Fuji Phosphoimager, FLA7000 (PSC 543)
Multi Gauge Analysis Software (PSC 543)

Every time you use the phosphoimager, you MUST sign the logbook.
Do not leave the Imaging Plate (IP) on the eraser any longer than necessary.
Once you have erased your IP, take it back to your lab.
Do not store any images on the hard drive. If you need to, save your image to a flash drive.
Do not under any circumstance, pull the IP out of the scanner drawer – let it come out on it's own (see below **)

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General Information
2 kinds of holders.

1. Fluor Stage. For fluorescent samples and radioactive samples exposed to imaging plates that are not magnetic on the back. Glass, costs $2400, so be careful. Keep in the plastic storage case. It is stored in NSC 516 and can be checked out from Kyu.
   a. gel and membrane. Set sample gently on the glass, with the side to be scanned touching the glass. Note the location on the grid.
   b. Gel with glass. Set on the glass gently. Fix the glass with one glass holder in the front position and two glass holders in the rear position.
   c. IP that are not Fuji and not magnetic. Place on glass, white side (exposed side) down.
2. IP Stage. For radioactive samples exposed to imaging plates that are magnetic on the back. All of the old BAS2500 IP are magnetic. The stage has 2 sides – magnetic side and a side with handles. Place the IP on the magnetic side with the notch of the IP lining up with the notch on the stage – the white surface should be visible.

Imaging Plates (IP) Information

1. 10X more sensitive compared to X-ray film.
2. Reusable
3. Clean with Kimwipes. Do not use H2O.
4. Store in the dark and flat.

Reading IP and Fluorescent Images

1. Turn on instrument. Switch is on the left side of the instrument near the back and near the power cord. Do not place IP inside instrument until the power light comes on. The IP may be damaged during the self diagnostic steps.
2. Let instrument warm up. The power lamp on the upper left panel will be lit when warm up is complete.
3. Double Click on Image Reader software. See below for software details.
4. Place the IP onto the IP stage or Fluor stage

5. Open instrument door. Place IP stage or Fluor stage so that the arrow lines up with the arrow on the main body. When the stage edge is placed on top of the arrow, there is a tab that comes up. Make sure everything fits snug.
6. Close instrument door gently.
7. As the instrument is reading the IP, the busy light is lit. When the read is complete, the busy light goes out.
8. Open the door and remove the IP.

Image Reader

A. Home page

1. IP Mode. Reading Imaging plates
2. Fluorescence Mode. Reading fluorescent samples
3. Digitizing Mode. Select when digitizing. (This is used for silver stained/Coomassie stained gels).
4. Method. Use to register or delete lasers or filters
5. Filter Module. Use to register or change filters
6. Preference. Set display menu, file format or image data type
7. Filter. The loaded filters are displayed
8. Laser. Loaded lasers are displayed as well as their status (ready to use – OK; or not ready to use – No)
9. Status. Status of the instrument is displayed – whether or not it is ready to scan.

Reading IP

1. Select IP button.
2. Reading Settings Window. Make all the following selections before reading IP.
   a. Image Folder. Specify where you want to save image. Use Browse button to go to appropriate folder.
   b. File Name. Name the image.
   c. Comment. Comments are saved with the image.
   d. Laser/Filter. Make sure LD650 laser and IP filter is displayed. These are automatically selected when you selected the IP button
   e. Sensitivity. 3 types of sensitivity; voltage applied to laser – S10,000 (highest sensitivity), S4000(default), and S1000 (lowest).
   f. Pixel size. The smaller the size, the better the resolution but the files are huge. 50 μm or 100 μm are good for most images.
   g. Latitude. This is the dynamic range. L4 is the default. If the band intensity is similar, then L4 is fine. If the band intensity is faint or over a broad range, select L5.
   h. Sampling Area. When you put your IP into the instrument, note the location of your sample on the grid. A vs. 1. Use either Grid (size using grid lines) or Free (size arbitrarily). Try to size as close to exact as you can so that the file is smaller. No matter what you size, the scan doesn't take too long.
   i. Top (at bottom of window). Returns to the Image Reader home page.
   j. Save Condition (top of window). Save settings.

k. Load Condition (top of window). Load saved condition (settings).

3. Load IP on the IP stage
4. Place IP stage into the instrument.
5. Start Scan. The scanned area is read from the left to the right. You can Stop the reading any time you want. The image is saved at the point when you stop the scan. However, the unscanned part will not be saved. You can't restart the scan at this point.
   a. You can change the display parameters of the real-time window (on the right top). You can change the type of tone curve (Exponential, Linear or Sigmoid).
   b. You can adjust the density of the image by dragging the adjuster. Data of low intensity on the left will be white and data of high intensity on the right will be black.
   c. Auto Range Scope if checked will automatically correct to optimum tone.
   d. Magnification. You can change the display area by selecting a magnification ratio. You can also use the +, - buttons.
6. **Save as.** Save the data with a different name.
7. **Launch.** Launch the Multi Gauge analysis software.
8. **Return.** Scan multiple IP. Do not open the door until the Return button is no longer grayed out.

Reading Fluorescent Samples

1. Select Fluorescence button.
2. Reading Settings Window.
   a. Image Folder. Specify where you want to save image. Use Browse button to go to appropriate folder.
   b. File Name. Name the image.
   c. Comment. Comments are saved with the image.
   d. Method Selection. From the pull-down menu, select the Method that corresponds with the sample. The appropriate laser and filter combination is displayed.
   e. PMT. You may set the voltage to be applied to the PMT. The larger the value, the higher the sensitivitly.
   f. -/+. Click the + button to increase the # of scans (up to 4). Click the – button to reduce the # of scans.
   g. Pixel size. The smaller the size, the better the resolution but the files are huge. 50 μm or 100 μm are good for most images.
   h. Latitude. This is the dynamic range. L4 is the default. If the band intensity is similar, then L4 is fine. If the band intensity is faint or over a broad range, select L5.
   i. Sampling Area. When you put your IP into the instrument, note the location of your sample on the grid. A vs. 1. Use either Grid (size using grid lines) or Free (size arbitrarily). Try to size as close to exact as you can so that the file is smaller. No matter what you size, the scan doesn't take too long.
   j. Top (at bottom of window). Returns to the Image Reader home page.
   k. Save Condition (top of window). Save settings.

l. Load Condition (top of window). Load saved condition (settings).

