Getting Started Manual GeneSys



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Chapter One- Introduction

GeneSys image capture software provides you with an application driven and time-saving workflow for running a variety of life science applications.

In GeneSys software there are two modes Auto and Manual capture. Auto capture mode assumes that you will know exactly what your application is and how you have prepared your gel or blot. You then simply enter this information into GeneSys (or recalls this information from a saved configuration) and the system takes over the rest.

GeneSys software has an extensive database which contains data relating to a very wide range of applications e.g. fluorescence, chemiluminescence and chemifluorescence. Once the software knows what sample to expect then GeneSys determines the optimal hardware configuration (camera control, exposure time, sensitivity setting, lighting requirements, lens control and filter selection) for your system and sets the system ready for image capture.

Manual capture mode provides you with the ability to take complete control of every function of the system.

1.1 Software Overview

The Home screen is one of the main windows used in GeneSys software. From this screen you can access a wide range of functions such as, Auto or Manual capture, Browse images and View images. In addition user configurations can be found on the Home screen and access to the hardware set-up screen where you can enter your additional accessories (**Figure 1**).

Manual Capture Browse Images	Aane: protein gel Created by: demoadmin Last used: 14-F	ar 11:17 Last used: 22-Feb 11:46 Name: [js etbr Greated by: demoadmin Last used: 13-Dec 12:13	Last used: 14-Feb 11:56 Name: L35 JUNK Created by: demoadmin Last used: 26-Nov 14:31
View Images		Camera: Synopti GeneSys Version: 1.0.7.1 Database Version: 1.49	ics 5.0MP 54

Figure 1- Home screen

Auto 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.

Browse Images

Allows you to browse all your saved images and upload images from USB devices and enter the View or Edit screens to work on previously stored images.

View Images

Allows you to browse all open images whether saved or unsaved. Images can be directly taken to the Image Edit screen or to the View comparison screen where up to four images can be compared at the same time.

User Configurations

User configurations can be set up for individual users only (Locked) or made public for all users.

1.2 Hardware screen



This screen can be used to inform the software which Transilluminator you have and any accessories you may have (e.g. converter screens).

1.3 Darkroom and Camera status

This icon shows the status of the darkroom and camera. If the darkroom or camera become disconnected a red cross will appear. Simply click the refresh button and this should reconnect the system (**Figure 2**).



Figure 2- Darkroom and Camera status icon

When the darkroom and camera are connected a green tick will appear on the icon next to the camera. If the darkroom and camera are not connected red crosses will appear on the icon. When the darkroom door is open the icon will change to show the inside of the darkroom.

The camera status icon not only informs you whether the camera is connecting (**Figure 3d**) or connected (**Figure 3b**) or not (**Figure 3c**) but also indicates if the camera is at the correct temperature.

If the camera status icon is red (**Figure 3a**) 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.



Figure 3- Camera Status icons

Chapter Two- Software Installation

2.1 Operating Requirements

2.1.1 Minimum system requirements

GeneSys can operate on the following Operating Systems Windows XP Professional SP3 (32 bit version only) or Windows 7 Professional.

Please note that Home versions of Windows operating systems are not supported.

Please refer to the Syngene website FAQs Gel documentation and analysis section for the up- to- date minimum system requirements.

2.2 Software Installation

This section provides information regarding the installation of GeneSys image capture software.

You will need to install the software from an account that has Administrator rights.

Installation is performed in the following sequence

- 1. Hardware Information
- 2. Select destination location
- 3. Select Start Menu Folder
- 4. Device Driver Installation Wizard
- 5. Protection Method
- 6. Your Installation
- 7. Security code

2.2.1 Installation of Hardware Information

Insert the GeneSys CD and run the set-up program.



Click 'Next'



Select from the list the hardware that you have then click 'Next'

In this example the hardware chosen was G:BOX now enter your specific model from the list then click 'Next'

Setup - GeneSys	
Hardware Information What G:Box are you using?	R
Please specify the associated G:Box, then click Next.	
④ G:Box EF2	~
G:Box Chemi-XR5	
G:Box Chemi-XT4	E
G:Box Chemi-XX8	
G:Box Chemi-XL1.4	
◯ G:Box	
◯ G:Box EF	
O G:Box iChemi	
O G:Box Chemi	~
<u> </u>	
< Back Next >	Cancel

2.2.2 Select Destination Location

Please select the folder in which you would like GeneSys to be installed. Select Browse to explore more folder options. Click 'Next'

🛔 Setup - GeneSys 📃 🗖 🗙					
Select Destination Location Where should Gene5ys be installed?					
Setup will install GeneSys into the following folder.					
To continue, click Next. If you would like to select a different folder, click Browse.					
C:\Program Files\Syngene\Gene5ys Browse					
At least 80.7 MB of free disk space is required.					
<pre></pre>					

2.2.3 Select Start Menu Folder

Please select where you would like to create the shortcuts for the program. To select a different folder click the browse button. Click 'Next'.



The software is now ready to install. Click 'Install' to continue the Installation process.

📩 Setup - GeneSys	
Ready to Install Setup is now ready to begin installing GeneSys on your computer.	
Click Install to continue with the installation, or click Back if you want to review or change any settings.	r
Destination location: C:\Program Files\Syngene\GeneSys	
Start Menu folder: Syngene	
<	× >
< Back Install	Cancel

2.2.4 Device Driver Installation Wizard

This wizard will now install the software drivers that some computer devices need in order to complete the installation. Click 'Next'.



Click 'Finish' to complete the Device Driver Installation Wizard.

