

XDotsReader

User's Guide



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Introduction.

XDotsReader is designed for biological applications. The software allows to analyze and compare images with spots organized in matrix form. The relations between the spots are drawn up according to specific biologic geometry (notions of grid, block, pair ...).

The spots detection procedure is automatic : the operator must only indicate the specific geometry, and find a suitable algorithm for spots detection. A detected spot is modeled according to a circle. If the image is very noisy, the operator can help the software to detect grids or/and spots, and even correct the spots one by one (position and modeled circle size).

A lot of tools allows to analyze the results (evaluations according different parameters, presentations through several graphics, access to measurements, and to computed values). To compare images, a lot of methods are available for background noise estimation and normalization. When the images are comparable, all kind of operations can be applied on them, and between them.

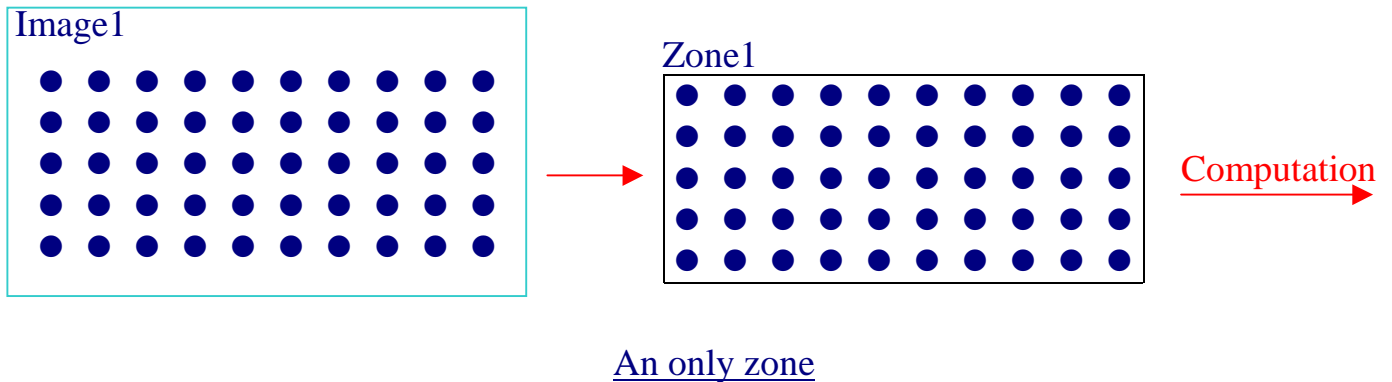
After a general presentation, the geometry description of the spots organization, and a section about images manipulations, two main parts compose the document :

First allows to realize simple basic operations on an example. Second explains in a detailed way the menus, and describes sequentially the buttons, from left to right, and from top to bottom, with - when it's necessary - some illustration examples about typical applications.

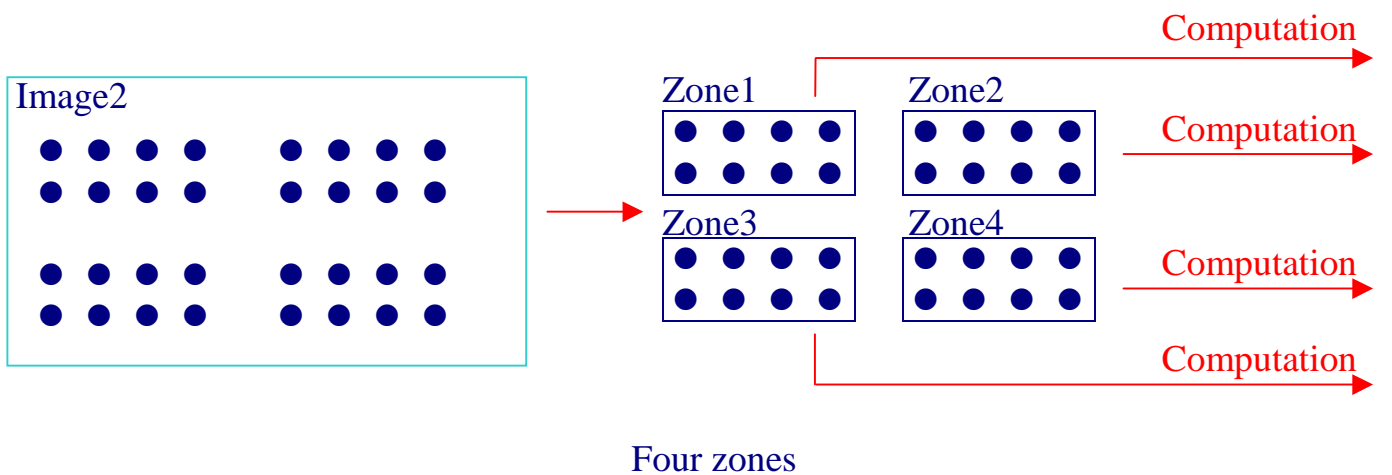
General presentation.

The principals of software working are as follows :

- ◆ Whatever the image to analyze, it's necessary to define zones - even if an only zone is definable - because the computation works exclusively with zones. For example :

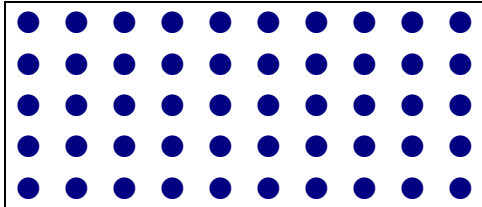


or still :



- ◆ When the zones are defined, a computation can be start on each zone. To start a computation, it's necessary to set some parameters. In general, it's only necessary to specify the detection algorithm type, and the geometric parameters (Read in priority the next section about the geometry description).
- ◆ When a computation on a zone is finished, the indexing grids are positioned, and the spots - detected or not (if a spot is not detected, it's possible to force the detection, by its theoretical position) - are modeled like a circular area. The spots characteristics are calculated according to this area :

Zone1



Computation →

Zone1 : Indexing Grids

	A	B	C	D	E	F	G	H	I	J
1										
2										
3										
4										
5										

Zone1 : Spots

○	○	○	○	○	○	○	○	○	○	○
○	○	○	○	○	○	○	○	○	○	○
○	○	○	○	○	○	○	○	○	○	○
○	○	○	○	○	○	○	○	○	○	○
○	○	○	○	○	○	○	○	○	○	○

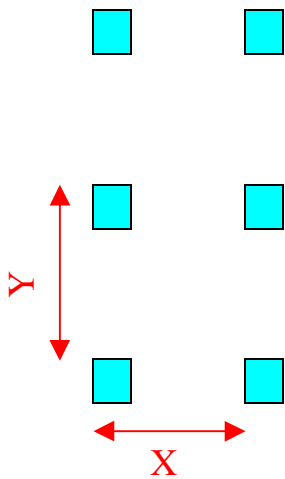
The indexing grids allow to assign coordinates to the spots (according to a chosen coordinates system), and are useful for some spots detection algorithm types. The modeled spots are colored according to their levels.

- ◆ Afterwards, it's possible to improve the results in different ways (other detection algorithms, matching through a manual intervention, or through a comparison with a reference image).
- ◆ Finally, when a zone is suitably treated, it's possible to compare it with other zones.

Geometry description.

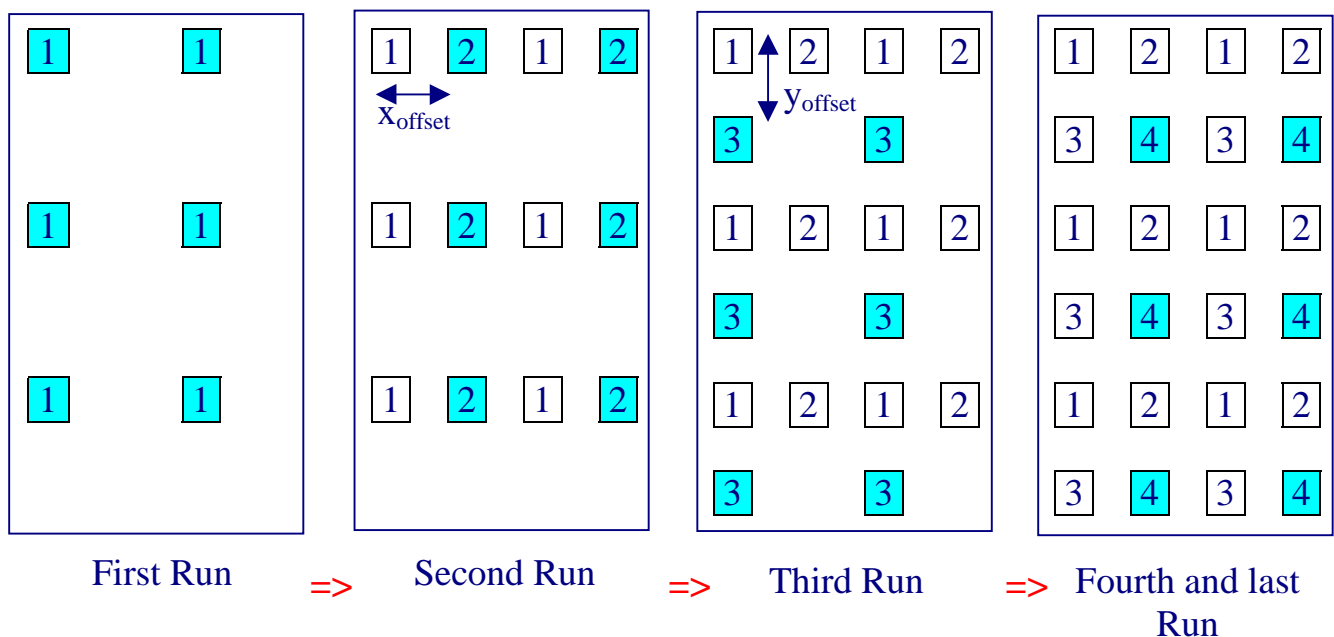
The software analysis refers to a specific description of spots repartition, in relation with the membranes industrial manufacture.

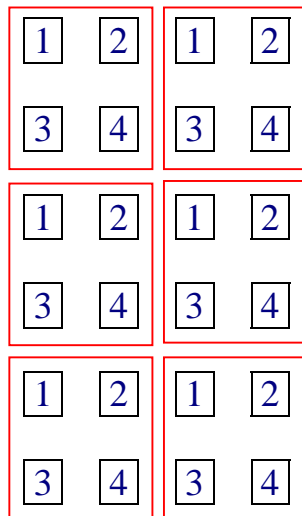
Spots are deposited by means of one or several runs. For each run, a needles grid lays spots. For the next run, a grid - with an identical geometry - lays others spots, shifted to the preceding of an horizontal or/and a vertical increment (let x_{offset} be and let y_{offset} be, respectively). The hypothesis with which the software works is the following : The runs are translated from left to right, then, when the right limit is reached, a new run begin on the line below, on the left. For example, for a grid with six needles :



- ◆ Let **X** be the distance between two needles on a line.
- ◆ Let **Y** be the distance between two needles on a column.

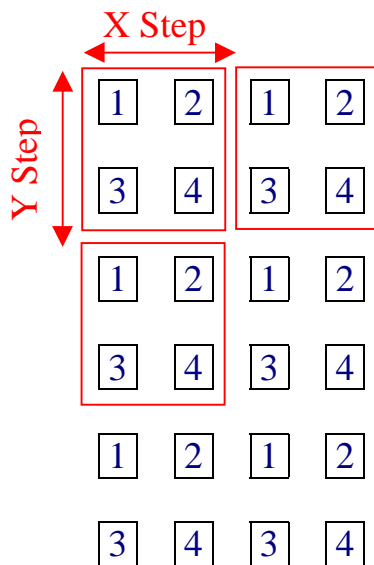
A membrane (named zone in the software) may be built as follows :





A block is constituted of an only spot of each run.

In the software are defined the parameters Number of lines, and Number of columns. These parameters correspond to the blocks lines number, and to the blocks columns number. In our example, Number of Lines = 3, and Number of Columns = 2.

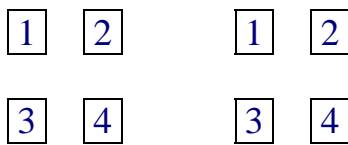
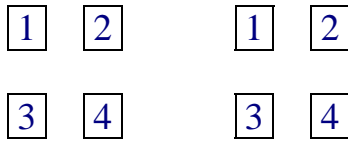


X Step and Y Step are defined :

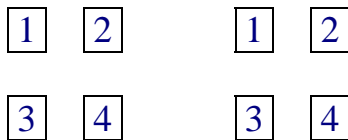
X Step : horizontal distance in pixel unit, between two consecutive blocks, i.e. between two spots of the same grid (corresponding to the distance **X** between two needles for a probe).

Y Step : vertical distance in pixel unit, between two consecutive blocks, i.e. between spots of the same grid (corresponding to the distance **Y** between two needles for a probe).

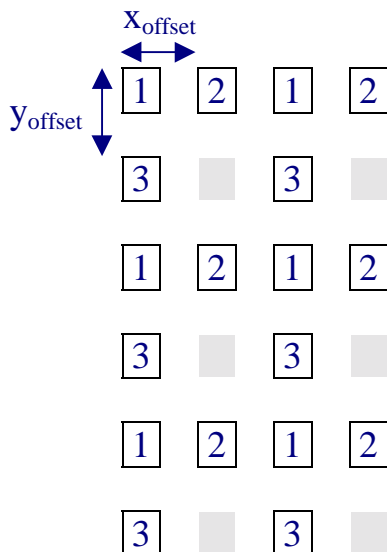
A spacing of one increment is possible to separate blocks constituted with a spot of each run :



Spacing = 1



A block has a maximum size. It's the maximum runs number that a given probe can realize on a given zone, the run offsets x_{offset} and y_{offset} being fixed. This maximum runs number is called density. It's possible to have a grid runs number, called grid, inferior to parameter density. For example :

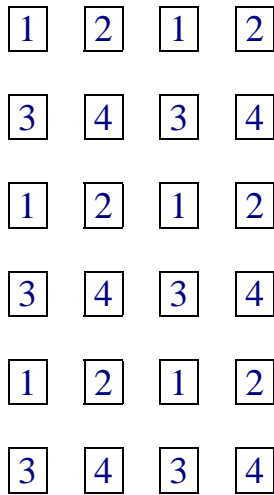


Density = 4

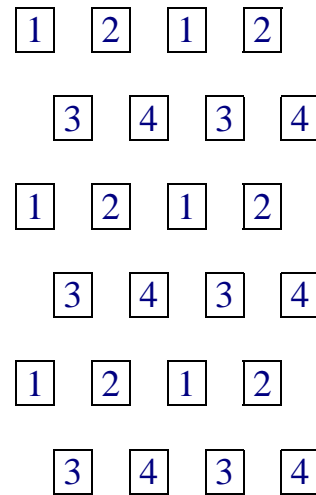
Grid = 3

Spacing = 0

There is another geometric configuration : when the spots of contiguous lines are shifted of an half increment. To take this case in consideration, the density is noted according to different ways. The density of a spots organization with no shifting is noted (n X m), else, the density is noted (p), with $p=n \times m$. For example :



Density = 2 X 2



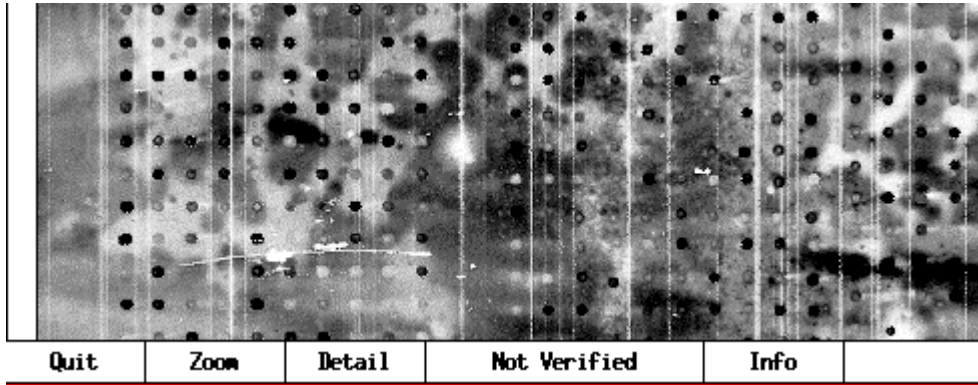
Density = 4

To summarize, the parameters to define a zone geometry are :

<u>X Step</u> :	Distance in a row between two blocks, in pixels unit.
<u>Y Step</u> :	Distance in a column between two blocks, in pixels unit.
<u>Grid</u> :	Number of probe runs.
<u>Density</u>	Maximum number of probe runs.
<u>Spacing</u> :	Value : 0 or 1. A spacing of 1 corresponds to an empty row and an empty column of spots, between blocks.
<u>Number of Lines</u> :	Blocks lines number.
<u>Number of Columns</u> :	Blocks columns number.

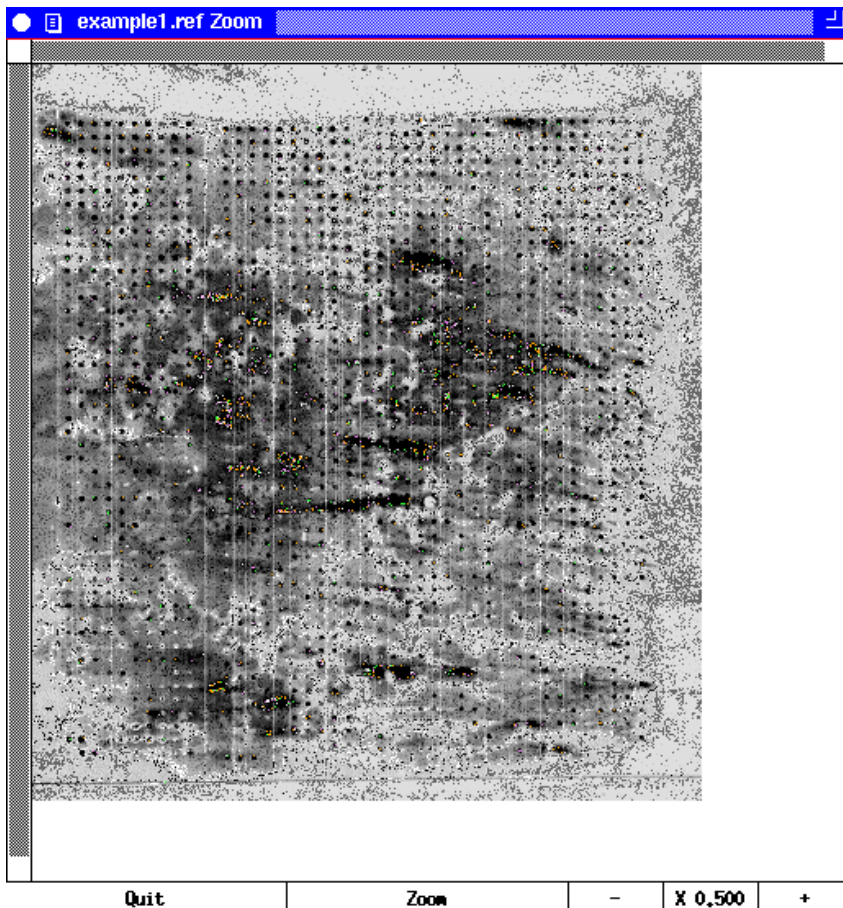
Manipulate images.

The images and the zones can be opened with the Open menu. At the bottom, a bar proposed several buttons :



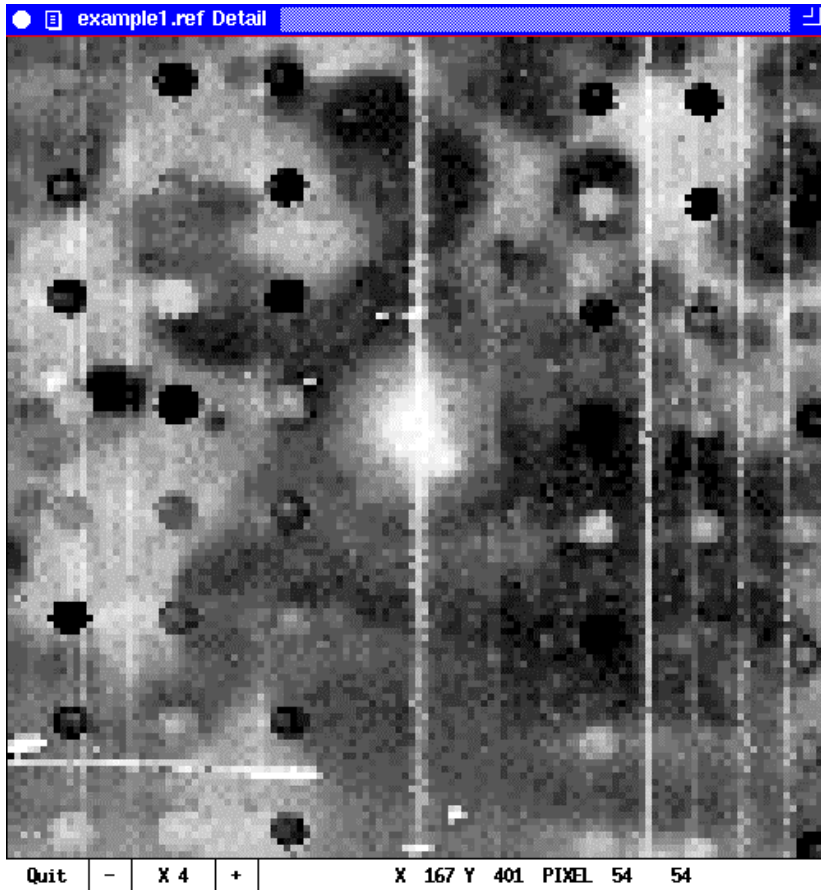
♦ Quit button : to close the image.

♦ Zoom button : Click on this button : a window named "Zoom" opens with the previous reduced image. This Zoom image is useful to draw approximately the zones boundaries, when the image is more large than the window.



- Quit button : to close this window.
- Zoom button : to applied the zoom factor.
- "-" button : to decrease the zoom factor.
- Display field : zoom factor.
- "+" button : to increase the zoom factor.

- ◆ Detail button : Click on this button : an empty window named "Detail" opens. Click on the image with the **right** mouse button : the area around the mouse pointer appears in the Detail window, with an enlargement factor :

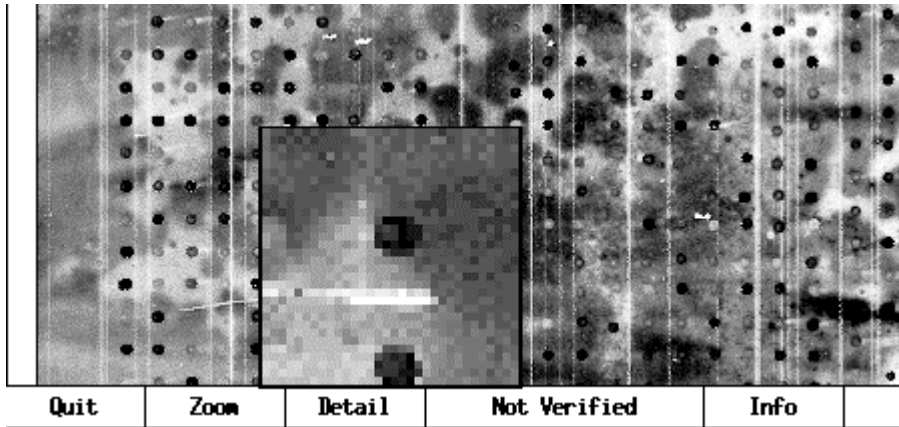


- Quit button : to close this window.
- "-" button : to decrease the enlargement factor. After a modification, click again in the image with the **right** mouse button.
- Display field : zoom factor
- "+" button : to increase the enlargement factor. After a modification, click again in the image with the **right** mouse button.

- ◆ Not Verify button : ??
- ◆ Info button : the Info menu opens.
- ◆ Information field : Set the mouse pointer on the image : the pixel coordinates and the pixel values (value, and normalized value) display.

Zoom function.

Click on the right button on an image : a zoom appears (if the Zoom image is closed) :



To begin easily : an example step by step.

In this part, an example is described step by step. The chosen example is an image with four different zones. Each zone has 48 spots lines and 10 spots columns. The density is 1, the grid number is 1, (so the blocs number is the spots number, and the spacing between two blocks is 0), and there are no pairs. See Geometry Description section for the essential understanding of these parameters (but you can execute this example without refer to this section).

Through this example, we will learn how to :

- ⇒ open an image.
- ⇒ lighten the image to improve its legibility, and define the spots zones as precisely as possible.
- ⇒ define the zones to analyze.
- ⇒ set the parameters the most important to analyze the zones.
- ⇒ analyze the zones, i.e. compute indexing grids and spots (indexing grids are useful to compute and to index the spots).
- ⇒ display indexing grids and spots.
- ⇒ verify the spots detection by means of spot enlargements, and spot characteristics reading.

Note : By default, the expression "Click on" means : "Click on with the left mouse button".

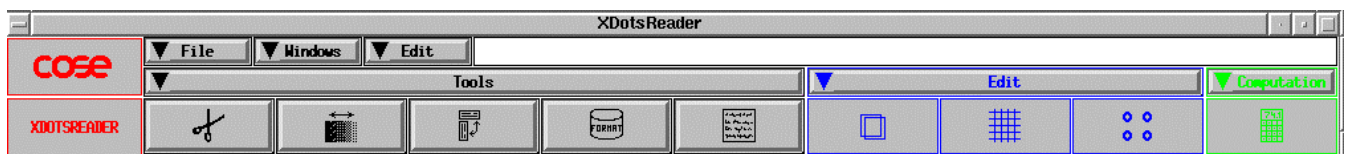
Recommendation : A lot of menus may be opened during an application. To facilitate the application use, close the unnecessary windows : click on the "Quit" button placed in each menu.

To move a menu, or a window, click on the menu or window title bar. Hold down and drag towards another location.

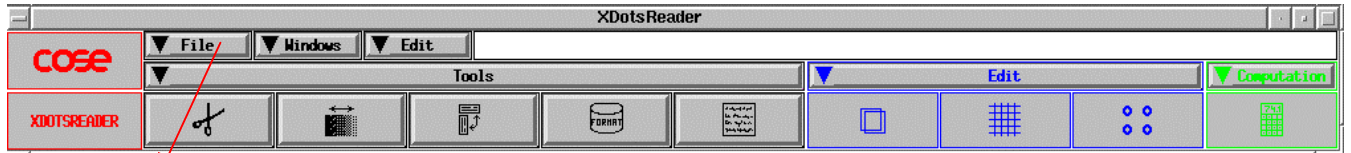
Some paragraphs are informative : you can execute this example without read these.

Set you in the directory "/DEMO".

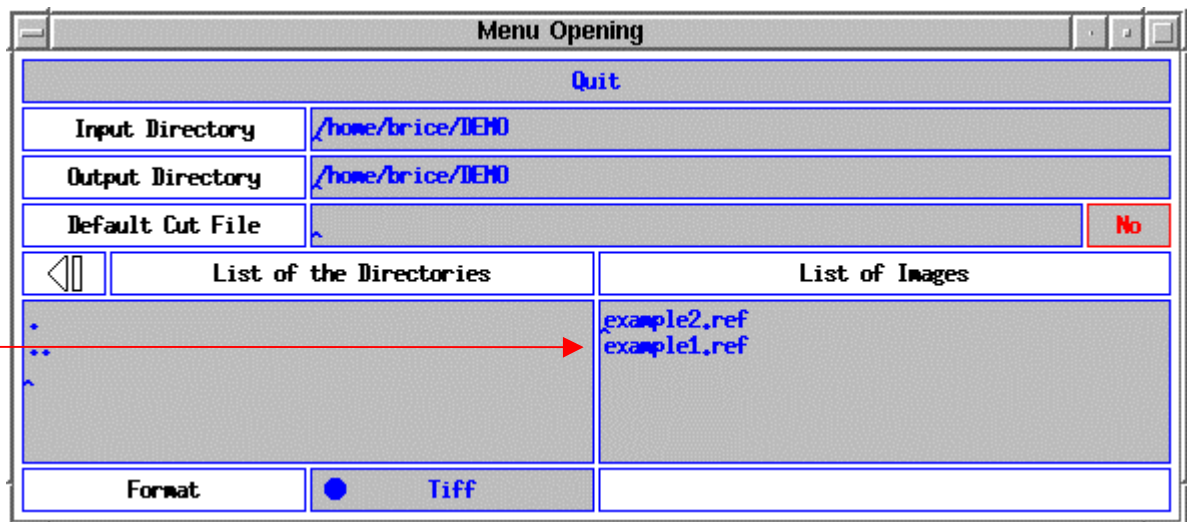
After the prompt, key **XDotsReader**, and press the Enter key. The main menu opens :




Open an image.



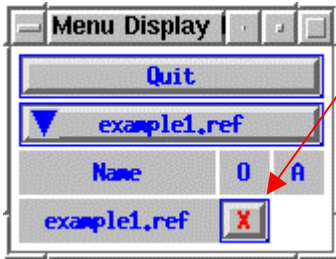
- ◆ In the main menu XDotsReader, click on the drop-down menu File. Hold down and drag to select the keyword Open (inverted image : black background, and white characters). Drop the mouse button : the Opening menu appears :




Information paragraph :

To select an image, it's necessary to select a directory and a file. It's possible to navigate in the directory tree just with the mouse. To go up in the tree directory, simple-click on the arrow button :  To go down in the tree directory, double-click on the directory name, in the zone List of the Directories. The image names are displayed in the zone List of Iimages.

- ◆ Double-click on the filename example1.ref. The Display menu appears :

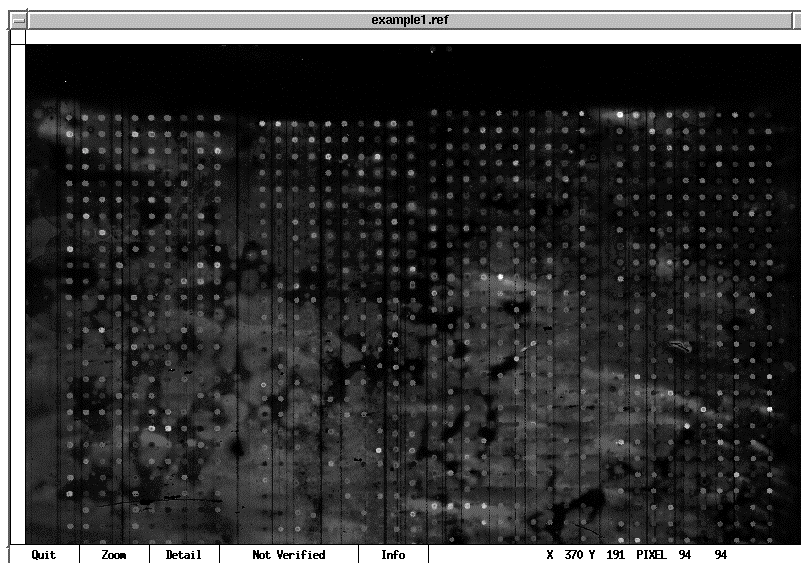


♦ Click on the red cross button, facing the chosen file.

The button displays a green O (Open) : , and the selected image appears.

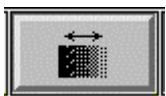
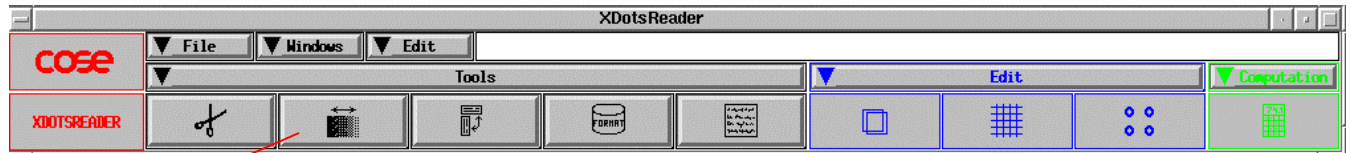
♦ Close this menu : click on the Quit button

The appeared image is this one (example1):

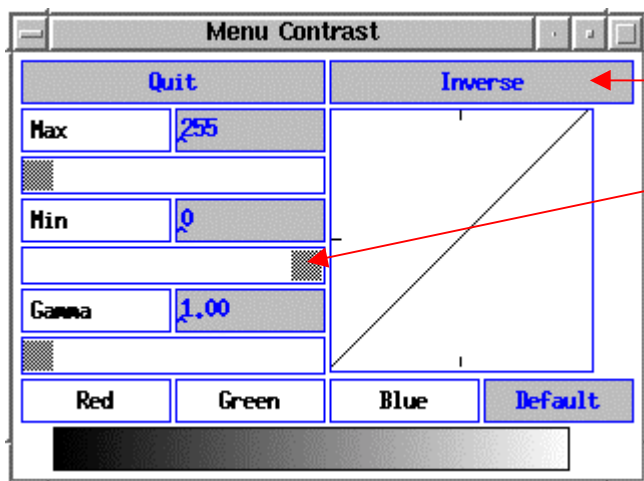


Modify the image contrast.

Before to define zones (Cut menu), it's often necessary to modify the contrast function to get a best spots visualization.

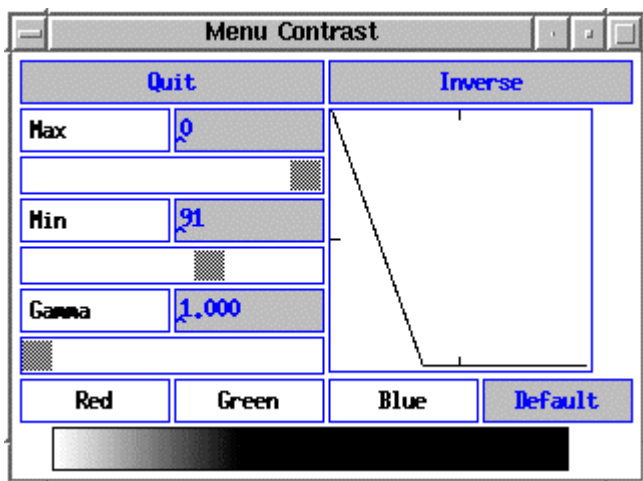


- ◆ In the main menu XDotsReader, click on the contrast icon. The Contrast Menu appears :



- ◆ Click on the inverse button.

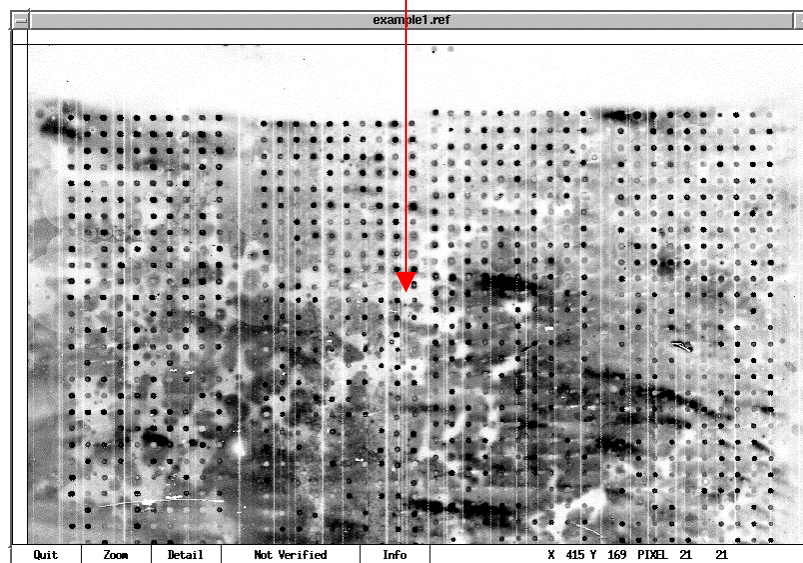
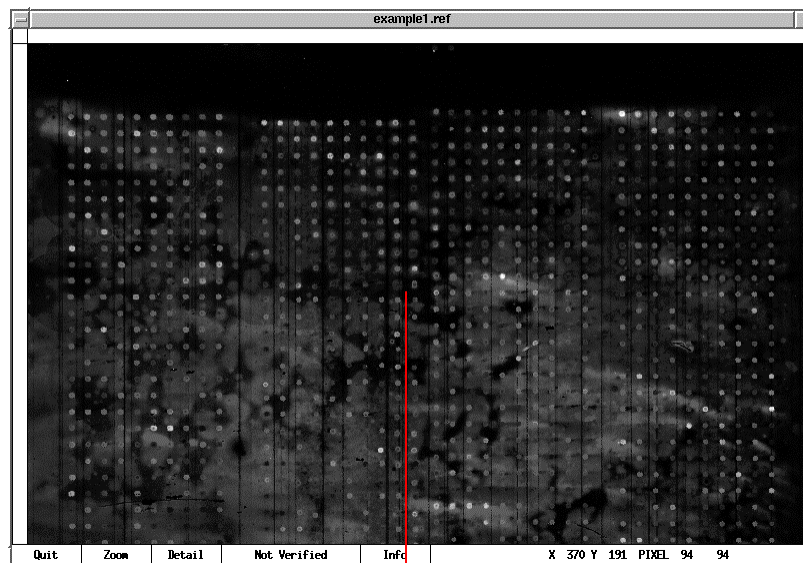
- ◆ Click on the Min slider until the 91 value.



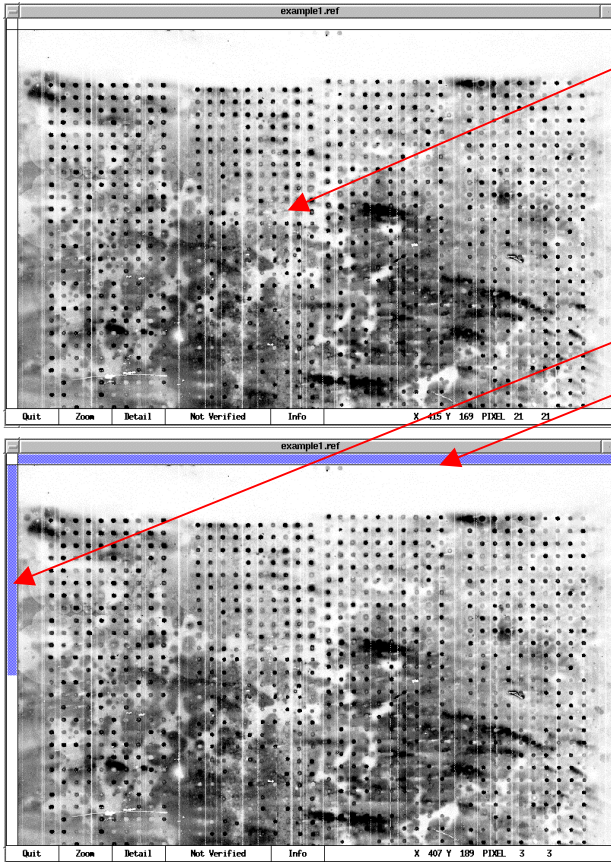
The Contrast menu is updated :

- ◆ To close the menu, click on the Quit button (the modifications are preserved).

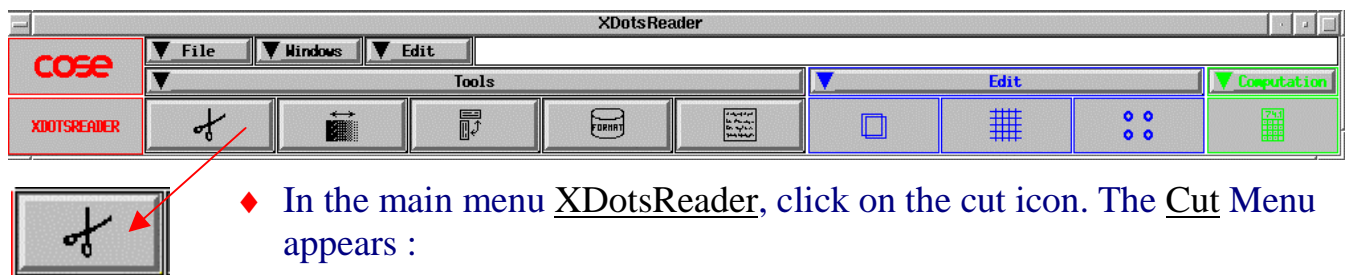
The effect on the image is the following :



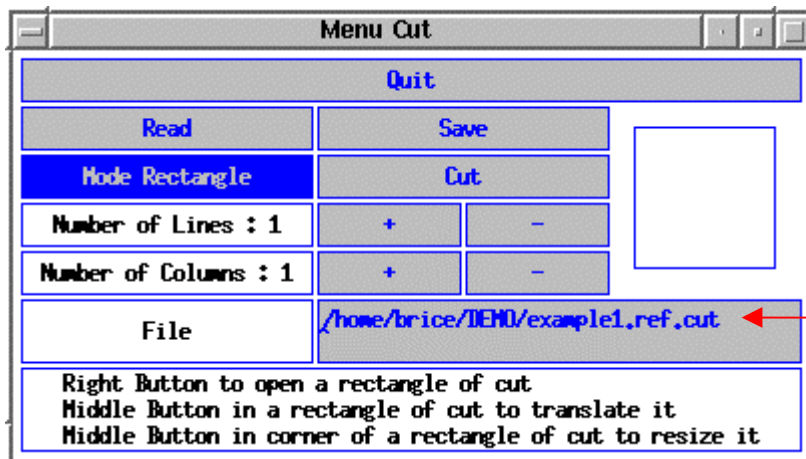
Create zones in an image.



