# KREX Protein Array

# Image Scanning Settings For 1- and 4-plex Array Format

## Instruction Manual

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## 1. Introduction

The KREX-based 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 ranges of antigens between 100+ and 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 KREX Protein Array experiment will be saved in a 16-bit TIFF format. 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. 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. Settings Procedure

### 2.1 Quick Guide

**Setting up slides scan region: Under the 'Tools' tab**

1. Create scan region by choosing 'Tools > Scan Region Editor'.
2. Input the scanning dimensions and save.

**Setting up slides scan protocol: Under the 'Tools' tab**

1. Select an existing protocol as a template and click 'Save As'.
2. Write a new name and click 'Save'.
3. Choose scan settings for the protocol.
4. Click 'Save'.

**Setting up Sengenics slides barcode and Agilent scan control program.**

1. Click 'Open Door' and click 'Tools>Input Barcode'.
2. Scan the slide barcode, load the slide holder into the cassette and click 'Set'.
3. Next, click 'Close Door' after all slide holders have been loaded in the cassette.
4. Enter the slides barcode if the scanner unable to read it.
5. Choose 'Scan Protocol' and 'Output Folder'.
6. Click 'All to Queue' and 'Yes' to add the slides to the queue.
7. Click 'Start Scan' to begin scanning the slides.

**Image Analysis**

Data extraction from TIFF images using GenePix software.

## 2.2 Setting up scan region: Under the 'Tools' tab

The scan region determines the area of the slide that is scanned. Users can create their own scan region or change a scan region. The larger the region, the longer the scan time. The new region appears as a selection in the Protocol Editor.

**1.** Click 'Tools > Scan Region Editor' (Figure 1).

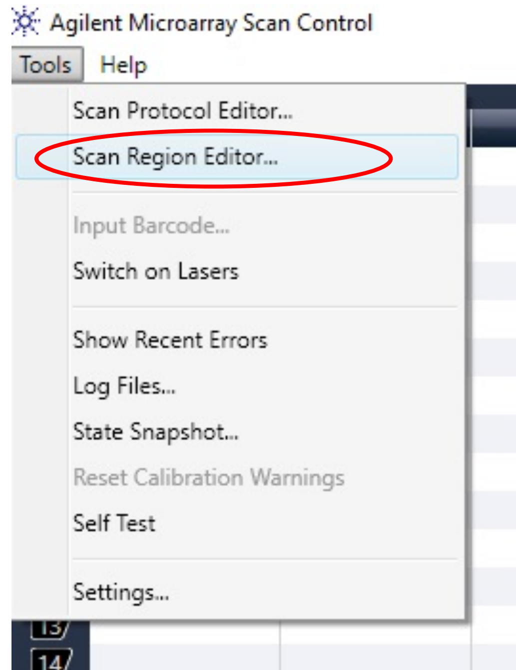


**Figure 1:** Scan Region Editor

i. In the list next to 'Scan Region', choose 'FullStandardSlide' as a template.

ii. Click 'Save As' and create a new name. Next click 'OK' (Figure 2).

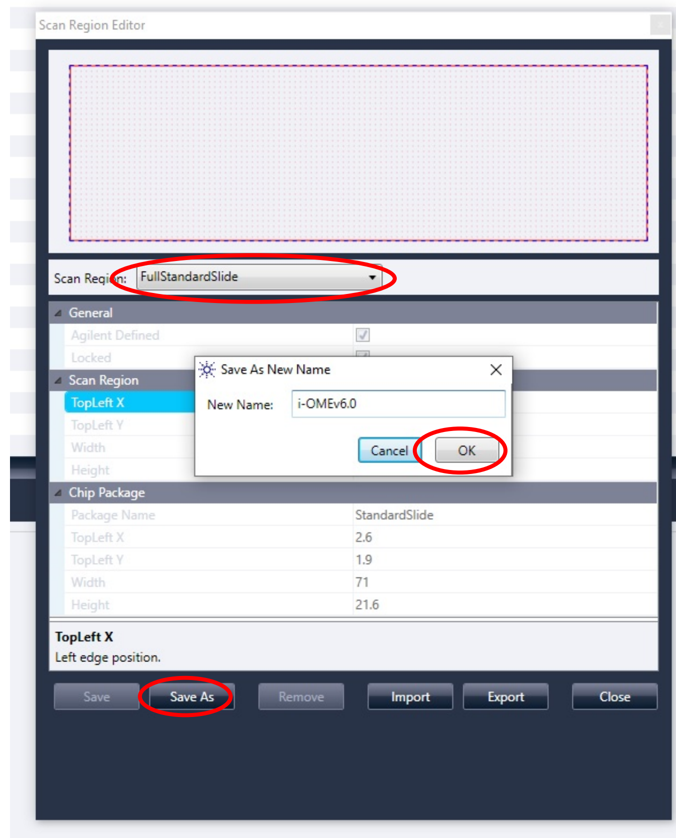


**Figure 2:** Create a new scan region.

i. In 'Scan Region' section, input the measurements for the relevant array scanning region as depicted in Table 1 and Figure 3-6 below:

**Table 1:** Details of scan area for the following 4-plex array format

| Dimension (mm) | | | | |
|---|---|---|---|---|
| Region | 1-plex Array i-Ome Discovery (version 6.0) | 4-plex Array CTA | 4-plex Array OncoREX p53 | 4-plex Array Pan Autoimmune |
| Top left X | 6.00 | 3.30 | 3.30 | 3.30 |
| Top left Y | 2.97 | 3.00 | 3.00 | 3.00 |
| Width | 67.00 | 70.00 | 70.00 | 70.00 |
| Height | 20.00 | 19.00 | 19.00 | 19.00 |

ii. Click 'Save' to save the changes for the selected scan region.

