Image Extraction

Instruction Manual

Contents

1. Introduction 3
2. Setting up the Software 6
3. Performing a Batch Image Analysis 11
4. Performing a Manual Image Analysis 14
5. Troubleshooting 19

1. Introduction

The KREX Protein Array is a slide based high-density protein microarray based on Sengenics patented KREX™ protein folding technology [1]. The product enables highly multiplexed detection and relative quantification of autoantibodies circulating in human blood and is intended primarily for disease biomarker discovery. The array content comprises 1800+ immobilized, full-length, correctly folded human proteins. The proteins are immobilized on a proprietary, planar hydrogel surface supported by a glass slide. KREX™ technology [1] ensures that only correctly folded proteins are immobilized onto the surface and the aqueous environment of the hydrogel helps the proteins to maintain their native conformation. The arrayed proteins represent major protein classes such as protein kinases and transcription factors, signalling molecules as well as proteins acting at the extracellular environment, such as cytokines.

Each array image from your i-Ome® Discovery Protein Array experiment will be saved in a 16-bit TIFF format. An example of an image of the array is shown in Figure 1 below. Each spot on each array represents a protein or control probe immobilized on the array. Depending on the type of microarray scanner you are using, the amount of serum or plasma IgG bound to each protein on the array is typically represented in relative fluorescence units (RFU) or median fluorescence intensities (MFI). For the purpose of this manual the term relative fluorescence units (RFU) will be used.

To obtain the RFU for each spot on the array, you will need to analyse each TIFF image using a compatible image analysis software*. This process involves converting each pixel within each spot on the array image into numerical values, i.e. RFUs. A GenePix Array List (GAL) file will be required to perform the image analysis. The GAL file contains the names and positions of all the proteins and control probes on each array. Our Support Team would have provided you with the relevant GAL file. You may also download the latest i-Ome® Protein Array GAL file from *https://sengenics.com/resources/product-information/*.

References

[1] N. Beeton-Kempen et al., "Development of a novel, quantitative protein microarray platform for the multiplexed serological analysis of autoantibodies to cancer-testis antigens," Int J Cancer, vol. 135, pp. 1842–1851, 2014, doi: 10.1002/ijc.28832.

* We do not provide any microarray image analysis software with any of our products. We highly recommend using GenePix® Pro 7 Software (https://support.moleculardevices.com).

Table 1. Pre-requisite checklist

Description	Remarks
KREX Protein Array scanned images	Scanner output - Please ensure this is in TIFF (16 bit) format
KREX Protein Array GenePix Array List (GAL) file	You may download this from https://sengenics.com/resources/product-information/
GenePix® Pro 7 Software	Not provided by Sengenics

Glossary:

TIFF = Tagged Image File Format represents raster images that are meant for usage on a variety of devices that comply with this file format standard. The extensible feature of this format allows storage of multiple bitmap images having different pixel depths, which makes it advantageous for image storage needs. Since it introduces no compression artifacts, the file format is preferred over others for archiving intermediate files. The file extension for this file is '.tif'.

GAL = GenePix Array List file extension is used by GenePix. It is a biological data format in the form of an array list. Microarray technology is capable of extracting fluorescence measurements from thousands of features at once. It is necessary that each feature has the appropriate ID and name assigned for carrying out accurate analysis. It aids in the description of the size and position of blocks, layout of feature-indicators consisted within, and the name and IDs of the printed substances linked with the respective feature-indicators. The names and locations (block, row, and column) of features existing on a microarray slide are saved in the GAL file. This data is useful in quantitative analysis of the fluorescent spots on microarrays. The file extension for this file is '. gal'.

GPR = GenePix Results data are saved as GPR files, which are in Axon Text File (ATF) format. A Results file contains general information about image acquisition and analysis, as well as the data extracted from each individual feature. The GPR file is primarily associated with the GenePix Pro that is used for analysis of biological experiments. The data gathered by biological experiment is saved in GPR files for further analysis. The data processed during analysis is also stored in GPR files along with results of experiment conducted by GenePix Microarray Systems. The file extension for this file is '.gpr'.

