**GeneGnome Imager training guide**
**using V1.8.10.0 software**
Aloke- (Revised March-2025)

**The GeneGnome Imager measures chemiluminescence generated from a Western blot.**

**Helpful suggestions:**

1. If saturated signals are a problem, try incubating with less secondary antibody (decrease by 100-fold for a 1 second film exposure).

2. Soy milk can give a lower background than cow milk.

3. Low background is more important than high chemi signal.

4. The background should be no more than twice the counts of the non-background (off the gel).

5. Binning is a way to decrease the time it takes to collect data. However, increasing the binning will lower resolution.

6. Increase exposure times and decrease binning to enhance quality of images.

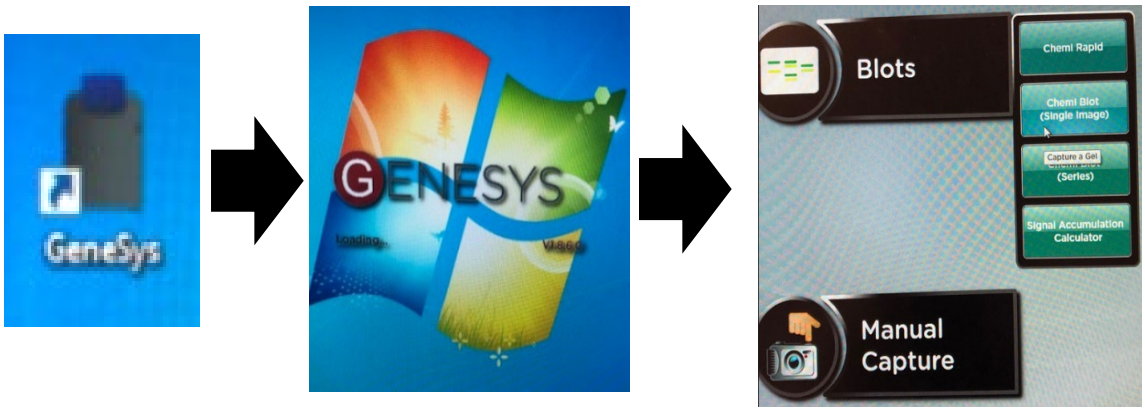
7. The blot needs to be no larger than 8 x 11 cm and can be placed directly on the tray.

8. The camera is a CCD (charge coupled device) and needs to be cooled to lower temperature. This will occur automatically when the software is opened and takes about **two minutes**.

9. The **white light** is used to take an image of the standards/visible markers (please turn it on in some mode).

**Start GeneSys software (V 1.8.10.0)**

Login to the computer and click on the icon and that will open GeneSys



**Home screen:**
You can use either “Blots” or “Manual Capture”.

**Blots Capture**
The software configures the optimal imaging protocol for your hardware configuration using the extensive database.

**Manual Capture**
Provides you with the ability to take control over every function of the system.

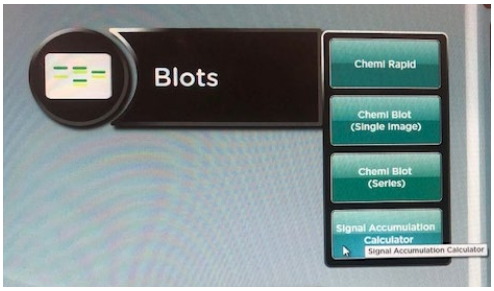
**Camera Status:**
The camera status icon not only informs you whether the camera is connecting (Figure d) or connected (Figure b) or not (Figure c) but also indicates if the camera is at the correct temperature.

If the camera status icon is red (Figure a) then the camera is too warm and needs to cool. To cool the camera, keep Genesys software running as this will power the camera fan and wait until the camera icon turns black.