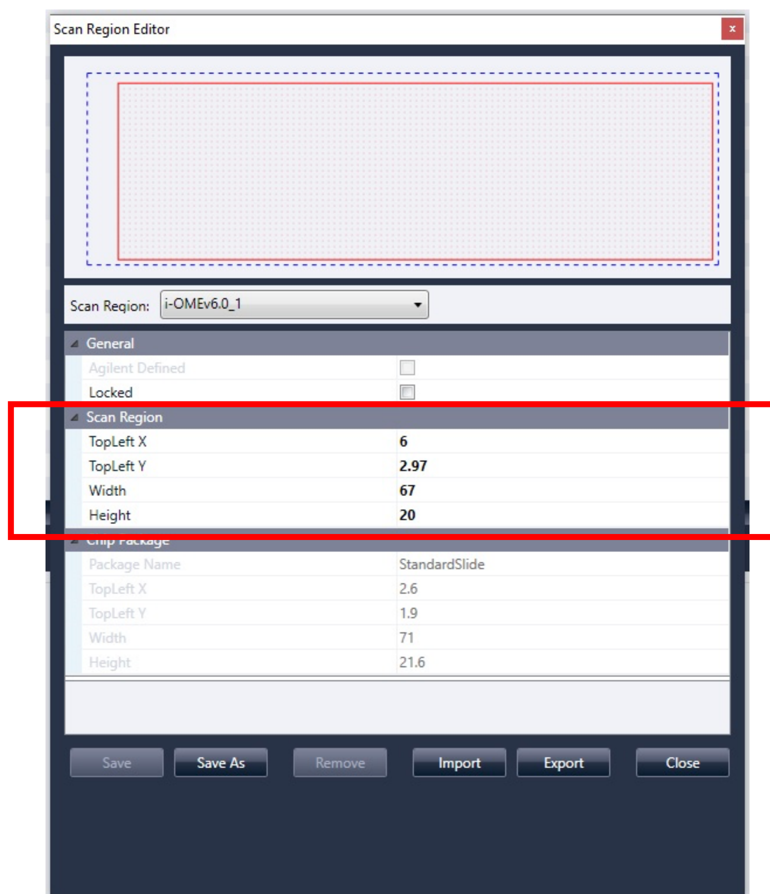


**Figure 3:** Setting up scan region for 1-plex array format, i-Ome® Discovery Protein Array

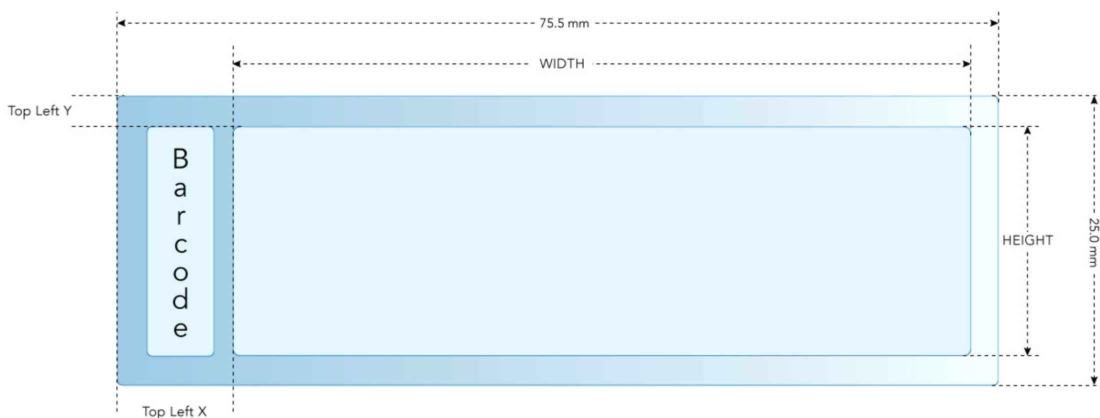


**Figure 4:** Dimension of scan region for 1-plex array

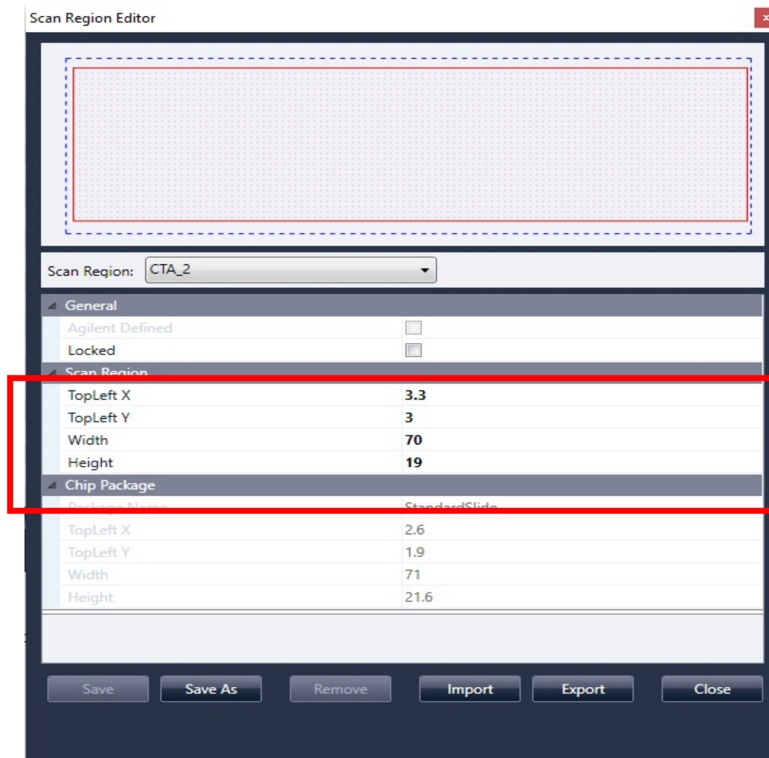


**Figure 5:** Setting up scan region for 4-plex array format

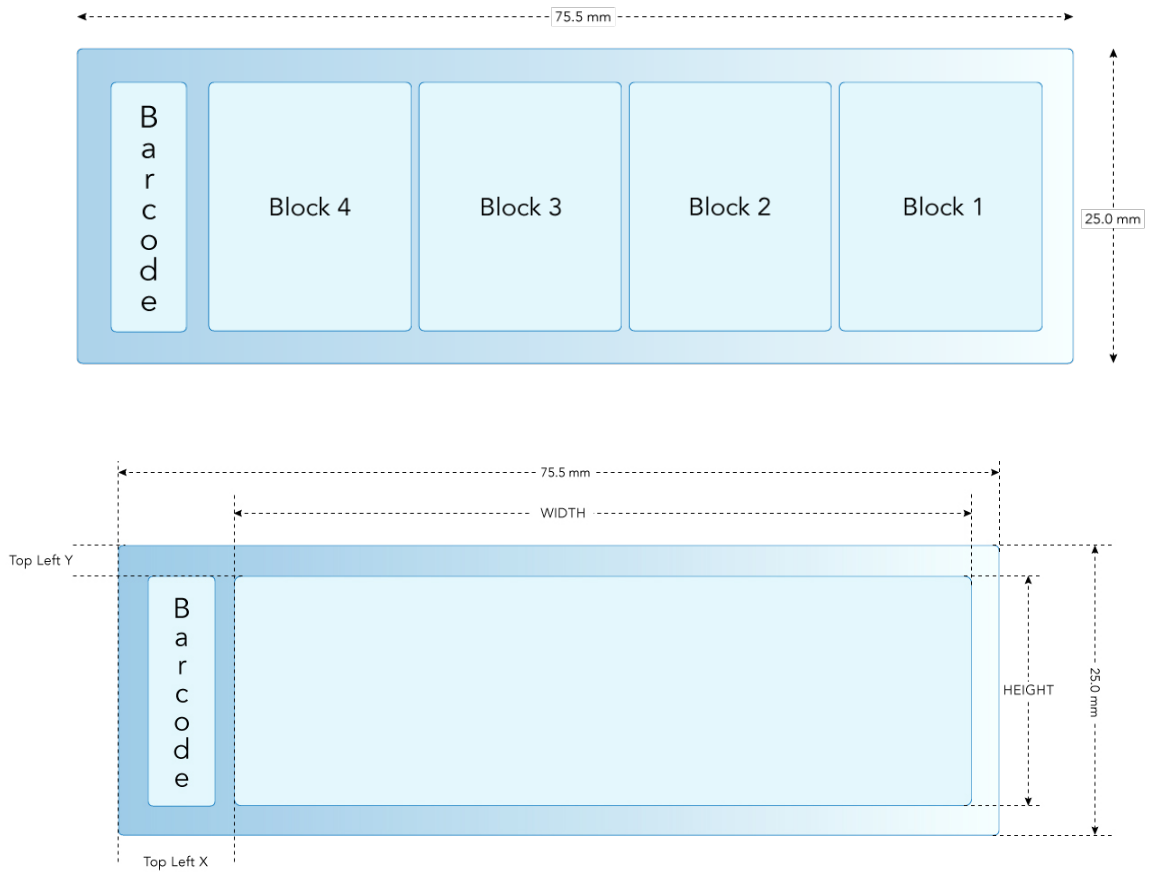


**Figure 6:** Dimension of scan region for 4-plex array format.

## 2.3 Setting slides scan protocol: Under the 'Tools' tab

A scan protocol is a predefined set of scan settings. Several default scan protocols are provided with the software. The 'Scan Protocol Editor' program allows users to select the fluorescence (dye) channels, scan regions, resolution, dynamic range, PMT gain and slides naming settings based on type of slides and assays.

