microarray systems

VersArray® Analyzer 5.0 Image Analysis Software

User Manual

BIO-RAD

VersArray™ Analyzer 5.0 Software

User's Guide

The VersArray Analyzer Software is for research purposes only. It is not intended or approved to be used for diagnostic purposes.

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Notice

Limitations and Liability:

Bio-Rad is not responsible for the misinterpretation of results obtained by following the instructions in this guide. As with all scientific studies, we recommend that you repeat your experiment at least once before making any significant conclusions for presentation or publication.

Bio-Rad Listens

The staff at Bio-Rad is receptive to your suggestions. Many of the new features are enhancements in this version of VersArray™ Analyzer 5.0 system software and are a direct result of conversations with our customers. Please let us know what you would like to see in the next version of VersArray Analyzer software by faxing, calling, or e-mailing our Technical Services staff.

Contents

Introduction to VersArray Analyzer 5.0 Software

VersArray Analyzer 5.0 Software is Bio-Rad Laboratories solution for a fast and accurate microarray image analysis software system. With VersArray Analyzer 5.0 Software, researchers can generate quantified data within seconds or minutes while at the same time generating extensive quality control information.

VersArray Analyzer 5.0 Software performs microarray image analysis for researchers. Microarray image analysis is used to quantify the relative expression levels that exist within a microarray scan. VersArray Analyzer 5.0 Software is used in the post hybridization and scanning step of the microarray process. The results of VersArray Analyzer 5.0 are quantified expression values that are saved to a text file. These results can then be used in a data analysis package, such as Bio-Rad's GeneGazer™ Software for further analysis.

Quick Start Guide

VersArray Analyzer 5.0 Software is designed to be the fasted and easiest software solution for quantification of microarray images. The description below outlines the essential steps required to setup VersArray Analyzer 5.0 Software. For additional information, please view the remaining sections of this user manual.

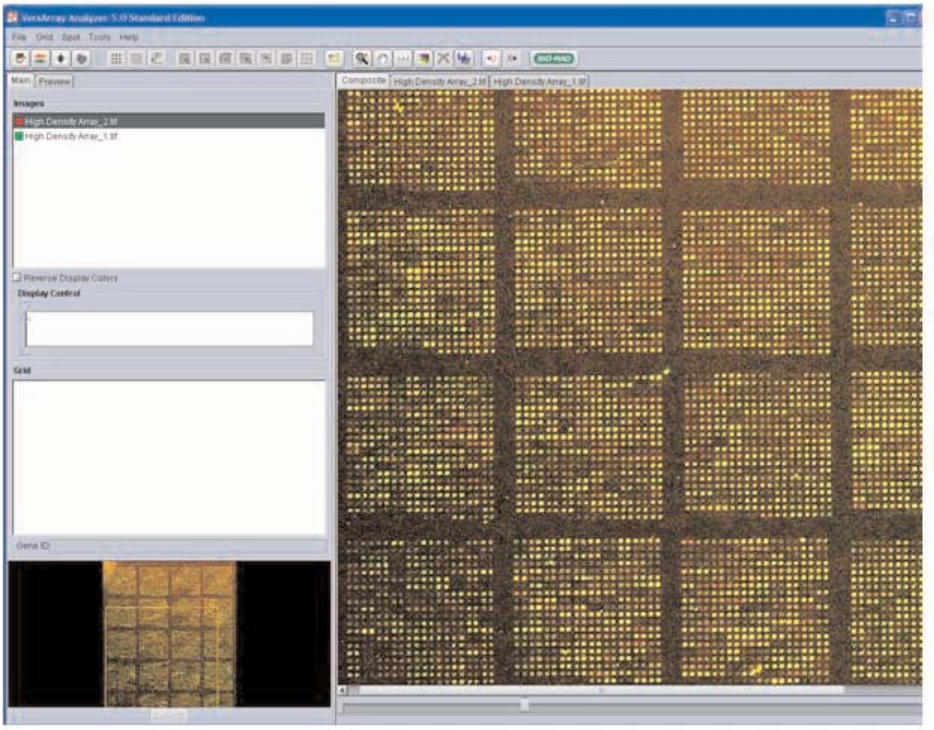
To setup VersArray Analyzer 5.0 Software:

1. Install VersArray Analyzer 5.0 Software
2. Submit registration information and request a license from Bio-Rad
3. Start VersArray Analyzer 5.0 Software
4. Load an image
5. Establish Settings
6. Perform grid placement
7. Quantify the Image

Part 1 – Lab Users Guide

1.1 VersArray Analyzer 5.0 Software Main Window Overview

The VersArray Analyzer 5.0 Software Main window is the primary program interface. It serves as the focal point of all work within VersArray Analyzer 5.0. You use this window to load images, place grids, produce quantified data and review results. This chapter identifies the components of this window and explains how each area fits into the array analysis and data extraction process.



- **Menu Bar** – Located along the top of the window. Click on one of the menus (File, Grid, Spot, Tools, or Help) to view the program commands available on that menu. The following options are available from the Menu Bar.

File

- **Load Images** – Allows selection of images to be loaded from the file system.
- **Remove Selected Image** – The image which is selected, or highlighted, from within the images panel is removed, or unloaded, from analysis. Multiple images can be removed by highlighting several images then selecting Remove Selected Images.
- **Review Results** – Allows selection of a snapshot, or sst file, which will allow for the results of previous analysis to be reviewed or reexamined. Simply browse to and select the desired sst file and VersArray Analyzer 5.0 Software will load previously created settings, data, segmentation and flagging information.
- **Batch Editor** – Allows creation of batches to automatically process data. Selecting this option will open the Batch Editor Window allowing for entries to be added to a batch.
- **Save Display Image** – Allows a 24-bit tiff image of the composite overlay to be created and saved to the file system. The image can be useful in post processing or generation of publication.
- **Settings** – The Settings option opens the VersArray Analyzer 5.0 Software Parameter Settings Window. This window and the tabs within the window contain all the parameters within VersArray Analyzer 5.0 Software for Quality Measures, Spot Finding and Alert Logging.
- **Exit** – Closes the VersArray Analyzer 5.0 Software program.

Grid

- **Load Grid** – Displays the Load Grid dialog box. Use this interface to select and open a previously saved grid (.grd) file.
- **Save Grid** – Displays the Save Grid dialog box. Use this interface to save a grid.
- **Clear Grid** – Removes all displayed grids from the Image Display Panel.
- **Load Gene IDs** – Displays the Load Gene ID File dialog box. Use this interface to select and open a gene ID (.txt) file.
- **Clear Gene Ids** – Displays the Confirm Deletion dialog box. Use this interface to verify that you want to remove the displayed gene ID information.
- **Load Template** – Displays the Load Template dialog box. Use this interface to select and open a template (.tpl) file. A template is a grid file, which contains gene ID information. Gal files are loaded through this option.
- **Save Template** – Displays the Save Template dialog box. Use this interface to save a template.

Spot

- **Adjust Metagrid** – Allows you to select and move an entire metagrid.

- **Adjust Subgrid** – Allows you to select and move an individual portion of the metagrid (subgrid).
- **Adjust Spot** – Allows you to select and move one spot in a metagrid.
- **Lasso Adjust** – Allows you to select and move a specific (free form) area of the metagrid.
- **Rectangle Adjust** – Allows you to select and move a specific rectangular area of the metagrid.
- **Auto Adjust Spots** – Tells VersArray Analyzer 5.0 Software to automatically adjust each spot to better align them with the corresponding image.
- **Wrangle** – Enforces new local spot flexibility parameters. Essentially, reduces the distance used in spot finding without requiring spot finding to be reapplied.

Tools Menu

- **Zoom** – Turns the cursor into a magnifying glass. Use this tool to adjust the on-screen display size of images.
- **Scroll** – Turns the cursor into a hand. Use this tool to scroll all images and grids at the same time.
- **Undo** – Cancels the last executed command. For example, if you moved a spot, you could select Tools > Undo to cancel this action and, in effect, deselect the spot.
- **Redo** – Restores the last canceled command. For example, if you used the Undo command to cancel a spot movement, you could select Tools > Redo to move the spot again.
- **Translate Images** – Turns the cursor into a cross with arrows. Use this tool to move selected images.
- **Rotate Images** –Turns the cursor into a circle. Use this tool to move selected images in a circular path based on a manually set anchor point in the image.
- **Ruler** – Turns the cursor into a small ruler. Use this tool to measure the size of spots or distances between them.
- **Image Intensities** – Turns the cursor into a small light bulb. Use this tool to measure the intensity of spots.
- **Tag Spots** – Turns the cursor into the letter X. Use this tool to manually flag spots. Right click with this tool to see a list of available flagging options.

Help

- **VersArray Analyzer 5.0 Software Help** – Displays the VersArray Analyzer 5.0 Software Online Help documentation.

- **Wizard On/Off** – Turns on and off the VersArray Analyzer 5.0 Software wizard. The wizard provides guidance on the proper steps to be performed within VersArray Analyzer 5.0 Software.
- **Change Skins** – launches a dialog for choosing between different skins for VersArray Analyzer 5.0 Software interface (using skinlf). When a specific skin is selected, the change takes effect next time you launch VersArray Analyzer 5.0 Software.
- **Support Center** – launches Web Browser with customer support page for VersArray Analyzer. Through that web-site you can access latest technical documentation, request a new feature, obtain sample images or templates etc.
- **About VersArray Analyzer 5.0 Software** – Displays the About VersArray Analyzer 5.0 Software dialog box. This interface contains information (license number, mode, etc.) about your copy of VersArray Analyzer 5.0 Software.
- **Toolbar** – Located directly beneath the menu bar. This region is composed of multiple buttons that provide a one-click method for executing program commands.

- **Context Menu** – Context Menus are created by right clicking on the various elements within the VersArray Analyzer 5.0 Software graphical user interface (GUI). Not all elements support context menus. Elements which do support this feature are listed below:

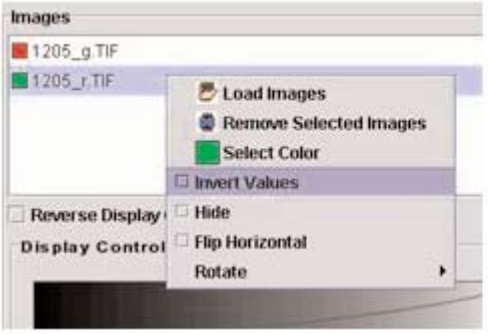
  Images Panel

  Grid Panel

  Image Display Panel

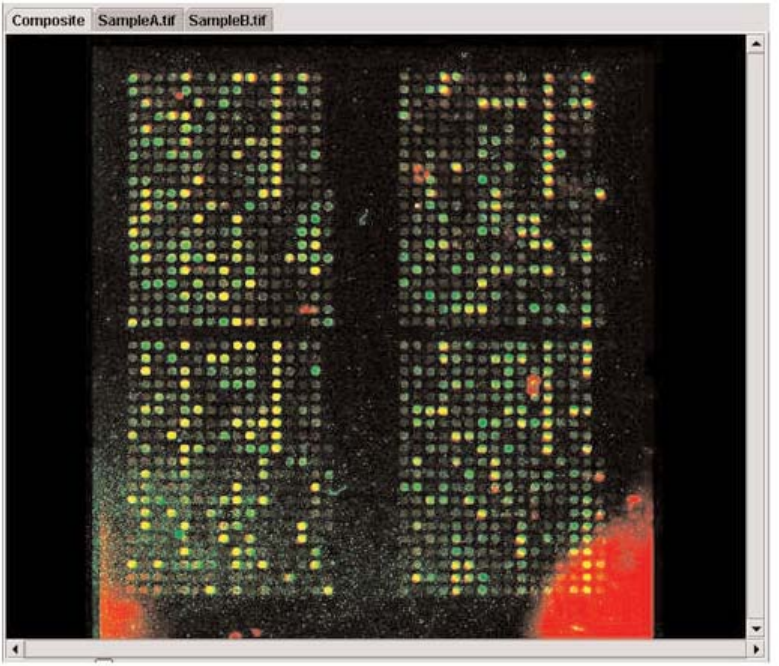
  Gene ID Selector

  Flagging Tool



- **Control Tabs** – Located to the left of the window. The control tabs provide information immediately relevant to the images being quantified. Please see section 1.2 for additional information on the control tabs.

- **Image Display Panel** – Located to the right of the window. This panel displays loaded images and is where grid placement takes place.



- **Status Bar** – Located at the bottom of the window. The status bar provides feedback to the user regarding loading and handling of images. Also, other tools such as the Ruler and Image Intensity display their information here.

1.2 Control Tabs

Located along the left of the main VersArray Analyzer 5.0 Software window are two tabs containing essential information about the analysis.

1.2.1 Main Tab

The Main Tab is used to perform the essential steps of microarray image analysis, loading the image(s) to be processed and placing a grid on the image and its corresponding structure. The following Panels are contained within the Main Tab.

Images Panel

The images panel displays the names of images currently loaded within VersArray Analyzer 5.0 Software. Each loaded image is listed here as well as its corresponding Composite color. The Composite Color is the color the image is displayed as when seen within the Composite Tab of the Main Image Panel.

The Images Panel fully supports context menus and as a result provides the following menu choices:

- **Load Images** – Allows browsing to and selection of images from the file system.
- **Remove Selected Images** – The images which are selected, or highlighted, from within the images panel are removed, or unloaded, from analysis.
- **Select Color** – Select the color to be used for the select image when seen under the Composite Tab. The default color for the first two images are red and green; however, these can be changed to any color desired. Changing the colors here in no way affects the resulting quantified values.
- **Invert Values** – This will invert all pixel intensities within the selected image. When the image is loaded, VersArray Analyzer 5.0 Software automatically determines which end of the grayscale spectrum is the high value. This information is typically available within the image file itself. However, in rare cases, this information is not present within the file and as a result, VersArray Analyzer 5.0 Software requires this information to be manually set. Should you determine the expected values for the signal and the background measurement to be opposite to what is expected, selecting Invert Values will solve the problem.
- **Hide** – The selected image will no longer be visible under the Composite Tab. While not visible, if quantified, data will be generated for the image.
- **Rotate** – Rotates the image 90, 180 or 270 degrees around the top, left of the image.

How to Load an Image(s)

The following steps describe the process of loading Images within VersArray Analyzer 5.0 Software:

From the menu bar, select File followed by Load Images. Alternatively, click the first icon on the toolbar or right click on the Images Panel and select Load Images.

From the Load Images dialog that appears, browse to and select the desired file. Multiple files may be selected by holding the <shift> or <ctr> keys and left clicking on the image name.

