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User's guide

INNOPSYS
Parc Activestre – 31390 CARBONNE – France
Tel : +33 561 971 974 / Fax : +33 561 971 975 - support@innopsys.fr

MAPIX USER'S GUIDE

Welcome to MAPIX, your high-performance, easy to use microarray image analysis software.

With MAPIX you will be able to:

- Control Innopsys' Innoscan scanners
- Display images in real time during acquisition
- Control display parameters: zoom, image properties, contrast, balance, etc...
- Save acquired images in 16-bit or 20-bit TIFF format together with all acquisition parameters (pixel size, laser output, etc...) and JPEG format.
- Save settings (scan and analysis parameters)
- Perform fast image analysis using a GAL file
- Display results and save them in a GPR format
- Display any 16/20-bit TIFF image
- Manage multiple users account

This guide will help you discover all MAPIX features and enable you to perform easy image analysis.

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1. Installation

1.1 Hardware requirements

Please contact INNOPSYS to get the latest information about the main hardware specifications required by MAPIX.

1.2 Installation Procedure

Place the software installation CD in the PC's CD-ROM drive and refer to the *install.txt* file for installation details.

2. Starting Mapix

2.1 User workspace

When the program starts, the following dialog box is displayed:

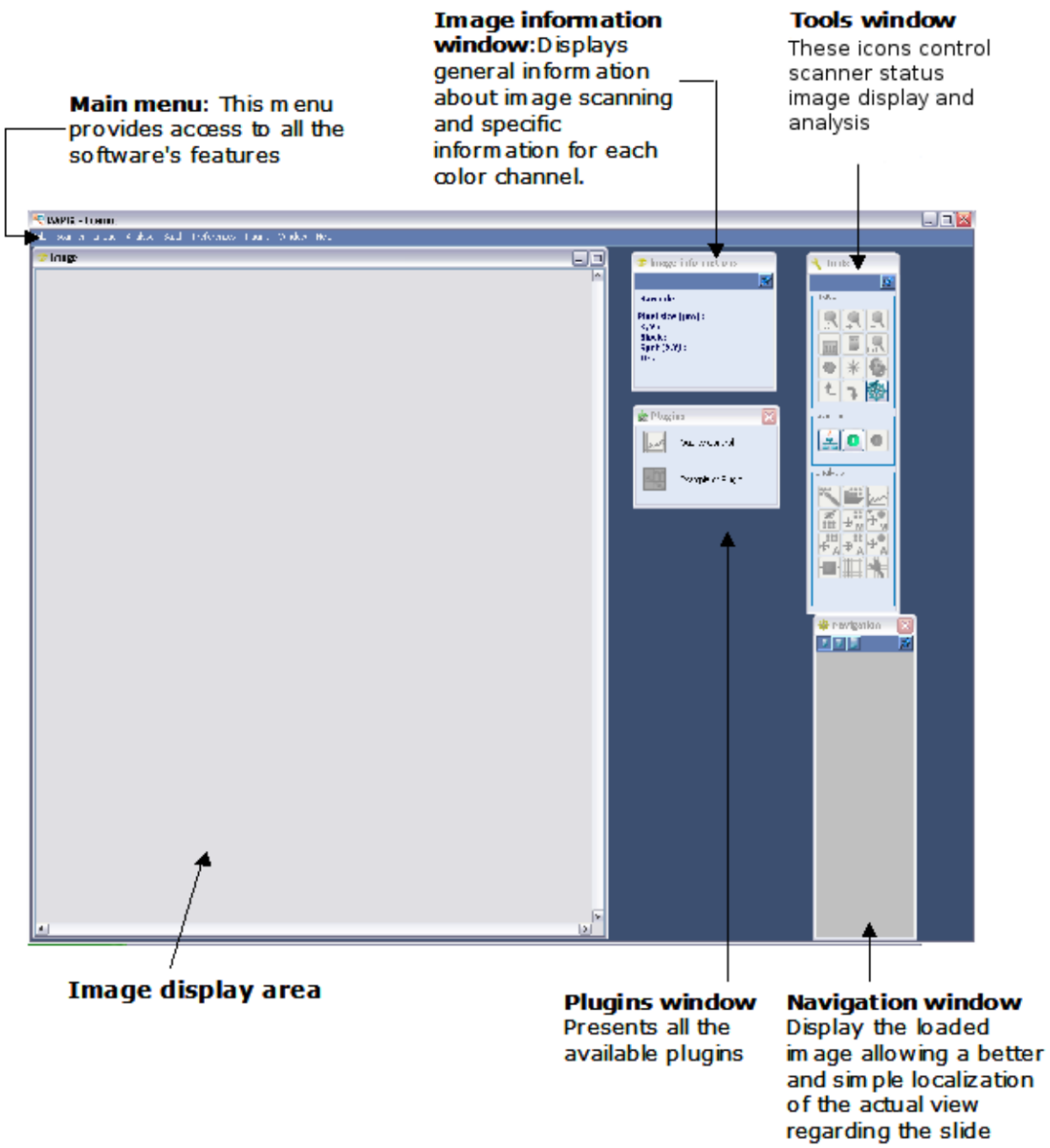
When using the software for the first time, you need to create a user account by clicking on the button **«Create».**

> **Note:** If this feature is unnecessary for your work environment, a single user mode is available which does not ask you to log on whenever the program is run. See the *Preferences* section.

Use the button «**Start»** to access the program from the workspace of the selected user.

2.2 The software application main window

Once a user's workspace is selected, the user interface displays the Main Window on the monitor screen. This is your starting point for sending commands, entering information or receiving scan status information. This is also where scanned images and quantification results are displayed.



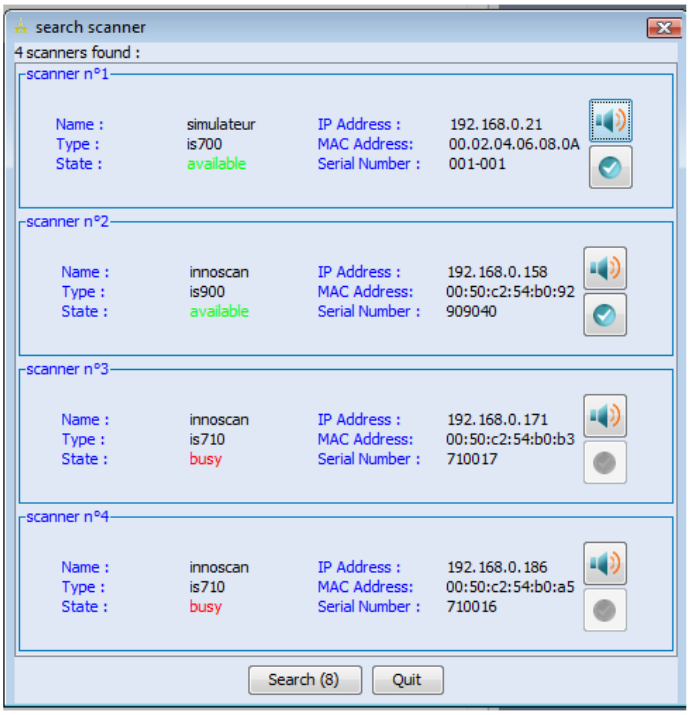
3. Displaying images in real-time

In this first step you will learn how to display a microarray image in real time and how to set the image acquisition parameters before the scan.

3.1 *Connecting* the scanner

MAPIX software controls any/all InnoScan scanners via a TCP/IP protocol over the network. Each scanner has an IP address (192.168.0.254 is the default).

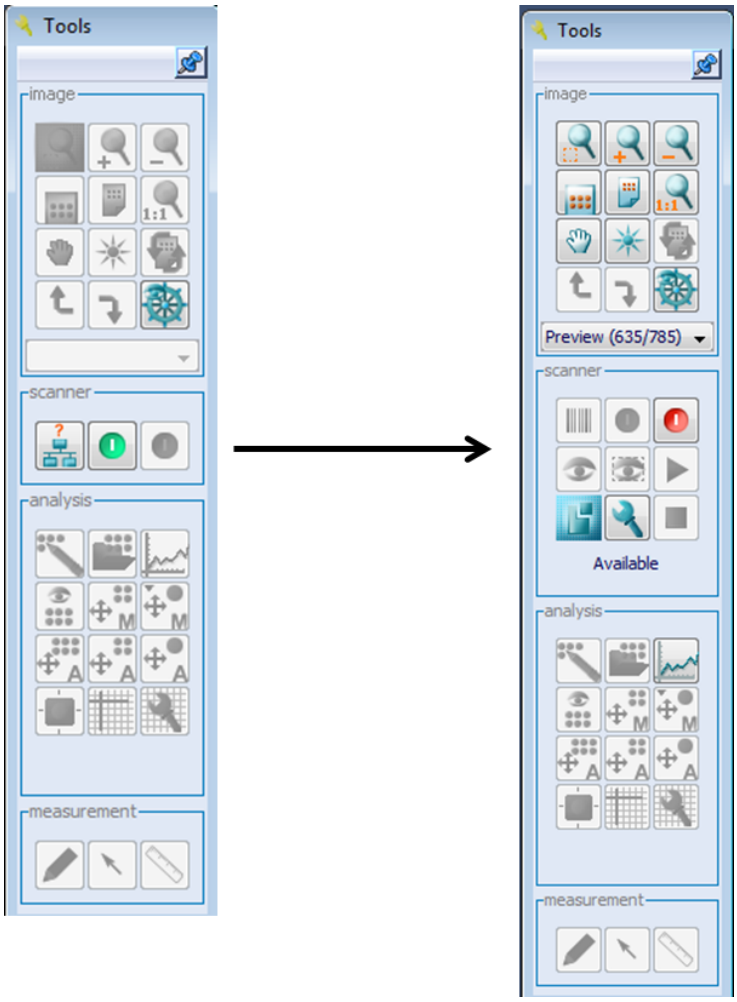
To search for an available scanner click on the  «**search**» icon situated either on the Tools window or in the scanner tab of the Main menu. A window containing the available scanners is opened:



If you work with multiple scanners connected to the same computer, you can recognize a scanner by its IP Address. If you do not know this address, you can use the  «**beep**» icon to identify the scanner.

Click on the  «**select**» icon to connect to the selected scanner.

The  «**Connect to the scanner**» icon in the tools window connects to the scanner used in the last session as defined by its IP address.



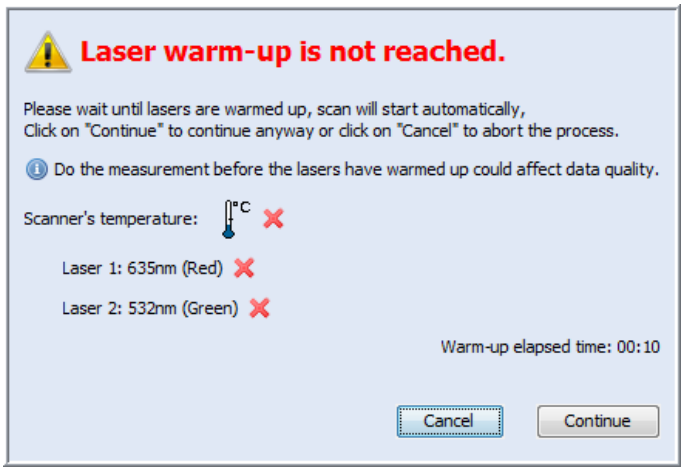
The Scanner parameters icons become visible once the scanner is connected.

Use the  «**disconnect from the scanner**» icon to disconnect the scanner.

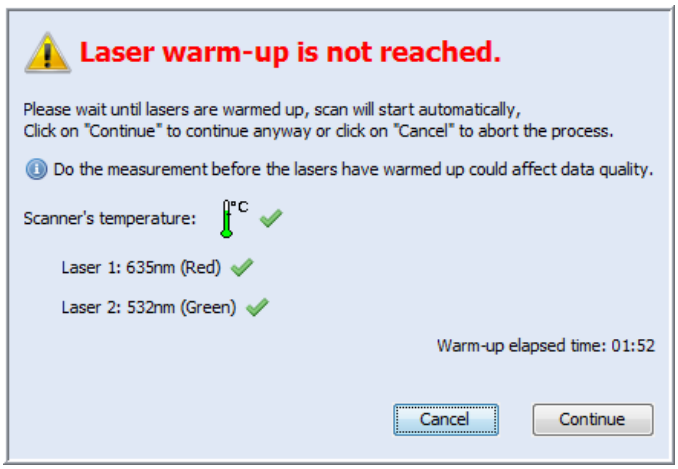
3.2 Scanner Warm-up

To get optimal performance, the scanner has to warm-up for around 10 minutes after being connected to Mapix. The scanner operating temperature is from 19°C to 26°C (air conditioning recommended).

Laser warm-up and scanner temperature are tested before each scan. If the laser and or scanner temperature are not adequate, the following message is displayed:

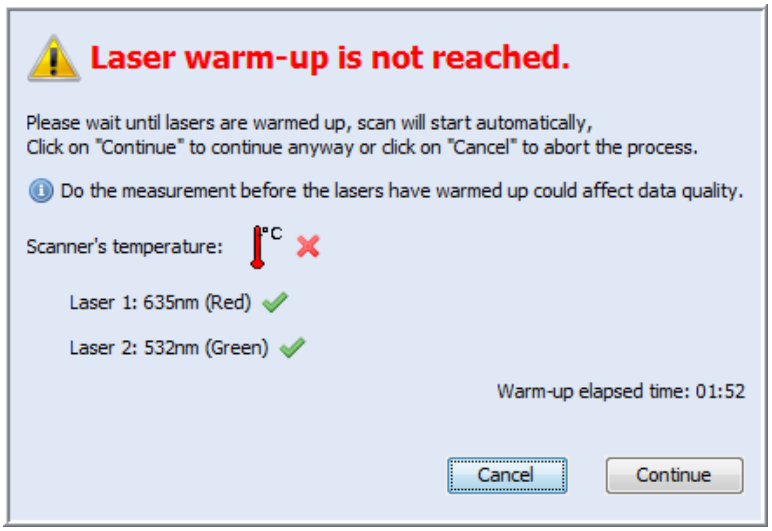


After 5 to 10 minutes, the lasers will reach the adequate operating temperature. One can see that the message display changes to the following:



The scan begins automatically after laser temperature is reached and if the scanner operating temperature is between 19°C and 26°C.

If room temperature is higher than 26°C the Laser warm-up message will stay until the scanner temperature decreases:



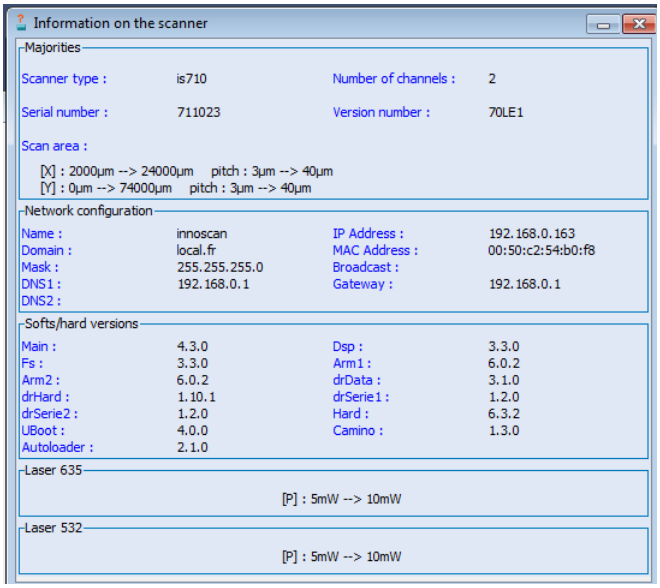
To decrease the scanner temperature you should ventilate the scanner and control the room temperature below 25°C.

At any time you can skip this message and begin the scan by selecting the option **«Continue»**, however **high temperatures can disturb scanner performance and image acquisition, directly impacting microarray results**.

When the scanner temperature check out is not needed, one can disable this option from the preference tab on the main menu. Please refer to the *preferences* section in this manual to learn how to disable this option.

3.3 Scanner Information

To display the information about the scanner you are working with, click the **«about the scanner»** option situated on the **«scanner»** tab of the main menu:

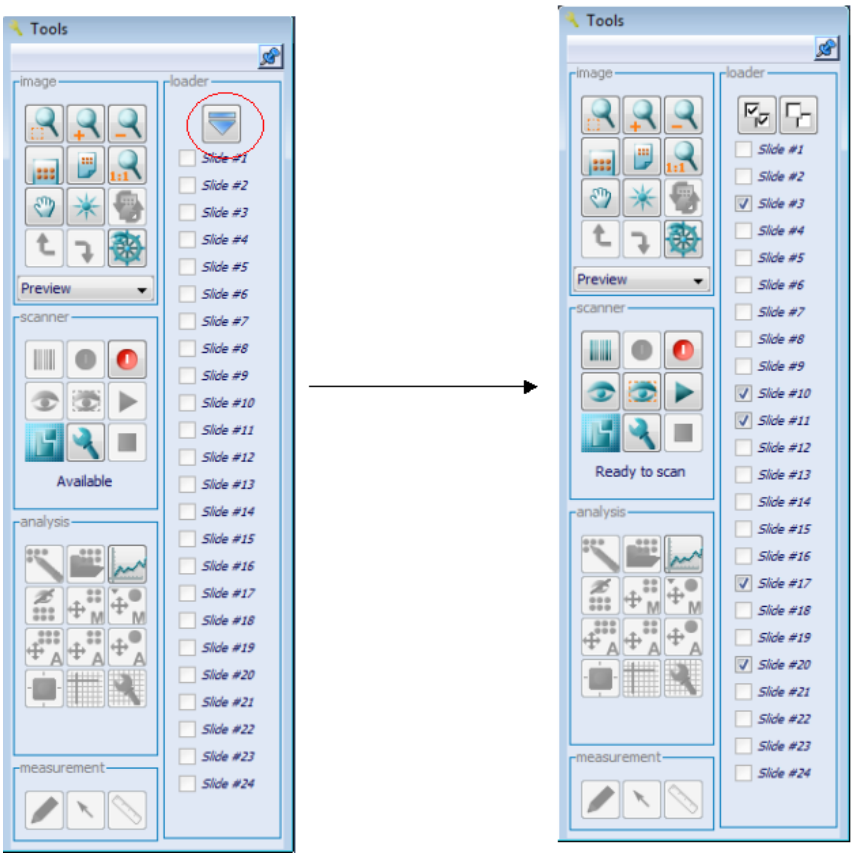


Information on the scanner to which you are connected, including the version of the software currently used is displayed.

3.4 Slide detection in the Auto-loader: only for InnoScan*XXX* AL models

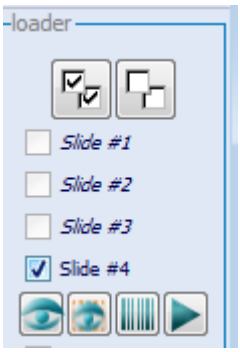
If your scanner has an auto-loader system you can load your slides in the autoloader's holder without having to put them in a sequential order. Mapix detects the position in which the slides are loaded.

Once Mapix is connected to the scanner, the auto-loader zone in the tools window becomes visible (see figure). To detect the location in which slides were loaded, click on the «**read loade**r» icon:

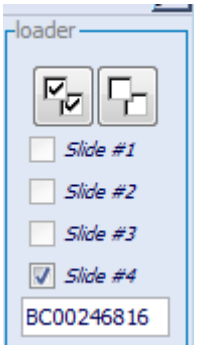


Note: To enable scanning your slides, you **must** perform this detection step. Once the slides are detected, *Preview* and *Scan* icons become visible.

Once the slides have been detected by the scanner, one can ask Mapix to ***read the barcode*** for each slide in order to know the position of each loaded slide. When passing the mouse on the slide position, the following scan parameters appear:



Click on the read the barcode icon to ask Mapix to detect the slide's barcode. The barcode will be shown under the slide position number:



3.5 Preview Scan

A preview gives you a fast, comprehensive image of the microarray. Use preview to adjust laser output and PMT gain to prevent saturation, and to define the region of interest before initiating slide scanning.

Click on  «**Preview**» icon to begin the preview scan of the whole slide.

Preview scan uses the following default parameters:

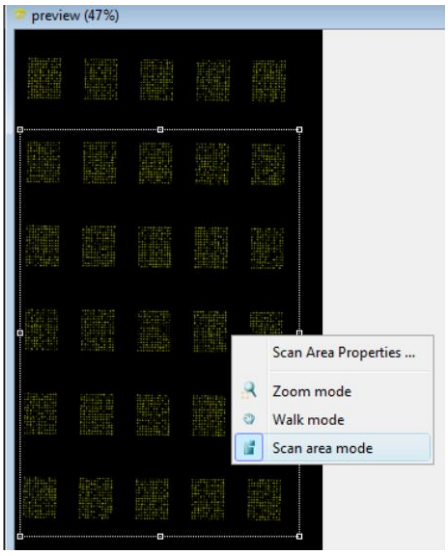
Pixel size: 40µm

Speed: 35 lines/s

Scan area: whole slide 22mm x 74mm

To change preview parameters click on  **«Scan parameters»** in the Main menu and then on the **«Misc»** tab.

You can define a region of interest directly in the preview image by using the mouse. Click on the  «**Select area mode**» icon on the tools window or directly in the image by using the right-click menu.

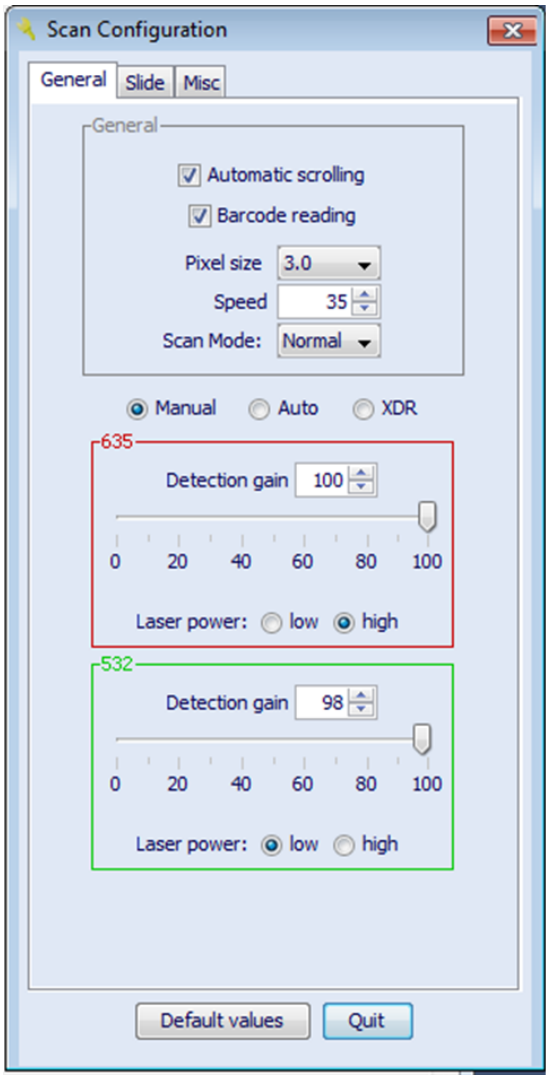


If you are using a slide with a scan region predefined in Mapix you can choose it directly, please refer to *Defining scan parameters* section for details.