1. Click 'Tools > Scan Protocol Editor' (Figure 7).

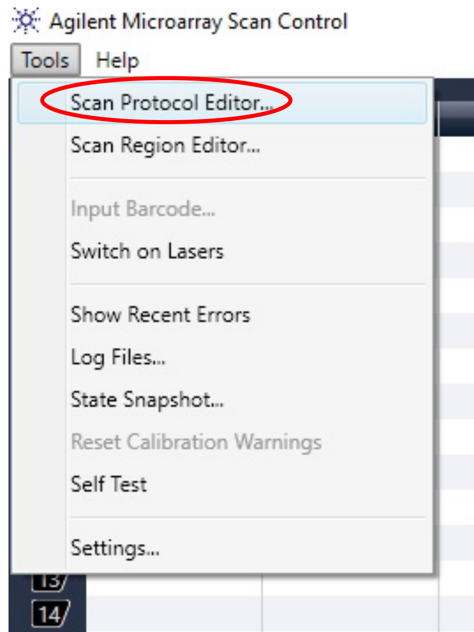


**Figure 7:** Scan Protocol Editor

i. Select any existing protocol as a template to create a new scan protocol.

ii. Click 'Save As' to save the existing protocol with a new name. Then 'Save As New Name' dialog box opens. Enter a new name for the protocol, and then click 'Save' (Figure 8).

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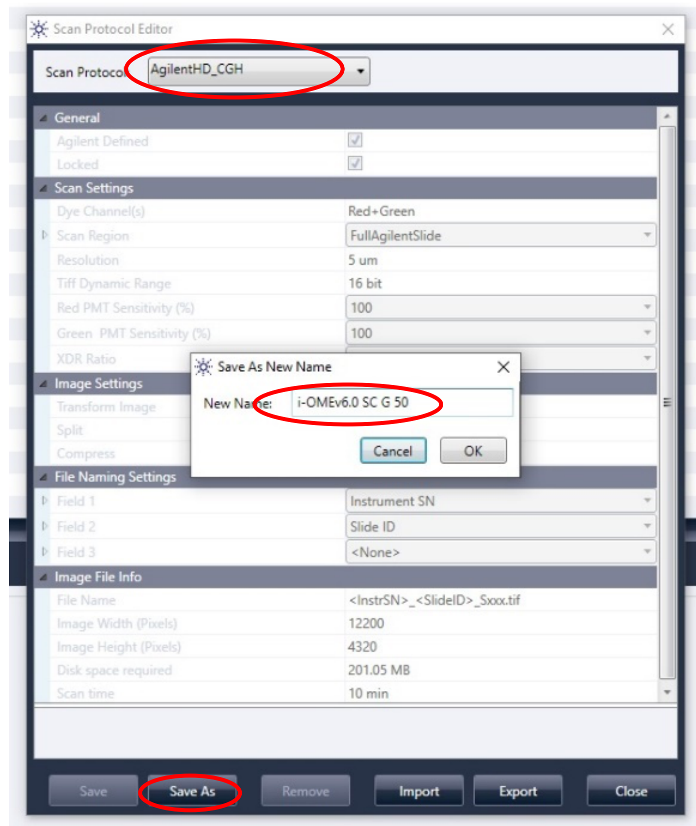


**Figure 8** Create a new scan protocol.

iii. Change the scan, image, and file naming settings according to the slides and assay types. For example, if user run i-Ome® slides and Single Colour assay, the scanning protocol settings will be set as shown in Figure 9 below.

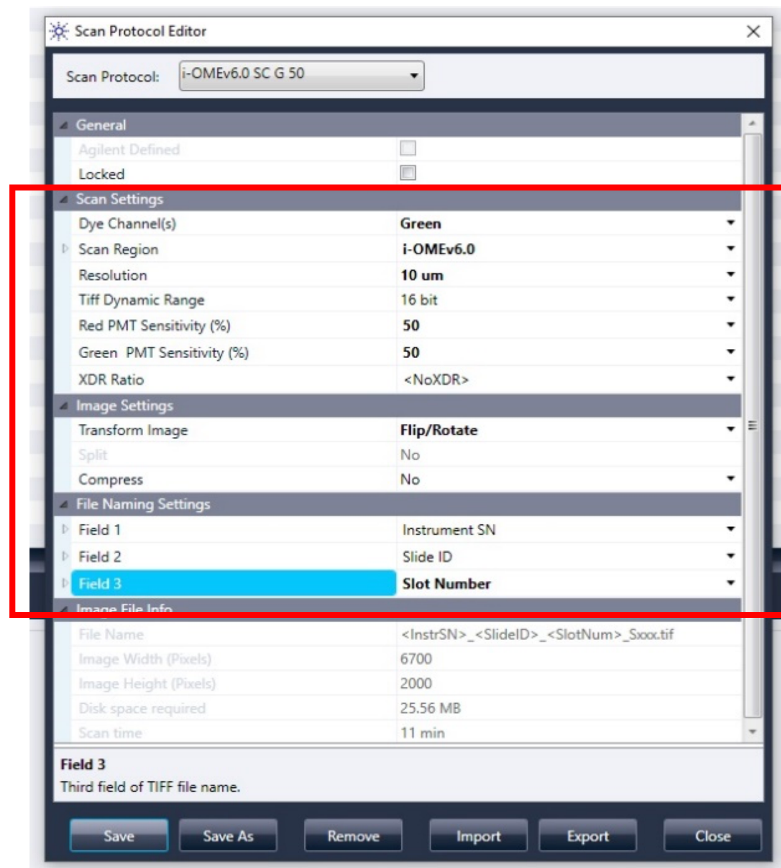


**Figure 9** Scan protocol settings (Image example for 1-plex array format, single colour)

NB: For standard scans, the Scan Control program uses up to three user-defined name prefixes to compose the file name. These prefixes are defined in the scan protocol. Scan files are named using the following rules: 'Field1_Field2_Field3_ScanNumber.tif'.

