

ImaGene®

# User Manual

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**Version 7**

 **BioDiscovery, Inc.**

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## Introduction to ImaGene

ImaGene is BioDiscovery's solution for a fast and accurate microarray image analysis software system. With ImaGene, researchers can generate quantified data within seconds while generating extensive quality control information.

Microarray image analysis is utilized to quantify the relative expression levels existing within a microarray scan. As such, the role of ImaGene within the microarray process is post hybridization and scanning. The subsequent results of ImaGene are quantified expression values that are saved to a text file. These results can then be used in any data analysis package, such as BioDiscovery's GeneSight for further analysis.

## Quick Start Guide

ImaGene was designed to be the fastest and easiest software solution for the quantification of microarray images. The description below outlines the essential steps required to set up ImaGene. For additional information, please view the remaining sections of this user manual.

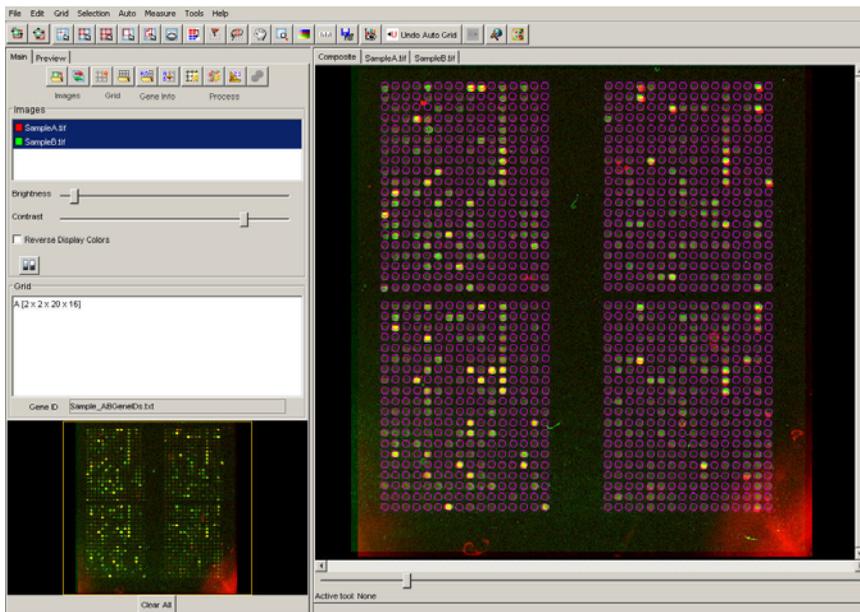
### Setting Up ImaGene:

1. Install ImaGene
2. Submit registration information and request a license from BioDiscovery
3. Start ImaGene
4. Load an image
5. Establish Settings
6. Perform Grid Placement
7. Quantify the Image
8. Run the Analysis

# Part 1: Lab Users Guide

## 1.1 ImaGene Main Window Overview

The ImaGene Main window is the primary program interface. It serves as the focal point of all work within ImaGene. This window is utilized to, among other things, load images, place grids, produce quantified data and review results. This chapter identifies the components of this window and explains how each fits into the array analysis and feature extraction process.



**Menu Bar** - Located along the top of the window. Click on one of the menus (*File, Edit, Grid, Selection, Auto, Measure, Tools, or Help*) to view the options available on the selected menu. The following options are available from the Menu Bar.

File Edit Grid Selection Auto Measure Tools Help

## 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 ImaGene will load previously created settings, data, segmentation and flagging information along with the images processed.
- **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 future generation of publications.
- **Settings** – The Settings option opens the ImaGene Parameter Settings Window. This window and the tabs within the window contain all the parameters within ImaGene for Quality Measures, Auto Flagging, Spot Finding and Alert Logging.
- **Exit** – Closes the ImaGene program.

## Edit

- **Undo** – Cancels the last executed command. For example, if you moved a spot, you could select Edit > 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 Edit > Redo to move the spot again.

## Grid

- **Create Grid** – Launches the Create Grid window where the essential information about the design of the grid must be entered.
- **Load Grid** – Displays the Load Grid dialog box. This is utilized to select and open a previously saved grid (.grid) file.

- **Save Grid** – Displays the Save Grid dialog box. This is utilized to save the displayed grid to a grid (.grd) file.
- **Clear Grid** – Removes all displayed grids from the Grid Display Panel.
- **Load Gene IDs** – Displays the Load Gene ID File dialog box. This is utilized to select and open a gene ID (.txt) file.
- **Clear Gene IDs** – Removes the displayed gene ID information.
- **Load Template** – Displays the Load Template dialog box. This is utilized to select and open a template (.tpl) file. (A template is a grid file, which contains gene ID information)
- **Save Template** – Displays the Save Template dialog box. This is utilized to save the displayed template to a template (.tpl) file.
- **Import template as GAL, GEML or MAGE** – Displays the Import Template dialog box. This is utilized to select and open a template file as:
  - Array Lists (.gal)  
[http://www.moleculardevices.com/pages/software/gn\\_genepix\\_file\\_formats.html#gal](http://www.moleculardevices.com/pages/software/gn_genepix_file_formats.html#gal)
  - GEML v1.0 (.xml)  
<http://www.rosettahp.com/tech/geml/default.htm>
  - MAGE-ML (.xml) format  
<http://www.mged.org>
- **Export template as GAL, GEML or MAGE** – Displays the Export Template dialog box. This is utilized to save a displayed template to a template file in Array Lists (.gal), GEML v1.0 (.xml), or MAGE-ML (.xml) format.

## Selection

- **Adjust Metagrid** – Utilized to select and move an entire metagrid.
- **Adjust Subgrid** – Utilized to select and move an individual portion of the metagrid (subgrid).
- **Adjust Spot** – Utilized to select and move one spot in a metagrid.
- **Lasso Adjust** – Utilized to select and move a specific (free form) area of the metagrid.
- **Rectangle Adjust** – Utilized to select and move a specific rectangular area of the metagrid.

## Auto

- ***Auto Align Images*** – Aligns the displayed images automatically if they are misaligned.
- ***Auto Adjust Grids*** – Automatically places the displayed grids globally onto the image spots.
- ***Auto Adjust Spots*** – Automatically adjusts each spot to better align them with the corresponding image.
- ***Wrangle*** – Enforces new local spot flexibility parameters. This, essentially, reduces the distance used in spot finding without requiring spot finding to be reapplied.

## Measure

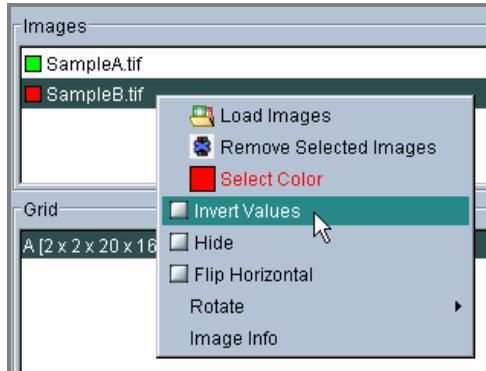
- ***Make Measurements*** – Quantifies the image spots to generate the intensity values for signals and backgrounds as well as quality control results such as flags.
- ***Analyze*** – Applies data analysis and runs the chosen normalization procedures (configured using the settings panel) to determine for 1) the **Gene Expression Module** whether the genes are up-, down-regulated, or have no change against a pre-determined fold threshold Or 2) the **CGH Module** whether there is regional copy number variations or amplifications and deletions.

## Tools

- ***Zoom*** – Turns the cursor into a magnifying glass. Utilized to adjust the on-screen display size of images.
- ***Scroll*** – Turns the cursor into a hand. Utilized to scroll all images and grids at the same time.
- ***Translate Images*** – Turns the cursor into a cross with arrows. Utilized to move selected images.
- ***Rotate Images*** – Turns the cursor into a circle. Utilized 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. Utilized to measure the size of spots or distances between them.
- ***Image Intensities*** – Turns the cursor into a small light bulb. Utilized to measure the intensity of spots.



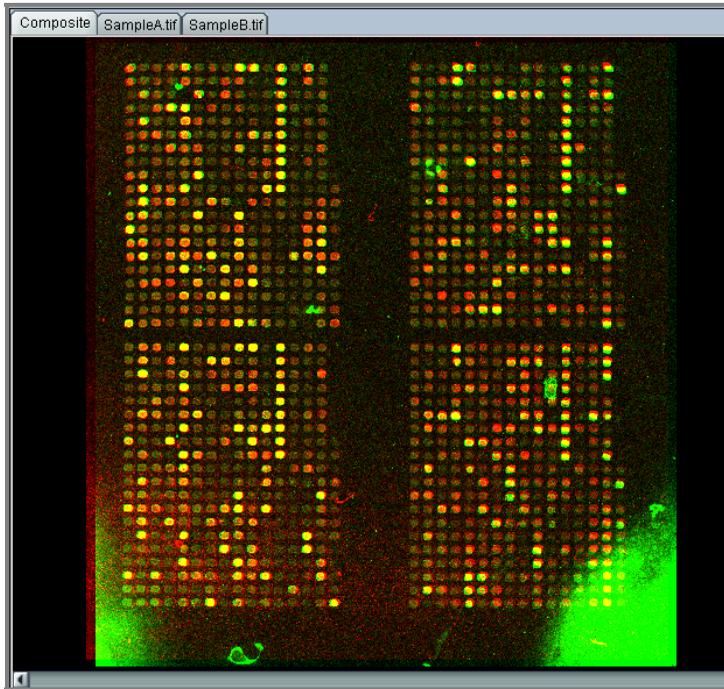
- Results panel in Raw Data tab



**Control Tabs** – Located at the left-hand side 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.



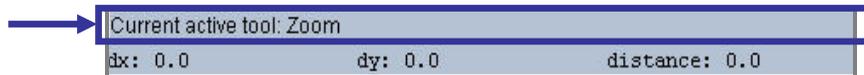
**Image Display Panel** – Located at the right-hand side of the window. This panel displays loaded images and is where grid placement takes place.



**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.



**Current Active Tool Bar** – Located directly above the Status Bar at the bottom of the window. Displays the tool currently being used in ImaGene.



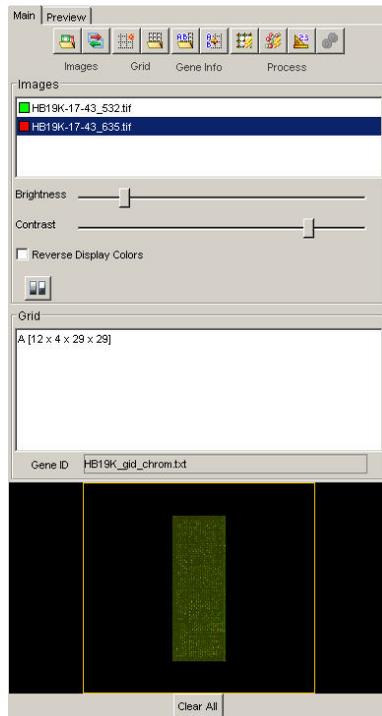
## 1.2 Control Tabs

Located along the left-hand side of the main ImaGene window are two tabs containing essential information about the images and quantification results.



### 1.2.1 Main Tab

The Main Tab is utilized to perform the essential steps of microarray image analysis. There are a group of icons on the top to streamline the image processing steps. The workflow can be easily followed by selecting the icons from left to right. Below them, there are different sections for images, grids, and gene IDs, as well as controls for image display and clearing.



## Image Analysis Workflow Icons



The essential tools for image analysis are grouped together as a line of icons to load and align images, to create or load grids, to load templates or Gene IDs, to auto adjust grids or spots, and to quantify the image spots.



**Load Images** – Opens the Load Images Dialog and allows the selection of desired image files. Multiple files can be selected by holding the <shift> or <ctrl> keys and clicking on the image names.

*Note: Imagen supports the following image file formats:*

- *Tiff (uncompressed) – 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*



**Auto Align Images** – Aligns the displayed images automatically if they are misaligned to each other after being loaded



**Create Grid** – Launches the Create Grid window where the essential information about the design of the grid must be entered



**Load Grid** – Displays the Load Grid dialog box. Use this interface to select and open a previously saved grid (.grid) file



**Load/Import Template** – Displays the Load Template dialog box. Use this interface to select and open a template (.tpl, .gal, .xml for GEML and MAGE-ML) file. A template is a grid file, which contains gene ID information



**Load Gene IDs** – Displays the Load Gene ID File dialog box. Use this interface to select and open a gene ID (.txt) file



**Auto Adjust Grid** – Automatically places the displayed grids globally onto the image spots



**Auto Adjust Spots** – Automatically adjusts each spot to better align them with the corresponding image



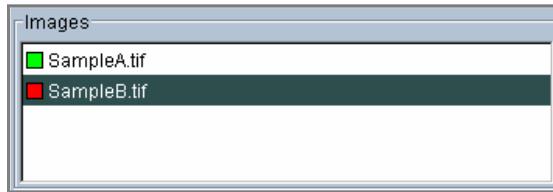
**Make Measurements** – Quantifies the image spots to generate the intensity values for signals and backgrounds as well as quality control results such as flags



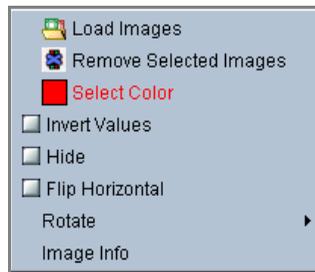
**Analyze** – Is activated upon generating measurements and results. This will run the normalization and Analysis methods and configurations specified in the Settings Panel tabs of *Normalization* and *Analysis*.

## Images Panel

The images panel displays the names of images currently loaded within ImaGene. 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** – Selection of images from the file system to load into ImaGene
- **Remove Selected Images** - The images selected, or highlighted from the images panel are removed, or unloaded from analysis
- **Select Color** – Select the color to be used for the selected image when seen under the Composite Tab. The default colors 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, ImaGene 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 ImaGene requires this information to be manually set. Should it be determined that 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.
- **Image Info** – Provides information about the image read directly from the image file, such as the file name, directory location, width, height, type, and resolution.

## Grid Panel

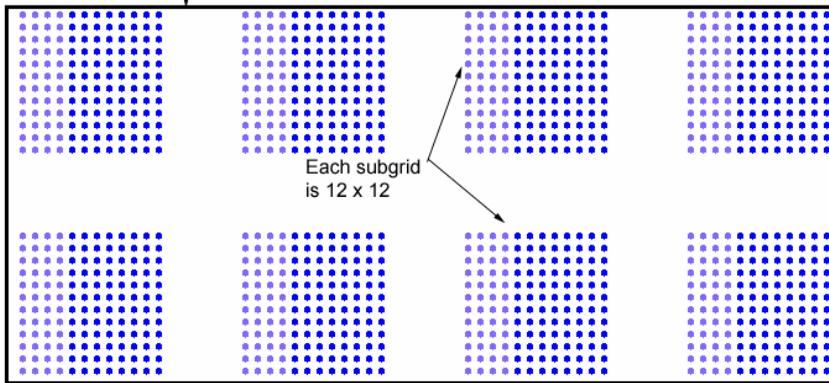
The Grid Panel lists all fields that have been created and placed on the image. Typically, only a single field will be required; however, depending on the design of the array being quantified, several fields may be required. As a new field is created, it is added to the list.



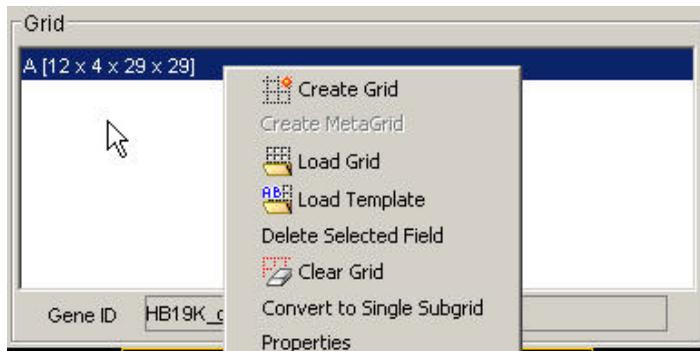
Before explaining how and why to create a **Field**, let us explore some of the definitions and structures of a field. A field is the largest design element within a slide. A field typically consists of the arraying done by a single print head on the slide. For example, if the arrayer has a print head 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 that are printed by a single pin, 12x12 for example. The accompanying diagram demonstrates the relationship between the three levels of structure.

The bounding box represents the field.

The MetaGrid is 2 x 4



Like other components of ImaGene, the Grid Panel fully supports context sensitive menus. Right clicking on the Grid Panel provides the following options:

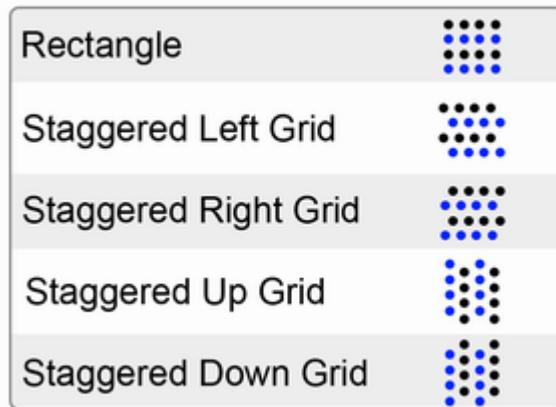


- **Create Grid** – The option launches the Grid Creation Window where the essential information about the design of the grid must be entered. Remember that within ImaGene the grid (subgrid) is the most elemental structure, followed by the metagrid and finally field. The following parameters must be specified:

- **Field Name** – Specify the name of the field here. The name may be any name desired. By default ImaGene 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 with the image to provide gene names or accession numbers within the text output file of ImaGene, the fields name, if used, must match between what is specified here and that 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 ImaGene.
- **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

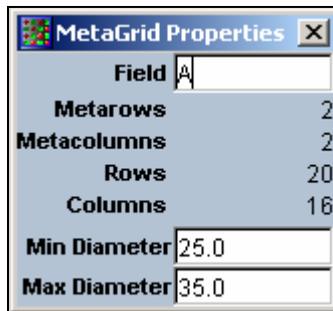
determined by use of the **Ruler** Tool. Due to the variety of array types and the variability of individual arrays there is no set procedure for determining the minimum diameter here. The most common rule of thumb is to specify the size of approximately 10 % of the smallest spots of the array. Depending on the type of array, if the spots are highly uniform, then the minimum diameter specified here would 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 Min Diameter, using the top 10% of large spots and measuring the sizes with the Ruler Tool can approximate this value.
- **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 position. While rectangular is the most common, all the possible selected are:



- **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. The Create MetaGrid window requires the following parameters to be specified.
  - **Metarows** – The number of rows of subgrids.
  - **Metacolumns** – The number of columns of subgrids
- **Load Template** – This will launch the template file browser panel.

- **Delete Selected Fields** – The current highlighted field will be removed.
- **Clear Grid** – All fields that have been created or loaded 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 2x2 metagrid with 15x10 subgrids, after selecting this conversion, the resulting subgrid size will be 30x20. During this conversion nothing changes except for the how the individual spot locations are represented. This feature is optional and may be used if required to construct a multi-level metagrid, for example.
- **Properties** – This option opens a window displaying the parameters for the current selected field. Only certain options can be modified while the remainder requires the grid to be deleted then re-created as 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.
- **Max Diameter** – The maximum expected diameter of the spots to be used during spot finding. This value may be changed.

## **How to Create Grid**

Perform the following steps to create a subgrid:

1. Load the desired images into ImaGene
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.

Perform the following steps to create a metagrid:

1. Click on and select the desired subgrid to use as a basis for the metagrid. This can be done by using a left-click on the grid within the Grid Panel.
2. Right-click and select Create MetaGrid from the menu.
3. In the Metagrid Parameters Window, specify the number of rows and columns of subgrids contained within the metagrid.
4. Click Place Metagrid and click on the top left spot in each of the corner subgrids. Typically, this process will require four clicks.

## **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 Dialog browse to the location where you wish to save the grid file.
3. Specify a file name. ImaGene 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, browse to and select the desired grid file.
3. Click on one of the following radio buttons to select a grid placement method:
  - a. Place Manually - Select this option to set the location and size of the saved grid manually. 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.
  - b. 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
4. Finally, click the Open Button to place the grid.

## Gene ID

The Gene ID file is utilized 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.

Note that for CGH analysis, the Gene ID format requires more information as described in the appendix 5.1.2.

If a Gene ID file was 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 IDs – Selecting this option will open a window allowing for the selection of the gene ID file to be used. 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 IDs – If a gene ID has already been selected, this option will remove the selected file from use.

## 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 BioDiscovery'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.

[http://www.moleculardevices.com/pages/software/gn\\_genepix\\_file\\_formats.html#gal](http://www.moleculardevices.com/pages/software/gn_genepix_file_formats.html#gal)

GEML (v 1.0) – XML standard representing a “pattern” that cannot fully describe a grid structure, but rather provides location and gene ID info for every spot.

<http://www.rosettatabio.com/tech/geml/default.htm>

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.

<http://www.mged.org>

**Reverse Display Colors** - Mark this check box to reverse the displayed colors of all loaded raw images, e.g. changing intensity scale from black to white to white to black. This feature has no effect on the Composite tab display. Changing the appearance of an image does not affect the pixel intensity of the original data. This is utilized to visualize dim spots in the image display.



## Display Control

The Display Control allows manual adjustment of how the images are displayed. By moving the Brightness (Max pixel value threshold adjuster) and Contrast (Minimum pixel value threshold adjuster) sliders located beneath the image panel, the scanned slide images can be lightened and darkened in the main panel views. The tool is designed solely to enhance viewing of the image and does not affect the quantified values generated by ImaGene. When adjustments are made to these sliders, they are applied only to the Image(s) selected within the Images Panel.



The specific elements of the Display Control and their roles are defined below:

- ***Brightness Adjuster*** (Top Slider) – Sets the Maximum intensity value threshold to be displayed. Moving the slider to the right, will decrease the threshold and increase the saturated appearance. This does not alter the true pixel values of the selected image(s).
- ***Contrast Adjuster*** (Bottom slider) – Moving the bar all the way to the left corresponds to a linear brightness scale (e.g. if pixel 1 has value 100 and pixel 2 has value 1000, pixel 2 will be 10 times brighter, up to the saturation point). As the slider bar is moved to the right, the brightness scale changes nonlinearly (faster for smaller values, slower for larger values), so a pixel value of 100 may have brightness of 80 and a pixel value of 1000 might have a brightness of 110 for example.
-  **Auto Adjust Contrast/Brightness** – Clicking on this icon automatically adjusts the contrast/brightness of the images to have the optimal settings for display.

## Map View Display

The Map View (lower left display on Main Panel) provides a comprehensive and unabridged view of the image while indicating exactly where within the image the primary image display panel is zoomed. 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 and zoom in for easier gridding and spot adjustments.



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.



**Clear All** – Clears everything currently loaded in ImaGene, including images, grids, gene IDs, etc.

## 1.2.2 Preview /Raw Data Tab

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

- **Segmentation Preview**
- **Quantification Preview**

The screenshot shows the 'Raw Data' tab in ImaGene. At the top, there is a metadata section with the following information:

- Gene ID: RP11-591L21 (with a link to [Ensembl](#))
- Field: A (with a link to [UCSC](#))
- Metarow 1, Metacolumn 1 (with a link to [google](#))
- Row: 18, Column: 8
- Diameter: 30.0

Below the metadata is a preview grid with four columns: 'Original', 'Segmen...', 'Composite', and 'Histogram'. The grid shows two rows of data, labeled 'CGHSampleA...' and 'CGHSampleB...'. Each row contains a grayscale image, a segmented image with red and green regions, a composite image, and a histogram.

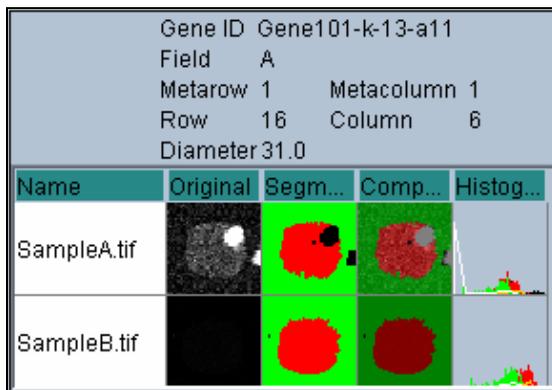
At the bottom of the interface is a data table with the following columns: 'Field', 'Me...', 'Met...', 'Row', 'Col...', 'Ge...', and 'Chro...'. The table contains 16 rows of data, with the first row highlighted in yellow. The data is as follows:

Field	Me...	Met...	Row	Col...	Ge...	Chro...
A	1	1	1	1	RP11-1...	X
A	1	1	1	2	RP11-1...	2
A	1	1	1	3	RP11-4...	5
A	1	1	1	4	RP11-2...	20
A	1	1	1	5	RP11-3...	7
A	1	1	1	6	RP11-3...	16
A	1	1	1	7	RP11-7...	9
A	1	1	1	8	RP11-5...	10
A	1	1	1	9	RP11-9...	2
A	1	1	1	10	RP11-5...	X
A	1	1	1	11	RP11-1...	5
A	1	1	1	12	RP11-7...	X
A	1	1	1	13	RP11-1...	5
A	1	1	1	14	RP11-9...	10
A	1	1	1	15	RP11-1...	5
A	1	1	1	16	RP11-1...	12
A	1	1	2	1	RP11-1...	5
A	1	1	2	2	RP11-2...	6

At the bottom of the interface, there are buttons for 'Save', 'Export XML', and 'Close'. Below the buttons, it says 'Selected rows: 1' and 'Right-click to "freeze" selection'.

## Segmentation Preview

Use the Segmentation Preview to view the effects of current parameter settings on the segmentation both before and after quantifying the data. Select the spot to view and the corresponding information about the spot will be displayed in the segmentation panel. Concurrently, the selected spot can be seen on the image and four of the plots (Scatter plot, M-A plot, Box plot, and GenePie). This interface also supports dynamic analysis within ImaGene. You can adjust settings, such as the maximum and minimum signal values, and see the effects of these changes in real time via the dialog box.



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

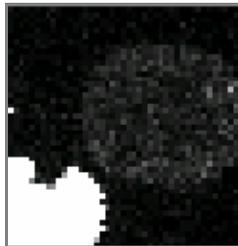
- **Gene ID** - Lists the corresponding information, typically name or accession from the Gene ID file (only available if a Gene ID file was previously loaded into ImaGene)
- **Field** - Lists the field that the selected spot belongs to. This name is specified when the grid was first constructed.
- **Metarow** - 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.

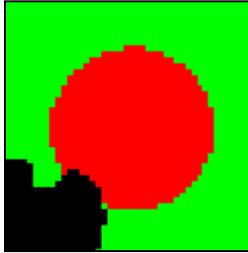
*Note: Segmentation involves the partitioning of a microarray image into a set of regions that convey a specified meaning. For microarrays, the purpose of segmentation is to decompose a scanned optical image into regions that are meaningful in terms of spot signal and spot background.*

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

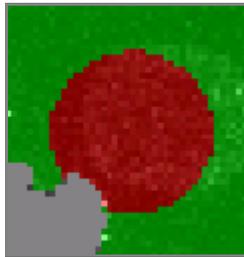
- **Name** - Lists the name of the source image file for the spot.
- **Original** - Displays the spot and its surrounding background without any segmentation information.



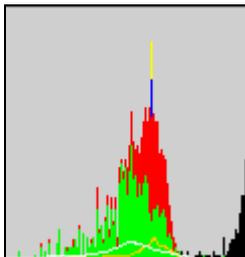
- **Segmented** - Displays an image of the segmentation, or determination of background and signal, that will be performed during quantification. The red pixels represent signal values and the green pixels represent background values. Black means the pixel is ignored.



- **Composite** - Displays an overlay between the *Original* and *Segmented* images. This image is *key* in 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 colors are used to indicate pixels to be included within the signal and background measurements. If a pixel is not colored, 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
- Black – Indicated the pixel is not used in either calculation of signal or 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
- Yellow – Quality criteria failures and controls warnings

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
- Optional Probe Annotation Fields for the spot.
- Flag – Numeric code of the flag for the spot (0 – no flag, flag codes 1,...,7)
- 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 peak 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 the signal area of a spot is inscribed into a circle. Then the number of non-signal pixels that fall within this circle is computed and divided by the circle's area. This ratio is subtracted from 1
- Ignored Area – Area of ignored regions directly neighboring (“touching”) the signal area is computed
- Spot Area – Signal Area plus Ignored Area (pixels)
- Ignored Median – Median pixel intensity computed over the local ignored region
- Area To Perimeter – This quality measure defines spot's circularity. The area of a spot is divided by the square of spot perimeter and multiplied by  $4\pi$ . 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 inscribed into the spot's signal region
- Control – Name of a control type for current spot (See Settings Controls/Alerts panel)
- Failed Control – 0 if the control passed all tests, 1 if at least one of the tests failed
- Background contamination present – 0 if the spot passed background contamination test, 1 if it did not (see section 1.4)
- Signal contamination present - 0 if the spot passed signal contamination test, 1 if it did not (see section 1.4)
- Ignored % failed – 0 if the spot passed ignored percentage test, 1 if it did not (see section 1.4)
- Open perimeter failed – 0 if the spot open perimeter test, 1 if it did not (see section 1.4)
- Shape regularity failed – 0 if the spot passed shape regularity test, 1 if it did not (see section 1.4)
- Perim-to-area failed – 0 if the spot passed perimeter-to-area test, 1 if it did not (see section 1.4)
- Offset failed – 0 if the spot passed offset test, 1 if it did not (see section 1.4)
- Empty spot – 1 if the spot was qualified as empty, 0 if it was not (see section 1.4)
- Negative spot – 1 if the spot was qualified as negative, 0 if it was not (see section 1.4)
- Saturated spot – 1 if saturation was detected, 0 if no saturation was found for the spot (see section 1.4)

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 results are satisfactory, the final step is to *Save* the data. ImaGene will save the data to common tab delimited text files, which can easily be opened in other programs such as Microsoft Excel or text editors. ImaGene will save each image’s data to a separate file and will automatically name the file based upon the image name.

### **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, or mode for export as signal/background value.

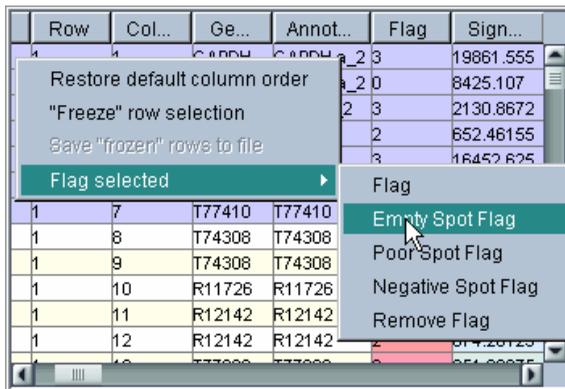
***MAGE-ML format (XML)*** – the most complete format, exported file will contain QuantificationType\_package, BioAssay\_package and BioAssayData\_package of MAGE standard. All ImaGene measurements will be exported. For more details go to: <http://www.mged.org>

### **Close**

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

## Context Menu

You can select several spots at a time pressing “Ctrl” or “Shift” keys when making selection in the results table. Right-clicking the selection and the context menu will be displayed:



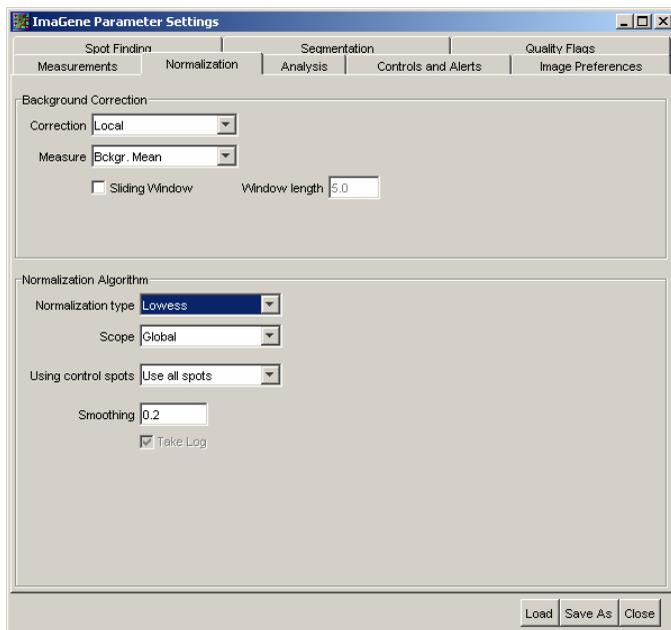
Row	Col...	Ge...	Annot...	Flag	Sign...
1	7	T77410	T77410		19861.555
1	8	T74308	T74308		8425.107
1	9	T74308	T74308		2130.8672
1	10	R11726	R11726		652.46155
1	11	R12142	R12142		16452.625
1	12	R12142	R12142		

The context menu is open over the first row. The 'Flag selected' option is highlighted, and its sub-menu is visible, showing 'Empty Spot Flag' as the selected option.

“**Restore default column order**” returns the columns to the default order if the columns have been reshuffled. “**Freeze row selection**” memorizes current row selection for future use. When this command is activated, the table rows will be highlighted in dark blue color and flags of “1” for these rows will appear in the “Selected” column at the right-hand end of the table. Clicking on the “Selected” column heading will move the selected rows to the bottom of the table. Meanwhile, “**Freeze row selection**” toggles to “**Unfreeze selection**”. Selecting “**Unfreeze selection**” cancels the grouping of the selected rows and they will become highlighted with a light-blue selection color. This feature is useful if spots were selected using one of the plots (for example: selected regulated genes using Histogram) and want to analyze their images, segmentations and quantifications results. The selected rows can be saved into .txt file using “**Save frozen rows to file**”. Also, user can manually flag the selected rows (spots) by selecting “**Flag selected**” and its subsequent flagging options. Using this option user can remove flags as well. If automatic flags are removed through this option, they can be re-generated by changing flagging options or re-quantifying images.

## 1.2.3 Normalization Tab

This functionality as of ImaGene version 7.0 has been moved to the Settings tool panel. In this tab, the raw data can be transformed by taking background correction, log transformation, and/or normalization. There are a variety of data transformation options.



### Background Correction

Background mean, median, or mode can be used in background correction. After selecting the measure, background correction can be applied locally, globally, or not at all (Local, Global, or None).

When applying the background correction locally, a check box “**Sliding Window**” is displayed. When it is not checked, the local background value of each spot is used for correction. If the “**Sliding Window**” box is

checked, a value for the **“Window length”** must be specified. The window length identifies a small square region of spots with the spot being background corrected as the center spot. The median of the background values of all spots is used for background correction.

When applying the background correction globally, users can select between **“Subgrid based”**, **“Using signal from control spots”**, or both. If **“Subgrid based”** is checked, the median of the background values of all the spots in a subgrid is used for background correction. If **“Using signal from control spots”** is checked, the median of the signal values of all the control spots is used (see detailed explanations about control spots in Section 1.4 **Controls and Alerts** subsection). When both boxes are checked, the median of signal values of the control spots within the same subgrid of the spot being background corrected will be used.

### **Log Transformation**

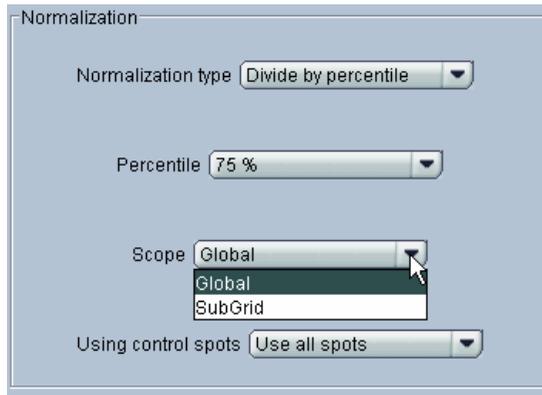
Log transformation with bases of 2 can be applied to the raw or background corrected data.

### **Normalization**

There are four options for normalization type: **None**, **Divide by percentile**, **Subtract percentile**, and **Lowess**. When either **Divide by percentile** or **Subtract percentile** is selected, a percentile value has to be set to 50% (median), 25%, or 75%. A scope of **Global** or **Subgrid** also has to be chosen for normalization.

In addition user has to select whether all spots should be used for computing normalization or utilize dedicated control spots (see detailed explanations about control spots in **Controls and Alerts** of **1.4 ImaGene Parameter Settings Window**). . Normalization coefficients can be computed based on:

- All spots
- Exclude Control Spots (for Lowess normalization)
- Use Control spots (for % normalizations)



For Lowess normalization, not only a scope of Global or Subgrid has to be set, a smoothing factor has to be specified as well. A smoothing factor determines how many neighboring data points will be used for the normalization of each data point.

The data is **Normalized** when the **Analysis** button is selected in the tool bar or Measurement Menu.. After the analysis process is complete, a Normalized Data tab appears in the Image Display panel along with the Analysis Results Panel and for CGH analysis the respective CGH report and result plots. The normalized data can be saved as a text file by clicking on the **Save Report** icon at the bottom.