Device Driver Installation	Wizard
	Completing the Device Driver Installation Wizard
	The drivers were successfully installed on this computer.
	You can now connect your device to this computer. If your device came with instructions, please read them first.
	Driver Name Status
	✓ FTDI CDM Driver Packa Ready to use ✓ FTDI CDM Driver Packa Ready to use
	< Back Finish Cancel

2.2.5 Protection Method

You must now license your software either by a Software key or a Hardware key. Click 'Next' when you have chosen the software licensing method that you have.



2.2.6 Your Software key Installation

Enter your media key which you can find on the inside cover of the CD case and your name and your organisation. Click 'Next'

Install Wizard: Your insta	allation	x
Please en	VARE	
Media Key:		
Please enter your name Your Name:		
Your Organization:	Microsoft	
S Y N	G E N E	
	< Back Next >	Cancel

2.2.7 Security Code

Please send a message to the Syngene Installation desk. Please send the highlighted text as a saved text file. You can contact installations by email <u>installations@syngene.com</u> or by telephone:+44 1223 727123.



Click 'Next'

To complete the installation you need to enter your security code which you will have received from Installations at Syngene.

Install Wizard: Security Code
The next step in the installation process requires that you enter a security code supplied by SynGene.
UNTIL YOU HAVE ENTERED THE CODE DO NOT MAKE CHANGES TO YOUR MACHINE CONFIGURATION AS THAT CAN STOP THE CODE WORKING
Please enter the security code returned to you by the Installation Desk.
Security Code:
To review the Installation Request press ' <back'< td=""></back'<>
< Back Next > Cancel

Click 'Next' to complete the installation of the software.

2.2.8 Installation of GeneSys Software using a Hardware Key

Follow the software media key instructions until section 2.2.5 Protection Method.

 ${\bf N.B.}$ DO NOT CONNECT THE KEY UNTIL YOU ARE TOLD TO DO SO BY THE SOFTWARE

Select Hardware key from the list then click 'Next'.



Choose the type of hardware key you will be using between USB key and Parallel key. Click 'Next'.





Remove all connected keys now then click 'Next'

The install wizard will now show you a Key installation summary- select 'Next'. Connect the hardware security key and follow the instructions in the dialog box shown below.



Press 'Next' to perform a quick check that the key is functioning properly. When the installation is complete, do not forget to eject the CD-ROM.

You can now use the Syngene software that you have installed. The hardware key contains all of your purchased options and must always be connected when using this software.

N.B. For Windows XP and Windows 7 systems with USB keys, if the system is to be used by normal (unprivileged) users, the key should be connected to each possible USB port in turn whilst an Administrator is logged on. This will allow normal users to move the key between ports as required in future sessions.

2.3 Enabling secure user accounts

To enable a secure user account you must use your Microsoft Windows account log in. Secure user accounts provide you with the full functionality of the software but will only show the configurations set up by you or the configurations which have been made public.

2.4 Setting up hardware

When using GeneSys software for the first time or when you have purchased additional accessories such as converter screens you need to tell GeneSys software this information.



To enter this information click on the 'View available hardware' icon from the home page. Once this information has been entered by selecting the appropriate buttons the selected items will turn red. Ensure that you click Save.



Figure 4- Hardware page

Chapter Three- Automatic Capture

The basis of auto capture mode is a series of steps that form a structured workflow. The workflow is very easy to navigate through by using the green bouncing arrow present in the bottom right hand corner of the screen to move forward to the next screen (**Figure 5**). The grey status bar shows the progress of the workflow, select any of the icons to move between any of the stages that make up the workflow (**Figure 6**).





Figure 5- Navigation arrow

Figure 6- Workflow status bar

The first step of the workflow is Sample Selection.

3.1 Sample Selection

The sample selection screen provides you with a choice of Sample Format. This refers to the type of sample with the options being Gel, Blot or Other. **N.B. If you select 'Other' this will take you straight to the Manual capture screen.**

3.1.1 Example of setting up sample selection for imaging a gel

Select Gel as the sample format. The second selection is of Sample Type- this refers to the sample that was loaded on the gel. You can select Protein, DNA or RNA (**Figure 7**).

If DNA is selected then the third selection is the Matrix Type with options of Acrylamide or Agarose (**Figure 7**).

I Sample Selection			G:Box Chen	ni-XR5 GENE SYS	
	Sample Format	Blot	Other		
	Sample Type Protein	DNA	RNA		
	Matrix Type	Agarose			
1		6 -			

Figure 7- Sample Selection screen with Gel as sample format

If protein is selected as the sample type then the third selection is the Dye Type with the options of Fluorescent dye or Visible stain (**Figure 8**).



Figure 8- Protein Dye type

3.1.2 Example of setting up sample selection for imaging a blot

Select Blot from the sample format tab, the next tab to appear will be blot type. The user selects between Chemiluminescent blot (chemi), Fluorescently labelled blot or visible blot (sample visible by eye) (**Figure 9**).

Sample Selection			G:Box Chemi-	xr5 GENE <i>Sys</i> (- • ×
	Sample Format				
	Gel	Blot	Other		
	Blot Type		_		
	Chemi	Fluorescence	Visible		
		6 -			
	_	- H	_	_	Arrest and

Figure 9- Sample selection blot as sample format

3.2 Dye Selection

GeneSys software has an extensive database containing a vast list of dyes covering a variety of applications from fluorescence, chemiluminescence, chemifluorescence and visible stains.

Dye Selection		G:Box Chemi-XR5 GENESYS	
Ethidium Bromide SYBR Gold I I	SYBR Safe		
Selected Items	Clear		
Search for dye here	4 dye(s) found	Recent selections only	
	<u></u>	_	

There are several ways to limit the number of dyes that you view, one way is to use the search box where you simply type in the name of the dye you wish to select (**Figure 10**). If you regularly use the same dyes it is recommended that the 'Recent selections only' box is checked (**Figure 10**).

