Imaging Systems

UVP Imaging System, new (PSC 543, 657, NSC 338, 460) No EtBr
UVP Imaging Systems, old (PSC 637) EtBr allowed
Ultra-Lum Omega Imaging Systems (PSC 543, NSC 338) EtBr allowed
Ultra-Lum Omega Imaging Systems (Kell 405) No EtBr
Alpha Innotech Imaging Systems (Kell 405) EtBr allowed

Every time you use the any of the Imaging system, you MUST sign the logbook.
DO NOT use EtBr under any circumstances on the new UVP imaging sytem or the Kell Hall 405 Ultra-Lum system.
Clean up any mess you make including wiping off the light box with H2O and paper towels.
Do not cut bands on the light box. On top of the box is a plastic tray, place your gel on the tray and then place on top of the transilluminator.
Be sure to turn off power when you are done.
Do not store any images on the hard drive.

Contact: Debby Walthall (404) 413-5363; dwalthall@gsu.edu

UVP Imaging System, new (PSC 543, 657, NSC 338, 460) No EtBr

Turn all 3 off – camera, software and transilluminator

1. Turn on camera (right hand side, top near the back), and transilluminator.(right hand side, front, top of instrument). This will turn on the lower switch.
2. Place gel on transilluminator. Choose wavelength – 365, 302 (short wave selected in NSC imagers) or 254.
3. Monitor is touch screen. I find it easier to use the stylus or end of an unopened pen.
4. Double click on TS software icon. Once software comes up, press Live.
5. Select EXP WARN. This will tell you (yellow image) when the image is overexposed. You need to increase the exposure until you see yellow and then just backoff. This will get you the full dynamic range of the camera.
6. Adjust exposure using the large + or – buttons on the bottom right and left of screen. There are 3 levels – far right – milliseconds; middle – seconds; far left – minutes. Just touch level you need (the lt. purple bar will shift to the your selection). Touch + or – to increase or lower exposure time.
7. Or adjust image with rings on camera:
   a. Top ring – exposure
   b. Middle ring – zoom
   c. Bottom ring – focus
8. Press SNAP
9. To adjust image (invert, turn image), go back to Live. Select Preferences. Select PostProcessing tab (near the top).
10. Rotate image – use arrow to select; Invert – will change the image from black to white. OK.
11. Snap and now will see results of Preferences selection.
12. Press PRINT to print your image.
13. To save your image.
    a. Put a USB drive into USB port.
    b. Go to Preferences. Select Saving tab.
    c. Save images to: select usb
    d. Other selections do not change: autoprint – never; autosave – off; do not save images to computer or desktop
    e. OK
    f. Select save button on screen
14. Turn off ALL 3 – software, camera and transilluminator
15. Clean Transilluminator with H2O.

16. Turn off software – large X on lower left of window.

To Analyze UVP captured image using IQTL

1. Capture image on UVP.
2. Save as Tiff file. The image is in color scale even though it looks like gray scale. IQTL can only import Gray scale images.
3. Open the Tiff file in any graphic software, such as Photoshop or Irfanview (free download).
4. Convert the image from "color scale" to "Gray scale".
5. Save as different Tiff file name.
6. Open in IQTL.

Ultra-Lum Omega 10g

General information

1. The 305 nm transilluminator is fixed and can't be pulled out.
2. Coomassie gels. Remove the white tablet from one of the drawers under the system. On the right hand side of the transilluminator is a cord and plug. Plug into the white tablet and place on top of the transilluminator.
3. Filter selection. There is a laminated list of available stain in a drawer under the system. Locate your stain and then what filter you will need to use. Be sure to look at the filter column, NOT the epiluminescence column.

Imaging Box

1. Power on button is on the right.
2. Far left – Door interlock disabled. Punch this button so that you can have the transilluminator light on when you cut out a band. Since you are using filters instead of UV light, you should be safe.
3. Camera ready. Comes on when you turn the power switch on.
4. Epi Illumination buttons, with UV and also WL(white light) buttons. You shouldn't need any of these. Epi-Illumination is used when you require fluorescent excitation different than 300nm UV to reflect light off the sample. Usually the samples are opaque, ex. TLC plates.
5. Transillumator. When the power button is turned on, the Transillumator is off. After you turn it on, the default is 10. The #'s 1 (weakest) – 10 (strongest) are how bright the bulbs are. If you are going to cut a band out, you need to have this # as low as you can and still see the band. You use this button (up and down) as well as the Iris button (open and close) to get a clear image of your gel.
6. Zoom. Up and down.
7. Focus. Up and down.
8. Iris. Open and Close. This controls the camera aperture (exposure). You use this button (Open and close) as well as the Transilluminator button (up and down) to get a clear image of your gel.
4. Filter Selection. When the power button is turned on, the default is none. Select appropriate filter for whatever stain you are using. There is a laminated list of available stain in a drawer under the system. Locate your stain and then what filter you will need to use. Be sure to look at the filter column, NOT the epiluminescence column.