a)  b)  c)  d)¶ 

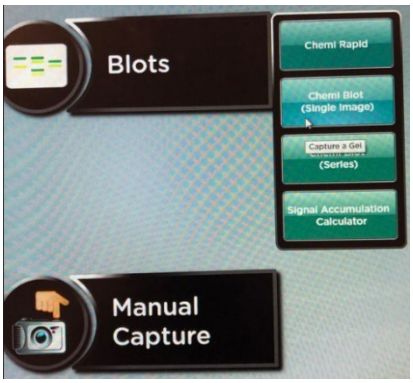
**Select Blot.**

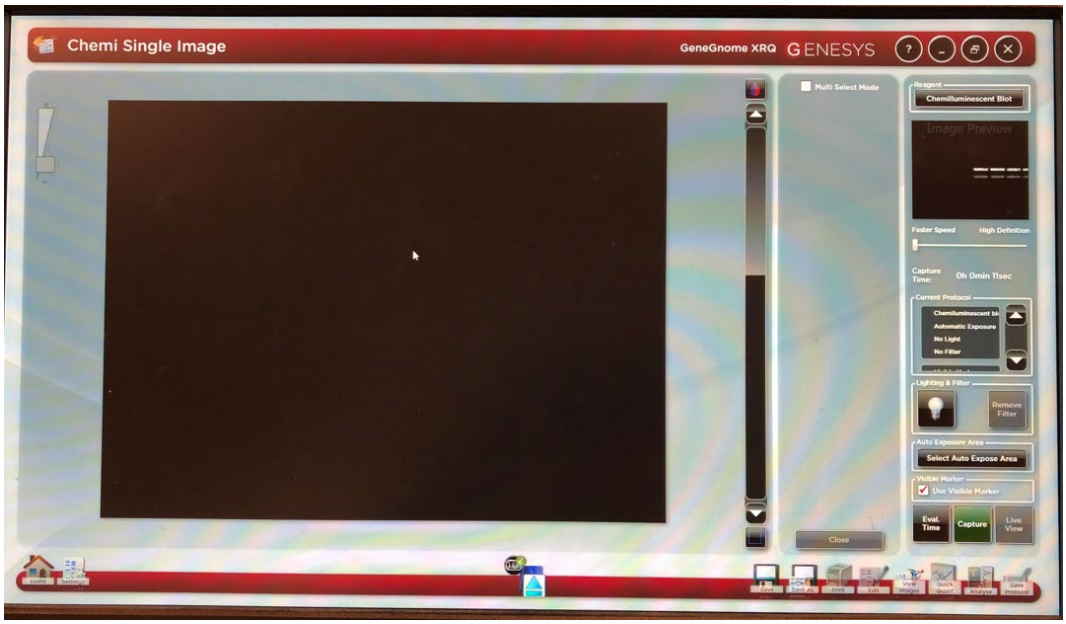
When the **Blots** icon is clicked, a menu will appear with **four** choices: We will discuss all the modes.




**A: <u>Chemi Blot</u> (Single image)**
The chemi (single image) mode is ideal for laboratory users who are new to chemi and unsure of what settings to use. To ensure maximum resolution use the sample size pop-up to select your sample size to ensure your sample size fills the screen. For this example, we will select 7X8.4 cm. Do not worry, if you select the wrong size, you can always adjust the zoom later.

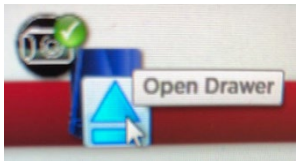
1. Select “Chemi Blot (Single image)” (option #2) even you have more blots, then following screen will appear:




2. **Open the drawer and put your blot.**

To open the drawer, press the blue arrow. Most of the time the door is open.




Place the blot inside the chamber (here a fake gel card is used for demonstration).

To close the drawer, push the drawer shut. After shutting the drawer, it will calculate the capture time (like, shows 57 seconds, in picture below)


3. **Data collection**:
    a. **Dye selection**: The top panel will show you the dye selection- "Chemiluminescent Blot". The software will save the dye you selected previously – if it is a different dye, select from the box in the lower right to display all dye choices (picture above).

    b. **Capture time** depends on the type of blot you have and amount of dye.

    c. **Current protocol**: You will not see any differences between "No light" and white light (do not change current protocol.

    d. **Lighting & filter:** You can turn the yellow light on for a live view, otherwise it will turn automatically. When positioning your sample, you can either do this with the door slightly open or closed. You can select to remove the filter that is in place if required

    e. **Auto Exposure**: GeneSys software has an **autoexpose area** function allowing you to define an area of interest. This function is particularly useful if you have faint bands. By selecting an autoexpose area this will result in a longer exposure, with faint bands becoming more visible. However, more prominent bands may become overexposed (saturated).