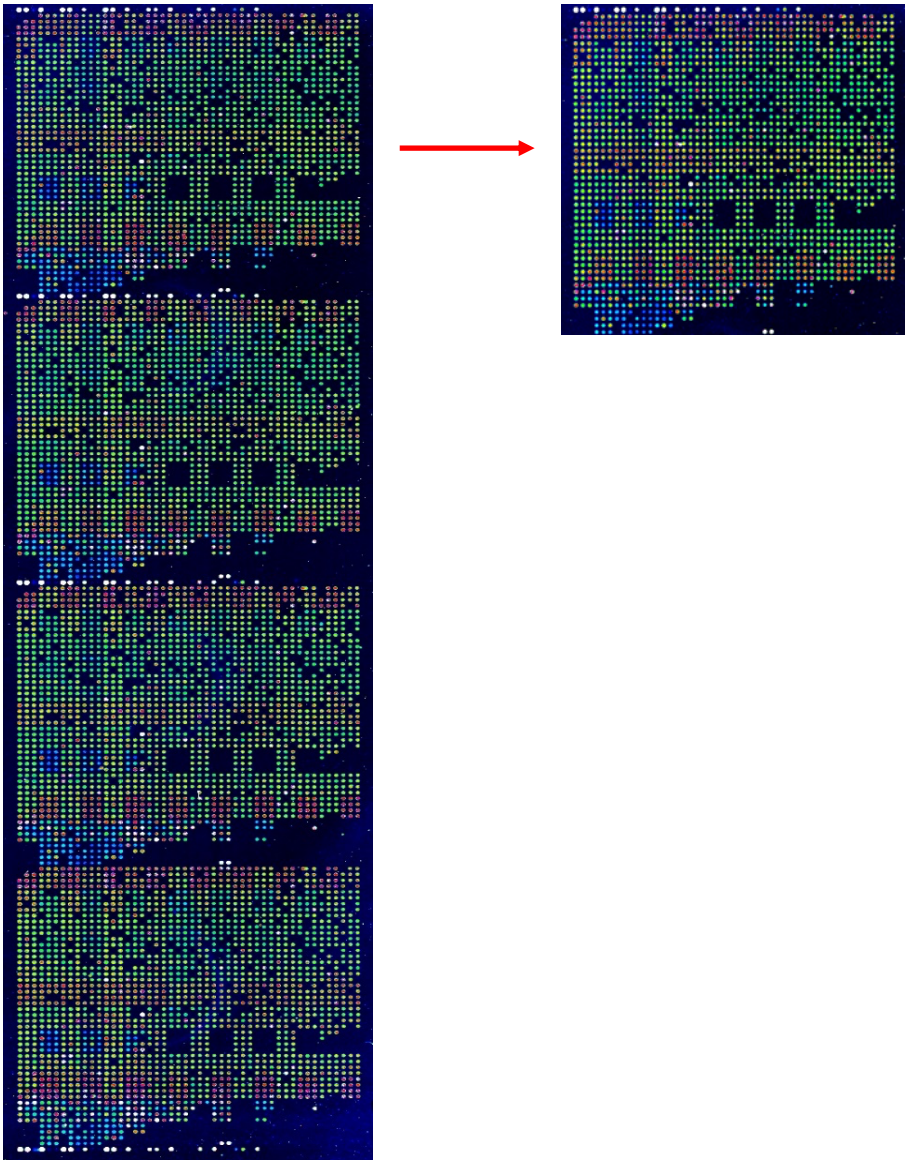


Figure 1. An example of an image of the i-Ome® Discovery Protein Array

References

1. Beeton-Kempen, N., Duarte, J., Shoko, A., Serufuri, J.-M., John, T., Cebon, J., & Blackburn, J. (2014). Development of a novel, quantitative protein microarray platform for the multiplexed serological analysis of autoantibodies to cancer-testis antigens. International Journal of Cancer, 135, 1842–1851

2. Setting up the Software

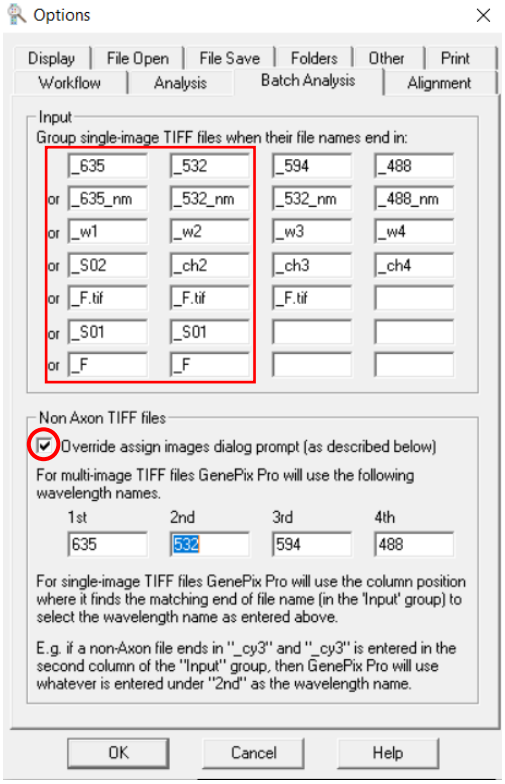
This manual instruction is based on i-Ome Discovery Array. Other KREX Protein Array would apply similar to this protocol. The GenePix® Pro 7 Software will allow you to perform microarray image analysis either manually or in batches of up to 1000 TIFF files. A manual extraction only allows you to analyze one image at one time and this will require a manual input from the user for each step of the process. A batch extraction will allow you to automatically analyze multiple images in one process. To perform the image analysis for the i-Ome® Discovery Protein Arrays, please follow the recommended settings below:

1) Launch the software:
   i. Plug the GenePix® Pro dongle into your computer. This is provided with the purchase of GenePix® Pro 7 Software.
   ii. Launch GenePix® Pro 7 Software.

2) To access the setting functions, click the 'Options' icon on the right-hand side of the main window.

3) Automatically set the wavelength name for batch analysis: Typically, a scanned array or slide will have the following name format: '***scannerID_SlideID_ScanNo.tiff***'. Click on the 'Batch Analysis' tab and make sure that the suffixes of image file names (_S01, _F.tif, _532, _635) are added into the 'Input' boxes under the column corresponding to user's scanner wavelength setting.

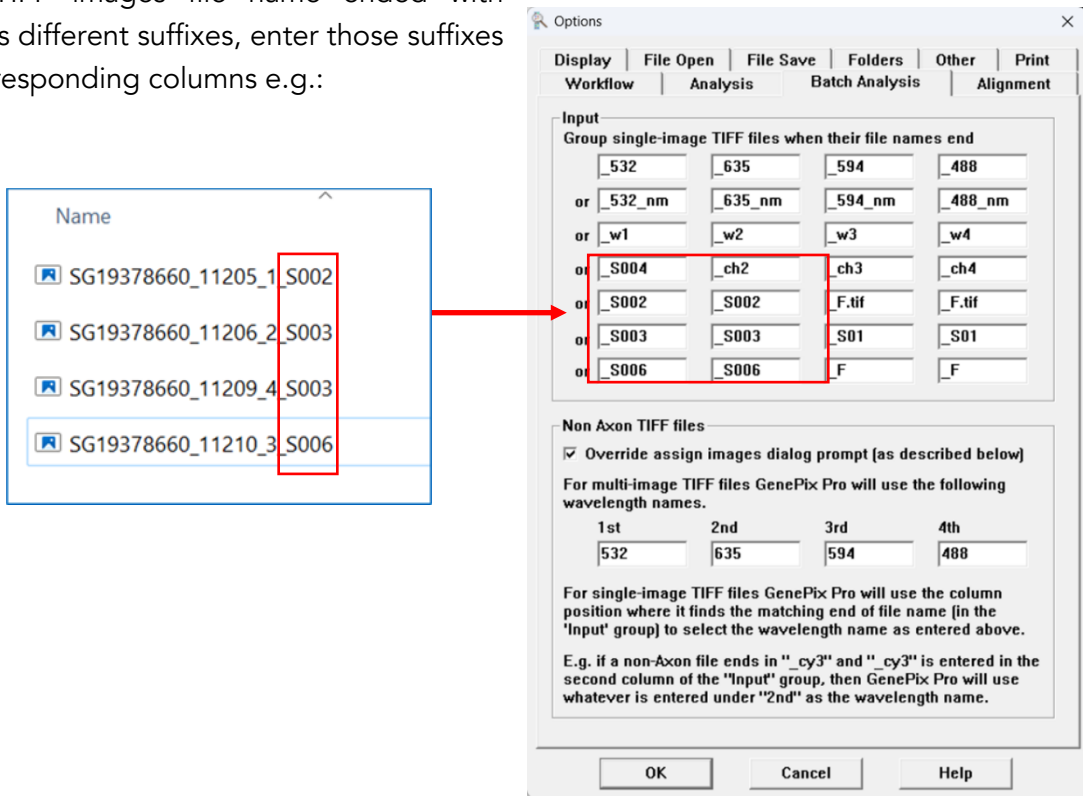
4) By default, Genepix read the suffix of image as scan 01 (double digit number), of which a suffix will be labelled as 'S01'. When a slide is scanned using Agilent G4900D, this suffix is standardised with the Genepix format. However, different scanner may indicate a different suffix. I this instance, the section in input 4 (image on the right, red box) needs to be amended accordingly, and this should be applied to each wavelength, if the slides was processed with dual colour assay.



