

# i-Ome<sup>®</sup> Protein Array Image Analysis

**Instruction Manual** 



## Contents

1.	Introduction	3
2.	Setting up the Software	6
3.	Performing a Batch Image Analysis	10
4.	Performing a Manual Image Analysis	13
5.	Troubleshooting	18



#### **1. Introduction**

The i-Ome® Protein Array is a slide based high-density protein microarray based on Sengenics patented KREX<sup>™</sup> 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 1600+ immobilized, full-length, correctly folded human proteins. The proteins are immobilized on a proprietary, planar hydrogel surface supported by a glass slide. KREX<sup>™</sup> 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® 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/.* 

\* 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
i-Ome® Protein Array scanned images	Scanner output - Please ensure this is in TIFF (16 bit) format
i-Ome® 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:

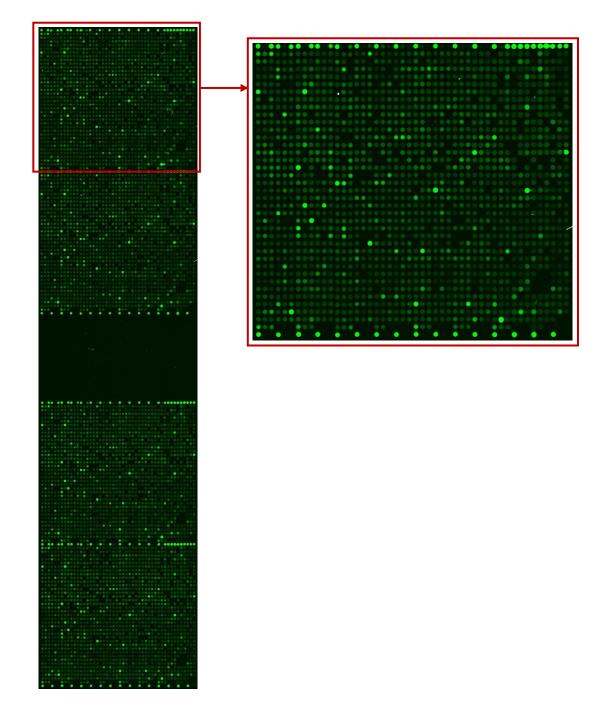
**TIFF** = Tagged Image File Format represents raster images that are meant for usage on a variety of devices that comply with this file format standard. The extensible feature of this format allows storage of multiple bitmap images having different pixel depths, which makes it advantageous for image 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® Protein Array



#### References

 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

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 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.
- 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 suffix of your image file names are added into the "Input" boxes under the column corresponding to your scanner's wavelength setting. Make sure that the "Override assign image dialog prompt" check box is ticked. Click "OK" to save your settings.

In the example image on the right, if your file name ends with "\_S001.tiff", and since "\_S001" is entered into the first column of the Input group, the software will use the wavelength name under "1st", i.e. "532".

*NB: Our lab protocol uses Cy3-fluorescence, corresponding to a wavelength of 532nm.* 

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or _635_nm	_594_nm	_532_nm	_488_nm
or _w1	_w2	_w3	_w4
or _ch1	_ch2	_ch3	_ch4
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- 4) 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 40.
  - iv. Select "Align Blocks/Estimate warping and rotation when finding blocks".
  - v. Click "OK".

🔹 Options 🛛 🕹
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Limit feature movement during alignment Max translation (um): 40
Flag features that fail background threshold criteria: Not Found  Composite pixel intensity (CPI) threshold to include a pixel in a feature during alignment:
Align Blocks
Automatic Image Registration Max translation (pixels): 10 - Allow sub-pixel registration
OK Cancel Help



- 5) Setting up the image display (recommended):
  - i. Under the "Display" tab, select "Colour Mode/One-color mode" and click OK.

R Options ×
Workflow Analysis Batch Analysis Alignment Display File Open File Save Folders Other Print
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Maintain zoom when opening images  Color Mode  One-color mode  Two-color mode  Four-color mode
OK Cancel Help

ii. Select the "Color Selection" icon. Click "Use wavelength colors" and the "Manual" option, followed by selecting the color "Green" and click "OK".

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C Custom	Define
Invert colors	
OK Cancel	Help



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

R Laser Settings	$\times$
Pixel Size (μm):	
Wavelength #1 (nm): 532	
Wavelength #2 (nm): 532	
OK Cancel He	p

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".

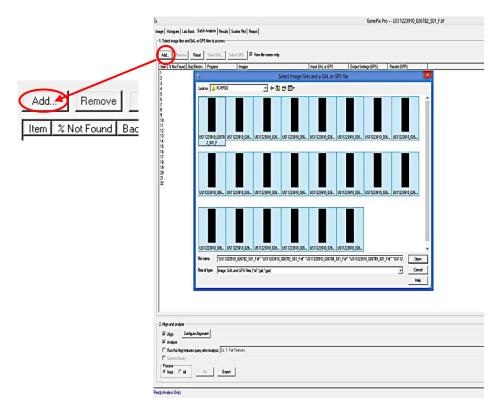


## 3. Performing a Batch Image Analysis

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

🕆 GenePix Pro Analysis Only (4000	B)
Image Histogram Lab Book Batch	Analysis Results Scatter Plot Report
C Preview (635/532) C Wavelength 635	

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) You will 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".

