Cytiva Amersham Typhoon5 Biomolecular Imager

STANDARD OPERATION PROTOCOL

Cytiva Amersham Typhoon5 Imager

Standard Operating Protocol

I. Basic Specification:

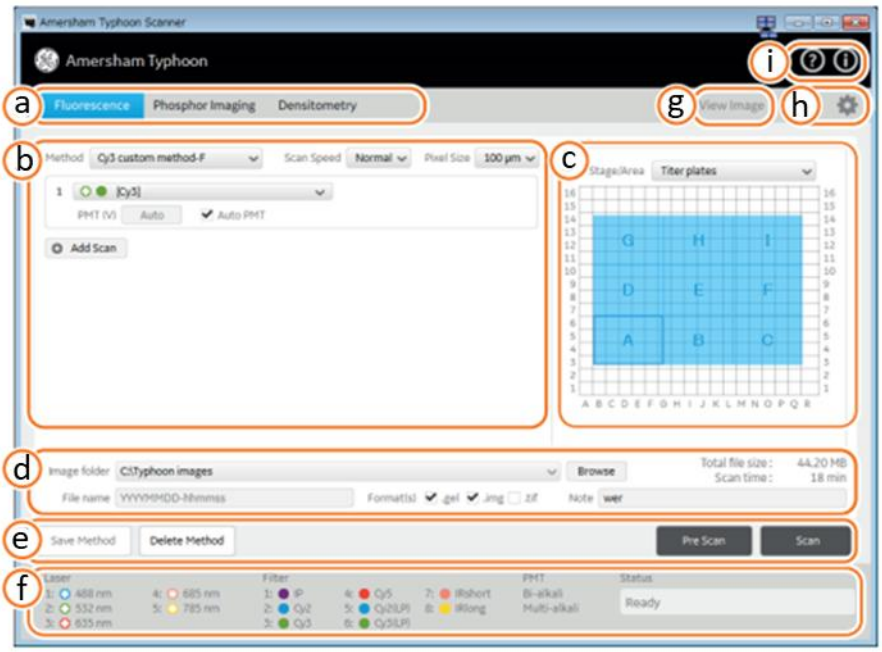
	Typhoon5 Imager
Detection Modes	OD measurements RGB Fluorescence NIR Fluorescence Phosphor imaging
Sample types	Gels (up to 20pcs) Membranes (up to 20 pcs) DIGE Gels Microplates (up to 9 plates) Phosphor imaging Screens Animal Tissues
Application	Western blots (Coomassie blue staining/ chemiluminescence / fluorescence) 2-D DIGE Fluorescence of whole tissue Assay detection Phosphor imaging

II. Initialization

1. Turn on the power buttons according to the numbers (②)
   ①: Main switch for Typhoon5 (at the right back of the instrument, keep always on)
   ②: Power switch for Typhoon5 (at the front of the instrument)
   It takes around 5 mins for the system to be ready (steady white light on the machine).

2. Log in PPMS tracker.

3. Start the Typhoon Software on the desktop. Ensure that there is no error for all the lasers and filter (f).



4. Choose appropriate stage for the capture and but your samples (signals facing **downward**) onto the stage. Remember the location of the sample for later steps. Samples are suggested to be put from lower left corner.

5. Insert the stage into the machine until a 'click' sound & the two arrow tips are clearly seen. Close the door firmly.

6. Choose the corresponding capture mode **(a)**.

7. Choose existing method/create a new method **(b)** for the lasers you wanted to capture. *For chemiluminescence/bioluminescence, select 'Dark Scan'* To save tailor-made method, press 'Save method' **(e).**