Search for dye here... 🗹 Recent

Recent selections only

Figure 10- Search or select recent selections only

If you wish to multiplex then simply select the dyes you wish to multiplex with from the list, the software allows you to multiplex up to 5 dyes in one sample.

Selected dye(s) will be shown in the selected items box. The clear all button removes all the currently selected dye(s). To remove a single selected dye then click on the red square on the top right hand corner (**Figure 11**).



Figure 11- Selected Items box

3.3 **Protocol selection**

GeneSys software has an extensive database which calculates the optimal imaging conditions for your sample based on the excitation and emission curves of the majority of dyes currently commercially available.

As a default setting GeneSys shows you the best imaging condition of the sample for your hardware set-up (**Figure 12**).

Protocol Selection	G:Box Chemi-XR5 GENESys
Ethidium Bromide Filter: UV06. Lighting: TLUM - Mid Wave	
More Options	Non-UV protocols only

Figure 12- Protocol Selection screen

If you prefer you can select to see all the imaging conditions available with your hardware then check the 'More options' box (**Figure 13**).

With the increasing trend towards using Safe dyes such as SYBR Green, SYBR Gold etc. which can be imaged using blue light to prevent damage to your sample you can select to show 'Non UV protocols only' (**Figure 13**).



Figure 13- Viewing all available imaging conditions

If you have selected multiplex imaging, the software will calculate the best imaging conditions for each of the dyes selected taking into consideration the imaging conditions of all selected dyes. This permits the capture of multiple dyes with minimal crossover (**Figure 14**).



Figure 14- Protocol Selection for multiplexing dyes

3.4 Sample Positioning

The sample positioning screen shows a live image of your sample (Figure 15).

Sample Positioning	G:Bo	IX EF2 GENESYS	
	Sananaan Katangga Anangga Angga Rangganga Baganaan Katang		Lens Control 5.5 347 139 4 4 4 4 4 4 4 4 4 4 5 5 347 139 139 4 4 4 5 6 4 4 4 6 4 6 4 6 6 6 6 6 6 6 6 6 6 6

Figure 15- Sample Positioning screen

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 (**Figure 16**).



Figure 16- Displaying Grid in Live mode

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 (**Figure 17**).



Figure 17- Sample Positioning Lighting and Filter buttons

When live controls that control the camera are present on screen, they will differ depending on system. If a feedback lens has been fitted slider bar controls will be present (**Figure 18**).





Figure 18- Lens Controls

The Iris control () adjusts the iris so that the sample is clearly visible.

Move the slider upwards to open the iris and let more light reach the camera sensor (\circ) and move slider down towards \bullet to close the iris. Use the arrows to adjust the iris for systems without a feedback lens.

Use the Zoom control (\searrow) to zoom in on the sample to obtain maximum resolution.

To adjust the focus ()) to see a crisp image by either focusing on the wells or on the edge of a gel.

N.B. Use the arrows to adjust the iris, zoom and focus for systems without a feedback lens.

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 be become overexposed (saturated).



Figure 19- Autoexpose function

GeneSys software also has the ability to Neutral field. The neutral field function is based on powerful algorithms which corrects for uneven illumination. This results in an image with a flat, even background whilst maintaining GLP compliance. To neutral field check the box (**Figure 20**) then follow the on-screen instructions (**Figure 21**)



Figure 20- Neutral Fielding button

Depending on which type of sample (UV or transilluminator white light) you would like to neutral field you may get either of the bottom two messages shown below.



Figure 21- Neutral Fielding dialog boxes

3.5 Capture screen

The capture screen offers a wide range of functions including the ability to save, print, edit images and analyse the captured image (GeneTools is required).

The captured image appears in the main window with the summary of the protocol used present on the right hand side as shown in **Figure 22**.



Figure 22- Automatic Capture screen

The Current Protocol box summarises the lighting and filter combination used and the exposure time (**Figure 23**).



Figure 23- Current Protocol summary

To ensure that the image you are capturing is not saturated select the 'Show Saturation button' (**Figure 24**). 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.



Figure 24- Show Saturation button.

The histogram tool is very useful for illustrating the distribution of grey scales within the image (**Figure 25**). The level of grey scales produced is dependent on exposure time. Up to 65536 grey scales will be displayed (**Figure 26**).



Figure 25- Histogram Tool Icon



Figure 26- Graphical representation of the number of greyscale levels captured

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 (**Figure 27**). 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.



Figure 27- Slider bars for brightness and contrast and digital zoom

To view more information about the captured image select the image information icon. Once selected the pop up window will display information on dye, filter, light source, iris F number, exposure time and image size. The image information button also provides information on the range of black and white levels. You can also point the mouse arrow over a band and the black and white levels and XY coordinates appear in this box (**Figure 28**).



Figure 28- Image Information Icon

The capture button enables another image to be captured using the exact conditions previously set (**Figure 29**).



Figure 29- Capture button

Once you have pressed the capture button you can navigate away from the Capture screen if you wish to browse, view and edit previously captured images. When your captured image has been acquired the exposure time icon (**Figure 30**) will flash green. To return to the captured image simply press or click on the exposure time icon which will return you to the capture screen.



Figure 30- Exposure time icon

GeneSys software can be used to run repeatable workflows containing a variety of settings which can be retrieved at any time (**Figure 31**). These repeatable workflows are configurations, to save a configuration select the following icon (refer to Chapter 6 for more information on how to set up a configuration).