Composite				CGHSampleACv5.tif			CGHSampleBCY3.tif			CGHSampleACv5.tif seq			
CGHSampleBCY3.tif seg				Plots	Normalized Data		Overview	Whole Genome		Chromosome	CGH Report		Summary
Fl...	MR	MC	R...	C...	GeneID	Chromos...	Base Pair	Flag	N. Signal Me...	N. Signal Me...			
A	1	1	1	1	RP11-124H12	X	31561116	0	10.026101	12.545955			
A	1	1	1	2	RP11-185M20	2	15197943	0	9.1759615	9.555748			
A	1	1	1	3	RP11-40N8	5	67274446	0	8.844778	9.800584			
A	1	1	1	4	RP11-274E3	20	45337121	0	9.408482	10.071046			
A	1	1	1	5	RP11-331D5	7	156680451	0	11.006078	13.927979			
A	1	1	1	6	RP11-39L24	16	14140801	0	9.1033325	9.071795			
A	1	1	1	7	RP11-79G7	9	79580442	0	8.897433	10.227885			
A	1	1	1	8	RP11-592B15	10	50857416	0	8.896872	9.132032			
A	1	1	1	9	RP11-91A12	2	122387436	0	14.52886	15.655639			
A	1	1	1	10	RP11-663P13	X	29593395	0	14.407039	15.235259			
A	1	1	1	11	RP11-103B21	5	158743671	2	8.874845	8.702867			
A	1	1	1	12	RP11-787J9	X	144452630	0	11.098706	12.369519			
A	1	1	1	13	RP11-1126E19	5	37209691	0	9.124718	9.031169			
A	1	1	1	14	RP11-951C8	10	116909066	0	10.5045	12.647262			
A	1	1	1	15	RP11-151K14	5	66908167	0	9.047106	8.969404			
A	1	1	1	16	RP11-184M3	12	43754451	2	6.9619064	6.943245			
A	1	1	2	1	RP11-135M13	5	15627305	0	9.660476	11.15629			
A	1	1	2	2	RP11-272G1	6	104150829	0	9.29753	9.683414			
A	1	1	2	3	RP11-316J16	3	23759859	0	8.717261	9.940787			
A	1	1	2	4	RP11-410B3	2	99915960	0	9.259812	9.459071			
A	1	1	2	5	RP11-56J3	11	107487025	0	9.350916	10.896739			
A	1	1	2	6	RP11-58K15	X	126442170	0	9.158521	9.589938			
A	1	1	2	7	RP11-91E5	4	87167643	0	9.1018915	9.131852			
A	1	1	2	8	RP11-662H24	6	143160905	0	8.904604	10.505361			
A	1	1	2	9	RP11-1036M24	6	22821642	0	9.339095	10.8700285			
A	1	1	2	10	RP11-784O17	17	70842883	2	8.158911	8.441132			
A	1	1	2	11	RP11-1123E2	1	204192811	2	8.499459	8.254031			
A	1	1	2	12	RP11-92F6	13	103782901	0	11.31989	12.743311			
A	1	1	2	13	RP11-151C15	X	62874378	0	7.999847	8.67277			
A	1	1	2	14	RP11-14M15	7	144390069	0	9.42138	11.220811			
A	1	1	2	15	RP11-26M6	14	34404822	0	8.854649	9.20664			
A	1	1	2	16	RP11-24J14	1	107639467	2	7.021699	6.733376			
A	1	1	3	1	RP11-124F2	11	81504791	2	8.411947	9.157609			

Row= 1

Save Report

## 1.2.4 Analysis Tab (Expression and CGH Modules)

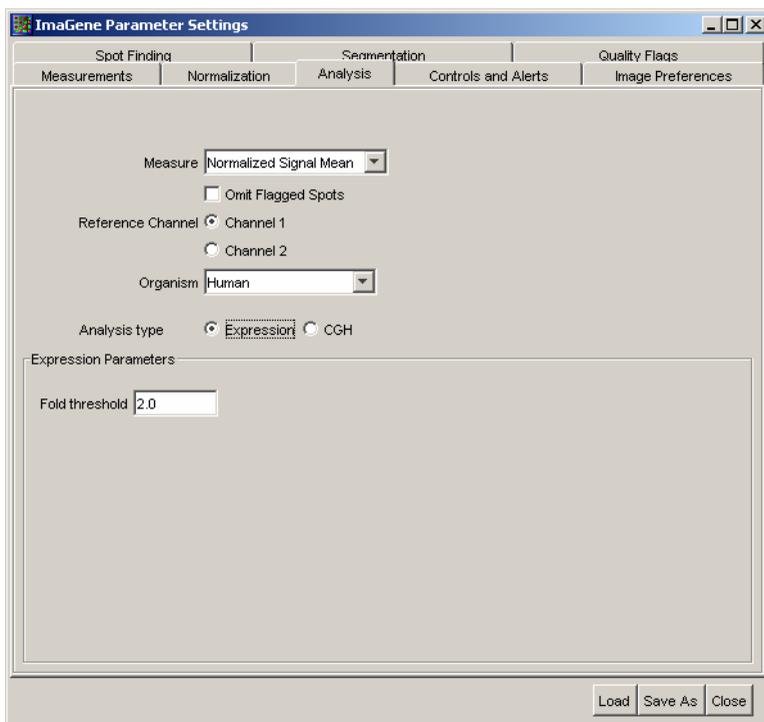
Starting with ImaGene 7, the normalization settings have been moved to the Analysis tab of the ImaGene Parameter Settings window. Depending on the type of ImaGene license purchased, the Analysis tab will either indicate Expression or CGH settings. For users licensed for both Expression and CGH modules, an radio buttons allow easy switching between the 2 types of analysis

The Analysis tool, in Expression mode, will select up and down regulated genes according to a fold threshold set by the user. CGH mode calculates insertion /deletion clustering based on user defined thresholds. A reference channel is selected and the user chooses normalized results, e.g. Normalized Signal Mean, Median, or Mode. Spot replicates on the slide are combined (their mean value is used) and flagged spots can be disregarded in the analysis (“Omit flagged spots”).

To begin the Analysis, click the Analysis button along the processing toolbar or select Measurements > Analyze.



*Note: Channel 1 and 2 refer to the order of the loaded images. For example, the first image in the image panel corresponds to Channel 1.*

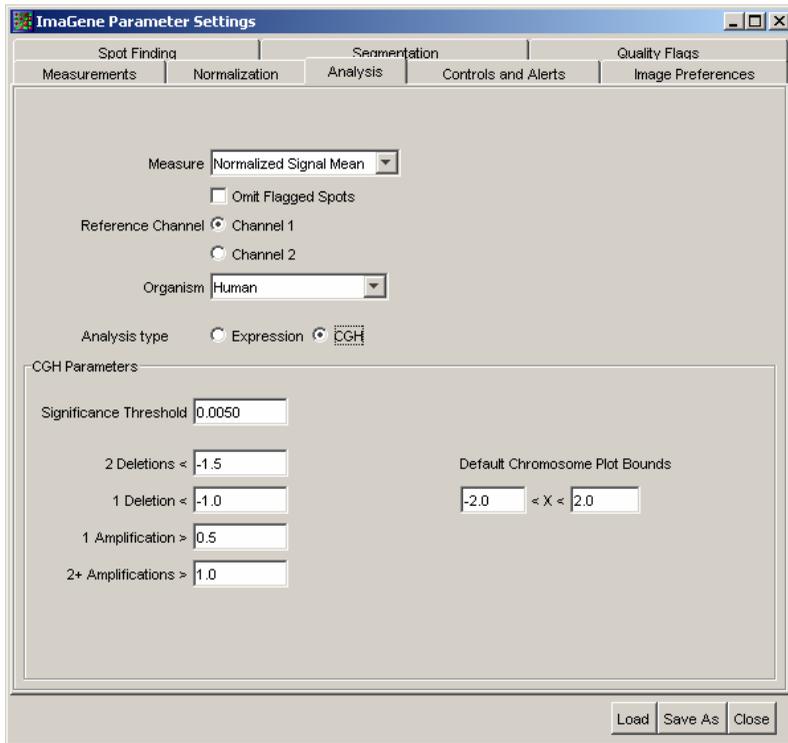


### **Expression Analysis Mode Settings Panel**

When the Expression Analysis complete, an Analysis tab appears in the image display panel with labels of genes for up (red color) and down (green color) regulations. The results can be saved in a text file by clicking on the Save Report button at the bottom of the panel.

Composite		CGHSampleACy5.tif		CGHSampleBCy3.tif		CGHSampleACy5.tif sea		CGHSampleBCy3.tif sea	
Plots	Normalized Data	Overview	Whole Genome	Chromosome	CGH Report	Summary	Analysis		
G...	Chrom...	Ba...	Ref...	Exp...	Difference	Significan...			
RP11...	X	315611...	0.01921...	1.29158621	2723665	SIGNIFICANT			
RP11...	2	1519179...	-0.0962...	-0.09310...	0.0031804256	UNCHANGED			
RP11...	5	672744...	-0.7194...	-0.550484	0.1689735	UNCHANGED			
RP11...	20	453371...	1.45102...	1.8827416	0.43171883	UNCHANGED			
RP11...	7	156680...	1.41081...	1.839704	0.42898936	UNCHANGED			
RP11...	16	141408...	0.18330...	-0.26444...	0.44775835	UNCHANGED			
RP11...	9	795804...	-0.2792...	0.6550926	0.9343169	UNCHANGED			
RP11...	10	508574...	-0.0875...	-0.570786	-0.48319483	UNCHANGED			
RP11...	2	122387...	2.88228...	3.5640779	0.6817961	UNCHANGED			
RP11...	X	295933...	3.198697	3.675418	0.47672066	UNCHANGED			
RP11...	5	158743...	0.24620...	0.58295...	0.33675843	UNCHANGED			
RP11...	X	144452...	1.56000...	1.6446016	0.08459973	UNCHANGED			
RP11...	5	372096...	1.22082...	0.8942299	0.3265946	UNCHANGED			
RP11...	10	116909...	0.80958...	1.4856436	0.6760549	UNCHANGED			
RP11...	5	669081...	0.01065...	-0.63696...	0.6476203	UNCHANGED			
RP11...	12	437544...	-2.4064...	-3.33061...	-0.92414635	UNCHANGED			
RP11...	5	156273...	3.25066...	4.0219164	0.7712507	UNCHANGED			
RP11...	6	104150...	0.26463...	-0.35535...	-0.61998814	UNCHANGED			
RP11...	3	237598...	2.45870...	3.126356	0.66764945	UNCHANGED			
RP11...	2	899159...	-0.4727...	-0.92840...	-0.45569374	UNCHANGED			
RP11...	11	107487...	-0.1000...	0.24771...	0.3478012	UNCHANGED			
RP11...	X	126442...	0.06606...	-0.41005...	-0.4761293	UNCHANGED			
RP11...	4	871676...	-0.5915...	-1.63204...	-1.0404966	SIGNIFICANT			
RP11...	6	143160...	0.25022...	0.322477	0.07225031	UNCHANGED			
RP11...	6	228216...	0.77019...	1.4616354	0.6914431	UNCHANGED			
RP11...	17	708428...	-0.7693...	-1.30523...	-0.5358994	UNCHANGED			
RP11...	1	204192...	-1.6766...	-1.72217...	-0.04550457	UNCHANGED			
RP11...	13	103782...	3.49133...	3.7791252	0.28778958	UNCHANGED			
RP11...	X	628743...	-0.1463...	0.7881948	0.93449837	UNCHANGED			
RP11...	7	144390...	0.43217...	1.7974485	1.3652742	SIGNIFICANT			
RP11...	14	344048...	-0.5366...	-0.66396...	-0.12732777	UNCHANGED			
RP11...	1	107639...	-0.3137...	-0.51408...	-0.20036542	UNCHANGED			
RP11...	11	815047...	0.53213...	0.5807904	0.04865569	UNCHANGED			
RP11...	X	161802...	0.42773...	-0.16081...	-0.58854544	UNCHANGED			
RP11-40W8									
Save Report									

## Expression Analysis Results Panel



The required CGH Parameters include the following:

**Significance Threshold:** This is a p-value below 1, related to the probability that a region is an amplification or deletion based on the levels chosen.

**2 Deletions <:** This is the log ratio threshold for which regions of two deletions are called. This is expected to be a negative value.

**1 Deletion <:** This is the log ratio threshold for which regions of just 1 deletion are called. This is expected to be a negative value.

**1 Amplification >:** This is the log ratio threshold for which regions of more than one copy are called. This is expected to be a positive value.

**2+ Amplifications >:** This is the log ratio threshold for which regions of two or more copies are called. This is expected to be a positive value.

**Default Chromosome Plot Bounds:** These are the minimum and maximum log ratio values displayed on the CGH plots. Note that any value falling outside this range will be represented as a small “x” mark at the left or right edge of the plot

area. These values have no effect on the call algorithm and are exclusively for display purposes only.

*Note: Please refer to the CGH Tutorial later in the manual for additional details and usage examples.*

## CGH Analysis Mode Settings Panel

When the CGH Analysis is complete, six new tabs appear in the image display panel. The tabs containing plots allow users to print or save the information. The tabs containing numerical data can be saved to tab delimited text files.



The **Normalized Data** panel is produced when the analysis is run and is identical to that produced in the Expression mode, but includes the Base Pair and Chromosome annotations along with any custom annotation fields.

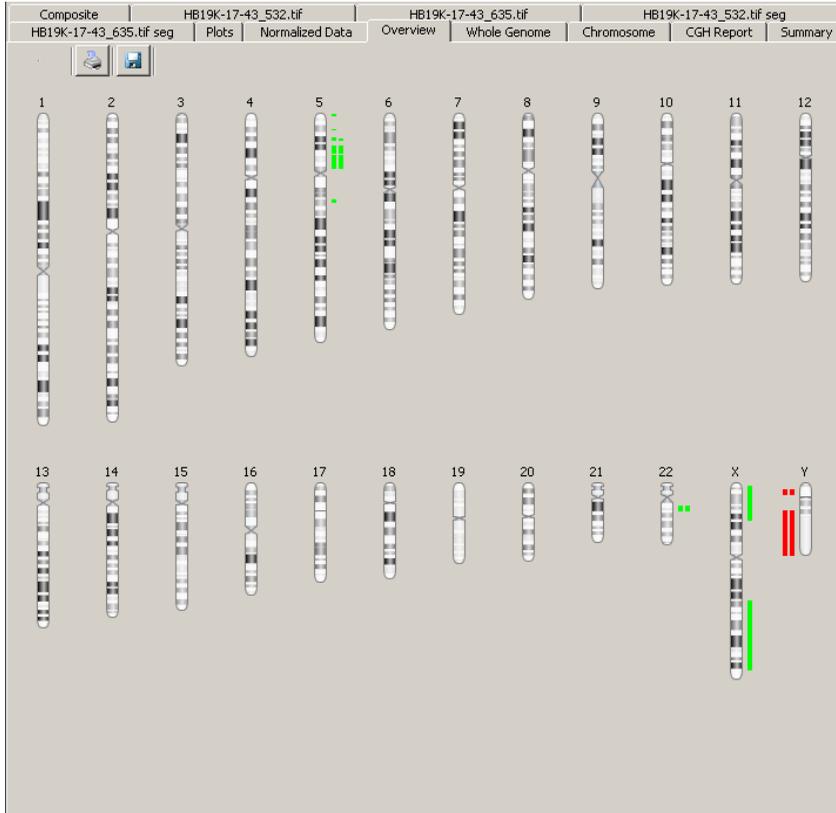
Composite			CGHSampleACy3.tif				CGHSampleACy5.tif			CGHSampleACy3.tif seg		
CGHSampleACy5.tif seg			Plots		Normalized Data		Overview	Whole Genome	Chromosome	CGH Report	Summary	
F...	MR	MC	...	...	GeneID	Chromos...	Base Pair	Flag	N. Signal Mean, ch1		N. 5	
A	1	1	1	1	RP11-124H12	X	31561116	0	9.083269		8.7577	
A	1	1	1	2	RP11-14M15	7	144390069	0	7.821996		7.9313	
A	1	1	1	3	RP11-91J20	X	74278977	0	9.530163		9.2771	
A	1	1	1	4	RP11-150L20	18	32257431	0	8.119185		8.1831	
A	1	1	1	5	RP11-31H2	13	24103018	0	10.189214		10.414	
A	1	1	1	6	RP11-151D4	X	96737813	0	7.841626		7.1432	
A	1	1	1	7	RP11-205C23	13	72218390	0	9.156329		9.2064	
A	1	1	1	8	RP11-151N18	14	71457074	0	7.2463737		7.0715	
A	1	1	1	9	RP11-274K8	X	27669953	0	9.632531		9.2863	
A	1	1	1	10	RP11-162E1	4	139575905	0	6.7721334		6.6680	
A	1	1	1	11	RP11-110J14	X	114124406	0	9.815309		9.4016	
A	1	1	1	12	RP11-163A20	19	10476965	0	6.8393		6.6684	
A	1	1	1	13	RP11-53F19	13	39905442	0	9.695714		9.8671	
A	1	1	1	14	RP11-163L23	13	51473628	0	8.53726		8.4690	
A	1	1	1	15	RP11-79I4	13	73284060	0	9.736278		9.8270	
A	1	1	1	16	RP11-164G18	11	84184899	0	7.587011		7.4353	
A	1	1	1	17	RP11-173E7	15	34024177	0	9.493796		9.5034	
A	1	1	1	18	RP11-162F14	15	73956624	2	5.6447306		5.2501	
A	1	1	1	19	RP11-13J23	15	58718215	0	9.026563		9.1110	
A	1	1	1	20	RP11-163E12	6	24795721	0	8.454347		8.2466	
A	1	1	1	21	RP11-60J21	15	78566480	0	9.119134		9.1934	
A	1	1	1	22	RP11-163O13	6	52904636	0	7.6761723		7.5775	
A	1	1	1	23	RP11-2K1	14	30669243	0	8.676882		8.6624	
A	1	1	1	24	RP11-164H13	14	95199492	0	8.063788		8.0674	
A	1	1	1	25	RP11-2D17	15	40014739	0	9.246788		9.2449	
A	1	1	1	26	RP11-183H19	8	72654725	0	8.286472		8.2031	
A	1	1	1	27	RP11-100N8	15	61375336	0	9.584351		9.7574	
A	1	1	1	28	RP11-184M3	12	43754451	0	8.527163		8.3924	
A	1	1	1	29	RP11-131K19	15	85959354	0	9.382862		9.4900	
A	1	1	2	1	RP11-185M20	2	15197943	0	7.064263		7.3183	
A	1	1	2	2	RP11-26M6	14	34404822	0	9.247893		9.2426	
A	1	1	2	3	RP11-187N24	4	119026135	0	9.020368		8.9836	
A	1	1	2	4	RP11-182C19	5	116875069	0	9.306953		9.3956	
A	1	1	2	5	RP11-184B6	9	87724919	0	8.834726		8.7939	
A	1	1	2	6	RP11-188F5	9	113307084	0	10.176981		10.044	
A	1	1	2	7	RP11-185B11	5	150687195	0	8.262149		8.6137	
A	1	1	2	8	RP11-71F8	10	92575444	0	9.860801		9.9372	
A	1	1	2	9	RP11-186B12	10	7623848	0	9.21714		9.2577	
A	1	1	2	10	RP11-252C8	12	102581524	0	9.918912		10.066	
A	1	1	2	11	RP11-188E14	5	23621470	0	9.971543		9.8563	
A	1	1	2	12	RP11-67P19	6	95111865	0	8.787525		8.8067	
A	1	1	2	13	RP11-212P7	7	127674031	0	10.067266		10.051	
A	1	1	2	14	RP11-29D1	10	8461380	0	8.788827		8.9664	
A	1	1	2	15	RP11-21H8	13	50211927	0	6.433919		6.0816	
A	1	1	2	16	RP11-156N9	10	129311918	0	8.669387		8.7141	

Meta Row= 1

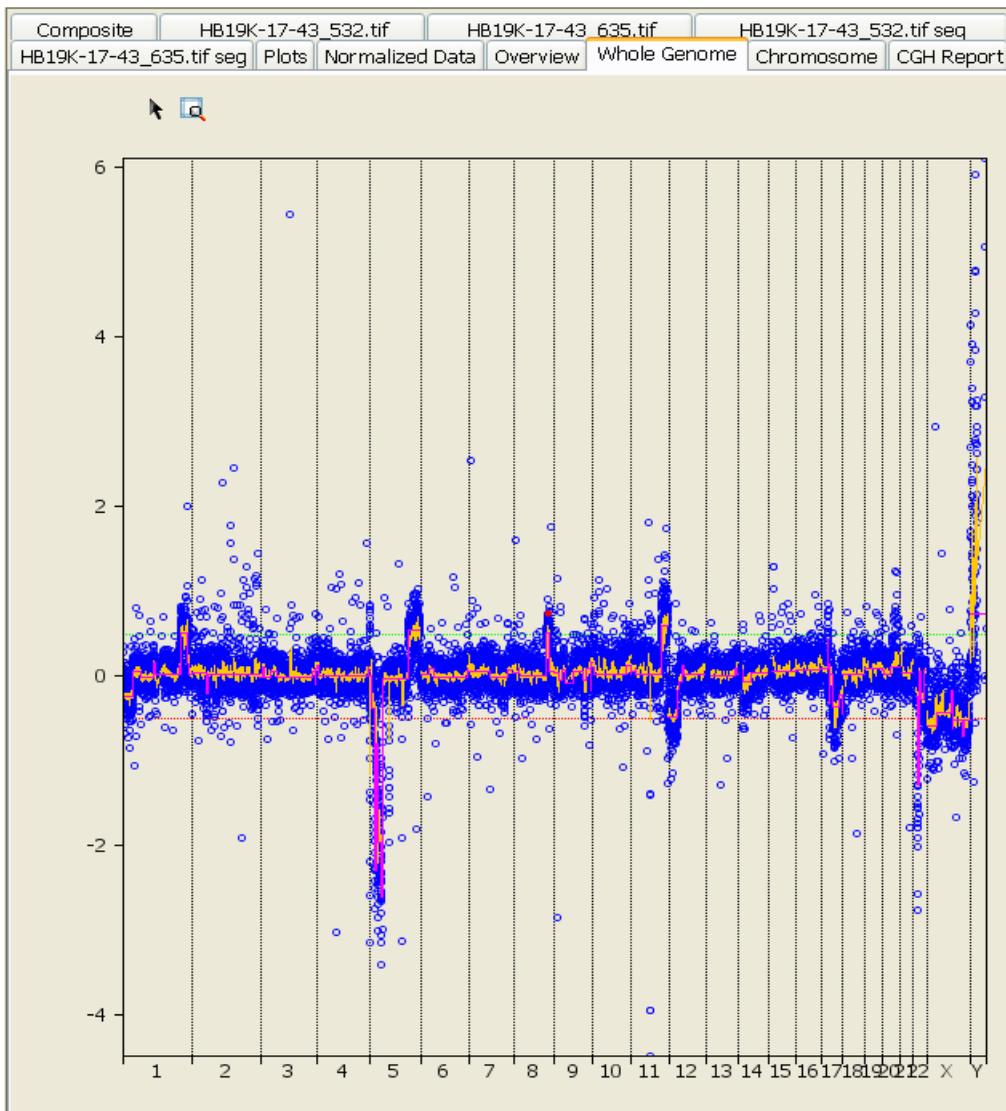
Save Report

The **Overview** panel displays the organism's chromosomes, depending on the organism selected from the drop-down list in the Analysis tab, with amplification/deletion calls marked as shown. Amplifications are marked by a green line to the right of the ideogram and deletions are shown as red marks on the left side of the ideogram. By clicking on any of the ideograms, the chromosome will be

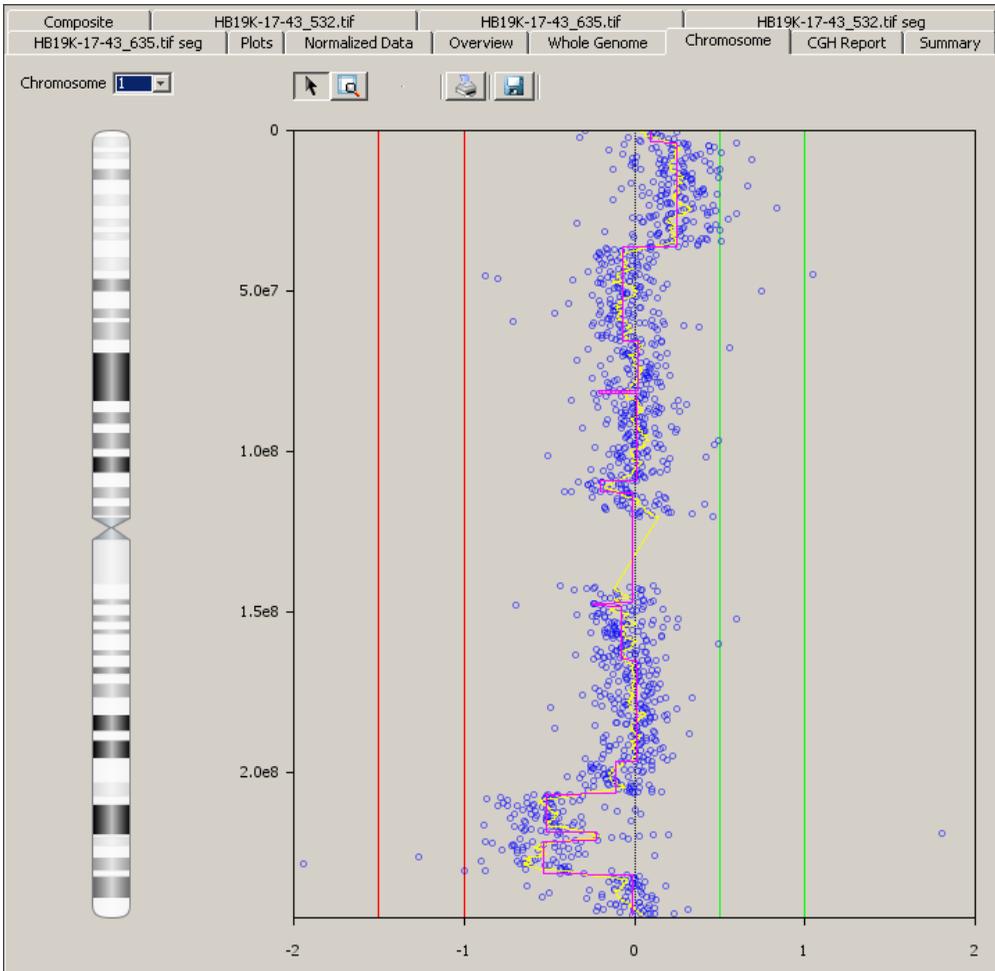
selected and the display will change to the single Chromosome view described later in this section.



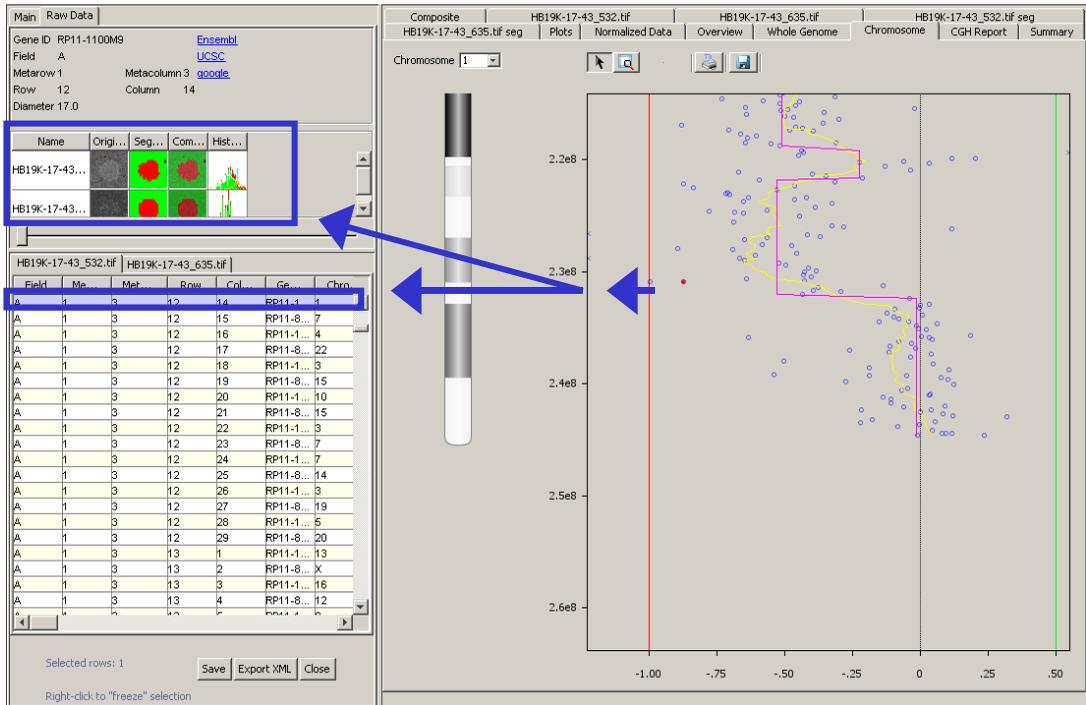
The **Whole Genome** panel displays the entire genome with chromosomes lined end to end as shown below. Each open circle corresponds to a probe on the array. If the same probe is spotted in multiple locations (replicates), the circles shown in the plots below are the combined value of all the replicates. The “call” thresholds for single and higher copy gain or loss are shown as green and red horizontal lines, respectively. The yellow line in the plot depicts a moving average value and the magenta colored line is the output of BioDiscovery’s call algorithm.



The **Chromosome** panel enables the user to zoom in and view selected chromosomes as shown. The “zoom” tool can be used to zoom into an area on the plot or on the ideogram.



A very useful feature in all the graphic plots is the ability to select a point (open circle in the plot) and then be able to “drill-down” in other screens as shown below. As can be seen, the original spot image, segmentation overlay, and quantification values are all immediately accessible. If there are multiple spots combined together, all the spots are selected in the quantification table at the bottom of the “Raw Data” tab.



Another useful feature of the program is the ability to launch a web browser and immediately go to Ensemble, UCSC, or Google for a search on the selected probe ID.

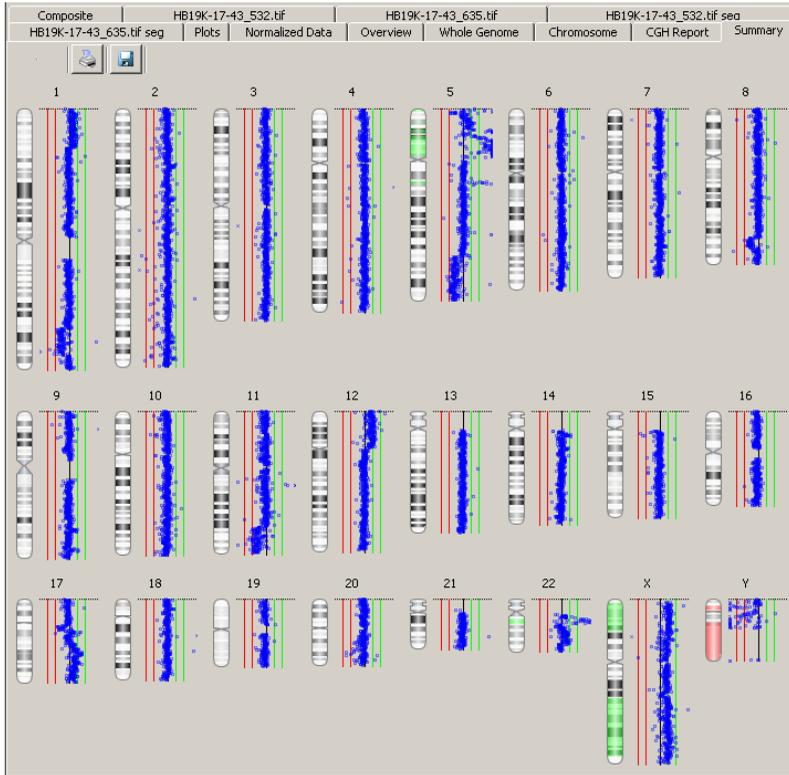
The **CGH Report** results table can be saved in a text file by clicking on the “**Save Report**” icon at the bottom of the panel. The results contain fields for Gene ID, Chromosome, Base Pair, Value (normalized, transformed, and replicate combined ratio values), Cluster Value and Algorithm Calls.

CGH Sample BCy3.tif seg	Plots	Normalized Data	Overview	Whole Genome	Chromosome	CGH Report	Summary
Gene ID	Chromosome	Base Pair	Value	Cluster Value	Call		
RP11-718D19	1	3927454	0.42118334770202637	0.42118334770202637	0.0		
RP11-629G14	1	8659133	-0.6035451889038086	-0.6035451889038086	0.0		
RP11-353D18	1	12138316	-0.530672550201416	-0.530672550201416	0.0		
RP11-799P1	1	16256010	0.23256301879882812	0.23256301879882812	0.0		
RP11-875G4	1	17648591	-0.3046150207519531	-0.3046150207519531	0.0		
RP11-14K12	1	18184421	-0.12620019912719727	-0.12620019912719727	0.0		
RP11-145C4	1	18771772	-1.3313205242156982	-1.3313205242156982	-1.0		
RP11-1058B2	1	20249583	-0.3050556182861328	-0.3050556182861328	0.0		
RP11-57F20	1	23544768	0.22323107719421387	0.22323107719421387	0.0		
RP11-16P4	1	34053907	0.4997291564941406	0.4997291564941406	0.0		
RP11-1128N1	1	38412432	0.2555885314941406	0.2555885314941406	0.0		
RP11-1146G4	1	81044501	-0.6169955730438232	-0.6169955730438232	0.0		
RP11-747H21	1	84718902	0.2985529899597168	0.2985529899597168	0.0		
RP11-246O5	1	88884053	-0.24956512451171875	-0.24956512451171875	0.0		
RP11-89P12	1	99238716	0.09523129463195801	0.09523129463195801	0.0		
RP11-813L21	1	102517234	0.25427746772766113	0.25427746772766113	0.0		
RP11-24J14	1	107639467	0.07250690460205078	0.07250690460205078	0.0		
RP11-355G24	1	107852158	0.3029289245605469	0.3029289245605469	0.0		
RP11-252A16	1	108159981	0.27843761444091797	0.27843761444091797	0.0		
RP11-605I3	1	154325812	-0.7407321929931641	-0.7407321929931641	0.0		
RP11-108I23	1	163025013	-3.4547557830810547	-3.4547557830810547	-2.0		
RP11-1102D8	1	163548394	-0.02298450469970703	-0.02298450469970703	0.0		
RP11-51N9	1	182246372	-0.37342333793640137	-0.37342333793640137	0.0		
RP11-80N24	1	195477228	-1.358424186706543	-1.358424186706543	-1.0		
RP11-288G13	1	202146767	-0.383756160736084	-0.383756160736084	0.0		
RP11-1123E2	1	204192811	-1.4044792652130127	-1.4044792652130127	-1.0		
RP11-142G24	1	216148774	0.9857568740844727	0.9857568740844727	1.0		
RP11-111N3	1	216258282	0.08598709106445312	0.08598709106445312	0.0		
RP11-42C8	1	216557598	-0.6951894760131836	-0.6951894760131836	0.0		
RP11-124J24	1	217175606	1.1878228187561035	1.1878228187561035	2.0		
RP11-956N16	1	218473004	-0.34867191314697266	-0.34867191314697266	0.0		
RP11-76K24	1	219911336	0.23750877380371094	0.23750877380371094	0.0		
RP11-52F17	1	224323596	-0.07935738563537598	-0.07935738563537598	0.0		

Value = 0.2985529899597168

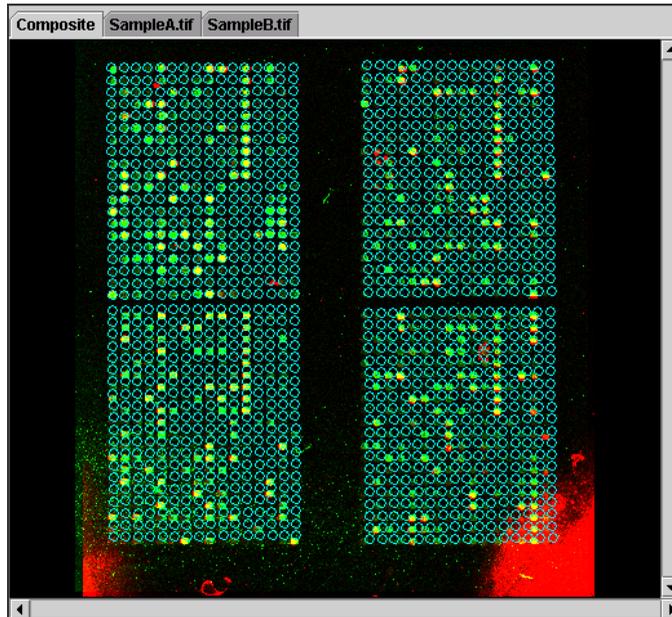
Save Report

The **Summary** panel shows a view of all the chromosomes individually shown in the Chromosome Panel.



## 1.3 Image Display Panel

This panel displays loaded images. A tab appears along the top of the panel for all current 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 that ImaGene can load is limited based upon the computer hardware specifications.



All image manipulation tools, such as Zoom and Rotate, 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.*

## Segmentation Tabs

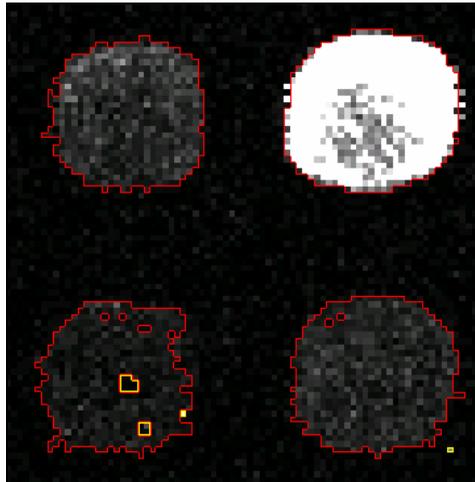
Once the image(s) have been quantified, additional tabs, one for each image plus one for plots will appear along the top of the image display panel. The segmentation tabs (with the name of the image file name followed by “seg”) displays the segmentation as it had been performed for the given image. From this view, you have the ability to see the macro view of the image and analyze any large defects and the corresponding segmentation. Typical usage 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:*

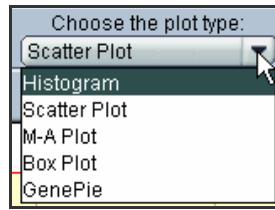
- Red – All pixels within the red lines are signal values.
- Yellow – All pixels within the yellow lines are ignored pixels.



