CIAN Typhoon trio + scanner:

mini-manual for fluorescence scanning

Note: This mini-manual is somewhat superficial because the procedure depends on your sample type (gel in glass, gel without glass, wet membrane, dry membrane) and the fluorochrome you are scanning. For more detailed information, ask me directly, or check in the manual.

Elke

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Warm-Up and Cleaning

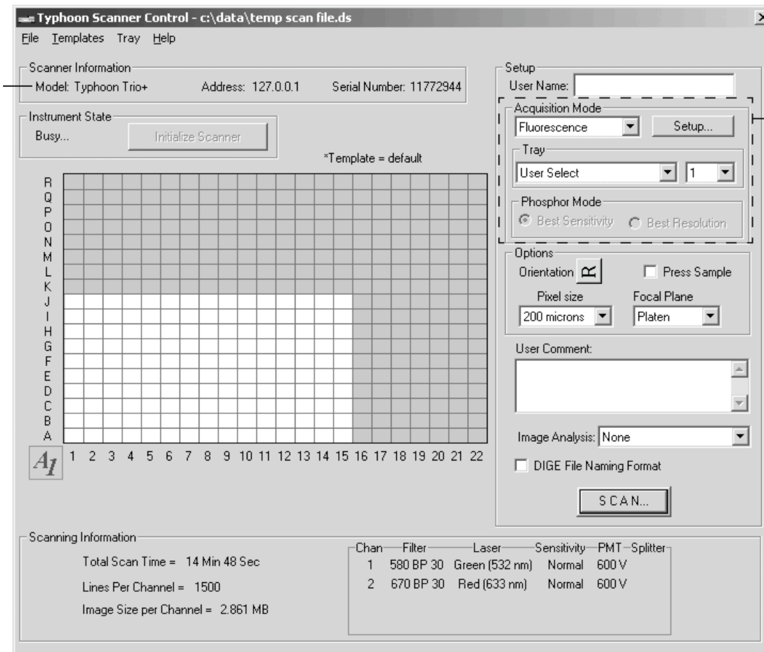
- The scanner needs to warm up about 5 min from stand-by, and 30 min from shut off. To start the warm up, open the scanner control software. The on/off button is on the right side, lower edge toward the front of the scanner. Don't turn off unless specifically instructed.
- Clean the glass platen with 70% ethanol, followed by destilled water. Use only Kimwipes, never paper towels.
  If fluorescent dyes have come in direct contact with the platen, use 10% H_2O_2, followed by water.

Sample Placement

- Place your gel or membrane on the platen, fluorescent side down. To keep your sample from drying out, you can put a little water or appropriate buffer on the platen before. Smooth out any bubbles. Wet membranes should be covered with some plastic wrap.
- To save scan time, place one corner of your sample into the 'A-1' corner of the platen, flush with the edges, and use the front-to-back dimension before the left-to-right dimension.
- Note the position of your sample's top right corner to determine the scan area.
- Close scanner lid.

Scanner Control Set-Up

- In the **scanner control software**, load the appropriate settings **template** from ‘templates--load’.
  Templates can change, so check all settings.
  If no template for your scan is available, adjust another one to your liking and save it later under an easy-to-understand name for future use.

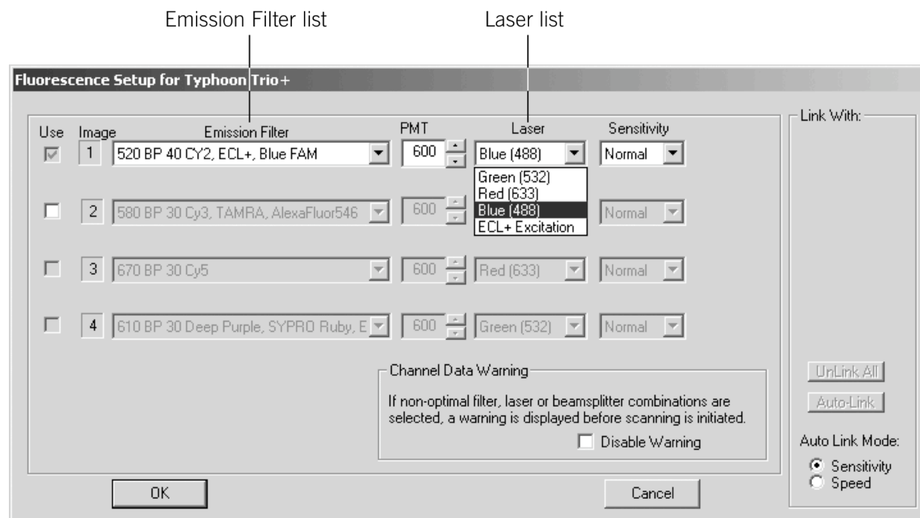


- On the right hand side of the window, set up your scan.
  - **User Name**: irrelevant, CIAN user is entered by default
  - **Acquisition Mode**: set to ‘Fluorescence’
  - **Setup… button**: use to set lasers, filters, and sensitivity; see below
  - **Tray**:
    - *User select*: scan area is selected by clicking on the A-1 field and then dragging up and to the right; everything is scanned into one file
    - *All other tray names*: pre-defined scan areas, e.g. for up to two DALT-size 2D gels that are always placed on the scanner in the same way; number of gels/membranes can be set in the pull-down on the right, each gel is scanned into its own file, scan will always start from the left; new trays can be defined in the ‘Tray’ menu
    - Phosphor Mode: n/a
  - **Options**:
    - *Orientation*: The ‘R’ represents your sample, seen on the scanner platen from the front edge of the scanner, from the

