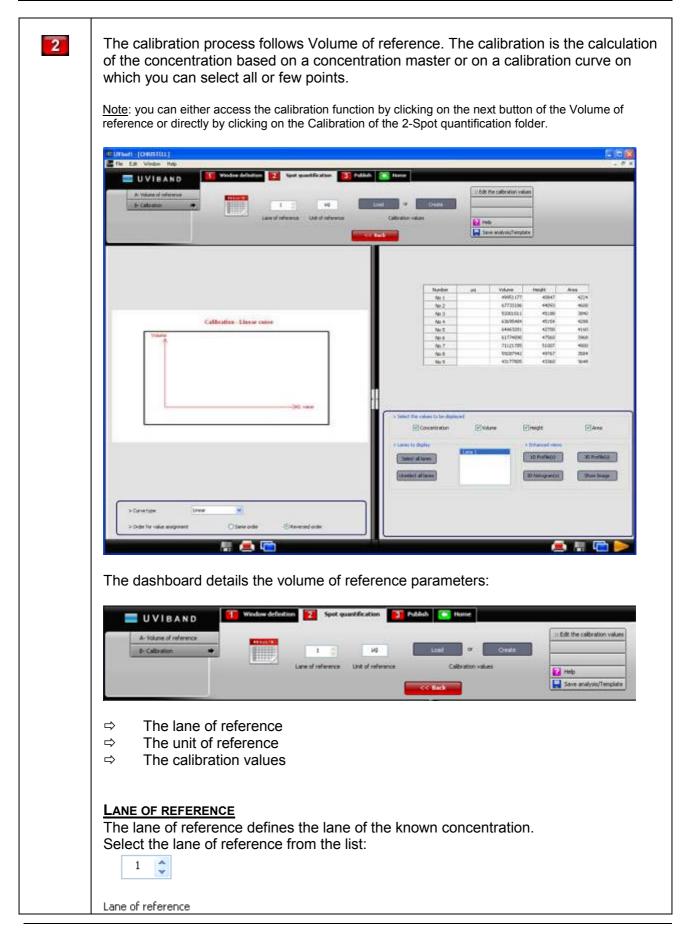
Note: Click on Print to print the 1D profile window

<u>Note</u>: Click on Send to clipboard to save the graph in the Windows $^{\ensuremath{\mathbb{R}}}$ clipboard (Past/copy Windows $^{\ensuremath{\mathbb{R}}}$ feature).

OPTION FOLDER
The option folder gathers the following functions:
⇔ Help
Save the analysis or the template
🔁 Help
Save analysis/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
Help
Index
About UVIsoft
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:
Save analysis/Template
A pop-up window displays the following menu:



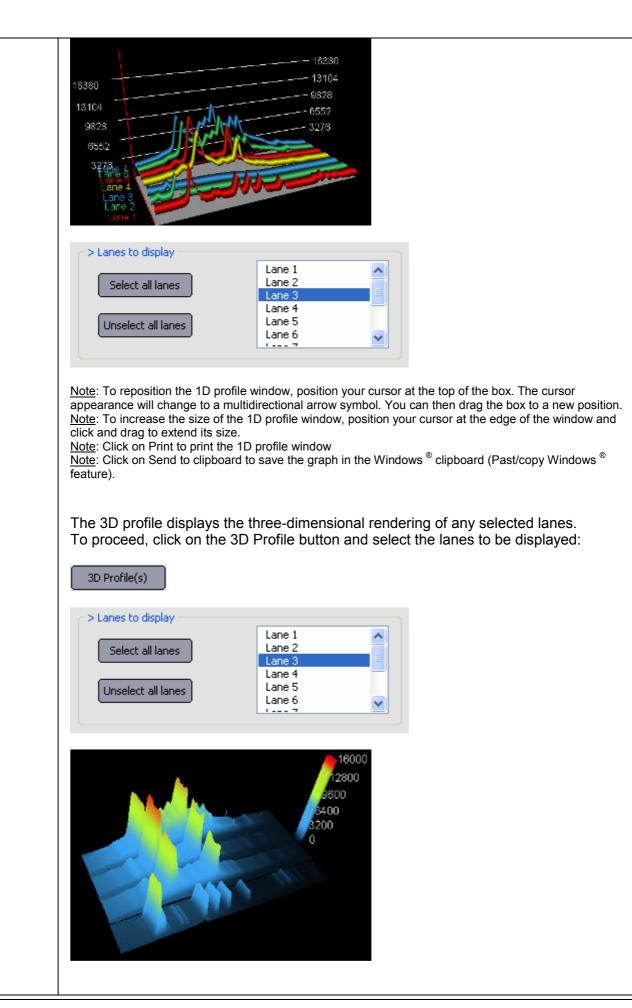
➔ B – Calibration

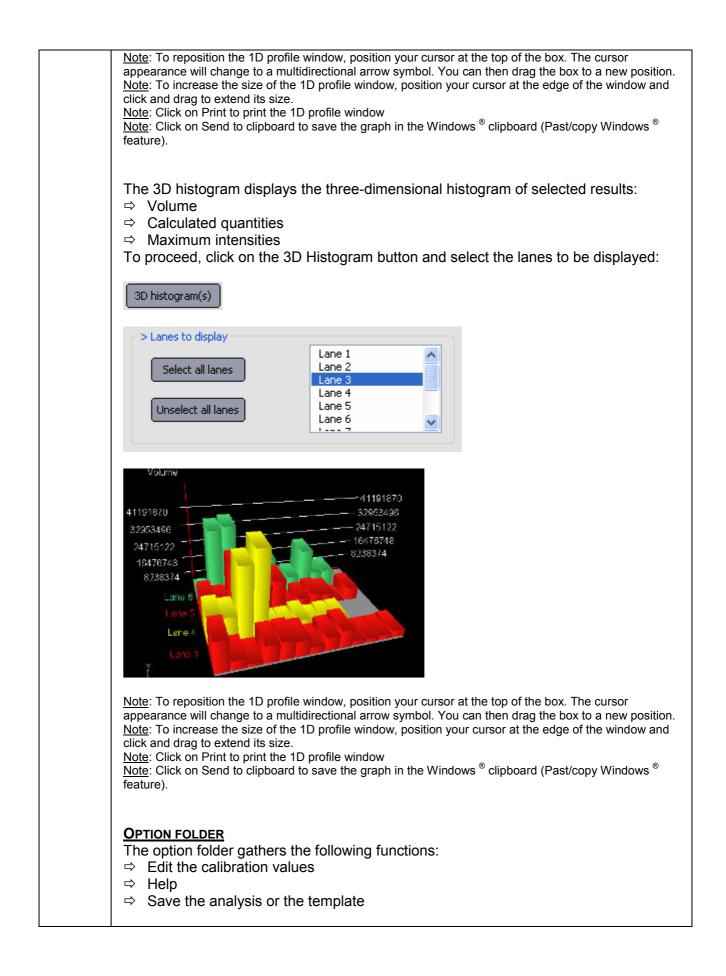


UNIT OF REFERENCE The unit of the concentration. You can define your own header such as % or μ g.	
In the "Unit of reference" edit field, type the unit you want to be displayed in the results table:	
THE CALIBRATION VALUES Click on the "Load" or "Create" button to enter calibration's values.	
Load or Create	
For "Create", a pop-up window displays the following menu:	
Value editor - Master Add value Add value Cancel Total bands Cancel Canc	
Type your values, band to band, in a descending order. The OK button validates your data.	
<u>Note</u> : 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:	
Save	
A pop-up window displays the following menu:	
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\Rightarrow Browse to specify the directory	

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21304167		_		
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> Curve type	e Linear	~		
> Order for \	value assignment	🚫 Same order	 Reversed or 	ler
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mathematical	ntal: the curve simp model,		es (point to point),	without any
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⇒ Logarithm	iic curve: displays a	a model with loga	arithmic regressior	ו
Linear	~			
Experimental				
Linear				
Linear Smooth				
Linear Smooth Logarythmic	select the order for			

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:
> Select the values to be displayed ✓ Concentration ✓ Height ✓ Area
> Lanes to display > Enhanced views Select all lanes Lane 1 Lane 2 In Profile(s) Lane 3 Lane 4 Lane 5 Lane 6 Lane 6 >
GRAPHICAL VIEW In the results parameter window, you can select the graphical results tables: ⇒ 1D profile ⇒ 3D profile ⇒ 3D histogram
Select the values to be displayed ✓ Concentration ♥ Volume ♥ Height ♥ Area ♥ Molecular Weight
> Lanes to display Lane 1 > Enhanced views Select all lanes Lane 2 1D Profile(s) 3D Profile(s) Unselect all lanes Lane 4 Lane 5 3D histogram(s) Show Image
<u>Note</u> : 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: 1D Profile(s)





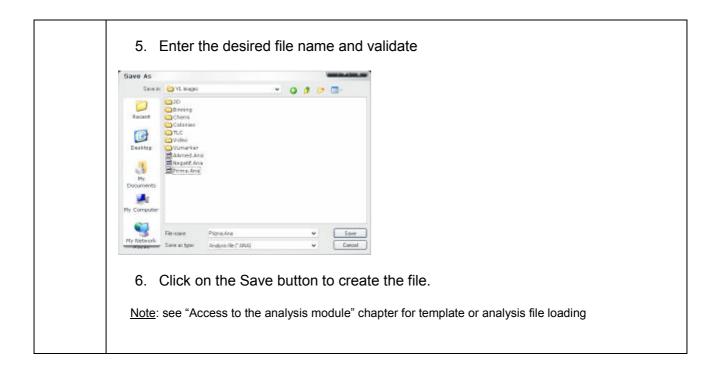
:: Edit the calibration values Help Save analysis/Template
EDIT THE CALIBRATION VALUES
Click on the "Edit the calibration values" button.
:: Edit the calibration values
A pop-up window displays the following menu on which you can modify the calibration values:
Value editor - Master
Add value Cancel
You can add, remove, and save your marker's value;
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
Help
About UVIsoft
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: 📑 Save analysis/Template 2. A pop-up window displays the following menu: Save As Save in: 😂 VI. integer · o / P 🖬 Dinning Recent Deaktop 3 0.00 . Save File name Carical Same at how on file (* 4NA) denie Re L'AN 3. Select analysis file or template file: Analysis file (*.ANA) Analysis file (*.ANA) Template File (*.PAR) Note: the software proposes Analysis file by default 4. Browse to specify the file directory Save As Savel -5 O # 🕫 🖽 Recent 0 Desktop 4 My 1 e Compa • PiperaAna Denne Sava

Cancel

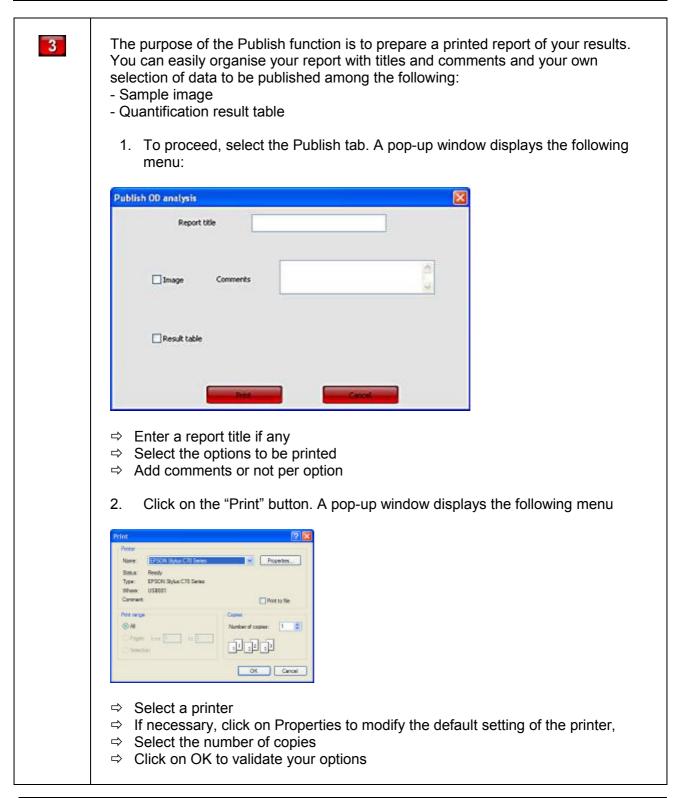
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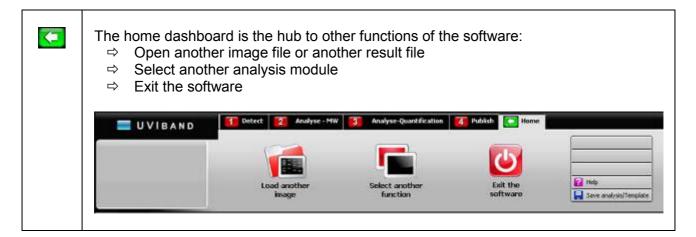
Publish

➔ Introduction

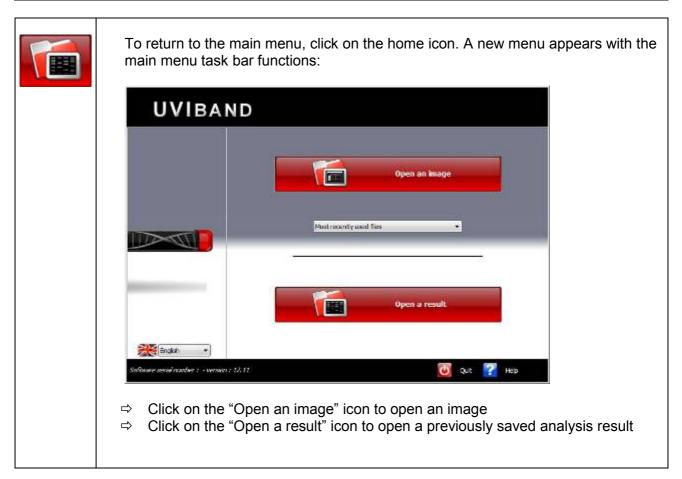


Return to Home

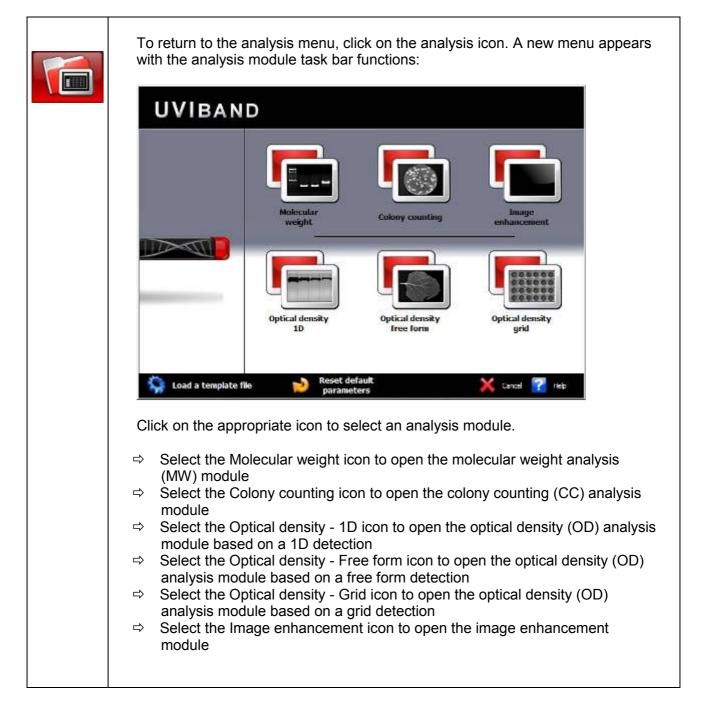
➔ Introduction



➔ Load another image



Select another function

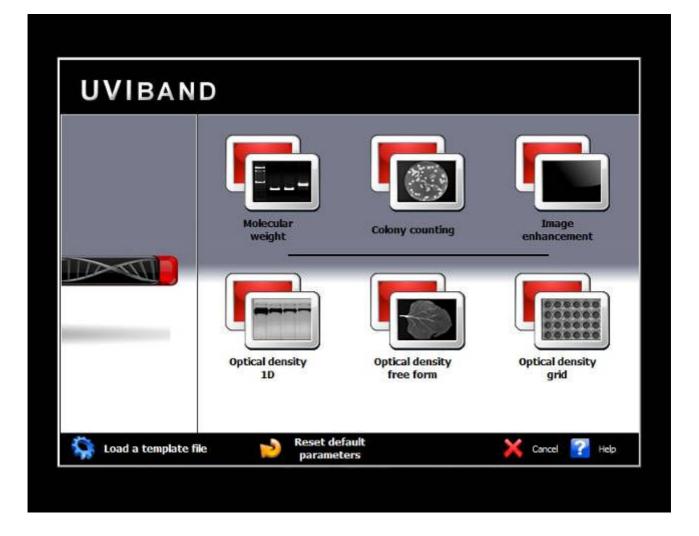


➔ Exit the software

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

You will be prompted to save your analysis.