Figure 30- Configuration Icon

The image pool present 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 (**Figure 32**).



Figure 32- Saved and unsaved images

To remove an image from capture screen image pool, select the Close button (Figure 33). 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.



Figure 33- The Close button

The image pool also permits you to select multiple images by checking the Multi Select Mode box (**Figure 34**).



Figure 34- Multi Select Mode check box

The selected image(s) can then be saved, printed, sent to the View screen (refer to Chapter 5 section 5.8) for comparison or to the Edit screen (refer to Chapter 5 section 5.1) for further enhancement (**Figure 35**).



Figure 35- Save, print, view and edit image icons

a) Save icon, b) print icon, c) View Images icon and d) Edit screen icon

Single images can be sent to GeneTools for analysis (Figure 36).



Figure 36- Send to GeneTools icon

Chapter Four- 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.



Figure 37- Manual capture screen

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 (**Figure 38**).

4. Manual Capture



Figure 38- Selecting Lighting and Filters in Manual mode

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 (**Figure 39**).



Figure 39- The Grid icon

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 (**Figure 40**). 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.



Figure 40 - Setting Exposure times

Auto Capture is designed to capture the maximum available grey scales of the sample without actually allowing saturation. Therefore, this feature is ideal for quantifying data (**Figure 41**).



Figure 41- Auto Capture button

To ensure that the image you are capturing is not saturated select the 'Show Saturation button' (**Figure 42**). Saturation will only show when the slider bars have been moved to each end of the brightness/contrast slider. Over exposed (saturated) white bands will show red and over-exposed black areas in blue (**Figure 43**).



Figure 42- Show Saturation button



Figure 43- Image of a gel showing saturation

The bands coloured red shows that these bands are saturated

GeneSys software also has the ability to Neutral field. The neutral field function is based on powerful algorithms which corrects for uneven illumination. This results in an image with a flat, even background whilst maintaining GLP compliance. To Neutral field check the box for neutral fielding (**Figure 44**) and then follow the on-screen instructions (**Figure 45**)



Figure 44- Neutral Fielding check box

Depending on which type of sample (UV or transilluminator white light) you would like to neutral field you may get either of the bottom two messages shown below.

	Neutral Fielding		
	No valid neutral field imag and apply it	ge. Do you wish	Yes No
0	Neutral Fielding Please remove your sample and then EITHER: Place the neutral field screen(s) on the transilluminator OR use the white light screen/pad OR use the NovaGlo screen as appropriate AND then press OK	Or	Neutral Fielding Please remove your sample and then : Place the blue neutral field screen on the transilluminator AND the 'frosty' screen on top. THEN press OK

Figure 45- Neutral Fielding Dialog boxes

To freeze the image press (if you have a touch screen monitor) or click the Capture button (**Figure 46**). The image is no longer Live. If you wish to abort capture you can press the 'Stop' button at any time (**Figure 46**).



Figure 46- Capture and Stop buttons

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 (**Figure 47**).



Figure 47- Binning factors

4.1 Manual series and multiplex capture set-up

The software guides you through a series of steps to effortlessly configure the system to capture a series of images or to capture multiplexed images (**Figure 48**).



Figure 48- Capture set-up button

4.1.1 Series Capture set-up

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 (**Figure 49**).



Figure 49- Manual series capture selecting the number of images to capture

If you wish to perform binning select the level of binning from the drop-down menu (Figure 47).

The software will now ask you 'Is this multiplexed?' As a series capture is being set up you need to select the green cross (**Figure 50**).



Figure 50- Series capture set-up multiplexing question

The software will then require you to answer the following question 'Add contents of the previous image?' Adding the images together gives a longer overall exposure. If you grab 3 images at 1 minute and add them together the final image will be the equivalent of a 3 minute exposure.



Figure 51- Series capture set-up adding previous contents question

Select the appropriate lighting, filter and exposure time (Figure 52).



Figure 52- Selecting the appropriate lighting, filter and exposure time for manual series capture

If you wish to alter the lighting, filter or exposure time of one of the images in the series then press the green arrow then deselect 'same as previous' (**Figure 53**).



Figure 53- Same as previous tick box

Press the green arrow to change between images. Prior to pressing the capture button you can review the protocol. When finished press the capture button (**Figure 54**).



Figure 54- Manual Series capture navigational arrows and capture button

Once the capture button has been pressed you will be shown the Current Protocol. When each image of the series has been captured a green tick will appear in the summary box (**Figure 55**).



Figure 55- Current protocol information for manual series capture

4.1.2 Multiplex Capture set-up

To perform a multiplex capture of a multiplexed Western blot for example, you need to select the number of images you wish to capture (i.e. the number of dyes in the multiplex (**Figure 56**).



Figure 56- Manual multiplex selecting number of images to capture
«Evposure —

If you wish to perform binning select the level of binning from the drop down menu (Figure 47)

The software will now ask you 'Is this multiplexed?' As a multiplex capture is being set up you need to select the green tick (**Figure 57**).



Figure 57- Manual Multiplex capture multiplexing question

Select the appropriate lighting, filter and exposure time (Figure 58).

e Filters ————	Lighting	A	ito		Manual
No Filter	No Light	+	+	+	+
		00	00	00	080
		-	-	-	-

Figure 58- Selecting the appropriate lighting, filter and exposure time for manual multiplex capture

If you wish to alter the lighting, filter or exposure time of one of the images in the series then press the green arrow then deselect 'Same as previous' (**Figure 59**). If you wish the system to set an optimal exposure time then leave 'Auto' selected (**Figure 58**).



