Typhoon scanner

Every time you use the scanner, you MUST sign the logbook.
Be sure to clean the platen before and after every use.
Don't forget reference markers if you are going to use the Spot Picker.

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The Typhoon scanner can acquire fluorescent and chemiluminescent images. from samples labeled with Fluorescent dyes, (red – 633 nm; green – 532 nm; blue – 457 or 488 nm; and multi-labeled samples),

Stain	Excitation		Emission	
SYPRO Ruby	280/450	Blue	610	
Deep Purple	532	Green	LP 560	
Krypton	520		580	
Coomassie				
Silver Stain				

Fluorescence scanning

Notes.

- Wear powder free gloves (powder fluoresces).
- Avoid fluorescent tracking dyes (bromophenol blue or xylene cyanol). Either put the tracking dye in a separate well or use nonfluorescent dyes.
- Avoid excessive exposure to light.

Excitation/Emission

1. Each fluorochrome will have 2 #'s. One for excitation and the other for emission. Some product information sheets will also give the laser to use for excitation. The fluorochrome excitation # and emission # is not a single #, but is actually a curve. The # is the center of that curve.
2. Select wavelength that your fluorochrome is excited. The Typhoon has 3 lasers.
    a. red laser – dyes that are excited at 633nm

b. green laser - dyes that are excited at 532nm
c. blue laser - dyes that are excited at either 457 nm (Blue 1) or 488 nm (Blue 2).

3. Select emission filter for your fluorochrome. After the dye is excited, the optical system directs the light through the emission filter you have selected. Each filter allows onlly the emitted light within the filter's bandwidth to pass through to the PMT (photomultiplier tube). The PMT converts the light to an electric current (analog signal) which is then converted to a digital signal that is stored in the computer. Following are the available filters with some of the fluorchromes they can be used for. The 1st # is the transmission peak center. BP stands for band pass. The 2nd # is divided in half; the band width is that # + the divided # above and below the first #.
   a. 520 BP40. Transmission peak at 520. Transmits light between 500 nm and 540 nm. Use for Cy2, ECL Plus or Blue FAM.
   b. 555 BP20. Transmission peak at 555. Transmits light between 545 nm and 565 nm. Use for R6G, HEX or AlexaFluor 532.
   c. 580 BP30. Transmission peak at 580. Transmits light between 565 nm and 595 nm. Use for Cy3, TAMRA or AlexaFluor 546.
   d. 610 BP 30. Transmission peak at 610. Transmits light between 595 nm and 625 nm. Use for ROX, EtBr, Deep Purple, SYPRO Red and SYPRO Ruby.
   e. 670 BP 30. Transmission peak at 670. Transmits light between 655 nm and 685 nm. Use for Cy5.
   f. 526 SP. Short Pass, transmits light below 526 nm. Use for fluorescein, Cy2 or AlexaFluor 488.
   g. 560 LP. Long Pass, transmits light above 560 nm. Use with TRITC.
   h. 390 BP. Used for radioactive samples.
   i. None

Setup

1. Prepare the sample using fluorescent dyes.
2. Run samples in 1D or 2D polyacrylamide gels. Assays in microtiter plates must be low fluorescent plates. Make sure glass plates are absolutely clean. Fingerprint oils or grease can

attract dust or fluorescent dyes. The glass plate must be 3 mm thick. The +3mm focal plane parameter is designed for 3 mm thick glass.

3. Reference Marker – if using Spot Picker. It is much easier to put markers on plate before it is poured. If you didn't put reference markers on when you poured your gel, you need to do it now. Put them on the right and left sides of the bottom gel plate (you need the marker to still be there when you remove the top plate for the Spot Picker), about 1.5 cm in, and in the middle. If your not sure where it needs to go – put the gel on top of the gray Spot Picker tray. The markers need to be within the 2 white lines. You also don't want it too far in from the sides and have it interfere with your spots. Pick a marker dot off the sheet, place the sticky side on the bottom glass plate. It should have enough stick to stay on the plate during the scan, store o/n and pick. If the marker moves during any of these steps before picking, you will have to rescan and do a new pick list.
4. Open scanner lid. Push up the lid release under the center front of the sample lid until the lid opens.
5. Clean the glass platen and sample lid. Clean before and after you scan each sample. Wear gloves.
    a. Clean the glass with EtOH using a KimWipe.
    b. Then clean the glass with dH2O using a KimWipe. This step is important – EtOH fluoresces.
    c. Do not use window cleaners because these contain some ingredients that fluoresce.
    d. Use only soft tissues to prevent scratches.
    e. Clean the sample lid the same sequence as platen.
    f. To prevent liquid from seeping inside the lid or rolling down onto the glass, do not spray the lid.
6. Place long black holder on end of platen, side next to you. Place short piece on other side of gel plate. These are the gel alignment guides. These guides are not necessary for microtiter plates. These are spacers that raise the gel 0.2 mm above the glass platen, which prevents optical interference and eliminates the need to use dH2O or buffer on the glass platen. When selecting the Scanner Control parameters, make sure you select the +3 mm parameter for the focal plane, the Press Sample check box and the correct tray definition.