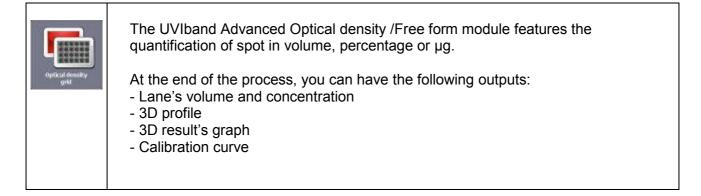




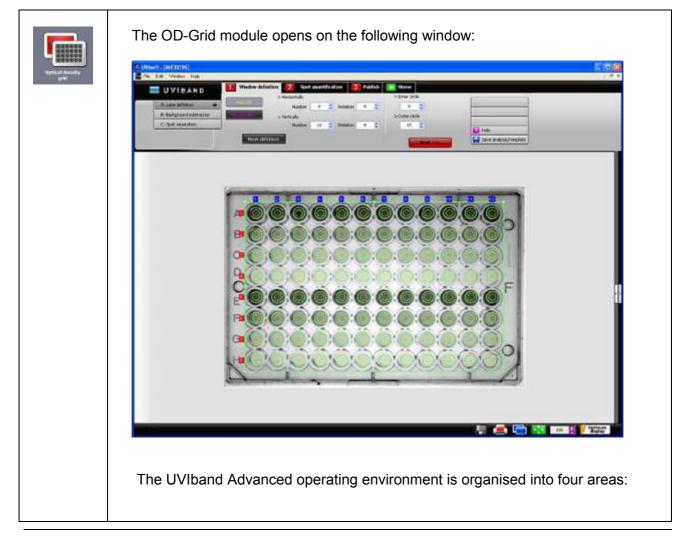
Optical density - Grid → OD-Grid Analysis module

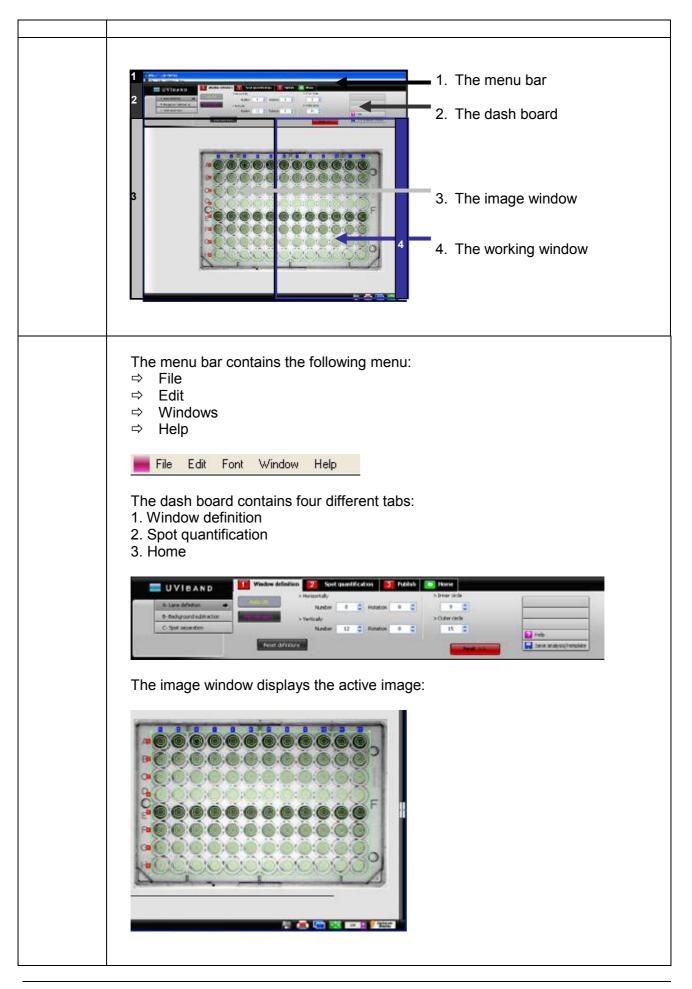
Optical density / Grid introduction

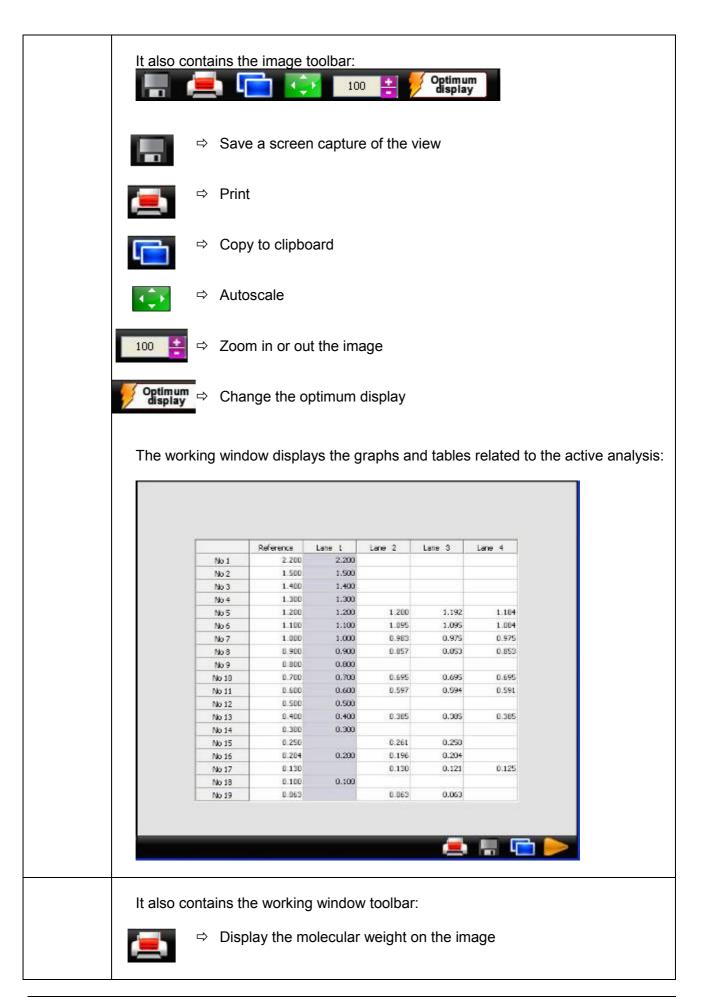
Objectives and output

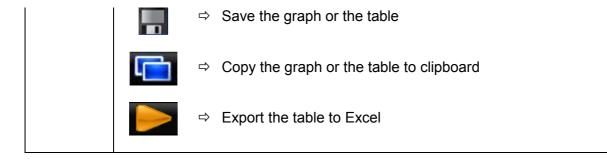


➔ Optical density / Grid operating environment

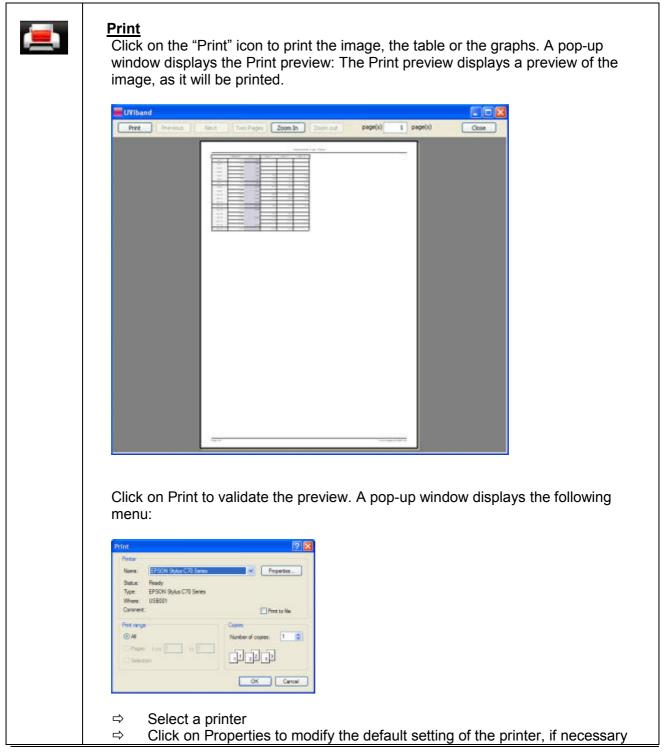








➔ Toolbar in details

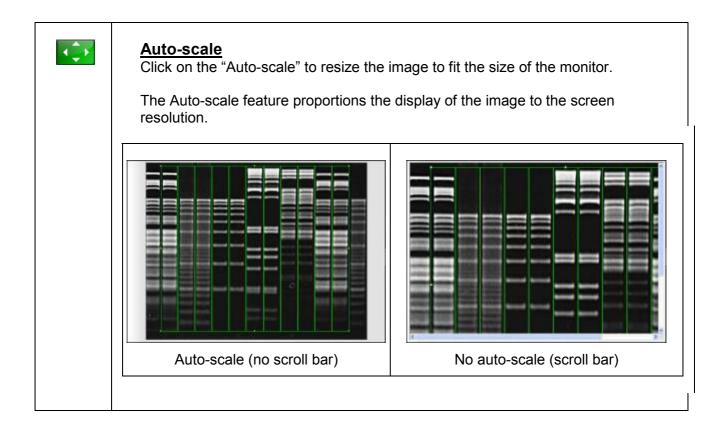


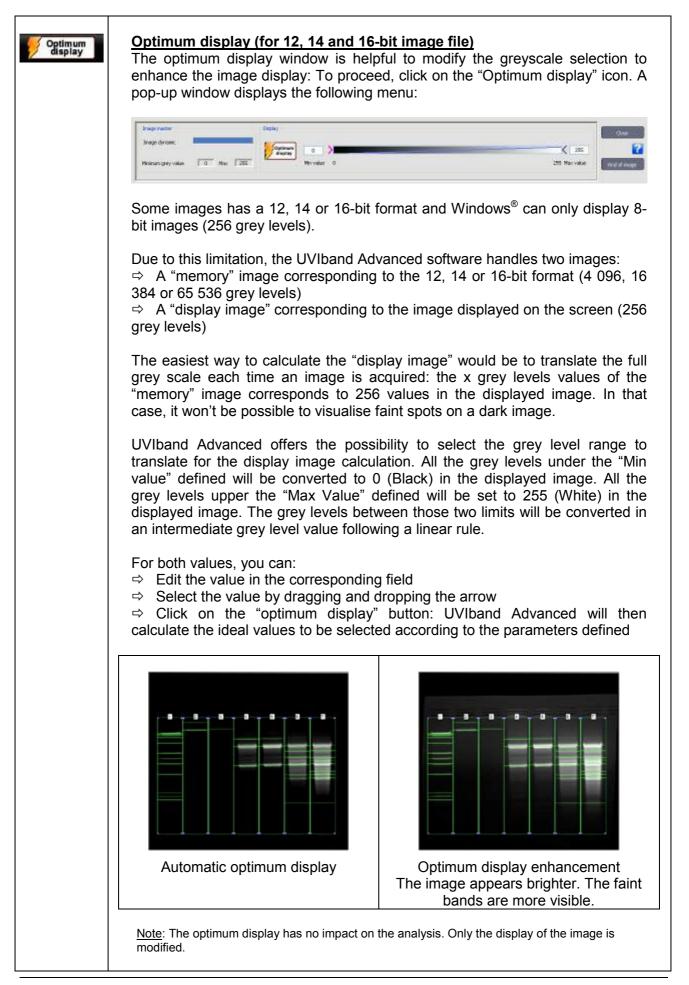
 ⇒ Select the number of copies ⇒ Click on OK to validate your options
Note: You can also access the Print menu from the Menu bar (File\Print).

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:						
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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:						
Excel file (*.XLS) Excel file (*.XLS) Text file (*.TXT) DBase file (*.DBF)						
The graphs can only be saved on a BMP format: Bitmap file (*.BMP) Bitmap file (*.BMP)						

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).

Т



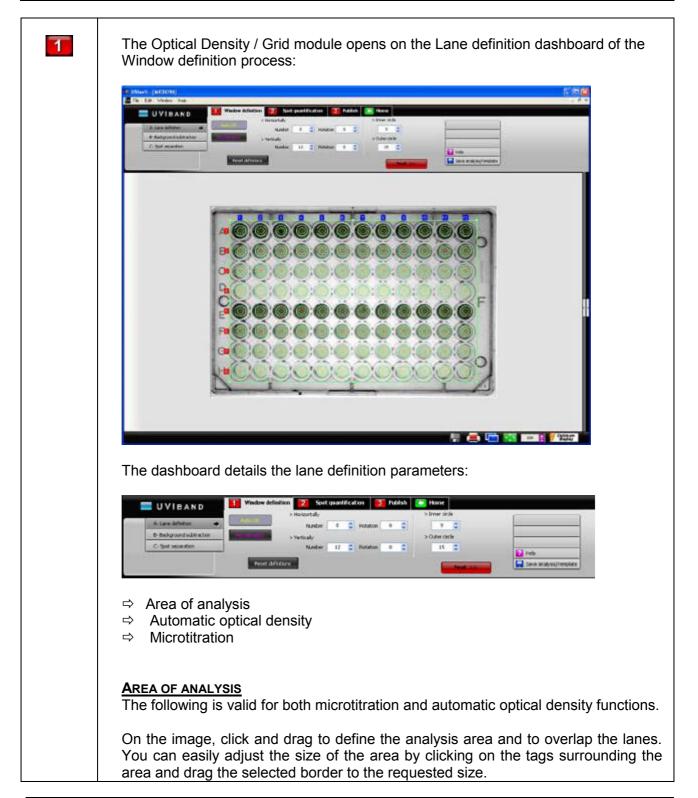




Send to Excel[™] This function transfers the results table to Windows Excel[™].

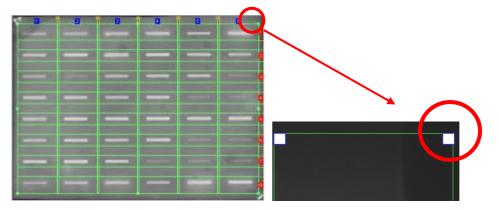
To proceed, click on the Send to Excel^{TM} icon. The Excel software is automatically opened by the UVIband Advanced and the table is transferred to Excel^{TM} .

➔ A – Lane definition



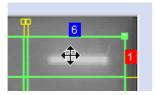
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The lanes are defined by green lines, overlaid on the gel image. The gel area is surrounded by square anchors:



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:



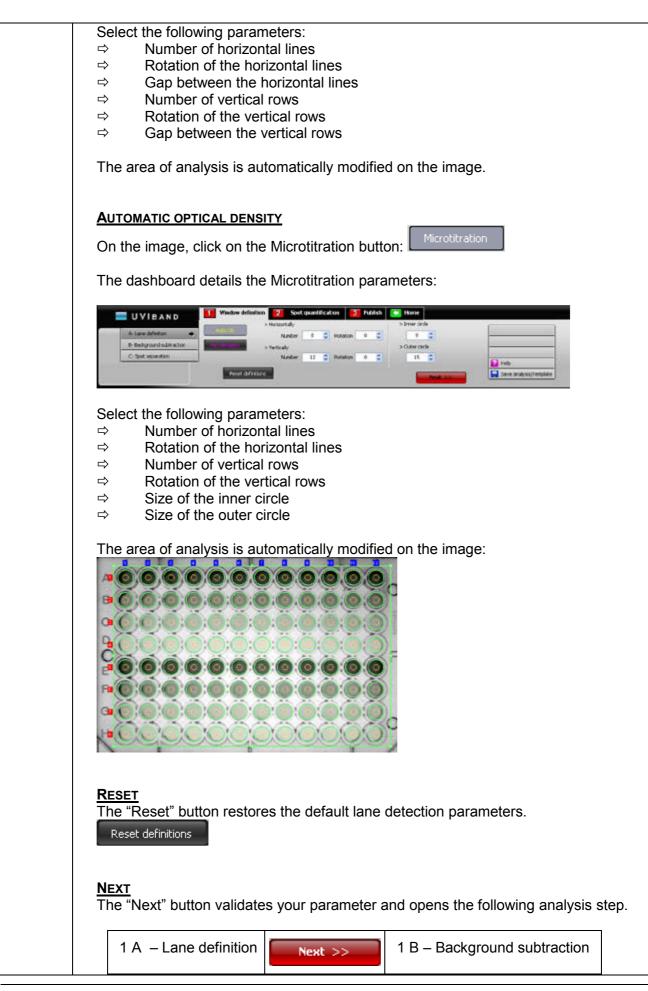
Click and drag the cursor to move the entire frame.

AUTOMATIC OPTICAL DENSITY

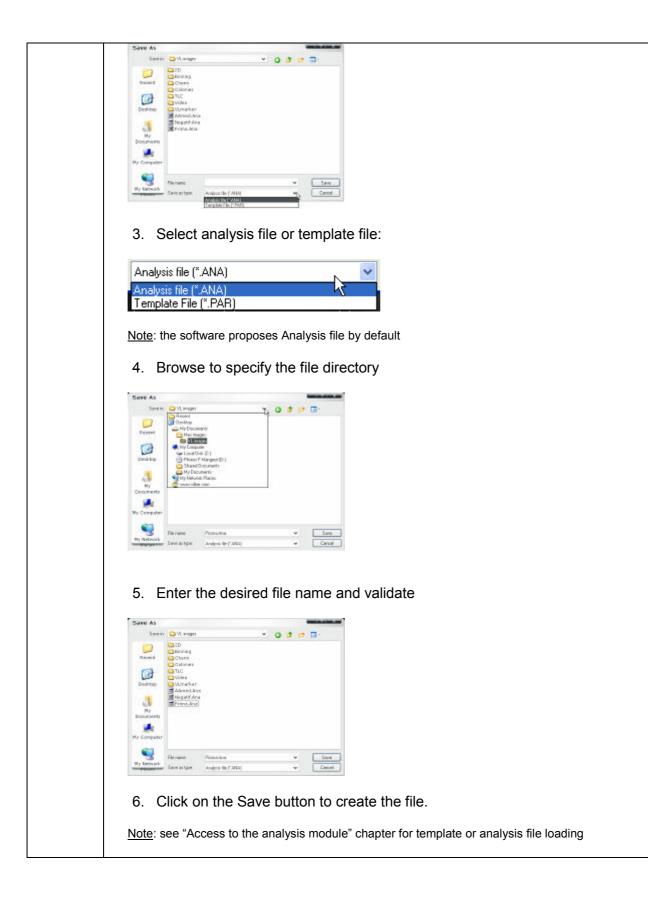
On the image, click on the Automatic OD button:

The dashboard details the Automatic Optical density parameters:

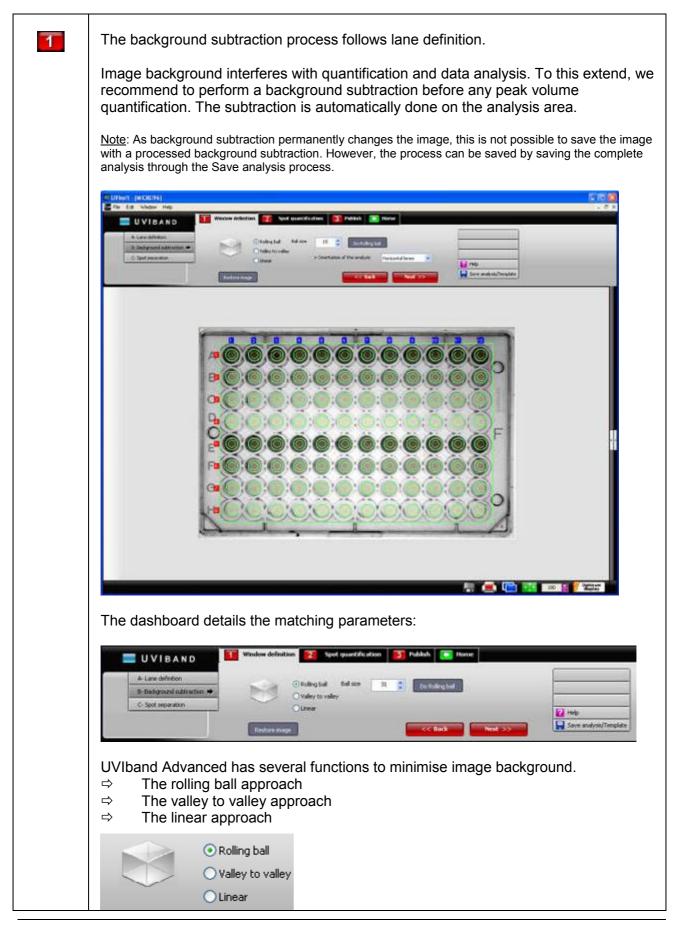
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	Save analysis/Template
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	k on the "Help" button. You automatically access the user manual at the
	oter corresponding to the function
_	Help
You	can access the help file index through the File\Help from the Menu bar
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	About UVIsoft
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This the interpretended in the interpretend	function saves the current analysis. The analysis file will contain the results image and all the parameters defined to obtain the results. analysis could also be saved as a template for automated analysis routines plate offers the user the ability to automate many of the repetitive tasks becauted with analysis and processing. As a result, you can spend more time uating and analysing results, and less time manipulating set-ups, variables a resettings. template automates a task or set of tasks that you perform repeatedly or on allar basis. It stores all the analysis commands and parameters of an analysis can run these parameters with another image whenever you need to perfore we analysis based on the same parameters. 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 temple e modifying it for a slightly different result, with minimal effort Click on the "Save analysis/Template" button:



➔ B – Background subtraction



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.