For example, US4510PP02_251485023883_S001_S03.tif

Field 1: Instrument Serial # = US4510PP02
Field 2: Slide ID = 251485023883
Field 3: Slot Number = S001
S03 is a Scan Number which indicates the third scan file in the folder with the same Instrument Serial # and Slide ID.

iv. Click 'Save' when the settings are completed. See example in figure 10-11 below:

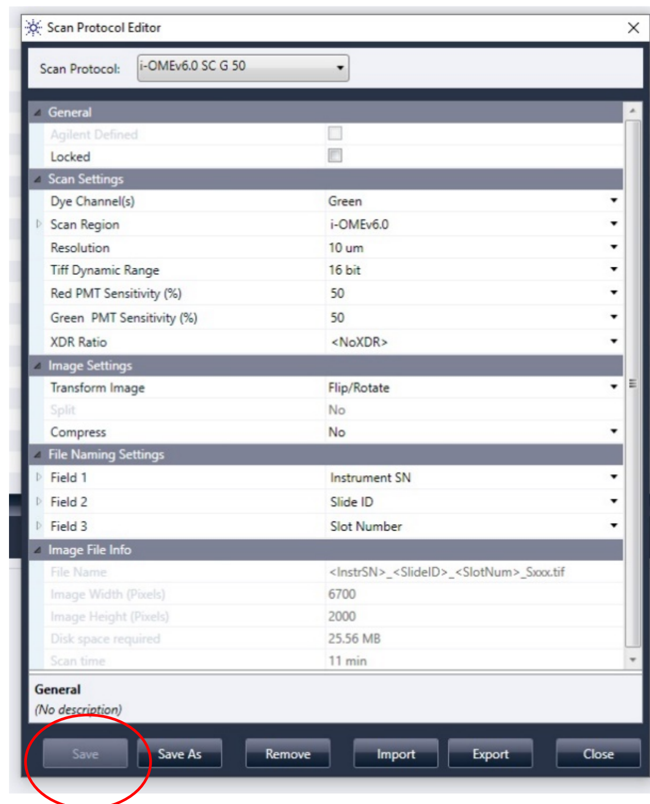


**Figure 10:** Saved scan protocol (image example for 1-plex array, i-Ome Discovery Array)

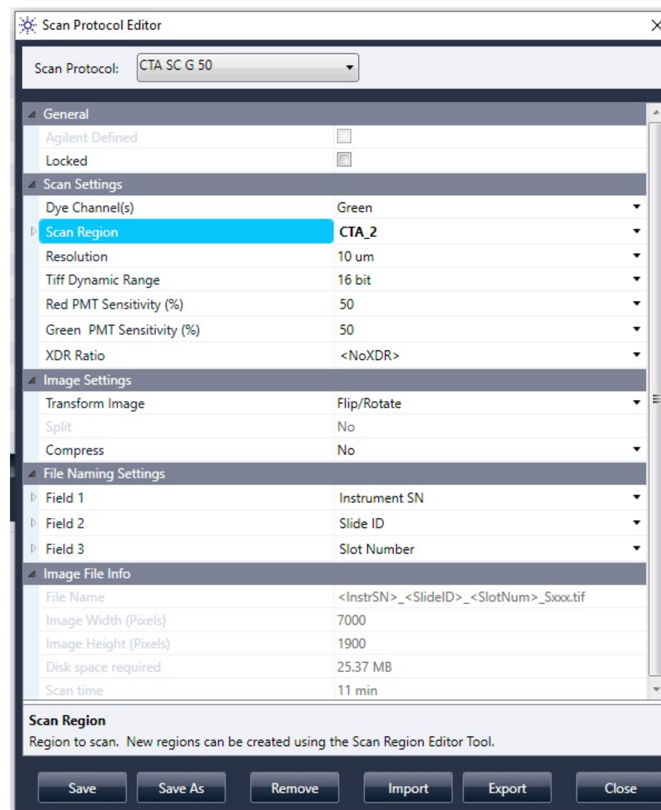


**Figure 11:** Saved scan protocol (image example for 4-plex array, CTA Array)

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## 2.4 Setting up Sengenics slides barcode and Agilent scan control program.