Note: VersArray Analyzer 5.0 Software supports the following image file formats:

Tiff – The file extension is tif

MD Gel – The file extension is gel

Fuji Bas – This is a two file format with one file ending in inf and the other ending in either img or bas.

- **Reverse Display Colors** – Mark this check box to reverse the displayed colors of all open images. Activating this feature has no affect on the Composite tab. Changing the appearance of an image does not affect the pixel intensity of your original data. This tool just makes it easier to see dim spots in an image.

Display Control

The Display Control allows adjustment of how the images are displayed. By moving the triangle located at the top and bottom the display control, the image can be lighted and darkened. The tool is designed solely to enhance viewing of the image and does not affect the quantified values generated by VersArray Analyzer 5.0 Software. When adjustments are made to the Display Control, these are applied only to the Image(s) selected within the Images Panel.

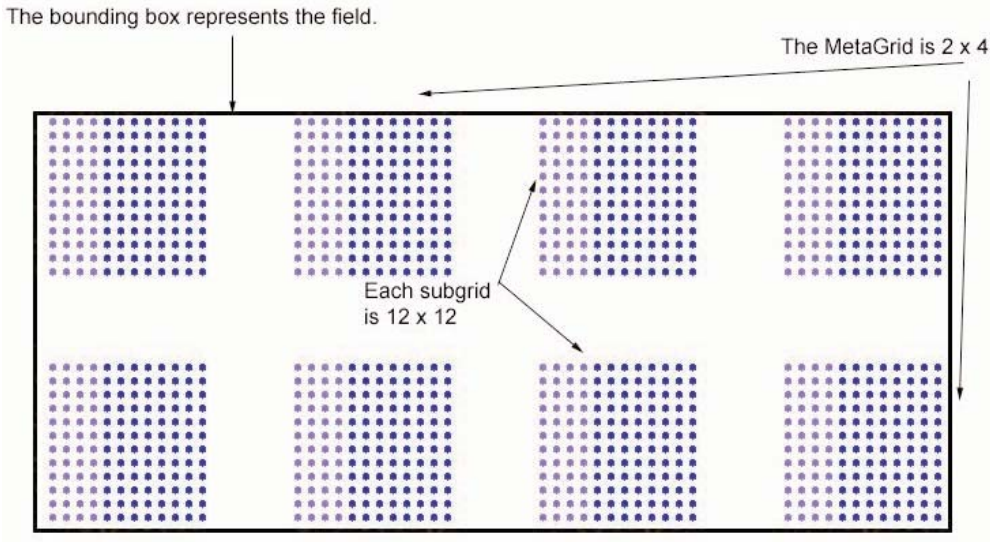
The specific elements of the Display Control are and perform the following:

- **Top Triangle** – This sets the minimum intensity to be displayed.
- **Middle Triangle** – Allows the rate of change of pixel intensities to be specified by changing the curvature of display curve.
- **Bottom Triangle** – This sets the maximum intensity to be displayed.

Grid Panel

The Grid Panel lists all fields that have been created and placed on the image. Typically, only a single field will be required; however, depending on the design of the array being quantified, several fields may be required. As a new field is created, it is added to the list.

Before explaining how and why to create a Field, let us explore some of the definitions and structures of a field. A field is the largest design element within a slide. A field typically consists of the arraying done by a single print head on the slide. For example, if the arrayer has a print head with 8 pins in a 2 x 4 conFIGuration, the region of the slide containing the resulting printing is a field. The metagrid in this example would be 2 x 4 as the resulting printing would generate 2 rows by 4 columns of subgrids. The subgrid is not defined here, but would be whatever rows and columns of spots are printed by a single pin, 12 x 12 for example. The accompanying diagram demonstrates the relationship between the three levels of structure.



The Grid Panel like other components of VersArray Analyzer 5.0 Software fully supports context sensitive menus. Right clicking on the Grid Panel provides the following options:

- **Create Grid** – The option launched the Grid Creation Window where the essential information about the design of the grid must be entered. Remember that within VersArray Analyzer 5.0 Software the grid is the most elemental structure, followed by the metered and finally field. The following parameters must be specified:
- **Field Name** – Specify the name of the field here. The name may be any name desired. By default VersArray Analyzer 5.0 Software provides the letter 'A' to the first fields, 'B' to the next field and so on. If a gene ID file is being used in conjunction the image to provide gene names or accession numbers within the text output file of VersArray Analyzer 5.0 Software, the fields name, if used, must match between what is specified here and what is contained within the Gene ID file. For example, within the gene ID file, if I have a column where I specify the field and that field has the name "Top", then I must enter "Top" for the field name within VersArray Analyzer 5.0 Software. If the name does not match, then VersArray Analyzer 5.0 Software will NOT use the gene name within the data output file.
- **Rows** – The number of rows of spots contained within the grid. Typically, this is simply counted visually.
- **Columns** – The number of columns of spots contained within the grid. Typically, this is simply counted visually.

- **Min Diameter** – This specifies the minimum expected diameter of the spot and is measured in pixels. The size of the spot can best be determined by use of the Ruler Tool. Due to the variety of array types and the variability of individual arrays there is no set procedure for determining the minimum diameter here. The most common rule of thumb is to specify the size of approximately the 10 % of the smallest spots of the array. Depending on the type of array, if the spots are highly uniform, then the minimum diameter specified here will be close or equal to the maximum diameter specified next.
- **Max Diameter** – Similar to the Min Diameter specified above, this parameter reflects the maximum anticipated spot size measured in pixels. As with the minimum diameter this value can be approximated by using the top 10% of large spots and measuring the sizes with the Ruler Tool.
- **Spot Orientation** – Spot Orientation reflects how each row is located relative to the row that preceded it. The selections here reflect how the row above the subsequent row is positioned. While rectangular is the most common, selections possible include:

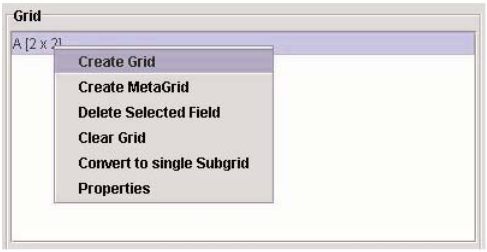
  Rectangular

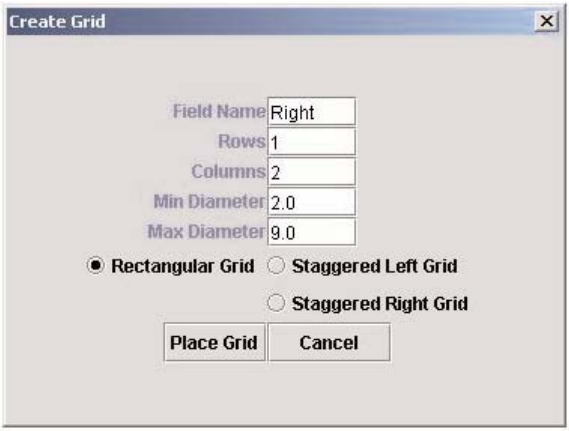
  Staggered Left Grid

  Staggered Right rid

- **Create MetaGrid** – This selection allows subgrids to be used to form a metagrid structure. Before creating a metagrid, at least one subgrid must be created. To create a metagrid, first highlight the desired subgrid, then select “Create Metagrid”. The Create MetaGrid Window appears and requires the following parameters to be specified.
- **Metarows** – The number of rows of subgrids.
- **Metacolumns** – The number of columns of subgrids
- **Delete Selected Fields** – The currently highlighted field will be removed.
- **Clear Grid** – All fields that have been created will be removed.
- **Convert to single Subgrid** – If a metagrid structure exists and has been used to “grid” the image, selecting this option will convert the metagrid structure to a simpler subgrid structure. For example, if we have a 2 x 2 meta grid with 15 x 10 subgrids, after selecting this conversion, the resulting subgrid size will be 30 x 10. During this conversion nothing changes except for the how the individual spot location are represented. This feature is option and may used if required to construct a multi-level metagrids for example.
- **Properties** – This option opens a window, which displays the parameters for the currently selected field. Only certain options can be changed while the remainder required the grid to be deleted then recreated a new. The following parameters are displayed within the properties window:
- **Field** – The name of the field. This value may be changed.
- **Metarows** – The number of metarows previously specified.

- **Metacolumns** – The number of metacolumns previously specified.
- **Rows** – The number of rows of spots within the subgrid that was previously specified.
- **Columns** – The number of columns of spots within the subgrid that was previously specified.
- **Min Diameter** – The minimum expected diameter of the spots to be used during spot finding. This value may be changed but the results will not be visible until spot finding has been performed again.
- **Max Diameter** – The maximum expected diameter of the spots to be used during spot finding. This value may be changed but the results will not be visible until spot finding has been performed again.





How to create Grid

Perform the following steps to create a subgrid within VersArray Analyzer 5.0 Software:

1. Load the desired images into VersArray Analyzer 5.0 Software
2. Right click with the mouse on the Grid Panel and select Create Grid
3. Specify the parameters within the create grid window. If a Gene ID file is being used then the field name must match the name of the field within the gene ID file. The min and max diameter can be calculated with the Ruler Tool.
4. Click the Place Grid Button and click on the four corners of a subgrid. If you make a mistake, right click with the mouse to remove the last placement.
5. Perform the following steps to create a metagrid within VersArray Analyzer 5.0 Software:
6. Click on and select the desired subgrid to use as a basis for the metagrid. This can be done by left clicking on the grid within the Grid Panel.
7. Right click and select Create MetaGrid from the menu.
8. In the Metagrid Parameters Window, specify the number of rows and columns of subgrids contained within the metagrid.
9. Click Place Metagrid and click on the top left spot in each of the corner subgrids. Typically, this process will require four clicks, but never more.

How to Save a Grid

Once a grid has been created the following steps will save the grid for later use.

1. From the menu bar select Grid followed by Save Grid.
2. In the Save as Dialog browse to the location where you wish to save the grid file.
3. Specify a file name. VersArray Analyzer 5.0 Software will automatically add the .grd file extension to the end of the name.

How to Load a Grid

To load a previously created grid, perform the following steps:

1. From the menu bar select Grid followed by Load Grid.
2. From the Load Grid Dialog that appears, browse to and select the desired grid file.
3. Click on one of the following radio buttons to select a grid placement method:

- **Place Manually** – Select this option to set the location and size of the saved grid yourself. This is useful when images have been scanned at different resolutions or the overall position of the array shifted between scans. To place the grid manually, left-click on the four corners of the entire array structure. The grid will then be resized and positioned based on this information.
- **Place in Saved Position** – Select this option to place the grid in the identical position of the original grid. Use this option if the size and resolution of the images has not changed

Finally, click the Open Button to place the grid.

Gene ID

The Gene ID file allows you to track information about the genetic material spotted at each location within the array. This information will be saved along with the quantified values in the text output file and visualization tools.

If a Gene ID has been selected the name of the corresponding file is displayed here. The Gene ID also supports right click context menus with the following options:

- **Load Gene ID's** – Selecting this option will open a window allowing for the selection of the gene ID file to be used. Likewise, you may also select "Grid" from the File Menu followed by "Load Gene IDs" to accomplish the same task. Note that while advantageous for a number of reasons, loading a Gene ID file is not mandatory. Please see the section of Gene IDs later within this manual for additional information on Gene IDs as well as appropriate file format.
- **Clear Gene ID's** – If a gene ID has already been selected, this option will remove the selected file from use. Likewise, you may also select "Grid" from the File Menu followed by "Clear Gene IDs" to accomplish the same task.

Template

You can load and save a template file that will contain both grid structure and corresponding gene IDs. For this purpose you can use VersArray's serialized data format *.tpl, or you can import and export the template using several well-known formats.

- **GAL** – tab-delimited text file containing location and structure of every subgrid (called "Block" within this format) and gene IDs.
- **GEML (v 1.0)** – XML standard representing a "pattern" that can not fully describe a grid structure, but rather provides location and gene ID info for every spot.
- **MAGE-ML** – the most complete XML format, imported/exported file will contain DesignElement_package and ArrayDesign_package of MAGE standard. This format can support multiple subgrids and metagrids.

Map View Panel

The Map View provides a comprehensive and unabridged view of the image while indicating exactly where within the image the primary image display panel is zoomed to. The Map View displays the entire image exactly as it appears along the main image display's composite view. Any corrections to contrast or rotation will likewise be visible within the Map Window. The Map View allows users with large arrays to more easily scan the image for proper gridding and segmentation.

The part of the image currently being displayed within the main image display is bound by a yellow rectangle within the Map View. You may also zoom using the Map View by left clicking and dragging with the mouse to select the desired region. To zoom completely out and display the entire image within the main image panel, double right click with the mouse.

Quantify

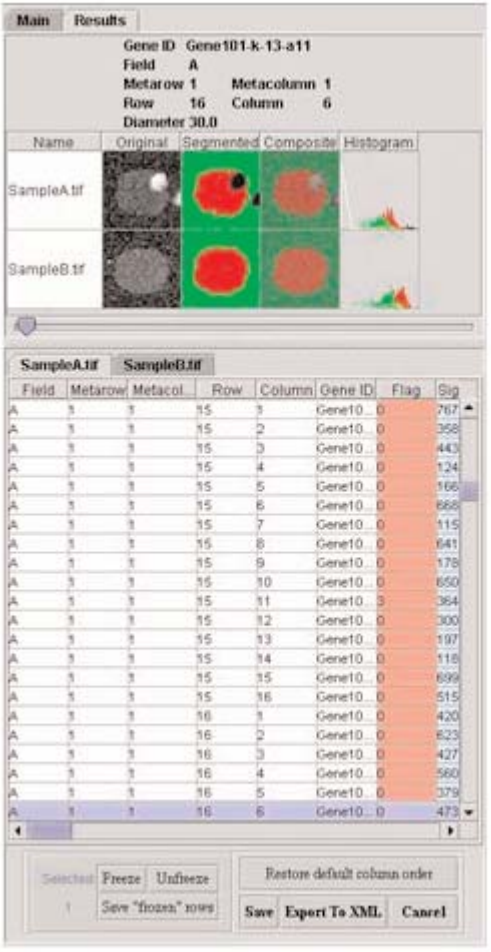
The act of quantification coverts the visual pixel intensities into numerical values to be used later in expression analysis. The Quantify Button starts this computation within VersArray Analyzer 5.0 Software and should be performed after all other parameters have been set and the grid has been placed. The amount of time quantification takes is directly proportional to the speed of the computer hardware. Once quantification is completed, the Preview Tab becomes highlighted and the numerical values become visible.

Additionally, after quantification is complete, an additional tab for each image becomes available over the main image panel. This tab is the segmentation tab and displays the segmentation, which has been performed across the entire image.