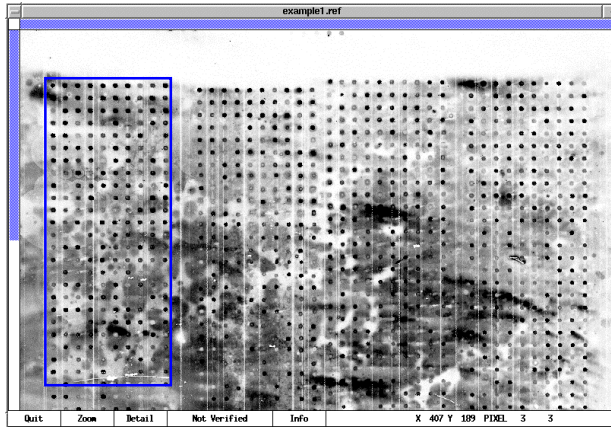
- Click with the left mouse button on the image, to set active the image. (The vertical and horizontal blue shading lifts appear on the left and on the top of the window.)



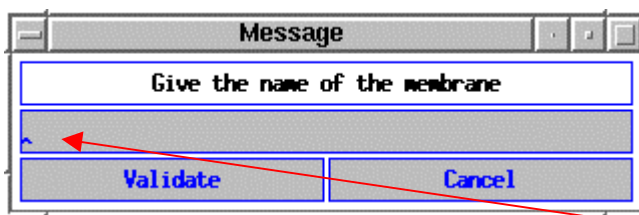
- In the main menu XDOTSREADER, click on the cut icon. The Cut Menu appears :



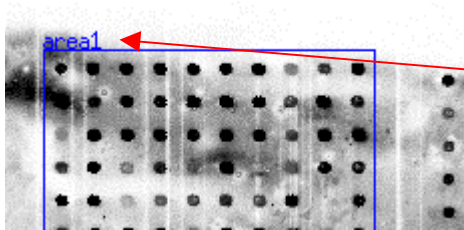
The zones filename is generated, and displayed facing the File label. The filename is built as following : "image name" with the extension **.cut**, i.e. example1.ref.**cut**



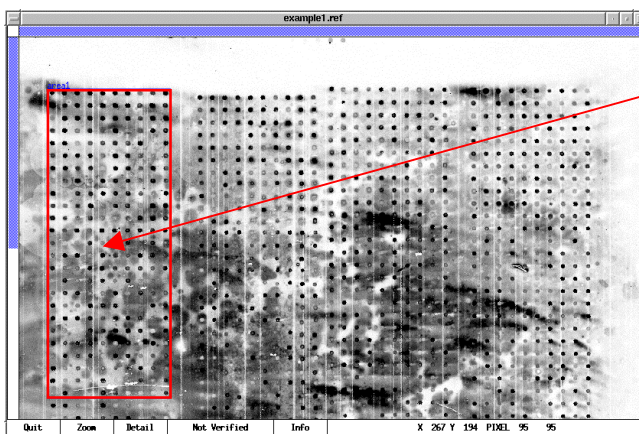
- ◆ Click with the **right** mouse button to open a rectangle. Hold down and drag to select the zone to cut. The rectangle lines are blue.



As the **right** mouse button is released, the Message menu appears.

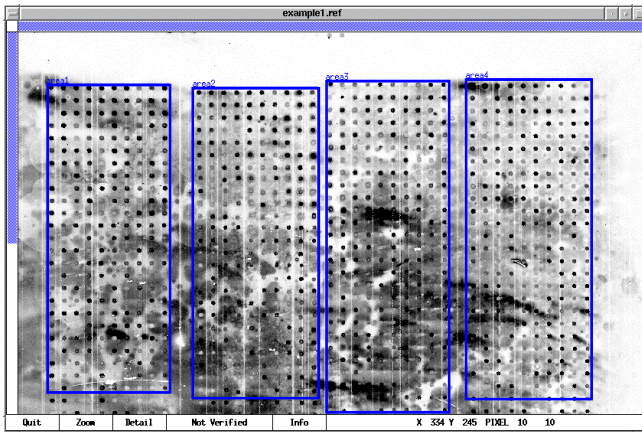


- ◆ Set the mouse pointer in the field below the message field Give the name of the membrane. Key in the zone name (area1 for example). Click on the Validate button. As the button is released, the menu closes, and the name of the zone appears above the rectangle. If not, click on the Cancel button, and begin again.



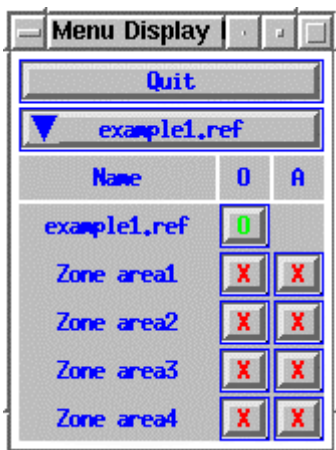
- ◆ To modify the position of the rectangle, click with the **middle** button on the rectangle. The rectangle is selected and its color becomes red. Then, click with the **middle** mouse button, hold down, and drag to translate the rectangle.

button close to the rectangle corner, hold down, and drag to resize the rectangle. Particularly, it's necessary to execute this operation if the image is taller than the window size. In this case, use the lift to display the remaining part, and resize the rectangle. For very large images, it's easier to open a Zoom image (button on the bottom of the image), and to draw roughly a rectangle. Then, fit the coins through the image.



- ◆ To draw new zones, all the rectangles drawn previously must be unselected (blue colored) : click on the image outside a rectangle, with the **middle** mouse button. Then, begin again the operations : click with the **right** mouse button on the image, and give a new zone name (area2 for example).

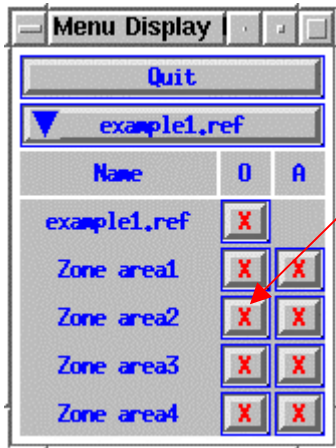
- ◆ When all the zones are suitably drawn, click on the Save button in the Cut menu :



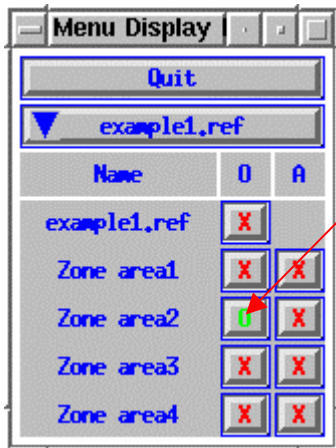
- The Display menu is updated : the zones names appear.
- The file example1.ref.cut is created : it contains the zones coordinates (and not the data inside).

- ◆ Quit the Cut menu : Click on Quit button in the Cut menu.
- ◆ Quit the image : Click on the Quit button at the bottom of the image example1.ref.

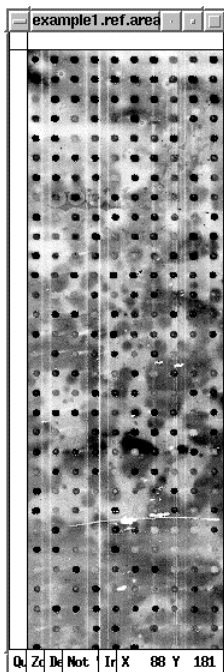
To display a zone.



- ♦ To display a zone : in the Display menu, click on the button facing the chosen zone (zone area2, for example) in the "O" column ("O" like "Opening") :



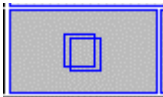
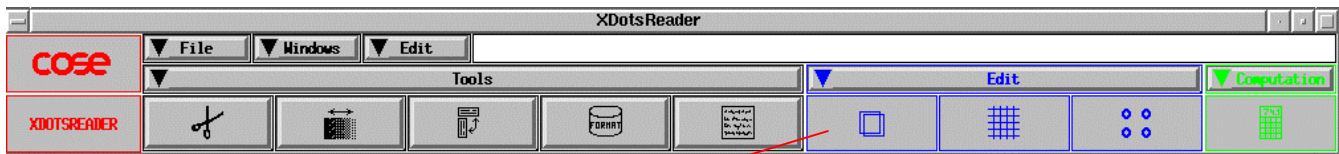
- The Display menu is updated.



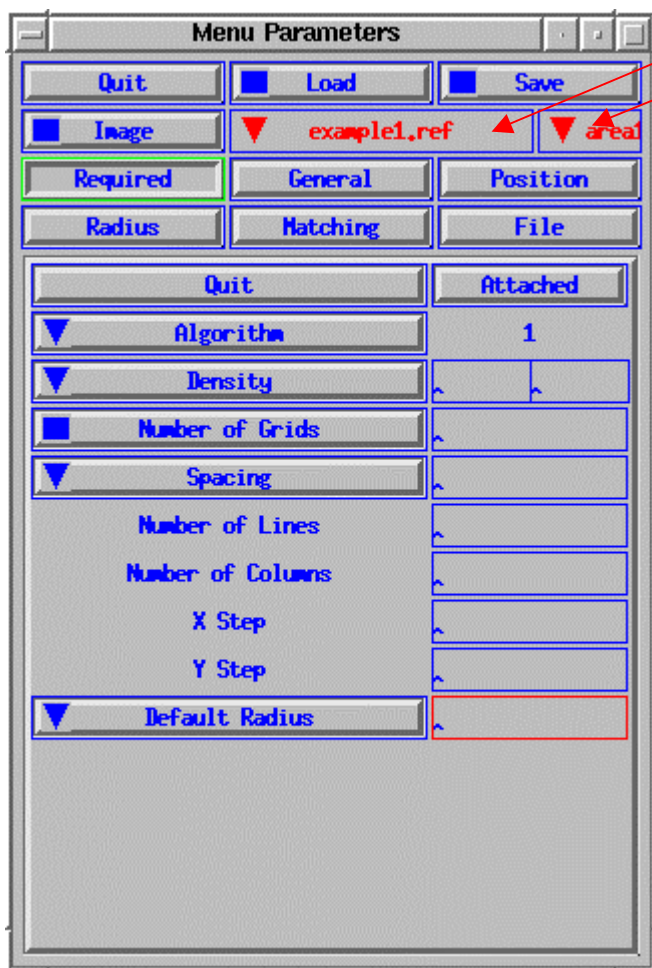
- The zone opens.

To set the required parameters.

- ◆ Click on the zone to select it (The vertical and horizontal blue shading lifts appear on the left and on the top of the window).



- ◆ In the main menu XDotsReader, click on the parameters icon. The Parameters menu appears :



The image name and the zone name appear in the red drop-down menus.

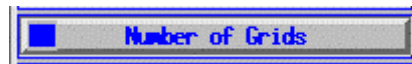
Fill all the required parameters (See next page)



- ◆ Open the drop-down menu Algorithm, and select Algorithm 2. This algorithm is robust, and presumes a good result. The choice is displayed on the right.



- ◆ Open the drop-down menu Density, and select 1x1. (The density of the zone is 1).



- ◆ The software fills the field with 1 (only possibility for a density 1).



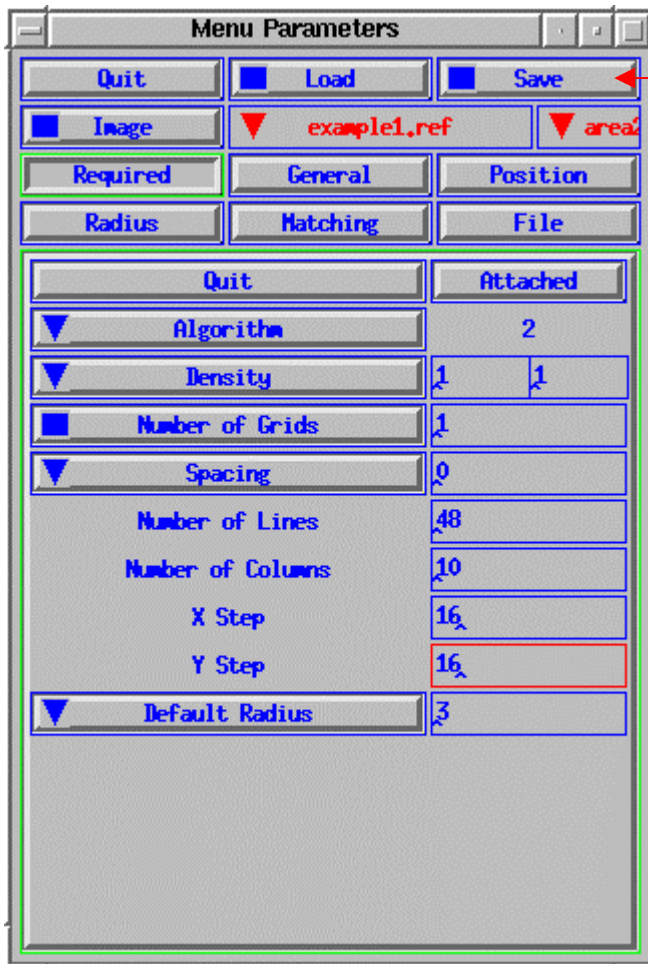
- ◆ Open the drop-down menu Spacing. Select 0. (This parameter specifies the spacing between two blocks. In our example, one block is constituted with an only spot : so, the spacing is 0).
- ◆ Input field Number of Lines : To set the blocks lines number of the selected zone. As the window is shorter than the image (in our example), count the lines from top to bottom of the window. Set the mouse pointer on the last line, and note the Y-coordinate, displayed in the bar on the bottom of the window. With the vertical lift, visualize the bottom of the image. Find again the last counted line with the Y-coordinate, and ended the counting. The zone of our example has 48 lines. Set the pointer on the field and key 48.
- ◆ Input field Number of Columns : To set the blocks columns number of the selected zone. The zone of our example has 10 columns. Set the pointer on the field and key 10.
- ◆ Field X Step : To set the pixels number between two blocks in a line. As one block, in this example, is constituted with an only spot, the distance between two blocks is the distance between two spots. To estimate this distance, place the mouse pointer on a spot. Note the X-coordinate displayed in the bar on the bottom of the window, and set the mouse pointer on the next right spot. The difference gives the X Step in pixel unit. In our example, the distance between two spots is 16. Set the pointer on the field and key 16.

- ◆ Field Y Step : Idem X Step. Set the pointer on the field and key 16.

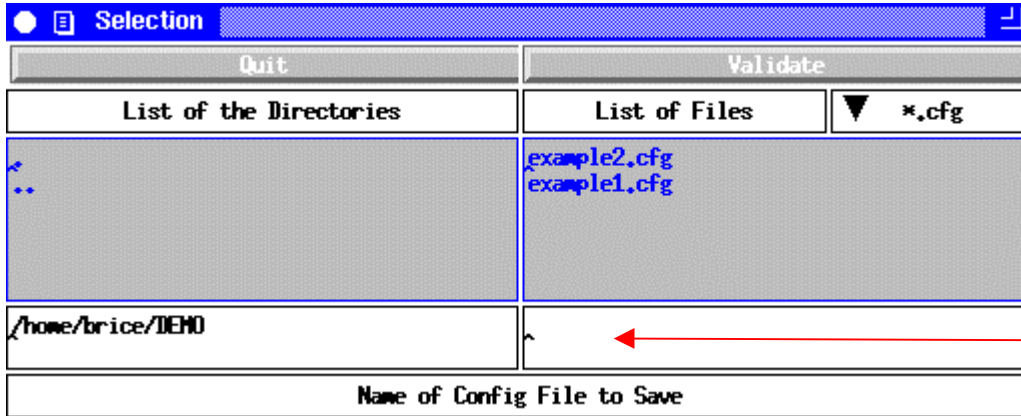


- ◆ Radius set by default, if the algorithm doesn't find a spot close to a grid intersection. For our example, the spots have approximately a 3 pixels radius. Open the drop-down menu and select 3.

Finally, the Parameters menu is filled as following :

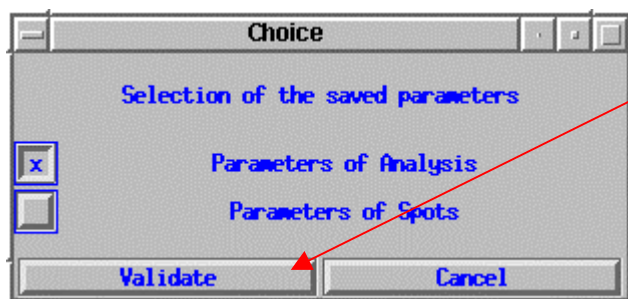


- ◆ It's very useful to save these parameters, to recall them after the application closing, or to reuse them for the others zones. So, click on the Save button : the Selection menu opens :



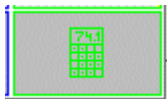
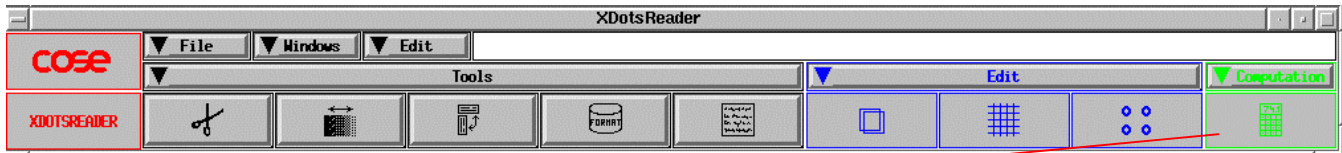
- ◆ Set the mouse pointer on the field on the right, above message "Name of Config File to Save".
Key a filename.

- ◆ For instance, type **example1**, and click on the Validate button. The Choice menu appears :

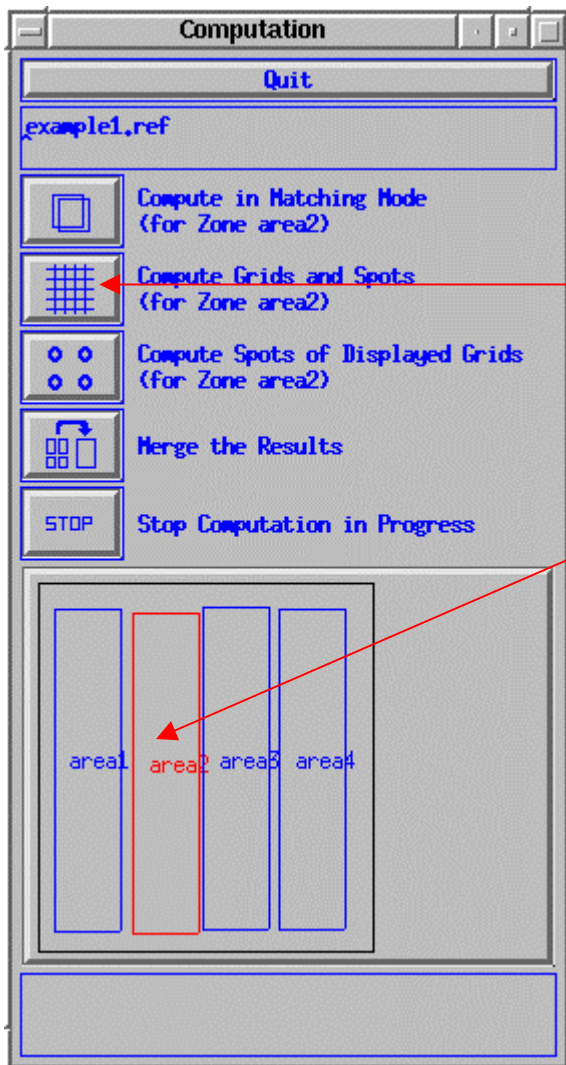


- ◆ Click on the Validate button. The analysis parameters are saved in the file **example1.cfg**.

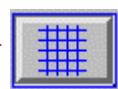
Compute indexing grids and spots.



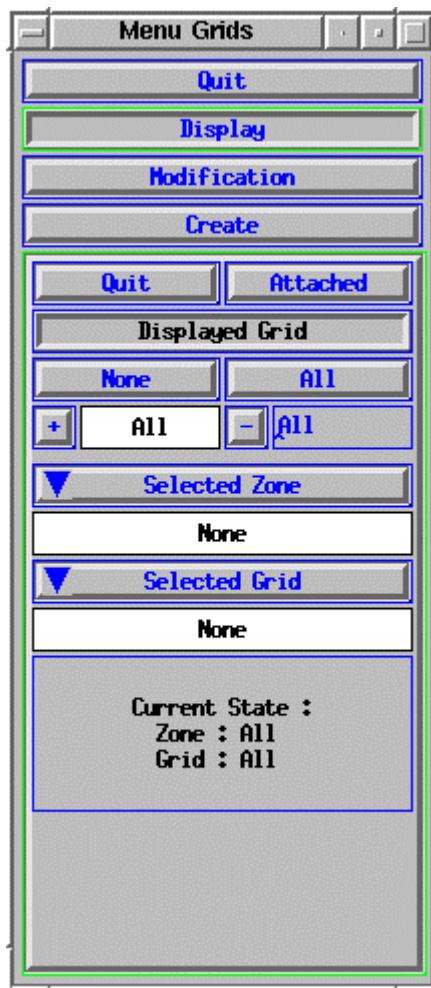
- ◆ In the main menu XDotsReader, click on the computation icon. The Computation menu appears :



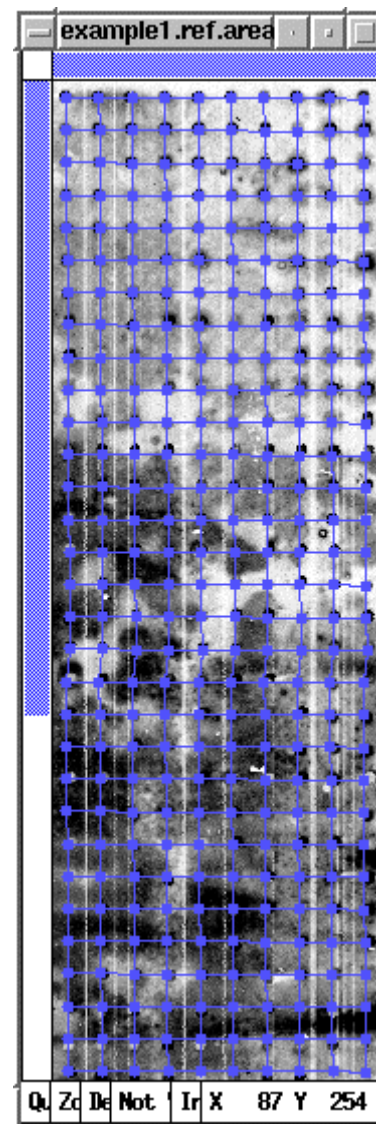
In our example, the image is cut in four zones. Click on the chosen zone to select it (the same as the required parameters fields are filled) : the rectangle lines become red.



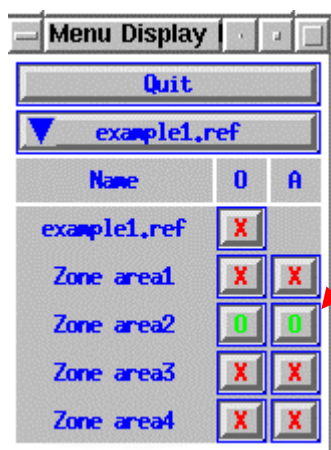
Click on the Compute Grids and Spots icon to start the computation. When the process is finished, the Grids menu appears, the Display menu is update, and the indexing grid displays in foreground of the image :



Grids menu

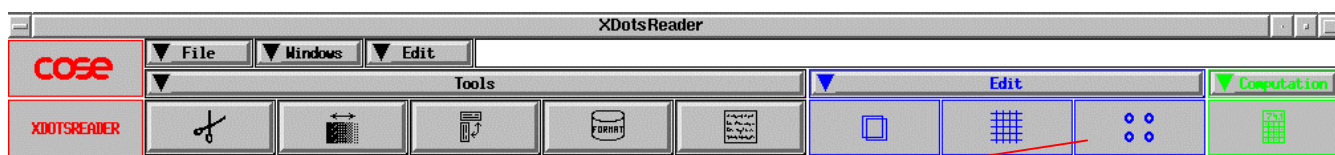


The indexing grid displays on foreground on the zone.

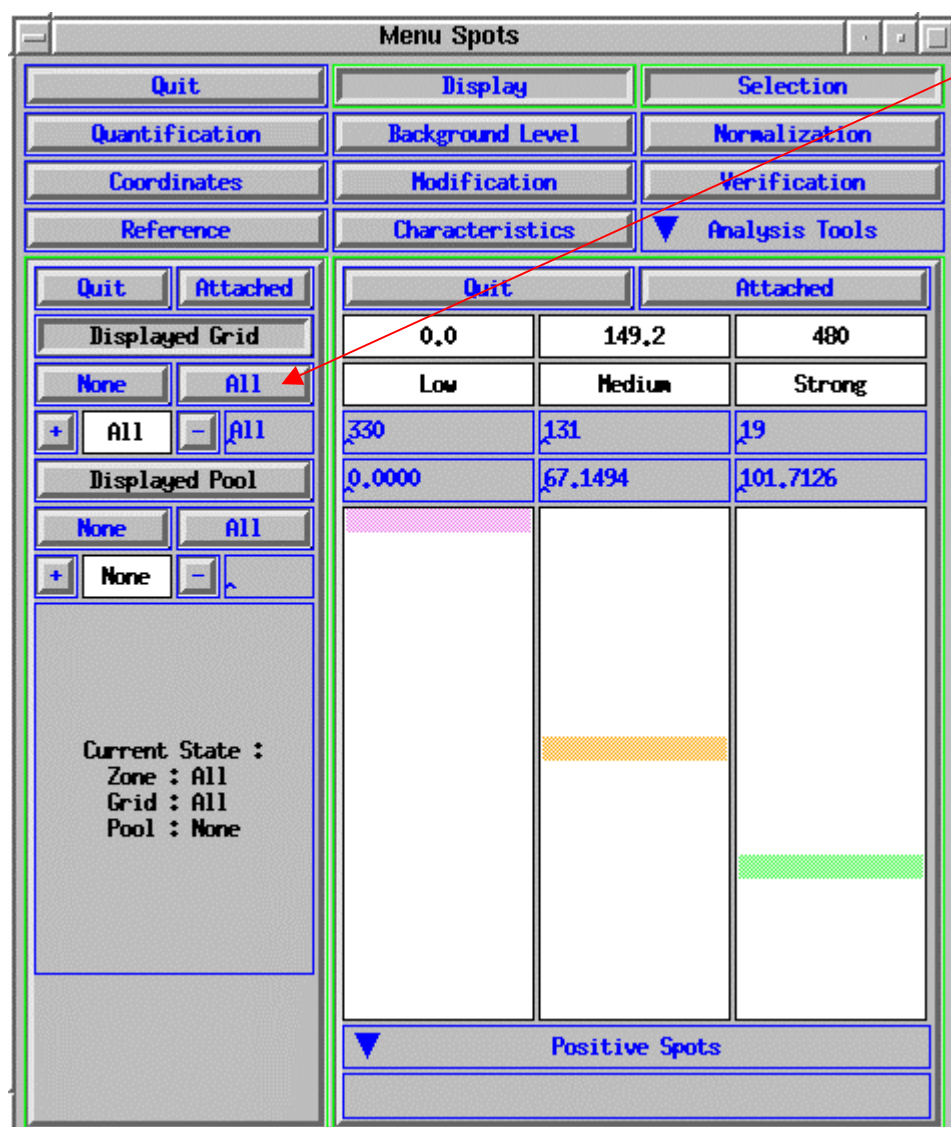


The Display menu is update : in the "A" column (A like Analyze), the button facing Zone area2 changed it with a green "O". It means that the zone is analyzed. A new file containing the analysis results is created. The filename is constituted with the image name, and the extension *."zone name".G*. In this example, the filename is *example1.ref.area2.G*.

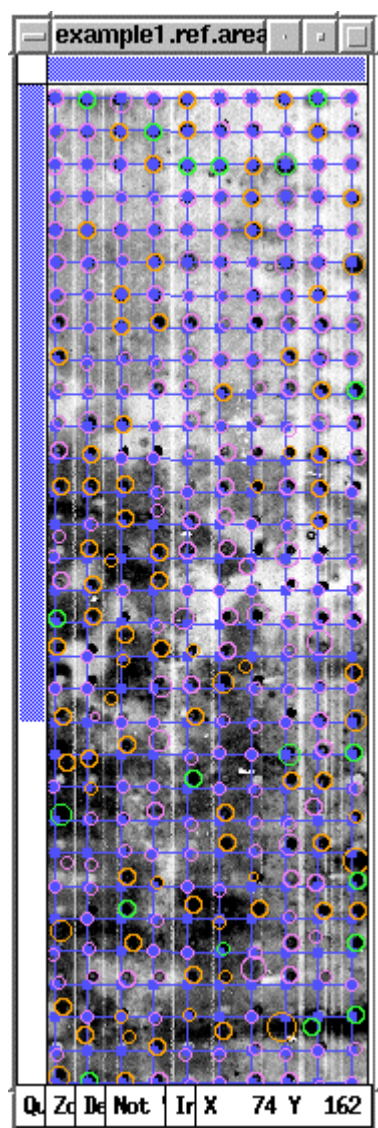
Display spots.



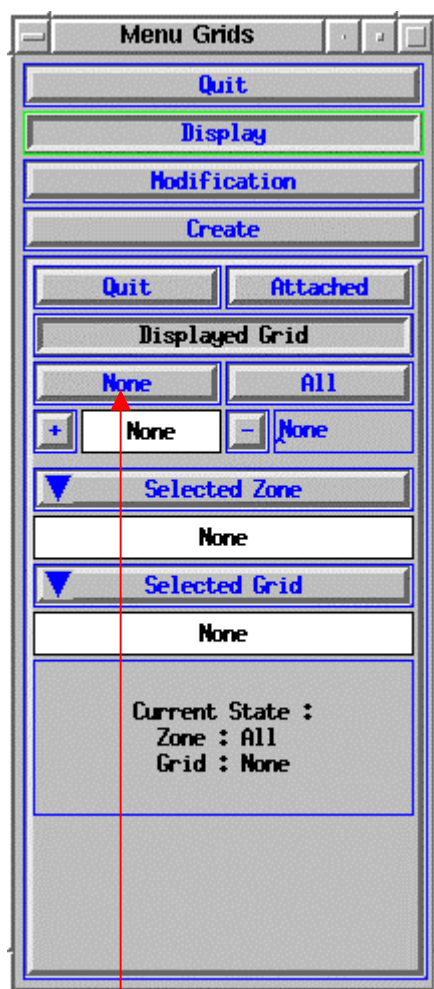
Click on the spot icon to open the Spot menu :



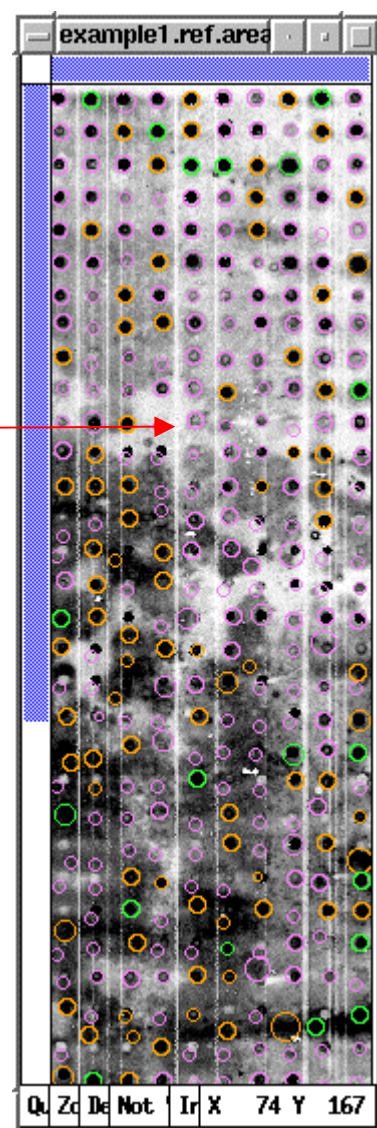
Click on the All button, under the Displayed Grid label to display the spots on the selected zone : all the spots corresponding to all the displayed grids are visualized (see image next page). In this case, there is only one grid.



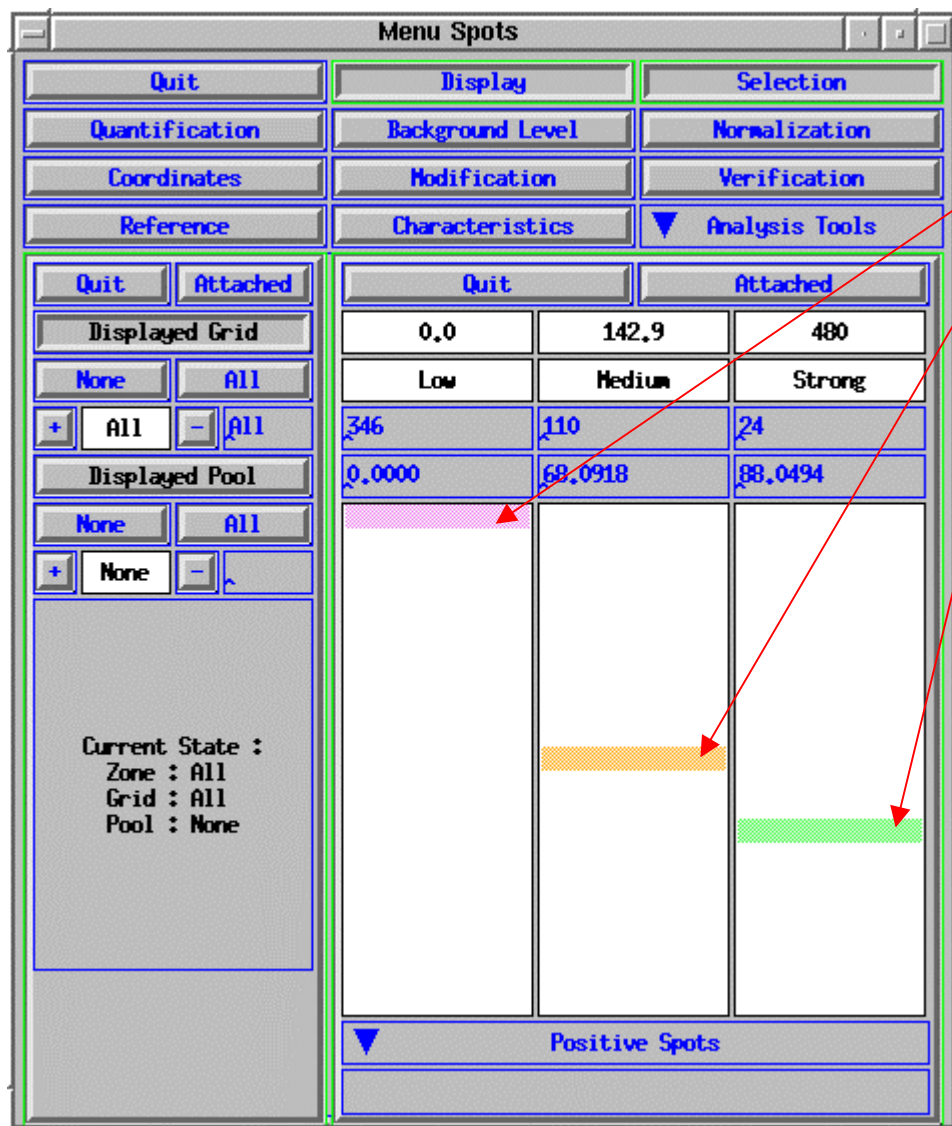
The spots appears foreground, and are modeled with circles.



For a best spots visualization ,clear the displayed grids : In the Grid menu, click on the None button under the Displayed Grid label



The indexing grid is cleared



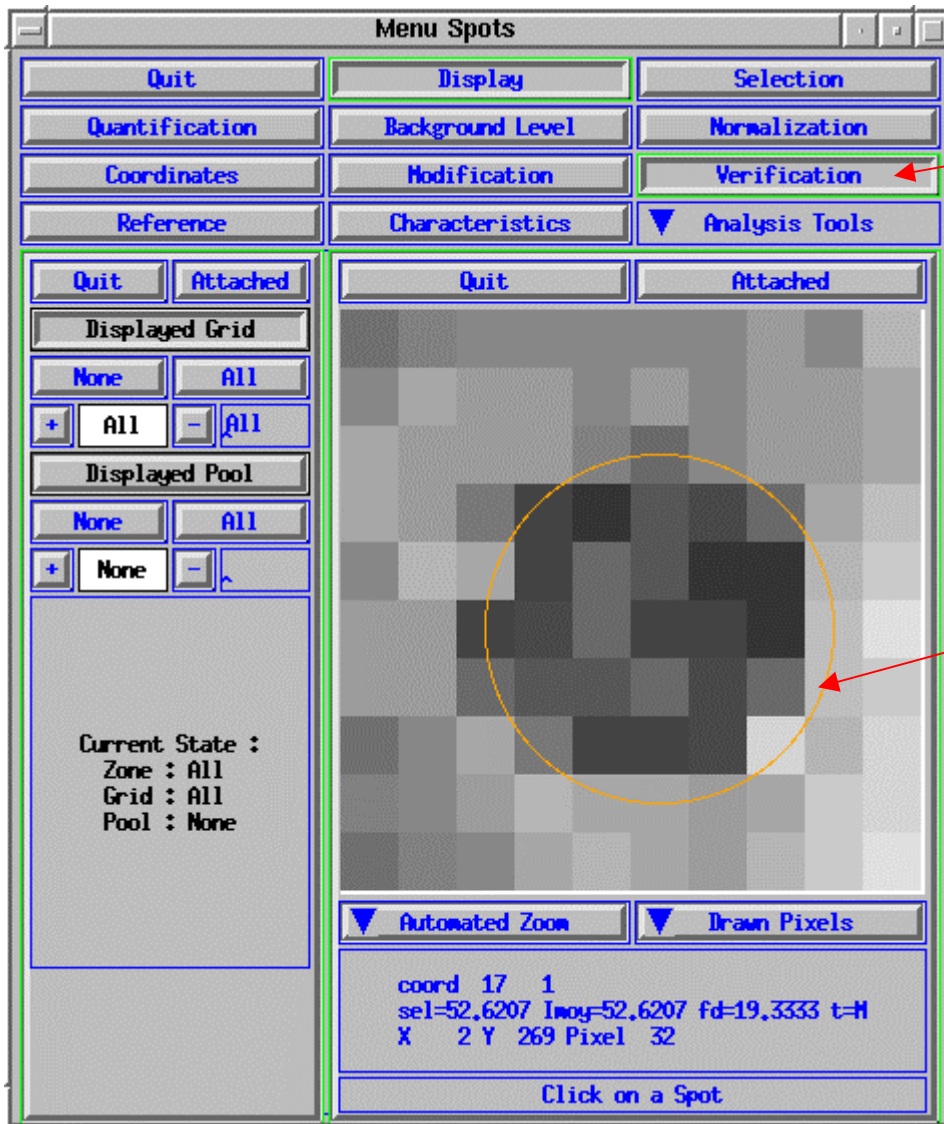
The spots circles are colored according to their intensities.

Three thresholds are set by default. They are adjustable with the mouse : Click on a colored bar in the Sspots menu. Hold down and drag to modify the threshold. At the same time, the spots colors change in the selected zone.

In this case, there are 346 spots (pink circles) between the levels 0. and 68.0918, 110 spots (orange circles) between the levels 68.0918 and 88.0494, and 24 spots (green circles) above the level 88.0494.

To verify the spots detection.

Having detected the spots, it's possible to display the spots one by one.

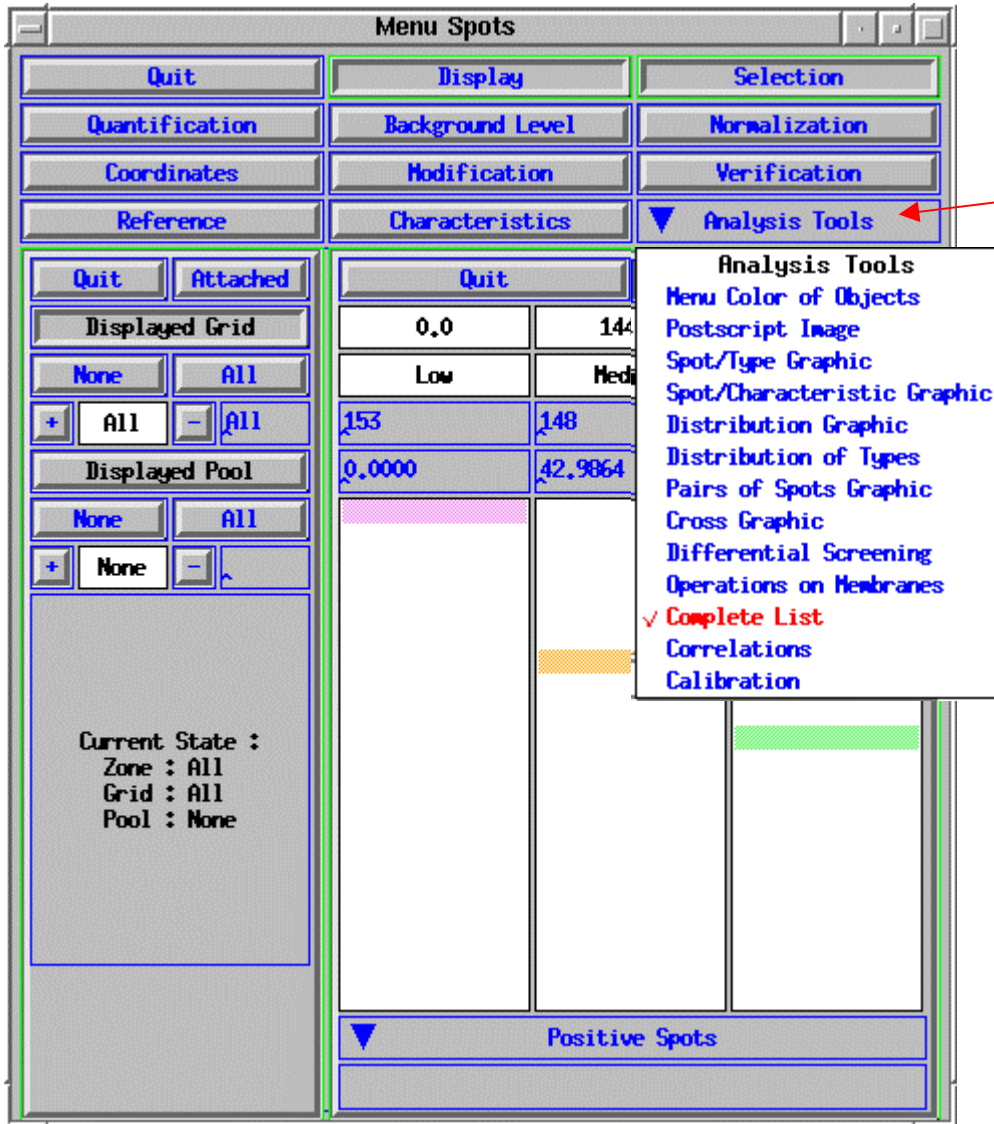


♦ In the Sspots menu, click on the button Verification.