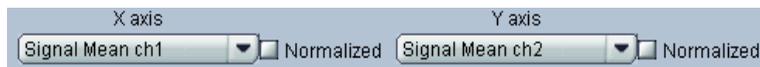
*Note: The description and use of colors between the Preview Panel and the segmentation tab vary slightly. The segmentation tab includes the use of yellow to indicate ignored pixels whereas the Preview Panel uses black to indicate ignored regions. Also, the segmentation tab does not use a color to indicate the background region; however, within the Preview Panel, background values are indicated by a green color. The differences between the two displays are accounted by the fact that per customer requests, the segmentation tab is designed to prevent eyestrain during extensive visual inspection.*

## Plots Tab

This tab appears only after image has been quantified. It contains a set of useful data visualization tools that can aid the analysis process.



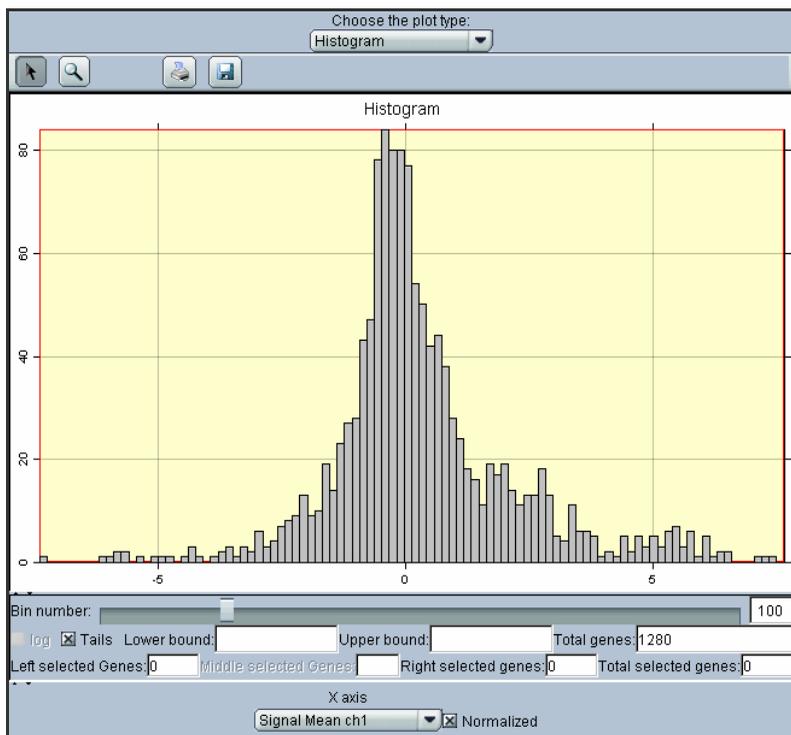
Any measurement available from the results table can be plotted in one of the tools: Histogram, Scatter Plot, M-A Plot, Box Plot, and GenePie. Once a desirable type of visualization is chosen, choices of the measurements become available. Measurement coming from a specific channel (image) will be denoted by suffix “ch1”, “ch2” etc. Also normalization options are available if signal mean, median or mode is chosen for the plot.



Change in measurement selection or normalization option will be followed by update of the current plot.

Any plot can be printed or saved as an image file.

- **Histogram.** Histogram allows users to choose only one measurement. Normalized data can be displayed if the **Normalized** box is checked.

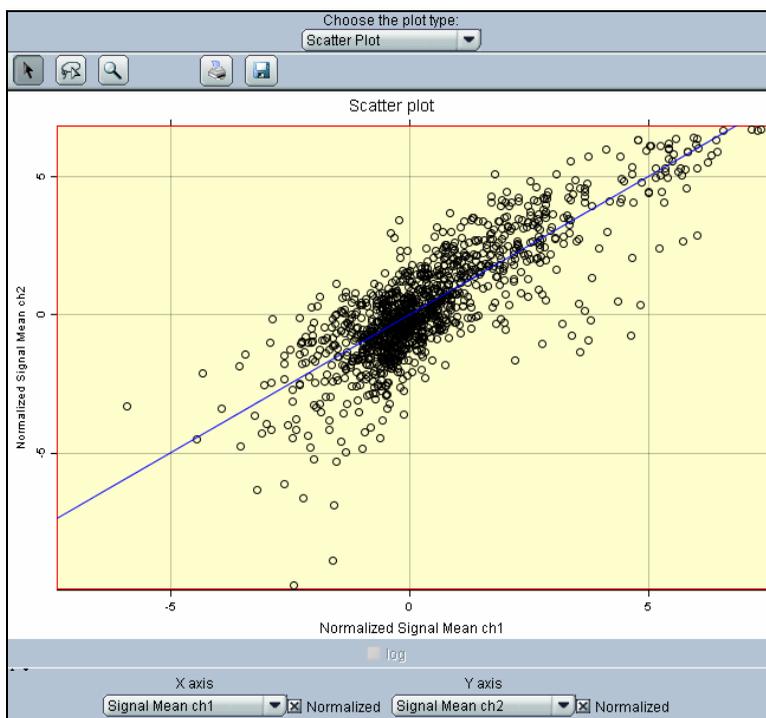


The histogram demonstrates distribution of the measurement values over their domain. One can make an interval selection on the histogram and visualize how many genes fall into that selection. By changing selection of “Tails” check box genes will be selected either inside the interval or outside. Toggling between untransformed measurements and log-transformed measurements can be accomplished by using “log” check box (however, this option will become available only if logarithm transformation was not applied to the chosen measurement during normalization). Changing bin density, printing the histogram and saving the image (this feature is especially useful for publications) can also be accomplished. The Histogram provides not only the ability to plot any of the measurements available in quantification table, but also provides access to useful values such as inter-channel signal ratios (including normalized). Using these values in combination with the “logarithmic” option, a histogram of a log-ratio (natural logarithm will be used) can be obtained. Selection of the tails of

such distribution provides 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 analysis of the selected spots in full scale. Ch1 and ch2 stand for Channel 1 and 2 that refer to the order of the loaded images. For example, the first image in the image panel corresponds to Channel 1.*

- **Scatter Plot.** Scatter plot offers visualization of one measurement plotted against any other. The normalized measurements can be used by checking the **Normalized** box next to either measurement.

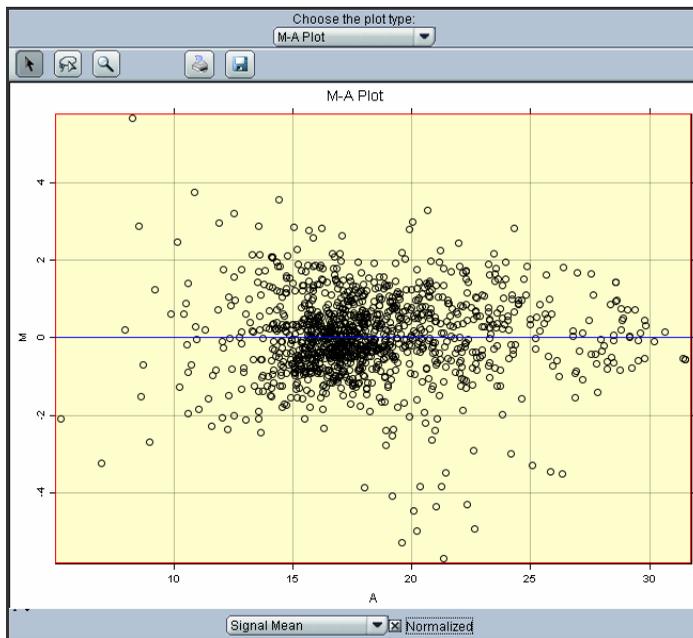


If multiple images are loaded, the user can plot not only two measurements within one channel, but also two measurements belonging to different channels. For instance, plot of signal means can show the difference between fluorescence characteristics in two channels or even

can give a rough idea about regulation of the genes. Selection of 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.

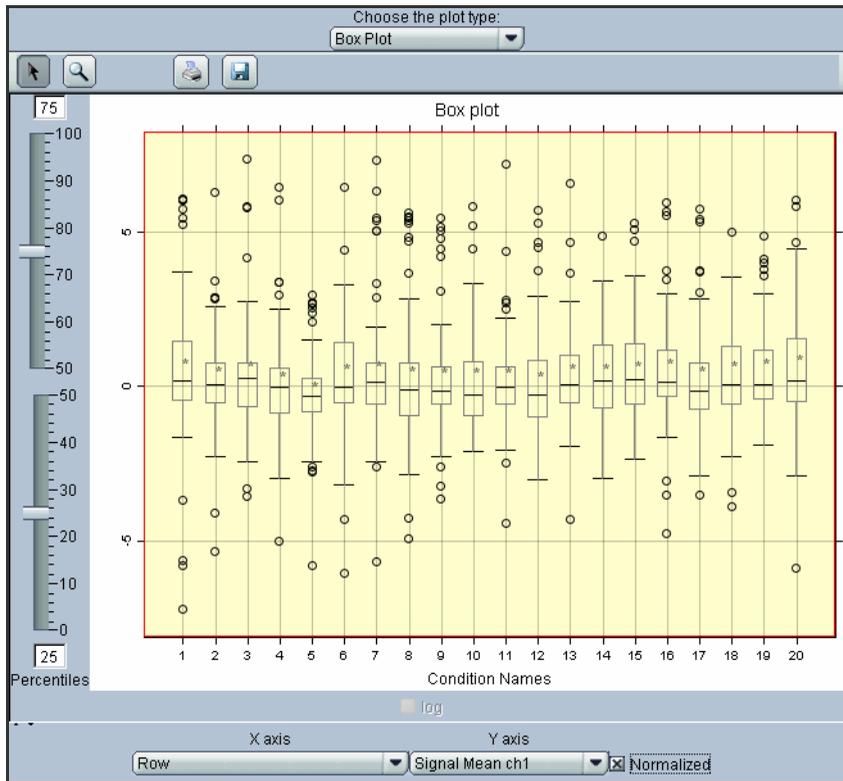
*Note: More spots can be added to the current selection by simply continuing to select other groups of spots. To start a new selection, right-click on the plot to clear the previous selection.*

- **M-A Plot.** M-A plots can show the intensity-dependent ratio of raw microarray data. The plot uses M as the y-axis and A as the x-axis where:  $M = \text{Log}_2(R/G)$  and  $A = \frac{1}{2} \text{Log}_2(R*G)$ . This plot is utilized to check how good the normalization method is. Ideally, if the normalized data is intensity independent, M-A plot will show the data points distributed along the horizontal zero line (Blue color). For this reason, the **Normalized** box should be checked.

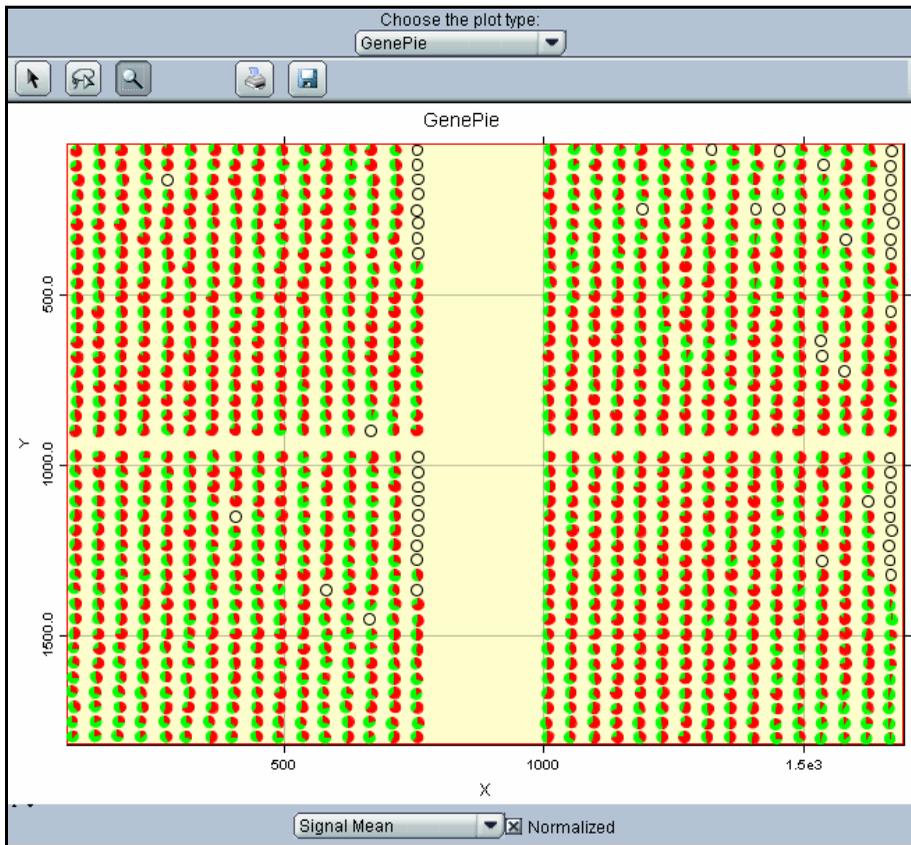


- Box Plot.** This type of plot is utilized to visualize the measurement's distribution between different categories of spots (belonging to different subgrids, different rows, having different flags, etc). The different categories may be used for X axis and different measurements for Y axis and with or without normalization. The box visualizes lower and upper percentiles of distribution. These percentiles can be changed using scroll bars on the left hand side. Box plot also visualizes distribution outliers. This is utilized when looking for abnormalities in the data due to special or other categorization.

*Note: for more detailed description of Box plot refer to GeneSight manual provided with ImaGene.*



- GenePie.** This plot displays the spot's expression values for each channel as portions of a circle. The colors within a pie correspond to the individual channels. The most common use of the GenePie chart is to plot differential expression patterns between channels. Different measurements may be used for GenePie plot with or without normalization. The values on X- and Y-axes represent the spot coordinates in pixels. The empty circles represent the spots where the expression value is not available (NaN) for either or all the channels, usually after taking log ratios or normalization.



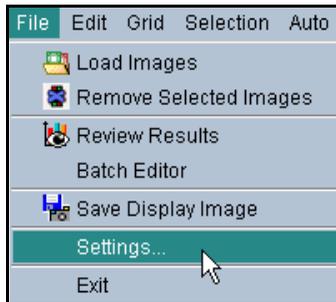
*Note: any selection made using one of the plots will be common for all other plots, results table and image tab. The one exception is if a group of spots is*

*selected using Scatter plot, Box plot or Results table, the selection will not be visible on Histogram plot because of its specifics. But if the selection is made using the Histogram plot, it can be viewed in all other tools.*

*Note: Mouse-over any of the data elements on any plot and see a tool-tip containing short info about gene ID and measurement values.*

## 1.4 ImaGene Parameter Settings Window

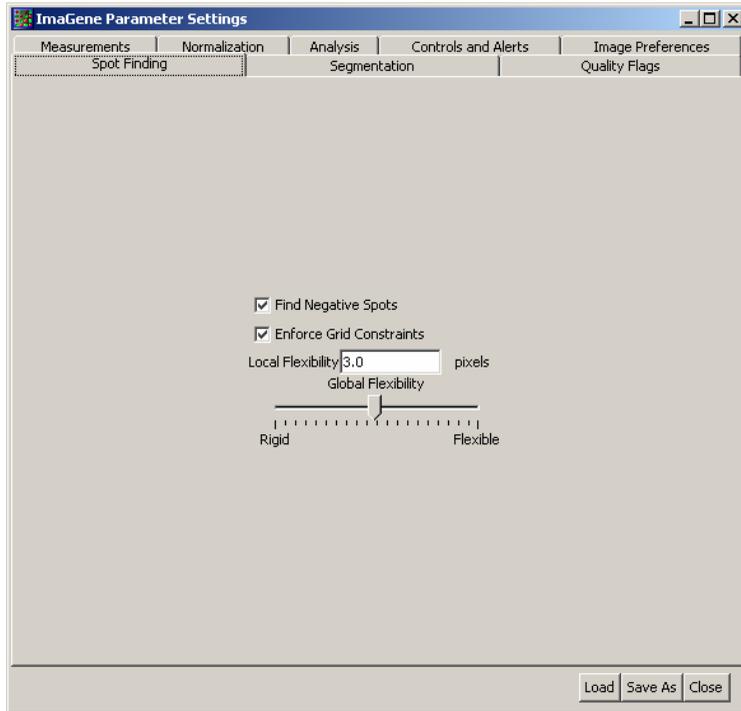
ImaGene encapsulates most of its parameters and settings within one common interface, the ImaGene Parameter Settings Window. Within this window are tabs, which control virtually all aspects of the array image analysis. From initial spot finding settings to complicated auto reporting of alert values,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



The window is organized into several tabs which group related parameters together. The tabs available and their parameters include:

### Spot Finding

Spot finding involves the localization of the array signal as printed on the array medium. Due to the warping of the medium or potentially bent pins during printing, spots and sometimes entire sub-grids 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 inaccurate.



The following parameters affect how ImaGene performs spot finding:

- ***Find Negative Spots*** – While not a problem for most arrays, negative spots can potentially cause problems for ImaGene spot finding. If the image may contain negative spots, BioDiscovery recommends leaving this option checked. When Find Negative Spots is enabled, ImaGene will look for negative spots while also looking for regular spots.

*Note: If all the signal values within the image are negative due to specific scanner settings, then it may be a necessity 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 ImaGene 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, ImaGene will use Local and Grid Flexibility when performing spot finding. When not selected, ImaGene will perform spot finding with no constraints. Please see below for additional information regarding Local and Grid Flexibility.

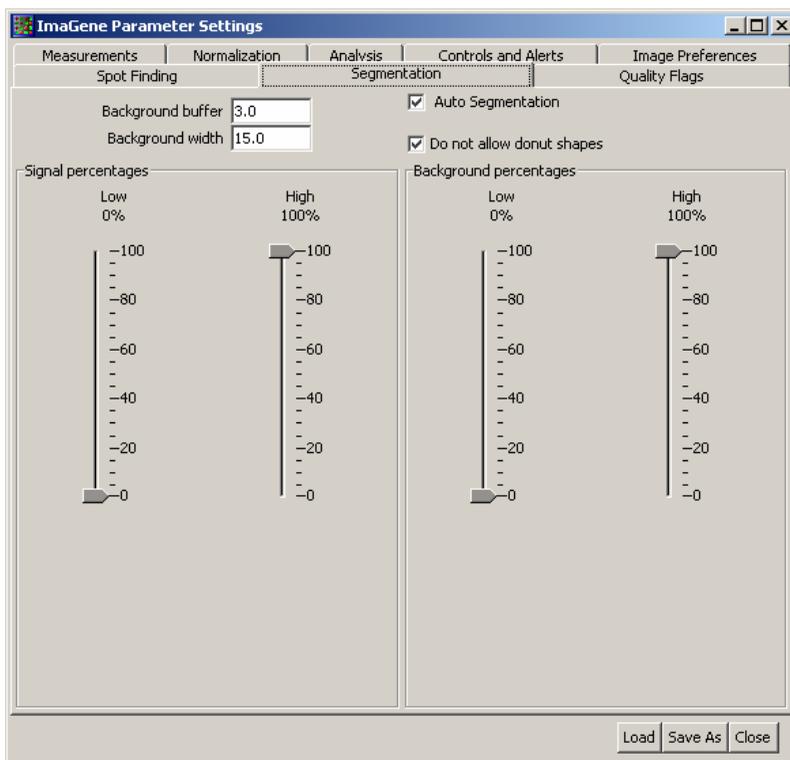
*Note: Under most circumstances, enforce grid constraints should be selected as this will limit the movement of circles during spot finding and help prevent erroneous spot finding due to dust and other contaminations.*

- **Local Flexibility** – Local Flexibility defines the radius, measured in pixels that ImaGene is allowed to search for spots. The origin for the search is the initial spot location as determined by grid placement. From here, ImaGene 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 ImaGene should deform the grid to match a given set of spots. The measurement is qualitative based on the unique properties of the image. Most users should set this to the middle values.

*Note: Use 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 effects.



ImaGene supports two primary types of segmentation, manual and automatic. With Manual Segmentation, the appropriate values must be specified for ImaGene to use when analyzing the image. Often these proper values can be arrived at through experimentation or experience. Alternatively, through a robust patented statistical approach, ImaGene can calculate the appropriate segmentation parameters automatically. Under normal circumstances, BioDiscovery recommends use of the Automatic Segmentation due to its superior contamination removal capability. Regardless of the segmentation used, both methods assist in providing the highest quality data available.

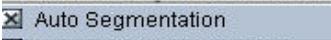
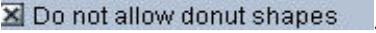
Listed under the Segmentation Tab are the following parameters:

- **Background Buffer** - 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. ImaGene should not be used with no background values as these values are required in numerous quality measurements.

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

- **Auto Segmentation** - . Selecting this option will turn on BioDiscovery's patented automatic segmentation algorithm. Under most circumstances, this should be selected. Under Auto Segmentation, the only parameter that ImaGene will use is the Background Buffer. The typical value should be slightly less than ½ the radius. Under Auto Segmentation, the slider bars for the signal and background percentages become disabled.
- **Do not allow donut shapes** - . When this option is selected, ImaGene 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 than signal's. This option is available for auto segmentation only.
- **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 value can be set as high as 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 smaller value, like 95%. This way, the pixels associated

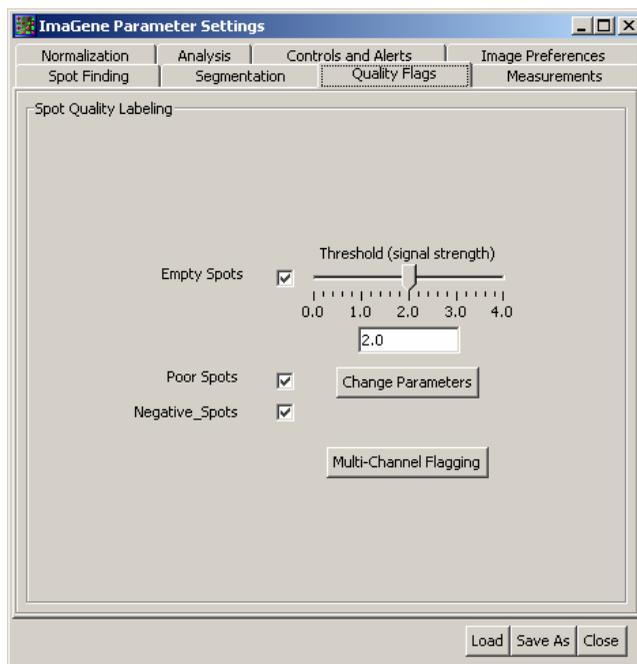
with a dust particle, assuming they will be fluorescing at a high intensity level, will be filtered out.

- **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%

*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 a more accurate segmentation.*

## **Quality Flags**

Once the segmentation is completed and the spot expressions are extracted, the next question would be how reliable is the expression data. In ImaGene, the user can use various types of automated spot flagging schemas to help remove suspicious spots from consideration. Alternatively, manual flags of different types can be applied for quality control.

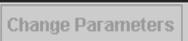


- **Empty spots**  – this option is utilized to automatically flag low-expressed spots or missing spots. This tool needs to have a sensitivity threshold determined. For each spot the following ratio is compared to the threshold.

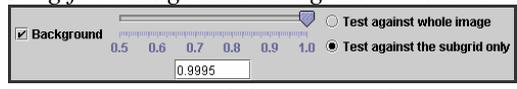
$$R = \frac{\text{signal mean} - \text{background mean}}{\text{background standard deviation}}$$

If for some spot this ratio appears to be lower than the threshold value, the spot will be flagged with a green “X” mark on image. It will also have flag value 2 assigned in the corresponding column in results table. Threshold values can be chosen anywhere between 0 and 4. The user can either use a slider on Quality Flags panel of Settings dialog box or type the threshold value directly in the text field.

- **Negative spots**  – This tool will automatically flag any spot with the signal mean lower than background mean. A green “.” symbol will be displayed on this spot on the image and corresponding flag in the results table will have value of 4.

- **Poor spots**   – ImaGene has a complex tool for detecting low-quality spots. It is designed to accommodate various possible interpretations of spot quality. This tool includes 7 different criteria for spot fail/pass test that can be used in combination as well as separately. To change configuration of this tool, click “Change Parameters” button  on Quality Flags panel of Settings dialog box. Once you click it, the new dialog box will pop up, and you will be able to select any combination of the following flagging tools.

1. *Flag for background being contaminated*



The top-most check box on quality parameters dialog corresponds to the flagging method based on background abnormality detection. The background level of each individual spot is compared to the local background level distribution over the image through a t-test. The resulting p-value is subtracted from 1 and is called “confidence in contamination presence”. This threshold can be set at any level between 0.5 and 1. A flag will be issued whenever a particular contamination confidence goes over that threshold. Background mode is used as a background level estimate. Background level can be tested against the information from all spots on the image or against information extracted only from those spots that fall within the same subgrid as the current spot. The latter approach can be useful if subgrids are large enough (>100 spots) and some kind of acceptable background level change is observed from one subgrid to another.

2. *Flag for the signal being contaminated*



The next check box corresponds to the signal abnormality detection method. This approach was designed to flag any spot with unusually high volatility in pixel-wise intensities within the signal area. For every individual spot, the signal variance is compared to the distribution of signal variance measurements across the image. However, before a t-test analogous to the one for background contamination is performed, spots are grouped according to their signal mean measurements. This is performed to compensate for a well-known observation that intra-spot intensity volatility tends to be different for different expression levels. This method will ensure that each spot is tested only against spots similar in their expression. The resulting confidence number is then compared to the threshold similar to the previous procedure. Additionally, one can check “combine signal test and background tests” and two confidence numbers will be added together with equal weights 0.5 and compared to the background contamination threshold.

3. *Flag for high ignored percentage*

Ignored percentage

As mentioned before, some pixels may not be assigned either to the signal or to the background in the presence of local contamination. Such contamination will be excluded from the measurements and called an “ignored” region. The ignored percentage flagging works in the following manner: It computes the area of ignored regions directly neighboring (“touching”) the signal area. If it is denoted as a  $I$ , then the following ratio is computed.

$$R = \frac{I}{I + S} \times 100\%$$

“ $S$ ” is equal to the signal area.

If for some particular spot, “ $R$ ”, is higher than the pre-set threshold, this spot gets flagged for low quality.

4. *Flag for high open perimeter percentage*

Open perimeter percentage

This tool was designed to capture a specific type of spot deformation. It computes the percentage of the signal perimeter that touches the border of rectangular snip. Such a deformation can appear if the spot is significantly shifted from its expected position or if its shape has an abnormal form. Whenever

the percentage crosses the pre-set threshold, the spot is flagged as low quality.

5. *Flag for abnormal shape regularity*  **Shape regularity** This algorithm computes shape regularity – the measure that characterizes the closeness of a spot’s border to a circular shape. The first step of this algorithm inscribes a signal area of a spot into a circle. Then the number of non-signal pixels that fall within this circle is computed and divided by the area of the circle. This ratio is then subtracted from 1 (value of “shape regularity”). This variable will range from 0 (highly non-circular shape) to 1 (a perfect circle). Whenever this ratio falls below the pre-set threshold, spot is flagged as low quality.
6. *Flag for low Area-to-Perimeter ratio*  **Area-to-Perimeter ratio** The measure of the spot’s quality plays a role similar to shape regularity mentioned in Item 5 above. The area of a spot is divided by a square of spot perimeter and multiplied by  $4\pi$ . As a result, this measure ranges from 0 (highly non-circular shape) to 1 (a perfect circle). Whenever this ratio falls below the pre-set threshold, the spot is flagged as low quality.
7. *Flag for significant offset from expected position*  **Offset** Expected position of a spot in the current grid is computed by fitting least square lines to columns and rows of the grid. Whenever the offset becomes larger than pre-set percentage of the distance between grid nodes, the spot is flagged as low quality.
8. *Flag for saturation.* One may want to flag spots that have significant number of pixels saturated at the level of maximum scanner sensitivity. ImaGene flags a spot for saturation if more than half of spot pixels have their intensity at the level of maximum image intensity.

*Note: The user can use any combination of the above quality measures with arbitrary thresholds. However, the quality parameters dialog has a Default button for a default configuration for the thresholds. Whenever at least one*

of selected criteria fails, the spot is highlighted with “+” mark on image and receives flag value 3 in the results table.

*Note: Multi-Channel Flagging button can be utilized to change the logic for combining automated flags between multiple channels. For all three types of automated flags (Empty, Poor and Negative) a flag can be assigned to a spot if it is flagged in at least one channel or in all channels.*

- **Manual flagging** – Utilized to manually flag spots for the following reasons:
  1. No particular reason – code 1, “X” mark
  2. Empty spot – code 5, “X” mark
  3. Poor spot – code 6, “+” mark
  4. Negative spot – code 7, “-” mark

The manual flag can be placed using a Tag Spots tool from the tool bar . Click on the Tag Spots button, then right-click on the image to select a type of flag. Place flags by clicking on the corresponding spots. The manual flag marks will be red in color.

## Flagging Logic

The unusual spots are flagged with the following logic:

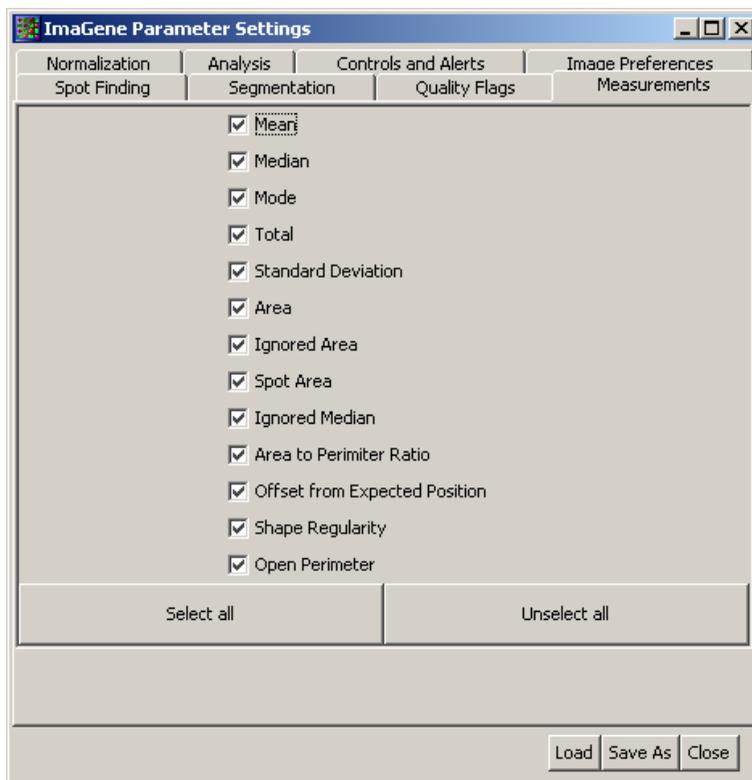


**All flagged:** In order for a spot to be flagged, it has to be found flagged in all the images.

**At least one flagged:** If a spot is found flagged in any one of the images, it will be flagged in all the images quantified together.

## Measurements

Various spot measurements can be computed by ImaGene and outputted to a tab-delimited text file. To select measurements that you are interested in you can use the Measurements panel of Settings dialog.



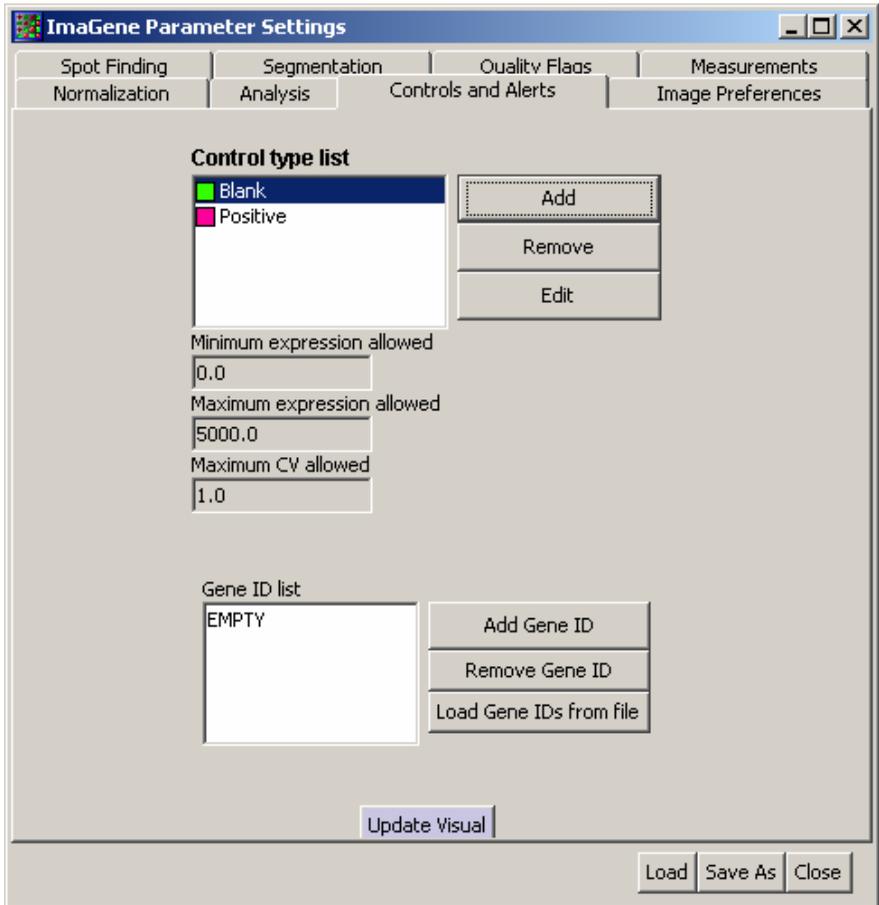
- Mean – signal and background mean intensity
- Median – signal and background median intensity
- Mode – signal and background mode intensity (mode corresponds to the peak of corresponding histogram)
- Total – signal and background total intensity (all pixel intensity summed up)

- 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 areas segmented as ignored and directly touching signal area
- Spot area – ignored area plus signal area
- Ignored median – median of pixel intensity in ignored area
- Area to perimeter ratio – see “Quality Flags” section above
- Offset from expected position – multiple offset measures get outputted, including X-, Y- and overall offset from expected position for the spot’s adjusted circle and spot’s center of mass.
- Shape regularity – see “Quality Flags” section above
- Open perimeter – see “Quality Flags” section above, measure is outputted as a decimal fraction corresponding to Open perimeter percentage (0%=0.0, 100%=1.0).

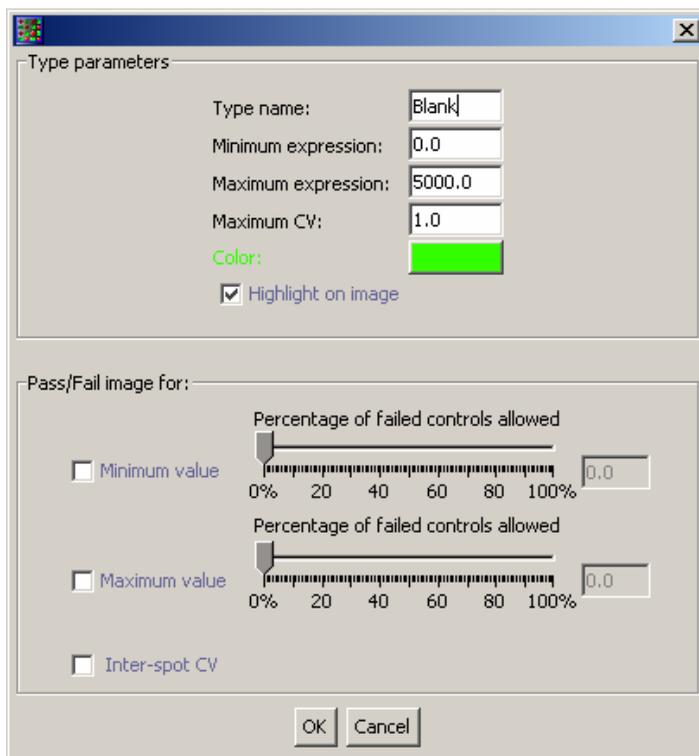
These measurements may be checked and unchecked at any time; results table will be updated immediately.

## Controls and Alerts

ImaGene incorporates a powerful tool for testing expressions of control spots located on image. This tool allows groupings of several gene IDs into a unique control type and to set up multiple test parameters for the type.



Using 'Add' button on the panel, a new control type is created by specifying several parameters.



1. Type name – provide a meaningful name for the type
2. Minimum expression – provide a minimum value for signal mean
3. Maximum expression – provide a maximum value for signal mean
4. Maximum CV – provide a maximum coefficient of variation between signal means for the spots.(should be >0)
5. Highlight on image – specify if all spots of this type are to be highlighted on the image. If this box is checked, the selection of a color for presentation of this type will be enabled. Spots of this type will be marked with squares; the problematic spots corresponding to violation of above thresholds will be highlighted with diamond shapes inside the squares.
6. Pass/Fail image for minimum value – whenever the number of spots belonging to this type that violate the minimum expression criterion become higher than the specified percentage, the warning box will pop up after the quantification procedure is completed or “*Update Visual*” button is pressed.

7. Pass/Fail image for maximum value – whenever the number of spots belonging to this type that violate the maximum expression criterion become higher than the specified percentage, the warning box will pop up after the quantification procedure is completed or “*Update Visual*” button is pressed.
8. Pass/Fail image for inter-spot CV – whenever the inter-spot CV of signal means within this type violates the maximum CV criterion, the warning box will pop up after the quantification procedure is completed or “*Update Visual*” button is pressed.