You can do a preview scan of the selected region by clicking on the  **«preview scan area»** icon.

3.6 Defining scan parameters

Click on  **«Scan parameters»** to define the parameters used during image acquisition.



Use this panel to define the scan parameters related to pixel size and acquisition mode.

You can define the laser power and PMT gain manually using this panel (*see table below*). For automatic definition of scan parameters or for scanning within an extended dynamic range, you can use the auto and the XDR options, respectively.

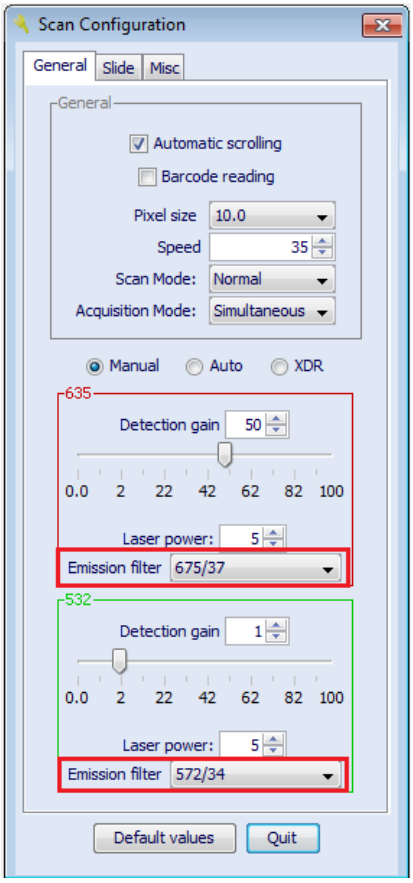
For more information about manual settings please refer to table below. For details on auto-settings- and XDR-scan mode please go to *Scanning within scan auto-settings mode* and to *dynamic range extension* sections, respectively.

ITEM	DESCRIPTION
Barcode reading	Enables barcode reading. If the slide has no barcode, disable this feature so the message «barcode reading error» won't be displayed.
Automatic scrolling	Scrolls the image window automatically during the scanning process.
pixel size	Selects a scan resolution. Scan resolution represents the individual pixel size in the scanned image. Pixel size can be set from minimum to maximum resolution according to the scanner model specifications.
Speed	Refers to scan speed. Speed can be set from minimal to maximal speed according to the scanner model specifications.
Scan Mode	Click on scan mode to choose between «**normal**» or «**median**» scan mode. In normal mode the scan is done in the defined pixel size, while in median mode the scan is done in a step equal to a half of the defined pixel size. In median scan mode, for each image pixel, four values are considered and the pixel value is set as the median of these four values. In this way, scanning is done in a higher resolution but with smaller image size. **Note**: Because scanning is done in a higher resolution than set, scan time is doubled in median scan mode. This scan mode is only available for **even** resolutions.
Detection gain	Sets a value for the gain percentage for each color channel (wavelength). The default value is the value used in the last scan session. Changing gain one can avoid saturation.
Laser power	Depending on the scanner model, one (*InnoScan 710-G and InnoScan 710-R*), two (*InnoScan 710, InnoScan 710-IR and InnoScan 910*) or three (*InnoScan 1100AL*) laser sources are available, each having two laser power. For 532 nm, 635 nm, 488 nm and 670 nm excitation lasers «**Low**» stands for 5 mW while «**high**» stands for 10 mW; while for 785 nm excitation laser «**Low**» stands for 5 mW while «**high**» stands for 20 mW .
Default values	Sets the parameters to their default values. These values are given by the scanner itself.

Scan parameters can only be adjusted during or after the preview scan.

3.6.1. Emission filter wheel: only for InnoScan 910 and InnoScan 1100 models

The InnoScan 910, InnoScan 910 AL, InnoScan 1100 and InnoScan 1100 AL scanners contain a 7-position filter wheel. The standard scanners are provided with a standard emission filter for each color channel, a standard optical density (OD) filter and 5 other positions for customized filters. Use the "**Emission filter**" option to select the filter to be used during the scan:



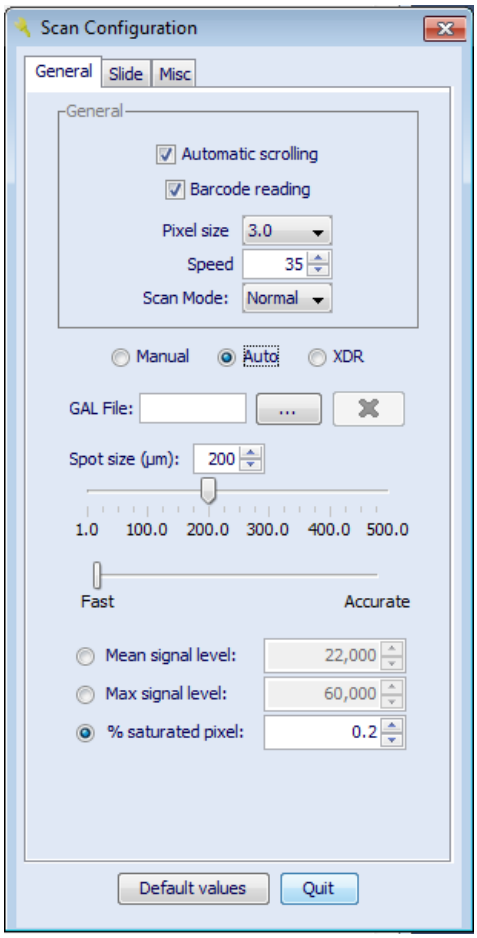
Note: The filter 676/37 corresponds to an emission wavelength range of 657.5 – 694.5 nm, while the filter 579/42 corresponds to an emission wavelength range of 558 – 600 nm. In the case of the InnoScan 1100 model, the filter 520/5 corresponds to an emission wavelength of 517.5 – 522.5 nm. These filters are standard filters compatible with fluorophores primarily used with these wavelengths.

This option is only available for InnoScan 910 and 1100 AL scanners.

3.6.2. Scanning within auto-settings mode

Using the scan auto-settings mode, Mapix searches automatically for the laser power and PMT gain that best fit your specifications: i.e. % saturated pixels, mean signal level or maximal signal level.

To work within scan auto-settings mode, choose the «**Auto**» option on the general tab of the configuration window, the following menu appears:



Option	Action
GAL File	Select the GAL file corresponding to your slides. By selecting the GAL file, the required parameters will be considered only for those pixels contained in the features defined in the GAL file. This excludes the pixels of regions with no spots whose intensity will be considered as background noise.
Spot size (µm)	If you do not have a GAL file, Mapix will apply the required parameters to the totality of pixels that make up the scan area. However, to better fit the parameters to the spots of your image, background noise estimation is calculated. For this you must indicate the theoretical spot size in µm.
Fast/Accurate	You can choose to get faster results by limiting the accuracy. If the signal is mainly homogeneous across the slide, the option «**Fast**» would be enough to get good results; in the other hand if your slide shows heterogeneous signal you should choose the «**Accurate**» option.
Mean signal level	To define the desired mean signal level for each of the wavelengths of the image
Max signal level	To define the maximum signal level of the image
% saturated pixel	To define the percentage of saturated pixels on the image

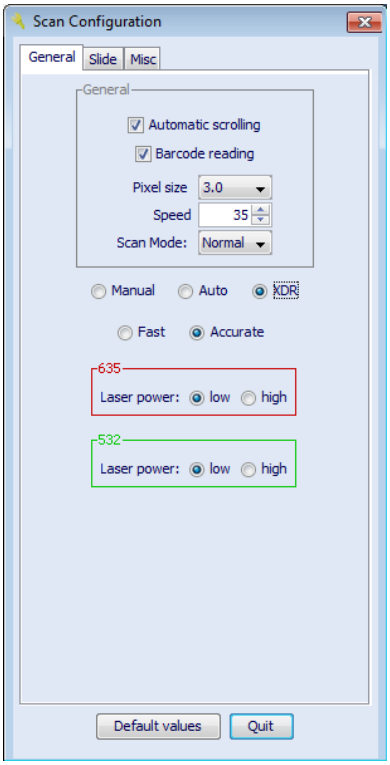
Note:

For two color images: The selected parameters will be used to set the laser power and PMT of each wavelength in parallel.

3.6.3. Extended Dynamic Range (XDR)

When samples have a wide dynamic range it is useful to use the dynamic range extension to avoid saturation. This option will make 20-bit images with a dynamic range of >10^6.

To scan within an extended dynamic range select the XDR option on the scan configuration window:



Set the laser excitation power to be used during the XDR scan: For 532 nm, 635 nm and 670 nm excitation laser the "Low" laser power corresponds to 5mW while "High" laser power defines 10 mW; for the 785 nm excitation laser the "Low" laser power corresponds to 5 mW while "High" laser power defines 20 mW.

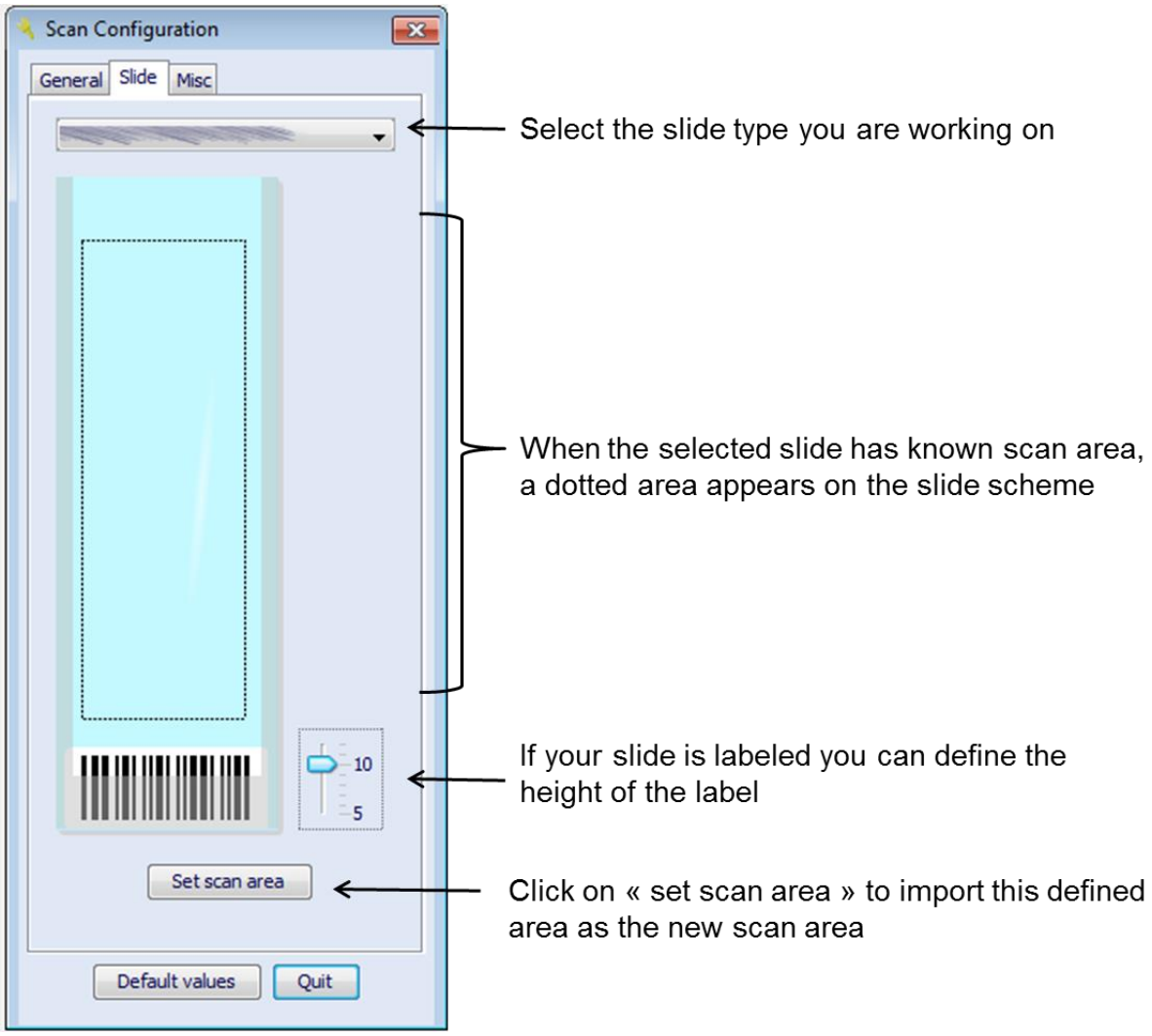
One can choose between «**Fast**» or «**Accurate**» mode. To choose between both options you should first make a preview scan to know the approximate signal in the slide; if the slide has strong signals getting a lot of saturated spots during the preview scan we recommend to use the accurate option. In the other hand, if the slide has moderate signals you can use the fast option.

3.7 Defining the Scan Area

You can define the desired scan area by either defining a known type of slide or directly in the image display area of the main menu by using the mouse.

Click on the  « **scan area**» icon to draw a scan area directly in the preview image by using the mouse.

To define a known type of slide with known scan area, click on the «**slide**» tab on the configuration window:



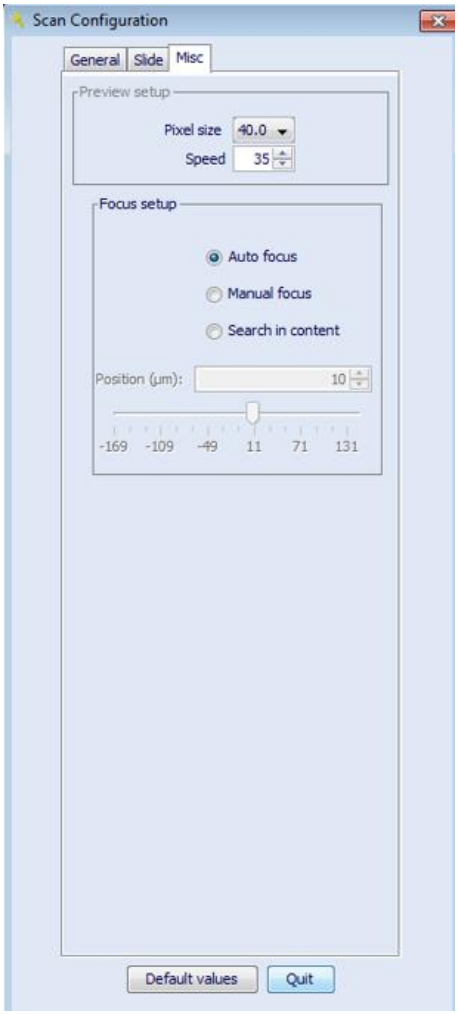
Click on  to do a preview scan of the **selected** area

3.8 Focus setup

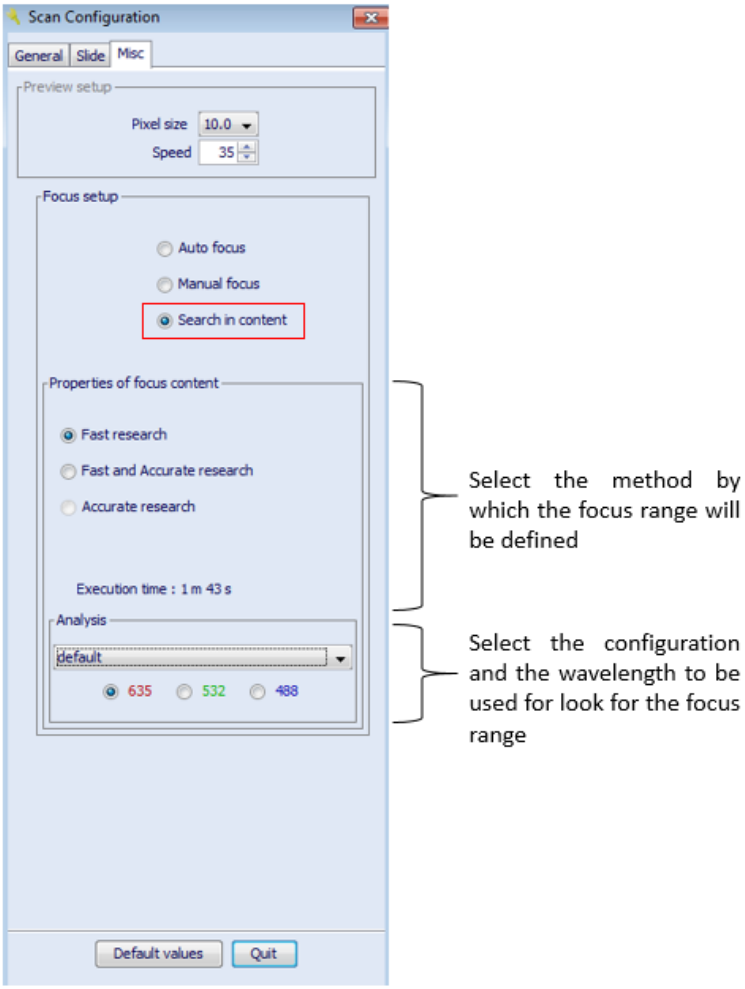
The InnoScan scanners work with either a **dynamic auto-focusing system** (or **Auto focus**) which greatly reduces background noise and gives uniform images, or a manually set focusing **(Manual focus)**. Additionally, the InnoScan 910 and the InnoScan 1100 AL scanners can use the **focus in content** module to scan specific samples such as cell microarrays or tissues.

To select the focus setup, go to the **Misc** tab on the scanner configuration window:

By the fault, the Auto focus option is selected. To manually set the focus position select the "**Manual focus**" focus option and define the focus position in µm:

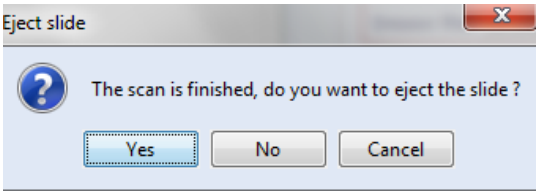


In the case of specific samples such as cell microarrays or tissue samples in which the thick is not homogeneous all along the slide, one can use the option search in content:



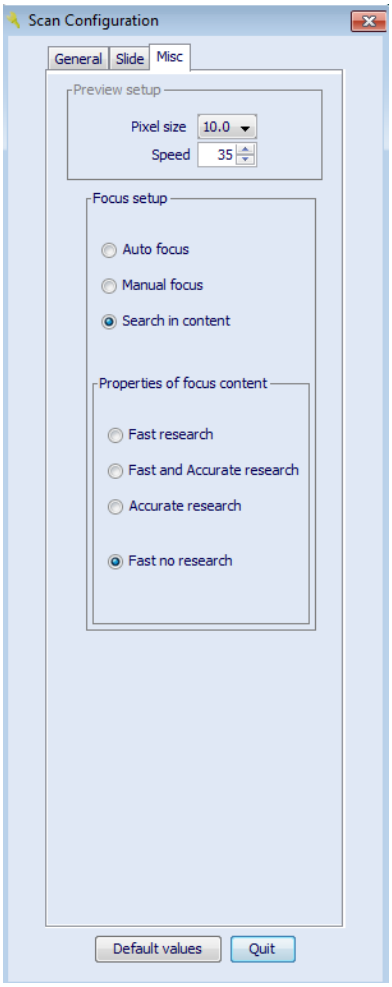
Select the configuration and the wavelength in which the focus will be done from the **Analysis** panel, then select the method of focus research: **Fast research** corresponds to a rapid definition of the points of focusing which are defined by the neatness of the fluorescence. In the **Accurate research** the focus position of each points of focusing is done in a more precise way, increasing the execution time. When the option **Fast and Accurate research** is selected, a first rapid selection of the focus position range is done, then a second more precise research in the defined range is done, in this case the execution time is doubled.

Once the scan is done, and in order to keep the focus position range for further scans, the slide is kept inside the scanner, the following message is displayed at the end of each scan:



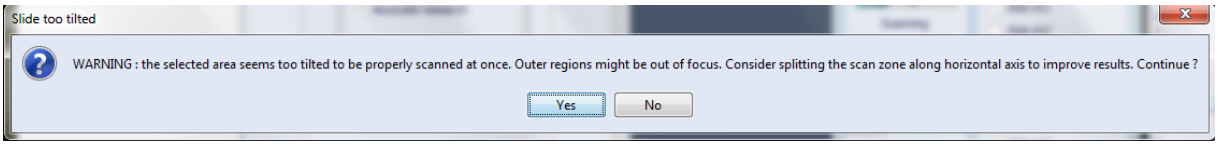
Select YES to eject the slide, in this case the focus range will be lost and the process of focusing should be done again. Select NO to keep the focus range defined on the current focus in

content process. To continue the scans under these focus proprieties select the option **Fast non research** or change the properties of the focus content process on the Misc window:



Note:

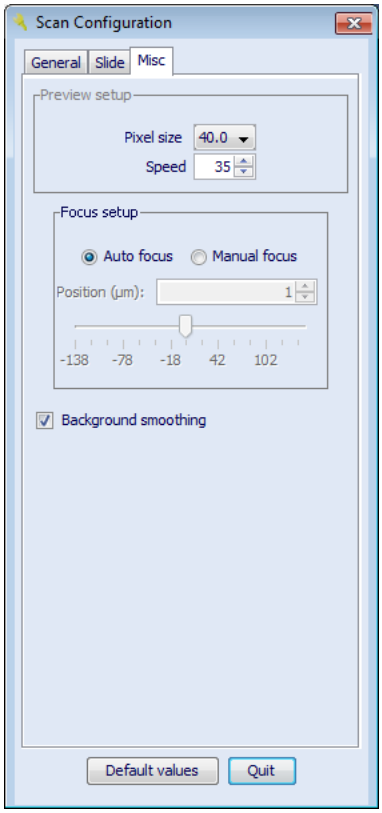
If the focus points are too far of the scanner focus range specification, or the scan area is too large with focus points to far from each other, the following message may appear:



A smaller scan area can be useful to avoid this message, please contact Innopsys to further information about the focus in content options.

3.9 Background smoothing

During the image acquisition a background smoothing is done automatically. You can remove the background smoothing option by deselecting it at the miscellaneous tab of the scan configuration window:



Note: Removing the smoothing background makes feature seeking more efficient for slides with very low signal. Remove the smoothing background when your slide has very low signals.

3.10 The histogram

Click on the  **«histogram»** icon to display the histogram.

The histogram displays pixels on a graph. Axis-X represents pixel values (from 0 to 65535). Axis-Y represents the number of pixels which are equal to the value given by Axis-X.

At the left hand side of the graphic area, scale parameter may be manually adjusted. To retrieve automatic scale, just check the box at the left of the scale. Log scale is available for each axis. You can also display the Y ordinate axis in percentage.