♦ In the zone with the displayed spots detections, click on a spot with the **m**iddle mouse button. A zoom of the spot is displayed, with the detection circle.

To analyze the spots detection.

Having detected the spots, it's possible to examine the spots detection characteristics.



In the Spots menu, click on the drop-down menu Analysis Tools. Hold-down and drag to the label Complete List. Release the mouse button

The menu Complete List appears.



- ◆ Click on the List button in the Complete List menu. The results displays in the window. Click on the lifts to visualize the results.

The header of this menu give general parameters about the results :

```

Experience      : example1
Exposition      : ref
Zone            : area2
Grid            : All
Pool            : None
Threshold       : Mean Intensity
With
No Normalization
Spots Coord., Line/Column
Positive Spots  : 480 m 54.80 54.7991 ec 26.76 26.7589
Low Spots       : 330 m 40.83 40.8340 ec 17.34 17.3398
Medium Spots    : 131 m 80.58 80.5761 ec 8.74 8.7414
Strong Spots    : 19 m 119.63 119.6250 ec 13.02 13.0200
Low Threshold   : 0.00 0.0000
Medium Threshold : 67.15 67.1494
Strong Threshold : 101.71 101.7126
Min Value       : 0.0
Max Value       : 149.2
  
```

Then, the abbreviations used in the analysis array are explained.

The analysis array is displayed after. Facing the coordinates are displayed a characteristics set :

coord	def.coord	pos	sel	r	Ic
1 1	1 A 1	6.0 7.0	133.9310	3.0	18
1 2	1 A 2	22.0 7.0	144.8276	3.0	20
1 3	1 A 3	38.0 8.0	100.4828	3.0	9
1 4	1 A 4	55.0 7.5	70.7000	3.5	8
1 5	1 A 5	71.0 7.0	115.3793	3.0	13
1 6	1 A 6	88.0 7.0	96.3448	3.0	9
1 7	1 A 7	105.0 7.0	42.6552	3.0	4
1 8	1 A 8	121.0 8.0	77.0816	4.0	9
1 9	1 A 9	136.0 8.0	68.7407	5.0	12
1 10	1 A 10	154.0 7.0	60.0690	3.0	!
2 1	1 B 1	6.0 23.0	55.9655	3.0	5
2 2	1 B 2	22.0 24.0	67.0000	4.0	9
2 3	1 B 3	38.0 23.0	85.7347	4.0	12
2 4	1 B 4	55.0 22.0	115.4505	2.5	12

A lot of options are available to modify the array contents. An interesting option is to change the coordinates system :



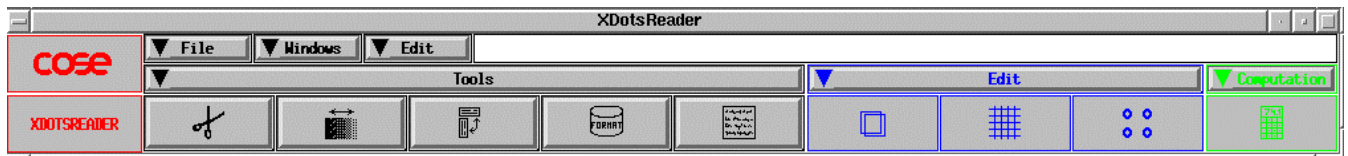
- ◆ In the Spots menu, click on the Coordinates menu. The drop-down-menu Coordinate Mode appears in the Spots menu.



- ◆ Click on the drop-down menu. Hold down and drag to select the new coordinates system. The chosen system name is displayed under the drop-down menu label.

Menus description.

Main menu.



Three bars constitute the menu :

- ◆ The superior bar includes the three drop-down menus File, Windows, Edit, and a messages area.
- ◆ The middle bar includes the three drop-down menus Tools, Edit, and Computation. Each menu allows to access to several functions.
- ◆ The inferior bar includes icons. These icons correspond to the functions of the over drop-down menus Tools, Edit, and Computation.

Superior bar.



Drop-down menu File. Click on the button. The following menu appears :

Drop-down menu File.

File	
Open	Control + o
Configuration	Control + C
Open In List Mode	Control + L
Save Selected Image	Control + s
Save and Quit Program	Control + S
Quit Program	Control + q

In the left column, six actions are proposed :

- Open (open an image).
- Configuration (configure the detail zoom).
- Open in List Mode (open an image in an images list).
- Save Selected Image.
- Save and Quit Program (save the selected image and quit the application).
- Quit Program (quit the application).

In the right column, are displayed the corresponding shortcut keys.

Open an image

To open an image, double-click on an image name in the Opening menu. This menu can open only the file which the syntax is :

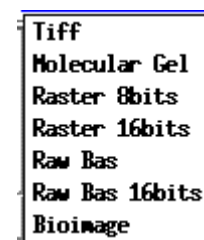
name.ref
name.1
name.2
 .
 .
name.10
name.tif (tif format)
name.gel (tif format)
name.pii (tif format)
name.b8
name.b16

The menu Opening is :

Menu Opening		
Quit		
Input Directory	/home/brice/DEMO	
Output Directory	/home/brice/DEMO	
Default Cut File		No
<	List of the Directories	List of Images
↖ ..		example2.ref example1.ref
Format	<input checked="" type="radio"/> Tiff	

- ◆ Button Opening : to close the menu.
- ◆ Field Input Directory : displays the current directory name. It's possible to key in directly in this field : just before, set the mouse pointer in this field.

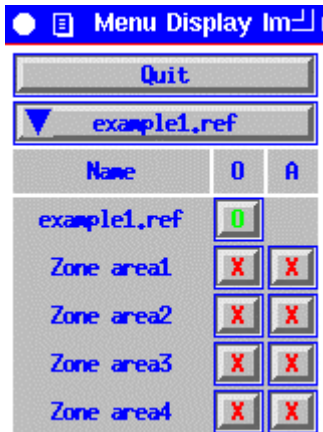
- ◆ Field Default Cut File : displays the automatic procedure filename to cut an image, if this one exists. When a file is selected, this procedure starts automatically. To refuse this action, click on the red No button.
- ◆ Arrow button : to go up in the tree directory. The fields List of the Directories and List of Images are updated.
- ◆ Field : List of the Directories : to go up in the directory tree, double-click on "..". To go down in the directory tree, double-click on a sub-directory name. For any change, the fields List of the Directories and List of Images are updated.
- ◆ Field : List of Images : to select an image, double-click on the image name. The Display menu appears.
- ◆ Drop-down menu : on the right of the Format label. The selected format is displayed. To open the menu, click on the drop-down menu. A list of format appears :



To select a format, hold down the mouse button, and drag.

Display menu.

This menu is called through the Opening menu, after a double-click on an image name in the Opening menu.

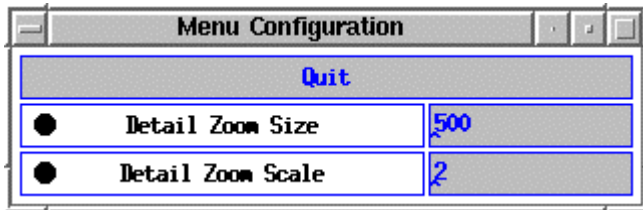


- ◆ Button Quit : To close this menu.
- ◆ Drop-down menu image name : In the Opening menu, after to click on an image filename, or a zone filename, the drop-down menu label is the image filename. Click on this drop-down menu to access all the opened images since the session starting. Hold down and drag to select an image. When the image is selected, the image filename and the zones filenames are displayed in the field below.
- ◆ Buttons : Below the Name label are displayed the image filename and the relative zones filenames. Facing the filenames, there are two columns. The first one concerns the opening files, the second, the analysis files.
 - O column :
 - If the file is closed, the button displays a red cross.
 - If the file is opened, the button displays a green O.
 - A column :
 - If the file has never been analyzed, the button displays a red cross.
 - If the file has been already analyzed, the button displays a green "O".

To open an image or a zone, **click on the button facing the chosen file**. The red cross turns into a green O, and the chosen image displays.

To close an image, click on the Quit button, on the image button.

Configure the detail zoom



This menu affects the windows named Detail Zoom. This window opens by means of the Detail button, locate at the bottom of each image.

Quit button : to close the Configuration menu.

Detail Zoom Size

200
250
300
350
400
450
500

Drop-down menu Detail Zoom Size : to select a predefined size for the detail image. Click on the drop-down menu, hold down and drag to select (inverse video) the size.

Field Detail Zoom Size : facing the drop-down menu. It's possible to key in directly the image size. Before to key, set the mouse pointer on the field.

Detail Zoom Scale

2
4
8
16
32

Drop-down menu Detail Zoom Scale : to select a predefined enlargement factor for the detail image. Click on the drop-down menu, hold down and drag to select (inverse video) the factor.

Field Detail Zoom Scale facing the drop-down menu. It's possible to key in directly the image size. Before to key, set the mouse pointer on the field.

Open an image in a list.



Quit button : to close the menu List.

List field : to specify the filename of the list which contains a list of images to display. The extension of a list is **.lst**. To write a list name, set the mouse pointer on the filename field and enter the filename.

Open button : to open the selected file.

Previous button : to select the preceding file.

Next button : to select the next file.

Experience button : display the experience name of the selected file.

Exposition button : display the image name of the selected file.

Membrane button : display the zone name of the selected file.

Tiff
Molecular Gel
Raster 8bits
Raster 16bits
Raw Bas
Raw Bas 16bits
Bioimage

File format : To select the image format (available option : Tiff, Molecular Gel, Raster 8 bits, Raster 16 bits, Raw Bas, Raw Bas 16 bits, Bioimage)

Save the selected image

A selected image has shading blue lifts : when you open an image, click inside it. If the image is fully hidden by others images or menus, click on the Windows button (Superior bar), and select the chosen image.

When an image is saved, the contrast specifications are preserved.

Save the active image and quit the application.

Realize the same operation than "Save the selected image", then, close all the windows.

Quit the application :

Close all the windows (images are not saved).

Drop-down menu Windows

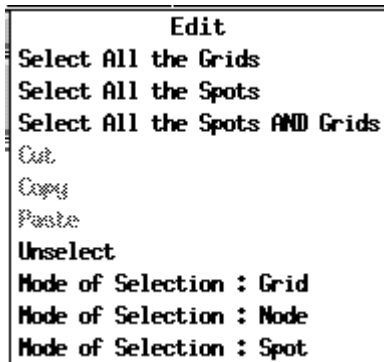


Drop-down menu Windows : Set a window in foreground. Click on the button to open the menu. Hold down and drag to select a window. Release, and the window appears.

Drop-down menu Edit



Drop-down menu Edit. Click on the button. The following menu appears :



Hold down and drag to select an available action (the available actions are black).

- ♦ Select all the Grids : Select all the indexing grids. The indexing grids color becomes red. The option Cut and Copy are active and change in black color in the drop-down menu Edit.
- ♦ Select All the Spots : Select all the spots. The spots color becomes red. The option Cut and Copy are active and change in black color in the drop-down menu Edit.
- ♦ Select All the Spots AND Grids : Select all the indexing grids and spots. The indexing grids and spots color becomes red. The option Cut and Copy change in black color in the drop-down menu Edit.

- ◆ Cut : to cut the selected grids or spots. The Paste option becomes available.
- ◆ Copy : to copy in buffer memory, the selected grids or/and spots. The Paste option becomes available.
- ◆ Paste : to paste the grids or the spots from the memory buffer to the selected image.
- ◆ Unselect : Unselect the selected indexing grids or/and the selected spots. The indexing grids become blue, and the spots take again their initial colors.
- ◆ Mode of selection : Grid : To select an indexing grid, and move it through the Grid menu :
 - Open the Grid menu.
 - Select an indexing grid.
 - Click on Modification button.
 - Click on the toggle button Visualization to set Modification.
 - Click on the indexing grid : it's becoming red. Hold down and drag the indexing grid.

Remark : When an indexing grid is moved, the spots in relation with the indexing grid are destroyed.

- ◆ Mode of Selection : Node : To select a node, or a group of nodes, and move them through the Grid menu : (See also the example at the end of this menu description).
 - Open the Grid menu.
 - Select an indexing grid.
 - Click on the Modification button.
 - Click on the toggle button Visualization to set Modification.
 - Click on a node, hold down and drag the node.
 - Or : delimit the zone with the middle mouse button : click on the zone, hold down and drag to open the wanted area (red rectangle). Release the mouse button.
 - Click on the red rectangle, hold down and drag the group of nodes.

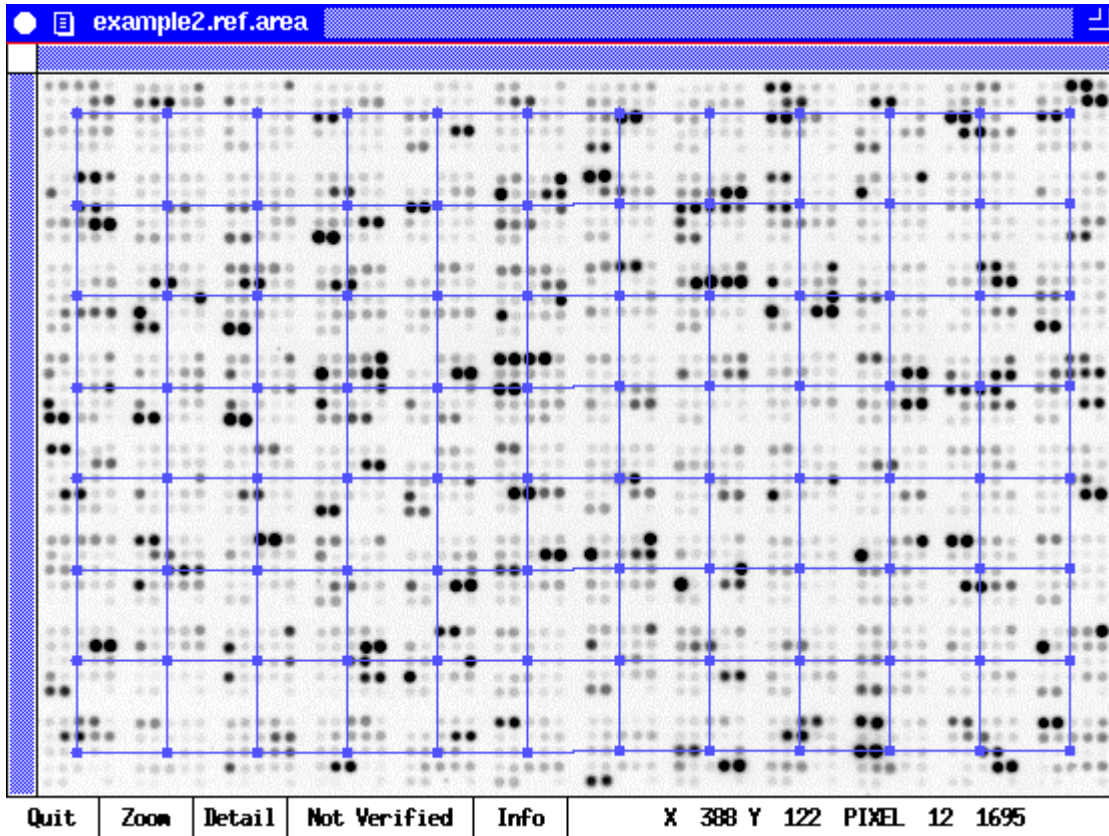
Remark : When an nodes are moved, the spots in relation with the corresponding indexing grid are destroyed.

- ♦ Mode of selection : Spot : To select a node, or a group of nodes, and move them through the Grid menu :
 - Open the Grid menu.
 - Select an indexing grid.
 - Open the Spot menu.
 - Click on the Modification button.
 - Click on the toggle button Visualization to set Modification.
 - Click on a spot, hold down and drag the spot.
 - Or : delimit the zone with the middle mouse button : click on the zone, hold down and drag to open the wanted area (red rectangle). Release the mouse button.
 - Click on the red rectangle, hold down and drag the group of spots.

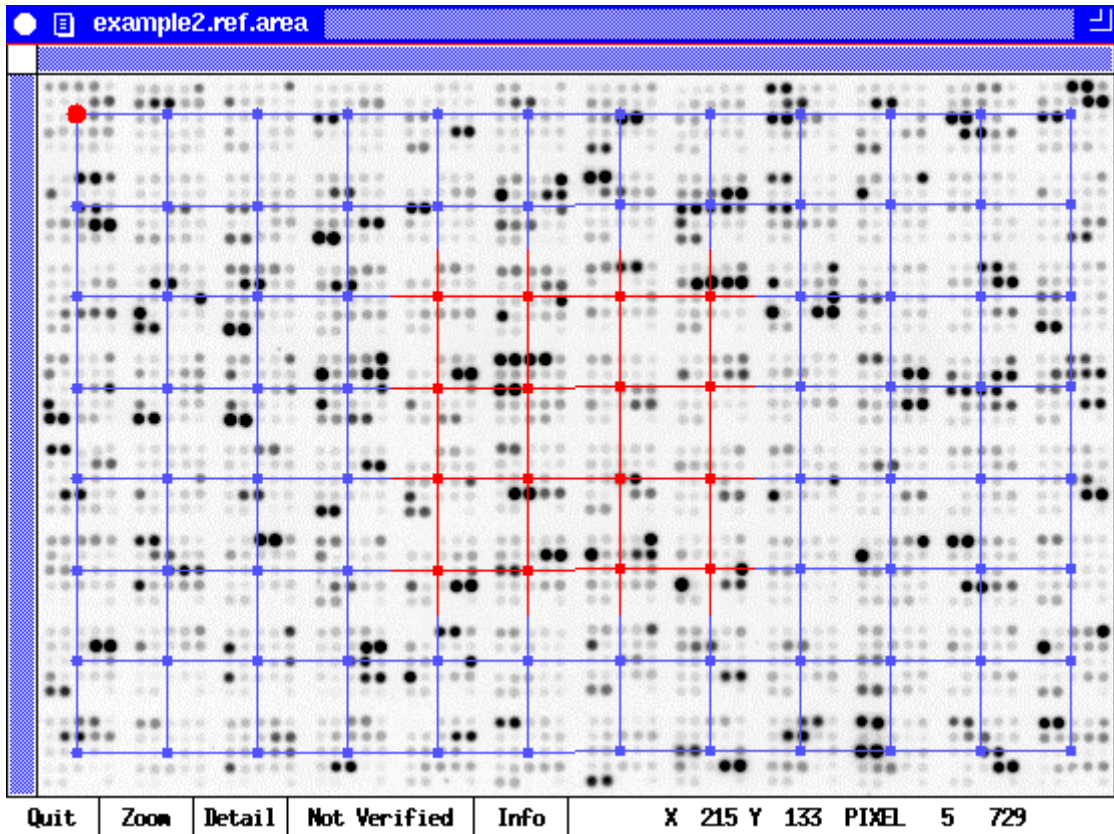
- ♦ Messages area.

An example : to move a group of nodes.

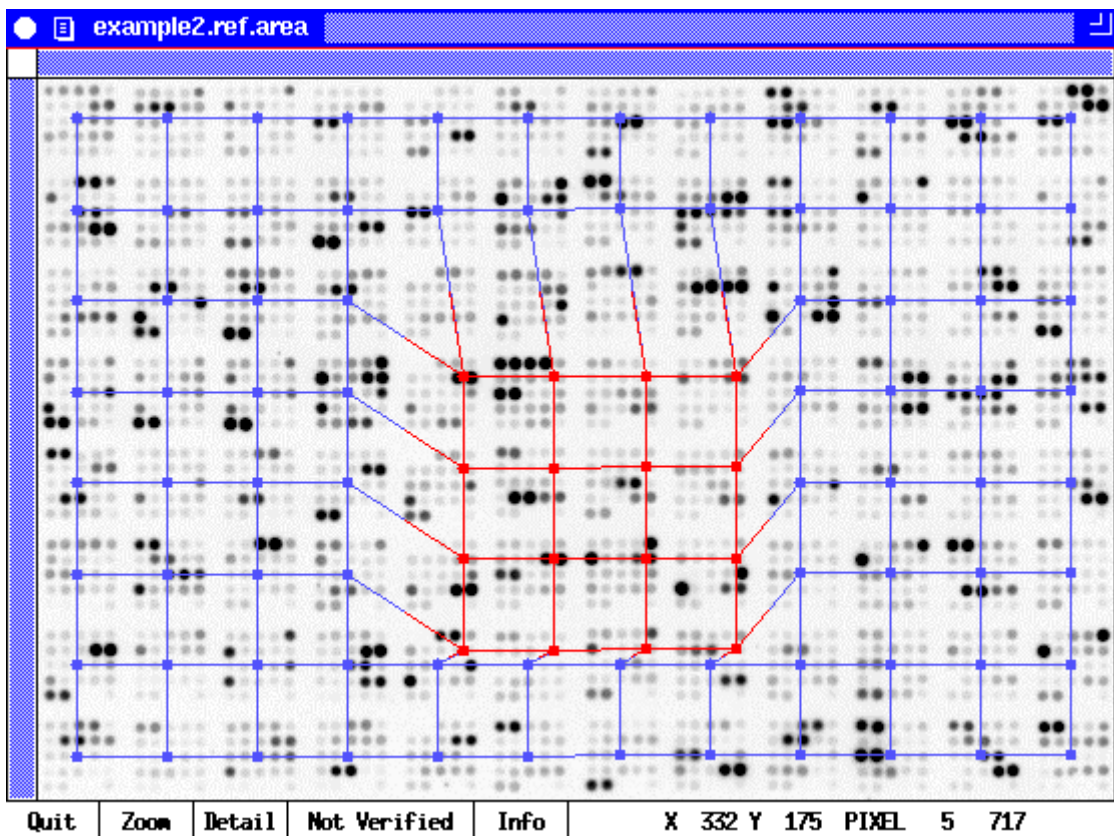
- Open a zone (here, the zone is extracted of example2) :



- Select Mode of Selection : Node through the Edit drop-down menu.
- Open the Grid menu.
- Select an indexing grid (The number 13, for example)
- Click on the Modification button.
- Click on the toggle button Visualization to set Modification.
- Click on the zone with the middle mouse button. Hold down and drag to delimit the zone with the middle mouse button : a red rectangle opens. Release the mouse button : the selected area becomes red :



- Click on the red rectangle, hold down and drag the group of nodes to the wanted location (This example is here just to illustrate, and is not realistic) :



Middle bar.

- ◆ Tools bar : Drop-down menu. Click on the button. The following menu appears :

Tools	
Cut	Control + d
Contrast	Control + c
Geometry	Control + g
Format	Control + f
Directory	Control + r
Help	Control + a

In the left column, six actions are proposed :

- Open the Cut menu.
- Open the Contrast menu.
- Open the Geometry menu.
- Open the Format menu.
- Open the Directory menu.
- Open the Help menu.

The corresponding shortcut keys are displayed in the right column.

These actions are symbolized by means of icons, locate below the Tools label, in the third bar of the main menu.

- ◆ Edit bar : Drop-down menu. Click on the button. The following menu appears :

Edit
Parameters
Grids
Spots

Three actions are proposed :

- Open the Parameters menu.
- Open the Grids menu
- Open the Slots menu.

These actions are symbolized by means of icons, locate below the Tools label, in the inferior bar of the main menu.

- ◆ Computation bar : Drop-down menu. Click on the button. The following menu appears :

Computation

The proposed action is to compute. This action is symbolized by means of an icon, locate below the Computation label, in the inferior bar of the main menu.

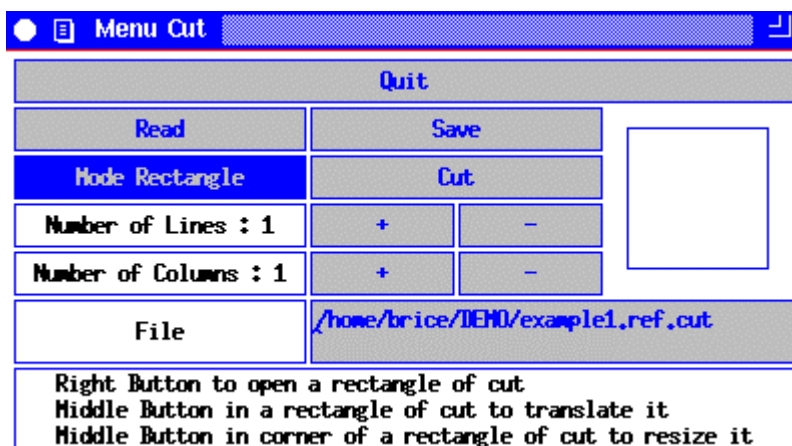
Inferior bar.

Cut menu.

The Cut menu allows to draw and save the lines to delimit the zones to analyze, and to cut images for archiving. To use this menu, you must open an image before, and click on with the left mouse button to select it.

A zones boundaries filename has the extension **.cut**

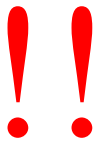
An archiving filename has the extension **_name of the zone.ref**



- ◆ Quit button : to close the menu. If no saving done previously, the zones boundaries are not saved.
- ◆ Read button : to display saved zones boundaries on the image. Only one zones boundaries file is linked with an image file. To activate this function, it's necessary to open previously an image.
- ◆ Save button : to save the current boundaries zones in a file. If boundaries zones have been saved previously, the file is overwritten. Moreover, all the analysis files are destroyed to keep the data coherency, because results depends of boundaries zones.

- ◆ Toggle button of modes : Two modes are available : the Rectangle mode and the Point mode. The rectangle mode allows to draw one or several rectangular zones. The point mode allows to draw one or several quadrilateral zones.

- ◆ Cut button : This button allows to save the data zones according to the boundaries zones.



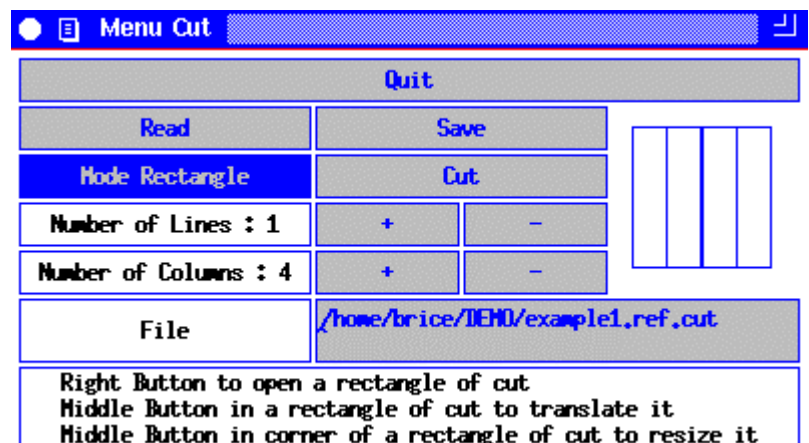
Notice that the Cut button is not useful to compute grids and spots : it's only for archiving. A new file is created : the dates inside the boundaries zones are duplicated, and so, the free disk space is reduced.

- ◆ Number of Lines button , and Number of Columns button : Although it's possible to draw the rectangles (or quadrilaterals) one by one, it's faster to draw several rectangles (or quadrilaterals) at once. The + and - buttons allow to set the rectangles (or quadrilaterals) number. Click on the image with the **right** button. Hold down and drag to get the chosen size.

- Number of Lines : to define the zones number in rows.
- Number of Columns : to define the zones number in columns

- ◆ Skeleton display area : This area displays the number and the scheduling of the quadrilaterals or rectangles.

Example : Viewing of the Cut menu, after have set one lines and four columns, in the rectangle mode.



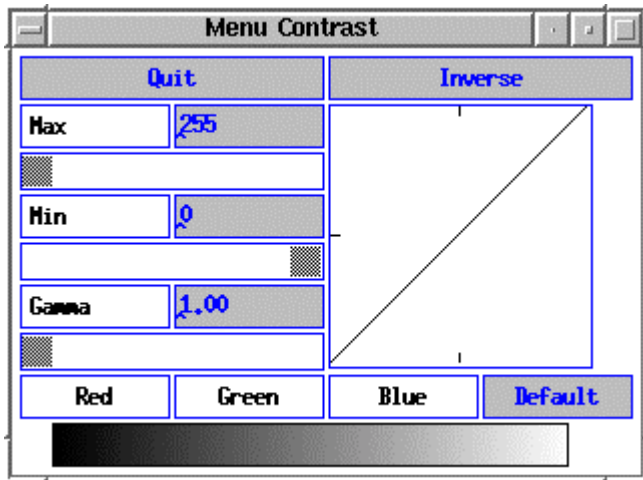
- ◆ File field : facing the label File, the path and the name of the zones boundaries file is displayed. If the path is too long, click on the horizontal lift, to read the whole name.

- ◆ Display area : recalling of the principal functions to can draw the zones boundaries.

To draw rectangles/quadrilaterals :

- To create zones boundaries : Click on the image with the **right** button. Hold down and drag : a blue rectangle is drawn. Release when the wanted size is reached.
- To translate zones boundaries : Click on the zone with the **middle** button, and hold down : the zones boundaries become red. Drag to translate. Release when the wanted position is reached.
- To resize zones boundaries : Click on the zone with the **middle** button : the lines become red. Click near a corner with the middle button, hold down and drag. Release when the wanted size is reached.
- To delete zones boundaries : Click on the zone with the **right** button : lines become red and a menu appears :

Contrast menu.

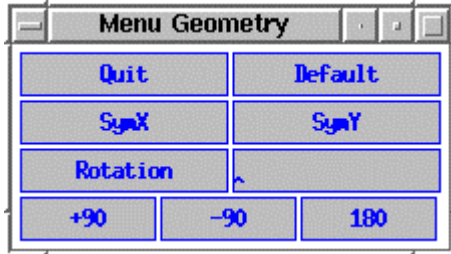


This menu allows to adjust contrast with grey levels or false colors.

By default, the contrast function is linear : the grey levels of the displayed pixels correspond to the saving levels of raw data. On the other hand, whatever the raw data coding, the displayed data coding is done with 8 bits, i.e. with 256 different values at most.

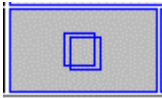
- ◆ Quit button : to close the menu.
- ◆ Inverse button : to inverse the contrast function : The minimum level of raw data is coded in white, and the maximum level is coded in black.
- ◆ Max slider : to assign the black level to a given value, if Max value superior to Min value. If the Max value is superior to the Min value, the data values higher than Max have the black level. If the Max value is inferior to the Min value, the data values lower than Max value have the white level. Max value varies between 0 and 255.
- ◆ Min slider : to assign the white level to a given value, if Min value inferior to Max value. Min value varies between 0 and 255.
- ◆ Gamma slider : To modify the contrast function between Min value and Max value.
- ◆ Contrast function area : display the contrast function : the x-axis is the raw data values, and the y-axis is the grey levels.
- ◆ Red / Green / Blue buttons : to suppress a component color (RGB system).
- ◆ Default button : Min value : 0, Max value : 255, linear function, and grey levels scale.
- ◆ Levels bar : display the pixel value or the pixel color according to the data value.

The Geometry menu.



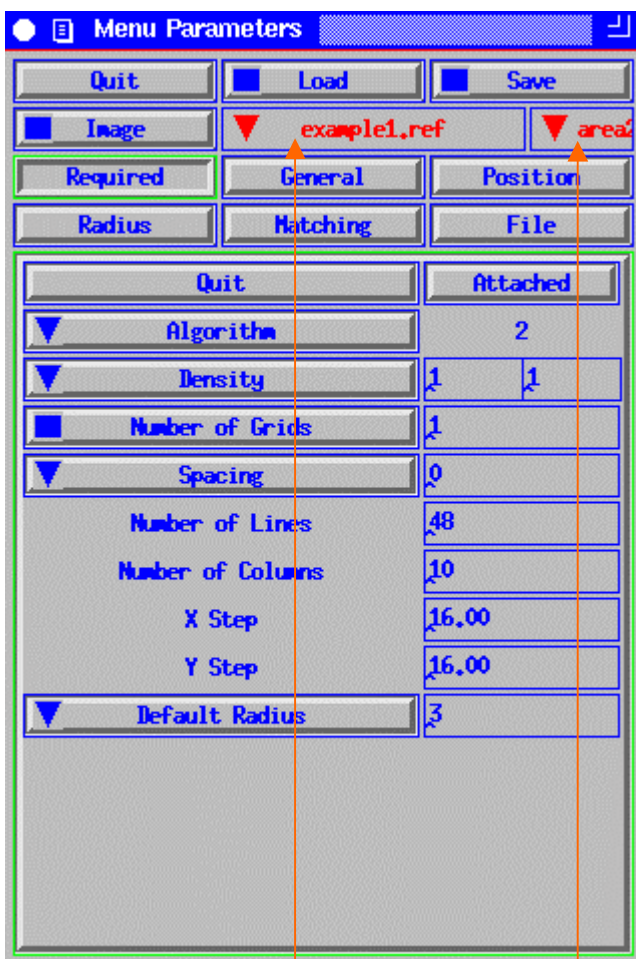
- ♦ Quit button : to close this menu.
- ♦ Button Default : return to the initial configuration.
- ♦ Button SymX : compute and display a symmetrical image relatively to the x-axis.
- ♦ Button SymY : compute and display a symmetrical image relatively to the y-axis.
- ♦ Input field for rotation angle : Key-in, in degree unit, the trigonometric rotation angle.
- ♦ Button +90 : compute and display an image with a trigonometric rotation of 90° , the center being the image upper left corner.
- ♦ Button -90 : compute and display an image with an antitrigonometric rotation of 90° , the center being the image upper left corner.
- ♦ Button 180 : compute and display an image with a rotation of 180° , the rotation center being the image upper left corner.

Parameters menu.



To open the Parameters menu, click on this icon in the XDotsReader menu.

If you activate an image (open an image, an click on it), and if a computation has been realized previously, the Parameters menu displays the last parameters used, and the X Step and Y Step values estimated by the software.



Quit button : To close the Parameters menu.

Load button : load the saved parameters. Click on this button. A Selection menu appears (See generic menu part). The configuration files list appears (.**cfg** extension). Double-click on the chosen file. If it doesn't exist configuration file, fill the required parameters fields, set the other parameters, and click on the Save button.

Save button : Save in a configuration file the set parameters. Click on the Save button. A Selection menu appears (See generic menu part) For a release, double-click on the chosen configuration filename. For a creation, select the directory, and key in the filename with the .**cfg** extension. Then, keystroke the return key.

Image button : to select an image, if no image is opened. Click on the button. A Selection menu opens.

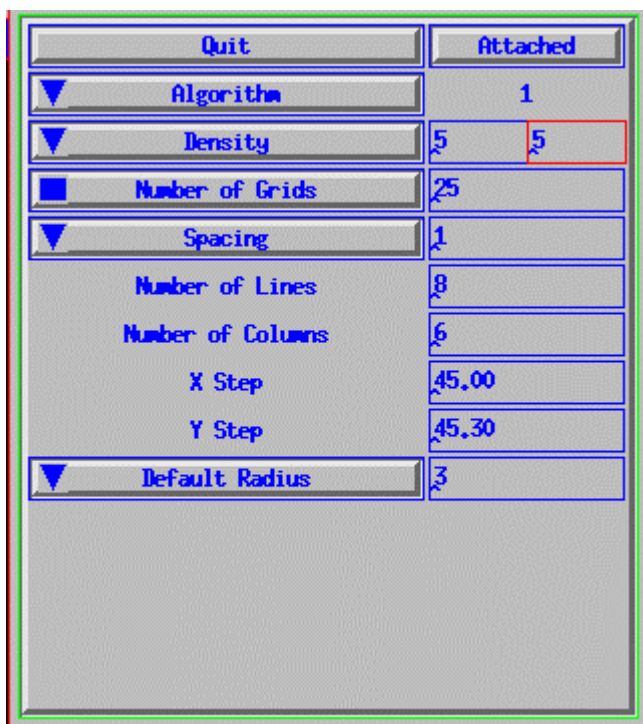
When an image is selected, the image name displays in the drop-down menu, on the right. To select another image in the same directory, open the drop-down menu and select it.

By default, a zone name is displayed in the second drop-down menu (on the right) and selected. To change the zone, open the drop-down menu, and select a new.

Required sub-menu.



By default, this sub-menu is displayed for the Parameters menu opening. When another sub-menu is opened, click on this button to return to the Required sub-menu.



♦ Button Quit : to close the sub-menu Required.

♦ Toggle button Attached/Detached :

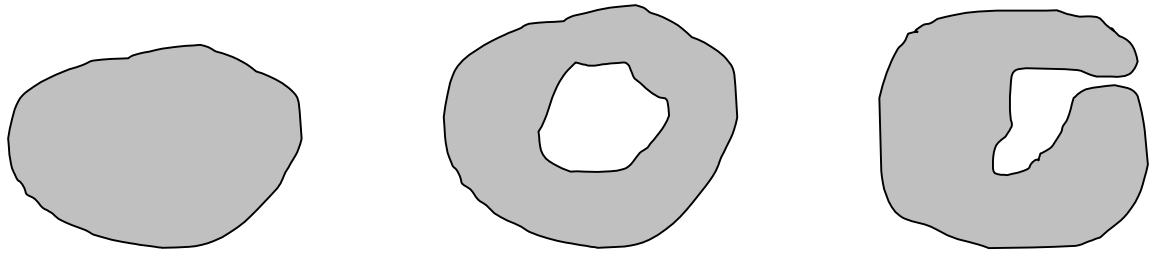
Attached : Means that the sub-menu Required is attached to the Parameters menu. To detach the sub-menu, click on Attached.

Detached : Means that the sub-menu Required can be moved, independently of the Parameters menu. To move the sub-menu, click on its title bar, hold on and drag. The Parameters menu can be close, without closing the sub-menu Required. To attach the sub-menu again, click on Detached.

♦ Drop-down menu Algorithm : To set the algorithm type used for the analysis. To chose an algorithm type, click on the menu, hold down, and drag to select. Two main algorithms are available, with additional constraints options. The both main algorithms are :

- Algorithm 1 : The spots detection is based on the hypothesis that the spots intensity distribution is Gaussian. Then, the indexing grid is set according to detected spots.
This algorithm is adapted for a gaussian spots intensities distribution (regular shape), not too noisy.
- Algorithm 2 : The spots detection is based on a morphological processing. There is no hypothesis about the spots intensities distribution. The Algorithm 2 is more robust than Algorithm 1; but slower.

- Algorithm 3 : The spots detection is based on the contour detection. This algorithm allows to detect ellipsoidal spots, with an irregular intensity distribution (as "hollow" spots). It's preferable the spots have a radius superior to 4 pixels. For example, the following spots may be detected :



- Additional constraints :

- Label : 1 constraint :

Algorithm 1 with constraint : the indexing grid is set independently of the detected spots located. Then, a processing merges the results, and gives a new spots mapping. This algorithm is adapted for **few regular shape spots**, and for an intensities distribution not too noisy.

- Label : 1 strong constraint :

Algorithm 1 with strong constraint : The algorithm search only **small** spots. The indexing grid is set independently of the detected spots located. Then, a processing merges the results, and gives a new spots mapping. This algorithm is adapted for **few regular shape small spots**, and for an intensities distribution not too noisy.

- Label : 1 with pairs :

Algorithm 1 used the fact there is at least a spots pair inside each block. This algorithm is only use with specific images with very few spots.

- Label : 2 constraint 1.1

Algorithm 2 with constraint : the indexing grid is set independently of the detected spots located. Then, a processing merges the results, and gives a new spots mapping. This algorithm is adapted for **few irregular shape spots**.

- Label : 2 constraint 2.1

Algorithm 2 with strong constraint : The algorithm search only **small** spots. The indexing grid is set independently of the detected spots located. Then, a processing merges the results, and gives a new spots mapping. This algorithm is adapted for **few irregular shape small spots**.

- Label : 2 constraint 1.2

Algorithm 2 with strong constraint : The indexing grid is set independently of the detected spots located. Then, a processing merges the results, but **only** around the indexing grid intersection points. A new spots mapping is given. This algorithm is adapted for **very few irregular shape spots**. This constraint option is faster than constraint 1.1

- Label : 2 constraint 2.1

Algorithm 2 with strong constraint : The algorithm search only **small** spots. The indexing grid is set independently of the detected spots located. Then, a processing merges the results, but **only** around the indexing grid intersection points. A new spots mapping is given. This algorithm is adapted for **very few irregular shape small spots**. This constraint option is faster than constraint 1.2.

- Label : 2 with pairs :

Algorithm 2 used the fact there is at least a spots pair inside each block. This algorithm is only use with specific images with very few spots.

- Label : 3 constraint :

Algorithm 3 with constraint : the indexing grid is set independently of the detected spots located. Then, a processing merges the results, and gives a new spots mapping. This algorithm is adapted for **few regular shape spots**.