Image   Hatogram   Lab Book   Batch Anstyris   Results - 1. Select image Res and GAL or GPS Res to process	cation of Generated Files Same as images 💿 Use fol	Input GAL OF GPS	Location of Generated Fies           C Same as images         © Use lotter options
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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.

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5) Select the GAL file and click "Open".

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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.

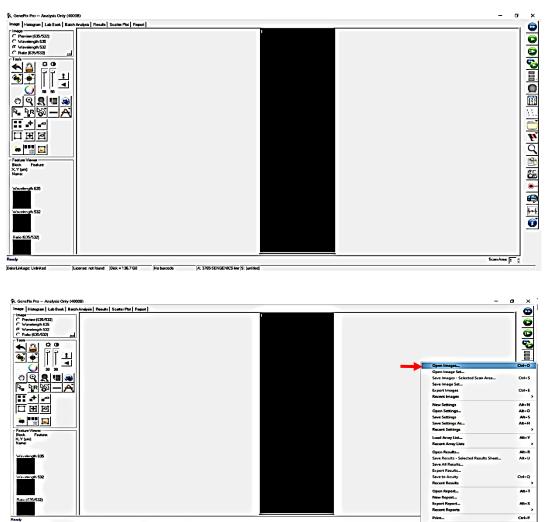
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7) Click "Go" to initiate the batch analysis. There are 3 output files that will be generated from the image analysis; GPR (GenePix Results containing numerical values from the extracted images), GPS (GenePix Settings file) and JPEG (the image file 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.

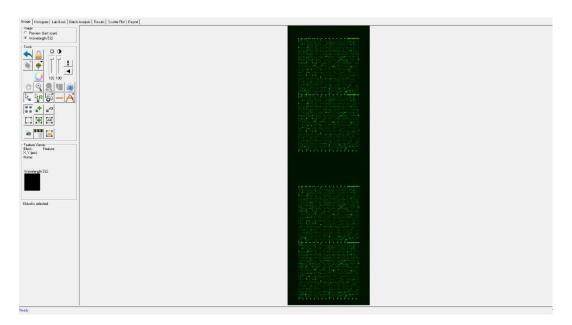


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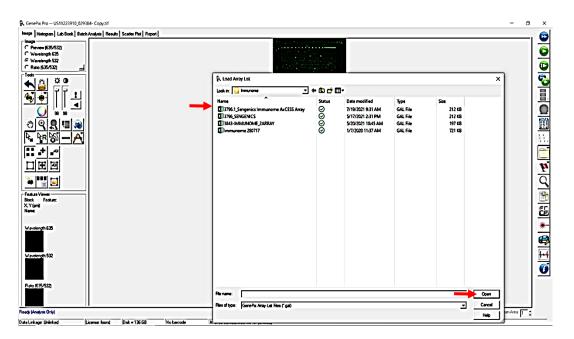




3) Upload your GAL file: Click on the file icon again and select "Load Array List" to browse to the location of the GAL file.

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





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

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

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

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         Cr20         Cr20           S         Cr20	$\label{eq:constraints} \begin{array}{ c c c c c } \hline X &   \mbox{ mode } mbox{ mod } mbox$	Audely         V         Dis         Fib         Media           3         30         76         3         30         76           3         30         76         3         30         76           3         20         77         3         20         76           3         20         76         3         20         76           3         20         76         3         20         76           3         20         76         3         20         76           3         20         76         3         20         76           3         20         76         3         20         76           3         20         76         3         20         76           3         20         77         64         3         20         76           3         20         76         3         20         76         3         20         76           3         20       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        017      0	Pass 50         Pass 50         Pass 50           3012         50         50           3013         50         50           3013         50         50           3013         50         50           3013         40         40           3014         50         40           3015         40         40           3016         40         40           3017         40         40           40         40         40           40         40         40           41         40         40           40         40         40           40         40         40           41         40         40           41         40         40           41         40         40           41         40         40           101         40         40           102         40         40           111         41         40           112         40         40           113         40         40           114         40         40           115	desit         8132 (M           0         30      <	50 2 16 2 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2020 /</th> <th>B Paule         Catala           10         10           10</th> <th>J. Page         PS32 1.544           2027541         2027541           2027541         2027541           202605         20057           202757         2027541           202757         2027541           202757         2027541           202757         2027541           202757         202757           202757         202757           202757         202757           202757         20275           20277         20275           20244         20249           20249         2049           20249         2049           20249         2049           20240         2049           20240         2049           20241         2049           20241         2049           20241         2049           20402         20417           20417         20417           20418         20417           20419         20417           20419         20417           20417         20417           20417         20417           20417         20417           20417         20417</th>	2215 / Freedom 2020 /	B Paule         Catala           10         10           10	J. Page         PS32 1.544           2027541         2027541           2027541         2027541           202605         20057           202757         2027541           202757         2027541           202757         2027541           202757         2027541           202757         202757           202757         202757           202757         202757           202757         20275           20277         20275           20244         20249           20249         2049           20249         2049           20249         2049           20240         2049           20240         2049           20241         2049           20241         2049           20241         2049           20402         20417           20417         20417           20418         20417           20419         20417           20419         20417           20417         20417           20417         20417           20417         20417           20417         20417

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 16bit 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 10µm.

#### **Contact Information**

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