Agarose gels

1. Open darkroom door.
2. Place gel on UV box. Position the gel appropriately.
3. Close darkroom door completely and turn knob. The transilluminator will not work if the door isn't closed properly.
4. Turn power on light box (button on right).
5. Make appropriate adjustments.
   a. Turn Transilluminator on. Lower # to ~5.
   b. Set filter to whatever your stain is – see laminated list.
6. Go to computer. Open UltraQuant 6.0.
7. Select Camera button.

8. Select Preview. Select #1button to reset to default settings (200mm for preview and acquire). Check saturation warning button.
9. Make appropriate adjustments for your image.
    a. Zoom in and out to image size you want.
    b. Focus up or down as needed.
    c. Iris – open or close as needed to visualize your bands. Use both the Iris and the transilluminator #'s to set your gel image
    d. You should not see any red (saturated). If you see red, you are past dynamic range and can lose resolution.
    e. Adjust the Iris and transilluminator until you see red and then back off until the red goes away.
    f. You can also adjust the exposure on the Capure Dialog window. Change the exposure by either clicking on the MM; ss; mm, the #'s will be highlighted and just type in the # you want; use the up or down arrows; use the slide bar below the Exp Pvw or Exp Acq. Notice that there is a lock on the left side of the Exp Pvw and Exp Acq. With this lock closed, any change to Exp Pvw or Exp Acq will change the other.
10. Stop preview.
11. Snap; turn power off light box.
12. There are several things you can do now to improve brightness and/or contrast.
    a. Enhance/Display range. Move bar on right to left with cursor.
    b. Button. Image Enhancement – move brightness, contrast and gamma slides.
    c. Button. Optimize Image. Automatically optimizes.
    d. Button. Reset Image – goes back to original.
13. Clitch in software to print. Save image. Close image. Reopen image – can now print. Print – use either print button or file/print. Do not use the print button on the printer. Select # of copies. Setup button will change usual print parameters (portrait vs. landscape). You can press Feed button on printer to get extra clearance before tearing off.
14. When finished:
    a. Close out all boxes
    b. Close out your image (save changes/no). Do not save ANY images on the hard drive.
15. Remove gel

*Turn power off light box!!!!!!

1D-Gels – Label, Quantitate. Consult with core personnel for instructions.

If you don't see an image or it is faint, be sure you adjust the following:

1. Transilluminator. Increase #'s.
2. Iris. Increase aperature on camera.
3. Exposure time. Increase exposure time - when you open the window to preview, the default (#1) is 200. Increase this #.

If you try to use Display Range (UVP) to see your image, the image maynot print. Use the above three to get your image set. You can still use Display Range, but don't use it exclusively.

Saving image to use in Adobe Photoshop.

1. Edit/Convert to/select either Gray Scale 8 or Gray Scale 16.
2. File/Save as/TIF.

Images to Adobe Photoshop

1. Edit/ Convert to/ Select Gray scale 8 or Gray Scale 16
2. File/ Save as/ Select TIF or JPEG/name file/Save

UVP directions, old

Agarose gels

16. Open darkroom door with an ungloved hand.
17. Place gel on UV box with a gloved hand. Position the gel appropriately.
18. Confirm that UV box is on (green switch) and set at 302 (button on transilluminator box, right hand side). Use an ungloved hand to change either of these. The other choice is 365, which is used for DNA bands that will be cut out for various experiments. The longer wavelength is supposed to prevent DNA nicking.
19. From now on – no gloves for any reason.

20. Close darkroom door completely.
21. Turn power on light box (button on right).
    a. Knob next to power stitch should be turned to safety switch
    b. Next knob should be turned to EtBr
    c. Next knob should be on off – this is only an overhead light source and isn't used for gels
22. Go to computer. If program not up, open Labworks 4.0.
23. You can capture your image 2 ways:
    a. Select either F8 (DNA/RNA/protein gels) or F7 (chemiluminescence). Follow the wizard instructions. Screen Capture/OK/(follow instructions)/Next/(follow instructions/Next/Select standard/Next/Enter Exp. Time/OK.
    b. Camera icon. Select Preview button. Toggle the Prev exp. To increase or decrease exposure until you have a picture
24. At camera lens –
    a. Bottom ring; <u>do not touch</u>; only need to focus if someone did chemiluminescence
    b. Turn top ring of camera until fuzzy blue disappears and little or no red showing
    c. Turn middle ring by tiny handle bar to zoom in and out. If you need to move your gel, you can put 1 glove on to move it, just don't forget to not touch any parts of the darkroom or light box with the glove.
25. Do not enhance image until after capture; this will change the dynamic range of the camera and any quantitation will be messed up.
26. Stop preview.
27. Capture; turn power off light box
28. Edit/Display range. Move bar on right with mouse to adjust intensity and contrast
29. Print – use either print button or file/print. Do not use the print button on the printer. Select # of copies. Setup button will change usual print parameters (portrait vs. landscape). You can press Feed button on printer to get extra clearance before tearing off.
30. When finished:
    a. Close out all boxes
    b. Close out your image (save changes/no). Do not save ANY images on the hard drive. If you want to save your image, you can save it to disk or there's a CD burner. Use Adaptec to burn a CD on the 522 system and use Nero Burning Room (icon on desktop) to burn a CD on the 460 system.
    c. Remove gel

*Turn power off light box!!!!!!

