GenePix Training

Molecular Devices

June 29, 2015

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Training Module Purpose

- **This presentation will guide you through steps to enable you to:**

1) **Get the best data out of your scans using proper scanning techniques.**
2) **Improve data quality through use of more accurate Feature Finding.**
3) **Orient you to data types provided by Genepix Pro and how these can be used for better ascertaining information about the array.**
4) **Use the ScatterPlot tab to graph up data.**
5) **Automate and standardize array quality control.**

Support Resources

- F1/Help within GenePix Pro 7 Software
- Support and Knowledge Base: http://mdc.custhelp.com
- Email Technical Support: support@moldev.com
- Telephone Technical Support: 800-635-5577, select options for Technical Support → Microarray Products → GenePix

GenePix Pro 7 Software Orientation

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Tabs in GenePix Pro 7

The software is organized into 7 tabs.

Image: Set up new scans, adjust laser power/PMT gain, generate preview & full res scans, align GAL, adjust display settings for array images

Histogram: Monitor/review graphical representation of fluorescence intensity distribution across your array as it scans or after completion.

Lab Book: Keeps a running task list of all of the functions you have accessed in the software. Useful for GLP applications.

Batch Analysis: Set up & run analysis (including GAL alignment & automated flagging if desired) on a batch of array images with selected GAL files or GPS settings.

Results: Review, sort, normalize, and/or flag raw data points.

Scatter Plot: Generate scatter plot or histogram views of raw data points, including regression line if desired.

Report: View/save preconfigured analysis and hardware diagnostic report types. Editing possible via VB script.

Common Terminology

GPS: GenePix Settings File; encompasses ALL settings used to acquire and analyze your array in a specific run.

GAL: GenePix Array List File; contains orientation information and spot annotation information for your array. If using a commercial array, will likely come with this file. You can also create one using GP software and edit in Excel.

GPR: GenePix Results File; contains images and any raw data from your array once scanned/analyzed.

16-bit TIF: Raw image file type generated by the GenePix scanner; this is the only image file type that is analyzable.

24-bit TIF or Overlay: Image file type generated by the GenePix Pro software; suitable for display only.

PMT: Photomultiplier tube; this is the detection hardware in the GenePix instrument. Adjusting the gain of the PMT(s) is part of optimizing sensitivity for each fluor during the array acquisition setup.

Alignment: Process of orienting the GAL file overlay for detection of spots on the array.

Common Buttons Overview

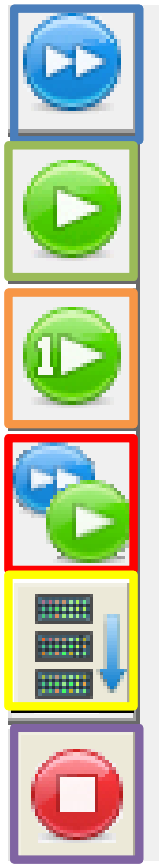
You will see these buttons on the right side of the window in any tab you view within GenePix Pro 7.

Note that not all of the buttons will be active on all tabs.

These key to commonly used functions for:

- **Scanning arrays**
- **Analyzing data**
- **File handling (open, save, etc.)**
- **Flagging features**
- **Searching data**
- **Copying the current view**
- **Accessing the Options dialog**
- **Laser settings**
- **Hardware settings**
- **Hardware diagnostics**
- **Accessing help resources**

Common Buttons – Scanning Arrays



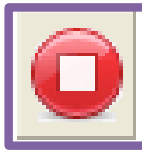
Preview Scan – Click to generate a low resolution (40 um pixel size) scan of your array (all wavelengths).

Data Scan – Click to generate a full resolution (at your chosen pixel size) scan of your array (all wavelengths)

Single Wavelength Scan – Click to generate a full resolution scan of your array with a single selected wavelength.

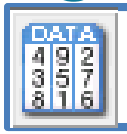
Preview Scan then Data Scan – Click to sequentially generate a low then a full resolution scan of your array (all wavelengths)

Data Scan All– Click to analyze all scan areas highlighted on your array. Note that this button will only be active if you have selected multiple scan areas, otherwise it will be greyed out.



Stop Scan– Click to stop a scan in progress. Note that this button will be active only during a scan (greyed out when not in progress).

Common Buttons – Analysis & File Handling

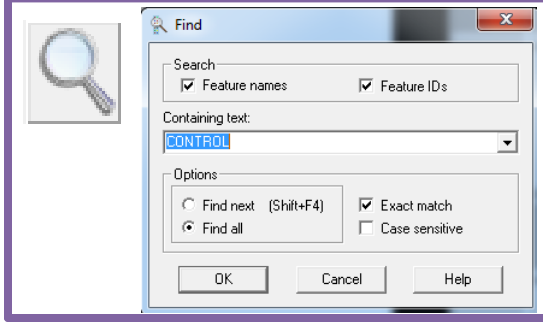


Analyze– Click to analyze your current array image with your selected GAL or GPS.

Analyze All – Click to analyze all selected scan areas. Note that this button will only be active if multiple scan areas were created.

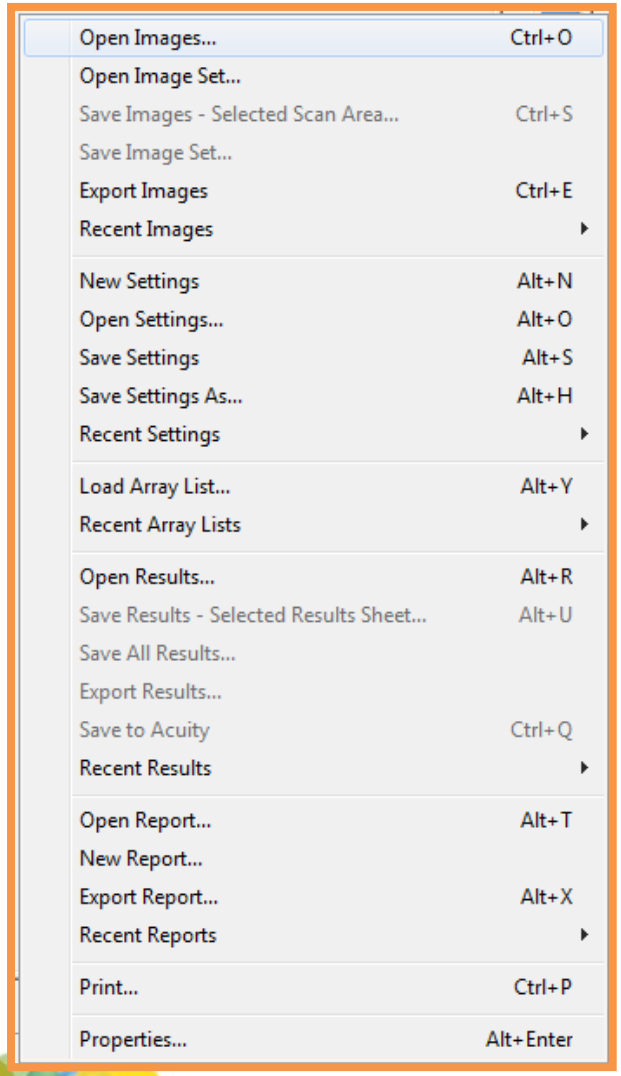
File – Click to access a menu of file handling options (see next slide for more details).

Feature – Click to access feature flagging capabilities (see subsequent slide for more details).



Find – Opens a dialog that allows you to search for features (spots) by feature | names or ID.

Common Buttons – File



File – You will see this menu when you click on the File button.

You can open, save, or export images or image sets.

You can also open or save settings, array lists (GAL files), results (GPR files), and reports (.htm/.html files).

You can also print from this menu.

If you are on the Image tab, you can view properties of your currently displayed array image.

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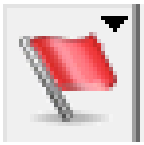
Common Buttons – Feature



Feature – You will see this menu when you click on the Feature button.

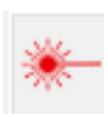
Go to Web will direct you to the Stanford Genome Database database (http://genome-www4.stanford.edu/). Note that if your spot ID does not key to one of the genes in their list, you will not see any info on the web page.

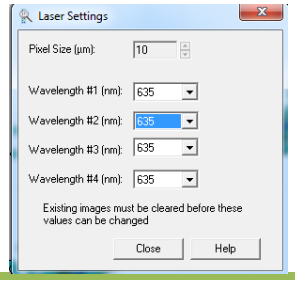
When working in the Results or Scatter Plot tabs, you can flag data points using pre-defined or user-defined flags (or clear existing flags).

You can also choose to include or remove selected points from data normalization.

Common Buttons – Settings & Help

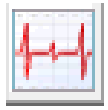
Options– Click to access the Options dialog, which allows you to customize multiple functions within GenePix Pro 7 (see next slide).





Laser Settings – Launches a dialog that allows you to assign wavelengths # to the lasers in your system and adjust pixel size. Options will depend on scanner type and configuration.

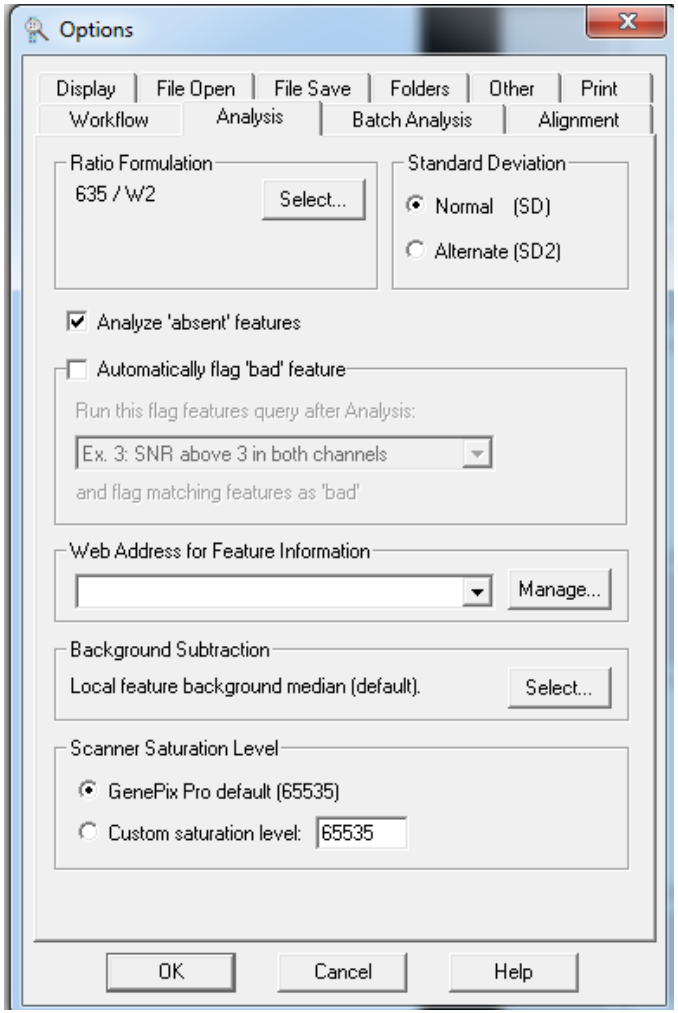
Hardware Settings – Opens a dialog allowing you to select lasers for a scan as well as adjust laser power and PMT settings for each color (more details on subsequent slide).



Hardware Diagnostics– Launches a dialog allowing your to monitor system performance and perform calibration (more details on subsequent slide).

Help – Access help resources including instrument manual & tutorial, searchable help file, and web based help (more details on subsequent slide).

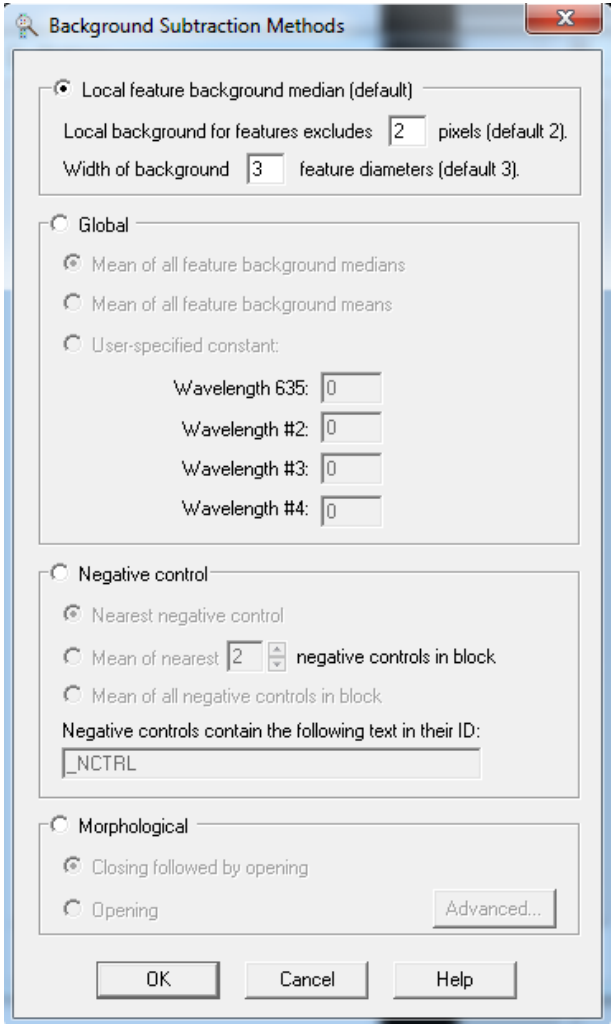
Options Dialog – Analysis Options



The Analysis tab in this dialog allows you to:

- Choose a ratio formulation (for multi-color analyses)
- Select one or two standard deviations (Normal vs Alternate) for analysis
- Enable analysis of features marked as “absent” by the software
- Automatically flag bad features by your definition of choice
- Add and reference web URLs for information on your array contents (features/spots)
- Manage background subtraction methods (see next slide for details)
- Choose default or custom pixel intensity saturation level (65535 is the default, which is max intensity of the instrument detectors)

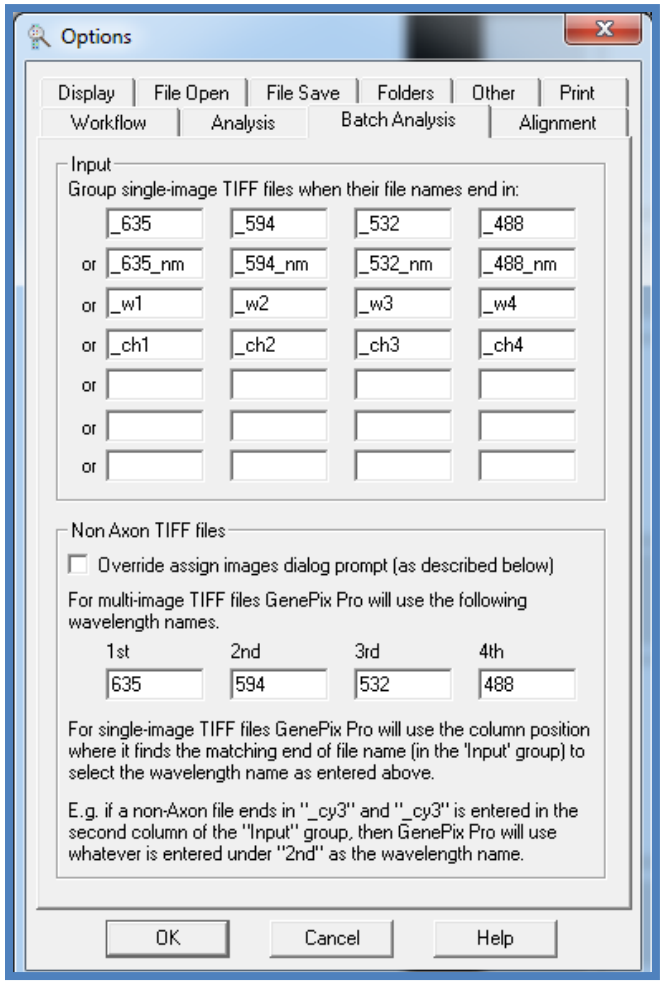
Analysis Options – Background Subtraction



The Background Subtraction Methods option within the Analysis tab in this dialog allows you to choose between and configure 4 different background subtraction methods:

- **Local feature background median: This is the default method. Local background is subtracted for each feature (spot) within a defined pixel range of the edge of the feature (2 pixels ore more). You can modify the # pixels to exclude and the width of the background region.**
- **Global: Subtracts either the mean of all background medians or means, or uses a user specified constant for each wavelength.**
- **Negative Control: Subtracts either the nearest negative control, the mean of the nearest x negative controls in the block, or the mean of all negative controls in the block (as specified in the GAL file).**
- **Morphological: Applies an image analysis method (choose from 2) that enhances each spot vs background.**

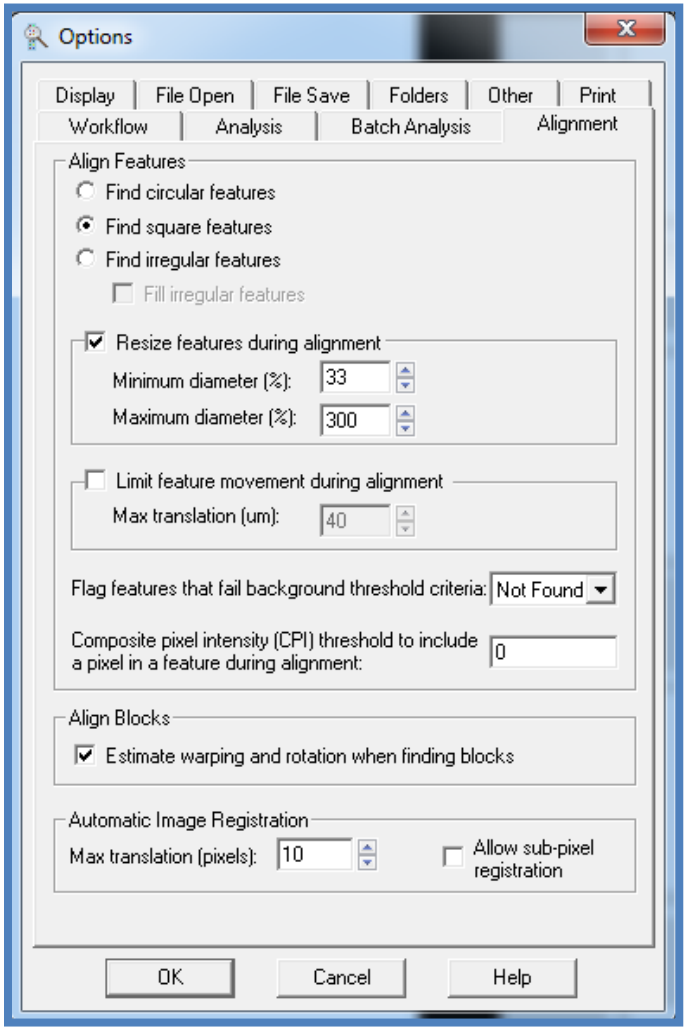
Options Dialog – Batch Analysis



The Batch Analysis tab in this dialog gives you control over:

- **Grouping of single image (TIFF) files on import/opening according to filename**
- **Wavelength assignments for non-Axon (i.e. non-GenePix) array images imported into the software.**

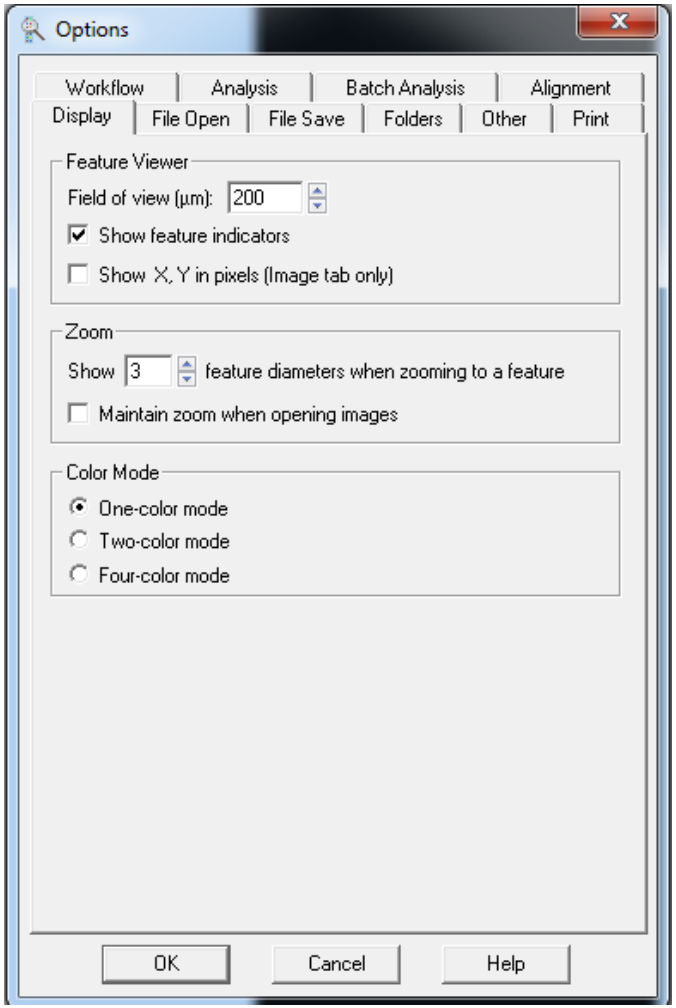
Options Dialog – Alignment



The Alignment tab in this dialog allows you to:

- Choose a **feature shape** that makes sense for the spots on your array (circular, square, or irregular)
- Set parameters to allow **resizing of feature detection** during automated alignment (min/max diameter)
- Set a tolerance for **feature movement** during automated alignment
- Automate **flagging of features (spots) that fail background criteria** – i.e. very dim spots
- Impose a **pixel intensity threshold to call a given pixel as a feature** during automated alignment
- Enable the software to **estimate warping and rotation of blocks** during automatic alignment
- Set a pixel threshold to **compensate for shift during automatic image registration**

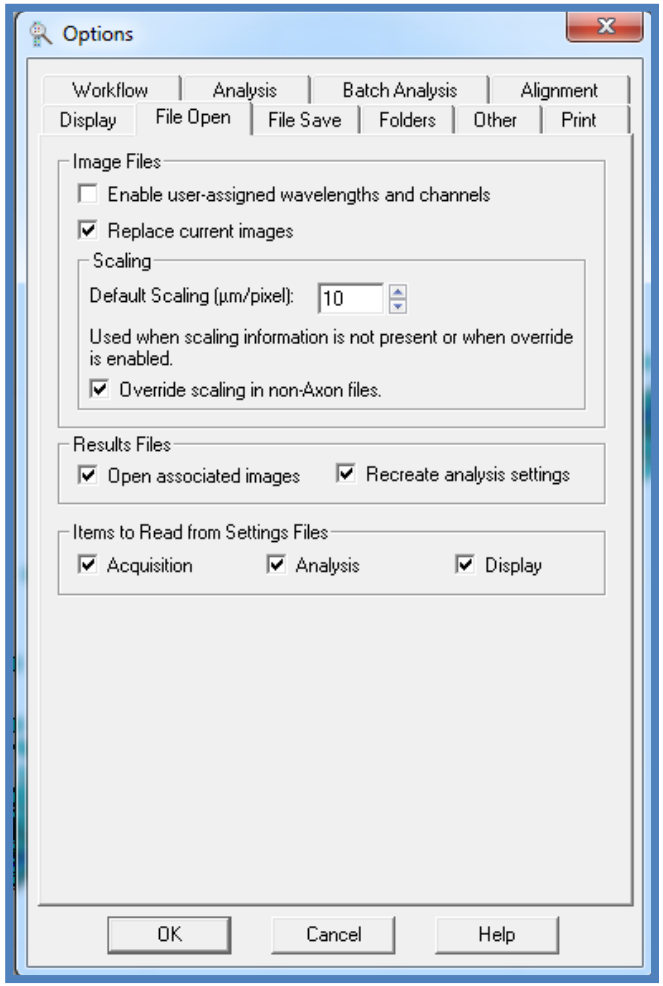
Options Dialog – Display Options



The Display tab in this dialog allows you to:

- Set field of view and information to display for the Feature Viewer.
- Specify zoom levels
- Choose color mode (one, two, or four color). Note that you must restart the software if you select a new color mode for that change to take effect.

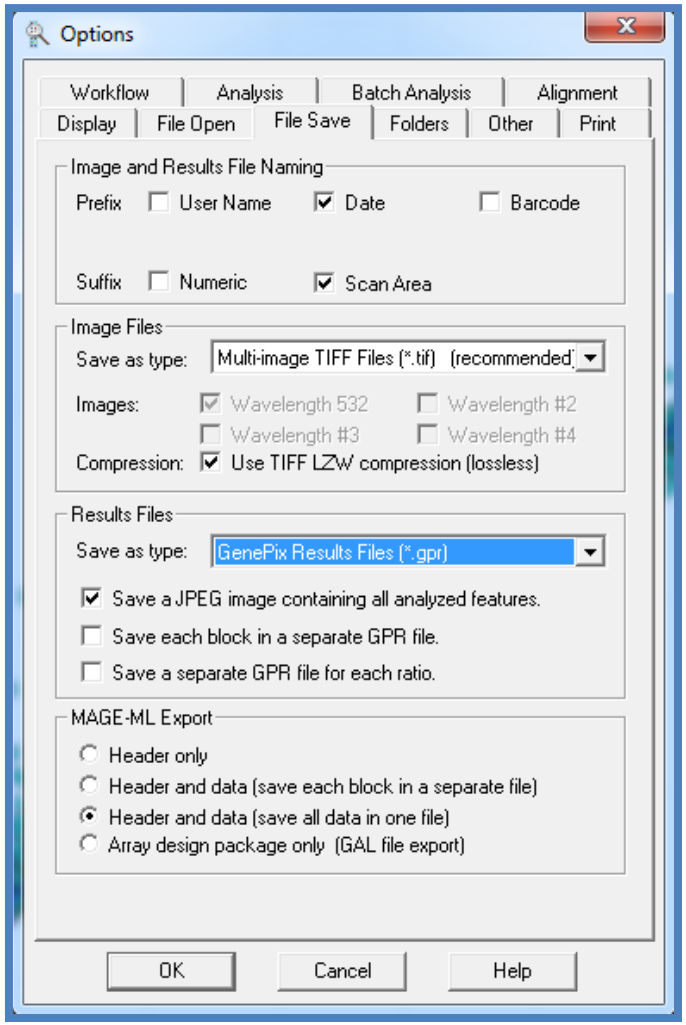
Options Dialog – File Open Options



The File Open tab in this dialog gives you control over:

- Ability to assign wavelengths and channels to image files you open
- Whether to replace current images with new ones by default
- Assign default pixel scaling for imported images
- Enable opening of associated images with result files
- Allow recreation of analysis settings when opening results files
- Specify which settings to read from GPS files.