Whenever the new type is added, its name shows up in the type list. Next step would be to add gene IDs that belong to this type.

- a) Select a type from the list, click on “Add gene ID” button.
- b) A new dialog box will pop up. If the gene ID file was previously loaded, all of those gene IDs will be listed on the right-hand side of the dialog box. A selection is made by highlighting the gene ID and clicking *Add*. Alternatively, a text field may be used to enter a gene ID and click *Add*.
- c) This field can also be utilized to designate a key for assigning gene IDs. The key is created using a “\*” symbol. For example by adding a key “BLANK\*” all gene IDs that start with “BLANK” are assigned to the current control type.
- d) When finished adding gene IDs, click *Close*.

Alternatively, the  button may be utilized to load a list of gene IDs from a text file. Such file can contain a column of multiple gene IDs, one gene ID per line. Once loaded, these gene IDs will be assigned to their corresponding control type. This feature is useful for bringing a list of “selected” genes from some “alien” program (GeneSight for instance) and analyze corresponding spots on microarray images. There is a maximum limit of 500 unique gene names for a given control type.

To highlight all the spots on the image belonging to the specified control types, click “*Update Visual*”. This action will also show the control violations. If the image fails the controls test, a corresponding warning box will pop up. Control type(s) for their corresponding spots will be shown in the results table in a column.

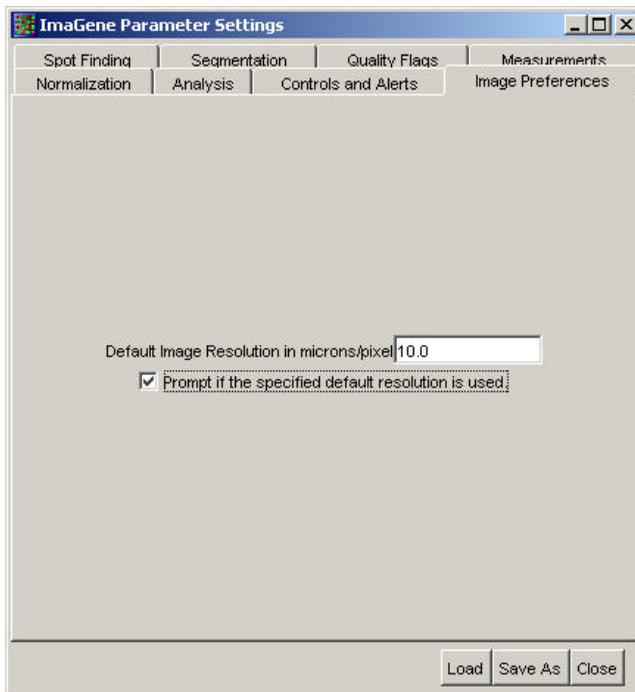
*Note: Control spots play important role in automated grid finding procedure. By specifying all spots that are not meant to contain any signal (negative controls) within a control type named “BLANK” or “EMPTY” you can help ImaGene place grid automatically with higher success rate. Also if you want ImaGene to base its grid placing procedure on bright control spots only, you can create a control type “BRIGHT” and auto grid finding will look for brightest spots on the image to match them to the dedicated bright control spots from “BRIGHT” type.*

*Note: Control spots can be used during background correction and normalization. During background correction you can use dedicated negative control spots to estimate “on-feature” background from their signal values. During normalization you can compute normalization coefficient based on a normalization control type (make sure you create one before normalization) or you can perform normalization based on all spots excluding controls.*

## Image Preferences

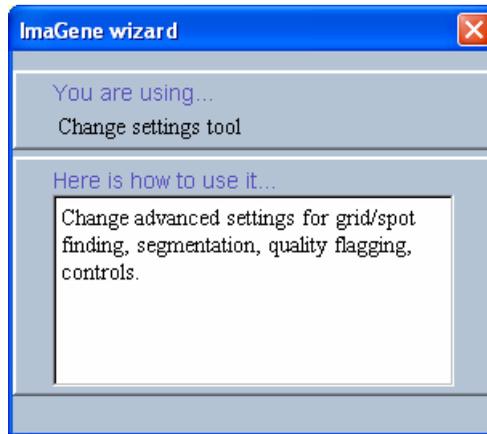
When ImaGene loads an image, it needs to obtain the resolution of the image in order to load array templates correctly (applies to GAL, GEML and MAGE-ML templates). Normally, it reads the resolution information from the header of the image file. However, some scanners do not store this type of information in the image files. In these cases, ImaGene has to apply a default resolution to the images. The Image Preferences tab allows users to enter the image resolution (10.0 micron/pixel by default). It can also prompt users when the set resolution is being used.

*Note: Only when there is no resolution information in the image file should the resolution setting in the Image Preference tab be used. The resolution in the image file will make ImaGene ignore the setting in this tab.*



## 1.5 ImaGene Wizard

The ImaGene Wizard was designed to assist the users to better understand ImaGene tools and aid them in going through the necessary steps. The Wizard can be switched on and off at any moment through the Help menu. Even when the Wizard window is switched on, it does not limit user's access to any of the ImaGene tools.



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

The ImaGene Wizard works in two modes. First mode switches on whenever Wizard detects that user performed some action that should be logically followed by another action. Then the Wizard will prompt what action was performed and what should be done next. To follow this advice, the user can either use the tool that the Wizard refers to, or just click on “*Follow Advice*” button in the bottom part of Wizard window. The latter will result in the recommended action being performed automatically. If the recommended action is not necessary, the user will have an option to go to the next advice. A sequence of recommended actions will start immediately after ImaGene launches with the advice to load the image. Thus, virtually any user can just follow the Wizard prompts to learn how to use ImaGene.

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

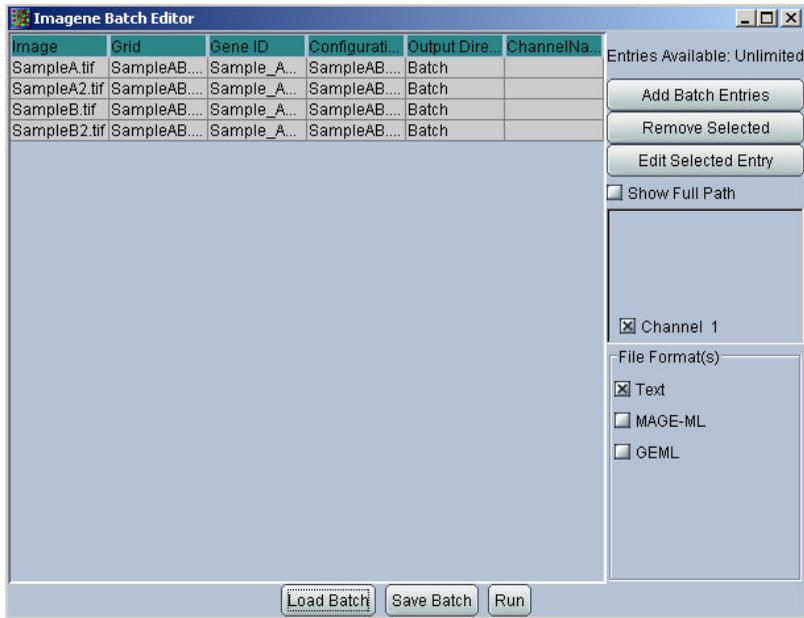
Using ImaGene Wizard, the user has an opportunity to learn how to use the software in an interactive fashion.

## 1.6 Batch Editor and Batch Processing

The ability to batch process images is an optional component that can be added to the basic ImaGene. A batch is simply a series of similar images with the same settings to be processed automatically. With this module, ImaGene can be used in high-throughput environments where large numbers of arrays are processed everyday. The same data are exported from ImaGene whether performed in batch mode or not. During batch mode, ImaGene display a simple, intuitive progress window indicating information about the current image and state of the batch.

### 1.6.1 The Main Batch Editor Window

The ImaGene Batch Editor Window allows batches to be created, saved and loaded. Batches can be created and saved for later use or as a template that may be modified at a later date. This window allows for the selection of channels of multi-channel/image composite for processing.



## Batch Table

Once a batch has been created, the information about the batch is displayed. The following information is contained within the table:

- Image – The name of the image
- Grid – The name of the grid
- Gene ID – The name of the Gene ID file
- Configuration – The name of the configuration file
- Output Directory – The name of the output directory
- Channel Name – The Channel to be processed when using composite images.

Image	Grid	Gene ID	Configur...	Output Di...	Channel...
Sample...	Templat...	Templat...	Config.xml	Desktop	
Sample...	Templat...	Templat...	Config.xml	Desktop	
Sample...	Templat...	Templat...	Config.xml	Desktop	

## Buttons

From within the Batch Editor Window, buttons exist to perform the following tasks:



- **Entries Available** – This field indicates the total number of images that are available to be processed. For most users, the field will indicate an unlimited number of images to be processed.
- **Add Batch Entries** – Adding batch entries allow several images to be associated with settings and a grid file for processing. For example, if 10 images were added to be processed, then the button only needs to be clicked once. Specify the 10 images, and all the images will be added to

the batch. If an additional 13 images of a different type is to be added, this process of adding batch entries needs to be repeated until all images are added. As the batch entries are added, information is displayed within the Batch Table to the left of the button.

- **Remove Selected** – This will remove the selected entry from the batch. Multiple entries may be highlighted and removed through the use of <shift> and <ctrl> keys.
- **Edit Selected Entry** – This opens up a window that allows the batch parameters to be changed. For example, if the location of the images changes, this can be used to update this field. If a batch file template is made, this tool would be used to modify any default values, if needed.
- **Show Full Path** – By default, ImaGene will only display the name of the files within the Batch Table. Select this option to display the entire path within the table. For example, select this checkbox to display the Grid file as:

*C:\ImaGene\Batch\ImaGeneGridFile.grd*



- **Load Batch** – This loads a batch file from the file system that had been previously saved. ImaGene batch files are simple XML files with a .bch file extension. Please see Section 5.1 regarding ImaGene file specification for additional information on ImaGene batch files.
- **Save Batch** – The batch, including information about all the entries will be saved to a file on the hard disk. This file can later be reloaded using the “**Load Batch**” Button.

*Note: When saving the batch, only basic information about the location of configuration files and image files is included. The actual files remain unchanged in their original location. Do not confuse saving the batch file with saving the configuration files used to run the batch.*

- **Run** – The batch and all its entries will be started. Soon after clicking Run, the Batch Editor Window will disappear and the Batch Progress Indicator becomes visible.

## Channel Selector

Located along the middle right side of the ImaGene Batch Editor Window is the Channel Selector. The channel selector is designed for multi-channel images, also known as composite images. Essentially, a composite image is a single file, which contains several images within. This makes it possible to load a single file into ImaGene containing 2, 4 or more images. Several scanner manufacturers produce such file formats. While most of the scanner software exports the images in a single, one image per file format, ImaGene is flexible enough to support both formats.



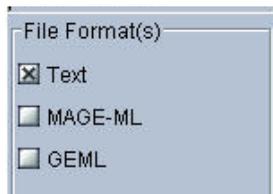
There may be times when only a single channel of a multi-channel scan is of interest. If this is the case then only processing the desired channel will save time.

*Note: Whenever multi-channel images are processed in batch, grid finding, spot adjustment and measurement extraction are performed separately on each channel (unlike processing of multi-channel images in usual mode of ImaGene, where you can align channels and use same grid for all channels at the same time). However, two channels (or even two image files) can be aligned and processed with a single grid in batch mode through loading a manually created XML batch file of a specific format described in section 5.1.5.*

## File Format(s) to Export for Batch Processing

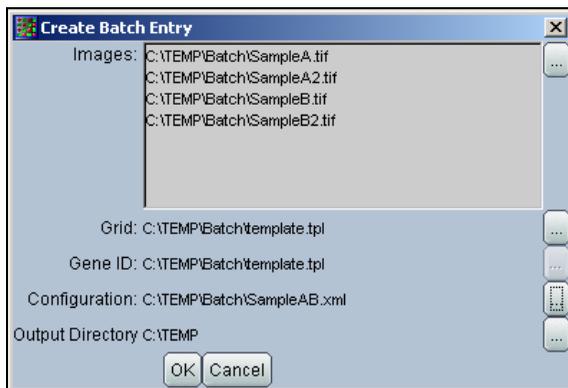
ImaGene allows exporting the results of batch image processing into text, MAGE-ML, and GEML formats. The options for these various file format

export are provided in the batch editor window. By default, the results would only be exported in text file format. The users can optionally choose to export results in any or all of these formats by checking their option boxes. MAGE-ML and GEML file formats would be saved in their respective destination folder using the same name as the text file.



## 1.6.2 Create Batch Entry Window

The Create Batch Entry Window allows entries to be added to a batch. The window appears as a result of clicking either the **“Add Batch Entries”** Button or the **“Edit Selected Entry”** Button. This window requires four parameters to be entered in order to batch a set of Images. All images belonging to a common set can be added to the batch as a group. For example, if 5 images were generated with Molecular Dynamics hardware, then all five images may be added here since they contain the same settings and grid within ImaGene. Thus only a single settings and template file will be required for several similar images.



The required parameters include:

- **Images** – The images to be included with the batch and to be associated with the Grid file. Typically, while the hybridization information about the images may be unique, the geometry of the arrays is the same. If this is the case, then multiple images can be loaded here.
- **Grid** – The grid file that matches the images to be batched. A template file may also be used as this includes a grid. If a template file is used, then a gene ID file does not need to be specified.

*Note: A template file contains the grid as well as the corresponding gene ID info. The grid file does not contain gene IDs.*

- **Gene ID** – The Gene ID file is utilized 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 output text file and visualization tools. Please see section 5.1 for the proper file specification for an ImaGene gene ID file.
- **Configuration** – The configuration file contains information about the parameters and their corresponding values used during analysis within ImaGene. Common examples of stored parameters include local flexibility for spot finding and the desired quality measures for the output text file. The file itself is an XML file that can be saved and loaded to the ImaGene Parameter Settings Window.
- **Output Directory** – ImaGene requires the location where to place subsequent output quantification text files after image processing. Click, browse and select the output directory. The location can be either be local or on a network drive.
- **OK** – Once all the required parameters have been specified, the OK Button becomes enabled and the entries can be added to the batch.
- **Cancel** – Closes the window and the entries are not added to the batch.

### 1.6.3 Creating and Running a Batch

Setting up batch processing within ImaGene takes only a few minutes and requires only a few pieces of information to be defined. The description below covers the basics for creating and running a batch as well as common tips and tricks for success.

1. Setup the initial parameters for use within ImaGene. This needs to be done the first time prior to running a batch. To do this, perform the following:
  - a. Load a sample image
  - b. Load a gene ID file
  - c. Create and place a grid on the image
  - d. Save the result as a template
  - e. Set the parameters on the ImaGene Parameter Settings Window
  - f. Save these configurations. You will probably want to save this to the same location as the template previously created.
2. From the ImaGene Menu Bar select File then Batch Editor.
3. Click the Add Batch Entries Button
4. Specify the images to be batched
5. For Grid, specify the template previously created.
6. Likewise, for configuration, specify the configuration previously saved.
7. Specify the output directory. This is the directory, which will store both the data and the snapshot file (or sst file).
8. Click the Run Button

### 1.6.4 Saving a Batch

Once entries have been added to the batch, the batch can be saved and recalled later. To save the batch to a file, perform the following:

1. Click the Save Batch Button
2. In the Save As Dialog that appears, navigate to the target location for the file
3. Specify the name of the file. A .bch file extension will automatically be added to the file name.

## 1.6.5 Loading a Batch

If a batch file was saved to the file system previously, it can be easily recalled and used again. The following steps outline the process for loading a batch file:

1. Click the Load Batch Button
2. Browse to and select the desired batch file
3. Select Open to load the file
4. The batch entries are now visible within the Batch Editor

## 1.6.6 Editing a Batch

Once entries are added to a batch they can be easily modified, thus eliminating the need to create a new batch file. To modify an existing entry within the Batch Editor, follow these steps:

1. Select or highlight the desired entry by left clicking on its row within the Batch Table. Only one row at a time may be modified.
2. Click Edit Selected Entry Button to open the Create Batch Entry Window for editing.
3. Modify the desired parameters
4. Click OK to commit the changes or cancel to return to the Batch Editor without saving any changes.

## 1.6.7 Normalization and analysis in batch mode

When batch procedure is designed through Batch Editor, no normalization or analysis is performed on extracted measurements. However, normalized data or analysis results can be produced in batch through usage of batch XML file of a specific format described in section 5.1.5.

## **1.7 Reviewing Results**

One of the most powerful features of ImaGene is the ability to review results from the previously processed data. ImaGene was designed to load and display data exactly as it was processed irrespective of time.

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

### **1.7.1 The Snapshot File (sst file)**

The snapshot file is the result of quantification within ImaGene. Upon saving the quantified data to the file system, ImaGene also saves another file, which contains a snapshot of the data after quantification. This snapshot file, ending with an .sst file extension, is utilized by ImaGene to load and allows the results to be reviewed.

The snapshot file itself is a proprietary binary file format. Due to the large amount of information contained within this file, its size can grow large, often surpassing the image size itself.

The snapshot file contains the following components within it:

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

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

## **1.7.2 Loading Results to Review**

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

1. From the ImaGene menu bar, select File followed by Review Results. The Review Results icon located along the toolbar may also be used.
2. With the Open dialog, browse to and select the snapshot file
3. Depending on the file, ImaGene may give a prompt to specify the location of the original images.
4. The information and data should now be visible within the main ImaGene user interface. If reprocessing the images is desired, click the Cancel button along the bottom of the Results tab and use Quantify button of the main panel.

## 1.8 *ImaGene Tools*

ImaGene includes a number of tools designed to help facilitate array analysis. These tools are designed to allow manipulation, quantification and analysis of array images.

### 1.8.1 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, ImaGene can handle up to 10 degrees of rotation as well.

The Auto Alignment tool is intended to provide quick and highly accurate process to solve a common problem of array image analysis, arrays which have been scanned slightly out of alignment. If the images have a high degree of rotation or another extreme problem, then the manual manipulation tools of transposing and rotation may be required.

*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.*

### 1.8.2 Auto Grid Placement Tool



ImaGene's Auto Grid Placement Tool automatically repositions a grid structure to the proper location within the image. Either a grid or a template file is required to be loaded before this tool can be utilized. If the geometry of an array stays the same through and between experiments, the use of a common template for all experiments would be beneficial. However, due to the differences in arraying and scanning, the location of the array within the image may vary across experiments and time. The solution is to use the Auto Grid placement tool once the image and the grid have been loaded. The tool will automatically move the grid to the proper location.

Because the Auto Grid Placement Tool is based on the same grid placement algorithm that Batch Processing uses, the tool is also beneficial to test grid placement on a set of images prior to setting up a batch. For example, instead of wasting time trying to process a poor quality image through the batch processing module, first try the Auto Grid placement on the image. If the Auto Grid Placement cannot locate the proper array geometry then the mage will probably need to be processed manually.

*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, please assign the gene IDs starting with “BLANK” or “EMPTY” or include their IDs using the Paramater Settings panel as a control type called “BLANK” or “EMPTY”. This way ImaGene will take possible absence of the signal in those spots into account and produce reliable results.*

*Note: If image contains bright control spots designed to provide reliable grid placement and that are brighter than any other spots throughout the image, assign corresponding gene IDs to a control type named “BRIGHT” and ImaGene will base auto grid placement on spots from that control type only.*

## 1.8.3 Save Display Image Tool



ImaGene’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. ImaGene saves the image as a high quality 24-bit tiff format. The image can later be recalled for reference for use in publications.

### 1.8.3.1 Saving the Display Image

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

1. From the menu bar, select File then Save Display Image. Alternatively, click the Save Display Image icon from the toolbar.
2. From the Save As Dialog that appears, browse to a location
3. Specify a file name. ImaGene will automatically add the .tif file extension to the end of the name.
4. The image is now saved and available to be viewed in virtually any graphics program such as Microsoft Imaging or Adobe Photoshop.

## 1.8.4 Zoom Tool



The Zoom Tool , allows the zooming of a specific region of the image. The zoom methods that ImaGene currently supports include:

- After selecting the Zoom Tool, left click with the mouse and drag a rectangle around the section of interest from within the main image panel.
- After selecting the Zoom Tool, left click with the mouse and drag a rectangle around the section of interest from within the Image Map View.
- Hold the <alt> key on the keyboard, left-click the mouse and drag a rectangle around the section of interest from within the either the main image panel or the Image Map View.
- Drag the Zoom slider bar located directly below the Main Image Panel.

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

## 1.8.5 Scroll Tool



The Scroll Tool  provides a convenient way to move about the image without zooming out of the current zoom level. 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 within the image the current view is situated, 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.

## 1.8.6 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 maximum diameter for spot sizes and distances between the spots. The tool can also be utilized 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 displayed 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

## 1.8.7 Intensity Tool



The Intensity Tool displays the raw pixel intensities within 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 will be displayed.

*Note: The values provided here are raw intensities meaning they are not mean or median values but rather single pixel values.*

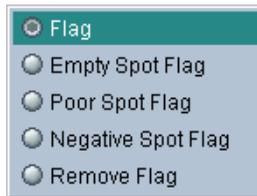
## 1.8.8 Flagging Tool



The Flagging Tool provides a method of manually flagging spots within ImaGene. 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 denoted in the output along with the quantified data. The flag value can then be used in any data analysis software, such as BioDiscovery's GeneSight, or can be used in post-analysis quality control measures. In addition to the numerical value, a symbol is also placed on the spot to make the flag visually unique. Each symbol corresponds to a different flag.

ImaGene supports a variety of automatic and manual flagging types. To change the type of flag: right-click the mouse and select from the context sensitive menu the desired manual flag. The following is a list of possible flags and the corresponding values:



### **Flagging codes:**

Manual flags (color: red)

- 1 - Flag spots (x)
- 5 - Empty spots (x)
- 6 - Poor spots (+)
- 7 - Negative spots (-)

Automatic flags (color: green)

- 2 - Empty spots (x)
- 3 - Poor spots (+)
- 4 - Negative spots (-)

*Note: Manual flags override auto flags.*

*Note: For flagging of a group of spots you can use lasso flagging tool*



## 1.8.9 Undo and Redo Tools

ImaGene supports an unlimited number of undo and redo operations. Anytime clearing the previous action is desired, this tool will provide means to do so.



*Note: The action to be undone or redone is displayed next to the text within the menu bar. For example, the grid was moved by mistake. To undo this action, from within the Tools Menu, the text will read “Undo Move Grid”. This is a convenient means to visualize what action will be reversed.*

## 1.8.10 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 each subgrid. The subgrids can be moved, stretched or resized.



### **Adjust Spot**

This tool allows for individual spots to be moved and resized.



### **Lasso Adjust**

This tool is utilized to select irregularly shaped regions of circles for adjustment. Instead of shifting entire subgrids, unusually shaped regions of circles can be selected for fine-tuning. For example, this tool is ideal for use with membrane arrays because edges could become warped quite often.



### **Rectangle Adjust**

This tool, which is similar to the lasso adjust tool, is utilized to sub-select circles to adjust from within the array. However, this tool employs a rectangular shape to highlight given circles. A possible use of this tool would be with microarrays produced by using a bent pin mounted on the pin head. In such a case an obvious rectangular anomaly might exist within the array making it ideally suited for the Rectangle Adjust tool.



### **Duplicate MetaGrid**

Duplicating an existing metagrid can save time and produce consistent results rather than manually re-creating the metagrid several times. To use it click on the button and select a metagrid to duplicate by left-clicking on it. Then this metagrid can be copied to any place of the image by left-clicking there. To stop copying perform a right-click.



### Show/Hide Grid

ImaGene allows the grid to be hidden enabling a better view of the spots behind. Hiding the grid hides lines, circles and flagging that may exist within the Main Image Panel.



### Auto Adjust Spots

Select this option to have ImaGene perform automatic spot localization. ImaGene searches the area around 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 spots with irregular signal values are present and the grid flexibility is set with a high number of pixels, ImaGene will tend to find pixels with high expression values. For full automation, use this tool in conjunction with auto grid placement tool.



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

### Wrangle

The wrangle feature of ImaGene applies new, stricter constraints to the results of spot localization without requiring further spot finding. Essentially, this allows users to reduce the spot search radius without re-performing 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: ImaGene Tutorials

### 2.1 Basic Analysis

This tutorial demonstrates performing basic quantification using ImaGene. The tutorial covers the essential steps from loading the initial array images until a report is generated via GeneSight-Lite. The following lessons will be presented within this tutorial.

- **Image Loading and Manipulation** - Describes how to load an image into ImaGene 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 properly.
- **Quantifying, Normalizing Image or Image Pairs, Analyzing paired images and Saving the Data** – Describes the quantification and subsequent output of the data.
- **Generating a Report via GeneSight-Lite** – Describes report generation using ImaGene built in data analysis module GeneSight-Lite

#### Image Loading and Manipulation

1. Launch ImaGene from the desktop button or from the ImaGene folder within the Windows Start Menu.
2. Click on Load Images icon under the Main tab.



3. Browse to the Samples folder within the ImaGene folder. The location is typically, C:\ImaGene\SampleData.
4. Select 17hrs1.gel and 17hrs2.gel images. Multiple selections can be performed by holding the <ctrl> key on the keyboard and left-click.

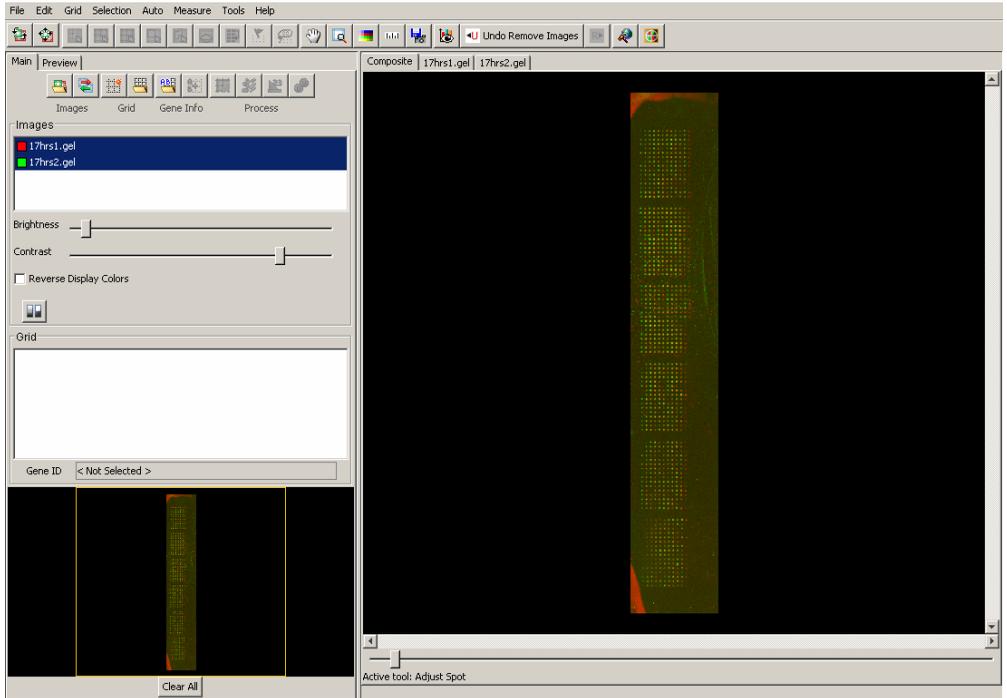


5. Click the Open Button to complete the image loading.
6. Once the images are loaded and are visible within the Main image display located along the right-hand side of the main ImaGene window, the images need to be overlaid. Not all images will require this action, but due to variances in scanning, some will. To overlay the Images Automatically,

click on the Auto Align Images button  under the Main tab.

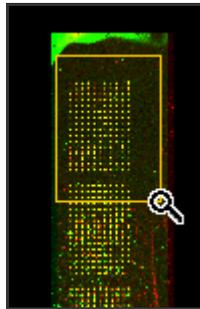


In addition to auto alignment, ImaGene offers manual tools for rotation and translation of images.



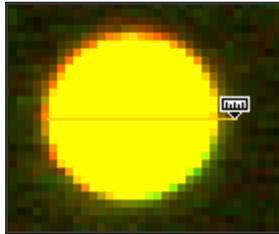
## Grid Placement and Manipulation

1. From the menu bar, select Tools followed by Zoom.
2. Drag a rectangle around the top sub-grid 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 later.

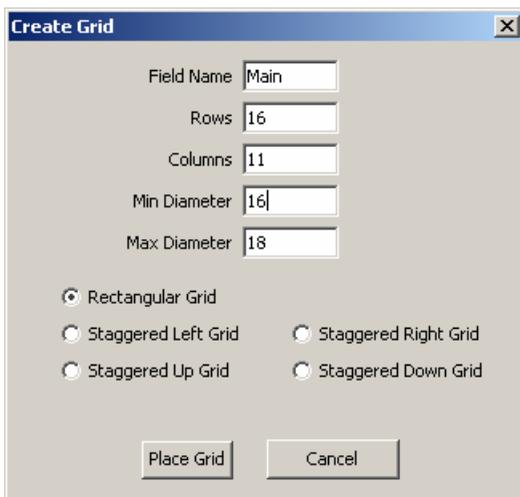
4. With the ruler tool, click and drag from one side of a spot to the other side of the spot. The distance is displayed in a status bar located beneath the main image panel. This distance is measured in pixels.



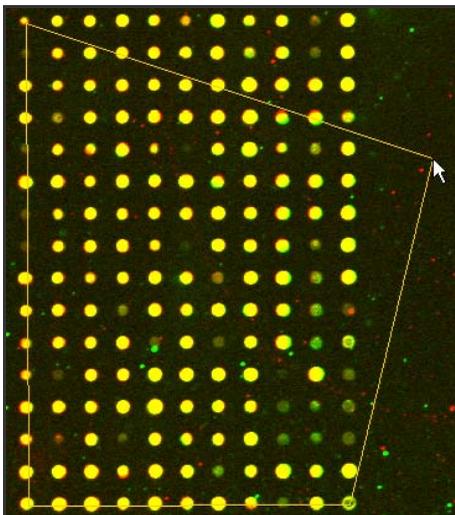
5. Next, count the number of rows and columns of spots within this sub-grid.
6. Click on the Create Grid button under the Main tab.

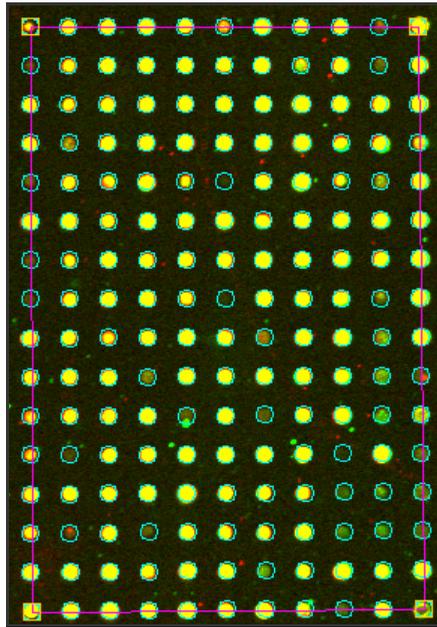


7. Enter the following information into the Create Grid Window that appears.
  - a. Field Name - "Main"
  - b. Rows - "16"
  - c. Columns - "11"
  - d. Min Diameter - "16"
  - e. Max Diameter - "18"
  - f. Rectangular Grid is selected

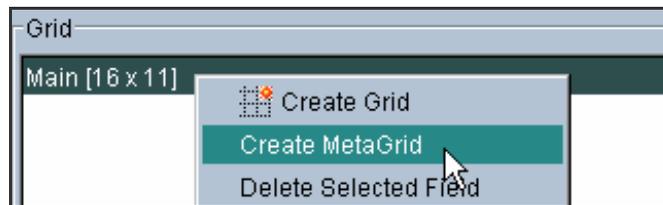


8. Click the Place Grid Button in the Create Grid Window.
9. Use the mouse cursor and click on the four corner spots of the sub-grid.  
Upon placement of the fourth spot, the grid becomes visible on the sub-grid.

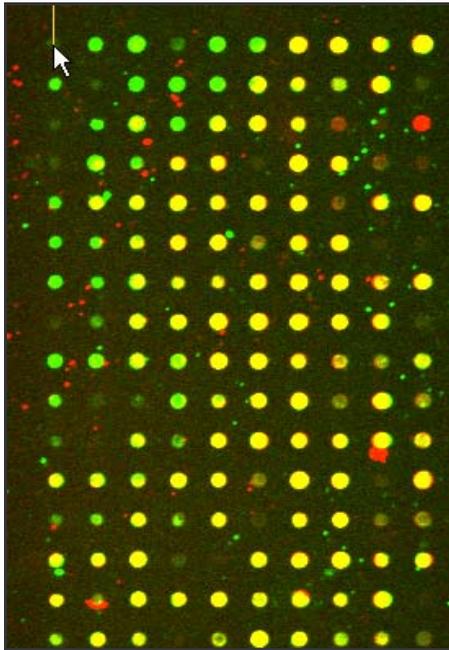




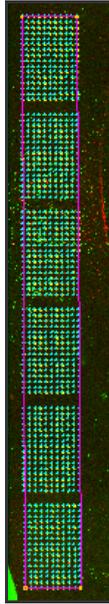
10. Click on the name Main in the Grid Panel.



11. With "Main" selected, right click and select Create Metagrid.
12. In the window that appears, enter the following information
  - a. Metarows – "6"
  - b. Metacolumn – "1"
13. Click Place MetaGrid
14. In the top sub-grid 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 sub-grid is reached



16. Click the top, left most spot within this sub-grid as well. ImaGene automatically places the remaining sub-grids in the proper locations.



17. Using the scroll bar along the right of the main image panel, scroll up so that the second to last sub-grid is visible.

18. Click on the Adjust Subgrid button under the main tab.



19. Click on and drag this sub-grid slightly to the left to provide a better fit.

20. Click the Adjust MetaGrid Button from the toolbar and click on any sub-



grid.

21. Alternatively, click on the Auto Adjust Grid button under the Main tab and the sub-grids will be adjusted automatically.



22. Click next on the Auto Adjust Spots button under the Main tab to perform automatic spot adjustment.

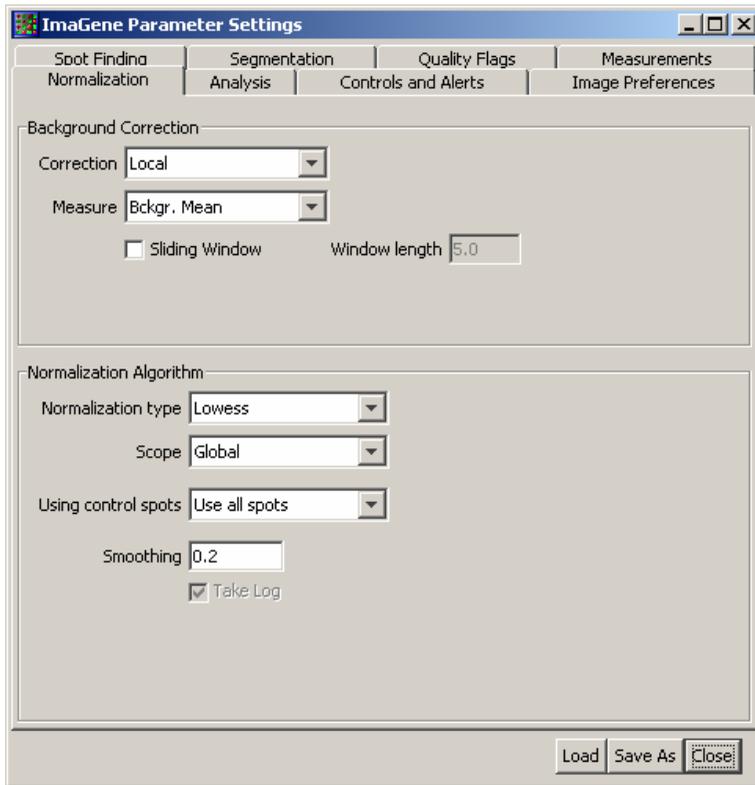


## Quantifying, Pre-Processing, Pre-Analyzing and Saving the Data

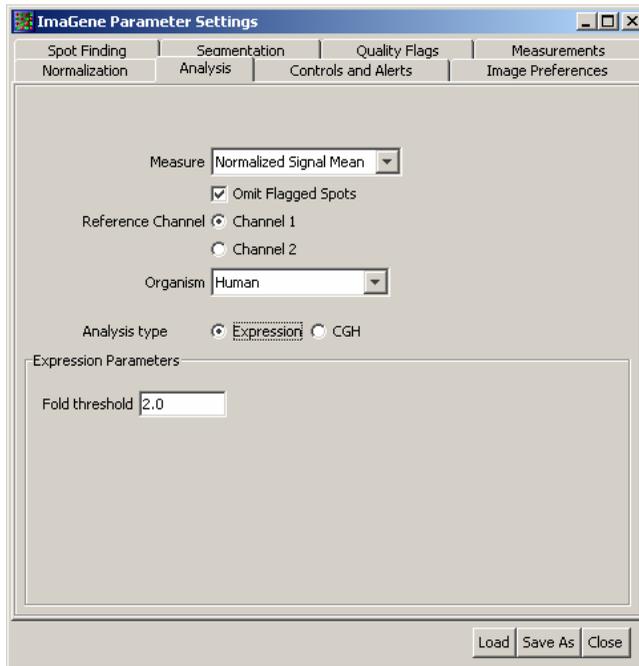
1. Click on the Make Measurements button under the Main Tab.



2. After a few moments the quantified data is visible under the Raw Data tab.
3. Select Plots tab on the Image Display panel.
4. Select Histogram on the Plots panel.
5. Select “Signal Mean Ratio” 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 ‘Mark Selected Rows’ option by a right-click on the selected rows.
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. To normalize and analyze the data you need to set the appropriate parameters. Go to the *Settings tool* from the File menu.