For example, in a slide processed using dual colour assay, if the file name ends with '_S001.tiff', change the information in input 4'_S01' to '_S001'. Enter '635' in the first column followed by '532'. If the file name ends with '_S001.tiff', change '_S01' to 'S001'. Make sure that the 'Override assign image dialog prompt' check box is ticked. Click 'OK' to save the settings.

NB: Our lab protocol uses AF55-equivalent and AF647-equivalent fluorescence, corresponding to wavelengths of 635 nm and 532 nm respectively.

If the TIFF images file name ended with multiples different suffixes, enter those suffixes into corresponding columns e.g.:



5) Setting up the spot alignment (recommended): Under the 'Alignment' tab:

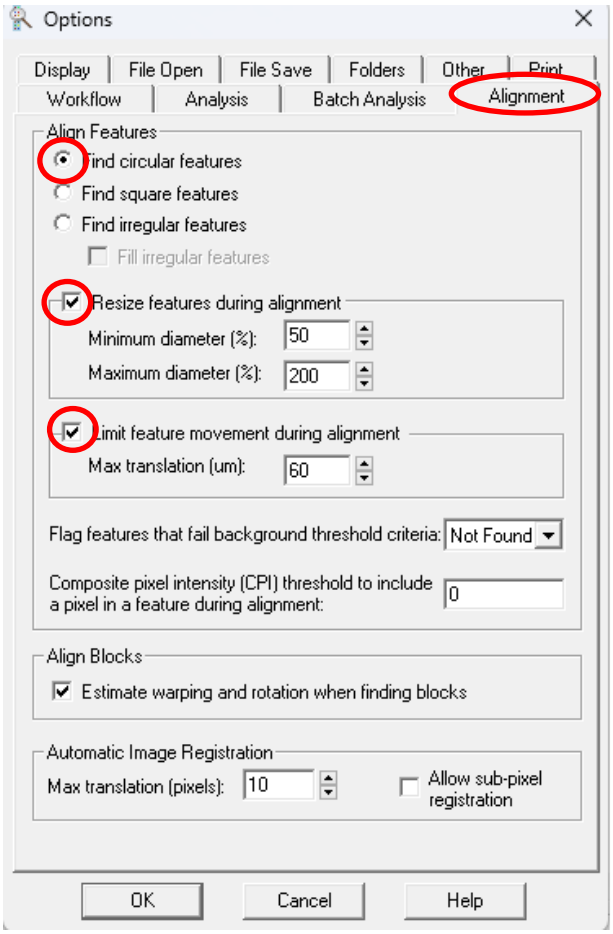
   i. Select 'Align Features/Find circular features',

   ii. Select 'Resize features during alignment'. Set the minimum and maximum diameter % to 50 and 200 respectively.

   iii. Select 'Limit feature movement during alignment'. Set the 'Max translation' to 60.

   iv. Select 'Align Blocks/Estimate warping and rotation when finding blocks'.

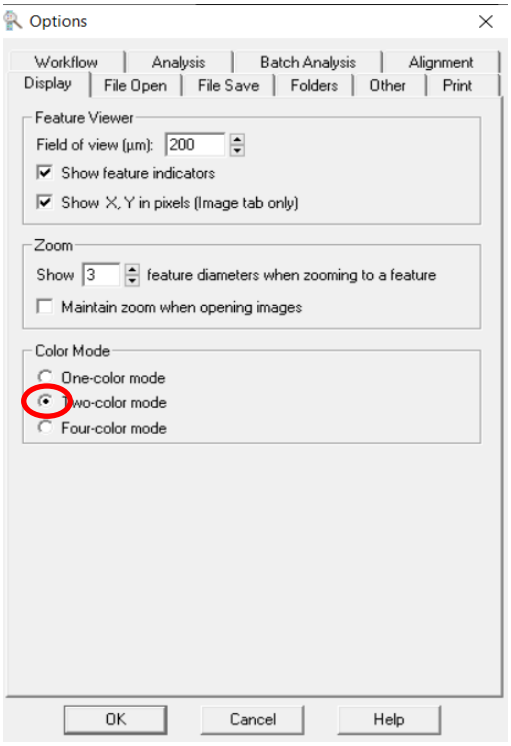
   v. Click 'OK'.



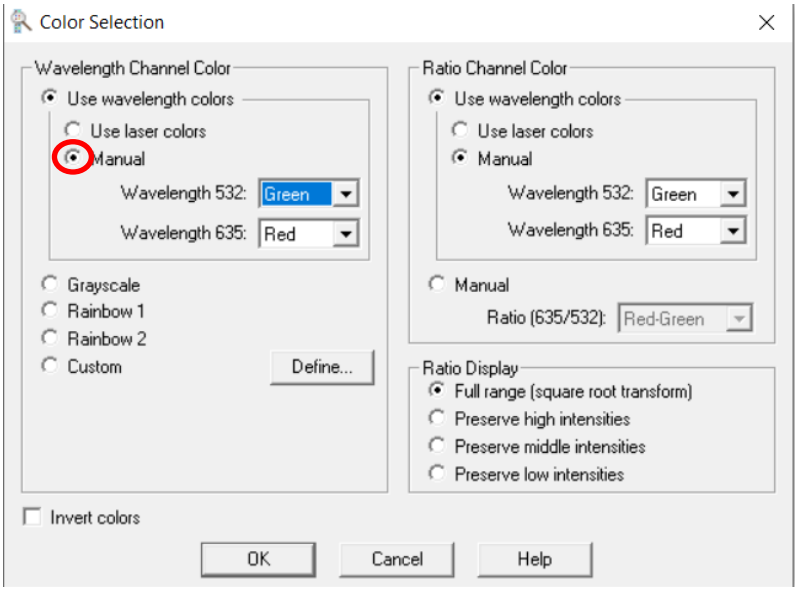
6) Setting up the image display (recommended):

i. Under the 'Display' tab, select 'Colour Mode/Two-color mode' and click OK.