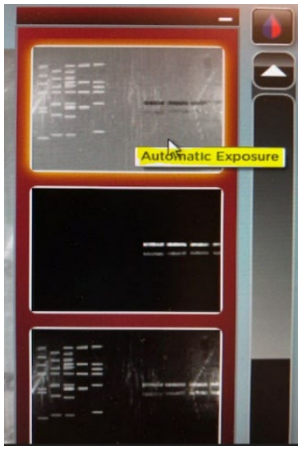
    f. Check the "**Visible marker**" box if you have visible standards. A white light image of the blot will be taken.

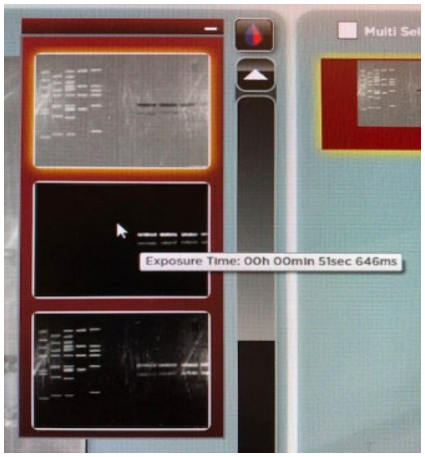
    g. Make sure you place the blot on the tray, insert the tray in the machine.

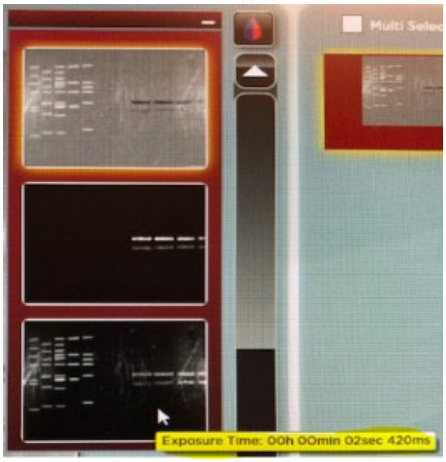
    h. Refer to the figure on the next page. The instrument will take a quick scan and calculate the time for optimal exposure. This will be displayed on the slide bar on the right panel directly under the two black boxes. You have the option of increasing or decreasing the exposure time by moving the slide bar.

    i. When the camera is live it is possible to check the position of your sample, to aid positioning, it is possible to display a grid. The grid helps to position the gel or blot so that it is straight.

    j. Click on "**Capture**".





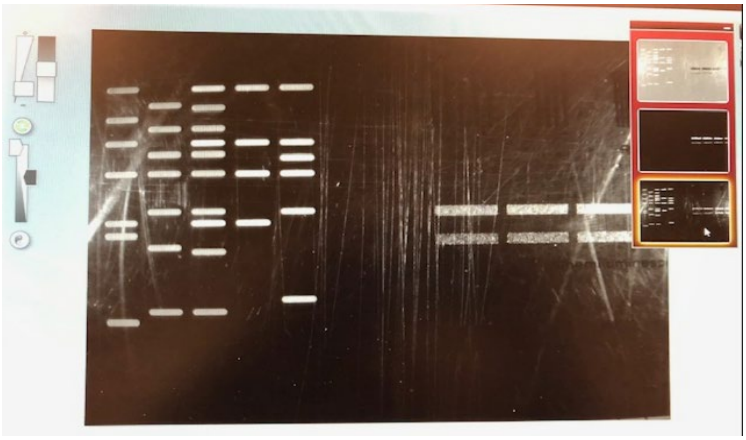



k. The instrument will take **three** pictures (above). One will be the chemi picture (Auto Exposure), the second will be without white light and the third one is a good picture with visible markers or standards. Select the image that have both chemi and markes. You can save all images also.

l. Select the best picture that you want. On the top left side is the zoom button. Under the zoom is the brightness and contrast buttons. Slide the button up and down. If you'd like to return to the computer optimized value, click the **green circular** arrows button.