1.2.2 Preview Tab

The Preview Tab displays information about the image both prior to and after quantification. Numerical values and segmentation can easily be viewed and reviewed to determine that optimum settings are established. The Preview Tab is divided into two primary parts:

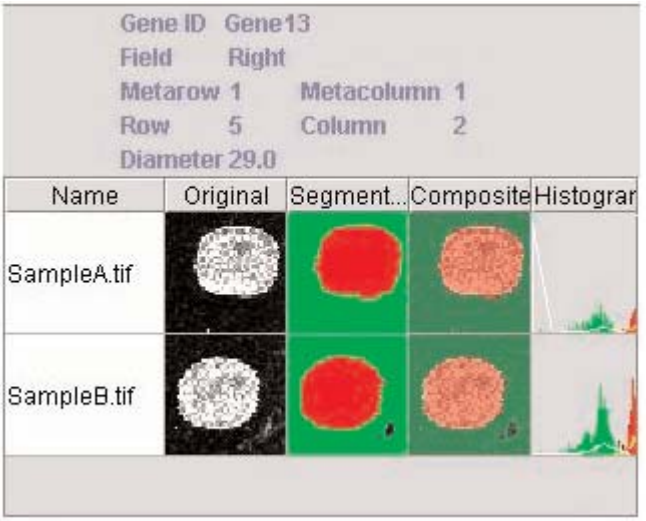
- Segmentation Preview
- Quantification Preview



Segmentation Preview

Segmentation involves the partitioning of a microarray image into a set of regions that convey a specified meaning. For microarrays, the purpose of segmentation is to decompose a scanned optical image into regions that are meaningful in terms of spot signal versus background.

Use the Segmentation Preview to view the effects of current parameter settings on the segmentation both before and after quantifying the data. You select the spot to view and the corresponding information about the spot displays in the segmentation panel. At the same time the selected spot can be seen on the image and two of the plots (Scatter plot and Box plot). This interface also supports dynamic analysis within VersArray Analyzer 5.0 Software. You can adjust settings, such as the maximum and minimum signal values, and see the effects of these changes in real time in the dialog box.



The following textual spot information is available via the Segmentation Preview:

- **Gene ID** – Lists the corresponding information, typically name or accession, from the Gene ID file (if you imported a Gene ID file into VersArray Analyzer 5.0 Software.
- **Field** – Lists the field that the selected spot belongs to. This name is specified when the grid was first constructed.
- **Metarow** – Identifies the row within the metagrid where the selected spot is located.
- **Metacolumn** – Identifies the column within the metagrid where the selected spot is located.
- **Row** – Identifies the row within the subgrid where the selected spot is located.

- **Column** – Identifies the column within the subgrid where this spot is located.
- **Diameter** – Lists the diameter, measured in pixels, of the selected spot. The diameter is determined during spot finding when various spot sizes are attempted.

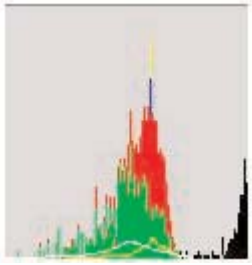
The following visual spot information is available via the Segmentation Preview:

- **Name** – Lists the name of the source image file for the spot.
- **Original** – Displays the spot and its surrounding background without any segmentation information.

- **Segmented** – Displays an image of the segmentation, or pixel determination, that will be performed during quantification. The red pixels represent signal values and the green pixels represent background values. Black means the pixel is ignored.

- **Composite** – Displays an overlay between the Original and Segmented images. This image is key to determining if the best settings have been entered on the Measurements tab. Use this view to modify the settings until the desired signal and background values are included while removing contaminants.

- **Histogram** – Represents the distribution of pixels along the intensity scale for the spot. The y-axis (vertical) represents the number of pixels and the x-axis (horizontal) represents the range of intensities of pixels across the spot. The histogram is intended for a simple qualitative preview and should not be used for any form of data analysis. Within the histogram, colored vertical bars indicate specific values. The colors and their meaning are:
- **Blue** – Signal Mean Value
- **Yellow** – Signal Median Value
- **Cyan** – Signal Mode Value



Under the Segmented, Composite, and Histogram Views color is used to indicate pixels to be included within the signal and background measurements. If a pixel is not color, then the pixel is being ignored and is not being used for calculation of either signal or background values.

The Preview Panel colors include:

- **Green** – Indicated the pixel is used in the background calculation
- **Red** – Indicated the pixel is used in the signal calculation
- **No color (or black on histogram)** – Indicated the pixel is used in neither calculation of signal nor background

Quantification Table

The Quantification Table displays the quantified numerical intensity values for each of the spots prior to being saved. =All measurements previously selected to be quantified, including all quality measures are indicated here. Additional information regarding flagging and spot location information is provided.

Each section of the data, as presented by a grouping of columns, is color coded to facilitate easy review of the analysis. The following color codes describe the table:

- **Red** – Quality flag code
- **Cyan** – Quantified intensity values -– mean, median, quality measures etc
- **Purple** – Spot location related information

Gene ID	Flag	XCoord	Control	Background Mode
Gene34	0	282.0...		343.733333333...
Gene42	0	325.7...		71.8
Gene50	0	369.5...		47.2263157894...
Gene58	0	413.2...		55.5615763546...
Gene66	0	457.0...		66.2060301507...
Gene74	0	500.7...		41.796875
Gene82	0	544.5...		47.3885350318...
Gene90	0	588.2...		149.97
	0	632.0...		72.4493392070...
	0	675.7...		68.8995215311...
	0	719.5...		51.8888888888...
	0	763.2...		24.8266666666...
Gene3	0	107.2...		116.180602006...
Gene11	0	151.0...		103.349593495...

Note: By default these colors are grouped together, but you can drag and drop the column headers to change this order. Should the default order of the columns change, you can restore the columns to the default order by using "Restore default column order" button in the bottom of the table.

The default measurement order in the table is as follows.

- **Field** – Name of a field where the spot is located
- **Metarow** – Number of metarow in the metagrid where the spot is located
- **Metacolumn** – Number of metacolumn in the metagrid where the spot is located
- **Row** – Number of row in the subgrid where the spot is located
- **Column** – Number of column in the subgrid where the spot is located
- **GeneID** – Gene ID information for the spot

- **Flag** – Numeric code of the flag for the spot (0 - no flag, flag codes 1,…,4)
- **Signal Mean** – Pixel intensity averaged over the local signal region
- **Background Mean** – Pixel intensity averaged over the local background region
- **Signal Median** – Median pixel intensity computed over the local signal region
- **Background Median** – Median pixel intensity computed over the local background region
- **Signal Mode** – Mode pixel intensity computed over the local signal region (mode corresponds to the pick location in intensity distribution)
- **Background Mode** – Mode pixel intensity computed over the local background region
- **Signal Area** – Number of pixels in the local signal region
- **Background Area** – Number of pixels in the local background region
- **Signal Total** – Total pixel intensity summed over the local signal region
- **Background Total** – Total pixel intensity summed over the local background region
- **Signal Stdev** – Standard deviation of pixel intensities over the local signal region
- **Background Stdev** – Standard deviation of pixel intensities over the local background region
- **Shape Regularity** – First signal area of a spot is inscribed into a circle. Than number of non-signal pixels that fall within this circle is computed and divided by circle's area. This ratio is subtracted from 1 as is called "shape regularity"
- **Ignored Area** – Area of ignored regions directly neighboring ("touching") the signal area is computed
- **Spot Area** – Signal Area plus Ignored Area
- **Ignored Median** – Median pixel intensity computed over the local ignored region
- **Area To Perimeter** – This quality measure defines spot's circularity. Area of a spot is divided by a square of spot perimeter and multiplied by. As a result, this measure ranges from 0 (highly non-circular shape) to 1 (a perfect circle)
- **Open Perimeter** – Computes the proportion of signal perimeter that touches the border of rectangular snip around the spot
- **XCoord** – X coordinate (in pixels) of grid circle corresponding to the spot
- **YCoord** – Y coordinate (in pixels) of grid circle corresponding to the spot
- **Diameter** – Diameter (in pixels) of grid circle corresponding to the spot
- **Position Offset** – Offset (in pixels) of the center of the grid circle from the expected position in the grid

- **Offset X** – X offset (in pixels) of the center of the grid circle from the expected position in the grid
- **Offset Y** – Y offset (in pixels) of the center of the grid circle from the expected position in the grid
- **Expected X** – X coordinate of expected position of the circle in the grid. Expected position in the grid is computed fitting least square lines to circle centers in every row and column
- **Expected Y** – Y coordinate of expected position of the circle in the grid. Expected position in the grid is computed fitting least square lines to circle centers in every row and column
- **CM-X** – X coordinate of the center of the mass of spot's signal region
- **CM-Y** – Y coordinate of the center of the mass of spot's signal region
- **CM Offset** – Offset (in pixels) of the spot's center of the mass from the expected position in the grid
- **CM Offset-X** – X offset (in pixels) of the spot's center of the mass from the expected position in the grid
- **CM Offset-Y** – Y offset (in pixels) of the spot's center of the mass from the expected position in the grid
- **Min Diam** – Diameter of the circle inscribed into the spot's signal region
- **Max Diam** – Diameter of the circle, the spot's signal region can be inscribed in

Some of the measures can be excluded from or added to the table at any moment through "Measurements" panel of Settings dialog box.

Individual spots can be selected for review, either by selecting the row from within the Quantification Table or by selecting the spot within the image. If the spot is selected from the image, the Quantification table will automatically scroll to the proper location and the corresponding spot row will be highlighted. Notice also that the Segmentation Preview automatically updates and displays the segmentation information for the selected spot.

Save

Once the data has been quantified and you are satisfied with the results, the final step is to Save the data. VersArray Analyzer 5.0 Software will save the data to common tab delimited test files, which can easily be opened in other programs such as Microsoft Excel or Notepad. VersArray Analyzer 5.0 Software will save each image's data to a separate file and will automatically name the file based upon the name of the image.

Note: By default, VersArray Analyzer 5.0 Software does not compute ratio values between images; however, there is access to ratio data directly from VersArray Analyzer 5.0 Software through the Histogram plot under the "Plots" tab in the Image Display Panel. To compute ratio information, please use VersArray GeneGazer Software. If additional and more advanced computations are required, Bio-Rad recommends VersArray GeneGazer Software for full data analysis and visualization.

Export to XML

Before saving the data you also can export the measurements in GEML or MAGE-ML format.

- **GEML (v 1.0) format** – GEML profile export, only four values per spot are available (signal/background value and standard deviation). User can choose between mean, median, mode for export as signal/background value. For more details go to http://www.rosettabio.com/products/conductor/geml/default.htm.
- **MAGE format (XML)** – the most complete format, exported file will contain QuantificationType_package, BioAssay_package and BioAssayData_package of MAGE standard. All VersArray Analyzer 5.0 Software measurements will be exported. For more details go to http://www.mged.org.

Note: once you export the data, the results table will not be closed, you can continue analyzing the data or save the results in usual format.

Cancel

Cancel clears the quantified data values without saving them. Once the quantification is cancelled, the image will need to be reprocessed before the data may be saved again. Upon selecting Cancel, you will be prompted to verify your action. Clicking yes will then clear the data. The Quantification table will no longer be visible.

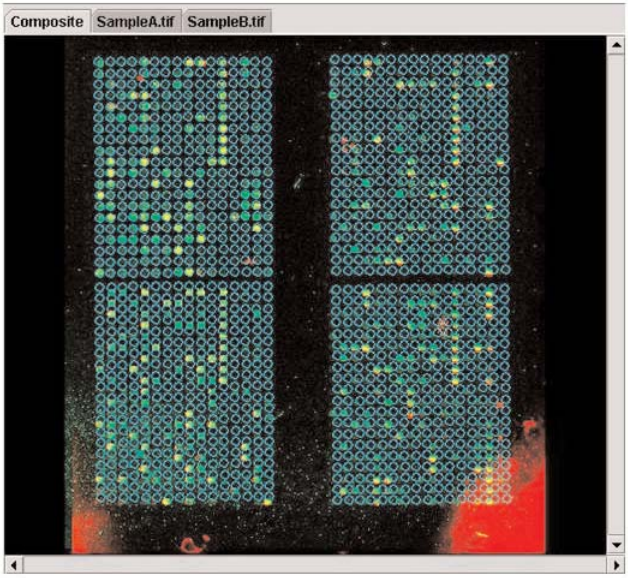
Selection

VersArray Analyzer 5.0 Software allows multiple spot selection. You can select several spots at a time pressing "Ctrl" or "Shift" keys when making selections in the results table. The indicator in the bottom of the table will show you how many spots are selected.

You can also "freeze" the selection using corresponding buttons. When "frozen", table rows will be highlighted in dark blue and will have a flag "Selected" in the right-hand end of the table turned assigned as "1". When you "freeze" the spots you can analyze them inividually by selecting only one row at a time. You can "unfreeze" the selection at any time and the spots will become highlighted with the usual selection color. This feature is useful if you selected the spots using one of the plots (selected regulated genes using Histogram, for instance) and want to analyze their images, segmentations and quantifications results. You also can Save the frozen rows into .txt file.

1.3 Image Display Panel

This panel displays loaded images. A tab appears along the top of the panel for all currently loaded images. Click on a tab to display the corresponding image. The Composite tab displays a false color overlay for all loaded images. You can use this tab to overlay multiple images prior to analysis. The number of images which VersArray Analyzer 5.0 Software can load is limited based upon the computer hardware specifications.



All image manipulation tools, such as zoom and rotation, can be applied within the image panel. Once zoomed into a region of the image, scroll bars become available along the sides of the panel.

Note: There is a Zoom slide bar located at the bottom of the panel. Move it to the left to zoom out of the image or move it to the right to zoom into an image. The Zoom slide bar is one of four tools available for zooming.

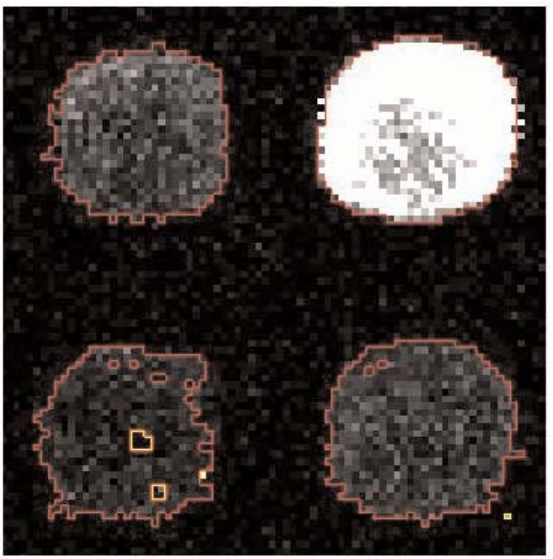
Segmentation Tabs

Once the image(s) have been quantified additional tabs, one for each image plus one for plots, will appear along the top of the image display panel. Some of these new tabs display the segmentation as it has been performed for the given image. From this view, you have the ability to see the macro view of the image and notice any large defects and the corresponding segmentation. Typical use of the segmentation tabs is for detailed post processing quality assurance analysis.

