

7. Acquisition Mode – Fluorescence

8. User Select –DIGE SE 600 small plates

9. Select the number of gels (= 4, for example)

10. Orientation –

a. Hold down on this button and drag it to the orientation of choice.

b. Choosing “**R**” will scan the gel in the same orientation as you see it on the platen.

11. Pixel Size – Start with 1000 microns. (This is the TEST scan to find the proper settings)

12. Focal Plane

a. 3mm for glass plates

b. Platen if the gel is sitting directly on the scanner surface

13. Select Press Sample ONLY WHEN you have a glass cover on the gel

14. Image Analysis

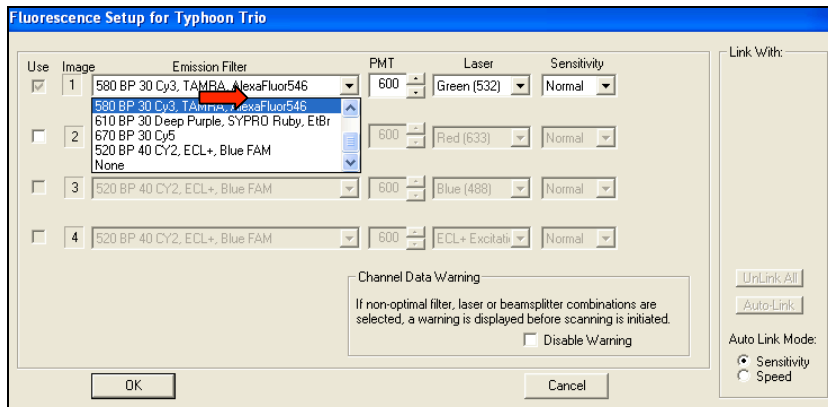
a. Scroll down the menu bar and select IMAGE QUANT

b. This automatically opens your scan into Image Quant once it is done scanning.

15. Set Up – There are 4 boxes

a. Cy 2 520 BP 40, Cy2, Blue FAM

- b. Cy 3 580 BP 30 580 TAMRA Alexfluor, 540
 - c. Cy 5 670 BP 30 Cy5
 - d. For Coomassie use red laser with no filter
16. PMT – Start at 600 for each laser
 17. Sensitivity = normal
 18. Click “Ok”



19. SCAN

- a. Click on file name to highlight it
 - b. Edit Sample file name (may remain as TEMP for TEST scans)
 - c. Edit – give it a name and a storage folder
 - d. You will generate three different gels with a **.gel** extension. This is a TIF file.
 - e. Standard – generally Cy2, but if you don't have a standard check none
20. After scan, IMAGE QUANT automatically opens.
 21. Click on all three channels (Labeled 1, 2, 3... at the top of menu bar)
 - a. This shows the overlay of the filters used in the scan.
 22. Click side by side icon – top tool bar (square box with a vertical line through it).
 23. Use the contrast graph at top toolbar to see which spot shows most intensity
 24. Determine if PMT voltage is appropriately set
 - a. Click on the Gray/Color Adjust button at the top of the menu bar on the right
 - b. Move the “slider” to the left until one red pixel appears
 - c. Read the High Volume number. Ideally, it should be between 60K-90K
 - d. IF volume is too high, go back to typhoon control and decrease PMT voltage and rescan. Repeat until in proper range
 - e. Proceed to Final Scan

25. Perform final scan at 100 microns

26. SET UP

- a. Decrease previously determined PMT voltage (Step 24) by 10% (i.e. If the ideal setting was determined to be 650 volts, decrease it to 585 volts)
- b. Pixel size 100 microns

27. Scan – go through the naming process again. This is your final Filename. Be careful and follow file naming protocol

28. Naming protocol is as follows for Cy Dye Images

a. Name the images as follows:

- i. gel# condition (additional description) Cy3.gel
- ii. gel# condition (additional description) Cy5.gel
- iii. gel# STANDARD Cy2.gel

b. The gel # is the letter “g” followed by the number from the gel tab

c. The condition would be a description of the sample (control or treated)

d. The additional description (in parenthesis) could be other important info you need (ex. 6 hours, 18 hours)

e. Be sure to add the proper extension. Cy3 and Cy5 need to be Cy3.gel and Cy5.gel Cy2 just needs to have STANDARD Cy2.gel after the gel #.

f. An example for a gel taken from a project with a control set and treated set that had samples taken over a time course would look something like this:

- i. g1546 Control (8 hours) Cy3.gel
- ii. g1546 Treated (16 hours) Cy5.gel
- iii. g1546 STANDARD Cy2.gel

g. For cropped images use the gel# and add crop after it. This is what your crop directory and .ds file for each cropped gel will be named. The individual images will keep their uncropped names.

i. g1546 crop.dir

h. Please try and keep the file names in one project as similar as possible with their formatting . If you capitalize the first in your conditions, do it in all of your gel images.

- i. Please avoid using all caps, except for the STANDARD in Cy2 images. It makes it harder to read if the file names are in all caps.

29. After scanning check volume report again and make sure it's still OK

30. Go to overlay mode – top toolbar colored box

31. Tools – define region of interest by clicking and dragging across the gel
 - a. Region of interest should exclude spacers, bottom edge, and stacking gel.
CONSISTENCY is very important
32. File – Save region of interest as: Give it a name
33. This is what you will import into DeCyder

Using the gel orientation guide:

Place the 2-D gel onto the Typhoon platen ready for scanning. Hold the gel alignment guide over the gel ensuring that the IEF strip and molecular weight orientation of the guide match that of the gel. The appearance of the letter “R” as seen from the front of the Typhoon shows which letter “R” to select in the Scanner Control Software for the image to be normally oriented.

RULE OF THUMB

To utilize maximum dynamic range of instrument (1 to 100,000)
Pre-scan at 1000 um (quick scan) – aim for 70,000 to 90,000 counts
for most intense peak.
Real scan is performed at 100 um.

10 Volts (PMT) equals 10,000 counts
1000 um to 100 um equals 10,000 counts

Eg Pre-scan at 500 V – max intensity is 50,000

Thus:

To get 90,000 counts;

Scan at 530 V (add 30,000 (10,000 x 3) + 10,000 = 40,000