Bin size may also be manually set. The lower the bin size is, the larger the number of dots will be displayed. Thus it is better to work with high bin size to speed up display.

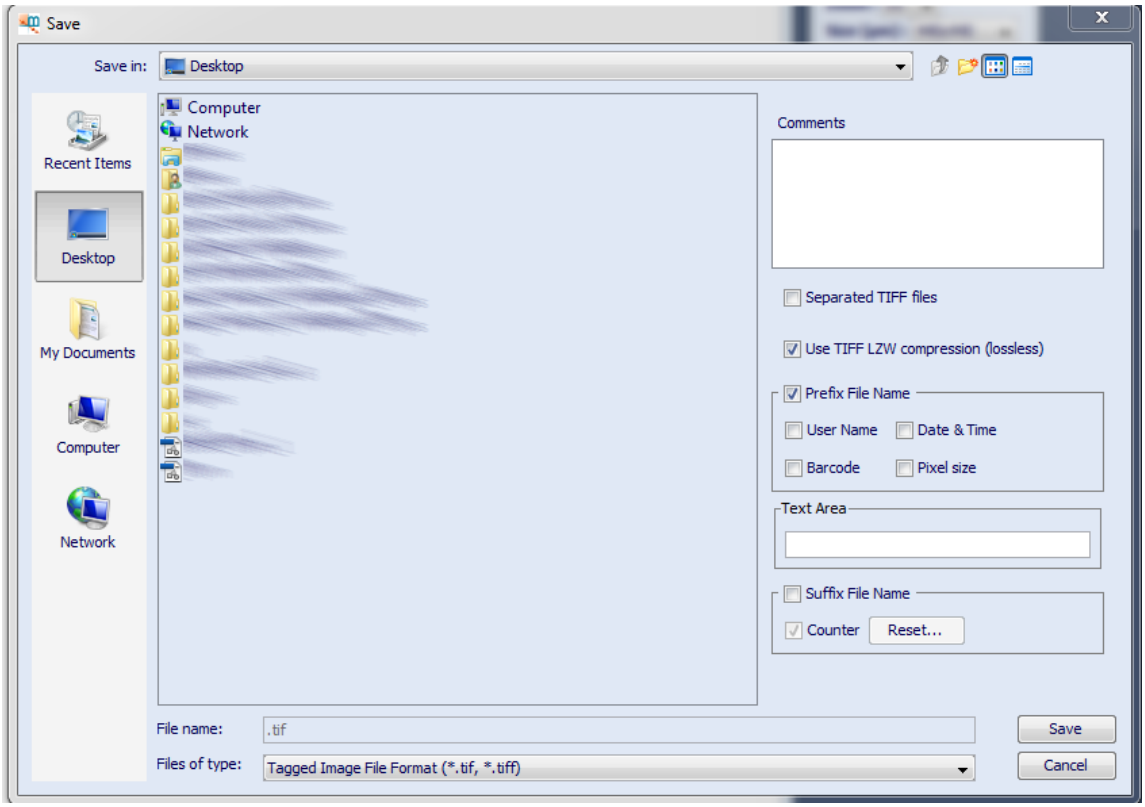
The saturation percentage is indicated for each of the wavelengths, the red value corresponds to the percentage of saturated pixels on the first channel (either 635 nm or 670 nm according to the scanner model) while the green value indicates the percentage of saturated pixels on the second channel (either 532 nm or 785 nm according to the scanner model).

The histogram helps you visualize the number of saturated pixels in each channel. You can modify laser intensity and PMT gain % in the «general» tab of the «configuration» window.

3.11 Scanning an image

«Scan» reads the slide, within the area of interest, using the current acquisition parameters.

Images are saved automatically after the scan. Before the scan begins you have to choose where to save your image(s):



For Windows users

If you use Windows and want to save your images or results in Windows folders named «*My documents*», «*My images*»... you have to check first if those directories are not "*read only*" (which is the default of these folders). Mapix indeed checks if directories are writable or not.

To modify read/write attributes of folders, right click on the folder, then click on «**Properties**» and in the «**Attributes**» area, uncheck the «**read only**» attribute.

For information about saving parameters refer to the table below:

ITEM	DESCRIPTION
Comments	You can use the comments box to add specific experiment parameters or other comments about your slides
Separated TIFF files	For two *color slide scans*, you can save the image as: ***A single TIFF file* containing both color images (default)**: To do this, keep the **«separate files»** box unchecked ***Separate TIFF files*:** the default name will have the suffix _xxx, where xxx is the relevant wavelength You can also choose to keep either one, two or three colors **Note**: ***a single TIFF file containing all color images is easier to manage, and guarantees image correspondence*.**
Use TIFF LZW	You can compress your TIFF image using LZW compression. LZW leads to a lossless image compression which guarantees no quality loss due to compression (see *Preferences* section).
Prefix File Name	Select the prefix file name options that you want to be automatically set on the file name (see *Preferences* section).
Suffix File Name	For similar file names you can add the file number by clicking on the suffix file name box. Counter can be reset
Text area	Use this area to manually write the file name
File Type	Images are saved on Tagged Image File Format (TIFF). As a format, TIFF offers specific features such as lossless image restitution and the ability to save one or more images together with related information called tags

Click on «**save»** to start the scan.

Click on  **Stop** icon to stop the current image acquisition sequence.

4. Working with images

Once images are acquired you can work on them to set the quantification parameters.

You can work with TIFF files created by Mapix or by any other compatible software. These files may contain one or multiple images. Mapix only handles files containing 1, 2 or 3-color images and 1 or 2 previews.

Note: Previews are not displayed

4.1 Open an existing image

To open an image, go to the «**File**» tab on the Main Menu. Click on  **«open image»** and search for the images you want to open.

If you have saved your images in separate TIFF files, you can open them either separately or in a single image.

To open two separated TIFF files in a single ratio image you can either:

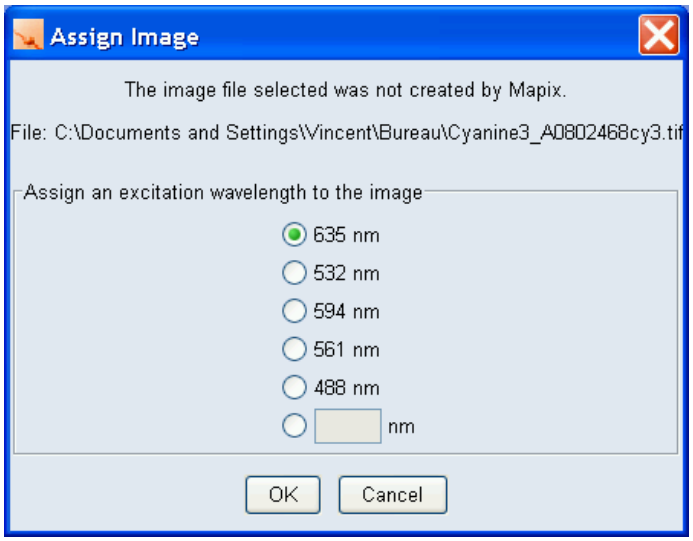
- Hold the «**Ctrl**» button down when selecting the images

- Open one image in first and then open the second one

 **Notes**:

To open two separated TIFF files and generate ratio images; images must have the same dimensions, the same pixel size and different color channels. Otherwise, only one of the selected images will be displayed.

When opening images created by other compatible software, if there is no wavelength indicated in the TIFF file, Mapix will ask you to assign a wavelength value for each channel to create the ratio metric image:

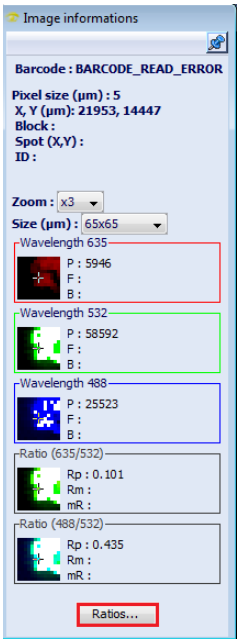


If you do not know these values click on «**Cancel**», then the default values will be 1 and 2 for the first and second channel, respectively.

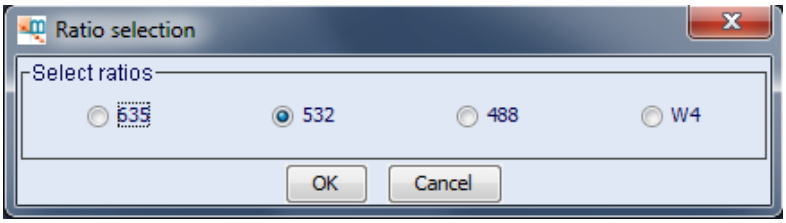
4.2 Image ratios

Image ratios use 3 channels: Red, Green and Blue. They are commonly called RGB images. Each channel uses 8 bits, so each channel comprises values from 0 to 255.

Ratio images are formed using two channels to display. Click on the "ratios" button on the image information window to select the wavelength channel that will be used to generate the image ratio:



The following window is displayed:



Select the wavelength that will be used as the common image from which the ratio image(s) will be generated. For example, when choosing the wavelength 532 nm, the ratio image(s) that will be created are 635/532 (In the case of the InnoScan 710 and 910 scanner models); and both 635/532 and 488/532 in the case of the InnoScan 1100 AL scanner.

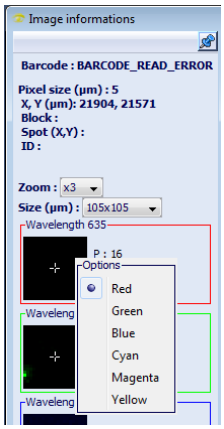
Notes:

The red channel corresponds to intensities at the first wavelength channel (either 635 nm or 670 nm according to the scanner model) while the green channel represents the intensities at the second wavelength channel (either 532 nm or 785 nm according to the scanner model). In the case of the InnoScan 1100 AL model the third wavelength channel is blue (488 nm). Please refer to the "personalized colors" section to learn how to change the colors for each wavelength.

For images taken at a pixel size of 1 µm; sometimes it is not possible to display both channels at once due to memory issues. However, this affects the image display only; image analysis and quantification are done in both channels, even when only one color is displayed.

Personalized colors

In order to define the colors for each of the different channels right click on the desired wavelength panel in the image information window and a window with the color options is displayed:



Once the image is opened, you can navigate through it by using the mouse. To change the mouse mode use the icons on the «**tools**» window or right-click in the image display area.

MODE	ACTION
 Zoom in window/ Zoom mode	Zoom into the selected area. When this icon is selected click the image to zoom in. You can also zoom out by holding the "Ctrl" key down and clicking on the image. You can also use the mouse wheel to zoom in and out by holding the "Ctrl" key down.
 Drag image/ Walk mode	Move through the image inside the "image display" window. This is the equivalent of using the sliders in the window

You can also change image display using the following resources:

ITEM	ACTION
 Zoom	Zooms the entire image by 20%
 Unzoom	Zooms out the entire image by 20%
 Undo	Returns you to the previous zoom
 Redo	Returns you to the zoom just canceled
 Fit to page width	Fits image width to the display window
 Full image	Fits the entire image into the display window
 Real size	Displays the image with a 100% zoom factor. One pixel on the screen corresponds to one image pixel.
 Display options	Lets you change the parameters by which an image is displayed. Please see *Image Display Options* section
 Transform Image	Allows user to rotate the image 90°, 180°, 270°, flip the image vertically, flip the image horizontally, automatically register two single wavelength images, and generate a negative image.
 /	Show/hide the navigation window

4.3 Image information

The «**About image**» option on the «**Image**» tab of the main menu displays the information contained in the TIFF images such as the serial number of the scanner that acquired the image, acquisition parameters, etc. as shown below:

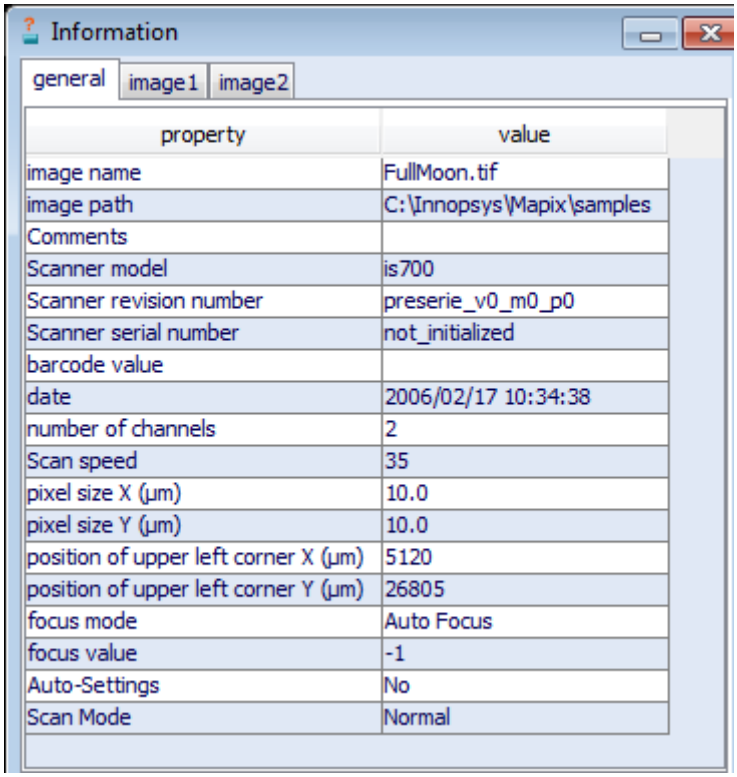


Image 1 and Image 2 tab contain the information of laser power and PMT gain percentage of each wavelengths; image 1 corresponding to red channel image (either 635 nm or 670 nm according to the scanner model) while image 2 to green channel image (either 532 nm or 785 nm according to the scanner model). This option is also accessible by passing the cursor over the image and typing "i".

Note: If you have scanned using auto-settings, use this to obtain the optimal scan settings in terms of PMT gain and laser power for future manual scans, assuming that the slides are homogeneous.

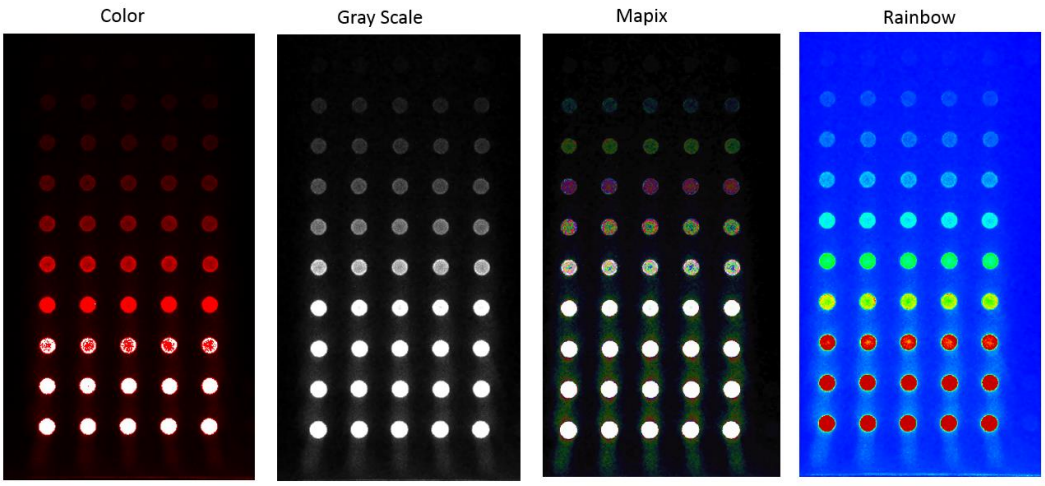
4.4 Image Display Options

Opens a menu by which you can choose the image display options

Displayed values are those used in the last Mapix session.

These options can be used to adjust image «**brightness**», «**contrast**», and the proportion of red or green in the ratio image by using «**balance control**».

Color palettes can also be adjusted when displaying single wavelength images. The «**Color**» option displays the color selected for the displayed wavelength; usually, 635 nm is displayed in red while 532 nm and 488 nm are displayed in green and blue, respectively. «**Gray scale**» option displays the image in a gray scale, while «**Rainbow**» and «**Mapix**» options display in a continuous color pallet representing signal levels. In «**Rainbow**» the color pallet goes from blue representing low signals to red representing high signals (see figure). This is contrary to the «**Mapix**» option in which low signals are represented in dark colors while high signals are represented in bright colors, and saturation is represented in white.



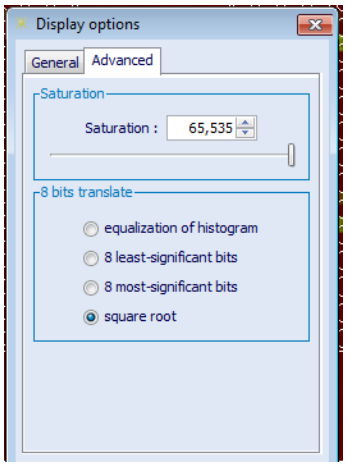
Notes:

Changing these options only affects the way an image is displayed: **Display options have no effect on results or image data.**

When the balance control cursor is located in the extremes values (completely to the left or right) only one color channel is displayed. Red or green channels are displayed respectively and saturation in the invisible channel is no longer displayed.

Click on «**Auto»** to allow Mapix for defining the best display parameters for your image. To be able of modifying the display parameters you should deselect this option.

Click on «**advanced»** tab to modify the saturation value and to select the parameters used for image conversion



ITEM	DESCRIPTION
Saturation	The maximum saturation value is 65535. This means that the white color used to show saturation on images will appear as soon as this new value will be reached.
8 Bits translate	The images displayed are 8-bit images converted from 16-bit images. 16-bit images can be converted to 8-bit images
Equalization of histogram	Adjust optimum 8-bit conversion using histogram equalization to display all the 16-bit values. It shows saturation on the most luminous pixels, while the least luminous pixels are visible
8 less-significant bits (LSB)	Displays only the 8 least significant bits showing the least luminous pixels while saturating all pixels with a value exceeding 255
8 most-significant bits (MSB)	Displays only the 8 most significant bits showing up the most luminous pixels
Square root	Converts the 16-bit image using the square root method, which compresses image dynamics

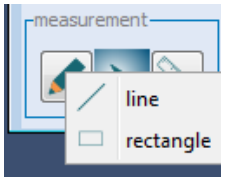
Note:

When an image is acquired by an InnoScan scanner, pixel depth is 16-bit or 20-bit, meaning that pixel values range from 0 and 65535 or from 0 to 1480575 respectively. Because of the hardware limitations of standard graphics boards, these images are only displayed with an 8-bit pixel depth, meaning that on-screen pixel values range from 0 to 255. The software also uses the 16-bit and 20-bit values for processing the images. 8-bit images are only used for display purposes.

4.5 Measurement tools

Using the measurement tools you can measure the number of pixels and their intensities inside specific zone on the image.

You can choose either a line or a rectangular zone on your image by clicking on  icon:



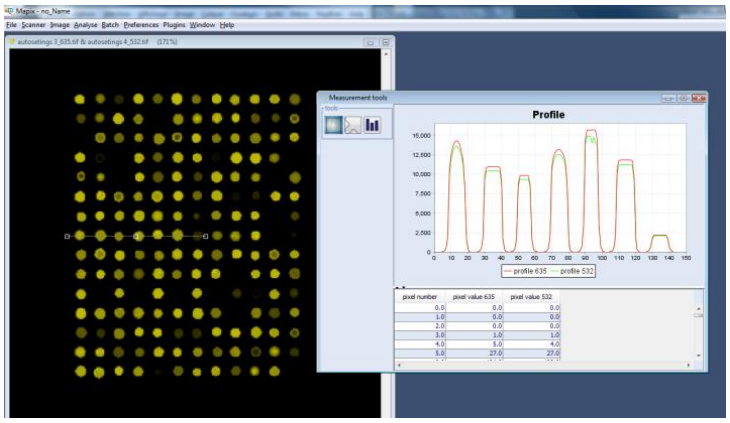
Once you have drawn a line or a rectangular zone, select one using the  icon.

Click on  icon to choose a measurement tool:

There are three available tools:

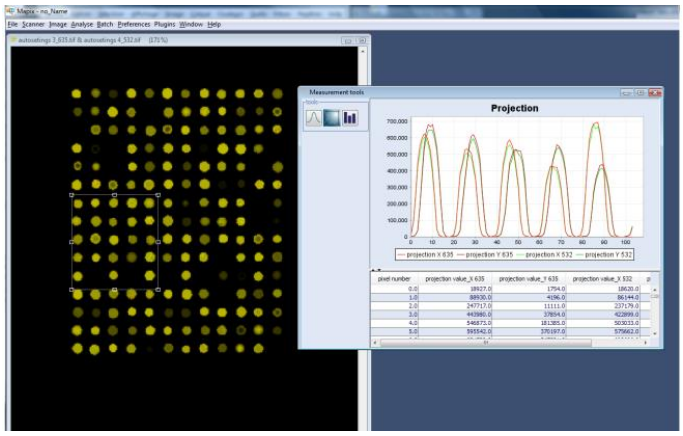
1.  **Profile measurement**: shows the intensity for each pixel inside the drawn line. You can only use this option when selecting a line and not when selecting rectangular zones.

The intensity profile for the selected line is shown in the window:



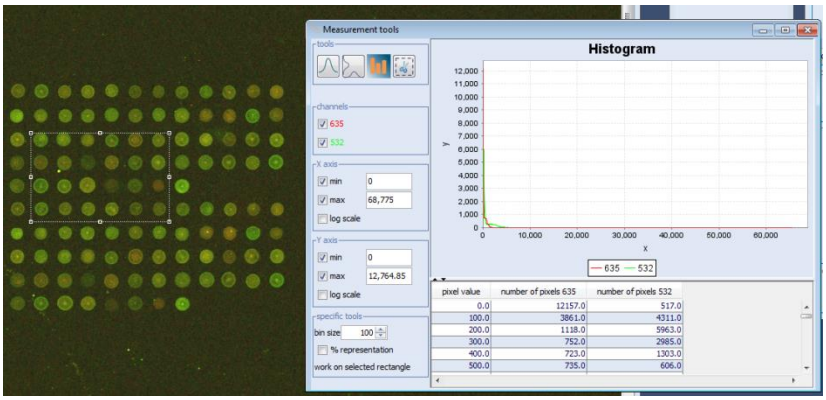
2.  **Projection measurement**: shows the projection in X and in Y for each wavelength. A projection is the addition of the number of pixels on each column

(X) and each row (Y) in which the rectangular zone is built. This option is only used for rectangular zones.



3.  **Histogram**: The histogram displays pixels on a graph. Axis-X represents pixel values (from 0 to 65535). Axis-Y represents the number of pixels which are equal to the value given by axis-X.