Unlike the Preview Panel, the Segmentation tabs use lines to indicate the segmentation's signal and background regions. All signal regions are surrounded by RED lines. All ignored regions, values not counted as signal or background, are surrounded by YELLOW lines. The remaining pixels within the image are all background regions.

The Segmentation Tab line colors:

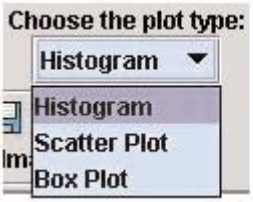
- **Red** – All pixels within the red lines are signal values
- **Yellow** – All pixels within the yellow lines are ignored pixels



Note: The description and use of colors between the Preview Panel and the segmentation tab does vary slightly. The segmentation tab includes the use of yellow to indicate ignored pixels where as the Preview Panel uses no coloring to indicate ignored regions. Also, the segmentation tab does not use a color to indicate the background region; however, within the Preview Panel, background values are indicated by a green color. The differences between the two displays is accounted by the fact that due to customer requests, the segmentation tab is designed to prevent eyestrain during extensive visual inspection.

Plots Tab

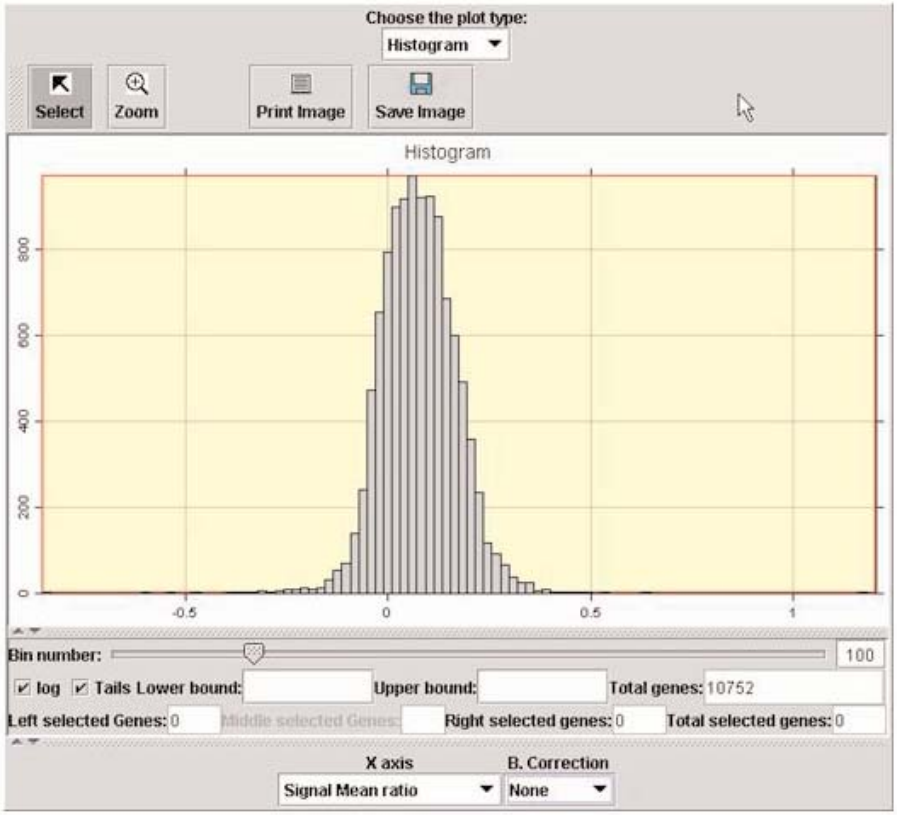
This tab appears also only when the image is quantified. It contains a set of useful data visualization tools that can help speed up the analysis process.



You can take virtually any measurement available from the results table and plot it in a manner corresponding to one of three tools: Histogram, Scatter Plot, Box Plot or GenePie. Once you choose the desired type of visualization, choices of measurement for X- and Y-axes become available. Background correction options are available for both axes if signal mean, median, mode or their inter-channel ratio is chosen for the plot. If Histogram is selected, only the measurement choice for the X-axis will be available. Correspondingly, only background correction for that measurement will be accessible.

A Change in measurement selection will be followed by an update of the current plot. Any plot can be printed or saved as an image file.

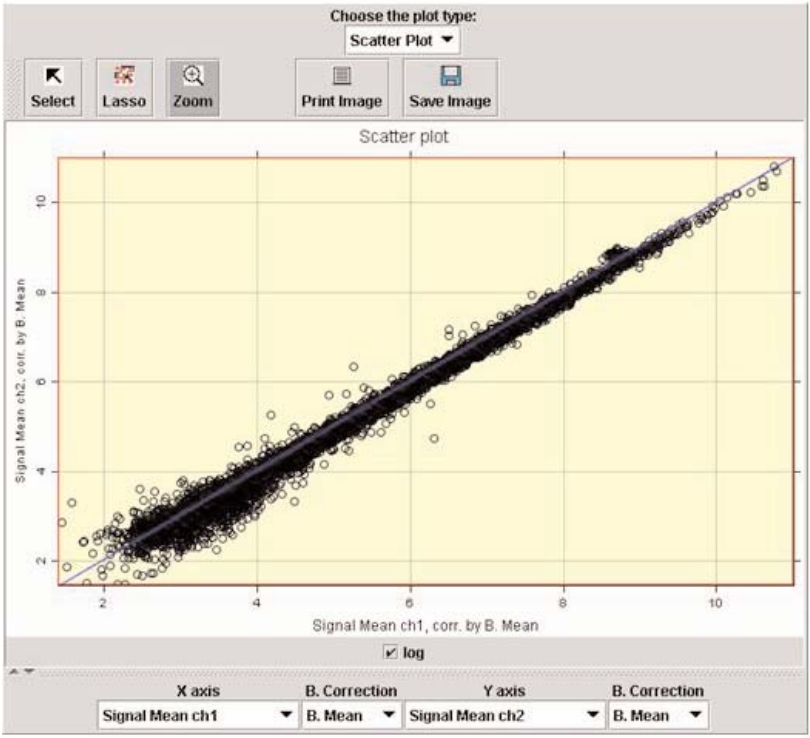
Histogram. As we mentioned previously, Histogram allows you to choose only one measurement.



The histogram demonstrates distribution of the measurement values over their domain. You can make an interval selection on the histogram and see how many genes fall into that selection. By changing the selection of "Tails" check box, you can select genes either inside the interval or outside. You can switch between untransformed measurement and log-transformed measurement using "log" check box. You can also change bin density, print the histogram and save its image (this feature is especially useful for publications). Histogram provides not only the ability to plot any of the measurements available in the quantification table, but also gives you access to such useful values as inter-channel signal ratios (including background corrected). Using these values in combination with "logarithmic" option you can obtain a histogram of a log-ratio (natural logarithm will be used). Selection of the tails of such distribution provides you a quick way to analyze up- and down-regulated genes.

Note: when a selection is made on the histogram, the same spots will be selected in measurements table and on the image, allowing you to analyze selected spots in full scale.

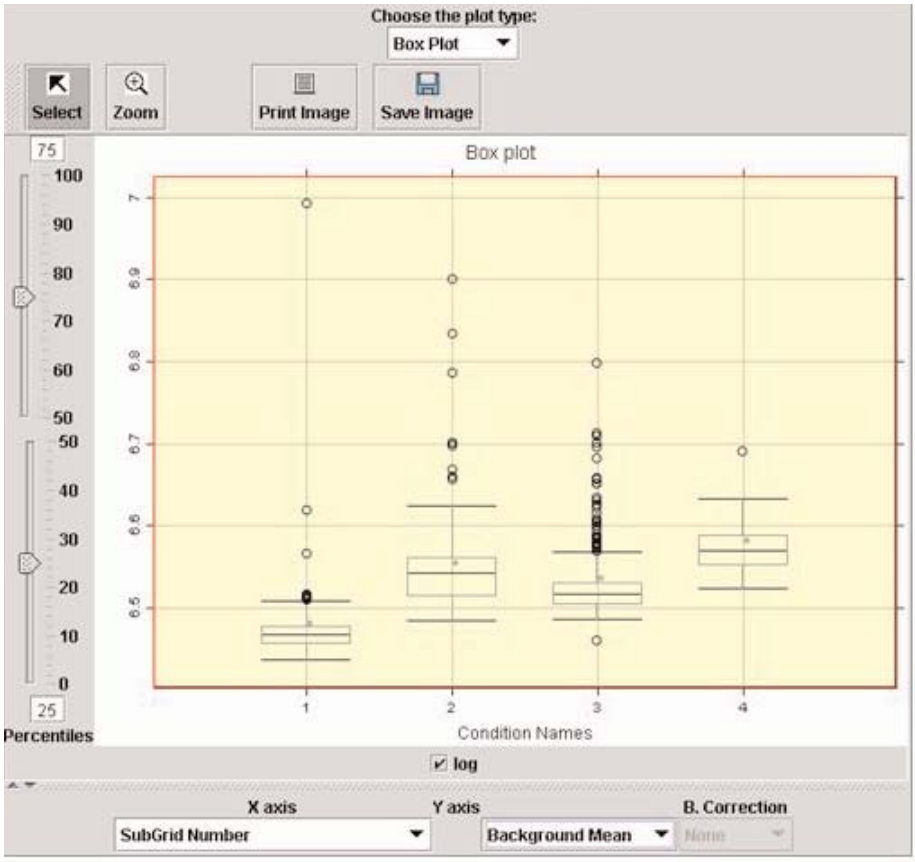
Scatter Plot. The scatter plot option offers visualization of one measurement plotted against any other.

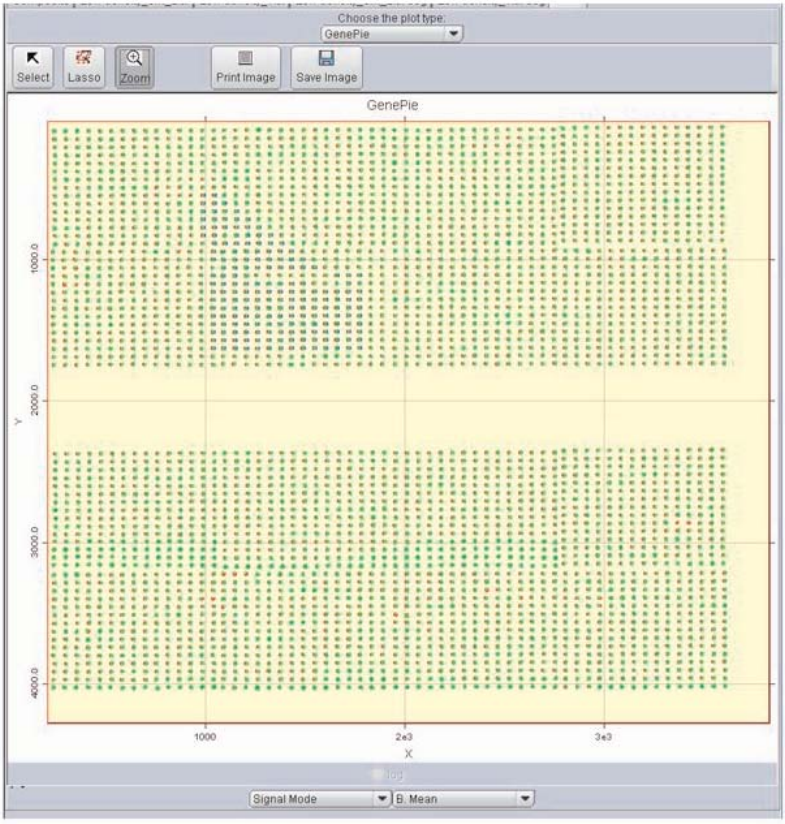


If multiple images are loaded, you can plot not only two measurements within one channel, but also two measurements belonging to different channels. For instance, a plot of signal means can show the difference between fluorescence characteristics in two channels or can even give a crude estimation of the regulation of the genes. Selecting points on the scatter plot will result in the selection of corresponding rows in the results table and highlighting of selected spots on the image.

Note: you can add to your current selection by simply continuing to select other groups of spots. To start a new selection, right-click on the plot first to clear the previous selection.

Box Plot. This option allows you to visualize the distribution of the measurement between different categories of spots (belonging to different subgrids, different rows, having different flags etc). You choose between different categories for the X axis and between different measurements for the Y axis. The box on such a graph visualizes displays the lower and upper percentiles of the distribution. These percentiles can be changed using the scroll bars on the left hand side. The box plot also allows visualization of the distribution of outliers, which can be useful when looking for abnormalities in the data due to special categorization.





GenePie. This plot displays the spot's expression values for each channel as portions of a circle. The colors within a pie correspond to thesignal intensity of the individual channel. The most common use of the GenePie chart is to plot differential expression patterns between channels. Different measurements may be used for GenePie plot. The values on X- and Y-axes represent the spot coordinates in pixels.

Note: any selection that you make within one of the plots will be common for all other plots, results table and image tab. The only exception is that if you select a group of spots using Scatter plot, Box plot or Results table, the selection will not be visible on Histogram plot. If you make the selection using the Histogram option, it can be viewed by all other tools.

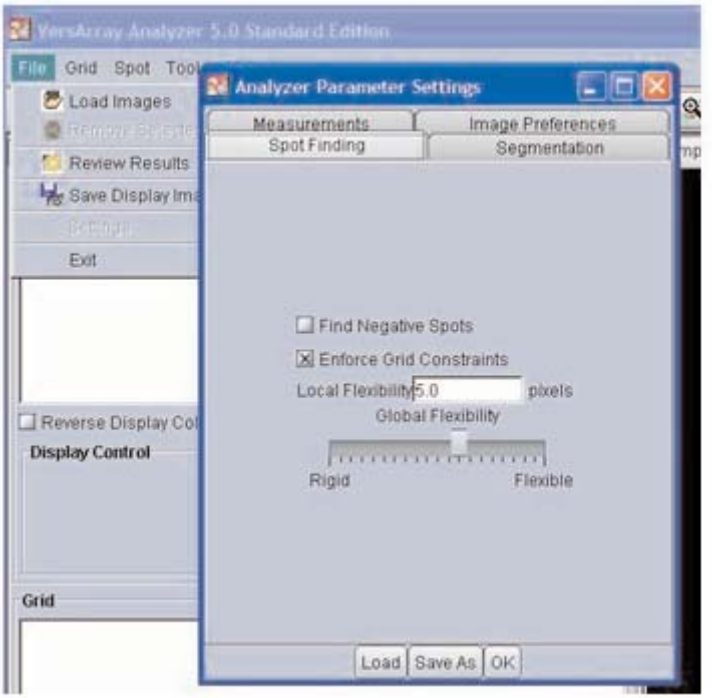
Note: you can move the cursor over any of the data elements on any plot and see a pop-up tip containing short information about the gene ID and measurement values.