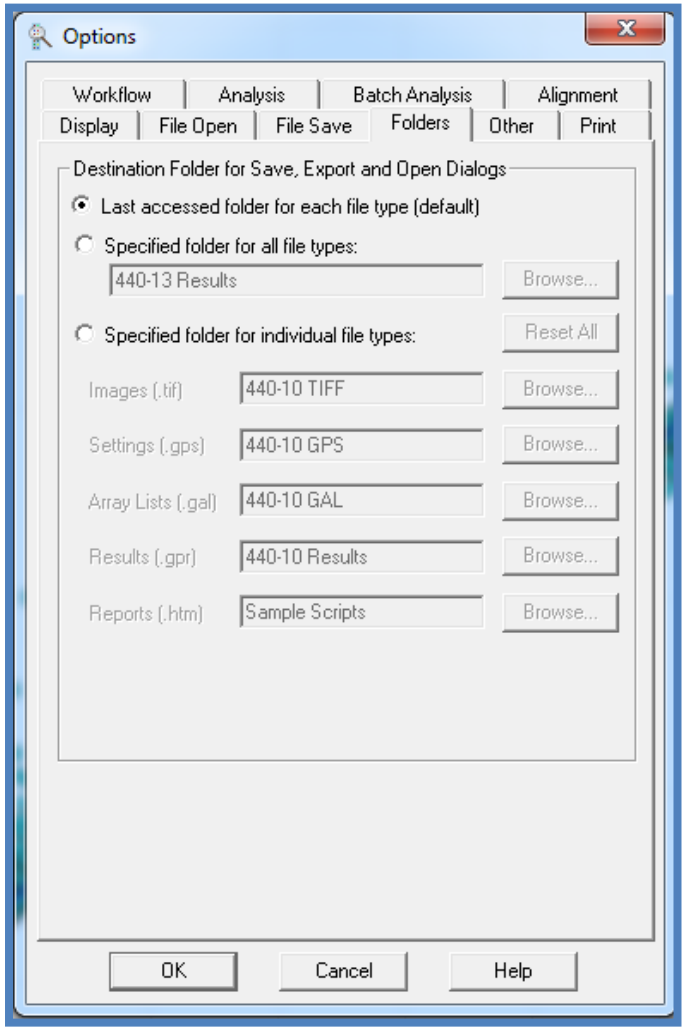
Options Dialog – File Save Options



The File Save tab in this dialog gives you control over:

- Default file naming for images (.tif) and results (.gpr)
- Image file type for saving (single or multi-image .tif)
- GPR file formats (GenePix Pro 7 or earlier versions)
- Option to save a JPG image of your array with analyzed features
- Split individual block results into separate GPR files
- Save separate GPR files for each ratio calculated
- MAGE-ML export file options (http://www.genomebiology.com/2002/3/9/research/0046)

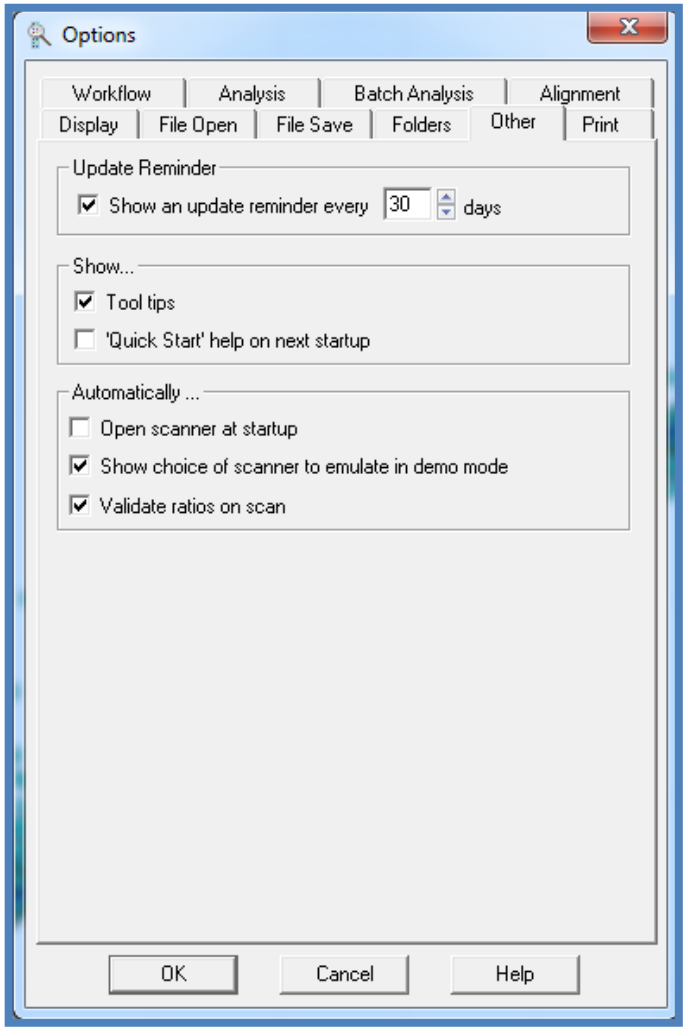
Options Dialog – Folder Options



The Folders tab in this dialog gives you options to automatically save your files to specified locations:

- **Last accessed folder for each file type (default).**
- **Specify a single folder to save all files**
- **Specify folders for individual file types (images, settings, GAL, results, and reports).**

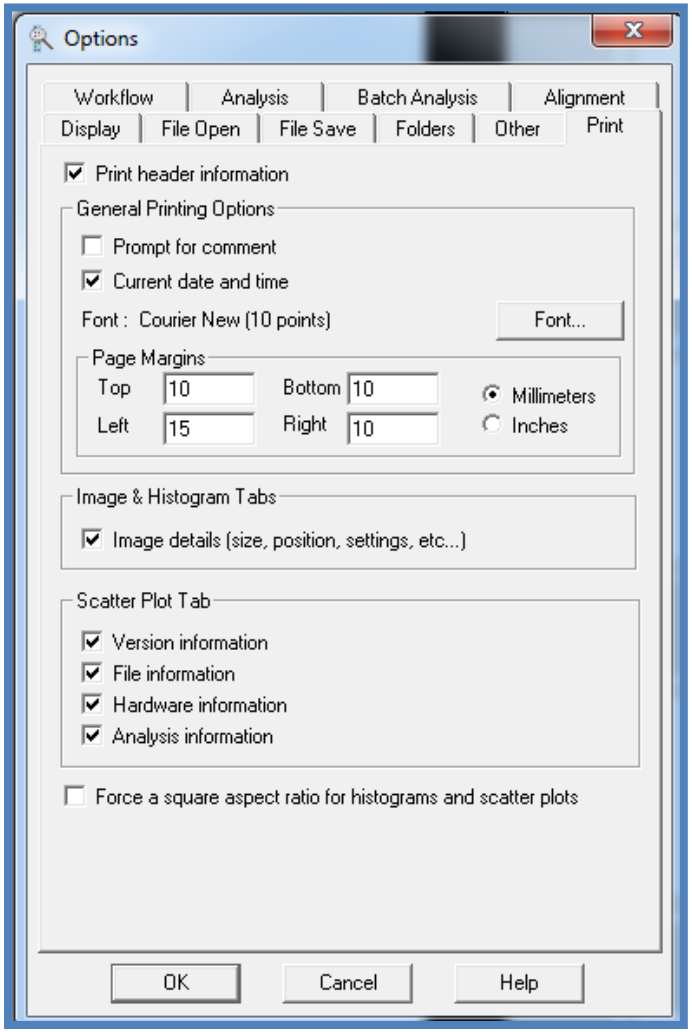
Options Dialog – Other Options



The Other tab in this dialog allows you to:

- **Schedule a software update reminder**
- **Toggle tool tips and Quick Start Help (launch the Help file at startup) on or off**
- **Automatically open scanner at startup, show scanner choice while in emulation mode, and validate ratios on scan.**

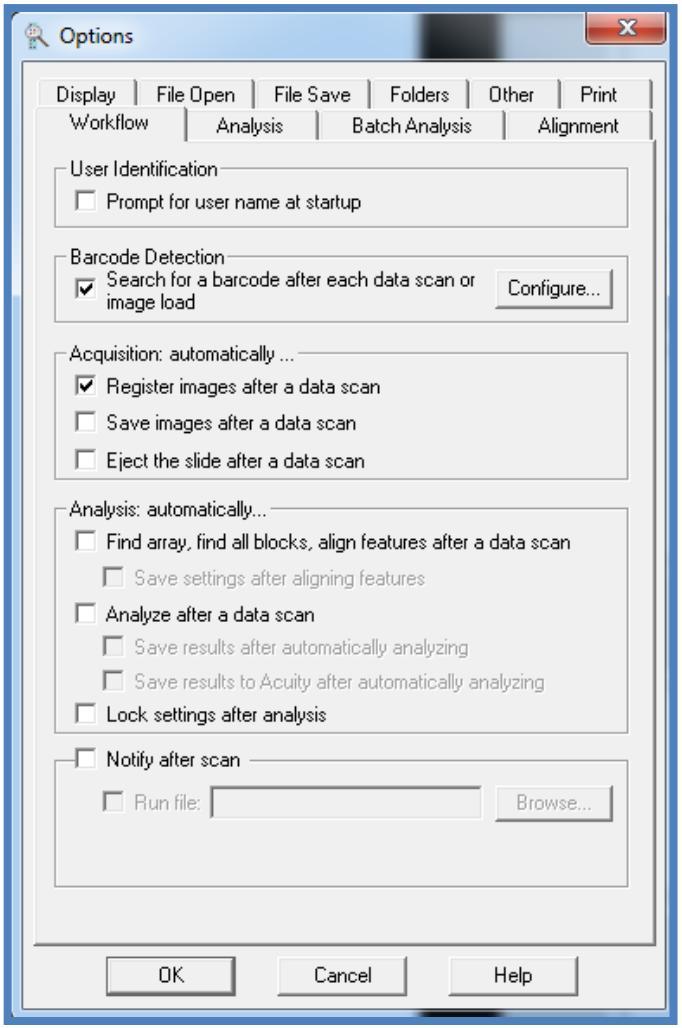
Options Dialog – Print Options



The Print tab in this dialog allows you to:

- **Opt to include header information on printouts**
- **Add a date stamp**
- **Adjust margins**
- **Include image details when printing from the Image and Histogram tabs**
- **Include software version, file info, harware info, and analysis info when printing from the Scatter Plot tab**
- **Force a square aspect ratio when printing histograms and scatter plots**

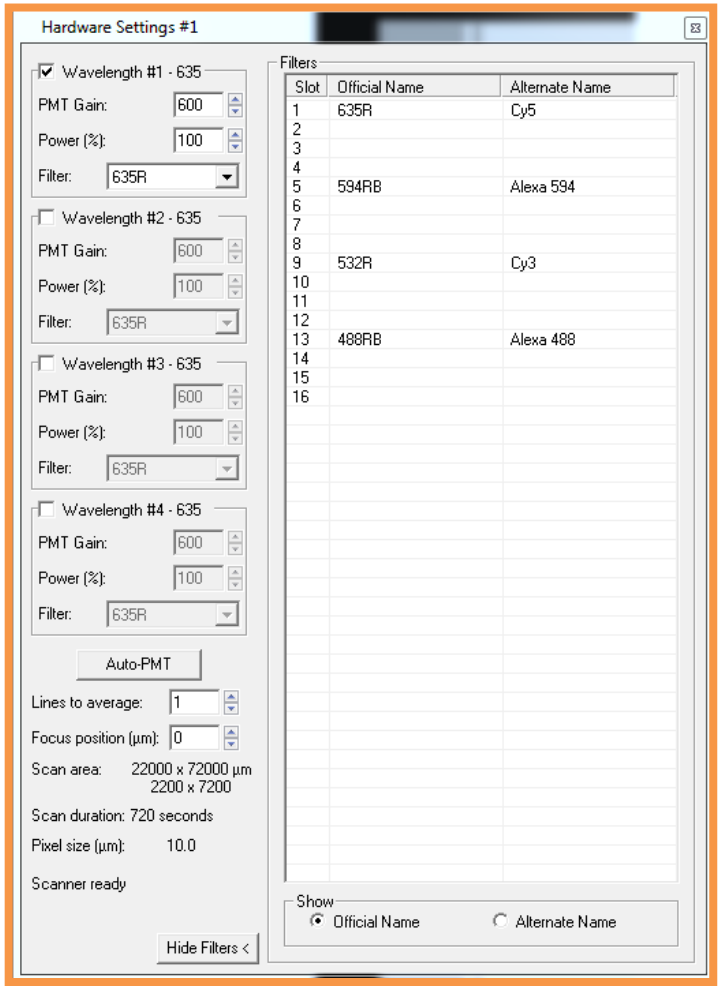
Options Dialog – Workflow Options



The Workflow tab in this dialog allows you to:

- Prompt for a user name at software startup
- Automatically look for a barcode during a data scan or image upload
- Enable automatic image registration, image saving, and ejection of a slide after a data scan
- Enable automatic array/block finding and features alignment by default after a data scan
- Enable automatic analysis after a data scan (with option to also save results .gpr file or Acuity file)
- Default to lock settings after analysis
- Run a specific file of your choice after scan (Notify after scan function)

Hardware Settings Dialog



Hardware Settings – Opens a dialog allowing you to select lasers for a scan as well as adjust laser power and PMT settings for each color. From this dialog you can:

- **Enable and assign your available lasers to each wavelength number**
- **Set the PMT Gain and laser power** for each wavelength here. You will likely be doing this during preview scanning when you initially acquire an array.
- Opt to use the **Auto PMT** setting for selected wavelengths.
- **View filter configuration(s)** on your system (this will vary with GenePix scanner model).

Image Tab Overview

This Image tab opens by default upon launch of GenePix Pro 7. If an array was scanned recently, its image may still appear in the center of this window.

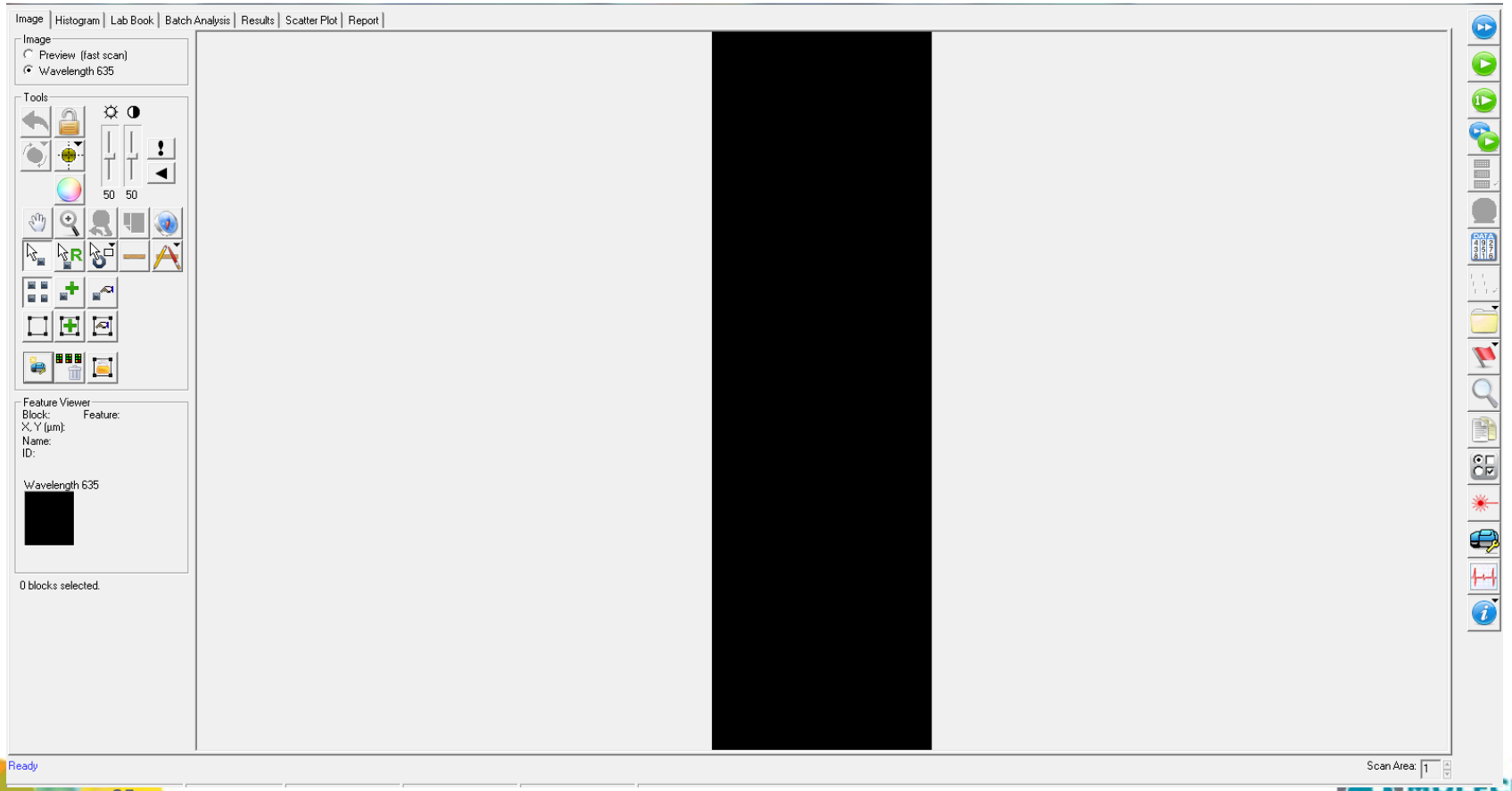
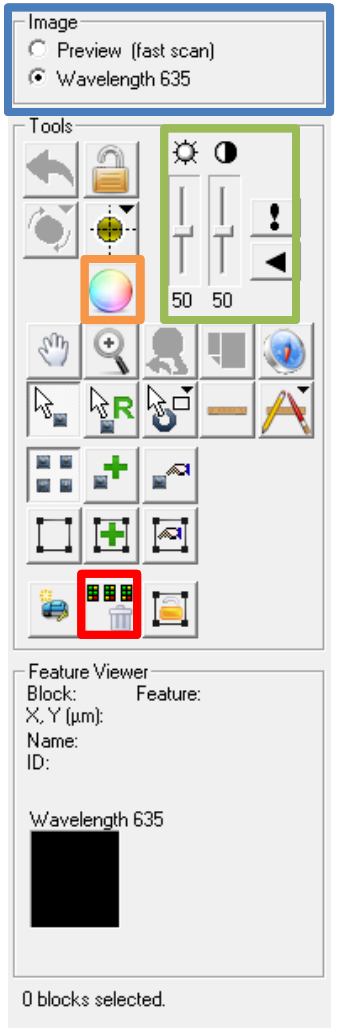


Image Tab Left Panel Icons – Image Display



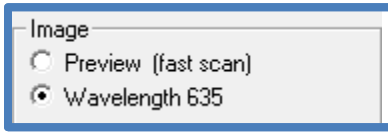
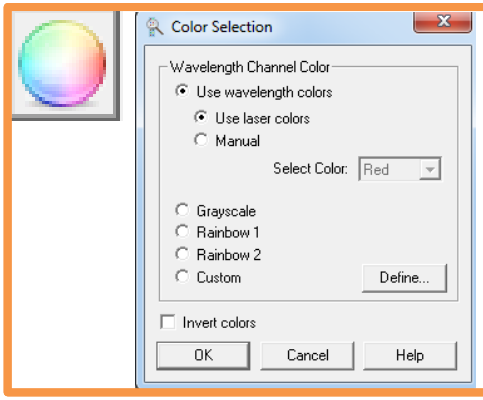


Image Selector – Radio buttons will update depending on images present. Select an image to display in the window.

Image Display Controls – Left slider controls brightness, right slider controls contrast. The exclamation point button auto-scales both brightness and contrast. Arrow button resets the display to original settings. ***NOTE: these controls do NOT alter intensity of the signal, just the display of those images onscreen/to your eye.***



Color Selection – Launches dialog allowing choice of pseudocolor to display for each image. Default is to color by wavelength/laser used at acquisition. Note that Rainbow options will heat map the array by intensity across a defined color range, where low intensities are in the blue range and higher intensities are in the red range.

Erase All Scan Area Images – Trashes all currently open images.

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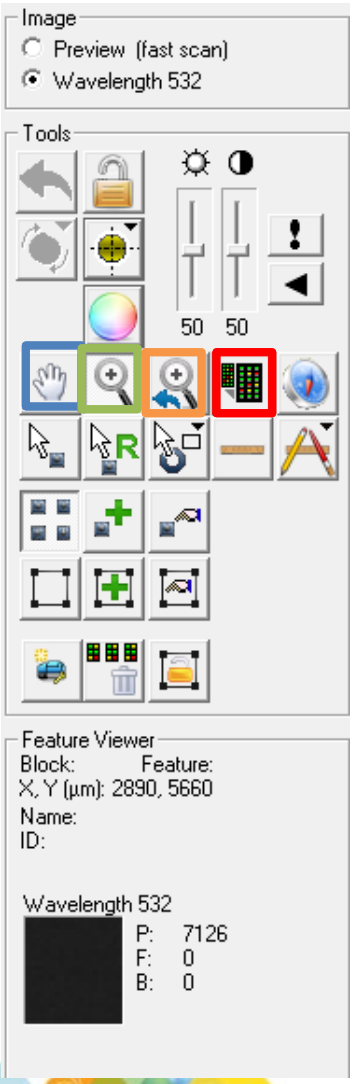
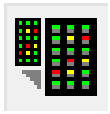
Image Tab Left Panel Icons – Image Display



Hand Mode– Activates hand tool, click on image and drag to pan around. GAL alignment will not be affected in this mode.

Zoom Mode– Activates zoom tool; click and drag a region on the array image to zoom to that area.

Undo Zoom– Click to reset the image display to the last zoom level. Click multiple times to return to the full view of the array.



Zoom Full Scale– A single click returns the display to the full view of the array.

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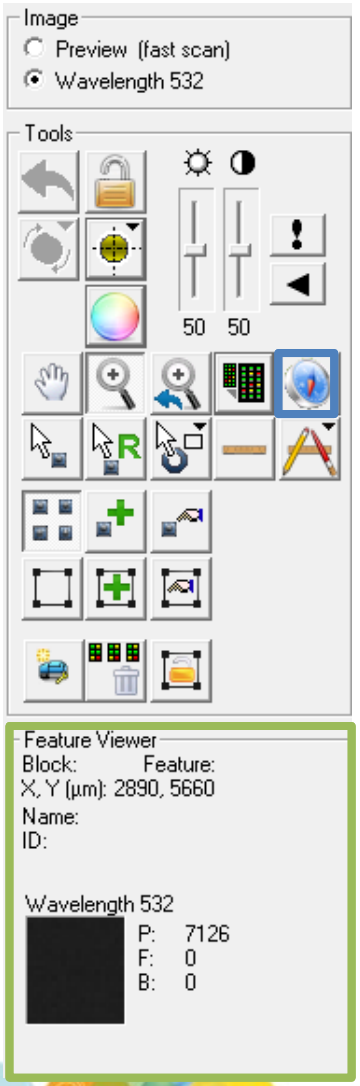
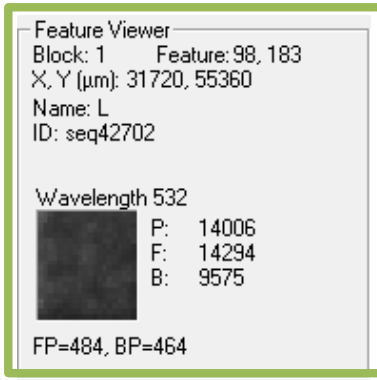
Image Tab Left Panel Icons – Image Display

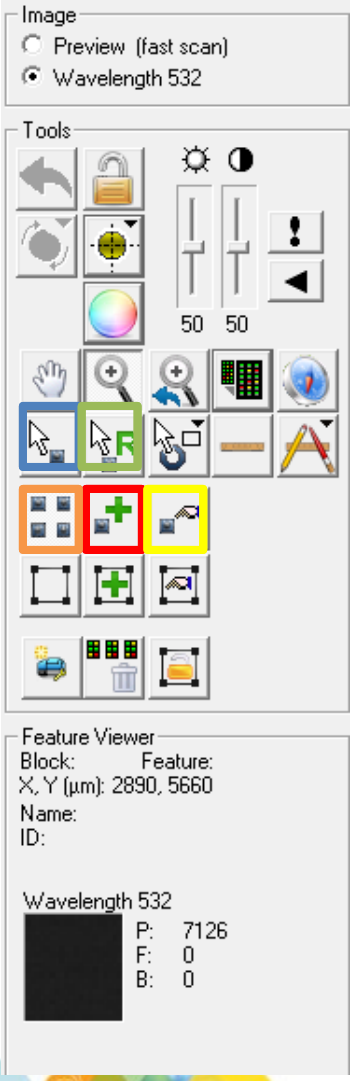


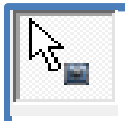
Navigation Tool– Opens a full array view that you can click and drag a region on to zoom to your chosen area in the main image window.



Feature Viewer – Interactive viewer, shows a zoom of the last spot you moused over on the array image. Information about the feature will be displayed including: number of current block (Block), XY coordinates (X, Y [um]), spot ID from GAL file (Name, ID), Wavelength, pixel intensity (P), feature intensity (F), background intensity (B).

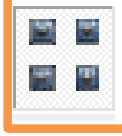
Image Tab Left Panel Icons – Block Mode Functions





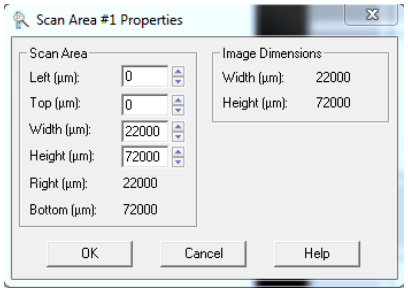
Block Mode – Activates block mode. You need to be in this mode to create or modify blocks.

Replicate Block Mode – Choose this mode to copy an existing block configuration to another region of your array image.



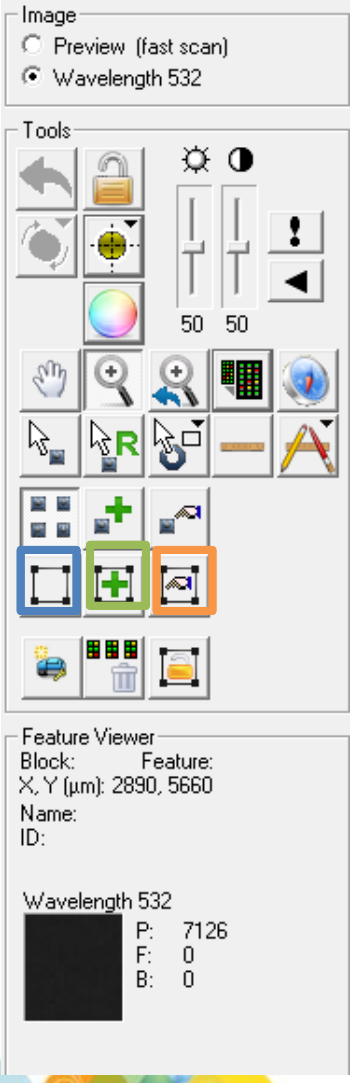
View Blocks – Toggles the block overlay on and off when viewing the array image.

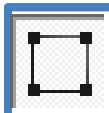
New Blocks – Allows you to create new blocks on the array image by clicking and dragging.



Block Properties – Click to view and modify properties of a selected block. Note that these properties are extracted from the GAL file initially.

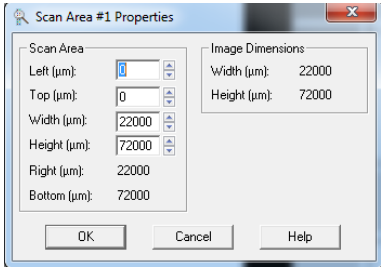
Image Tab Left Panel Icons – Scan Area Functions





View Scan Area – Allows you to visualize scan area(s) that you have already set up on the array.

New Scan Area – Enables you to set a new scan area by clicking and dragging on the preview scan image of your array.



Scan Area Properties – Click to view and modify properties of a selected scan area.

MOLECULAR DEVICES

Image Tab Left Panel Icons – Image Registration & GAL Alignment Functions

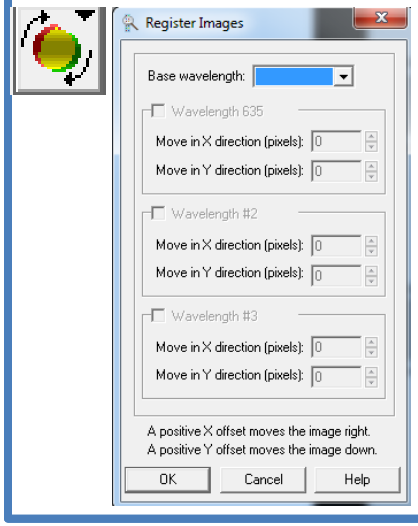
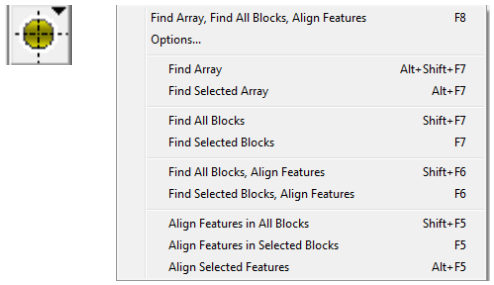
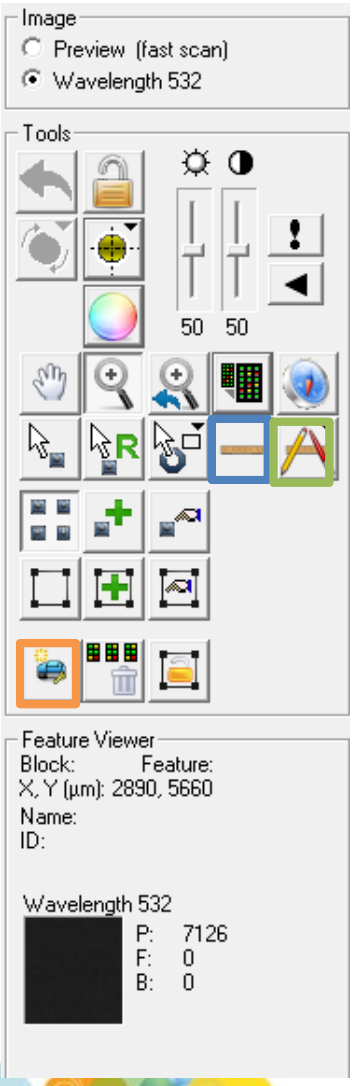


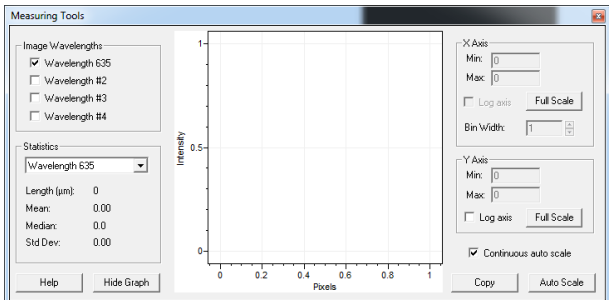
Image Tools – Allows you to register images either automatically or applying parameters entered in the associated Register Images dialog. Note that this too will only be active when you are working with a multi-wavelength image set.



