Bauer Core Standard Protocol		
Title: Using the Amersham/GE Typhoon Imager		
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1. Purpose

This protocol provides instructions for using the GE (Amersham) Typhoon Trio Imager for storage phosphor autoradiography, and fluorescent or chemiluminescent imaging. Three excitation wavelengths are available for fluorescent imaging: red (633), green (532) and blue (488). The Typhoon can acquire images from phosphor screens, gels, and microtiter plates, and it has an adjustable focal plane to allow for imaging gels between glass plates. The resolution can be adjusted down to 25 micron pixels.

2. Materials

2.1. Cassette and Screen (e.g. Amersham/GE # 63-0034-86)
2.2. Intensifying screen cleaner and antistatic solution (Kodak #106 4930)
2.3. Labeled gel or membrane

3. Instrumentation

GE (Amersham) Typhoon Trio Imager

4. Reagent preparation

none

5. Procedure

5.1. Expose radiolabeled samples to a phosphor screen

5.1.1. Sign out a screen and cassette from the Bauer Core or use your own.
5.1.2. Place your radiolabeled membrane in the cassette.
5.1.3. Place the screen face down on top of the membrane .
Do not fold or bend the screen – treat it as if it were glass.
5.1.4. Exposure should take 1/10th of the time that film exposure takes (usually 1-2hr).

5.2. Scan the Image

5.2.1. The power to the Typhoon is usually left on (the power light will indicate this).
5.2.1.1. The power switch is on the right hand side of the instrument.
5.2.2. Place the sample face down on the bed of the scanner.
5.2.3. Open the Typhoon Control Software.
5.2.4. Choose the type of sample: phosphor, chemilumicescent or fluorescent.
5.2.4.1. Phosphor: choose best sensitivity for most applications.

5.2.4.2. Chemiluminescence: click setup

Choose sensitivity - number of times to scan and average each line.

Normal = 1, Medium = 4, High = 8.

Don't use beam splitter (this mode is not yet optimized)

5.2.4.3. Fluorescence: click setup

Choose up to four fluors to be scanned.

7 band pass filters are available.

3 lasers (488, 532 and 633) are available.

The software will warn of a bad filter/laser combination.

The PMT is linear between 400-1000.

The "Link" feature allows fluors to be scanned simultaneously.

The default is to scan sequentially to prevent crosstalk.

5.2.5. Choose the scan area (called the "tray").

5.2.5.1. User defined: manually select the area on the grid.

5.2.5.2. Several pre-defined "trays" exist for gels and microarrays.

Numbered areas in the pre-defined trays are saved as separate files.

5.2.6. Choose the pixel size

5.2.6.1. Use 1000 or 500 for low resolution pre-scans

5.2.6.2. Use a sharper resolution (200 or better) for real scans.

5.2.7. Click "Scan" and save data

5.2.7.1. Save directly to the titan server or transfer files immediately.

5.3. Image Analysis

5.3.1. Open the ImageQuant TL software.

5.3.2. Choose the type of analysis to perform.

5.3.2.1 1D Gel Analysis for standard analysis.

5.3.2.2. Use Array Analysis for microtiter plates or microarrays

5.3.2.3. Colony Counting can be used for colonies or 2D gels

5.3.2.4. Analysis toolbox has line and object tools for manual quantification.

5.3.2.4.1. This application is similar to Image Quant on the Storm.

5.3.3. Open the image file to be analyzed.

5.3.4. Use Edit Image, Colour and Contrast to alter image appearance.

5.3.5. Use the bar at the left of the screen to quantify bands.

5.3.5.1. Edit options as needed using the Preferences tab.

5.4. Clean Up

5.4.1. Remove the sample from the scanner.

5.4.1.1. Remember to clean the bed if necessary.

5.4.2. Close the software and log out of the computer.

5.4.3. Phosphor Screen Care.

5.4.3.1. Erase screen using the light box.

5.4.3.2. If a screen needs cleaning, use only the provided cleaning solution.

Be sure that the screen is not contaminated with radioactive material.

5.4.3.3. Always store screens in the cassette to protect it.

Do not fold or bend the screen and do not store anything on top of it.