- Label : 3 strong constraint :

Algorithm 3 with strong constraint : The algorithm search only **small** spots. The indexing grid is set independently of the detected spots located. Then, a processing merges the results, and gives a new spots mapping. This algorithm is adapted for **few regular shape small spots**.

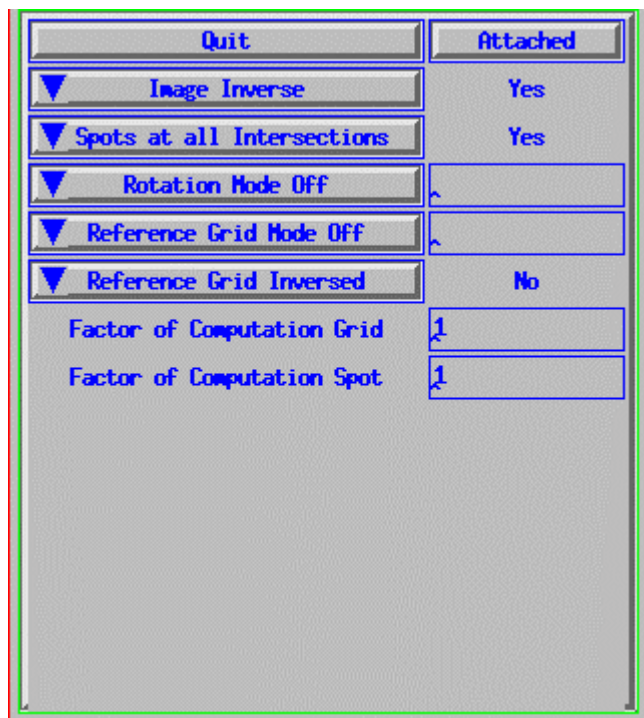
- ◆ Drop-down menu Density : To set the density parameter. The choices are : 1x1, 2x2, 5, 8, 3x3, 4x4, 5x5, 6x6. When the choice is done, the field Number of Grids is automatically filled with the maximum value of grids, i.e. a value equal to the density. For instance, the density choice 5x5 give a grid number equal to 25.
- ◆ Input Fields (facing drop-down menu Density) : It's possible to enter directly the density. Set the mouse pointer on a field, and key in (the first field corresponds to the block lines number, and the second field correspond to the block columns number). When the fields are filled, and when the mouse pointer go out of the fields, the field Number of Grids is automatically filled with the maximum value of grids
- ◆ Button Number of Grids : to display the block geometry. Fill before the field facing Number of Grids.
- ◆ Field Number of Grids : To enter the grid parameter. This number must be inferior or equal to density parameter. When the both fields are filled, the spots are horizontally and vertically aligned.
- ◆ Drop-down menu Spacing : To set the spacing parameter. The choices are 0 or 1.
- ◆ Field Number of lines : To set the blocks rows number used by the software analysis. If you don't know the blocks rows number, open the zone to analyze, and count the rows. If the window is shorter than the image, count the rows from top to bottom of the window. Set the mouse pointer on the last row (same spot position), and note the Y-coordinate, displayed in the bar on the bottom of the window. With the vertical lift, visualize the bottom of the image. Find again the last counted row with the Y-coordinate, and ended the counting.
- ◆ Field Number of columns : To set the blocks columns number used by the software analysis. If you don't know the blocks columns number, open the zone to analyze, and count the blocks columns. If the window is more narrow than the image, count the blocks columns from left to right of the window. Set the mouse pointer on the last blocks column (same spot position), and note the X-coordinate, displayed in the bar on the bottom of the window. With the horizontal lift, visualize the right of the image. Find again the last counted blocks column with the X-coordinate, and ended the counting.

- ◆ **Input Field X Step** : To set the pixels number between two blocks in a blocks row. To estimate the distance between two blocks, place the mouse pointer on a block spot. Note the X-coordinate displayed in the bar on the bottom of the window, and set the mouse pointer on the next right block (same spot position). The difference give the X Step in pixel unit.
- ◆ **Input Field Y Step** : To set the pixel number between two blocks in a blocks column. To estimate the distance between two blocks, place the mouse pointer on a block spot. Note the Y-coordinate displayed in the bar on the bottom of the window, and set the mouse pointer on the next below block (same spot position). The difference give the Y Step in pixel unit.
- ◆ **Drop-down menu : Default Radius** : To set the radius default used in the mode Spots at all Intersections (General button in the Parameters menu). The choices are from 2 to 10.
- ◆ **Input Field : (facing drop-down menu Default Radius)** : It's possible to enter directly an integer value (even superior to 10).

General sub-menu



Click on this button. The General sub-menu displays in the Parameters menu. Some of these parameters are imperative to get coherent results.



♦ Button Quit : to close the sub-menu General.

♦ Toggle button Attached/Detached :

Attached : Means that the sub-menu General is attached to the Parameters menu. To detach the sub-menu, click on Attached.

Detached : Means that the sub-menu General can be moved, independently of the Parameters menu. To move the sub-menu, click on its title bar, hold on and drag. The Parameters menu can be close, without closing the sub-menu General. To attach the sub-menu again, click on Detached.

♦ Drop-down menu Image Inverse : the two choices are :

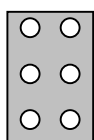


Image Inversion Forced : The software searches light spots upon dark background. Indicator Yes is set facing the drop-down menu.

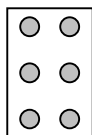


Image Inversion Forbidden : The software searches dark spots upon light background. Indicator No is set facing the drop-down menu.



The contrast adjustment, especially the Inverse button, has no effect on this function. The software works on the raw data.

♦ Drop-down menu Spots at all Intersections. The two choices are :

- Spots at all Intersection : If the software doesn't detect spot around a grid intersection, a spot is created : the center is the intersection, and the radius is the default radius, defined in the sub-menu Radius, accessible in the Parameters menu. Indicator Yes is set facing the drop-down menu.
- Automatic Spots : The software sets a detection circle around a grid intersection, only if a spot is detected around a grid intersection. Indicator No is set facing the drop-down menu.

♦ Drop-down menu Rotation Mode. The two choices are :

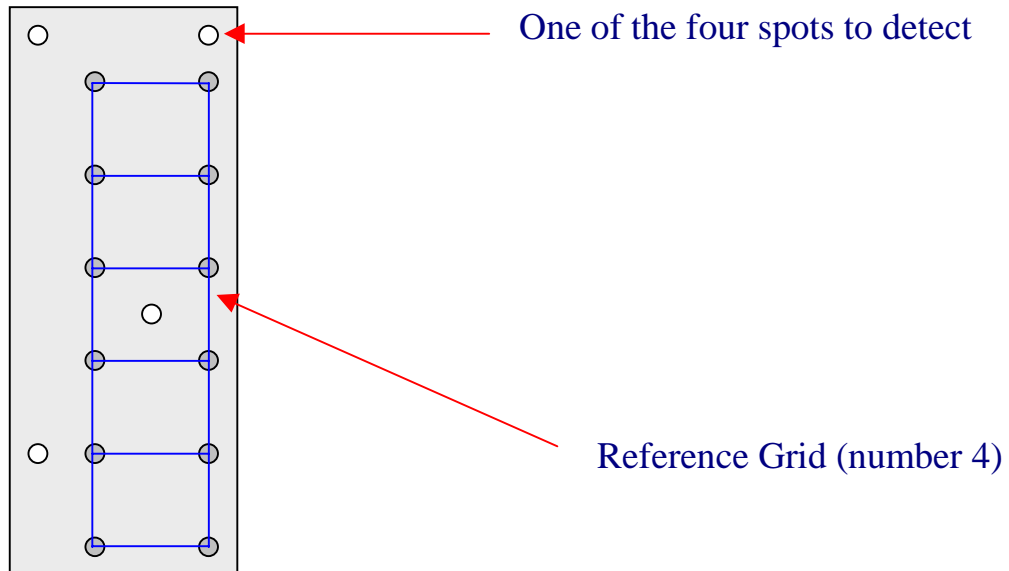
- Rotation Mode Off : The software values the angle between the grid and the image direction. Then, grids computation and spots detection are worked out according this parameter.
- Rotation Mode On : If the angle grid with the image direction is great (over 10°), it's better to specify an image rotation angle. Click on the field on the right of the drop-down menu. The frame becomes red. Key in the angle value.

♦ Drop-down menu Reference Grid Mode The two choices are :

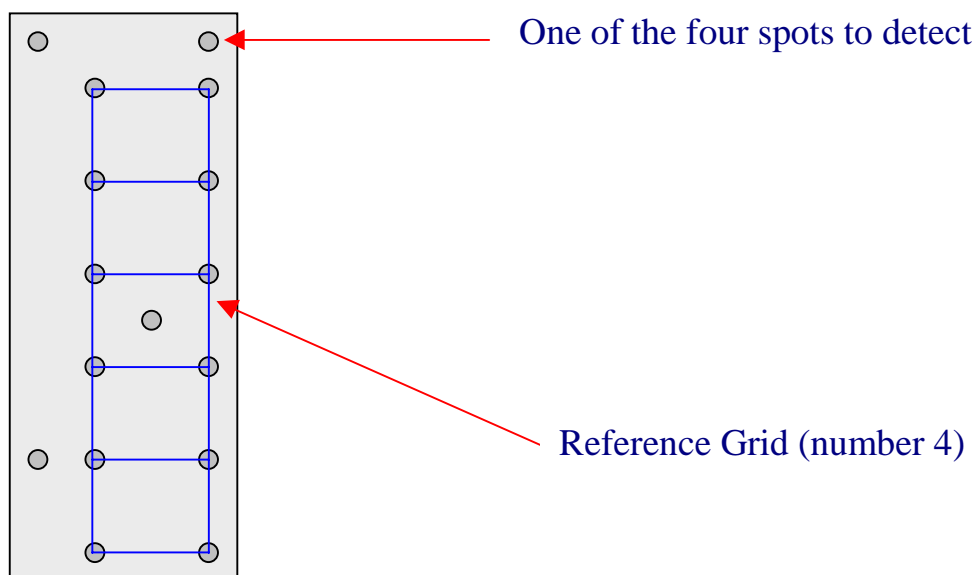
- Reference Grid Mode On : A reference grid is present. If there are few spots excepted the spots assigned to the reference grid, this option is necessary to built the others indexing grids. (An example is exposed at the end of this sub-menu description).
- Reference Grid Off : There is no reference grid.

- ◆ Drop-down menu Reference Grid Inversed (an example is exposed at the end of this sub-menu description). the two choices are :

- Reference Grid Inversed : Indicator Yes is set facing the drop-down menu. The spots of the reference grid are inverted in relation with the spots to detect (light reference spots and dark spots to detect, or dark reference spot and light spots to detect). Illustration : density : 4; grid : 4; reference grid number : 4



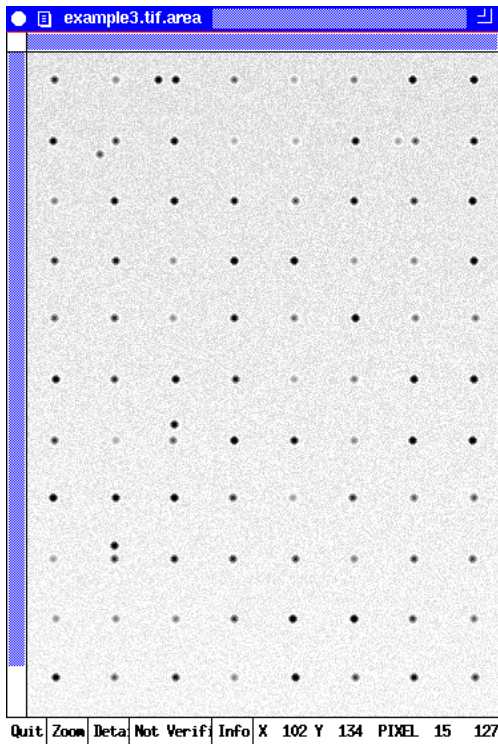
- Reference Grid not Inversed : Indicator No is set facing the drop-down menu. The spots of the reference grid are not inverted in relation with the spots to detect (reference spots and spots to detect are both light or dark) :



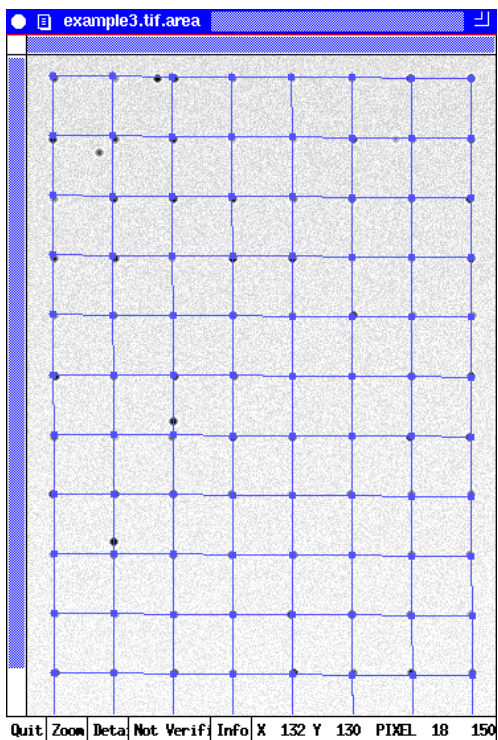
- ◆ Input field Factor of Computation Grid : Integer number. Use only with a constraint algorithm. Greater is the number, speedier is the algorithm, but less accurate is the result.
- ◆ Input field Factor of Computation Spot : Integer number. Use when the spots size is greater than 10 pixels. Greater is the number, speedier is the algorithm, but less accurate is the result.

An example with a reference grid.

- This example is an image with the following parameters : Density = 9; Grid = 9; Spacing = 1; Lines number = 12 Columns number = 8; Reference grid number = 5 :



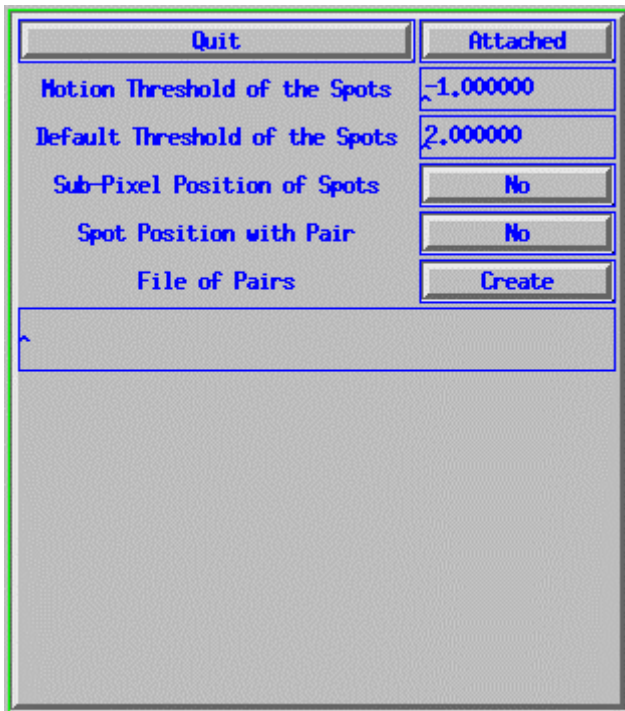
- After to compute, the zone displayed with the indexing grid number 5 give :



Position sub-menu.



Click on this button. The Position sub-menu displays in the Parameters menu.



- ♦ Quit : To close the Position sub-menu.
- ♦ Toggle button Attached/Detached :
 - Attached : Means that the sub-menu Position is attached to the Parameters menu. To detach the sub-menu, click on Attached.
 - Detached : Means that the sub-menu Position can be moved, independently of the Parameters menu. To move the sub-menu, click on its title bar, hold on and drag. The Parameters menu can be close, without closing the sub-menu Position. To attach the sub-menu again, click on Detached.

- ♦ Motion Threshold of the Spots : Detection window in pixel unit around the theoretical position, i.e. around an intersection grid. To detect a spot, the criteria set must be respected, inside the window. The value -1 or 0 means that the window size is adjusted by the software.
- ♦ Default Threshold of Spots : When no spot is detected around an intersection grid, the software try to set a spot inside a window defined by this parameter, according to the default radius.
- ♦ Toggle button Sub-Pixel Position of Spots :
 - Yes label : The spot center position is computed with a half-pixel precision.
 - No label : The spot center position is computed with a one pixel precision.

♦ Toggle button Spot Position with Pair :

- Yes : indicate that the detection algorithm must use the pairs information to compute spots. The algorithms to chose in the Parameter menu are Algorithm 1 with pairs, or Algorithm 2 with pairs. Use this option carefully (effective for very particular cases).
- No : the detection algorithm doesn't use the pairs information, even if there are pairs.

♦ Create button (facing File of Pairs label) : Open the Create Pair File menu :

1. Give the density
2. Give the number of areas
3. Give the current area number
4. Define pairs of spots with left button (Right button to destroy a pair)
5. Give the file name
6. Press the Create button

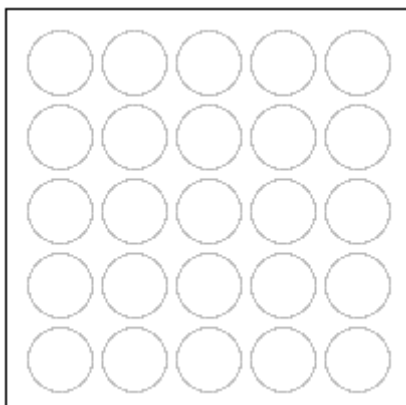
- Quit button : To close this menu.
- Create : create the file containing the pairs geometry characteristics, necessary for the specific algorithm using the pairs information.
- File of Pairs area : Enter the filename of the file containing the pairs geometry characteristics.
- Input field Density : Enter the density. When the density is entered, and when the mouse pointer go out this field, the area below display the schematized spots in a block. Click on the spots which constitute pairs. First click assigns a number (displayed inside the spot), and the second click assign the same number : a pair is defined.
- Input field Number of Areas : 1 ??
- Input field : Area Number : 1 ??
- Area to define the pairs geometry.

An example to create a pairs file for a specific algorithm using pairs.

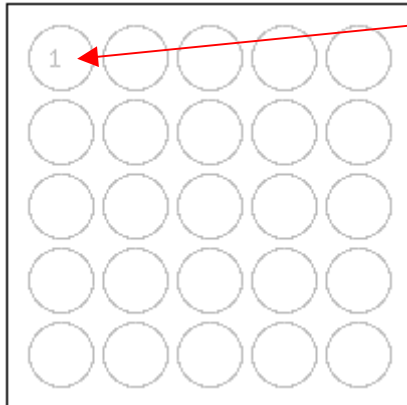
- ◆ Click on the Create button in the Position menu. The following menu appears :

1. Give the density
2. Give the number of areas
3. Give the current area number
4. Define pairs of spots with left button
(Right button to destroy a pair)
5. Give the file name
6. Press the Create button

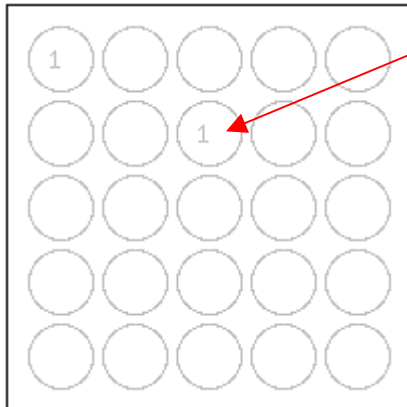
- Input the filename of the pairs geometry file, without extension (the extension **.pair** is automatically added.)
- Input the density parameter. For example : 25
- Input the Number of Areas and Area Number parameters (1 and 1 for example).



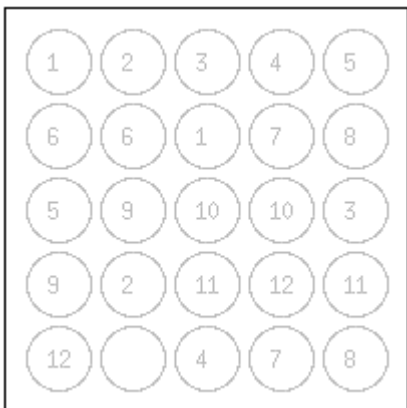
- Go out the mouse pointer : the area visualizes the schematized spots in a block (Here, the density is 25)



- Click on the first spot of the first pair. A number is assigned.



- Click on the second spot of the second pair. The same number is assigned.



- Begin again until all the spots pairs are specified.

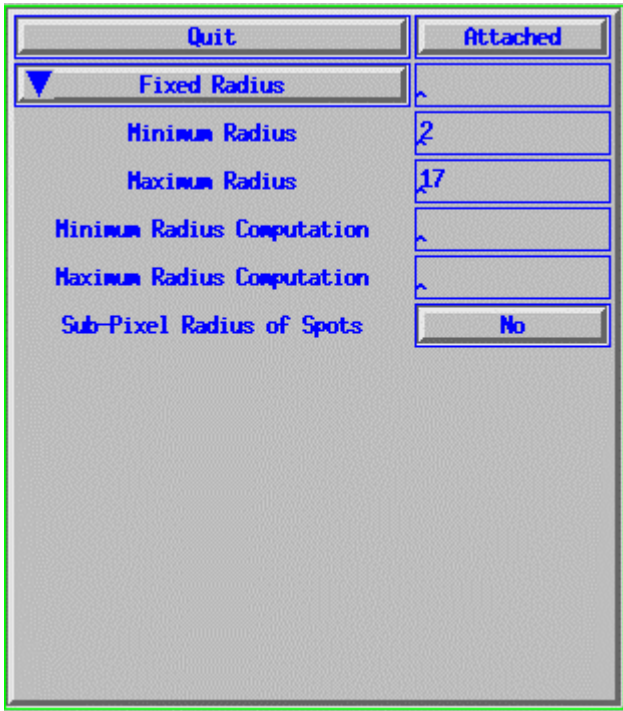


- Click on the Create button : The pairs geometry file is saved, and the filename appears in the Position sub-menu.

Radius sub-menu.



The software computes modeled spots (a center and a radius). This sub-menu defines the radius management.



- ♦ Quit button : to close this menu.
- ♦ Toggle button Attached/Detached :
 - Attached : Means that the sub-menu Position is attached to the Parameters menu. To detach the sub-menu, click on Attached.
 - Detached : Means that the sub-menu Position can be moved, independently of the Parameters menu. To move the sub-menu, click on its title bar, hold on and drag. The Parameters menu can be close, without closing the sub-menu Radius. To attach the sub-menu again, click on Detached.

- ♦ Drop-down menu Fixed Radius : The same radius is assigned for all the spots.
- ♦ Input field : Minimum Radius : Minimum radius of the modeled spot circle. If the computerized radius is inferior to Minimum Radius, the software sets the modeled spot radius to Minimum Radius.
- ♦ Input field : Maximum Radius : Maximum radius of the modeled spot circle. If the computerized radius is superior to Maximum Radius, the software sets the modeled spot radius to Maximum Radius.
- ♦ Input field : Minimum Radius Computation : Only used for algorithms of type 2. The morphological algorithm doesn't search spots which the radius are inferior to Minimum Radius Computation.

- ♦ Input field : Maximum Radius Computation : Only used for algorithms of type 2. The morphological algorithm doesn't search spots which the radius are superior to Maximum Radius Computation.
- ♦ Toggle button Sub-Pixel Radius of Spots :
 - Label No : The software computes a radius with a one pixel precision. (Readable with the option Complete List in Spots menu)
 - Label Yes : The software computes a radius with a half pixel precision. (Readable with the option Complete List in Spots menu).

Matching sub-menu.



When automatic analysis fails with all algorithms (this possibility may occur when there are few spots), the user can help the automatic process. The additional information given by the user are two constrained passing points, or a reference zone name, or a reference image name. Typically, this sub-menu is very useful when different measurements are made on the same membrane.

- ◆ Button Quit : To close the sub-menu Matching.
- ◆ Toggle button Attached/Detached :
 - Attached : Means that the sub-menu Position is attached to the Parameters menu. To detach the sub-menu, click on Attached.
 - Detached : Means that the sub-menu Matching can be moved, independently of the Parameters menu. To move the sub-menu, click on its title bar, hold on and drag. The Parameters menu can be close, without closing the sub-menu Matching. To attach the sub-menu again, click on Detached.

- ◆ Button First Point : Click on this button to enter the first point coordinates. To get these coordinates, click with the **middle** mouse button on the chosen point. The pixel coordinates displays facing Pixel label.
- ◆ Input field Grid : Key-in the indexing grid number (integer between 1 and the total grids number) of the selected first point.
- ◆ Input field Line : Key-in the line number of the selected first point (integer between 1 and the total lines number), for the specified indexing grid.
- ◆ Input field Column : Key-in the column number of the selected first point (integer between 1 and the total columns number), for the specified indexing grid.

- ◆ Button Second Point : Like First Point.

- ◆ Button Image of Reference : To select a reference image. When this button is clicked, a Selection menu appears. (See Selection menu section). Select the filename which displays in the field just below. The software uses all the grids of all the reference image zones to analyze the current image.

- ◆ Toggle button Yes/No : To accept the matching through a reference image.

- ◆ Button Zone of Reference : To select a reference zone. When this button is clicked, a Selection menu appears. (See Selection menu section). Select the filename which displays in the field just below. The software uses all the grids of the reference zone to analyze the current zone.

- ◆ Toggle button Yes/No : To accept the matching through a zone image.

- ◆ Toggle button Yes/No (facing Improvement of Points label)
 - No : The software searches the specified indexing grids, passing exactly by the both constrained points.
 - Yes : It's thought the constraint points are inside spots. So, the software search, according to the intensities distribution, the best constraint points. If a best solution is found, the new points are kept.

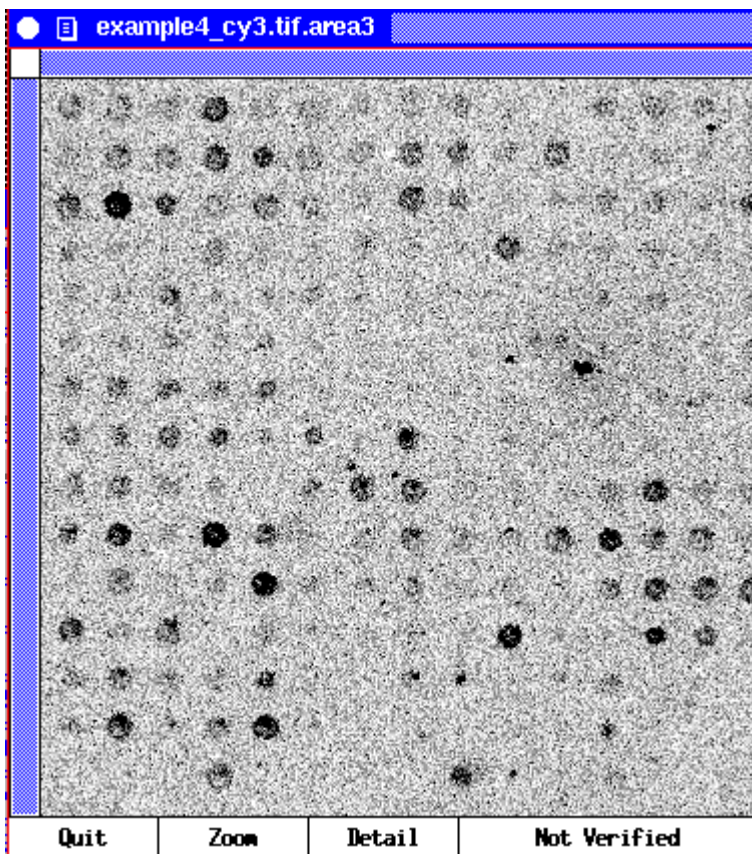
- ◆ Toggle button Yes/No : (facing : Improvements of Positions of Grids label) :
 - No : The software searches indexing grids. The indexing grids notified in the First Point field and in the Second Point field don't move.
 - Yes : The software searches indexing grids. The indexing grids notified in the First Point field and in the Second Point may move, according to the reference zones

♦ Toggle button Yes/No : (facing : Improvements of Positions of Spots label) :

- No : If the indexing grids are not improved, the spots remain fixed according to them.
- Yes: The software searches spots, according to the references zones. A typical use is to compare the same membrane before and after get a new treatment : The spots positioning remains the same, but the spots reaction differs.

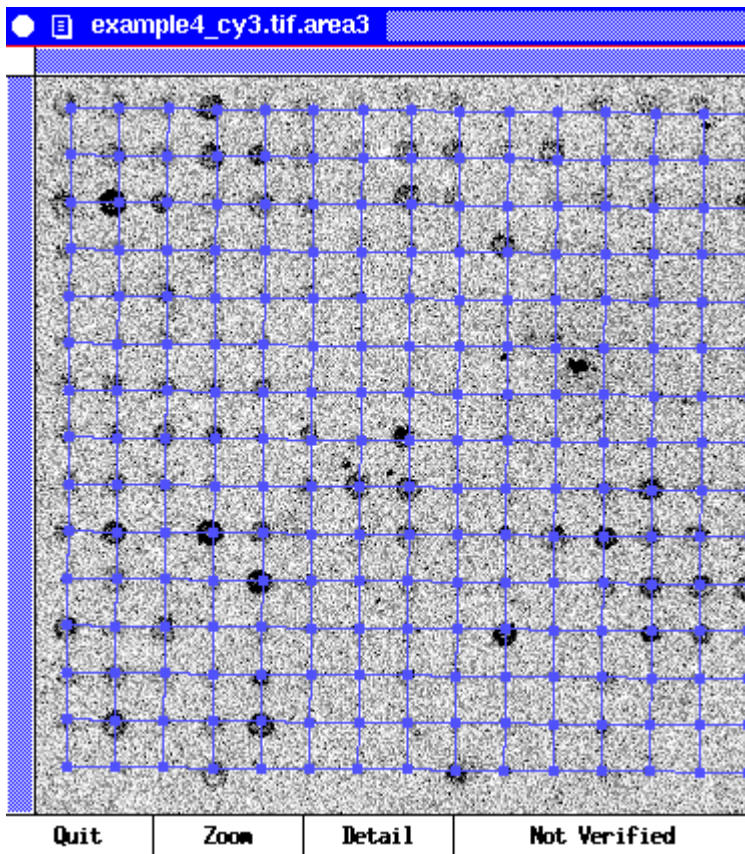
An example using a reference zone.

In this example, the membrane has been lighted successively with two different wavelengths : the membrane has exactly the same position in the both images. As the spots are more visible with the first image, an automatic search is applied, then, the founded indexing grids and spots are used for the second image :

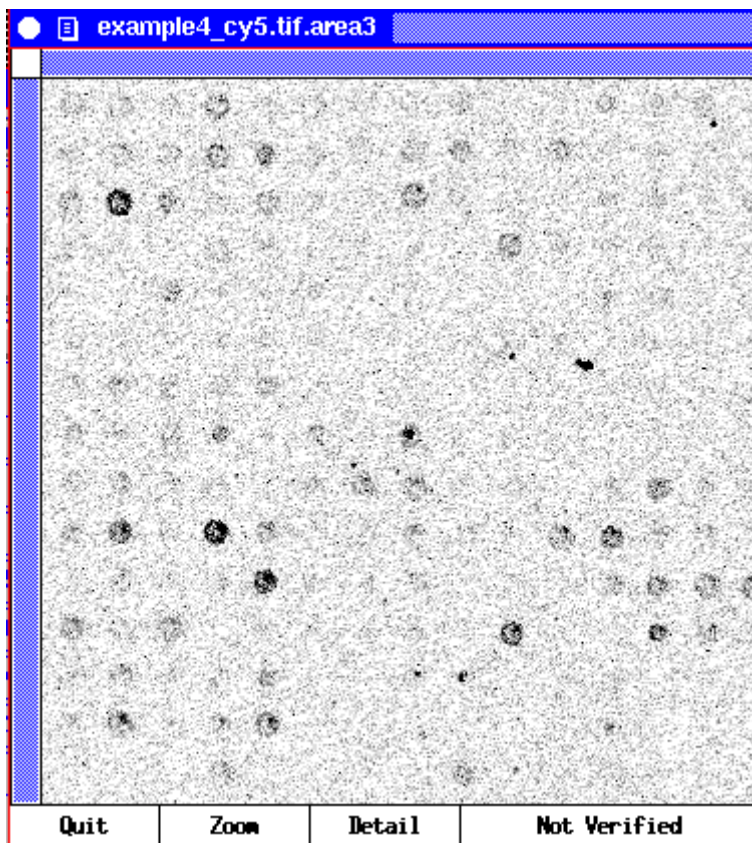


In the example4_cy3, the zone geometry is : Density 1, Grid 1; Spacing 0; Nb lines 15; Nb columns 36.

Algorithm2 is applied :

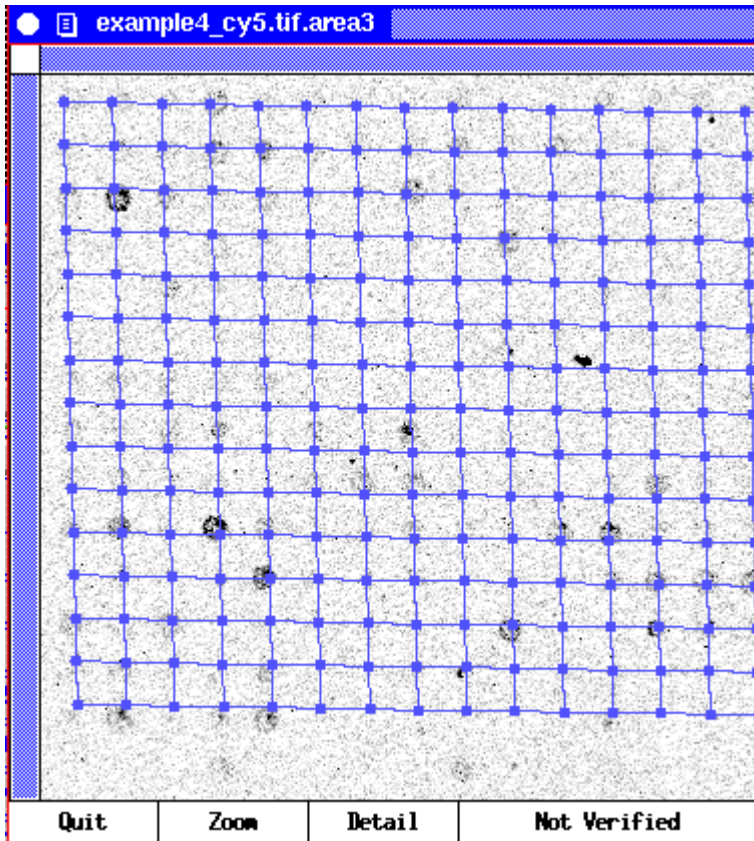


There are few spots, but the indexing grid is correctly positioned. Here, the right part of the image.

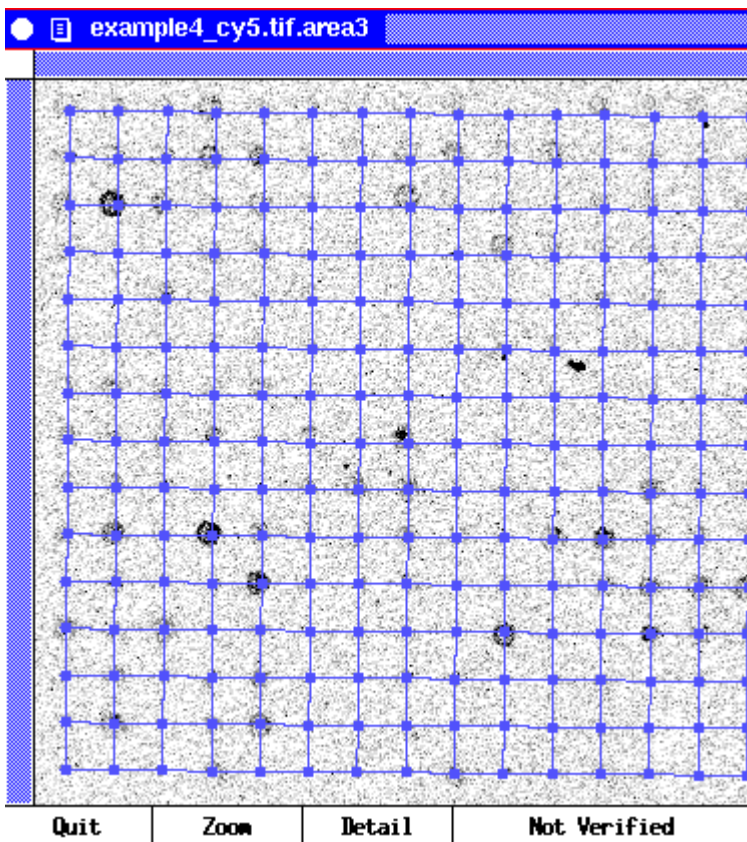


The spots of the image example4_cy5 are less visible.

The algorithm 2 is applied :



The algorithm 2 is not satisfactory.



Here, example4_cy3 has been taken as reference zone. To obtain this result, execute the following operations :

- Compute indexing grids and spots for example4_cy3.
- Fill the required parameters for example4_cy5.
- Select the reference zone in the Matching sub-menu, and set the toggle button to Yes.
- Set the three toggle buttons Improvement of Points, Improvement of Positions of Grids, Improvement of Positions of Spots to No.



- Click on Compute in Matching Mode in the Computation menu.

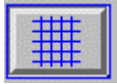
File sub-menu.



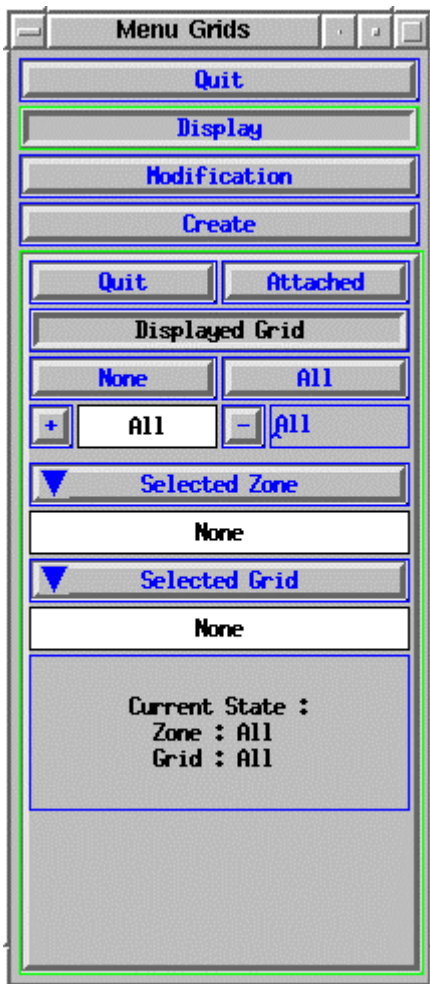
File of Command		Quit
Edit	XDots.Cmd	Save
^		
Analysis	Dots.Cmd	Save
^		
Automated Analysis		Automated Cutting
Directory	/home/brice/DEMO	<input checked="" type="radio"/> csh
Batch File Name	/home/brice/DEMO/Dots.Batch	Save
Type of Selection	<input checked="" type="radio"/> Chosen Selection	
List of the Directories	List of the Membranes	
^ ..	example1.ref.area1 example1.ref.area2 example1.ref.area3 example1.ref.area4	
List of the Selected Membranes		
^		
DotsReader -Experience \$Expe -Exposition \$Expo -Membrane \$Mb -FileOfCut \$Dir/\$Expe.\$Expo.cut -dirIn \$Dir -dirOut \$Dir -Format t -GridsComputation -Algorithm 1 -Density -1 -NumberOfGrids -1 -Spacing -1 -NumberOfStep -1 -1 -Step -1 -1 -MotionThreshold -1.0 -Force 1 -Inverse 1 -DefaultRadius 2 -DefaultThreshold 2.0 -MinRadius 2 -MaxRadius -1 -ZoomComputationGrid 1 -ZoomComputationSpot 1 -MinRadiusComputation -1 -MaxRadiusComputation -1 -SubPixelSpots 0 -SpotsPaire 0 -RotationComputation 0.000000 -ReferenceComputation -1 -GIFOutput 0 -Background 0 -Relative 0 -RelativeVal 0.0000 -CoordSpots 12 -Language us		

Grids menu.

To display or to modify the computed indexing grid (See Compute Grids and Spots section in the Computation menu), or, if the computation is not possible (very noisy zone, or very few spots), to create manually the indexing grids.



To open the Grids menu, click on this icon in the main menu. This menu appears automatically, when Compute Grids and Spots is run through the Computation menu.

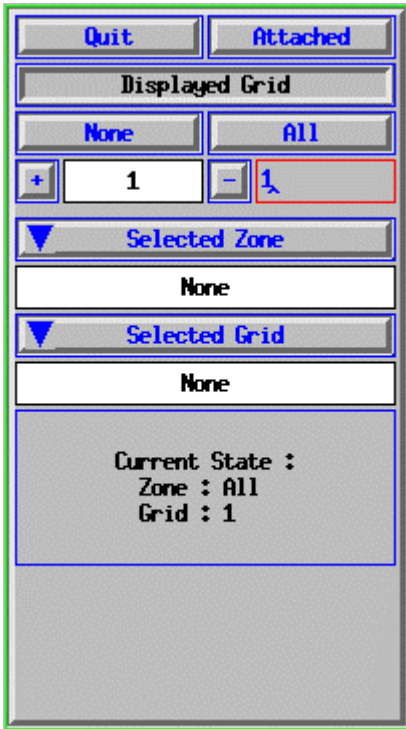


♦ Button Quit : To close the Grids menu.

Display sub-menu.



Button Display : sub-menu (displayed by default) : To visualize the indexing grids.