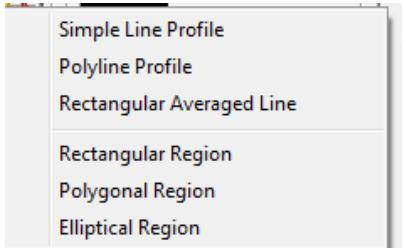
Align Blocks– Gives you multiple options for finding array, finding blocks, and aligning features within those blocks. The menu lists functions in order of most to least automated from top to bottom. Selecting the Options… item from this menu directs you to the Options dialog – Alignment tab described in previous slides.

Image Tab Left Panel Icons – Other Tools





View Measuring Tools – Launches the Measuring Tools dialog which allows you to visualize statistics such as length, area, and pixel intensity measurements of spots on your array made with measuring tools (see next item).



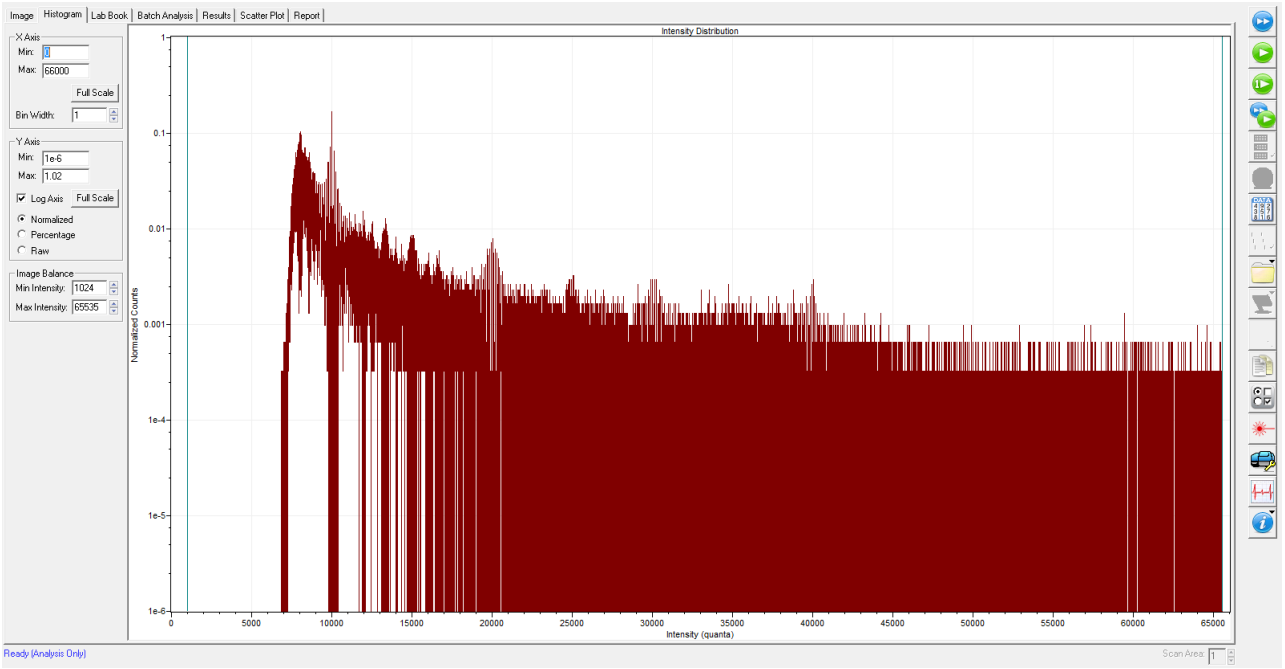
Measuring Tools – Gives you access to a panel of tools that you can use to draw regions directly on your array image for making basic measurements such as length, area, and intensity. You can choose from a simple line, polyline, rectangular average line, rectangular region, polygonal region, or elliptical region.

New Settings – Closes the current settings and opens a fresh settings file. The software will prompt you to save your current settings first.

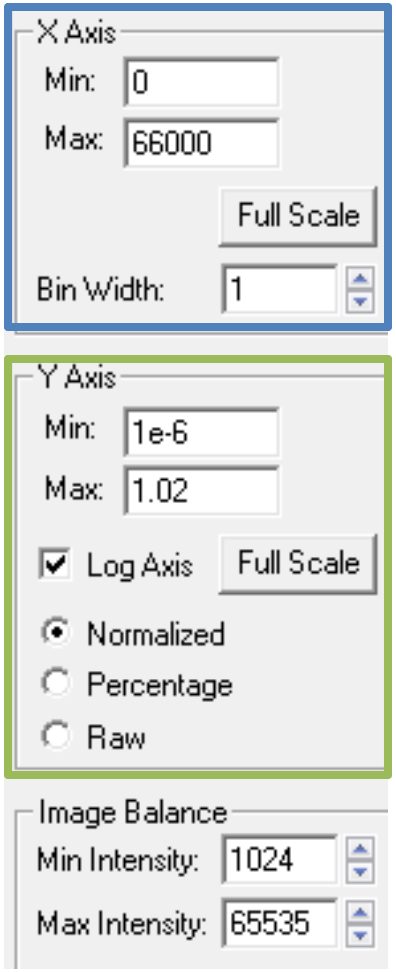
MOLECULAR DEVICES

Histogram Tab Overview

This tab displays a histogram plotting all of the raw pixel fluorescence intensity values for each color in your current array. Note that the histograms for each color are coded to the pseudocolors you choose in Color Selection. This graph updates as the array is being scanned. It is especially useful to monitor during scan setup to ensure PMTs are balanced between colors.



Histogram Tab – Manipulate View



X Axis: You can scale the X axis of the graph by manually entering desired Min, Max, and Bin Width (1-2048) values in the indicated fields. You can also click the Full Scale button to revert to the default view.

Y Axis: You can scale the Y axis of the graph by manually entering desired Min and Max values in the indicated fields. You can change this axis to a Log scale by checking the box next to Log Axis. The default view is Normalized - intensity counts graphed on the Y-axis are scaled so that the highest point of the histogram is set to 1, and all other points on all histograms are scaled relative to this. If you select Percentage, the scale will change accordingly. Raw plots all raw pixel values (not normalized).

Histogram Tab – Manipulate View

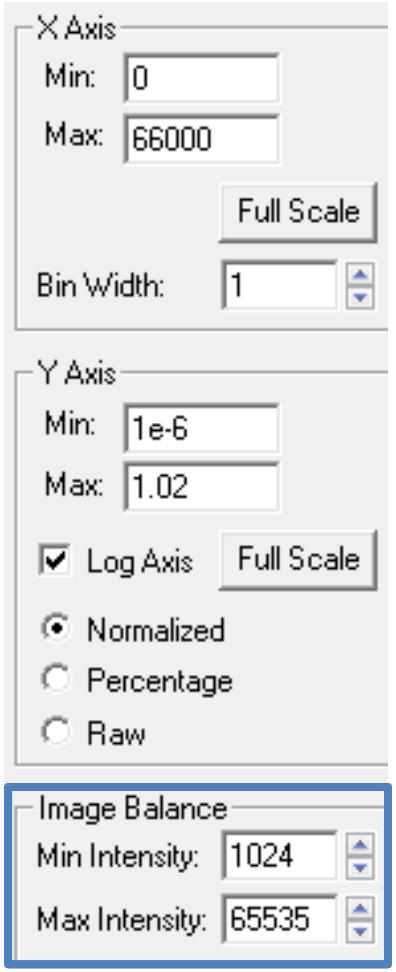
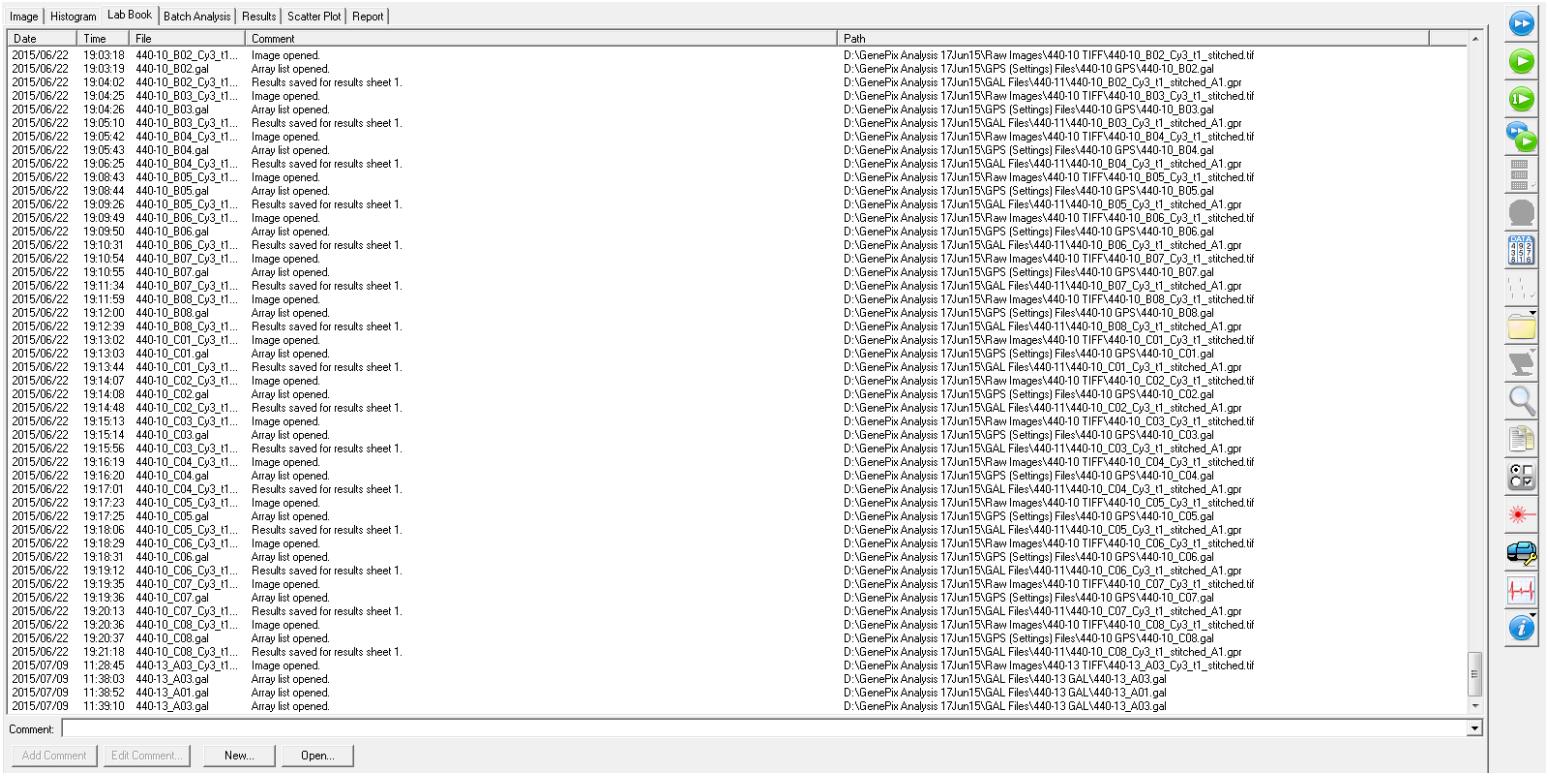


Image Balance: Use the Min Intensity and Max Intensity fields to set the region of the Histogram used to calculate the Count Ratio for each ratio that you have defined. The Count Ratio (W1/W2) is defined as the quotient of the sums over the specified intensity range of the Normalized Counts for wavelengths W1 and W2: in other words the ratio of the areas under the W1 and W2 wavelength histograms.

Lab Book Tab Overview

This tab displays a log of all actions of the GenePix Pro 7 software as they are run, such as accessing GAL or settings files. This may be useful for troubleshooting or simply keeping a record of your activities within the software.

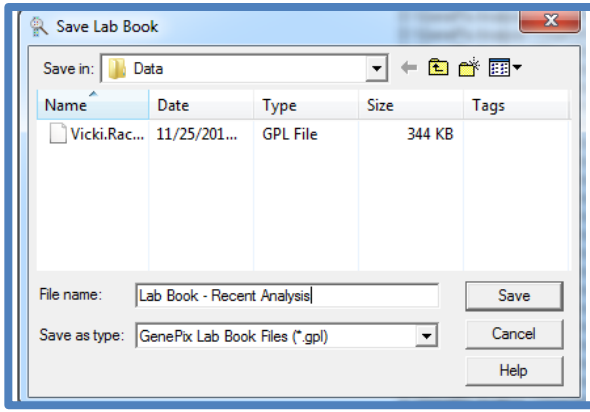


Lab Book Tab – Available Actions

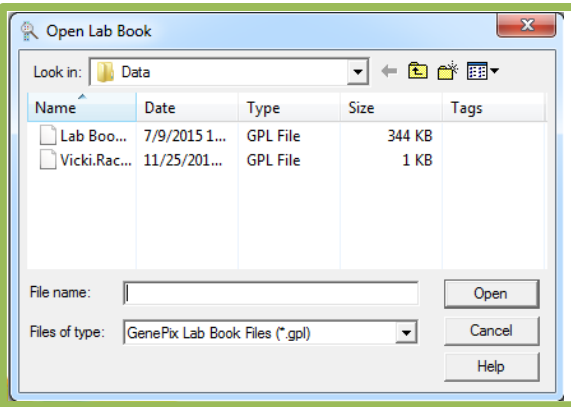
You can add a comment to the log by filling in the Comment field, then clicking Add Comment.

You can edit an existing comment by highlighting it in the log, then clicking Edit Comment.

Lab Book Tab – Available Actions



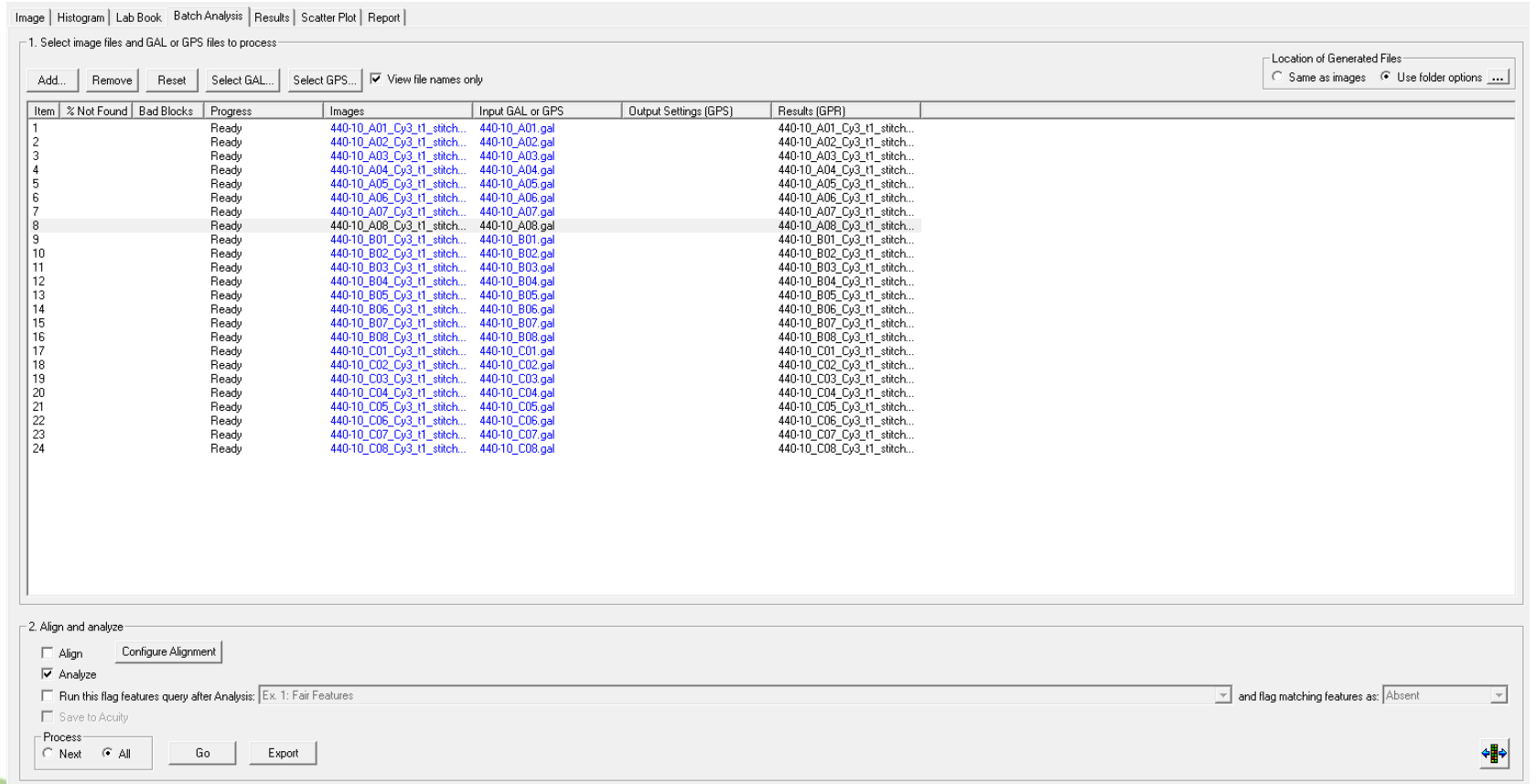
You can save the current Lab Book by clicking on New. A browser will appear that will allow you to save as a .gpl file to the location of your choice. Once you click Save, a new empty Lab Book will appear in the tab.



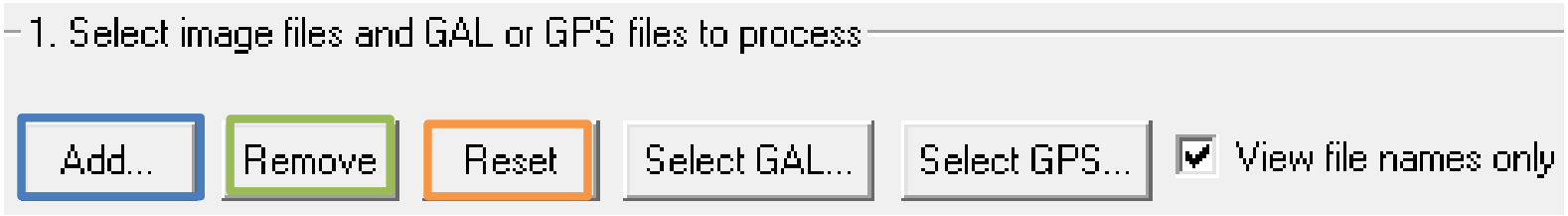
You can open an existing Lab Book file by clicking on Open. A browser will appear that will allow you to navigate to a saved .gpl file.

Batch Analysis Tab Overview

This tab allows you to select multiple array images to analyze with selected GAL or GPS (settings) files. You can also opt to perform automatic alignment of GAL files as part of the processing.



Batch Analysis Tab Functions – Choose Array Images & Settings

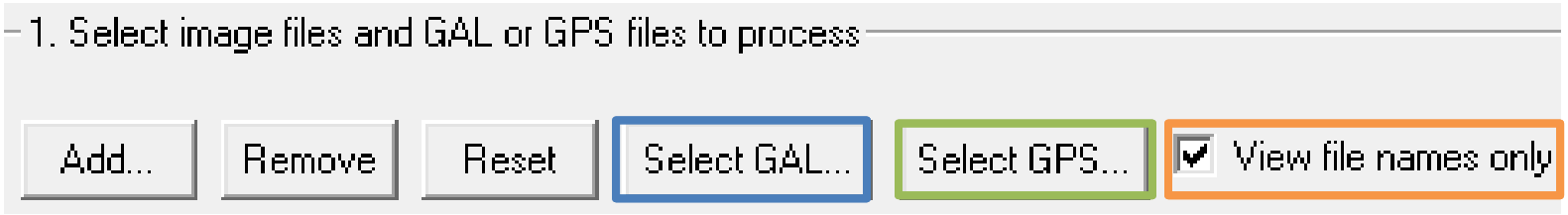


Add – Opens a browser that will allow you to navigate to array image files (.tif) and add to the analysis queue.

Remove – Removes an image file that you've highlighted from the analysis queue.

Reset – Resets the status of analyzed array images back to "Ready" (i.e. clears the analyzed status tag).

Batch Analysis Tab Functions – Choose Array Images & Settings



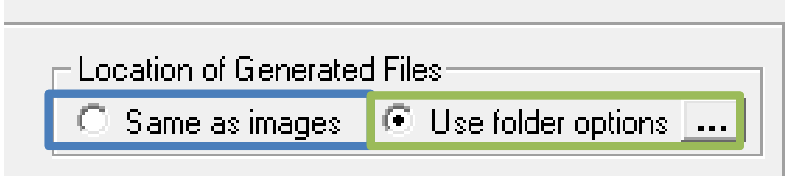
Select GAL… – Opens a browser that will allow you to navigate to GAL files for analysis. If you highlight a selection of images, it will assign the same GAL file to your selected array images.

Select GPS… – Opens a browser that will allow you to navigate to .GPS files for analysis. If you highlight a selection of images, it will assign the same GPS file to your selected array images.

View file names only – When enabled hides the full file path of the array images you are analyzing, displaying only the file name.

NOTE: You can select EITHER a GAL file OR a GPS file for analysis.

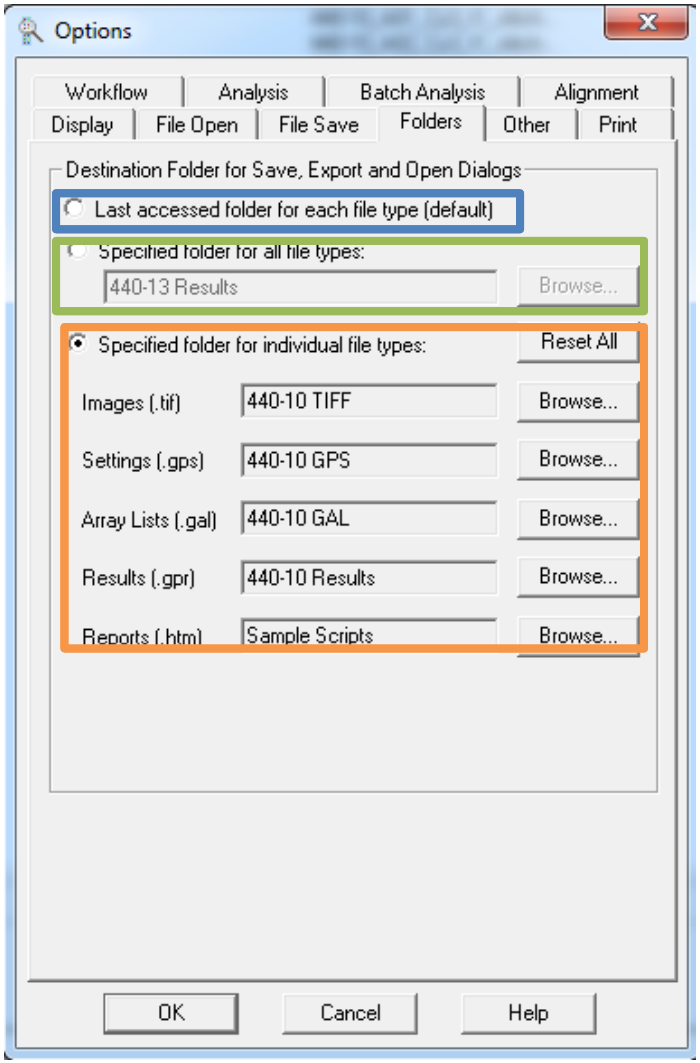
Batch Analysis Tab Functions – Save Options for Results



Same as images – Choose this option to save your results (.gpr files, overlay images) to the same location that your array images are being pulled from for the batch analysis.

Use folder options – Choose this option to specify where you would like your results files saved. Click on the elipsis (…) button to launch the Options dialog. The Folders tab in this dialog will appear (see next slide for details).

Batch Analysis Tab Functions – Save Options for Results

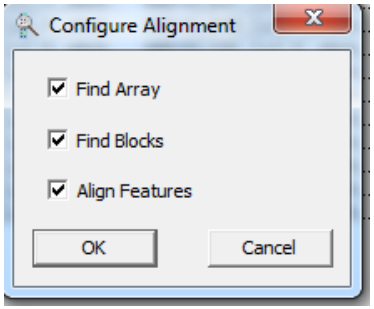


<u>Last accessed folder for each file type</u>: This is the default setting. If you choose this option, your .gpr (results) and overlay images will be saved to the same location as your input array images.

<u>Specified folder for all file types</u>: Choose this option to save ALL of your .gpr (results) files and overlay images to the SAME specific location (use the Browse button to navigate).

<u>Specified folder for individual file types</u>: Choose this option to save your .gpr (results) files and overlay images to a specific location for EACH file (use the Browse button next to each selection to navigate to desired locations). *NOTE: For batch analysis, you only need to specify locations for Results (.gpr) and Images (.tif) files. Any changes you make here will apply to manual analysis as well.*

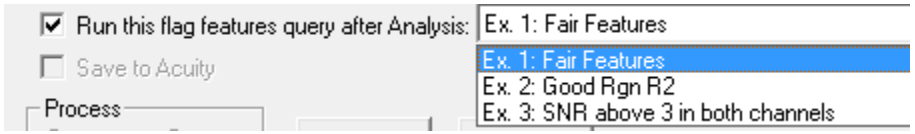
Batch Analysis Tab Functions – Align & Analyze

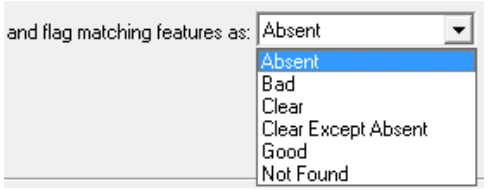


Align/Configure Alignment– When enabled, performs automated alignment of selected GAL file(s) with your array image(s) during the batch processing using the method you select. Click Configure Alignment to select automated alignment options.

Analyze – Flags all of your array images in the queue for analysis using the GAL or GPS file settings that you have selected.

Batch Analysis Tab Functions – Align & Analyze





<u>Run this flag features query after Analysis</u>– Allows you to automatically flag features (spots) as Absent, Bad, Clear, Clear Except Absent, Good, or Not Found based on a defined query as in the example shown.

<u>Process</u> – Select Next to process one array in the queue then the next (when you click GO). Select All to process all arrays in the queue.

