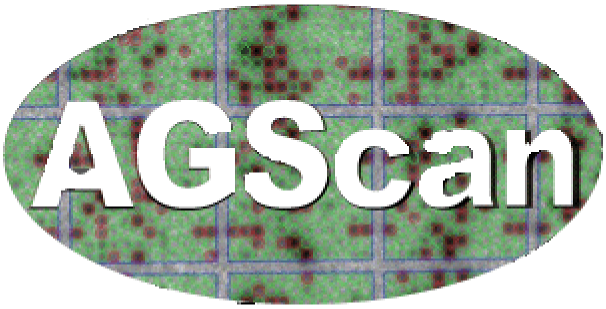
AGScan
USERS MANUAL

Version 0.1 / February 2006 Edition

Rémi Cathelin



Revision table :

Version	Date	Major modifications	Author(s)
0.1	February 15th 2006	First release	R. Cathelin

Acknowledgment :

I wrote this guide in English having the aim to give access to AGScan to the largest possible scientific community. But English is not my mother language, so any remark about the orthography or the grammar is welcome.

INDEX

I. Introduction

This manual is aimed to provide the necessary information to biologists using AGScan image processing software. The document is split in four parts :

- Introduction
- General overview
- Processing steps
- Plugins presentation
- Frequently asked questions

I.1. A bit of history

AGScan is a micro-array image processing software developed by the SIGENAE team.
http://www.sigenae.org/
It is an evolution of BZScan : BZScan is a radioactive DNA micro-array image processing software based on the JAI library . It was developed by the TAGC (http://tagc.univ-mrs.fr/) from 2002 to 2005.

In 2005, the SIGENAE team decided that it was worthwhile to go on with the development of BZScan, and mainly by going through the new Sigenae version named AGScan.

This software is freely available using the following address:
http://mulcyber.toulouse.inra.fr/projects/agscan/

You can also download the source code, report bugs and propose enhancements at the same address.

I.2. Main features of AGScan

The main features of AGScan are :

1. Grid conception
2. Able to use single or multiple (up to 3) channel images as input
3. Image manipulation and processing based on ImageJ
4. Manual and automatic global alignment
5. Automatic local alignment
6. Automatic quantification
7. Batch processing
8. Data export
9. Plugin support, to add new file formats, alignment or quantification methods...
10. Multi-language support

These different features will be presented in the rest of this document.

I.3. Vocabulary

Image : is a file produced by a micro-array scanner and sometimes modified (cut,...).

Grid : is first a file generated by AGScan describing the way the array has been spotted. From this it can be aligned on an image and be a part of an alignment. You will see later the importance of the grid structure.

Alignment : is composed of an image and a grid. It can have several states like 'not aligned', 'globally aligned' and 'locally aligned'.

Quantification : once the alignment is finished the quantification is the process of extracting one or more figures for each spot giving an intensity value for the spot.

Batch : is a group of alignments processed by AGScan from global alignment to quantification through local alignment.

II. General Overview

This chapter presents an overview of the whole process, some information about image file types and quality and gives the software install procedure.

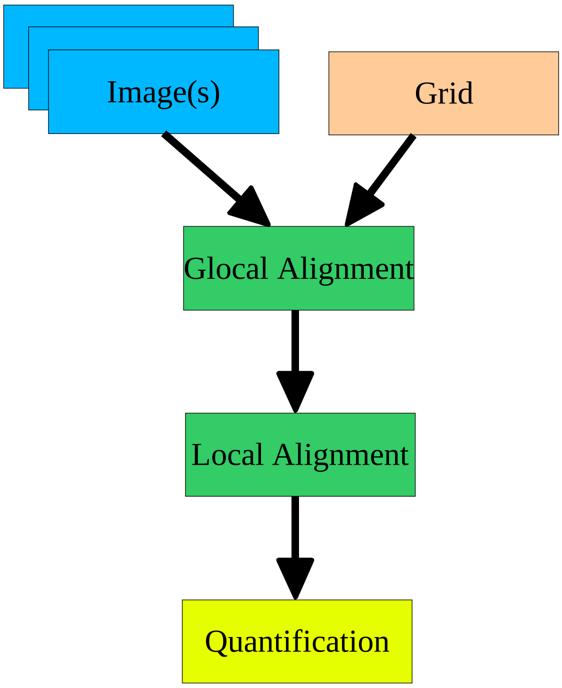
II.1. The process

To start the process you need at least :

- a micro-array image (different formats can be used),
- a grid file (generated in AGScan see next chapter section),
- AGScan installed (see install procedure below)

Notice that AGScan works with 16bits-unsigned grayscale images.
By default, AGScan input image format is the TIFF format but ISAC format (INF/IMG) is also recognized by a plugin automatically provided into all AGScan versions.

The grid file can be generated with AGScan or provided by someone having generated it. To share a grid you have to be sure that the micro-array and the definition used to scan the image are the same.



The process can be split in five steps :

- experiment image(s) loading

- grid loading

- global alignment : This step can be done by AGScan or by the user. It corresponds to the general alignment of the designed grid on the image(s).

- local alignment : This step is done by AGScan. Starting from the global alignment AGScan tries to figure out what the small local changes are to fit the sub-grids as well as possible to the image.

- quantification : This step is done by AGScan. Starting from the local alignment coordinates AGScan calculates for each spot several quantification values. These values will be presented later on.

As presented before, AGScan is able to process an image and a grid without any human help. This is a very important and user asked AGScan feature.

II.2. File formats

AGScan can load and save several file formats depending on the element. See table hereunder.

Element	***file extension***	***comments***
image [1]	**.tif**	Standard format of compression of image without loss of information,
	.inf	This file is the information file of the corresponding ".img" file. Both files have to be in the same directory to be used by AGScan. This file type is named ISAC and is for example produced by the Fuji BAS5000 scanner.
grid	**.grd**[2]	This file type is produced and used by AGScan. The format is kind of XML compliant.
experiment: Alignment / quantification	**.zaf**	This file type is produced and used by AGScan. It's an archive file that contains a ".ali" file (experiment data file), a manifest file and may contains the image(s), The ".ali" format is kind of XML compliant.[3]
batch	**.bzb**[4]	This file type is produced and used by AGScan. The format is kind of XML compliant.

Notice that AGScan provides also non-openable export formats like a ".txt" output, easily usable with your favorite spreadsheet.

II.3. Image quality

Even if AGScan proposes methods for automatically align grids on image, the result depends of a lot of things like the quality of the image, the grid structure and parameters used for the alignment.

1- Be aware that the image files have to be a 16 bits format to be processed by AGScan.

2- Be careful: BZScan and AGScan .grd grid files and .bzb batch files are incompatible!

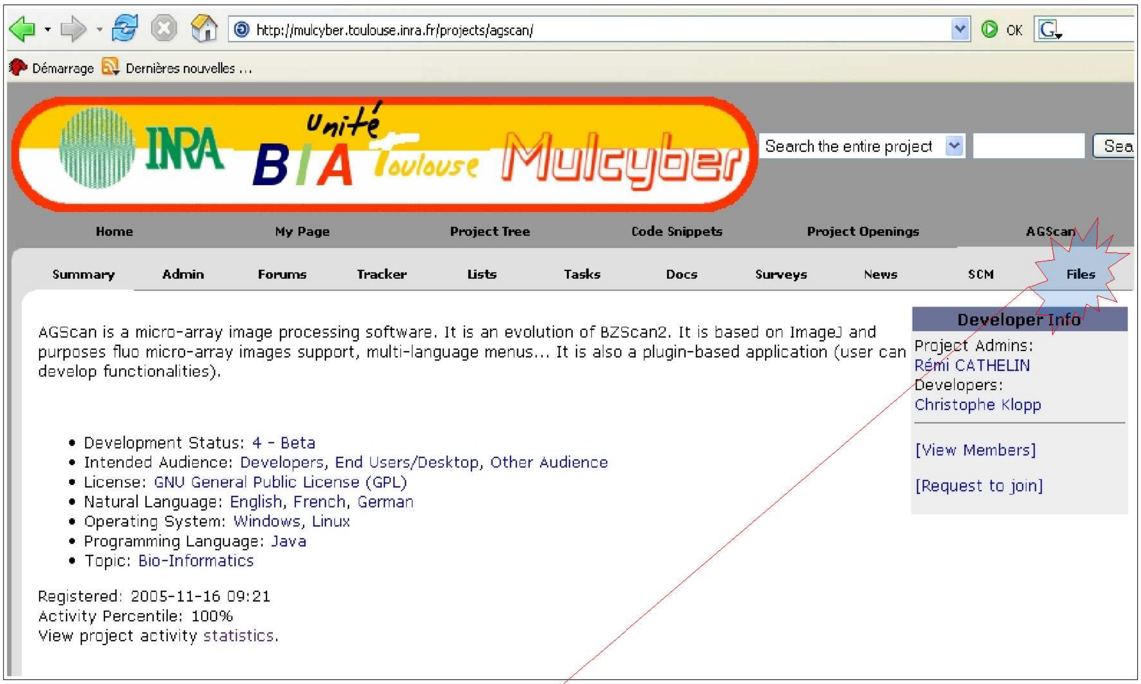
3- Never try to open a .zaf file: .ali files are not alone support

4- Be careful: BZScan and AGScan .grd grid files and .bzb batch files are incompatible!

II.4. Installing AGScan on your Computer

II.4.a. Download

AGScan is freely available at:
http://mulcyber.toulouse.inra.fr/projects/agscan



AGScan is a micro-array image processing software. It is an evolution of BZScan2. It is based on ImageJ and purposes fluo micro-array images support, multi-language menus... It is also a plugin-based application (user can develop functionalities).

- Development Status: 4 - Beta
- Intended Audience: Developers, End Users/Desktop, Other Audience
- License: GNU General Public License (GPL)
- Natural Language: English, French, German
- Operating System: Windows, Linux
- Programming Language: Java
- Topic: Bio-Informatics

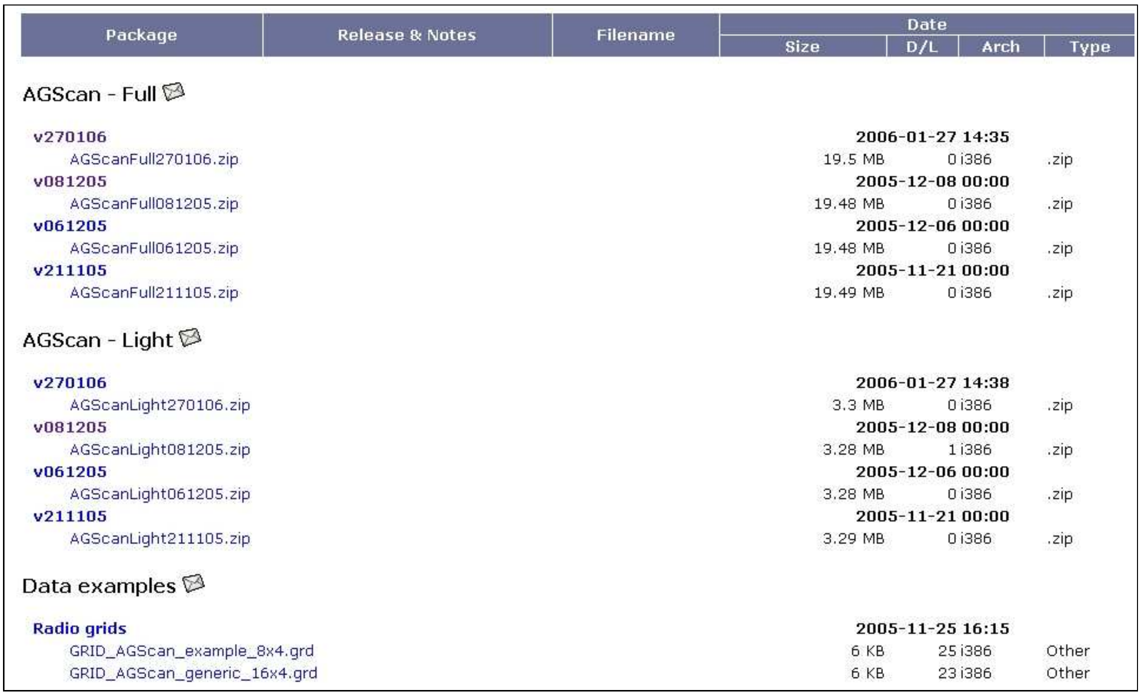
Registered: 2005-11-16 09:21
Activity Percentile: 100%
View project activity statistics.