3. Load Fluorescent sample on the Fluor stage.
4. Place Fluor stage into the instrument.
5. Start Scan. The scanned area is read from the left to the right. You can Stop the reading any time you want. The image is saved at the point when you stop the scan. However, the unscanned part will not be saved. You can't restart the scan at this point.
   a. On the window, the scan results of the different scans (up to 4) are displayed.
   b. The current settings are also displayed.
   c. You can change the display parameters of the real-time window (on the right top). You can change the type of tone curve (Exponential, Linear or Sigmoid).
   d. You can adjust the density of the image by dragging the adjuster. Data of low intensity on the left will be white and data of high intensity on the right will be black.
   e. Auto Range Scope if checked will automatically correct to optimum tone.
   f. Magnification. You can change the display area by selecting a magnification ratio. You can also use the +, - buttons.
6. **Save as.** Save the data with a different name.
7. **Launch.** Launch the Multi Gauge analysis software.
8. **Return.** Scan multiple IP. Do not open the door until the Return button is no longer grayed out.

Analysis Software. Multi Gauge v. 3.x; Science Lab 2006

Following is a very simple overview. You can borrow the user's manual for more details. The software is setup so that you will step through each mode in sequence.

1. Multi Gauge is installed on the FLA7000 computer. There are limited # of copies available. See Debby for details.
2. Open Multi Gauge icon or setup FLA7000 scan to open image in Multi Gauge.
3. Different modes. Image Mode has navigation panel, operation panel and image window. Measure Mode (profile) has the same panels as Image mode as well as a profile window. Analysis Mode has the same panels as Image mode as well as a results table window.
4. Navigation panel buttons.
   a. Image mode. Allows you to open and trim image. If you need to analyze multiple images, open in this mode first and then proceed to the next mode.
   b. Display Mode. Allows you to adjust the contrast of images opened and to make crosstalk correction.
   c. Process Mode. Allows you to perform image processing such as rotaion, filtering or noise reduction.
   d. Annotation Mode. Allows you to put comments and arrows on image
   e. Measure Mode. Allows you to set measurement regions, such as lanes, bands and titer plates.
   f. Calibration Mode. Allows you to draw the calibration curve of concentration and/or molecular wt.
   g. Analysis Mode. Allows you to display measuring results and to analyze data.

h. Save Mode. Allows you to save images and results data table.
i. Print.

5. Image Mode.
   a. Images can be opened in 3 ways. Buttons for each are on side panel below image display. Open image directly (OPEN), trim the image (Trimming) and then open trimmed image or combine images, then open overlaid image (Compose).
   b. +/- buttons. Magnify or reduce image
   c. Hand button. Scroll the image.
   d. Density Picker tool. Display the density value of a pixel.
   e. Next. Proceed to the next mode.
6. Display Mode. Following are just the differences from Image Mode.
   a. Channel color. Color icon, change the display color. The appearance of the icon changes depending on the type of image.
   b. Matching. Adjusts the positions of the overlaid images.
   c. Crosstalk. Correct the crosstalk.
   d. Range Scope. Enhance the contrast.
   e. Contrast Scale Type. Magnifying the image of the contrast panel being displayed.
   f. Scale Type. Adjust the contrast.
   g. Next. Proceed to the next mode.
7. Process Mode. Following are just the differences from Image Mode. 4 options.
   a. Rotate. Rotate/Flip image and inverts pixel values.
   b. Filter. Reduces noise and displays sharp images
   c. FFT. Analyzes the frequencies of image components.
   d. Etc. Clips, joints or positions images.
8. Annotation Mode. Following are just the differences from Image Mode.
   a. Pointer tool.
   b. Arrow tool. Allows you to draw arrows.
   c. Text tool. Add text to image.
   d. Information tool. You can add the scan settings onto the image. Window opens so you can select what you want.
   e. Scale bar tool. Window opens that lets you setup the details of the scale bar.
   f. Next. Proceed to the next mode
9. Measure Mode. Following are just the differences from Image Mode. Four Options.
   a. Quant. Allows you to set the region of interest so that it fits the size of teha sample as well as specifying the background value so it can be subtracted.
   b. Profile. Allows you to create the profile region in the migrating direction.
   c. Plate. Set the region of the plate. For the background, a blank well can be designated.
   d. Distance. Allows you to measure the diameter of a circle, the distance between 2 points and angles.
10. Calibration Mode. Following are just the differences from Image Mode. 4 options.
    a. Quant. Used for measuring band concentration

b. Profile. Used for measuring band concentration, molecular wt. and isoelectric point
c. Plate. Used for measuring titerplate concentration.
d. Distance. No setup.

11. Analysis Mode. Following are just the differences from Image Mode. Quantitative results are displayed in table mode.
    a. Table format varies depending on the quantifying mode used.
    b. To change the quantifying modes, click the desired tab.
    c. Table option. Set items displayed on the results table.
    d. Export.
    e. Setting. Sets the way to calculate the background.
    f. Optional tables. Quant/Profile/Plate All – Displays the quantitative results in order; Comparisons – Arranges the quantitative results so that the corresponding bands are adjacent to each other. Distance – displays the results in order.