

ARRAYSUITE 2.0

Note: It is much faster and easier to copy all files from Outbox onto the desktop and subsequently save all files there. You can then split the files into the proper folders as described in Steps 32-34 when finished with QA/QC.

- 1) Opening your files from Witchfire.
 - a. If your images were obtained using either of the Axon scanners, go to “File”, “Open As”. Select each of your images individually and open as “Tiff” files.
 - b. If your images were obtained using the Agilent scanner, go to “Ext”, “ImageReader”, and select your image. Image Reader automatically separates the two channels and knows what scanner was used. These files are named by default as pxxsxx.Red and pxxsxx.Green.
- 2) Adjust the contrast of both channels under the “Enhance” menu.
- 3) Go to “Ext”, “Grid-On-Array”. This function now takes the place of the flip and mark. Select the correct Gipo from witchfire/quincy/chipsahoy/gipos/arraysuite2_0 according to the print. Information specific to that print is shown in the top section and automatically changes to reflect this Gipo.
- 4) In the bottom half of the box, select a “Vertical” orientation of the scanned image and select “Create new grids”. Click on the “original Tiff image” and unclick “GIPO file” to do this. See picture below.

The screenshot shows the 'Grid-On-Array' software interface with the following sections:

- Array Configuration Info:**
 - Use attached resources
 - Use GIPO File (Click ↘)
 - Field: [p47tox [NHGRI-1 96 2x2 22x22]]
- Pen Config.:** rows [2] x columns [2]
- Sub-array:** rows [22] x columns [22]
- Print Mode Info:** NHGRI-Print-Mode #1
96-well Plate placed in normal orientation.
Pens are configured in 2x2,
Pick-up clone from A1->H1 of micro-plate first,
Print along short-side first, 1 times each clone.
- Print Mode:** [NHGRI-1]
- Plate type:** [96 Well-plates]
- Duplication:** [Normal Spotting]
- Orientation of Scanned Image:**
 - Horizontal
 - Vertical
- Initial grids:**
 - Use existing grids
 - Use grids from last array
 - Use grid-template from GIPO
 - Create new grids
- Attach grid/clone info to Resource-Fork of**
 - original TIFF image
 - GIPO file
- Note:** The first printed spot must be aligned at upper-left corner.
- Buttons: [Exit] [OK]
- Footer: GridOnArray, 2.0, © 2000, NIH/NHGRI/CGB

Click OK.

- 5) Boxes will appear on one of your gray scale images. The easiest way to align the grid is to first move the entire grid so that one of the spots in the

corner of the first block is aligned which is done by clicking and holding somewhere in the middle of the grid until it is correctly positioned.