Developer Info
Project Admins:
Rémi CATHELIN
Developers:
Christophe Klopp

[View Members]

[Request to join]

Just click on the “Files” subsection:



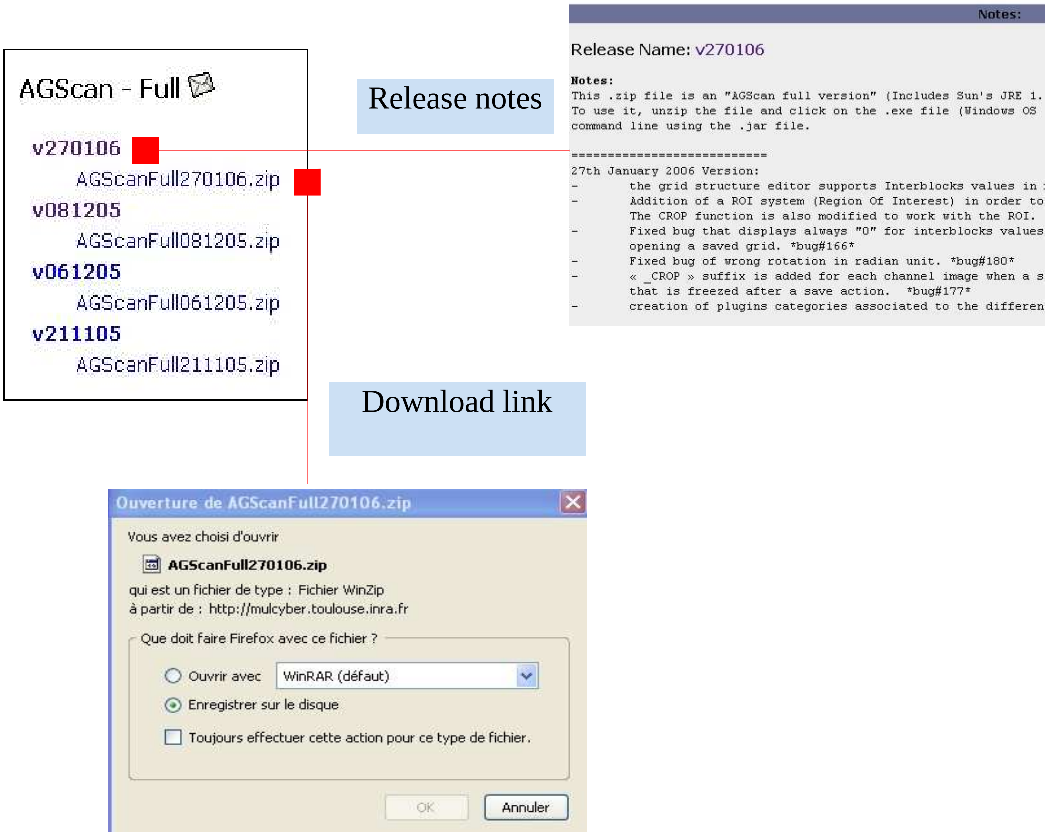
Package	Release & Notes	Filename	Date			
			Size	D/L	Arch	Type
AGScan - Full						
	v270106		**2006-01-27 14:35**			
		AGScanFull270106.zip	19.5 MB	0	i386	.zip
	v081205		**2005-12-08 00:00**			
		AGScanFull081205.zip	19.48 MB	0	i386	.zip
	v061205		**2005-12-06 00:00**			
		AGScanFull061205.zip	19.48 MB	0	i386	.zip
	v211105		**2005-11-21 00:00**			
		AGScanFull211105.zip	19.49 MB	0	i386	.zip
AGScan - Light						
	v270106		**2006-01-27 14:38**			
		AGScanLight270106.zip	3.3 MB	0	i386	.zip
	v081205		**2005-12-08 00:00**			
		AGScanLight081205.zip	3.28 MB	1	i386	.zip
	v061205		**2005-12-06 00:00**			
		AGScanLight061205.zip	3.28 MB	0	i386	.zip
	v211105		**2005-11-21 00:00**			
		AGScanLight211105.zip	3.29 MB	0	i386	.zip
Data examples						
	Radio grids		**2005-11-25 16:15**			
		GRID_AGScan_example_8x4.grd	6 KB	25	i386	Other
		GRID_AGScan_generic_16x4.grd	6 KB	23	i386	Other

Mulcyber AGScan page

All versions of the software are available. They are named using the date of the release.

The software releases are available in two different packages: light and full versions. With the light version, Java Runtime Environment needs to be installed previously on your operating system. The full version does not need any other software, just to click on the icon!

Downloading AGScan is very easy.
First choose a release of the package. For each release, notes are available by clicking on the release name.
Next you can download the corresponding “AGScanxxxxxx.zip” file.



AGScan - Full

- v270106
  - AGScanFull270106.zip
- v081205
  - AGScanFull081205.zip
- v061205
  - AGScanFull061205.zip
- v211105
  - AGScanFull211105.zip

Notes:

Release Name: v270106

```
Notes:
This .zip file is an "AGScan full version" (Includes Sun's JRE 1.
To use it, unzip the file and click on the .exe file (Windows OS
command line using the .jar file.

===========================
27th January 2006 Version:
-       the grid structure editor supports Interblocks values in
-       Addition of a ROI system (Region Of Interest) in order to
        The CROP function is also modified to work with the ROI.
-       Fixed bug that displays always "0" for interblocks values
        opening a saved grid. *bug#166*
-       Fixed bug of wrong rotation in radian unit. *bug#180*
-       « _CROP » suffix is added for each channel image when a s
        that is freezed after a save action.  *bug#177*
-       creation of plugins categories associated to the differen
```

Ouverture de AGScanFull270106.zip

Vous avez choisi d'ouvrir

AGScanFull270106.zip

qui est un fichier de type : Fichier WinZip
à partir de : http://mulcyber.toulouse.inra.fr

Que doit faire Firefox avec ce fichier ?

○ Ouvrir avec WinRAR (défaut)
● Enregistrer sur le disque
☐ Toujours effectuer cette action pour ce type de fichier.

Download AGScan

II.4.b. packages contents

The “AGScanFullxxxxxx.zip” archive file contains the following directory:

AGScanFull270106

The “AGScanLightxxxxxx.zip” archive file contains the following directory:

Package contents are similar except 3 elements added to the Full version:

- the install directory: contains a Java Runtime Environment
- the “AGScan.cfg” file: the launcher configuration file
- the “AGScan.exe” file : the icon to launch AGScan (on Windows OS)

Note: the Full version launcher is only usable for Windows OS.

The file structure of files is composed of:

- the “AGScanxxxxxx.jar” file is the java executable archive file
- the languages directory containing language files available in AGScan
- the “lib” directory containing AGScan libraries and dependences used by the software (ij.jar for example). Notice that the subdirectory called “plugins” of this directory contains imageJ plugins that can be used by AGScan
- the “plugins” directory containing AGScan “.class” plugins files
- the “ChangeLog.txt” file containing the history of release notes
- the “parameters.properties” file containing the current user preferences of the software

II.4.c. Launching AGScan

On Linux OS, use the command line:

```
java -Xss10m -Xmx420m -jar AGScanxxxxxx.jar
```

On Windows OS:
If you choose a full version: just click on the AGScan.exe icon!
You can also run the command line (or write it in a .bat file):

```
"java.exe" -Xss10m -Xmx420m -jar "AGScanxxxxxx.jar"
```

The “.jar” file name is the release name of AGScan.
Note: the “-Xss” and the “-Xmx” parameters correspond to the memory allocation given to the Java Virtual Machine(JVM) in order to support the application. If you encounter “java OutOfMemory errors” then increase the “-Xmx” value.
By default, AGScan full version is configured with 420Mo of memory allocated for the JVM.You can modify this value in the launcher configuration file (“AGScan.cfg”file).

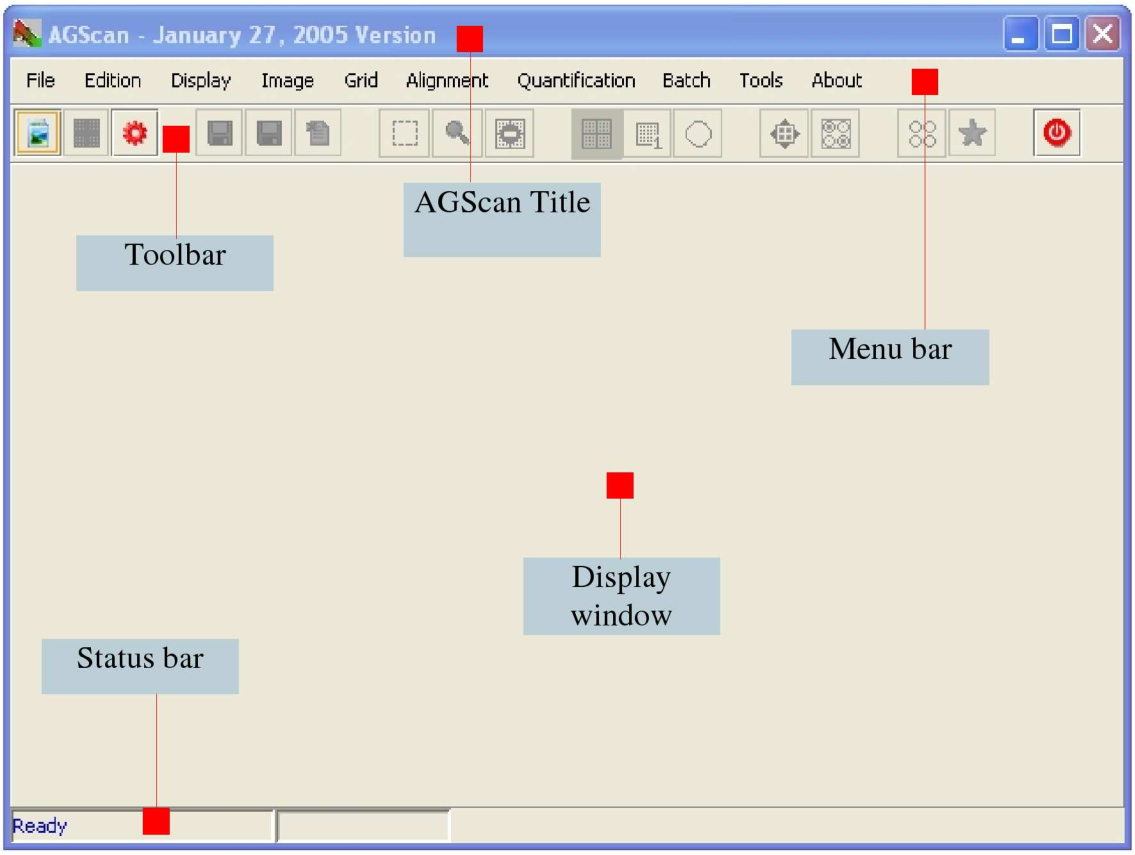
III. Processing steps

This guided tour will walk us through the different parts listed hereunder :

- User interface,
- Loading an image file,
- Creating a grid,
- Aligning a grid on an image,
- The table,
- Quantifying an alignment,
- Analyzing quantification results,
- Processing a batch.

First it will start by presenting the user interface layout:

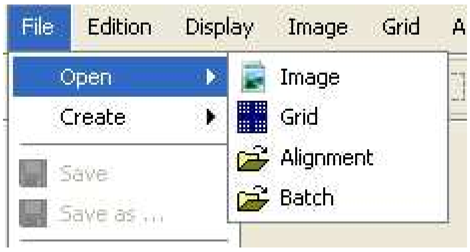
III.1. User interface

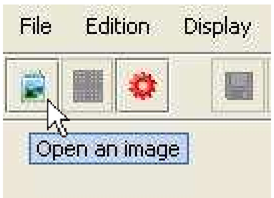


The user interface of the software wants to be simple, readable and as ergonomic as possible.
It is composed of five elements:

- The “title window”: presenting the used AGScan version, in the selected language.
- The “menu bar”: containing the different menus of the application.
- The “toolbar”: icons wich are shortcuts to some sub-menu actions.
- The “display window”: the main window containing experiment view. Several elements may be displayed in the same time (images, grids, alignments). They are open in different tabs.
- The “status bar”: displays current information like status and progress bar.

As we want the software to be simple to use we tried to place the menus in a logical order from left to right. We hope that menus are explicit enough to be easily memorized. Shortcuts to common actions have been added below the menus in order to open, align and quantify an image in three clicks! Shortcut icons are also duplicated next to each menu title to ease memorization. Notice that if you point your mouse on a shortcut, you will obtain a little description of the associated action.





Example : the “Open Image” action menu (and related toolbar item)

The first menu you can use is the “Edition>Language” one. It allows to change the language of the application menus.

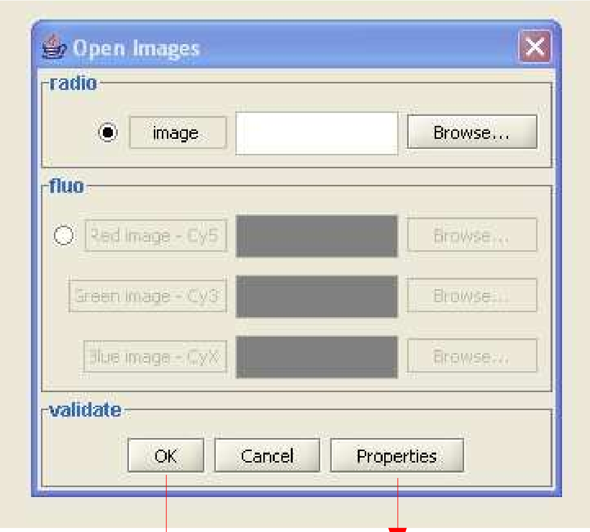
Notice that you need to reload the application to apply a new language.
Note: in the current version, three languages are available: English, French and German.
There can be spelling mistakes. However it is easy to modify these faults. Indeed, all the words are stored in text files (named “messages_en.properties”, “messages_fr.properties” and “messages_de.properties”). We can even easily generate new ones in other languages. For that, it is necessary to add the new “messages_xx.properties” file into the subdirectory of AGScan named “languages”. It will be automatically recognized by the software.

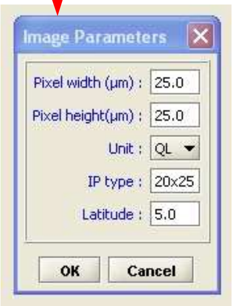
Now Let us present the different steps needed to quantify an image.

III.2. Loading an image file

Preliminary remark: an experiment can be composed from 1 to 3 images, but in the following chapters we will refer to an experiment including only one image.