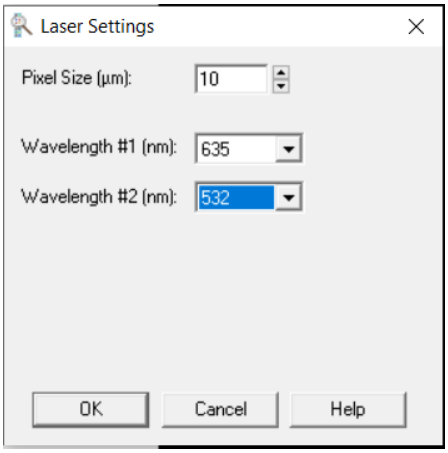
ii. Please re-start the software after changing the colour mode to reboot the software.



iii. Select the 'Color Selection' icon. Click 'Use wavelength colors' and the 'Manual' option, followed by selecting the color 'Green' for 532 and 'Red' for 635. Click 'OK'.



iv. Next, click the 'Laser Settings' icon and input '635' for 'Wavelength #1' and '532' for 'Wavelength #2' as shown in the image below. Click 'OK'

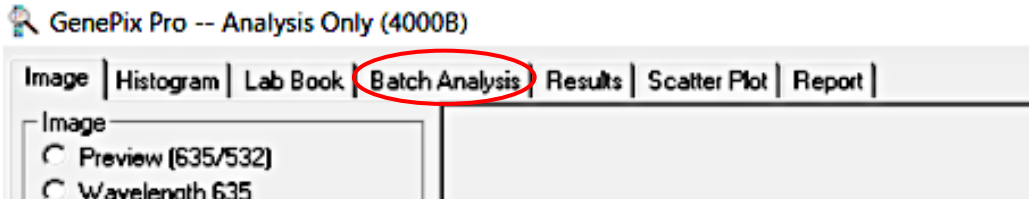


NB: The default setting for GenePix® Pro 7 Software will output the GPR files to include all the information required for you to do your downstream data processing and analysis. When using the software for the first time, we would advise to check that the following columns are available in your GPR files:

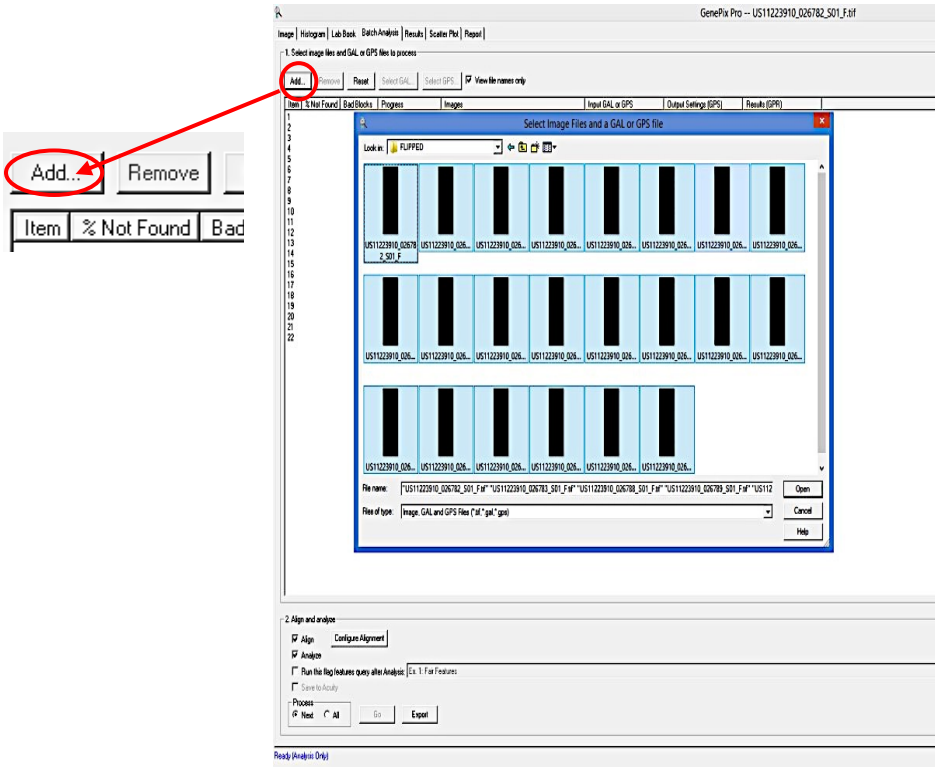
- **GeneSymbol** – this will contain the feature identifier (i.e. names of all the antigens and control probes on the array)
- **Block** – this corresponds to the block number(s) on the array.
- **F532 Median** – this corresponds to the foreground median RFU of each spot on the array
- **B532 Median** – this corresponds to the background median RFU of each spot on the array. Maintain the default setting for the background calculation as 'Local feature background median'.