m. To ensure that the image you are capturing is not saturated. Saturation will only show when the slider bars have been moved to each end of the slider. Over exposed (saturated) white bands will show red and over-exposed black areas in blue. This function is particularly useful when you have chosen the 'Select AutoExpose Area' and you want to see if the bands outside the chosen area have become saturated or not.

n. The histogram  tool is very useful for illustrating the distribution of grey scales within the image. The level of grey scales produced is dependent on exposure time. The slider bars present on the left-hand side of the screen can be used to adjust the brightness and contrast of an image and to digitally zoom in or out. Adjust the brightness and contrast by moving the slider tabs up and down. To return to original settings press the reset button (green arrows). To zoom in move the slider bar towards the (+) sign and to zoom out move the slider bar towards the (–) sign.



4. **Saving the data**:


Images captured with GeneSys software can be saved in several formats. To save an image simply press (touch-screen) or click on the following icon. A dialog box will pop up where you can enter a file name for your image You can also use the 'Save as type' drop down menu and select to save the image as a SynGene Data (*sgd), TIFF image file (*tif), Windows Bitmap image file (*bmp) or a JPEG image file (*jpg)

The default is set to SynGene Data (*.sgd) which is a secure file and GLP compliant. These files can only be opened or altered in Syngene software packages such as GeneTools. SGD files contain all the capture information such as lighting, filter, exposure times and many more complex details i.e. the camera serial number.

You can select to save the raw image as a TIFF (16 bit uncompressed or 8 bits compressed). This format is used when you require all the image data to be retained. This file option creates a larger file but will allow you to analyze the image in other software packages.

To save the displayed image select between *.bmp and *.jpg formats N.B. *.BMP and *.jpg formats will save as 8-bit files and you will therefore lose quantitative data. This format should only be used for the export of visual information to presentation or word processing software, such as Microsoft PowerPoint or Microsoft Word.

When the Save icon is clicked, the software will ask whether you want to save "Together" or "Individually." Selecting "Together" will save the Multiplex as one image in .sgd format. This is the format required for further analysis in GeneTools. If you want a tif of the chemi image, select "Individually" and export the file as .tif or jpg

The image pool presents on the right-hand side of the screen contains saved and unsaved images that have been recently captured in this session. The images **outlined in red** are images that have not been saved; images **outlined in green** are images which have been saved and the file name will be present.

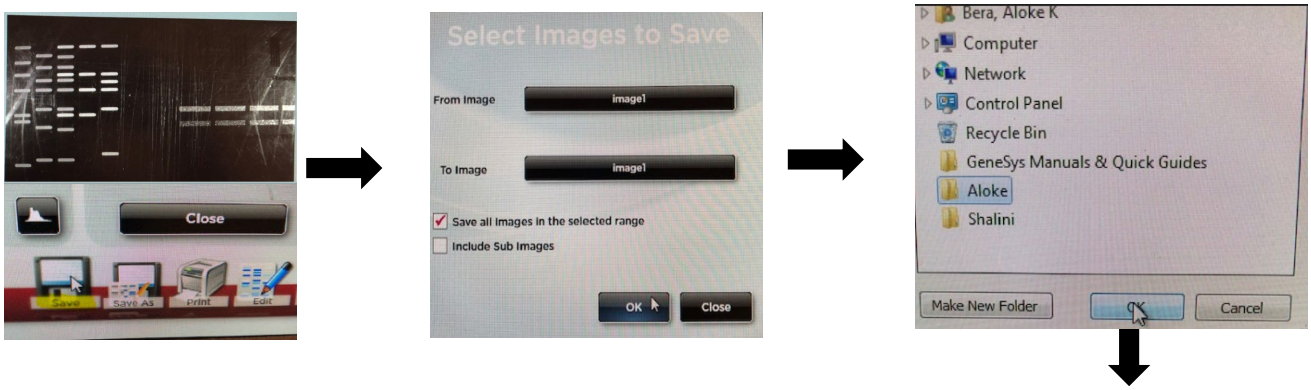


To remove an image from capture screen image pool, select the Close button 

. N.B. This will remove the image from every image pool. If the image has not been saved the software will prompt you to save the image before removing it.

**sdg format**:

Select Best Image> Click Save> Make or select folder> Change file name> after saving it has green outer boader.