12. Select the Normalization tab and from the different drop-down options, choose the parameters for background correction and normalization.
13. Select the Analysis tab and select either *Expression* or *CGH* analysis. Based on this selection, provide desired parameter settings.



14. When Normalization and Analysis settings are chosen, the Analyze tool can be run using this button in the menu bar.



15. The normalized data will be displayed in a new tab Normalized Data in the right panel.

Composite		17hrs1.gel	17hrs2.gel	17hrs1.gel seg	17hrs2.gel seg	Plots	Normalized Data		Analysis	
F...	MR	MC	...	...	GeneID	Flag	N. Signal Me...	N. Signal Me...	N. Signal Medi...	N. S
A	1	1	1	1	Gene101-c-1...	D	NaN	NaN	NaN	NaN
A	1	1	1	2	Gene101-c-1...	D	10.04264	11.307187	9.913355	11.354
A	1	1	1	3	Gene101-e-1...	D	10.855072	11.637711	10.966904	11.64
A	1	1	1	4	Gene101-g-1...	D	11.322279	12.310238	11.326308	12.05
A	1	1	1	5	Gene101-i-1-a9	D	12.038672	11.342288	11.855591	11.33
A	1	1	1	6	Gene101-k-1...	D	11.026041	10.48532	11.219604	10.46
A	1	1	1	7	Gene101-m-1...	D	10.141575	12.143713	10.264398	12.18
A	1	1	1	8	Gene101-o-1...	D	12.474608	10.711523	12.393939	10.73
A	1	1	1	9	Gene101-a-3...	D	10.712369	11.517187	10.832352	11.53
A	1	1	1	10	Gene101-c-3...	D	11.466678	9.733537	11.509806	9.628
A	1	1	1	11	Gene101-e-3...	D	9.263853	11.896776	9.285154	12.08
A	1	1	2	1	Gene101-a-5...	D	4.832106	9.166543	4.9105926	8.922
A	1	1	2	2	Gene101-c-5...	D	8.791435	11.409499	8.697711	11.56
A	1	1	2	3	Gene101-e-5...	D	10.649933	11.750508	10.720339	11.63
A	1	1	2	4	Gene101-g-5...	D	11.55451	11.787683	11.493683	11.83
A	1	1	2	5	Gene101-i-5-a9	D	11.401009	11.761473	11.434829	11.80
A	1	1	2	6	Gene101-k-5...	D	11.336598	11.170411	11.414387	11.19
A	1	1	2	7	Gene101-m-5...	D	11.262131	11.987861	11.312622	12.17
A	1	1	2	8	Gene101-o-5...	D	11.786624	10.299189	11.891319	10.40
A	1	1	2	9	Gene101-a-7...	D	10.269091	11.741798	10.432623	11.85
A	1	1	2	10	Gene101-c-7...	D	11.489634	9.432495	11.611429	9.471
A	1	1	2	11	Gene101-e-7...	D	9.387245	11.459543	9.464306	11.66
A	1	1	3	1	Gene101-a-9...	D	NaN	NaN	NaN	NaN
A	1	1	3	2	Gene101-c-9...	D	11.380755	11.074423	11.319586	11.09
A	1	1	3	3	Gene101-e-9...	D	10.543212	11.491205	10.408863	11.50
A	1	1	3	4	Gene101-g-9...	D	10.913564	12.198295	10.835863	12.23
A	1	1	3	5	Gene101-i-9-a9	D	11.577277	11.665299	11.40261	11.66
A	1	1	3	6	Gene101-k-9...	D	11.366541	11.767567	11.409842	11.78
A	1	1	3	7	Gene101-m-9...	D	11.474043	12.177996	11.579144	12.05
A	1	1	3	8	Gene101-o-9...	D	11.832004	12.753475	11.849381	12.51
A	1	1	3	9	Gene101-a-11...	D	12.624221	11.998506	12.378552	12.03
A	1	1	3	10	Gene101-c-11...	D	11.7825	11.976402	11.833366	12.01
A	1	1	3	11	Gene101-e-11...	D	11.857233	11.624554	11.625214	11.72

Row= 3

Save Report

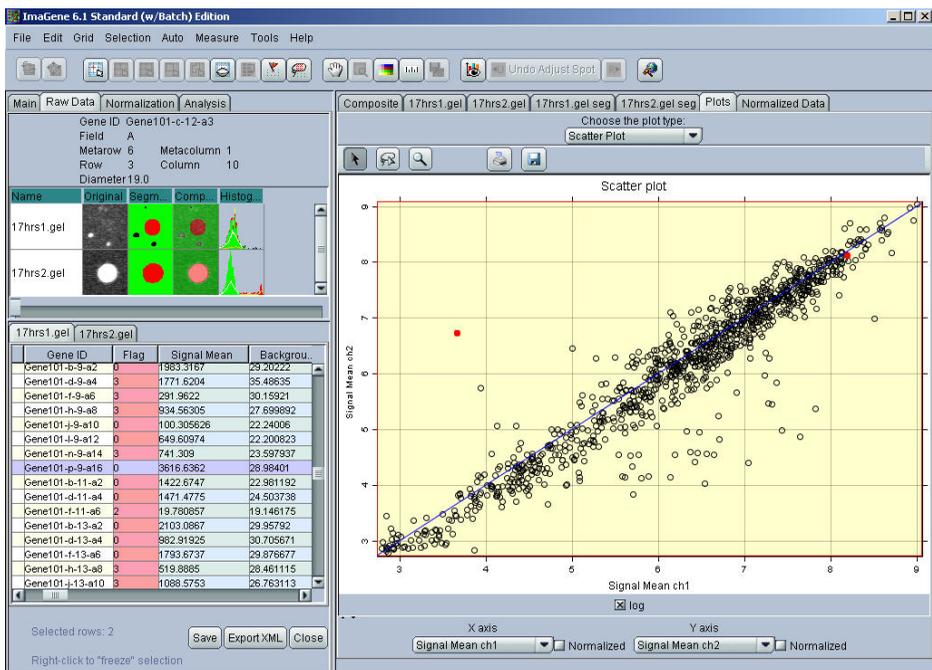
16. The Analyzed data will be displayed in a new tab Analysis in the right panel.

GeneID	Ref...	Experiment	Difference	Significance
Gene101-c-1-a1	5.0159287	8.949533	3.9336047	SIGNIFICANT (UP)
Gene101-c-1-a3	10.656332	10.849661	0.1935494	UNCHANGED
Gene101-e-1-a5	10.6213875	11.796173	1.1747859	SIGNIFICANT (UP)
Gene101-g-1-a7	11.48449	11.833193	0.34870243	UNCHANGED
Gene101-i-1-a9	11.746229	11.057189	-0.68903995	UNCHANGED
Gene101-k-1-...	11.142246	10.525988	-0.61625934	UNCHANGED
Gene101-m-1-...	10.4574585	11.270718	0.8132596	UNCHANGED
Gene101-o-1-...	11.339281	10.2339325	-1.1053488	SIGNIFICANT (DOWN)
Gene101-a-3-a1	10.388395	10.926994	0.53859925	UNCHANGED
Gene101-c-3-a3	11.077978	9.797095	-1.2808628	SIGNIFICANT (DOWN)
Gene101-e-3-a5	9.495159	10.802787	1.3076272	SIGNIFICANT (UP)
Gene101-a-5-a1	4.4687414	10.222038	5.753296	SIGNIFICANT (UP)
Gene101-c-5-a3	9.418478	11.294889	1.8764113	SIGNIFICANT (UP)
Gene101-e-5-a5	10.819314	11.451538	0.63222384	UNCHANGED
Gene101-g-5-a7	11.2805195	11.219816	-0.0607028	UNCHANGED
Gene101-i-5-a9	11.032477	11.56387	0.53139305	UNCHANGED
Gene101-k-5-...	11.385519	11.491124	0.1056056	UNCHANGED
Gene101-m-5-...	11.41889	11.763578	0.3446877	UNCHANGED
Gene101-o-5-...	11.727753	11.181665	-0.546087	UNCHANGED
Gene101-a-7-a1	11.327107	11.0385475	-0.2885599	UNCHANGED
Gene101-c-7-a3	11.141273	9.652801	-1.4884725	SIGNIFICANT (DOWN)
Gene101-e-7-a5	9.616791	10.483963	0.8671727	UNCHANGED
Gene101-a-9-a1	3.6624207	10.949913	7.287492	SIGNIFICANT (UP)
Gene101-c-9-a3	10.945151	11.885287	0.9401362	UNCHANGED
Gene101-e-9-a5	11.545335	11.564067	0.018731833	UNCHANGED
Gene101-g-9-a7	11.25489	10.801059	-0.45383215	UNCHANGED
Gene101-i-9-a9	10.588286	11.615666	1.0273798	SIGNIFICANT (UP)
Gene101-k-9-...	11.447912	11.080713	-0.36719918	UNCHANGED
Gene101-m-9-...	11.043566	11.724286	0.6807201	UNCHANGED
Gene101-o-9-...	11.671056	11.808783	0.1377263	UNCHANGED
Gene101-a-11-...	11.869293	10.479076	-1.3902168	SIGNIFICANT (DOWN)
Gene101-c-11-...	10.4799385	10.40886	-0.071077585	UNCHANGED
Gene101-e-11-...	11.336381	11.758776	0.42239523	UNCHANGED
Gene101-a-13-...	3.2154708	12.26371	9.048239	SIGNIFICANT (UP)
Gene101-c-13-...	4.264227	12.26371	8.000000	UNCHANGED

SIGNIFICANT (UP)

Save Report

- Click the Save Button to save the Quantified data and snapshot file to an output directory of your choice. To save the normalized and Analysis Reports, you must select the *Save Report* button from their result panels.



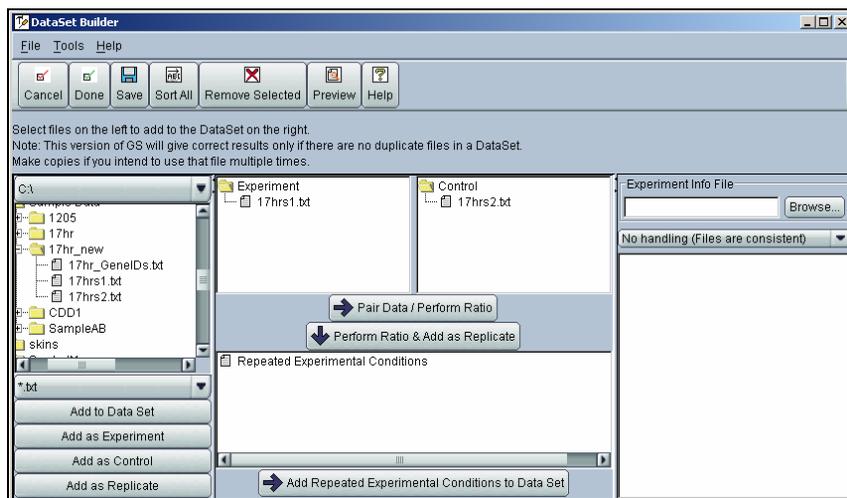
## Generating a Report Via GeneSight-Lite

1. If further analysis is needed, click the GeneSight-Lite icon from the toolbar.



After a brief moment GeneSight-Lite and the Dataset Builder will appear. (For complete information on GeneSight-Lite, please see the GeneSight User's Manual.)

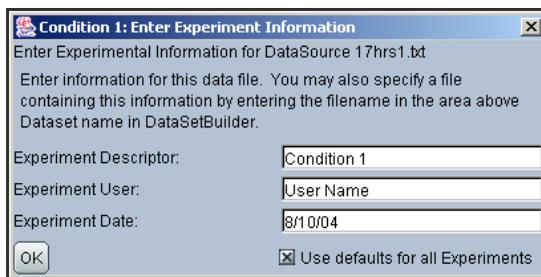
2. With the file system window along the left of the Dataset Builder, browse to the data file previously generated within ImaGene.



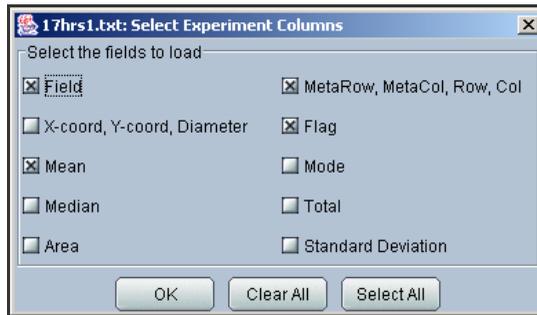
3. Select and drag 17hrs1.txt to the Experiment Panel.
4. Select and Drag 17hrs2.txt to the Control Panel.
5. Click the Pair Data / Perform Ratio Button.
6. Click Done from the Dataset Builder Toolbar.



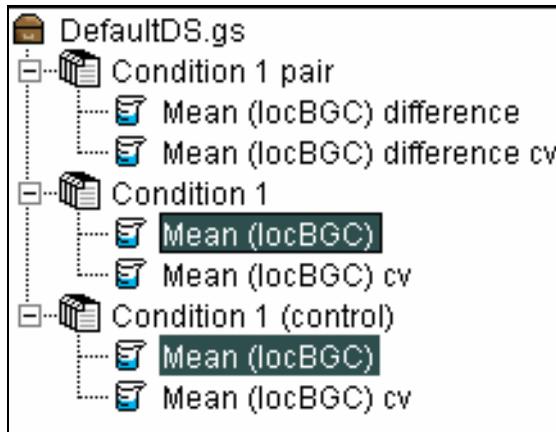
7. Select User Defaults for all Experiments and click the OK Button.



8. Click OK on the next window to use all experimental conditions available within the data files.



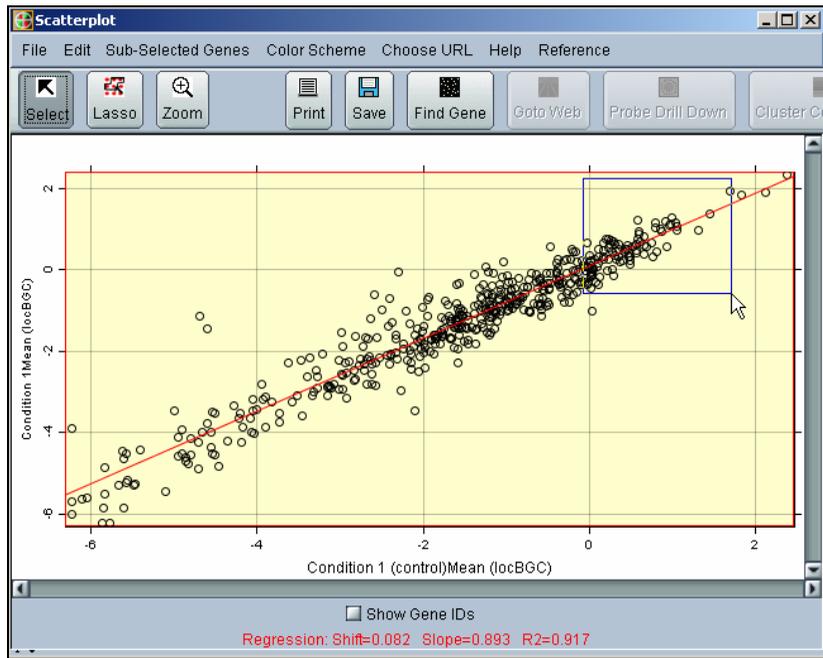
9. Click on while holding the <ctrl> key Mean listed under Condition 1 (control) first, and then Condition 1 in the GeneSight-Lite main window. Be certain both “Mean” labels are highlighted.



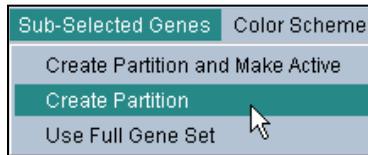
10. Click the Scatter Plot Button from the toolbar.



11. Drag with the mouse to select all the genes located along the top right of the plot, or those genes with high intensity values.



12. From the menu bar, select Sub-Select Genes followed by Create Partition.



13. Enter "Hot Genes" for the name of this sub selection.
14. Close the Scatter Plot Window
15. From the menu bar of GeneSight-Lite, select Utilities then Generate Report.
16. Click Save Report to save the selected genes to a text file on the file system.  
The file is a common tab delimited text file, which can be opened by programs such as Microsoft WordPad or Excel.

Select the look of the Report

DefaultDS.gs  Show Only Selected Genes

Plot Reports

Select the reports from the available plots to add to your report.

Scatterplot

Row No.

Gene ID

Condition 1 pairMean (locBC

Condition 1Mean (locBC

Condition 1 (control)Me:

Condition 1 pairMean (locBC

Condition 1Mean (locBC

Condition 1 (control)Me:

Hot Genes

Select All Columns

Deselect All Columns

Update Table View

Save Report

Cancel

Row No.	Gene ID	Condition 1...	Condition 1...	Condition 1...	Condition 1...
2	Gene101-a...	-0.4922771...	-0.3698183...	0.1224587...	-1.5524668...
5	Gene101-a...	0.1210529...	0.2999103...	0.1788574...	0.2740591...
15	Gene101-a...	-0.3781497...	0.9496428...	1.3277926...	-1.1615517...
30	Gene101-b...	-0.1877631...	-0.1585189...	0.0292441...	-1.0245052...
38	Gene101-b...	0.3550547...	0.6067184...	0.2516637...	
40	Gene101-b...	0.2851841...	0.5282284...	0.2430443...	
49	Gene101-b...	-0.2833074...	0.3791777...	0.6624852...	
63	Gene101-c...	-0.1934036...	0.2906671...	0.4840707...	
64	Gene101-c...	-0.3132908...	-0.1830466...	0.1302441...	-1.4486891...
76	Gene101-d...	-0.1772153...	0.2246307...	0.4018460...	-1.1062391...
80	Gene101-d...	-0.3877111...	0.5532506...	0.9409618...	-0.8660327...
84	Gene101-d...	-0.1341477...	0.6285017...	0.7626494...	
88	Gene101-d...	-0.4616990...	-0.1572884...	0.3044106...	
124	Gene101-f...	0.1805044...	0.2011584...	0.0206539...	0.8427057...
127	Gene101-f...	-0.0475235...	0.0479679...	0.0954914...	-2.2789508...
131	Gene101-f...	-8.2535989...	0.2426557...	0.2434810...	
132	Gene101-f...	-0.1210380...	0.9464245...	1.0674626...	
134	Gene101-f...	0.0149428...	0.3241027...	0.3091599...	35.589990...
136	Gene101-f...	-0.2567415...	-0.0404948...	0.2162467...	
154	Gene101-g...	-0.1826571...	-0.1383636...	0.0442935...	-1.4444261...
167	Gene101-h...	0.2660851...	0.6196911...	0.3538060...	
179	Gene101-i...	-0.2963549...	-0.0146570...	0.2816978...	-1.1495416...
186	Gene101-j...	0.3777735...	0.6813168...	0.3035433...	0.1223344...
192	Gene101-j...	0.0290404...	0.9124788...	0.8834383...	
221	Gene101-...	-0.0980410...	-0.0632792...	0.0347618...	-5.2269766...
224	Gene101-...	-0.4708484...	0.2997654...	0.7706138...	-1.1783400...
233	Gene101-n...	0.1087322...	1.0954607...	0.9867284...	
236	Gene101-n...	0.3130406...	0.3288873...	0.0158466...	

## 2.2 Pre-manufactured Array Analysis

ImaGene provides the ability to load templates for many commercially available arrays. A template within ImaGene is the combination of a grid and the gene list, or Gene IDs. These are required to easily quantify and name the various probes on the array. BioDiscovery provides a selection of these templates to its customers as a service to aid their array analysis. For a complete listing of templates currently available, contact BioDiscovery support. The following Lessons will be covered:

- **Image Selection** - Describes how to load an image into ImaGene 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 BioDiscovery pre-made templates.

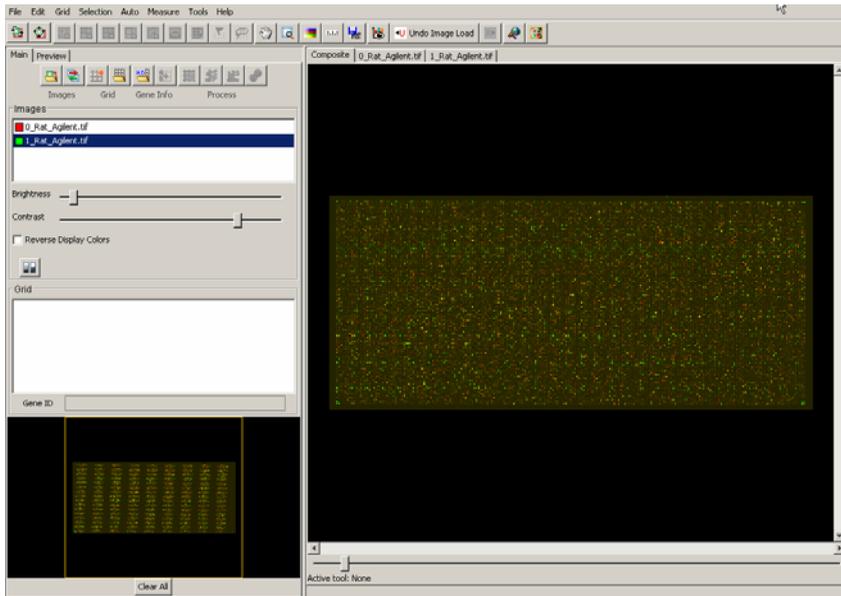
- **Quantifying, Pre-Processing, Pre-Analyzing and Saving the Data** - Describes the quantification and subsequent output of the pre-processed and pre-analyzed data.
- **Loading GAL, GEML or MAGE templates**

## Image Selection

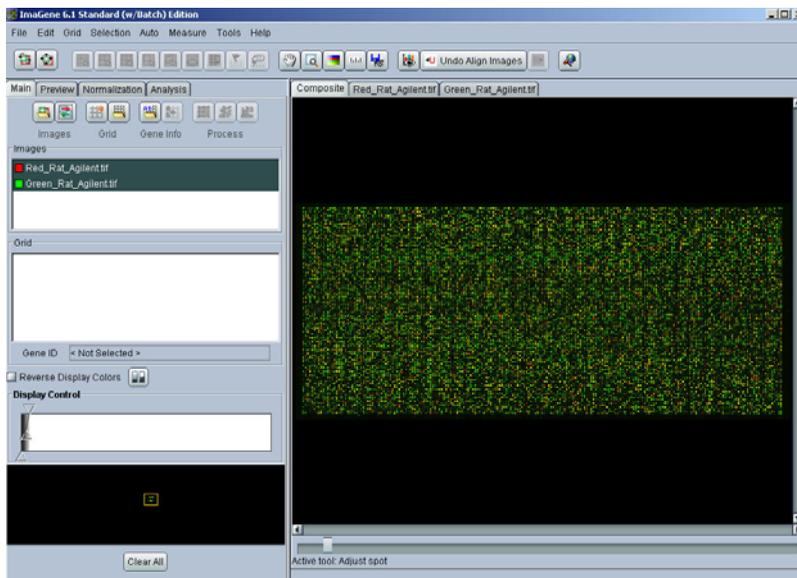
1. Launch ImaGene from the desktop icon or from the ImaGene folder within the Windows Start Menu.
2. Click on Load Images icon under the Main tab.



3. Browse to the Samples folder within the ImaGene folder. The location is typically, C:\ImaGene\SampleData.
4. Select Rat\_Agilent.tif from the Agilent folder.
5. Click the Open Button to complete the image loading. A progress bar displays time remaining.



6. Select both the images in the Main tab and click on the ‘Auto align images’ button above.



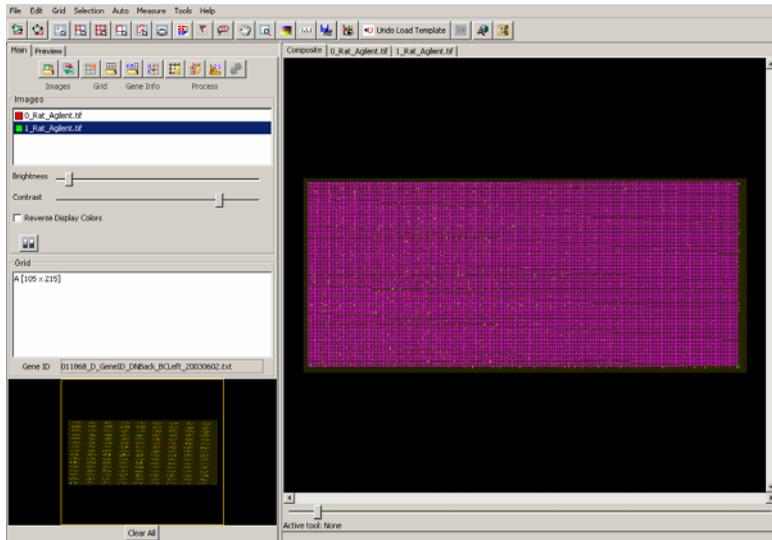


## Template Selection

1. Click on the Load Template button in the Main tab.



2. Browse to the Agilent folder, and select the template.tpl template.

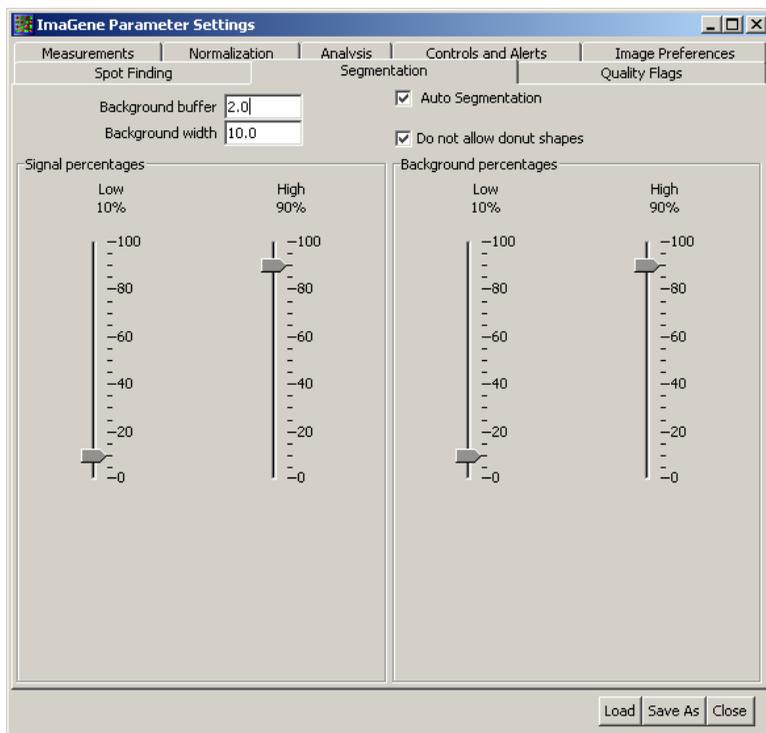


3. From the File menu, select Settings.
4. In the settings window, click on Load and select/open the settings.xml file from the Agilent folder. Close the settings window.

*NOTE: ImaGene version 7 runs Orange packing segmentation algorithm automatically when staggered array designs are used. This can be seen from the preview panel where the selected feature is diamond shaped. In ImaGene 6, if the array is a high density one, e.g. Agilent 44k array, make sure to check the box for **Orange Packing Segmentation** in the **Segmentation** tab in the settings window.*



ImaGene version 6



ImaGene version 7

To verify that Orange Packing segmentation is being applied, view a selected spot in the Preview panel.

Name	Original	Segmented	Composite	Histogram
0_U5225026...				
1_U5225026...				

## Grid Spot adjustment

1. Click on the 'Auto adjust grid' button in the 'Main' tab.

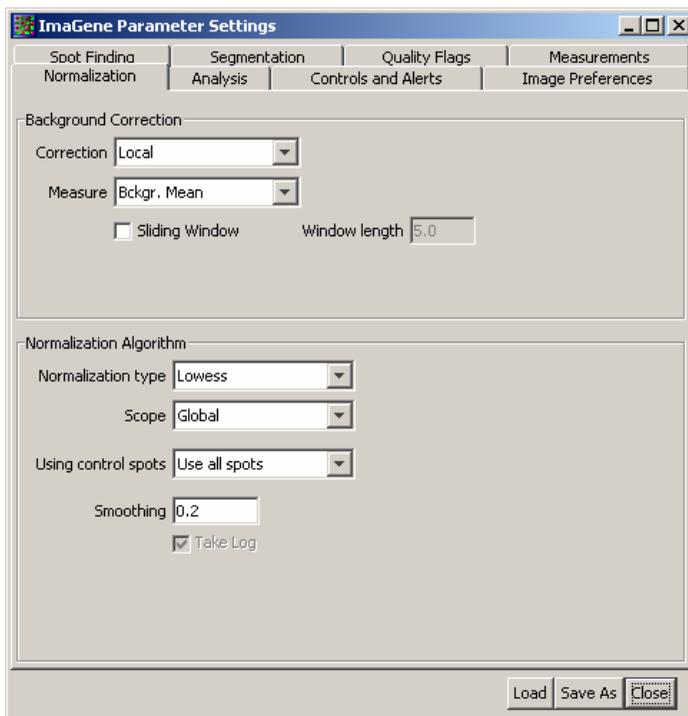


2. Click on the 'Auto adjust spots' button in the 'Main' tab.

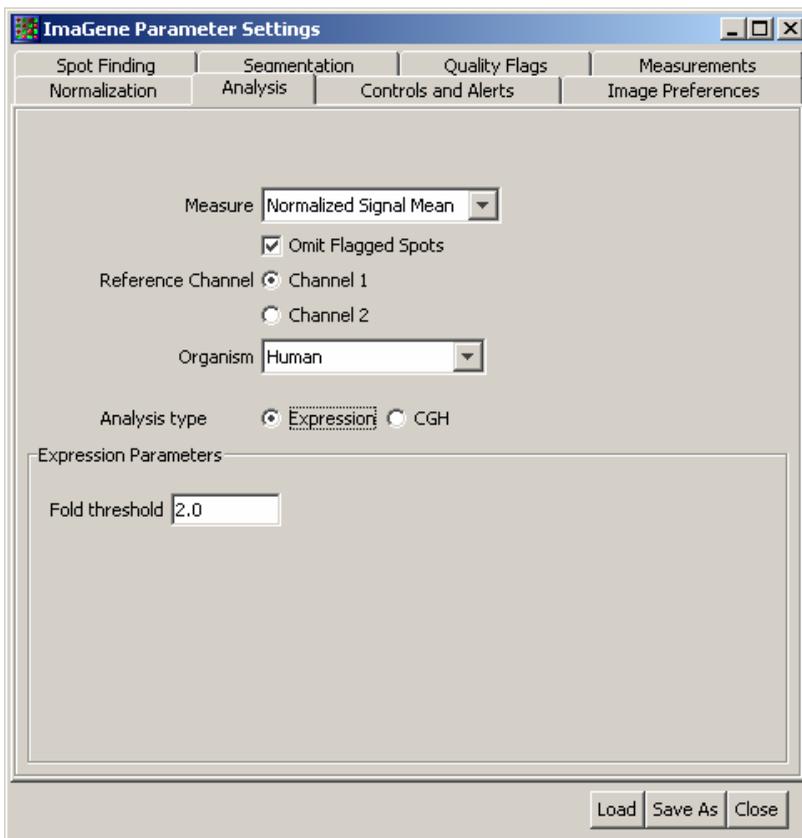


## Quantifying, Normalizing Image or Image Pairs, Analyzing paired images and Saving the Data

1. Enter the Normalization and Analysis parameters in the two Settings Tool panels. These panels are described above in sections 1.2.3 and 1.2.4.



Normalization Settings



## Analysis Settings

2. Click on the 'Make measurements' button in the Main Tab.



3. Once the quantification completes, the *Analyze tool* is activated. However the settings for Normalization and Analysis must be chosen from the Settings Tool, see sections 1.2.3 and 1.2.4 for a full description.



Composite		Red_Rat_Agilent.tif		Green_Rat_Agilent.tif		
Red_Rat_Agilent.tif seg		Green_Rat_Agilent.tif seg		Plots	Normalized Data	Analysis
GeneID	Annotati...	Reference	Experim...	Difference	Significance	
(+)Pro25G-03	q0055--COX1 ...	8.329507	14.4029455	6.073438	SIGNIFICANT (UP)	
(-)3xSLV1	q0055--COX1 ...	3.8919137	5.150032	1.2581183	SIGNIFICANT (UP)	
A_43_P21252	q0075--COX1 ...	8.090106	8.048014	-0.042092323	UNCHANGED	
A_42_P534203	q0075--COX1 ...	9.7179	7.9723334	-1.7455668	SIGNIFICANT (DOWN)	
A_43_P22195	q0110--mRNA...	7.21053	7.5427637	0.3322339	UNCHANGED	
A_43_P16421	q0110--mRNA...	7.2909627	7.371367	0.08040428	UNCHANGED	
(+)Pro25G-02	q0140--mitoch...	7.0594006	14.568673	7.509272	SIGNIFICANT (UP)	
A_43_P13118	q0140--mitoch...	7.9471636	8.229355	0.28219128	UNCHANGED	
A_43_P19445	q0250--cytoc...	6.0012627	6.1011553	0.099892616	UNCHANGED	
A_43_P11302	q0250--cytoc...	10.595569	11.660409	1.0648403	SIGNIFICANT (UP)	
A_43_P22361	ybr011c-r--ino...	6.8682256	7.0017905	0.13356495	UNCHANGED	
A_43_P10152	ybr011c-r--ino...	7.062393	7.3165107	0.2541175	UNCHANGED	
A_42_P573643	yal061w--simil...	8.813372	10.742812	1.9294405	SIGNIFICANT (UP)	
A_43_P13367	yal064w--hyp...	11.939107	11.260348	-0.6787586	UNCHANGED	
A_43_P20241	yal064w--hyp...	7.2563596	7.334912	0.078552246	UNCHANGED	
A_43_P21201	Ara1--	8.045874	8.154196	0.10832214	UNCHANGED	
A_43_P11033	Ara1--	6.7275124	6.768035	0.040522575	UNCHANGED	
A_43_P13501	yar002c-a--p2...	9.009765	8.93575	-0.07401466	UNCHANGED	
A_43_P22765	yar002c-a--p2...	6.4552083	7.9995527	1.5443444	SIGNIFICANT (UP)	
A_42_P694253	yar008w--trN...	11.595569	8.982265	-2.6133032	SIGNIFICANT (DOWN)	
A_43_P20815	yar019c--prot...	6.3047214	6.529222	0.22450066	UNCHANGED	
A_43_P19256	yar019c--prot...	7.1947255	11.537289	4.342563	SIGNIFICANT (UP)	
A_43_P11341	ybl049w--hyp...	11.173158	9.575088	-1.5980701	SIGNIFICANT (DOWN)	
A_43_P23211	ybl049w--hyp...	6.6017127	6.9481993	0.34648657	UNCHANGED	
A_43_P10868	ybl053w--que...	9.020153	8.850221	-0.16993237	UNCHANGED	
A_42_P512232	ydr054c-r--E2...	9.984394	10.130186	0.145792	UNCHANGED	

- To Save the Raw quantification table results, click on the Save button located under the Results Tab.



- Specify a file name and location to save the data file.
- Using Windows Explorer or My Computer, navigate to the data file within the file system.
- Open the quantified data file using either MS Excel or Microsoft WordPad.

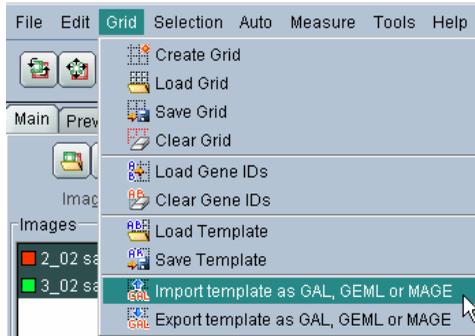
	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Begin Header													
2		version	6.0.0 Beta											
3		Date	Tue Aug 24 10:08:25 PDT 2004											
4		Image File	C:\TEMP\Agilent\Rat_Agilent.tif											
5		Page	1											
6		Page Nam	Green											
7		Inverted	FALSE											
8		Begin Field Dimensions												
9		Field	Metarows	Metacols	Rows	Cols								
10		A	1	1	105	215								
11		End Field Dimensions												
12		Begin Measurement parameters												
13		Segmentat:auto												
14		Signal Low	0											
15		Signal Higl	0											
16		Background	0											
17		Background	0											
18		Background	1											
19		Background	10											
20		End Measurement parameters												
21		Begin Alerts												
22		Control Ty	Minimum t	If tested	Percentage	If failed	Maximum	If tested	Percentage	If failed	CV thresh	If tested	If failed	
23		End Alerts												
24		Begin Quality Flags												
25		Begin Flagging Settings												
26		Empty Spc	TRUE	Threshold:	2									
27		Poor Spots	FALSE											
28		Negative S	TRUE											
29		End Flagging Settings												
30		Begin Flagged spots												
31		# of Empty Spots: 500												
32		# of Poor Spots: 0												
33		# of Negative Spots: 0												
34		# of Manually Flagged Spots: 0												
35		End Flagged spots												
36														
37		End Quality Flags												
38		End Header												
39		Begin Raw Data												
40		Field	Meta Row	Meta Colu	Row	Column	Gene ID	Flag	Signal Me	Background	Signal Me	Background	Signal Me	Background
41		A	1	1	1	1	(+)Pro25G	0	22062.05	46.8561	24797.5	46.26089	39	45.0789

- To save the normalized data or analysis results, choose the *Save Report* options beneath those respective data tables.