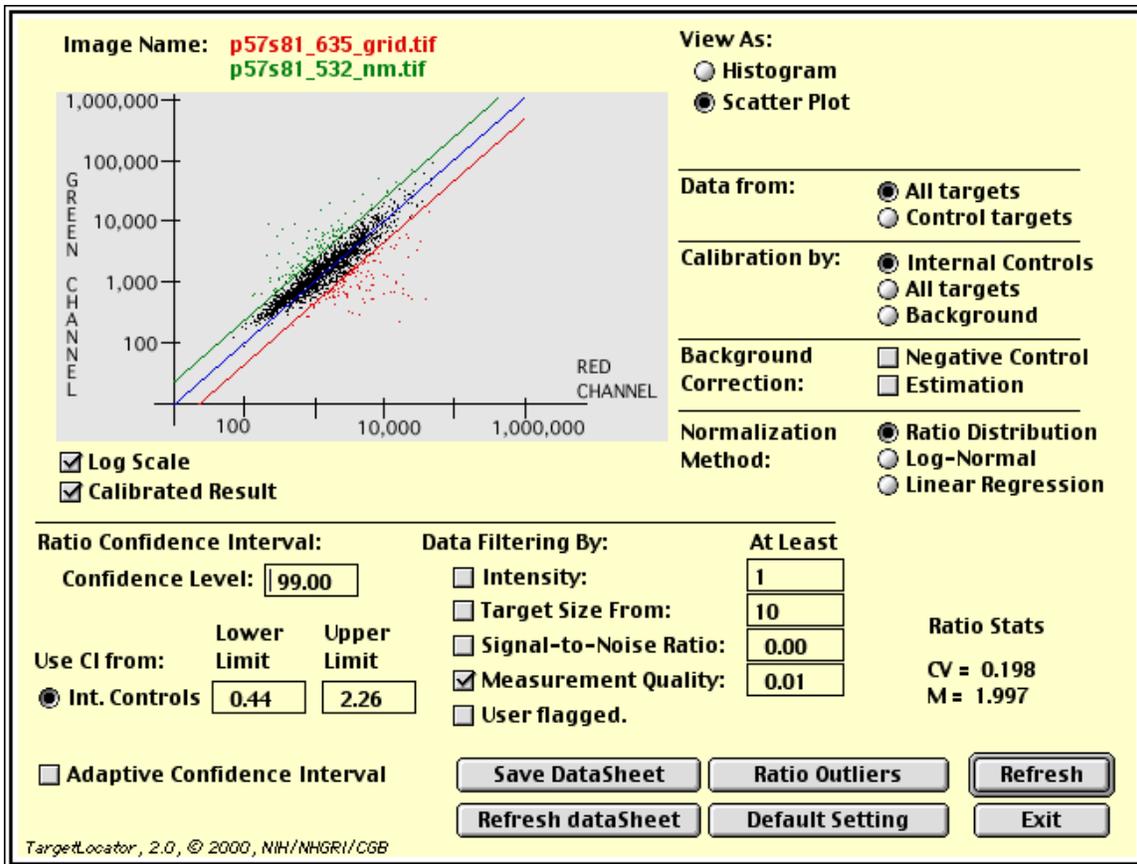
- 6) If the grid is not the size you need (i.e., the other corners did not align with the first one), click and hold the edge of the grid. You can make the grid larger or smaller this way. Align all four corners. You can use < to zoom in or > to zoom out. To move to the next block to align, press the shift key, click and hold an area of the image and move around with the mouse.
- 7) Once grids are aligned in all blocks, press Q to quit. If a warning box appears, just click okay. We will take care of this later.
- 8) **A.** For **Axon**, save the gray scale image that was gridded as pxxsxx_532_grid.tif (Green) or pxxsxx_635_grid.tif (Red). The other tif file does not to be saved again. When renaming these images, be sure to select file format as TIFF. Click OK to “Drawings & objects will not be saved” box.
B. For **Agilent**, save both gray scale images as pxxsxx_###_grid.tif, as above.
- 9) Click on “DeArray” under “Ext”. Select which image you want for top and bottom. Gipo information should automatically be displayed. Make sure that the Ratio Confidence Interval is set to 99.00%.

The screenshot shows the DeArray software interface with the following sections:

- Microarray Image Source:**
 - Sample: #1 p47s10_bottom_635_ni #2 p47s10_bottom_532_n
 - Scan: Primary Secondary
 - Probe Type: Fluor. Rad. (repeated for both samples)
 - Image scanned: Same slide Vertical
- Microarray Specifics:**
 - GIPO File (Click Box): p47tox [NHGRI-1 96]
 - Pen Config. rows columns: 2 x 2
 - Sub-array rows columns: 22 x 22
 - PrintMode/Spotting: NHGRI-1 Normal Spotting
- Processing Specifics:**
 - Target Detection:**
 - Target size: Auto Fixed circle
 - Min. target size: 10 pixels.
 - Detection Method: Thresholding Mann-Whitney
 - Target Analysis:**
 - Analysis for: Gene Expression
 - Ratio Confidence Interval: 99.00 %
 - Background Subtraction:** Local Global Neg. Controls No
 - Intensity:** Average Within Target Within Box Avg. Duplicates

Buttons: Cancel, Go...
DeArray, 2.0, © 2000, NIH/NHGRI/CGB

- 10) Do not save the file at the prompt when DeArray is finished.
- 11) Click on "Ext", "TargetLocator".
- 12) Press the "S" key once to balance the color image (balanced 1), press the "E" key to enhance the color image (enhanced).
- 13) Take a snapshot of the image. You can toggle the zoom with the "<" or ">" keys. Determine if the background is clean or noisy, or if there are blackholes. Record this on the Label/Scan/QA/QC sheet.
- 14) Press the "R" and "G" keys to visualize the location of outliers. If the red and green boxes are evenly distributed throughout the entire array, select a Hyb distribution of "Uniform" on your Label/Scan/QA/QC sheet. If the distribution of either color box seems to be skewed, select a Hyb distribution of "Patchy". Press "R" and "G" for the boxes to disappear.
- 15) Press the "F" key and change the search type to Clone ID. In the box, type "xxxx" (or "yyy" for newer arrays) and click on "Search" to get the values from blanks. Find the highest value for the blanks and record this. Also make a rough estimate of the average of the blanks and record this.
- 16) Press "D" to enter the dialog box. The default leaves only a measurement quality of 0.001 checked as shown below.