A	Josho (
Rolling ball	Background subtraction

The centre of gravity of the ball describes a curve:

- \Rightarrow This curve represents the noise to be subtracted.
- \Rightarrow 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:

31	-
	31

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

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

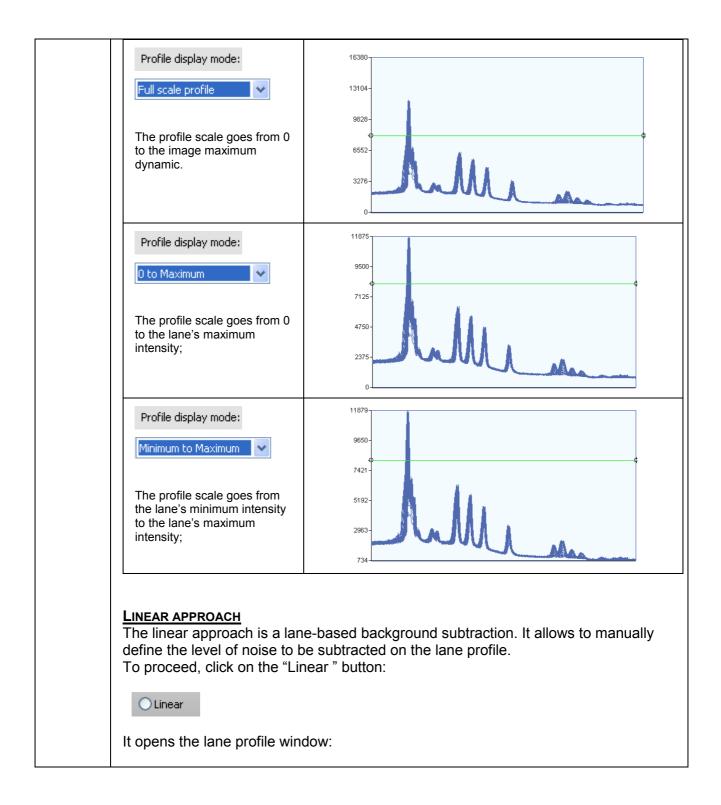
The valley-to valley approach is a lane-based background subtraction. It allows to manually define on the lane profile the level of noise to be subtracted.

Click on the "Valley to valley " button: O Valley to valley

It opens the lane profile window:

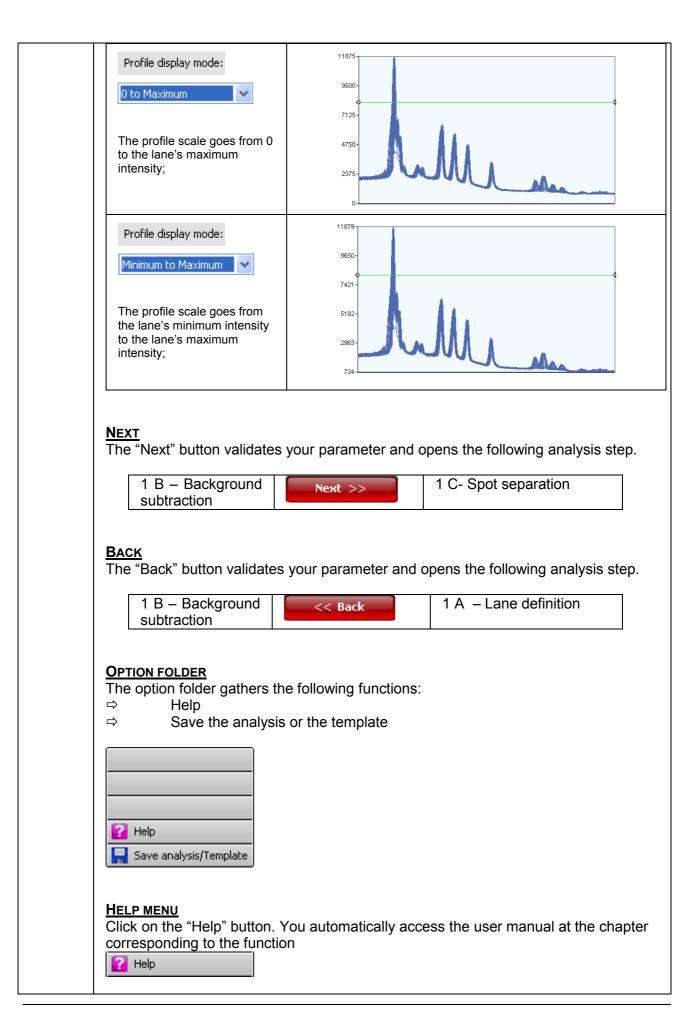
Vyher': [DHSTILL] In Lot Wriden Hill UVIB AND A Loss defetors Cool reported Co
Bategrood subtracting Votey to votey - Line 1
Rollin deploy node: "nå sode porfie 💌 Okeenge polis: Ohd polis
In the profile parameters window, select the lane to perform the valley-to-valley approach
Lane number: 1 Subtract noise Apply to all lanes
Profile display mode: Full scale profile 🔹 🔿 Average profile 💿 Full profile
On the profile, click to define the background profile you want to remove:
Then, click on Subtract noise: Subtract noise
The changes will be automatically applied to the image and to the profile:

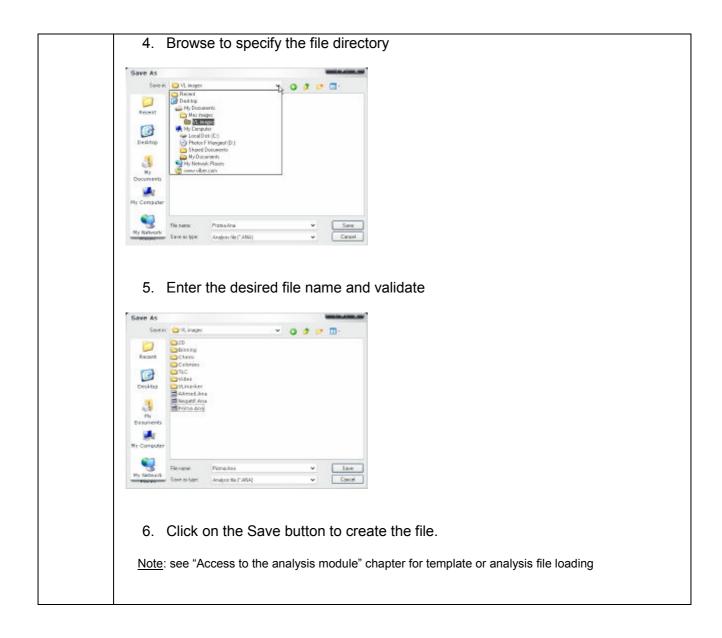
the same subtraction level fo the selected lane. Any chang	h is a lane-based background subtraction. You can set or all lanes or specify an individual subtraction level for ges you make will be automatically applied to the image.
To apply the same subtractic button:	on level for all lanes, click on the "Apply to all lanes"
Apply to all lanes	
You can easily adjust the pro	file displays settings as follows:
• Full profile	Lulli man



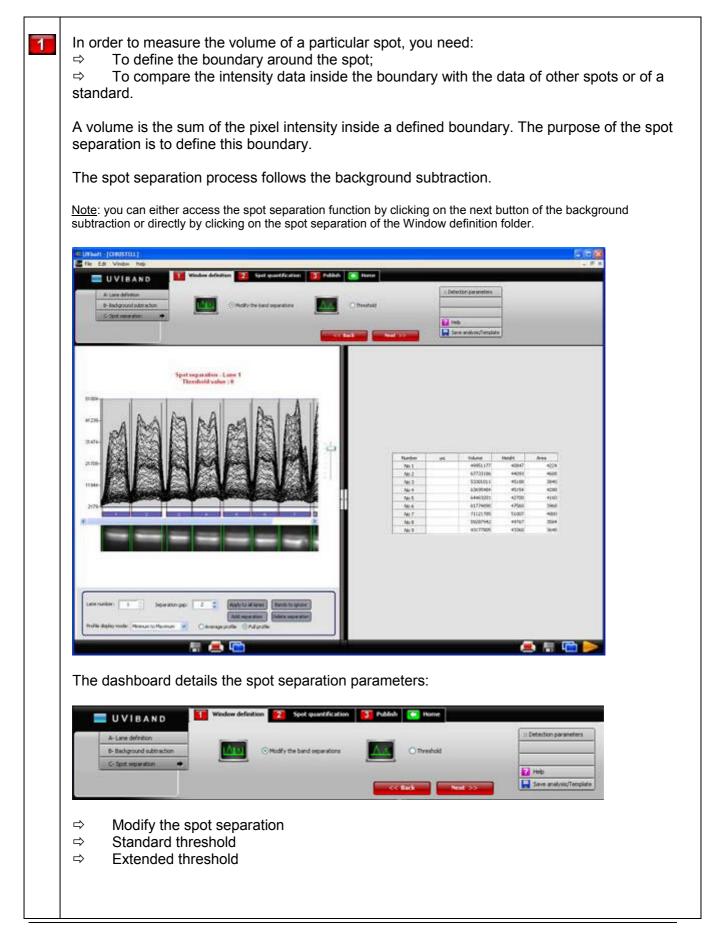
C UNINOT CORT	en Heb BAND Adda secadatector	21227		Mile Ton and/outTanglets	
				Background subtraction Valley to colley - Laws 1	
In the p	rofile parameters win	dow, select th	Funded depice model (indicate part	ھ	
	umber: 1	Subtract i		⊙ all lanes ⊙ Full profile	
On the p	profile, click to define	e the backgrou	nd linear leve	el you want to re	move:
~	htt	M			
	lick on Subtract noise Inges will be automa			and to the profile	e:

MM	M
subtraction level for all lanes	e-based background subtraction. You can set the same or specify an individual subtraction level for the selected e will be automatically applied to the image.
To apply the same subtraction button:	on level for all lanes, click on the "Apply to all lanes"
Apply to all lanes	
You can easily adjust the pro	ofile displays settings as follows:
⊙ Full profile	Autor and
O Average profile	<u> </u>
Profile display mode:	16380
Full scale profile 🛛 👻	13104- 9828-
The profile scale goes from 0 to the image maximum dynamic.	6552- 3276- 0-





➔ C – Spot separation

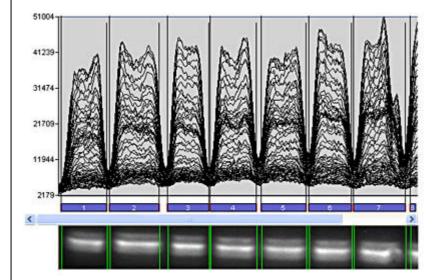


MODIFY THE SPOT SEPARATION

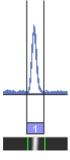
UVIband Advance proposes by default an automatic predefined spot separation based on the band detection. You can modify the default spot separation by selecting the "Modify the spot separation" option.



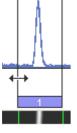
The default separation is illustrated on the lane's profile:



The brackets illustrate the bands boundaries:

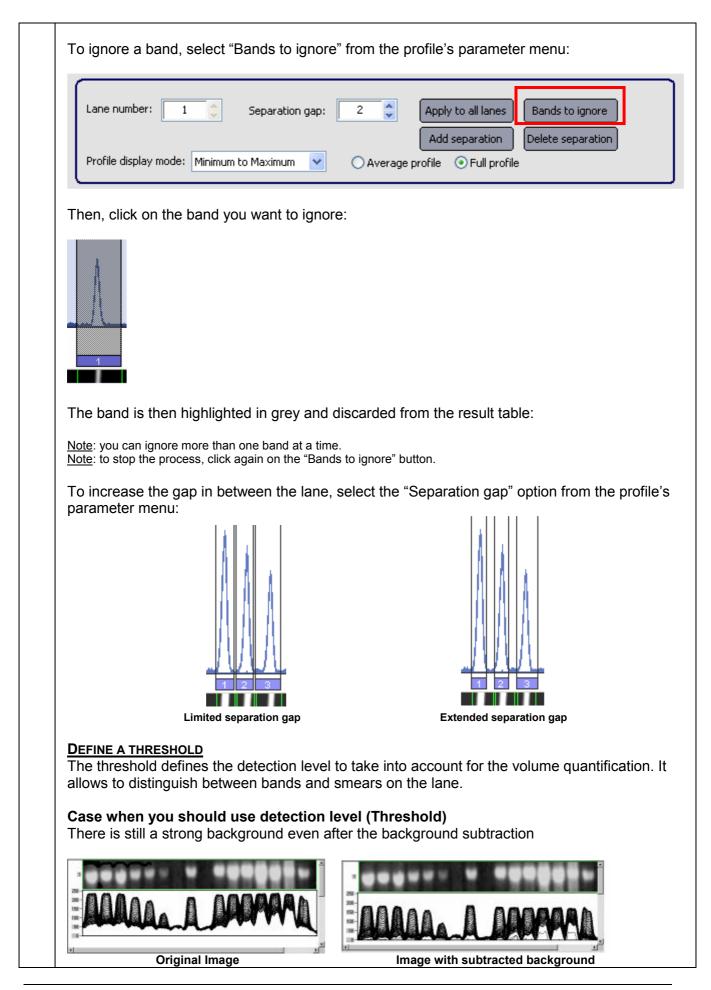


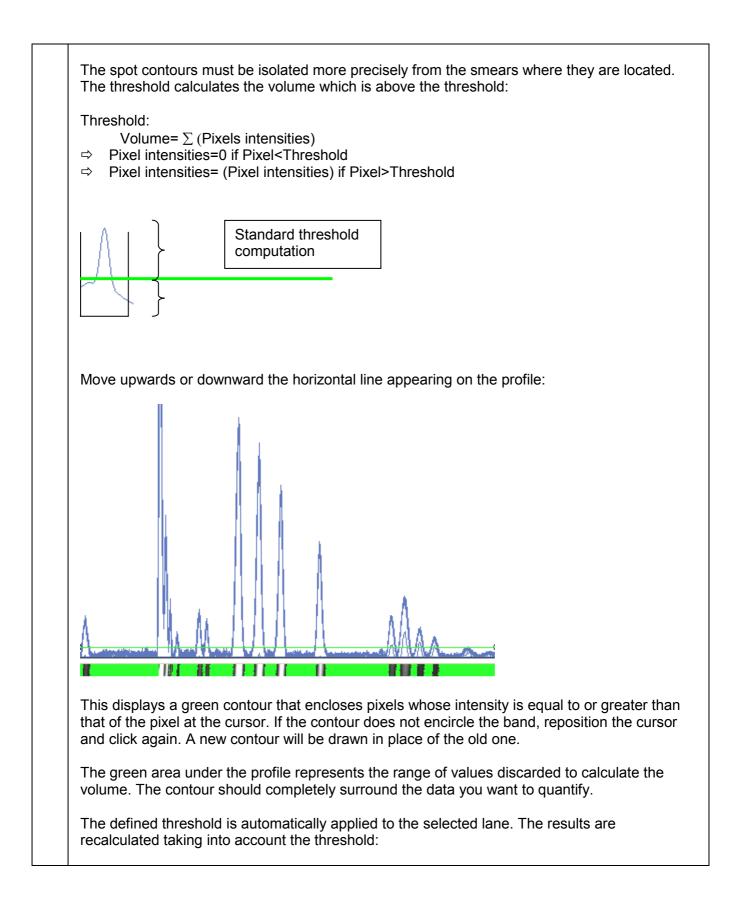
You can easily reposition a band's boundaries. In order to do so, click on the bracket and drag the cursor:



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.





Number	11	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

 \Rightarrow The volume is the sum of intensities included in the spot area of analysis.

 \Rightarrow The height is the maximum spot intensity, in grey levels.

 \Rightarrow 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:

Apply to all lanes

<u>Next</u>

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

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

<u>Васк</u>

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:

⇒

김 Help

⇒

Save the analysis or the template

:: Detection parameters

Help

Save analysis/Template

HELP MENU

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

김 Help 👘

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

Help	1
	Index

About UVIsoft...