7. Lay gel on glass platen with + end/top left corner towards you. Positioning the gel is important. To minimize scan time – place top + end/top left corner of the gel near the A1 corner of the grid. Position a rectangular gel so the shortest edge is along the numbered side of the glass platen. This minimizes the scan time. To minimize the image file – scan only the number of grid squares covered by the sample (see below for instructions). If you do not want to scan the whole gel, scan the squares that contain the part of the gel you are interested in analyzing. You can record multiple gels – for instructions, see manual.

Scan

1. Turn instrument on, switch on the lower right side of the instrument. After initialization and a self-test, the green light will be solid. Let the instrument warm up for ~30 min.
2. Turn on computer or log in.
3. Double click the Typhoon Scanner Control icon on desktop. If it's not there, go to C:/Scanner/Typhoon Scanner.
4. Select a template if you want. If a template exists with the parameters you want to use, select the template. Templates/Load/select template name from the list; Cy 235 or sypro, etc. If you want to set up a new Template, you can borrow the manual for instructions.
5. On the right side of window are all the setup parameters.
6. The first is the user name - whatever name you logged in on.
7. Select the acquisition mode. Select Fluorescence. Note Setup button to right. You will need this for #18.
8. Tray – Ettan DALT (2D CyDye gels) or User Select. See #17 for instructions about user defined scan area.
9. # gels – 1 or 2
10. press sample box – check
11. Gel orientation. With the top + side of the gel at A1, have the "R" sideways with the long end laying down and top of "R" towards Typhoon. You can select other orientations, just by clicking on the "R" button. There are 4 choices for how the top of the gel is oriented. You want the "R" pointing in the direction of the top of the gel.
12. Pixel size. Start with 1000 μ until the PMT voltage is optimal (see #28). Then change to 100μ. Choose the largest pixel size that provides the best resolution for your sample. Using the

smaller pixel sizes increases the scan time and the image file size.

13. User comment – if you want
14. Focal Plane - +3 mm (Default) – leave; if
15. Image Analysis – Image Quant. Select DIGE File naming format box if using CyDyes. You need the files to have certain extensions to be able to crop in Image Quant and be able to use these images in Decyder.
16. User Defined Scan area. Click on gray scan area and the white squares will turn gray. Select squares where you have your gel – note A -> R and also 1 -> 22 on the platen edges. Click, hold and drag across gel location. The drag is one way though – Click and drag up towards end of alphabet and towards end of #'s. You can't go backwards. As you drag, the gray squares will turn from gray to white.
17. Select Setup (up by Acquisition mode, button on the right. See #7).
18. Select the dyes you used in the gel. Following are the standard ones used. Start at the bottom, uncheck all boxes until you have only the top box checked (you can't change anything until the either all the lower ones are unchecked or you work from the bottom up). Check box to the left and then select the dye you used in the pull down menu. Once you have selected the first box, the next box if checked will be enabled.
19. CyDyes:
    a. Cye 3 Control
    b. Cye 5 Sample
    c. Cye 2 1/2 control and 1/2 sample (internal control)
20. Deep Purple. Laser to select and wavelength on product sheet (610 BP works best) – one of the choices on pull-down menu. When you select 610 BP – the correct laser and wavelength will be selected. Only 1 dye, so deselect all the other lines.
21. Select sensitivity not speed (bottom right of window); Speed is the Default, so every time you close and open the software, you will have to change this.
22. OK
23. SCAN. Depending on the # of scans, there is a different Save sequence.
24. More than 1dye (CyDyes) 3 scans:

a. Edit sample file name (button to right)/Edit (button to right, top)
b. C: Drive/Data (want Data folder that has a hand on it or the data can't be shared or accessed through network)/your folder (if you don't have one, create one)
c. Type in Date and what it is/Save. .ds (data set) file
d. Save.
e. You are back at the Multiple Sample Name window. Go to Common Setting for all samples - lower part of window. 1st row – folder, Browse to folder from #b./click on set
f. 2nd line, base file name, Browse to folder from #b. (data folder does not have hand) and retype (you won't see the name you did in #c) the same name that you did in #c./click on set.
g. After scan, see Decyder instructions below for cropping and saving image correctly.
h. a .gel and .Dset file are created. Important for Decyder analysis. These names have to be the same and can not be changed.