To open an image, use the “File>Open>Image” menu or the following short icon of the toolbar:





Pixel width (µm) : 25.0
Pixel height(µm) : 25.0
Unit : QL
IP type : 20x25
Latitude : 5.0

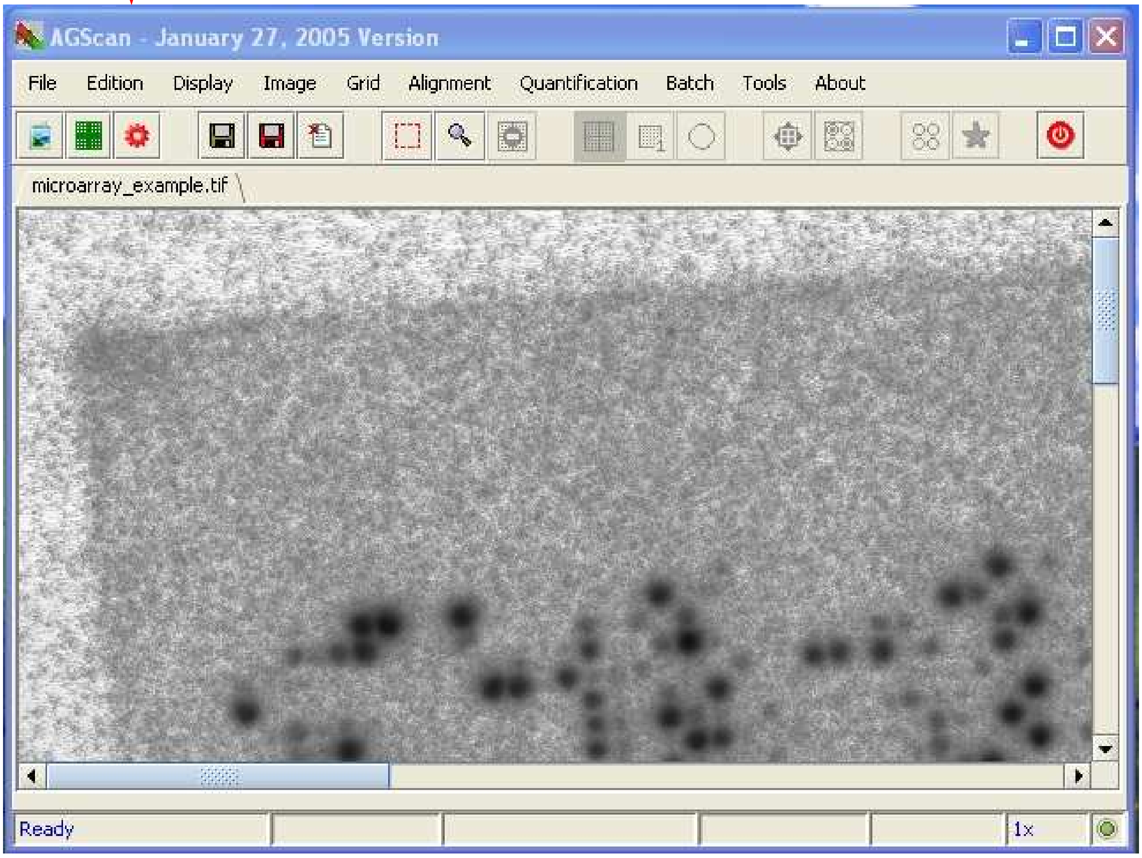
A selection window appears. Choose your type of experiment (one channel: radio or multi-channels: fluo) and load the image(s) thanks to the Browse button.
Notice that only the images files extensions that AGScan supports are visible in the file chooser, (By default ".tif" and ".inf").

The "Properties" button allows you to give some initial parameters of the image (produced by the scanner). These parameters are stored into the general parameters of the application.

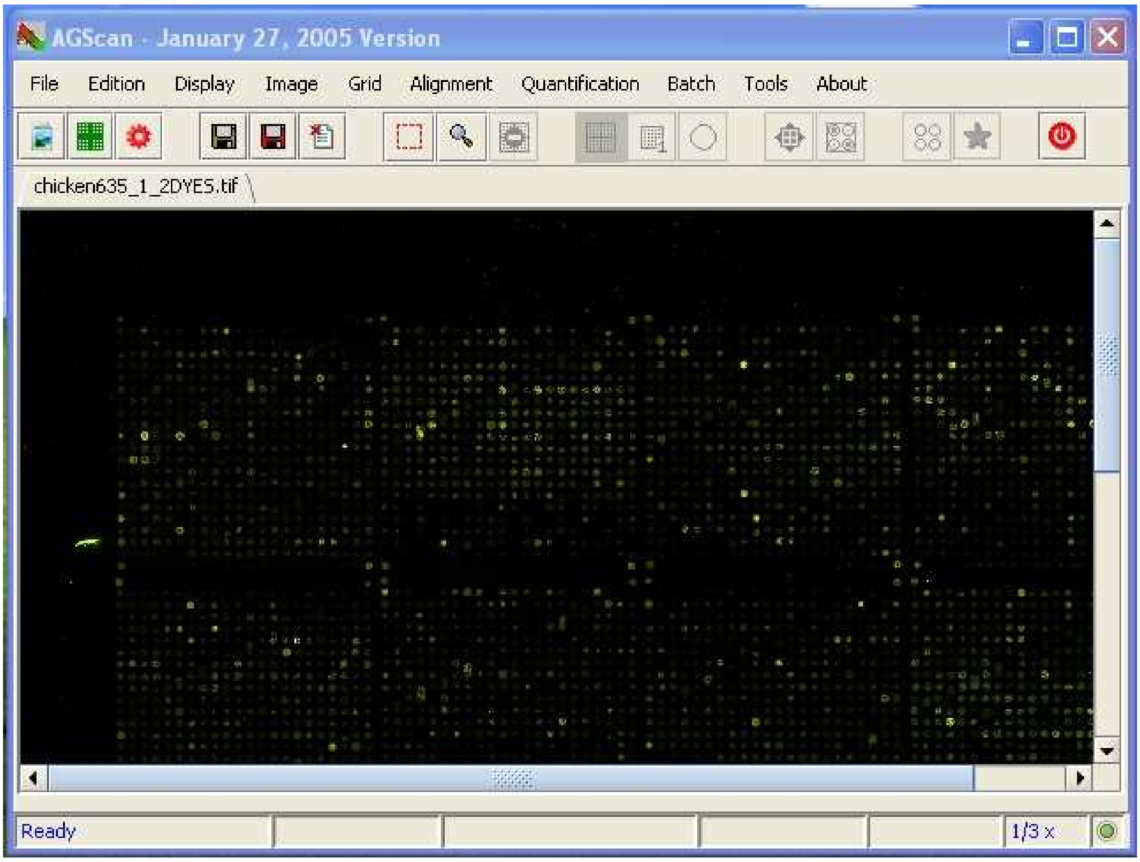
-Pixel width : width of a pixel, micrometers
-Pixel height : height of a pixel, micrometers
-Unit: image unit (QL or PSL)
-IP type: Image Plate size ,centimeters
-Latitude: dynamic range of "above number" power of 10

If you don't know where to find these parameters, ignore them (default values will be apply).

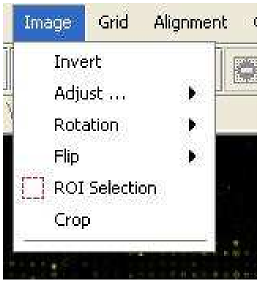
Click "OK" to load the experiment!



The image is loaded into the “Display window”.
In the case of a multi-channels experiment, the RGB image is displayed :



Once opened, you can now modify it using the different widgets disposed in the “Image” menu.

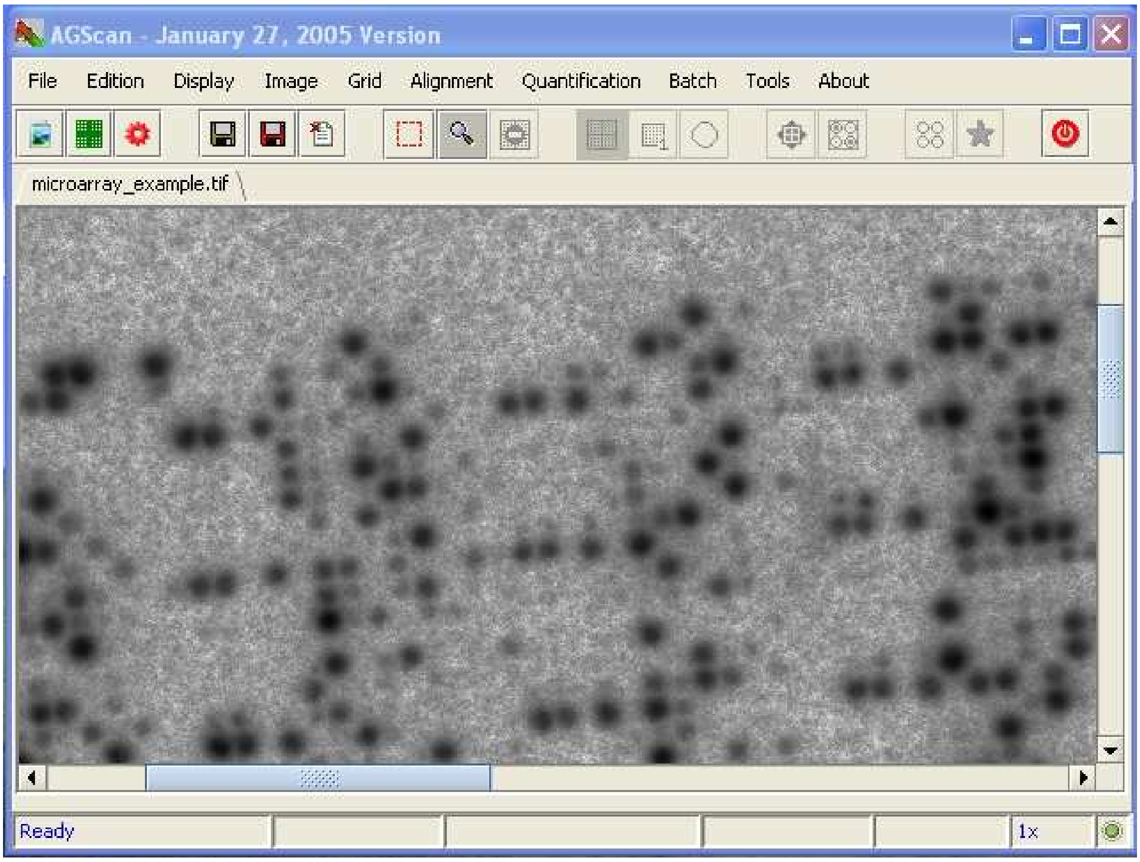
	They allow to invert the LUT, to modify the contrast and the brightness, to rotate the image, to flip it horizontally or vertically. Some of these options are very interesting when you have scanned your array the wrong way. You can also select a rectangular region and crop it to create a new sub-image. Sometimes due to low contrast or quality, the spots can be quiet difficult to locate. All these image tools can help you to visualize them. **However, you have to know that the modification of the contrast and the brightness of the image doesn't affect pixel values of the image and isn't save.**

Other general functionalities can be used in the “Display” menu.

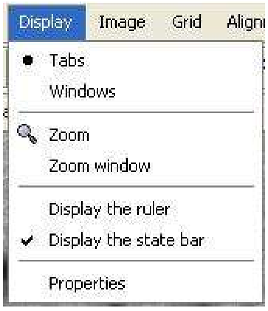
<u>An example:</u> the “zoom”: 🔍

Working with images, we sometimes need a general view and sometimes a very precise one. This can be obtained by using the zoom . Clicking on the left of the progress bar will zoom out of the image, clicking in the right will zoom in the image.

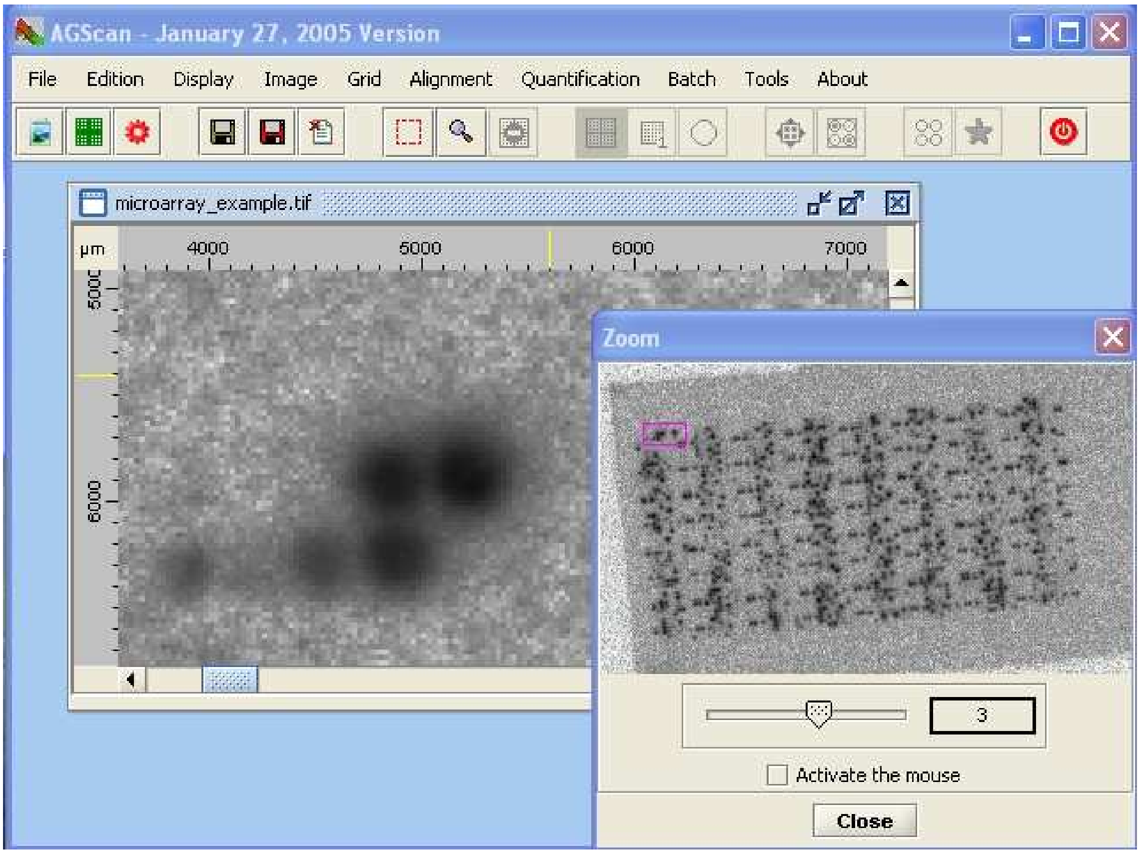
When the zoom is active, the icon background becomes gray. By clicking a second time on the icon, the zoom is disable.



Example: image with zoom action enabled.

	The "display" tools are : - "tabs" or "windows": the display mode. Each item (image, grid, alignment) is on a tab or on a distinct window, Tabs are the default display mode. - "zoom": disable/enable the zoom, - "zoom window". Allows to display a little window containing the entire image, the zoom factor applied and that show the part of the image zoomed. - "Display the ruler": disable/enable a ruler. Disabled by default. - "Display the State bar": disable/enable the status bar. Enabled by default. -"Properties": allows to display the properties (name,size...) of the current element displayed into the current tab or window.

By default, the display mode is "tabs", the zoom isn't active, the ruler is disabled and the status bar is displayed. (see the image above).