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

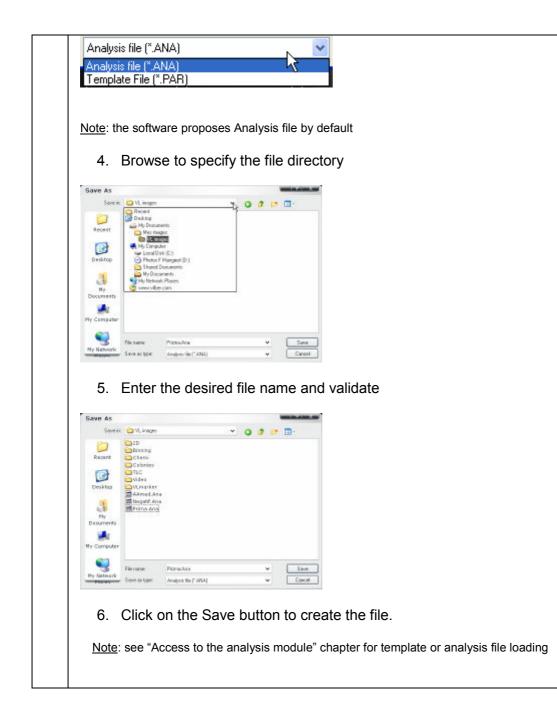
1. Click on the "Save analysis/ Template" button:

📑 Save analysis/Template

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

Save As						
Ser.	😂 VL avages		v	0	1 10	10
Recard Desktap Desktap My Documents My Computer	2D Dinning Coloniai TUC Vurenkor Mospitf Ana Prona Ana					
He Matheorik	Plename				v	- Sere
	Severet type	Analysis file ("ARM Analysis file ("ARM) Template File ("TWA			1	Canod

3. Select analysis file or template file:

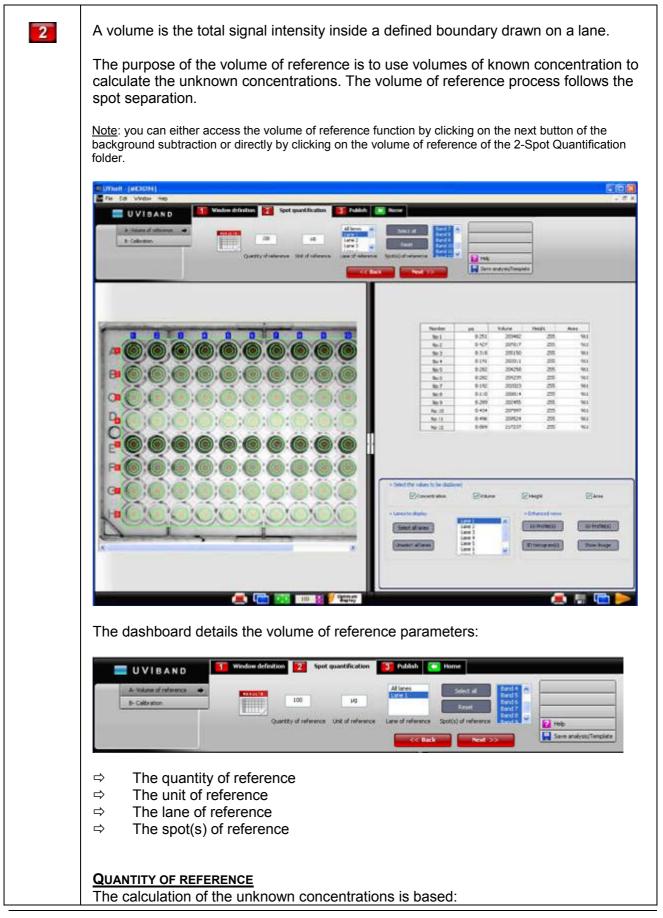


2- Analyse – Quantification

➔ 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 $V = \Sigma$ ni li
	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



 ⇒ 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:
100 Quantity of reference
<u>UNIT OF REFERENCE</u> The unit of reference is the header unit of the concentration. You can define your own header such as % or μ g.
In the "Unit of reference" edit field, type the unit you want to be displayed in the results table:
% µg Unit of reference Unit of reference Percentage as unit of reference µg as unit of reference
<u>LANE OF REFERENCE</u> The lane of reference defines the lane of the known concentration. Select the lane of reference from the list:
All lanes Lane 1 Lane 2 Lane 3 Lane 4 Lane 6
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 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 0
No 5 3.574 530507 0
No 6 1.840 273100 0 No 7 0.835 123860 0
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:

Band 1 Band 2 Band 3	
Band 4 Band 5 Band 6	~

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	μα	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

Quar	100 ntity of reference	% Unit of reference	All lanes	Select Resel e Spot(s) of re	Band 2 Band 3 Band 4 Band 5
he results t	able indicates	the followin	g 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
<mark>IEXT</mark> īhe "Next" b	utton validate	s your parar	neter and op	ens the follo	owing analysis
he "Next" b	utton validate olume of refe		neter and op Next >>		owing analysis - Calibration
he "Next" b 2 A – V <u>Back</u> he "Back" b	olume of refe	rence	Next >>	2 B -	

> Select the values to be dis	played Volume	🗸 Height	🗸 Area	Molecular Weight	
 Lanes to display Select all lanes Unselect all lanes 	Lane 1 Lane 2 Lane 3 Lane 4 Lane 5 Lane 6		> Enhanced views 1D Profile(s) 3D histogram(s)	3D Profile(s)	
GRAPHICAL VIEWIn the results parame⇒ 1D profile⇒ 3D profile⇒ 3D histogram	eter window,	you can sele	ect the graphical	results tables:	
 Select the values to be dis Concentration 	played 🗸 Volume	🗸 Height	🗸 Area	Molecular Weight	
> Lanes to display Select all lanes Unselect all lanes	Lane 1 Lane 2 Lane 3 Lane 4 Lane 5 Lane 6		> Enhanced views 1D Profile(s) 3D histogram(s)	3D Profile(s)	
 <u>Note</u>: 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: 					
1D Profile(s)					
16380 13104 9828 8552 3276 7 Lane 4 Lane 4 Lane 2 Lane 1	16360 13104 9928 6552 3278				

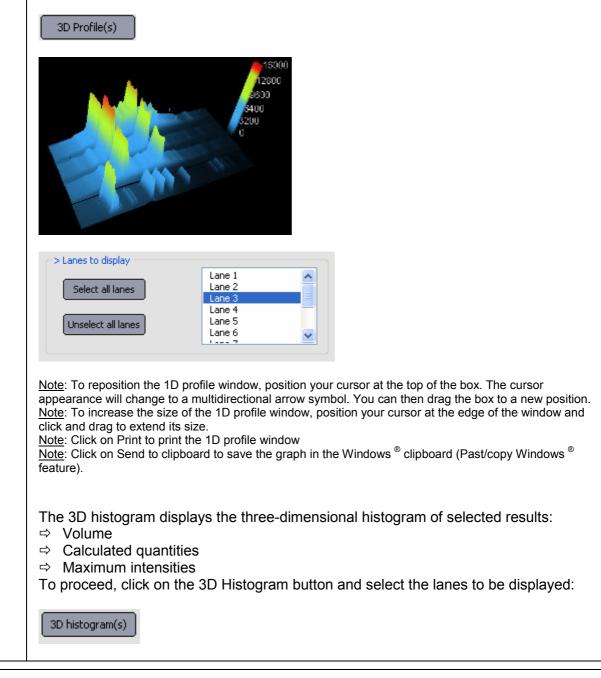
	Lane 1	~
Select all lanes Unselect all lanes	Lane 2	
	Lane 3	=
	Lane 4	_
	Lane 5	
	Lane 6	~
	1 7	

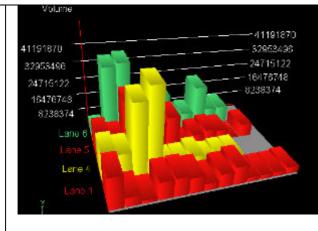
<u>Note</u>: To reposition the 1D profile window, position your cursor at the top of the box. The cursor appearance will change to a multidirectional arrow symbol. You can then drag the box to a new position. <u>Note</u>: To increase the size of the 1D profile window, position your cursor at the edge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

Note: Click on Print to print the 1D profile window

<u>Note</u>: 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:





<u>Note</u>: 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. <u>Note</u>: 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

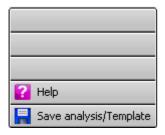
<u>Note</u>: Click on Send to clipboard to save the graph in the Windows $^{\ensuremath{\mathbb{B}}}$ clipboard (Past/copy Windows $^{\ensuremath{\mathbb{B}}}$ feature).

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



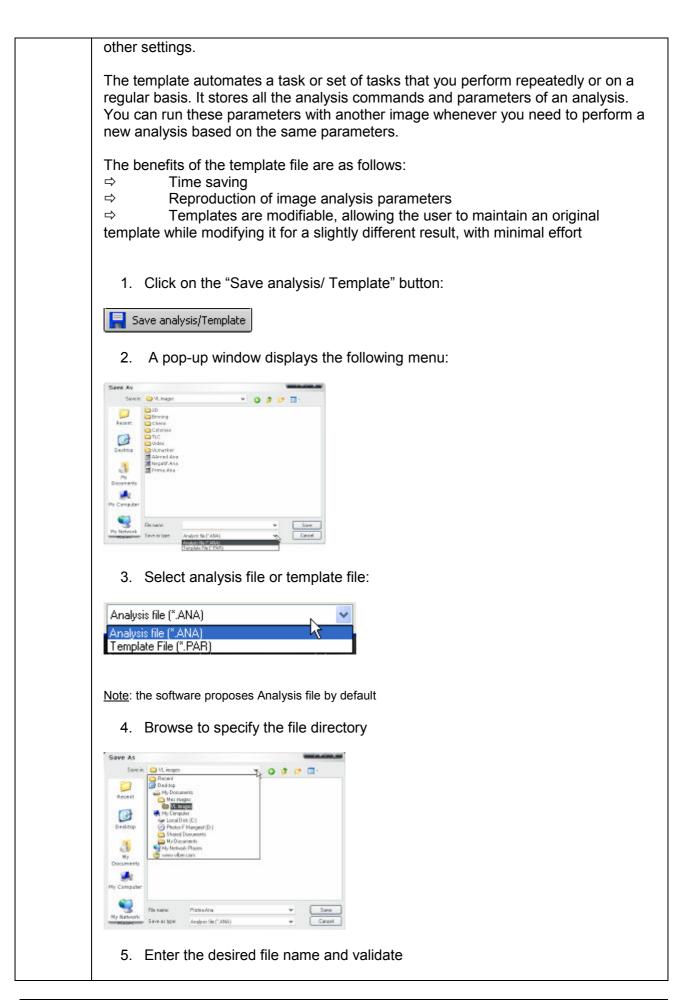
You can access the help file index through the File\Help from the Menu bar

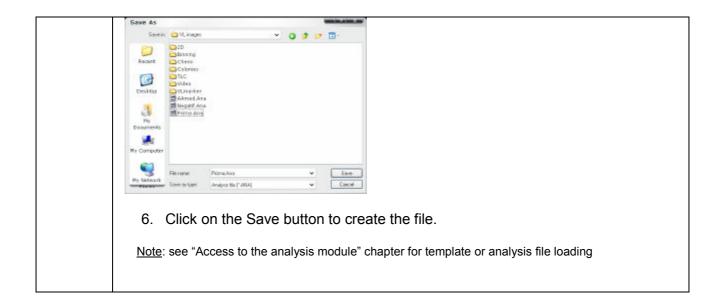
Help	
	Index
	About UVIsoft

SAVE ANALYSIS / TEMPLATE

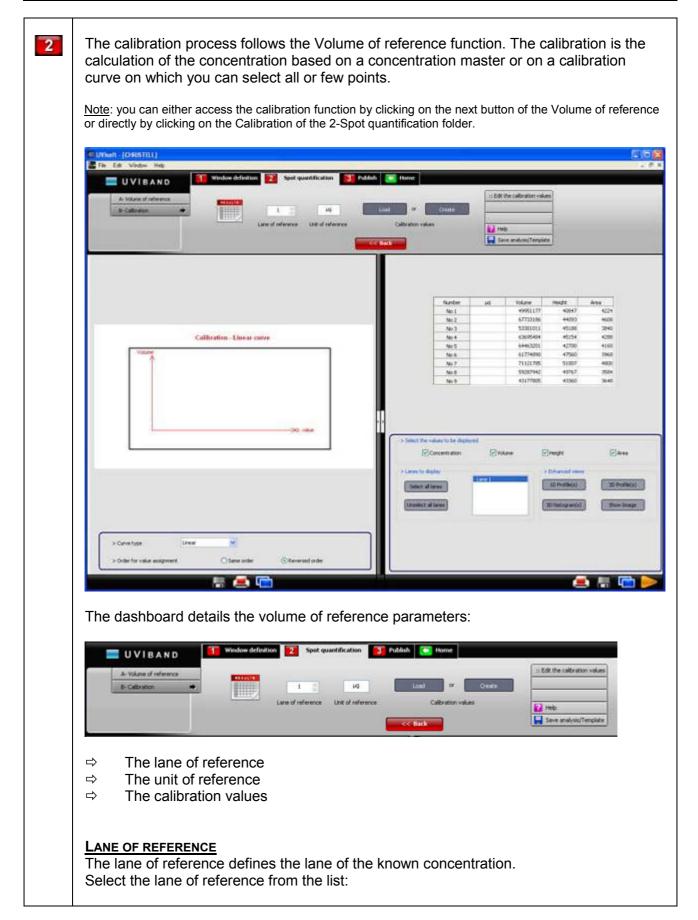
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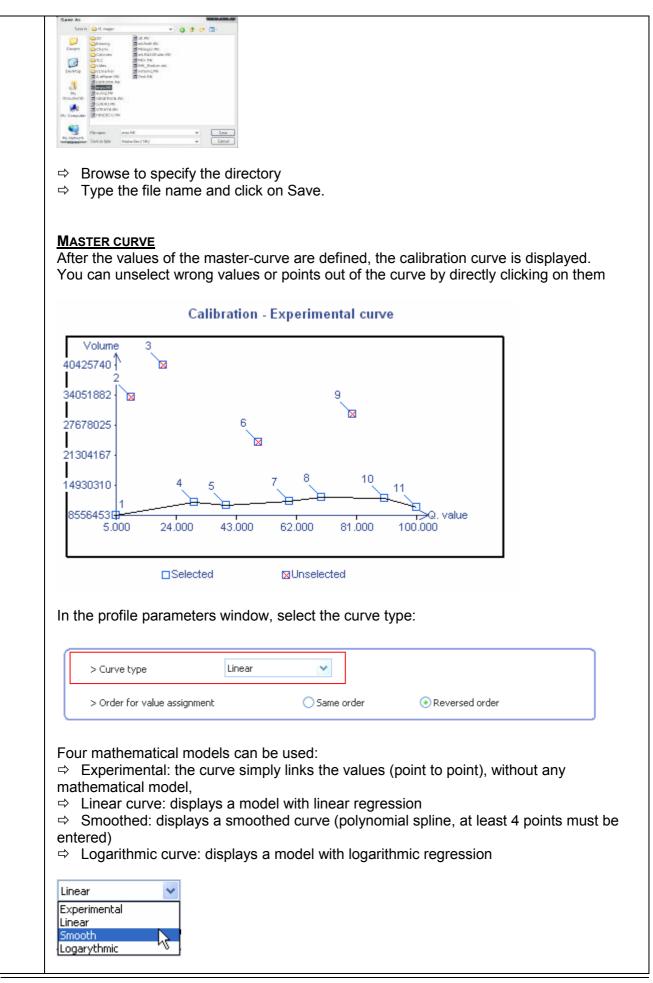




➔ B – Calibration

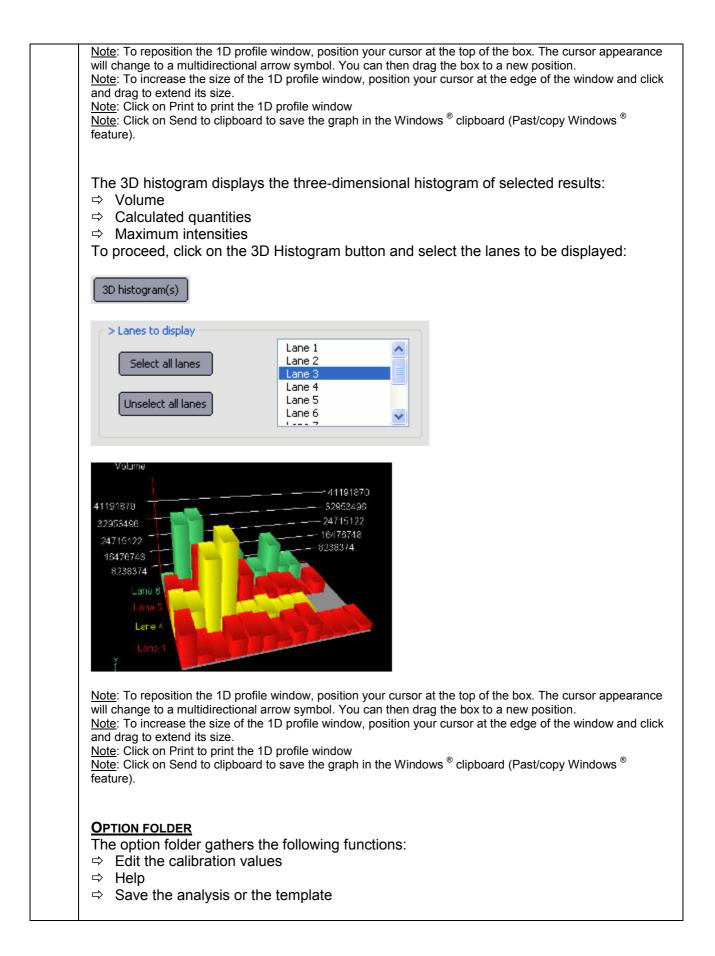


All lanes Lane 1 Lane of reference
UNIT OF REFERENCE
The unit of reference is the header unit of the concentration. You can define your own header such as % or μ g. In the "Unit of reference" edit field, type the unit you want to be displayed in the results table:
% µg Unit of reference Unit of reference 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.
Load or Create
For "Create", a pop-up window displays the following menu:
Value editor - Master Value 100.000 90.000 90.000 80.000 70.000 60.000 Total bands 6 Save
Type your values, band to band, in a descending order. The OK button validates your data.
<u>Note</u> : 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:
Save
A pop-up window displays the following menu:



	Linear	*		
> Order for value assi	gnment	◯ Same order	 Reverse 	ed order
 the results tables Concentration Volume The maximum in The area 	ntensity			ne values to be disp
o select your displ	•	on the approp	oriate selection:	
Concentration	Volume	🗹 Height	🗹 Area	Molecular Weight
> Lanes to display		h (> Enhanced views	
Select all lanes	Lane 1 Lane 2		1D Profile(s)	3D Profile(s)
Unselect all lanes	Lane 3 Lane 4 Lane 5 Lane 6		3D histogram(s)	Show Image
the results param 1D profile 3D profile		you can select ♥ Height	✔ Area	esults tables: ✓ Molecular Weight
 the results param 1D profile 3D profile 3D histogram Select the values to be only and the values to be values	displayed	✓ Height	Area Enhanced views	Molecular Weight
 the results param 1D profile 3D profile 3D histogram Select the values to be of Concentration 	Volume Volume Lane 1 Lane 2 Lane 3		✔ Area	
 3D profile 3D histogram > Select the values to be of Concentration > Lanes to display 	displayed Volume Lane 1 Lane 2	✓ Height	Area Enhanced views	Molecular Weight

Ϋ́.	
16380 13104 9828 6552 3738 6552 3738 6552 3738 6552 3738 6552 3738 6552 3738 6552 3738 6552 3738 6552 3738 6552 3738 6552 3738 6552 3738 6552 3738 6552 3738 6552 3738 6552 3738 6552 3738 6552 3738 6552 3738 6552 3738 6552 3738 6552 3738 6552 3738 6552 3738 6552 3738 6552 3738 6552 3738 6552 3738 6552 3738 6552 3738 6552 3738 6552 3738 6552 3738 6552 3738 6552 3738 6552 3738 6552 3738 6552 3738 6552 3738 6552 3738 6552 3738 6552 3738 6552 3738 6552 3738 6552 3738 6552 3738 6552 3738 6552 3738 6552 3738 6552 6552 3738 6 6 6 7 7 7 8 7 8 7 8 7 8 7 8 7 8 7 8	
> Lanes to display Select all lanes Unselect all lanes	Lane 1 Lane 2 Lane 3 Lane 4 Lane 5 Lane 6 Lane 7
Note: To increase the size o and drag to extend its size. Note: Click on Print to print to Note: Click on Send to clipbo feature). The 3D profile displays To proceed, click on the	onal arrow symbol. You can then drag the box to a new position. of the 1D profile window, position your cursor at the edge of the window and click the 1D profile window oard to save the graph in the Windows [®] clipboard (Past/copy Windows [®] o the three-dimensional rendering of any selected lanes. e 3D Profile button and select the lanes to be displayed:
3D Profile(s)	
> Lanes to display Select all lanes Unselect all lanes	Lane 1 Lane 2 Lane 3 Lane 4 Lane 5 Lane 6 Lane 6



:: Edit the calibration values
EDIT THE CALIBRATION VALUES 1. Click on the "Edit the calibration values" button.
:: Edit the calibration values
A pop-up window displays the following menu on which you can modify the calibration values:
Value editor - Master

		Value editor	- Master	
R-			Add value	
100.000 90.000 80.000 70.000			Delete value(s)	Cancel
60.000 50.000	Total bands	6	Save	2

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

🕜 Help

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

Help 📗

Index
About UVIsoft

SAVE ANALYSIS / TEMPLATE

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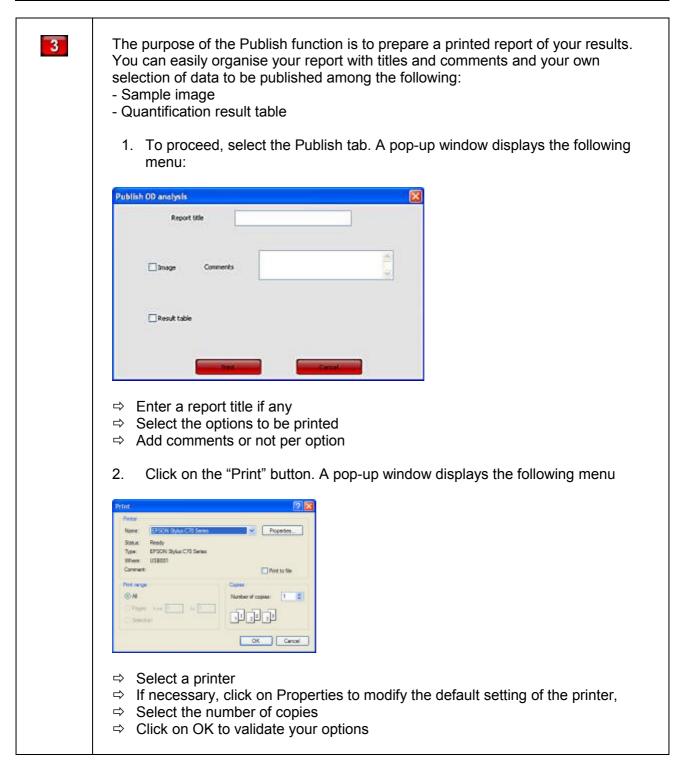
The template automates a task or set of tasks that you perform repeatedly or on a regular basis. It stores all the analysis commands and parameters of an analysis. You can run these parameters with another image whenever you need to perform a new analysis based on the same parameters.