25. Single Scan: Deep Purple
    a. C: Drive/Data (want Data folder that has a hand on it or the data can't be shared or accessed through network)/your folder (if you don't have one, create one)
    b. Type in Date and what it is/Save. .ds (data set) file
    c. OK
26. Scan takes about 20 min. The data file (.gel) is a single image file. The dataset file (.ds) is an image file required for viewing multichannel images.
27. PMT 600 V (default); Important. For accurate quantitation of high-intensity samples, the pixel intensities in your scanned image should be within a specific range to take advantage of the full dynamic range of the software. To determine the correct PMT voltage:
    a. scan a test sample or scan the real sample using 1000 μm pixel size. Open into Image Quant.
    b. Two ways to determine whether any spots/bands/wells are satured.
        1) Draw a line (tool bar for line to the left)through darkest spot/spots. Select Create Graph button

(histogram on button; bottom bar, 3rd from the right). The top of the peak should be between 60,000 and 90,000. The top of the peak should not be cut off. You need for it to be less than 90,000 because when you do the 100 μ scan, the # will go up.

2) If you aren't sure which spot is the darkest, draw a square around an area of the gel. Analysis/Volume review. The Max Volume value is the # for the darkest peak. The # needs to be between 60,000 and 90,000.
3) If the # is too high or low, change the PMT voltage and rescan. Keep changing the voltage until the # is optimal. For weak samples, increase the PMT voltage in 10 V increments (you might lose quantitation accuracy at the upper end of the signal range). Do not go over 700. For high-intensity samples that saturate the system, decrease the PMT voltage to bring the high-intensity signals into the linear range of the instrument in 10 V increments (the image might lose sensitivity and quantitation accuracy at the lower end of the signal range). For the best sensitivity, use the High sensitivity parameter with a medium PMT voltage (600-800V).

28. Monitoring the scan progress. After you start the scan, the ImageQuant window opens and the green Scan light flashes. The image will appear as it scans along with # of data lines scanned and the total scan time remaining.
29. At the end of the scan, the preview window will display a Complete message and the green light will turn off. At the end of the scan, the image will automatically appear in Image Quant or whatever analysis program you selected for Image Analysis. Be patient, the window goes away and after a short time, a dialog box comes up that the image is being saved. As soon as it is saved, Image Quant will open.
30. If you need to find your data, C:/Data/your folder/name of scan.
31. Clean the platen and sample lid well with EtOH and H2O.

Image Quant

Gel image launches into Image Quant if you selected it under Image Analysis. If you didn't, open image in C:/Data/your folder/name. You need to trim the edges off of your image so it doesn't interfere with the spot detection in whatever analysis program you are using. The image must be treated/trimmed differently depending on whether you are using Image Master or Decyder.

Image Master.

1. Select the square button on left panel. Place the cursor on the upper left corner of the gel area you want to box. Run square to right and then down to bottom of gel. Be sure to include reference markers. Try to not include any dark areas usually on the edges – this will interfere with the spot detection. Edges of box are red.
2. Select Define Region of Interest button, last button to right of second row of buttons (has hatched square in middle). Click anywhere in box you made. Edges of box are now blue and red.
3. Adjust gray and contrast. Select Gray/Color adjust button, 2nd button from right on 2nd row of buttons (next to ROI button).
    a. click on button in the middle of the window to right. Has a curve and black arrow on it. This opens bend and bright slide bars.
    b. Move these 2 up and down until you are happy with the image. You can see your changes in the small window at the top.
    c. Once you are happy, select Apply and your changes will be applied to your gel.
1. File/Save Region of Interest as.
2. Name image and place in appropriate drive and folder. Change save as filetype to TIFF (.tif). Save. A .gel file cannot be opened in ImageMaster.

Decyder

1. Close image that opens after scan.
2. All images for a gel (Cy3, Cy5 and Cy2) must be cropped the same and must be saved in the correct format for Decyder to work.
3. After scan, browse to My computer/C:/Data (with hand)/ your folder. Your folder should have a .dir folder and .Dset file are

created. Important for Decyder analysis. These names have to be the same and can not be changed. This file and folder are created when the window opens after selecting Scan button asking about where and how to save images, see Scan #24.

4. Open folder. Folder has a .Dset and .dir folder (3 dyes/3images). Open Dset.
5. Opens the overlay option in Image Quant. Only 1 image appears, select the book button and you can see all 3. Select 1, 2 or 3 buttons to see each color. Select all 3 to see all 3 colors.
6. Crop in dset.
7. Select Region of Interest button and draw a square. Place the cursor on the upper left corner of the gel area you want to box. Run square to right and then down to bottom of gel. Be sure to include reference markers. Try to not include any dark areas usually on the edges.
8. File/Save Region of Interest as. Name. This creates 2 files again, .dsf and a .dir folder. Export the .dir folder to Decyder. The folder has the 3 images and a dset file.