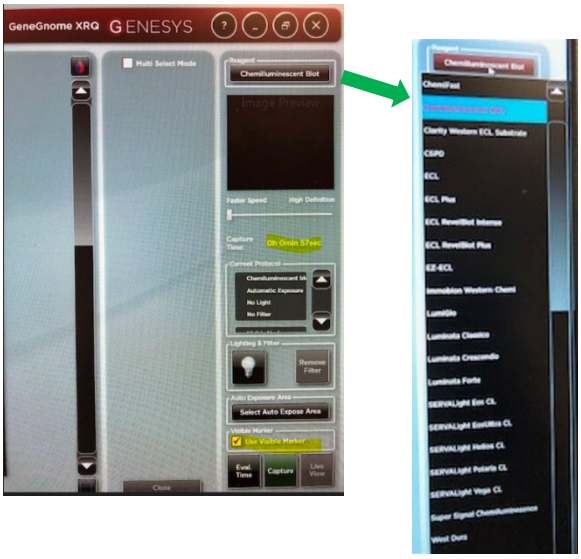



If you prefer to save your image as a Tiff or jpeg then use the “save as”icon to export images “as displayed” or “as captured” To export a captured or saved image simply select the ‘open selected images in GeneTools’ button For .tif format: Select the image> Click save as > Export as txt


Make sure save all images (in case you need it in future).

To remove the blot, click on the blue arrow button directly in the center of the lower panel.


If you want to use another dye- right, click on all dye list and select dye (for Example ECL)




**Save Another image:** To capture additional images, simply open the door, place your sample, and then close the door. The system will automatically calculate your exposure times and capture them for you.

To save the image select the save icon and from the pop-up you can edit the file name. This will save the image in the sgd file format that ensures image and data integrity.

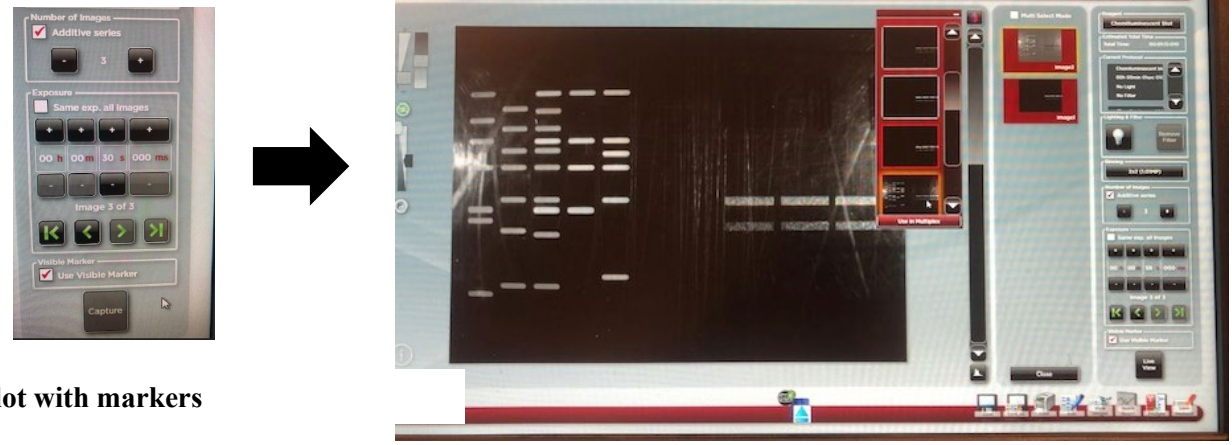
## B: Chemi Rapid:

Chemi Rapid is the easiest method of capturing a chemi Western, as Genesys automatically calculate expisure times and captures simply by closing the door to the darkroom. It gives three images: The top image is the overlay of the chemi image and the marker image and then the next image is the chemi image and then the marker image.

## C: Chemi Blot series:

Chemi blot (series) allow you to take a series of images and lets the user choose the best image from the range. This mode is for users who are used to putting their blot to film and wish to see it develop over time or for time course intensity studies. To perform a series capture of a chemilluminescent blot for example, you need to select the number of images you wish to capture then select the green arrow to move to the next stage. Select to capture a visible or a color marker and then close the door. Press capture.