Figure 59- Manual multiplex same as previous check box

Press the green arrow to change between images. Prior to pressing the capture button you can review the protocol. When you are ready press the Capture button (**Figure 60**)



Figure 60- Manual Multiplex capture navigational arrows and capture button

Once the capture button has been pressed you will be shown the Current Protocol. When each image of the series has been captured a green tick will appear in the summary box (**Figure 61**).

Curr	Current Protocol			
	1.534 seconds			
ľ	Filt 525			
	Automatic Europeuro			
	Red LED Module (multiplexed)			
	Filt 705M			

Figure 61- Current protocol information for manual multiplex capture

The histogram tool is very useful for illustrating the distribution of grey scales within the image (**Figure 62**). The level of grey scales produced is dependent on exposure time up to 65536 grey scales will be displayed (**Figure 63**).



Figure 62- Manual Histogram tool button



Figure 63- Graphical representation of the number of greyscale levels captured

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 (**Figure 64**). 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.



Figure 64- Manual capture slider bars for brightness and contrast and digital zoom

To view more information about the captured image select the image information icon. Once selected the pop up window will display information on dye, filter, light source, Iris F number, exposure time and the image size. The image information button also provides information on the range of black and white levels. You can also point the mouse arrow over a band and the black and white levels and XY coordinates appear in this box (**Figure 65**).



Figure 65- Image Information Icon

GeneSys software can be used to set up custom protocols containing system settings (lighting, filter and camera settings) which can be repeated and retrieved at any time. These repeatable custom protocols are configurations, to save a configuration select the following icon (refer to chapter 6 for more information on how to set up a configuration).



Figure 66- Configuration Icon

The image pool present 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 (**Figure 67**).



Figure 67- Saved and unsaved images

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 (Figure 68).



Figure 68- The Close button

The image pool also permits the user to select multiple images by checking the Multi Select Mode box (**Figure 69**).



Figure 69- Multi Select Mode check box

The selected image(s) can then be saved, printed, sent to the View screen (refer to Chapter 5 section 5.8) for comparison or to the Edit screen (refer to Chapter 5 section 5.1) for further enhancement (**Figure 70**).



Figure 69- Save, print, view and edit image icons a) Save icon, **b)** print icon, **c)** View Images icon and **d)** Edit screen icon

Single images can be sent to GeneTools for analysis (Figure 71).



Figure 71- Send to GeneTools icon

Chapter Five- Browse, Edit and View Screens

5.1 Edit screen

The Edit screen offers a variety of functions ranging from image enhancement and 3D view to changing the resolution of the image for publication purposes.



Figure 72- The Edit screen

The Edit screen has a panel of 3 buttons present on the top left hand side of the screen allowing you to switch between annotation, enhancement tools and 3D view (**Figure 73**).



Figure 73- Edit screen annotation, enhancement and 3D icons The icons from left to right are annotation, enhancement and 3D.

5.2 Annotation tools

There are several annotation tools currently available with GeneSys software. The annotation tools will appear automatically when you enter the Edit screen to access the Enhancement and 3D tools you will need to select the appropriate icon from figure 73.



Figure 73- Annotation tools



Use the mouse arrow as an selection tool



Use the Abc button to add text to your image. Simply click on the image where you wish to write. Now you can type your text directly onto the image. To edit the text, double click on it. A window will appear enabling selection of font, size and style (**Figure 75**). The text box can be dragged around the screen as can all the annotation.

Text		Please se Font	elect a font to use	
Diraction	Select Font O Horizontal	Size Style	24 Normal	Normal
Direction	OVertical OK Cancel			OK

Figure 75- Abc dialog boxes



This tool can be used when you wish to draw a filled shape. Press the Fill button then select the shape you wish to draw from the icons shown in figure x.

To draw rectangles, circles, freehand shapes, lines and arrows click the appropriate icon shown below (**Figure 76**).



Figure 76- Draw icons

The colour of any annotation can be changed using the colour drop-down menu (**Figure 77**). **N.B.** When using the black and white thermal printer it is recommended to use white or black annotation for clarity.



Figure 77- Select line and shape colour drop-down menu

The style of annotated objects i.e. the line thickness and style can be altered using the drop-down menu (**Figure 78**).

	_					- •	-
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-							
1		_	_	-	_	 	-
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Figure 78- Select line thickness drop-down menu

Any annotations made will be automatically saved when images are in SGD format. When saving in other formats, you must check the engrave box to ensure annotations are saved. See section 7.1 for more information on saving images.

To save any annotation that you have made to your image in a separate file that can be re-opened over another image, press on (touchscreen) or click the following icon (**Figure 79**)



Figure 79- Save/Load Annotations icon

When you have selected the icon the following dialog box will appear. Select to save, load or cancel (**Figure 80**).



Figure 80- Save/Load Annotations dialog box

5.3 Image Rotation

The rotate function is available when the annotation and enhancement tools have been selected. To use the rotate function select the following icon (**Figure 81a**). Once this button has been selected the mouse cursor will change to show two arrows. This cursor can then be used to freely rotate the image (**b**). To finalise (fix) the rotation click button (**a**). To reset the orientation of the image press (**c**).



Figure 81- Rotation icons

a) rotation/fix button , b) arrow cursor permitting free rotation and c) reset button

5.4 Image source

If you have made enhancements to your image such as sharpening, smoothing and cropping an area this will alter the image source and the image will now be processed (**Figure 82**). To view the original image press original image button (**Figure 82a**). To go back to the processed image press the processed image button (**Figure 82b**)



Figure 82- Image source icons

a) Original image and b) Processed image

5.5 Saturation button

To show saturation move the slider bars to each end of the brightness/contrast slider. Over-exposed (saturated) white bands will show red and over-exposed black areas in blue



Figure 83- Saturation button

GeneSys offers a range of enhancement tools. Select this icon to access the enhancement tools.