On this example, the window mode is active,the status bar is hidden, the ruler is displayed and the zoom window is open (the current zoom factor is 3),

The next part will show you how to create a grid.

III.3. Creating a grid

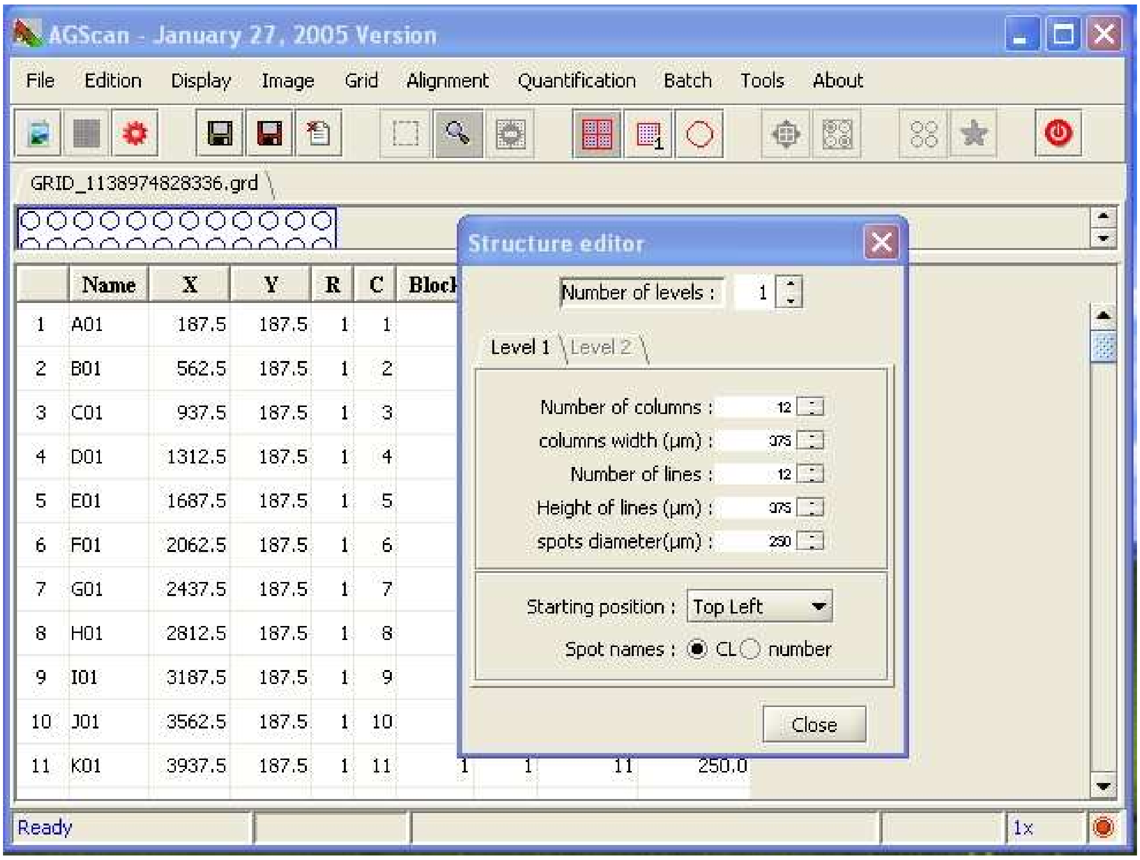
The grid is a very important element for the image analyze. After opening an image, you have to load a grid on it and therefore you must have already define it.
Note that you can use the same grid for all your experiments that use the same spotting scheme.

So this part will show you how to create, open and modify a grid.

At first, choose the creation grid menu found in the “File>Create>New Grid” menu or in the “Grid>New Grid” menu.

A new tab and the “structure editor” will appear. The main window is divided into two parts:

- the **grid**: the graphical representation of the grid
- the **table**: a table with all the characteristics of the grid spots (number, name position). It will be describe later.



	Name	X	Y	R	C	Block			
1	A01	187.5	187.5	1	1				
2	B01	562.5	187.5	1	2				
3	C01	937.5	187.5	1	3				
4	D01	1312.5	187.5	1	4				
5	E01	1687.5	187.5	1	5				
6	F01	2062.5	187.5	1	6				
7	G01	2437.5	187.5	1	7				
8	H01	2812.5	187.5	1	8				
9	I01	3187.5	187.5	1	9				
10	J01	3562.5	187.5	1	10				
11	K01	3937.5	187.5	1	11	1	1	11	250.0

Number of levels : 1

Number of columns : 12
columns width (µm) : 375
Number of lines : 12
Height of lines (µm) : 375
spots diameter(µm) : 250

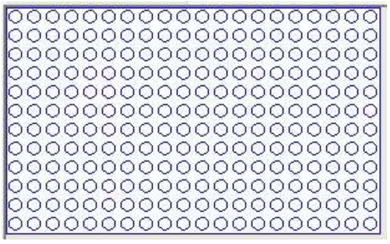
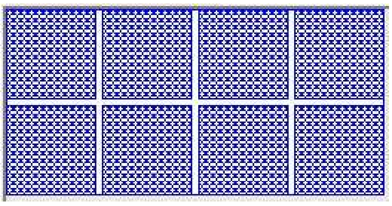
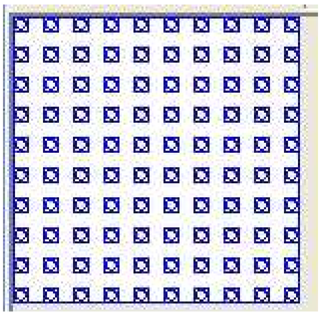
Starting position : Top Left
Spot names : ● CL ○ number

By default, the editor appears on a “one level” grid, So we have to introduce you the notion of **level** in the grid. A grid can have two levels representing the block and the grid.

- level1 = the block level. In fact, the spotting is usually made using a block pattern. This is usual but not always the case. The level defines a block of spots. Information needed is the dimension of a block (number and size of columns and lines) and the diameter of a spot.
- level2 = the grid level. A grid can often be compared to a set of blocks. So if you have this second level, you just need to inform how many blocks you have vertically and horizontally. So you can also notify inter-blocks space size.

To sum up, a grid is seen by AGScan as a set of elements. The smallest element is the spot. When you have two levels, a set of spots forms a block and a set of block a grid .

In the next table the first line gives you number of levels used, the second gives you the structure of the first level and the third line gives you the structure of the second level. In the last line, you can see the graphical view of the type of spotting pattern you have used.

1 level	***2 levels***	***2 levels***
20 x 12 spots	**12 x 12 spots**	**1 x 1 spots**
-	**4 x 2 blocks**	**10 x 10 blocks**
		

Examples of grids

The grid, the table and the editor are linked. So, be aware that you need to move on an other box or hit <ENTER> (carriage return) after having entered a value in each text box of the "grid structure editor" in order to synchronize the view of the grid .

You have now define your grid and you can save it. The default proposed name of the saved file has no sense. It is just created randomly in order to be unique.
The resulting file has a ".grd" extension and is in XML format.

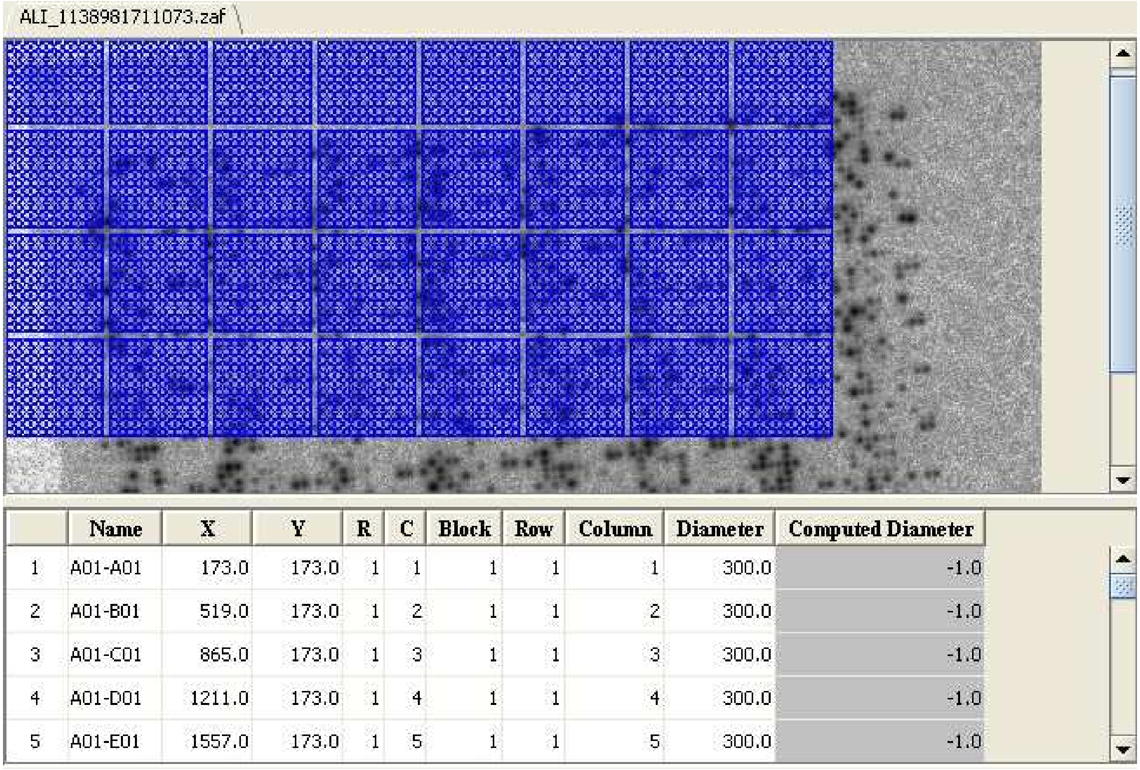
Notice that you cannot create a grid directly on a loaded image. You need before to load the image and after the grid above.

III.4. Aligning a grid on an image

III.4.a. Loading a grid

This is where all really begins. After having opened an image, you can load a grid on it.
To do this, you can use the "Grid>Load" menu or the following icon :
▦

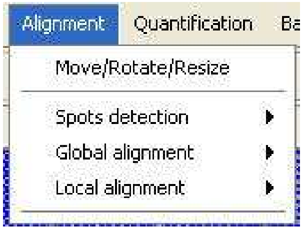
The selected grid will be loaded on the image, placed on the top left and the tab is divided into two parts, in order to add the table of spots characteristics.



	Name	X	Y	R	C	Block	Row	Column	Diameter	Computed Diameter
1	A01-A01	173.0	173.0	1	1	1	1	1	300.0	-1.0
2	A01-B01	519.0	173.0	1	2	1	1	2	300.0	-1.0
3	A01-C01	865.0	173.0	1	3	1	1	3	300.0	-1.0
4	A01-D01	1211.0	173.0	1	4	1	1	4	300.0	-1.0
5	A01-E01	1557.0	173.0	1	5	1	1	5	300.0	-1.0

The next step consists in correctly aligning the grid on the image.
You have several possibilities to do it. This step can be done manually or automatically by launching alignments algorithms.

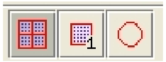
All the alignment functionalities are available in the “Alignment” menu:



III.4.b. aligning manually a grid

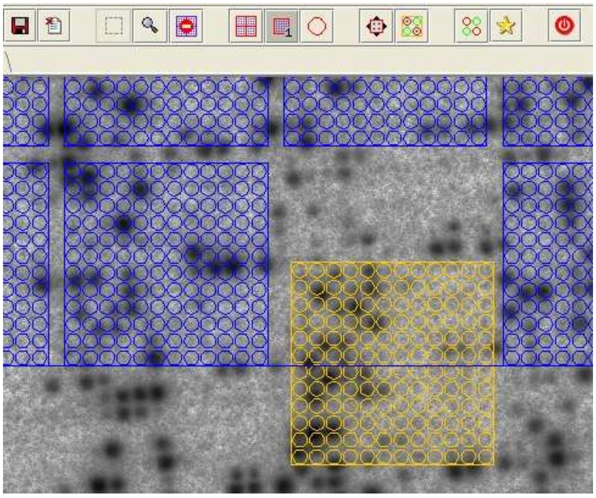
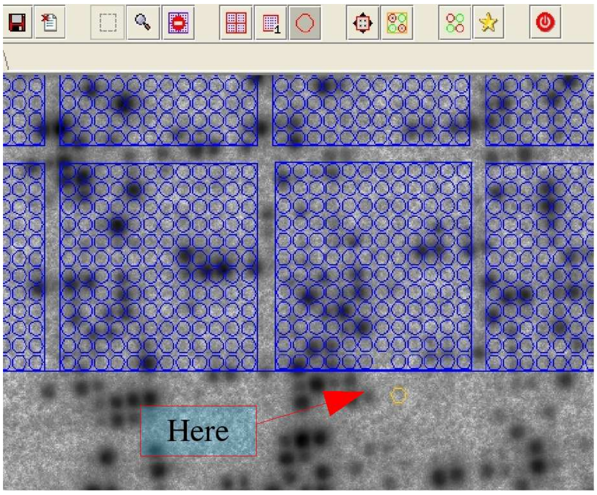
How to manually place the grid?
You can either use the mouse and the “Move/Rotate/Resize” menu.
Let us present to you the “color code” of a grid: a loaded grid on an image appears in blue if it is not selected and orange if you select it.