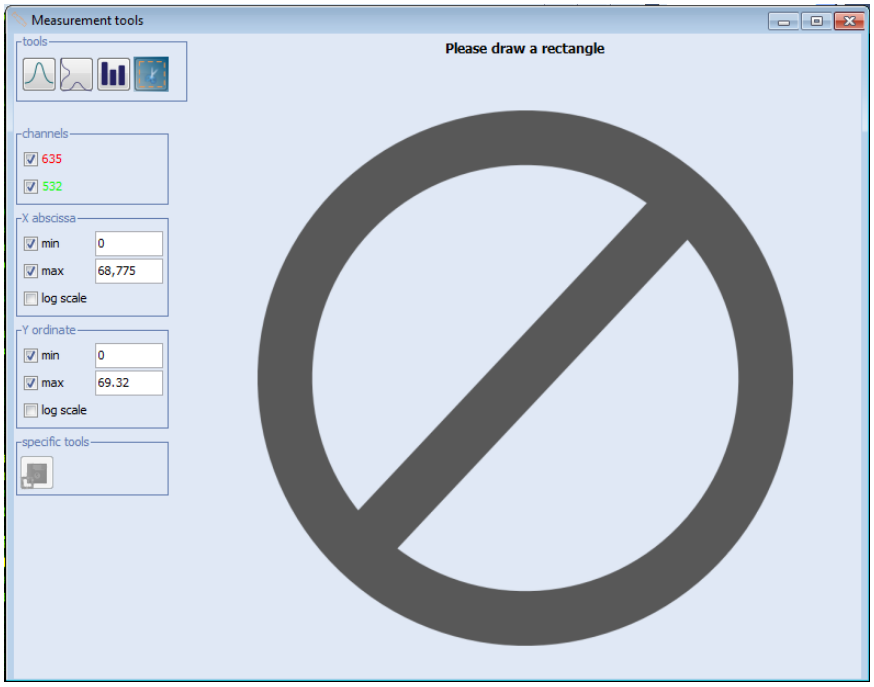
While the histogram option in the tools window displays the pixels that compose the whole image, this option shows the pixels contained in the selected area.



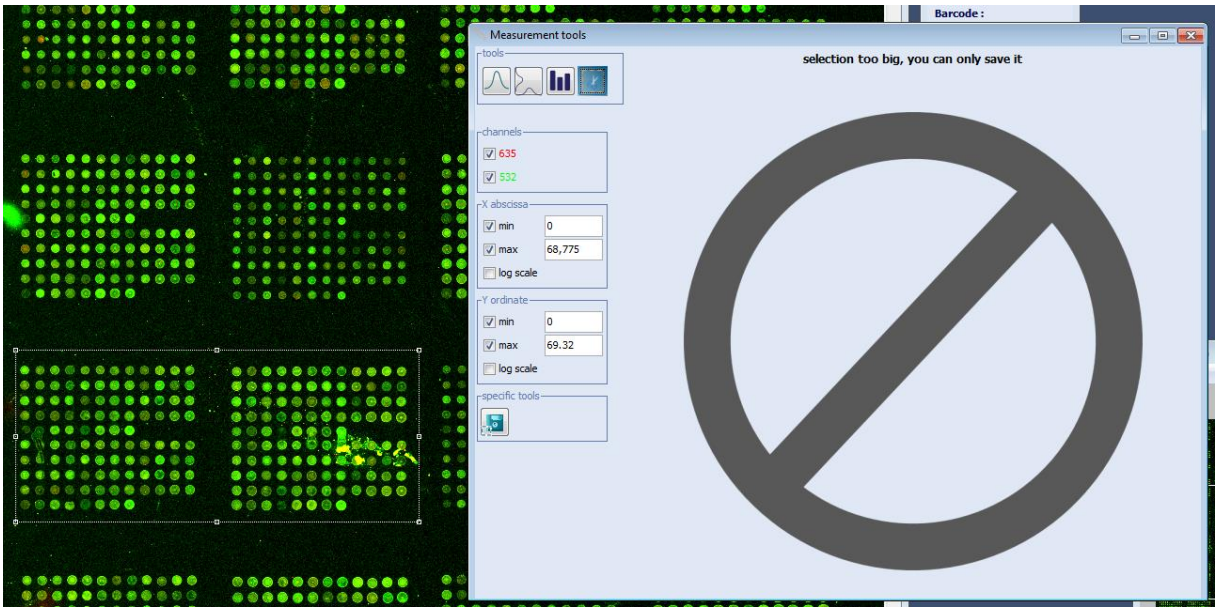
4.6 Crop an image

One can export a part of the images using the option  "extract image sample". This option is useful to separate the blocks of an images.

Click on the icon  , the next message is displayed:



Select the image zone that you want to export, to do this, click on the icon  and then select the option "rectangle", then draw a rectangle corresponding to the image you want to export. Once the rectangle is drawn, the next message will be displayed:



Click on the icon  "save" and select the file where you want to save the new image.

4.7 The grid

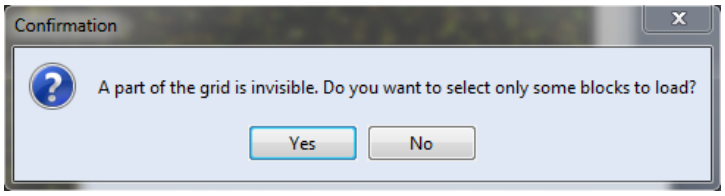
To determine the position of each spot, a grid containing the parameters (spot spacing, diameter, etc...) used at slide creation must be assigned.

To assign a grid, you can use either a GAL file, which is a standard ATF file generated by the spotter itself, or the grid creation tool.

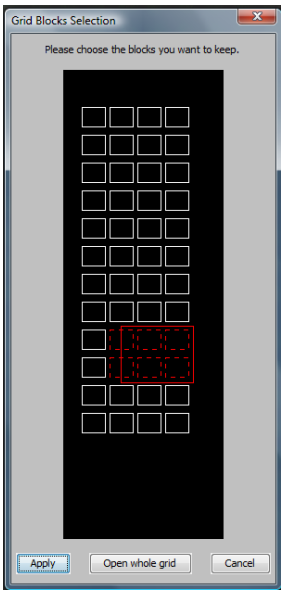
4.7.1. Open an existing grid file (GAL file)

You can import an existing GAL file by using the icon  «**Open a grid / Import a grid from a GAL file**» at the tool window or at the «**Analyze**» tab of the Main Menu.

If the image corresponds only to a part the slide, and the GAL file contains the complete grid a message will ask to confirm for loading the grid:



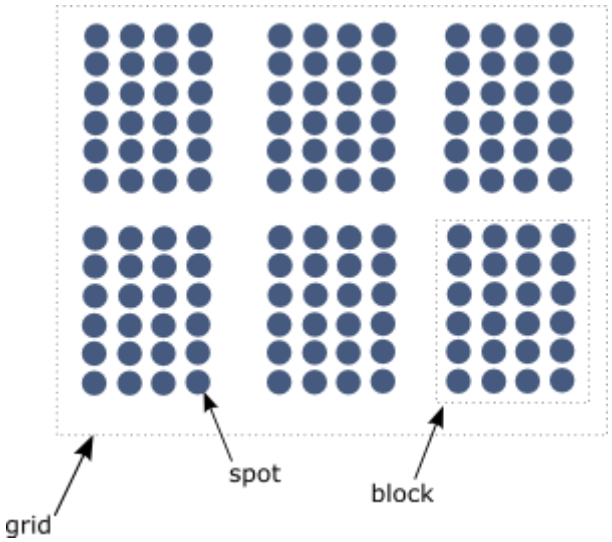
Select «**Yes**» to continue, then select the block corresponding to the image:



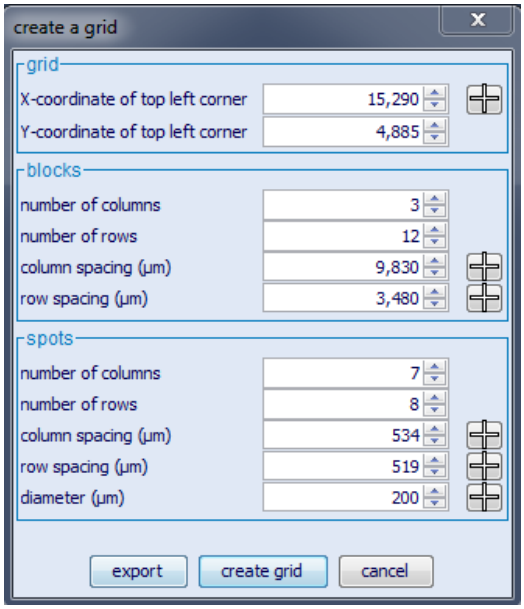
Selecting the "Apply" option, Mapix will automatically open the part of the grid corresponding to the selected block. With the option "Open whole grid" the whole grid will be charged independently of the blocs present in the image. Finally, the option "Cancel" will stop the grid opening process.

4.7.2. Creating a grid

A grid is a set of evenly spaced blocks; a block is a set of evenly laid out spots, forming an array:



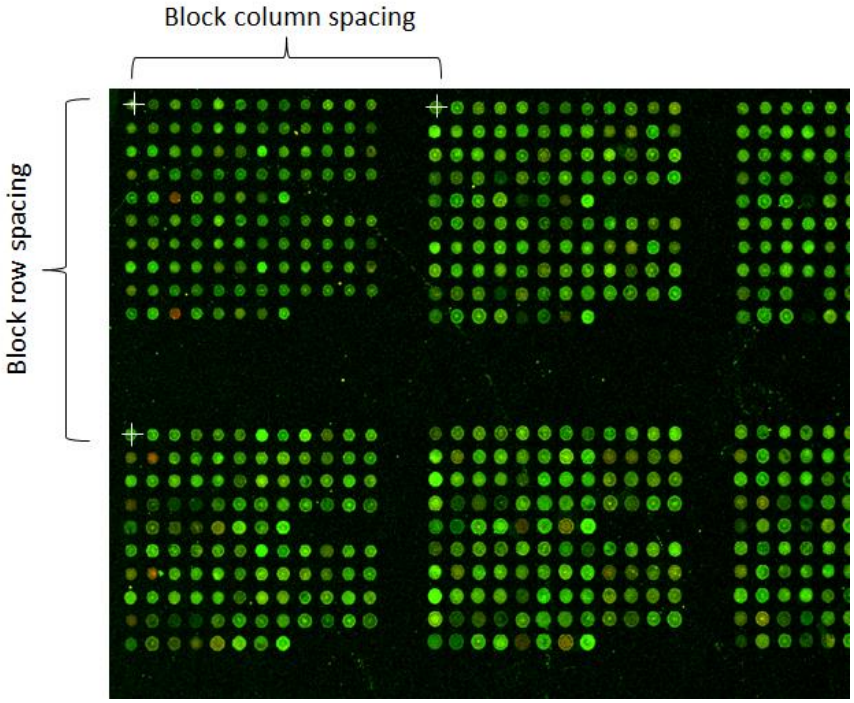
To create a grid use the  **grid drawing** tool either in the tools window or in the «**Analyse**» tab of the Main Menu. You can then specify the grid parameters in the following window:



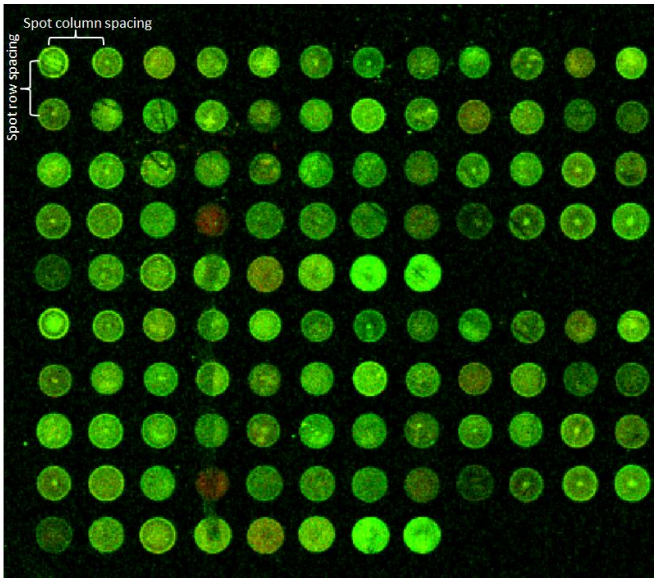
Use the icon to establish the X-Y coordinates of the top left spot of the grid by clicking the center of the spot in the top left corner of the top left block of the microarray.

In the blocks panel the column spacing corresponds to the distance between the first spot of the first block and the first spot of the second block along the X axis. In the same way the row

spacing corresponds to the distance between the first spot of the first block and the first spot of the second block along the Y axis as is shown in the following image:



In the case of the spots, the column spacing refers to the distance between the spots on the X axis, while the row spacing corresponds to the distance between the spots on the Y axis:

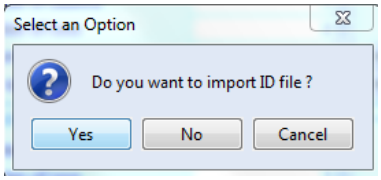


Use the icon to directly select the corresponding spots in the image.

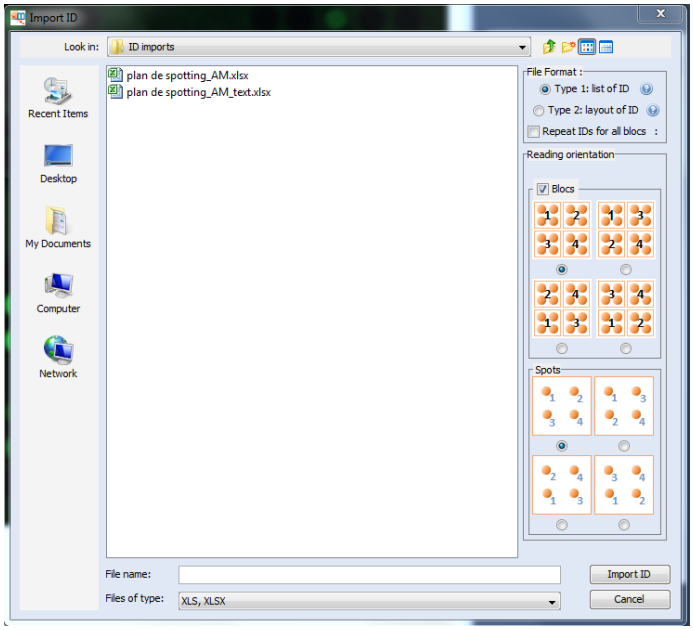
When selecting the export option, a grid file is saved in a GAL file format that can be further uploaded for other images. One can import the sample IDs corresponding to the image when exporting the grid.

4.7.3. Importing Sample IDs to the GAL file

Once the grid parameters are defined, one can import the sample IDs corresponding to the image by choosing the option "Yes" when exporting the GAL file:



The following window is displayed:

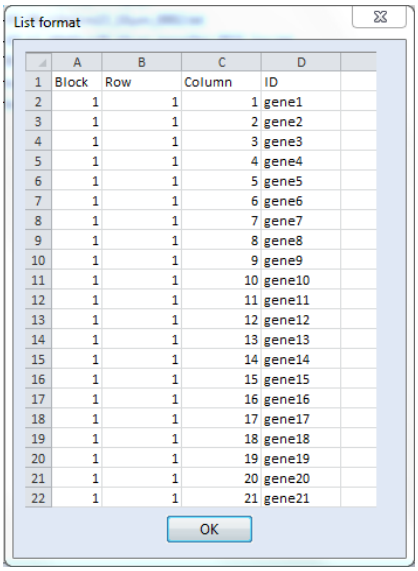


Choose the file containing the ID list either from an Excel file or from a tabulation-separated text file (txt).

ID file format

The file containing the ID list must have one of the two following formats:

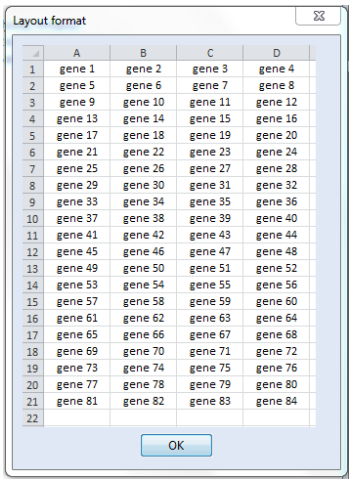
Type 1: In which the **IDs are listed** as in the following matrix:



	A	B	C	D
1	Block	Row	Column	ID
2	1	1	1	gene1
3	1	1	2	gene2
4	1	1	3	gene3
5	1	1	4	gene4
6	1	1	5	gene5
7	1	1	6	gene6
8	1	1	7	gene7
9	1	1	8	gene8
10	1	1	9	gene9
11	1	1	10	gene10
12	1	1	11	gene11
13	1	1	12	gene12
14	1	1	13	gene13
15	1	1	14	gene14
16	1	1	15	gene15
17	1	1	16	gene16
18	1	1	17	gene17
19	1	1	18	gene18
20	1	1	19	gene19
21	1	1	20	gene20
22	1	1	21	gene21

This option is available for both txt and Excel files.

Type 2: In which the ID matrix correspond to the ***exact layout*** of the image:



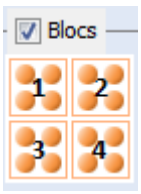
	A	B	C	D
1	gene 1	gene 2	gene 3	gene 4
2	gene 5	gene 6	gene 7	gene 8
3	gene 9	gene 10	gene 11	gene 12
4	gene 13	gene 14	gene 15	gene 16
5	gene 17	gene 18	gene 19	gene 20
6	gene 21	gene 22	gene 23	gene 24
7	gene 25	gene 26	gene 27	gene 28
8	gene 29	gene 30	gene 31	gene 32
9	gene 33	gene 34	gene 35	gene 36
10	gene 37	gene 38	gene 39	gene 40
11	gene 41	gene 42	gene 43	gene 44
12	gene 45	gene 46	gene 47	gene 48
13	gene 49	gene 50	gene 51	gene 52
14	gene 53	gene 54	gene 55	gene 56
15	gene 57	gene 58	gene 59	gene 60
16	gene 61	gene 62	gene 63	gene 64
17	gene 65	gene 66	gene 67	gene 68
18	gene 69	gene 70	gene 71	gene 72
19	gene 73	gene 74	gene 75	gene 76
20	gene 77	gene 78	gene 79	gene 80
21	gene 81	gene 82	gene 83	gene 84
22				

Please note that the ID matrix mustn't contain empty lines or columns. This option is only available for Excel files.

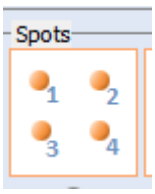
Reading orientation

Choose the orientation in which the matrix IDs should be read according to the image. First choose the block orientation and then the spot orientation.

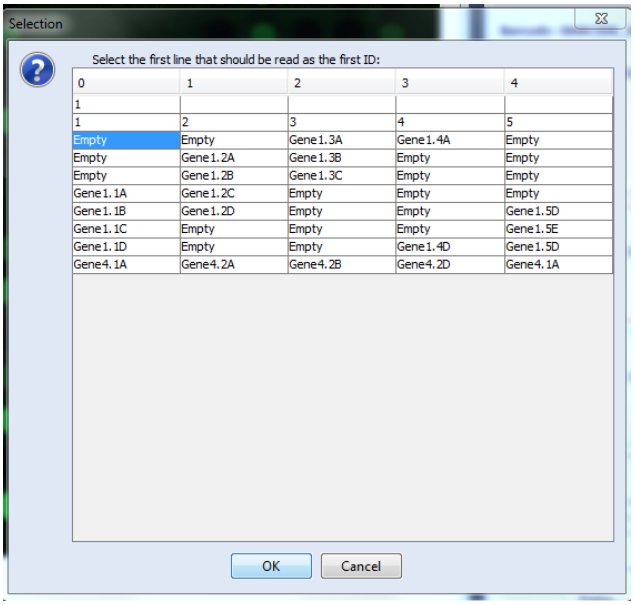
Image orientation in Mapix should be read as follows:



and



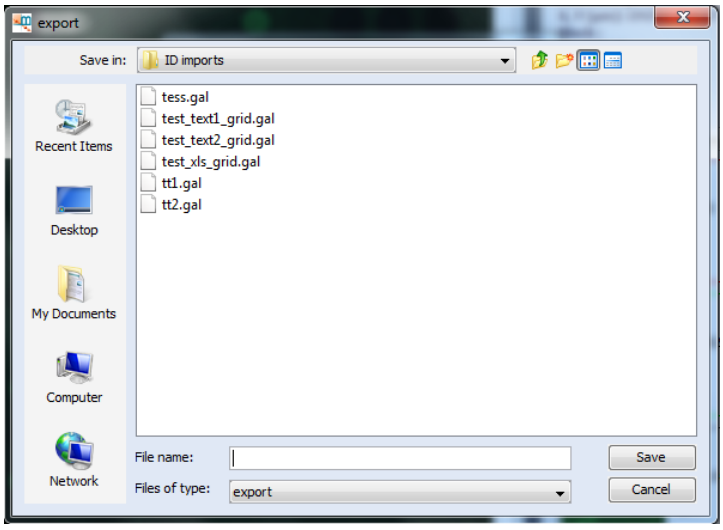
Once you have selected the format of the file containing the IDs, click on **« Import ID »** to continue. The following window is displayed:



0	1	2	3	4
1				
1	2	3	4	5
Empty	Empty	Gene1.3A	Gene1.4A	Empty
Empty	Gene1.2A	Gene1.3B	Empty	Empty
Empty	Gene1.2B	Gene1.3C	Empty	Empty
Gene1.1A	Gene1.2C	Empty	Empty	Empty
Gene1.1B	Gene1.2D	Empty	Empty	Gene1.5D
Gene1.1C	Empty	Empty	Empty	Gene1.5E
Gene1.1D	Empty	Empty	Gene1.4D	Gene1.5D
Gene4.1A	Gene4.2A	Gene4.2B	Gene4.2D	Gene4.1A

Please check that the file format corresponds to the image layout. Select the first line that should be read as the first ID in the image by clicking on the first ID of your list.

Select the folder in which you want to save the GAL file:



4.7.4. Positioning the grid

Once the grid file is opened a grid is positioned on the image. To hide the grid click on the  **«hide the grid»** icon, to see the grid click on the  **«show the grid»** icon.

Block selection / spot selection

To align the grid to its correct position with respect to the features, you can move the blocks and spots manually by using  «**blocks modification**» and  «**spot modification**» icons of the tools window, or you can perform it automatically by following the next steps:

 Find grid automatically: this option locates the position of the entire grid without moving blocks in relation to each other. This tool does not take into account any selection of blocks or spots and then acts directly on the entire grid.