- ◆ Button Quit : to close the Display sub-menu.
- ◆ Toggle button Attached/Detached :
 - Attached : Means that the sub-menu Display is attached to the Grids menu. To detach the sub-menu, click on Attached.
 - Detached : Means that the sub-menu Display can be moved, independently of the Grids menu. To move the sub-menu, click on its title bar, hold on and drag. The Grids menu can be close, without closing the sub-menu Display. To attach the sub-menu again, click on Detached.

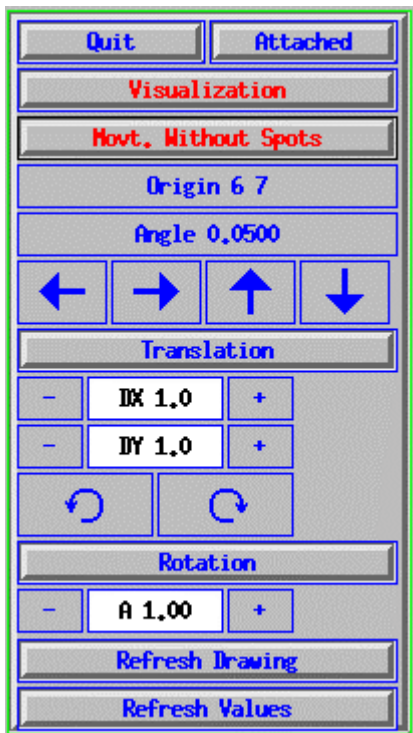
- ◆ Button None : No indexing grids in foreground. The information field below displays None.
- ◆ Button All : All the indexing grids are visualized in foreground.
- ◆ Toggle button "+" : To increment the indexing grid number to visualize.
- ◆ Display field : Displays the indexing grid number.
- ◆ Toggle button "-" : To decrement the indexing grid number to visualize.
- ◆ Input field : To key-in directly the indexing grid number to visualize.
- ◆ Drop-down menu Selected Zone : to select a zone number, or none zone, or all the zones, to display the associated grids.

- ◆ Display field : displays the selected zone number.
- ◆ Drop-down menu Selected Grid : to select one or all the indexing grids for modifications. The indexing grids take the red color.
- ◆ Display field : displays the selected fields number.
- ◆ Display field Current State : Display which are the visualized grids.

Modification sub-menu.



Button Modification : sub-menu : to modify position and direction indexing grid. To modify an indexing grid, it's necessary to select a grid through the sub-menu Display of the Grids menu.



♦ Button Quit : to close the sub-menu Modification.

♦ Toggle button Attached/Detached :

- Attached : Means that the sub-menu Modification is attached to the Grids menu. To detach the sub-menu, click on Attached.
- Detached : Means that the sub-menu Modification can be moved, independently of the Grids menu. To move the sub-menu, click on its title bar, hold on and drag. The Grids menu can be close, without closing the sub-menu Modification. To attach the sub-menu again, click on Detached.

♦ Toggle button Visualization/Modification :



Option by default : Translation and rotation buttons off. Click on Visualization to activate these buttons. The label Modification displays.



Translation and Rotation buttons on. Click on Modification to disactivate these buttons. The label Visualization displays.

♦ Toggle button Movt Without Spots / Movt With Spots :



Option by default : Translation and rotation are active only on the selected grids, and the associated spots are destroyed.



Translation and rotation are active on the selected grids and on the associated spots.

To active the following buttons, the toggle button Visualization/Modification must display :



- ♦ Field Origin : Display in pixel unit the selected indexing grid origin coordinates.
- ♦ Field Angle : Display in degrees the selected indexing grid angle, in relation to the initial position.



Click on this button to translate the selected indexing grid of one pixel on the left.



Click on this button to translate the selected indexing grid of one pixel on the right.



Click on this button to translate the selected indexing grid of one pixel on the top.



Click on this button to translate the selected indexing grid of one pixel on the bottom.

- ♦ Button Translation : To translate the indexing grid according to the translation vector (DX, DY), defined in the both fields below.
- ♦ DX step size : Click on "-" button to decrement the step size value DX.
Click on "+" button to increment the step size value DX.
- ♦ DY step size : Click on "-" button to decrement the step size value DY.
Click on "+" button to increment the step size value DY.



Click on this button to rotate the indexing grid of 0.1 degree anticlockwise.



Click on this button to rotate the indexing grid of 0.1 degree clockwise.

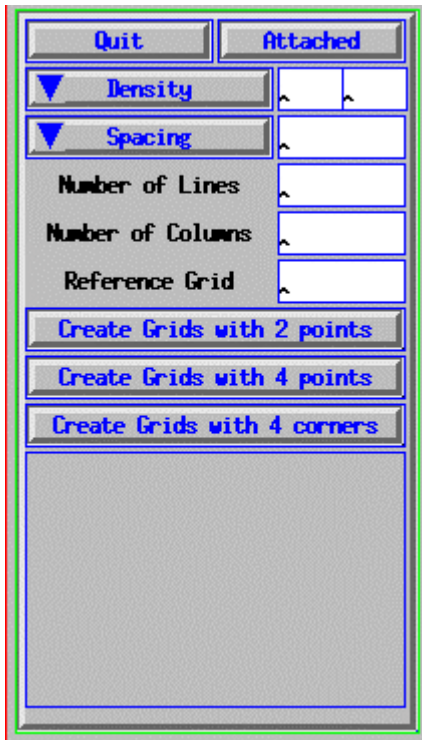
- ◆ **Button Rotation** : To rotate the indexing grid according to the angular step size A. By default, the rotation center is the origin of the first indexing grid (on the upper left-hand corner).
To change the rotation center, click on an intersection with the middle mouse button. A big red point appears : it's the new rotation center.
- ◆ **A angular step size** : Click on "-" button to decrement the angular step size (0.1 by 0.1 degree)
Click on "+" button to increment the angular step size (0.1 by 0.1 degree)
Click on "-" button to decrement the angular step size (0.1 by 0.1 degree)
- ◆ **Button : Refresh Drawing** : Click on this button when former image pieces are not clear upon the grid image.
- ◆ **Button : Refresh Value** : After a movement with the Movt With Spots option. Click on this button when former image spots are not clear

Create sub-menu.



Create : sub-menu : The user has the possibility to create the indexing grids, by defining manually one grid. To compute the spots in relation with these created indexing grids, use the Compute Spots of Displayed Grids function, in the Computation menu.

(An example is exposed at the end of this sub-menu description).



- ◆ Button Quit : to close the sub-menu Create.
- ◆ Toggle button Attached/Detached :
 - Attached : Means that the sub-menu Create is attached to the Grids menu. To detach the sub-menu, click on Attached.
 - Detached : Means that the sub-menu Create can be moved, independently of the Grids menu. To move the sub-menu, click on its title bar, hold down and drag. The Grids menu can be close, without closing the sub-menu Create. To attach the sub-menu again, click on Detached.

- ◆ Drop-down menu Density : Click on this drop-down menu. Hold down and drag to the chosen density. If the wanted density is not suggested, key-in it directly in the facing field.
- ◆ Display and input field for Density parameter : If the wanted density is not suggested in the drop-down menu, key-in it directly.
- ◆ Drop-down menu Spacing : Click on this drop-down menu. Hold down and drag to the chosen spacing.
- ◆ Display and input field for Spacing parameter : It's possible to key in directly. The only choices are 0 or 1.

- ◆ Input field Number of Lines : Key-in the blocks lines number of the zone.
- ◆ Input field Number of Columns : Key-in the blocks columns number of the zone.
- ◆ Input field Reference Grid : Key-in the grid number to create a given grid.

Create Grids with 2 points

Click on this button to create a grid by means of two points. According to the specified grid number, click on a spot of a grid corner, with the middle mouse button. Then, click on the opposite corner spot. All the others grids are computed and displayed. If the grids are not suitable, it's possible to destroy them with the functions Select All the Grids and Cut.

Create Grids with 4 points

Click on this button to create a grid by means of four points. This button is useful if the spots repartition is not straight. So, it's possible to define grids not rectangular. According to the specified grid number, click on a spot of a grid corner with the middle mouse button. Then, click on the others grid corners spots. All the others grids are computed and displayed. If the grids are not suitable, it's possible to destroy them with the functions Select All the Grids and Cut.

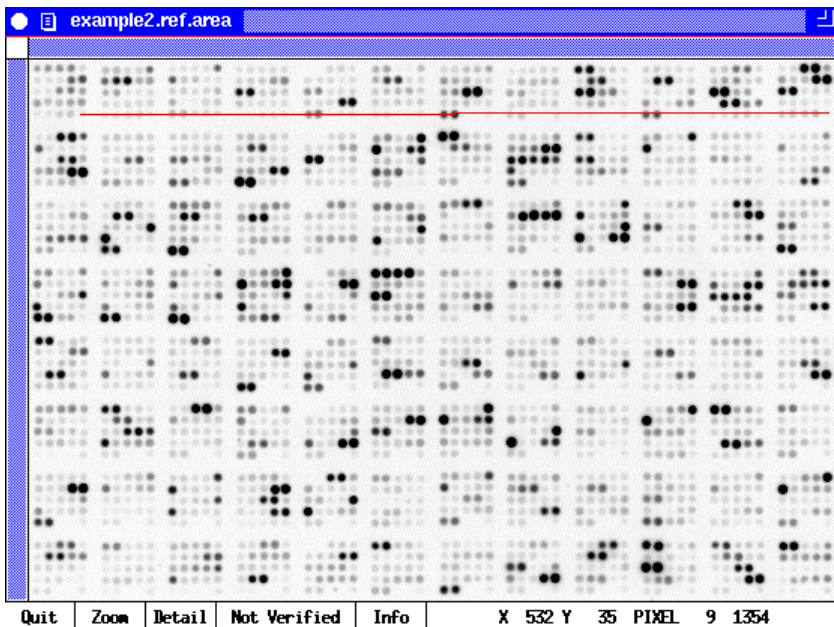
Create Grids with 4 corners

Click on this button to create grids by means of points of the four external corners. The specified grid number is useless in this case. Click on a spot of a grid corner with the middle mouse button. Then, click on the others corners spots. All the others grids are computed and displayed. If the grids are not suitable, it's possible to destroy them with the functions Select All the Grids and Cut.

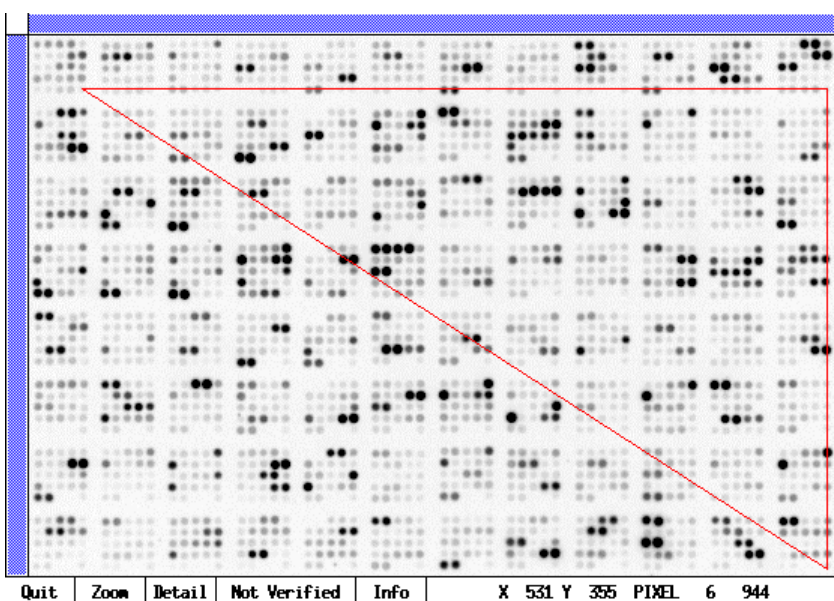
An example to create manually indexing grids.

Example with a zone which the density is 5x5. The chosen option is Create Grids with 4 points.

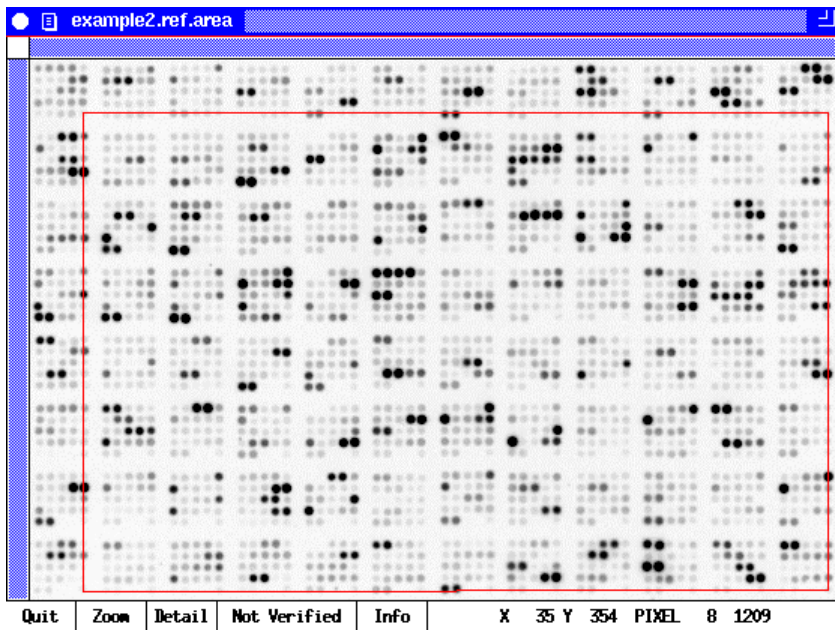
- ♦ Open a zone to analyze (Here, from the image named example2.ref). Select it : set on the mouse pointer, and click.
- ♦ Fill the parameters : Density = 5x5; Spacing = 1; Number of lines = 8; Number of Column = 12. We chose, for example, Reference Grid = 25; i.e., the last grid corresponding to the spot in the lower right-hand corner of a block.



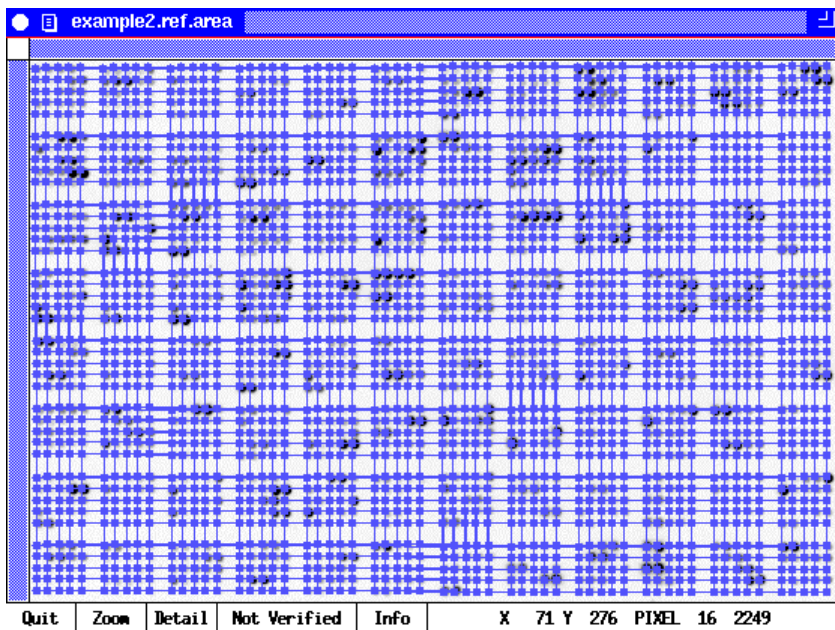
- ♦ Click on the button Create Grids with 4 points : the mouse pointer becomes a cross. Click with the **middle** mouse button on the last spot of the first block (on the upper left-hand corner, for example). Move to the upper right-hand corner. A red line appears. Click on the last spot of the block.



- ♦ Move to the lower right-hand corner. Click on the last spot of the block.

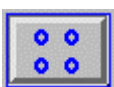


♦ Move to the lower left-hand corner.



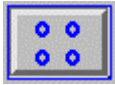
♦ Click on the last spot of the block.
The indexing grids are computed and displayed.

♦ To compute spots in relation to these indexing grids, open the Computation menu.
Click on the zone in the selection area to select it.



Then click on the icon Compute Spots of Displayed Grids.

Spots menu.



To open the Sspots menu, click on this icon in the main menu. This menu appears automatically, when Compute Sspots and Displayed Grids is run through the Computation menu. Furthermore, the zone appears with the modeled spots foreground.

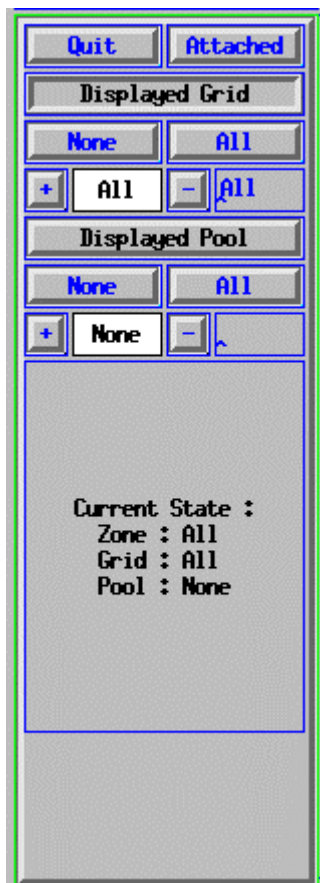
♦ Button Quit : to close the Sspots menu.

Menu Spots		
Quit	Display	Selection
Quantification	Background Level	Normalization
Coordinates	Modification	Verification
Reference	Characteristics	▼ Analysis Tools
Quit	Attached	
Displayed Grid		
None	All	
+ All	- All	
Displayed Pool		
None	All	
+ None	-	
Current State : Zone : All Grid : All Pool : None		
0.0	144.6	480
Low	Medium	Strong
153	148	179
0.0000	42.9864	64.2585
Positive Spots		

Display sub-menu.



By default, this sub-menu is displayed in the left pad :



- ◆ Button Quit : to close this sub-menu.
- ◆ Toggle button Attached/Detached :
 - Attached : Means that the sub-menu Display is attached to the Spots menu. To detach the sub-menu, click on Attached.
 - Detached : Means that the sub-menu Display can be moved, independently of the Spots menu. To move the sub-menu, click on its title bar, hold on and drag. The Spots menu can be close, without closing the sub-menu Display. To attach the sub-menu again, click on Detached.
- ◆ Button Displayed Grid : (default option). Display the spots in relation with the indexing grids. To click on this button disables the Displayed Pool button.
- ◆ Button None : remove the spots display.
- ◆ Button All : (default option) Display the spots of all the indexing grids.
- ◆ Button "+" : Increase the selected indexing grid number, and display only the corresponding spots.
- ◆ Button "-" : Decrease the selected indexing grid number, and display only the corresponding spots.
- ◆ Input field All : To key-in directly the indexing grid number.

- ◆ Button Displayed Pool : Display the spots in relation with the spots pool. To click on this button disables the Displayed Grid button.
- ◆ Button None : remove the spots display.
- ◆ Button All : (default option) Display the spots of all spots pools.
- ◆ Button "+" : Increase the selected spots pool number, and display only the corresponding spots.
- ◆ Button "-" : Decrease the selected spots pool number, and display only the corresponding spots.
- ◆ Input field All : To key-in directly the spots pool number.

Selection sub-menu.

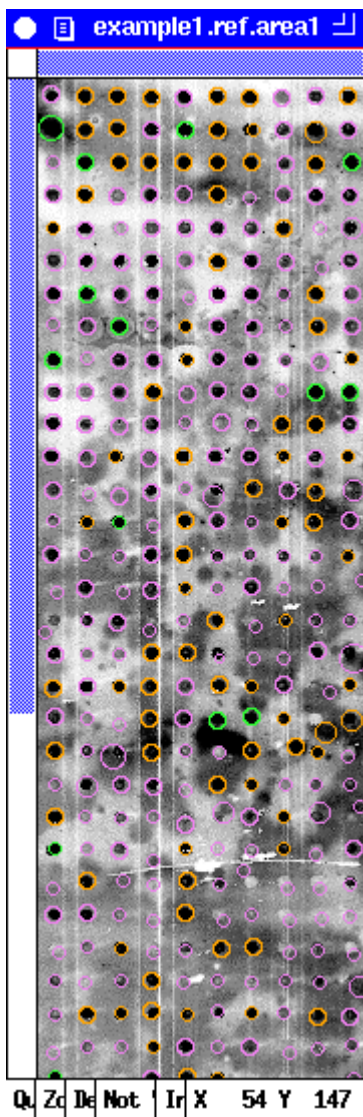


By default, this sub-menu is displayed in the right pad :

Quit		Attached
0,0	144,6	480
Low	Medium	Strong
153	148	179
0,0000	42,9864	64,2585
▼ Positive Spots		

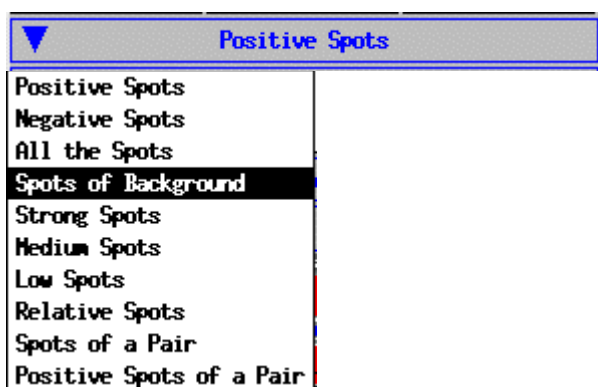
- ♦ Button Quit : to close this sub-menu.
- ♦ Toggle button Attached/Detached :
 - Attached : Means that the sub-menu Selection is attached to the Spots menu. To detach the sub-menu, click on Attached.
 - Detached : Means that the sub-menu Selection can be moved, independently of the Spots menu. To move the sub-menu, click on its title bar, hold on and drag. The Spots menu can be close, without closing the sub-menu Selection. To attach the sub-menu again, click on Detached.
- ♦ Display bar :
 - First cell : Minimum spot value.
 - Second cell : Maximum spot value.
 - Third cell : Total spots number.

- ♦ Low column : column concerning low spots. A low spot is a spot which has a value superior to the low threshold, and inferior to the medium threshold.
 - First cell : low spots number.
 - Second cell : low threshold value.
 - Third cell : pink cursor to modify the low threshold value. On the zone image, the modeled spots have a pink color. To move the cursor, click on the cursor, hold down the mouse button, and drag.



- ♦ Medium column : column concerning medium spots. A medium spot is a spot which has a value superior to the medium threshold, and inferior to the superior threshold.
 - First cell : medium spots number.
 - Second cell : medium threshold value.
 - Third cell : orange cursor to modify the medium threshold value. On the zone image, the modeled spots have an orange color. To move the cursor, click on the cursor, hold on the mouse button, and drag.

- ♦ Strong column : column concerning strong spots. A strong spot is a spot which has a value superior to the medium threshold.
 - First cell : strong spots number.
 - Second cell : strong threshold value.
 - Third cell : green cursor to modify the low threshold value. On the zone image, the modeled spots have a green color. To move the cursor, click on the cursor, hold on the mouse button, and drag.



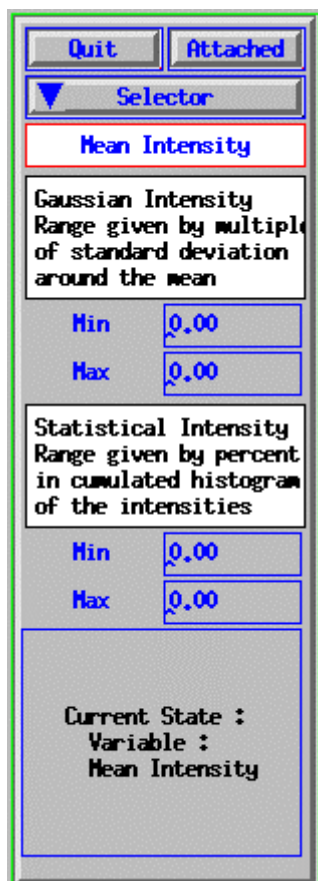
- ♦ Drop-down menu for the modeled spots display on the zone image. The different options are :
 - Positive Spots : The spots which the value is superior to the low threshold.
 - Negative Spots : The spots which the value is inferior to low threshold.
 - All the Spots.

- Strong Spots : The spots which the value is superior to the medium threshold.
- Medium Spots : The spots which the value is superior to the medium threshold, and inferior to the superior threshold.
- Low Spots : The spots which the value is superior to the low threshold, and inferior to the medium threshold.
- Relative Spots : The reference spots for the normalization computation.
- Spots of a Pair : All the spots paired.
- Positive Spots of a Pair : The spots paired which the value is superior to the low threshold.

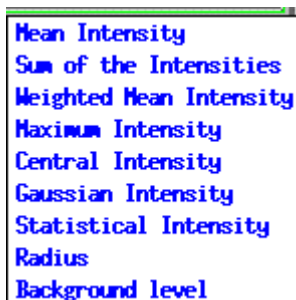
Quantification sub-menu.



To select a criteria (value type) for the modeled spots display. By default, the value type is Mean Intensity.



- ♦ Button Quit : to close this sub-menu.
- ♦ Toggle button Attached/Detached :
 - Attached : Means that the sub-menu Quantification is attached to the Spots menu. To detach the sub-menu, click on Attached.
 - Detached : Means that the sub-menu Quantification can be moved, independently of the Spots menu. To move the sub-menu, click on its title bar, hold on and drag. The Spots menu can be close, without closing the sub-menu Quantification. To attach the sub-menu again, click on Detached.



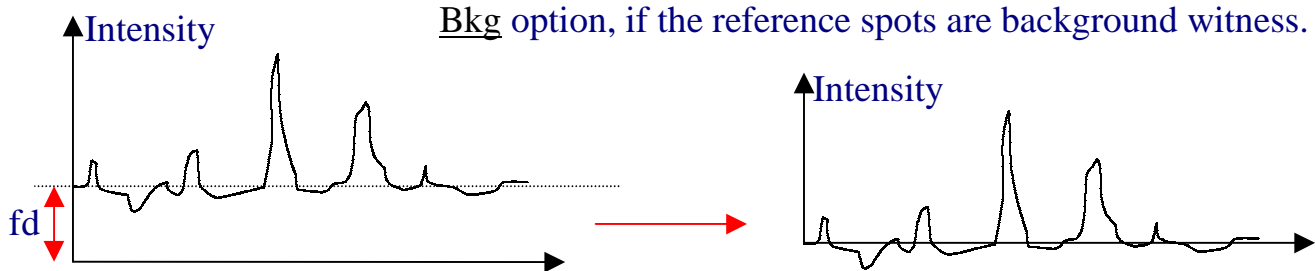
- ♦ Drop-down menu Selector : Select the criteria to display the modeled spots, according the color code. This description requires the understanding of the spots modeling section. By default, the criteria is Mean Intensity. Click on the drop-down menu to modify the value type. Hold down the mouse button and drag to select the criteria.
 - Mean Intensity : Mean of the pixels values inside the modeled spot circle.
 - Sum of the Intensities : Sum of the pixels values inside the modeled spot circle.

- Weighted Mean Intensity : Mean of the pixels values inside the modeled spot circle, weighted by their distance with the spot center.
 - Maximum Intensity : Value of the maximum intensity pixel inside the modeled spot circle.
 - Central Intensity : Value of the center pixel of the modeled spot circle.
 - Gaussian Intensity : Sum of spot pixels values, according to a selection inside a gaussian intensity range (see the corresponding input fields Min / Max)
 - Statistical Intensity : Sum of spot pixels values, according to a selection inside a statistical intensity range (see the corresponding input fields Min / Max)
 - Radius : to select the spots by means of size.
 - Background level : Local background level around the spot : (Mean intensity outside the spot)
-
- ◆ Display field : display the selected criteria.
 - ◆ Information field : about Gaussian Intensity
 - ◆ Input field Min (Real value) : lower bound of the range. This number is a standard deviation multiple. (If the lower bound is inferior to the mean, enter a negative value).
 - ◆ Input field Max (Real value) : upper bound of the range. This number is a standard deviation multiple. (If the upper bound is inferior to the mean, enter a negative value).
 - ◆ Information field : about Statistical Intensity.
 - ◆ Input field Min : (value between 0 and 100) : lower bound of the range. This number is a percentage of the intensities cumulated histogram.
 - ◆ Input field Max : (value between 0 and 100) : upper bound of the range. This number is a percentage of the intensities cumulated histogram.
 - ◆ Summarize field : display the current state, according to the selected variable.

Background Level sub-menu.

To select a background estimation method. In all cases, the estimation is done from one or several pixel values. The computation of this value has two functions :

- First, to can compare zones with different exposure times : the background level (noted fd) can be subtracted from the level spot.
- Second, to class the spots in positive and negative type, especially with the One Spot Bkg by Block option, or the File Bkg option, if the reference spots are background witness.



Click on this button. The Background Level sub-menu appears. By default, the background type is Local Bkg.

- ♦ Button Quit : to close this sub-menu.
- ♦ Toggle button Attached/Detached :
 - Attached : Means that the sub-menu Background Level is attached to the Spots menu. To detach the sub-menu, click on Attached.
 - Detached : Means that the sub-menu Background Level can be moved, independently of the Spots menu. To move the sub-menu, click on its title bar, hold on and drag. The Spots menu can be close, without closing the sub-menu Background Level. To attach the sub-menu again, click on Detached.

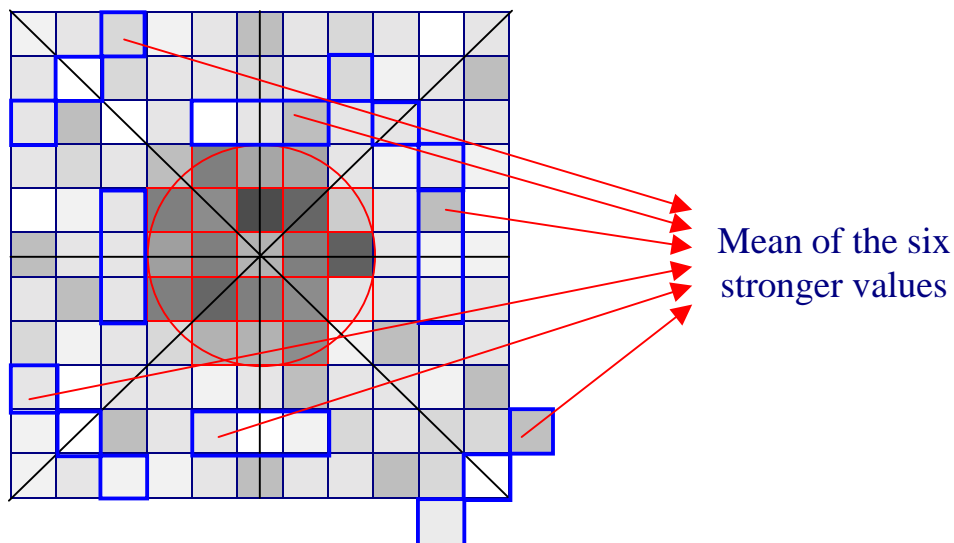


- ♦ Drop-down menu Background Type : To select a background type, click on the drop-down menu, hold down and drag. By default, the background type is Local Bkg.

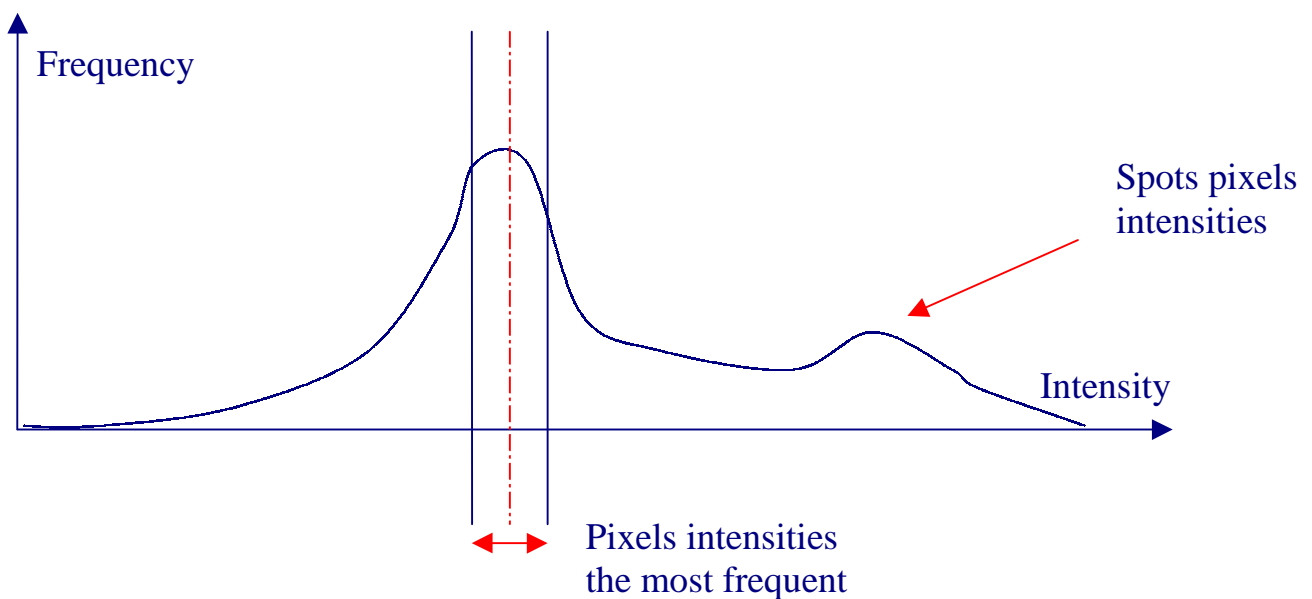
- ♦ Display field : display the selected background type.

- Local Bkg : The local background level is computed around each spot. Each spot has a different background level. Calculus method :

The spots of 4 axes (medians and diagonals), passing by the spot center, are examined. For each side of the 4 axes, the spot with the minimum value is selected (so, 8 spots in all). For each selected spot, the both adjacent spots in the orthogonal direction are also selected. Among these 24, the six stronger are selected. The mean is calculated on these six values : it's the local background level.



- Global Bkg : The global background level is the average of all the local background levels.
- Bkg Average on Block : The block background level is the average of all the local background levels of one block. (block : see geometry section). Each block spot has the same background level.
- One Spot Bkg by Block : The block background level is the local background level of one reference spot of the same block. Each block spot has the same background level. (?? choix du spot)
- Bkg Around Block : The block background level is the average of the local background levels of all the blocks around. (so, 9 blocks, if the block is not on a side, or a corner)
- Image Bkg : Statistical analysis on the entire zone. An histogram is built with the whole pixels of a zone. The maximum gives the pixels intensities the most frequent. The background level is the mean of these pixels :



- File Bkg : To create a background file. Click on this option : The Create File menu appears :

- ☞ Quit button : to close this menu.
- ☞ Create button : to create the background file. (Before to press it, fill the fields below).
- ☞ Input field : Under the Background File Name label : Enter the normalization filename (extension : **.nrm**)
- ☞ Drop-down menu Density : Click on the drop-down menu, hold down and drag to select the wanted option. The options are : 1x1; 2x2, 8, 3x3; 4x4; 5x5; 6x6. Once selected, schematized spots appear in the display area below.
- ☞ Input field Density : It's possible to key in directly the density. Once filled, schematized spots appear in the display area below.
- ☞ Drop-down menu Number of Lines. Click on the drop-down menu, hold down and drag to select the wanted option.
- ☞ Input field Number of Lines : It's possible to key in directly the density.

☞ Drop-down menu Number of Columns. Click on the drop-down menu, hold down and drag to select the wanted option.

☞ Input field Number of Lines : It's possible to key in directly the density.

- ☞ Drop-down-menu Background Computation Mode : to choose the background mode. The choices are : Global Mean, Global Maximum, Mean by Block, Maximum by Block.

Global Mean : for the entire zone. The background value is the mean of all the selected spots.

Global Maximum : for the entire zone. The background value is the maximum value of all the selected spots in the zone.

Mean by Block : for each block. The background value is the mean of selected spots in the block.

Maximum by Block : for each block. The background value is the maximum value in the block.

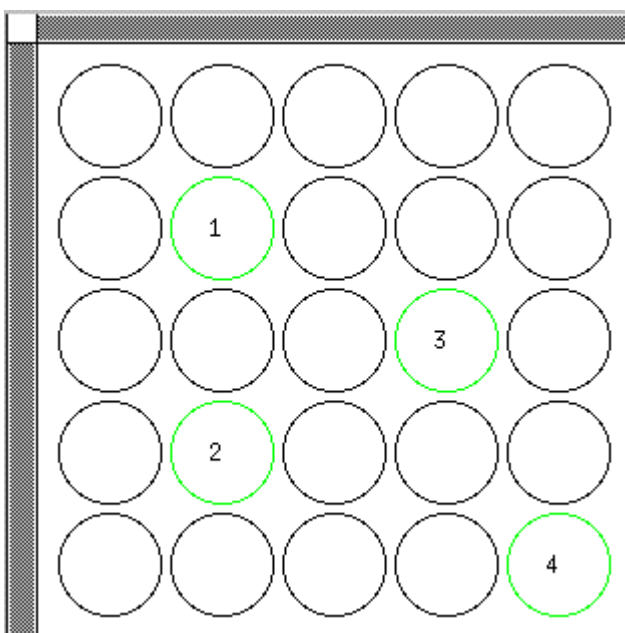
- ☞ Next Block button : To select the next block, and select spots. Each block can have different selected spots.

- ☞ Previous Block button : To select the preceding block. It's possible to modify the selected spots.

- ☞ Input field Line Number : To select a block : enter its line number

- ☞ Input field Line Column : To select a block : enter its column number.

- ☞ Copy on All the Blocks button : To copy the selected spots from first block to all the others blocks.



- ☞ Display area : click on the spots to select them. To unselect a spot, click on it with the right button. Example of selection :

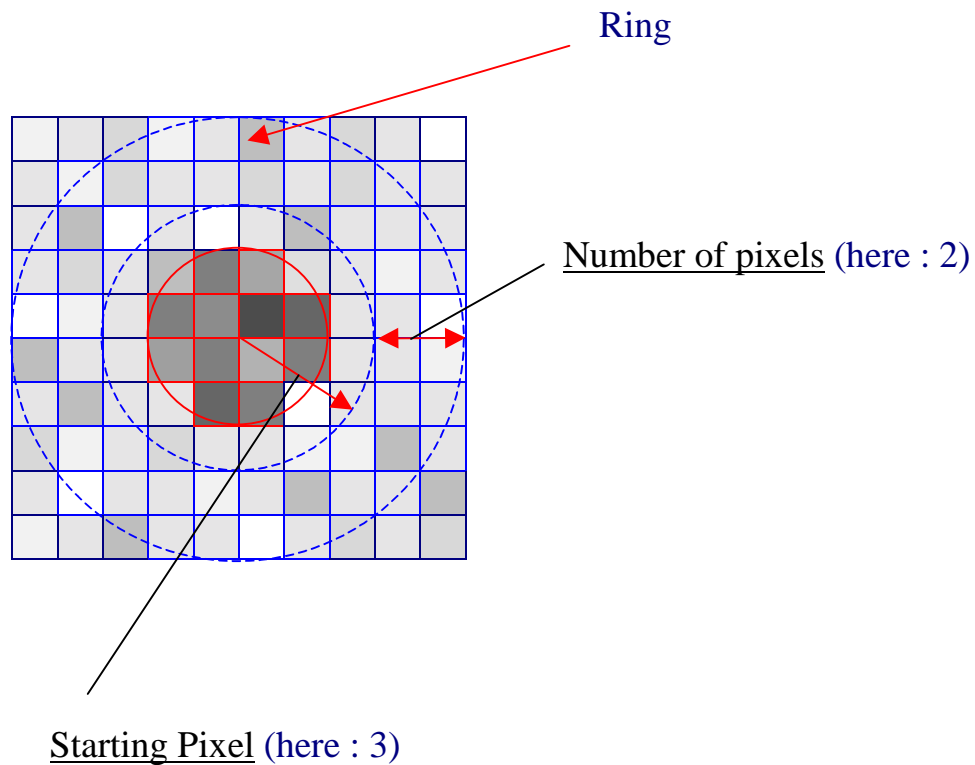
- Statistical Bkg : Statistical analysis in a ring around the spot center. The ring is defined by its lowest radius (Starting Pixel parameter), and its width (Number of Pixels parameter). In this area, pixels are selected according a level criteria (Min and Max parameter). Statistical Bkg is the selected spots mean. (See drawings both next pages).

♦ Toggle buttons Y / N for Background Level :

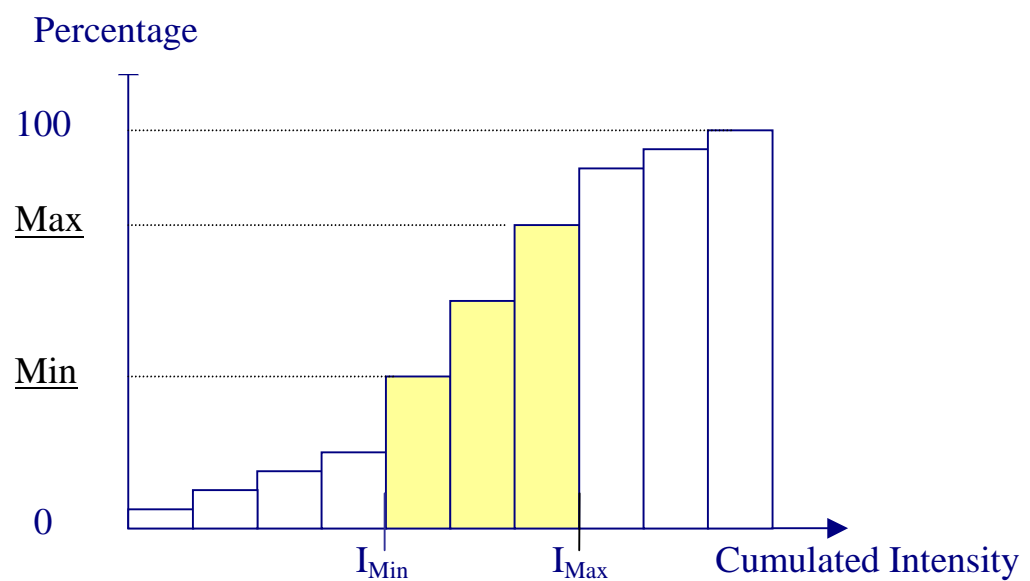
- Subtracted : Toggle button Y : Background level subtract.
- Not Subtracted : Toggle button Y : Not background level subtract.