To select in the grid, just click on it.
Note that you can easily only move a sub-grid by changing the selection mode:



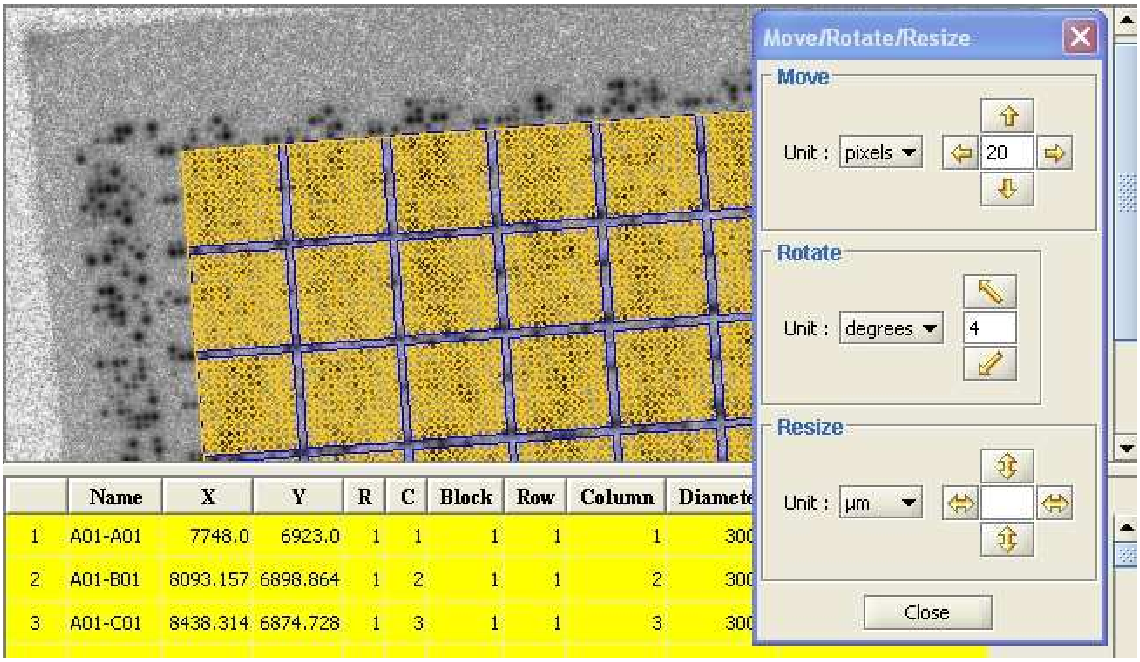
These three icons of the toolbar allow to change the grid selection mode. By default, the first is enabled. It corresponds to the entire grid. If you select the second icon, you turn into the block mode that allows you to only select a block. The third icon corresponds to the spot mode that allows to only select one spot.

A selected item (spot, block or the grid) can be moved using the mouse. For this, move it by maintaining it selected (left mouse button down).
Notice that the table view corresponding selected spots lines are yellow.

Block selection mode	Spot selection mode
	

You can also deform the grid by placing your mouse at the corners and drag them.

A specific and useful tool allow to easily manually align a grid :the “Move/Rotate/Resize” menu.
Click on it: a window appears, offering you the possibility to rotate, move and resize the grid of given values:



Move — Unit : pixels, 20
Rotate — Unit : degrees, 4
Resize — Unit : µm

	Name	X	Y	R	C	Block	Row	Column	Diamete
1	A01-A01	7748.0	6923.0	1	1	1	1	1	30
2	A01-B01	8093.157	6898.864	1	2	1	1	2	30
3	A01-C01	8438.314	6874.728	1	3	1	1	3	30

Example: the grid has been moved and rotated.

You can choose the unit of your rotation (degrees, radian), the unit of your translation or resizing (pixels, µm or spots).

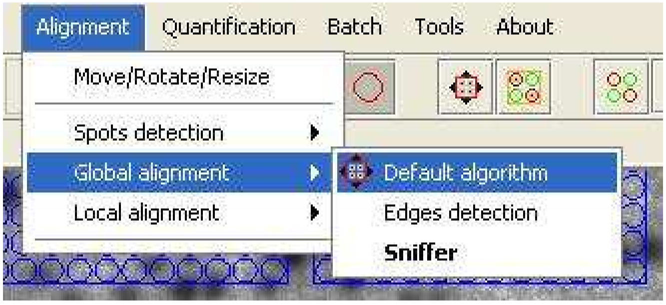
Thanks to this tool, you can manually correctly place the grid on the image. In order to verify the position of each spot, you can apply a “spot detection” (presented below).
Note that it is possible to perform an automatic local alignment on a manually aligned grid.

It is interesting to know how manually place a grid but in the most cases, you can perform automatic global and local alignments.
In the next pages we will see how to perform these automatic alignments.

Let us first introduce the notions of “global” and “local” alignments. The global alignment places the grid (as good as possible) on the entire image. The local alignment searches to correctly align each block separately.

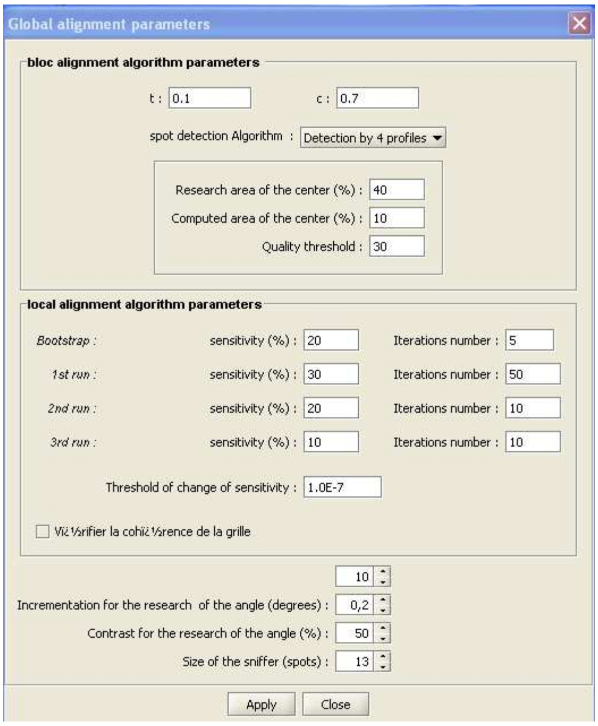
These two algorithms have been initially developed for the BZScan software. So they have a lot of parameters that can be modified and memorized.

III.4.c. Global alignment algorithm



The “Global alignment” sub-menu contains two different algorithms, the “sniffer” and the “edges detection” global algorithms. The default algorithm is the “sniffer” one (in bold in the menu). You can also call it thanks to the following toolbar icon :

If you click on “Default algorithm”, it is launched without proposing you to change the parameters. To modify them, you need to choose the “Sniffer” menu. So the parameters windows appears:



Global alignment parameters

bloc alignment algorithm parameters

t : 0.1 c : 0.7

spot detection Algorithm : Detection by 4 profiles

Research area of the center (%) : 40

Computed area of the center (%) : 10

Quality threshold : 30

local alignment algorithm parameters

Bootstrap : sensitivity (%) : 20 Iterations number : 5

1st run : sensitivity (%) : 30 Iterations number : 50

2nd run : sensitivity (%) : 20 Iterations number : 10

3rd run : sensitivity (%) : 10 Iterations number : 10

Threshold of change of sensitivity : 1.0E-7

☐ Vï¿½rifier la cohï¿½rence de la grille

10

Incrementation for the research of the angle (degrees) : 0,2

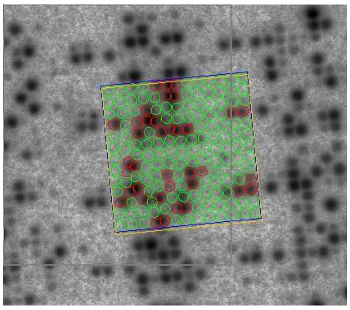
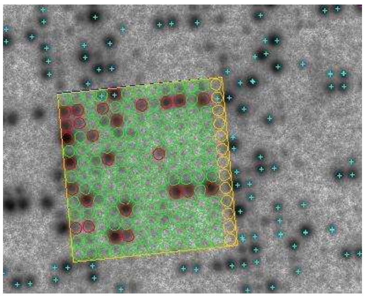
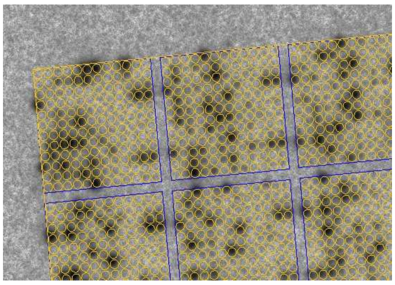
Contrast for the research of the angle (%) : 50

Size of the sniffer (spots) : 13

Notice that all these parameters can be changed and saved. It is also always possible to restore the default parameters. So don't hesitate to change them. For more information about their meaning, please contact us.

<u>“Sniffer” algorithm quick presentation:</u>

At first, the angle of the image is calculated. Then (about 10% of the process), the algorithm try to parse all the image with a sniffer block to find all spots. To do this, it already searches to place the “sniffer block” (a defined block of spots) at 40% in width and in height of the image, the condition to place it is that the center spot must be a detected one. When the algorithm detects this spot, it considers this position as the first position of its route (pict1). The sniffer block moves after in all the direction of the image and flags every spot it finds. Each time it will find something considered a spot, it will put a little colored cross on its center (pict2). After having recovered the entire image, the algorithm computes how to place the grid in order to include the most of detected spots.
At the end of the process, you will have a yellow (selected) grid aligned on the image(pict3).

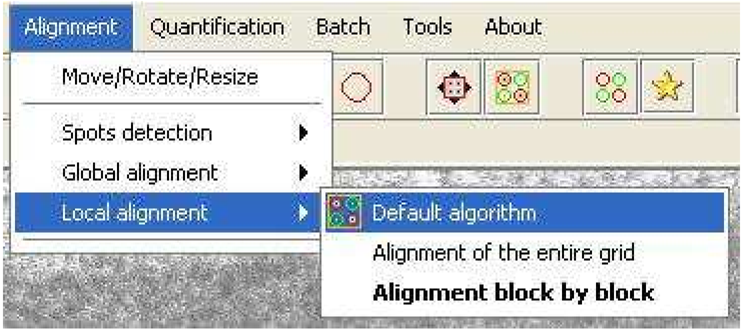
The Sniffer block	***Spots are found***	***Grid is placed***
		

<u>Some visual steps of the sniffer global alignment.</u>

Our grid is now globally aligned.
The next step of the process is the local alignment.

III.4.d. Local alignment algorithm

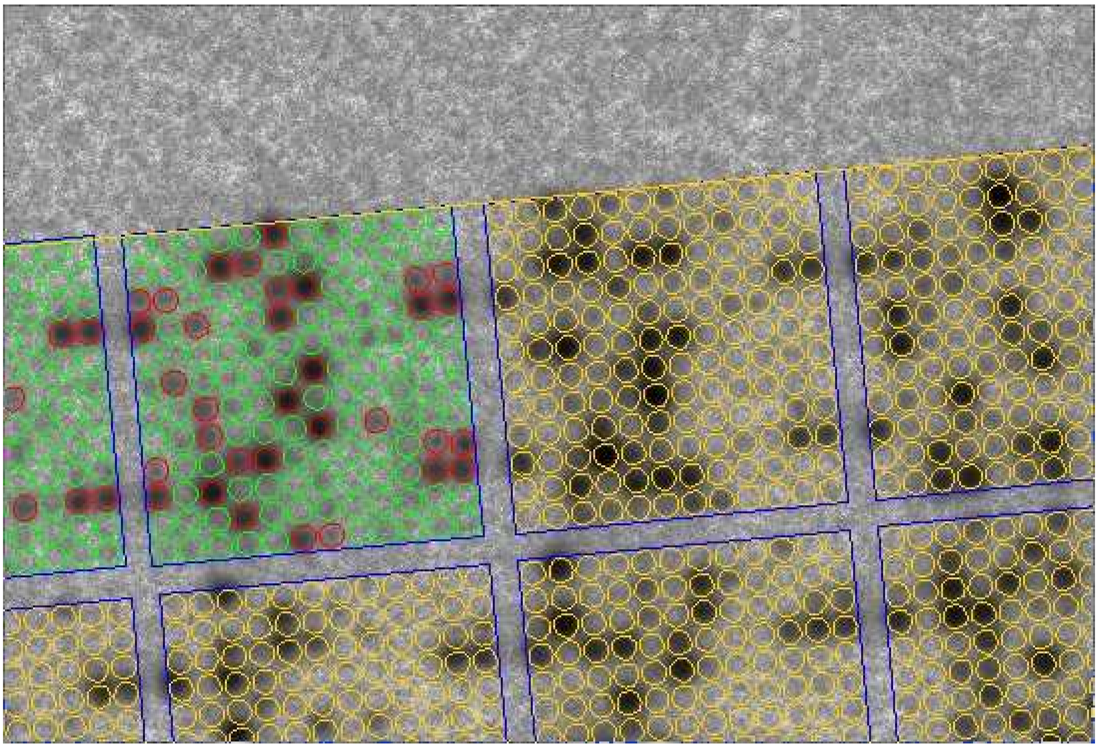
A local alignment is an alignment that you must launch on a “already aligned” grid. It allows to improve the alignment quality.
In the actual version, two different local alignment algorithms are available.



The “Local alignment” sub-menu contains two different algorithms, the “entire grid” and the “block by block” local alignment algorithms. The default algorithm is the “block by block” one (in bold in the menu). You can also call it thanks to the following toolbar icon :

If you click on “Default algorithm”, it is launched without proposing you to change the parameters. To modify them, you need to choose the “Alignment block by block” menu.

“Alignment block by block” algorithm quick presentation:
Each block is processed individually. The position of each spot is verified and adjusted. The block may be moved from the initial global alignment place.