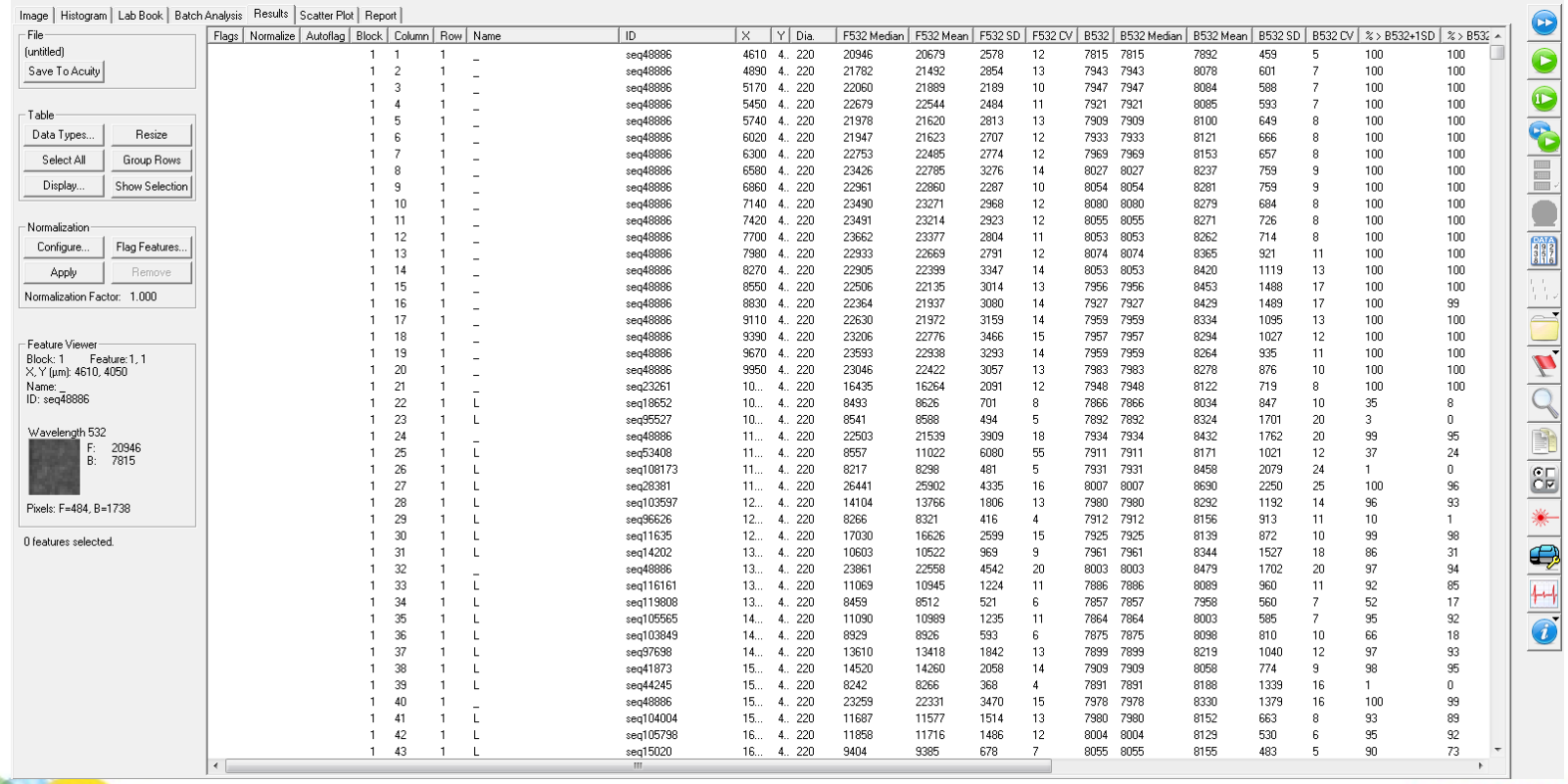
Batch Analysis Tab Functions – Align & Analyze

Go – Click to run your selected batch analysis on the array images in the queue.

Export – Select to export your current list of array images/analysis files in the queue as well as % not found and # bad blocks statistics (if analysis is complete). A browser will open that will prompt you to save a .txt file containing this information.

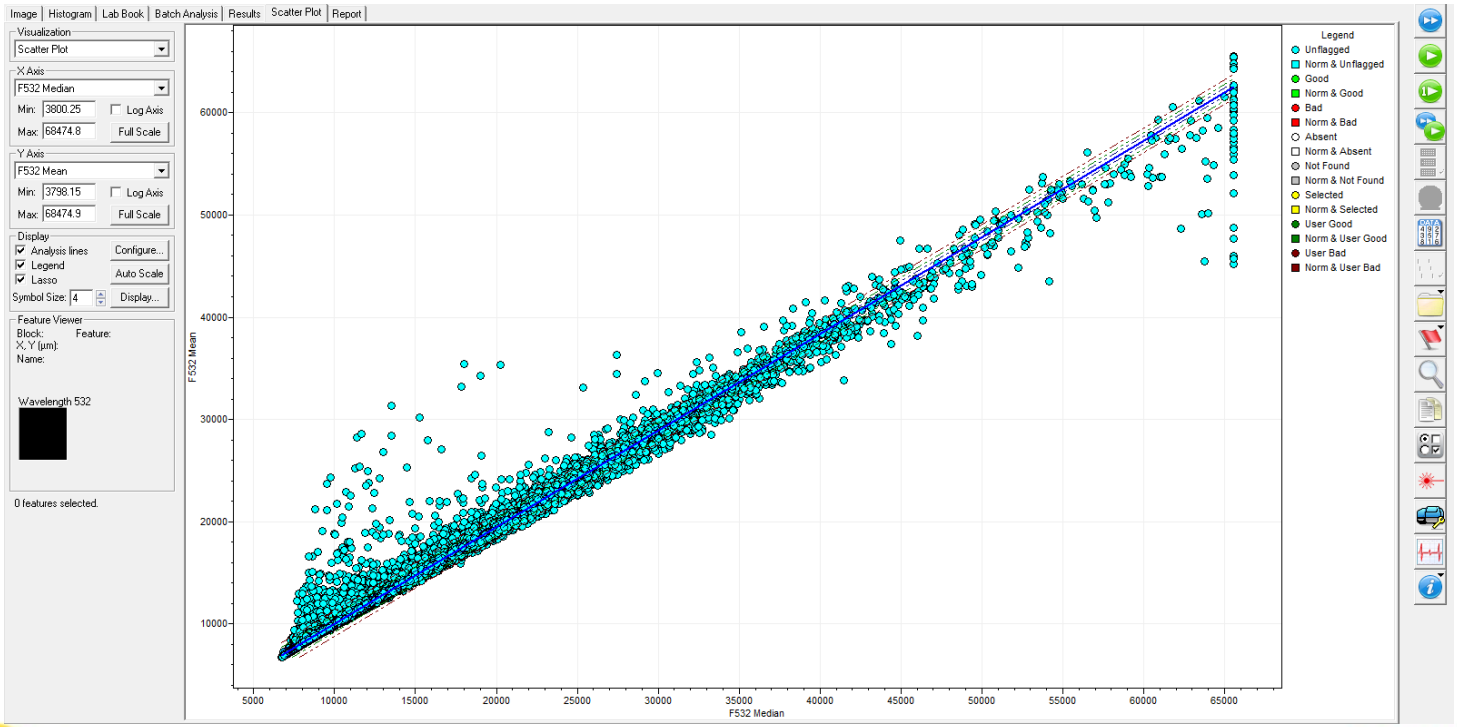
Results Tab Overview

This tab contains the results view from an analyzed array. You can sort, flag, and normalize data from this tab. You can also access the feature viewer to compare the image/raw intensity values of each spot to the calculated data.



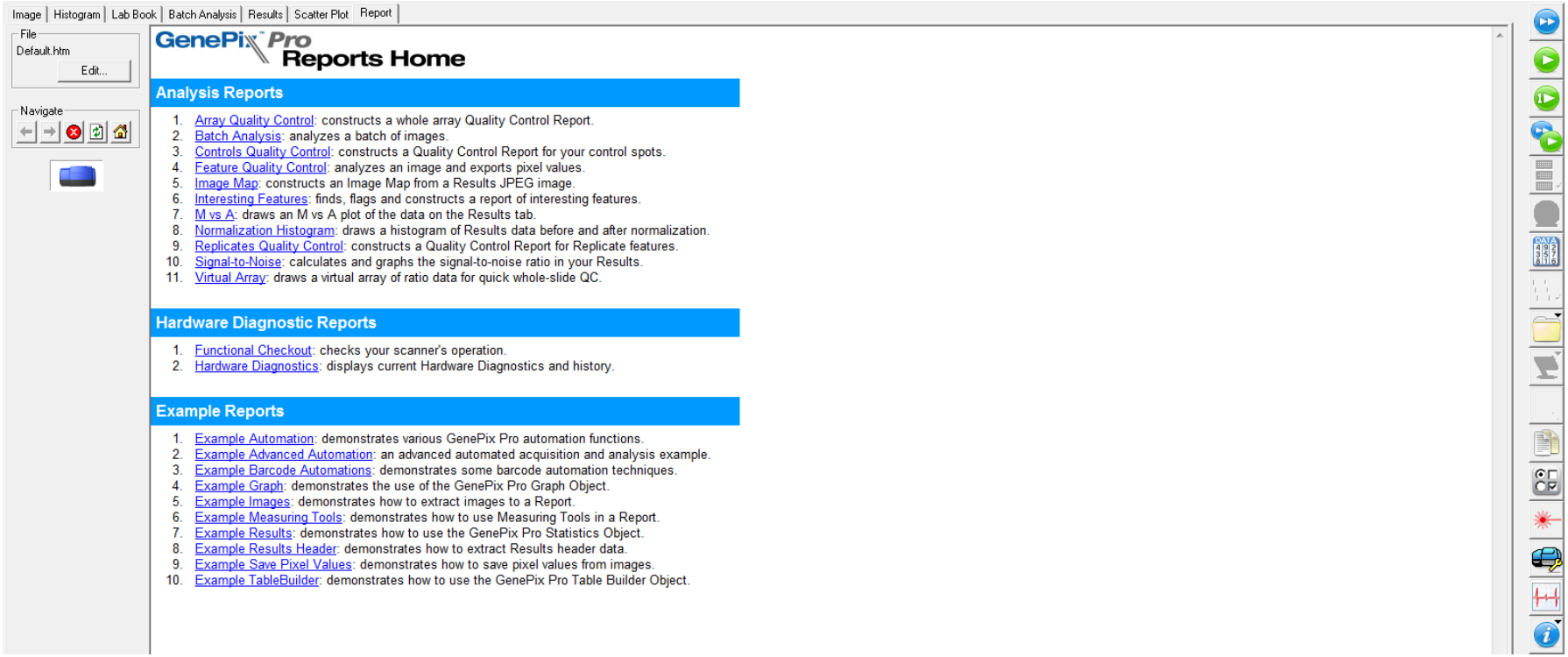
Scatter Plot Tab Overview

This tab contains a graphical view of data from an analyzed array. You can choose a scatter plot or histogram view, change graph axes, add regression lines, flag data points, etc. You can also access the feature viewer to review images of spots for selected data points.



Report Plot Tab Overview

You can access automatically generated analysis and hardware diagnostic reports from this tab. If you have experience with Visual Basic scripting, you can also create custom report views based using the edit function.



Scanning Arrays

Preparing for Scanning Arrays

1) **Power on your GenePix scanner at least 30 minutes prior to scanning your first array.**

Hold for Picture of On Switch on GenePix Scanner

TIP: Be sure to install your scanner in an area of the lab where temperature fluctuations are infrequent (<5 degrees F overall). You will see a warning in the software when temperature fluctuates significantly.

Preparing for Scanning Arrays

2) **Launch the GenePix Pro 7 software by double-clicking on the icon on your instrument computer's desktop.**

Preparing for Scanning Arrays

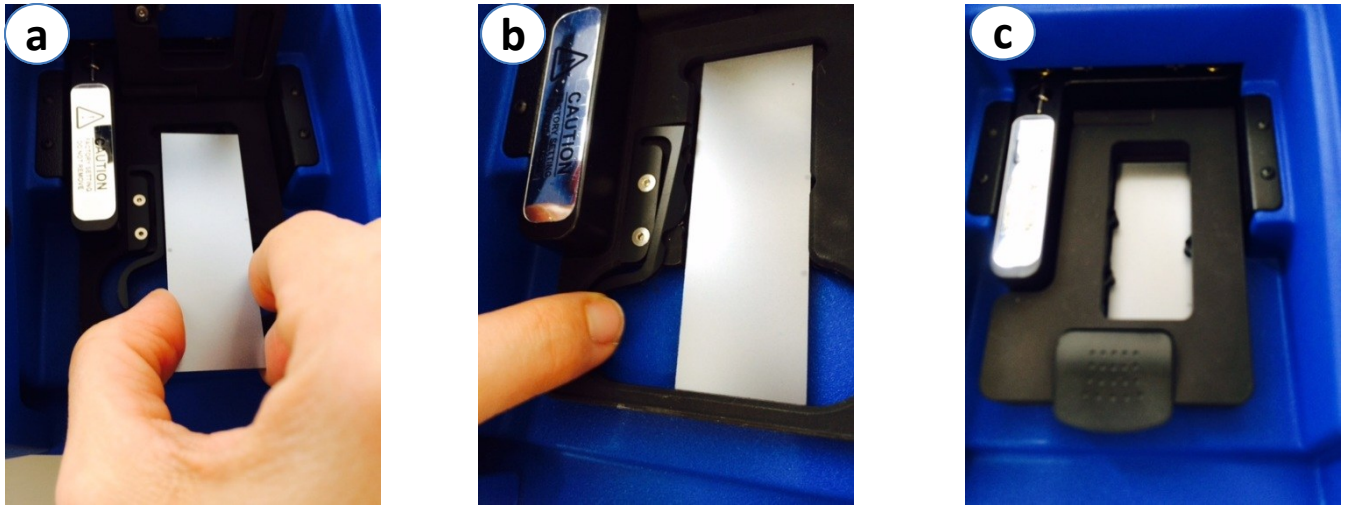
3) Open the door to your scanner.

- *For the GenePix 4000B, manually slide the door to the left to access the slide holder.*
- *For other GenePix models, open the door and eject the slide holder using the eject button on the scanner.*

Hold for Picture of Open Button on Scanner

Preparing for Scanning Arrays

4) Insert array face down into the slide holder (a). If your holder has a clamp (such as GP 4000B pictured) hold it so that slide falls into place (click) before closing the top clamp (b, c).



NOTE: The scanner fires the laser from below, so sample surface must be facing down in the holder.

Preparing for Scanning Arrays

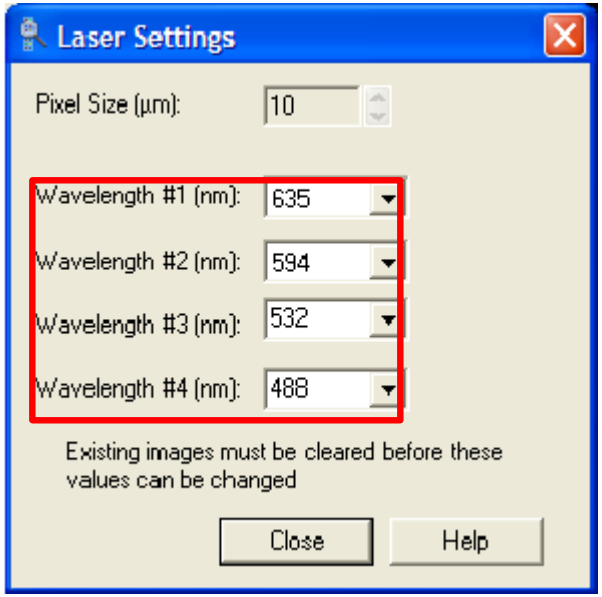
4) Close the cover on your GenePix scanner. For the 4000B, you can push the cover manually to the right. For other models, press the load button on the scanner.

Defining Laser & Pixel Size Settings

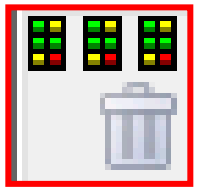
5) Within the GenePix Software, click on the Image tab Image and then the Laser Settings button 

Defining Laser Settings

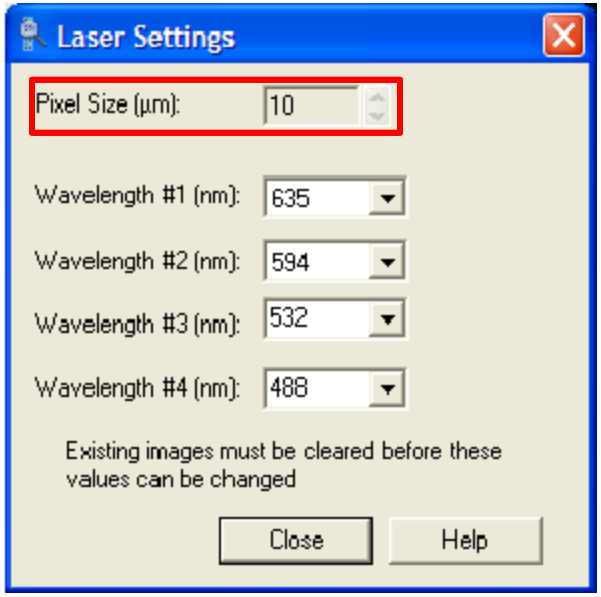
6) The Laser Settings dialog appears. First assign wavelength order. Options will depend on your scanner model and the lasers it is equipped with.



HINT: The software will automatically assign these settings based on the last scan performed. To adjust these settings for a new scan, be sure to clear any existing images first by clicking the ***Erase All Scan Area Images*** *button first.*



Choosing Pixel Size Settings



7) Pixel size is actually the resolution at which you would like to scan your array.

The size you should choose depends on the size of the spots on your array.

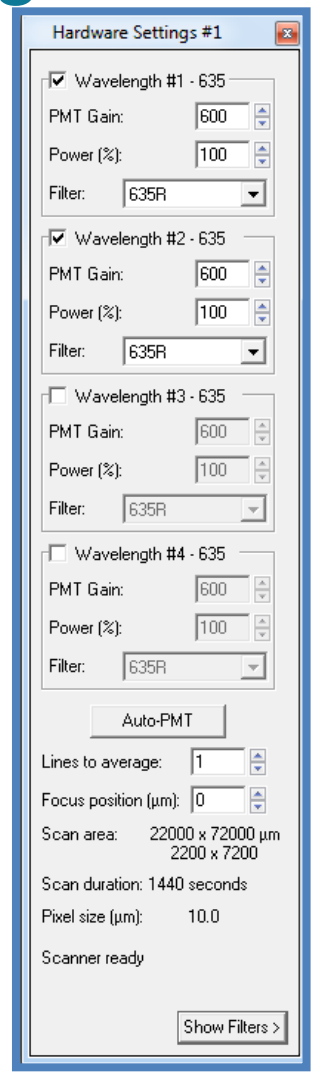
General Guidelines:

>100 um spot – use 10 um Pixel Size

<100 um spot – use 5 um Pixel Size

For reference: at 10 um Pixel Size setting you will capture ~80-100 pixels per spot.

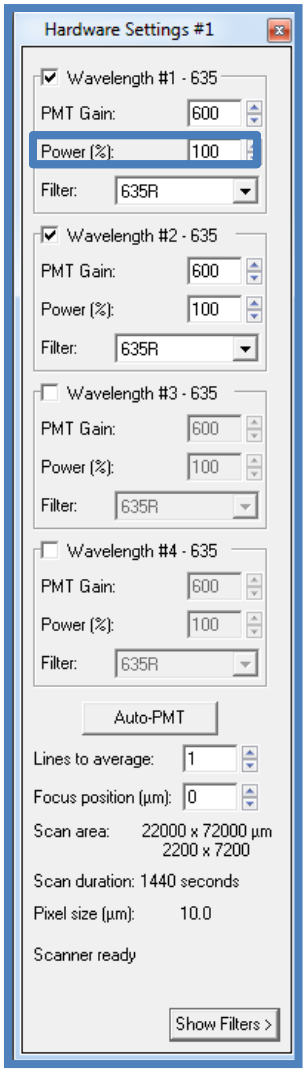
Setting Laser Power, PMT Gain, Lines to Average, and Focus Position



8a) Use the Hardware Settings dialog in combination with the Preview Scan function to define appropriate laser power, PMT gain, and lines to average settings for your array.

First, click on the Hardware Settings icon to launch the dialog that allows you to adjust these settings (see next slides for guidelines).

Guidelines for Setting Laser Power



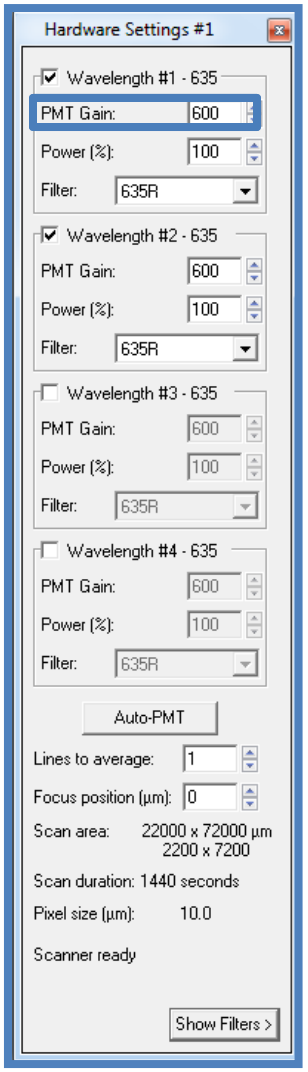
Laser Power: Typically this value is set to 100% for optimal signal to noise.

Depending on your scanner model, you may be limited to specific settings options for laser power (e.g. 100, 33, 10 percent only for the GenePix 4000B).

Photobleaching can be a concern, however the laser beam is ~ 5 um wide so in theory you should be able to scan your array 5 times before you see evidence of this.

HINT: Cy5 can be particularly affected by environmental conditions such as ozone exposure, humidity, etc. We recommend use of Alexa dyes for optimal stability.

Guidelines for Setting PMT Gain

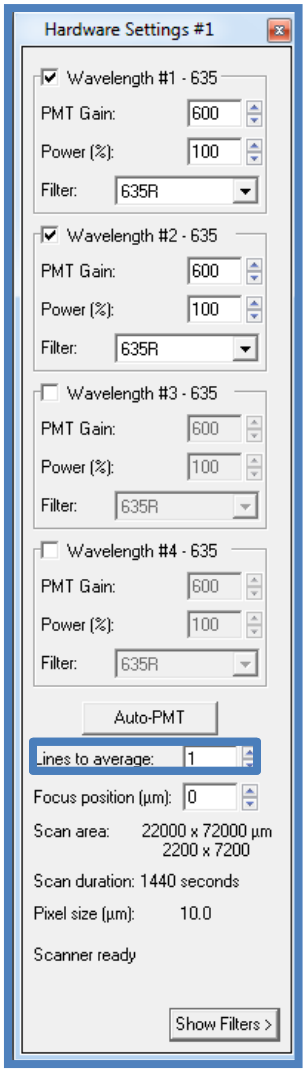


PMT Gain: PMT (Photo Multiplier Tubes) are detectors of photons of light, i.e. emission fluoresence.

The gain setting determines the sensitivity of your PMT(s) to fluorescence signal from your array.

The full range of the PMT is 100-100, however we recommend you use settings between 250-900 which is the linear range of the PMT(s).

Guidelines for Setting Lines to Average



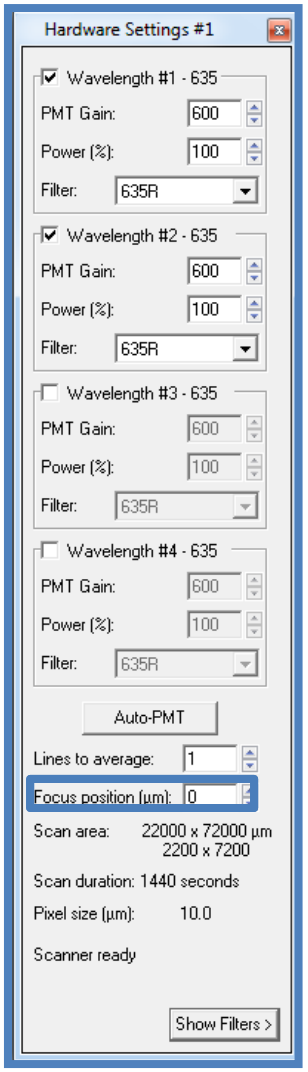
Lines to average: This setting determines the number of times the laser line passes over your sample at each scan position on your array.

Increasing lines to average will result in smoothing of the background on your array image, however will increase scan time by a factor of the value you choose.

Note that increasing lines to average may also result in faster photobleaching of your array.

We recommend scanning with averaging of 1 vs increased value and comparing data.

Guidelines for Setting Focus Position



Focus Position: This setting allows you to control the position in Z at which your array is scanned.

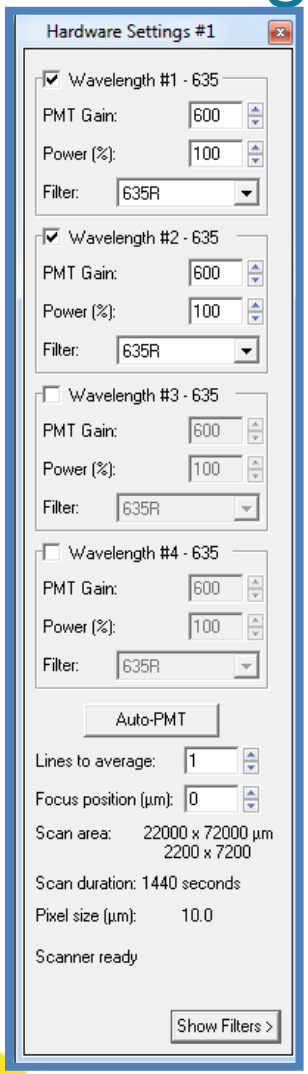
The depth of focus of your scanner is +/- 32 um, where 0 is equivalent to the surface of the array.

You can set the range between -50 to +200 um.

Typically for a microarray you will leave at 0 um.

You may want to adjust focus position for thicker samples such as tumor microarrays (TMAs) or chamber slides.

Interactively Setting Laser Power & PMT Gain During a Preview Scan

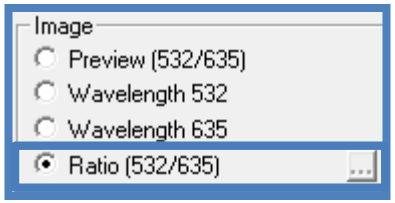


8b) With the Hardware Settings dialog active, now click on the Preview Scan button.

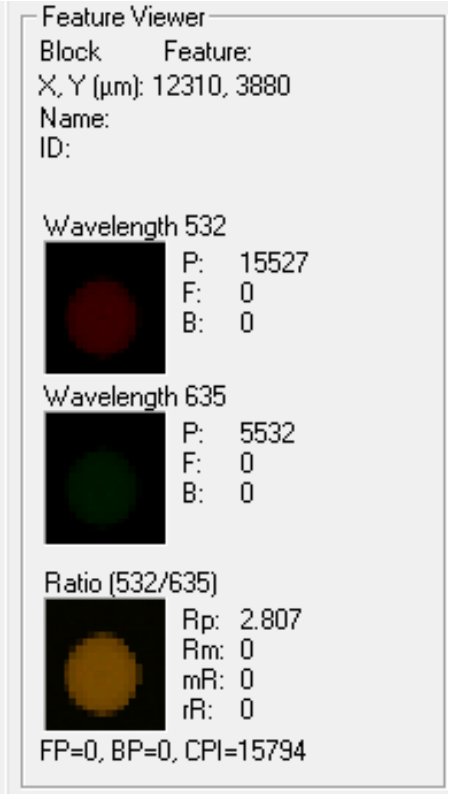
A low resolution (40 um pixel size) overlay preview image of your array will begin to appear.

Make sure that the Ratio Image is selected for display so that you can visualize the overlay image of your wavelengths.

Note that you can only view overlays of 2 channels at a time; click the elipsis (…) button to select.



Interactively Setting Laser Power & PMT Gain During a Preview Scan



8b) Mouse over the spots on your array image to see the intensity values for each wavelength (see P value in Feature Viewer).

Adjust the PMT settings so that you see equivalent signal in your selected wavelengths.

For instance, for a 2 color (red/green) array you will want to see a yellow color in the overlay preview image for your positive control spots.

Note that if you see lots of saturated pixels (will appear white, with value of 65535 intensity), drop the PMT setting.

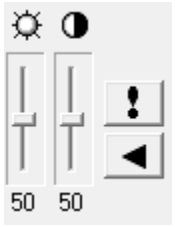
If you reach the lower recommended limit (250) for your selected PMT then you may need to drop the laser power for that wavelength.

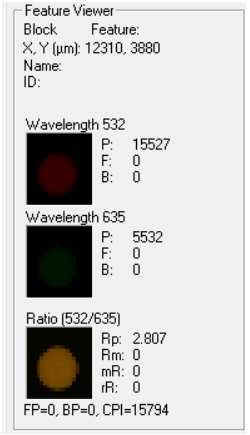
Additional Tips for Setting Laser Power & PMT Gain During a Preview Scan

The detectors on your scanner will generate a 16 bit image of your array, with a dynamic range of 0-65535 intensity values.

Ideally your laser/PMT settings will result in your control spots with intensity falling within 50-75% of the total dynamic range (so 30000-45000) in each channel.

Be aware that the brightness and contrast controls can affect the display, so be sure to check the actual intensity (P) values in the Feature Viewer for your control spots.