 **Find blocks automatically**: this option finds the best position of each block. The following options are available:

«Find all blocks» locates the position of each block without moving the spots in relation to each other

«Find selected blocks» locates the position of selected blocks without moving the spots in relation to each other

«Find all blocks and spots» locates the position of each block and the position and diameter of each spot

«Find selected blocks and spots» locates the position of selected blocks and the position and diameter of spots in selected blocks

 **Find spots automatically**: accurately locates the position of each spot, and determines their diameter. The following options are available:

«Find spots in all blocks» finds each spot without taking into account any block selection

«Find spots in selected blocks» finds each spot in selected blocks

«Find selected spots» finds each selected spot

The  «**Global search / find all automatically**» icon allows to perform all steps 1-3 automatically in only one step. You can use this option if the blocks are correctly aligned with each other and the image matches the theoretical grid closely. This can be useful when processing a large number of slides spotted simultaneously.

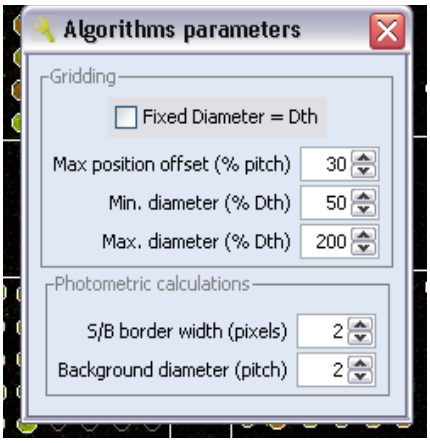
Once the grid is correctly positioned you can quantify the image.

5. Image quantification

5.1 Configuring the analysis parameters

Each scan starts with standard parameters; however, you can define some analysis parameters that better fit your image.

Click on  «**grid algorithm settings/configuration**» icon on the tools window or on the «**Analyse**» tab of the Main Menu. The following window is opened:



In the «**gridding panel**» you can adjust parameters related to automatic spot search, whereas in the «**photometric calculations**» panel parameters are used to produce the results of the image quantification.

Refer to the following table for information:

Panel	ITEM	DESCRIPTION
Gridding	Fixed Diameter	Use this option when you want to keep a defined spot diameter (theoretical diameter defined in the gal file, or the manually defined diameter). The real spot diameter will not be measured but stated as the defined spot diameter.
	Max. position offset (% pitch)	Each spot has a mean position defined by the geometric parameters of the block in which it is located, and an actual position (observed). This parameter defines the maximum distance tolerated between the mean position and the observed position (this distance is expressed as a percentage of average spot pitch). **Note**: This value should never exceed 50%, and 30% is generally about right
	Min. diameter (%Dth)	Defines the minimal spot diameter as a percentage of the theoretical diameter (DTH), given by the gal file, that will be used to find a spot. If one spot is smaller than this value it will be flagged as "Not found"
	Max. diameter (% Dth)	Defines the maximum spot diameter as a percentage of the theoretical diameter (DTH), given by the gal file, that will be used to find a spot. If one spot is larger than this value it will be flagged as "Not found"
Photometric Calculations	S/B border width (pixels)	Each spot is framed in a gridding circle, overlaid above the images. This circle defines the border between spots and background. The pixels in the immediate vicinity of this circle have an indeterminate status because they are located at the border between spot and background. The width of the exclusion area is defined by this parameter. An average value of 2 pixels is generally acceptable: the first circle 1 pixel thick inside and 1 pixel thick outside the gridding circle are ignored (Figure 1).
	Background diameter (pitch)	Background value is calculated as a local value estimated within a circular area centered on the spot (excluding all the spots located within this circle). This parameter defines the diameter of this circular area. It is expressed by the number of pitch units between spots (Figure 1).

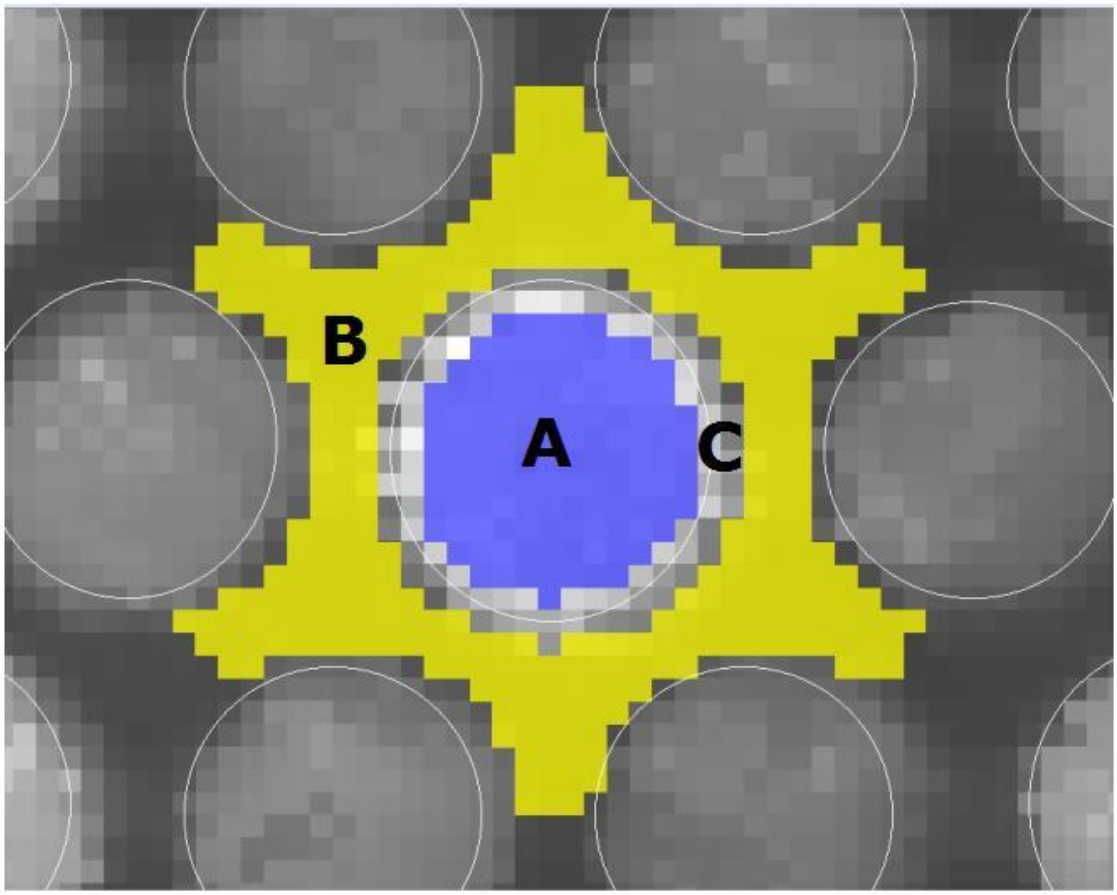


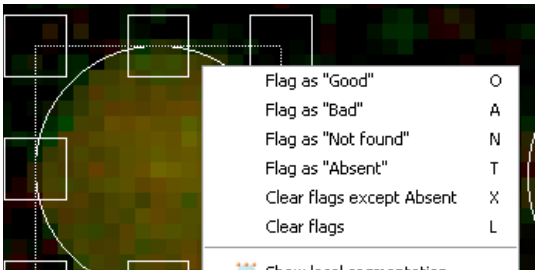
Figure1. Spot signal and background calculations. Each spot is framed in a gridding circle (white circles), overlaid on the images. This circle defines the border between spots and background. Because the pixels around this gridding circle are uncertain by definition, no processing is performed on the pixels located in the immediate vicinity of the gridding circle. The spot / background transition zone is defined in pixels by an adjustable parameter set at 2 pixels in this figure. **A:** Feature pixels. **B:** Background pixels. **C:** 2 pixel exclusion region.

Note:

To visualize the local segmentation around the spot, click the «**spot modification**» button, then select and click on the «**show local segmentation**» option.

Once the grid is in place, spots can be flagged. To do this, select a spot or group of spots (be sure that the «**spot selection/modification tool** » is selected) and right-click within the selection. A menu appears under the cursor allowing you to flag the selected spots:

5.2 Flagging spots

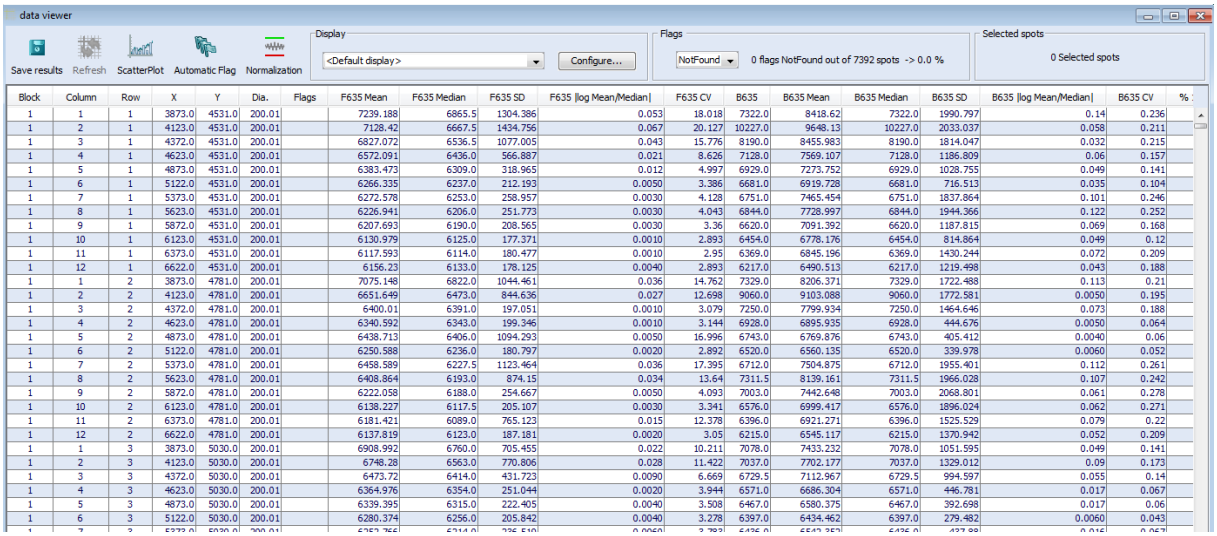


Flagging lets you sort these spots when analyzing the results. The results file includes a Flag column containing values to identify each flag:

FLAG	VALUE
 Good	100
 Bad	-100
 Not found	-50
 Absent	-75 **Notes**: Absent spots cannot be modified by Mapix. These spots are defined in the GAL file or designed with an "empty" ID. Absent spots are not handled by the user interface and are ignored by the automatic search algorithms
Not flagged spot	0

5.3 The results table

Use  «**Photometric Calculations**» icon located either in the tools menu or in the «**Analyse**» tab of the Main Menu to begin the image quantification process. The resulting table is displayed in a spreadsheet:



Block	Column	Row	X	Y	Dia.	Flags	F635 Mean	F635 Median	F635 SD	F635 |log Mean/Median|	F635 CV	B635	B635 Mean	B635 Median	B635 SD	B635 |log Mean/Median|	B635 CV	%
1	1	1	3873.0	4531.0	200.01		7239.188	6865.5	1304.386	0.053	18.018	7322.0	8418.62	7322.0	1990.797	0.14	0.236	
1	2	1	4123.0	4531.0	200.01		7128.42	6667.5	1434.756	0.067	20.127	10227.0	9648.13	10227.0	2033.037	0.058	0.211	
1	3	1	4372.0	4531.0	200.01		6827.072	6536.5	1077.005	0.043	15.776	8190.0	8455.983	8190.0	1814.047	0.032	0.215	
1	4	1	4623.0	4531.0	200.01		6572.091	6436.0	566.887	0.021	8.626	7128.0	7569.107	7128.0	1186.809	0.06	0.157	
1	5	1	4873.0	4531.0	200.01		6383.473	6309.0	318.965	0.012	4.997	6929.0	7273.752	6929.0	1028.755	0.049	0.141	
1	6	1	5122.0	4531.0	200.01		6266.335	6237.0	212.193	0.0050	3.386	6681.0	6919.728	6681.0	716.513	0.035	0.104	
1	7	1	5373.0	4531.0	200.01		6272.578	6253.0	258.957	0.0030	4.128	6751.0	7465.454	6751.0	1837.864	0.101	0.246	
1	8	1	5623.0	4531.0	200.01		6226.941	6206.0	251.773	0.0030	4.043	6844.0	7728.997	6844.0	1944.366	0.122	0.252	
1	9	1	5872.0	4531.0	200.01		6207.693	6190.0	208.565	0.0030	3.36	6620.0	7091.392	6620.0	1187.815	0.069	0.168	
1	10	1	6123.0	4531.0	200.01		6130.979	6125.0	177.371	0.0010	2.893	6454.0	6778.176	6454.0	814.864	0.049	0.12	
1	11	1	6373.0	4531.0	200.01		6117.593	6114.0	180.477	0.0010	2.95	6369.0	6845.196	6369.0	1430.244	0.072	0.209	
1	12	1	6622.0	4531.0	200.01		6156.23	6133.0	178.125	0.0040	2.893	6217.0	6490.513	6217.0	1219.498	0.043	0.188	
1	1	2	3873.0	4781.0	200.01		7075.148	6822.0	1044.461	0.036	14.762	7329.0	8206.371	7329.0	1722.488	0.113	0.21	
1	2	2	4123.0	4781.0	200.01		6651.649	6473.0	844.636	0.027	12.698	9060.0	9103.088	9060.0	1772.581	0.0050	0.195	
1	3	2	4372.0	4781.0	200.01		6400.01	6391.0	197.051	0.0010	3.079	7250.0	7799.934	7250.0	1464.646	0.073	0.188	
1	4	2	4623.0	4781.0	200.01		6340.592	6343.0	199.346	0.0010	3.144	6928.0	6895.935	6928.0	444.676	0.0050	0.064	
1	5	2	4873.0	4781.0	200.01		6438.713	6406.0	1094.293	0.0050	16.996	6743.0	6769.876	6743.0	405.412	0.0040	0.06	
1	6	2	5122.0	4781.0	200.01		6250.588	6236.0	180.797	0.0020	2.892	6520.0	6560.135	6520.0	339.978	0.0060	0.052	
1	7	2	5373.0	4781.0	200.01		6458.589	6227.5	1123.464	0.036	17.395	6712.0	7504.875	6712.0	1955.401	0.112	0.261	
1	8	2	5623.0	4781.0	200.01		6408.864	6193.0	874.15	0.034	13.64	7311.5	8139.161	7311.5	1966.028	0.107	0.242	
1	9	2	5872.0	4781.0	200.01		6222.058	6188.0	254.667	0.0050	4.093	7003.0	7442.648	7003.0	2068.801	0.061	0.278	
1	10	2	6123.0	4781.0	200.01		6138.227	6117.5	205.107	0.0030	3.341	6576.0	6999.417	6576.0	1896.024	0.062	0.271	
1	11	2	6373.0	4781.0	200.01		6181.421	6089.0	765.123	0.015	12.378	6396.0	6921.271	6396.0	1525.529	0.079	0.22	
1	12	2	6622.0	4781.0	200.01		6137.819	6123.0	187.181	0.0020	3.05	6215.0	6545.117	6215.0	1370.942	0.052	0.209	
1	1	3	3873.0	5030.0	200.01		6908.992	6760.0	705.455	0.022	10.211	7078.0	7433.232	7078.0	1051.595	0.049	0.141	
1	2	3	4123.0	5030.0	200.01		6748.28	6563.0	770.806	0.028	11.422	7037.0	7702.177	7037.0	1329.012	0.09	0.173	
1	3	3	4372.0	5030.0	200.01		6473.72	6414.0	431.723	0.0090	6.669	6729.5	7112.967	6729.5	994.597	0.055	0.14	
1	4	3	4623.0	5030.0	200.01		6364.976	6354.0	251.044	0.0020	3.944	6571.0	6686.304	6571.0	446.781	0.017	0.067	
1	5	3	4873.0	5030.0	200.01		6339.395	6315.0	222.405	0.0040	3.508	6467.0	6580.375	6467.0	392.698	0.017	0.06	
1	6	3	5122.0	5030.0	200.01		6280.374	6256.0	205.842	0.0040	3.278	6397.0	6434.462	6397.0	279.482	0.0060	0.043	

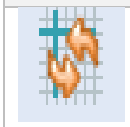
Each column corresponds to the results of the photometric calculations performed at each wavelength (see *Results description* section below for details).

The number (532, 635, 670 or 785) present in some column titles indicates the corresponding wavelength.

Data can be sorted by a chosen column in ascending or descending values by clicking on the desired column header. A small arrow indicates the sort status. You can define a sub-sort on other columns by holding the "Ctrl" key down.

When clicking in a row the corresponding spot is selected and zoomed in the image window.

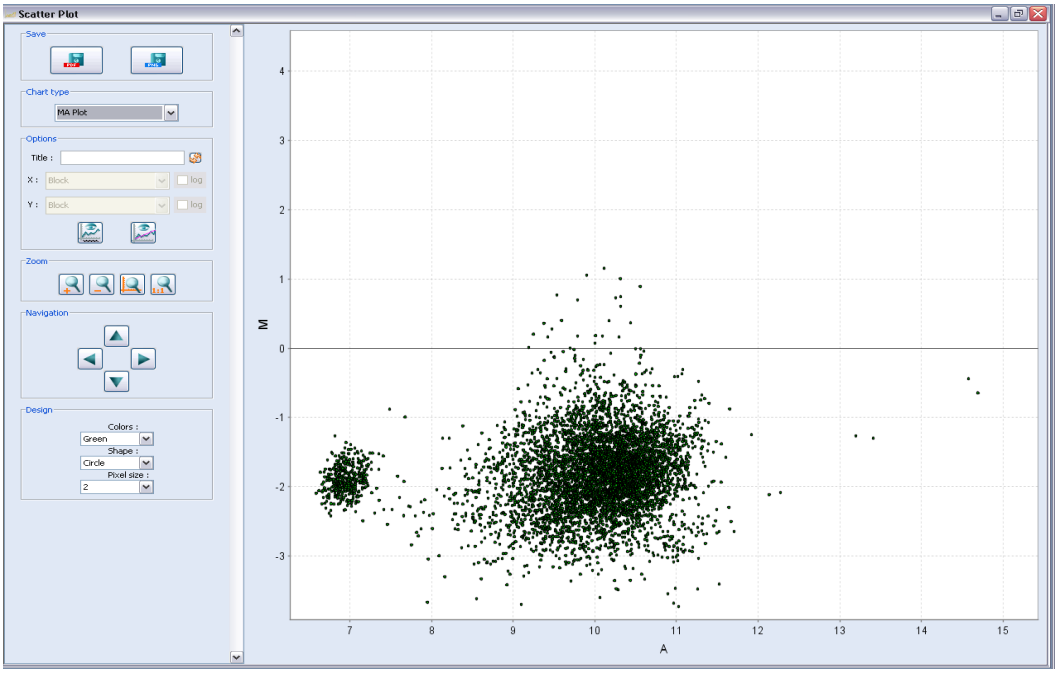
The **data viewer menu** gives access to further data analysis and result displays. Refer to the table below for brief description; each of the functions are detailed later in the manual:

ITEM	DESCRIPTION
 Save results	You can save your results in txt or gpr format
 Refresh	**Note**: If the grid, a block or a spot is modified after the quantification process, an asterisk will appear in the display window title and the icon Refresh becomes active to indicate that the data is not in accordance with the grid Use the refresh button to re-quantify the spots
 Scatter plot	Opens the scatter plot window
 Automatic flag	Opens the automatic flag window
 Normalization	Opens the normalization window
Display	Changes the display into the data viewer window as well as the form in which data is saved in the 'results' files
Flags	Counts the number of spots flagged in each category (good, bad, not found, absent) as stated in the *flagging spots* section

5.3.3. The scatter plot window

Click on the  "scatter plot" icon to open the scatter plot window.

This window allows you to draw plots from generated data. You can generate either a scatter plot of all spots on the array, a histogram or a MA-plot analysis.

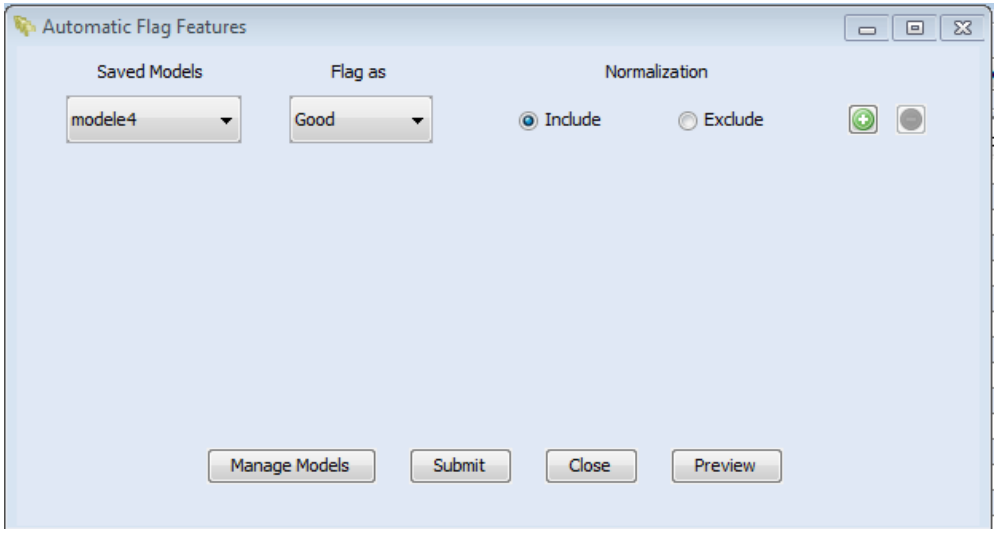


When generating a scatter plot, choose the data to be plotted on the X and Y axis in the options dialog of the scatter plot window.

5.3.2. The automatic flag window

As seen in the flagging spots section, spots can be flagged as "*good*", "*bad*" or "*not found*". You can perform an automatic flag by introducing flag parameters in this window.

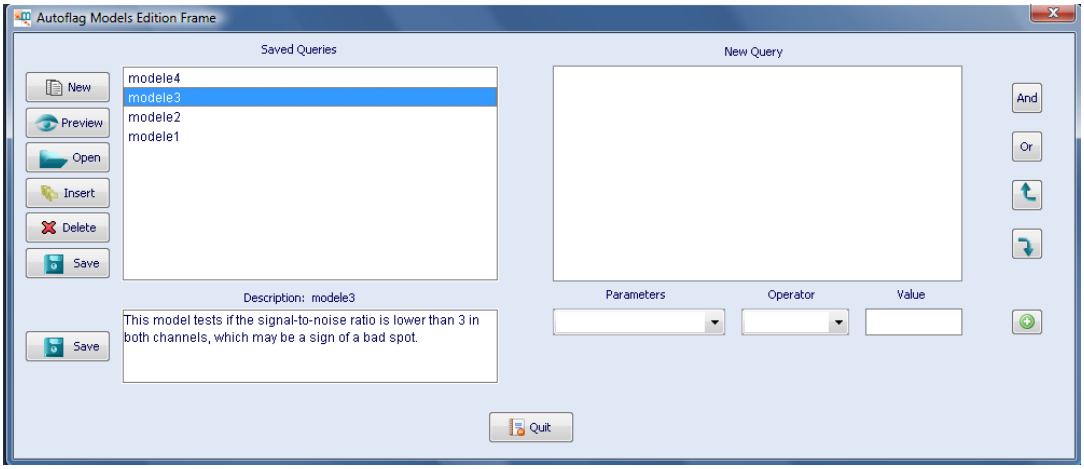
When clicking on  the following window opens:



ITEM	DESCRIPTION
Saved Models	Shows the existing queries used for flagging a spot
Flag as	Defines the flag associated to a query
Normalization	Defines whether flagged spots will be used during the normalization process or not
Manage Models	Opens the **«Autoflag Model Edition»** frame. Use this window to add new- or edit existing queries
Preview	Shows the parameters and the description of each query
	Add a new query
	Delete a query
Submit	Applies the query to the results
Close	Exit the automatic flag window

Adding a new- or editing a query

When clicking on «**Manage Models**» the **«Autoflag Model Edition»** frame opens:



Use this window to add new queries, modify or delete an existing query.