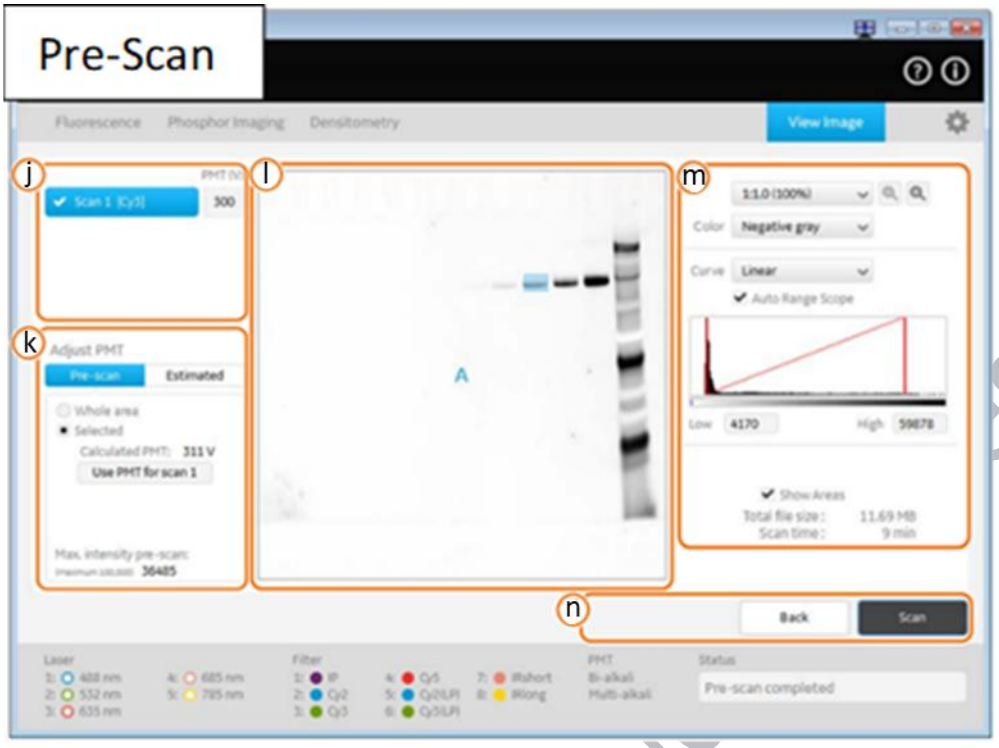
8. Adjust the PMT (manually/automatic), pixel size (resolution), scan speed. Recommended PMT: 500-600V; Max. PMT: 800V, if not there will be high background.

9. Choose the sample position by dragging the box **(c)**. There is some preset stage/area, choose from the drop-down list. To increase number of images, simply drag the area wanted. To remove unwanted area or extra setting, right click or press 'delete' on keyboard.

10. Choose the save location (*C:\Typhoon\Department\PI\user*) and name the file name as *'PI initial_user name_date_details'*.**(d)**. Files will be saved as capturing date-time if not specified. File size and estimated time will be shown at the right corner.

11. Choose the file type (recommended to tick all three) .img: Allows preservation of broad dynamic range .gel: Typhoon-specified; Accurately represent small differences in data .tif

12. Press 'Pre-Scan' or 'Scan'



13. After pre-Scan, one can have a quick look about the intensity, and determine which PMT is the best for the capture using selected area/whole area. **(k)**
    Typhoon5 provide 'Estimated' tab to simulate images using different PMT settings.

14. Click 'Start' to start capturing. Captured image will be save automatically into the selected folder.
    When pressing 'Stop', the scanned image will be saved and the scan CANNOT be re-started from the stopped position.

15. Edit image (contrast, zoom) with 'View Image' tab.
    Right click to copy the displayed image or save displayed setting with 'Save display'.

16. Transfer data by data transfer server.

III. Turning off the system

17. Remove any stage from the scanner.

18. Clean the stage with kimwipes with (1) 70% Ethanol (2) water thoroughly and (3) dry with a kimwipes.

19. Place the stage back in the corresponding stage cabinet.

20. Turn off the power button ②
    *No need to turn off ①.
21. Log out PPMS tracker.

IV. ImageQuant

Detail instructions are available in video located in desktop/under 'Help' section of the software.

1. Open the software IQ in desktop.

2. Choose the corresponding analysis mode.
   - 1D gel analysis: Western analysis
   - Analysis Toolbox: For multiple sample types
   - Colony Counting: Petri dish colony counting
   - Array analysis: Multi-well plate assay analysis

3. Open image wanted (must be .tif).

4. Perform corresponding analysis. Can edit image's contrast, merge images, …

V. Supplementary notes:

Fluorescence & Densiometry Imaging

Gels/membranes

1. Place gels/membranes onto the 'Fluor sage' with signals facing **down**.
2. Remove any air bubbles between the gel/membrane and stage.
3. If the sample is a membrane, place a membrane weight on top of the membrane to reduce vibrations during the scan.