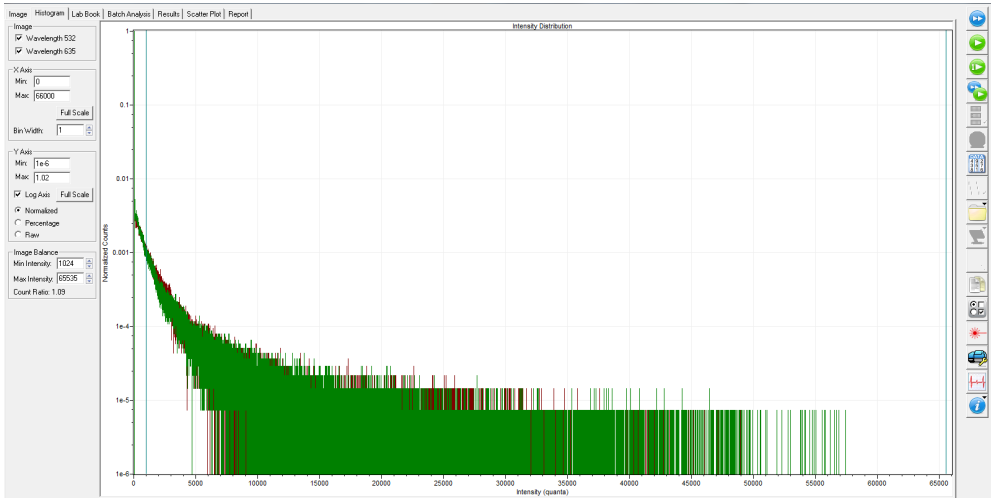


Additional Tips for Setting Laser Power & PMT Gain During a Preview Scan

As an additional check on your laser/PMT settings for each channel, you can also click on the Histogram tab.

This tab will automatically plot all of the fluorescence intensity values across your array for each wavelength as the scan progresses.

You will see that the plots for each wavelength will overlap well when your settings are close to ideal as in the example below. Note that the plots are color-coded by wavelength and that the zoom level on the array image is reflected in the plot.

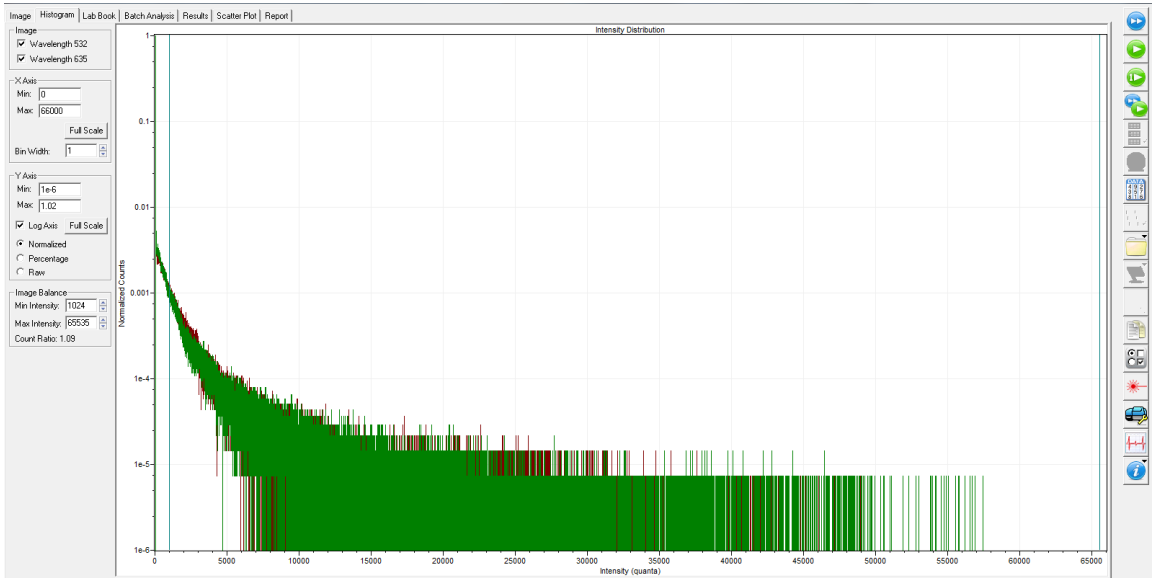


Additional Tips for Setting Laser Power & PMT Gain During a Preview Scan

The plot is organized such that the pixel intensities are plotted on the X axis from dimmest to brightest (left to right).

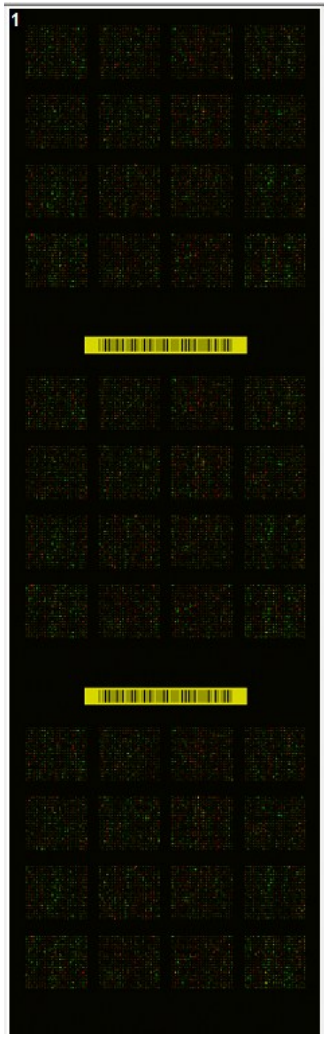
The Y axis is normalized to the bin with the most pixels in it. Each bin is 32 intensity values wide.

In this plot the background pixels generally outnumber the data pixels. These background pixels "anchor" the histogram.

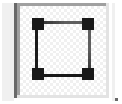


MOLECULAR DEVICES

Setting Scan Area(s)



9) You can now use the low resolution preview scan image to set your scan area(s).

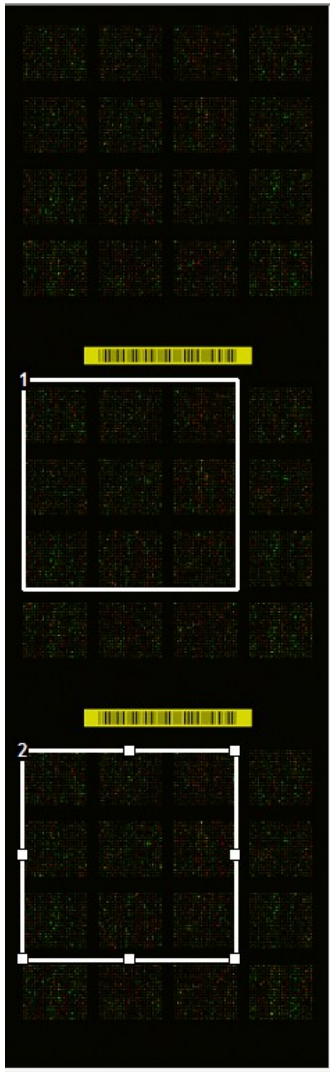
To do this, on the Image tab first click on the View Scan Area button 

You will now see an outline of the default scan area appear on your array image with a number 1 in the upper left corner.

When you mouse over the array image, you will now see the cursor appear with a white box attached to it.

You can now click on the borders of the scan area and drag to adjust its size and position to match the borders of your array.

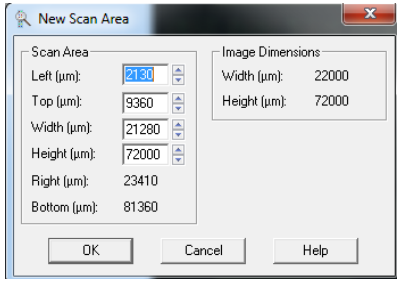
Setting Scan Area(s)



9) You can also choose to set multiple scan areas on your array.

To do this, on the Image tab click on the New Scan Area button 

The New Scan Area dialog box appears. You can manually set values within, or just click OK to add a new scan area and adjust on the image itself. Note that the initial values will default to those for the first scan area set.



The new scan areas will be numbered sequentially in the upper left in the order that they are added.

Performing a Data Scan

10) You are now ready to capture a full resolution scan of your array.

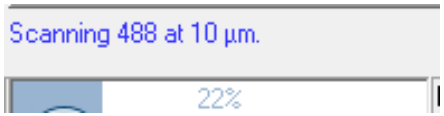
To do this, on the Image tab click on the Data Scan button. 

If you have selected multiple scan areas, click the Data Scan All button to capture an image of all areas 

Otherwise the scanner will only capture an image of the last scan area you selected.

Depending on the model of your scanner, your wavelengths will either be scanned simultaneously or sequentially.

If scanning sequentially with the ratio image display selected, you will see the image of the first wavelength appear, then the next.

To monitor the progress of your scan, check the status bar in the lower left corner of the Image tab. 

Performing a Data Scan – Additional Tips

- **The Preview Scan function always captures an image of the entire slide**

- **The Data Scan and Data Scan All functions capture an image of only the scan area(s) that you have defined.**

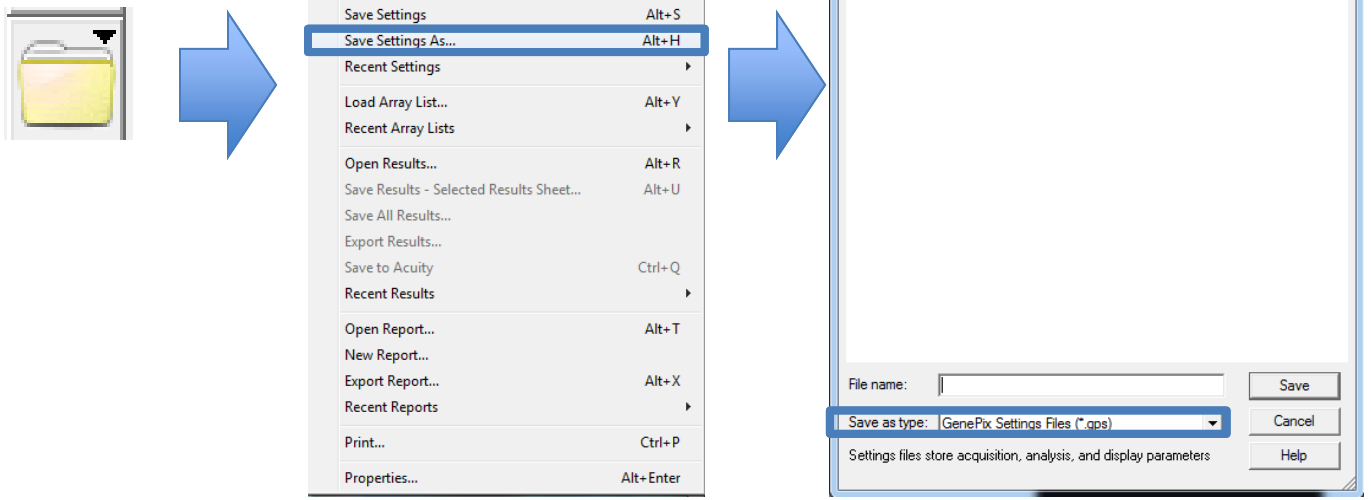
- ***HINT: You can set multiple overlapping scan areas and define different PMT settings for each one during the assay optimization process. This way you can directly compare data for the same scan area with different settings.***

- **You can also lock your scan settings by clicking the Lock Settings button on the Image tab. Note that this will also lock your alignment settings.** 

Saving Your Scan Settings

11) Once you have defined optimal scan settings for your array, you should save these as a .gps file.

To do so, click on the File… button, then select Save Settings As from the menu. A browser will appear, you should save as the default file type (GenePix Settings Files .gps)

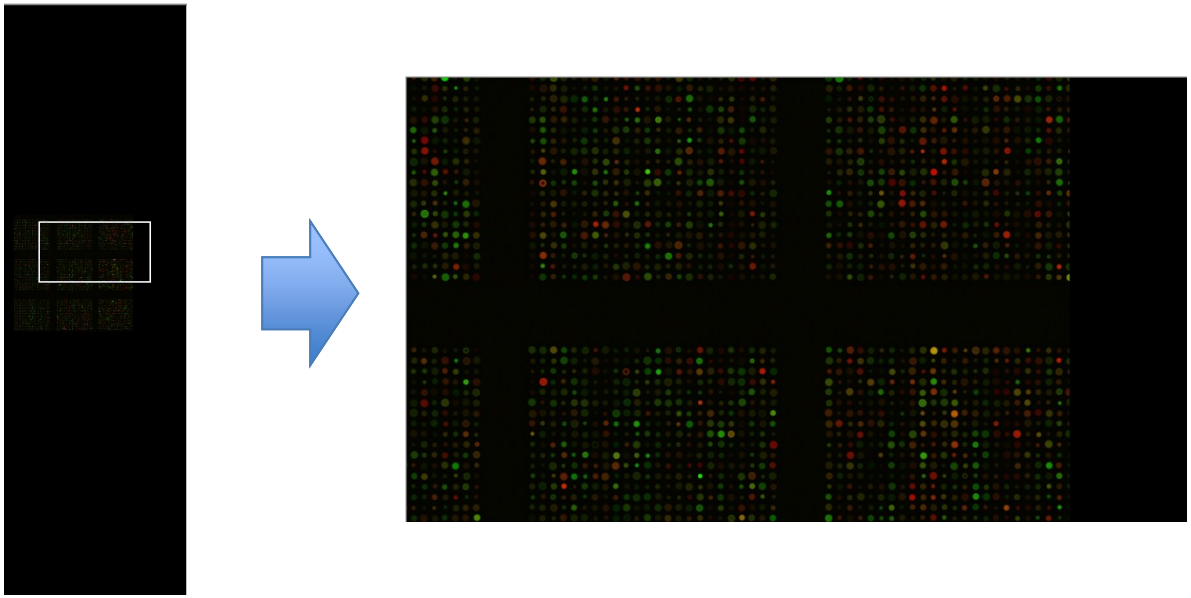


Viewing & Saving Array Images

Navigating the Array Image – Zoom Mode

To zoom in on an area, click on the Zoom mode button.

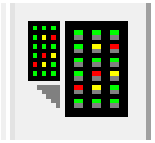
You will see the cursor change to a magnifying glass when you mouse over your array image. Click and drag a box on a desired area to zoom to that region.



Navigating the Array Image – Undo Zoom/Zoom Full Scale/Hand Mode

To go back 1 zoom level, click on the Undo Zoom button.

To return to the full array image view, click on the Zoom Full Scale button.



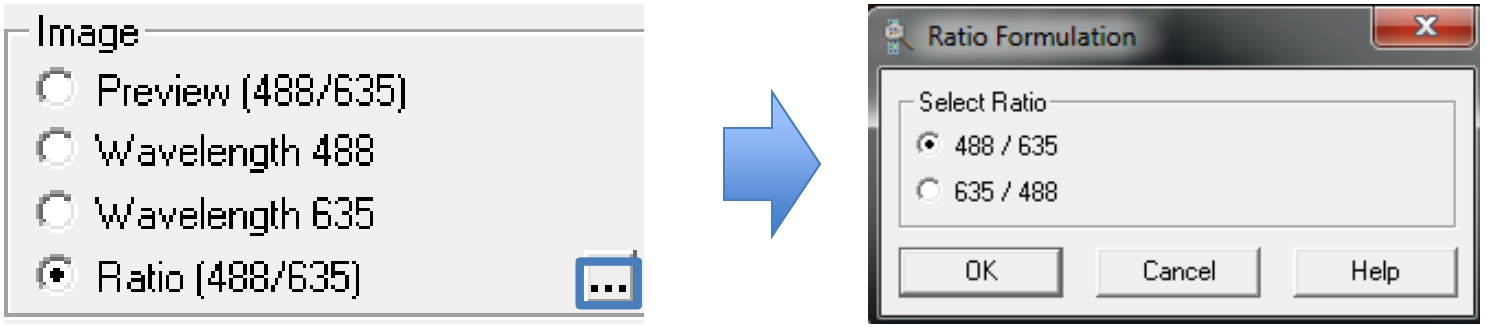
To move the entire array image within your current zoom level, click on the Hand Mode button, then click and drag the array image. ***Note that if you have an alignment (GAL) file overlaid on the image, the overlay will move along with the image.***

Changing the Image Display – Image Selector

Depending on the wavelengths you have scanned and whether you have captured a preview image, you will see several options in the Image pane on the Image tab.

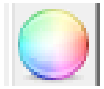
Select the radio button next to your desired image to display it.

If you have captured more 2 or more wavelengths, you can choose your desired Ratio image by clicking the elipsis (…) button and choosing a ratio in the Ratio Formulation dialog.

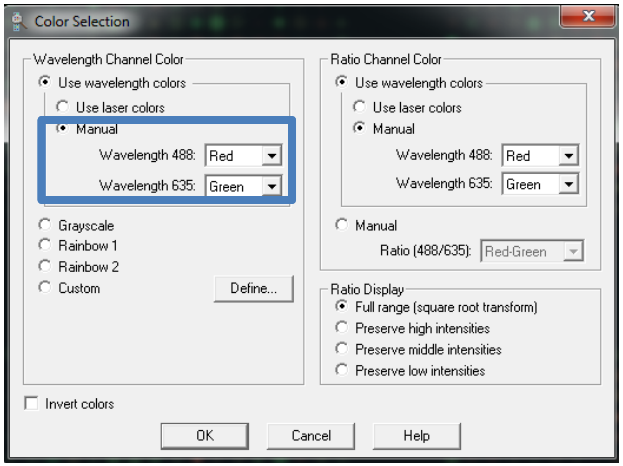


Changing the Image Display – Modify Colors

The array images captured by your scanner are greyscale, however you can choose to display them with a chosen color overlay.

To modify this setting for your selected image, click on the Color Selection button. 

The Color Selection dialog appears. You can opt to color by laser wavelength, or manually select desired colors from the dropdowns.



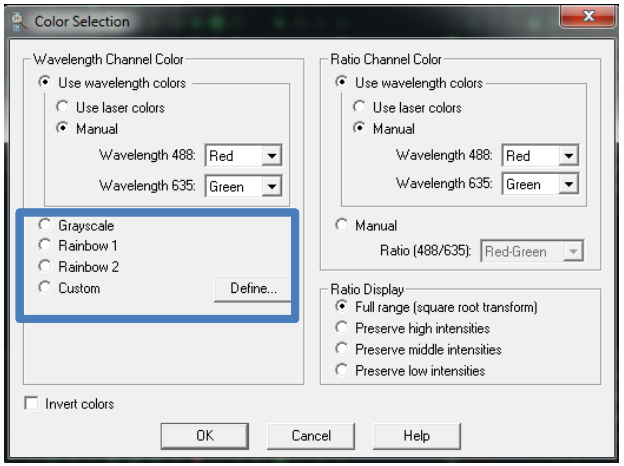
Changing the Image Display – Modify Colors

You can also opt for Greyscale, Rainbow, or Custom colors.

HINT: Rainbow mode generates a heat map view of your array image. This can make it easier to distinguish lower intensity spots vs background on your image.

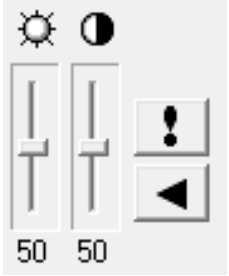
The Rainbow 1 option is better suited for viewing array images that have spots with primarily high intensity ranges.

The Rainbow 2 option works best for visualizing array images with lower spot intensities.



Changing the Image Display – Adjust Brightness & Contrast

You can adjust the brightness and contrast of your array image using the sliders on the Image tab.



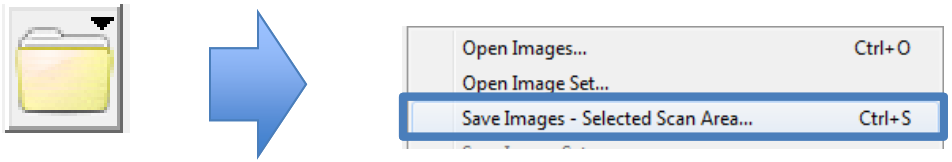
Clicking the exclamation point button will trigger the software to automatically scale the image.

Clicking the back button will reset the display back to the default brightness/contrast levels.

Note that these controls affect display of the image ONLY, they do not impact the intensity values of your array images.

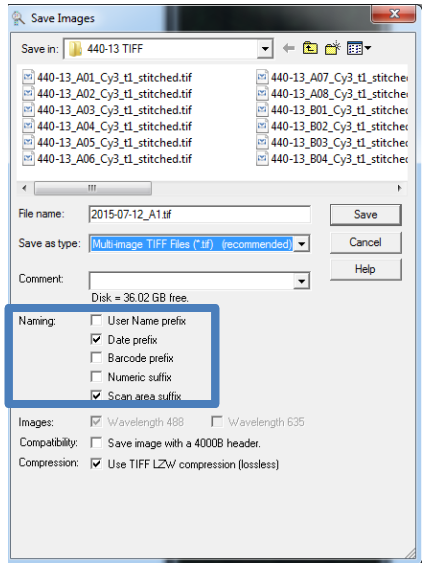
Saving Array Images

To save your array images, click on the File button, then select Save Images - Selected Scan Area from the menu.

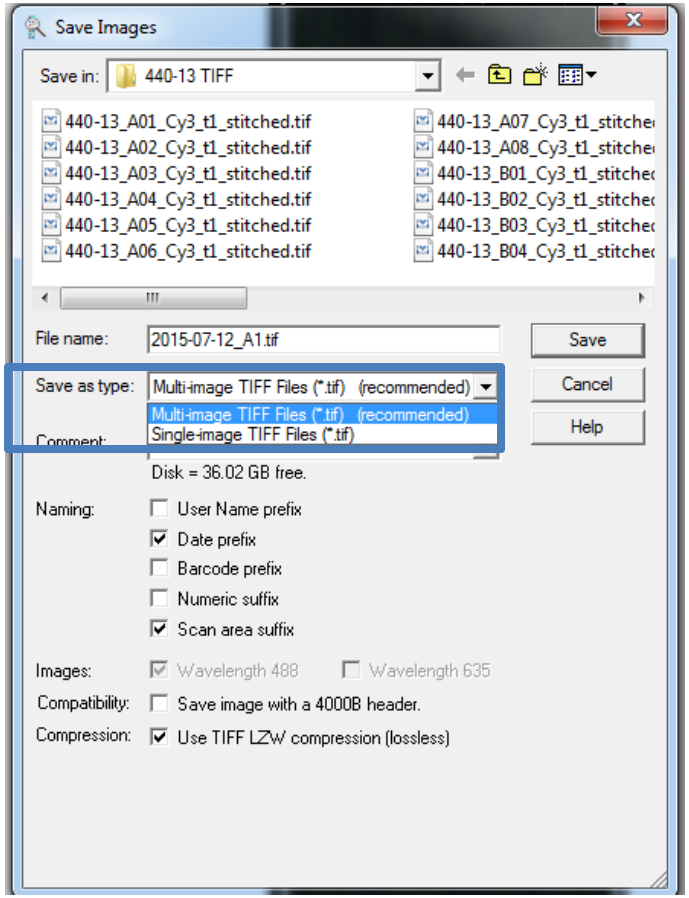


You have several options for saving your images.

You can choose to name your image manually, or opt for automatic tagging with User Name, Date, Barcode etc. by enabling the appropriate check boxes in the Naming field of the browser.



Saving Array Images – Image Formats



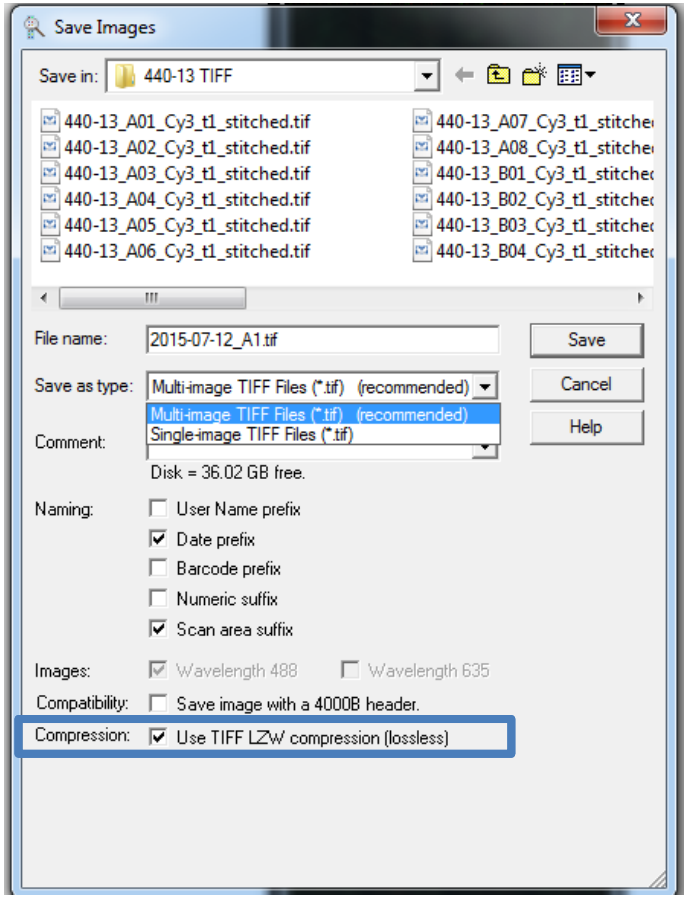
You can choose from two different file formats, Multi Image TIFF or Single Image TIFF.

If you choose Multi Image TIFF (the default), then all images will be saved including individual wavelengths, preview scan, and overlays in 1 file.

If you would like to access or analyze your images with a program other than GenePix Pro 7, you should save as single image TIFF files.

Note that for single image TIFFs, you can only save the individual 16-bit wavelength images, not the overlay or preview images since these are 24 bit color images (not analyzable).

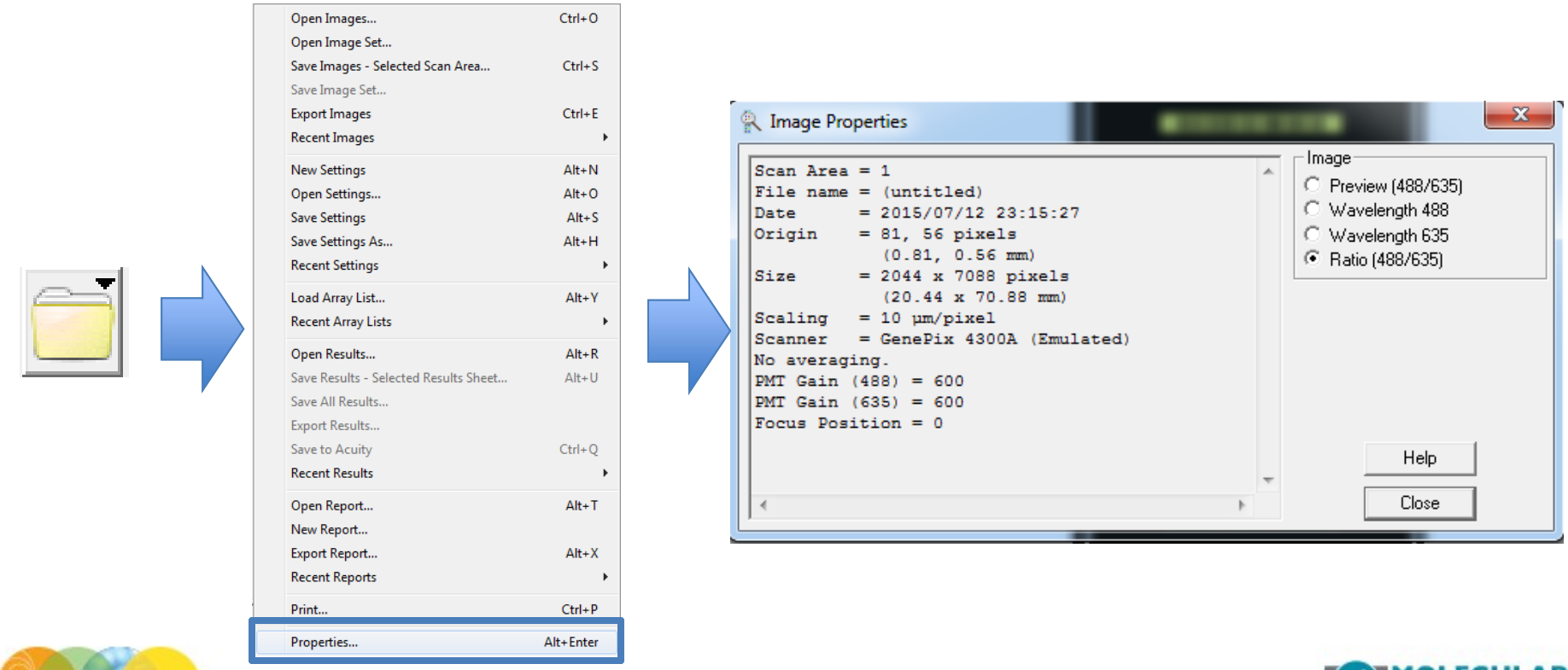
Saving Array Images – Additional Tips



- Use the Comment field to add notes to your images – these will be saved along with the file.
- The software automatically tags all Single-Image TIFF files with the laser color used to capture.
- Ratio & Preview Scan images are for display only. Note that it is risky to judge the quality of an array or scan from these as they reflect brightness/contrast/color settings when you created them.
- If you choose to save your Single-Image TIFF files with LZW compression, this may make it difficult for other programs to open.