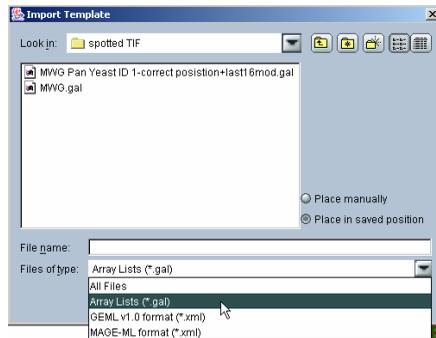
## Loading GAL, GEML or MAGE templates

The use of templates through ImaGene provides a convenient and simple way to perform grid placement and associate gene information with clone locations. Templates can also be imported as GAL, GEML or MAGE format. In order to do so, follow the steps below:

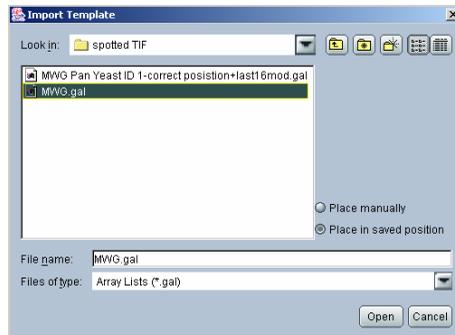
- From the Grid menu, select the option Import template as GAL, GEML or MAGE.



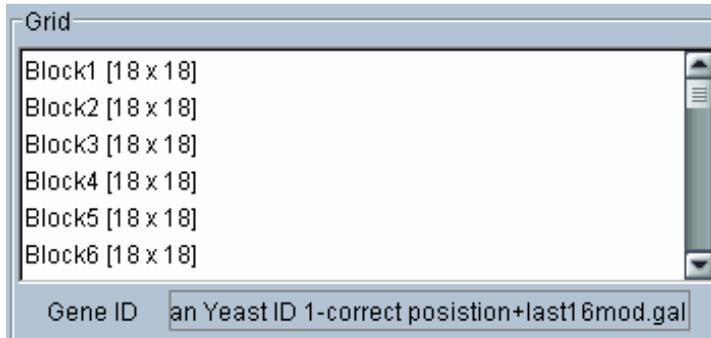
2. In the next window, select the appropriate option among GAL, GEML or MAGE for the file types. GAL is the default option here.



3. Browse and select your template file and click on Open.



4. The template will be displayed in the Grid and Gene ID panels in the Main tab.



## 2.3 CGH Tutorial

This tutorial demonstrates performing basic CGH quantification using ImaGene. The tutorial covers the essential steps from loading the initial array images until the final CGH report is saved to disk. For users experienced with standard ImaGene expression analysis, many of these topics will be familiar. The following lessons will be presented within this tutorial.

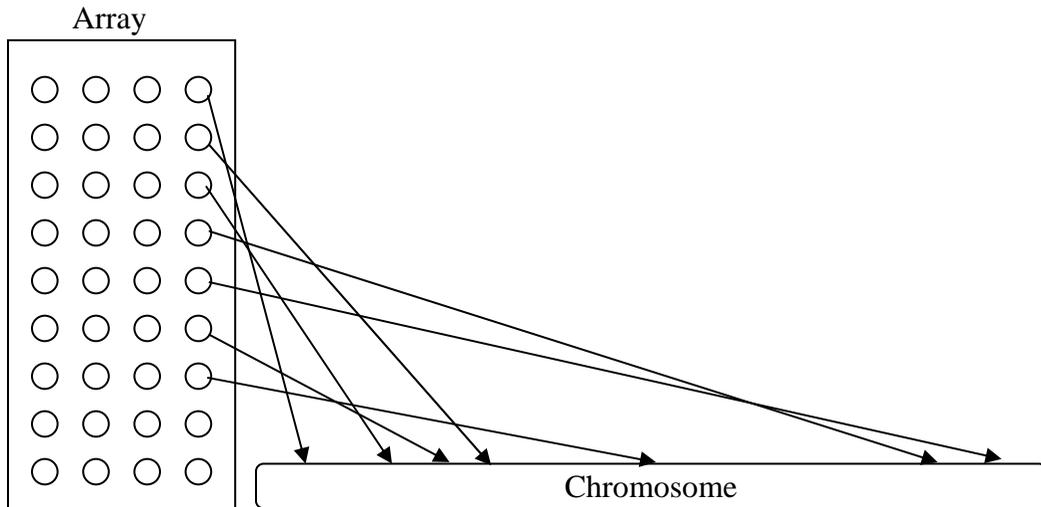
- **Image Loading and Manipulation** - Describes how to load an image into ImaGene and use the controls on the Image tab to enhance the on-screen display of the image.
- **Grid Placement and Manipulation** – Describes how to create a grid and adjust it to fit the image properly.
- **Quantifying, Normalizing Image or Image Pairs, Analyzing paired images and Saving the Data** – Describes the quantification, analysis and subsequent output of the CGH data.

### CGH Overview

Array CGH is a powerful method for detection of regions of chromosomal gain (or amplification) and loss (homozygous and heterozygous deletion). The

basic principal behind array CGH is to label genomic DNA from a sample under study and hybridize this onto an array. Typically a different label is used for a control DNA sample. The labeled extracts are then competitively hybridized on an array where each spot on the array (each probe) represents a particular region on the chromosome. The probes can be either BACs or oligos. ImaGene can process either platform.

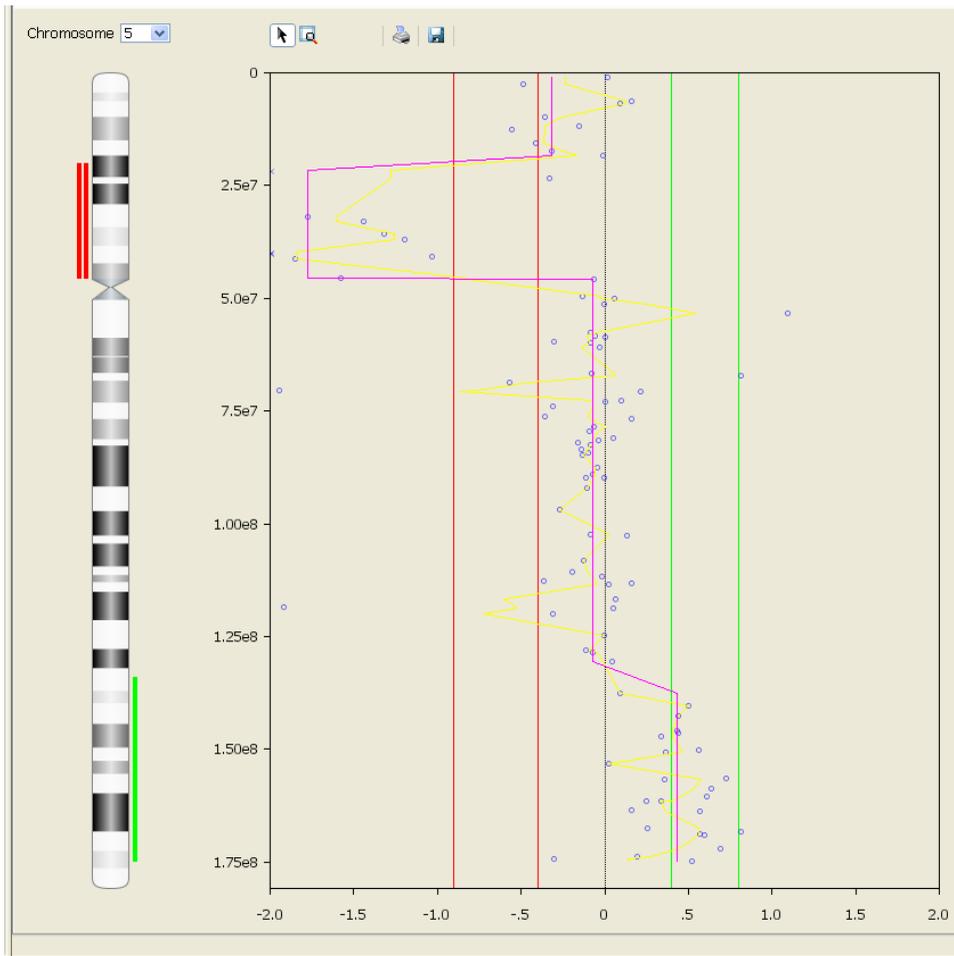
The CGH analysis is different from expression analysis in one crucial aspect – The location of the probes on the genome is an important aspect of the analysis and is therefore taken into consideration during the analysis. The figure below depicts how probes are mapped to specific locations on the genome. In ImaGene 7, you specify this mapping information in the GeneID file. As described in Section 5.1.2, each row of the GeneID file corresponds to a spot on the array. The columns with headers Chromosome and Base Pair are used to specify this genome location information for each probe.



### CGH Analysis Details

Once ImaGene quantifies the spot values for each channel, it creates log (base 2) ratios for each spot. If there are multiple spots with the same GeneID, ImaGene automatically combines the ratios from these spots by taking an average. It will then arrange the ratios according to their position

along the chromosome, see the figure below. Here, you can see each probe as a small blue circle along the length of a chromosome. Please note that the color of each circle (probe) can be specified in the GeneID file using a column with header *Color*. This can be a useful feature allowing you to color code control probes or those with questionable annotation. In the figure below, you can also see the two user specified calling thresholds as green and red vertical lines. These lines specify the threshold for calling a certain region as a one copy gain, multiple copy gain, single loss, or two copy loss. The most trivial calling algorithm would be to simply use these thresholds and the probe locations. This will cause a very noisy output marking any probe that exceeds these thresholds as a copy number change event. To improve on this very basic approach, we can use multiple probes that are relative neighbors of each other on the genome to create a “moving average” value. You can see the result of a moving average as a yellow line in the figure below. We could use the moving average to make calls. However, this approach is sensitive to the window size and outliers in the data. BioDiscovery’s approach is to use a statistically based algorithm, similar in spirit to the Circular Binary Segmentation (CBS) algorithm developed by Adam Olshen at Sloan-Kettering Institute. We have modified the CBS algorithm and significantly improved the processing speed by using a Normal distribution function for testing for change points as oppose to the non-parametric permutation based statistics used in the original CBS algorithm. The result of the algorithm is to segment the genome into clusters of uniform ratios. The cluster values are plotted as the magenta line in the figure below. The calling algorithm then uses the cluster values and the user defined thresholds to establish regions of copy number variations.



### Reasonable parameter values for the CGH algorithm

Please note that the recommendation here will not be appropriate for every image and the selection of parameters should be done by the user based on their own images and knowledge of the underlying biology. The following recommendations should be used as a starting point.

***Significance Threshold:*** This value is used to adjust the sensitivity of the clustering algorithm described above. The smaller the number, the less sensitive

the algorithm is in creating a new cluster. So, if some known aberrations are not being called because they are too small, increase this value. We have found a value of 0.005 to provide a good balance.

**2 Deletions <:** This will indicate the threshold for a homozygot loss (both copies deleted). Theoretically, this would be  $\log(0/x)$  or a very large negative number. A threshold of -0.9 offers a good performance.

**1 Deletions <:** This will indicate the threshold for a heterozygot loss (one copy of the pair is deleted). Theoretically, this would be  $\log(1/2)$  or -1. Due to the inherent noise in the experiment a threshold of -0.4 offer a good performance.

**1 Amplification>:** This will indicate the threshold for a single copy gain. Theoretically, this would be  $\log(3/2)$  or 0.585. Due to the inherent noise in the experiment a threshold of 0.4 offer a good performance.

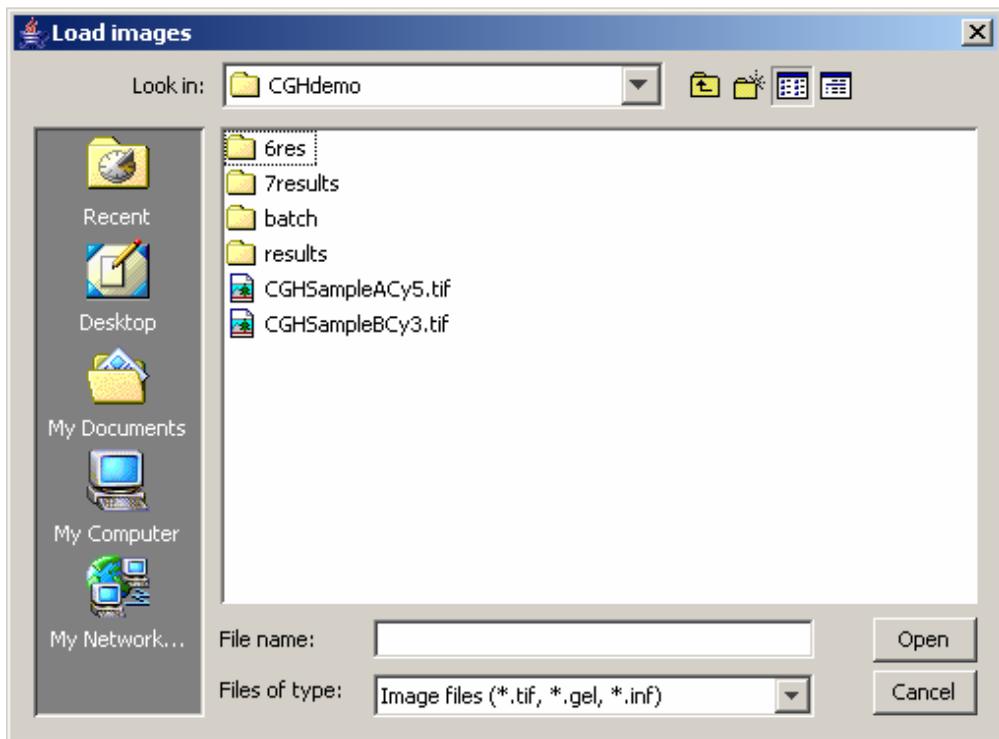
**2+ Amplification>:** This will indicate the threshold for two or more copy gain. Theoretically, this would be equal or greater than  $\log(2)$  or 1. Due to the inherent noise in the experiment a threshold of 0.8 offer a good performance

## Image Loading and Manipulation

1. Launch ImaGene from the desktop button or from the ImaGene folder within the Windows Start Menu.
2. Click on Load Images icon under the Main tab.

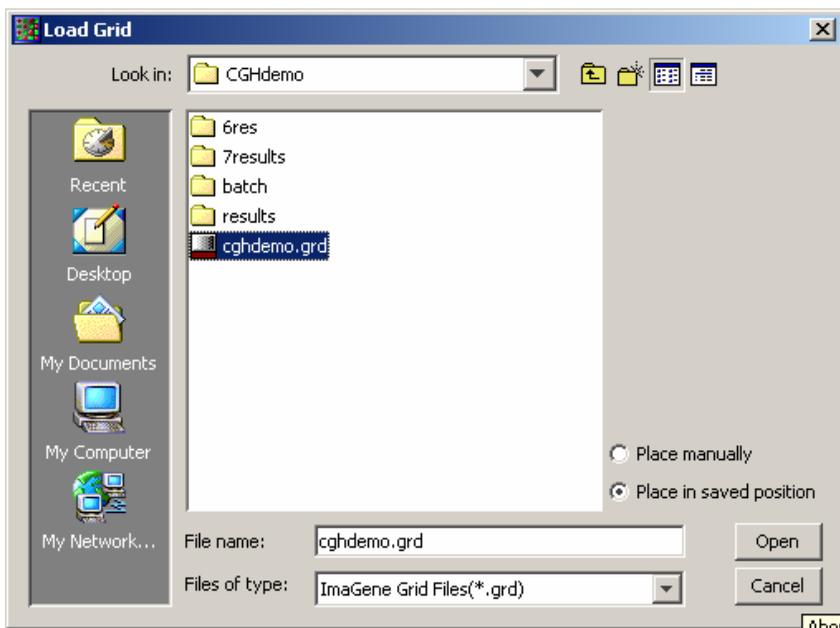


3. Browse to the Samples folder within the ImaGene folder. The location is typically, C:\Program Files\BioDiscovery\ImaGene\SampleData\CGHdemo.
4. Select CGHSampleACy5.tif and CGHSampleACy3.tif images. Multiple selections can be performed by holding the <ctrl> key on the keyboard and left-click.



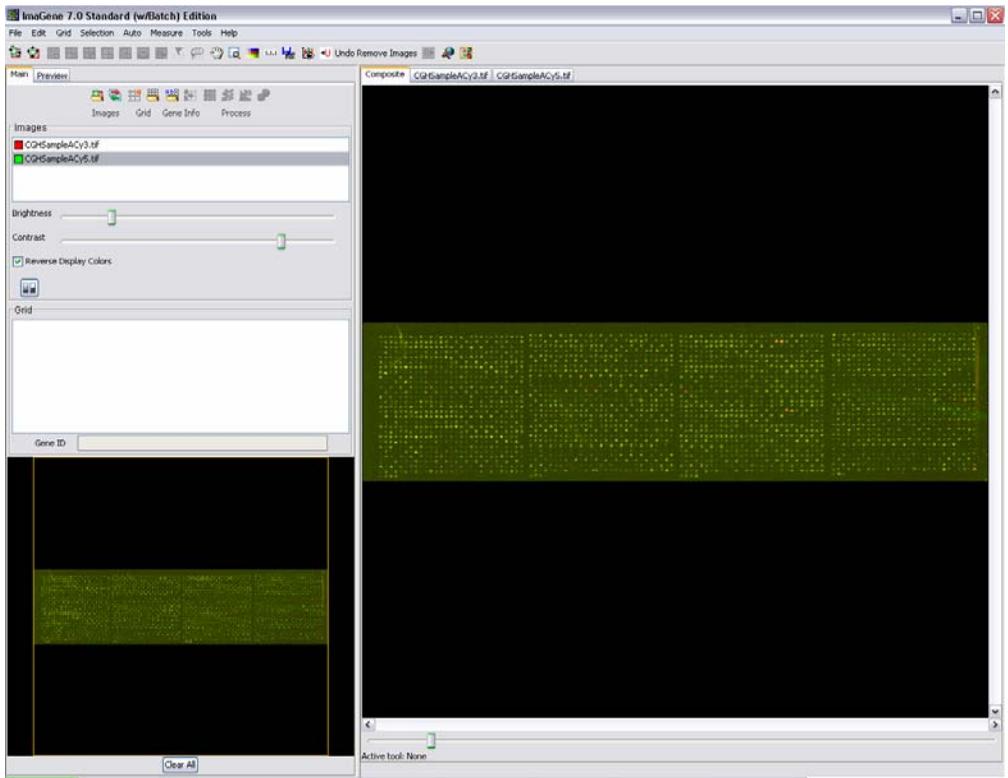
5. Click the Open Button to complete the image loading.
6. Select the Load Grid tool and choose from the same directory the file, cghdemo.grd. Select the “Place in saved position” option.



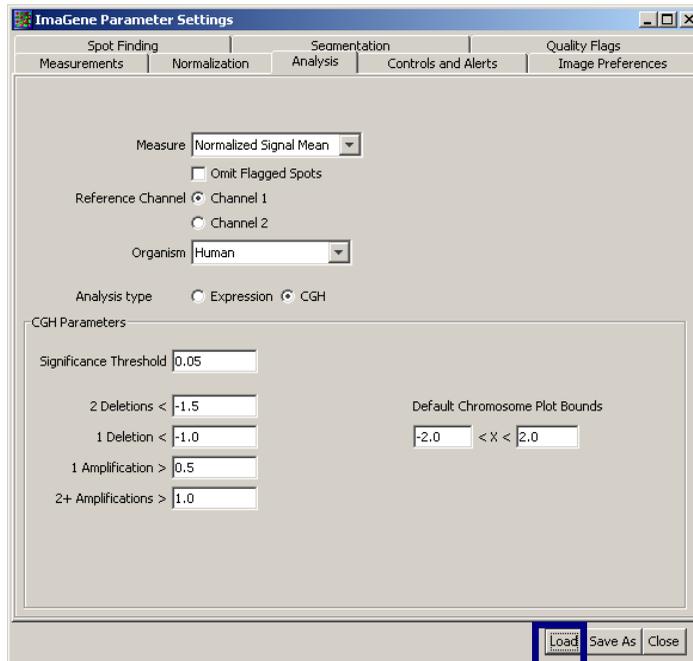


7. Load the CGH gene ID file, cghdemo-geneid.txt from the menu bar.

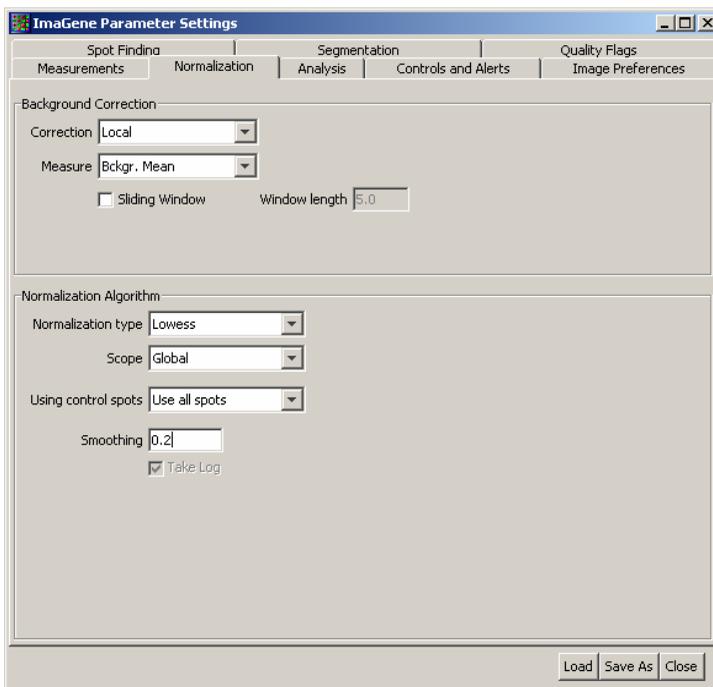




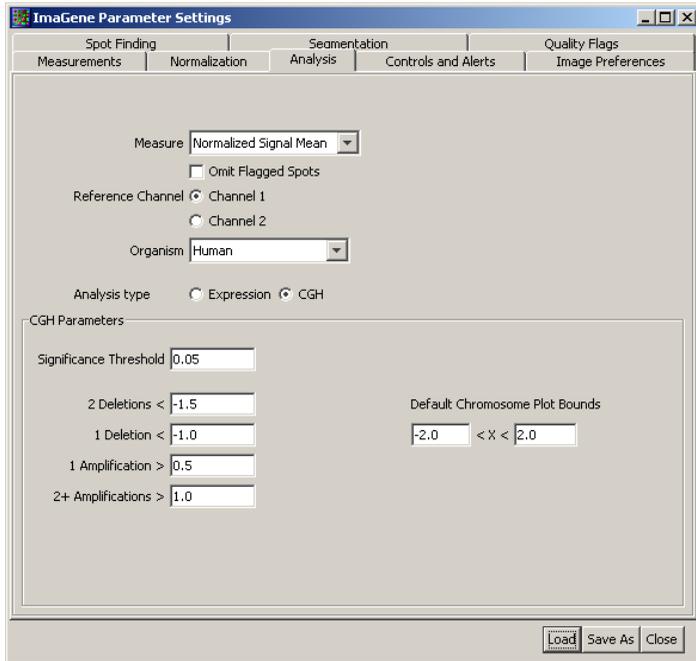
8. Open the CGH Settings tool and select the Load button. Then browse and select the CGH settings file, cghdemo-settings.xml.



9. From the Settings tool, go to the Normalization tab and select desired normalization parameters.



10. From the Settings tool, go to the Analysis tab and select desired Analysis parameters, see section 1.2.4 for full descriptions.



- Click on the Auto Adjust Grid button under the Main tab and the sub-grids will be adjusted automatically.



- Click next on the Auto Adjust Spots button under the Main tab to perform automatic spot adjustment.



## Quantifying, Normalizing CGH Image Pairs, Analyzing CGH copy number and Saving the Data

1. Click on the Make Measurements button under the Main Tab.



2. After a few moments the quantified data is visible under the Raw Data tab.
3. You can then run your CGH analysis by selecting the Analyze button under the Main panel. This will run the Analysis using your chosen normalization and analysis settings.



4. When finished running, 6 new tabs will appear on the right side panel. These include, Normalized Data, Overview (opens first by default), Whole Genome, Chromosome, CGH Report and Summary.

Composite	CGH5sampleACy5.tif	CGH5sampleBCy3.tif	CGH5sampleACy5.tif seg	CGH5sampleBCy3.tif seg
Plots	Normalized Data	Overview	Whole Genome	Chromosome
			CGH Report	Summary



5. The plots allow users to drill down by selecting elements to obtain ever more specific information. For example, start by selecting the Overview tab. To view the gains and losses for the number 5 chromosome, left click on that chromosome. ImaGene instantly displays the single Chromosome tab with the corresponding ideogram and plot. As with all ImaGene plots, selection of a single probe causes selection of the raw data and spot image. See section 1.2.4 for full descriptions of the various CGH panels including a description of the colors and lines within the plots.
6. Save the Raw results by selecting the Save button under the Raw Data table. Save the Normalized Data and CGH Report by choosing the Save Report button beneath the tables. Pictures may be printed or saved using the tools available in the various plotting panels.

## 2.4 Batch Processing

This tutorial demonstrates how to use batch processing within ImaGene. Batch processing is an additional module, which is not included by default with the base ImaGene. Please contact BioDiscovery support with any question regarding eligibility. The lessons learned within this tutorial include:

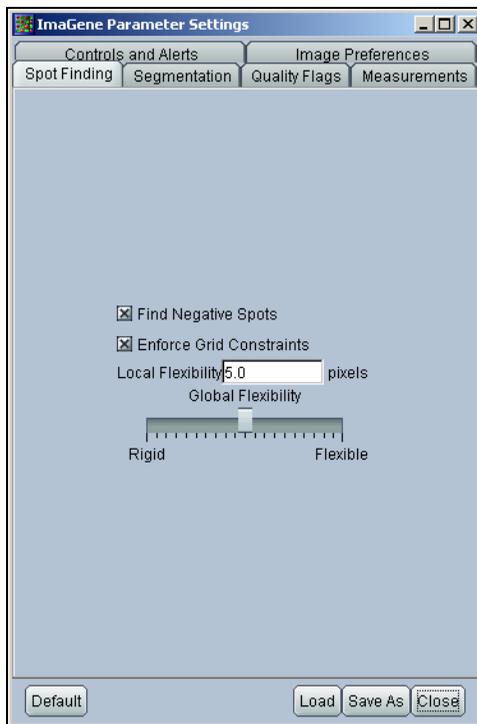
- **Preparing for batch processing** – Prior to performing batch processing, several files need to be made available to ImaGene.
- **Adding Entries to the Batch** – Entries are the images and the corresponding settings to be used when performing batch processing
- **Post Processing** – Start times, stop times and any errors that occurred during the batch are recorded to a simple text file for later analysis. Likewise, results can be reviewed to determine processing quality.

### 2.4.1 Preparing for batch Processing

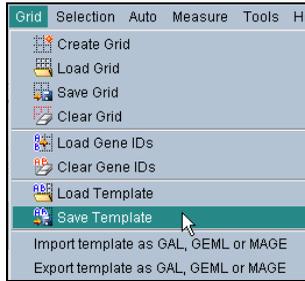
Prior to performing batch processing a configuration file and either a grid or a template file must exist. To generate these files, perform the followings:

1. Launch ImaGene if the software is not already started.
2. Load a sample image that is indicative of the group of images to be batch processed.
3. The next step is to setup the configurations to be used. To do this, open the ImaGene Parameter Settings Window by selecting File followed by settings from the menu bar. For this example, be certain AutoSegmentation is checked, located under the segmentation tab, and that all the measurements are checked, located under the measurements tab. Go to Quality Flags tab, check Empty Spots, set empty spot threshold to 1. Check Poor Spots and click Change Parameters button and click Default on Quality Parameters dialog, close dialog. Finally, click the Save As button to save the

configuration file to the file system to a location you choose. Remember this location as we will recall it shortly.



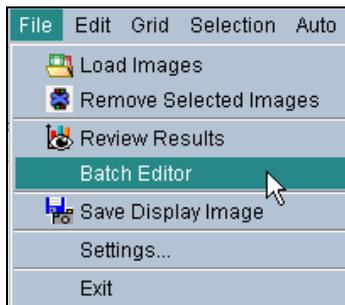
4. Click the Close Button to exit the ImaGene Parameters Settings Window.
5. The next step is to perform grid placement. To do so, right click on the Grid Panel and select Create Grid. Enter the parameters for creating the grid.
6. Click the Place Grid Button and click on the four corner spots of the array.
7. Specify a gene ID file to be used with this array. To do so, select Grid from the menu bar followed by Load Gene IDs. Locate and select the study Gene ID file within the ImaGene samples directory. Note that the Gene ID, while helpful, is not required for batch processing.
8. The final step is to save a template that can be used when setting up the batch. To save a template, select Grid followed by Save Template from the menu bar. Specify a name and save this to the location of your choice.



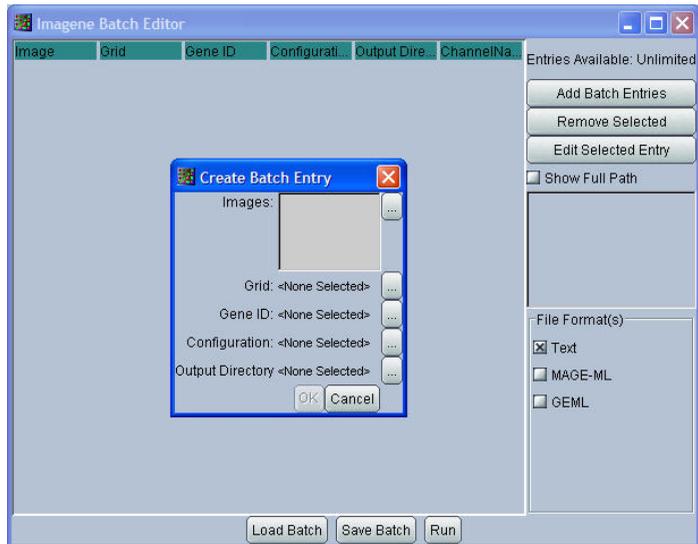
## 2.4.2 Adding Entries to a Batch

Once the configuration file and the template files exist, these can be combined with one or more images to create a batch. To add entries to a batch:

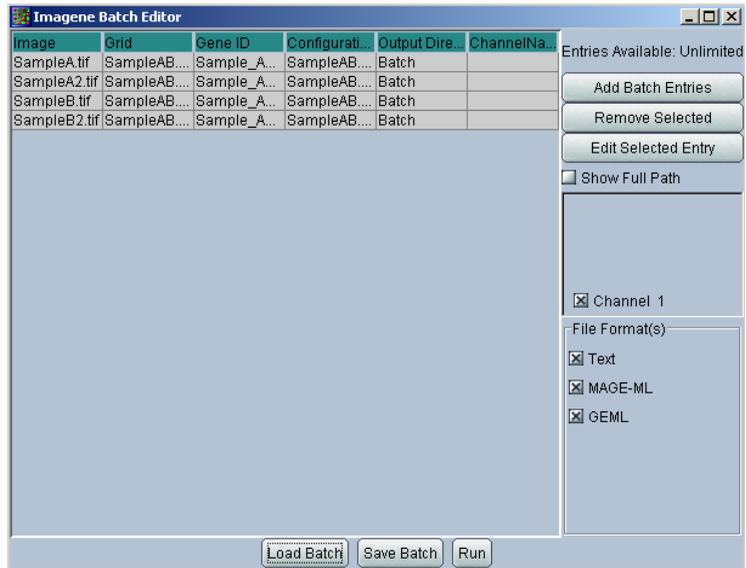
1. From the menu bar, select File followed by Batch Editor.



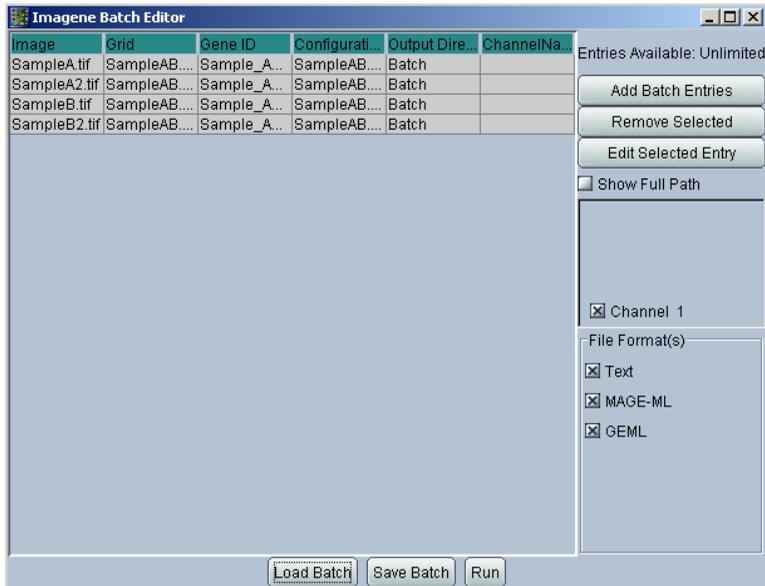
2. Click the Add Batch Entries Button on the Batch Editor Window.
3. The Create Batch Entry window appears allowing us to specify the files previously created as well as other information. First, click the Images button and browse to the Imagen samples directory. Select all image files that you plan to batch process. Holding the <ctrl> key while selecting the images allows several files to be selected at once.



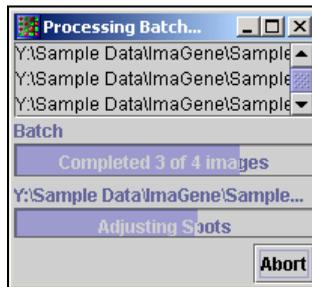
4. Next, click the Grid Button. Either a grid or a template may be specified here. Browse to and select the template we created previously.
5. Notice the Gene ID selection below the Grid is now not enabled. This is because we previously specified a gene ID and associated it with this template. Hence there is no need to do so again.
6. Click the Configuration button and specify the configuration file previously created.
7. The last parameter to specify is the Output Directory. Imagen needs to know where to store the quantified data files as well as the \*.sst snapshot files.
8. Click the OK Button to add the entries to the batch. Notice within the main Batch Editor window, there are four entries corresponding to the four images previously specified. If another set of images is to be batch processed, this process of adding entries can be repeated as many times as needed.



9. Click the Save Batch button to save this batch for possible reuse later. From the Save As window, specify a location and file name for the batch file.
10. In the **File Format(s)** panel, select any or all of the formats that you want the batch results to be saved in by checking their option boxes.



11. Finally, click the Run Button to begin the batch. A small Batch Processing Window appears displaying both the progress through the entire batch and the progress through the current image. Upon completion of the batch, this small window closes.



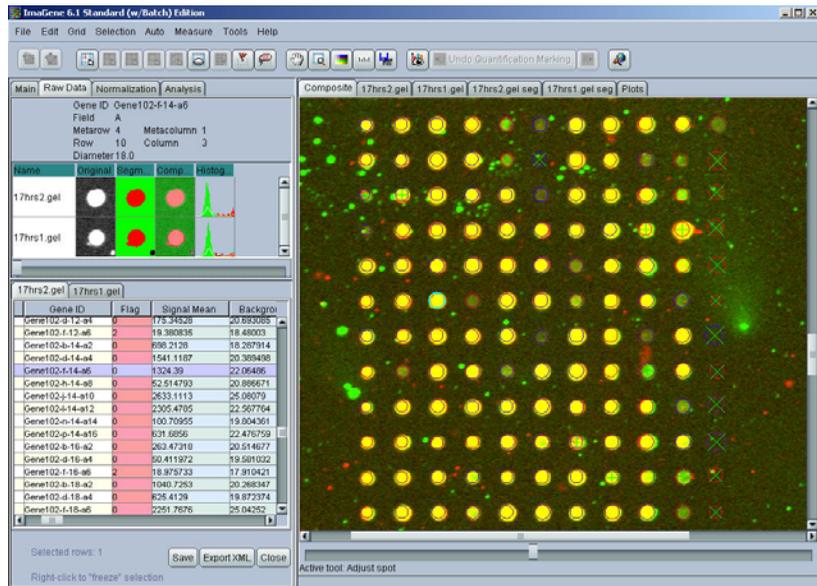
### 2.4.3 Post Batch Processing

To view the results of Batch Processing, ImaGene includes two tools, the results reviewer and the ImaGene log file.

1. Launch the ImaGene software.
2. From the File menu, select Review Results.



3. Browse to and select any one of the four snapshot files, or files ending with a .sst extension, which were produced during the previous batch processing. The file will be located within the data output directory specified. Click the Open Button.
4. ImaGene loads the images, data, quality measures, and flagging as occurred though batch processing. From here, you are free to determine the quality of the batch processing and whether or not the image needs to be manually processed.



- Also located within the data output directory is a file called log.txt. This file contains start and stop times for any or all the batches. It also contains any errors that may have occurred.

Begin Batch Fri Aug 20 16:20:02 PDT 2004

Begin Run

Info: current image: C:\TEMP\Batch\SampleA.tif

Info: channel name:

Info: # of Empty Spots: 0

Info: # of Poor Spots: 51

Info: # of Negative Spots: 7

End Run

Begin Run

Info: current image: C:\TEMP\Batch\SampleB.tif

Info: channel name:

Info: # of Empty Spots: 0

Info: # of Poor Spots: 60

Info: # of Negative Spots: 24

End Run

End Batch Fri Aug 20 16:21:41 PDT 2004

Batch processing is an easy and flexible way to provide image analysis in a high throughput environment, or walk away convenience for overnight processing. The previous example demonstrates the steps required to perform a batch. The overall success of the batch depends on a number of factors including the quality of the images, the accuracy of the settings and the uniformity of the images to one another.

