

PROCEDURE FOR USING QUANTARRAY SOFTWARE (VERSION 3.0)

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
Typically, label control sample with cy3 and experimental sample with cy5

1. Open QuantArray version 3 software
2. The first time you scan a slide with a new layout, you must create a new protocol:
 - a. Open an image
 - b. Go to File menu and chose Protocol Wizard.
 - c. Step 1: Either input the spacing (distance in um) of rows, columns, or spots) or Select Pattern Wizard and follow steps 1-12 (to define the spot pattern based on your image).
 - d. Step 2: Use default Grid/ Spot Elasticity values (50 for each)
 - e. Step 3: Select Adaptive Quantitation. Select the “Browse” button and pick the “Gene Database file”, which is a tab delimited text file, containing a list of spot coordinates and 1 column with a spot ID (clone ID, for example).
 - f. Format of Gene Database file (each line indicates a spot, and comments can be added by placing a '#' character before the text). Do not keep the first row of header names.

Array Row	Array Column	Spot Row	Spot Column	Gene descriptor
1	1	1	1	Gene 73389
1	1	1	2	Gene 87390

ETC...

- g. Step 4: Use default values
- h. Step 5: Use default file name (you can change the file name when you export your data)
- i. Hit the Finish button
- j. You can also put in quality score cut-offs, which will flag those spots that are poor according to your quality values. You will need to do a few arrays first before determining what values to use.

3. To use an existing protocol, open the protocol file= *.pro (File -> Open Protocol). Then open the image(s) (File -> Open Images). Always open the image for the control sample first (we say Cy3 is always the control image) because it will be labeled as “control” in the output data file (the first image is false-colored red). The second image opened is colored green. The third image opened is colored yellow.
4. After opening both images, adjust the Contrast (button that is a circle with half black and half white) and then select Apply to All.
5. Follow the Analysis steps, listed in the left hand window:
 - a. Register Images:. Select the Register window (Window menu -> Register), which is the two images overlaid.
 - b. Each image can be moved individually using the arrows so the spots overlap well.
 - c. Specify Location: Put the + (crosshairs) in the center of the upper left-most spot of the upper left-most subarray (if the spot is not visible, you must estimate the location).
 - d. Edit Pattern: Circles appear around the spots in a grid to mark the subarray pattern. Move the subarray grids if they are not close the spots (you can also move the rows or columns individually). It is sometimes easier to see the spots on the green image.
 - e. Locate Spots: Hit the “Start Locate” button (With large images, this can take 5 minutes or more and it may seem that the computer has frozen. Just be patient!) When the spots are located, then a box with a + sign appears over  each one:
 - i. The middle of each + should be in the center of a spot. Adjust and move the green +'s if needed. You should view both red and green images to see all the spots.
 - ii. To Flag spots: Right click (and then pick “Ignore”) or simple double click. (Flagged spots will turn orange and have a cross through the box.) Flag spots when you see hair, dust or bleeding of one spot into the next. To select many boxes at once, left-click on one, then press the Ctrl button while left-clicking on many squares. Keep holding down the Ctrl button, and double

click on the spots to ignore all the them. (When you flag spots, they will have a “1” in the ignore filter column of the output data file).

- f. View Reports: Skip
- g. Export: Hit the Export Button, then type path and file name of data file (such as C:\filename or to save to zip disk E:\filename). Include in the filename: the lab book page number, the date, and the slide number).

Note: To capture a composite of the two images (as a bmp file), first adjust the contrast as needed. Then go the “Register Images” on the Analysis step list. Choose from Tools list “Annotate”. MS Paint will open with the composite image. You can add text to the image and save it as a .bmp file.

Note: To change the quantitation method or Gene Database File: Go to Edit -> Quantitation Method. Select either histogram, fixed circle or adaptive based methods. Hit the Browse button to select the Gene Database file (text file).