Viewing Image Properties

- **GenePix Pro 7 automatically saves details of image capture along with your array images. To view these for your current image, click on the File button then select Properties.**



Analyzing Array Images

What is a GAL File?

ATF	1			
5	5			
Type=GenePix ArrayList V1.0				
BlockCount=1				
BlockType=0				
URL=				
Block1=4616.3, 4056.33, 220, 336, 281.1, 392, 280.916				
Block	Row	Column	Name	ID
1	1	1	_	seq48886
1	1	2	_	seq48886
1	1	3	_	seq48886
1	1	4	_	seq48886
1	1	5	_	seq48886
1	1	6	_	seq48886
1	1	7	_	seq48886
1	1	8	_	seq48886
1	1	9	_	seq48886
1	1	10	_	seq48886
1	1	11	_	seq48886
1	1	12	_	seq48886
1	1	13	_	seq48886
1	1	14	_	seq48886
1	1	15	_	seq48886
1	1	16	_	seq48886
1	1	17	_	seq48886
1	1	18	_	seq48886
1	1	19	_	seq48886
1	1	20	_	seq48886
1	1	21	_	seq23261

GAL stands for Genepix Array List.

The GAL file contains a map of all of the spots on your array.

If you have purchased your array from a vendor, they will typically provide this file with your array.

In addition to positioning of spots, the GAL file may also contain information about what is spotted on the array, for instance gene or protein IDs.

If you are working with a homemade array, you can generate a GAL file within the GenePix Pro 7 software and add your own annotation in Excel (opens as tab delimited text file).

GAL (Alignment) Terminology

ATF	1			
5	5			
Type=GenePix ArrayList V1.0				
BlockCount=1				
BlockType=0				
URL=				
Block1=4616.3, 4056.33, 220, 336, 281.1, 392, 280.916				
Block	Row	Column	Name	ID
1	1	1	_	seq48886
1	1	2	_	seq48886
1	1	3	_	seq48886
1	1	4	_	seq48886
1	1	5	_	seq48886
1	1	6	_	seq48886
1	1	7	_	seq48886
1	1	8	_	seq48886
1	1	9	_	seq48886
1	1	10	_	seq48886
1	1	11	_	seq48886
1	1	12	_	seq48886
1	1	13	_	seq48886
1	1	14	_	seq48886
1	1	15	_	seq48886
1	1	16	_	seq48886
1	1	17	_	seq48886
1	1	18	_	seq48886
1	1	19	_	seq48886
1	1	20	_	seq48886
1	1	21	_	seq23261

GenePix uses specific terms to refer to the organization of your array.

Array: This is the entire array.

Block: Sub-arrays or defined sections within the full array. (Note that your array may be organized into one or several blocks).

Feature: Individual spots within your array.

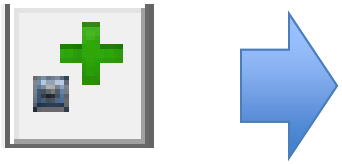
Name: This is the common name for a given spot. You may also see terms such as negative or positive control in this field of the GAL file.

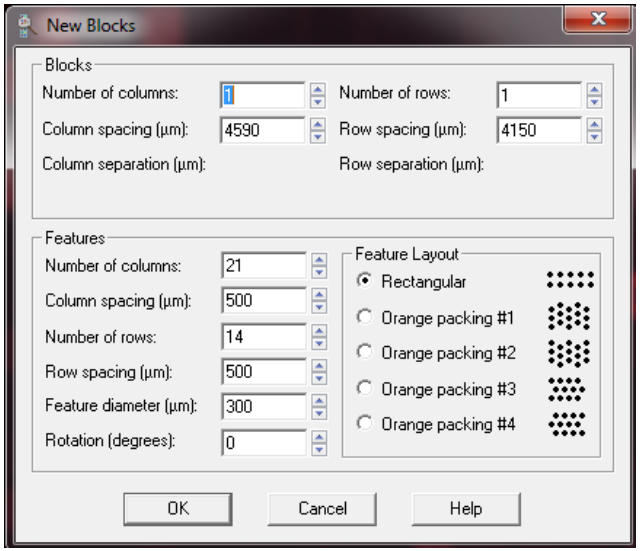
ID: This is generally the sequence ID for a given spot.

Analyzing Array Images – Creating a New GAL File

If your array is homemade or the manufacturer did not provide a GAL file, you will need to create one within the GenePix Pro 7 software using these steps:

1) **Click on the New Blocks button and enter the specifications for your array printing in the appropriate fields of the New Blocks dialog box.**





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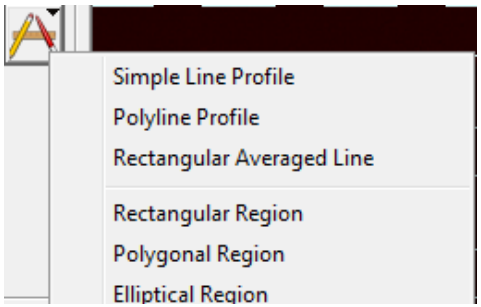
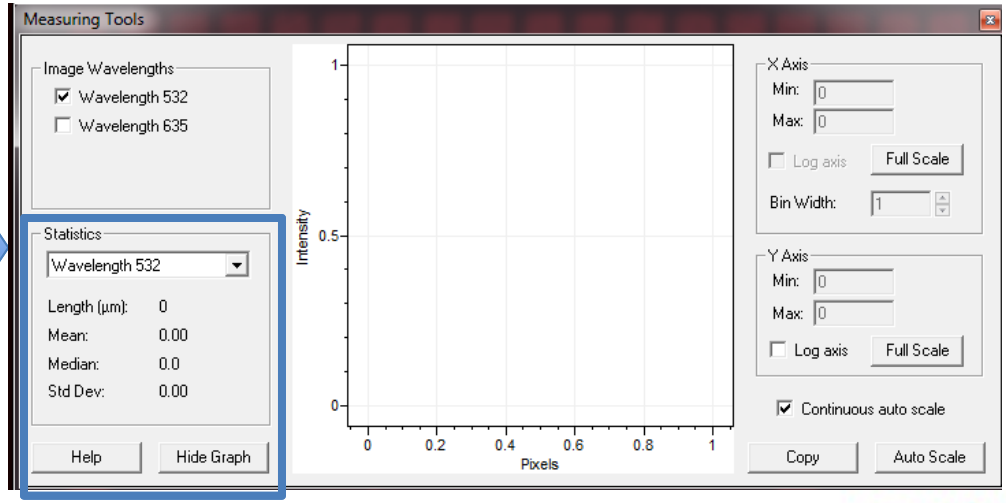
Analyzing Array Images – Creating a New GAL File

If you need help determining values (such as feature diameter) to populate the New Blocks dialog, you can use the Measuring Tools on the Image tab.

Click on the Measuring Tools icon and select a tool from the menu that is appropriate for your desired measurement. You will see the cursor change shape to reflect your choice and the Measuring Tools dialog will appear.

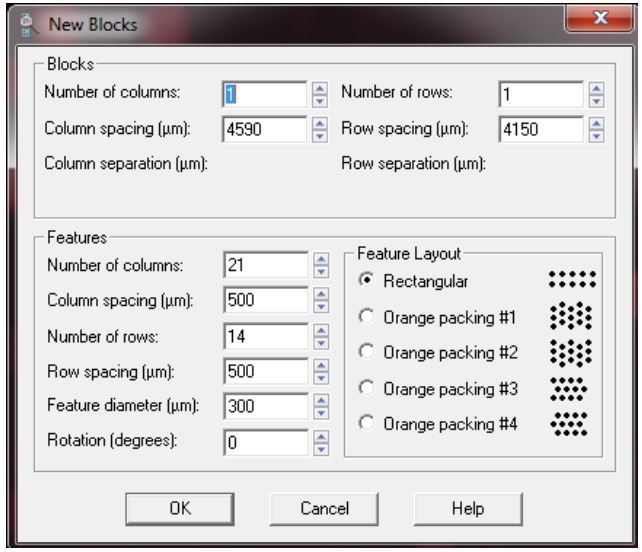
Next, click on the image to draw a region around your area of interest (i.e. a spot). You will see the statistics field populate in the Measuring Tools dialog.

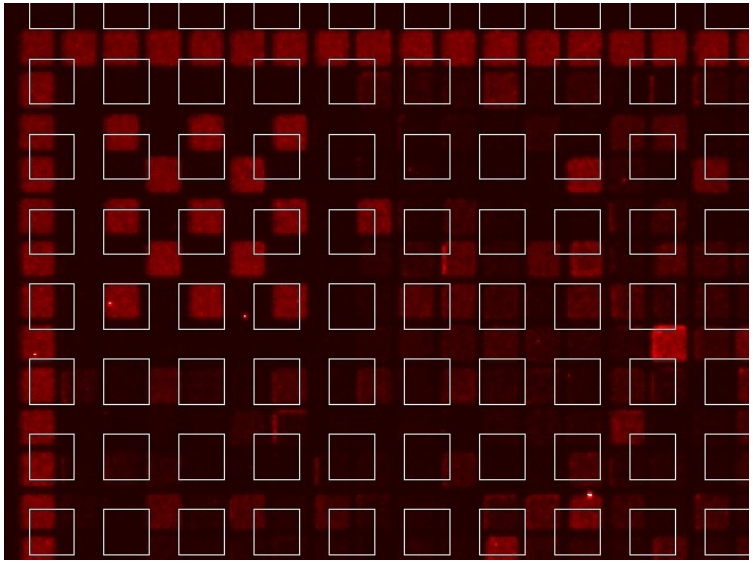




Analyzing Array Images – Creating a New GAL File

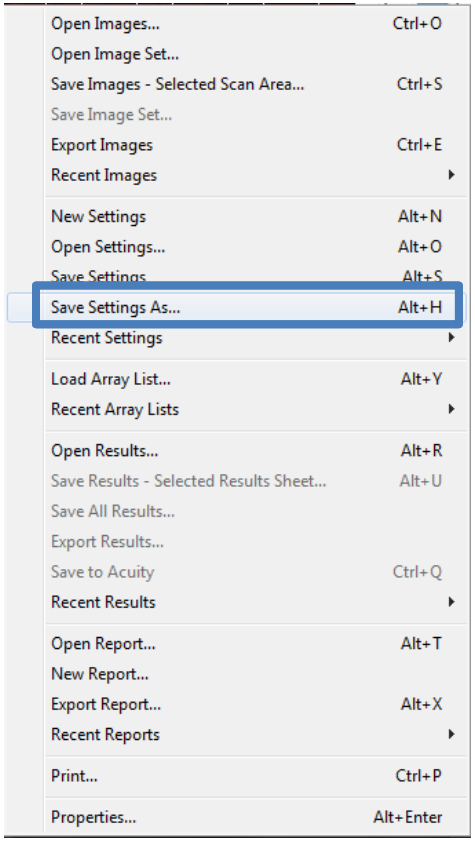
2) Click OK on the New Blocks dialog box. You will now see a white GAL file overlay appear on top of your selected array image. (See next slides for details on how to reposition and further optimize this overlay).

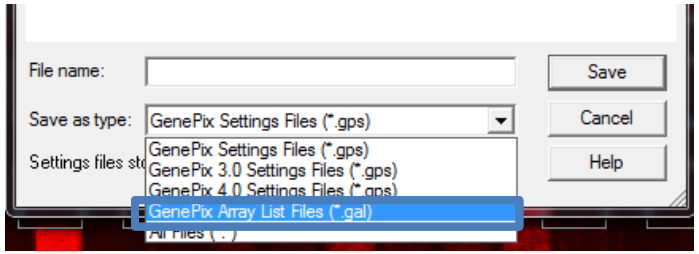




Analyzing Array Images – Creating a New GAL File

3) Save the GAL file by clicking the File button, then selecting GenePix Array List File (.gal) from the Settings files type dropdown in the browser.





Analyzing Array Images – Creating a New GAL File

ATF	1			
5	5			
Type=GenePix ArrayList V1.0				
BlockCount=1				
BlockType=0				
URL=				
Block1=4616.3, 4056.33, 220, 336, 281.1, 392, 280.916				
Block	Row	Column	Name	ID
1	1	1	_	seq48886
1	1	2	_	seq48886
1	1	3	_	seq48886
1	1	4	_	seq48886
1	1	5	_	seq48886
1	1	6	_	seq48886
1	1	7	_	seq48886
1	1	8	_	seq48886
1	1	9	_	seq48886
1	1	10	_	seq48886
1	1	11	_	seq48886
1	1	12	_	seq48886
1	1	13	_	seq48886
1	1	14	_	seq48886
1	1	15	_	seq48886
1	1	16	_	seq48886
1	1	17	_	seq48886
1	1	18	_	seq48886
1	1	19	_	seq48886
1	1	20	_	seq48886
1	1	21	_	seq23261

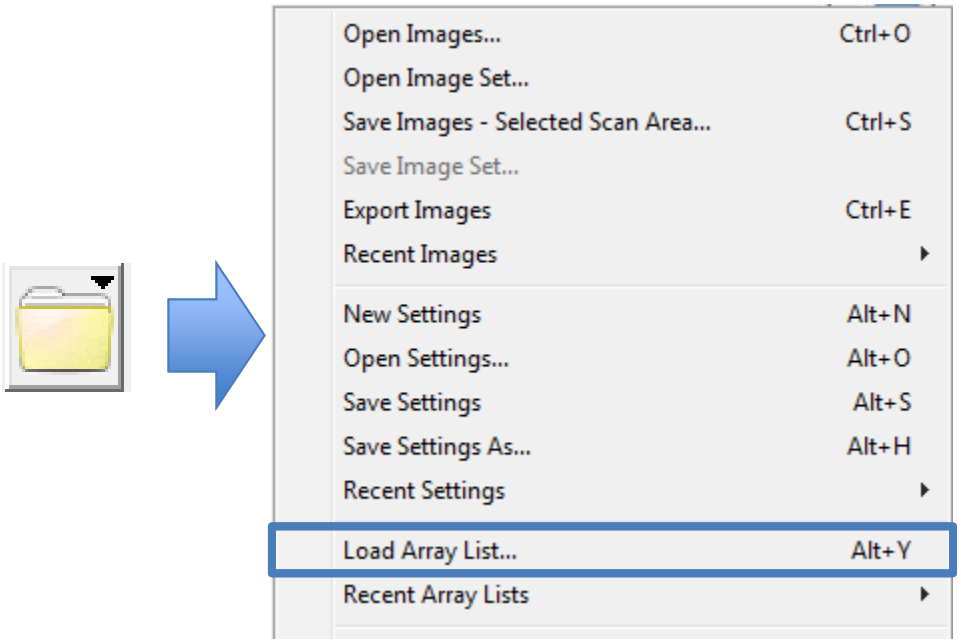
4) You can now open the GAL file in Excel as a tab delimited text file and populate additional information such as spot ID and name.

Be careful not to modify the header information or the block/row/column fields in this file.

Analyzing Array Images – Loading a GAL File

1) **To analyze an array image with an existing GAL file, click on the File button, then select Load Array List from the menu.**

A browser will appear that will allow you to navigate to your selected GAL file (.gal extension).

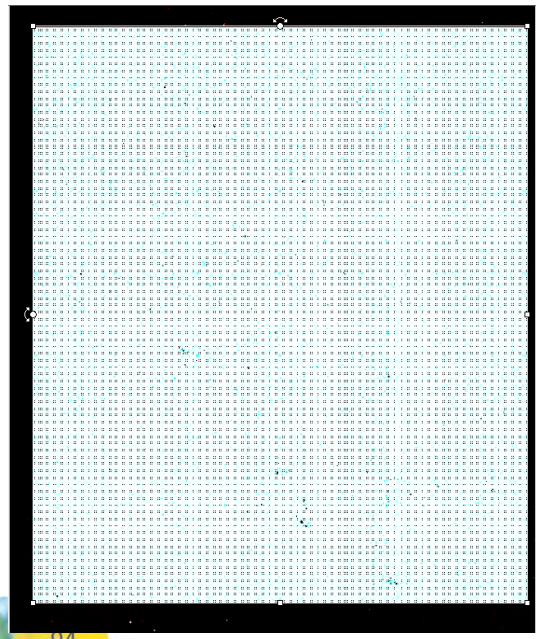


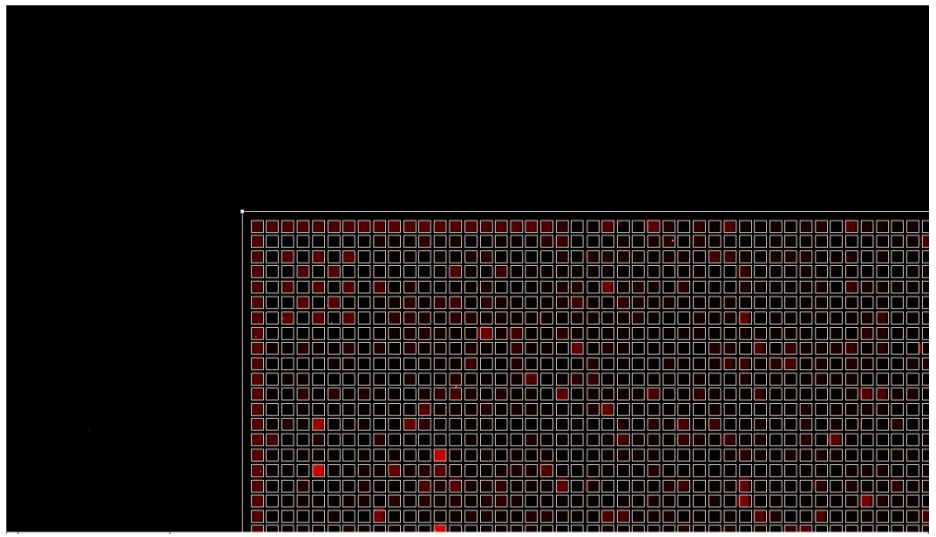
Analyzing Array Images – Checking GAL Alignment

2) **You will now see a white grid appear overlaid upon your array image.**

You can use the Zoom Mode tool in to check the alignment of the grid on your image. 

We recommend doing this even with pre-defined GAL files, as there can be some within and between batch variability in spotting of manufactured arrays.

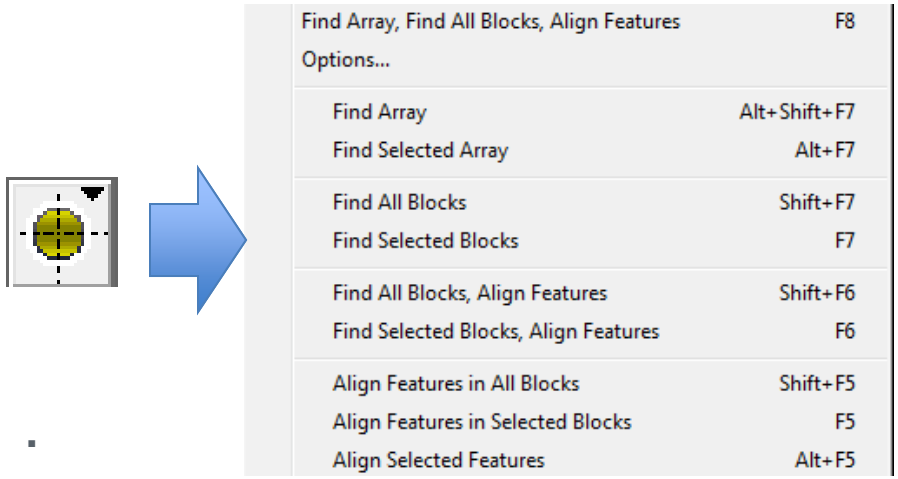




Analyzing Array Images – Automated GAL Alignment

3) **Adjust the alignment of the GAL overlay using any combination of the automated or manual tools available.**

To access the automated tools, click on the Align Blocks option and select from the methods listed.



The automated alignment options in this menu are listed from most automated to least automated (top to bottom)

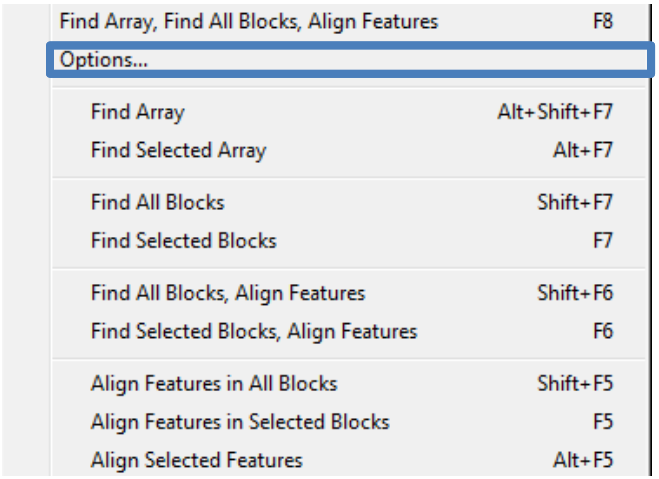
You can also access the Options dialog for alignment from this menu (see next slides for details).

Optimizing Automated Alignment

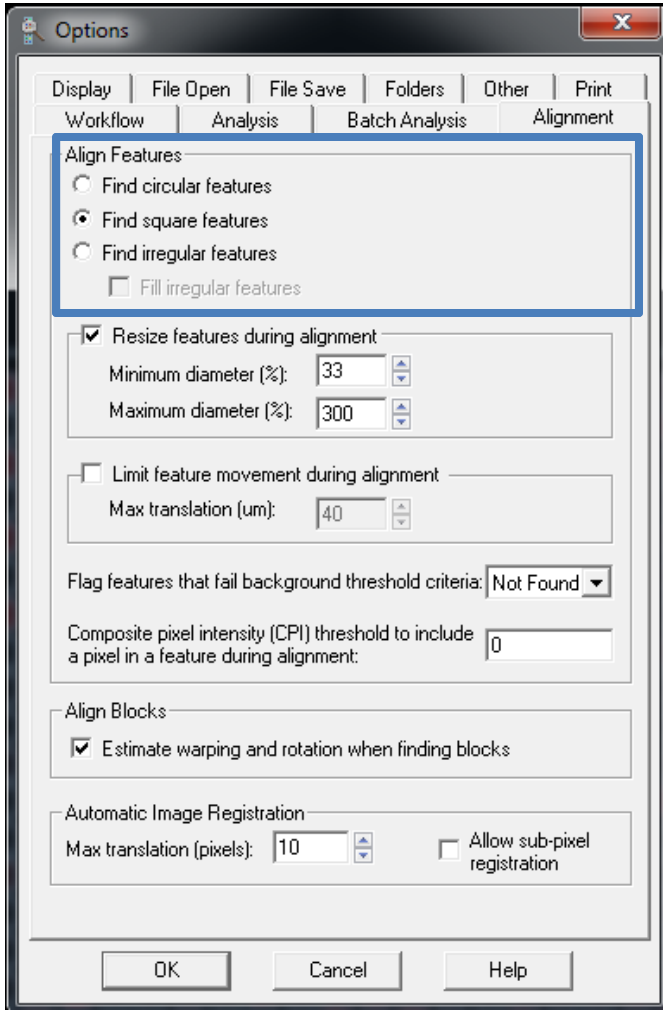
The GenePix Pro software uses image analysis-based algorithm to perform automated alignment of your array, blocks, and features (spots).

Automated alignment can therefore be affected by very dim signal as well as other factors such as skew in the array printing.

To refine automated alignment, click on the Align Blocks button and select Options from the menu.



Optimizing Automated Alignment



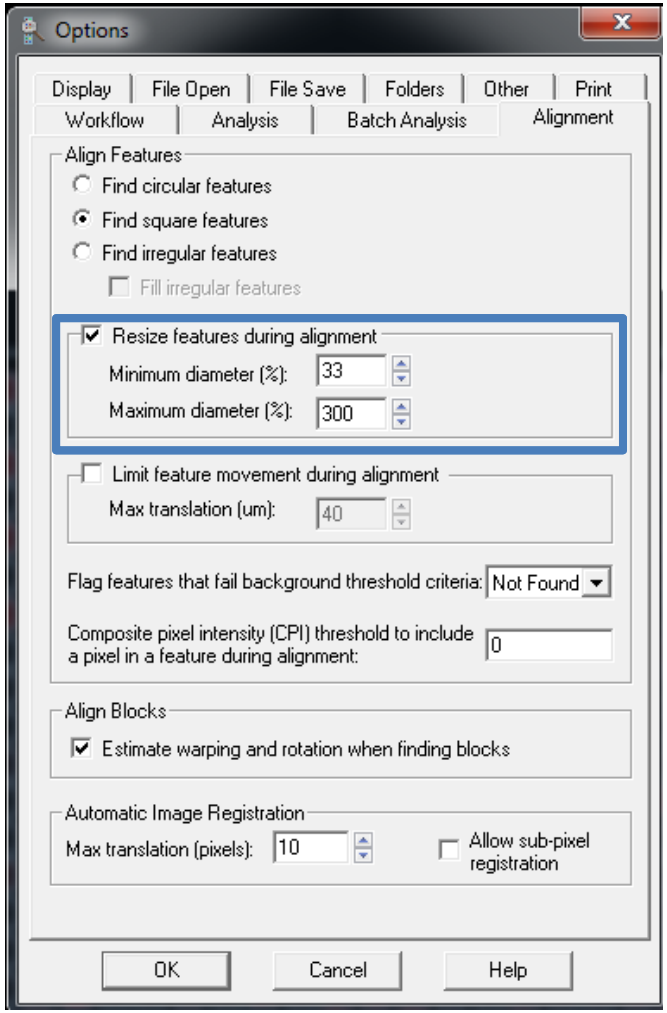
The Options dialog will appear with the Alignment tab selected.

<u>Align Features:</u> Here you can modify the shape of features – choose from circular, square, or irregular.

If you choose irregular, the software will automatically detect spot shape based on the fluorescence of the spot over its local background.

You can also opt to fill in irregular features by checking the appropriate box.

Optimizing Automated Alignment



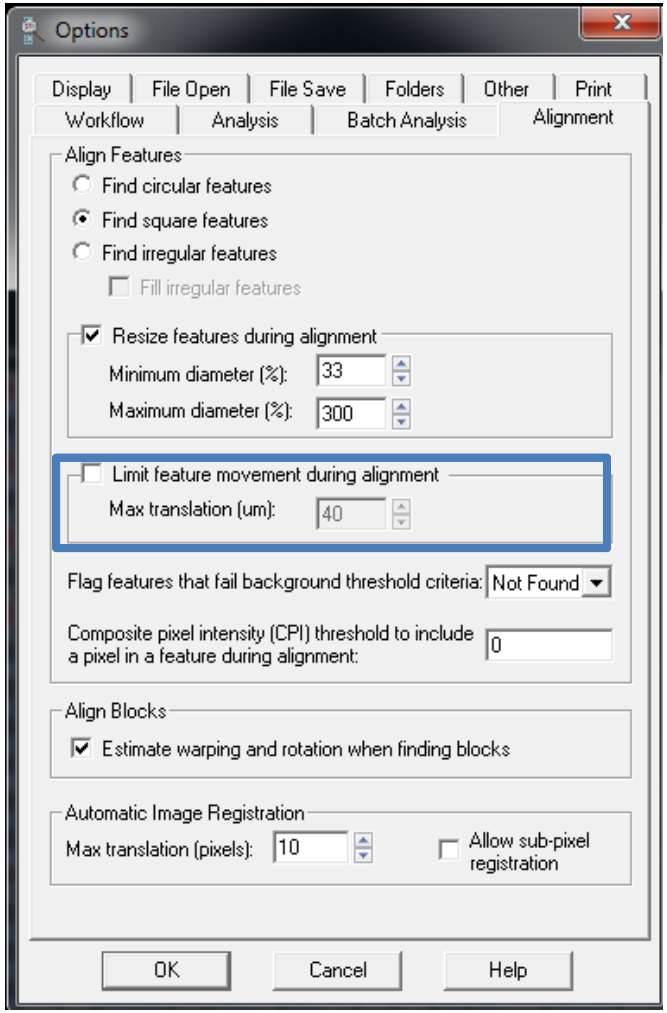
<u>Resize features during alignment</u>: You can also set a tolerance for variability in spot diameter in this dialog.

Select Resize features during alignment and enter percent minimum and maximum diameter values in accordance with your knowledge of the array.

The maximum recommended range for this feature is 50-200%

Features (spots) that fall outside this range will automatically be flagged as Not Found.

Optimizing Automated Alignment



Limit features movement during alignment: To accommodate variability in spotting, you can also set a limit for feature (spot) movement during alignment.