1.4 VersArray Analyzer 5.0 Software Parameter Settings Window

VersArray Analyzer 5.0 Software encapsulates most of its parameters and settings within one common interface, the VersArray Analyzer 5.0 Software Parameter Settings Window. Within this window are tabs, which control virtually all aspects of the array image analysis. From initial spot finding settings, to complicated auto reporting of alert values, the VersArray Analyzer 5.0 Software Parameter Settings window provides complete user control. Selecting File, then Settings, may open the window.

Spot Finding

Spot finding involves the localization of the array signal as printed on the array medium. Due printing inconsistencies, spots and sometimes even entire subgrids require spot finding in order to properly determine the location of the signal value. Depending on the type and characteristics of the array, if spot finding is not performed, the resulting quantification may be questionable.



The following parameters affect how VersArray Analyzer 5.0 Software performs spot finding:

- **Find Negative Spot** – While not a problem for most arrays, negative spots can potentially cause problems for VersArray Analyzer 5.0 Software spot finding. If you suspect that the image may contain negative spots, we recommend leaving this option checked. When Find Negative Spots is enabled, VersArray Analyzer 5.0 Software will look for negative spots while also looking for regular spots.

  Note: If you suspect all the signal values within the image are negative due to specific scanner settings, then you may need to invert value for the images. Occasionally, the scanner software does not save information about which values are high and low within the tiff image. The result is that VersArray Analyzer 5.0 Software does not know whether white or black pixels are the high values. If all signal values are negative, which can be deduced through quantification or use of the intensity tool, then select Invert Values and re quantify. The Find Negative Spots option is not intended for use in these situations.

- **Enforce Grid Constraints** – With this option selected, VersArray Analyzer 5.0 Software will use Local and Grid Flexibility when performing spot finding. When not selected, VersArray Analyzer 5.0 Software will perform spot finding with no constraints. Please see below for additional information regarding Local and Grid Flexibility.

  Note: Under most circumstances, you will want to enforce grid constraints as this will limit the movement of circles during spot finding and help prevent erroneous spot finding due to dust and other contaminations.

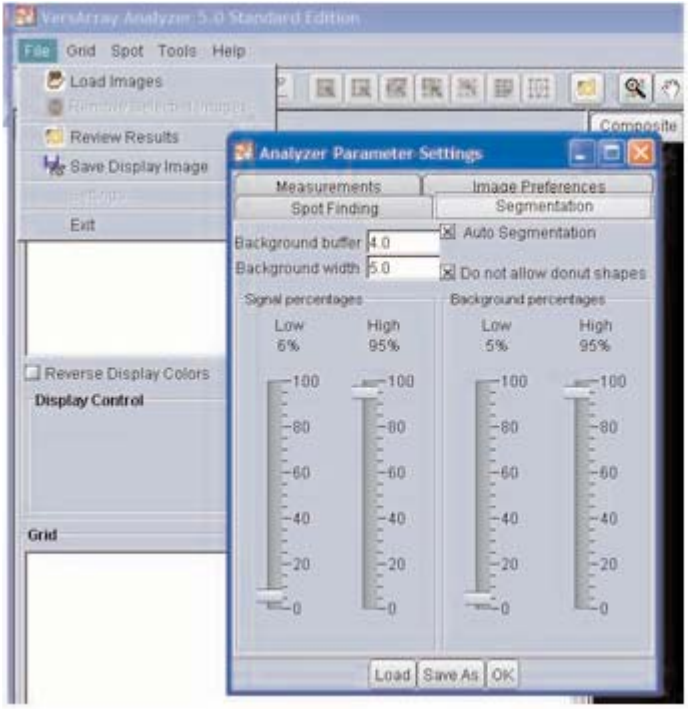
- **Local Flexibility** – Local Flexibility defines the radius, measured in pixels, that VersArray Analyzer 5.0 Software is allowed to search for spots. The origin for the search is the initial spot location as determined by grid placement. From here, VersArray Analyzer 5.0 Software will search for a spot with X pixels distance where X is defined in the local flexibility parameter.

- **Grid Flexibility** – Grid flexibility is an indication of the extent to which VersArray Analyzer 5.0 Software should deform the grid to match a given set of spots. The measurement is a qualitative notion based on a large part due to the unique properties of the image. Most users should set this to the middle values.

  Note: You can press the End key to move the slider all the way to the right or the Home key to move the slider all the way to the left.

Segmentation

Segmentation is the differentiation of signal and background values within the array image and constitutes one of the most important aspects of array image analysis. Proper segmentation yields more robust data free from contamination and other adverse affects.



VersArray Analyzer 5.0 Software supports two primary type of segmentation, manual and automatic. With Manual Segmentation, you must specify the appropriate values for VersArray Analyzer 5.0 Software to use when analyzing the image. Often these proper values can be arrived at through experimentation or experience. Alternatively, VersArray Analyzer 5.0 Software can calculate the appropriate segmentation parameters for you using a robust patent pending statistical approach. Under normal circumstances, we recommend use of the Automatic Segmentation due to its superior contamination removal. Regardless of the segmentation in use, both methods assist in providing the highest quality data available.

Listed under the Segmentation Tab are the following parameters:

Background Buffer – 

The distance, in pixels, between the signal and the background measurement regions. Within this region, all pixel values are ignored during quantification. Setting the proper buffer size helps to ensure accurate results. The desired size is dependent on several factors, including the spot size, density, image quality, and spot shape.

Background Width – 

The measurement, in pixels, to determine how far background measurements will extend from the buffer region. In other words, the measurement will extend X pixels from the end of the buffer. Remember that the background should include enough pixels to provide a sufficient sampling. VersArray Analyzer 5.0 Software should not be used with no background values as these values are required in numerous quality measurements.

Do not allow donut shapes – 

When you select this option, VersArray Analyzer 5.0 Software will make sure that a donut-shaped segmentation does not appear for any spot. A segmentation is considered to be donut-shaped if there are ignored pixels completely surrounded by signal pixels and their median intensity is lower then signal's. This option is available for auto segmentation only.

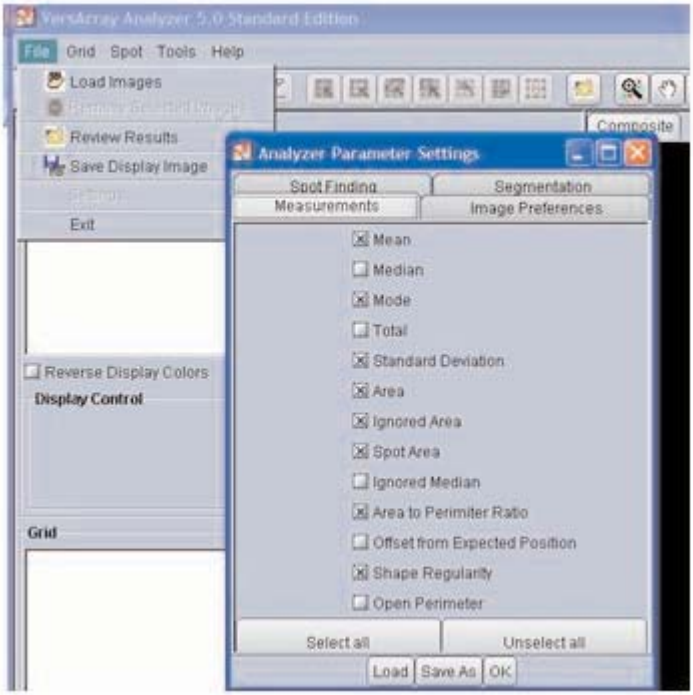
Note: The background width will not extend beyond the snip, or rectangular boundary around each spot. Even though you can set the background to an extremely high value, the background measurements will stop at this boundary and not include signal values from surrounding spots.

- **Signal Percentages** – These parameters are a percentage of all intensity levels within the signal region. The intensity ranges are raw values that do not include any statistical measurements (such as mean and median). The high percentage can to 100%, since the sample should contain the pixel with the highest intensity value. If you want to filter out possible noise sources, such as a speck of dust, set this percentage at a lower value, like 95%. This filters out the high intensity values associated with particle (assuming it will be fluorescing at a high intensity level).
- **Background Percentages** – These parameters are a percentage of all intensity levels within the background region. Set them the same way as described for the signal percentages. Press the Home key to set a slider to 0%. Press the End key to set a slider to 100%
- **AutoSegmentation** – Selecting this option will turn on a patented automatic segmentation method. Under most circumstances, this should be selected. Under AutoSegmentation, the only parameter, which VersArray Analyzer 5.0 Software will use, is the Background Buffer. The typical value should be slightly less than ½ the radius. Under AutoSegmentation, the slider bars for the signal and background percentages become disabled.

Note: An important difference between automatic and manual segmentation is the fact that under manual, the parameters and their corresponding values are applied uniformly across all spots of the image. Under automatic segmentation, each spot is calculated independently usually generating more accurate segmentation.

Measurements

The Measurements panel allows you to select the contents of the output data file.



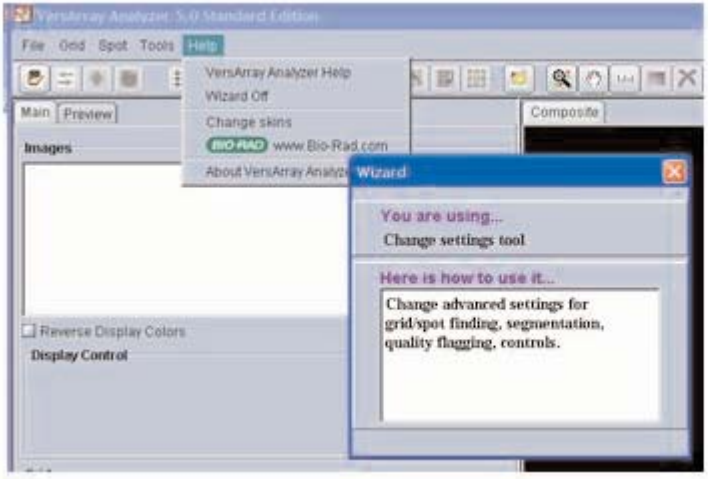
- **Mean** – signal and background mean intensity
- **Median** – signal and background median intensity
- **Mode** – signal and background mode intensity (mode corresponds to the pick of corresponding histogram)
- **Total** – signal and background total intensity (all pixel intensity summed up)
- **Standard deviation** – standard deviation of signal and background intensity distribution
- **Area** – number of pixels segmented as signal and background
- **Ignored area** – number of pixels in the segmented areas ignored and directly touching the signal area

- **Spot area** – ignored area plus signal area
- **Ignored median** – median of pixel intensity in ignored area

You can check and uncheck these measurements at any time and the results table will be updated immediately.

1.5 VersArray Analyzer 5.0 Software Wizard

VersArray Analyzer 5.0 Software wizard is designed to help you understand the VersArray Analyzer 5.0 Software tools better and to assist in navigating through the necessary steps. The Wizard can be switched on and off at any moment through Help menu. Even when the Wizard window is switched on, it does not limit your access to any of the VersArray Analyzer 5.0 Software tools.



To launch the Wizard, select the "Wizard On" option from the Help menu. The Wizard window will be shown in the bottom right part of your screen.

The VersArray Analyzer 5.0 Software Wizard works in two modes. The first mode switches on whenever Wizard detects that you have performed an action that should logically be followed by another action. The Wizard will display the action that was performed and indicate the next step. To follow this advice you can either use a the tool the Wizard refers to or click on the "Follow Advice" button in the bottom part of the Wizard window. The latter will result in the recommended action being performed automatically. If the recommended action is not necessary, you will have an option to go to next suggested action. The sequence of the recommended actions will start immediately after the VersArray Analyzer 5.0 Software launches with the advice to load the image. Thus, virtually anyone can learn to use VersArray Analyzer 5.0 Software by following the Wizard prompts.

If you try to apply a tool that does not fall into a pre-defined sequence of actions, the Wizard will switch to its second mode. In this mode the Wizard will prompt the user as to what tool is being used and how to use it.

Thus, the VersArray Analyzer VersArray Analyzer 5.0 wizard provides you with an opportunity to learn how to use the software in an interactive fashion.

1.6 Reviewing Results

One of the most powerful features of VersArray Analyzer 5.0 Software is the ability to review results of previously processed data. VersArray Analyzer 5.0 Software is designed to load and display data exactly as it was processed days or even months earlier. The capability to review the results provides the following benefits:

- Compare old data with recently processed data
- Review the results of batch processing
- Establish post image analysis quality controls and screening
- Reload and take screen captures for publication

1.6.1 The Snapshot File (sst file)

The snapshot file is a result of quantification within VersArray Analyzer 5.0 Software. Upon saving the quantified data to the file system, VersArray Analyzer 5.0 Software also saves another file, which contains a snapshot of that data as it existed after quantification. It is this snapshot file, ending with an .sst file extension, which VersArray Analyzer 5.0 Software loads allowing review of results.

The snapshot file itself is simply a proprietary binary file format which is not useful outside of VersArray Analyzer 5.0 Software. Due to the large amount of information contained within the file, its size can grow quite large, often surpassing the image size itself. The snapshot file contains the following components:

- Grid
- Gene ID
- Segmentation
- Quantified Data
- Quality Measures

While the sst file contains almost all the required information to reload and review data, it does not contain the actual images. To review results, VersArray Analyzer 5.0 Software must have access to the original images. By default, VersArray Analyzer VersArray Analyzer 5.0 Software will attempt to load the images from the location specified within the sst file. However, if the images have been moved and VersArray Analyzer 5.0 Software is not able to load them, VersArray Analyzer 5.0 Software will ask you to browse and select the appropriate images.