top. Select the orientation you put the sample on the platen, so that the final image will automatically be right-side up.

- *Press Sample*: Check this box for gels in glass plates, dry membranes, or wet membranes covered with foil. A foam pad in the scanner lid will press down on the sample to keep it in place.
- *Pixel Size*: Select pixel size, i.e. edge length of scanned pixels in μm, between 1000 (for pre-scan) and 100 (for final scan), or down to 10 (for extremely small samples like spotted micorarray slides)
- *Focal Plane*: 'Platen' for all samples that are directly on the glass platen; '+ 3 mm' for gels scanned in 3 mm low-fluorescence glass plates, or for thick agarose gels

  - **User Comment**: mostly n/a, can be used to put comments into scanned file; all scan conditions are already automatically recorded
  - **Image Analysis**: n/a, leave on 'None'
  - **DiGE File Naming Format**: Check for DiGE gels that will be analyzed in DeCyder



- **Fluorescence Setup Window**
  - Check '*Use*' for the number of fluorochromes you are scanning
  - *Emission Filter*: For each fluorochrome, select appropriate emission filter from drop-down menu
  - *PMT*: photomultiplier tube voltage, the higher the voltage, the more intense your signal will be; can be set between 0 and 1000 V, should be kept between 400 and 900 V for linearity, 500-600 V is a good starting point; a 50 V increase will roughly double the signal

- *Laser*: select one of the three lasers (or the special setting for ECLplus); the system automatically defaults to the recommended laser for the emission filter selected, but this can be overridden; especially for membranes, try different combinations to achieve minimal background signal
- *Sensitivity*: ‘Normal’ is good for most applications, ‘Medium’ and ‘High’ increase the number of times the scanner passes each pixel, subsequently increasing scan times four- to eight-fold, respectively
- *Link With*: allows linking scans of different fluorochromes via beamsplitters, not recommended for quantitative work, ignore settings

Typhoon trio+ emission filters and excitation lasers

filter	recommended laser	applications
390 BP 100	green (532)	phosphorimaging
520 BP 40	blue (488)	Cy2, ECL+, Blue FAM
526 SP	green (532)	Fluorescein, Cy2, Alexa 488
555 BP 20	green (532)	R6G, HEX, Alexa 532
580 BP 30	green (532)	Cy3, TAMRA, Alexa 546
610 BP 30	green (532)	DeepPurple, Sypro Ruby, EtBr
670 BP 30	red (633)	Cy5

Start scan

- Click ‘Scan’ button in scanner control window.
- Select output file location within C:\data; make sure to put all files into a user folder within your lab’s folder, data files in other locations may be deleted without prior notice
- Each fluorochrome scan creates one .gel file, and multi-channel scans also create a .ds (dataset) file that describes which ones belong together; the default name is ‘temp scan file’, which can be changed to your specifications

Monitor and evaluate scan

- During the scan, a preview window shows the progress. The contrast and brightness are adjusted automatically to what the computer thinks is optimal, they do not tell you anything about the actual pixel intensities. The only exception are oversaturated pixels, which appear in red.
- A scan in progress can be cancelled; because of the very short dwell times of the laser on each pixel, effects of photo-bleaching are so minimal that quantitation is not affected by partial scans.
- To assess the quality of a scan, open it in ImageQuant, Tool Box module. The maximum pixel intensity is 100000 (saturation level). Check some spots or bands of high intensity by mousing over them, and reading the intensity value in the lower left corner of the screen, or draw a box around an area of interest and read the maximum intensity value from the resulting table.
  To make best use of the system, try to adjust the PMT voltage so that your maximum pixel intensity is between 50 and 75k in a low-resolution scan.

Finishing up

- Remove your sample from the scanner, and clean the platen and lid.
- Close the lid. Scanner stays on. It will automatically go into stand-by mode.
- For further processing your Typhoon scanner images, see the ‘CIAN Viewing and Editing Typhoon images’ document. It is available on our web site.
- A mini-tutorial on ImageQuant for quantitatively analyzing your gel images can be arranged on demand.
- The files on the Typhoon PC are not backed up. Make sure to move a copy to a safe place. Let us know if you are interested in setting up an account on the CIAN server for this purpose.