## **Part 3: GeneSight-Lite**

### ***3.1 What is GeneSight-Lite?***

After intensity measurements are extracted from an image, there is a need for further data processing. ImaGene incorporates a powerful tool that provides creating simple types of experimental design, data cleansing, transformation, normalization, visualization etc. This tool is called GeneSight-Lite and can be launched at any time using its corresponding button on the toolbar. Please, be patient while it launches (might take up to 20 sec for some machines).

GeneSight-Lite will start with data set builder GUI, where you can build your data set for further analysis. Data set may include data from multiple images you processed using ImaGene. To build data set, you will have to arrange ImaGene output files (tab-delimited text files) into a specific experimental design.

Once data set is built, you can use Data Preparation tool for data cleansing, transformation and normalization. After all necessary preprocessing are applied to the data, you can start using various visualization and analysis tools of GeneSight-Lite. Aforementioned tools include:

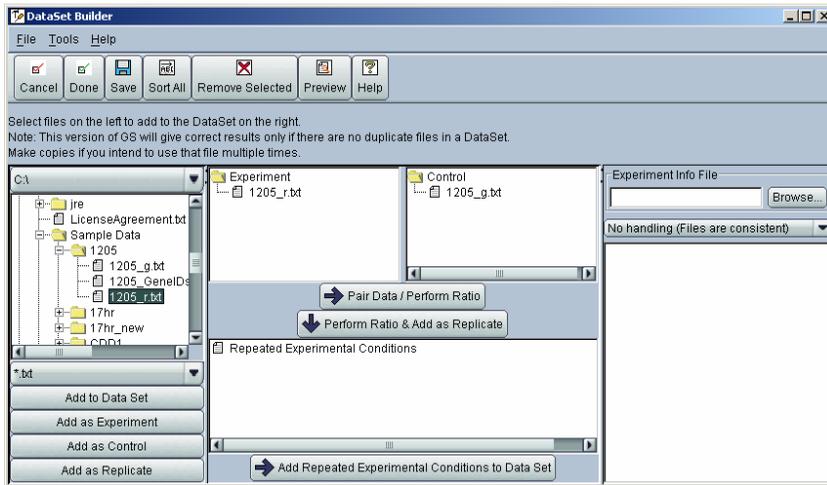
- Histogram
- 2-dimensional Scatter Plot
- Box Plot
- Partition Manager
- K-means clustering
- Time series visualization
- Reports generation

### ***3.2 How to use GeneSight-Lite***

For details on the usage of GeneSight-Lite, please refer to GeneSight-Lite user's manual included in ImaGene distribution package. In this section we will provide some guidelines for using basic visualization tools.

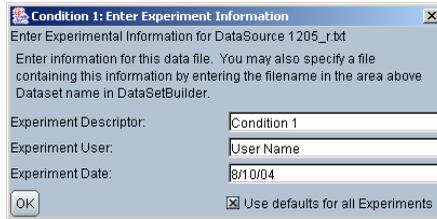
**Scatter plot.** To build a scatter plot on a data you quantified, you need to follow these steps:

1. Make sure that you save quantification results from current image (click Save button at the bottom of quantification table).
2. Click GeneSight-Lite button on the toolbar  and wait until GeneSight-Lite GUI pops up.
3. GeneSight-Lite will start with a DataSet Builder window. You can either use its Wizard or construct the data set using Builder window directly. We will explain how to use the latter. First, find the result files saved by ImaGene in the file browser window on the left-hand side. Drag and drop the control data file under the Control panel, and the experimental data file under the Experiment panel.

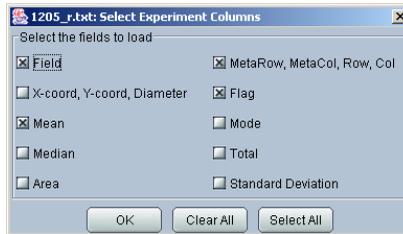


*Note: if you have only one file with measurements, drag this file directly into the right side panel of DataSet Builder and hit “Done”.*

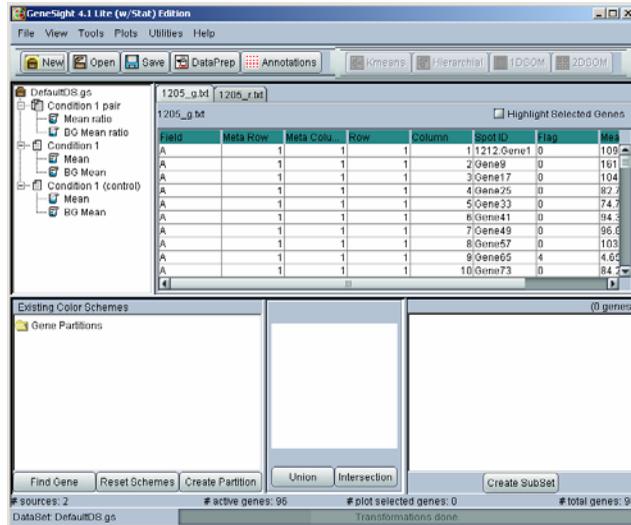
4. Click on “Pair Data/Perform Ratio” button, the new data set will show up in the panel on the right-hand side. Click “Done” button on Builder’s toolbar. Builder will ask you to enter such information as experiment date and user name to tag the data set.



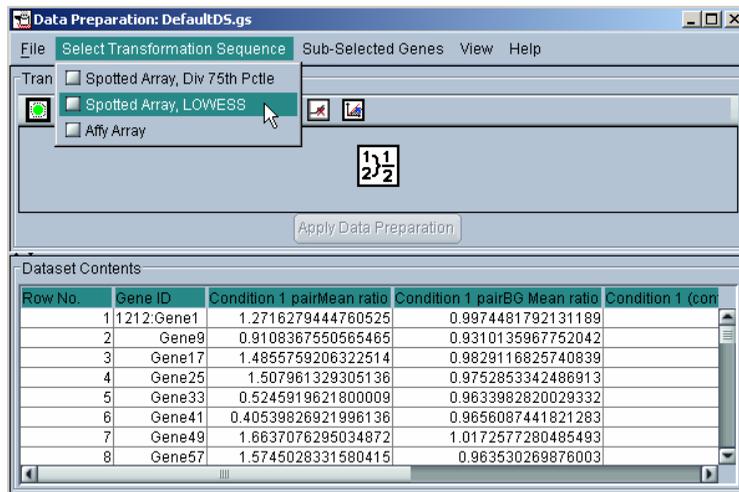
Specify the information and click “Ok”. Additional dialog box will pop up asking you to select measurements that you want to import into GeneSight-Lite.



Select measurements and click “OK”. New data set will be created and corresponding measurements will be shown on the upper-left side of the GeneSight-Lite window. For more information on how to build a new data set, please refer to GeneSight manual, Chapter 6.



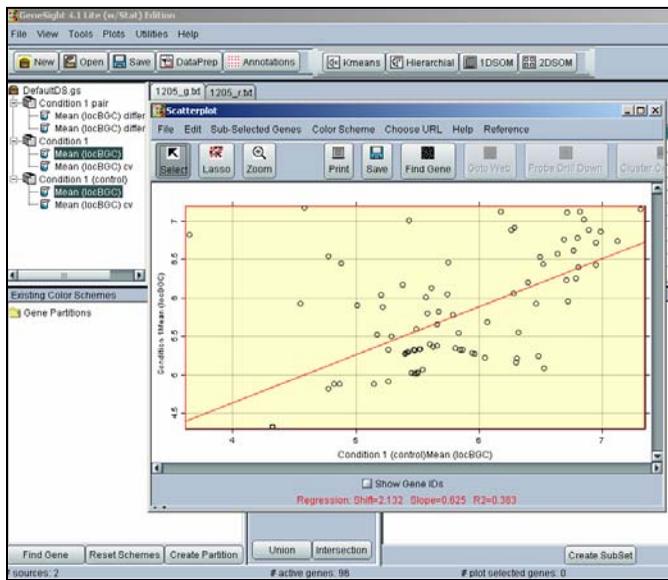
5. Select the data set and go to Tools menu of GeneSight-Lite. Select Data Preparation tool. The window for Data Preparation tool will pop up. Select one of the preset data preparation sequences from Select Transformation Sequences menu.



Or alternatively you can create your own sequence by dragging and dropping the preparation icons into the area under toolbar, where the

sequence will be displayed. Click on “Apply Data Preparation” button. Close data preparation window when the transformations have been applied. For more information on how to construct data preparation sequence, please refer to GeneSight manual, Chapter 7.

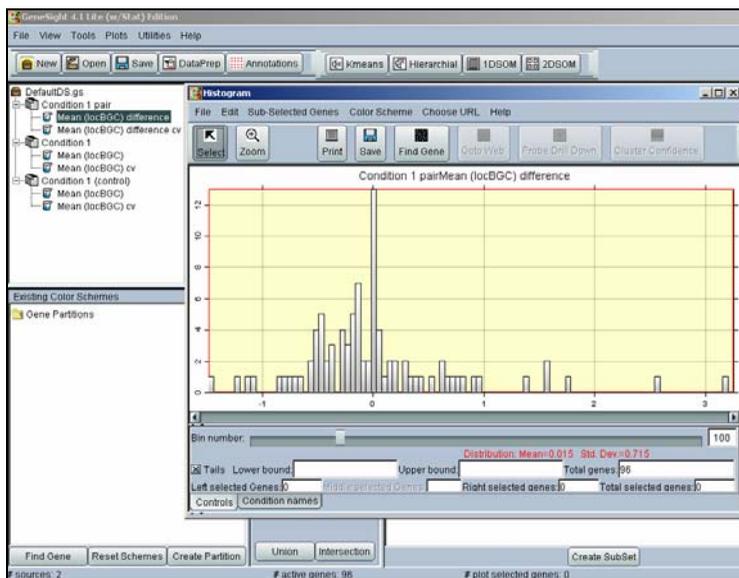
6. Select any pair of measurements in GeneSight-Lite main window.
7. Click “Scatter Plot” button on the toolbar . 2-D scatter plot window will pop up.



8. For manipulations within Scatter Plot window, refer to GeneSight manual, Chapter 9.

## Histogram

1. Follow steps 1-5 from the previous section.
2. Select the measurement of interest in GeneSight-Lite main window.
3. Click “Histogram” button on the toolbar . Histogram window will pop up.



- For manipulations within Histogram window, refer to GeneSight manual, Chapter 9.

## Go To Web

When using any of the tools mentioned above, select a gene or set of genes

and click on the “Go To Web” button . You will be automatically directed to the web based public genetic data.

## Report

GeneSight-Lite also has various ways of generating reports; please refer to GeneSight manual, Chapter 10.

*Note: all GeneSight-Lite tools can be used simultaneously and any gene sub-selection in one tool will propagate into all other tools.*

## Part 4: Installation and Licensing

### 4.1 ImaGene Installation

#### 4.1.1 Windows

##### Introduction

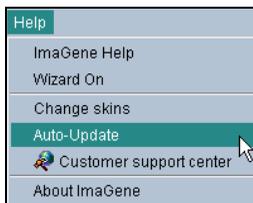
Most users are familiar with the standard windows installation system and will not encounter any difficulties. The ImaGene installation simply places the necessary files on the host computer and modifies the Start Menu.

##### ImaGene Installation

1. If you are installing ImaGene on a Windows NT, 2000, or XP operating system, *you MUST log in with an account that has Administrator access.*
2. Launch the installation by double-clicking on the exe file.
3. Review the version information on this screen, and then click the Continue button to unpack the installation files.
4. When the “Welcome to ImaGene Setup” screen appears, click the Next button.
5. Read the license agreement, and then click the Yes to agree to the terms and conditions.
6. Read the setup notes, and then click the Next button to continue.
7. Note the default name (ImaGene) of your program folder. This will be the location of the ImaGene shortcuts on your Start Menu. If you do not want to use this name, enter another name in the Program Folders field. Then click the Next button to begin the installation. An installation progress bar appears so that you can monitor the installation.
8. When the installation is complete a message box appears. Click the Finish button to complete the installation and close the install program.
9. Prior to use, please review the next section on licensing ImaGene.

#### 4.1.2 ImaGene Update

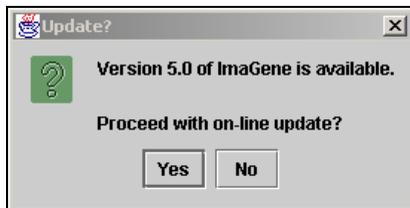
If computer is connected to the Internet, ImaGene automatically checks for any update available from BioDiscovery at the start-up. User can control frequency of update checks through menu item Help->Update.



Five different frequencies are available from checking at every start-up to not checking at all.



When ImaGene detects a new version available for download, the following message will be shown.



To proceed with the update press Yes. Your ImaGene will be updated and your old files will be backed up inside ImaGene installation directory. When update is complete, it will prompt you to restart the software.

## 4.1.3 Un-Installation Instructions

### Introduction

This section explains how to remove ImaGene from your computer. For example, if you are no longer using an older version of ImaGene, you can use this procedure to remove it from your computer.

*Note: If you have a licensed version of ImaGene, please contact BioDiscovery Technical Support at [support@biodiscovery.com](mailto:support@biodiscovery.com) before removing any version of ImaGene from your computer as un-installation may affect your licensing.*

### ImaGene Un-Installation

1. Click Start -> Settings -> Control Panel -> Add/Remove Programs.
2. Click on “ImaGene” to select this program from the Currently Installed Programs list.
3. Click the Change/Remove button to initiate the un-installation process and display the ImaGene confirmation dialog box.
4. Click the Yes button to uninstall ImaGene.  
A second ImaGene confirmation dialog box displays when the un-installation completes. Click the OK button to acknowledge that ImaGene has been removed from your computer.

## 4.1.4 Macintosh

Please see the release notes provided with your ImaGene software distribution.

## 4.1.5 Unix

Please see the release notes provided with your ImaGene software distribution.

## **4.2 License Management**

### **4.2.1 License Manager**

The License Manager is a small software service/daemon that controls which computers can run a particular BioDiscovery product and what features are available in that product. The main purpose of the license manager is to prevent software piracy, but there are additional benefits as well (such as user license tracking, time-limited demos, and advanced user network permission settings).

BioDiscovery has switched to GlobeTrotter's FlexNet license manager. This popular license manager provides an excellent balance of features, flexibility, and easy-of-use. It is purely software based, so there is no need for any sort of hardware (like a parallel port dongle) and it can handle licenses from multiple companies. If your institution/business is already running FlexNet-based products, the addition of BioDiscovery licenses will be very easy.

Many specific FlexNet questions are answered in this online FAQ:

<http://www.globetrotter.com/FlexNet/lmfaq.shtml>

It is not necessary to review this FlexNet FAQ, since the basic setup of FlexNet is covered in the accompanying text.

The FlexNet license manager can be configured in two possible ways: Node-Locked and Floating.

### **4.2.2 Node-Locked Licenses**

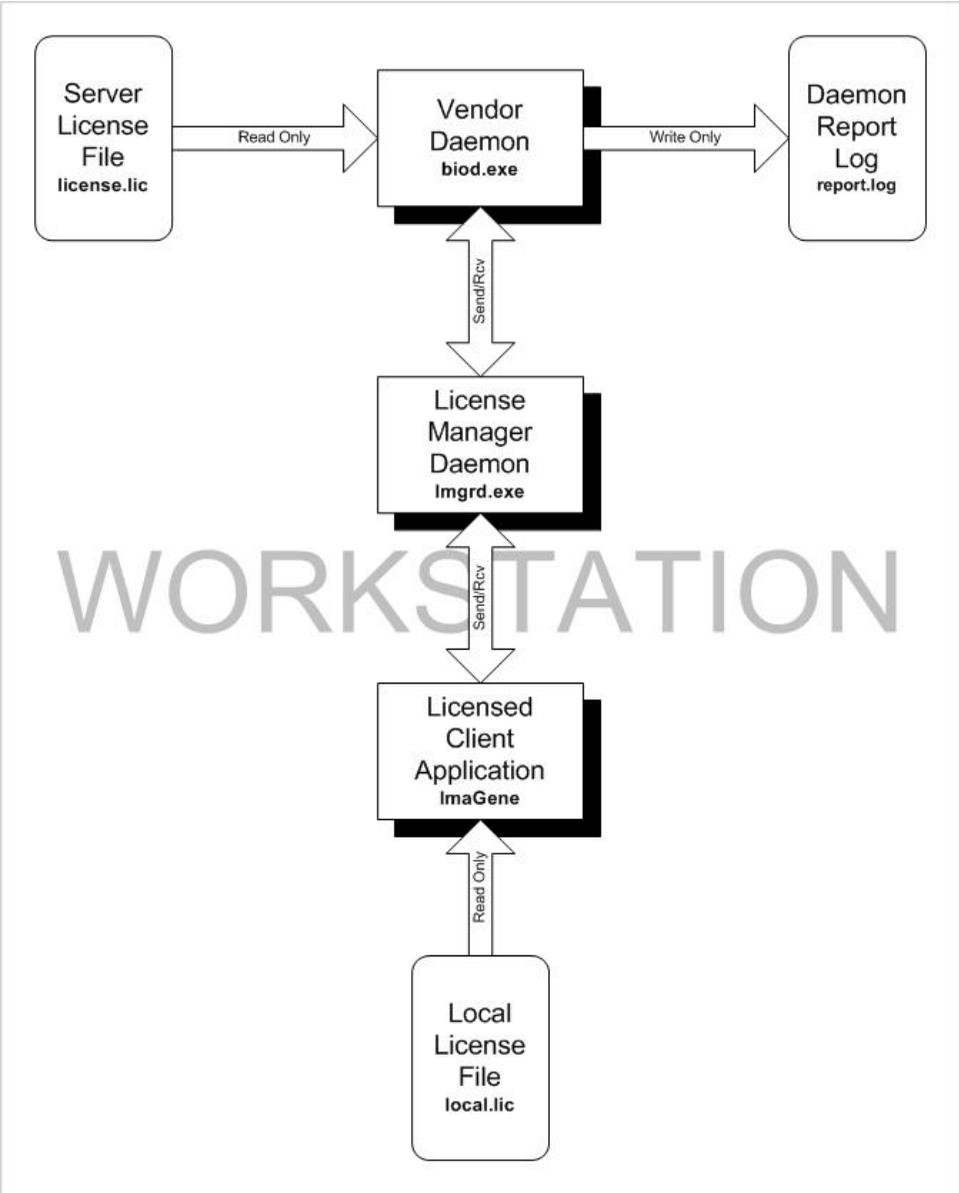
A node-locked license allows BioDiscovery products to run on a single, specific computer. The license is "hard-coded" to that specific computer's ID and will not run on any other computers. Node-Locked is the more common of the two possible configurations.

In order to run in the Node-Locked mode, the computer must be both a FlexNet license server and its own client. This requires the user to setup a server on the computer in addition to the regular installation of the software.

The FlexNet license manager service will run in the background at all times, regardless of whether or not a BioDiscovery product is also running. This may seem inefficient, but the overhead of the license manager is minimal and it would be inconvenient for the user to have to manually start the license manager service every time.

Below is an example of a node-locked computer being both a license server and a client. This diagram is also a good reference since it shows the file names for each part of the license.

# FlexNet Node-Locked Dataflow



### 4.2.3 Floating Licenses

A floating license allows BioDiscovery products to run on a set number of different computers with the limitation that no more than a certain number of computers can run the software simultaneously.

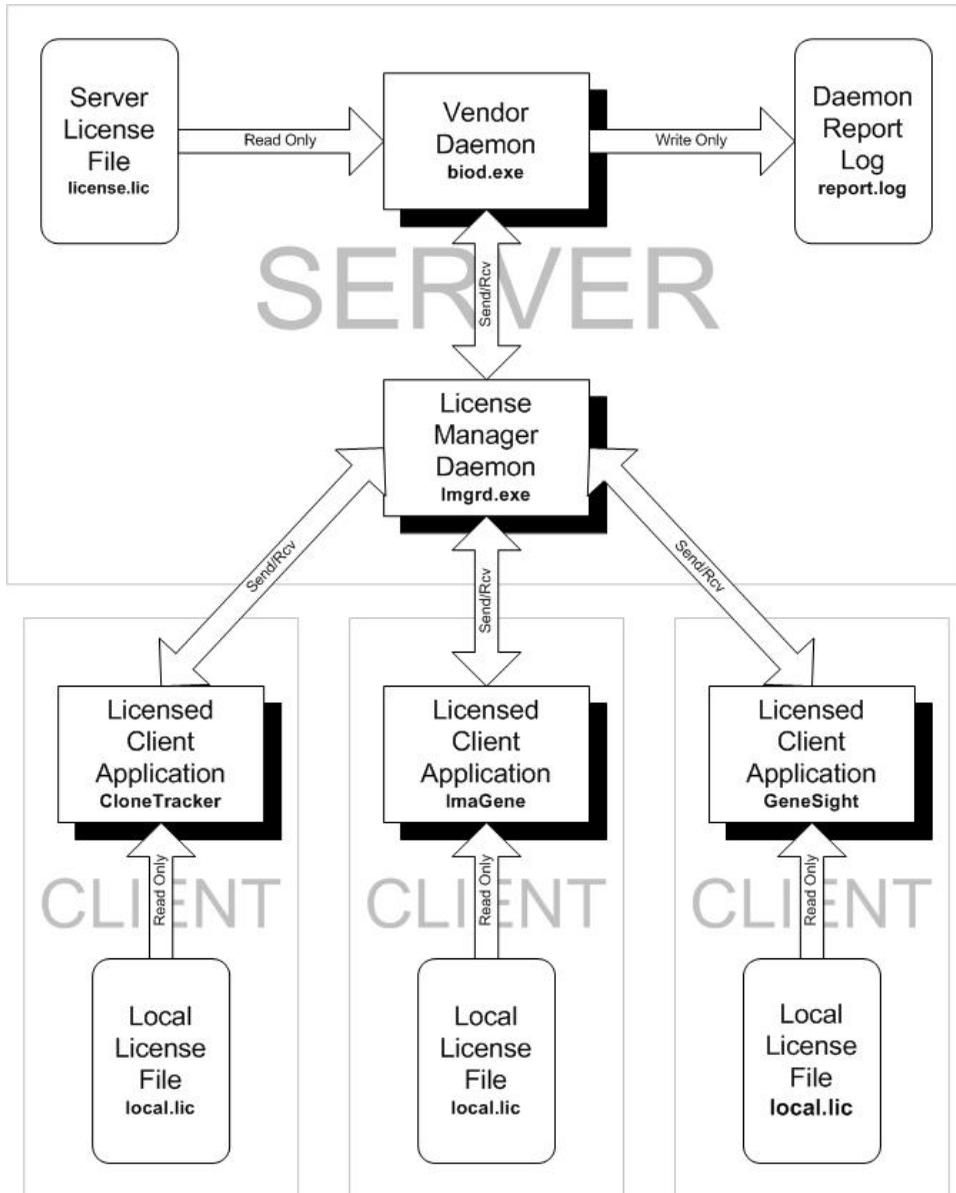
For example, a 5-seat floating license server would allow up to 5 computers access to a particular product. The user may install the software on as many computers as she wishes, but only 5 can run the software at one given time.

FlexNet provides distinct advantages over other licensing systems in that it can track license usage at the current moment or over an extended period of time. This allows system administrators to track who is checking out licenses most often and if additional licenses need to be purchased.

While less common, the floating license scheme is actually easier to set up than the node-locked scheme since only one server needs to be set up. Each of the client computers only needs to be told where that server is (on a network or even over the Internet).

Below is an example of three clients communicating with a single license server. This diagram is also a good reference since it shows the file names for each part of the license.

# FlexNet Floating (Client/Server) Dataflow



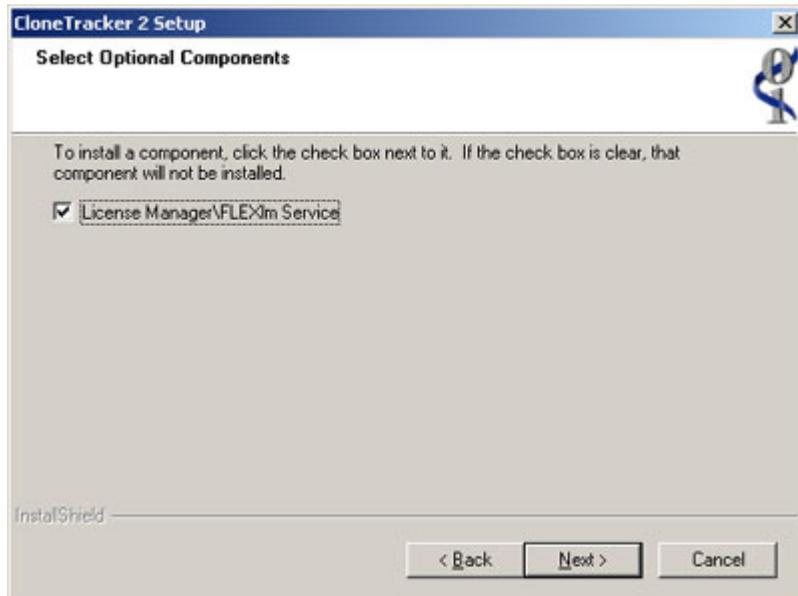
## 4.3 FlexNet NodeLocked Installation Guide (for Windows)

These instructions apply to all BioDiscovery products running on any supported platform. In some places the tag {Product} will appear. This represents the name of the BioDiscovery product being installed (like “CloneTracker” for example).

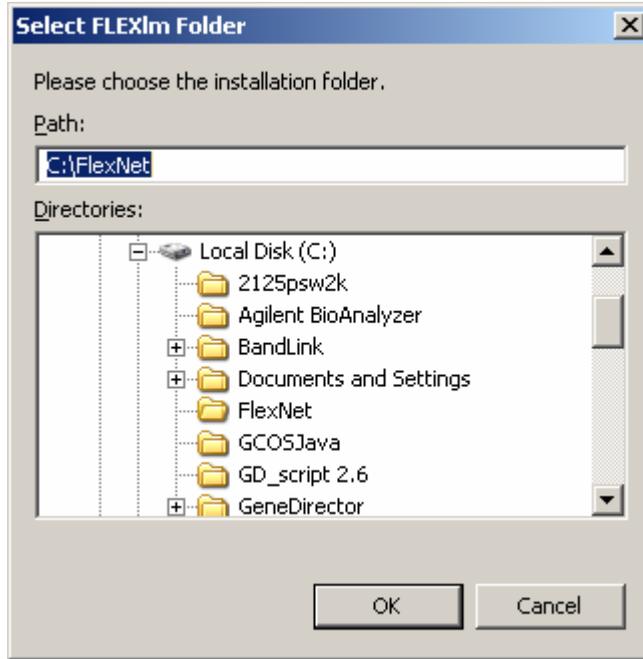
*The default install directory for FlexNet is C:\FlexNet. In the following examples, it is assumed that this is the directory being used. If FlexNet is installed into another directory then make the appropriate modifications to the path.*

### 1. Install the BioDiscovery software (with the FlexNet license manager service)

- a. Make sure that the account you are using has administrative privileges on this computer.
- b. Make sure that you select the option to install the FlexNet Service component during the software install.



- c. Remember the directory you've selected for FlexNet during the install. Note that "C:\FlexNet" is the default

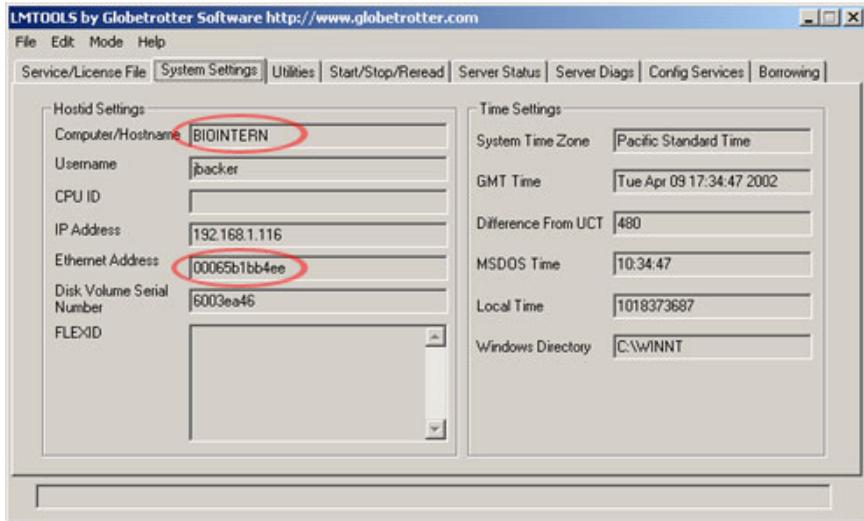


**3. Do you already have a license file called "license.lic"?**

- a. If yes, then skip to step #6.
- b. If no, then proceed to step #4.

**4. Determine the server's Ethernet Address and HostName.**

- a. Start the LMTools program by clicking on Start -> Program Files -> {Product} -> LMTools.
- b. Click on the System Settings tab.
- c. You will see the following screen:



- d. The computer's Ethernet Address is used as the computer ID.
- e. The Ethernet Address is a 12-digit hexadecimal number. *In this example the address is "00065b1bb4ee".*
- f. The computer's Hostname is used to identify the computer on the network. *In this example the hostname is "BIOINTERN".*
- g. You can close LMTools by select Exit from the File menu, or clicking the small X button in the top-right corner.

## 5. Request license file from BioDiscovery.

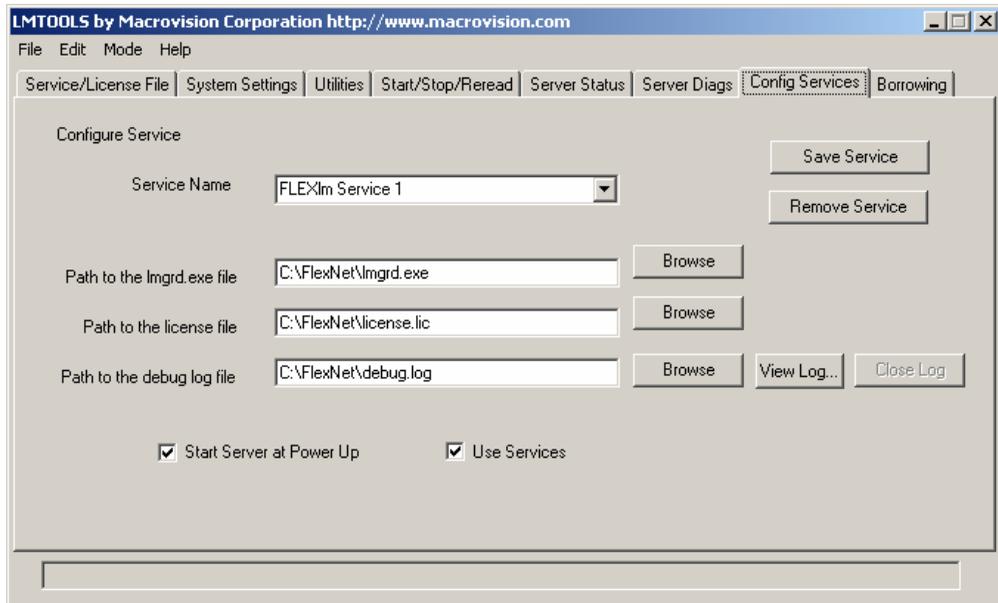
- a. Email [support@biodiscovery.com](mailto:support@biodiscovery.com) for your customized license file.
- b. Please include:
  1. The Computer's Ethernet Address from step #4.
  2. The Computer's Hostname from step #4.
  3. Your name (or the name of who purchased the software)
  4. Your company
  5. Your BioDiscovery Product Serial Number (if you've purchased a copy). You can find your serial number on the case of the CD shipped to you or in the accompanying User Registration form. If you haven't purchased a copy, just list the products you wish to evaluate.
- c. BioDiscovery will reply with a small text file called "license.lic". This file contains all of your licensing information.

## 6. Copy this license file into the FlexNet directory you specified in step #2.

You should keep a backup copy of this license file on another computer or floppy disk in case of an emergency.

## 7. Configure the license services

- a. Start the LMTools program by double-clicking on lmttools.exe in the FlexNet directory.
- b. Click on the Config Services tab.
- c. You will see the following screen:

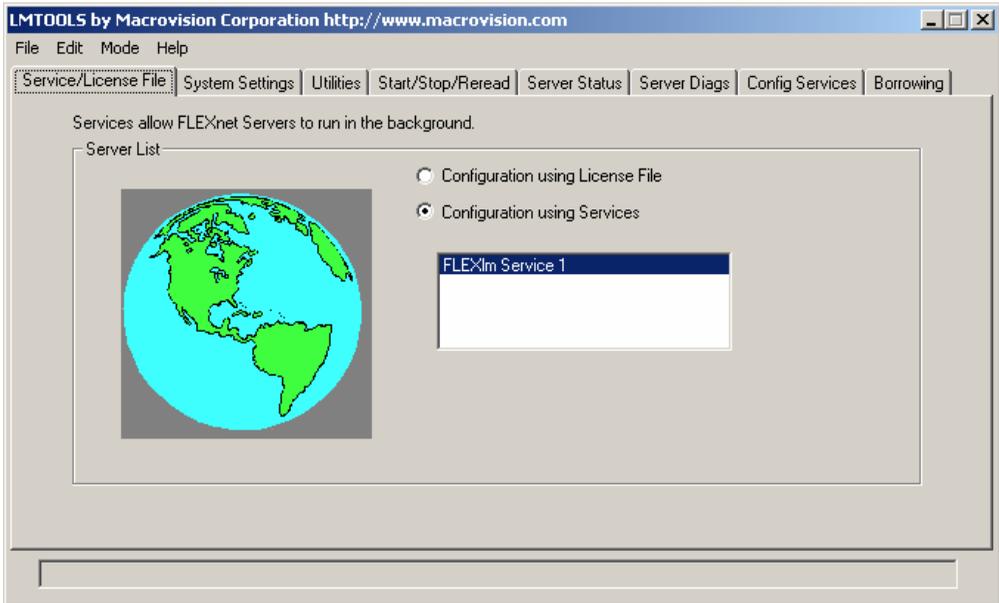


- d. You can leave the Service Name as its default: "FlexNet Service 1".
- e. Enter the Path to the lmgrd.exe file or click the Browse button to the right. In this example it is "C:\FlexNet\lmgrd.exe".
- f. Enter the Path to the license file or click the Browse button to the right. In this example it is "C:\FlexNet\license.lic".
- g. Enter the Path to the debug.log file or click the Browse button to the right. In this example it is "C:\FlexNet\debug.log".
- h. Make sure you place a check mark in both Start Server at Power Up and Use Services. *Note that the Use Services checkbox may be disabled under Windows 95/98/ME.*
- i. Click on the Save Service button.

- j. Answer “Yes” on the dialog that appears to confirm these settings.

## 8. Select the license services

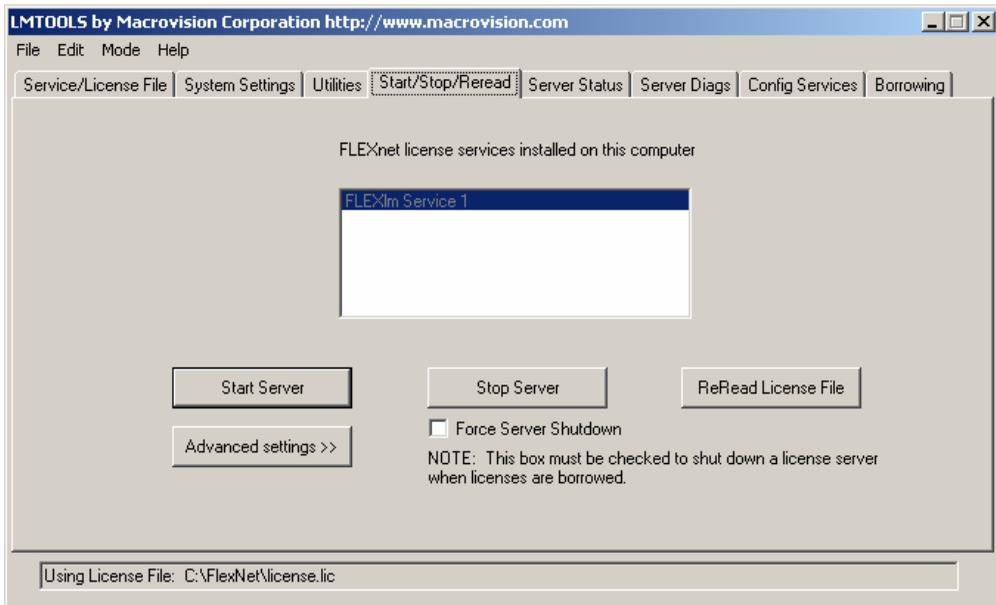
- a. Click on the Service/License File tab.  
b. The following screen will appear:



- c. Select the Configuration using Services radio button.  
d. Select the Service Name you used from step #7.

## 9. Start the License Server

- a. Click on the Start/Stop/Reread tab.  
b. The following screen will appear:



- c. Double-check that the Service Name selected is the same as the one you configured in step #7.
- d. Click on the Start Server button.
- e. The text at the bottom of the window should change from “Using License File:” to “Server Start Successful.”

### 9. Did the license server start successfully?

- a. If yes, then you’re almost done. Skip to step #11.
- b. If no, then you need to do some troubleshooting. Proceed to step #10.