1.6.2 Loading Results to Review

Loading data for subsequent review can be accomplished by performing the following steps:

1. From the VersArray Analyzer 5.0 Software menu bar, select File followed by Review Results. Likewise, you may click on the Review Results icon located along the toolbar.
2. With the Open dialog, browse and select the snapshot file you wish to open.
3. Depending on the file, VersArray Analyzer 5.0 Software may prompt you to specify the location of the original images as well.
4. The information and data should now be visible within the main VersArray Analyzer 5.0 Software user interface. If you wish to reprocess the images, click the Cancel button along the bottom of the Results tab and use the Quantify button of the main panel.

1.7 VersArray Analyzer 5.0 Software Tools

VersArray Analyzer 5.0 Software includes many handy tools designed to help facilitate array analysis. These tools are designed for manipulating, quantifying and analyzing array images.

1.7.1 Auto Alignment Tool

The Auto Alignment tool  automatically overlays several images on top of one another. If two or more images are loaded, activating this tool will attempt to overlay the spots from each image. While this tool is designed largely for transposing of images, or left, right, and up, down, movement, VersArray Analyzer 5.0 Software can rotate images up to 10°.

The Auto Alignment tool is intended to provide a quick and highly accurate means to solve the problem of analyzing arrays which have been scanned slightly out of alignment. If the images have a high degree of rotation or other extreme problems, then the manual manipulation tools of transposing and rotation may be used.

Note: The Auto Alignment tool is intended to account for shifts of the array in scanning and not for warping of the medium being printed upon.

1.7.2 Auto Grid Placement Tool

VersArray Analyzer 5.0 Software's Auto Grid Placement Tool  automatically repositions a grid structure to the proper location within the image. This tool is used with grid and template files. If the geometry of an array stays the same between experiments, the use of a common template for all experiments is beneficial. However, due to differences in arraying and scanning, the location of the array within the image may vary across experiments. The Auto Grid placement tool solves this problem, because once the image and the grid have been loaded, the tool will automatically move the grid to the proper location.

Note: it is important that at least 60% of the spots in each row and each column of every sub-grid are visually resolvable in order for the grid placement algorithm to find the correct grid location. In case the image has some spots that are a priori blank or empty, assign the gene IDs "BLANK" or "EMPTY". This way VersArray Analyzer 5.0 Software will take the absence of signal in those spots into account and produce reliable results.

1.7.3 Save Display Image Tool

VersArray Analyzer 5.0 Software's save Display Image Tool  provides the capability to save a screen capture of the overlaid, i.e. composite, images. The saved image includes only the overlay as it appears within the main image panel. VersArray Analyzer 5.0 Software saves the image as a high quality 24 bit tiff format. The image can later be recalled for reference of for use in publications.

1.7.3.1 Saving the Display Image

Perform the following step to save the current composite overlay to a tiff image file:

From the menu bar select File then Save Display Image. Alternatively, click the Save Display Image icon from the toolbar.

From the Save As Dialog that appears, browse to the location you wish to save the image.

Specify a file name. VersArray Analyzer 5.0 Software will automatically add the .tif file extension at the end of the name.

The image is now saved and is available to be viewed in virtually any graphics program such as Microsoft Imaging or Adobe Photoshop.

1.7.4 Zoom Tool

The Zoom Tool  allows you to zoom a specific region of the image. VersArray Analyzer 5.0 Software allows several methods tozoom, including:

After selecting the Zoom Tool, you can left click with the mouse and drag a rectangle around the section of interest in the main image panel.

left click with the mouse and drag a rectangle around the section of interest in the Image Map View.

Hold the <alt> key on the key board, left click with the mouse and drag a rectangle around the section of interest in either of the areas mentioned previously.

Drag the Zoom slider bar located directly below the Main Image Panel.

Note: To return to the original image size, double right click with the mouse.

1.7.5 Scroll Tool

The Scroll Tool  provides a convenient way to move about the image. To pan the image, simply click and drag the image around as you would any other object. The image will automatically scroll left and right as needed.

If at any time you wish to know where the current view is situated in the image, simply look for the location of the yellow box within the Map View. The yellow box indicates the region currently being viewed within the Main Image Panel.

1.7.6 Ruler Tool

The Ruler Tool  allows objects to be measured within the Main Image Panel. The most common use of this tool is to determine the minimum and the maximum diameter for spot sizes and distance between the spots. The tool can also be used to determine the general resolution of the image and how many pixels are included within each spot.

The Ruler Tool displays the results within the Status Bar located under the Main image Panel. The information provided includes:

Dx: the distance moved on the x-axis as measured in pixels

Dy: the distance moved on the y-axis as measured in pixels distance: the absolute distance moved as measured in pixels

1.7.7 Intensity Tool

The Intensity Tool  displays the raw pixel intensities in the Status Bar located under the Main Image Panel. After selecting the tool, simply position the cursor over the pixel of interest and the pixel intensity for each image is displayed.

Note: The values provided here are raw intensities representing single pixel values rather than mean or median values.

1.7.8 Flagging Tool

The Flagging Tool  provides a simple means of manually flagging spots within VersArray Analyzer 5.0 Software. While spots may be flagged or marked for a variety of reasons, the most common reason is due to a defect within the spot or array.

Once a spot has been flagged, a numerical value indicating the type of flag is then displayed with the quantified data. The flag value can then be used in later data analysis software, such as Bio-Rad's GeneGazer Software, or can be used in post analysis quality control measures. In addition to the numerical value, a shape is also added to make the flag visually unique. Each shape corresponds to a different flag as is explained below.

VersArray Analyzer 5.0 Software supports a variety of manual flagging types. To change the type of flag, right click with the mouse and select the desired manual flag from the context sensitive menu. The following is a list of possible flags and the corresponding values:

Flagging codes:

Manual flags (color: red)

1 – Flag spots

2 – Empty spots (cross)

3 – Poor spots (plus)

4 – Negative spots (dash)

1.7.9 Undo and Redo Tools

VersArray Analyzer 5.0 Software supports an unlimited number of undo and redo operations. Anytime you wish cancel an action, this tool provides means to do so.

Note: The action to be undone or redone is displayed next to the text in the menu bar. For example, if you move the grid and then wish to undo this action, the text in the Tools Menu will read "Undo Move Grid". This is a convenient means to see what action is reversed.

1.7.10 Grid Tools

Adjust Metagrid

This tool allows manipulation of entire metagrids. The metagrid can be moved, stretched or resized.

Adjust Subgrid

This tool allows manipulation of entire subgrids. The subgrids can be moved, stretched or resized.

Adjust Spot

This tool allows individual spots to be moved and resized.

Lasso Adjust

This tool allows you to select irregularly shaped regions of circles for adjustment. Instead of shifting entire subgrids, you can now specify unusually shaped regions of circles for fine-tuning.

Rectangle Adjust

This tool, which is similar to the lasso adjust tool, allows you to make a sub-selection of circles to adjust from within the array. This tool employs a rectangular shape to highlight given circles.

Duplicate MetaGrid

Duplicating an existing metagrid can save time and produce better results than manually recreating the metagrid several times. To use it click on the button and select a metagrid to duplicate by left-clicking on it. After that you can multiply this metagrid in any place on the image by left-clicking. To stop duplicating, right click on the mouse.

Show/Hide Grid

VersArray Analyzer 5.0 Software allows the grid to be hidden to allow a better view of the bare spots. Hiding the grid hides any lines, circles and flagging that may exist within the Main Image Panel.

Auto Adjust Spots

Select this option to have VersArray Analyzer 5.0 Software perform automatic spot localization. VersArray Analyzer 5.0 Software searches the area around the grid placement for a spot within the predefined minimum and maximum diameters. Adjustment accuracy also depends on grid constraints and the quality of your images. For example, if you have spots with irregular signal values and you set grid flexibility with a high number of pixels, VersArray Analyzer 5.0 Software will tend to find pixels with high expression values. For full automation use this tool in conjunction with auto grid placement button.

Note: If you use this button several times, spot searching will start from the position where previous attempt left it. Be careful: multiple auto adjustment can dramatically deform grids on low-quality images.

Wrangle

The wrangle feature of VersArray Analyzer 5.0 Software applies new, stricter constraints to the results of spot localization without requiring further spot finding. Essentially, this allows you to reduce the spot search radius without repeating the spot finding. The benefit of this feature is to assist processing for those with either slower computer hardware or for those with numerous spots.

A sample application would be to perform spot finding for grid geometry on an array image with a Local Flexibility set to a large number of pixels. After spot finding, if the resulting circle placement has high variability, the Local Flexibility can be reduced. After reducing Local Flexibility, click the Wrangle Button to apply the new setting without waiting for spot finding to be performed again.

Part 2: VersArray Analyzer 5.0 Software Tutorials

2.1 Basic Analysis

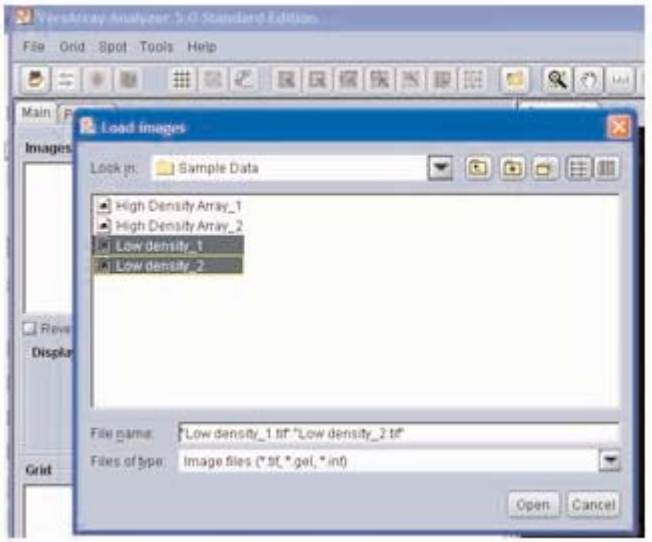
This tutorial demonstrates performing basic quantification through VersArray Analyzer 5.0 Software. The tutorial covers the essential steps from loading the initial array images until a report is generated. The following lessons will be presented within this tutorial.

- **Image Loading and Manipulation** – Describes how to load an image into VersArray Analyzer 5.0 Software and use the controls on the Image tab to enhance the on-screen display of the image.
- **Grid Placement and Manipulation** – Describes how to create a grid and adjust it to fit the image.
- **Quantifying and Saving the Data** – Describes the quantification and subsequent output of data.

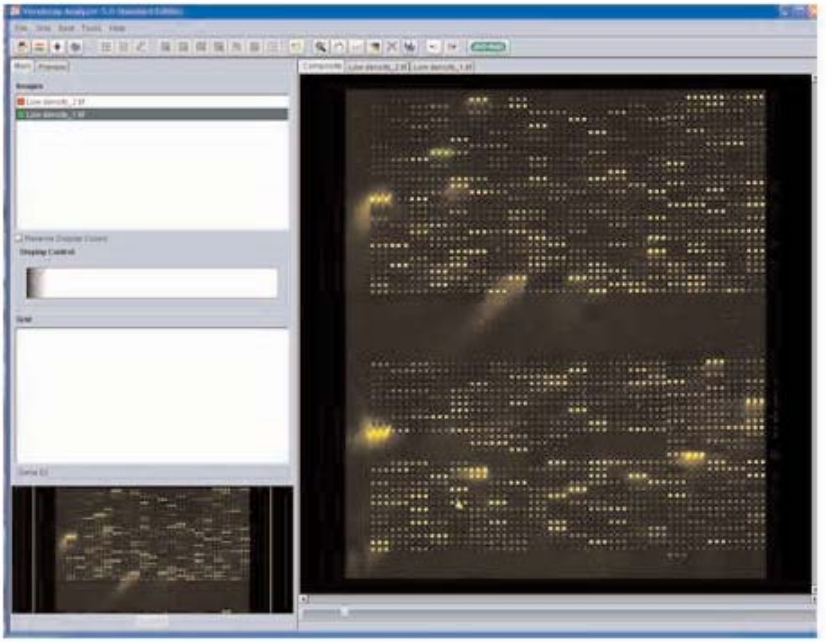
Image Loading and Manipulation

- Launch VersArray Analyzer 5.0 Software from the desktop icon or from the VersArray Analyzer 5.0 Software folder within the Windows Start Menu.
- From the menu bar, select File , then Load Images.

- Browse to the Samples folder within the VersArray Analyzer 5.0 Software folder. The location is typically, C:\VersArray Analyzer 5.0\Samples.
- Select lowdensity_1 or lowdensity_2. Multiple selections can be performed by holding the <ctl> key on the keyboard and left clicking.

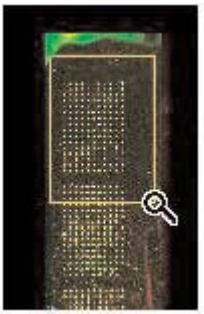


5. Click the Open Button to complete the image loading. A progress bar displays time remaining. The first file loaded will appear as the Cy5 image; the second, as the Cy3 image.
6. Once the images are loaded and are visible within the Main image display located along the right of the primary VersArray Analyzer 5.0 Software window, the images need to be overlaid. Not all images will require this action, but due to variances in scanning, some will. To overlay the Images Automatically, click the Align Images button from the toolbar. In additional to auto alignment, VersArray Analyzer 5.0 Software offers manual tools for rotation and translation of images.

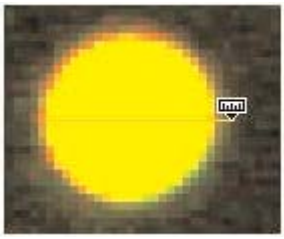


Grid Placement and Manipulation

1. From the menu bar, select Tools followed by Zoom.
2. Drag a rectangle around the top subgrid of the array to zoom into this region of the image.