3. Performing a Batch Image Analysis

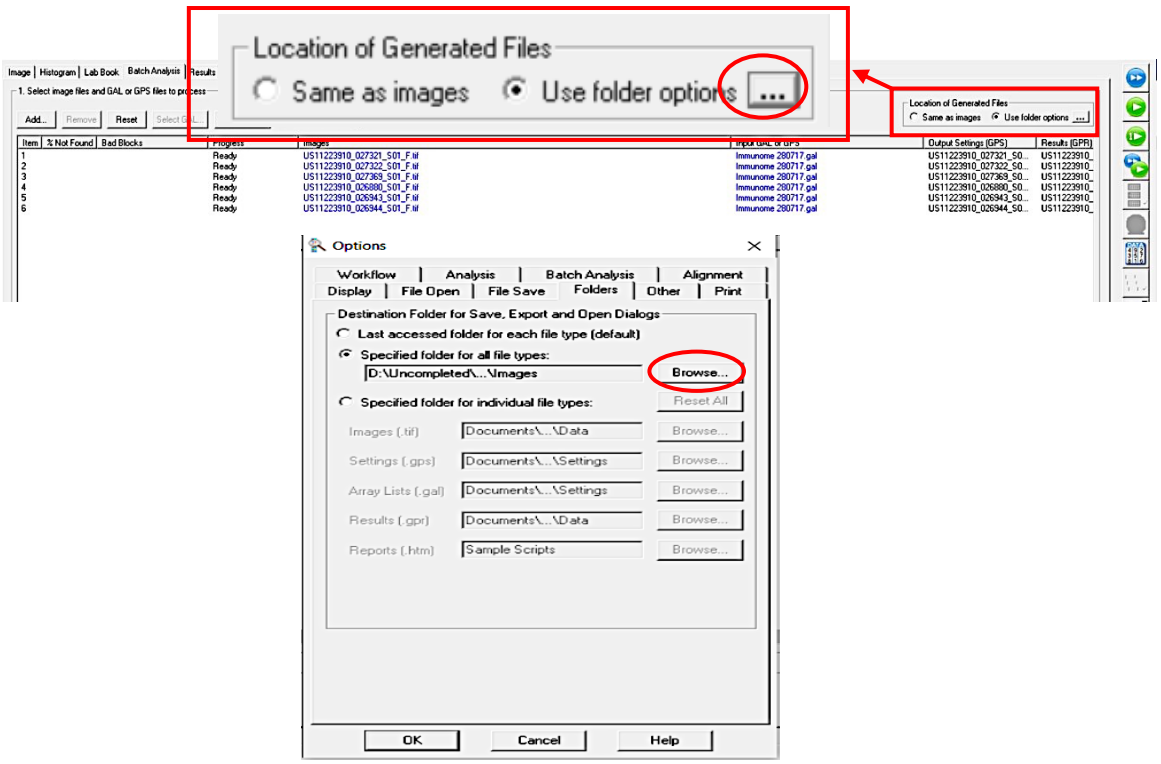
1) To upload your images, click on the 'Batch Analysis' tab.



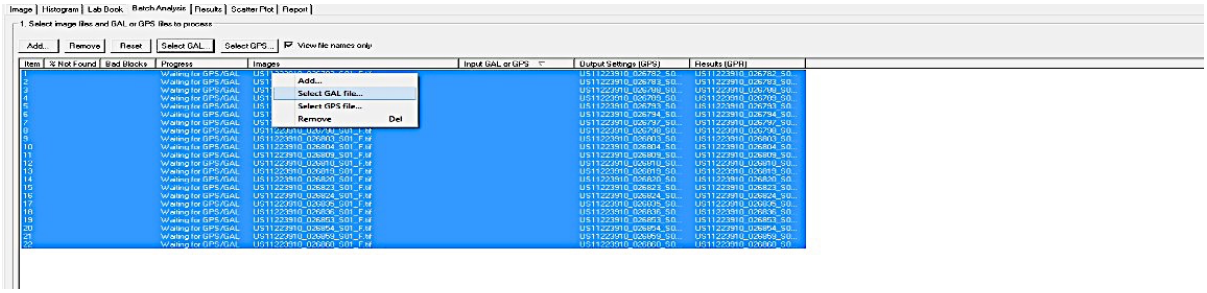
2) Click the 'Add' button and navigate to the folder where your TIFF images are stored. Select the TIFF images (you can select multiple images at one time) you wish to analyze and click 'Open'.



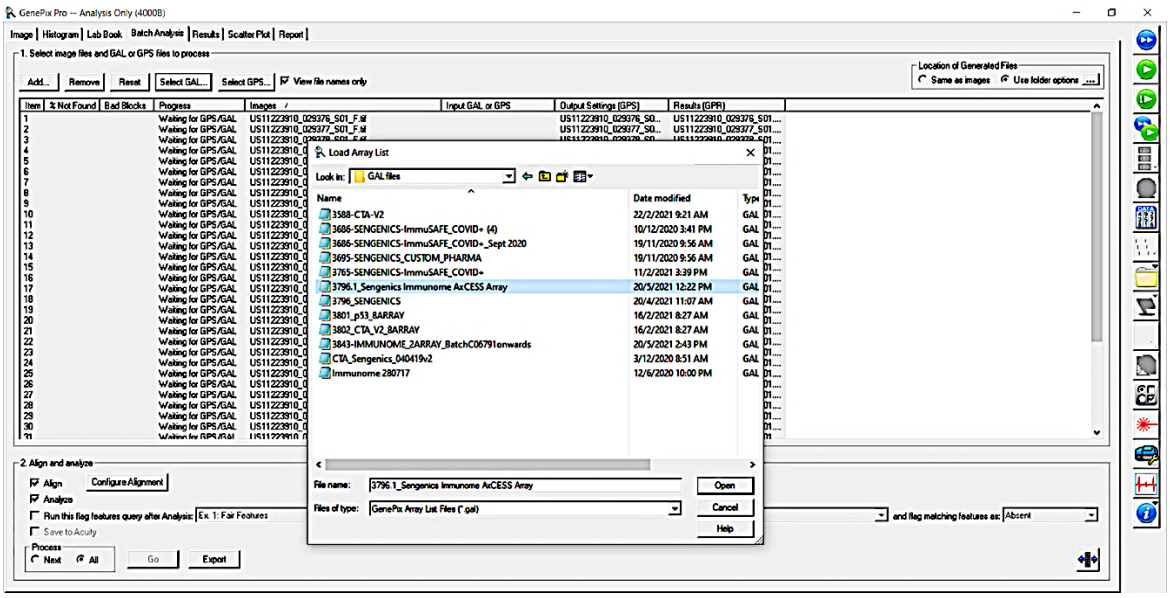
3) Users need to set up the location of the output files from the batch analysis. Recommendations: Create a folder named 'GenePix Output' under the same path as the location of your TIFF images. Select 'Use folder options' under 'Location of Generated Files'. Click the 3 dots (Options) button to select the location where the output files will be saved. Select 'Browse' to navigate to the location of the folder created and click 'OK'.