5.6 Enhancement tools

The image enhancement tools allows the user to invert, smooth, sharpen, flip and crop the image (Figure 84).

Enhancement Tools			
	1	0	

Figure 84- Image Enhancement tools

Click on this icon to apply a sharpening filter to your image. When this filter has been applied band edges should become more pronounced but you may also observe an increase in the graininess of the image.



Click on this icon to apply a smoothing filter. This is useful when the image has specks of dust or bubbles present. However, this will make bands appear less sharp.



The Reversal icon will reverse the image to give black bands on a white background or vice versa. This icon is particularly useful when trying to see faint bands.



Speckle correction icon will remove white speckles or 'hot pixels' from the image.



Flip vertical will mirror the image on the vertical plane. This is useful if the sample has been placed in the cabinet the wrong way round.



Flip horizontal will mirror the image on the horizontal plane. This is useful if the sample has been placed in the cabinet the wrong way round.



Select icon allows you to select an area of interest within the captured image.



The crop icon will crop the selected area of interest and paste into a separate window; when used together the select and crop processes will reduce the image size.

5.7 3D View

3D view is a function which enables the user to see a 3D view of their image, revealing gel thickness and the constrast between peaks on a gel. The 3D view can be used with any sample type.

To turn on the 3D viewer select this icon.





Figure 85- 3D View screen





To control the view use the following controls

Figure 86- 3D View controls

Use the blue circles to rotate the image and the green arrows to move the image left to right and up and down the screen. The buttons in the middle reset to the original position.

The 3D image can also be controlled by clicking and dragging with a mouse to position as required.

Use the Gamma slider to adjust the gamma of the image to see fainter bands (**Figure 87**).



Figure 87- 3D View gamma slider

Adding a false colour wash can improve the contrast. Select the 'Default' button and from the menu select the colour wash you wish to apply to the 3D image (**Figure 88**).

The colour wash button can also be used to emulate different dyes, select the green colour wash to emulate a SYBR Green DNA gel, the yellow colour wash imitates a SYBR Gold DNA gel. The Orange, blue and red makes a protein gel appear silver, Coomassie blue and SYPRO red stained respectively. The false colour can be used to better illustrate faint bands.



Figure 88- Colour Wash

The Image pool present on the right hand side of the Edit screen shows the images that you have brought into the Edit screen from the Browse screen or from any of the capture screens. To remove any of the images from this pool simply select the 'Remove' button. If you wish to remove all the images from the image pool select the 'Remove All' button (**Figure 89**)



Figure 89- Remove image buttons

The selected image(s) can then be saved, printed, sent to the View screen (refer to Chapter 5 section 5.8) for comparison or to the Edit screen (refer to Chapter 5 section 5.1) for further enhancement (**Figure 90**).



Figure 90- Save, print, view and edit image icons

a) Save icon, b) print icon, c) View images icon and d) Edit screen icon Single images can be sent to GeneTools for analysis (Figure 91).



Figure 91- Send to GeneTools icon

5.8 View Images Screen

The View Images screen allows you to view and compare the images that you have captured whether they are saved or unsaved and any images brought in from the Browse Images screen (**Figure 92**).



Figure 92- View Images Screen

The zoom slider can be used to increase or decrease the size of the image (Figure 93).



Figure 93- View Screen digital zoom slider bar

The View images screen also enables you to compare up to four images. To compare images simply highlight the images you wish to compare then select the icon with the magnifying glass (**Figure 94**).



Figure 94- Change Layout icons

Once you have clicked the 'Change Layout' icon the following screen will appear (Figure 95).



Figure 95- Change Layout Screen

The default setting is set to link all images that are being compared allowing the zoom and brightness and contrast all images to be altered to the same degree (**Figure 96**).



Figure 96- Change Layout screen Zoom and Range slider bars

To adjust the zoom setting of the image(s) use the slider on the left hand side. Moving the slider bar towards the (+) to zoom in and towards the (-) to zoom out. To adjust the brightness and the contrast use the slider bar on the right hand side.

To alter the zoom, brightness and contrast of individual images the images will need to be unlinked. To do this select the numbered icon of the image you wish to alter under the Link heading (**Figure 97**). To link images again select the linked chains icon (**Figure 97**).



Figure 97- Linking and Unlinking images

The key identifies which image you have selected and the position of each of the images that you are comparing (**Figure 98**).



Figure 98- Change Layout screen image key

To exit from the comparison screen simply select the icon without the magnifying glass from the change layout heading (Figure 94)

5.9 Browse Images screen

The Browse images screen allows you to open files that are already saved on your computer and to open files from external devices such as USB keys.

Use the left hand side of the screen to explore folders on your computer. All images will be organised by date (**Figure 99**).

To select images simply click or press (touchscreen) on the image thumbnails which will turn green when selected. Then select the View images icon to compare or edit images.



Figure 99- Browse screen

Chapter Six- Configurations

GeneSys software can be used to run specific applications with repeatable workflows and to design custom configurations with a wide variety of settings that are easily accessable, adaptable and re-usable at any time.

6.1 Saving Configurations

6.1.1 Saving configurations in Auto Capture mode

Setup your Protocol by working through the following screens Sample selection, Dye selection, Protocol selection and Sample positioning. Once you have captured an image you can save this as a configuration by selecting the following icon (**Figure 100**)



Figure 101- Configuration Icon

A pop-up window will appear where you enter a name for the configuration. You can at this point select to save the configuration as 'Lock' or 'Make public'. A 'locked' configuration means you cannot make any adjustments to the protocol 'Make public' configurations allow you to share configurations with other users (**Figure 101**).