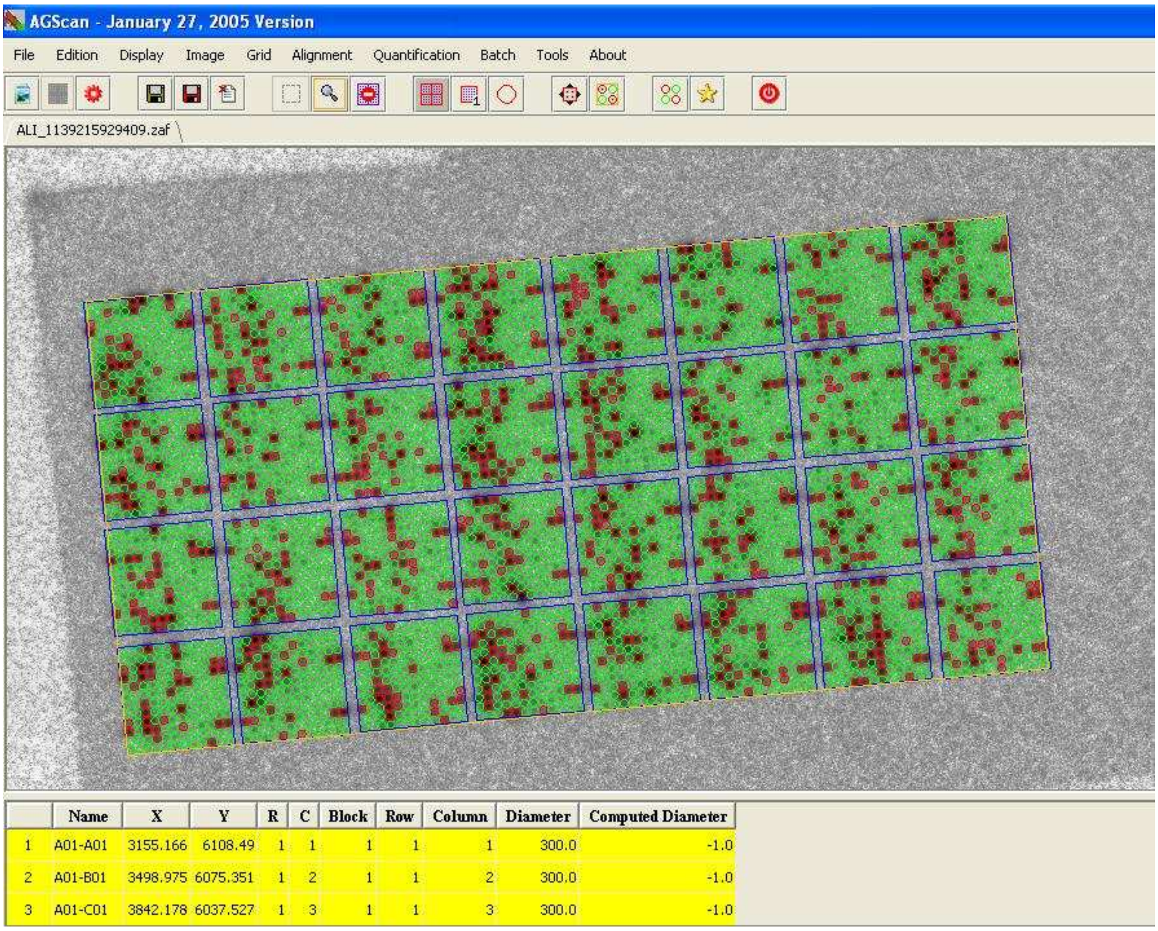


Progression of the local algorithm

In this process, AGScan takes each block and tries to fit it as precisely as possible to the spots it finds underneath. Each block is processed separately and can therefore move from the previously calculated global position to a new one. It is quiet amazing to see how a block is progressing towards its end position. Once the block position is optimized, AGScan goes on with the next block.
The red circles indicate that the program identify a spot ; on the opposite, the green ones indicate that the spot is deduced due to the grid structure and the red spots locations.

Note that the coordinate values of the spots have changed during the local alignment process. You can see values in the alignment table.

The progress of the alignment is given by a progress bar.
At the end of the process your display windows will look like this :

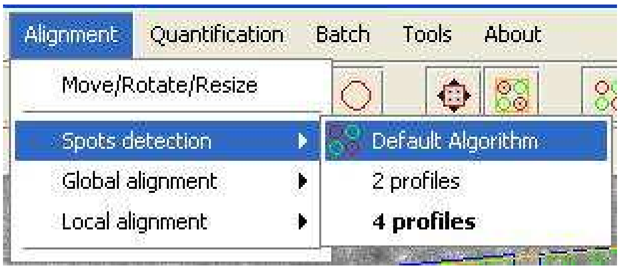


	Name	X	Y	R	C	Block	Row	Column	Diameter	Computed Diameter
1	A01-A01	3155.166	6108.49	1	1	1	1	1	300.0	-1.0
2	A01-B01	3498.975	6075.351	1	2	1	1	2	300.0	-1.0
3	A01-C01	3842.178	6037.527	1	3	1	1	3	300.0	-1.0

An aligned grid

III.4.e. Spot detection

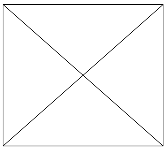
The spot detection is the method used to define if a spot is found (red) or estimate (green). A spot is recognize as one if its form fit the model.
You can call it on a selected a grid. Indeed, a selected grid becomes orange, so you don't see the spot nature.



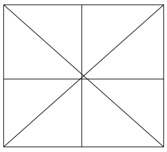
The “Spots detection” sub-menu contains two different algorithms, the “2 profiles” and the “4 profiles” algorithms. The default algorithm is the “4 profiles” one (in bold in the menu). You can also call it thanks to the toolbar icon :

If you click on “Default algorithm”, it is launch without propose you to change parameters. To modify them, you need to choose the “4 profiles” menu.

The “2 profiles” and the “4 profiles” spot detection algorithms are similar. The difference is that profiles of spots used to search the center of the spot since the center of the research zone are diagonal lines (for the “2 profiles” detection) and diagonals + vertical + horizontal lines (for the “4 profiles” detection).

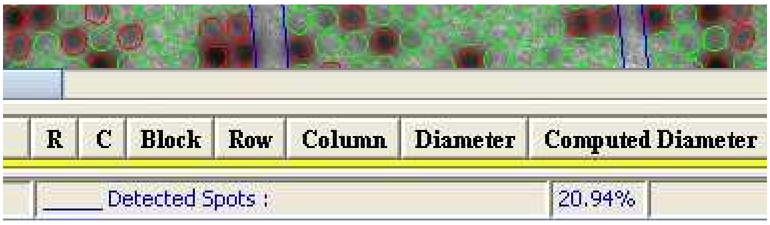


2 profiles



4 profiles

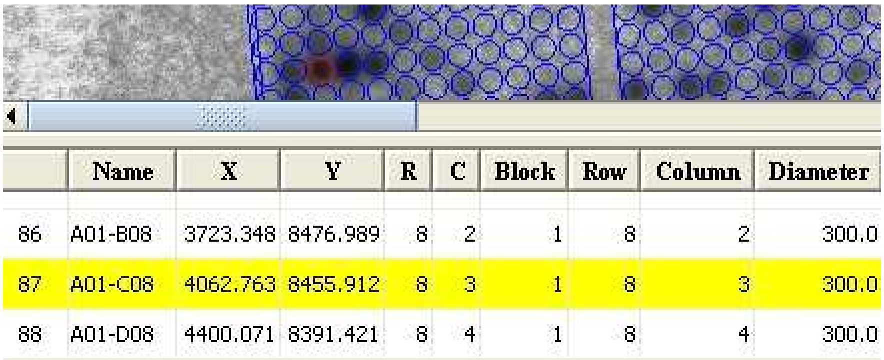
These two methods progress rapidly and give you the percentage of detected spot in the status bar:



Detected Spots : 20.94%

III.5. The table

When a grid is open, the main window is split into two parts, giving you access to a table view of the spot informations.
This table is synchronized with the alignment. So coordinates have been updated by the move of the grid. However, no new columns are added.



	Name	X	Y	R	C	Block	Row	Column	Diameter
86	A01-B08	3723.348	8476.989	8	2	1	8	2	300.0
87	A01-C08	4062.763	8455.912	8	3	1	8	3	300.0
88	A01-D08	4400.071	8391.421	8	4	1	8	4	300.0

A selected spot and its table description

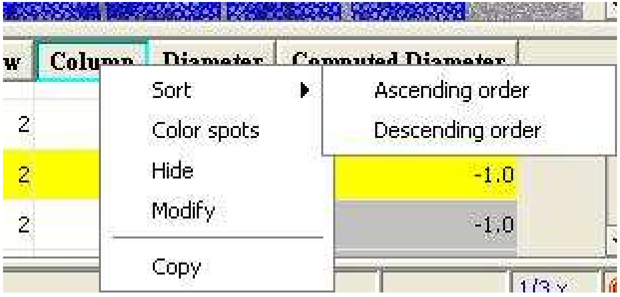
Base columns are:

- the first column is the number of the spot, determined by the spotting plan.
- Name: ID of each spot defined by AGScan: the first part is the block ID, the second part is the spot ID. The letter corresponds to the line position (A,B.....) and the number is the column position (1,2,3,,,).
- X : X coordinate of the center of the spot in the image(in micrometer).
- Y : Y coordinate of the center of the spot in the image(in micrometer).
- R: row number in the entire grid.
- C: column number in the entire grid.
- Block: number of the current block.
- Row: row number in the current block.
- Column: column number in the current block.
- Diameter: constant diameter of the spot defined in the grid structure.

Note: the “Computed diameter” column is present but not used (values are “-1”) until the quantification is processed.

The table and the alignment are interactive. You can select a spot in the table and see its location at the same time on the alignment: the spot (or the set of spots) are highlighted into the alignment (see the figure above).

So it's also possible to sort columns values, copy it,color some values...



The columns options can be active by clicking on the right click of the mouse.
The interest of these options will be describe to you after the quantification part.

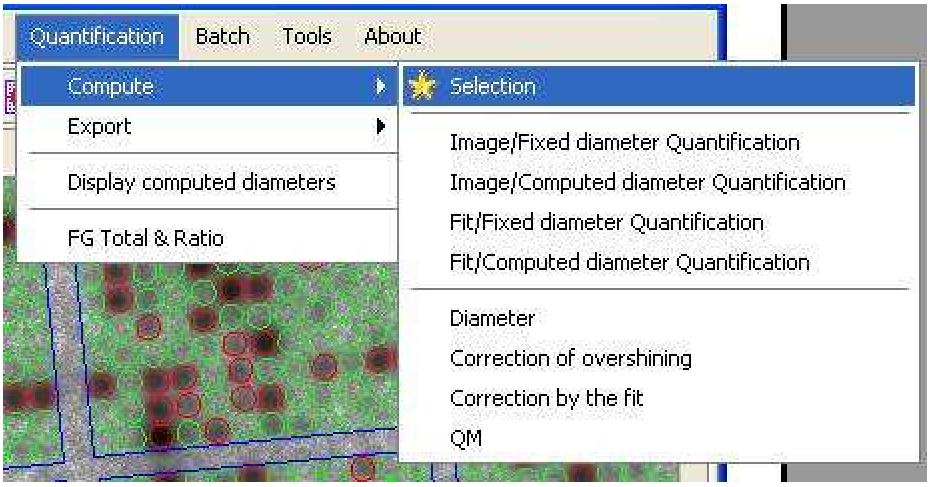
III.6. Quantifying an alignment

Now you have an aligned grid. So the next step is the quantification.

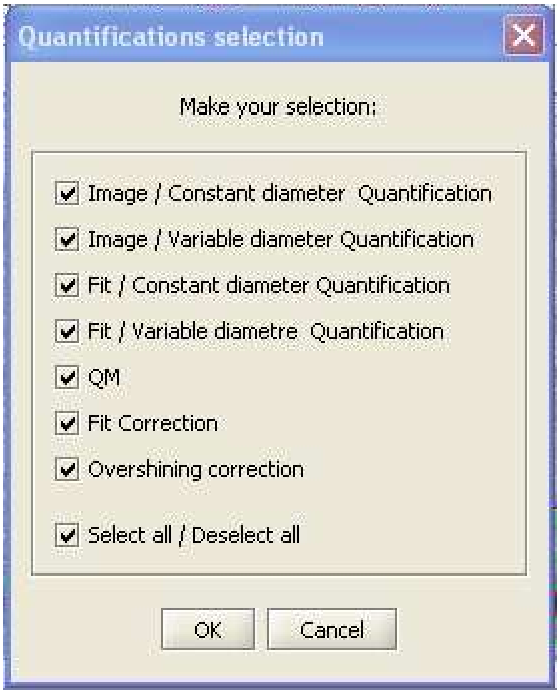
III.6.a. quantifications

Several computations are available. To launch a quantification, use the "Quantification>Compute" menu and select your action.
You can choose the type of quantification you want to call or select several of them with the "Selection" sub-menu or the following toolbar icon:

★

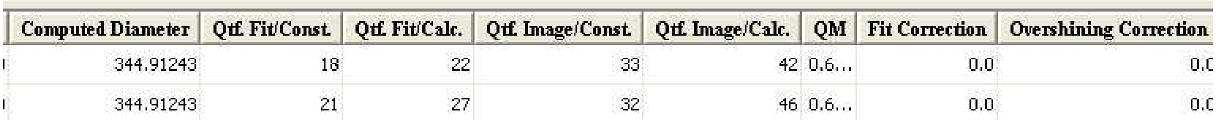


The selection menu opens the next window:



☑ Image / Constant diameter Quantification
☑ Image / Variable diameter Quantification
☑ Fit / Constant diameter Quantification
☑ Fit / Variable diametre Quantification
☑ QM
☑ Fit Correction
☑ Overshining correction
☑ Select all / Deselect all

After selecting the actions you want to process, click on "OK".
Each calculation is done one after the other. You can follow advance thanks to the progress bar.
Results are available into the table.



Computed Diameter	Qtf. Fit/Const.	Qtf. Fit/Calc.	Qtf. Image/Const.	Qtf. Image/Calc.	QM	Fit Correction	Overshining Correction
344.91243	18	22	33	42	0.6...	0.0	0.0
344.91243	21	27	32	46	0.6...	0.0	0.0

Example of columns added, results of quantifications

Note: the “Computed diameter” column is filled during quantifications that need this value.

By selecting the data column title, you can change its position in the table.

Available Quantifications:
Four quantification are available in AGScan:

- Image/Constant diameter Quantification: for each spot,the quantification value is computed thanks to the image pixels intensities and the constant diameter given by the grid structure.
- Image/Variable diameter Quantification: for each spot, the quantification value is computed thanks to the image pixels intensities and a variable computed diameter.
- Fit/Constant diameter Quantification:the quantification value is computed thanks to the estimated intensity of the spot (with a fit function) and the constant diameter given by the grid structure.
- Fit/Variable diameter Quantification: the quantification is computed thanks to the estimated intensity of the spot (with a fit function) and its variable computed diameter.

The QM value is an indication of quality of the spots. Each spot has a value of QM ranging between 0 and 1 (the closer the value is to 1, the more it is reliable).

The “fit correction” and the “overshining correction” are two corrections values that can be used with quantification. Be careful, to apply them, you need a specific formula.