Titer plates/glass slides

1. Align the guide plate with the grooves for **titer plate holder**.
2. Place the titer plate holder on the multi stage and lock the springs to hold the holder in place.
3. Place the titer plate on the desired position in the titer plate holder. Preferably form lower left.
4. For glass slides, insert the glass slides in the glass slide holder and place the holder on the titer plate holder, with the glass slides facing **down**.

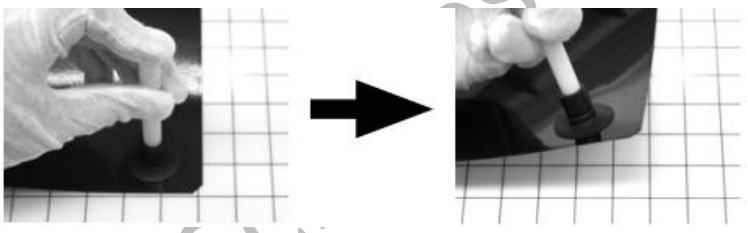
DIGE gels

1. Align the guide plate with the grooves for the **DIGE gels**.
2. Place the DIGE gels on the stage, signals facing **down**.
3. Close the spring locks that hold the DIGE gels in place

Phosphor imaging

- Protect storage phosphor screen from water (sensitivity of the storage phosphor screen will be reduced if water enters), volatile solvents (may deform the protective film on storage phosphor screen), light & dust.
- Remove the storage phosphor screen from Typhoon when not in use. Store the storage phosphor screens in a horizontal position, protected from moisture.
- Radioactive isotopes containing sample ***MUST NOT*** directly contact the phosphor screen. The sample should be dried and completely wrapped at least two layers of plastic film (properly folded) to avoid leakage.

Perform Step 1 – 7 in your own lab



1. Use the suction rod to transfer the storage phosphor screen out.
2. Clean the screen surface with kimwipes and the inside of the cassette with 70% ethanol to remove dust and stains.
3. Optional: Erase the phosphor screen completely with Amersham Eraser in 10-20min.
4. Wrap the radioisotope sample completely in two layers of plastic film. Make sure there are no wrinkles in the film.
   Avoid radioactive contamination and liquid leaks
5. Place the sample on the cassette, with the surface of the sample facing **up** and place the phosphor screen onto the sample, with the exposure surface (white side) facing the sample.
6. Close the cassette and leave the phosphor screen for the required exposure time.
7. Remove the sample and keep the phosphor screen in the cassette and transfer to L2-01.
   Must not bring radioactive samples into L2-01!!
8. Turn the 'Phosphor stage' **upside down**, so that the white print faces down.
9. Open the cassette and transfer the phosphor screen with the suction rod to the 'Phosphor stage' with the exposed surface facing **up**.
10. Turn the phosphor stage around and place the 'Phosphor stage' onto the stage holder, with the white print on the phosphor stage facing **up**, and the storage phosphor screen attached to the bottom of the phosphor stage. The exposed surface (white) of the storage phosphor screen should faces **down**.
11. After the scan, clean the surface of the screen and the cassette with kimwipes.
12. Erase the screen with Amersham Eraser in 10-20 min, if it is not overly exposed. The screen can be re-used after erasing.
    *A tritium storage phosphor screen can be used only once.

Amersham Eraser

13. Turn on the power switch at the back of the Eraser.
The indicator lights up and the Eraser enters standby mode.

14. Pull out the tray and put the phosphor screen with the exposure surface (white side) faces **up**.
15. Push the tray back and press the Run button to set erasure time and start erasing.
16. As erasing time elapses, the blue indicators will turn off one by one from right to left. If the tray is withdrawn during erasing, erasing will be canceled at that point.
17. Pull out the tray and remove the phosphor screen when finished.
18. Clean the tray with kimwipes with (1) 70% Ethanol (2) water thoroughly and (3) dry with a kimwipes.
19. Turn off the Eraser.

Condition	Erasure time
Normal	10 minutes or more
Over-exposed	40 minutes or more
Unknown	40 minutes or more

		Total run time
One	▶	10 min
Two	▶▶	20 min
Three	▶▶▶	40 min
Four	▶▶▶▶	60 min