

USING THE PHOSPHORIMAGER

Overview:

Use of the Phosphorimager represents a quicker and quantitative method of visualizing radioactive gels. The Phosphorimager screens need only be exposed for 1/10th the time normally exposed to film. The software package offers various features for quantitation of the data.

The procedure for using the Phosphorimager involves: exposing your dried gel to the Phosphorimager screen, scanning the screen, and analysis of the data.

Starting up:

- The ImageQuant software will automatically boot up when the computer is turned on; however, if for some reason the computer is in DOS, do the following to start up ImageQuant:

1. Type **WIN** to start Windows.
2. Double click on the **IQ** folder.
3. Double click on the **IQ** icon to start ImageQuant.

Exposing a gel:

- The gels should be completely dry. **DO NOT** expose the screens to wet gels or wet chemicals!

1. Erase the Phosphorimager screen by placing it on the eraser underneath the desk and pressing the ERASE button. A yellow light will come on and the erasing should take approximately 10 min. Note: the time dial should be set at 10 min or more.
2. Remove the screen, being careful to avoid exposing the screen to any direct light, and place it on top of your gel either in a cassette or on a flat surface. If you are using a flat surface, also place a weight on top of the screen.
3. Expose the screen for the times indicated below based on the type of gel and type of screen being used:

Screen Type	Gel Type	Exposure Time
MD Metal Backed	Gel Shift Assay P32	1 hour

	Sequencing Gel S35	1 hour
Homemade Glass-Backed	Gel Shift Assay P32	overnight
	Sequencing Gel S35	overnight
	CAT Assay C14	overnight
	Run-on Txn P32	overnight

Scanning the screen:

1. Remove screen from gel, being careful to minimize exposure of the screen to direct light.
2. Open lid and place screen onto the stage. If you have a small screen, then use the adaptor tray. Close the lid.
3. Go to the computer and select **Scan sample** under the **SCANNER** menu item.
4. A dialog box will appear asking for the coordinates for the scan and a filename. The default coordinates are for an entire small screen. Open the lid and check along the edges of the stage to see what coordinates you want to use. Note: there is also a number at the bottom of the dialog box which should be 176; if its not, change it to 176. This is the scanning resolution and 176 is optimal for the type of work we do.
5. Click on **SCAN**. It will take a few minutes for the scan to be completed.
6. There will be two windows: the right one shows the entire scanned region, the left one shows a selected region of the scan, outlined by a box in the right window. The left window is the “working” window in which a number of manipulations can be performed, quantitation in particular.
7. The size of the left window can be changed by dragging the lower right corner pressing the left mouse button. The sensitivity of the scan output can be changed by clicking on **VIEW** and selecting **Grey/Color Adjust**. A separate window will appear allowing you to change the upper and lower thresholds for the display window.

Note: most likely, the lower number will need to be changed to the minimum, 0.012, and the upper number can be gradually reduced until the desired band intensities result.