♦ Input field : about Statistical Background area.

- Input field Starting Pixel (Integer value) : lower radius of the ring for the statistical analysis.
- Input field Number of Pixels (Integer value) : Ring width for the statistical analysis.



- ♦ Input field : about Statistical Background estimation.
 - Input field Min (value between 0 and 100) : lower bound of the range. This number is a percentage of the intensities cumulated histogram.
 - Input field Max (value between 0 and 100) : upper bound of the range. This number is a percentage of the intensities cumulated histogram.

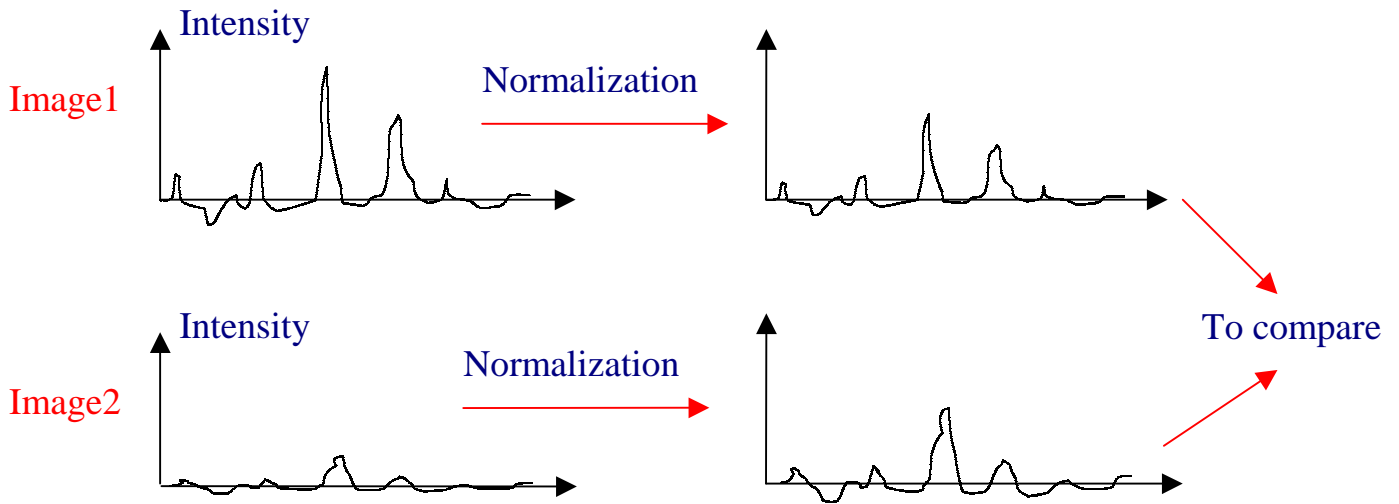


- ♦ Summarize field : display the current state.

Normalization sub-menu.

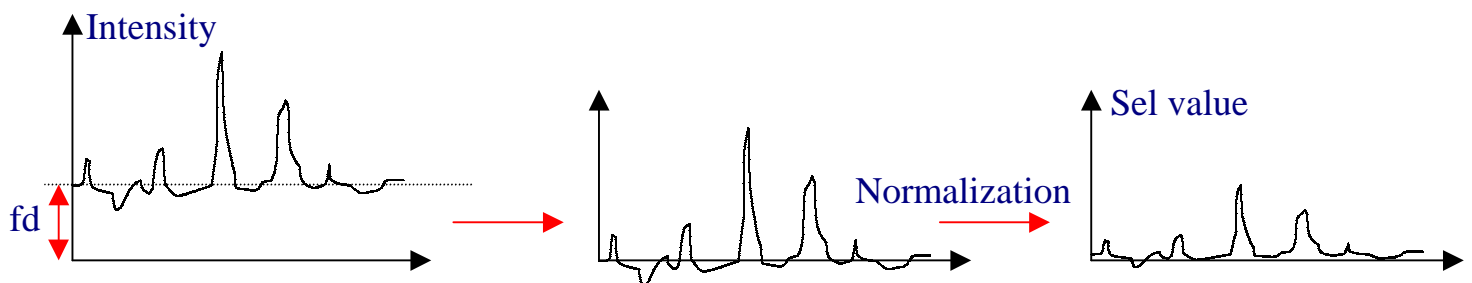
Normalization

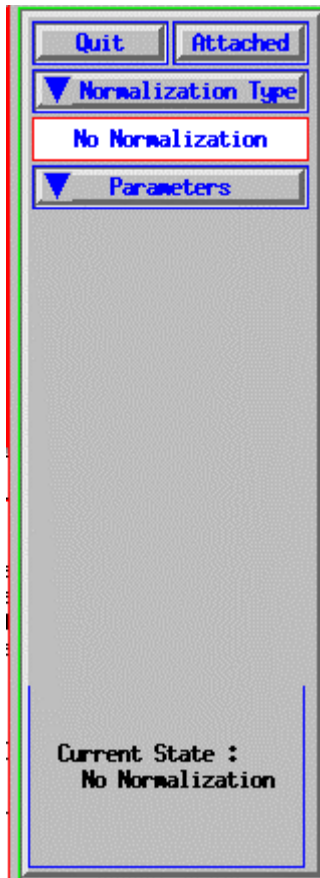
To select a normalization method. By default, there is no normalization. Normalization allows to compare spots level from different image : values are divided by the normalization value :



The Normalization value allows to calculate the Sel value :

$$\text{Sel} = (\text{spot value} - \text{background level}) / \text{normalization value}$$

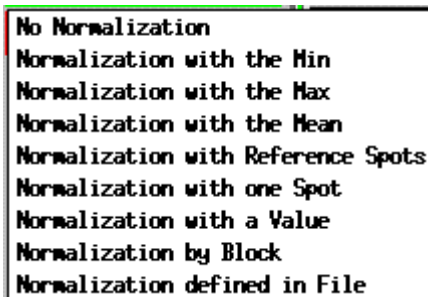




- ♦ Button Quit : to close this sub-menu.
- ♦ Toggle button Attached/Detached :
 - Attached : Means that the sub-menu Normalization is attached to the Spots menu. To detach the sub-menu, click on Attached.
 - Detached : Means that the sub-menu Normalization can be moved, independently of the Spots menu. To move the sub-menu, click on its title bar, hold on and drag. The Spots menu can be close, without closing the sub-menu Normalization. To attach the sub-menu again, click on Detached.

▼ Normalization Type

- ♦ Drop-down menu : to select a normalization method. Click on this menu, hold down the mouse button and drag to select. The different methods are :



- No normalization : spots keep the raw values.
- Normalization with the Min : the reference level to normalize is the lowest level in the zone, the reference spots levels being exclude. This normalization type gives spot levels superior to 1
- Normalization with the Max : the reference level to normalize is the highest spot level in the zone, the reference spots levels being exclude. This normalization type gives spot levels include between 0 and 1.
- Normalization with the Mean : the reference level to normalize is the mean level of all spots in the zone, the reference spots levels being exclude.
- Normalization with Reference Spots : the reference level to normalize is the mean level of all the reference spots in the zone.
- Normalization with one Spot : the reference level to normalize is the chosen spot level.

Normalization Spot

Grid	<input type="text"/>
Line	<input type="text"/>
Column	<input type="text"/>

The input menu appears to choose the spot :

- Field Grid : Key-in the grid number of the chosen spot.
- Field Line : Key-in the line number.
- Field Column : Key-in the column number.

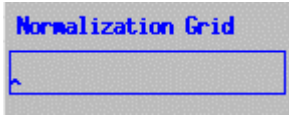
- Normalization with a Value : the reference level to normalize is the given value.

- The input field appears : key-in a value.

Normalization Value

<input type="text" value="1.000"/>

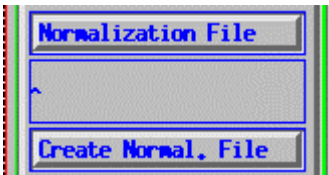
- Normalization by Block : the reference level is different for each block. A spot in a block, always in the same location, is taken as reference : Give a grid number to specify the spots.



- The input field appears : key-in a grid number.

- Normalization defined in a File : the reference level is the mean level of all the file spots.

The input menu appears :



- Button Normalization File : Click on the button to open a Selection menu (See Selection menu section). Select a normalized file.
- Input field : It's possible to key-in directly the normalization filename (the current directory is the directory where the application is running).

- Button Create Normal. File : To create a normalization file. Click on this button : The Create menu appears :

- Quit button : to close this menu.
- Create button : to create the normalization file. (Before to press it, fill the fields below).
- Input field : Under the Normalization File Name label : Enter the normalization filename (extension : **.nrm**)
- Drop-down menu Density : Click on the drop-down menu, hold down and drag to select the wanted option. The options are : 1x1; 2x2, 8, 3x3; 4x4; 5x5; 6x6. Once selected, schematized spots appear in the display area below.
- Input field Density : It's possible to key in directly the density. Once filled, schematized spots appear in the display area below.
- Drop-down menu Number of Lines. Click on the drop-down menu, hold down and drag to select the wanted option.
- Input field Number of Lines : It's possible to key in directly the density.
- Drop-down menu Number of Columns. Click on the drop-down menu, hold down and drag to select the wanted option.
- Input field Number of Lines : It's possible to key in directly the density.

- Drop-down-menu Normalization Computation Mode : to choose the normalization mode. The choices are : Global Mean, Global Maximum, Mean by Block, Maximum by Block.

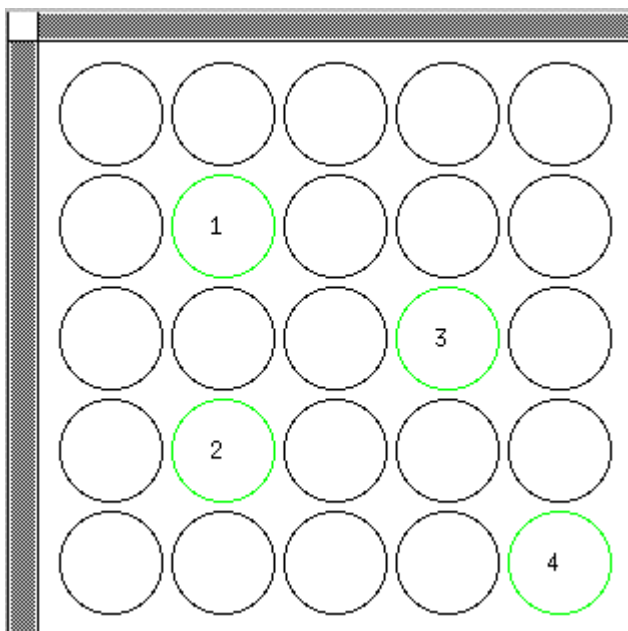
Global Mean : for the entire zone. The normalization value is the mean of all the selected spots.

Global Maximum : for the entire zone. The normalization value is the maximum value of all the selected spots in the zone.

Mean by Block : for each block. The normalization value is the mean of selected spots in the block.

Maximum by Block : for each block. The normalization value is the maximum value in the block.

- Next Block button : To select the next block, and select spots. Each block can have different selected spots.
- Previous Block button : To select the preceding block. It's possible to modify the selected spots.
- Input field Line Number : To select a block : enter its line number
- Input field Line Column : To select a block : enter its column number.
- Copy on All the Blocks button : To copy the selected spots from first block to all the others blocks.



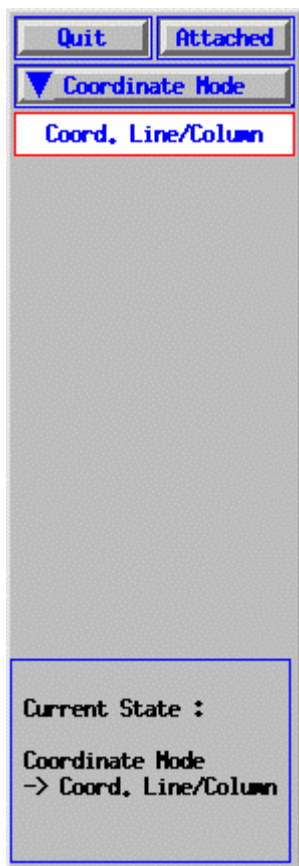
- Display area : click on the spots to select them. To unselect a spot, click on it with the right button. Example of selection :

- ◆ Drop-down menu Parameters : to access to the input fields of Normalization with one Spot, Normalization by Block, Normalization defined in a File, Normalization File.
- ◆ Summarize field : display the current state.

Coordinates sub-menu.



To select a coordinates mode. The coordinates are displayed in the Complete List option of the drop-down menu Analysis Tools in the Spots menu, and in the Characteristic sub-menu in the Spots menu. By default, the spots coordinates are the lines number, and the columns number. Click on this button. The following sub-menu appears :



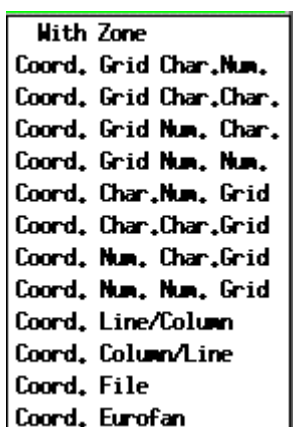
♦ Button Quit : to close this sub-menu.

♦ Toggle button Attached/Detached :

- Attached : Means that the sub-menu Coordinates is attached to the Spots menu. To detach the sub-menu, click on Attached.
- Detached : Means that the sub-menu Coordinates can be moved, independently of the Spots menu. To move the sub-menu, click on its title bar, hold on and drag. The Spots menu can be close, without closing the sub-menu Coordinates. To attach the sub-menu again, click on Detached.



♦ Drop-down menu : Click on this button. A coordinates mode list appears. Hold on and drag to select a coordinates mode.



With Zone : Add to the coordinates the zone name.

Coord. Grid Char. Num. : Triplet : Indexing grid number, line reference by character, column reference by number.

Coord. Grid Char. Char. : Triplet : Indexing grid number, line reference by character, column reference by character.

Coord. Grid Num. Char. : Triplet : Indexing grid number, line reference by number, column reference by character.

Coord. Grid Num. Num. : Triplet : Indexing grid number, line reference by number, column reference by number.

Coord. Char. Num. Grid : Triplet : line reference by character, column reference by character, indexing grid number.

Coord.Char. Char. Grid : Triplet : line reference by character, column reference by character, indexing grid number.

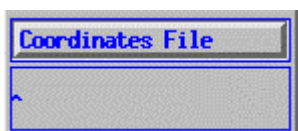
Coord. Num. Char. Grid : Triplet : line reference by number, column reference by character, indexing grid number.

Coord. Num. Num. Grid : Triplet : line reference by number, column reference by number, indexing grid number.

Coord. Line/Column : Doublet : line reference by number, column reference by number.

Coord. Column/Line : Doublet : column reference by number, line reference by number.

Coord. File : ??



The input menu appears :

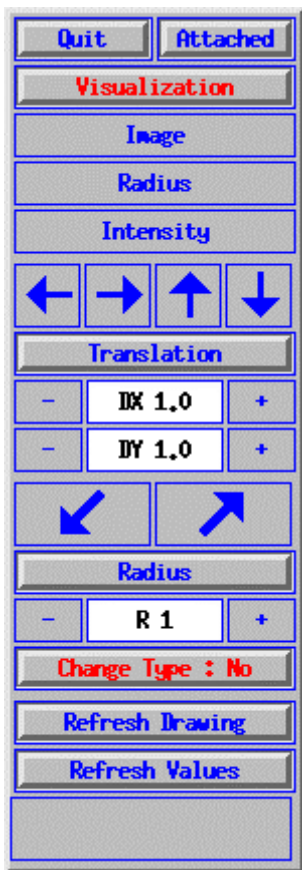
- Button Coordinates File : Click on the button to open a Selection menu (See Selection menu section). Select a coordinates file.
- Input field : It's possible to key-in directly the coordinates filename (the current directory is the directory where the application is running).

♦ Summarize field : display the current state.

Modification sub-menu.



To modify modeled spots one by one (position, radius). This function is used with a zone with displayed modeled spots (spots image). Open the sub-menus Verification or Characteristics for an optimal use : additional information or zoom are displayed in the right pad of the Slots menu.



♦ Button Quit : to close this sub-menu.

♦ Toggle button Attached/Detached :

- Attached : Means that the sub-menu Modification is attached to the Slots menu. To detach the sub-menu, click on Attached.
- Detached : Means that the sub-menu Modification can be moved, independently of the Slots menu. To move the sub-menu, click on its title bar, hold on and drag. The Slots menu can be close, without closing the sub-menu Modification. To attach the sub-menu again, click on Detached.

♦ Toggle button Visualization/Modification :

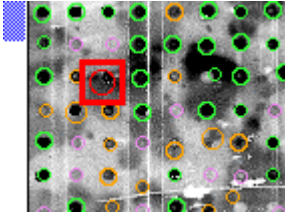


Label by default : Translations and radius modifications buttons off. Click on Visualization to activate these buttons. The label Modification displays.



Translation and Rotation buttons on. Click on Modification to disactivate these buttons. The label Visualization displays.

To active the following buttons, the toggle Visualization/Modification button must display :



To display data about one spot, or to modify the spot odelization, set the pointer mouse on the spots image . Then, **set the mouse pointer on the modeled spot**, and click on with the **middle** mouse button : the modeled spot circle becomes red, and the display fields Image, Radius, Intensity, updates.

- ◆ Display field Image : display the modeled spot center coordinates.
- ◆ Display field Radius : display modeled spot radius.
- ◆ Display field Intensity : display modeled spot mean intensity.



Click on this button to translate the spot of a half pixel on the left.



Click on this button to translate the spot of a half pixel on the right.



Click on this button to translate the spot of a half pixel on the top.



Click on this button to translate the spot grid of a half pixel on the bottom.

- ◆ Button Translation : To translate the spot according to the translation vector (DX, DY), defined in the both fields below.
- ◆ DX step size : Click on "-" button to decrement the step size value DX.
Click on "+" button to increment the step size value DX.
- ◆ DY step size : Click on "-" button to decrement the step size value DY.
Click on "+" button to increment the step size value DY.



Click on this button to decrease the modeled spot radius of a half pixel.



Click on this button to increase the modeled spot radius of a half pixel.

- ◆ Radius button : to set the modeled spot radius according to the displayed value, below this button.

"-" button : to decrease the radius value to assign to a modeled spot.

"+" button : to increase the radius value to assign to a modeled spot.

- ◆ Toggle button Change type : No/ Change type : Yes :

- Change type : No : Value button by default : Impossible to change the modeled spot type.
- Change type : Yes : the mouse pointer is a black ring. Set the mouse pointer on a spot, and click with the **middle** mouse button. For each click, the type change according to a loop : **Low** spot => **Medium** spot => **Strong** spot => **Negative** spot => then begin again. When a type spot is modified, the modeled spot circle becomes a **double** circle, and takes the defined type. By default, a low spot has the pink color, a medium spot has the orange color, a strong spot has the green color, and the negative spot has the cyan color.

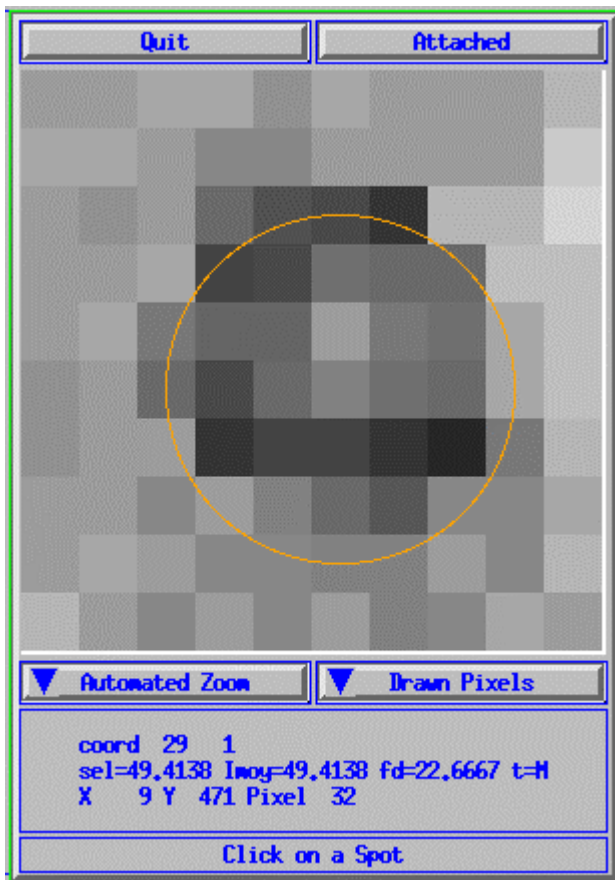
- ◆ Refresh Drawing button : To refresh the drawing if necessary.

- ◆ Refresh Values button : To refresh the values if necessary.

Verification sub-menu.

Verification

Display the modeled spot, superimposed on the raw values. Click on this button to open the Verification sub-menu. Click on a spot in the spots image with the **middle** mouse button : the modeled spot circle becomes red in the spot image, and is enlarged in the Verification sub-menu (right pad).



- ◆ Button Quit : to close this sub-menu.
- ◆ Toggle button Attached/Detached :
 - Attached : Means that the sub-menu Verification is attached to the Spots menu. To detach the sub-menu, click on Attached.
 - Detached : Means that the sub-menu Verification can be moved, independently of the Spots menu. To move the sub-menu, click on its title bar, hold on and drag. The Spots menu can be close, without closing the sub-menu Modification. To attach the sub-menu again, click on Detached.

- ◆ Display pad : By default, display the enlarged modeled spot circle. The contrast function modifies at the same time the display pad and the spots image. The circle color is the type color. The example above shows a medium spot (orange color by default).
- ◆ Drop-down menu Automated Zoom : To specify the zoom factor. Click on the button, hold down the mouse button and drag to select the chosen option. The zoom may be fixed (value 1 to 8), or automatic (default option).

- ◆ Drop-down menu Drawn Pixels : Click on the drop-down menu, hold on and drag to notch the chosen options :

All Pixels : Frame all the pixels inside a modeled spot circle, with the green color. The pixel where is the spot center is framed with the red color.

Pixel of Quantification : Exclusive option with All Pixel. Frame all the pixels selected to compute the modeled spot level, with the green color. Frame the others pixels, inside the modeled spot circle, with the yellow color.

Pixel of Background : Frame the pixels used for the background computing with the cyan color.

- ◆ Information field : give the following information :

- Coordinates of the spot, in the specified mode. (See Coordinates sub-menu)
- sel : This value is computed thus :

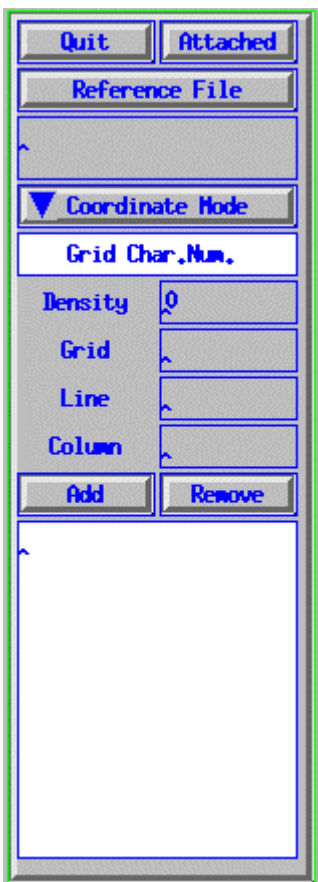
$$sel = (Imoy - fd) / \text{normalization value.}$$

- Imoy : average of modeled spot pixels.
- fd : background level.
- t (spot type) : low, medium, or strong.
- X and Y : pixel coordinates : set the mouse pointer on a pixel of the enlarged image.
- Pixel : pixel level : set the mouse pointer on a pixel of the enlarged image.

Reference sub-menu.



To mark spots reference, and set them in a reference file (extension .reper). The marked spots take the yellow color in the spots image. The reference file is used with the Normalization defined in File option of the Normalization sub-menu of Spots menu.



- ◆ Button Quit : to close this sub-menu.
- ◆ Toggle button Attached/Detached :
 - Attached : Means that the sub-menu Reference is attached to the Spots menu. To detach the sub-menu, click on Attached.
 - Detached : Means that the sub-menu Reference can be moved, independently of the Spots menu. To move the sub-menu, click on its title bar, hold on and drag. The Spots menu can be close, without closing the sub-menu Reference. To attach the sub-menu again, click on Detached.
- ◆ Button Reference File : To select an existing reference file (extension .reper). Click on this button to open a Selection menu (See Selection file section) and select the file.
- ◆ Input field : To create a reference file. Key-in the reference filename.
- ◆ Drop-down menu Coordinate Mode : To choose the coordinate mode of the reference file. Click on the drop-down menu, hold on and drag to select.
- ◆ Input field Density : Key-in the zone density value.
- ◆ Input field Grid : Key-in the grid number.
- ◆ Input field Line : Key-in the line number.
- ◆ Input field Column : Key-in the column number.



To add a reference spot, fill the field Density, Grid, Line and Column, then click on Add button.



To remove a reference spot, fill the fields Density, Grid, Line and Column, then click on Remove button.

- ◆ Display field : list the reference spots.

Characteristics sub-menu.

Characteristics

Display the selected spot characteristics : set the mouse pointer on a spot in the spots image. A red square frame the chosen spot. The characteristics display in the right pad of the Spots menu

Quit	Attached	Information
1 19 6	19 6	
sel 95,21		
Central Intensity 83 (124)		
Maximum Intensity 99 (140)		
Mean Intensity 53,71 (95,21)		
Sum of the Intensities 1557,50 (2761,00)		
Weighted Mean Intensity 57,03 (98,53)		
Gaussian Intensity -41,50 (0,00)		
Statistical Intensity 68,60 (110,10)		
Position 91,00 302,00		
Radius 3,00		
Background level : Local 41,50 Statistical 54,32		
Block 41,50 0,00 1,50		
Global 19,70 Image 35,12 File 0,00		
Type S		
Put the Mouse Pointer on a Spot		

♦ Button Quit : to close this sub-menu.

♦ Toggle button Attached/Detached :

- Attached : Means that the sub-menu Characteristics is attached to the Spots menu. To detach the sub-menu, click on Attached.
- Detached : Means that the sub-menu Characteristics can be moved, independently of the Spots menu. To move the sub-menu, click on its title bar, hold on and drag. The Spots menu can be close, without closing the sub-menu Characteristics. To attach the sub-menu again, click on Detached.

♦ Information button :

♦ Display field : Coordinates in the Grid Num Num system.

♦ Display field : Coordinates in the chosen coordinates mode. (Spots menu, Coordinates sub-menu)

♦ The following spots characteristics are displayed :

- sel : Normalized spot value. If the Background Level is subtracted (Spots menu, Background Level sub-menu), the computed sel value is :

$$\text{sel} = (\text{Imoy} - \text{fd}) / \text{normalization value.}$$

with :

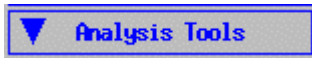
Imoy : Spot value, according to the selected criteria.

fd : Background level.

- Central Intensity : Normalized value of the center pixel level of the modeled spot circle. In bracket : Non normalized value.
- Maximum Intensity : Normalized value of the maximum level pixel inside the modeled spot circle. In bracket : Non normalized value.
- Mean Intensity : Normalized mean of the pixels values inside the modeled spot circle. In bracket : Non normalized value.
- Sum of the intensities : Normalized sum of the pixels values inside the modeled spot circle. In bracket : Non normalized value.
- Weighted Mean Intensity : Normalized mean of the pixels values inside the modeled spot circle, weighted by their distance with the spot center. In bracket : Non normalized value.
- Gaussian Intensity : Normalized sum of spot pixels values, according to a selection inside a gaussian intensity range. In bracket : Non normalized value.
- Statistical Intensity : Normalized sum of spot pixels values, according to a selection inside a statistical intensity range. In bracket : Non normalized value.
- Position : coordinates in pixel unit, in the zone. Origin : on the top, on the left.
- Radius : spot radius in pixel unit.
- Background level : display the different background values : see the Background level sub-menu, in the Spots menu.

♦ Type : Low, Medium, Strong or Negative.

Analysis Tools sub-menu.

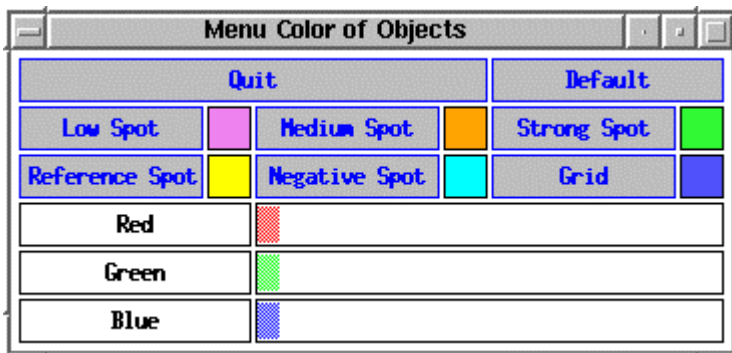


Tools to analyze the modeled spots. Click on the drop-down menu. Hold on the mouse button, and drag to select the chosen analysis tool.



Each choice opens a menu.

Tools : Menu Color of Objects



- ◆ Quit button : Close this menu.
- ◆ Default button : If a colors modification has been made, this button allows to return to default colors convention :
 - Low spots : pink
 - Medium spots : orange
 - Strong spots : green
 - Reference spots : yellow
 - Negative spots : cyan
 - Grid : blue.

-
- ◆ To modify a color : Click on object type button (Low Spot, Medium Spot, Strong Spot, Reference Spot, Negative Spot, Grid). Then, click on one of the three cursors, hold on and drag to move the cursor. The object colors are instantaneously update.
-

Tools : Postscript Image

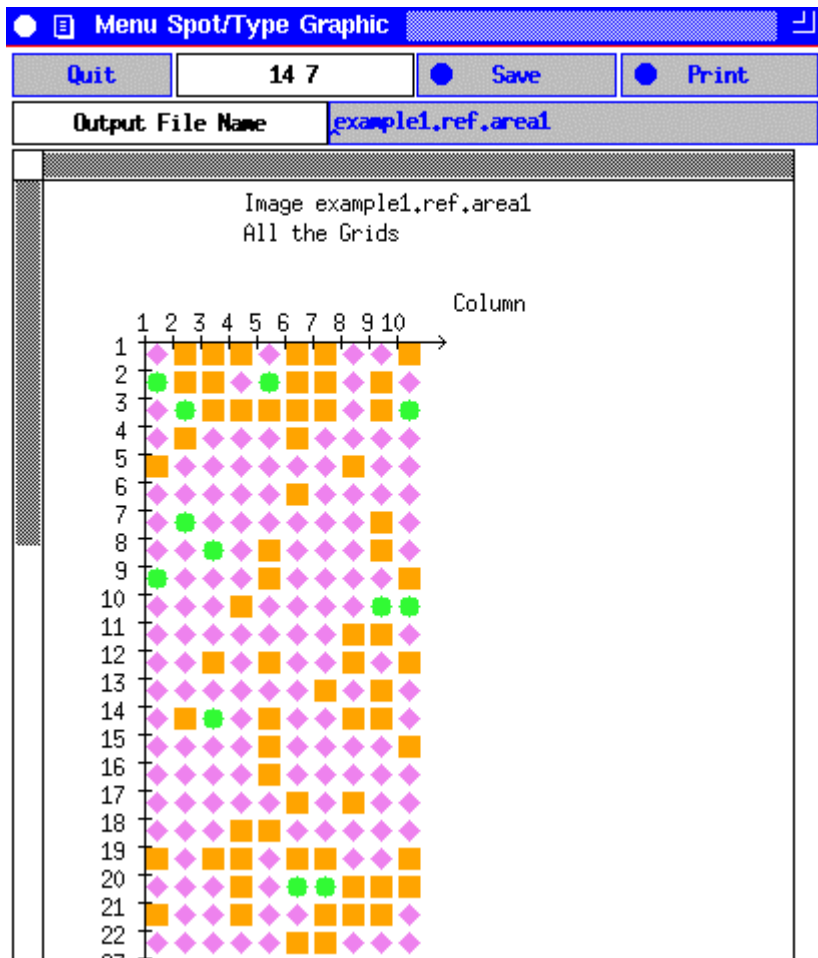
Postscript Image Menu			
Quit			
Output File Name		example1.ref.areal	
<input checked="" type="radio"/>	Postscript Format	<input checked="" type="radio"/>	Zoom 1
<input checked="" type="radio"/>	Header	<input checked="" type="radio"/>	Horizontal
<input checked="" type="radio"/>	Notation	<input checked="" type="radio"/>	Vertical
<input checked="" type="radio"/>	Border	<input checked="" type="radio"/>	Automatic
Save		Print	

- ◆ Quit button : to close this menu.
- ◆ Input field : facing the Output File Name label. Key in the file name.

- ◆ Drop-down menu Format : Click on this button, hold down and select the chosen format. The choices are : Postscript, Gif, Jpeg, Tiff.
- ◆ Toggle button : Y / N : To save the header or not.
- ◆ Toggle button : Y / N : To save the notation or not.
- ◆ Toggle button : Y / N : To save the border or not.
- ◆ Drop-down menu Zoom : Click on this button, hold down and select the enlargement factor. The choices are : x1; x2; x4; x8; x16.
- ◆ Toggle button : Y / N : To save the image in horizontal position.
- ◆ Toggle button : Y / N : To save the image in vertical position.
- ◆ Toggle button : Y / N : The software chooses the horizontal or vertical position.
- ◆ Toggle button : Y / N : To save the image or not.
- ◆ Toggle button : Y / N : To save the image and the spots, or not.
- ◆ Toggle button : Y / N : To save the image and the grids, or not.
- ◆ Toggle button : Y / N : To save the image, the grids and the spots, or not.
- ◆ Save button : to save the file (extension : .ps or .gif or .jpeg or .tiff)
- ◆ Print button : to print the file.

Tools : Spot/Type Graphic

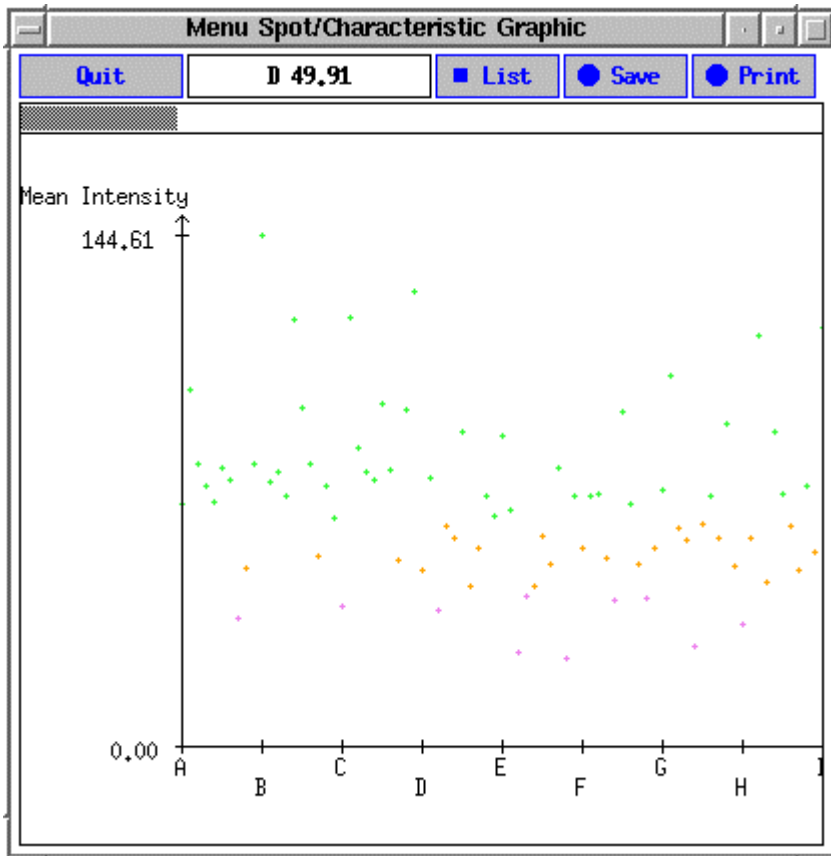
This tool allows to display spots types distribution, according to the chosen coordinates mode.



- ◆ Button Quit : to close this menu.
- ◆ Display field : display the spot coordinates : set the mouse pointer on the chosen spot.
- ◆ Button Save : Drop-down menu. The saving choices are : Black and White Graphic (.ps extension), Color Graphic (.cps extension), or Gif Graphic (.gif extension). (For example, the filename can be here : example1_type.cps)
- ◆ Button Print : Drop-down menu. The printing choices are : Black and White Graphic, Color Graphic, or Gif Graphic.
- ◆ Display field Output File Name : display the analyzed filename.
- ◆ Display Area : display spots types distribution, according to the color types convention.

Tools : Spot/Characteristic Graphic

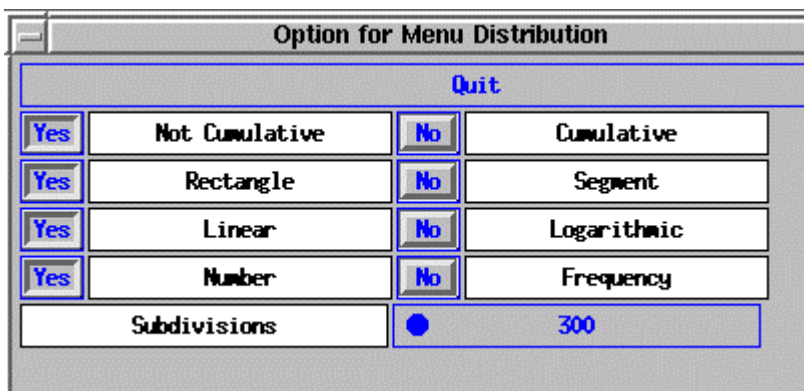
This tool allows to display spots mean levels, according to their position. The color type is displayed.



- ◆ Button Quit : to close this menu.
- ◆ Display field : display the spot coordinates : set the mouse pointer on the chosen spot.
- ◆ Button List : Display a list with the spot coordinates, the mean intensity (sel), and the type.
- ◆ Button Save : drop-down menu : the saving format choices are : Ascii, Excel, Postscript (extension **.liste**, **.liste.exl**, **.liste.ps**)
- ◆ Button Print : drop-down menu. The only printing format choice is Postscript).
- ◆ Display field Output File Name : display the analysed filename.
- ◆ Display Area : display spots types distribution, according to the color types convention.

This tool allows to display an histogram showing the spots number include inside spot mean level intervals. Several presentation options are available. The Distribution Graphic menu has four buttons : Quit, Option, Drawing, List.

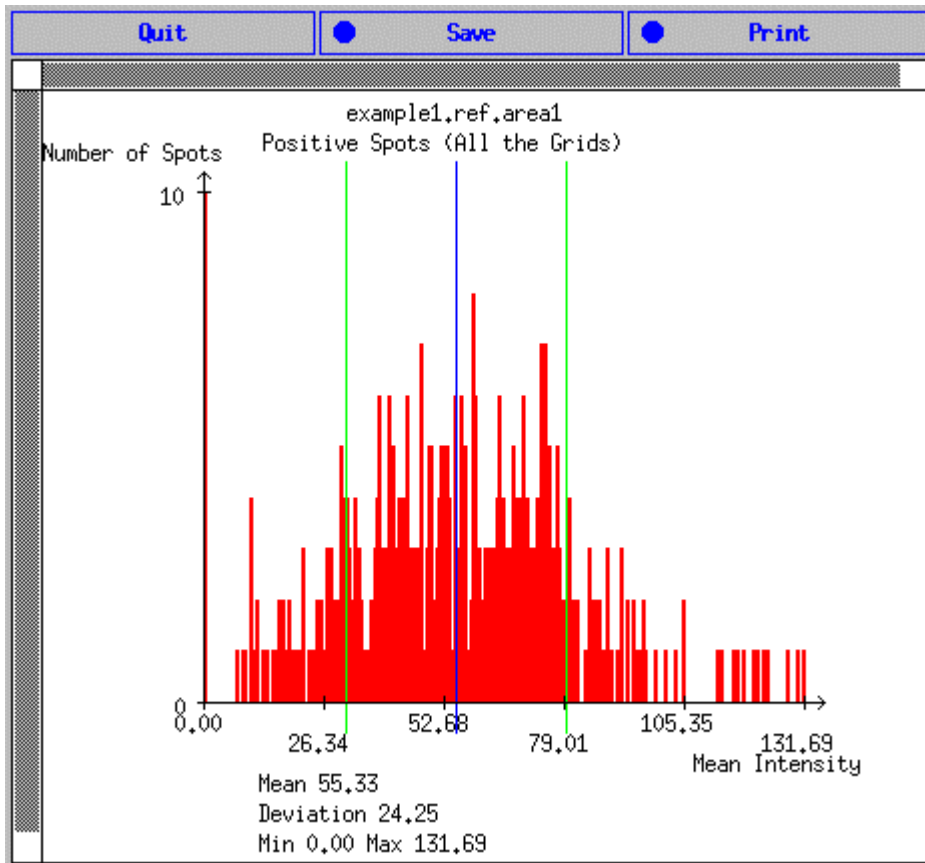
- ◆ Button Quit : to close the menu.
- ◆ Button Option : open the Option for Menu Distribution menu.