**Blot without markers**

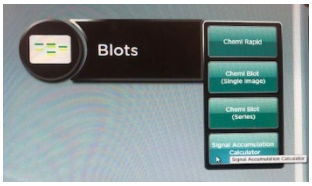




**Blot with markers**

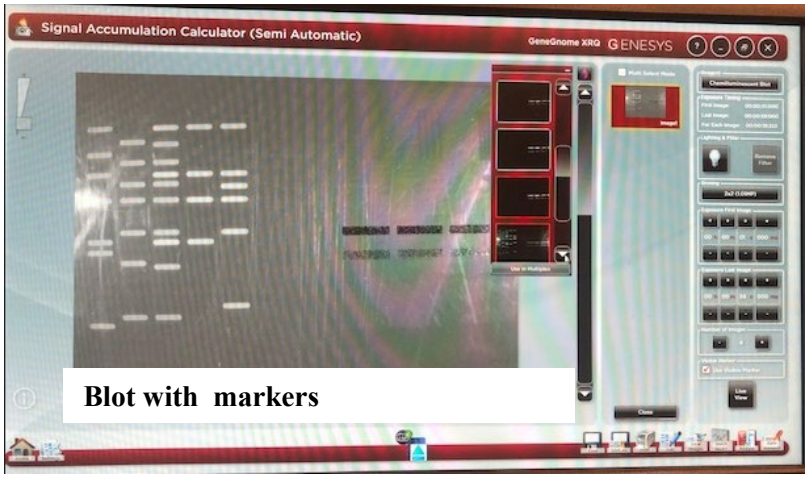
## D: Signal accumulation calculators:

Signal accumulation calculators allow you to take series of images and its useful for determining the optimal imagine time for chemiluminescent samples. Then you can pick the best image to capture again as a single image.

In this case select the exposure time for your first image for example 1 minute and choose the exposure time for the last image for 4 minutes, number of images and select to capture a visible marker








**Blot with markers**

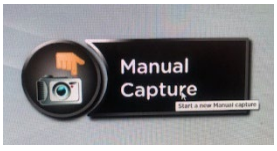
# Manual Capture:

Manual capture offers you the ability to take complete control of every function of the system. The manual mode is particularly useful for the more unusual applications and if you have known imaging parameters that you wish to use.

Once Manual capture has been selected the camera is live. In Live mode the Lens controls are present. To be able to position your sample the appropriate lighting and filter combination need to be selected. Both the lighting and filter menus have a drop-down menu where you can select from all the available lighting and filters present in your system.

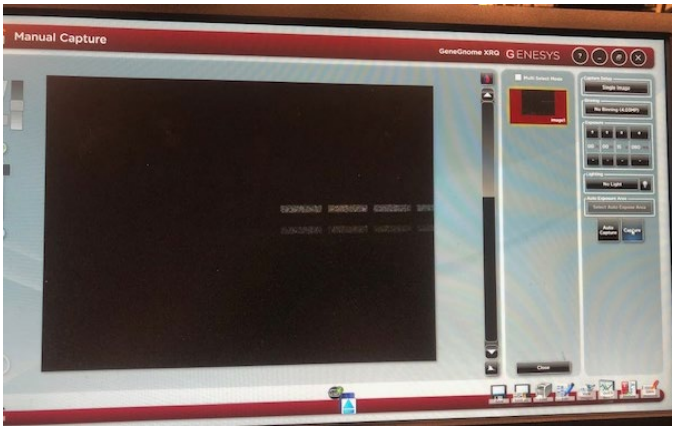
When the camera is live it is possible to check the position of your sample and to aid the positioning of the sample it is possible to display a grid. The grid helps you to position the gel or blot so that it is straight.

Once you have selected a light and filter you need to set the exposure time. Use the +/- buttons to increase or decrease the exposure time The exposure time can be entered manually by typing a number directly into the box. Please enter the exposure time in the following format hours:minutes:seconds:milliseconds (h:m:s:ms). There is a default exposure time of 80ms for fluorescent gels.

