DETAILED PROTOCOL FOR USING KODAK IMAGE STATION 440CF

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This is a protocol for capture, storage, analysis, and decoration of all images acquired by any light source including chemiluminescence, fluorescence, chromogenic detection, and densitometry (ECL Western blot, Silver staining, ethidium bromide gel, films..).

1. Choose the appropriate filter and illumination conditions using the Filter Selection Dial located at the top of the Capture System Chamber.

Note: Always turn the Filter Selection Dial clockwise.

> For ECL Western blot membrane, protein staining gel, and exposed film, turn the dial to #0 (nude) position.
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> Make sure that UV light is "OFF" (the UV light source is inside the cabinet, there is only one button you can hit; push downward → off). Turn on UV light source if needed (for ethidium bromide and fluorescent dyes only).

2. Lift the lid over and place your sample in the center. If you use ECL blot membrane cover the membrane with the sponge cushion with the white area directly on the top of your membrane. Close the lid tight. For absorbance (ambient) light source (protein staining gel and exposed film), open the lid and use the light diffuser instead of the sponge cushion on the top of your membrane to evenly distribute the light on your sample. Do not close the lid when using the imager for this purpose.

3. Start the image capture program.

4. Click File → Acquire → Image Station. Now you will see the Image Capture Settings on the monitor.

5. For preview, click "Preview" then "Expose". This provides a live-image during which sample formatting and capture adjustments (to locate the sample in the center, to enlarge or reduce the size of image, to focus and to adjust the aperture) can be accomplished. You may fully open the aperture (f-stop: 1.2) to do the sample adjustment (you may see better when the aperture is fully open). The aperture (f-stop) and zoom are inside the door of the imager (only one door!). The operation is similar to your camera.

6. Click "Stop" and uncheck "Preview". Set up the number of captures and exposure time using the Capture and Exposure Time Slider (for example, 32 captures -- 15 seconds for ECL Western membrane; 4 captures -- 15 seconds for ethidium bromide gel; 1 capture -- 0.07 second (f stop: 8-11) for exposed film). Always click Auto

Contrast and click Invert if preferred. Click “Expose” and enjoy your results. Click “Stop” any time if the captured images are satisfied.

NOTE: Best signal to noise ratios obtained if you do fewer captures for longer time periods. For example, for low intensity bands, do 6 five minute captures instead of 120 15 second captures.

7. If the image is underexposed → enlarge the diaphragm using the lower f stop located in the Capture System Chamber and/or check the X and Y Binning (but lose resolution) and/or increased the exposure time.

8. Now you can have a quick print of the image from the monitor by clicking the Quick Print Button or submitting to 1D Image Analysis software by clicking the Submit Button. Using the latter option, you can save your image and do cropping, orientation, contrast, annotation, pseudocolor, band densitometry, etc. To submit image, click Submit Button and key in the exposure information including f-stop, zoom setting, and light source (you can refer to the camera inside the door!).

9. After Submit, you can save the image by clicking “Save” from the File menu. Enter a new file name, find the folder in which you want to store your project. Click Image Display to adjust the contrast by moving the Max, Min, and Sigma bars. You may perform some simple graphic arts using the graphic tools (cropping, rotating etc). Click Annotation and apply your legends, arrows, molecular weights etc. together with your bands. Always click Help if you need help!

10. Please clean the image station, close the lid and turn off the UV light when you are done.