III.6.b. analyze your results

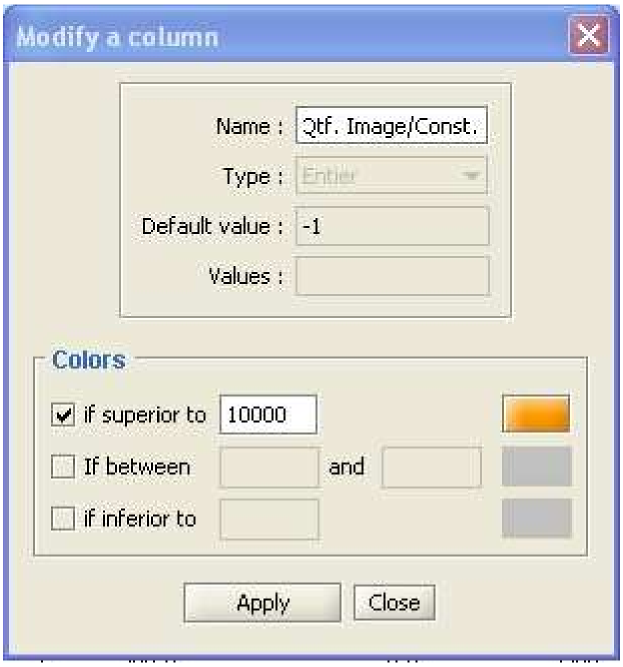
Thanks to the table columns options, you can sort the result values,
Example of a sorted column: click right on the column header and choose “Sort> Descending Order”.

Qtf. Image/Co...
13759
12058
11290
10656
9888
9840
9275

An useful option is also the “color” tool. You can choose to give a color to some spots of the alignment in order to locate them.

Example: we want to color in orange spots that have an “image/constant” quantification value higher than 10000. To do that:

1/select the “Modify” menu of the “Qtf Image/Const” column.
2/Check the “superior” case, fill the value (10000) and choose a color(orange).

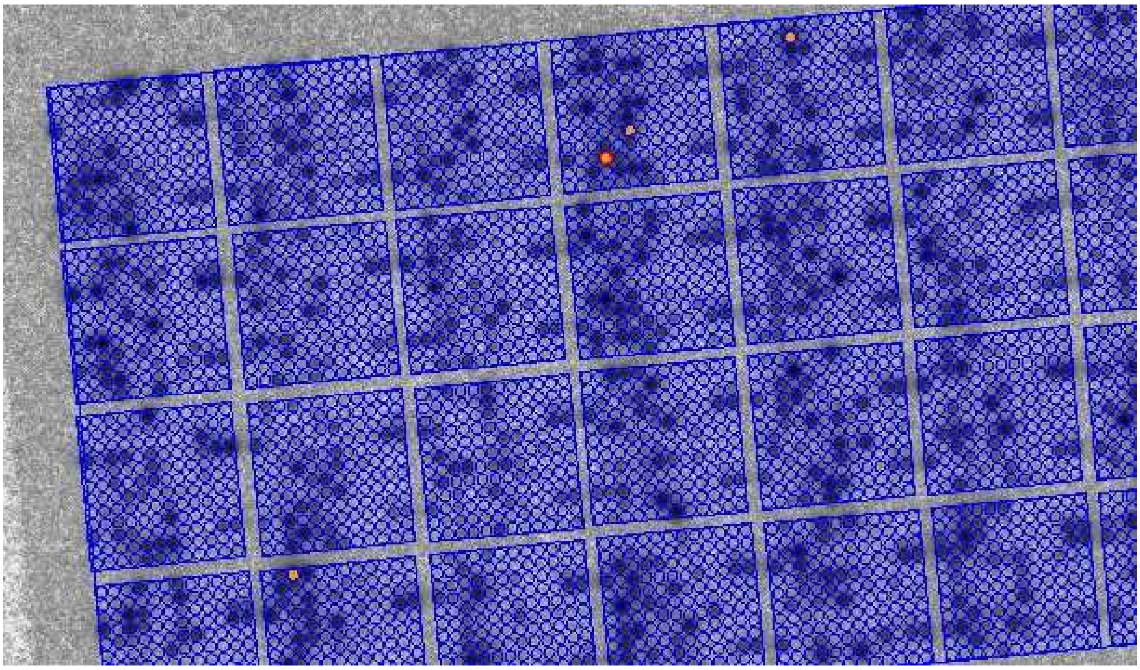


Name : Qtf. Image/Const.
Type : Entier
Default value : -1
Values :

Colors
☑ if superior to 10000
☐ If between and
☐ if inferior to

"Modify a column" window

3/Click on “Apply” and “Close”,
4/select the “Color spots” menu of the “Qtf Image/Const” column.
The text header of the column becomes red. Spots concerned by this condition will appear in orange on the aligned image.
In our example, only 4 spots are colored (as the sorted column shows us before).



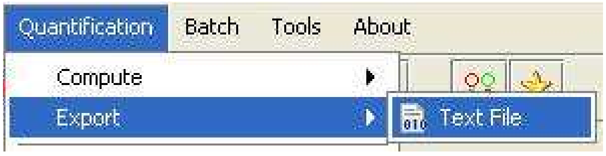
Spot coloration example

You can also color spots between two values or lower than an other one.

III.6.c. Export results

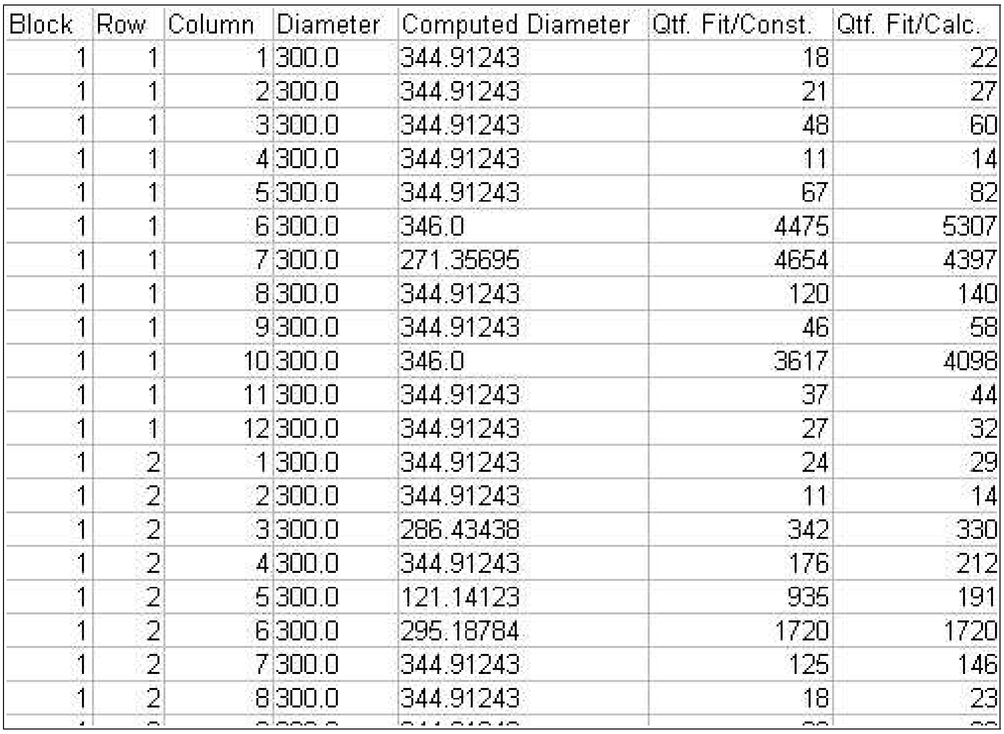
You can save the experiment in every process step. An alignment and a quantification are considered as the same thing. In fact, there are two “aligned grid with a table”. Only tables columns differ. So use the “save” menu to save it. The extension of an experiment file is “.zaf”.

You can also export only the table values in order to use them into a spreadsheet.
To do that, select the menu:



"Quantification>Export>Text File" menu

The file saved content looks likes:



Block	Row	Column	Diameter	Computed Diameter	Qtf. Fit/Const.	Qtf. Fit/Calc.
1	1	1	300.0	344.91243	18	22
1	1	2	300.0	344.91243	21	27
1	1	3	300.0	344.91243	48	60
1	1	4	300.0	344.91243	11	14
1	1	5	300.0	344.91243	67	82
1	1	6	300.0	346.0	4475	5307
1	1	7	300.0	271.35695	4654	4397
1	1	8	300.0	344.91243	120	140
1	1	9	300.0	344.91243	46	58
1	1	10	300.0	346.0	3617	4098
1	1	11	300.0	344.91243	37	44
1	1	12	300.0	344.91243	27	32
1	2	1	300.0	344.91243	24	29
1	2	2	300.0	344.91243	11	14
1	2	3	300.0	286.43438	342	330
1	2	4	300.0	344.91243	176	212
1	2	5	300.0	121.14123	935	191
1	2	6	300.0	295.18784	1720	1720
1	2	7	300.0	344.91243	125	146
1	2	8	300.0	344.91243	18	23

Text file output example

Now, you have done a complete analyze of your image.
Do you know that this process can also be repeat automatically, using the batch mode?

III.7. Processing a batch

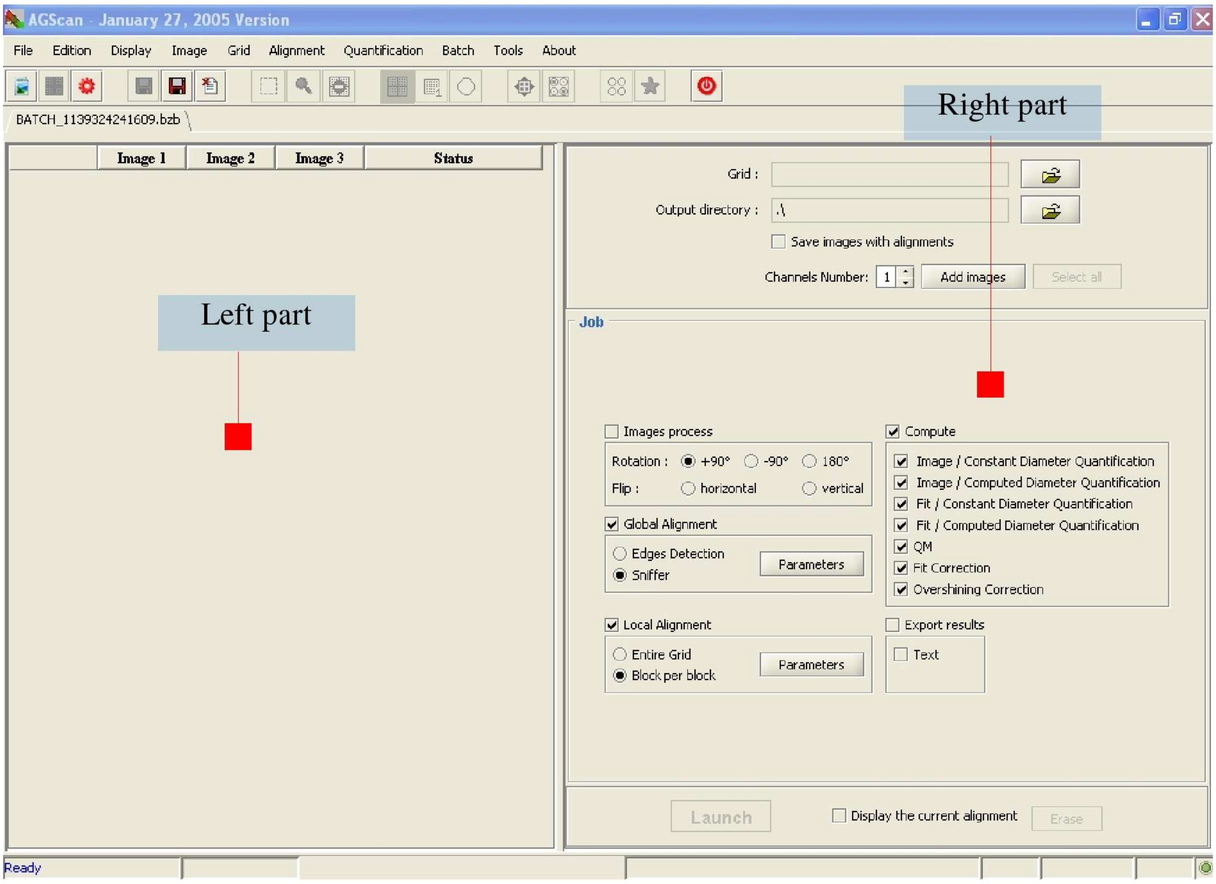
III.7.a. Batch tab

A batch allows to analyze automatically several experiments!

Important note: all these expermiments are analyzed with the same grid.

To create a batch, you can use the "File>Create>New Batch" menu or the "Batch>New Batch".
The associated icon of the toolbar is:
⚙

The batch tab contains two parts:



Grid :
Output directory : .\
☐ Save images with alignments
Channels Number: 1

☐ Images process
Rotation : ● +90° ○ -90° ○ 180°
Flip : ○ horizontal ○ vertical

☑ Global Alignment
○ Edges Detection
● Sniffer

☑ Local Alignment
○ Entire Grid
● Block per block

☑ Compute
☑ Image / Constant Diameter Quantification
☑ Image / Computed Diameter Quantification
☑ Fit / Constant Diameter Quantification
☑ Fit / Computed Diameter Quantification
☑ QM
☑ Fit Correction
☑ Overshining Correction

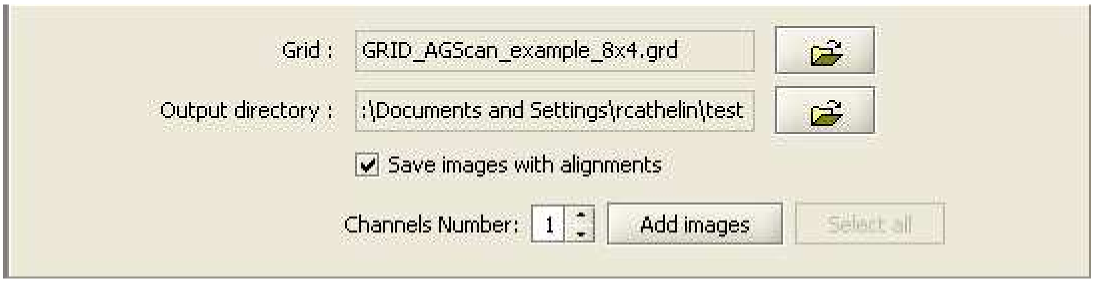
☐ Export results
☐ Text

☐ Display the current alignment

"Batch" tab

The tab is split into two parts, the left part that displays experiments status and images open and the right part that contains the configuration of the batch.
Note that the left part is by default hidden. You have to increase it with the mouse to see it better.