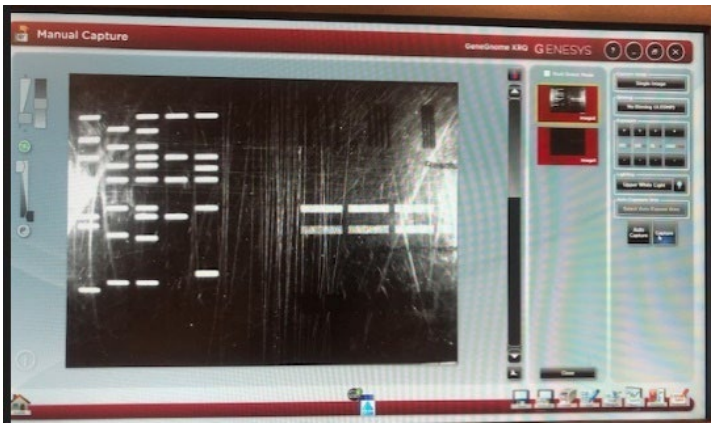





**Without Marker (no light)**

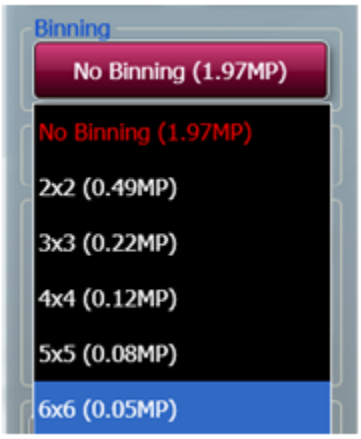



**Visible Marker- when Upper white light is on**




## Additional:

Pixel **binning** is a process that further enhances the sensitivity of a CCD sensor in terms of the speed of the image acquisition. The process of binning involves taking square groups of pixels and combining them into one 'super' pixel. This has the effect of reducing required exposure times. However, this will lead to a reduction in image resolution.




GeneSys software offers a range of pixel binning options which involve combining pixel groups 2x2, 3x3, 4x4, 5x5, 6x6. 8x8 (system dependant). If you wish to perform binning select the 'No Binning' button and choose the level of binning from the drop-down list

**Multiplex** is a combination of two images – typically the white light image showing the standards and the chemi image. When more than one chemi exposures has been captured, you can select which one that will be combined with the white light image (typically the best image) by clicking the "Use in MultiPlex" button.

When the Save icon is clicked, the software will ask whether you want to save "Together" or "Individually." Selecting "Together" will save the Multiplex as one image in .sgd format. This is the format required for further analysis in GeneTools. If you want a jpeg of the chemi image, select "Individually."

| PROBLEM | POSSIBLE CAUSE | SOLUTION |
|---|---|---|
| **Camera not connected or not responding** | Camera power cable not plugged in<br><br>Software driver for the camera is missing | Make sure all cables are connected as shown in the Installation quick guide<br><br>Install Camera driver<br><br>Press the refresh button on the G:BOX icon |
| **'Hardware not available'** | You do not have the correct filter or lighting for the dye you have selected<br><br>Check the 'hardware list' on the hardware screen | Contact bera@purdue.edu or text message @7654914394 to upgrade your system<br><br>Programme in any extra lights or filters that you may have on the hardware screen |
| **Dye not in list** | Your dye is not in the database | Contact Aloke |
| **Dye name is greyed out** | You do not have the appropriate hardware to image that particular dye<br><br>Check the 'hardware list' on the hardware screen | Contact 'Aloke' to upgrade your system<br><br>Programme in any extra lights or filters that you may have on the hardware screen |
| **Transilluminator will not turn on** | Make sure the transilluminator switch is in the 'on' position<br><br>Make sure the darkroom cabinet is completely closed<br><br>Check transilluminator tubes | If the transilluminator will not turn on then please contact "Aloke"<br><br>Transilluminator will not turn on when the cabinet door is open for safety reasons<br><br>The tubes may need replacing |
| **Unable to exit capture set-up in manual capture mode** | | You need to press the capture set-up button again and select 1 for the number of images you wish to capture. This will return to the manual screen |

Example: Western blot analysis of CTIR-APEX2 protein. The blot shows the presence of CTIR-APEX2 monomer (~50 kD), dimer, and trimer forms. Protein ladder (left) includes molecular weight markers ranging from 35 kD to 250 kD. The image was captured using this  GeneGnome instrument.