1. In the Scan Control program window, click 'Open Door' to open the scanner door (Figure 12).

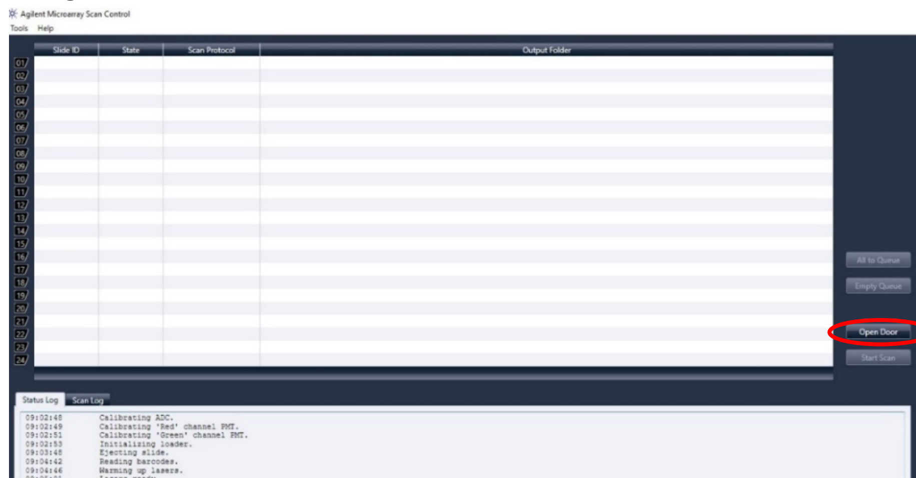


**Figure 12:** Agilent Scan Control window

2. Insert a slide into a slide holder.

3. Click 'Tools > Input Barcode' (Figure 13).
***Note:*** *If you are using external barcode reader, please ensure the barcode reader is connected to the computer. Please ensure to set or change scan data format to 'Data As Is' or 'Return to Factory Defaults'. Kindly please refer to the barcode reader manufacturer's manual.*

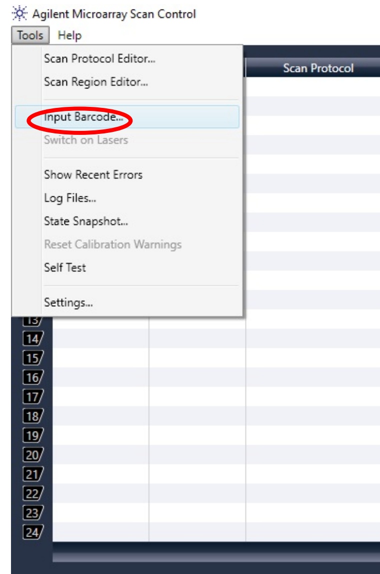


**Figure 13:** Input Barcode

4. A pop-up message will appear. Scan the barcode, load the slide holder into the cassette and click 'Set'. The barcode is displayed under Slide ID in the Scan Control software slot table (Figure 14-15).