Figure 101- Save Configuration box

You can also modify an existing protocol by selecting the configuration you wish to edit from the 'Save Configuration' dialog box.

6.1.2 Saving Configurations in Manual mode

Once you have selected the lighting and filter options you wish to use, positioned your sample so it is zoomed in and in focus then capture the image. Once you have captured an image select the configurations icon (**Figure 100**).

Then enter a name for your configuration and select if you want to 'Lock' or 'Make public' the configuration (**Figure 101**).

Opening Configurations

From the home page you can open saved configurations by simply pressing or selecting the configuration you wish to open. Once selected the notebook will enlarge (**Figure 102a**) enabling you to select whether to open the file (**Figure 102b**) or delete the file (**Figure 102c**)



Figure 102- Opening User Configurations

a) Enlarged configuration b) Open configuration and c) Delete configuration

Once the configuration has been opened you will be taken to the Sample Positioning screen.

Chapter Seven- Exporting, Saving and Printing Images

The most convenient way to archive or document the complete information about your experiments is to generate reports.

However, you may just want to export only your gel or blot image(s) for presentation or publication purposes.

This chapter describes all the ways you can export an image, save and print images.

7.1 Saving Images

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 (**Figure 103**).



Figure 103- Save icon

A dialog box will pop up where you can enter a file name for your image (**Figure 104**). 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) (**Figure 104**).



Figure 104- Save as dialog box

SynGene Data (*.sgd) 📃 💌
SynGene Data (*.sgd)
TIFF image file (*.tif)
Compressed TIFF image file (*.tif)
Windows Bitmap image file (*.bmp)
JPEG image file (*.jpg)

Figure 105- Save file type

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 bit 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 analyse 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.

7.1.1 Saving Annotations

Annotations can be saved as a .sgd file format. Select the Save/Load annotations icon to add annotations to an image from a saved .ANN file using the Load Annotation button (ANN. Annotation file) (**Figure 106**).



Figure 106- Save/Load Annotations icon

7.2 Printing Images

Images captured with GeneSys software can be printed using an thermal printer or any other printer. To print an image you need to press (touch-screen) or click the following icon (**Figure 107**).



Figure 107- Print Icon

A dialog box will appear showing a print preview. Select print to print the image (**Figure 108**).



Figure 108- Print preview

7.3 Exporting Images to GeneTools analysis software

Various file formats can be exported from GeneSys to GeneTools. Syngene recommends uncompressed TIFFor .sgd to maintain quantification.

To export a captured or saved image simply select the 'open selected images in GeneTools' button (**Figure 109**).



Figure 109- Send to GeneTools icon

Chapter Eight- Technical information

If your dye/reagent is not listed then please contact support@syngene.com

8.1 Technical Information

GeneSys software hardware installation guide

Please visit the Syngene website <u>www.syngene.com</u> to download the following technical literature (This is also available on your GeneSys software CD).

Quick Guides

G:BOX range fluorescence automated (GeneSys) Image capture

G:BOX range fluorescence manual (GeneSys) Image capture

G:BOX range manual series and multiplex (GeneSys) Image capture

G:BOX Chemi chemiluminescence (GeneSys) Image capture

Lighting modules (Installation)

8.2 Technical Assistance

Europe Office

Beacon House Nuffield Road Cambridge CB4 1TF United Kingdom **Tel:** +44 (0)1223 727123 **Fax:** +44 (0)1223 727101 USA Office

5108 Pegasus court, Suite M Frederick MD 21704 USA

Tel: 800-686-4407 (Toll free) 301-662-2863 Fax: 301-631-3977 Email: <u>support@syngene.com</u>

Email: support@syngene.com

Troubleshooting

PROBLEM	POSSIBLE CAUSE	SOLUTION
Camera not connected or	Camera power cable not	Make sure all cables are
not responding	plugged in	Installation quick guide
	Software driver for the camera is missing	Install Camera driver
		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	Contact support@syngene.com to upgrade your system
	Check the 'hardware list' on the hardware screen	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 support@syngene.com
Dye name is greyed out	You do not have the appropriate hardware to image that particular dye	Contact 'support@syngene.com' to upgrade your system
	Check the 'hardware list' on the hardware screen	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	If the transilluminator will not turn on then please contact support@syngene.com
	Make sure the darkroom cabinet is completely closed	Transilluminator will not turn on when the cabinet door is open for safety reasons
	Check transilluminator tubes	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

Glossary

The following glossary defines words used in this manual.

Autocapture- the software will configure the optimal exposure time for your sample using the maximum number of greyscales from your camera.

Binning- is a process that further enhances the sensitivity of the CCD sensor in terms of speed of the image acquistion. The process of binning involves combining square groups of pixels and making them into one 'super' pixel. This will lead to a reduction in image resolution.

Histogram- A graphed representation of brightness, or grey value, of an image.

Image source- there are two types of image sources, original and processed. If you have made enhancements to your image such as sharpening, smoothing and cropping an area this will alter the image source and the image will now be processed.

Iris- On a CCD camera the iris is an adjustable aperture used to control the amount of light coming through the lens.

Neutral Fielding- The neutral field function corrects for uneven illumination of light sources.

Speckle correction- will remove white speckles or 'hot pixels' (bright spots) from the image.

Appendix A- Quick Start DNA/RNA Gel Image Capture

This appendix is a step-by-step guide on how to capture an image of a DNA/RNA gel.