III.7.b. Preparing the batch



Grid : GRID_AGScan_example_8x4.grd

Output directory : :\Documents and Settings\rcathelin\test

☑ Save images with alignments

Channels Number: 1

Batch files configuration

In the top of the right part of the tab:
1/ Select the grid of the batch.
2/ Choose the output directory where results will be saved.
3/ Check or not the fact to save image into the archive created for each image. Notice that if the image isn't in the archive, you need to give its localization when you will open the experiment.
4/ Select the number of channels (1 to 3).
5/ Click on “Add Images” to select images.

Note: you can add images one by one or several in the same time in a multi-selection (only in “1 channel mode”).

After choosing several images: the left part is filled:

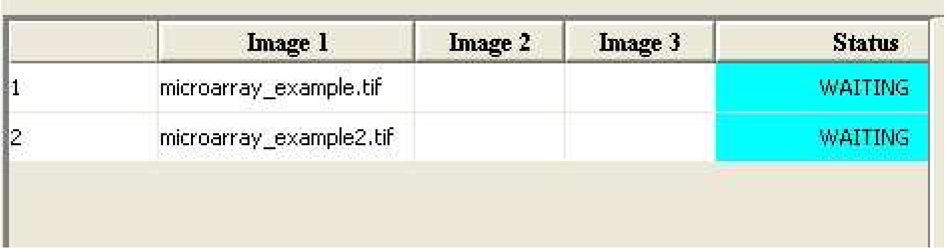
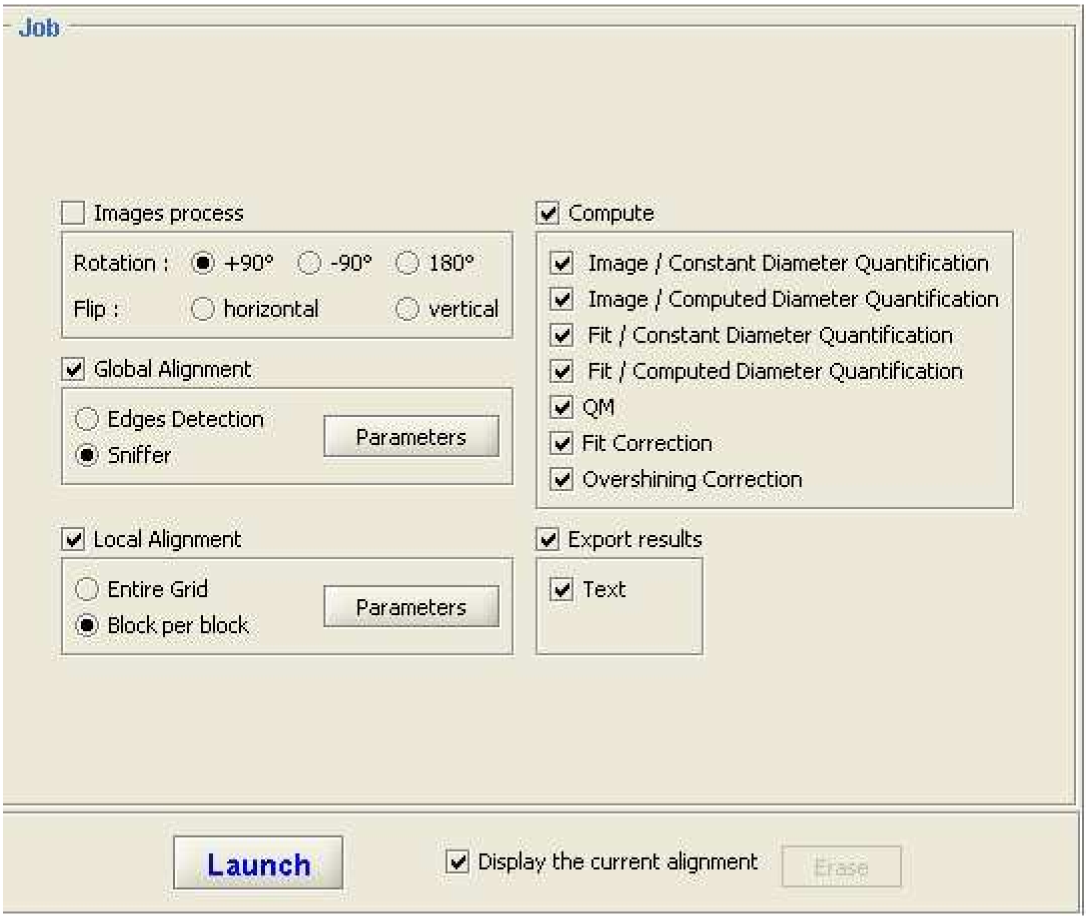


	Image 1	Image 2	Image 3	Status
1	microarray_example.tif			WAITING
2	microarray_example2.tif			WAITING

Batch experiment information

You can now choose all the actions you want to process for each image thanks to the job right part.



Job

☐ Images process

Rotation : ● +90° ○ -90° ○ 180°

Flip : ○ horizontal ○ vertical

☑ Global Alignment

○ Edges Detection

● Sniffer

☑ Local Alignment

○ Entire Grid

● Block per block

☑ Compute

☑ Image / Constant Diameter Quantification

☑ Image / Computed Diameter Quantification

☑ Fit / Constant Diameter Quantification

☑ Fit / Computed Diameter Quantification

☑ QM

☑ Fit Correction

☑ Overshining Correction

☑ Export results

☑ Text

☑ Display the current alignment

"Batch" parameters configuration

You just need to check or un-check process you want.
You can choose to display or not the current alignment processing by checking the last checkbox.
To launch the batch, just click on “Launch”.

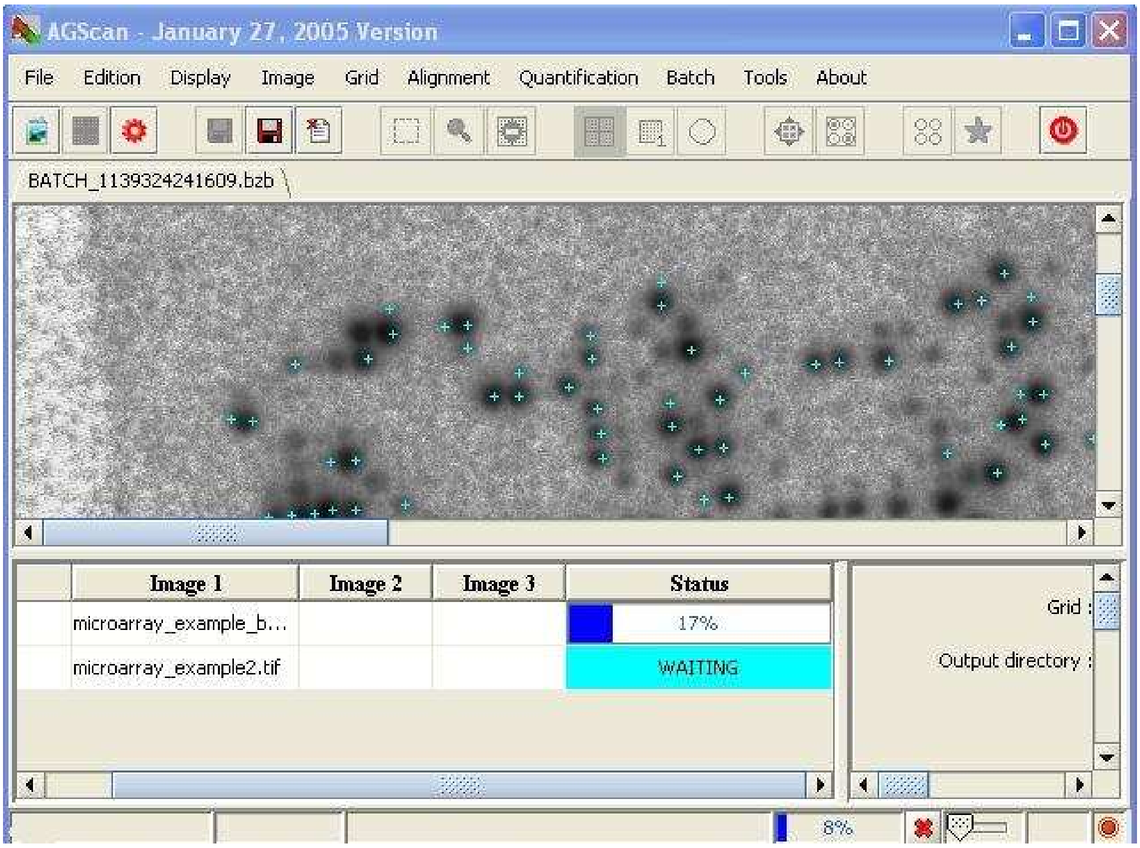


	Image 1	Image 2	Image 3	Status
	microarray_example_b...			17%
	microarray_example2.tif			WAITING

Following a "Batch" in progress

When you choose to display the process, you can follow it into the same tab: indeed a new part appears in the top of the current tab. As for the left window, you have to stretch it with the mouse.
The status of each image is updated.
In the statusbar of AGScan, the total percentage of the batch is given.

When the process is successfully finished, the image status is “DONE”.

	Image 1	Image 2	Image 3	Status
1	microarray_example_...			DONE
2	microarray_example2...			DONE

Batch progress finished

When the batch is finished, you can see results in the output directory.

For each experiment, you will have:
- the archive .zaf file
- the snapshot .tif file (rapid snapshot of the alignment)
- the export files (optional)

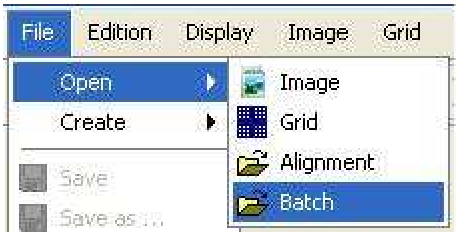
In our example, we have analyze two images and we asked for the text result file.So we have three files by image:

Elements of the output directory

III.7.c.Save/Open a batch

You can save a batch configuration in order to launch it several times or to re-open and modify it.
The extension of a batch file is a ".bzb". This file can only be used with AGScan. The format is kind of XML compliant.
Note:In the current version, it is not possible to record a batch in multichannel mode.

To open a batch, use the "File>Open>Batch" menu:



"File>Open>Batch" menu

IV. Plugins

IV.1. Presentation

One of the most interesting feature of AGScan is the plugins system.
It allows to easily add “functionalities” to AGScan.
For example, to add new file formats, alignment or quantification methods...
The only thing to do is to add plugins files in the agscan directory named “plugins”.

Some plugins are already available on the mulcyber following page:

Plugins

SPlotMatrixPlugin	**08/12/2005 14:28**		
SPlotMatrixPlugin.zip	18 KB	14 i386	.zip
SPlotPlugin	**08/12/2005 14:21**		
SPlotPlugin.zip	6 KB	12 i386	.zip
SAlignmentSnapshotPlugin	**06/12/2005 15:12**		
SAlignmentSnapshotPlugin.zip	3 KB	19 i386	.zip
SSurfacePlot_3DPlugin	**28/11/2005 16:04**		
SSurfacePlot_3DPlugin.zip	49 KB	17 i386	.zip
SSurfacePlot_3DPlugin_v2.zip	49 KB	0 i386	.zip
SQuantif1Plugin	**28/11/2005 15:13**		
SQuantif1Plugin.zip	27 KB	17 i386	.zip
SFilesMergePlugin	**24/11/2005 14:45**		
SFilesMergePlugin.zip	4 KB	27 i386	.zip
SMemoryPlugin	**24/11/2005 09:15**		
SMemoryPlugin.zip	6 KB	22 i386	.zip

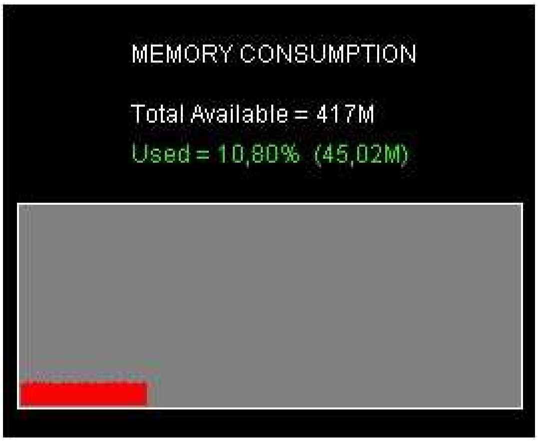
Plugins download page

On the website, you can click on the name of the plugin to watch its presentation and on the .”zip” file to download it.

IV.2. Example: the “Memory Plugin”

For example, we will present you how to download/install and run the SmemoryPlugin.
This plugin allows to add to AGScan a little window that give you the current memory consumption.

At first, click on the SMemoryPlugin.zip file to download it.
The .zip file contains sources, classes and compile notes (version of javac and AGScan used) of the Memory Controler plugin. Note that there are one source file SMemoryPlugin.java and 3 .class files (caused by embedded classes).
To use this plugin, just put .class files into the plugins directory of AGScan.
A "Memory Controler" submenu is added into the tool menu.



MEMORY CONSUMPTION

Total Available = 417M

Used = 10,80% (45,02M)

"Memory plugin" window

Notice that different categories of plugins exist. So, each plugin menu appears into its menu (alignment, tool, quantification...). There are also other plugins like “format plugins” that allow to read/save other images formats and that doesn't create menu into the application. You know that they are installed by showing the different type of files you can open. All these inforamtion are available int o the note provided with the plugin.

V. Frequently asked questions

(to be completed)

VI. Index

VI.1. Word Index

VI.2. Illustration index

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Version 2, June 1991

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