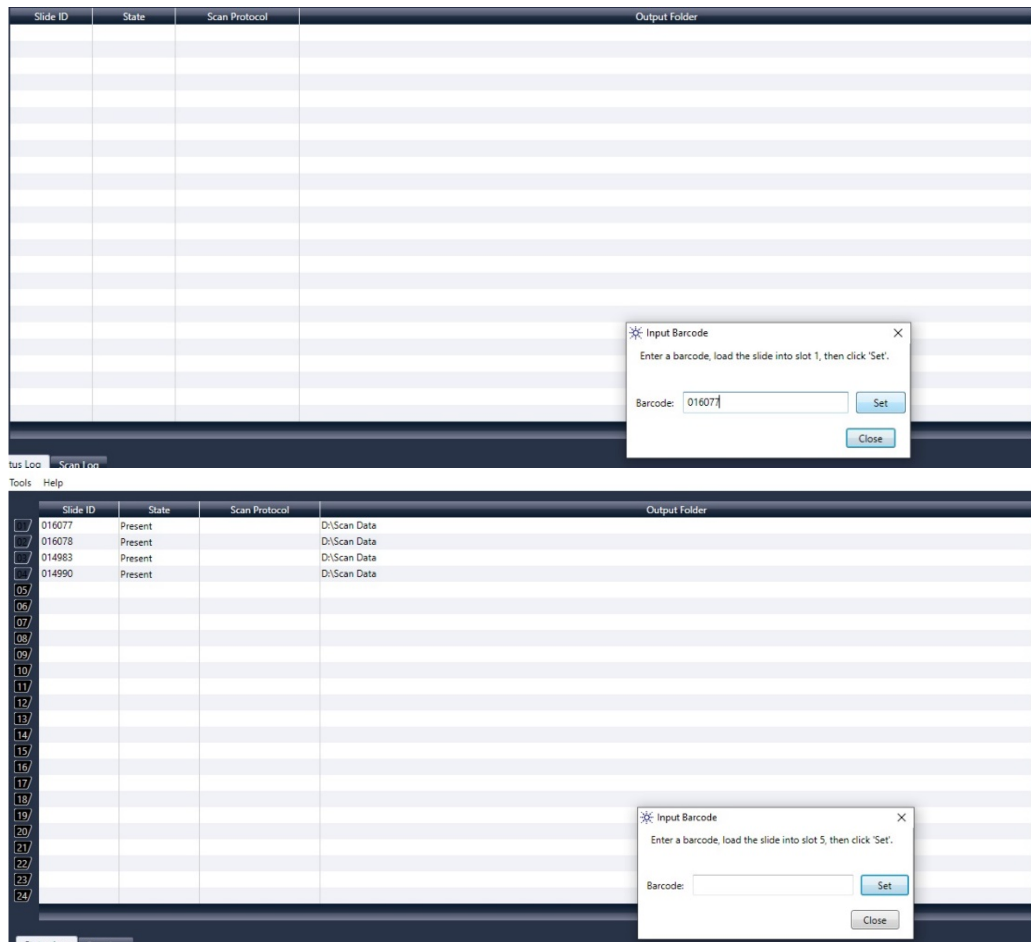


**Figure 14** Scan the slides barcode before start scanning.

**Figure 15:** Place the slide holder into the cassette.

5. Repeat step 2 and 4 until all slides are loaded in the cassette.
6. Next, click 'Close Door'.
7. If the scanner could not read the barcode on slides, enter the barcode manually by clicking the Slide ID cell for the specific slide.
8. For each slide in the slot table, click the 'Scan Protocol' and select a scan protocol to use for the scanning processes.
9. Next, choose the output folder. The output folder is a location of the scanned image files for slides are saved. By default, the output folder is directed to D:\ScanData.
10. Click the 'Output Folder' cell for a slide and click the browse icon.
11. Browse to the location where you plan to save the scanned image files and click 'OK'.
12. If all the scanning settings have been set up and the 'State' column showed 'Ready for queue', click 'All to Queue' and click 'Yes' to add the slides to the scanning queue.
    **Note:** To add a slide to the scan queue, its 'State' column must be select to 'Ready to Queue' (this status is typically autodetected)

13. Click 'Start Scan' to begin scanning the slides.
14. If the scanner status showed 'LaserOff', user need to warm up the laser first before proceeding with scanning (Figure 16).
15. Click 'Tools > Switch on laser' (Figure 17).