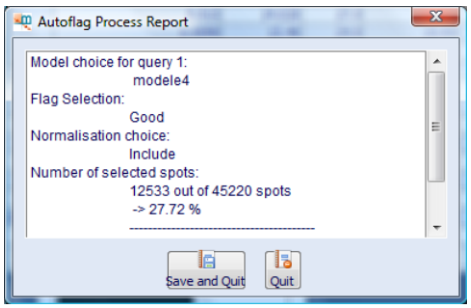
Note:

In a query the parameters used for flagging a spot are defined in the form of a model or a single query. In a model you can combine several queries at a time by using the «**And**» and «**Or**» buttons.

Refer to the table below for description of each command:

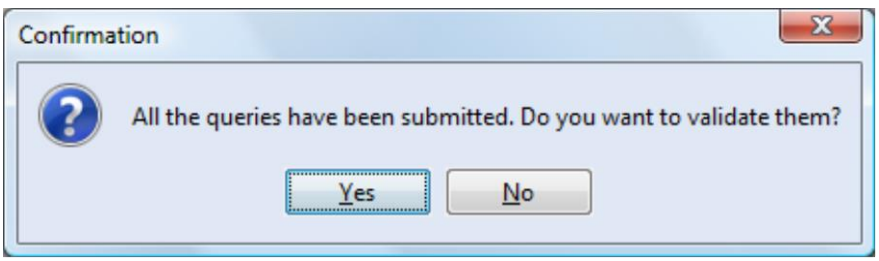
ITEM	DESCRIPTION
Saved Queries	Displays the existing models or queries
Description	Displays comments of the selected query
New Query	Initializes the query area to create a new query
Preview	Accesses information on all the queries
Open	Opens a selected model, the parameters used in the corresponding model are displayed in the query area.
Insert	Inserts the parameters used by a model into a new query.
Delete	Deletes a selected model
Save	Saves a new model or the modifications made in an existing model
Parameters	Chooses the quantifiers used in a query
Operator	Defines the operator to be applied in a query

Once the selected queries are submitted, a flagging report is displayed, describing each performed query and the number of spots selected by them:



When multiple queries are combined, the number of spots flagged by several queries is shown. Click on «**see details**» for detailed information of these spots.

Click on the «**Quit**» button to submit the query. A confirmation dialog is then displayed.



When validated, the query is then performed. You can see the results in both the image and the results table:

In the «**Flags**» column of the results table, the symbol of the last designated flag is displayed.

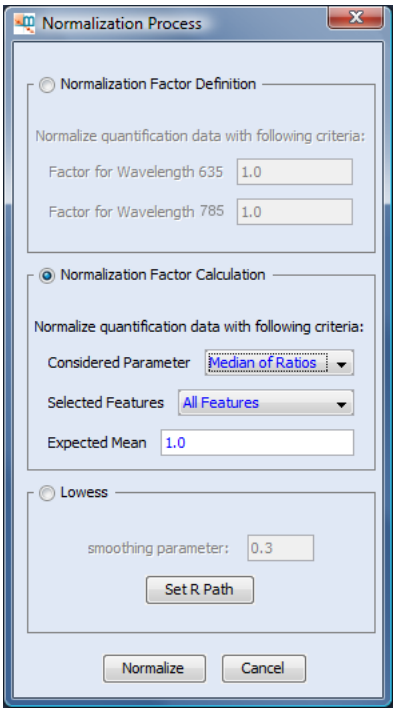
In the «**AutoFlag**» column, the legend indicates the query in the automatic flag function by which the flag was performed.

The «**normalization**» column indicates whether the flagged spot will be used (***"Include"***), or not (***"Exclude"***) for normalization calculations.

5.3.3. Normalization

In the case of two color microarrays, one can perform data normalization by either a fixed factor or using a global method.

Click on the  "normalization" icon to open the normalization process window



Refer to the table below for information

PANEL	ITEM	DESCRIPTION
Normalization Factor Definition	Factor for Wavelength 1 (635nm or 670nm)	By selecting this panel, the normalization is done with a fixed factor. You can define the factor for each wavelength
	Factor for Wavelength 2 (532nm or 785nm)	
Normalization Factor Calculation (Global method)	Considered Parameter	Selects the parameter to be used for normalization, you can choose among: Ratio of medians Ratio of means Median of ratios Mean of Ratios Regression ratios
	Selected Features	Choose which features will be taken to calculate the normalization factor. When selecting Included features, the spots used for calculating the normalization factor are those selected during the automatic flag function. In order to avoid the influence of extreme values only features with a ratio value between 0.1 and 100 are considered for normalization calculations.
	Expected Mean	Corresponds to the expected ratio mean
Lowess	Set R Path	Lowess normalization is performed in a R software-based algorithm. You must install R software to use this option. To download R software please go to the R-project website: *http://cran.r-project.org* For the first use of lowess normalization you have to define the complete Path to R software, either in the preferences menu (see preferences section) or directly by using the «Set R Path» option.

PANEL	ITEM	DESCRIPTION
	Smoothing parameter	Gives the proportions of points of the curve which influence the smoothness at each value. Smaller smoothing factor values give a better fit. When the smoothing factor is 1, all points influence the regression at each value of the X axis. You can modify this value to adjust the flexibility of the regression function used during lowess normalization. A smoothing parameter between 0.25 and 0.5 is recommended.

Note:

Once the normalization is performed, the title of the data viewer indicates that the data has been normalized, together with the normalization parameters. Also the Normalization icon becomes disabled indicating that the data has been normalized.

When using lowess normalization, a PDF file containing the graphs (MA plot and RG densities) before and after normalization is automatically created in the image directory.

Results description

Column Title	Description
Block	Block number
Column	Spot column number
Row	Spot row number
X	X-coordinate of the spot center in µm from the top left corner of the image
Y	Y-coordinate of the spot center in µm from the top left corner of the image
Dia.	Spot diameter in µm
Flags	Spot flag (See *Flagging spots section*)
Fxxx Mean	Spot pixels mean at the referenced wavelength
Fxxx Median	Spot pixels median at the referenced wavelength **Note**: The median is less sensitive to dust than the mean value
Fxxx SD	Standard deviation of spot pixels at the referenced wavelength
Fxxx |log Mean/Median|	Absolute value of the logarithm of the spot Mean/Median ratio at the referenced wavelength. This parameter indicates the consistency between mean and median values, normally very similar. When mean and median values are very different, |log Mean/Median| value tends to differ from zero. This may indicate dust effect on mean value or non-homogeneity in the spot
Fxxx CV	Coefficient of variation of the spot at the referenced wavelength. CV corresponds to the SD/mean ratio
Fxxx %Sat	Percentage of spot saturated pixels at the referenced wavelength. Saturation is defined at an intensity value of 65535.
Fxxx Median - Bxxx	The median spot pixel intensity with the background subtracted at the referenced wavelength
Fxxx Mean – Bxxx	The mean spot pixel intensity with the background subtracted at the referenced wavelength

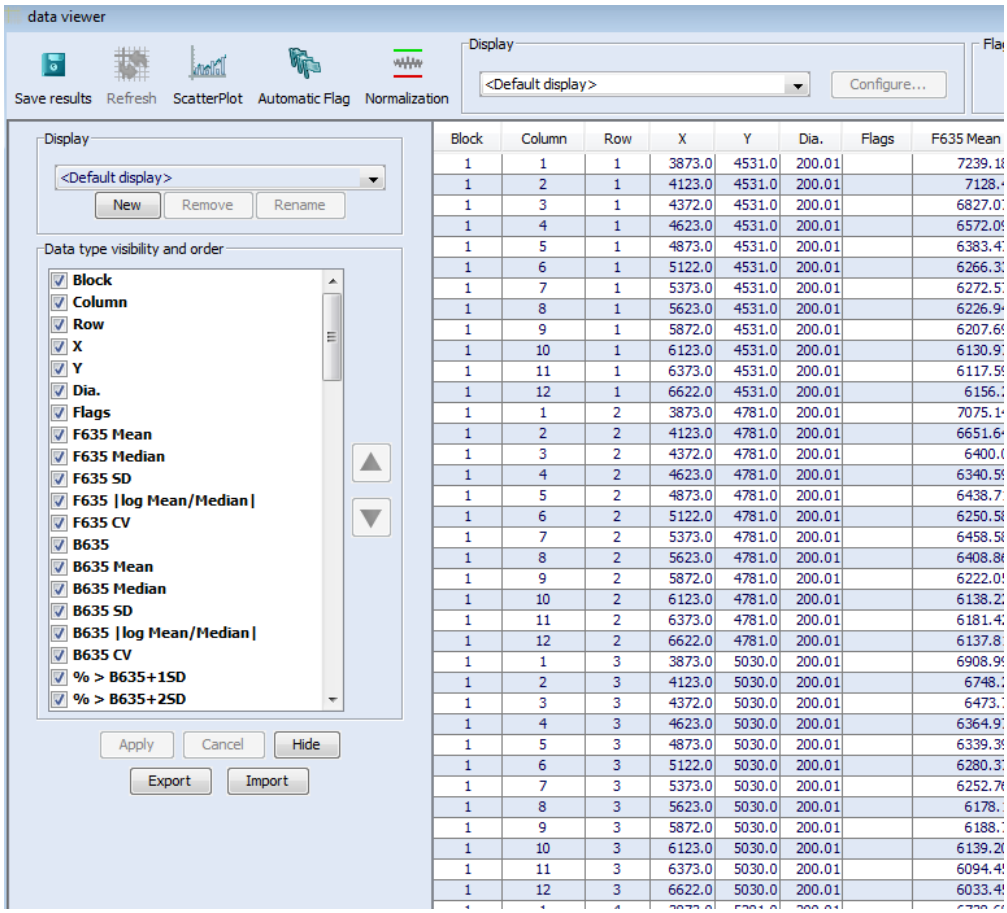
Fxxx Total Intensity	Sum of intensity values of spot pixels at the referenced wavelength
Bxxx Mean	Background noise pixel mean at the referenced wavelength
Bxxx Median	Background noise pixel median at the referenced wavelength
Bxxx SD	Background noise pixel standard deviation at the referenced wavelength
Bxxx |log Mean/Median|	absolute value of the logarithm of the background noise Mean/Median ratio
Bxxx CV	Background noise coefficient of variation at the referenced wavelength which corresponds to the background standard deviation/mean ratio
% > Bxxx +1 SD, % >Bxxx +2 SD	Percentage of background pixels at the referenced wavelength with an intensity values greater than 1 or 2 SD above the median background intensity value
SNRxxx	signal to noise ratio at the referenced wavelength defined by (spot mean – noise mean)/(standard deviation noise)
F Pixels	spot area in pixels
B Pixels	pixel area used to compute background noise
Circularity	Circularity measure from 0 to 100 (100 is most circular, 0 is most non-circular) ⚠ Mapix manages only circular features, this value is always 100
Ratio of Medians (w1/w2)	The ratio of the median intensities wavelengths w1 and w2, with the median background intensity subtracted
Ratio of Means (w1/w2)	The ratio of the arithmetic mean intensities wavelengths w1 and w2, with the median background intensity subtracted
Median of Ratios (w1/w2)	The median of pixel-by-pixel ratios of pixel intensities for wavelengths w1 and w2, with the median background intensity subtracted. **Note**: Ratios greater than 100 and less than 0.01 are excluded when calculating this value.

Mean of Ratios (w1/w2)	The mean of the pixel-by-pixel ratios of pixel intensities for wavelengths w1 and w2, with the median background intensity subtracted. **Note**: Ratios greater than 100 and less than 0.01 are excluded when calculating this value.
Ratios SD (w1/w2)	The standard deviation of the log of pixel intensity ratios for wavelengths w1 and w2. **Note**: Ratios greater than 100 and less than 0.01 are excluded when calculating this value
Sum of Medians (w1/w2)	The sum of the median intensities for wavelengths w1 and w2, with the median background intensity at each wavelength subtracted.
Log Ratio (w1/w2)	Log2 transform of the ratio of the medians for wavelengths w1 and w2

5.4 Customizing the results table

You can change the results table display and select the quantifiers of interest for your work by using the **«Display»** area on the data viewer menu.

Click on the «**Configure...**» button on the Display area to set the display configuration area



Note:

The **<default display>** cannot be modified. To change the display settings you have to create a new display.

Click on «**New**» button to create a new display.

You can create a new display based on an existing one. Once the new display name is created, you can customize the table results by selecting/deselecting columns.

Item	Description
New	Creates a new display
Remove	Removes an existing display
Rename	Changes the name of an existing display
Data type visibility and order	Shows the available columns and their order. Check/Uncheck the boxes to select/deselect a column.
	Allows you to change the column order in the table.
Apply	Validates the modifications
Cancel	Returns to the last display
Hide	Hides the display area. **Note:** If there are no applied modifications, a window will appear to save or ignore modifications
Export	Exports the display configuration into a mapix display configuration file (.mdp)
Import	Opens a mapix display configuration file (.mdp)

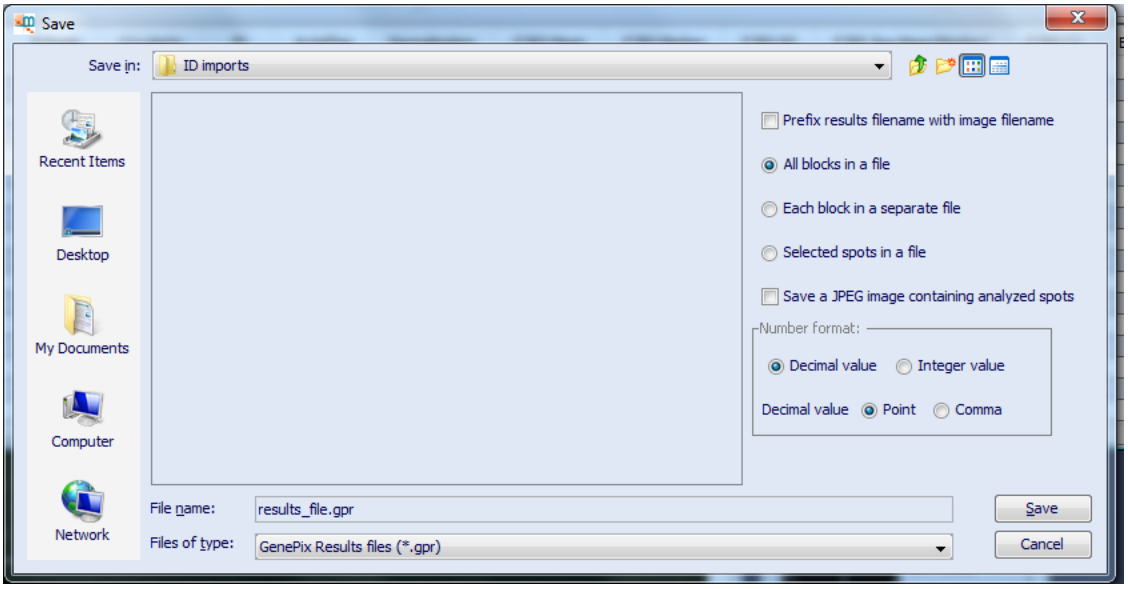
Click on «**Apply**» button to apply the desired modifications. The modifications are automatically saved.

Use «**Export**» button to save the results table display configuration as an independent file that can later be imported using the «**Import**» button. This would be useful when different users want to use the same results table display configuration.

5.5 Exporting the results table -GPR and TXT files-

You can save your data in a Mapix text format (.txt) or a GenePix Result format (.gpr)

Click on the  "**Save Results"** icon to save the table results. The following dialog window is opened



Refer to the following table for details:

Item	**Description**
Prefix results filename with image filename	Fix the image name as a prefix of the results file name.
All blocks in a file	If the slide contains several blocks, this option will keep the results for all blocks in the same results file.
Each block in a separate file	If the slide contains several blocks, this option generates as many resulting files as the number of blocks present in the slide. The files are named: *filename*_**bx**.txt or *filename*_**bx**.gpr; where x corresponds to the block number in the slide.
Selected spots in a file	The results file will contain the results corresponding to the selected spots.
Save a JPEG image containing analyzed spots	You can save an image containing the analyzed spots. When the «**Each block in a separate file**» option is selected, an image of each block is saved in separate files, one file for each of the wavelength images and one file for the ratio image. If «**All blocks in a file**» or «**Selected spots in a file**» options were selected, then an image of the complete slide is saved.
Number format	To choose the desired number format, either decimal or integer number
Decimal separator	To choose the desired decimal separator
File type	Two options are available: Mapix txt file (.txt) Gene pix results file (.gpr) **Note**: The quantification data contained in the results files are the same regardless of the file extension.

The results file contains the data displayed in the data viewer table together with the information on the origin of the results. This information is displayed in the first lines of the results file. If the data is sorted, the results file will keep the sorted result.

The results file is made in an ATF (Axon Text File) format with the following information:

	Item	Description
Headers	ATF 1.0	Indicates the file format (ATF= Axon Text File)
	XX YY	XX= Number of header lines YY= Number of result columns
Optional lines	Checksum_ImageFile	These values are calculated when the image and the results files are created.
	Checksum_ResultFile	
	Type	Identifies the exact results file type
	DateTime	Date when the image was scanned
	ConfigFile	Name of configuration file used
	GalFile	Name of GAL file used
	PixelSize	Pixel size in microns
	Wavelengths	List of wavelengths
	Image Files	Name of image file (two names if separate files)
	Jpeg Image	Shows the directory where the JPEG Image was saved. It is only present if a JPEG file was saved during analysis
	Ratio Formulations	Shows the direction by which ratio values were calculated: 635/532 or 532/635 for InnoScan 710 and 900 models and 670/785 or 785/670 for InnoScan 710-IR model
	Feature Type	Shows the type of analyzed feature, since Mapix can process only circular features, this item will be always "circular"
	Barcode	Barcode number if present on slide

	Item	Description
	Background subtraction	Describes whether or not the local background was substracted
	Normalization	Defines if a normalization of data was done, describing the normalization method used
	Image Origin	Position of top left corner of image in relation to top left corner of slide, in microns.
	Jpeg Origin	Position of top left corner of image in relation to top left corner of slide, in microns.
	Creator	References of the software that produced the file
	Scanner	References of the scanner that produced the image
	Temperature	Temperature during scan in °C
	Comment	Optional image comments
	Laser Power	Laser output during scan in mWatts
	PMT Gain	PMT Gain during scan

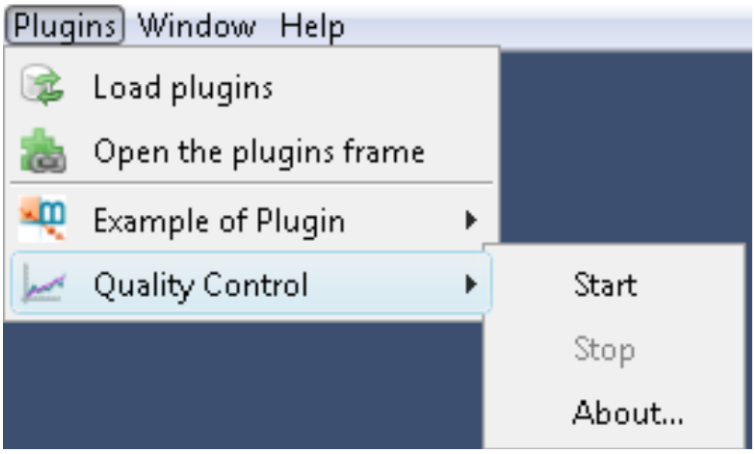
Note: Optional lines are also defined from the GAL file.

5.6 The plugins

A plugin (jar file) enables you to extend the capabilities of Mapix adapting them to your own needs. When Mapix (version 4.0.0 and later) is opened for the first time a directory named "plugins" is automatically created as a sub-directory of the Mapix folder.

To make a plugin available for use in Mapix it has to be located in the plugins sub-directory of the mapix folder.

To see the available plugins installed on Mapix, go to the «**Plugins**» tab at the Main menu:



«**Load plugins**» option allows you to search for plugins added to the plugins sub-directory after Mapix was started.

start: starts the plugin

stop: stops the plugin

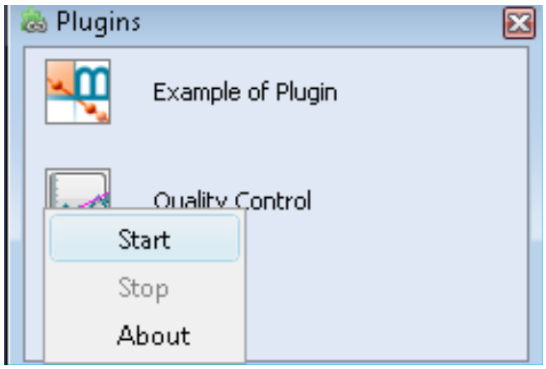
about: displays information about the plugin. Indicated are information needed to initiate the plugin (image, grid, quantification, etc).

Notes:

Only when all the information that is needed by the plugin is available in Mapix will the "start" option become enabled.

When a plugin is started, "start" is inaccessible whereas "stop" is enabled.

The «**Open the plugins frame**» option opens the Plugins frame displaying the available plugins in the desktop of Mapix.



This frame permits users to easily start the plugins by clicking on the appropriate icon. The icons are disabled until the needed objects are created in Mapix. When a plugin is started, the appropriate icon is disabled since it first has to be stopped.

Note:

Plugins can be created by the users. For more information about how to create a plugin please refer to the "***plugins creation documentation***" available in our website or in the installation CD-ROM.

5.7 Saving / Opening a Mapix work file

You can save your work at any moment on a Mapix work file (.mwk). This file will save all the Mapix settings: images, scan parameters, grid, flags, and the analysis made.

To save a work file, go to the «**File**» tab of the Main menu and click on «**Save work as**». In the same way, click on «**Open a work**» on the «**File**» tab of the Main menu to open an existing work file.

Note:

Images used for analysis are automatically linked to the work file, when the file is saved a link with the image is created. If you change the image location, when loading a work file, Mapix will ask for a new pathway to access the images.