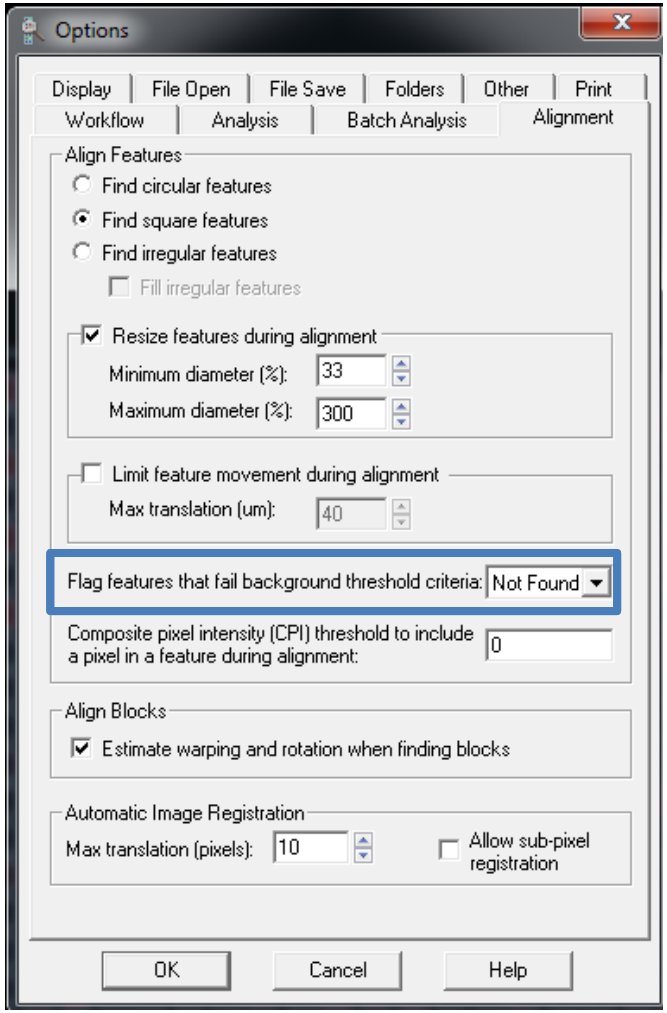
Use this feature to limit the distance that features move off grid during the Align Features process…like a leash for spots!

A good starting value would be 2X the pixel size you scan the array at. For example, try a value of 20 um for an array scanned at 10 um pixel size.

Features that move outside this limit are flagged Not Found.

This feature is useful if you have high-quality manufactured arrays where the spots are all exactly on the ideal grid, and some speckle in the background. By limiting feature movement, the spot-finding algorithm will not be distracted by background speckle.

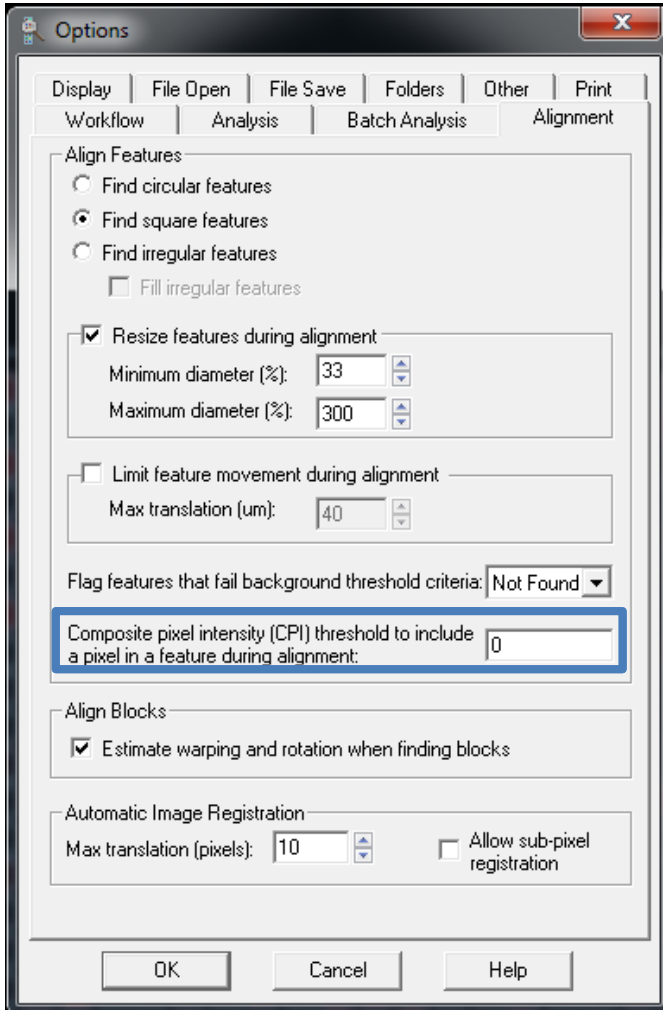
Optimizing Automated Alignment



<u>Flag features that fail background threshold criteria</u>: You can choose to have features (spots) that fail selection criteria flagged as either Not Found or to remain unflagged.

If you suspect that your "Not Found" features are still likely to be in the correct position with in the block (but just too dim to pass detection) then you may want to leave them unflagged so that the overall alignment of the block is not affected.

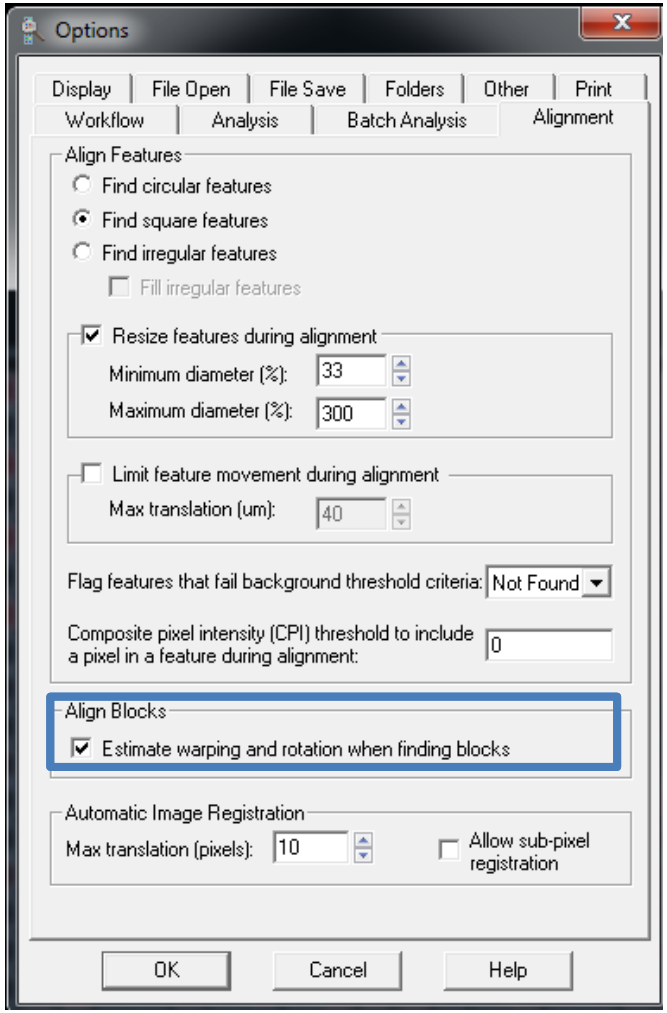
Optimizing Automated Alignment



<u>Composite pixel intensity (CPI) threshold</u>: If you see significant variation in background across your array image, you may want to set a CPI threshold.

The CPI Threshold should be set to a value below the average feature pixel intensity in the dimmest channel, and slightly higher than the average background speckle intensity in the brightest channel.

Optimizing Automated Alignment

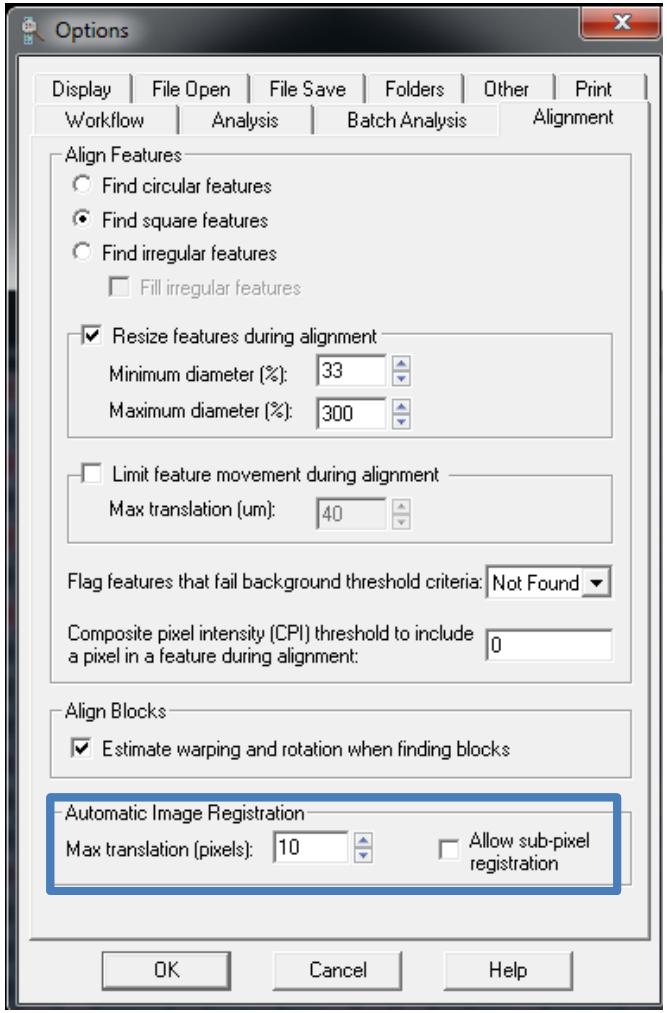


<u>Align Blocks</u>: Estimates the warping and rotation of blocks on the image when performing the alignment.

You should disable this if your array image has bright linear defects/background.

.

Optimizing Automated Alignment



Automatic Image Registration: This option allows you to set thresholds to guide registration of multi-wavelength image sets.

The Max translation (pixels) value sets a limit for aligning the starting point (in X and Y) of subsequent images vs the base wavelength image.

Selecting Allow sub-pixel registration enables registration of images using displacements of fractions of a pixel. Use this when whole pixel registration is not adequate.

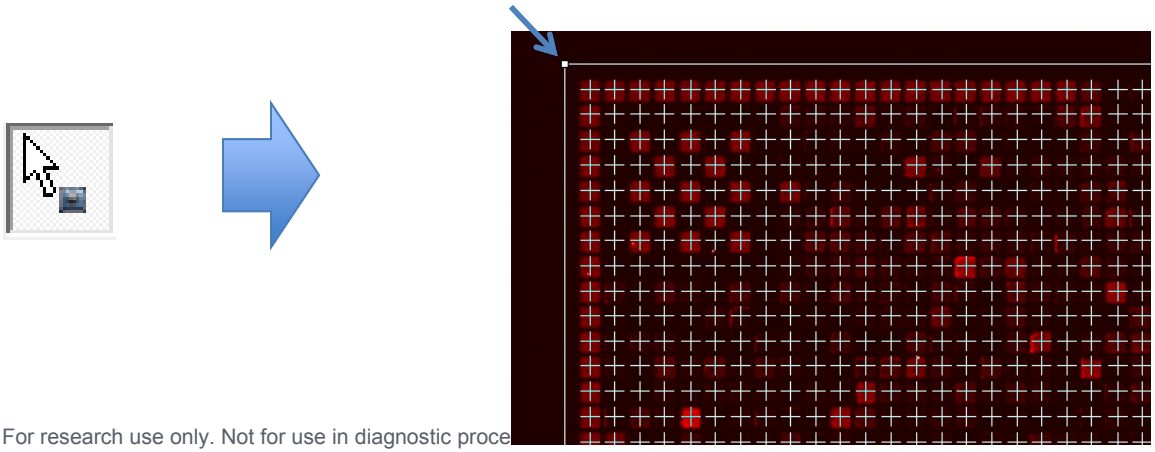
Analyzing Array Images – Manual GAL Alignment

3) To manually adjust alignment of the GAL overlay you can use the following tools/modes:

a) Block Mode – Click on the Block Mode button, then click and drag the handles at the borders of the GAL overlay to modify alignment of the block.

You will see plus signs appear over the features (spots) – use these like crosshairs to align the entire block to the centers of your features (spots).

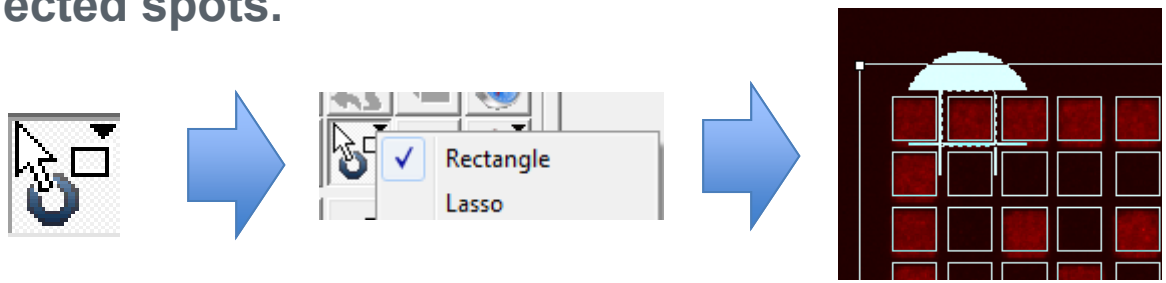
HINT: If you are adjusting for skew in the array, hold down CTRL key when clicking and dragging on a handle of the overlay.



Analyzing Array Images – Manual GAL Alignment

3) To manually adjust alignment of the GAL overlay you can use the following tools/modes:

b) Feature Mode: Click on the Feature mode button. You can choose from rectangle or lasso selection options to select a group of features, or simply click on individual features (spots) in the array to view and modify the overlay. You can click and drag to resize or reposition the GAL overlay for your selected spots.



You can also use the arrow keys on your keyboard to move selected spots.

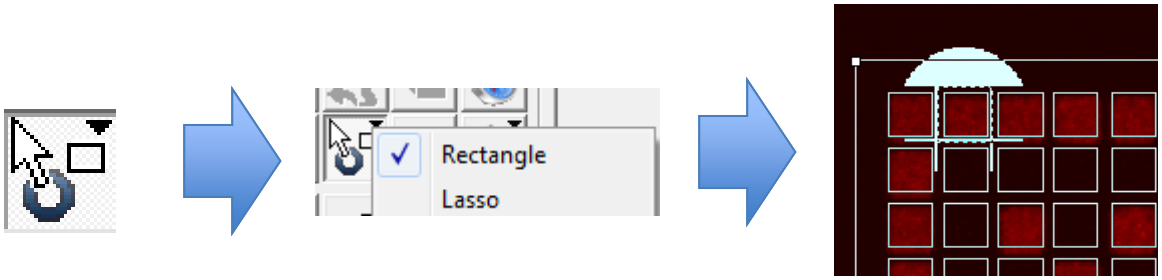
Hold down the CTRL key + arrow keys to resize selected spot(s).

Analyzing Array Images – Introduction to Background Subtraction

Note that for your selected spots in Feature Mode you will also see a solid white region around the borders of the feature.

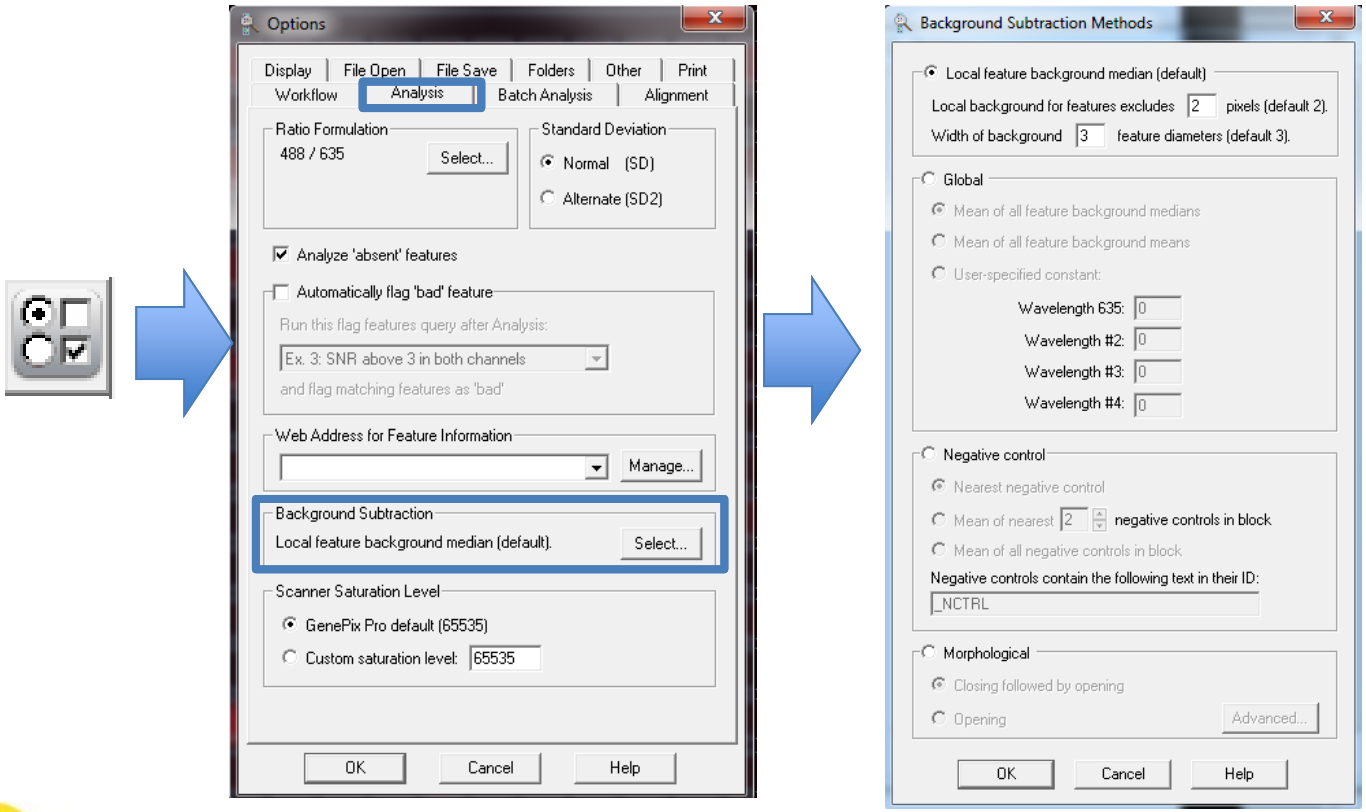
This represents the sampling of local background which the software references when calculating data from your array image (with the default method).

We will cover how to modify background subtraction settings in subsequent slides.



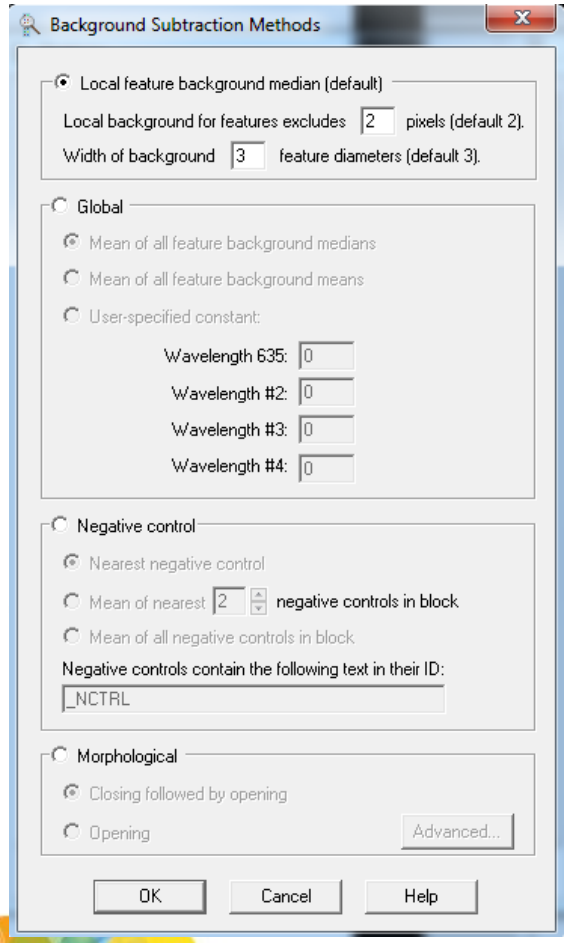
Analyzing Array Images – Background Subtraction

4) Choosing Background Subtraction methods: Access this dialog by clicking the Settings button, click on the Analysis tab, then on Select in the Background Subtraction subsection of the tab.



Analyzing Array Images – Existing GAL File

4) The software offers 4 different background subtraction options as follows:

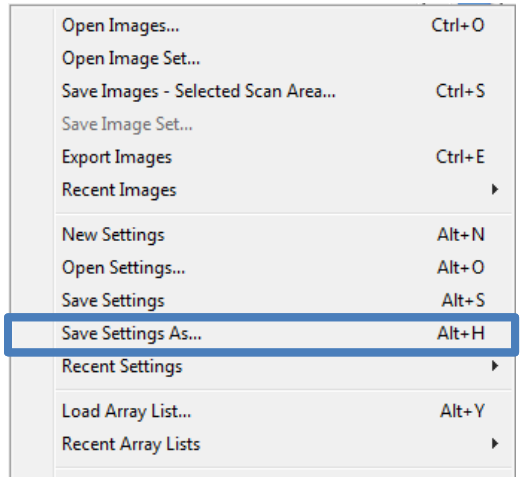


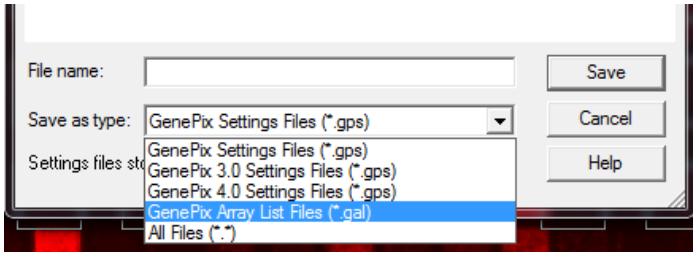
- **Local feature background median:** This is the default method. Local background is subtracted for each feature (spot) within a defined pixel range of the edge of the feature (2 pixels ore more). You can modify the # pixels to exclude and the width of the background region.
- **Global:** Subtracts either the mean of all background medians or means, or uses a user specified constant for each wavelength. Useful for arrays that have variable background.
- **Negative Control:** Subtracts either the nearest negative control, the mean of the nearest x negative controls in the block, or the mean of all negative controls in the block (as specified in the GAL file).
- **Morphological:** Applies an image analysis method (choose from 2) that enhances each spot vs background. (Not commonly used.)

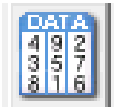
Running Analysis

5) You are now ready to save your setting and run analysis. First be sure to save any changes to the GAL file and GPS settings file. You can do this by clicking on the File button, then selecting Save Settings As.

   Select the appropriate file type (.gal or .gps) from the Settings files type dropdown in the browser before clicking save.





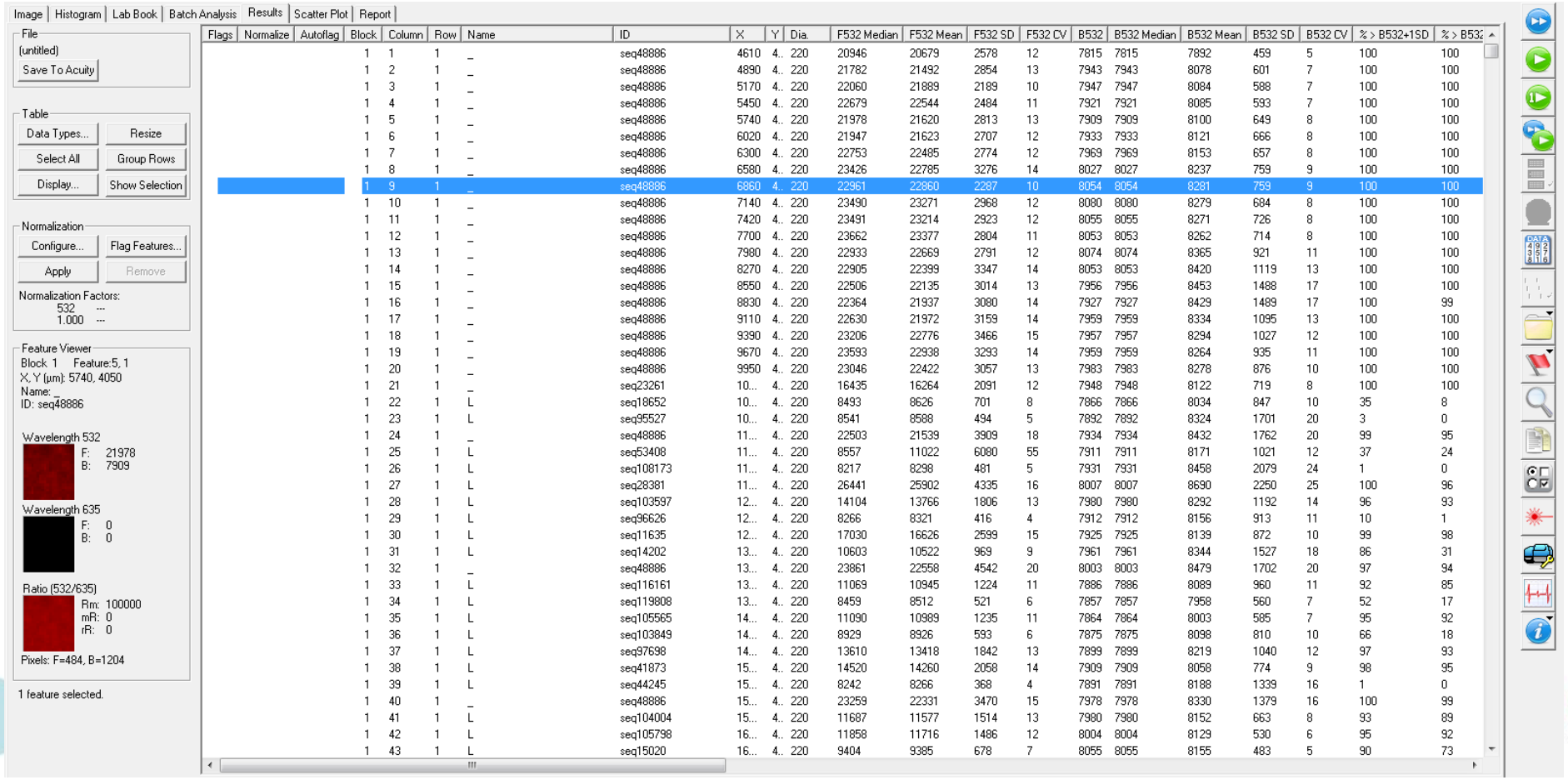
Finally click the Analyze button. 

Data will now be populated in the Results tab.

Reviewing Raw Data & Creating Data Flags

Reviewing Raw Data – Results Tab

To review your raw array data, click on the Results tab after your analysis is complete. You will see all of your raw data populated in the center table of this tab and will have access to tools for sorting, flagging, and normalizing data. (More details on subsequent slides).



