

Custom Array Preliminary Analysis: QuantArray

The data gathered using the ScanArray 4000 can be analyzed using QuantArray software. The scan files must be transferred between the two computers (ScanArray computer -> QuantArray computer). One way to transfer the files is using the cgrb server networked on both machines as the B: drive (incoming data). The pathway on all three computers should reflect the pathway developed for the Custom Array hierarchy:

Microarray/ArrayDesign/your_organism/your_arraydesign/output/PrintRun/Slidefolder.

Open up 'My Computer' and transfer the files from the incoming data disk (B:) to the equivalent location on the QuantArray machine. This is optional, as the software can work by pulling and pushing files to the cgrb server. Drawbacks for not transferring the files are 1) it may take more time and 2) if the cgrb server disconnects (e.g. goes down) the links to the image files will be disconnected.

Open up QuantArray software.

If you will be using a QuantArray protocol which has already been developed begin with step 21 below.

The first time you perform analysis on a new array design you will be defining the protocol which can then be used for subsequent slides of the same array design. If a protocol has already been saved for the array design of interest, begin at step 20 below.

1. Open up Cy3 image first (File -> Open Image Browse to find the Cy3 file of interest). You will be prompted to give a unique name for the analysis.
2. Open up Cy5 image (File -> Open Image...)
3. Under the View menu choose-> Composite Image
4. Change contrast (the icon above the image window with a circle split into dark and light sides) to some high number (e.g. 90), apply to all, close
5. Go to the Edit menu and choose-> Array Pattern
 - a. Input number of rows and columns for within each of the grids (i.e. the number of features a single pin makes [e.g. a 20x20 grid])
 - b. Input number of rows and columns of the array grids (e.g. using an 8 pin-tool you could have 4 rows and 2 columns in the array. If you have printed the twice (2 subarrays) on a single slide you would have either: 4 rows and 4 columns or 8 rows and 2 columns, depending on the orientation of the subarrays on the slide).
 - c. Input spot diameter (usu. 130 – 150 μm)
 - d. The spacing of the grids (2000-5000) will define how far apart the grids will be displayed at the Edit Pattern step below.
6. Go to the Edit menu and choose -> Quantification Methods
 - a. Browse and add the QuantArray output file from the Microgrid

13. The spots have been located once the green boxes appear. If you are using the Adaptive method (Quantification Methods) you have a little more freedom on where to center the feature within the green box. The cross in the center of the box needs to touch the feature. If you are using the Fixed Circle method of quantification, the cross must be in the center of the feature. Navigate around the entire slide and adjust the green boxes according to the best position representing each feature. Again, do not move the boxes away from their relative position to one another to maintain the correct identification from the QuantArray file (from Microgrid) linked in the Quantification Method (Step 6a above). These boxes do not respond to arrow keys.
14. Choose 'View Reports' to see the graph of the data. The scatter plot icon is one way of viewing the data, including the log plot.
15. Choose 'Export Data' and accept the pathway defined by the software. Once the export is complete, hit the button that says 'Open Recent Export file in Excel'. Save this file as **Text (tab delimited)** in the Custom Array hierarchy:
C:/Microarray/ArrayDesign/your_organism/your_arraydesign/output/PrintRun/ Slide_x/filename_export.txt and you may wish to save this export file on the cgrb server
(B://Microarray/ArrayDesign/your_organism/your_arraydesign/output/PrintRun/ Slide_x/filename_export.txt).
16. Save the protocol: Especially the first time you run this analysis and create the protocol for future slides with this array design; save it in your_arraydesign folder.
17. Save the experiment: After you have saved the Export Data save the Experiment (under the File menu). Note: the command 'Save' or 'Save as' references the protocol, not the experiment. You may want to 'Save the experiment' several times during the (e.g. after edit pattern, after locate spots).
18. You are then finished with that slide, and you can begin analysis of a new slide or log out of the software. To begin a different analysis, see step 21 below.
19. Loading everything into BASE:
 - a. Using Explorer log into BASE. Upload the export (data) file by using the QuantArray File Conversion Utility link BASEload on the Site Info page. Hit the 'Choose File' button to browse the local computer and find the export file you just created. Check the box for 'Upload data file to BASE' and hit the Reformat button. The file will be converted into the format needed for BASE, and it will be 'owned' by 'generic'.
 - b. Go to Uploads page and upload both scans used to create the data file (e.g. Cy3 and Cy5 for a given slide/array).
20. Once these three files have been loaded into BASE you can begin to connect them into a hybridization file for downstream analysis and viewing.

QuantArrayanalysis, cont.

21. To begin analysis of a new slide using a Protocol which has already been created:
 - a. Open the QuantArray software
 - b. Under the File menu 'Open Protocol'. Browse the local computer to open the protocol for the array design you are preparing to analyze.
 - c. Open the Cy3 image, give the new experiment a name
 - d. Open the Cy5 image
 - e. Under the View menu choose-> Composite Image
 - f. Change contrast (the icon above the image window with a circle split into dark and light sides) to some high number (e.g. 90), apply to all, close
 - g. Continue, beginning with step 8 above