- ◆ Button Quit : To quit this menu.
- ◆ Toggle buttons Yes/No for options:
 - Not Cumulative : spots number inside mean level intervals.
 - Cumulative : cumulate the spots number of all the preceding mean level intervals.

- Toggle buttons Yes/No for options:
 - Rectangle : full colored mean level intervals.
 - Segment : spots number pointed out by a segment.
- Toggle buttons Yes/No for options:
 - Linear : linear mean level scale.
 - Logarithmic : logarithmic mean level scale.
- Toggle buttons Yes/No for options:
 - Number : y-axis in spots number unit.
 - Frequency : y-axis in percentage.
- Drop-down menu Subdivision : to choose the interval size.

- ◆ Button Drawing : to draw the histogram according to the options.

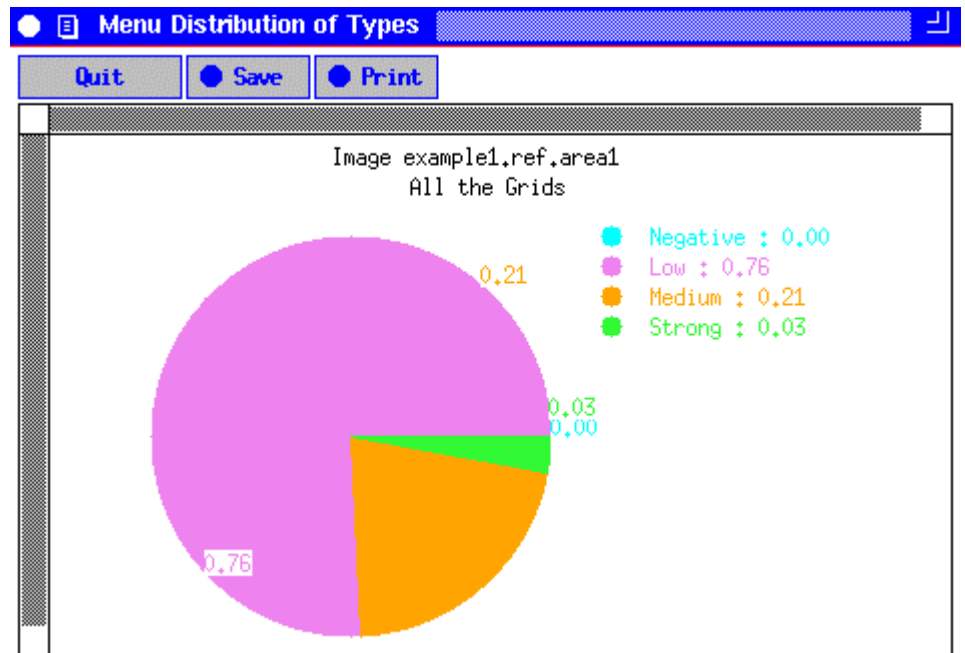


- ◆ Button Quit : to close this menu.
- ◆ Button Save : Drop-down menu. The saving choices are : Black and White Graphic(.ps extension), Color Graphic (.cps extension), or Gif Graphic(.gif extension).
- ◆ Button Print : Drop-down menu. The printing choices are : Black and White Graphic, Color Graphic.
- ◆ Display Area : histogram drawing.

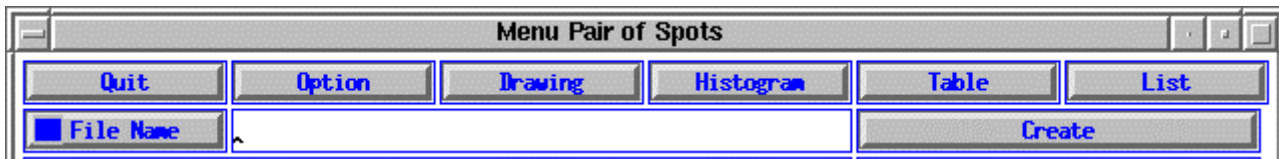
- ◆ Button List : list the data concerning the histogram.

Tools : Distribution of Types

This tool allows to display a pie graph according to spots type.

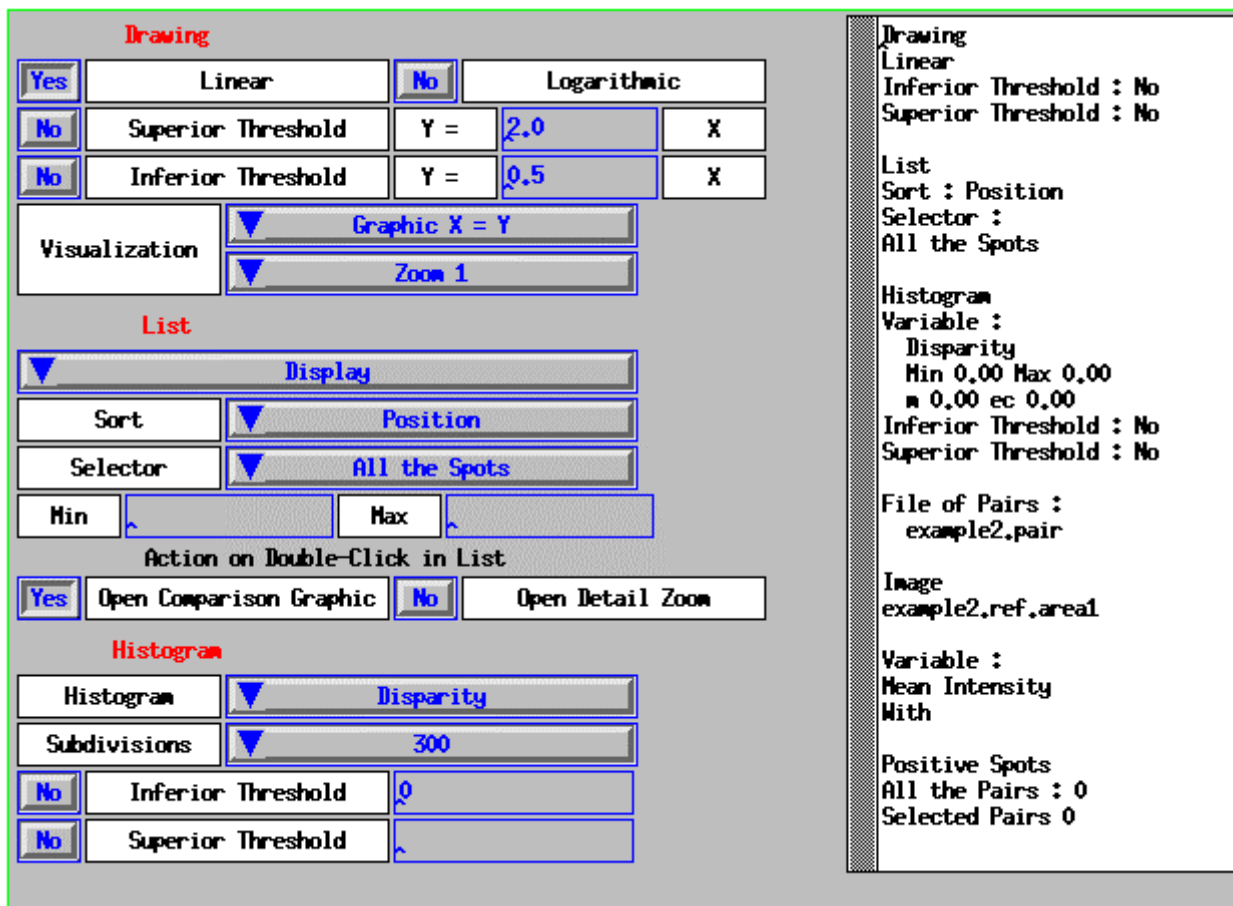


- ◆ Button Quit : to close this menu.
- ◆ Button Save : Drop-down menu. The saving choices are : Black and White Graphic (.ps extension), Color Graphic (.cps extension), or Gif Graphic (.gif extension).
- ◆ Button Print : Drop-down menu. The printing choices are : Black and White Graphic, Color Graphic.



This menu allows to examine and analyze zones with spots pairs. To use this menu, the first thing to do is to create the file which describe where are the pairs in the block (Create button). Then, adjust the parameters (Option button) about the drawing, the histogram, or the list to display (Drawing button, Histogram button, List button).

- ♦ Quit button : To close this menu.
- ♦ Option button : to parameter drawing, histogram, and list. Click on this button, the following menu appears :



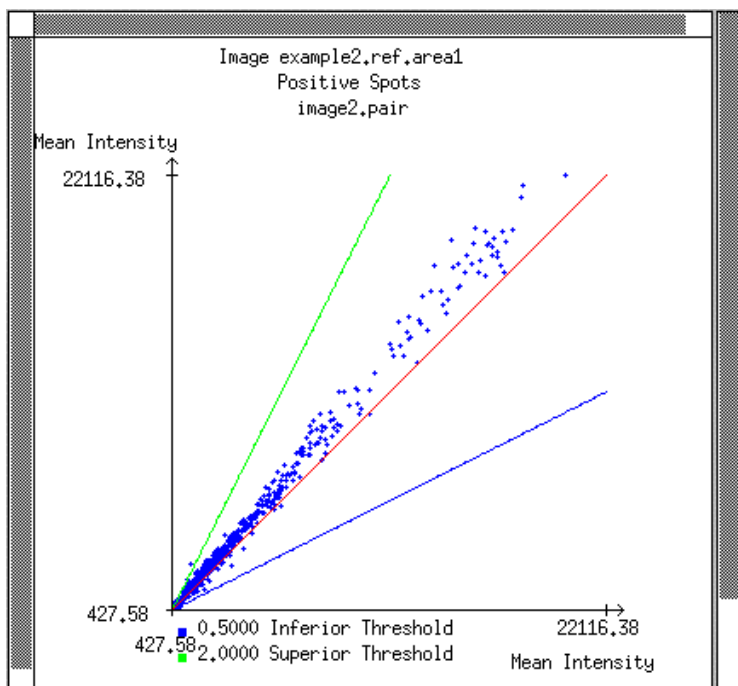
The left pad is divided in three parts : a Drawing part, a List part, and an Histogram part.

The right pad is an information area, in relation with the parameterization of Drawing, List, and Histogram parts.

⇒ Drawing part :

- Toggle button : Yes/No : For Linear or Logarithmic representation type, when the visualization Graphic X=Y is selected (See drop-down menu just below).
- Toggle button : Yes/No (Superior Threshold) : To visualize or not the superior threshold on the drawing. If the answer is Yes, set the mouse pointer on the input field on the right, and enter the coefficient of the equation ($Y = coefficient \times X$)
- Toggle button : Yes/No (Inferior Threshold) : To visualize or not the inferior threshold on the drawing. If the answer is Yes, set the mouse pointer on the input field on the right, and enter the coefficient of the equation ($Y = coefficient \times X$)

For example, set Graphics X=Y, with the parameters Linear Scale, Superior Threshold : 2.0; Inferior Threshold : 0.5. Click on Drawing button. The following graphic displays :



- First drop-down menu : (facing Visualization label) : several representation choices are possible :

Graphic X=Y (default choice) : The x-axes is graduated with the mean intensity of the first spot of a pair. The y-axis is graduated with the mean intensity of the second spot of a pair. For each spots pair, a point is drawn. If the measurements are the same for each spot for each pair, the points are aligned according to the equation $X = Y$.

Image : Disparity (3 colors) [Pixel] : The computation is realized for each pixels pair of each spots pair :

Pixel disparity of spot1 = $2 (\text{pixel1} - \text{pixel2}) / (\text{pixel1} + \text{pixel2})$

Pixel disparity of spot2 = $2 (\text{pixel2} - \text{pixel1}) / (\text{pixel1} + \text{pixel2})$

For each pixel, the representation is as follows :

(■ : Disparity < -0.25; ■ : Disparity > 0.25; ■ : -0.25 < Disparity < 0.25.)

If the measurements are near for each spot of each pair, the image will display spots in blue. If the pair pixel is red, the other pixel pair is green.

Image : Division (3 colors) [Pixel] : The computation is realized for each pixels pair of each spots pair :

Pixel division of spot1 = $\text{pixel1} / \text{pixel2}$

Pixel division of spot2 = $\text{pixel2} / \text{pixel1}$

For each pixel, the representation is as follows :

(■ : Division < 0.75; ■ : Division > 1.25; ■ : $0.75 < \text{Division} < 1.25$.)

If the measurements are near for each spot of each pair, the image will display spots in blue. If the pair pixel is red, the other pixel pair is green.

Image : Disparity (256 colors) [Pixel] : Identical to Image : Disparity (3 colors) [Pixel]
except for the representation :

(■ : Disparity = - Maximum negative disparity; ■ : Disparity = Maximum positive disparity). Between this two boundaries, intermediate colors in RGB coding are computed and displayed.

Image : Division (256 colors) [Pixel] : Identical to Image : Division (3 colors) [Pixel]
except for the representation :

(■ : Division = Minimum ratio ; ■ : Division = Maximum ratio) . Between this two boundaries, intermediate colors in RGB coding are computed and displayed.

Image : Superposition (256 colors) [Pixel] : The computation is realized for each pixels pair of each spots pair :

Pixels division of spot1 = Pixels division of spot2 = pixel1 / pixel2

For each pixel, the representation is as follows :

(■ : Division = minimum ratio; ■ : Division = 1; ■ : Division = maximum ratio)
Between these boundaries, intermediate colors in RGB coding are computed and displayed.

Image : Disparity (3 colors) [Spot] : The computation is realized for each spots pair :

Spot1 disparity = 2 (spot1 value - spot2 value) / (spot1 value + spot2 value)

Spot2 disparity = 2 (spot2 value - spot1 value) / (spot1 value + spot2 value)

For each pixel, the representation is as follows :

(■ : Disparity < -0.25; ■ : Disparity > 0.25; ■ : -0.25 < Disparity < 0.25.)

If the measurements are near for each spot of each pair, the image will display spots in blue. If a pair spot is red, the other spot pair is green.

Image : Division (3 colors) [Spot] : The computation is realized for each spots pair :

Spot1 division = spot1 value / spot2 value

Spot2 division = spot2 value/ spot1 value

For each spot, the representation is as follows :

(■ : Division < 0.75; ■ : Division > 1.25; ■ : 0.75 < Division < 1.25.)

If the measurements are near for each spot of each pair, the image will display spots in blue. If a pair spot is red, the other spot pair is green.

Image : Disparity (256 colors) [Spot] : Identical to Image : Disparity (3 colors) [Spot] except for the representation :

(■ : Disparity = - Maximum negative disparity; ■ : Disparity = Maximum positive disparity). Between this two boundaries, intermediate colors in RGB coding are computed and displayed.

Image : Division (256 colors) [Spot] : Identical to Image : Division (3 colors) [Spot] except for the representation :

(■ : Division = Minimum ratio ; ■ : Division = Maximum ratio) . Between this two boundaries, intermediate colors in RGB coding are computed and displayed.

Image : Superposition (256 colors) [Spot] : The computation is realized for each spots pair :

Superposition = Spot1 value / Spot2 value

For each spot, the representation is as follows :

(■ : Division = minimum ratio; ■ : Division = 1; ■ : Division = maximum ratio)
Between these boundaries, intermediate colors in RGB coding are computed and displayed.

- Second drop-down menu : (facing Visualization label) : scale factor choice. The choices are :
x 1; x 2; x 3; x 4.

⇒ List part :

- Drop down menu Display : To chose the information to display. Click on the menu, hold down and release to notch an option. The options are :

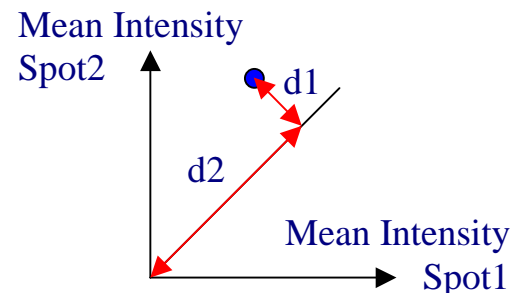
☞ Information : ??

☞ Characteristics : display general characteristics about type spots (mean, standard deviation), thresholds, min and max value.

☞ Notation : abbreviations meaning :

li :	line number.
co :	column number
g :	grid number
xc :	pixel x coordinate of the spot center.
yc :	pixel y coordinate of the spot centre.
Ic :	intensity of central pixel.
xm :	pixel x coordinate of the maximum intensity.
ym :	pixel y coordinate of the maximum intensity.
xb :	pixel x coordinate of the intensities barycentre.
yb :	pixel y coordinate of the intensities barycentre.
Ib :	intensity of barycentre pixel.
Imoy :	mean of the pixels intensities in the spot.
Is :	sum of the pixels intensities in the spot.
Ip :	weighted sum of the pixels intensities in the spot.
Ig :	central intensity of the intensities gaussian modelization.
r :	radius of the modeled circle.
fd :	background level.
var :	external variable. ??
s :	area.

t : type (low, medium, strong, reference, negative).
 L : low type.
 M : medium type.
 S : strong type.
 R : reference type.
 N : negative type.
 rl : relative value.
 m : mean value.
 ec : standard deviation.
 dif : subtraction (x-y).
 div : division (x/y).
 disp : disparity : $[(x-y) / (x+y)/2]$.
 d1 : distance to the line $x=y$.
 d2 : distance on the line $x=y$.



- ☞ Coord : notch by default. Coordinates of the first pair spot.
- ☞ Sel1 : assigned value of a the first spot in a pair: $[Sel1 = (Imoy - fd) / \text{normalization value}]$, with Imoy : average of modeled spot pixels, and fd : background level.
- ☞ Exp1 : $Sel1 / (Sel1 + Sel2)$
- ☞ t : type of the first spot in a pair.
- ☞ Coord : notch by default. Coordinates of the second pair spot.
- ☞ Sel2 : assigned value of a the second spot in a pair : $[Sel2 = (Imoy - fd) / \text{normalization value}]$, with Imoy : average of modeled spot pixels, and fd : background level.
- ☞ Exp2 : $Sel2 / (Sel1 + Sel2)$
- ☞ t : type of the second spot in a pair.
- ☞ Coord : ??

- ☞ d1 : distance to the line $x=y$.
- ☞ d2 : distance on the line $x=y$.
- ☞ Mean : in a pair : mean of the both spots $(Sel1 + Sel2)/2$
- ☞ Subtraction : in a pair : $Sel1 - Sel2$
- ☞ Division : in a pair : $Sel1 / Sel2$
- ☞ Disparity : in a pair : $(Sel1 - Sel2) / [(Sel1 + Sel2)/2]$
- ☞ Absolute disparity : in a pair : absolute value of disparity.

- Drop down menu about sort (facing the Sort label) : to sort according a criteria. Click on the List button to display the sort result. The possible choices among the criteria are :

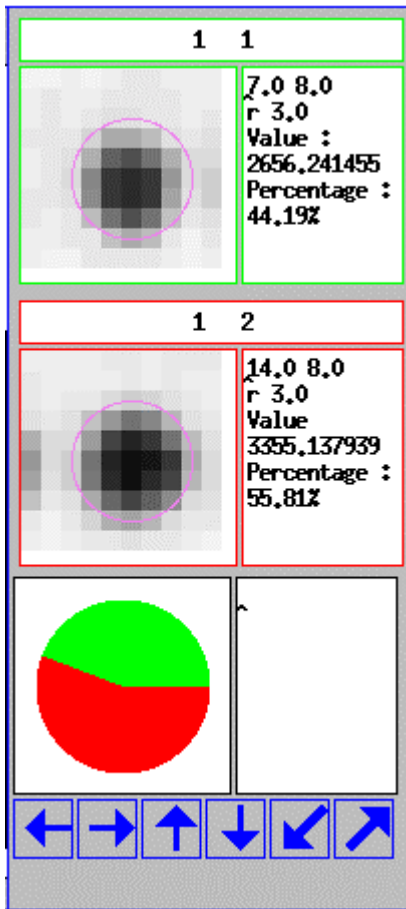
position :	spots coordinates (choice by default).
Sel1 :	for a pair : first spot value.
Sel2 :	for a pair : second spot value.
exp1 :	for a pair : first spot contribution.
exp2 :	for a pair : second spot contribution.
d1	distance to the line $x=y$.
d2	distance on the line $x=y$.
m	for a pair : mean of the both spots.
dif :	for a pair : difference between first and second spot.
div :	for a pair : ration between first and second spot.
disp	for a pair : disparity between first and second spot.
disp :	for a pair : absolute value of disparity.

- Drop-down menu about selection (facing the Selector label) : to select spots according to a criteria. Selection function and sort function are cumulative. Click on the List button to display the results. The possible choices among the criteria are :

all the spots :	Choice by default.
d1 :	distance to the line $x=y$.
d2 :	distance on the line $x=y$.
m :	for a pair : $\min < \text{mean of the both spots} < \max$. Before to use this function, fill the <u>Min</u> and <u>Max</u> fields locate just below.
dif :	for a pair : $\min < \text{difference of the both spots} < \max$. Before to use this function, fill the <u>Min</u> and <u>Max</u> fields locate just below.
div :	for a pair : $\min < \text{ratio of the both spots} < \max$. Before to use this function, fill the <u>Min</u> and <u>Max</u> fields locate just below.
disp :	for a pair : $\min < \text{disparity of the both spots} < \max$. Before to use this function, fill the <u>Min</u> and <u>Max</u> fields locate just below.
disp :	for a pair : $\min < \text{absolute value of disparity of the both spots} < \max$. Before to use this function, fill the <u>Min</u> and <u>Max</u> fields locate just below.

Pairs with two negative spots.
 Pairs with one negative spot.
 Pairs with two positive spots.
 Pairs with one positive spot.
 Pairs with two low spots.
 Pairs with one low spot.
 Pairs with two medium spots.
 Pairs with one medium spot.
 Pairs with two strong spots.
 Pairs with one strong spot.

- Input field Min : Fill this field for the selection criteria which required this. (d1, d2, m, dif, div, disp, |disp|). Click on the field. The blue frame becomes red. Enter the wanted value.
- Input field Max : Fill this field for the selection criteria which required this. (d1, d2, m, dif, div, disp, |disp|). Click on the field. The blue frame becomes red. Enter the wanted value.



- Toggle button Yes/No : Open Comparison Graphic : If Yes is selected, a double-click in the list opens an image in the right pad of the Pairs of Spots menu.
- ❖ First area : display the coordinates of the first spot of a pair.
- ❖ Second area : display an enlargement of the first spot of a pair, its coordinates in pixels ??, its radius, its value, and its percentage of intensity in relation with the intensities sum of the both pair spots.
- ❖ Third area : display the coordinates of the second spots of pair.
- ❖ Fourth area : display an enlargement of the second spot of a pair, its coordinates in pixels ??, its radius, its value, and its percentage of intensity in relation with the intensities sum of the both pair spots.
- ❖ Fifth area : display a pie graphic, with the intensities proportion of the both spots. The first spot portion is green (like the frame of the second area), and the second spot portion is red.

❖ Sixth area : ??

❖ Modification buttons : to modify the spot modelization :

- ☞ translate the circle toward the left, of one pixel.
- ☞ translate the circle toward the right, of one pixel.
- ☞ translate the circle toward the top, of one pixel.
- ☞ translate the circle toward the bottom, of one pixel.
- ☞ reduce the modeled circle of one pixel.
- ☞ increase the modeled circle of one pixel.

- Toggle button Yes/No : Open Detail Zoom : If Yes is selected, a double-click in the list opens a window named Detail. This image is an enlargement of the spot indicated when the double-click has been done. (The Configuration menu parameterizes the size and the enlargement factor.)

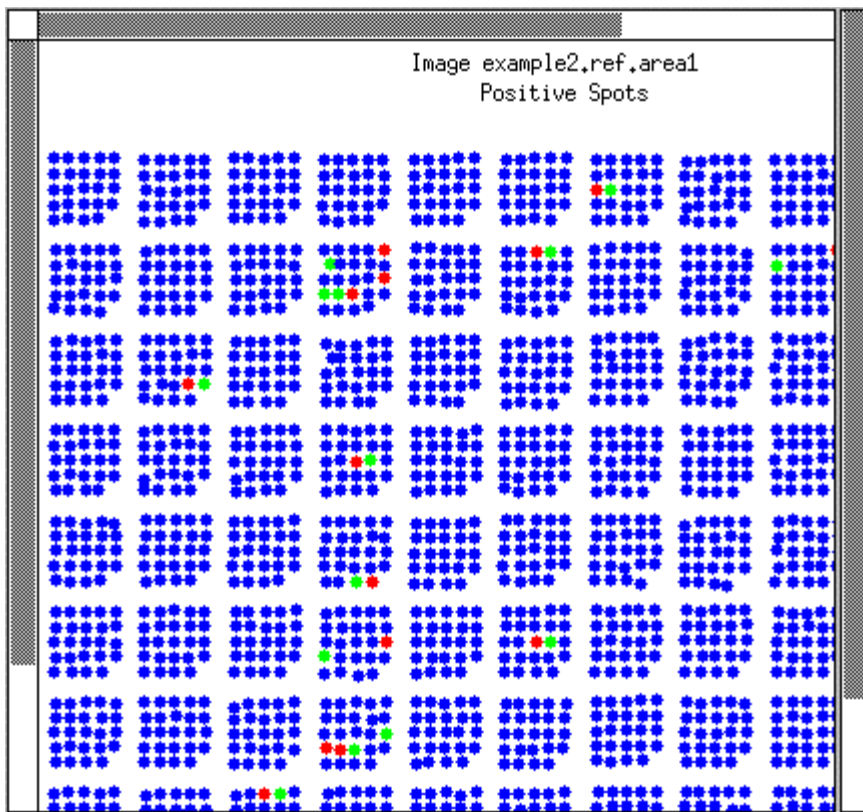
The bar at the bottom allows to close the window (Quit button), to modify the enlargement factor ("- " or "+" button) : click again in the list, on the same line. When you set the mouse pointer on the image, the coordinates in pixel unit are displayed in the bar, on the right, as well as the pixel value, and ??.

⇒ Histogram part :

- Drop-down menu about data type to analyze (facing the Histogram label) : the possible choices are :

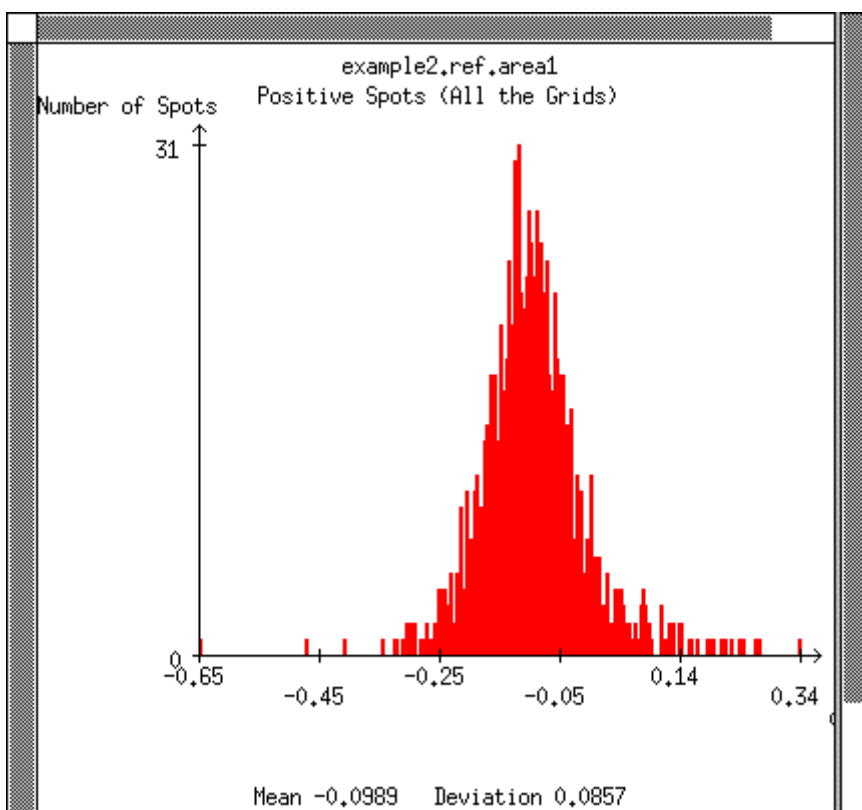
- ☞ Intensity Spot1
- ☞ Intensity Spot2
- ☞ Mean
- ☞ Subtraction
- ☞ Division
- ☞ Disparity
- ☞ Absolute disparity
- ☞ Expression Spot1
- ☞ Expression Spot2

- Drop-down menu Subdivisions : to build an histogram with a given number of subdivisions. The possible choices are : 300, 200, 100 or 50.
- Toggle button Yes/No : Inferior Threshold. If Yes is selected, a blue line is displayed on the histogram, according to the specified value in the right field.
- Input field (inferior threshold): Click in the field, enter a real number, and press the Enter key.
- Toggle button Yes/No : Superior Threshold. If Yes is selected, a green line is displayed on the histogram, according to the specified value in the left field.
- Input field (superior threshold): Click in the field, enter a real number, and press the Enter key.



- ◆ Drawing button : click on this button to displayed the drawing according the parameters adjusted with Option button. In the following example, the selected parameters are : Image : Division (3 colors) [Spot], Zoom x1.

(In red : spot ratio < 0.75
In green : spot ratio > 1.25
In blue $0.75 < \text{spot ratio} < 1.25$)



- ◆ Histogram button : click on this button to displayed the histogram according to the parameters adjusted with Option button. In the following example, the parameters adjustment are Datas type : Disparity and Subdivisions = 300.

▼ Tolerance		20.0%		+ -		99% / 1152		▼ Value	
	1	2	3	4	5		6	7	
1	1.26	1.26	1.12	1.12	1.03		1.13	1.13	
2	1.03	1.08	1.08	1.21	1.21		1.13	1.20	
3	1.03	1.03	1.09	1.09	1.08		1.23	1.23	
4	1.08	1.02	1.02	1.09	1.09		1.04	1.12	
5	1.04	1.04	1.08	1.08			1.14	1.14	
6	1.17	1.17	1.17	1.17	1.19		1.24	1.24	
7	1.19	1.05	1.05	1.04	1.04		1.10	1.13	
8	1.16	1.16	1.24	1.24	1.14		1.21	1.21	
9	1.14	1.11	1.11	1.16	1.16		1.27	1.14	
10	1.09	1.09	1.02	1.02			1.18	1.18	
11	1.16	1.16	1.11	1.11	1.08		1.19	1.19	
12	1.08	1.19	1.19	1.20	1.20		1.03	1.18	
13	1.19	1.19	1.16	1.16	1.01		1.04	1.04	
14	1.01	1.14	1.14	1.11	1.11		1.12	1.12	
15	1.11	1.11	1.10	1.10			1.06	1.06	
16	1.21	1.21	1.16	1.16	1.25		1.08	1.08	

♦ **Table button** : Display a table giving value type for each spot. In white are indicated the spots whose the values are out of the tolerance scale. In green are indicated the spots whose the values are in the tolerance scale.

- Drop-down menu **Tolerance** : Click on this menu, hold down and drag. Choose the wanted tolerance. The choices are : 0%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%. The tolerance is applied according to the mean value of the both spots.
- "+" button : To increase the tolerance by step of 1%.
- "-" button : To decrease the tolerance by step of 1%.

- Display field : Spots number in the tolerance scale, and whole spots number.
- Drop down menu **Value** : To select a criteria. The choices are :

- ☞ Division
- ☞ Disparity
- ☞ Mean
- ☞ Subtraction
- ☞ Intensity

- ◆ List button : click on this button to display the list according with Option button. In the following example, the notched options are : Sel1; t; Sel2; t, disp.

Save

Print

Zone : example2.ref.areal

sel :

Mean Intensity

With background level

No Normalization

Spots Coord. Line/Column

Number of Spots : 1151

Disparity Less than 0.25 : 1116 (0.97)

Disparity Less than 0.50 : 1150 (1.00)

Disparity Less than 1.0 : 1151 (1.00)

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- Drop-down menu Save : to choice the saving format of the list. The choice are : Ascii, Exel, Postscript.
- Drop-down menu Print : to choice the printing format. The only choice is Postscript.

- ◆ File Name button : If there is at least a file with a .pair extension. In this case, a Selection menu opens. Chose the file. A file with a .pair extension contains the pairs localization in a block.
- ◆ Input field : It's possible to enter directly the path and the name of a file.

- ♦ Create button : To create the menu to describe the pairs localization in a block. Enter a filename in the input field on the left, and click on this button. The menu appears :
 - Drop-down menu Density : The choices are : 1x1; 2x2; 5; 8; 3x3; 4x4; 5x5; 6x6. The display area on the right at the bottom visualizes the bloc geometry.
 - Number of Lines field : fill with the blocks lines number of the zone.
 - Number of Columns field : fill with the blocks columns number of the zone.

- Display area : The area displays the spots of one block. Set the mouse pointer on a spot, and click on. A number is assigned it. Click on the other spot of the pair : the same number is assigned. Begin again this operation for all the pairs, and click on the Create button on the bottom of the Pair of Spots menu.
- Information area : recalling how to create a file which described the pairs localization.
- Create button : To create the file which described the pairs localization.

Tools : Cross Graphic

This menu allows to compare two zones, which have the same geometry.



- Open a zone. Click on the zone with the mouse, display the spots through the Spot menu, and let the mouse pointer on the zone.
To specify the filename in the Cross Graphic menu, press together the third keys : (Alt + shift + 1). The first filename appears on the right of the label Image1.
- Open a new zone, to compare it. Click on the zone with the mouse, display the spots through the Spot menu, and let the mouse pointer on the zone.
To specify the filename in the Cross Graphic menu, press together the third keys : (Alt + shift + 2). The second filename appears on the right of the label Image2 :

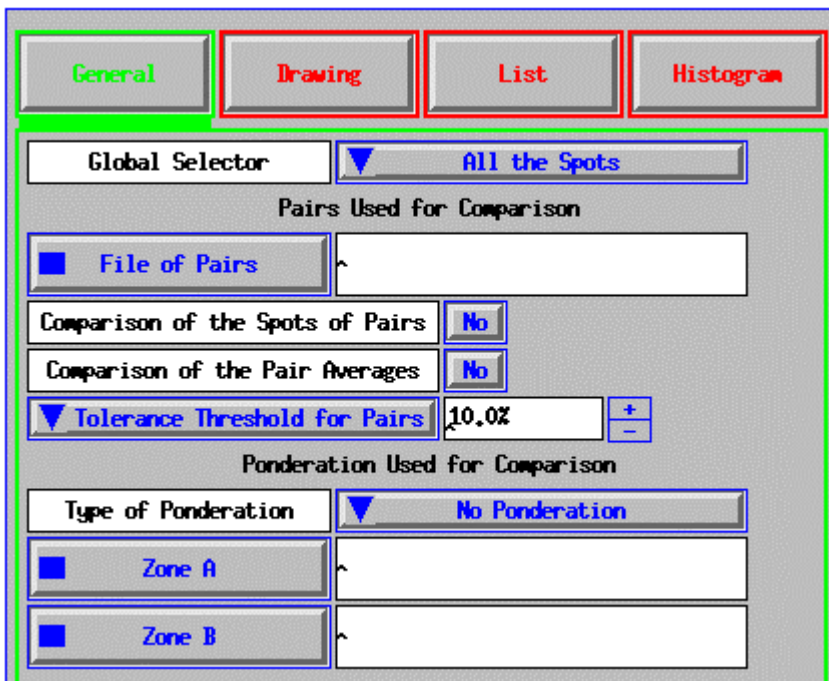


- ◆ Button Quit : to close this menu.
- ◆ Button Option : Open the Option menu : to set the parameters concerning the drawing, the histogram, and the list to display.
- ◆ Button Drawing : Open a window to draw a graphic, according to the Drawing parameters, defined in the Option menu.
- ◆ Button Histogram : Open a window to draw an histogram, according to the Histogram parameters, defined in the Option menu.
- ◆ Button List : Open a window to list spots characteristics, according to the List parameters, defined in the Option menu.

Option Menu.

This menu allows to parameterize the information to display, and how display them.

- ♦ Button General : The following menu appears :



- Drop-down menu Global Selector : Analyze All the Spots by default. To change, click on the drop-down menu, hold-down and drag to select another option. The options are:

All the spots.
 Positive spots
 Common positive spots
 Positive spots only in Image1
 Positive spots only in Image2
 Negative spots
 Common negative Spots
 Negative spots only in Image1
 Negative spots only in Image2

- Button File of Pairs : Click on this button to open a Selection menu. Choose the wanted pairs description file.
- Input field File of Pairs : To key-in directly a pairs description filename.
- Toggle button : Comparison of the Spots of Pairs :
- Toggle button : Comparison of the Pair Averages :
- Drop-down menu : Tolerance Threshold for Pairs : The choices are : 0%; 10%; 20%; 30%; 40%; 50%; 60%; 70%; 80%; 90%; 100%.
- +/- buttons : to adjust the tolerance threshold by step of 1%.
- Drop-down menu Type of Ponderation : Apply No Ponderation by default. To change, click on the drop-down menu, hold-down and drag to select another option. The options are :
 - ☞ No ponderation.
 - ☞ Membrane A/B : Each spot of the first zone is divided by the corresponding spot of the second zone. The zone (Image1) to compare is divided by these values.
- Input field Membrane A : Enter the name of the first zone (Membrane A/B option)
- Input field Membrane B : Enter the name of the second zone (Membrane A/B option)

- ◆ Button Drawing : To parameterize the drawings. The following menu appears :

- Toggle buttons Yes / No: To choose the scale type, in the case X by Intensity (Drop-down menu facing Visualization label).

To choose a linear scale : click and set Yes before the Linear label.

To choose a logarithmic scale : click and set Yes before the Logarithmic label.

- Toggle button Yes / No : To display the spots inferior to the superior threshold.

Without superior threshold : click and set No before the Superior Threshold label.

With a superior threshold : click and set Yes before the Superior Threshold label.

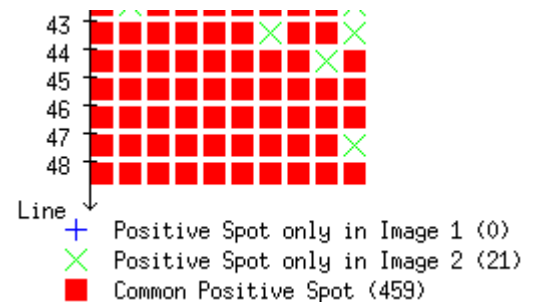
- Toggle button Yes / No : To display the spots superior to the inferior threshold.

Without superior threshold : click and set No before the Inferior Threshold label.

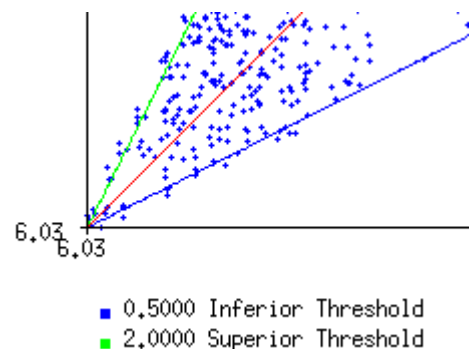
With a superior threshold : click and set Yes before the Inferior Threshold label.