17) Take a snapshot of the scatter plot (prior to any changes being made); take a snapshot of the ratio distribution, red channel, green channel and size histograms. Note how the red and green channel histograms compare to each other, either equal, Cy3 stronger or Cy5 stronger and record on QAQC sheet.

18) Click back on the scatter plot. Select a quality metric. This number can range up to one. The default is 0.01. Record this on the QAQC form. This quality metric feature usually filters out the small and noisy spots that the target size and intensity cut off formally did. Check your Target Size Histogram to be sure that it is consistent, make a cutoff if needed, making sure that the box is checked.

19) Determine what your intensity cutoff should be. Enter this number in the appropriate box. For your cutoff to be effective, the box next to "Intensity From" should be checked and you must click into another box. Record your cutoff.

20) Record your CV and Mean values. Take a snapshot of the scatter plot after cutoffs.

- 21) Record the 99% RUL and RLL on the QA/QC form. Click on “Refresh Datasheet” and then “Save Datasheet”. This file will be called pxxsxx_si_fci.txt.
- 22) Change the confidence to 95%, record the RUL and RLL .
- 23) Change the confidence back to 99%. Change the Normalization Method from “Ratio Distribution” to “Linear-Regression” and record the Linear 99% RUL and RLL. Click on “Refresh” and take snapshot. Click on “Refresh Datasheet” and “Save Datasheet”. This file will be called pxxsxx_si_lin.txt.
- 24) Change the confidence to 95%, record the Linear RUL and RLL.
- 25) Press the exit button and double click on the image to exit out of TargetLocator.
- 26) Save the color image as pxxsxx_color.tif.
- 27) Go to your hard drive where your snapshots are stored. There should be eight. Rename these by adding “pxxsxx_” to Picture 1, i.e. pxxsxx_Picture 1.
- 28) Picture files should be placed into a folder named “pxxsxxInvestigator name” in Quincy/ArraySnapshots_Drop folder.
- 29) The gray scale grid file, the sample intensity files (si_fci and si_lin at both 95% and 99%) and the color tiff image should be placed into the appropriate print’s folder in “inbox” on Witchfire (should be eight files total).
- 30) Enter Label and Hybridization information into MAPS. Give QA/QC sheet to appropriate QA/QC team.

NOTE: If you do not completely close out IPLab before beginning to QA/QC your next image, all of the information you entered previously will remain, i.e. when you open the dialog box, all of the cutoffs from the previous slide will be there. Therefore the best thing to do is to **COMPLETELY** quit IPLab between slides.

NOTE: When performing Grid-On-Array, be sure to pay extra attention if there are extra spots on one of the blocks. You will need to account for this extra row on **ALL** blocks.

APPENDIX I: Target Locator Key Code

APPENDIX II: Where to Store Files Generated in IPLab.

APPENDIX I

TargetLocator (v1.3) Key Code

<	Image Zoom Out
>	Image Zoom In
C	Control Genes Boundary Toggle
B	All Target Boundary Toggle
L	Array Layout Toggle
R	Red Target Toggle
G	Green Target Toggle
S	Background Subtraction Toggle
E	Enhance Image Toggle
D	Data Analysis Dialog Box
F	Find Gene
V	View in Array or Microtiter Plate mode
Option key	Will toggle magnifier to zoom out on IPLab images
Shift key	When held down one can use mouse to move images

APPENDIX II

File names	Where to Place in Witchfire
<p>AXON SCANNER: pxxsxx_532_grid.tif OR pxxsxx_635_grid.tif pxxsxx_color.tif pxxsxx_si_fci.txt pxxsxx_si_lin.txt</p> <p>AGILENT SCANNER: pxxsxx_532_grid.tif pxxsxx_635_grid.tif pxxsxx_color.tif pxxsxx_si_fci.txt pxxsxx_si_lin.txt</p>	<p>Inbox in Appropriate Print Folder</p>
<p>pxxsxx_Picture 1 (array) pxxsxx_Picture 2 (scatter) pxxsxx_Picture 3 (ratio hist) pxxsxx_Picture 4 (red hist) pxxsxx_Picture 5 (green hist) pxxsxx_Picture 6 (size hist) pxxsxx_Picture 7 (scatter after cutoffs) pxxsxx_Picture 8(lin scatter)</p>	<p>Quincy/ArraySnapshots</p> <p>Places files for each print together in a folder with pxxsxxInvestigator name (i.e.p54s33Afshari)</p>