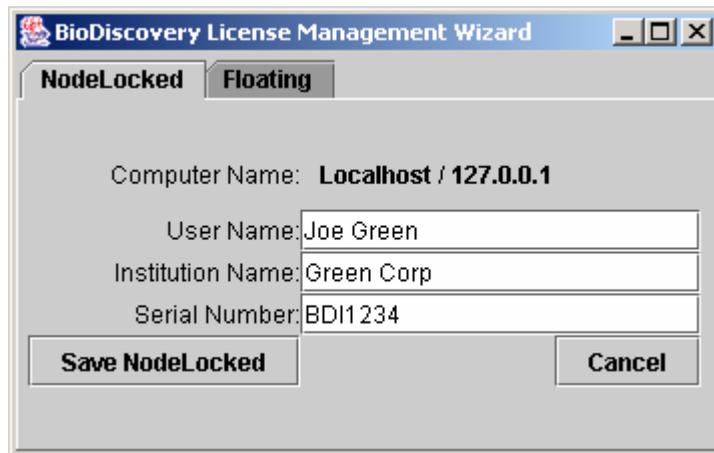
### 10. Troubleshooting the FlexNet license server

- a. Double-check that you entered the correct file paths in step #7.
- b. View the license file by right clicking on the “license.lic” in the FlexNet directory and selecting Open With...
- c. Select “Notepad” from the list.
- d. Place a checkmark next to “Always use this program to open these files”.
- e. Click the OK button.
- f. The text file will contain a line that looks similar to this:
- g. SERVER HOSTNAME 00065b1bb4ee

- h. Make sure that HOSTNAME is your computer's Hostname from step #4.
- i. Make sure that the following Ethernet Address matches your computer's Ethernet address from step #4.  
If it still doesn't start, then email the C:\FlexNet\debug.log file to [support@biodiscovery.com](mailto:support@biodiscovery.com)

## 11. Run the License Wizard (configure the local client)

- a. Start the License Wizard by clicking on Start -> Program Files -> {Product} -> License Wizard.
- b. Click on the NodeLocked tab.
- c. You will see the following screen:



- d. Enter your User Name, Company Name, and Serial Number.
- e. It is important that you enter your actual Serial Number since this information is used to determine eligibility for upgrades using the BioDiscovery Auto-Update feature.
- f. The serial number can be found on the case of the CD shipped to you or in the accompanying User Registration form.
- g. Click the Save NodeLocked button to save this information and exit.

## 12. Start the BioDiscovery software.

If the license server is running, the software will start up normally.

## 4.4 FlexNet Server Installation Guide (for Windows)

*The default install directory for FlexNet is C:\FlexNet. In the following examples, it is assumed that this is the directory being used. If FlexNet is installed into another directory then make the appropriate modifications to the path.*

### 1. Extract FlexNet Server files.

- a. Double-click on the self-extracting executable FlexNet.zip.
- b. You will see the following screen:



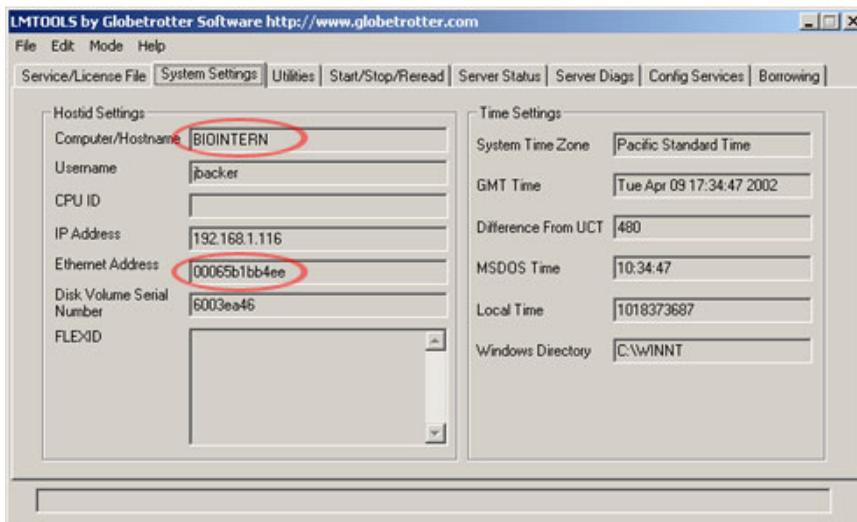
- c. Select “Unzip to folder” (C:\FlexNet by default).
- d. Click the Unzip button to extract the files.
- e. Click OK once the extraction is complete.
- f. Click Close to exit the extraction program

### 2. Do you already have a license file called “license.lic”?

- a. If yes, then skip to step 5.
- b. If no, then proceed to step 3.

### 3. Determine the server's Ethernet Address and HostName.

- a. Start the LMTools program by double-clicking on lmtools.exe in the FlexNet directory.
- b. Click on the System Settings tab.
- c. You will see the following screen:



- d. The computer's Ethernet Address is used as the computer ID.
- e. The Ethernet Address is a 12-digit hexadecimal number. *In this example the address is "00065b1bb4ee".*
- f. The computer's Hostname is used to identify the computer on the network. *In this example the hostname is "BIOINTERN".*
- g. You can close LMTools by select Exit from the File menu, or clicking the small X button in the top-right corner.

### 4. Request license file from BioDiscovery.

- a. Email [support@bioDiscovery.com](mailto:support@bioDiscovery.com) for your customized license file.
- b. Please include:
  1. The Computer's Ethernet Address from step #4.
  2. The Computer's Hostname from step #4.
  3. Your name (or the name of who purchased the software)
  4. Your company
  5. Your BioDiscovery Product Serial Number (if you've purchased a copy). You can find your serial number on the case of the CD shipped to

you or in the accompanying User Registration form. If you haven't purchased a copy, just list the products you wish to evaluate.

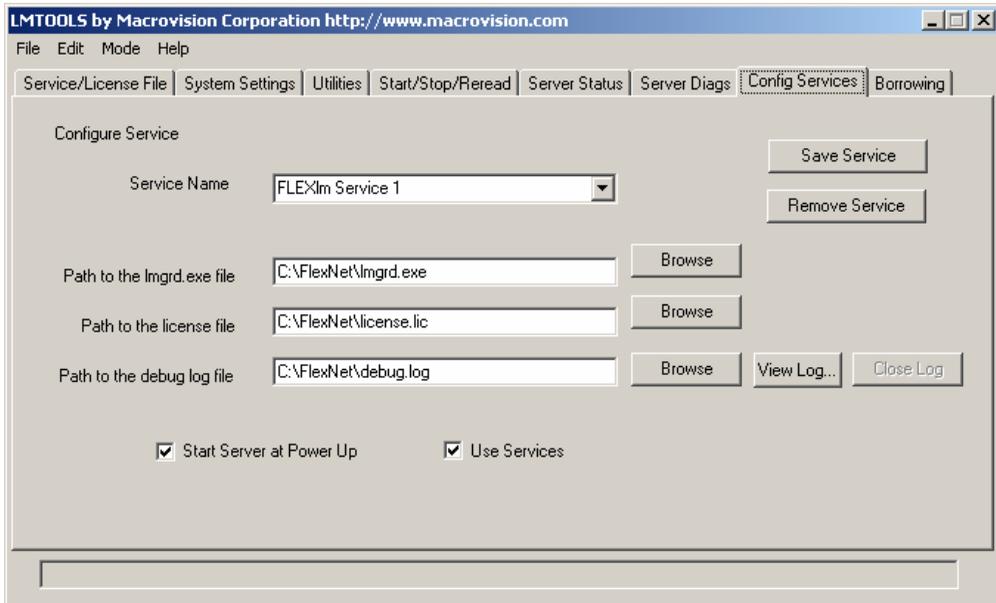
- c. BioDiscovery will reply with a small text file called "license.lic". This file contains all of your licensing information.

## 5. Copy license.lic into your FlexNet directory.

In truth, the license.lic file can be anywhere on your computer, but we put it in the C:\FlexNet directory to make it easier to keep track of.

## 6. Configure the license services

- a. Start the LMTools program by double-clicking on lmtools.exe in the FlexNet directory.
- b. Click on the Config Services tab.
- c. You will see the following screen:

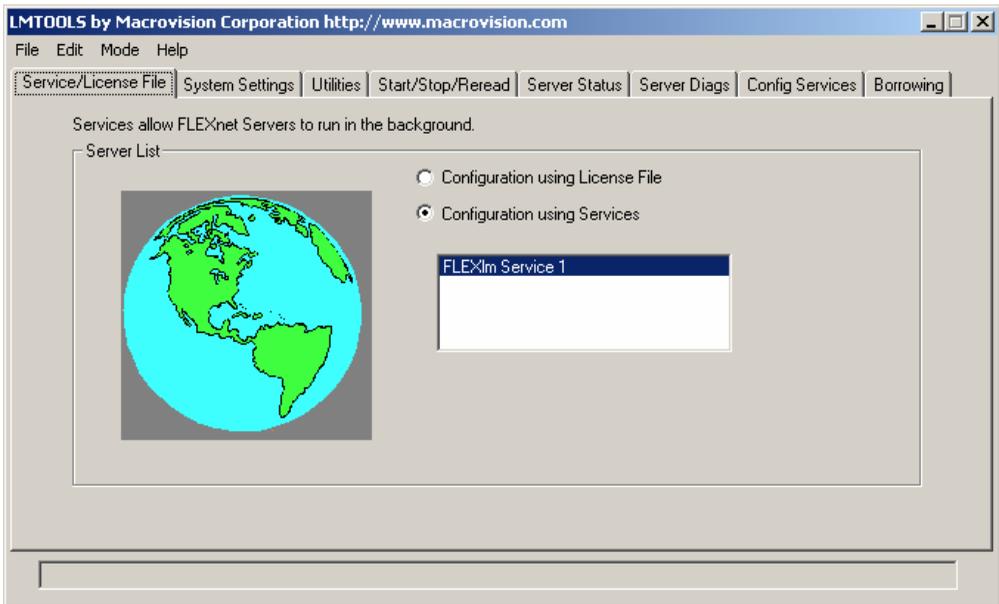


- d. You can leave the Service Name as its default: "FlexNet Service 1".
- e. Enter the Path to the lmgrd.exe file or click the Browse button to the right. In this example it is "C:\FlexNet\lmgrd.exe".
- f. Enter the Path to the license file or click the Browse button to the right. In this example it is "C:\FlexNet\license.lic".

- g. Enter the Path to the debug.log file or click the Browse button to the right. In this example it is “C:\FlexNet\debug.log”.
- h. Make sure you place a check mark in both Start Server at Power Up and Use Services. *Note that the Use Services checkbox may be disabled under Windows 95/98/ME.*
- i. Click on the Save Service button.
- j. Answer “Yes” on the dialog that appears to confirm these settings.

## 7. Select the license services

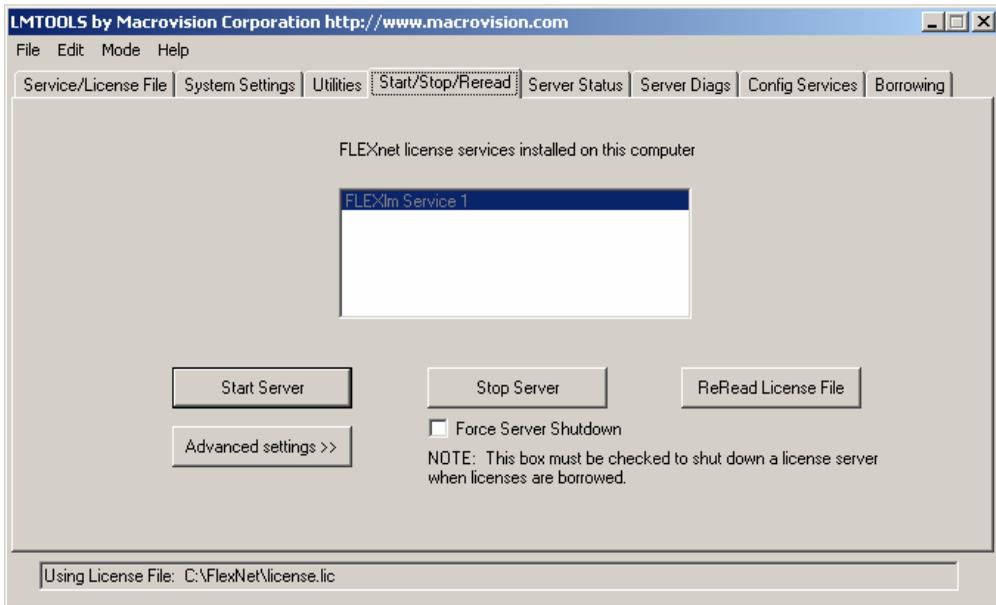
- a. Click on the Service/License File tab.
- b. The following screen will appear:



- c. Select the Configuration using Services radio button.
- d. Select the Service Name you used from step #7.

## 8. Start the License Server

- a. Click on the Start/Stop/Reread tab.
- b. The following screen will appear:



- c. Double-check that the Service Name selected is the same as the one you configured in step #6.
- d. Click on the Start Server button.
- e. The text at the bottom of the window should change from “Using License File:” to “Server Start Successful.”

## 9. Did the license server start successfully?

- a. If yes, then you’re almost done. All you need to do now is configure each of the clients to run the software. Please see the FlexNet Client Installation Guide for details.
- b. If no, then you need to do some troubleshooting. Proceed to step 10.

## 10. Troubleshooting the FlexNet license server

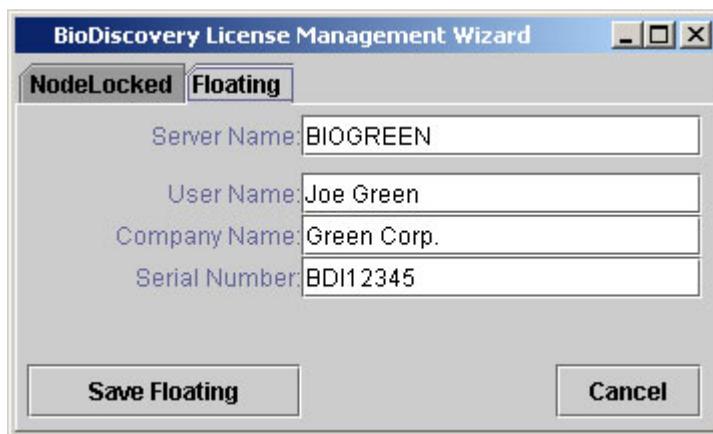
- a. Double-check that you entered the correct file paths in step #6.
- b. View the license file by right clicking on the “license.lic” in the FlexNet directory and selecting Open With...
- c. Select “Notepad” from the list.
- d. Place a checkmark next to “Always use this program to open these files”.
- e. Click the OK button.

- f. The text file will contain a line that looks similar to this:
- g. SERVER HOSTNAME 00065b1bb4ee
- h. Make sure that HOSTNAME is your computer's Hostname from step #3.
- i. Make sure that the following Ethernet Address matches your computer's Ethernet address from step #3.
- j. If it still doesn't start, then email the C:\FlexNet\debug.log file to [support@biodecovery.com](mailto:support@biodecovery.com)

## 4.5 FlexNet Client Installation Guide (all platforms)

*These instructions apply to all BioDiscovery products running on any supported platform. In some places the tag {Product} will appear. This represents the name of the BioDiscovery product being installed (like “CloneTracker” for example).*

1. Start the License Wizard by clicking on Start -> Program Files -> {Product} -> License Wizard.
2. Click on the Floating tab.
3. You will see the following screen:



The screenshot shows a dialog box titled "BioDiscovery License Management Wizard". It has two tabs: "NodeLocked" and "Floating", with "Floating" selected. The dialog contains four text input fields: "Server Name" with the value "BIOGREEN", "User Name" with "Joe Green", "Company Name" with "Green Corp.", and "Serial Number" with "BDI12345". At the bottom, there are two buttons: "Save Floating" and "Cancel".

4. Enter the name of the computer running the license server:
  - a. This can be the computer's network name (like BIOGREEN).
  - b. This can be the TCP/IP address of the computer (like 192.168.1.6).
  - c. This can be an Internet domain name (like [www.biodiscovery.com](http://www.biodiscovery.com)).
  - d. This can be this local computer. Use "THIS\_HOST" to indicate that this is the very computer serving the license files.
5. Enter your User Name, Company Name, and Serial Number.
6. It is important that you enter your actual Serial Number since this information is used to determine eligibility for upgrades using the BioDiscovery Auto-Update feature.
7. The serial number can be found on the case of the CD shipped to you or in the accompanying User Registration form.
8. Click the Save Floating button to save this information and exit.

9. Start the BioDiscovery software. If the license server is running, the software will start up normally.

## Part 5: Appendices

### 5.1 File Specifications

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

#### 5.1.1 Gene ID File (ExpressionModule)

##### **Expression Module:**

The Gene ID file is utilized for track information about the 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, if a spot is selected from a scatter plot within GeneSight-Lite, the specific Gene ID associated with the selected spot in addition to all the other detailed quantification information will be displayed. To accomplish this, ImaGene needs the Gene ID file to associate a reference ID to each spot in the array of spots in the image.

ImaGene supports two formats for the Gene ID file. The first format will be familiar to existing ImaGene users since the basic structure is the same across versions. The second format is based upon the new multi-level grid structure available in ImaGene. 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 ImaGene will not be able to process the hybrid file format.*

*Note: In ImaGene 6.0 you can add unlimited number of annotation columns after gene ID column in gene ID file and these are displayed in the results as Annotation1...N (For N such columns).*

## 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. For header information: this line must start with a percentage sign, “%.” The percentage sign is an indication to ImaGene 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 /Annotation 1/ Annotation 2/... . Separator: Tabs are utilized to delimit the fields in each row.

**Order** - ImaGene does not care about the order in which the rows are sorted. It's also unnecessary 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. A database entry can be specified followed by the gene name as one option.

**Comment Lines** - Use the percentage sign, “%,” to indicate comment lines.

**Creating the Gene ID File** - Any text processing program, spreadsheet, or database program may be used to generate the Gene ID file. CloneTracker software can also be utilized 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 ImaGene.

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 Annotation1 Annotatio2 ...**

*Note: You can optionally add Annotation columns after the Gene ID column. The number of Annotation columns is not limited.*

## Gene ID Format #2 (ImaGene 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 / Annotation1 / Annotation2 ...

### Header Information

Field MR MC SR SC Gene ID Annotation1 Annotatio2 ...

*Note: You can add optional Annotation columns after the Gene ID column. The number of Annotation columns is not limited.*

## 5.1.2 Gene ID File (CGH and Expression Module)

The format and content for this Gene ID file is required for CGH analysis, but may be used for Expression analysis. The new format is also a tab-delimited text file that may look as follows:

Metarow	Metacol	Row	Col	ID	Chromosome	Base Pair
1	1	1	1	RP11-124H12	X	31561116
1	1	1	2	RP11-185M20	2	15197943
1	1	1	3	RP11-40N8	5	67274446
1	1	1	4	RP11-274E3	20	45337121

It must contain a Header Row with the following required text strings headers in any order.

**“Metarow”**, **“Metacol”**, **“Row”**, **“Col”**, **“ID”**, **“Chromosome”** and **“Base Pair”**.

Optional headers include:

**Field** (as in earlier versions of ImaGene) and the new column **“Color”**, where color determines how this probe will display in the results plots.

Additional custom headers can include User defined annotations, which will also appear in the ImaGene results files.

## 5.1.3 Output File

ImaGene exports standard tab-delimited text files when saving data. The information below is intended to describe the general format of the test file and explain particular fields therein. ImaGene saves the quantified data from each image processed into individual text files. Due to the multi-image processing capability, ImaGene 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, BioDiscovery recommends importing the data files into a spreadsheet program such as Microsoft Excel.*

```

Begin Header
  version  6.0.0
  Date     Mon Sep 06 16:40:28 GMT-08:00 2004
  Image File      C:\ImaGene\Sample Data\CDD1\CCD1_cy3.tif
  Page         0
  Page Name
  Inverted     false
  Begin Field Dimensions
      Field      MetarowsMetacols  Rows      Cols
      A          2          2          21        22
  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              1.0
      Background Width              10.0
  End Measurement parameters
  Begin Alerts
      Control Type      Minimum thresholdIf tested  Percentage allowed If
failed  Maximum threshold  If tested  Percentage allowed If failed  CV threshold
      Bright           0.0      false      0.0%      false      10000.0  false
      0.0%            false      10.0      false      false
  End Alerts
  Begin Quality Flags

```

```

Begin Flagging Settings
  Empty Spots      true   Threshold:      2.0
  Poor Spots       false
  Negative Spots   true
End Flagging Settings
Begin Flagged spots
  # of Empty Spots: 262
  # of Poor Spots: 0
  # of Negative Spots: 0
  # of Manually Flagged Spots: 0
End Flagged spots

```

```

End Quality Flags
End Header
Begin Raw Data
  Field      Meta Row      Meta Column      Row      Column      Gene ID
  Annotation 1  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
  Selected spot      Saturated spot
  A      1      1      1      1      null      null      0
28161.3613      647.7194      15924.0      636.0      64460.6015      631.3684      83.0
278.0      2337393.0      180066.0      27675.3652      41.7804      0.7232      0.0      83.0
0.0      0.9577      0.0      37.7295      36.0467      9.0      0.5892      -0.4163
0.4169      38.1458      35.6297      39.3132      34.1084      1.9176      1.1674      -1.5212
9.0587      11.9599      0      0      0      0      0      0      0
0      0      0      0      0      0
A      1      1      1      2      null      null      0
7143.6123      645.0494      3064.0      630.0      15128.1542      624.7039      98.0
263.0      700074.0      169648.0      7305.3032      46.2776      0.649      0.0      98.0      0.0
0.8995      0.1351      55.8644      35.9673      9.0      0.5331      -0.3766      0.3772
56.241      35.59      57.9795      33.7959      2.4982      1.7385      -1.7941      9.4711
13.5513      0      0      0      0      0      0      0      0
0      0      0      0      0

```

## 5.1.4 Configuration File

The configuration file stores information about virtually all the parameters within ImaGene. This file is an XML file format which is easily saved and

loaded from the ImaGene Parameter Settings Window. While the contents of this file are not important to most users, be aware that this file is required for batch processing. Advanced users integrating ImaGene into high-throughput environments may also find the information useful.

Listed below is a sample ImaGene configuration file:

```
<?xml version="1.0" encoding="UTF-8"?>

<ImaGene_Parameters>
  <SPOT_AREA>true</SPOT_AREA>
  <MEASURE>Normalized Signal Median</MEASURE>
  <CONNECT_FLAG>false</CONNECT_FLAG>
  <CUT_OFF>0.0</CUT_OFF>
  <SIGNAL_LOW>0.0</SIGNAL_LOW>
  <CONTROL_TYPE_LOWTHRESH_2>0.0</CONTROL_TYPE_LOWTHRESH_2>
  <CONTROL_TYPE_UPTHRESH_1>500.0</CONTROL_TYPE_UPTHRESH_1>
  <CONTROL_TYPE_LOWTHRESH_1>1.0</CONTROL_TYPE_LOWTHRESH_1>
  <CONTROL_TYPE_COLOR_1>-16724992</CONTROL_TYPE_COLOR_1>
  <DONUT_FLAG>true</DONUT_FLAG>
  <EMPTY_THRESH>2.0</EMPTY_THRESH>
  <HOT_CHECK>false</HOT_CHECK>
  <FLEXIBILITY>50.0</FLEXIBILITY>
  <BLANK_LOW>0.0</BLANK_LOW>
  <BACKGROUND_FLAG>true</BACKGROUND_FLAG>
  <CONTROL_TYPE_IFLOWALERT_1>false</CONTROL_TYPE_IFLOWALERT_1>
  <TYPE_FOLD>false</TYPE_FOLD>
  <SIGNAL_HIGH>0.98</SIGNAL_HIGH>
  <MEASURE_MODE>true</MEASURE_MODE>
  <NEGATIVE_FLAG>false</NEGATIVE_FLAG>
  <IGNORED_AREA>true</IGNORED_AREA>
  <POOR_FLAG>false</POOR_FLAG>
  <PERIMETERTOAREA_THRESH>0.7</PERIMETERTOAREA_THRESH>
  <CONTROL_TYPE_UPTHRESH_0>100000.0</CONTROL_TYPE_UPTHRESH_0>
  <CONTROL_TYPE_COLOR_2>-65536</CONTROL_TYPE_COLOR_2>
  <CONTROL_TYPE_UPALERTTHRESH_0>0.0</CONTROL_TYPE_UPALERTTHRESH_0>
  <IGNORED_FLAG>true</IGNORED_FLAG>
  <TYPE_ERROR>false</TYPE_ERROR>
  <CONTROL_TYPE_UPALERTTHRESH_1>0.0</CONTROL_TYPE_UPALERTTHRESH_1>
  <POOR_AND>false</POOR_AND>
  <CONTROL_TYPE_IFCVALERT_2>false</CONTROL_TYPE_IFCVALERT_2>
  <BACKGROUND_HIGH>1.0</BACKGROUND_HIGH>
  <CONTROL_TYPE_ID_0_0>2000* </CONTROL_TYPE_ID_0_0>
  <STANDARD_LOW>5000.0</STANDARD_LOW>
  <CONTROL_TYPE_IFUPALERT_1>true</CONTROL_TYPE_IFUPALERT_1>
  <FIND_NEGATIVES>false</FIND_NEGATIVES>
  <EMPTY_AND>true</EMPTY_AND>
  <HOT_HIGH>100000.0</HOT_HIGH>
  <SIGNAL_THRESH>0.9995</SIGNAL_THRESH>
  <SATURATION_THRESH>50.0</SATURATION_THRESH>
  <PERIMETERTOAREA_FLAG>false</PERIMETERTOAREA_FLAG>
```

```

<GENES_DEDICATED_CTRL>false</GENES_DEDICATED_CTRL>
<OMIT_FLAGGED_SPOTS>true</OMIT_FLAGGED_SPOTS>
<IGNORED_THRESH>25.0</IGNORED_THRESH>
<HOT_CV>3.0</HOT_CV>
<TOLERANCE>5.0</TOLERANCE>
<CONTROL_TYPE_NAME_0>POSITIVE</CONTROL_TYPE_NAME_0>
<LOG_SCALE>false</LOG_SCALE>
<MEASURE_MEAN>true</MEASURE_MEAN>
<SHAPE_REGULARITY>true</SHAPE_REGULARITY>
<LOG>Base 2</LOG>
<SMOOTH>0.1</SMOOTH>
<CONTROL_TYPE_UPTHRESH_2>0.0</CONTROL_TYPE_UPTHRESH_2>
<OFFSET_FLAG>true</OFFSET_FLAG>
<CHANNEL2>true</CHANNEL2>
<OVERALL_FLAG>false</OVERALL_FLAG>
<CONTROL_TYPE_COLOR_0>-65536</CONTROL_TYPE_COLOR_0>
<CONTROL_TYPE_NAME_1>BLANK</CONTROL_TYPE_NAME_1>
<CONTROL_TYPE_ID_0_1>2000*</CONTROL_TYPE_ID_0_1>
<MEASURE_TOTAL>true</MEASURE_TOTAL>
<BACKGROUND_LOW>0.0</BACKGROUND_LOW>
<PERIMETER_THRESH>20.0</PERIMETER_THRESH>
<ENFORCE_GRID>true</ENFORCE_GRID>
<STANDARD_CHECK>false</STANDARD_CHECK>
<CONTROL_TYPE_VARTHRESH_0>10.0</CONTROL_TYPE_VARTHRESH_0>
<CONTROL_TYPE_ID_2_1>HOT</CONTROL_TYPE_ID_2_1>
<STANDARD_HIGH>10000.0</STANDARD_HIGH>
<PERCENT>50 % (median)</PERCENT>
<MEASURE_MEDIAN>true</MEASURE_MEDIAN>
<SLIDING_WINDOW>false</SLIDING_WINDOW>
<BACKGROUND_THRESH>0.9995</BACKGROUND_THRESH>
<CHANNEL1>false</CHANNEL1>
<PERIM_TO_AREA>true</PERIM_TO_AREA>
<BLANK_HIGH>1000.0</BLANK_HIGH>
<CONTROL_SPOT_LIST>No control spots defined</CONTROL_SPOT_LIST>
<DEFAULT_RESOLUTION>10.0</DEFAULT_RESOLUTION>
<GENES_ALL>false</GENES_ALL>
<CONTROL_TYPE_VARTHRESH_1>10.0</CONTROL_TYPE_VARTHRESH_1>
<PERCENTILE_CONTROL_SPOT_LIST>No control spots
defined</PERCENTILE_CONTROL_SPOT_LIST>
<LOWESS_CONTROL_SPOT_LIST>No control spots
defined</LOWESS_CONTROL_SPOT_LIST>
<CONTROL_TYPE_N>0</CONTROL_TYPE_N>
<REGULARITY_FLAG>true</REGULARITY_FLAG>
<REGULARITY_THRESH>0.6</REGULARITY_THRESH>
<BKGD>Local</BKGD>
<CONTROL_TYPE_ID_N_0>1</CONTROL_TYPE_ID_N_0>
<OFFSET_THRESH>60.0</OFFSET_THRESH>
<STANDARD_CV>5.0</STANDARD_CV>
<CONTROL_TYPE_LOWALERTTHRESH_0>0.0</CONTROL_TYPE_LOWALERTTHRESH_0>
<SCOPE>SubGrid</SCOPE>
<COMBINE_REPL>true</COMBINE_REPL>
<SUBGRID>false</SUBGRID>
<CONTROL_TYPE_ID_1_1>STANDARD</CONTROL_TYPE_ID_1_1>
<IGNORED_MEDIAN>true</IGNORED_MEDIAN>

```

```

<CONTROL_TYPE_LOWALERTTHRESH_2>0.0</CONTROL_TYPE_LOWALERTTHRESH_2>
<FOLD>1.0</FOLD>
<CONTROL_TYPE_IFCVAlert_1>>false</CONTROL_TYPE_IFCVAlert_1>
<CONTROL_TYPE_LOWALERTTHRESH_1>0.0</CONTROL_TYPE_LOWALERTTHRESH_1>
<HOT_LOW>10000.0</HOT_LOW>
<EMPTY_FLAG>>true</EMPTY_FLAG>
<CONTROL_TYPE_ID_2_0>* Pro*</CONTROL_TYPE_ID_2_0>
<CONTROL_TYPE_IFLOWALERT_2>>false</CONTROL_TYPE_IFLOWALERT_2>
<CONTROL_TYPE_IFUPALERT_0>>false</CONTROL_TYPE_IFUPALERT_0>
<CONTROL_TYPE_IFCVAlert_0>>false</CONTROL_TYPE_IFCVAlert_0>
<CONTROL_TYPE_UPALERTTHRESH_2>0.0</CONTROL_TYPE_UPALERTTHRESH_2>
<POOR_THRESH>0.995</POOR_THRESH>
<CONTROL_TYPE_NAME_2>HOT</CONTROL_TYPE_NAME_2>
<CONTROL_TYPE_ID_1_0>1999*</CONTROL_TYPE_ID_1_0>
<PERIMETER_FLAG>>true</PERIMETER_FLAG>
<BACKGROUND_WIDTH>5.0</BACKGROUND_WIDTH>
<OFFSET>>true</OFFSET>
<CONTROL_TYPE_VARTHRESH_2>0.0</CONTROL_TYPE_VARTHRESH_2>
<BKGD_MEASURE>Bckgr. Mean</BKGD_MEASURE>
<MEASURE_AREA>>true</MEASURE_AREA>
<LOG_ENABLED>>true</LOG_ENABLED>
<MEASURE_STDEV>true</MEASURE_STDEV>
<CONTROL_SPOT>>false</CONTROL_SPOT>
<BLANK_CHECK>>false</BLANK_CHECK>
<BACKGROUND_BUFFER>2.0</BACKGROUND_BUFFER>
<CONTROL_TYPE_LOWTHRESH_0>150.0</CONTROL_TYPE_LOWTHRESH_0>
<SATURATION_FLAG>>false</SATURATION_FLAG>
<AUTO_SEG>true</AUTO_SEG>
<OPEN_PERIMETER>true</OPEN_PERIMETER>
<BLANK_CV>5.0</BLANK_CV>
<CONTROL_TYPE_ID_N_1>1</CONTROL_TYPE_ID_N_1>
<NORM>Subtract percentile</NORM>
<CONTROL_TYPE_ID_N_2>2</CONTROL_TYPE_ID_N_2>
<CONTROL_TYPE_IFUPALERT_2>>false</CONTROL_TYPE_IFUPALERT_2>
<CONTROL_TYPE_IFLOWALERT_0>true</CONTROL_TYPE_IFLOWALERT_0>
<NEGATIVE_AND>true</NEGATIVE_AND>
<SIGNAL_FLAG>>false</SIGNAL_FLAG>
<WINDOW_LENGTH>5.0</WINDOW_LENGTH>
</ImaGene_Parameters>

```

## 5.1.5 Batch File

The ImaGene batch file is an XML file containing links to all the configuration files required to batch process images. The file is created, saved and loaded from within the Batch Editor Window. Similar to ImaGene configuration files, the contents are only relevant for advanced users and those who need to create a batch file from scratch. As ImaGene can also be launched from the command line, creating a batch manually and

passing in this information as a command line argument provides a viable alternative than simply running the software from the Batch Editor.

Listed below is a sample batch file. Note that the information contained here matched the information required within the Batch Editor.

*Note: There are options in the XML file below that are not accessible through Batch Editor GUI. These options can be used by advanced users through manual creation of the file.*

```
<?xml version="1.0" encoding="UTF-8"?>

<Batch>
  <Entry>
    <Image>C:\ImaGene\Sample Data\1205\1205_g.TIF</Image>
    <Image2>C:\ImaGene\Sample Data\1205\1205_r.TIF</Image2>
    <Template>C:\ImaGene\Sample Data\1205\1205_new.gal</Template>
    <Configuration>C:\ImaGene\Sample
Data\1205\1205_config_with_norm.xml</Configuration>
    <Destination>C:\ImaGene\Sample
Data\1205\Out_batch_norm</Destination>
    <Channel>0</Channel>
    <ChannelName></ChannelName>
    <Channel2>0</Channel2>
    <ChannelName2></ChannelName2>
    <SubstituteGridImage>null</SubstituteGridImage>
    <SubstituteGridChannel>0</SubstituteGridChannel>
    <AlignImages>true</AlignImages>
    <AdjustGrid>true</AdjustGrid>
    <AdjustSpots>true</AdjustSpots>
    <Normalize>true</Normalize>
    <Analyze>true</Analyze>
  </Entry>
  <Entry>
    <Image>C:\ImaGene\Sample Data\1205\1205_r.TIF</Image>
    <Template>C:\ImaGene\Sample Data\1205\1205_new.gal</Template>
    <Configuration>C:\ImaGene\Sample
Data\1205\1205_config_with_norm.xml</Configuration>
```

```

<Destination>C:\ImaGene\Sample
Data\1205\Out_batch_norm</Destination>
<Channel>0</Channel>
<ChannelName></ChannelName>
<SubstituteGridImage>null</SubstituteGridImage>
<SubstituteGridChannel>0</SubstituteGridChannel>
<AdjustGrid>true</AdjustGrid>
<AdjustSpots>true</AdjustSpots>
<AlignImages>false</AlignImages>
<Normalize>true</Normalize>
<Analyze>false</Analyze>
</Entry>
</Batch>

```

The file above contains options that you can recognize from working with Batch Editor (like Image, Channel, Configuration, Template etc), where you specify locations of all resources needed for processing an image. Also you can notice that some other features were added in ImaGene 6.0:

- <AlignImages> - specify whether to align images if a batch entry contains two images (parameters Image2 and Channel2 should be utilized for this purpose – see below). Default value “true”.
- <AdjustGrid> - specify whether to automatically adjust grid for the current image. Default value “true”.
- <AdjustSpots> - specify whether to automatically adjust spots for the current image. Default value “true”.
- <SubstituteGridImage> - specify an image name here from which to take adjusted grid and put it on top of the current image. The image, which will provide the substitute grid should be processed before current image. This feature is useful if you are sure that some images are perfectly aligned with each other. Default value “null”, i.e. no substitution will be made.
- <SubstituteGridChannel> - designates a channel in substitution image file from the previous option. Default value “0”.
- <Normalize> - determines whether to perform normalization on the output data. Settings for normalization will be taken from the configuration file. Normalized data will be outputted into a TXT file, “\_normalized” will be added to the name of this file. Lowess normalization is possible only if an entry contains two images (see option <Image2>). Default value “false”.

- <Analyze> - determines whether to perform ratio analysis on the normalized data. Settings for analysis will be taken from the configuration file. Analysis data will be outputted into a TXT file, “\_analyzed” will be added to the name of this file. Analysis is possible only if a batch entry contains two images (see two following options). Default value “false”.
- <Image2> - allows including another image in the same batch entry. This way ImaGene will load both images at the same time (Image and Image2), auto align them (if <AlignImages> parameter has value “true”), load just one grid for both images, adjust grid and spots based on composite image and process both images in one quantification, giving an opportunity to perform Lowess normalization or two-channel ratio analysis. This option will also be useful if one of the channels of the same array is too dim to provide reliable grid finding. By default not included in entries.
- <Channel2> - serves in combination with <Image2> option. By default not included in entries.
- <ChannelName2> - serves in combination with <Image2> option. Used to name output TXT file. If <Channel2> exists, but <ChannelName2> does not (or is empty), prefix with channel number will be used. By default not included in entries.

## **5.2 Technical Support**

BioDiscovery is available to answer any questions about ImaGene. Your questions will be addressed promptly so you can focus on what is most important - your research. Your ImaGene serial number will be requested when you contact technical support using any of the following methods:

E-mail - [support@biodiscovery.com](mailto:support@biodiscovery.com)

Phone - (310) 414-8100 (United States)

Fax - (310) 414-8111

Mail - 2121 Rosecrans Ave, Suite 3315, El Segundo, CA 90245, USA

*Note: Free technical support is available for one year from your date of purchase.*

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E-mail - [info@biodiscovery.com](mailto:info@biodiscovery.com)

Phone - (310) 414-8100 (United States)

Mail – 2121 Rosecrans Ave., Suite 3315, El Segundo, CA 90245, USA

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