6. Working in batch mode

One can use the batch mode to scan several slides with similar or different scan parameters and / or to automate image analysis. To open the batch editor go to the batch tab of the main menu and click on Batch Editor. The following window is displayed:

6.1 Batch scans

«Add a scan step» button permits you to define/edit parameters to be used in scanning and image analysis.

Use this option to either only scan or scan and analyze several slides using the same or different parameters.

This menu consists of six steps that follow the work flow from slide scan to image analysis:

Scan Parameters

Loader slide selection (only for InnoScanxxxAL models)

Filename & Directory

Analysis

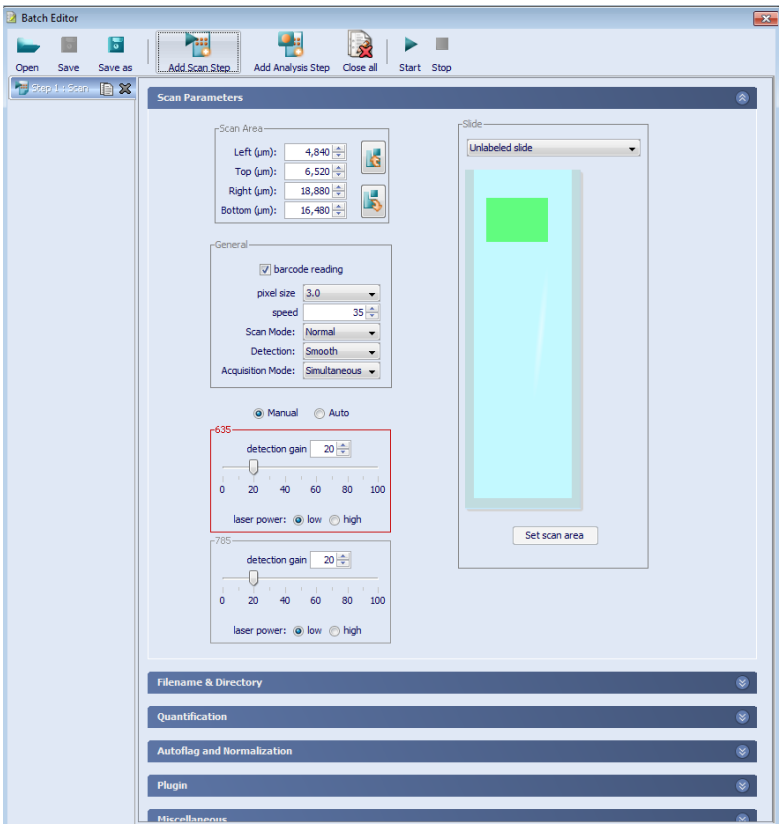
Plugin

Miscellaneous

Note:

Each of these steps has been previously described. Please refer to the referenced sections for details.

6.1.1. Scan Parameters



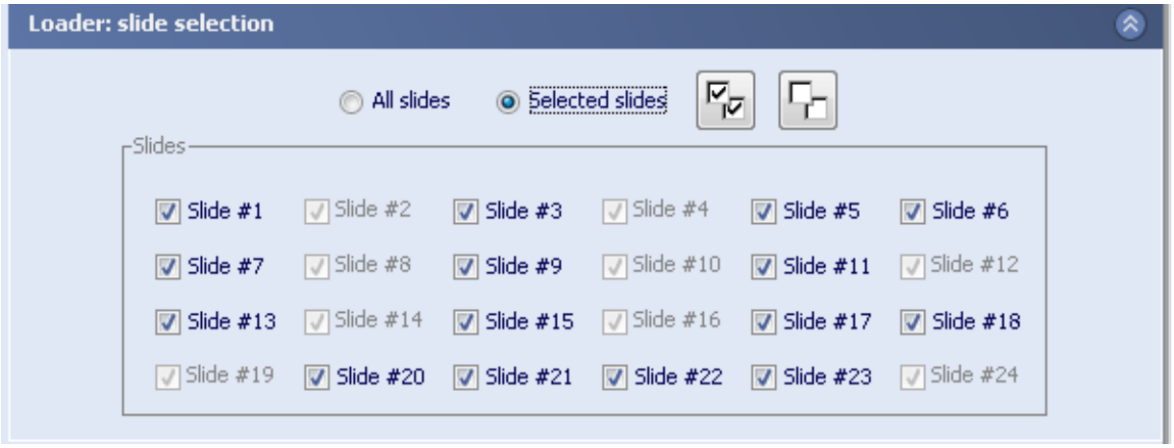
This step describes the definition of the scan parameters, here you can define the scan area and detection gain (laser power). Please refer to *Defining scan parameters* and *Defining the Scan Area* sections for details.

 Imports the scan area from the preview window

 Exports the scan area to the preview window

6.1.2. Loader slide selection (Only for models equipped with an auto-loader)

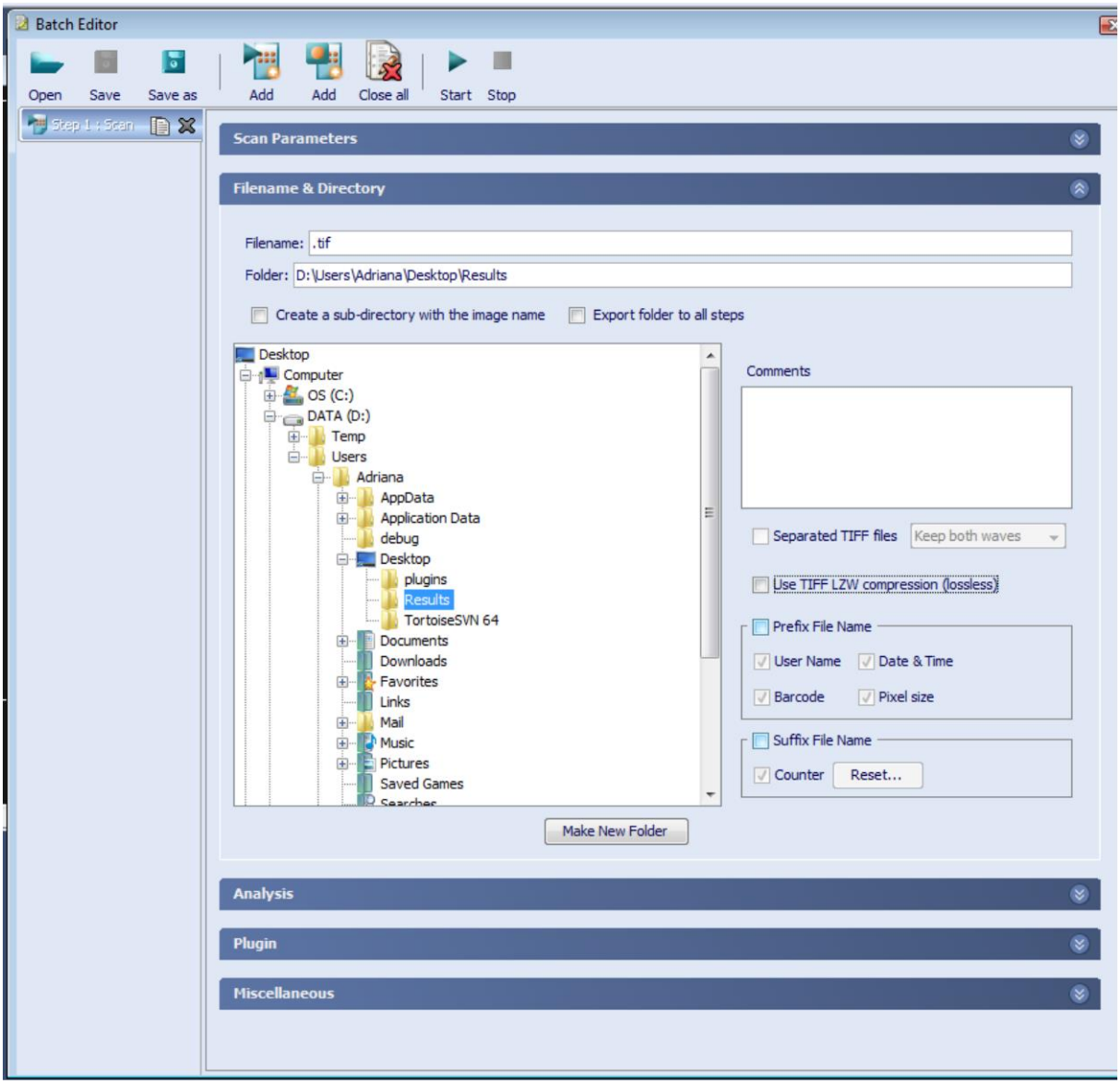
If the scanner is equipped with an auto-loader you can select the slides to be used in the batch.



Note:

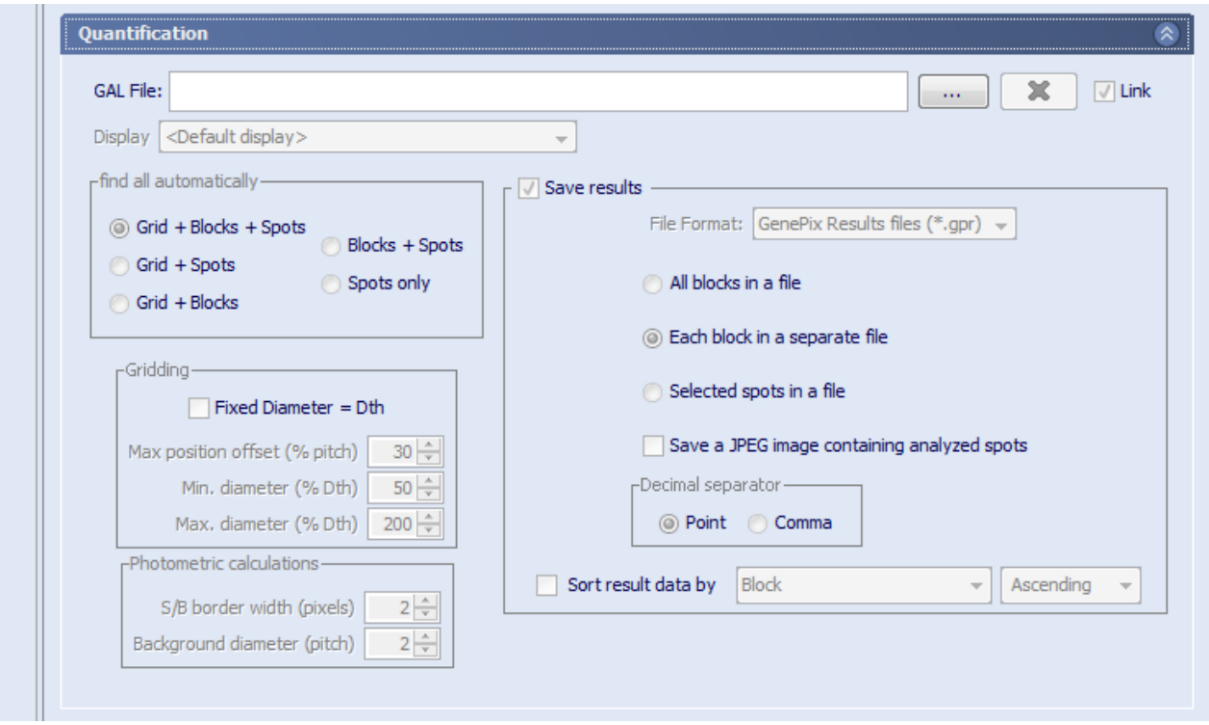
To scan the slide in an auto-loader scanner, you have first to perform a slide detection test. To do this click on the  icon in the tools window. Please refer to *Slide detection in the Auto-loader - only for scanners provided of an autoloader system -* section for more details.

6.1.3. Filename & Directory



In this step you can define the parameters to be used when saving the obtained images. Please refer to *Scanning an image* for details.

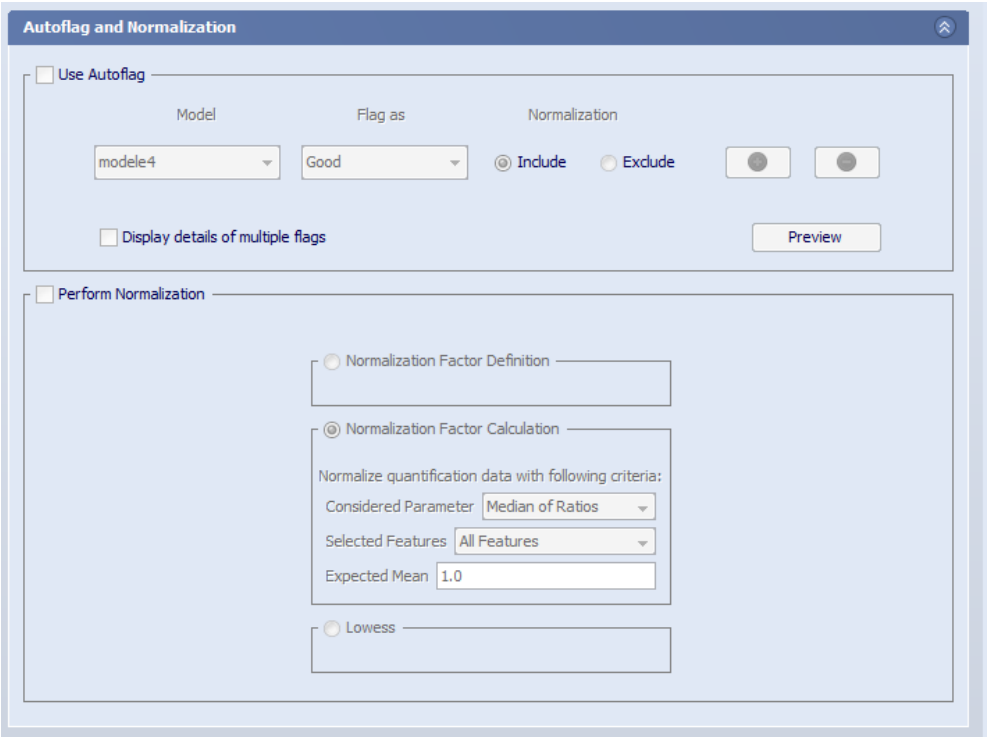
6.1.4. Quantification



Use this step to define the parameters to be used for feature quantification and analysis. Each panel corresponds to a specific step in the quantification process, as defined below:

Panel	Step in the quantification process	For details please go to section...
GAL file	Selection of the GAL file	*Open an existing grid file (GAL file)*
Find all automatically	Positioning the grid	*Positioning the grid*
Gridding	Parameters used for positioning the grid	*Positioning the grid*
Photometric calculations	Parameters used for photometric calculations	*Configuring the analysis parameters*
Save results	Save results as txt or gpr files	*Exporting the results table - GPR and TXT files-*

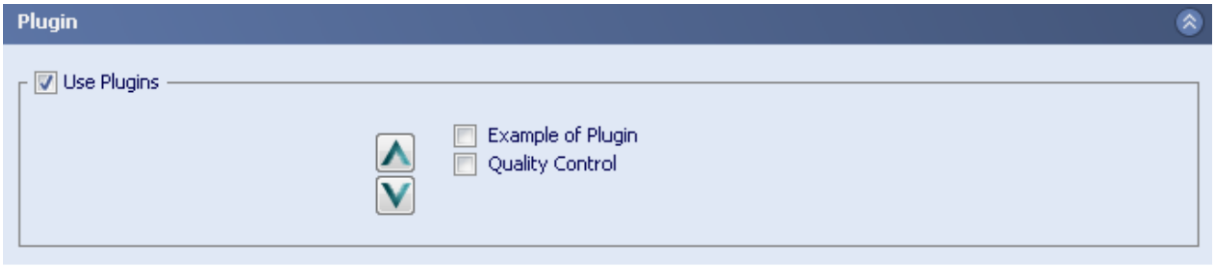
6.1.5. Autoflag and normalization



In this section you can choose to perform an autoflag step by using pre-defined models and / or to normalize your data. When "Perform Normalization" is selected, both raw-data and normalized results files are automatically saved.

For details on each of these options please go to the *automatic flag window* and *normalization* sections.

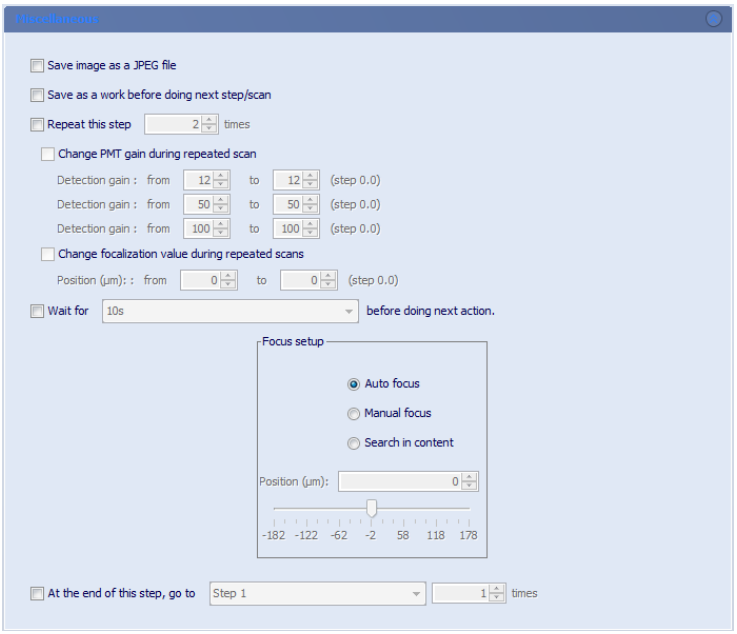
6.1.6. Plugin



This step contains the different available plugins. You can select the plugins to be used during the analysis. You can define the order in which the plugins will be launched by using arrows. If a plugin does not have access to the data it needs, it will not be able to start thus creating an error message in the batch report.

For details about plugins please refer to *the plugins* section.

6.1.7. Miscellaneous



Use this section to define the steps to be done between each scan.

Refer to the table below for information

Item	Description
Change the slide before scanning	When conducting the same analysis for different slides on a one-slide scanner model, you may select this option. This option is not available for the auto-loader scanner models.
Save image as a JEPG file	Saves an additional image in JEPG format
Save as a work before doing next step/scan	Saves each step before doing the next scan
Repeat this step	Defines the number of repetitions of the same batch step. Use this option when conducting the same analysis on different slides.
Change PMT gain during repeated scan	Changes the PMT gain during repeated scans.
Change focalization value during repeated scans	Changes focus level during repeated scans
Wait for ….. before doing next action	Defines wait time between executing each step
Focus setup	Defines the method for focusing. Please refer to the *focus setup* section.
At the end of this step, go to	You can define the order of batch step by using this option.

6.2 Batch analysis

«**Add an analysis step**» icon opens the options for defining/editing the parameters to be used during the analysis of images.

Use this option when you want to analyze several images at once, using the same or different parameters.

By clicking on this option a menu consisting of four sections for image analysis is opened:

Quantification

Autoflag and normalization

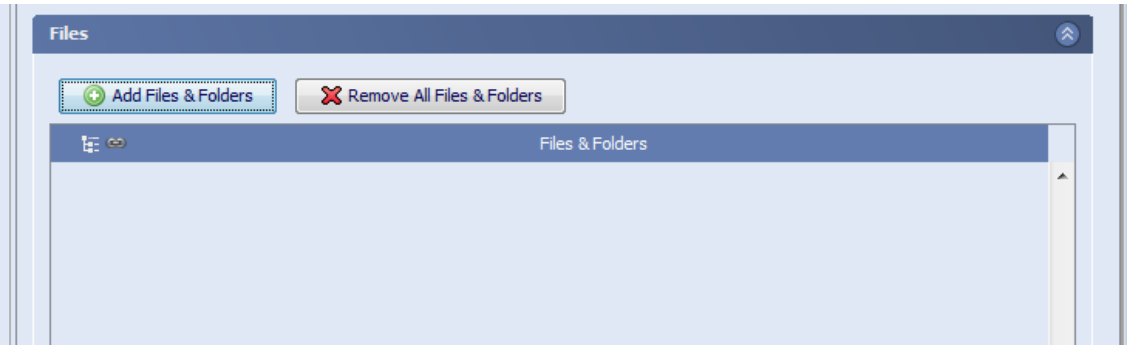
Plugin

Files

Miscellaneous

The "Quantification", "Autoflag and normalization", and "Plugin" steps are the same as those of the Batch scans menu. Please refer to these sections for details.

6.2.1. Files



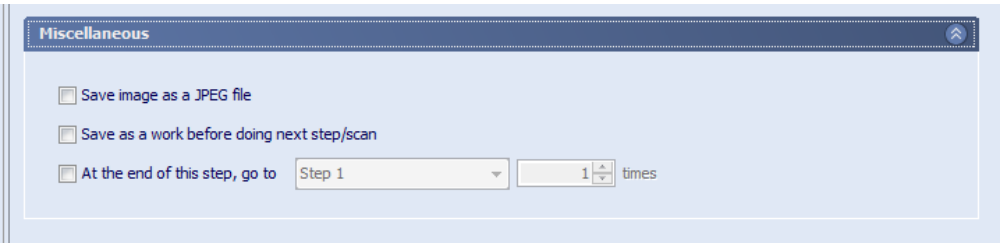
In this section you can add the files for the analysis.

Notes: when opening a folder

If the  recursive check box is selected, all the files contained in this folder and sub-folders will be included during the analysis. If it is not selected, only this folder will be considered in the study.

If the  link check box is selected, separate TIFF files of the same scan will be analyzed together in a single step; the intensity ratio is then computed and results will be saved in a single result file.

6.2.2. Miscellaneous

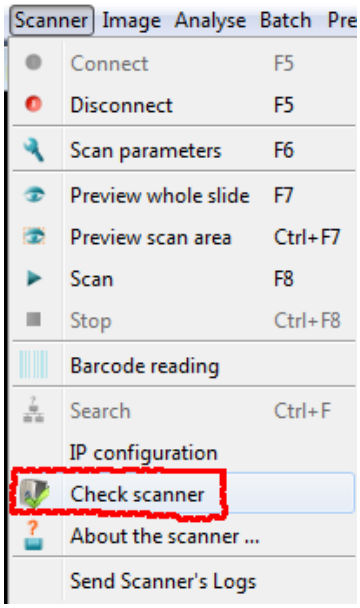


Use this option to define if you want to save an image in a JEPG file format and to save a work in a Mapix work file (mwk) file before launching the next batch step.