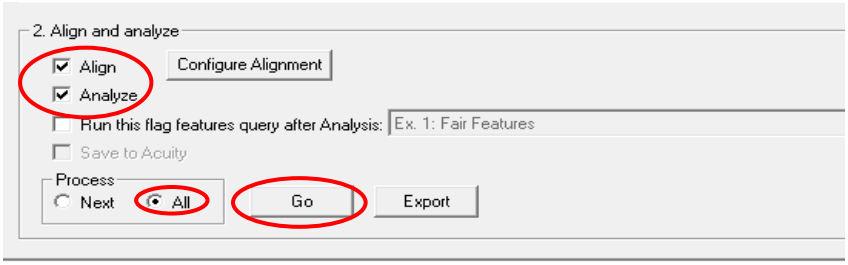
4) Upload the GAL file: Select all of the uploaded TIFF images (you may use the CTRL+A command to do this). Click 'Select GAL file…' on the top of the panel or right click on your mouse and click 'Select GAL file…' and navigate to the location of your GAL file.



5) Select the latest i-Ome® Discovery GAL file and click 'Open'.



6) Set your analysis parameters: At the bottom of the window, under '2. Align and analyze', select 'Align', 'Analyze' and select 'All' in the Process box.

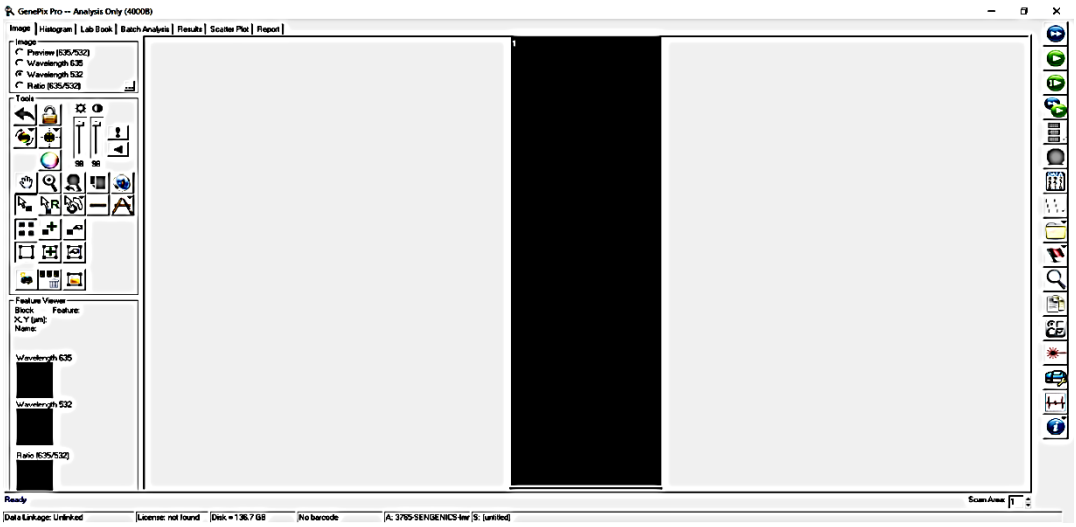


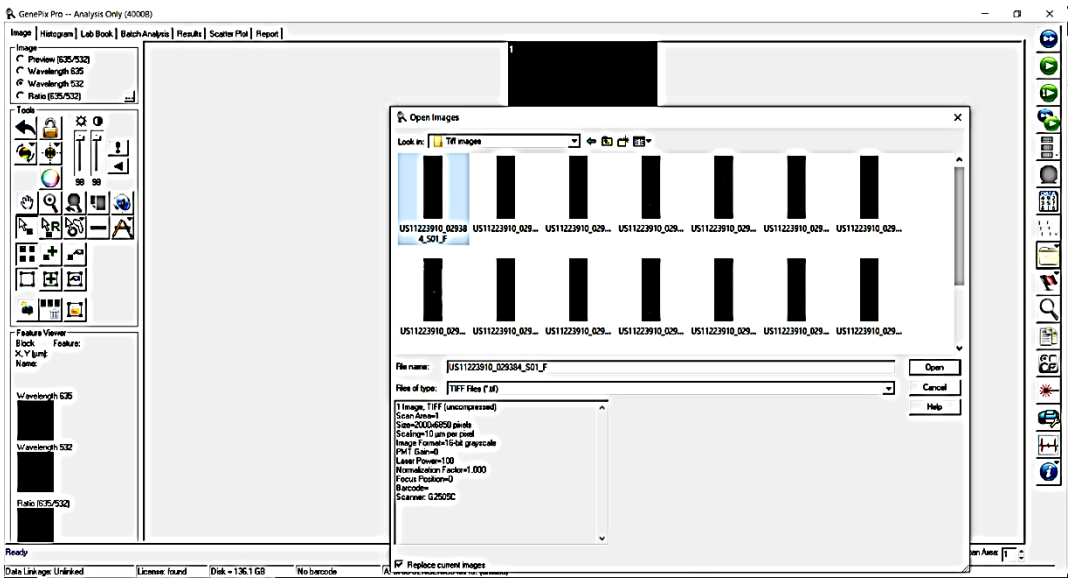
7) Click 'Go' to initiate the batch analysis. There are 3 output files that will be generated from the image analysis; GPR (GenePix Results containing numerical values from the extracted images), GPS (GenePix Settings file) and JPEG (the image file of the tiff images) files. Only the GPR files will be used for downstream processing and analysis. Each set of output files will have the same name as the TIFF images.