The	penefits of the template file are as follows:
⇔ ⇔ ⇔ while	Time saving Reproduction of image analysis parameters Templates are modifiable, allowing the user to maintain an original template 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:
Save As San Recent Desktop Desktop Ny Compet	
3.	Select analysis file or template file:
Analy	sis file (*.ANA)
<u>Note</u> :	the software proposes Analysis file by default
4.	Browse to specify the file directory
Save As Save Pocert Desistop My Desarrent My Py Compute	
Ph Arthor	e fore entree Andyst Ba (1864) v Cancel
5.	Enter the desired file name and validate

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	Dinning											
Recent	Colonies											
	C TLC											
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	AAmed Are Regatit Ana											
3	Prime Ana											
Ply Documents												
1												
My Computer												
07												
1	File name:	Plane.Ana		v		Save						
Pty Network	Save at type:	Andyois file ("ANA)				Cancel						
•												
6.	CIICK O	n the Sav	/e b	uttor	t tC	o creat	e the file.					
Note:	see "Ar	cess to the	anal	veie r	nor	dule" ch	anter for te	mnlate (or analysis	file loadi	na	
NOIC.	SCC AU		anai	y313 I	1100			inplate t	or analysis	nic loaun	ig	

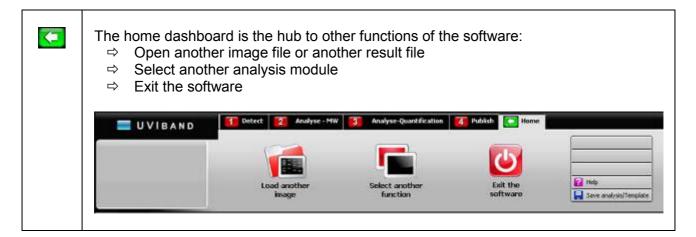
Publish

➔ Introduction

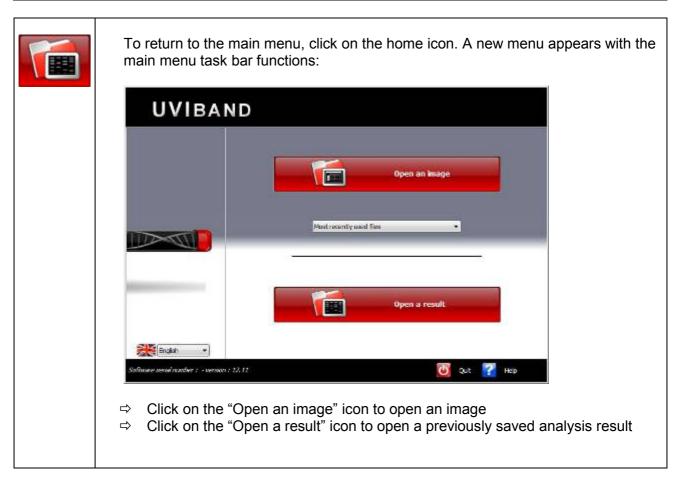


Return to Home

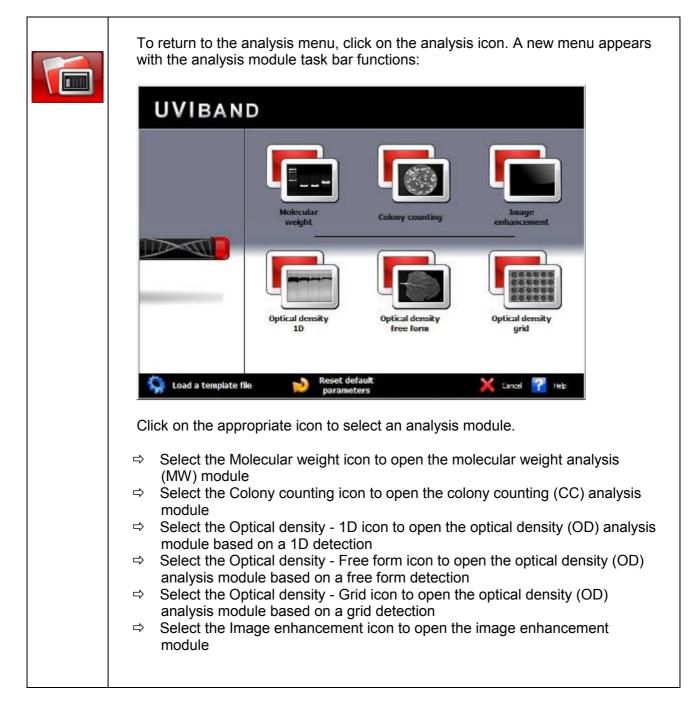
➔ Introduction



➔ Load another image



Select another function

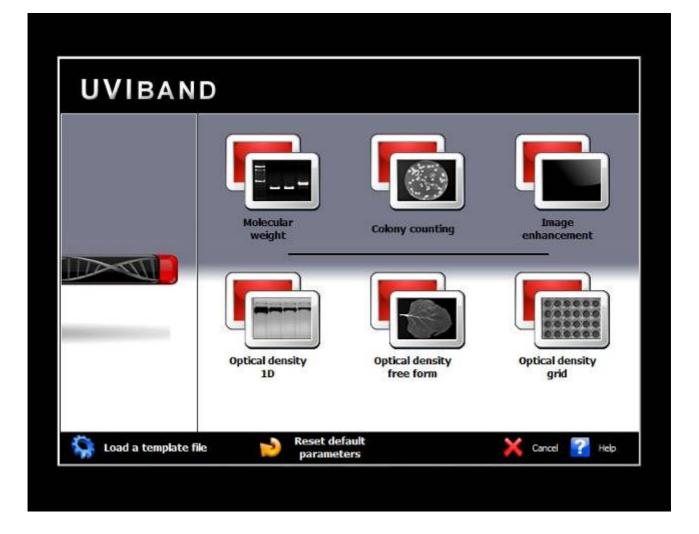


➔ Exit the software

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

You will be prompted to save your analysis.

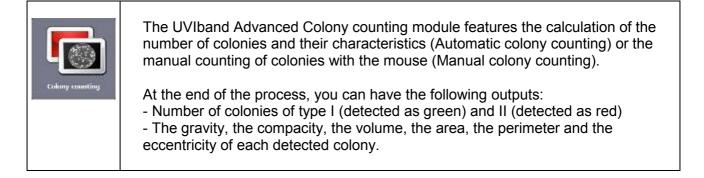




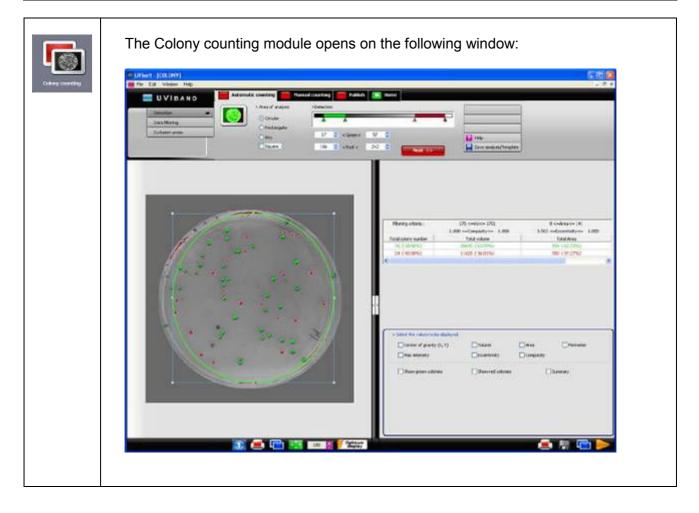
Colony counting → CC Analysis module

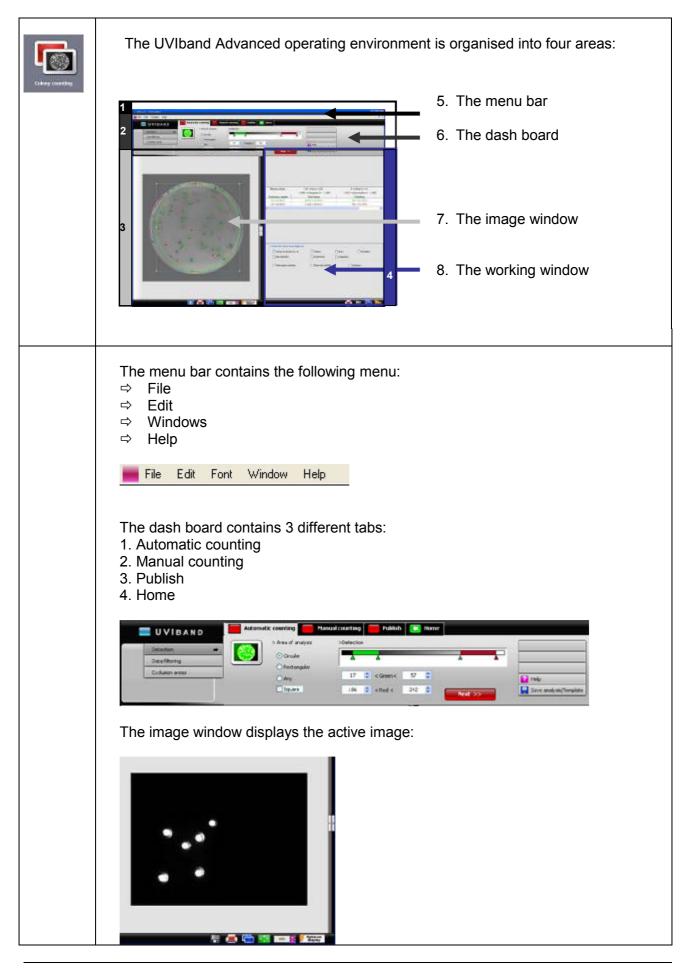
Colony counting introduction

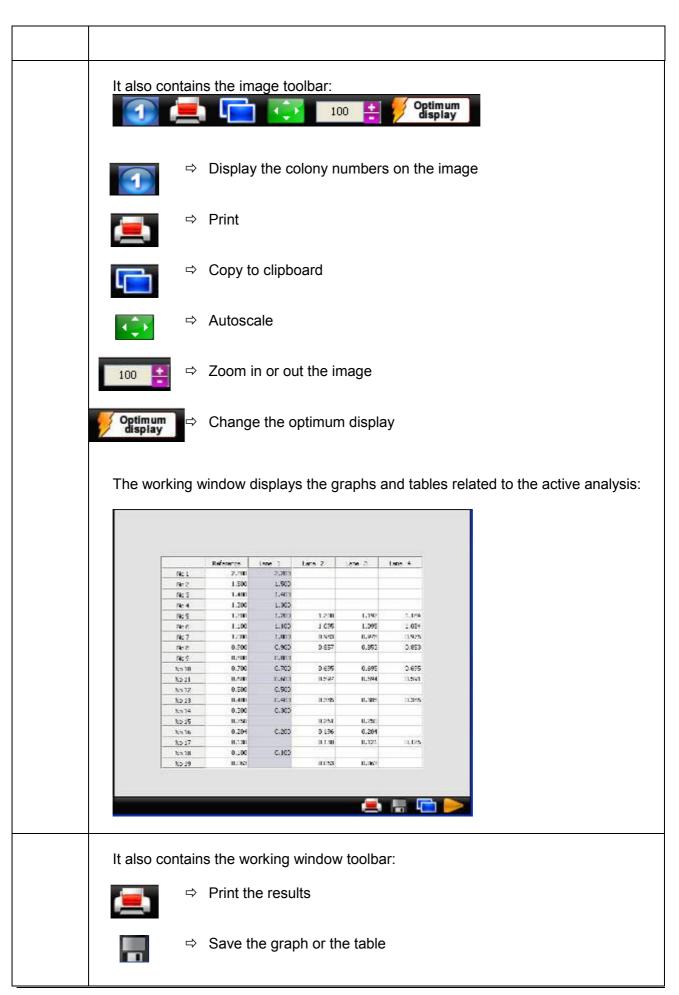
➔ Key features



→ Colony counting module (CC) operating environment









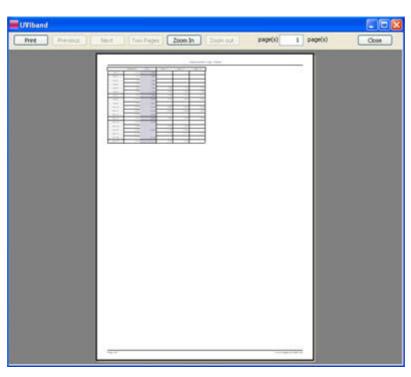
- ⇒ Copy the graph or the table to clipboard
- ⇒ Export the table to Excel

➔ Toolbar in details

-	-		
			-
-	-		
		-	

<u>Print</u>

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:

fint	2
Pretar Norre: EPSON Styles C70 Server	Popertos
Statue: Ready Type: EPSON Stylus C70 Series Where: USE001	
Connect	🎦 Prest, to file
Pertinge	Coper
⊙ Al	Number of copies: 1 🤹
Chape inc 1 is 1	
	OK Cancel

- ⇒ 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).

11	
 1	

<u>Save</u>

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:

Save As						
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Rocert Desktop Po Decurrents	2D Binning Columine Columine TUC Video					
Ny Computer	Filenatur Save as type	Exced rile (* 30,51			-	Silve Cwol
Here	THE PARTY OF	Excelled [2015] Test He (2017) D0ars No (2017)		b:	-	Lace

Browse to specify the file directory

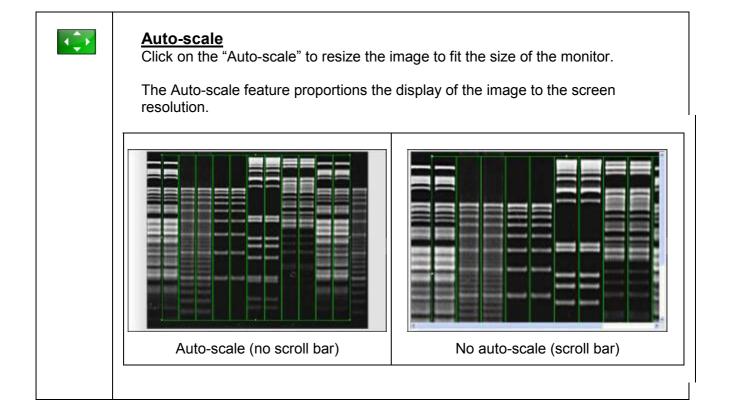
Save As						
	Photos -	gen - R¢ agen ten da (C) FMongent (D1) Otacuerati umenta di Robins	•	0	•	
He Computer	File some	Facel Me (*34.5)				Salve Cascal

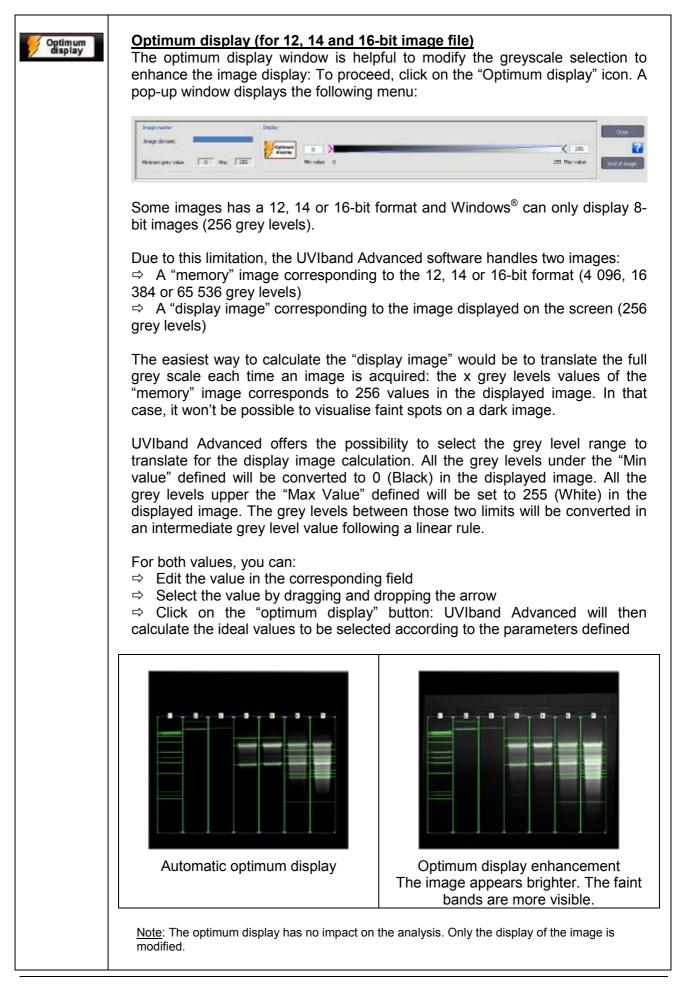
Enter the desired file name, select a file extension and validate

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

Excel file (*.XLS)	Ν	*
Excel file (*.XLS) Text file (*.TXT) DBase file (*.DBF)	12	
Text file (*.TXT)		
DBase file (*.DBF)		
The graphs can only be save	ed on a BMP	format
Bitmap file (*.BMP)		
Bitmap file (*.BMP)		N

[]	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).