31. **Do not turn camera off. Do not touch the knob holding the camera to the frame. If it gets loose, the camera drops onto the filter wheel and the wheel can't be changed then.**

SDS-PAGE gels

Most of instructions are the same for protein gels and agarose gels. Following are the differences only:

1. Place the SDS-PAGE gel on the white plate. In 522 it's usually in the drawer under the transilluminator; in 460 it should just be on the desk somewhere. You don't need to turn the light box on.
2. The filter wheel knob that is turned to EtBr for agarose gels, should be turned to Coomassie Blue.
3. You can capture your image 2 ways:
    a. Select F7 (chemiluminescence). Follow the wizard instructions. Screen Capture/OK/(follow instructions)/Next/(follow instructions/Next/Select standard/Next/Enter Exp. Time/OK.
    b. Camera icon. Select Preview button. Toggle the Prev exp. To increase or decrease exposure until you have a picture
4. You can crop your image. Select the rectangular AOI (area of interest tool), the 10th button from the right. Put a rectangle around the image size you want. Duplicate/Crop under Edit. The new image will be displayed in an untitled window. Be sure to save this image if you need the cropped image.

Tools

1. Edit/Annotate. You can add text to your images. These can be stored separately from your image. The text doesn't become part of the image until you click on the Burn button (last button on the right, second row). See reference manual, 2-89 for details on adding text to your image.
2. Edit/Display range. See above.
3. Edit/Contrast Enhancement. You can not only change the brightness and contrast, but also adjust the Red, Blue and Green color channel settings. To adjust brightness, run the slide up or down under the sun icon. To adjust the contrast, run the slide up or down under the circle with white/black icon. The color channels are the buttons under the 3 slides (if the image is not true color or Palette, the Luminance button, first one, is the only button you can select). Select apply when you are done.
4. Acquire/Video/Digital. This sets the parameters you want for your image acquisition. Usually you don't need to change these. See the reference manual, p. 2-159 for more details about options. Acquire also has the setup for creating a Macro.
5. 1D-Gels/Show toolbar. Tool Palette to manipulate your image.

a. You can rotate the image. Clicking on the rotate image button, opens a dialog box with lots of choices about how to rotate and how much.
b. Lanes. Automatically finds lanes and bands in the active image. You also get the Lanes dialog box so that you can add, delete and curve lanes. You can also label the lanes and bands. Click on the lane or band you want to label and enter the label text in the Label text box. You can change the Lane width and specify that when you change the width, it will change all the lane widths. To do this, position the mouse over the lanes left or right edge of the lane. An arrow tool appears, click and drag this until the lane is the size you want. You can do only 1 lane or have the change in all the lanes – either select or deselect the Uniform Lane width box.
c. Bands. Dialog box that lets you add, delete, filter, curve and label bands. The dialog boxes will walk you through how to do each of these. To curve a band, follow the instructions in the dialog box, and then curve the line using one of the contact points, clicking on the point and pull the point where you want it to go.
d. M.W. standard. This allows you to get an absolute MW of your samples. Select Standard and a Molecular Weight Standard dialog box will appear. Click on a lane in the list box. Select/unselect to add or remove lanes from the list. Select the MW standard you used from the drop-down list. Once you have selected a standard, the size of each of the bands will appear in the box. You can edit these #'s, add or delete a size. You can also create a new standard. See the Reference manual, p. 2-241.
e. Results. Once you have standard entered, you can select results to get the amounts (Optical density) and MW of each band.

6. Tools
    a. Colony Counting. You can use this to count colonies on a plate. See the Reference manual, p. 2-297.
7. Saving image to use in Adobe Photoshop.
    a. Edit/Convert to/select either Gray Scale 8 or Gray Scale 16.
    b. File/Save as/TIF.

Images to Adobe Photoshop

1. Edit/ Convert to/ Select Gray scale 8 or Gray Scale 16
2. File/ Save as/ Select TIF or JPEG/name file/Save

Alpha Innotech Imaging System

1. Turn computer on and login. User name: Biology, password: biology
2. Turn camera on (front of box to right of instrument), turn instrument on (back right of instrument) and turn printer on (front top left – green button)
3. Open software.
4. Select Acquire button on left top.
5. Select in software:
    a. UV gutton if doing EtBr or Fluorescent dye
    b. Select appropriate filter
        i. Chemiluminesence
        ii. EtBr
        iii. Green
        iv. Gold (labeled Gold, but don't have this filter)
        v. Red
6. Set AutoExpose – select box
7. Adjust Image
    a. Bottom ring – focus
    b. Middle ring – zoom
    c. Top ring - exposure
8. Acquire image to take printer
9. Contrast adjustment and tool box windows open. Adjust brightness and contrast as needed.
10. Save image on flash drive NOT computer.
11. Print image – select print button.
12. Clean Transilluminator with H2O.

13. Turn off software , camera and printer.