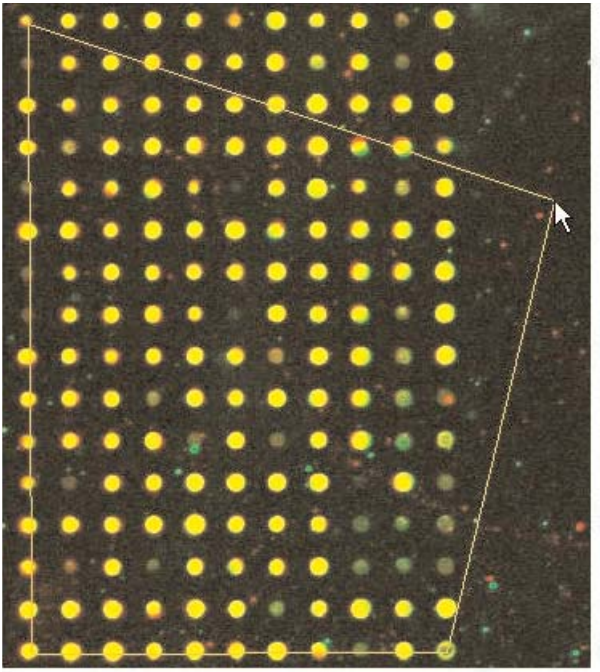


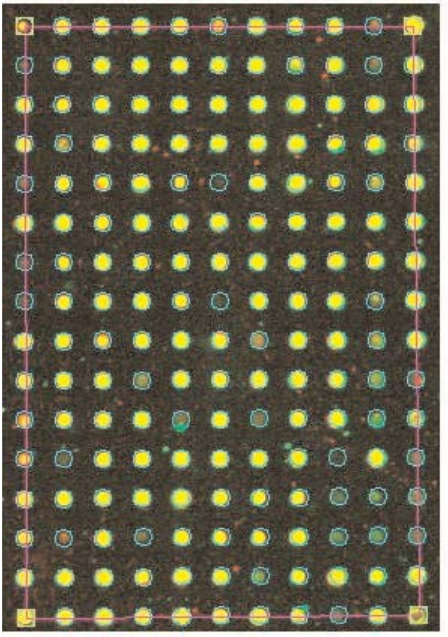
3. Select Tools then Ruler from the menu bar to measure the approximate sizes of the spots within this array. This information will be used slightly later.
4. With the ruler tool, click and drag from one side of a spot to the other side of the spot. The distance is displayed in a status bar located beneath the main image panel. This distance is in pixels.



5. Next count the number of rows and columns of spots within this subgrid.
6. Right click on the Grid Panel and select Create Grid from the menu that appears.

7. Enter the information obtained above into the Create Grid Window that appears. The values should be:
   a. Field Name – “Main”
   b. Rows – “30”
   c. Columns – “60”
   d. Min Diameter – “18”
   e. Max Diameter – “21”
   f. Rectangular Grid is selected
8. Click the Place Grid Button
9. Use the mouse cursor and click on each of the four corner spots of the subgrid in succession. Upon placement of the fourth spot, the grid becomes visible on the subgrid.





10. Click on the name Main in the Grid Panel.

11. With "Main" selected, right click and select Create Metagrid.
12. In the window that appears, enter the following information
    a. Metarows – "1"
    b. Metacolumn – "2"

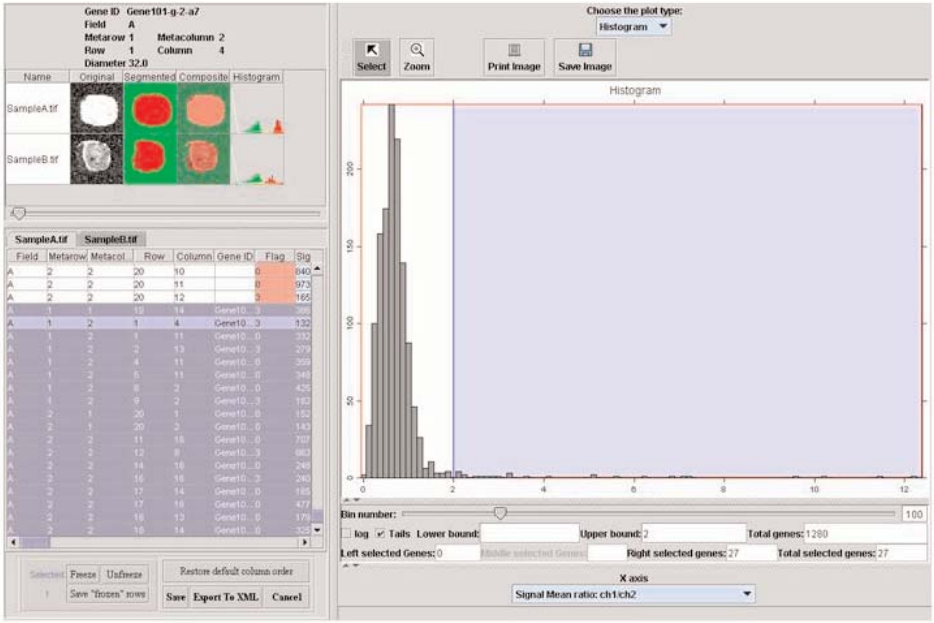
    (This indicates that your array has 2 total grids, and both are found in 1 row)

13. Click Place MetaGrid
14. In the top subgrid where we just placed the grid, click on the top, left most spot.
15. Using the scroll bar located along the right of the main image panel, scroll downward until the last subgrid is reached
16. Click the top, left most spot within this subgrid as well. VersArray Analyzer 5.0 Software automatically places the remaining subgrids in the proper locations.
17. Click the Adjust Subgrid button on the toolbar.
18. Click on and drag this subgrid slightly to provide a better fit.
19. Alternatively, click the Automatically Place Grid button to automatically place the subgrids.
20. Click the Adjust MetaGrid Button from the toolbar and click on any subgrid. 
21. Click the Auto Adjust Spots Button from the Toolbar to perform automatic spot finding. 

Quantifying, Pre-Analyzing and Saving the Data

1. Click the Quantify Button located along the Bottom of the Main Tab.

2. After a few moments the quantified data is visible under the Results Tab.
3. Select Plots tab on the Image Display panel.
4. Select Histogram on the Plots panel.
5. Select "Signal Mean Ratio ch1/ch2" from the list of measurements.
6. Make sure that "log" option is not selected for the Histogram.
7. Select 2-fold regulated genes by specifying 2 in the "Upper bound" text box.
8. "Freeze" the selection using the corresponding button in the bottom of the table.
9. Sort the data by column "Selected" clicking on its header. All selected genes will be in the bottom of the table.
10. Go through the spots one by one analyzing spot images.



11. Click the Save Button to save the Quantified data and snapshot file to an output directory of your choice.

2.2 Premanufactured Array Analysis

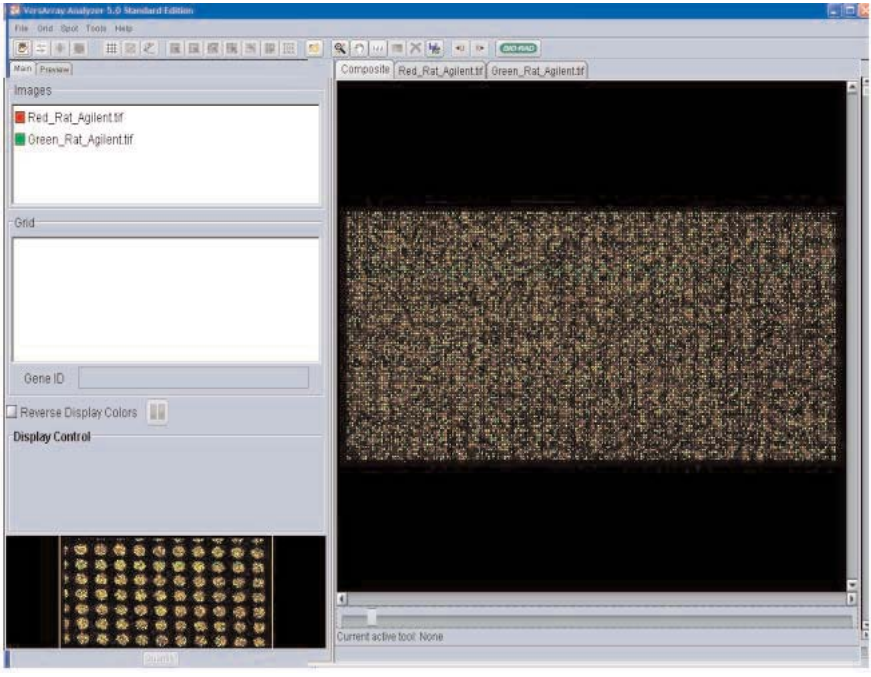
VersArray Analyzer 5.0 Software provides the ability to load templates for many commercially available arrays. A template within VersArray Analyzer 5.0 Software is the combination of a grid and the genelist, or Gene IDs, required to easily quantify and name the various probes on the array. A selection of these templates are provided to customers as a service to aid their array analysis. For a complete listing of templates currently available, contact Bio-Rad support. The following Lessons will be covered:

- **Image Selection** – Describes how to load an image into VersArray Analyzer 5.0 Software and use the controls on the Image tab to enhance the on-screen display of the image.
- **Template Selection** – Demonstrates the loading and placement of premade templates.
- **Quantification** – Describes the quantification and subsequent output of data.

Note: This procedure is also used for GAL, GEML, or MAGE files.

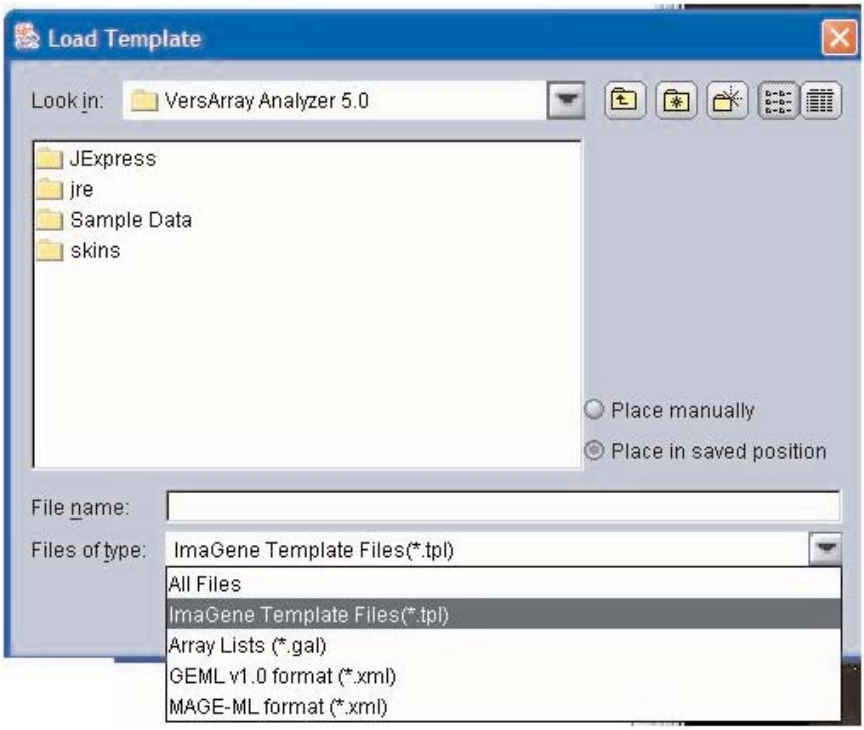
Image Selection

1. Launch VersArray Analyzer 5.0 Software from the desktop icon of from the VersArray Analyzer 5.0 Software folder within the Windows Start Menu.
2. From the menu bar, select File, then Load Images.
3. Browse to the Samples folder within the VersArray Analyzer 5.0 Software folder. The location is typically, C:\VersArray Analyzer5.0 Software\Samples.
4. Select Rat_Agilent.tif from the Agilent folder.
5. Slide the top triangle of the Display Control Panel to the left of the panel to see the image clearly.



Template Selection

1. From the menu bar, select Grid, then Load Template.

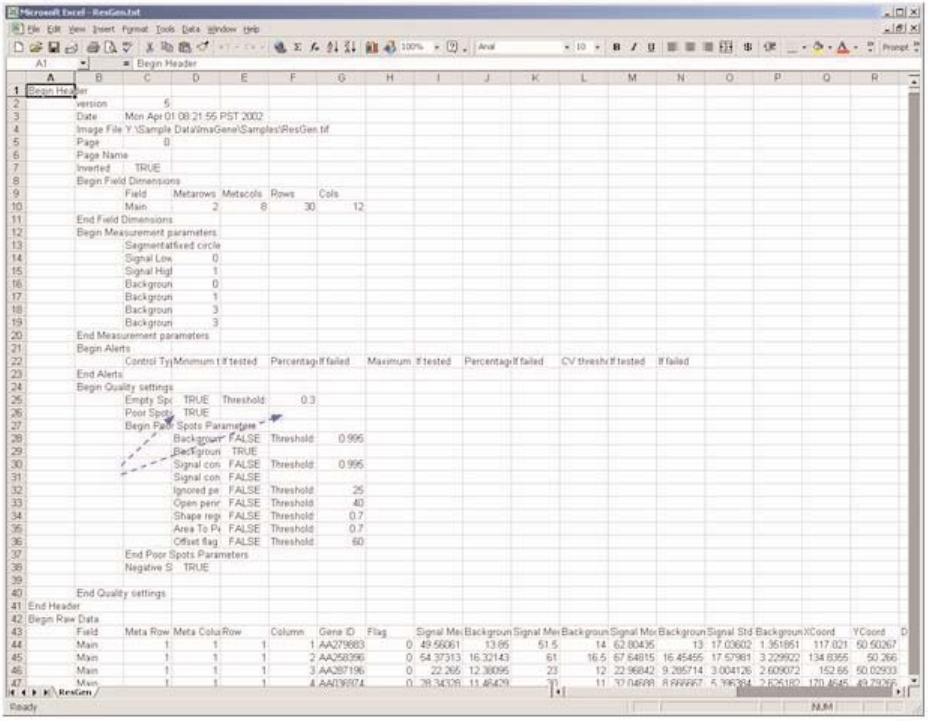


2. Browse to the Agilent folder, the Rat folder and select the template file.
3. Select Place Manually
4. Click the Open Button
5. Click the four corners of the entire array. The grid will be placed exactly on the array taking into account differences in scale and rotation.

Quantification

1. Click the Quantify Button under the Main Tab.
2. Once the quantification is complete, click the Save Button located under the Results Tab.
3. Specify a file name and location to save the data file.

4. Using Windows Explorer or My Computer, navigate to the data file within the file system.
5. Open the file using either MS Excel or Microsoft Wordpad. Notice the GeneID column contains gene names as defined by Agilent.



6. The use of templates through VersArray Analyzer 5.0 Software provides a convenient and simple way to perform grid placement and associate gene information with clone locations.

Part 3: Licensing

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Part 4: Appendices

4.1 File Specifications

While VersArray Analyzer 5.0 Software is designed to be flexible with regards to information imported and exported, you must adhere to certain standards regarding file formats.

4.1.1 Gene ID File

The Gene ID file allows you to track information about the genetic material spotted at each location within the array. This information will be saved along with the quantified values in the text output file and visualization tools.

For example, you will be able to click on a GenePie from within GeneSight-Lite and see the specific Gene ID associated with the selected spot in addition to all the other detailed quantification information. To accomplish this, VersArray Analyzer 5.0 Software needs the Gene ID file to associate a reference ID to each spot in the array of spots in the image.