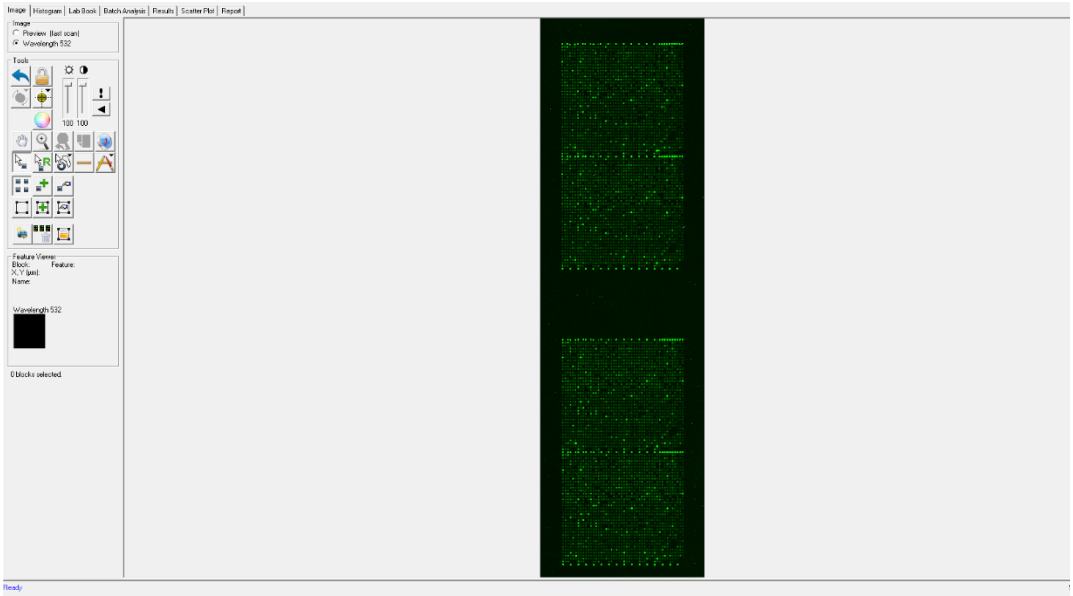
4. Performing a Manual Image Analysis

1) Repeat the steps outlined in section 3.3) above.

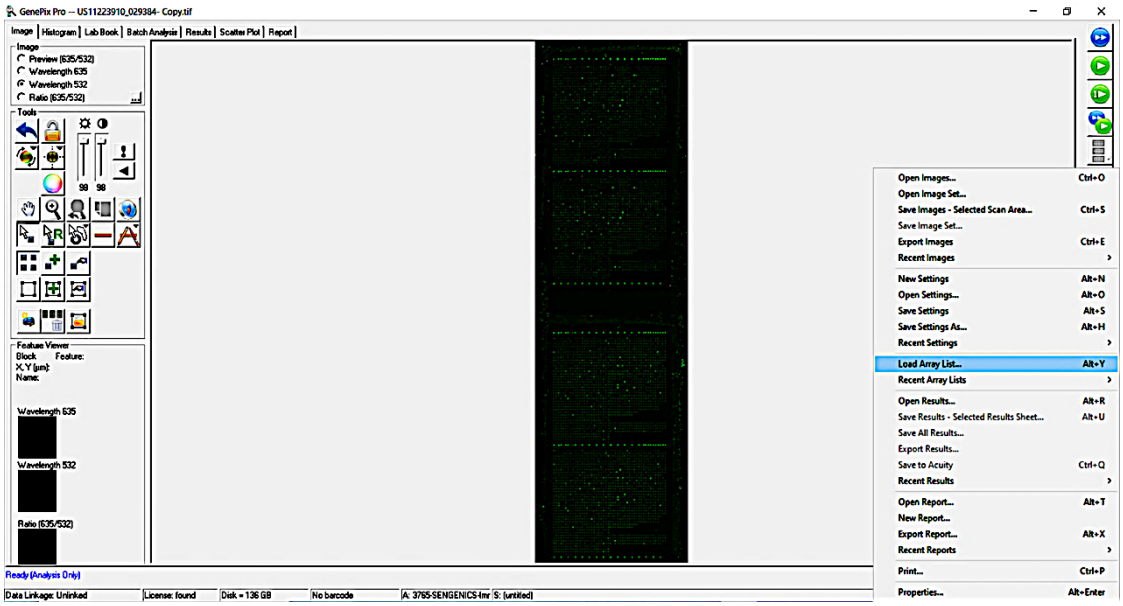
2) Upload your TIFF image: Click on the file icon and click 'Open Images…'. Navigate to the folder where your TIFF image is stored. Select the image to analyze. Only one TIFF image can be analyzed at a time.



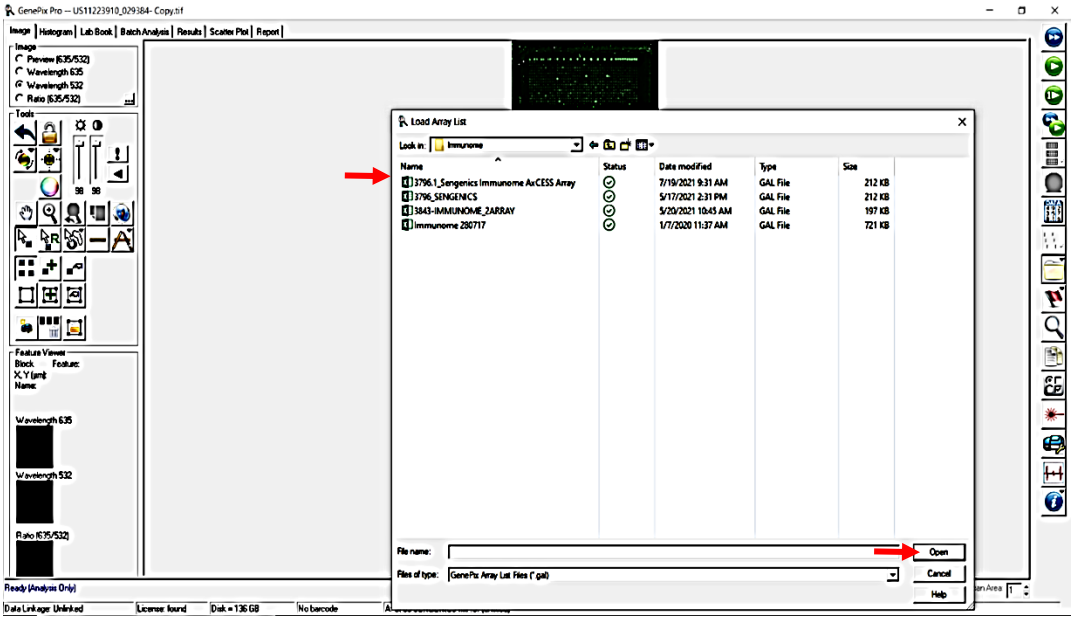

GenePix Pro -- Analysis Only (40008)
Image | Histogram | Lab Book | Batch Analysis | Results | Scatter Plot | Report
Open Images
Look in: Tif images
File name: US11223910_029384_S01_F
Files of type: TIFF Files (*.tif)
Open
Cancel
Help
Replace current images
Ready