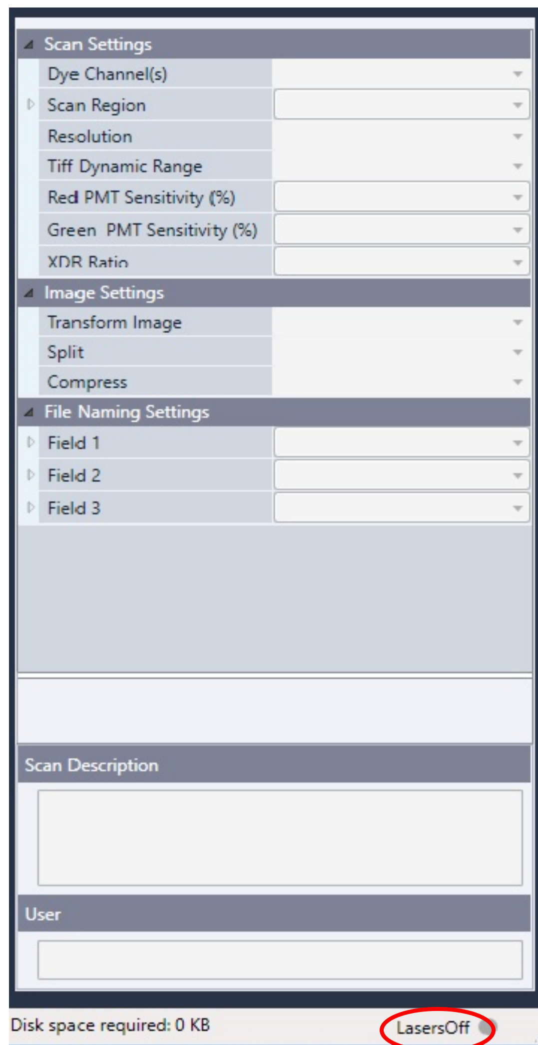


**Figure 16:** Lasers Off Status

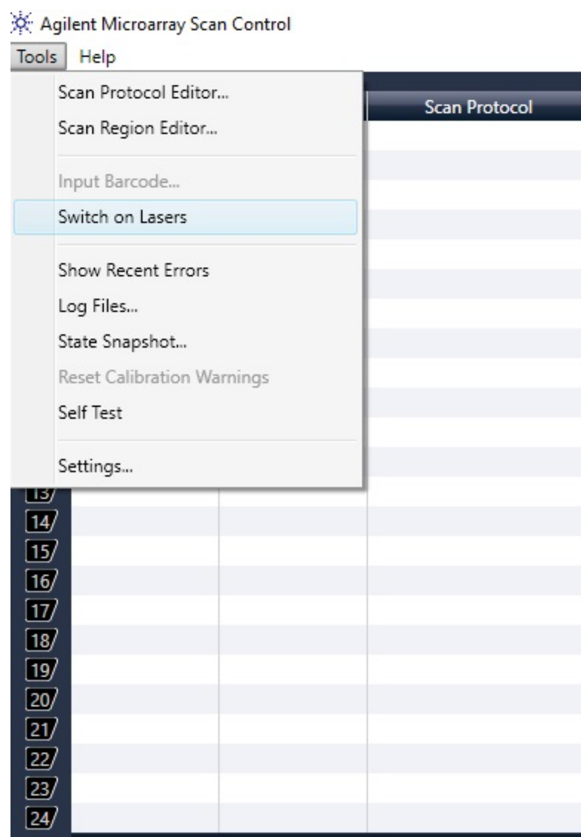


**Figure 17**: Switch on lasers

16. Proceed with Image Analysis after scanning process has completed.

    ** 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).*

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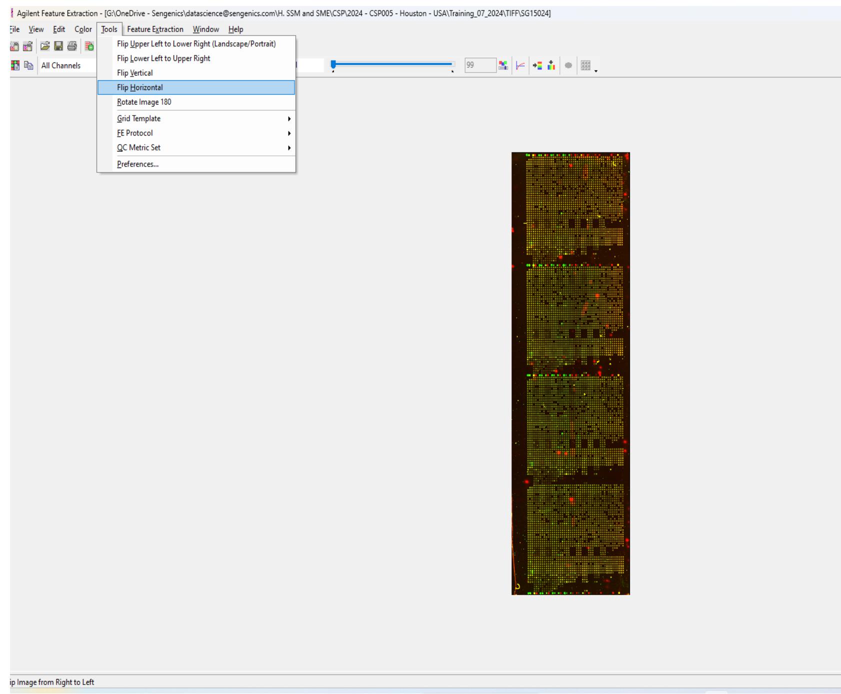
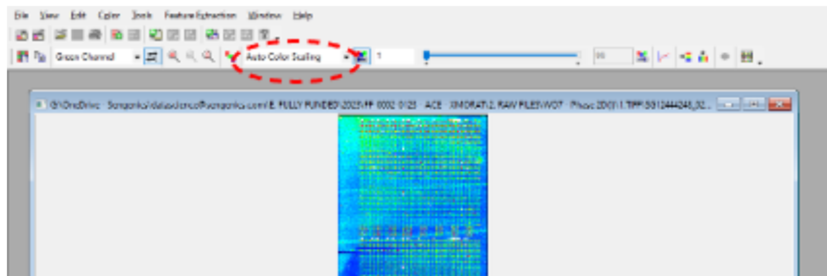
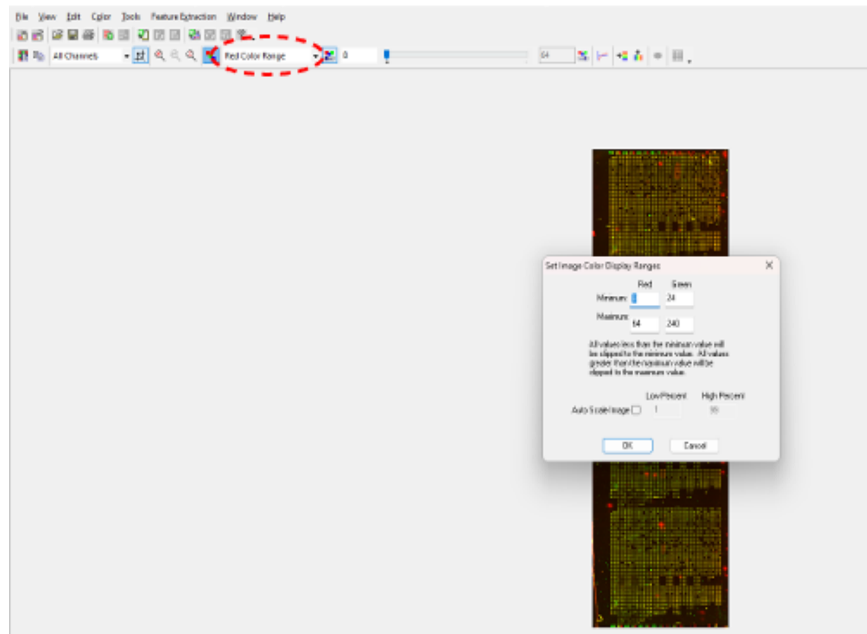
## 2.5 Troubleshooting

1. To flip an image in Agilent Feature Extraction software, open an image, go to 'Tools' in the menu bar and select the preferred direction of flip. Use the orientation marker as guideline:



2. To adjust the color contrast of an image in Agilent Feature Extraction software, select the Auto Color Scaling drop down menu and select the preferred laser channel to adjust the color contrast. Fill in various ascending or descending number under the 'Maximum' box at either red or green color scale until signal is observed. This will not change the image original image as this meant for you to inspect the slide post scan. Further color adjustment can be done in GenePix.





## Contact Information

Sengenics Corporation

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