VersArray Analyzer 5.0 Software supports two formats for the Gene ID file. The first format will be familiar to existing VersArray Analyzer 5.0 Software users since the basic structure is the same between versions. The second format is based upon the new multi-level grid structure available in VersArray Analyzer. This new format takes advantage of the use of fields, or unique grid/meta grid structures. To deal with the unlimited number of possible fields, and grid structures, an additional column needs to be added to the Gene ID file.

Note: Choose the Gene ID format based on your array structure and preferences. However, when creating the Gene ID file, do not combine the two formats in the same file, as VersArray Analyzer 5.0 Software will not be able to process the hybrid file format.

Gene ID Format #1 (Traditional Format)

Listed below is a brief description of the various parts of the Gene ID file as well as the precise syntax.

- **Header** – Anything other than the Gene ID information located at the beginning of the file. If you want to include header information, you must start each line with a percentage sign, "%." The percentage sign is an indication to VersArray Analyzer 5.0 Software not to read this line as gene information but rather continue until a line is encountered matching the structure found below.

- **Columns** – Metarow Number (MR) / Metacolumn Number (MC) / Subgrid Row Number (SR)/ Subgrid Column Number (SC) / Gene ID. Separator: You must use tabs to separate the fields in each row.
- **Order** – VersArray Analyzer 5.0 Software does not care about the order in which the rows are sorted. It's also not necessary to specify Gene ID information for all the spots in the array.
- **Gene ID** – The Gene ID field can be any alpha-numeric string (including blank spaces) following the Subgrid Column Number. You can specify a database entry followed by the gene name as one option.
- **Comment Lines** – Use the percentage sign, "%," to indicate comment lines.
- **Creating the Gene ID File** – You can use any text processing program, spreadsheet, or database program to generate the Gene ID file. You can also use the CloneTracker software tool to generate this file. CloneTracker manages the information about cDNA plates and the mapping of plates onto the slide. It can also generate the necessary gene ID file to be used by VersArray Analyzer.

  Insert the accession number followed by a colon and then insert the Gene ID name. For example, all of the following are valid formats for the Gene ID File:

  Header information

  MR MC SR SC Gene ID

  MR MC SR SC Ascension #

  MR MC SR SC Ascension #: Gene ID

  Note: The actual accession numbers are not provided nor determined by VersArray Analyzer 5.0 Software or Bio-Rad. The information must either be located by you or provided by the microarray chip manufacturer.

Gene ID Format #2 (VersArray Analyzer 5.0 Software Format)

The second format follows the same rules as the first except for the addition of a field column:

- **Columns** – Field/Metarow Number (MR) / Metacolumn Number (MC) / Subgrid Row Number (SR)/ Subgrid Column Number (SC) / Gene ID.

Header Information
Field MR MC SR SC Gene ID
Field MR MC SR SC Ascension #
Field MR MC SR SC Ascension #: Gene ID

4.1.2 Output File

VersArray Analyzer 5.0 Software exports standard tab delimited text files when saving data. The information below is intended to describe the general format of the text file and explain particular fields therein. VersArray Analyzer 5.0 Software saves the quantified

data from each image processed into individual text files. Due to the multi-image processing capability, VersArray Analyzer 5.0 Software no longer exports two channel ratio data. Researchers are required to either generate any desired ratio information themselves or import the raw data into a data analysis program such as the GeneSight software tool. The data file is divided into two main parts. The first part is header information about the parameters set for analysis. The second part of the file includes the data extracted from the images.

Note: Due to the nature of tab delimited text files, wrapping of columns is common. To easily view information, open the data files into a spreadsheet program such as Microsoft Excel.

```
Begin Header
        version 5.0
        Date    Tue Mar 26 09:19:34 PST 2002
        Image File      C:\BioDiscovery\VersArray Analyzer\Samples\1205_g.TIF
        Page    0
        Page Name
        Inverted false
        Begin Field Dimensions
                Field   Metarows        Metacols        Rows    Cols
                A       1       1       8       12
        End Field Dimensions
        Begin Measurement parameters
                Segmentation Method     auto
                Signal Low      0.0
                Signal High     0.0
                Background Low          0.0
                Background High         0.0
                Background Buffer       3.0
                Background Width        3.0
        End Measurement parameters
        Begin Alerts
                Control Type    Minimum threshold       If tested Percentage allowed
If failed Maximum threshold     If tested Percentage allowed    If failed CV threshold
If tested If failed
                hot     45000.0 false   0.0%    false   65000.0 false   0.0%    false
1.0     false   false
        End Alerts
        Begin Quality settings
                Empty Spots     true    Threshold:      0.3
                Poor Spots      true
                Begin Poor Spots Parameters
                        Background contamination flag   true    Threshold:      0.9995
                        Background tested against subgrid data only     true
```

Signal contamination flag false Threshold: 0.9995
Signal contamination test connected to background contamination threshold false
Ignored percentage flag true Threshold: 25.0
Open perimeter flag true Threshold: 25.0
Shape regularity flag true Threshold: 0.65
Area To Perimeter Ratio flag false Threshold: 0.65
Offset flag true Threshold: 60.0
End Poor Spots Parameters
Negative Spots true
End Quality settings
End Header
Begin Raw Data
Field Meta Row Meta Column Row Column Gene ID Flag Signal Mean
Background Mean Signal Median Background Median Signal Mode
Background Mode Signal Area Background Area Signal Total Background
Total Signal Stdev Background Stdev Shape Regularity Ignored Area Spot
Area Ignored Median Area To Perimeter Open Perimeter XCoord YCoord
Diameter Position offset Offset X Offset Y Expected X Expected Y CM-X
CM-Y CM Offset CM Offset-X CM Offset-Y Min Diam Max Diam
Control Failed Control Background contamination present Signal contamination present
Ignored % failed Open perimeter failed Shape regularity failed Perim-to-area failed
Offset failed Empty spot Negative spot
A 1 1 1 1 0 109.89393939393939
4.926565874730022 129.5 5.0 132.9090909090909 4.738562091503268
66.0 463.0 7253.0 2281.0 55.13623039946595 2.9050872336804874
0.584070796460177 0.0 66.0 NaN 1.0 0.0 33.427956043956044
21.692307692307704 9.0 0.3435990182607777 0.3298958928437372 -
0.09606760762402544 33.09806015111231 21.78837529993173
33.984848484848484 21.151515151515152 1.0917803796972039
0.8867883337361775 -0.6368601484165772 8.319720247072127
10.339058029748454 0 0 0 0 0 1 0
0 0 0
A 1 1 1 2 0 163.88571428571427
4.821350762527233 228.5 5.0 248.61111111111111 4.571428571428571
70.0 459.0 11472.0 2213.0 102.5569587883649 1.2165800511090508
0.6481481481481481 0.0 70.0 NaN 0.9773843811168245 0.0
55.77061338661339 21.72727272727274 9.0 0.2811541783968894
0.269943687738035 -0.07860074732736422 55.500669698875356
21.805873474600105 56.0 21.514285714285716 0.5782336652136307
0.49933030112464394 -0.29158776031438904 8.236983309361506
11.051234910005755 0 0 0 0 0 1 0
0 0 0
A 1 1 1 3 0 105.26470588235294 4.836776859504132
138.0 5.0 173.3 4.7482993197278915 68.0 484.0 7158.0 2341.0
68.92421619325587 1.4288247004403603

4.1.3 ConFIGuration File

The conFIGuration file stores information about virtually all the parameters within VersArray Analyzer. This file is a simple XML file format which is easily saved and loaded from the VersArray Analyzer 5.0 Software Parameter Settings Window. While the contents of this file are not important to most users, you should be aware that this file is required for batch processing. Advanced users integrating VersArray Analyzer 5.0 Software into high throughput environments may also find the information useful.

Listed below is a sample VersArray Analyzer 5.0 Software conFIGuration file:

<?xml version=”1.0” encoding=”UTF-8”?>

<VersArray Analyzer_Parameters>
<IGNORED_MEDIAN>true</IGNORED_MEDIAN>
<PERIMETERTOAREA_FLAG>false</PERIMETERTOAREA_FLAG>
<BACKGROUND_FLAG>true</BACKGROUND_FLAG>
<POOR_FLAG>true</POOR_FLAG>
<PERIMETER_FLAG>true</PERIMETER_FLAG>
<PERIMETERTOAREA_THRESH>0.65</PERIMETERTOAREA_THRESH>
<TOLERANCE>5.0</TOLERANCE>
<STANDARD_LOW>5000.0</STANDARD_LOW>
<OFFSET_FLAG>true</OFFSET_FLAG>
<SHAPE_REGULARITY>true</SHAPE_REGULARITY>
<CONTROL_TYPE_LOWTHRESH_0>45000.0</CONTROL_TYPE_LOWTHRESH_0>
<NEGATIVE_FLAG>true</NEGATIVE_FLAG>
<BLANK_CV>5.0</BLANK_CV>
<IGNORED_AREA>true</IGNORED_AREA>
<POOR_THRESH>0.995</POOR_THRESH>
<CONTROL_TYPE_ID_0_0>Gene17</CONTROL_TYPE_ID_0_0>
<OFFSET_THRESH>60.0</OFFSET_THRESH>
<HOT_CV>3.0</HOT_CV>
<CONNECT_FLAG>false</CONNECT_FLAG>
<EMPTY_THRESH>0.3</EMPTY_THRESH>
<HOT_CHECK>false</HOT_CHECK>
<MEASURE_MEAN>true</MEASURE_MEAN>
<BACKGROUND_THRESH>0.9995</BACKGROUND_THRESH>
<MEASURE_STDEV>true</MEASURE_STDEV>
<CONTROL_TYPE_VARTHRESH_0>1.0</CONTROL_TYPE_VARTHRESH_0>
<MEASURE_AREA>true</MEASURE_AREA>
<PERIMETER_THRESH>25.0</PERIMETER_THRESH>
<STANDARD_CV>5.0</STANDARD_CV>
<AUTO_SEG>true</AUTO_SEG>
<CONTROL_TYPE_UPALERTTHRESH_0>0.0</CONTROL_TYPE_UPALERT-
THRESH_0>
<CONTROL_TYPE_IFLOWALERT_0>false</CONTROL_TYPE_IFLOWALERT_0>

```
  <BLANK_LOW>0.0</BLANK_LOW>
  <BACKGROUND_BUFFER>3.0</BACKGROUND_BUFFER>
  <CONTROL_TYPE_NAME_0>hot</CONTROL_TYPE_NAME_0>
  <EMPTY_FLAG>true</EMPTY_FLAG>
  <MEASURE_MODE>true</MEASURE_MODE>
  <ENFORCE_GRID>true</ENFORCE_GRID>
  <OVERALL_FLAG>false</OVERALL_FLAG>
  <MEASURE_TOTAL>true</MEASURE_TOTAL>
  <SIGNAL_HIGH>1.0</SIGNAL_HIGH>
  <PERIM_TO_AREA>true</PERIM_TO_AREA>
  <SIGNAL_FLAG>false</SIGNAL_FLAG>
  <BACKGROUND_WIDTH>3.0</BACKGROUND_WIDTH>
  <BLANK_CHECK>false</BLANK_CHECK>
  <ON_TOP>false</ON_TOP>
  <CONTROL_TYPE_UPTHRESH_0>65000.0</CONTROL_TYPE_UPTHRESH_0>
  <SIGNAL_THRESH>0.9995</SIGNAL_THRESH>
  <FLEXIBILITY>0.0</FLEXIBILITY>
  <FIND_NEGATIVES>false</FIND_NEGATIVES>
  <SIGNAL_LOW>0.0</SIGNAL_LOW>
  <STANDARD_HIGH>10000.0</STANDARD_HIGH>
  <BACKGROUND_LOW>0.0</BACKGROUND_LOW>
  <OFFSET>true</OFFSET>
  <CONTROL_TYPE_IFUPALERT_0>false</CONTROL_TYPE_IFUPALERT_0>
  <SPOT_AREA>true</SPOT_AREA>
  <REGULARITY_THRESH>0.65</REGULARITY_THRESH>
  <LOG_SCALE>false</LOG_SCALE>
  <STANDARD_CHECK>false</STANDARD_CHECK>
  <HOT_HIGH>100000.0</HOT_HIGH>
  <REGULARITY_FLAG>true</REGULARITY_FLAG>
  <CONTROL_TYPE_N>1</CONTROL_TYPE_N>
  <MEASURE_MEDIAN>true</MEASURE_MEDIAN>
  <CONTROL_TYPE_IFCVALERT_0>false</CONTROL_TYPE_IFCVALERT_0>
  <CONTROL_TYPE_COLOR_0>-52429</CONTROL_TYPE_COLOR_0>
  <IGNORED_FLAG>true</IGNORED_FLAG>
<CONTROL_TYPE_LOWALERTTHRESH_0>0.0</CONTROL_TYPE_LOWALERT-
THRESH_0>
  <OPEN_PERIMETER>true</OPEN_PERIMETER>
  <CONTROL_TYPE_ID_N_0>1</CONTROL_TYPE_ID_N_0>
  <BLANK_HIGH>1000.0</BLANK_HIGH>
  <BACKGROUND_HIGH>1.0</BACKGROUND_HIGH>
  <HOT_LOW>30000.0</HOT_LOW>
  <IGNORED_THRESH>25.0</IGNORED_THRESH>
</VersArray Analyzer_Parameters>
```

4.2 Technical Support

Bio-Rad is available to answer any questions that you have about VersArray Analyzer 5.0 Software. Your questions will be addressed promptly so you can focus on what is most important - your research. Your VersArray Analyzer 5.0 Software serial number will be requested when you contact technical support using any of the following methods:

Bio-Rad Technical Service Department
Phone: (800) 424-6723, option 2, option 3
Fax: (510) 741-5802
E-mail: LSG.TechServ.US@Bio-Rad.com (U.S.)
LSG.TechServ.Intl@Bio-Rad.com (International)

4.3 Warranty Information

Bio-Rad guarantees VersArray Analyzer 5.0 Software to be free from defects up to 30 days from the date of purchase. Bio-Rad will promptly address any problems you may have through either technical support or by sending you a replacement copy of VersArray Analyzer 5.0 Software.

Bio-Rad Laboratories, Inc.

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Israel *03 951 4127* ***Italy*** *39 02 216091* ***Japan*** *03-5811-6270* ***Korea*** *82-2-3473-4460* ***Latin America*** *305-894-5950* ***Mexico*** *55-52-00-05-20*
The Netherlands *0318-540666* ***New Zealand*** *64 9 415 2280* ***Norway*** *23 38 41 30* ***Poland*** *+ 48 22 331 99 99* ***Portugal*** *351-21-472-7700*
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