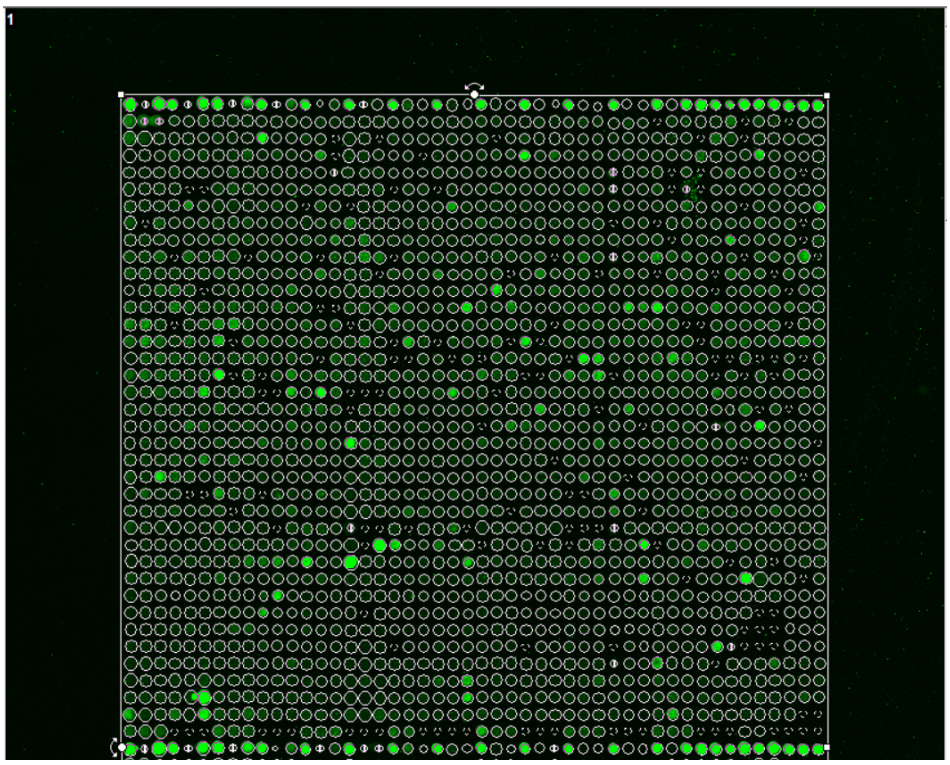
3) Upload the GAL file: Click on the file icon again and select 'Load Array List' to browse to the location of the GAL file.



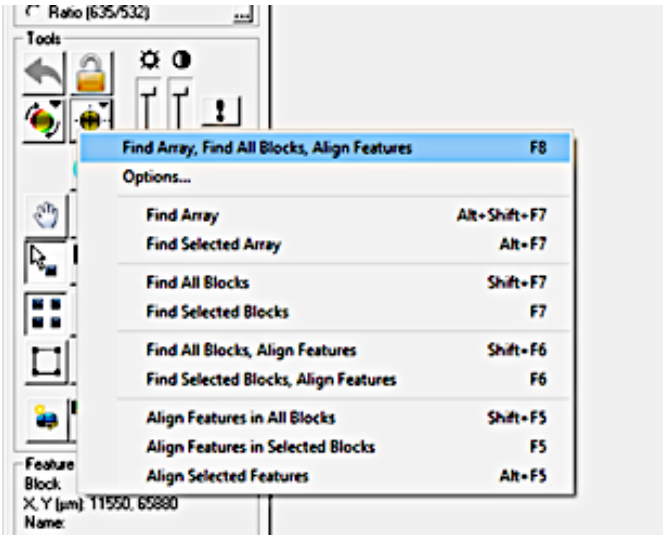
4) Select the GAL file and click 'Open'.



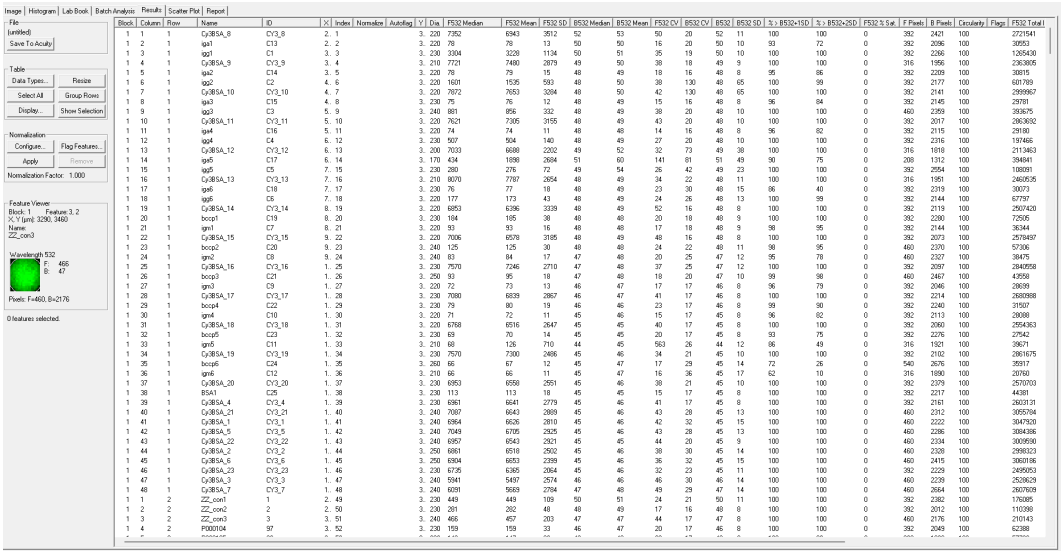
5) Align the GAL file correctly to each spot on the TIFF image.



6) For auto alignment, click the 'Align blocks' icon. Select 'Find Array, Find All Blocks, Align Features' (or press 'F8') to enable auto alignment.



7) Click on the 'Analyze' icon to perform the analysis. The subsequent computations are sent to the Results tab (indicated by the red circle.)



8) Click the file icon and select 'Save Results - Selected Results Sheets' to save the GPR and JPEG files. The GPR, JPEG and TIFF file names must match. Select 'Save a JPEG Image containing all analyzed features' check box and then click 'Save'.

5. Troubleshooting

The software is unable to load the TIFF image	Ensure that the TIFF images were generated and saved in a 16-bit format. You may need to re-scan the slides.
The GAL file does not fit scan area of the TIFF image	You may need to re-scan the slides at the correct scanner setting, i.e. resolution at 10 µm.

Contact Information

Sengenics Corporation

Technical support email: support@sengenics.com
www.sengenics.com