- Drop-down menu : (by default : X by Type) The options are :

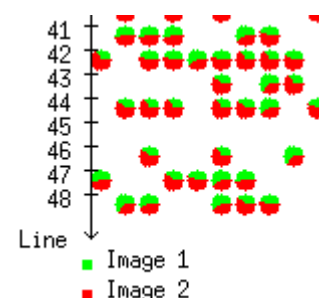
☞ X by Type : Map with the spots type : display a red square for the common positive spots, a blue cross (+) for the spots which are only positive on the first image, and a green cross (x) for the spots which are only positive on the second image. Example of a fraction of a map (Click on the blue Drawing button) :



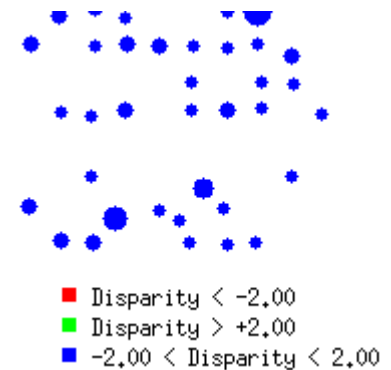
☞ X by Intensity : Example of a fraction of a graphic (Click on the blue Drawing button) :



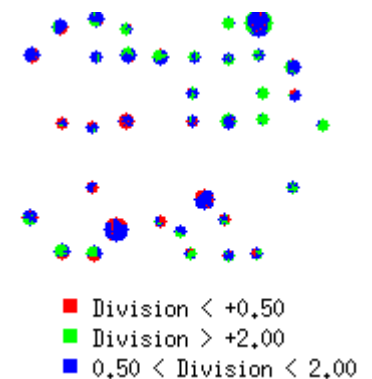
☞ X by Comparison : Map with spots as pies. The intensity contribution of a spot of the first image is green, and the intensity contribution of corresponding spot of the second image is red. Example of a fraction of a map (Click on the blue Drawing button) :



- ☞ Image : Disparity (3 Colors) : Map with spots disparities. For the spots which the disparity is inferior to -2 and superior to +2, the color is blue. For the spots which the disparity is inferior to -2, the color is red, and for the spots which the disparity is superior to +2, the color is green. Furthermore, the spots size is the maximum size between the corresponding spots. Example of a fraction of a map (Click on the blue Drawing button) :

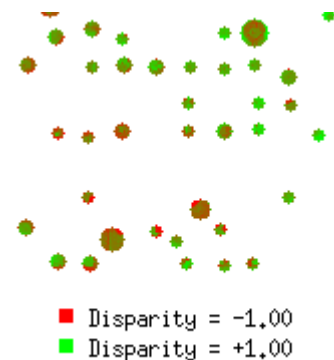


- ☞ Image : Division (3 Colors) : Map with spots disparities. For the spots which the division is inferior to 0.5 and superior to +2, the color is blue. For the spots which the division is inferior to -0.5, the color is red, and for the spots which the division is superior to +2, the color is green. Furthermore, the spots size is the maximum size between the corresponding spots. Example of a fraction of a map (Click on the blue Drawing button):



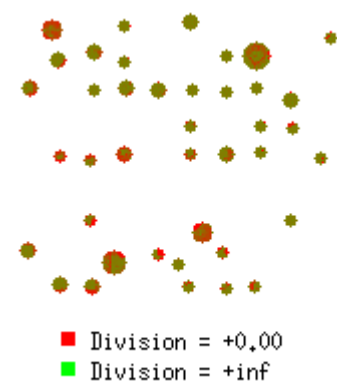
☞ Image : Disparity (256 Colors) : Identical to Image : Disparity (3 colors) except for the representation :

(■ : Disparity = - Maximum negative disparity; ■: Disparity = Maximum positive disparity). Between this two boundaries, intermediate colors in RGB coding are computed and displayed. Example of a fraction of a map (Click on the blue Drawing button) :



☞ Image : Division (256 Colors) : Identical to Image : Division (3 colors) except for the representation :

(■ : Division = Minimum ratio ; ■: Division = Maximum ratio) . Between this two boundaries, intermediate colors in RGB coding are computed and displayed. Example of a fraction of a map (Click on the blue Drawing button) :

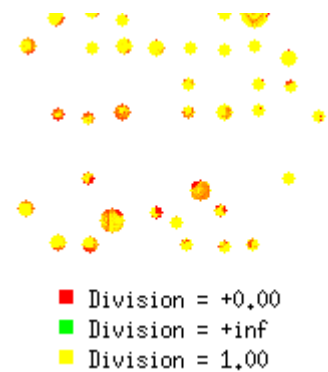


- ☞ Superposition of Spots : The computation is realized for each corresponding pixel in the corresponding spots :

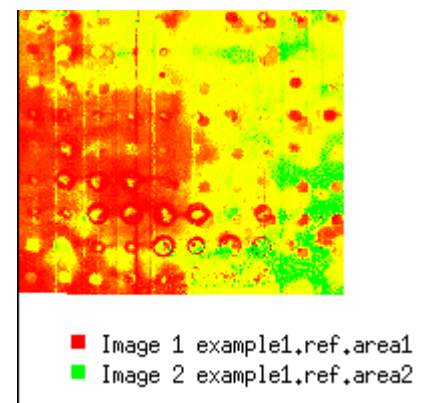
Superposition of spots = pixel1 / pixel2

For each pixel, the representation is as follows :

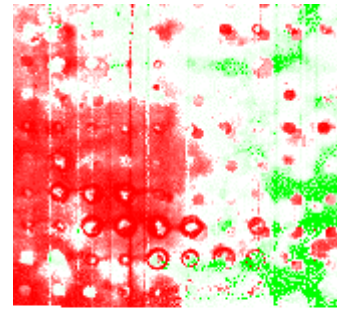
(■ : Division = minimum ratio; ■ : Division = 1; ■ : Division = maximum ratio)
Between these boundaries, intermediate colors in RGB coding are computed and displayed. Example of a fraction of a map (Click on the blue Drawing button) :



- ☞ Superposition of Images (Red, Yellow, Green) : Example of a fraction of a map (Click on the blue Drawing button) :

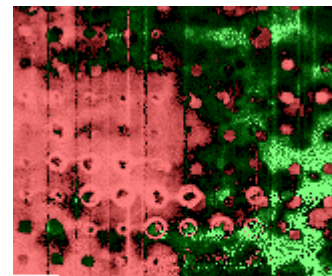


☞ Superposition of Images (Red, White, Green) : Example of a fraction of a map
(Click on the blue Drawing button) :



■ Image 1 example1.ref.area1
■ Image 2 example1.ref.area2

☞ Superposition of Images (Red, Black, Green) : Example of a fraction of a map
(Click on the blue Drawing button) :

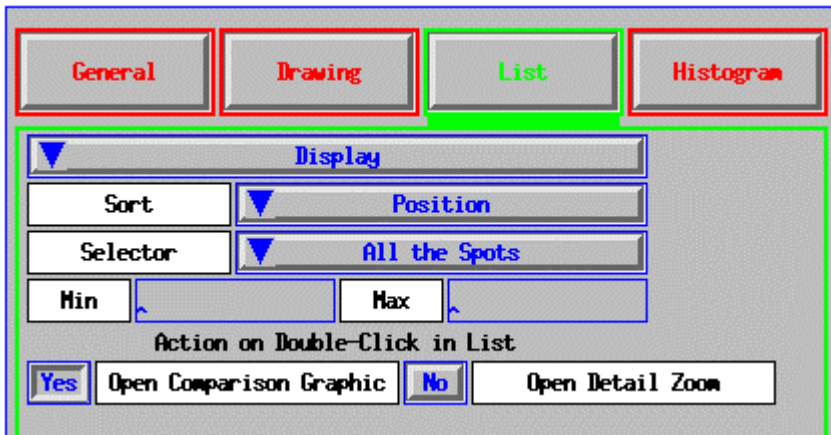


■ Image 1 example1.ref.area1
■ Image 2 example1.ref.area2

- Drop-down menu : (by default : Zoom1). The options are : Zoom1 (x1); Zoom2 (x2); Zoom3 (x3); Zoom4 (x4).
- Input field TX (0.0 by default) : point shifting in the x-axis direction. (See the drop-down menu below).
- Input field TY (0.0 by default) : point shifting in the y-axis direction. (See the drop-down menu below).
- Input field ANGLE (0.0 by default) : image rotation according to the specified point. (See the drop-down menu below).

- Drop-down menu (by default : Point 1 Image 1) : to superpose the spots between the two images to compare.
 - ☞ Computation with Spots : automatic images superposition : the fields TX, TY, ANGLE, are automatically filled.
 - ☞ Computation with Points : Enter the points :
 - ☞ Point 1 Image 1 : to define a first point in the first image.
 - ☞ Point 2 Image 2 : to define a second point in the first image.
 - ☞ Point 1 Image 2 : to define a first point in the second image.
 - ☞ Point 2 Image 1 : to define a second point in the second image.
- Input field (by default 0.0) :
- Input field (by default 0.0) :

- ◆ Button List : (After the parameters adjustment, click on the blue List button).



- Drop-down menu Display : to notch the display options. By default are notched : coord, sel1, t, sel2, t, Disparity. The options are :

- ☞ Information
- ☞ Characteristics
- ☞ Notation
- ☞ coord
- ☞ sel1
- ☞ exp1
- ☞ t
- ☞ sel2
- ☞ exp2
- ☞ t
- ☞ Ponderation
- ☞ Mean
- ☞ Substation
- ☞ Division
- ☞ Disparity
- ☞ Absolute Disparity

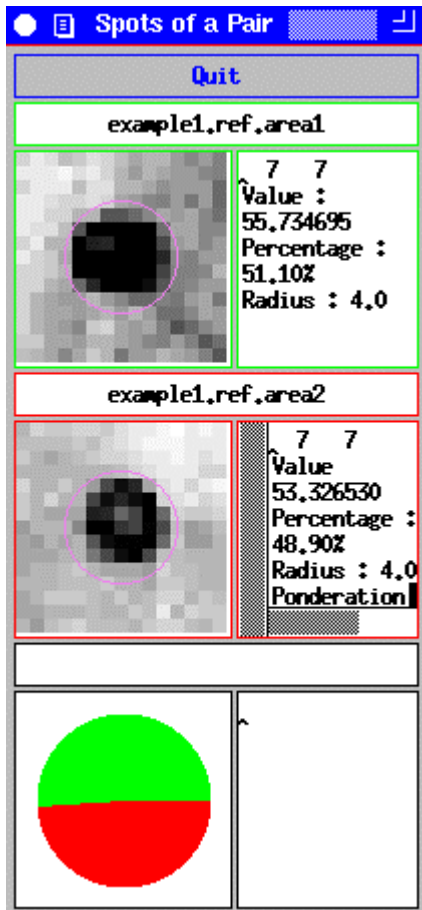
- Drop-down menu : Sort : to sort according to a criteria (by default, the criteria is Position). The options are :
 - ☞ Position
 - ☞ sel1
 - ☞ sel2
 - ☞ m
 - ☞ dif
 - ☞ div
 - ☞ disp
 - ☞ |disp|
 - ☞ exp1
 - ☞ exp2

- Drop-down menu Selector : Select spots according to a criteria (by default : no criteria : All the Spots). Before to select a criteria, fill the Min and Max fields below.
 - ☞ All the Spots
 - ☞ Min <= sel1 <= Max
 - ☞ Min <= sel2 <= Max
 - ☞ Min <= m <= Max
 - ☞ Min <= dif <= Max
 - ☞ Min <= div <= Max
 - ☞ Min <= disp <= Max
 - ☞ Min <= |disp| <= Max
 - ☞ Min <= exp1 <= Max
 - ☞ Min <= exp2 <= Max

- Input field Min : Enter a number.

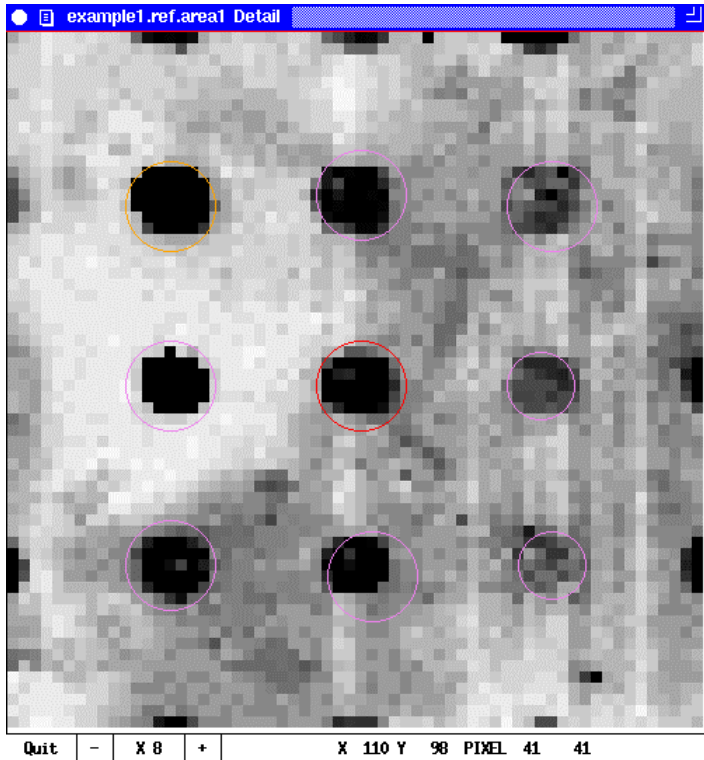
- Input field Max : Enter a number.

- Toggle button Yes (Open Comparison Graphic) : Click on a spot in the list, the window opens.

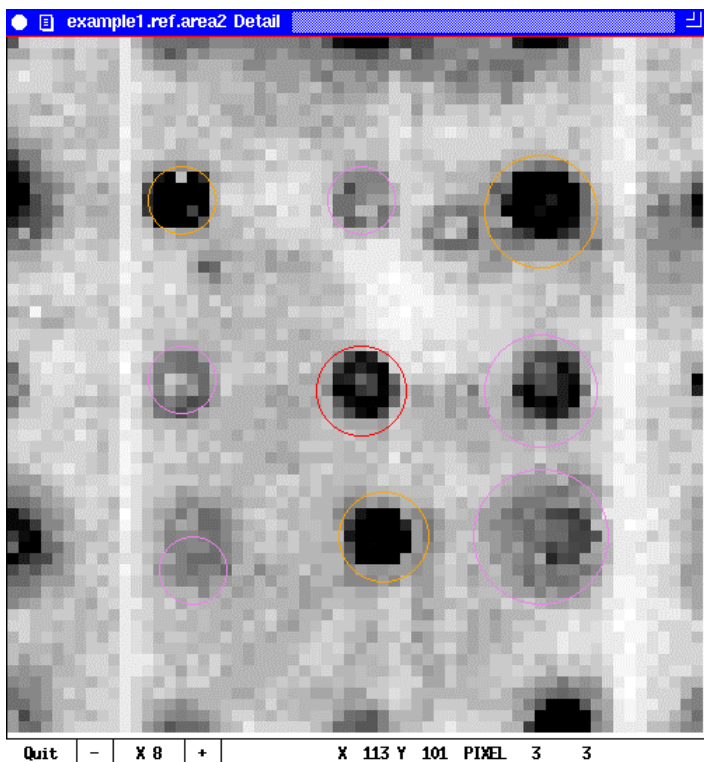


- ☞ First area : display the image name of the first spot.
- ☞ Second area : display an enlargement of the spot, its coordinates, its value, its percentage of intensity in relation with the intensities sum of the both pair spots, and its radius.
- ☞ Third area : display the image name of the second spot.
- ☞ Fourth area : display an enlargement of the spot, its coordinates, its value, its percentage of intensity in relation with the intensities sum of the both pair spots, and its radius.
- ☞ Fifth area : display a pie graphic, with the intensities proportion of the both spots. The first spot portion is green (like the frame of the second area), and the second spot portion is red.
- ☞ Sixth area : ??

- Toggle button No (Open Detail Zoom) : Click on the toggle button : Yes appears. Click on a spot in the list, two Detail windows open :

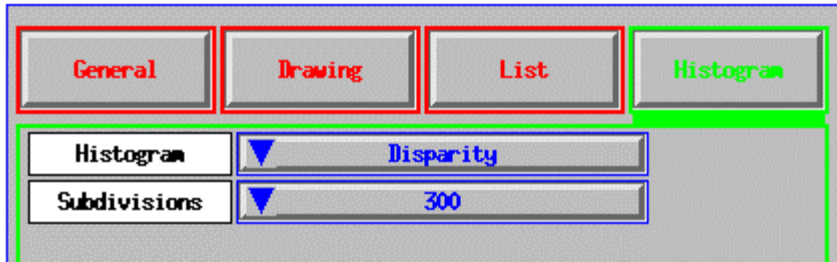


The first visualize an enlargement around the first spot.



The second visualize an enlargement around the second spot.

- ◆ Button Histogram (After the parameters adjustment, click on the blue List button) :



- Drop-down menu Histogram (by default : Disparity) : Draw an histogram with the chosen variable. The options are :
 - ☞ Intensity Spot1
 - ☞ Intensity Spot2
 - ☞ Mean
 - ☞ Subtraction
 - ☞ Division
 - ☞ Disparity
 - ☞ Absolute Disparity
 - ☞ Expression Spot1
 - ☞ Expression Spot2
- Drop-down menu Subdivisions (by default : 300) : Histogram bars number. The options are 300; 200; 100; 50

Tools : Differential Screening

To compare the spots of several zones.

Menu Differential Screening				
Quit	Help	Save	Print	
Directory	/home/brice/DEMO			
Result File Name	liste			
List of the Directories	List of the Membranes	List of the Selected Membranes	Choice	Characteristics
..	example2.ref.area.6 example1.ref.area2.6 example1.ref.area1.6 example1.ref.area3.6 example1.ref.area4.6			
Selector	Common Positive Spots			
Variable	Mean Intensity			
Value	Absolute Value			
Coordinates	Spots Coord. Line/Column	Number of Images	1	
<div>Information</div> <div>coord</div> <div>Values</div> <div>ec</div>				

- ♦ Quit button : to close this menu.
- ♦ Help button : open a Help menu, with the description of the principal functions.

- ◆ Drop-down menu Save : to choice the saving format of the list. The choices are : Ascii, Exel, Postscript.
- ◆ Drop-down menu Print : to choice the printing format. The only choice is Postscript.
- ◆ Input field Directory : By default, display and select the current directory. It's possible to key in directly in the field.
- ◆ Input field Result File Name : By default, define and display the filename of the saving file. The saving filename is : "*filename*" **.comp**
- ◆ Area List of the Directories : display the sub-directories. To go up in the tree directories, double-click on "..".
- ◆ Area List of the Membranes : display the analyzed zones names. Double-click on a zone to select it. The name is displaying on the right area List of the Selected Membranes.
- ◆ Area List of the Selected Membranes : display the selected analyzed zones names to compare.
- ◆ Choice : use with the criteria Spots of Chosen Type selected with the drop-down menu Selector. By default, all the spots are selected (display I). Double-click on the letter to change the spot type. The choices are in this order : I, N, L, M, S, P, I,...
- ◆ Display area : Characteristics : Display the following characteristics : density, lines number, columns number.
- ◆ Drop-down menu Selector : To select a criteria to extract spots. The criteria is applied about spots of same coordinates. The criteria are :
 - Common Positive Spots : extract a spot if the spots of all the zones are positive.
 - Common Low Spots : extract a spot if the spots of all the zones are low.
 - Common Medium Spots : extract a spot if the spots of all the zones are medium.
 - Common Strong Spots : extract a spot if the spots of all the zones are strong.
 - Common Negative Spots : extract a spot if the spots of all the zones are negative.

Positive Spots at least n Images : extract a spot if there is at least one positive spot in all the zones.

Low Spots at least n Images : extract a spot if there is at least one low spot in all the zones.

Medium Spots at least n Images : extract a spot if there is at least one medium spot in all the zones.

Strong Spots at least n Images : extract a spot if there is at least one strong spot in all the zones.

Negative Spots at least n Images : extract a spot if there is at least one negative spot in all the zones.

Positive Spots at most n Images : extract a spot if there is at most one positive spot in all the zones.

Low Spots at most n Images : extract a spot if there is at most one low spot in all the zones.

Medium Spots at most n Images : extract a spot if there is at most one medium spot in all the zones.

Strong Spots at most n Images : extract a spot if there is at most one strong spot in all the zones.

Negative Spots at most n Images : extract a spot if there is at most one negative spot in all the zones.

Spots of Chosen Type : extract a spot if the spots type is identical in comparison with the spots type specified with Choice.

Common Positive Spots and at least n Low Spots : extract a spot if all the spots are positive, with at least a low spot.

Common Positive Spots and at least n Medium Spots : extract a spot if all the spots are positive, with at least a medium spot.

Common Positive Spots and at least n Strong Spots : extract a spot if all the spots are positive, with at least a strong spot.

Common Positive Spots and at most n Low Spots : extract a spot if all the spots are positive, with at most a low spot.

Common Positive Spots and at most n Medium Spots : extract a spot if all the spots are positive, with at most a medium spot.

Common Positive Spots and at most Strong Spots : extract a spot if all the spots are positive, with at most a strong spot.

- ◆ Drop-down menu Variable : To select the variable type to display in the second area, for the extracted spots according to the Selector. The choices are :
 - Mean Intensity
 - Sum of the Intensities
 - Weighted Mean Intensity
 - Gaussian Intensity
 - Maximum Intensity
 - Central Intensity
 - Radius
 - Background level
 - Mean Intensity Without Background Level
 - Sum of the Intensities Without Background Level
 - Weighted Mean Intensity Without Background Level
 - Gaussian Intensity Without Background Level
 - Maximum Intensity Without Background Level
 - Central Intensity Without Background Level

- ◆ Drop-down menu Value : to apply a function on the spots values. The choices are :
 - Absolute Value.
 - Value relative to the Min : value divided by the minimum value.
 - Value relative to the Max : value divided by the maximum value.
 - Value relative to the Mean : value divided by the mean of the values.
 - Value relative to the Spot : ??
 - Value relative to an Interval : ??
 - Value relative to References : ??
 - Value relative to a Value : value divided by a value. The value is entered in the field facing Value.
 - Value relative By Block : ??

- ◆ Input field (facing value) : to enter a value used Value.

- ◆ Drop-down menu Coordinates : To choose the coordinates system.

- ◆ Information area : Number of Images : display the images number used to get the differential results.

- ◆ First display area : display the coordinates of the extracted spots.
- ◆ Second display area : display information about the extracted spots according to the choices defined with the drop-down menus Variables and Value. The information to display are selectable with the toggle buttons at the top of this area :
 - Toggle button Information : to display general information about the selected zones.
 - Toggle button Coord : to display the chosen coordinates system.
 - Toggle button Value : to display the selected spots values.
 - Toggle button m : to display the mean of spots (with the same coordinates) of all the selected zones.
 - Toggle button ec : to display the standard deviation of spots (with the same coordinates) of all the selected zones.

Tools : Operations on Membranes

Menu Operations on Membranes																							
Quit	Help	Save	Print																				
Result File Name	liste6																						
Directory	/home/brice/DEMO																						
List of the Membranes		List of the Directories																					
<pre>example2.ref.area.G example1.ref.area2.G example1.ref.area1.G example1.ref.area3.G example1.ref.area4.G example3.tif.area.G</pre>		<pre>DEMO_REF</pre>																					
<table border="1"> <tr> <td>C</td> <td>(</td> <td>)</td> <td>/</td> </tr> <tr> <td>7</td> <td>8</td> <td>9</td> <td>*</td> </tr> <tr> <td>4</td> <td>5</td> <td>6</td> <td>-</td> </tr> <tr> <td>1</td> <td>2</td> <td>3</td> <td>+</td> </tr> <tr> <td>0</td> <td>.</td> <td>+/-</td> <td>=</td> </tr> </table>				C	()	/	7	8	9	*	4	5	6	-	1	2	3	+	0	.	+/-	=
C	()	/																				
7	8	9	*																				
4	5	6	-																				
1	2	3	+																				
0	.	+/-	=																				
Coordinates	Spots Coord. Line/Column	Sort	By Line																				
Information	coord	Values																					
<pre>^</pre>																							

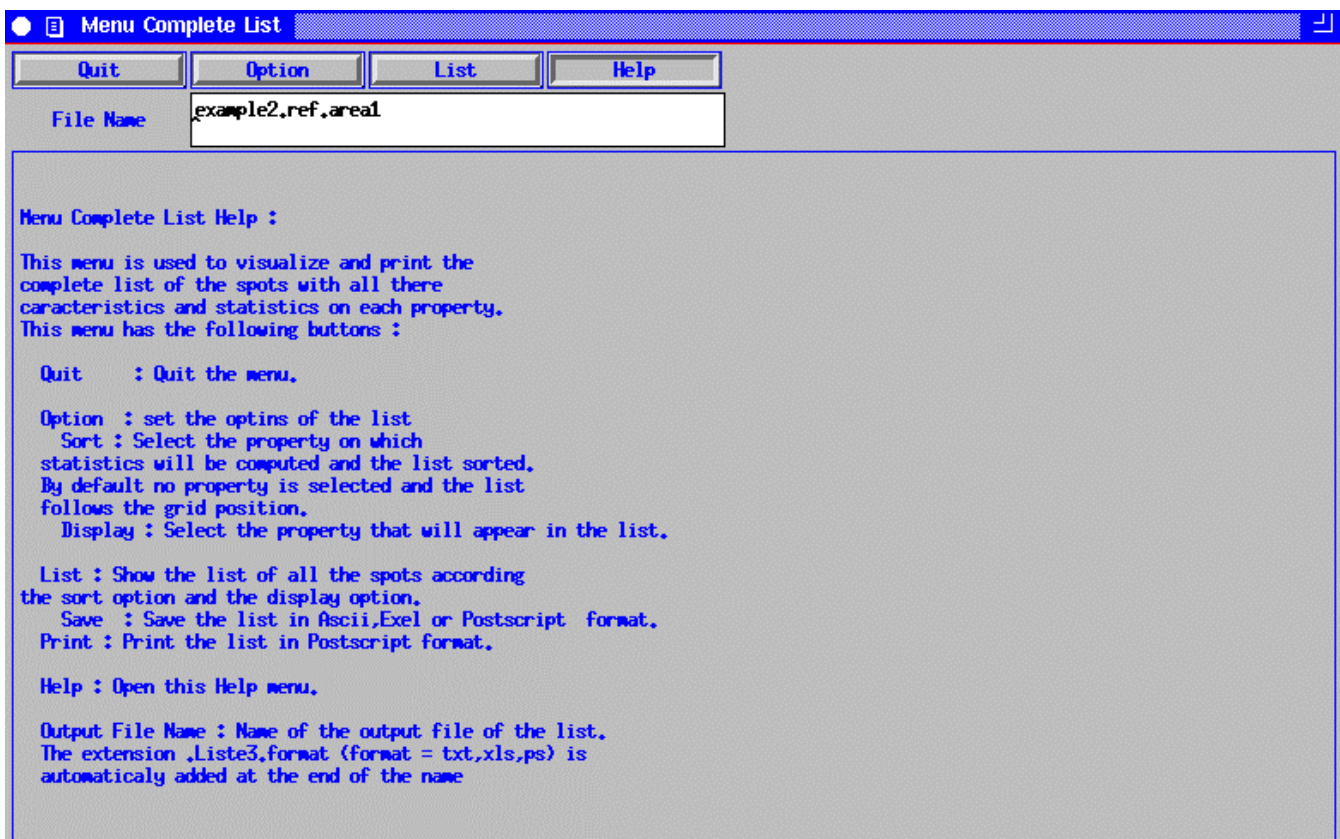
- ◆ Quit button : to close this menu.
- ◆ Help button : to get information about the main functions.
- ◆ Drop-down menu Save : to choice the saving format of the list. The choice are : Ascii, Exel, Postscript.
- ◆ Drop-down menu Print : to choice the printing format. The only choice is Postscript.

- ◆ Input field Result File Name : By default, define and display the filename of the saving file.
- ◆ Input field Directory : By default, display and select the current directory. It's possible to key in directly in the field.
- ◆ Area List of the Membranes : display the analyzed zones names. Double-click on a zone to select it. The name is displaying on the area facing the calculator pad.
- ◆ Area List of the Directories : display the sub-directories. To go up in the tree directories, double-click on "..".
- ◆ Display field : recalling the geometry parameters of the selected zone (Density; Number of Grids; Number of Lines; Numbers of Columns).
- ◆ Calculator pad : To realize operations on the membranes. Click on the key. (To get the result, key "=").
- ◆ Display area : display the operations sequence. To clear the sequence, key "C".
- ◆ Drop-down menu Coordinates : To choose the coordinates system to display.
- ◆ Drop-down menu Sort : To sort the results according four choices :
 - By Line
 - By Column
 - By Grid
 - By Value
- ◆ Toggle button Information : To display general information about zones.
- ◆ Toggle button coord : to display the coordinates.
- ◆ Toggle button Values : to display the operations result.
- ◆ Display area : display the operation result, according to the given choices.

Tools : Complete List

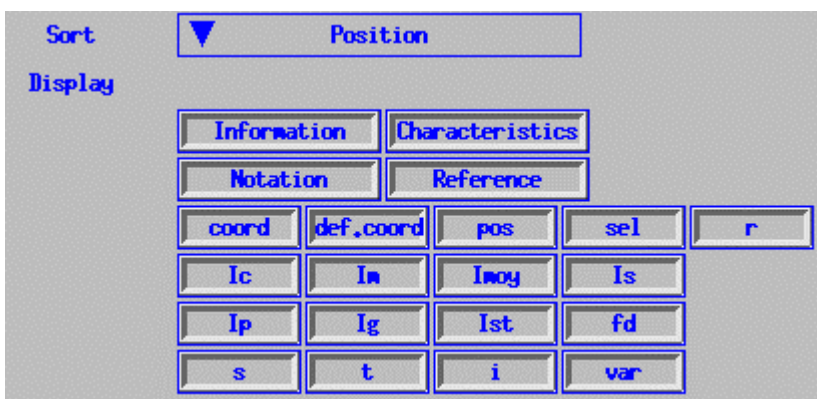
Select Complete List through the Analysis Tools menu, in the Spots menu. The menu opens with the Help button pressed.

The Complete List menu allows to display the spots parameters. It's possible to select the parameters to display, and to sort the list in relation with one chosen parameter.



♦ Quit button : To close the menu.

♦ Option button : The following menu appears :



- Drop-down menu to sort : facing the Sort label. To sort the list in relation with one chosen parameter. The parameters are :

position : (default parameter)
 radius
 central intensity
 maximum intensity
 mean intensity
 sum of the intensities
 weighted mean intensity
 gaussian intensity
 statistical intensity
 background level
 selector (not available)
 coord.file ??

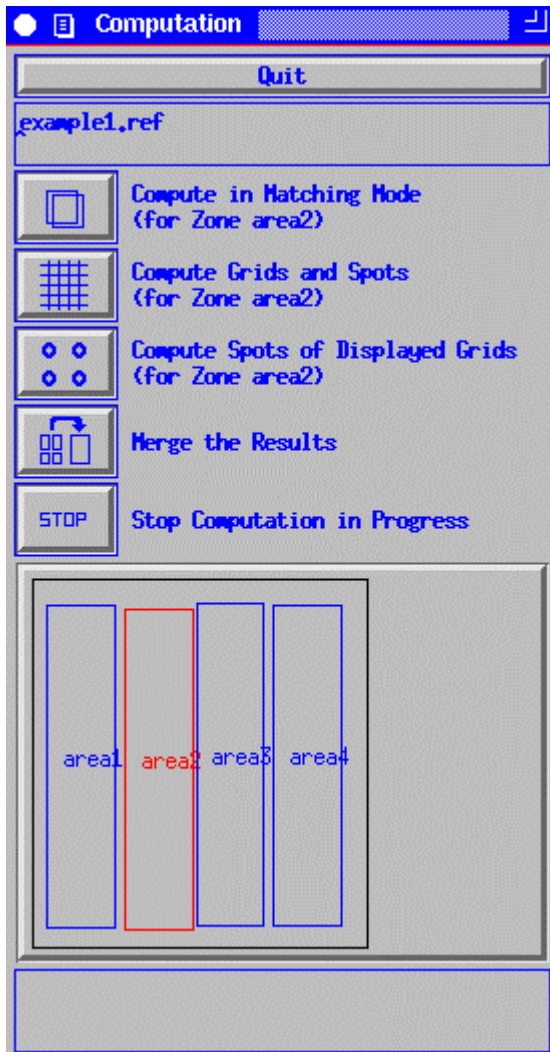
- Display buttons : to select the parameters to display.
 - Information : ??
 - Characteristics : display general characteristics about type spots (mean, standard deviation), thresholds, min and max value.
 - Notation : calling of the abbreviations meaning :
 - li : line number.
 - co : column number
 - g : grid number
 - xc : pixel x coordinate of the spot centre.
 - yc : pixel y coordinate of the spot centre.
 - Ic : intensity of central pixel.
 - xm : pixel x coordinate of the maximum intensity.
 - ym : pixel y coordinate of the maximum intensity.
 - xb : pixel x coordinate of the intensities barycentre.
 - yb : pixel y coordinate of the intensities barycentre.
 - Ib : intensity of barycentre pixel.
 - Imoy : mean of the pixels intensities in the spot.
 - Is : sum of the pixels intensities in the spot.
 - Ip : weighted sum of the pixels intensities in the spot.
 - Ig : central intensity of the intensities gaussian modelization.
 - r : radius of the modeled circle.
 - fd : background level.
 - var : external variable.
 - s : area.

t : type (low, medium, strong, reference, negative).
 L : low type.
 M : medium type.
 S : strong type.
 R : reference type
 N : negative type.
 rl : relative value.
 m : mean value.
 ec : standard deviation.
 dif : subtraction (x-y).
 div : division (x/y).
 disp : disparity : $[(x-y) / (x+y)/2]$.

- Reference ??
- Coord : coordinates system defined through the drop-down menu Coordinates in the Spots menu.
- Def. Coord : Default coordinates default : Grid Line Column
- pos : spot position in pixel unit.
- sel
- r
- Ic
- Im :
- Imoy
- Is
- Ip
- Ig
- Ist
- fd
- s
- t
- i : ??
- var : ??

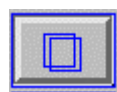
Computation menu.

To start a computation of indexing grids, or/and spots.



♦ Quit button : to close this menu.

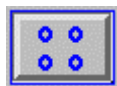
♦ Display field : Display the image filename.



Compute in Matching Mode : To compute with the matching parameters (Parameters menu).



Compute Grids and Spots : To compute Grids and Spots for the selected zones. The selected zones are specified in bracket. When the computation is finished, the grids menu appears (see Grids menu)



Compute Spots of Displayed Grids : To compute only the spots, from the displayed indexing grids, for the selected zones. This button is useful when parameters concerning the only spots are modified, like Position sub-menu, Radius sub-menu, or still, when indexing grids are manually defined. When the computation is finished, the spots menu appears (see Spots menu).



Merge the Results : Merge the analyzed zones of a same image. Click on the zones to merge in the selection area just below (The selected zones become red). To unselect a zone, click again on it. Then, click on the Merge button. The Display menu appears with a new zone : Zone all. (See the example at the end of this menu description).

After a merge, note that two files are created :

A text file, named *image_filename.merge.xls*, contains the whole spots data.

An image file, named *image_filename.all.G*, contains the merged selected images.



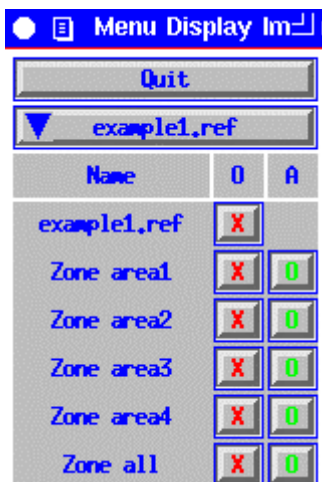
Stop Computation in Progress. : to stop the computation in progress.

- ♦ Selection Area : All the boundaries zones relating to the image to analyze are schematized in the selection area. Click on the chosen schematized zone to select it : the rectangle lines become red. it's possible to select others zones, or even, all the zones.

An example to merge zones.

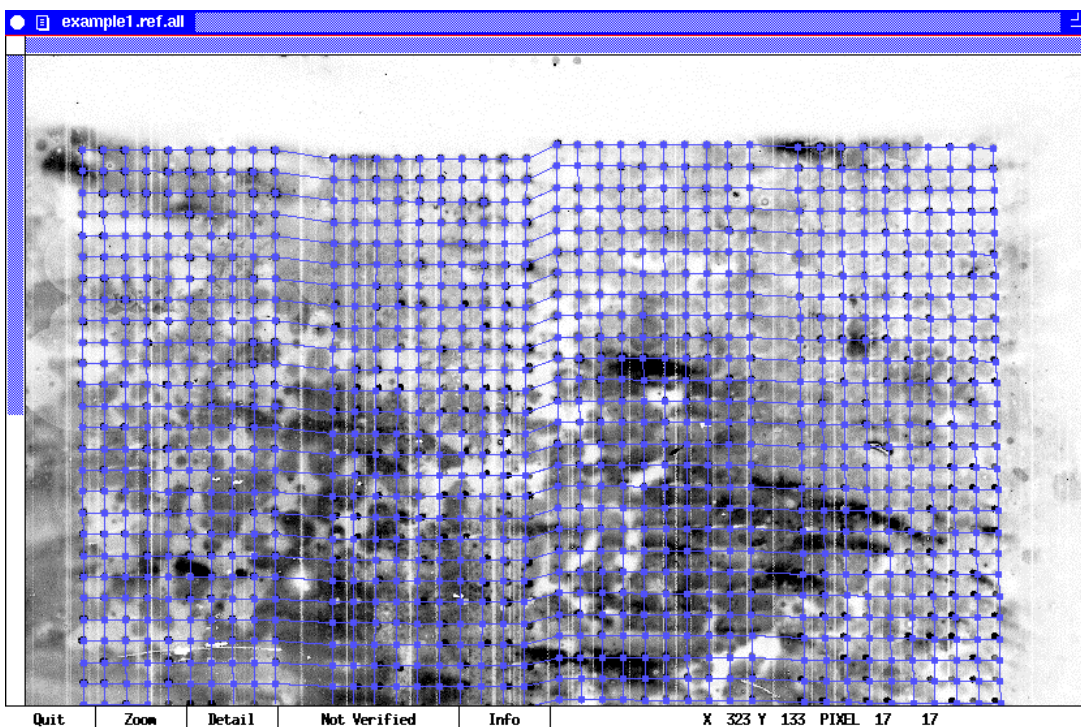
The example is example1. Having analyzed the four zones, click on the four zones in the Selection area : The four zones boundaries are red.

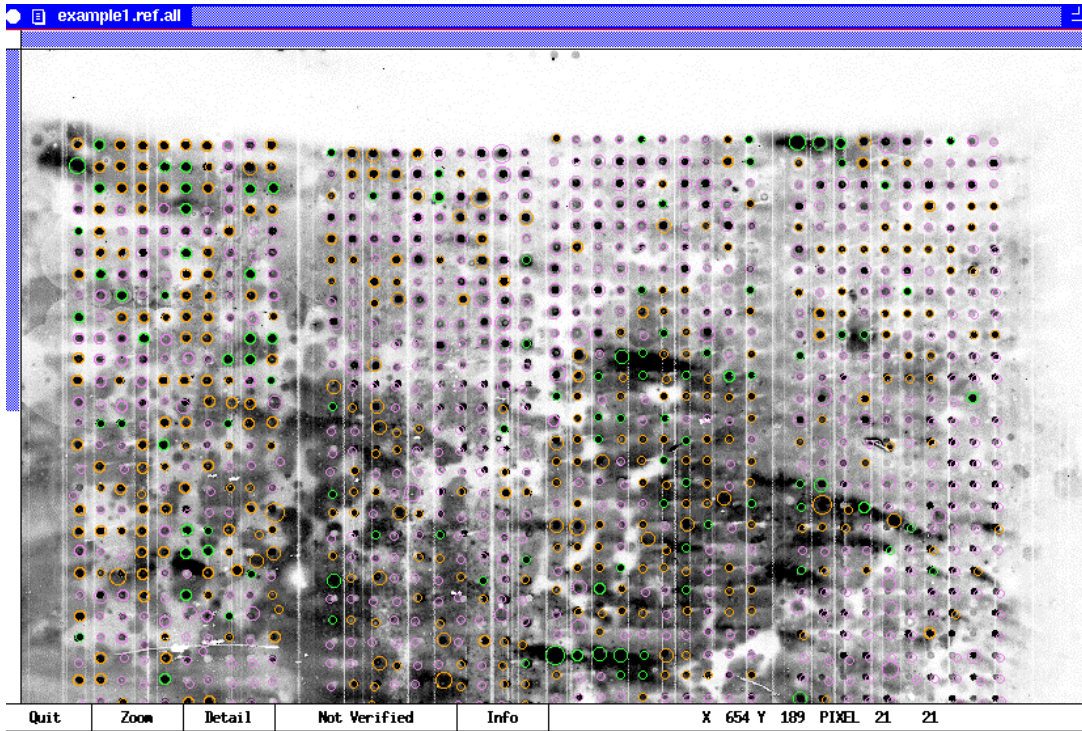
Click on the Merge button. The Display menu is updated with a new field : Zone All :



Click on the button with the red cross, facing Zone all : the whole image displays.

Click on Grid menu and display the indexing grids :





Click on Spot menu and display the spots.

Variables Notation.

co	column number.
d1	distance of a spot to the line $x=y$
d2	distance of a spot on the line $x=y$
dif	subtraction of values
disp	disparity of values
div	division of values
ec	standard deviation
exp	spot contribution
fd	background level
g	grid number.
Ib	intensity of the intensities barycentre for a spot.
Ic	intensity of the central pixel of a spot.
Ig	central intensity value of the intensities gaussian modelization.
Imoy	mean of the pixels intensities for a spot.
Ip	weighted sum of the pixels intensities for a spot.
Is	sum of the pixels intensities for a spot.
L	low spot type
li	line number.
M	medium spot type
m	mean value
N	negative spot type
r	radius of the modeled circle.
R	reference spot type
rl	relative value
s	area
S	strong spot type
sel	spot value according the background level and the normalization value
t	spot type
var	external variable
xb	x coordinate in pixel unit, of the intensities barycentre for a spot.
xc	x coordinate in pixel unit, of the spot centre.
xm	x coordinate in pixel unit, of the maximum intensity for a spot.
yb	y coordinate in pixel unit, of the intensities barycentre for a spot.
yc	y coordinate in pixel unit, of the spot centre.
ym	y coordinate in pixel unit, of the maximum intensity for a spot.

Variables signification.

column number.
 distance of a spot to the line $x=y$
 distance of a spot on the line $x=y$
 subtraction of values
 disparity of values
 division of values
 standard deviation
 spot contribution
 background level
 grid number.
 intensity of the intensities
 barycentre for a spot.
 intensity of the central pixel of a
 spot.
 central intensity value of the
 intensities gaussian modelization.
 mean of the pixels intensities for a
 spot.
 weighted sum of the pixels
 intensities for a spot.
 sum of the pixels intensities for a
 spot.
 low spot type
 line number.
 medium spot type
 mean value
 negative spot type
 radius of the modeled circle.
 reference spot type
 relative value
 area
 strong spot type
 spot value according the
 background level and the
 normalization value
 spot type
 external variable
 x coordinate in pixel unit, of the
 intensities barycentre for a spot.
 x coordinate in pixel unit, of the
 spot centre.

x coordinate in pixel unit, of the maximum intensity for a spot.

y coordinate in pixel unit, of the intensities barycentre for a spot.

y coordinate in pixel unit, of the spot centre.

y coordinate in pixel unit, of the maximum intensity for a spot.