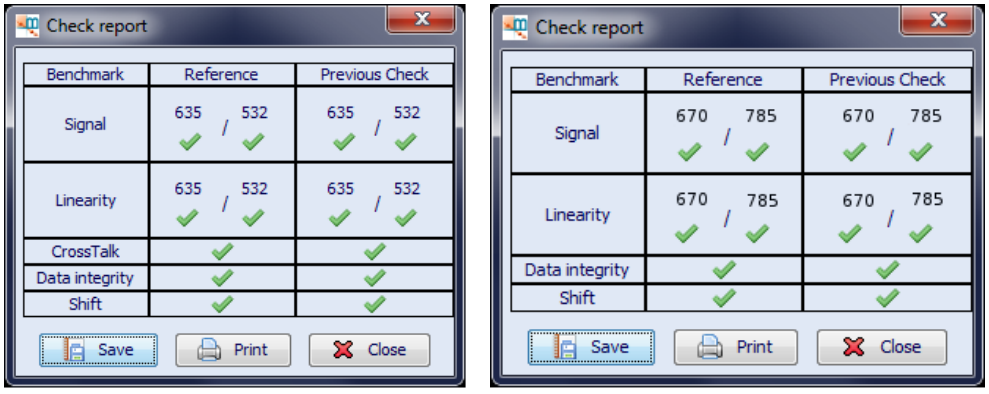
Once all the parameters have been defined for each batch step, use the  «**start**» button to start the application. You can stop the application at any time by using the  «**stop**» button. You can save the parameters in a *Mapix Batch File* (mbt).

7. Scanner Validation

InnoScan® scanners are provided with a validation slide which allows the validation of scanner performance. To run a check scanner, turn on the scanner and connect it to Mapix as described in the *Connecting the scanner* section. Insert the validation slide into the scanner. Select the «**check scanner**» option on the scanner Tab of the Main menu:



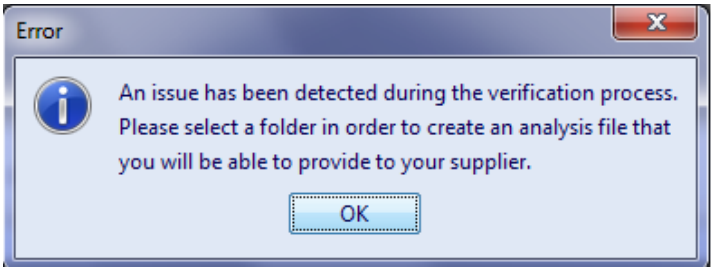
During this operation the scanner runs several scans in which its main functions are tested. At the end of the check scanner process a results report is displayed:



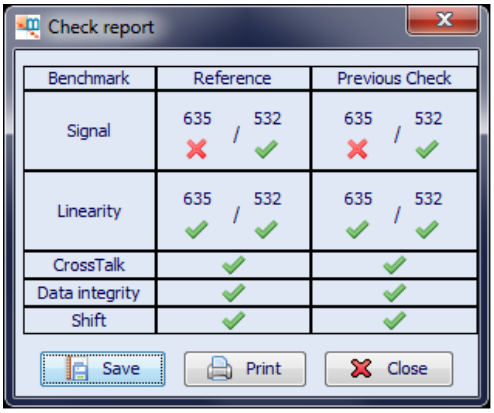
Please note that for the InnoScan 710-IR scanner model the CrossTalk function is not evaluated within the check scanner process.

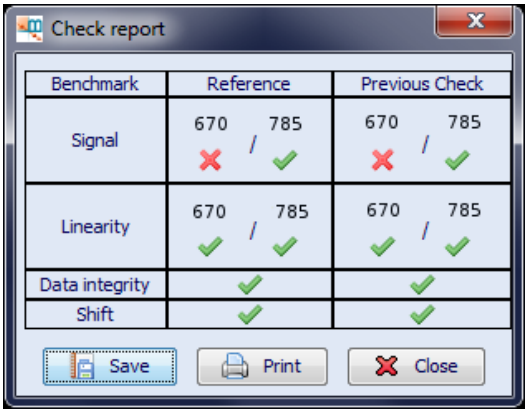
You can export the check scanner report as a pdf file.

If check scanner results don't conform to the expected results, the following message is displayed:



Select a directory to save the analysis file (.inl). Once the file saved the check scanner report is displayed:





Choose to save the check scanner report. Send the scanner report together with the analysis file (.inl) to your supplier for a rapid intervention.

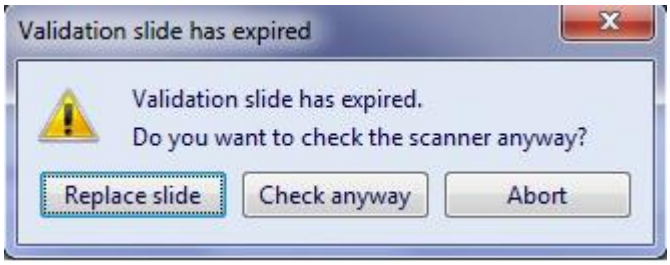
Notes:

Checking the scanner verifies that its main functions are working properly. It is recommended to run a check scanner at least once per month.

The validation slide has a lifecycle of one year at the end of which you should acquire a new validation slide. To get a new validation slide please contact your supplier.

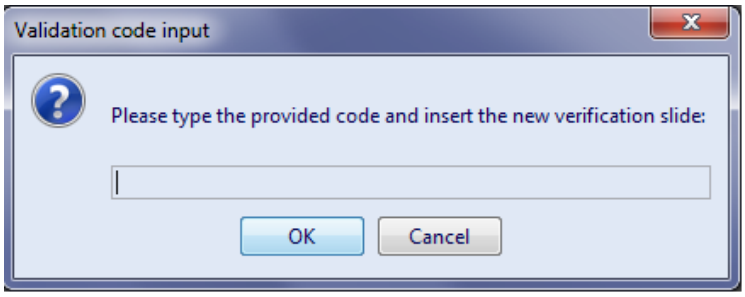
7.1 Replacing a validation slide

If the validation slide is expired, the following error message is displayed:



One can continue the check scanner with an expired slide by clicking on the «**Check anyway**» option. However, verification results might be influenced since the slide is no longer valid.

To acquire a new validation slide please contact your supplier. The validation slide is then provided with an exchange code. Write this code to perform the validation slide exchange:



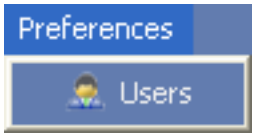
Insert the new validation slide and then click ok. The validation process will proceed as usual.

8. Preferences

Using the **«Preferences»** menu you can:

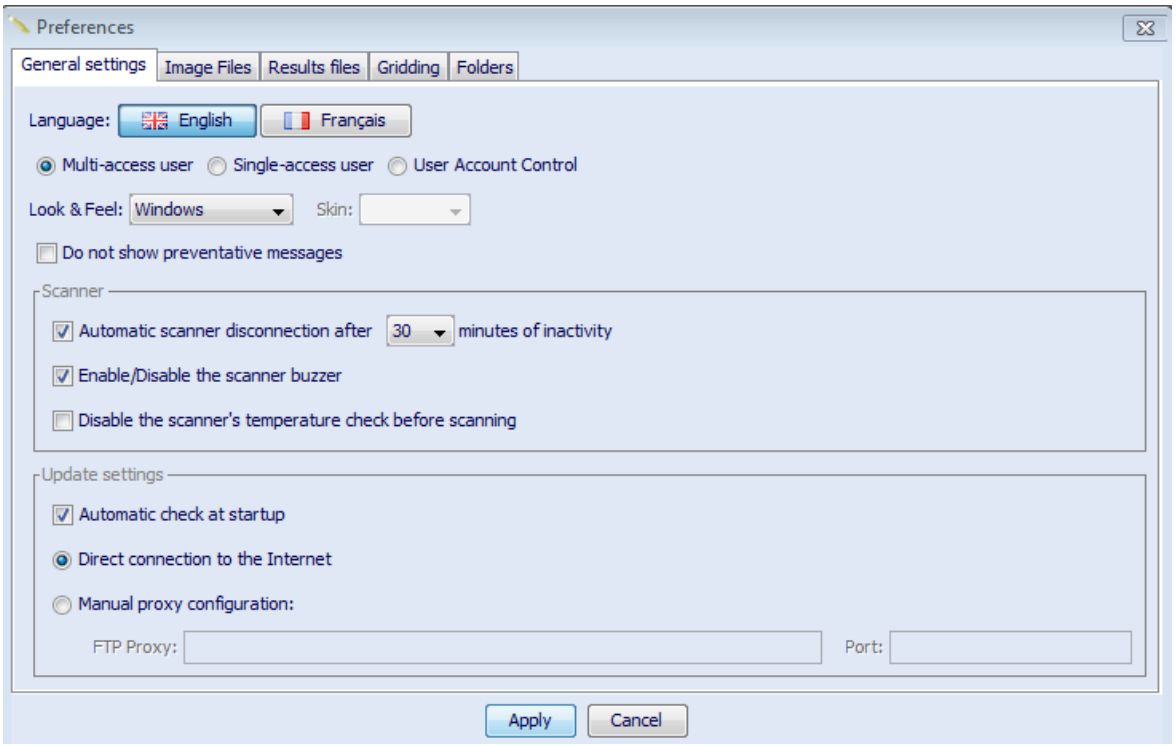
- ✓ Define the language and the appearance of Mapix
- ✓ Define the generic file name by which the image will be saved when created
- ✓ Define the format of results files and customize the results table
- ✓ Choose a user as the account manager
- ✓ Choose a password to protect your account

Click on the «**Users**» option to display the «**Preferences**» menu



8.1 General settings

Use this section to define different MAPIX options



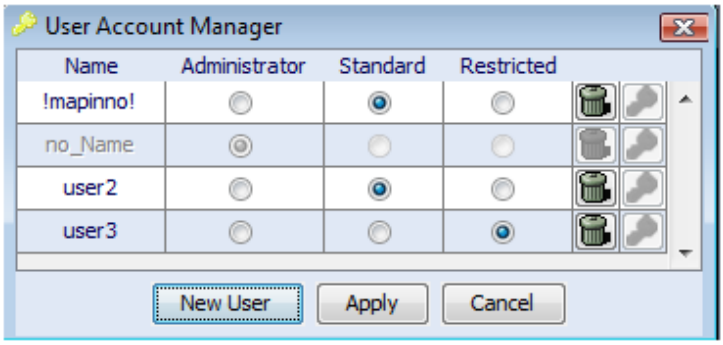
Item	Description
Language	You can choose the language of Mapix between French and English versions
Look & Feel	Use this option to change the appearance of Mapix
Do not show preventive messages	Preventive messages are displayed when a work or a configuration has been created or modified without having been saved. You can choose to turn this off or on.
Scanner	In this panel you can define some parameters on the scanner connection and testing. Select the option "disable the scanner's temperature check before scanning" if you do not want to check for the scanner temperature.
Update settings	To receive Mapix updates keep this option active.

You can handle the user accounts by choosing between «**single-access user**» and «**multi-access user**». When «**Multi-access user**» is selected, each user must choose his/her own session when Mapix starts on.

«**Users account control**» option allows an administrator account to be defined. Only the administrator can add, modify, or delete another user's accounts.

Once «**Users account control**» option has been selected the «**Users Account Manager**» is added in the preferences tab of the Main Menu:

Use this menu to control the access of different Mapix users:



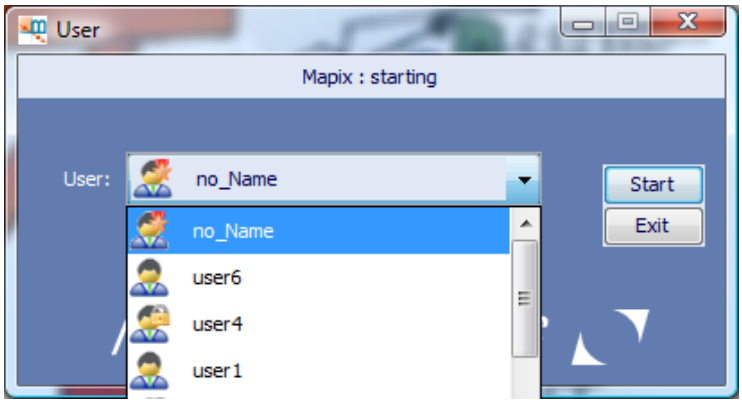
A user can be stated as administrator, standard or restricted user:

The **administrator** can access different user's accounts as well as create, delete or modify the status of other user's accounts.

A **standard** user has complete access to Mapix but cannot change or enter other user's accounts.

A **restricted** user has only restricted access to Mapix and cannot modify scan parameters or table results display.

The status of a user's account is displayed in the users dialog when Mapix starts:

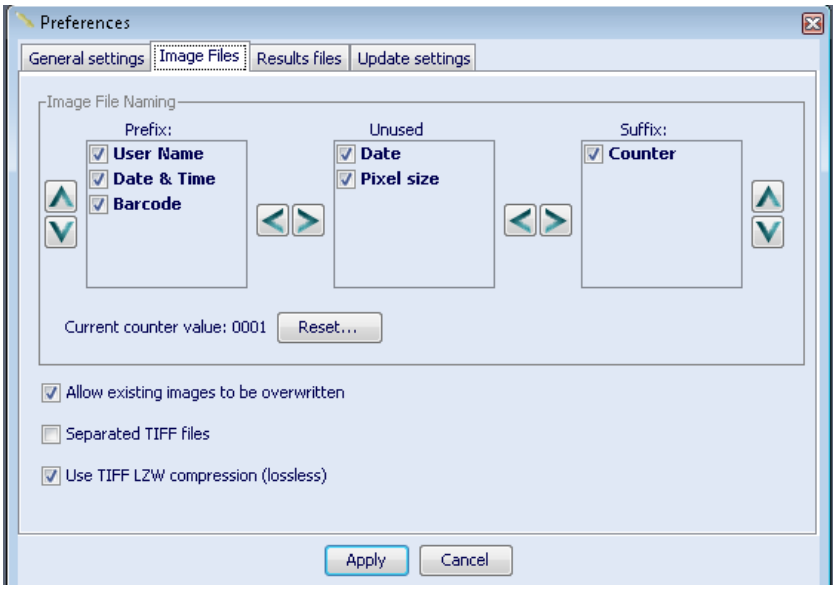


Each user can protect their account by defining a password. To define a password, go to the Preference tab on the main menu, and then click on «Password»

Note: The password must consist of at least 6 characters.

8.2 Image Files

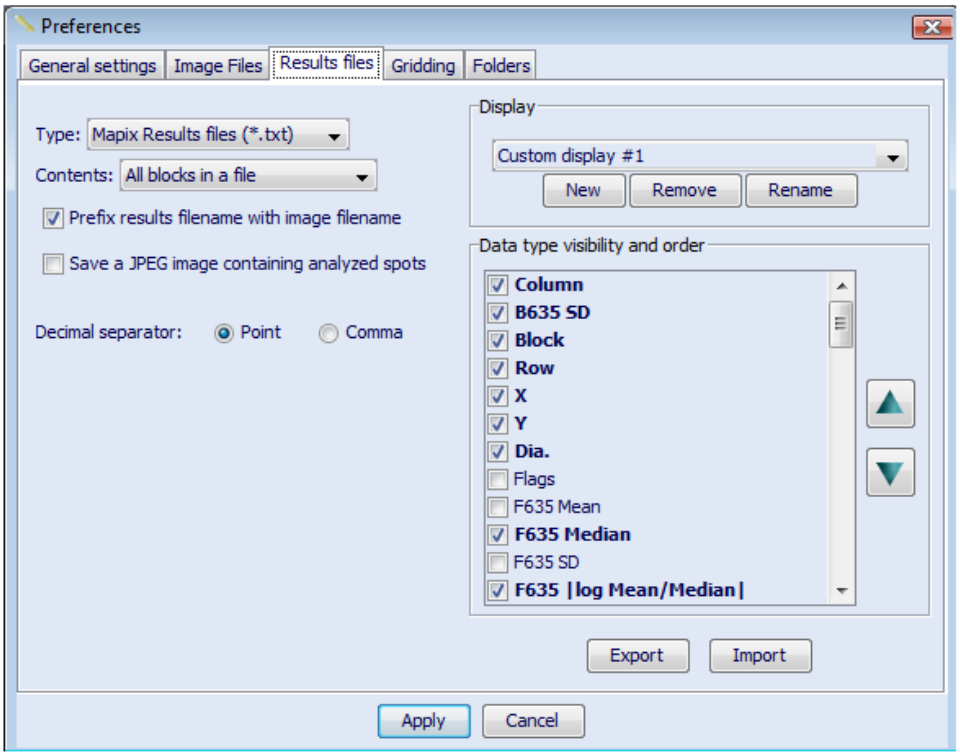
Use this section to define the parameters to be used during image acquisition and saving:



You can choose the parameters to be used as prefix or suffix of the image file name. Those parameters in the unused box will not be present as options in the image save dialog box. For details about each parameter please refer to section *Scanning an image*

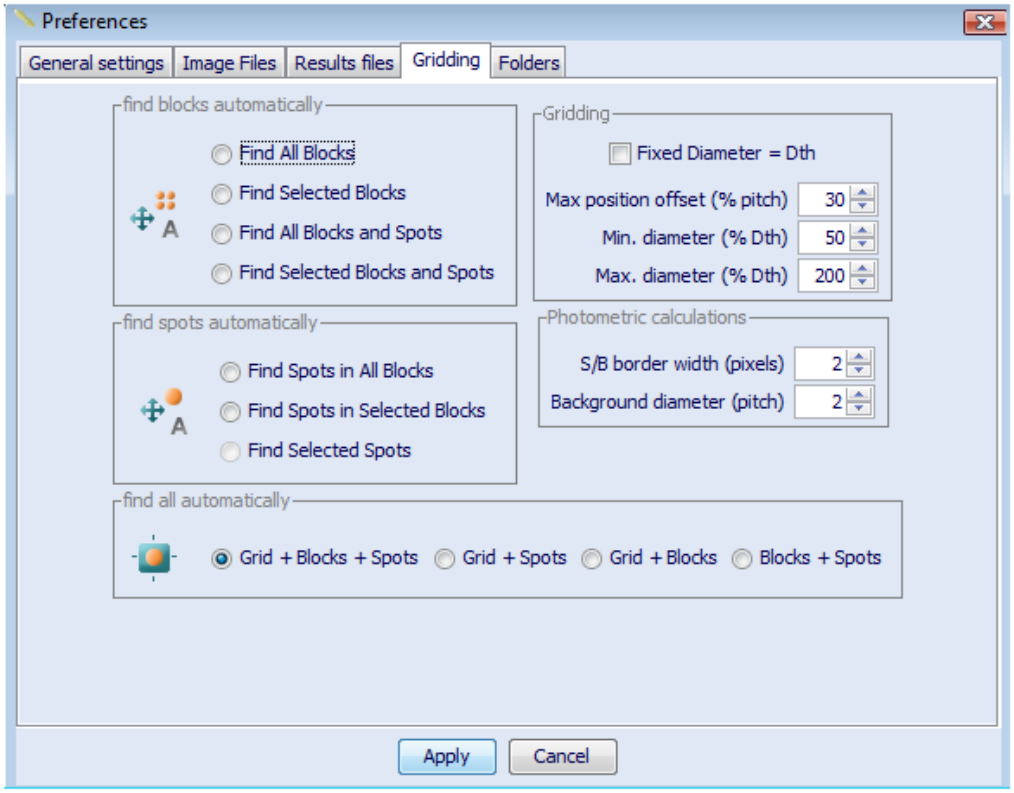
8.3 Results Files

In this section you can define your preferences to be applied when saving the results table. Please refer to *Customizing the results table-* and *Exporting the results table -GPR and TXT files*- sections for details.



8.4 Gridding

In this section you can define the actions associated with those buttons related to the grid. You can define default gridding and photometric calculation parameters.



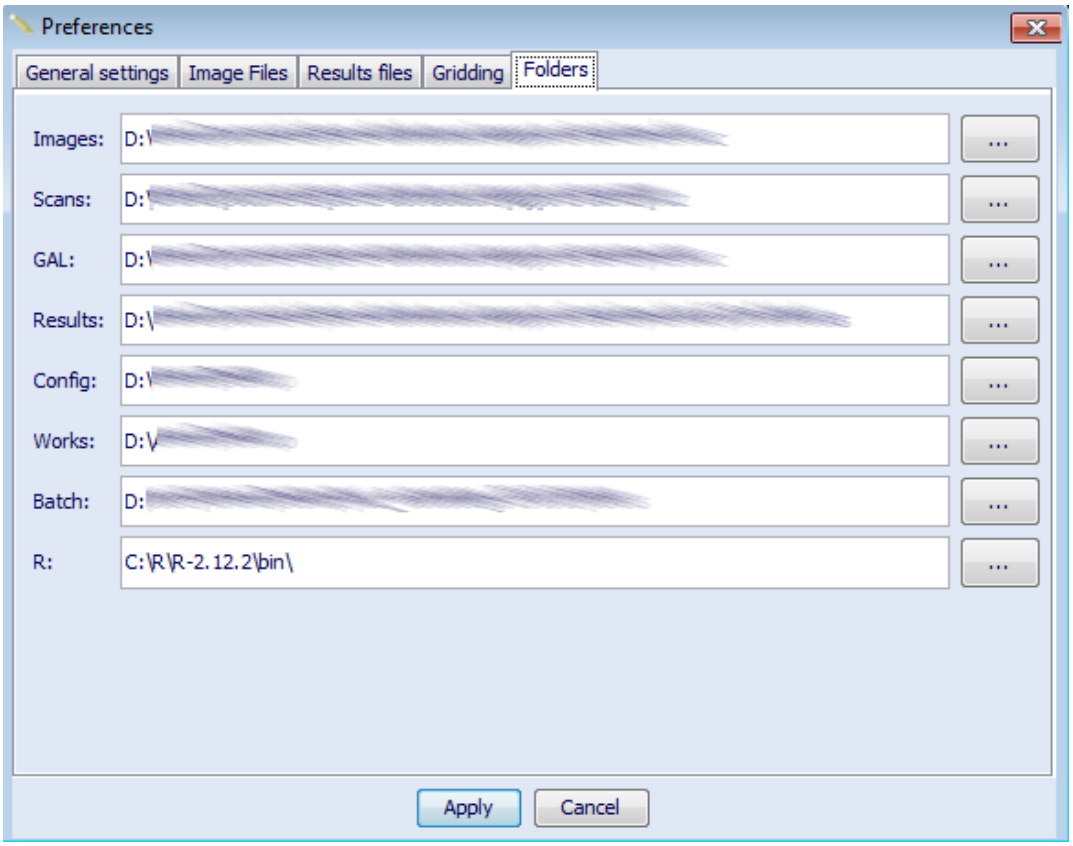
Please refer to *Positioning the grid* section for details.

8.5 Folders

In this section you can define:

The default folders for image, results and work file storage.

The folders presented by default when using grid files and configuration files



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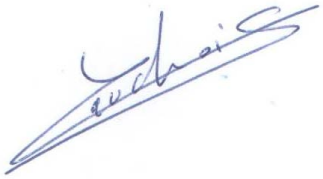
Approved by	Software development manager	

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INNOPSYS
Parc Activestre – 31390 CARBONNE – France
Tel : +33 561 971 974 / Fax : +33 561 971 975 - support@innopsys.fr