

Biology 324 Instructions for the Ultra Lum Gel Documentation System

Fluorescent dyes, such as ethidium bromide, are often used to tag nucleic acids. Ethidium bromide intercalates into the grooves of DNA and when excited by UV light (280 nm) becomes excited and emits a pink color. The traditional way of archiving a gel stained with ethidium bromide is to use a UV transilluminator and polaroid film. Instead, we will be routinely using an Ultra Lum system for digitizing the image. The CCD camera and framegrabber is controlled by a version of NIH Image called Scion Image.

The ultra lum system consists of :

- 1) a UV transilluminator
- 2) a white light transilluminator
- 3) a UV epilluminator
- 4) a white light epilluminator
- 5) a Cohu CCD camera
- 6) A macrolens
- 7) a Scion LG-3 framegrabber in a Powerbase 180 computer

To use this system:

A) Place the gel (with Saran Wrap) in the middle of the UV transilluminator. Keep the curtain open.

B) Open Scion Image Program:

- 1) Turn on the computer with the top right button on the keyboard
- 2) Launch "Scion Image" Program from the Apple Menu
- 3) Under the "Special" Menu, launch "Start Capturing".

C) Adjust Macrolens

Open the aperture to the most open position (F 1.4). (The aperture is controlled by the top lever on the macrolens). This will allow the highest amount of light into the camera.

D) Center Gel

The incident light should be high enough to see the outlines of the gel on the computer. If the light is still too low, turn on the overhead white light. (The controls for the overhead (epi) illumination are at the top right of the Ultra Lum.).

E) Adjust the zoom and focus.

The zoom and focus controls are the two lowest levers on the macrolens.

F) Close curtain and turn on UV transilluminator.

The controls are on the lower part of the Ultra Lum. **NEVER LOOK AT UV LIGHT WITHOUT WEARING UV PROTECTIVE GLASSES.**

G) Adjust framegrabber for appropriate brightness and contrast.

Launch "video control" in the "Special" menu. At the bottom of the video control there is an offset and a gain control.

The offset control is used to adjust the brightness of the image. Decreasing the offset will increase brightness.

The gain control is used to adjust contrast. Increasing gain will increase contrast.

If your gel is too dim, lower the offset until the bands are bright. Then increase the gain. Continue to adjust the offset and gain until you have reasonable contrast. To make sure you haven't saturated the image, select the "Highlight Saturated Pixels" box.

H) Collect a frame averaged image

Usually the image has a fair amount of random noise, primarily due to thermal noise of the CCD camera. To remove this noise, select "Average Frames" from the "Special" menu. At the bottom of the "Average Frames" menu is the default range for the pixel values (0- 4096). (The program apparently assumes that you are digitizing a 12 bit image.) Set the number of frames to be averaged to somewhere between 10 and 60. Be sure you understand how averaging works to remove random noise.

I) Save image in 324 folder on computer.

A Biol 324 folder will be on the desktop. Make a folder for yourself and your partner and save your gel.

J) Label gel.

You can cut the relevant portion of the image and paste into a new NIH Image document. If you wish to print the image, you should invert the pixel values (ie. make the black pixels white) as it will significantly save on toner on the printer. To invert pixels, select "LUT options" under the "Options" menu.

To label the gel, use the eye dropper to select a dark color. Then use the Font tool to print a label on your image.

K) Print image

You can use the fourth floor networked laser printer in the kitchen area. To select a printer, go to "Chooser" under the apple menu and select "HP printer" icon and "NW 416 HP 4M plus". Under "File" menu, select "Print image".

Note that also there is a video printer that can be used to print the image coming directly from the CCD camera.