Key to Results Types – Annotation & Naming Convention

- **Flag Status, Block, Column, Row, ID are annotations pulled from the GAL file.**
- **X and Y axis refers to the slide position**
- **Spot diameter also comes from the GAL file unless you have selected to detect irregular features.**
- **The calculated data points are named in accordance with a defined code. For example, the value "F635 Median":**
  - **-"F" stands for Feature (Spot)**
  - **"635" is the wavelength of the laser used for excitation**
  - **Median is the calculated median intensity (recommend using this value if you have debris on your array)**
  - **You will see the prefix "B" in other measures. This stands for Background.**

Key to Results Types – QC Measures

- **Several QC measures are also included:**
  - **%B(Wavelength Number) + 1SD – Indicates how clean the separation is between positive pixels inside the feature (spot) vs background pixels (the higher number, the better)**
  - **% Saturation – Measures precent of pixels in a given feature (spot) that are saturated**
  - **Total Intensity – accounts for size of feature (spot) where the Average Intensity value will not. This is important for users working with protein arrays as they may see large aggregates on their arrays.**
  - **Mean or median SD of ratios – Reports standard deviation of these base values**

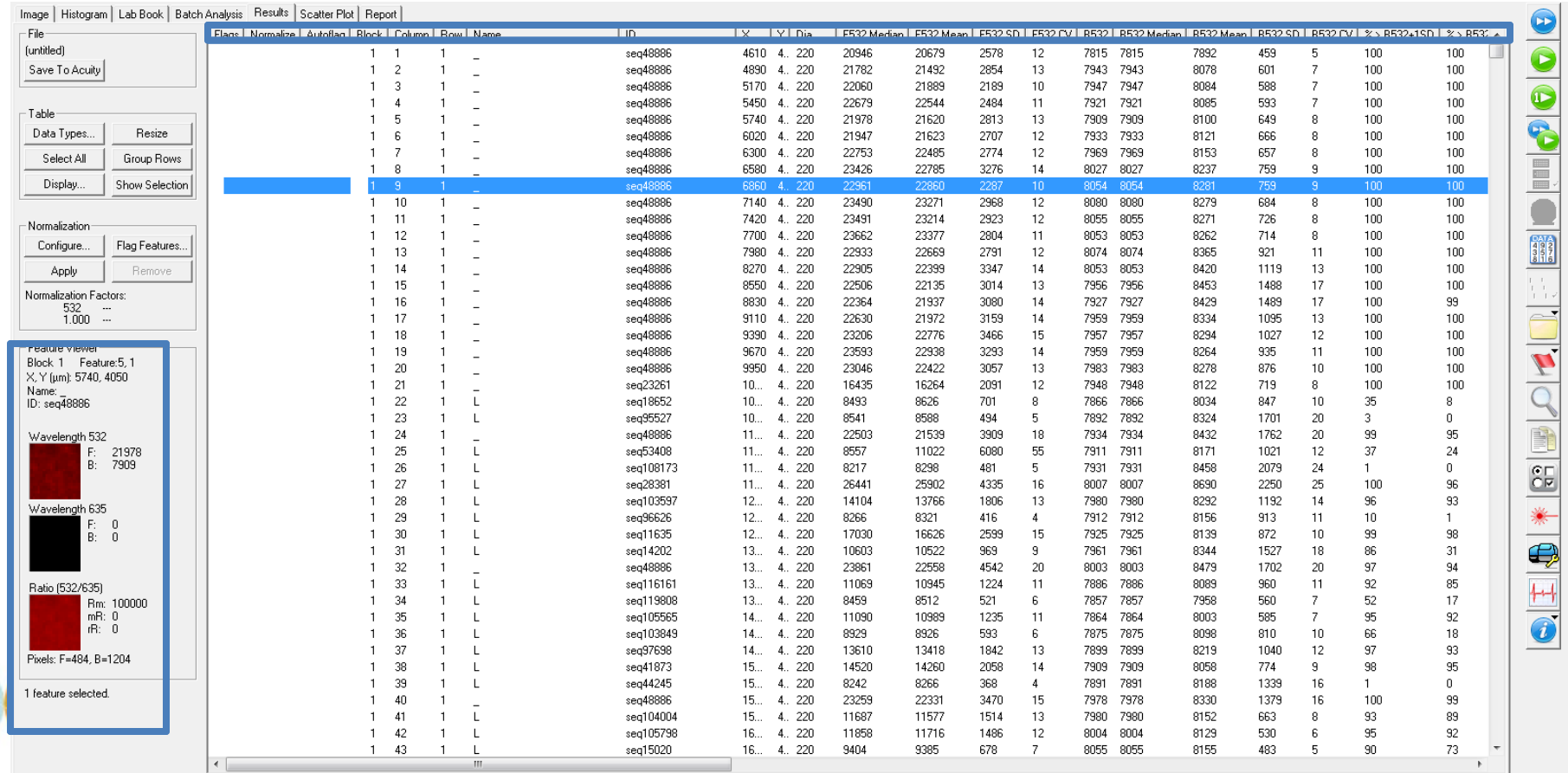
Key to Results Types – FAQ

- **What is the difference between the Ratio of Medians measurement vs the Median of Ratios?**
  - **The Median of Ratio value is calculated on a pixel by pixel basis for your feature (spot) of interest**
  - **The Ratio of Medians is calculated from the entire area of the feature (spot).**
- **What is sum of medians?**
  - **Sum of medians is a calculated average of one wavelength plus an average of a second wavelength (such as red and green).**
  - **This can be a useful QC measure indicating how reliable the spot is – if this value is too low, the spot is likely very dim intensity.**
- **What is Circularity?**
  - **Circularity is a measure of roundness of a given feature (spot).**
  - **This may be particularly useful for flagging partial spots on an array spotted with round features.**

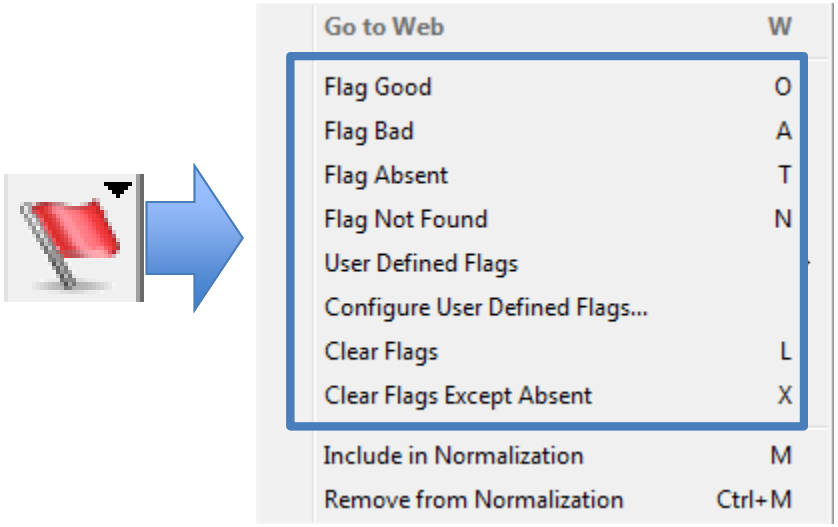
Navigating the Results Tab

Click on any of the column headers to sort the entire table by that data type.

Each line of data represents one feature (spot). If you select a line of data, you will see the spot image(s) appear in the Feature Viewer.



Flagging Data Points - Tools

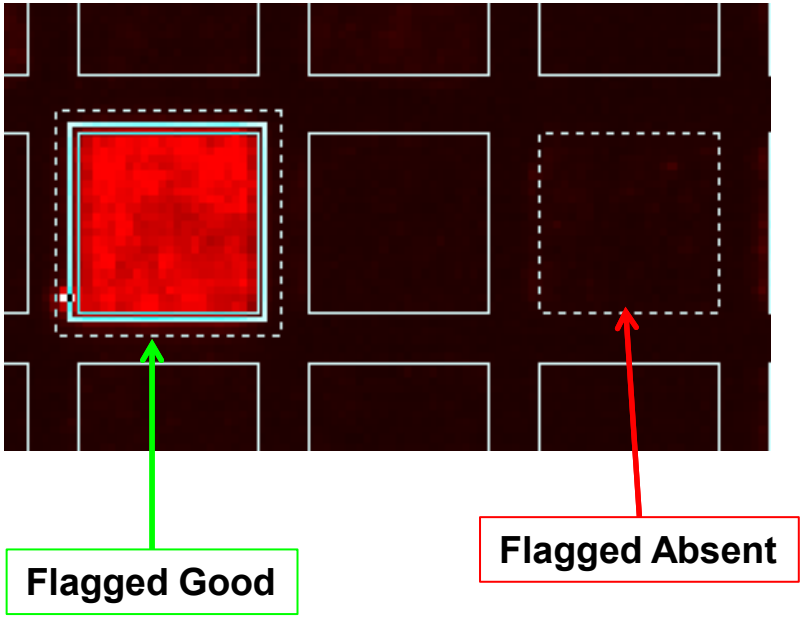


You can flag data points either pre-analysis in the Image tab or post analysis in the Results and Scatter Plot tabs using the tools available in the Feature Menu.

To access the flagging tools, click on the Feature button and choose an option from this menu.

You can opt to flag data as: good, bad, absent, not found, or create your own user-defined flags. (See next slides for more details).

Flagging Data Points – Image Tab



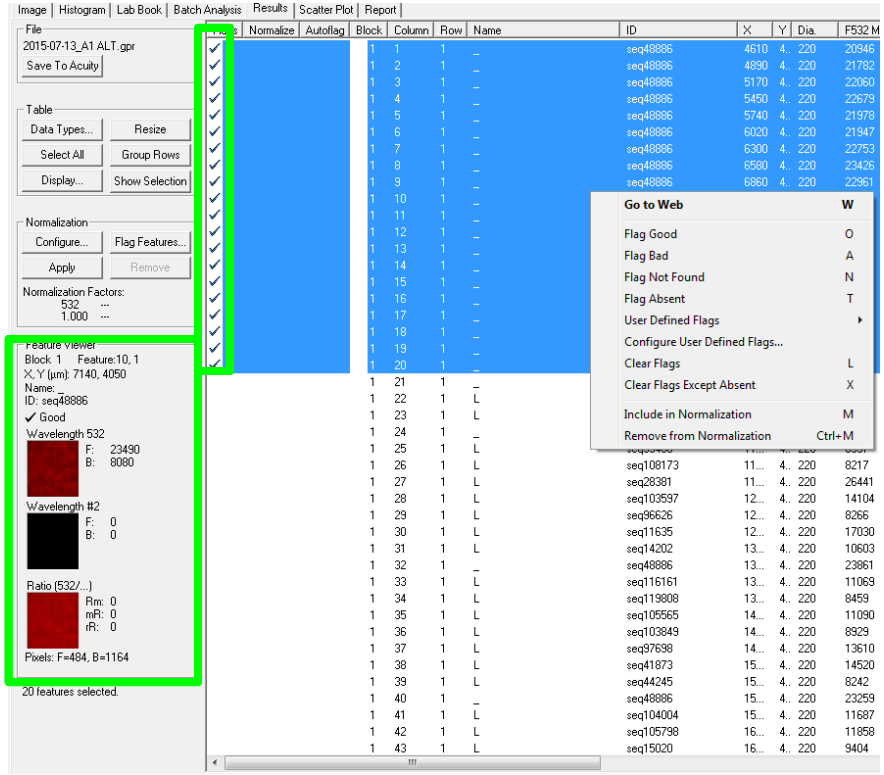
When in the Features Mode on the Image Tab, you can either use the Features button OR right click on a feature (spot) and access the flagging functions in that same menu.

Your selected feature(s) will then be flagged by a defined border as in the example on the left.

Flagging Data Points – Results Tab

On the Results tab you can Shift-click to select a subset of results to flag.

You can then either choose a flag from the Features menu either but clicking the button OR right clicking and accessing the menu while in the data window.



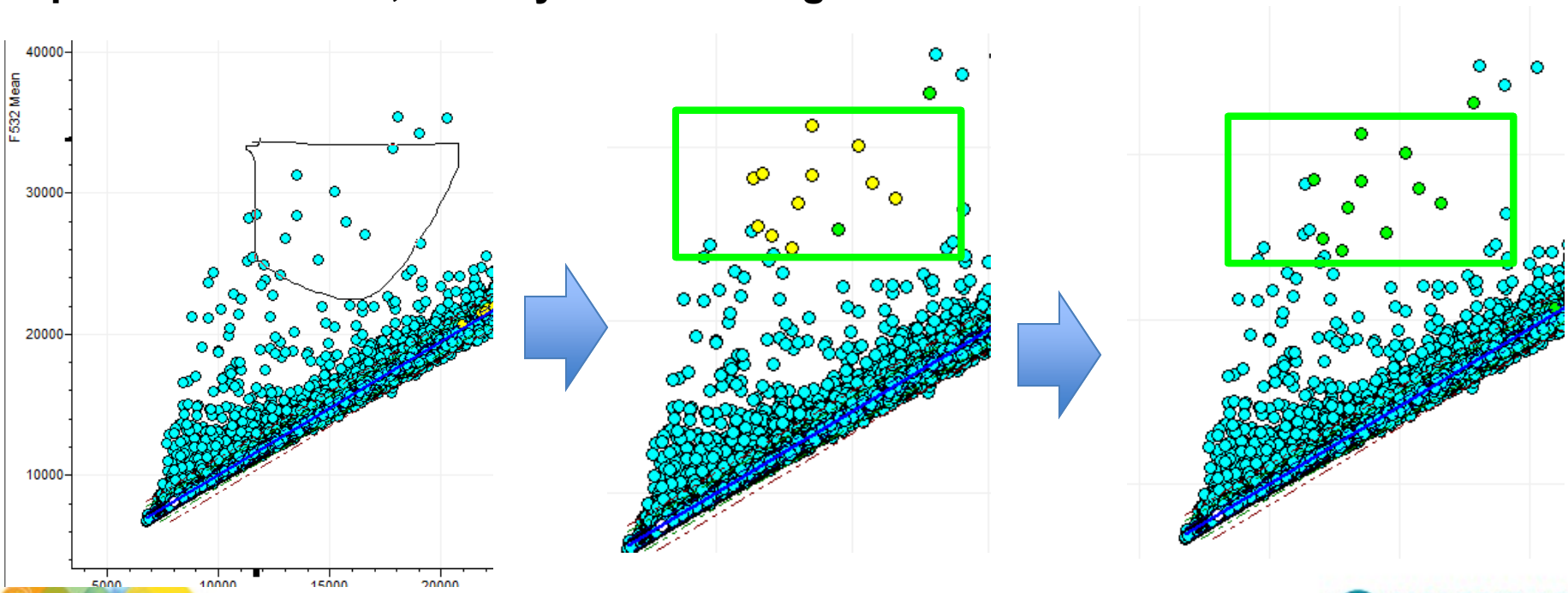
As in the example shown, you will see a check mark in the Flags data column for flagged data points.

You will also see the detailed flag ID in the Feature Viewer when you mouse over a line of data.

Flagging Data Points – Scatter Plot Tab

On the Scatter Plot tab you can drag a region around a selection of data points on the plot, then flag them by either clicking on the Feature button OR right clicking and choosing an option from the menu.

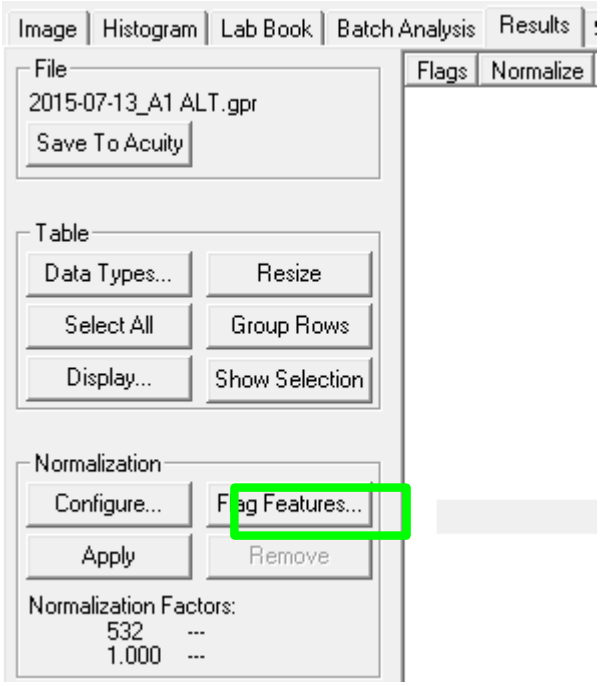
Selected points will appear yellow; once you've flagged them they will be color coded accordingly. In the example below, we've flagged a subset of points as "Good", so they are colored green.

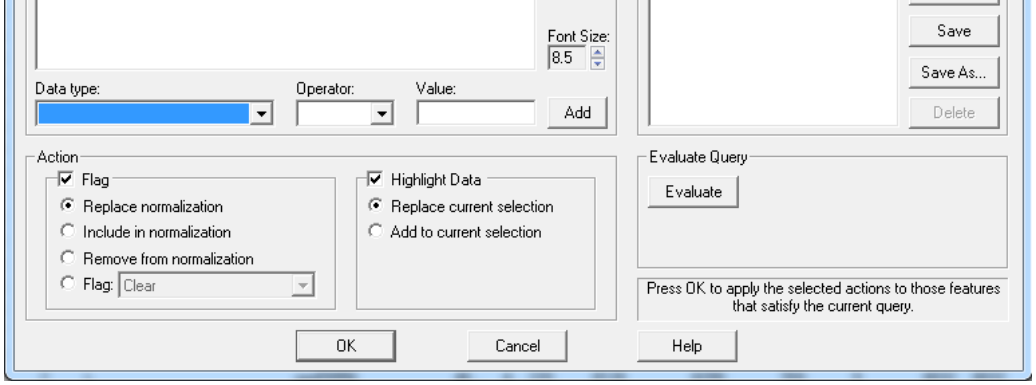


Flagging Data Points – Flag Features

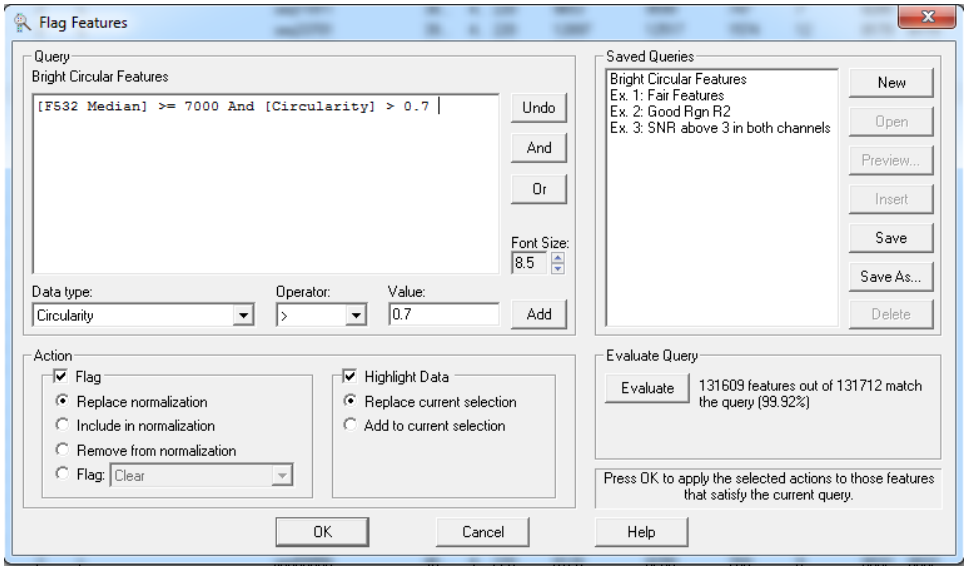
You have the option to create custom flags based on criteria you define, such as spot intensity, shape, etc.

To access this capability click on the Flag Features button in the Normalization field on the Results tab.





Flagging Data Points – Flag Features



Click on the Data type dropdown in the Flag Features dialog to select from available data filters.

Choose an Operator from the dropdown (such as >,<)and specify thresholds by populating the Value field.

Click the Add button to combine multiple filters with and/or operators.

Click the Evaluate button to preview the # of data points that fit your criteria.

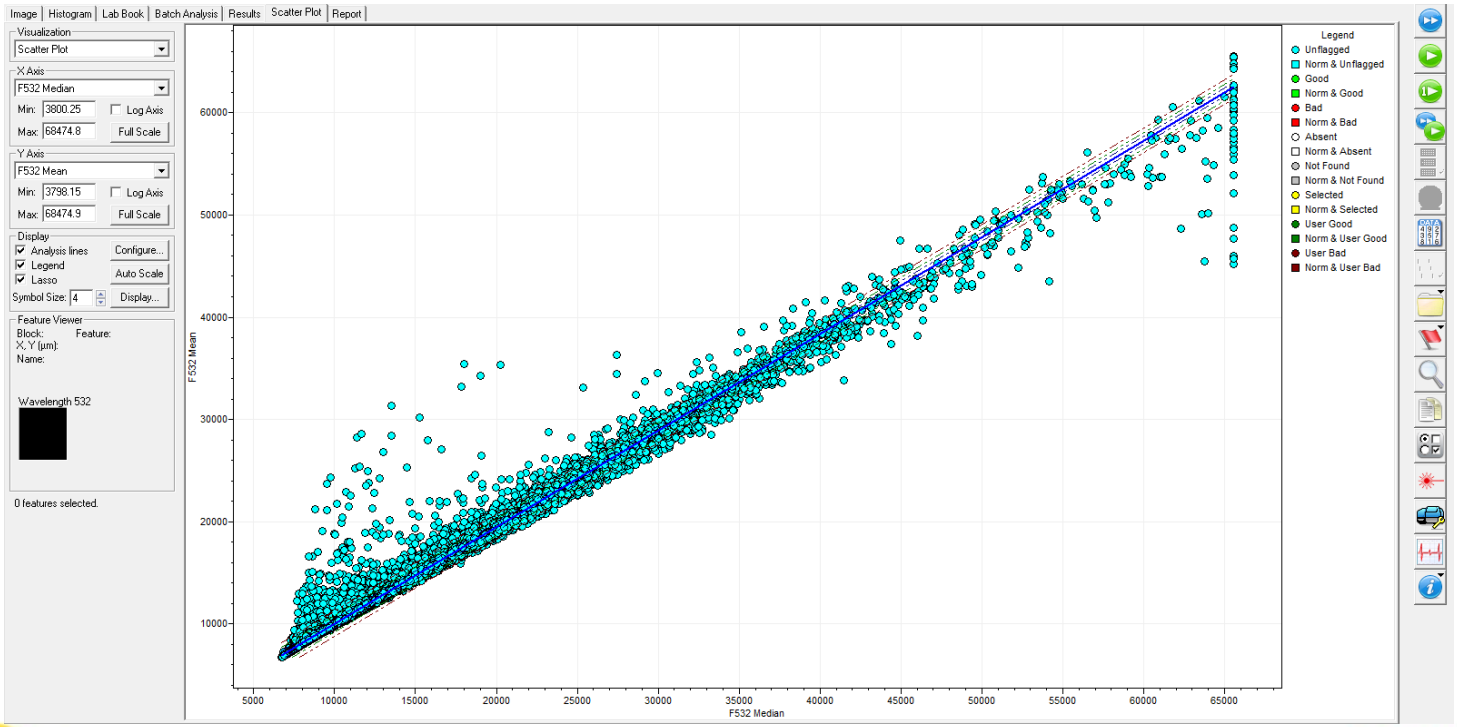
Click the Save As button to save your custom flag. It will now appear in the Saved Queries panel in the dialog.

Note that if you create this type of flag it will automatically be applied to the images/data that you have open at the time.

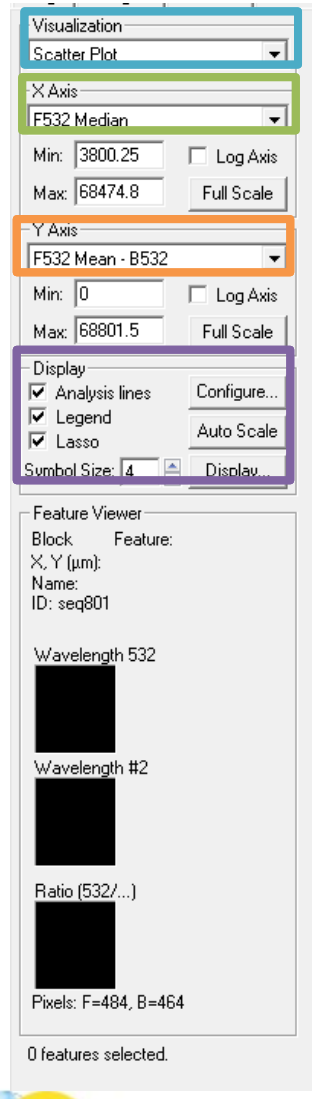
Configuring Scatter Plots

Scatter Plot Tab Overview

This tab contains a graphical view of data from an analyzed array. You can choose a scatter plot or histogram view, change graph axes, add regression lines, flag data points, etc. You can also access the feature viewer to review images of spots for selected data points.



Scatter Plot Tab Features



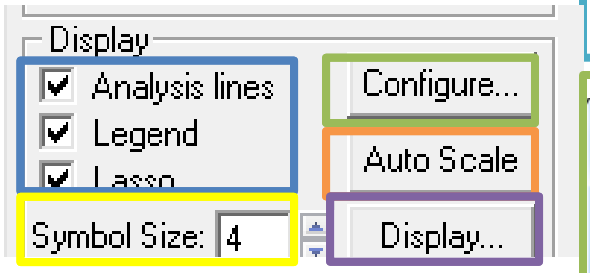
Visualization dropdown – click here to toggle between Scatter Plot and Histogram views of your data.

X Axis dropdown – click here to choose a value to plot on the X axis of your graph

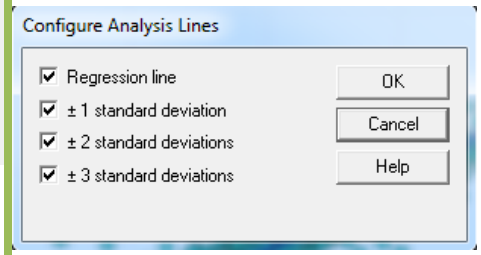
Y Axis dropdown – click here to choose a value to plot on the Y axis of your graph

Display section – Enable or disable display of analysis lines (regression), graph legend, and lasso tool for selection of data points (see next slide for more info)

Scatter Plot Tab Features - Display



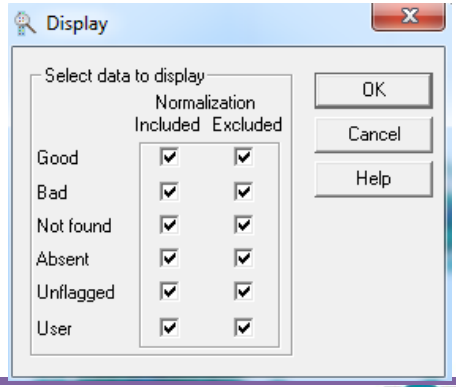
Toggle Checkboxes – put a check mark in any or all of these boxes to enable the listed options



Configure – Launches Configure Analysis Lines dialog; click to enable regression and SD lines.

Auto Scale – click this button to auto scale the graph to the full range of your displayed data

Display – Allows you to select normalized/flagged data points to display.



Symbol Size – changes the size of data point symbols on your plot

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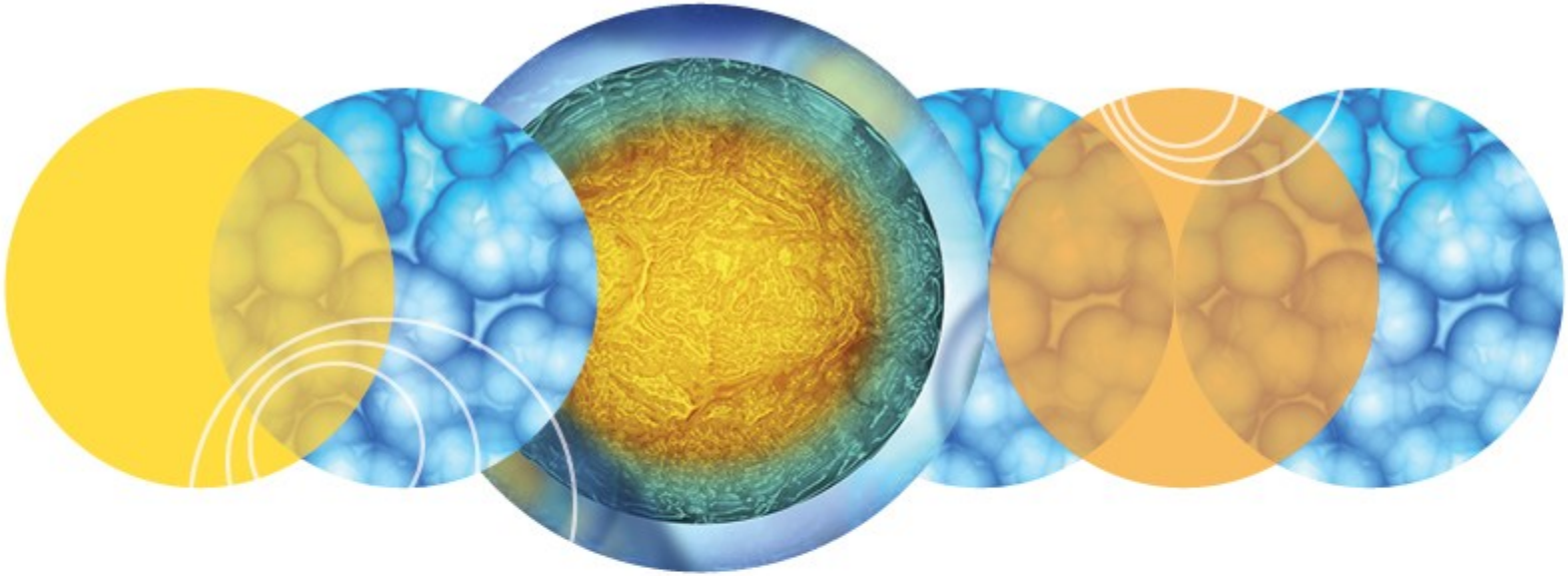
Thank you!



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