Send to Excel[™] This function transfers the results table to Windows Excel[™].

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

Automatic counting

Detection

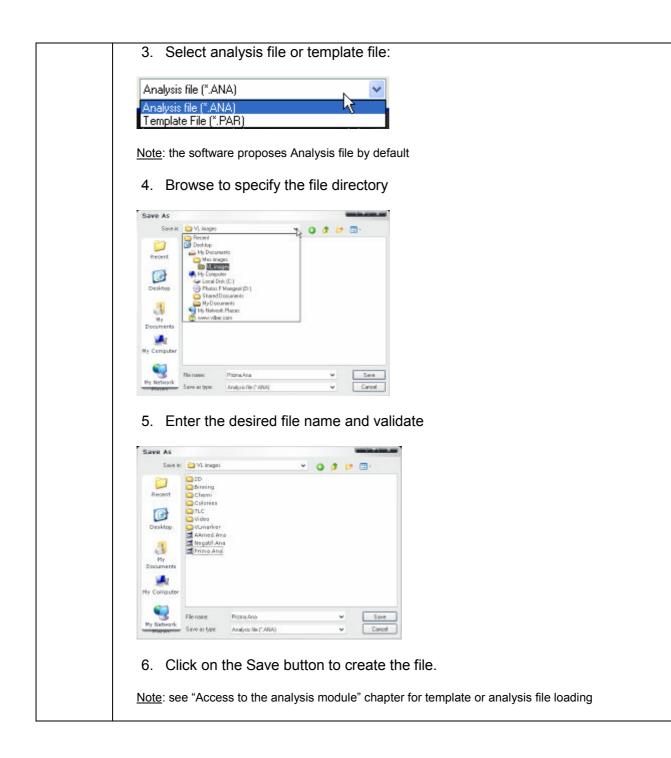
The detection process automatically identifies all the colonies for a defined area of analysis lanes. The colonies will be automatically detected when you first access the band detection process, based on default parameters. Two types of colonies can be detected: Type A called green type, overlaid on green on the image. • Type B, called red type, overlaid on red on the image. UVIBAND đ 9 Conte 2 4.99914 in tele Ote 104 2 attada 212 2 City.ers. 175 collect 170 1.000 ---3.512 e-6 01/1- L008 - 1.000 Intelestry narder Tatal volume TatalActa. 24 (45.02%) 0.620 (106-01%) 00 (7.175) Center of granty (5, 1) Desi 33 🛋 🖻 100 - Maging 💻 🐘 📭 The dashboard details the lane definition parameters: UVIBAND Orule т Chedangula 17 C «Greek 57 C O Are 🔽 this Seve analysis/Te ⇔ Define the area of analysis ⇔ Adjust the detection parameters

	define a circular, a rectangular or a free form area of analysis:
📀 Circular	
ORectangula	ar
○ Any	
Square	
To define a	a free form, click on the first point of the area on the image. Mo
	efine one edge of the area. Validate this edge by clicking once
repeats this	s procedure as many times as necessary.
Select the	"Square" option to obtain a circle instead of an ellipse or a
	a rectangle.
	E DETECTION PARAMETERS
The detect	ion parameters are summarised in the following bar graph:
A	
1	
	and red areas represent the grey level range used to determin
kinds of co	lonies.
Click on th	ne coloured triangle and drag them to a new place to mod
detection ra	ange. A preview of the detection is displayed on the image.
Note: vou cou	uld also type the grey level range in the detailed green and red parameters:
,	
35 💲	< Green < 66 🗘
145 🛟	< Red < 255 🛟
· · ·	
RESULT TAI	BLE It parameter window, you can select the lanes and the values to
	n the results tables:
	e of gravity
⇔ Volun	ne
⇒ Area⇒ Perim	leter
	num intensity
	•
⇒ Eccer	

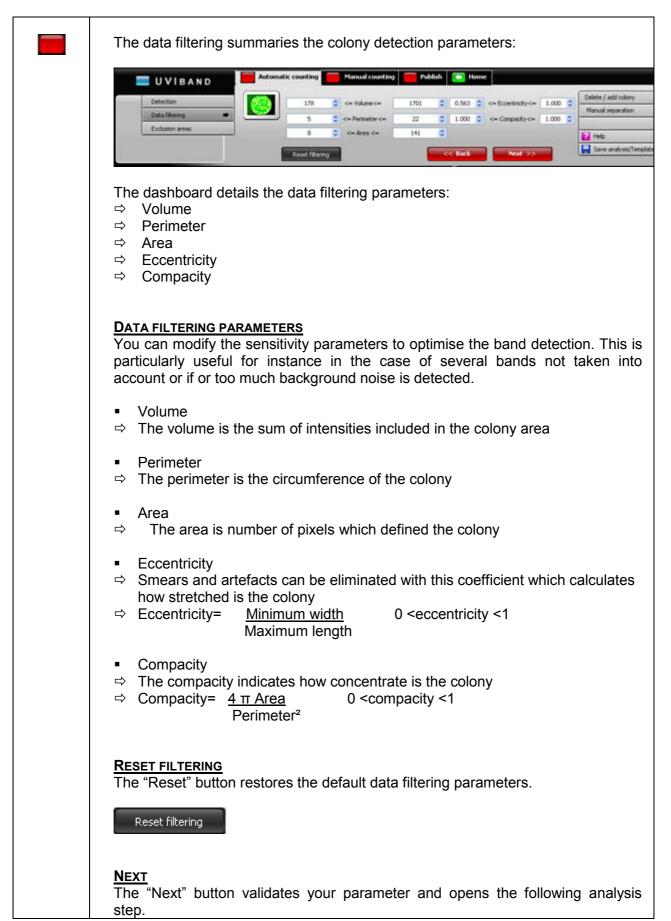
ſ	> Select the values to be displayed			
	Center of gravity (X, Y)	Volume	Area	Perimeter
	Max intensity	Eccentricity	Compacity	
	Show green colonies	Show red colonies	🗸 Su	ummary
■ ⇒	Centre of gravity Co-ordinates of the centre of	of gravity of the de	etected colony	
■	Volume The volume is the sum of ir	tensities included	in the colony a	rea
■	Area The area is number of pix	els which defined	the colony	
■	Perimeter The perimeter is the circum	ference of the cold	ony	
■ ⇒	Maximum intensity The maximum intensity is the	ne grey level heigh	nt of the spot.	
■ ⇔	Eccentricity Smears and artefacts can be how stretched is the colony Eccentricity = <u>Minimum</u> Maximum I	width 0 <ec< th=""><th>this coefficient</th><th>which calculates</th></ec<>	this coefficient	which calculates
		w concentrate is t 0 <compacity< th=""><th></th><th></th></compacity<>		
T	EXT he "Next" button validates y ep.	our parameter an		
	Detection	Next >>	Data filtering	
		-		

HELP ME	
	the "Help" button. You automatically access the user manual at the corresponding to the function
<table-cell> Help</table-cell>	
You can	access the help file index through the File\Help from the Menu bar
Help	
Inde	×
Abo	ut UVIsoft
_	
	IALYSIS / TEMPLATE ction saves the current analysis. The analysis file will contain the results,
the imag	ge and all the parameters defined to obtain the results.
	lysis could also be saved as a template for automated analysis routines. The offers the user the ability to automate many of the repetitive tasks
associat	ed with analysis and processing. As a result, you can spend more time
	ng and analysing results, and less time manipulating set-ups, variables er settings.
The tem	plate automates a task or set of tasks that you perform repeatedly or on
a regula	r basis. It stores all the analysis commands and parameters of an . You can run these parameters with another image whenever you need
	m a new analysis based on the same parameters.
The ben	efits of the template file are as follows:
	ne saving
	production of image analysis parameters mplates are modifiable, allowing the user to maintain an original template
while mo	odifying it for a slightly different result, with minimal effort
1. Clic	k on the "Save analysis/ Template" button:
📙 Save	analysis/Template
2. Ap	oop-up window displays the following menu:
Save As	
Sava in G	2 VL images V Q 2 🕫 🖻 s
Recent	Ditaria Colonia Colonias
Oesktop	Nideo Numaker Aamed. Ane Magddi Ane
	Prima Ana
My Computer	
My Network	de name Save
	Analysis (M. 2004)

Analysis Be (* ANA) Teoplate File (* FSUR)

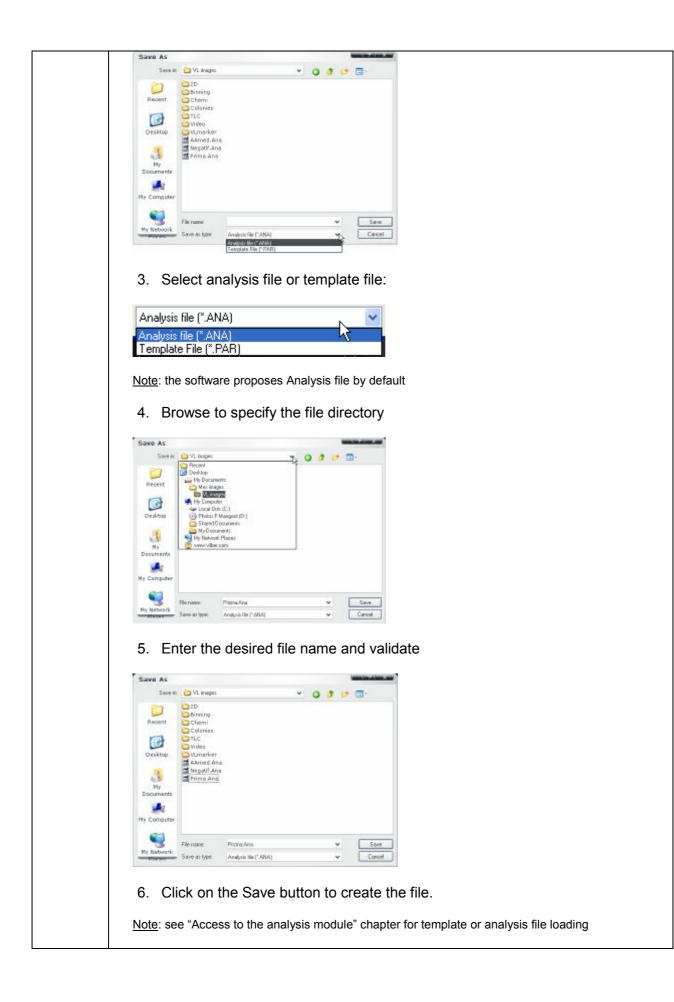


➔ Data filtering

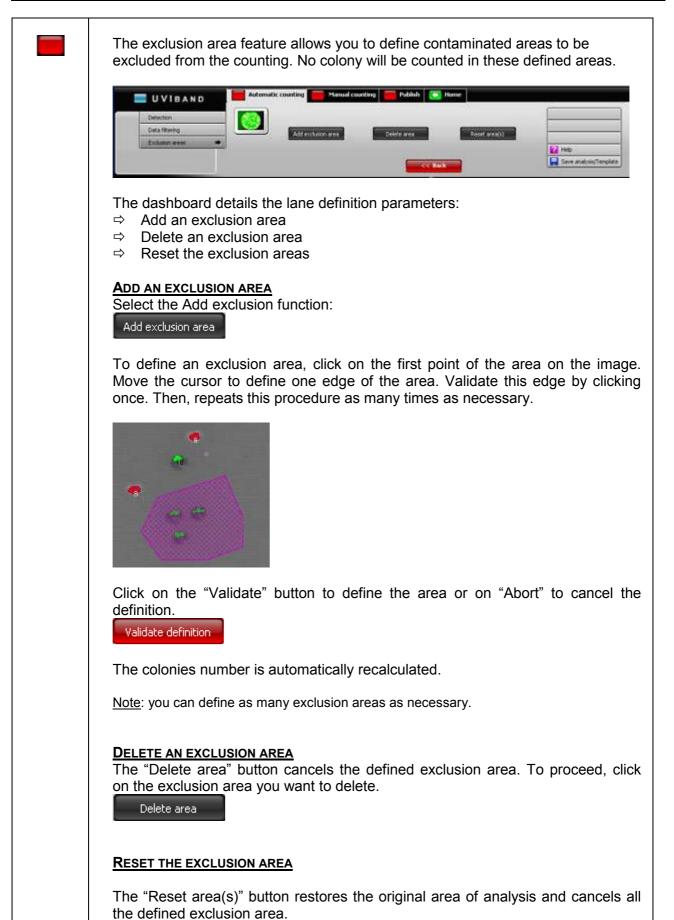


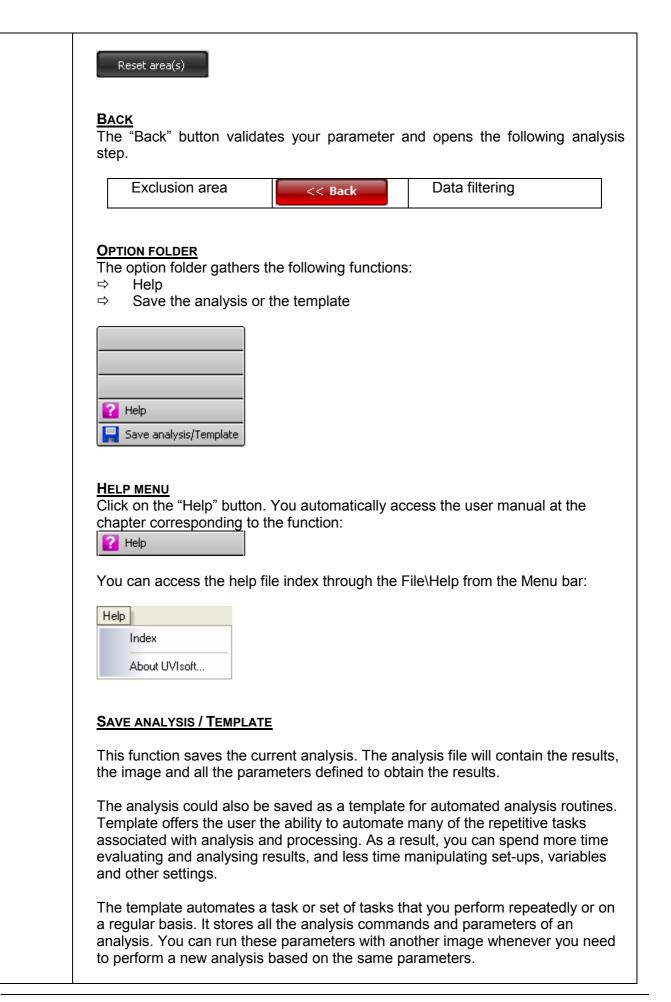
Data filtering Next >> Exclusion area
<u>BACκ</u> The "Back" button validates your parameter and opens the following analysis step.
Data filtering C<< Back Detection
OPTION FOLDER The option folder gathers the following functions: ⇒ Delete / Add colonies ⇒ Manual separation ⇒ Help ⇒ Save the analysis or the template
Delete / add colony
Manual separation
Save analysis/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.
Delete / add colony
 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.
Manual separation
 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.

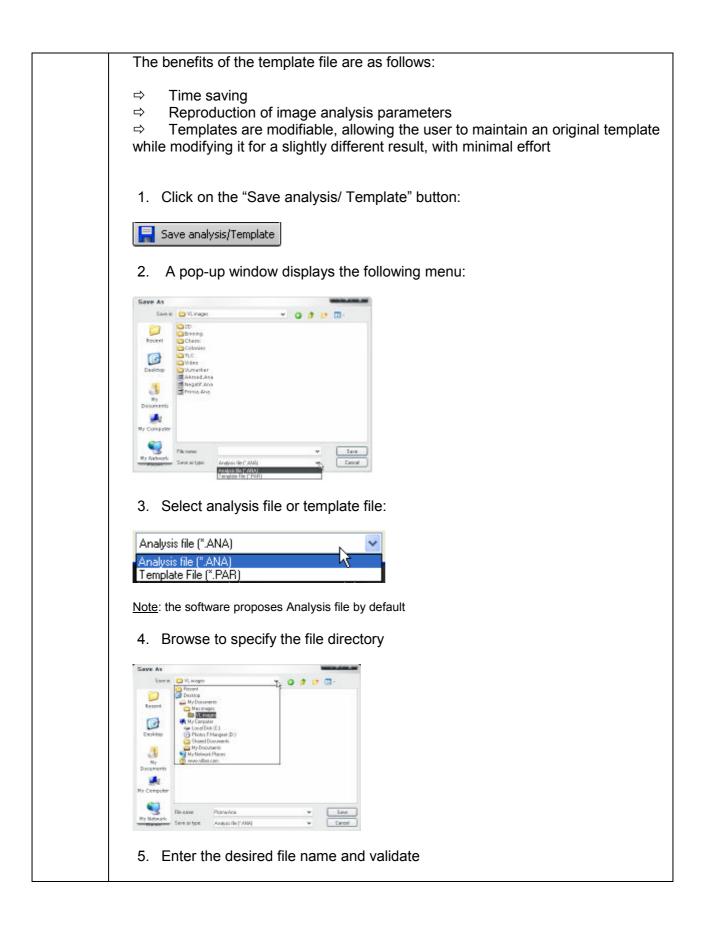
The number of colonies is then recalculated taking account this separation line.
No separation Separation line
Note: Click one more time on the "Manual separation" button to exit this function.
HELP MENU
Click on the "Help" button. You automatically access the user manual at the chapter corresponding to the function
P Help
You can access the help file index through the File\Help from the Menu bar:
Help Index
About UVIsoft
Abbut Ovisoit
0
<u>SAVE ANALYSIS / TEMPLATE</u> 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:
\Rightarrow Time saving
Reproduction of image analysis parameters
 ⇒ Reproduction of image analysis parameters ⇒ Templates are modifiable, allowing the user to maintain an original templat
 ⇒ Reproduction of image analysis parameters ⇒ Templates are modifiable, allowing the user to maintain an original templat
 Reproduction of image analysis parameters Templates are modifiable, allowing the user to maintain an original templat while modifying it for a slightly different result, with minimal effort
 Reproduction of image analysis parameters Templates are modifiable, allowing the user to maintain an original templat while modifying it for a slightly different result, with minimal effort Click on the "Save analysis/ Template" button:

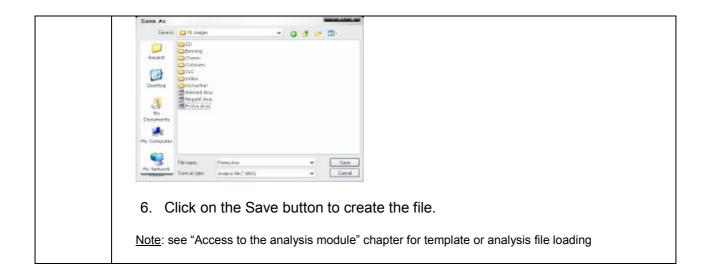


➔ Exclusion area



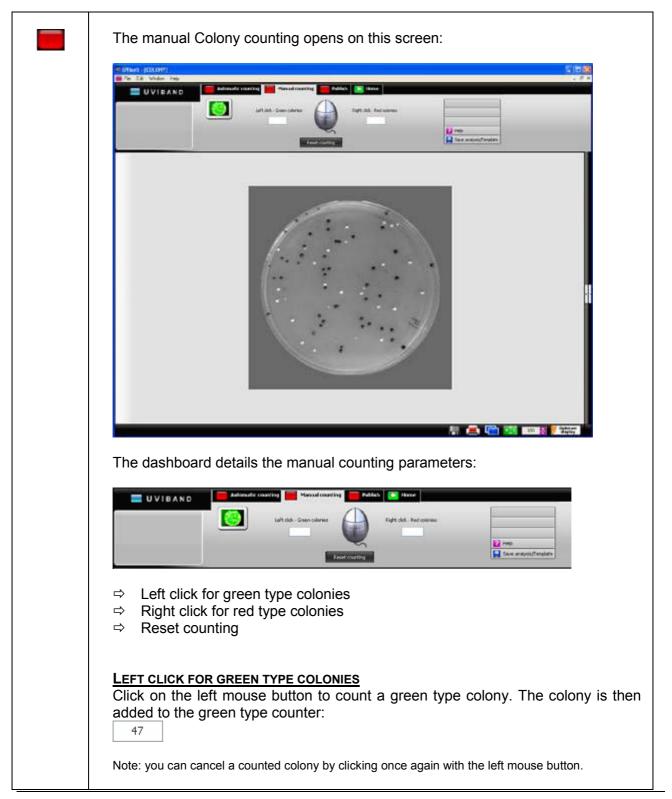




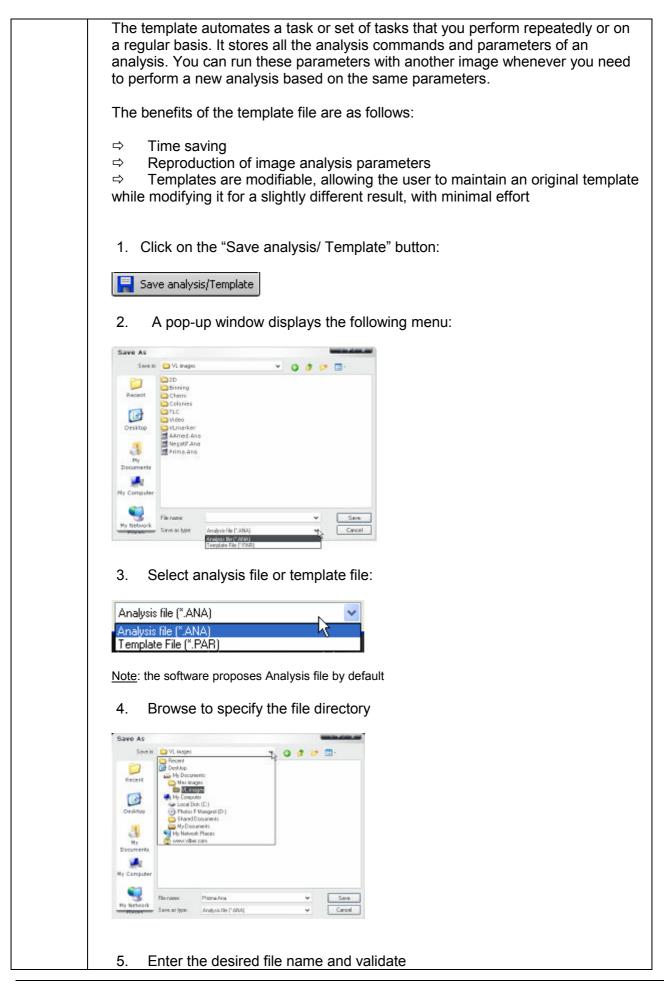


Manual counting

➔ Manual counting



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.
Reset counting
OPTION FOLDER
The option folder gathers the following functions:
 ⇒ Help ⇒ Save the analysis or the template
Help
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:
Help Index About UVIsoft
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.



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0	Colonies Colonies								
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(1)									
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Taking the second secon	Save as type	Analyziz ble (* ANA)		~	Careel				

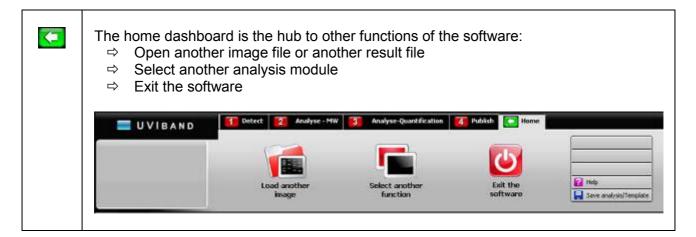
Publish

➔ Introduction

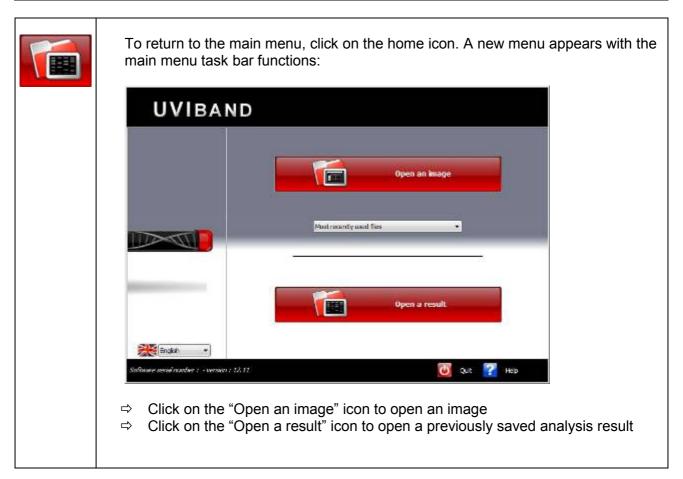
 The purpose of the Publish function is to prepare a printed report of your results. You can easily organise your report with titles and comments and your own selection of data to be published among the following: Sample image Quantification result table 1. To proceed, select the Publish tab. A pop-up window displays the following menu:
Colony counting publish Second
Danaga Commentes
Caree
 ⇒ 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
Print Pertor None Stons Stons Reduk Type: USE001 Commant: Project Stons Commant: Project Stons Stons Reduk Type: Stons
 ⇒ 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

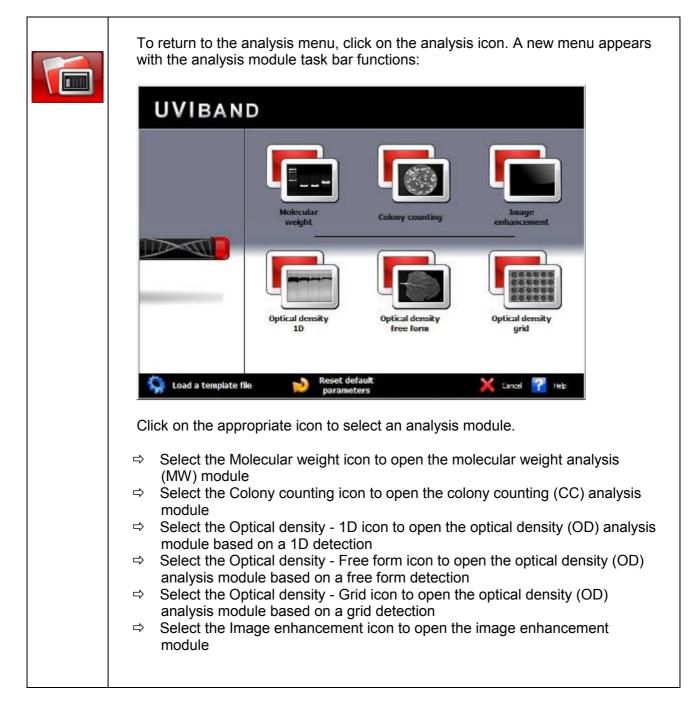
➔ Introduction



➔ Load another image



Select another function



➔ Exit the software

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

You will be prompted to save your analysis.



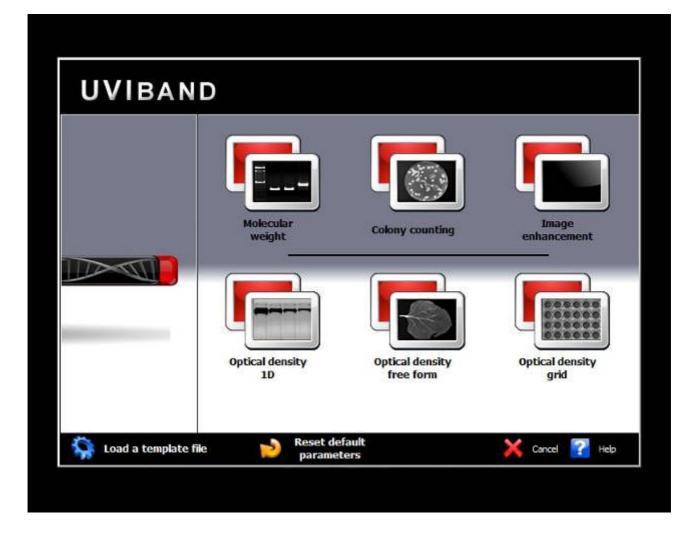


Image enhancement → Image enhancement module

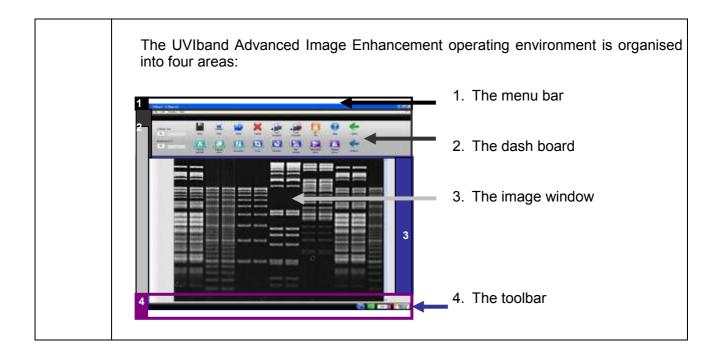
Image enhancement introduction

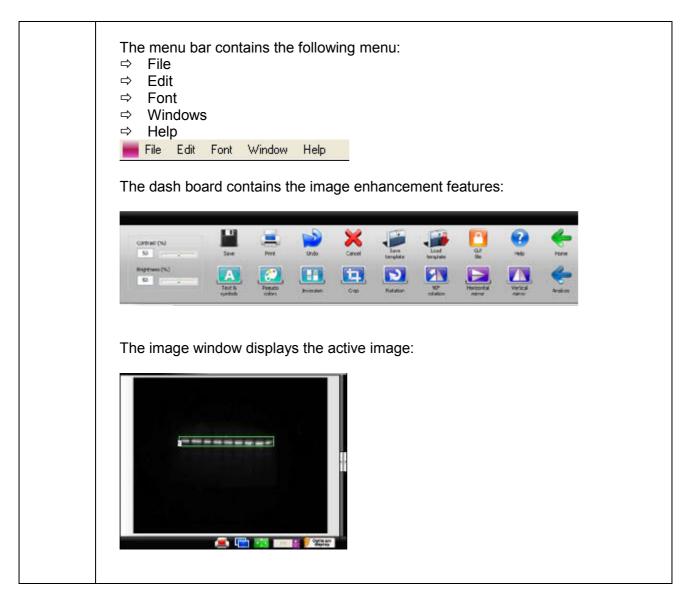
Objectives and output

 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







It also conta	ns the image toolbar:
100 🚼 🖯	Zoom in or out the image
<mark>∮ Optimum</mark> display	 Change the optimum display

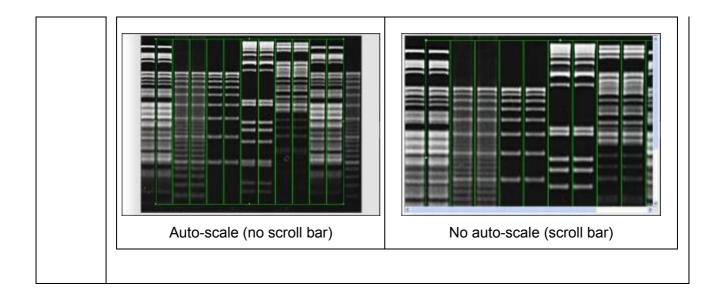
➔ Toolbar in details

<u>Copy to clipboard</u> 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.



Optimum display	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:
	Independent Deploy Independent Image denotes Pendentingery value 0 Minungery value 0 Minungery value 200 Minungery value
	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

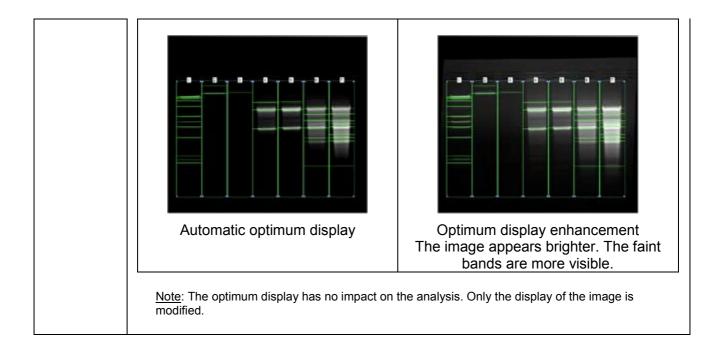
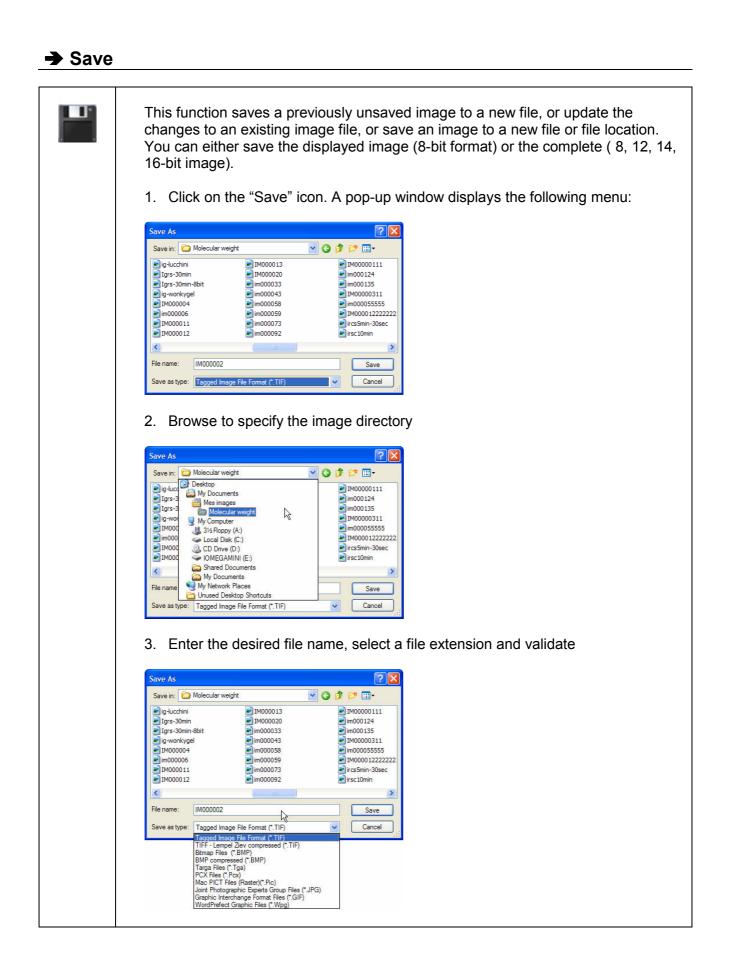


Image enhancement functions

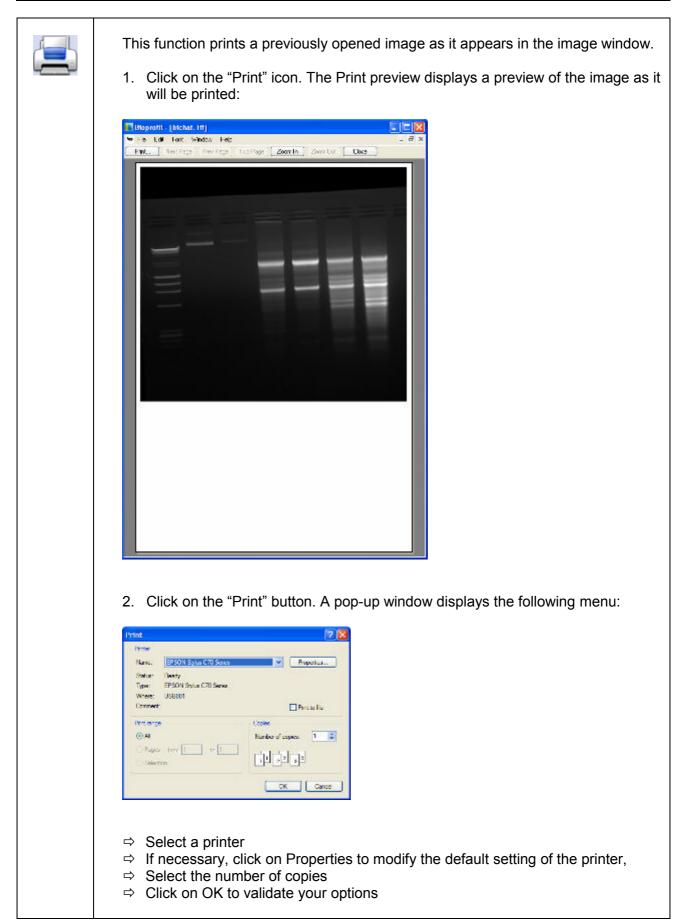
➔ Introduction

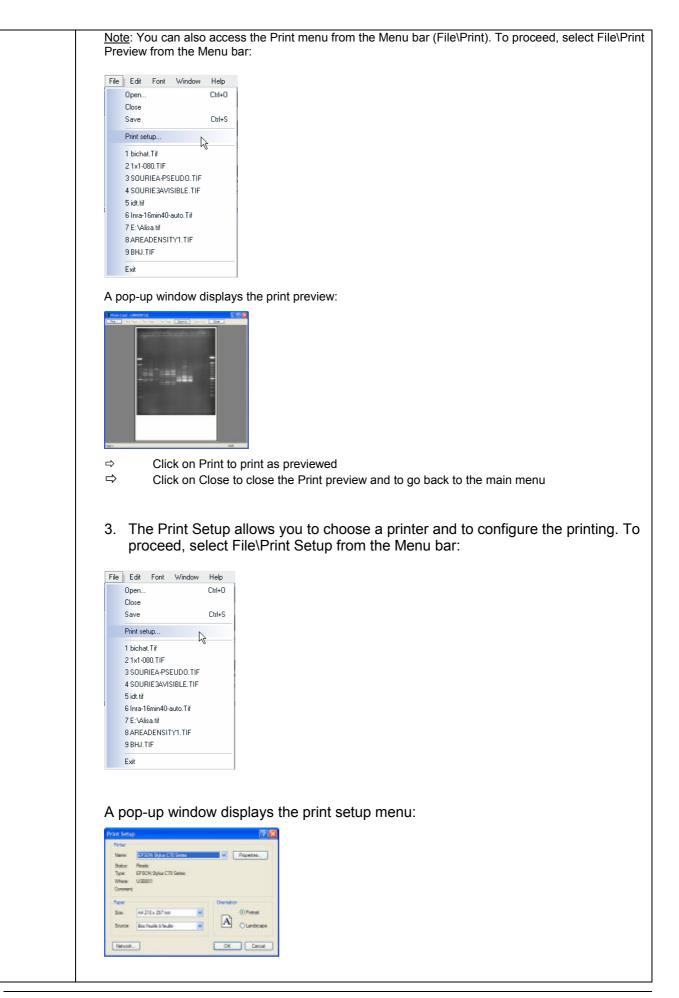
The dashboard contains all the image enhancement functions:

Carte	en 💾 🚊	N	K 🎜			0	4
50	ees (%) Tech & Ferd Tech & Ferdo	undo o	arcel Lary tanglida Cao Retator	Lood tergiate Mon estation	C) the Harbonical network	ND Visca New	Hone Aroline
⇒	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						



➔ Print





 ⇒ Select a printer ⇒ Click on Properties to modify the default setting of the printer, if necessary ⇒ Select the paper size and source; select the orientation ⇒ Click on OK to validate your options
<u>Note</u> : 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

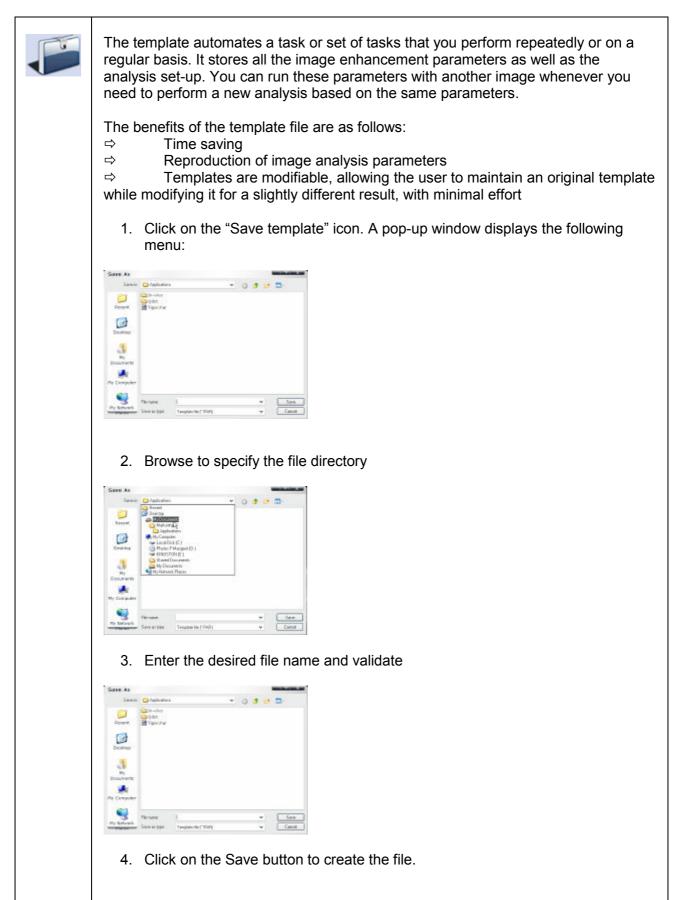
The undo function undoes the last image enhancement modification you made. The Undo option allows several levels of edits. In other words, you can undo step by step the preceding edit.
To Undo an action, click on the undo icon. The list of the last editing actions is displayed:
Vertical mirror Pseudo colors Image inversion Horizontal mirror Image Rotation (4°)
Undo 2 action(s)
Select from this list, the actions you want to undo. The Undo applies automatically on the image.

→ Cancel

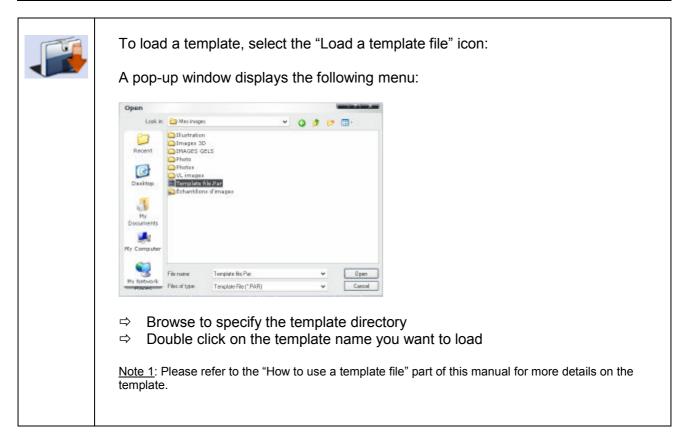


Click on the "Cancel image change(s)" icon to undo all previous image treatments. The original image is then displayed without all the further modifications.

➔ Save template



→ Load 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:
Good Laboratory Practice : GLP
Experiment title :
Comments:
Gip contents:
Date : 04/28/04 Time : 21:01:29 Image name :
Acquisition parameters : Real time Acquisition
Positive Acquisition
OK Cancel Print 🕐

 ⇒ Enter the experiment title and comments. ⇒ All other image acquisition parameters (Real time acquisition or integration time, positive or negative image) are recorded. ⇒ Treatments on frozen images (contrast, brightness, inversion) are also recorded.

➔ Help	
·	
?	The Help menu contains information about the UVIband Advanced software and enables you to access the on-line help system.
	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:
	Help
	About UVIsoft

→ Home

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

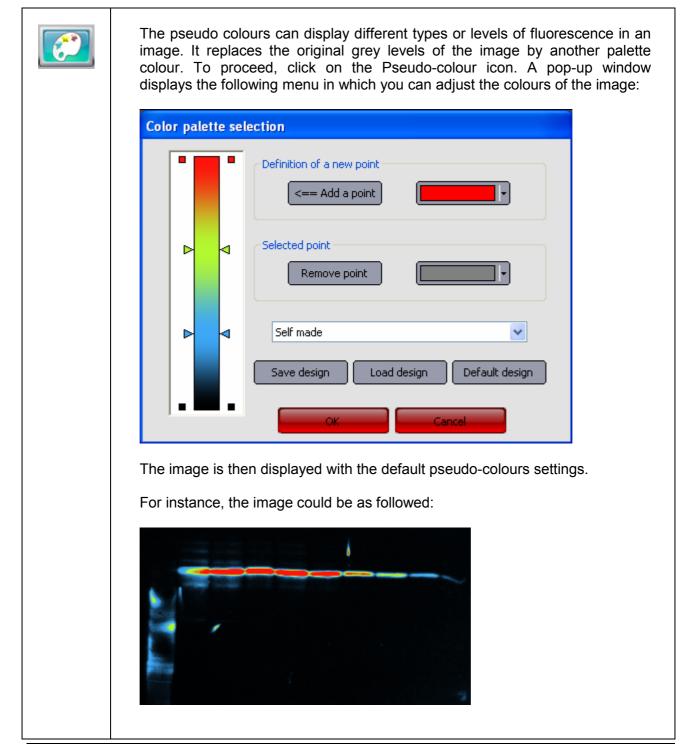
	Open an image
Most recently used files	•
	Open a result
Software serial number : - version : 12.11	🔯 Quiz 📝 Невр

Text and symbols

A	 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.
	 To annotate an image, click on the "Text and symbols" icon. A pop-up window displays the following menu:
	Type a comment Date Time Short file name Close Long file name Font selection Tahoma Size : 8 Wodify font Tahoma Size : 8 Vertical 1
	Add a new comment Delete the selected Clear the current text comment Add Symbol
	 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

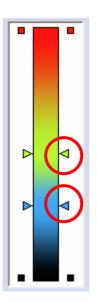
	the computer you are using. Time. Add the current time to the image. This time defaults to the time set on the computer you are using.
	Full image name. Add the image title to the image. The title defaults to the file name and location of the opened image.
⇔	Short image name. Add the image title to the image. The title defaults to the file name of the opened image.

➔ Pseudo colours



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:

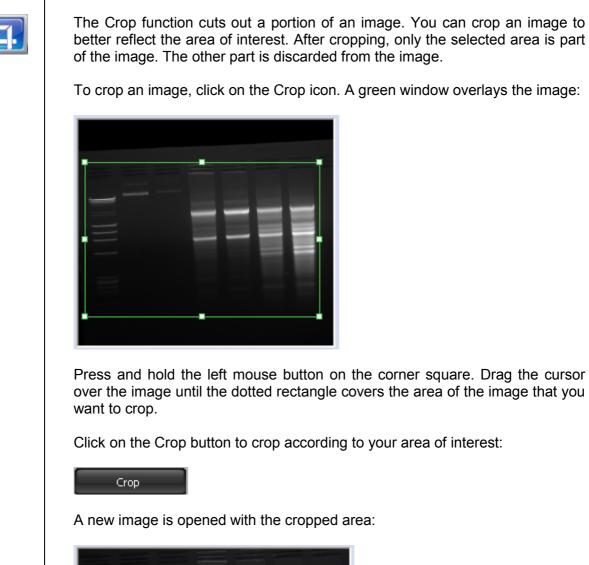
Self made		~
Self made		
Ascending grey levels		
Red palette		
Green fluorescent protein		
Coomacie blue	2	
Descending grey levels	. 0	

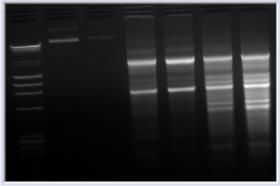
You can also save and load your own palette design. Define the set of colours you want to apply and click on Save to save the palette design. Click on Load to open your palette design.
Save design Load design
To come back to the default design, click on the default design button:
Default design

➔ Inversion





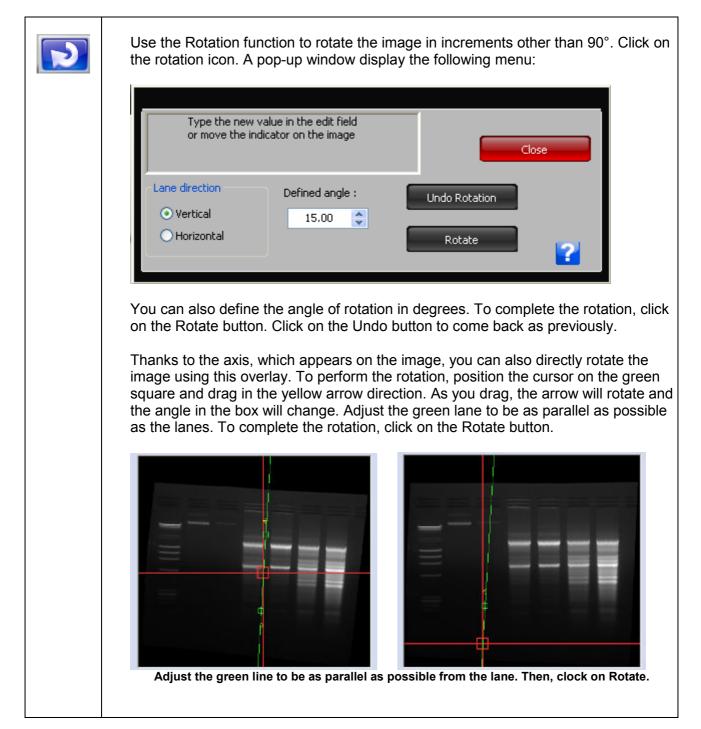




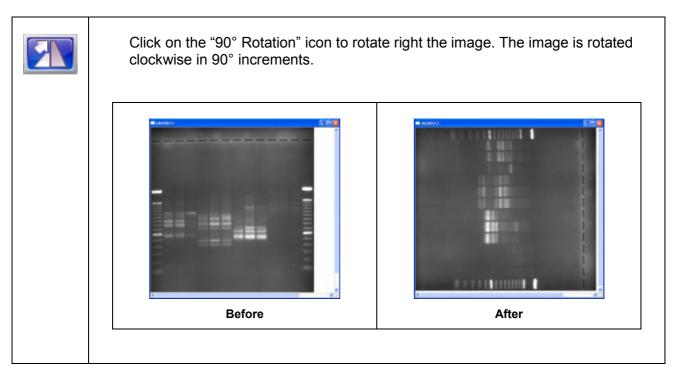
Note: this image is a new unsaved image. You need to save this image.

Note: The former image is still opened.

➔ Rotation



➔ 90° rotation



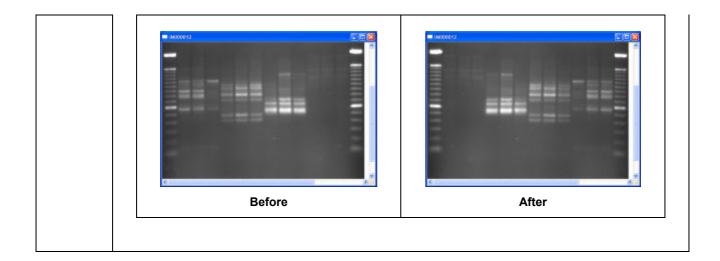
➔ Horizontal mirror



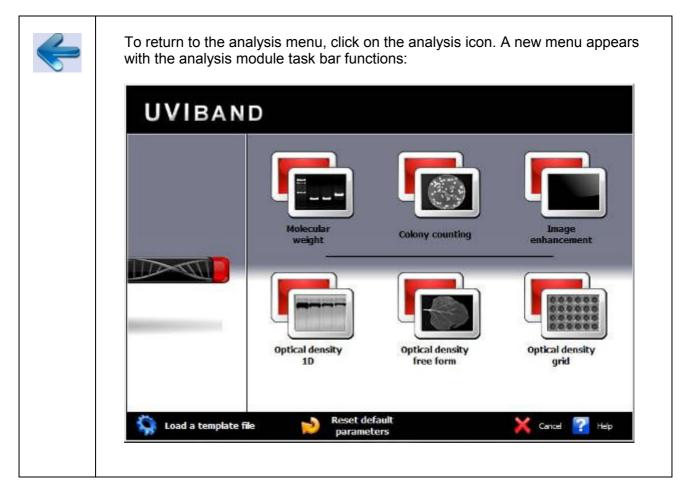
Vertical mirror



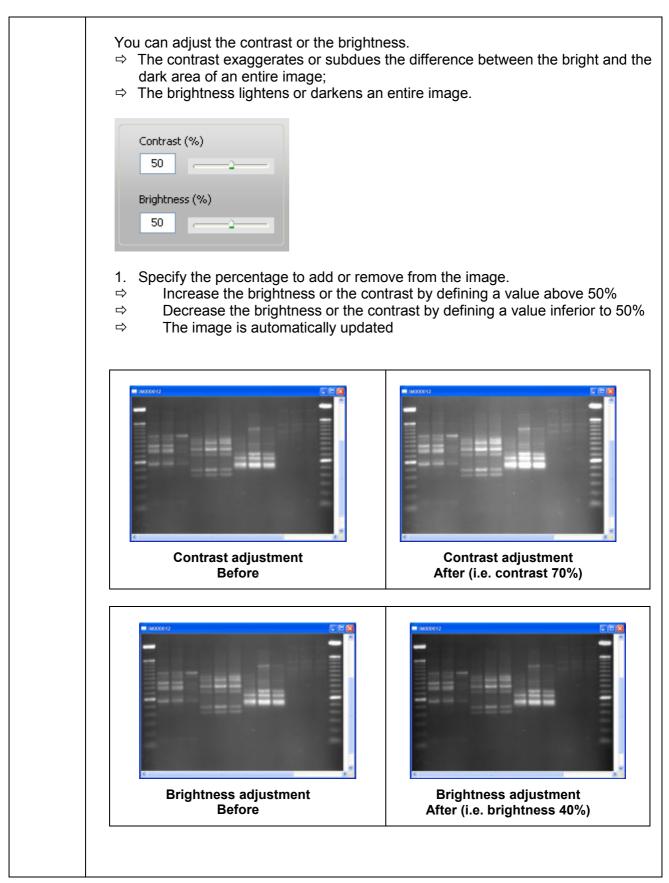
Click on the "Vertical mirror" icon to flip the image from right to left.



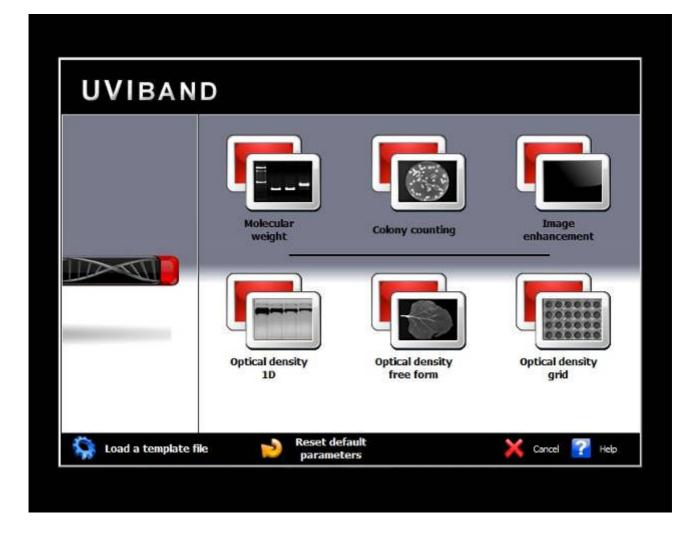
➔ Analyse



➔ Contrast and brightness







How to use a template file → UVIband Advanced

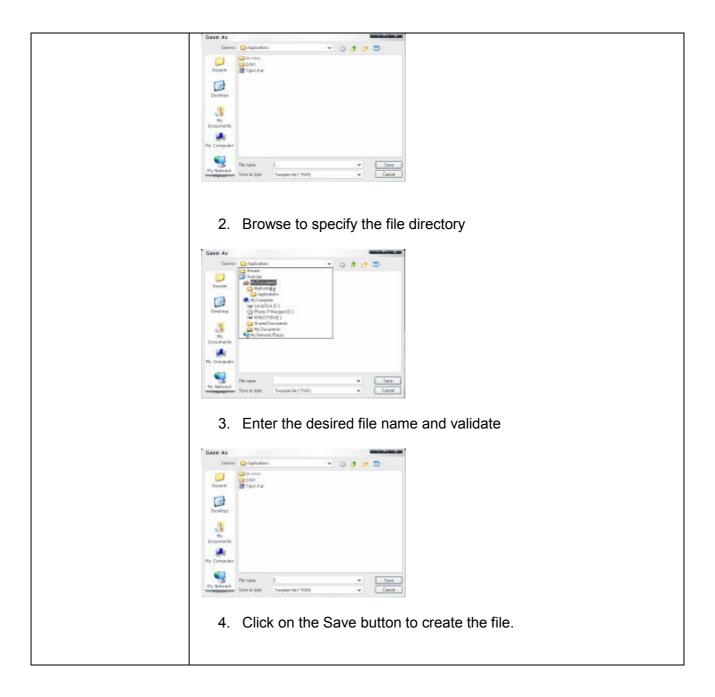
Automation with template file

➔ Introduction

Ş	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

	 Click on the "Save template" icon. A pop-up window displays the following menu:
or	
Save analysis/Template	



→ Load template