Step One

Select Auto Capture from the 'Home' screen.



Step Two

From the Sample Selection screen select the following Gel as Sample Format, the sample type is either DNA or RNA and finally select whether your gel is Acrylamide or Agarose.



Step Three

From Dye Selection screen select the dye that you are using either from your recent selections only or by searching for a dye (**N.B.** untick recent selections only to search the full list).



Step Four

As a default setting GeneSys shows you the best imaging condition for your hardware set-up.



To see more options check the 'More Options' box. To view the non-UV protocols check the 'Non-UV protocols only' box.

More Options	Non-UV protocols only

Step Five

Place your sample on top of the transilluminator and close the door. The Sample Positioning screen will allow you to adjust the Lens controls (focus, zoom and iris settings). For a Feedback lens you will have slider bar controls.



The Iris control -adjust the iris so that the sample is clearly visible. Move the slider towards top of the slider to open the iris and let more light reach the camera sensor ($^{\circ}$) and move slider down towards • to close the iris. Use the arrows to adjust the iris for systems without a feedback lens.



Zoom control- to zoom in on the sample to obtain maximum resolution.



Focus- adjust to see a crisp image by either focusing on the wells or on the edge of a gel.

Step Six

The captured image will appear in the main window. The image can now be saved in various file formats (.sgd, TIFF, JPG and BMP) or printed. You can also export the image to analysis software GeneTools.



Save



Appendix B- Quick Start Chemiluminescence Blot Image Capture

This appendix is a step by step guide on how to capture an image of a chemiluminescence blot

Step One

Select Auto Capture from the 'Home' screen.



Step Two

From the Sample Selection screen select the following blot as Sample Format, the sample type Chemi.



Step Three

From Dye Selection screen select the reagent that you are using either from your recent selections only or by searching for a dye (**N.B**. untick recent selections only to search the full list).



□ Visible marker

Step Four

Place your sample on top of the black plastic and place on top of the transilluminator and close the door. The Sample Positioning screen will allow you to adjust the Lens controls (focus, zoom and iris settings) For a Feedback lens you will have slider bar controls.



- Zoom control- to zoom in on the sample to obtain maximum resolution.
- Focus- adjust to see a crisp image by either focusing on the wells or on the edge of a gel.

Step Five

An image preview of your chemiluminescent blot can be found in the top right hand corner of the screen. Use the Time versus Quality slider bar to set the exposure time. For a high quality image move the slider bar towards the quality end of the slider bar.





Step Six

Press the Capture button. The image capture can be stopped at anytime by pressing the Stop button.

Step Seven

The captured image will appear in the main window. The image can now be saved in various file formats (.sgd, TIFF, JPG and BMP) or printed. You can also export the image to analysis software GeneTools.



Appendix C- Quick Start Coomassie Gel Image Capture

This appendix is a step-by-step guide on how to capture an image of a Coomassie blue gel.

Step One

Select Auto Capture from the 'Home' screen



Step Two

From the Sample Selection screen select the following Gel as Sample Format, the sample type is protein and then select whether your gel is fluorescently stained or visibly stained.



Step Three

From Dye Selection screen select the visible stain that you are using either from your recent selections only or by searching for a dye (**N.B**. untick recent selections only to search the full list).



Step Four

As a default setting GeneSys shows the user the best imaging condition your hardware set-up.

Step Five

If you have a white light converter then place your sample on top of the converter then place the converter on top of the transilluminator and close the door.

If you have a white light pad then simply place your sample on top of the pad if it is not already turned on, then turn it on.

The Sample Positioning screen will allow you to adjust the Lens controls (focus, zoom and iris settings). For a Feedback lens you will have slider bar controls.



The Iris control- adjust the iris so that the sample is clearly visible. Move the slider towards top of the slider to open the iris and let more light reach the camera sensor ($^{\circ}$) and move slider down towards • to close the iris. Use the arrows to adjust the iris for systems without a feedback lens.

Zoom control- to zoom in on the sample to obtain maximum resolution.

Focus- adjust to see a crisp image by either focusing on the wells or on the edge of a gel.

Step Six

The captured image will appear in the main window. The image can now be saved in various file formats (.sgd, TIFF, JPG and BMP)or printed. You can also export the image to GeneTools analysis software.



Save







GeneTools

Appendix D- Quick Start Multiplexed Western Blot Image Capture

This appendix is a step-by-step guide on how to capture an image of a multiplexed Western Blot.

Step One

Select Auto Capture from the 'Home' screen.



Step Two

From the Sample Selection screen select the following Blot as Sample Format, the blot type is Fluorescence.



Step Three

From Dye Selection screen select the dyes that you are multiplexing either from your recent selections only or by searching for a dye (**N.B**. untick recent selections only to search the full list).



Step Four

As a default setting GeneSys shows the user the best imaging condition for each dye selected to ensure minimal crossover and for the hardware set-up.

To see more options check the 'More Options' box.

Step Five

The Sample Positioning screen will allow you to adjust the Lens controls (focus, zoom and iris settings). For a Feedback lens you will have slider bar controls.



The Iris control- adjust the iris so that the sample is clearly visible. Move the slider towards top of the slider to open the iris and let more light reach the camera sensor (○) and move slider down towards • to close the iris. Use the arrows to adjust the iris for systems without a feedback lens.



Zoom control- to zoom in on the sample to obtain maximum resolution.



Focus- adjust to see a crisp image by either focusing on the wells or on the edge of a gel.

Step Six

The captured image will appear in the main window. The image can now be saved in various file formats (.sgd, TIFF, JPG and BMP)or printed. You can also export the image to GeneTools analysis software.







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