

Amersham ImageQuant 800 User Manual



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1 Introduction

About this chapter

This chapter contains important user information and a general description of the Amersham[™] ImageQuant[™] 800 and its intended use.

In this chapter

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1.1 About this manual

About this manual

The Amersham ImageQuant 800 User Manual provides instructions for setting up the Amersham ImageQuant 800 system, performing image capture, and handling image files using the Amersham ImageQuant 800 control software and Amersham ImageQuant CONNECT software. For detailed instructions on installing the Amersham ImageQuant 800 system, see the Amersham ImageQuant 800 Operating Instructions (29372604).

Scope of this manual

The User Manual covers the Amersham ImageQuant 800 instrument, Amersham ImageQuant 800 control software and Amersham ImageQuant CONNECT. If you work in a regulated environment and have purchased and installed Amersham ImageQuant 800 GxP license, refer to the user manual provided separately for additional information as software functions can vary. The illustration below shows the Amersham ImageQuant 800 system.



Note: Depending on your system configuration, not all options described in this manual may be available. It is possible to upgrade your system to increase the range of imaging options. See the Amersham ImageQuant 800 Operating Instructions (29372604) for more information.

Typographical conventions

Software items are identified in the text by **bold italic** text. Hardware items are identified in the text by **bold** text. In electronic format, references in *italics* are clickable hyperlinks.

Notes and tips

- **Note:** A note is used to indicate information that is important for trouble-free and optimal use of the product.
- *Tip:* A tip contains useful information that can improve or optimize your procedures.

1.2 The Amersham ImageQuant 800 instrument

About the Amersham ImageQuant 800

The Amersham ImageQuant 800 is an imaging instrument designed to detect and quantify proteins, DNA, and other biological samples, such as bacterial colonies. A cooled 8.3 megapixel CCD camera is used to capture high resolution digital images of electrophoretically separated protein and DNA bands in gels or membranes obtained through blotting methods. Use of the optional NP lens also allows for chemiluminescence image capture from multi-well plates and petri dishes. The instrument can capture images of chemiluminescent, fluorescent, and colorimetric samples, depending on the system configuration. It is also possible to capture optical densitometry (OD) images of stained gels to accurately measure amount of proteins in bands on a gel. Image files obtained from the instrument can be analyzed with standard image analysis software, such as Cytiva ImageQuant analysis software. For regulated environments, GxP functionality is available for the Amersham ImageQuant 800 control software. Contact a Cytiva representative to learn more about this option.

The instrument is designed to be used for research purposes in life science laboratories within academia and industry.

Instrument configuration

The Amersham ImageQuant 800 is available in several different configurations, which can be upgraded to improve functionality. Depending on the configuration of your Amersham ImageQuant 800 instrument, not all of the functions described in this manual will be available. For more information on Amersham ImageQuant 800 instrument configurations and upgrades, see the Amersham ImageQuant 800 Operating Instructions (29372604).

Imaging capabilities

A fully equipped Amersham ImageQuant 800 instrument has the following LED light sources:

Lightsource	Emission wavelength
UV Epi	365 nm
Blue Epi	460 nm
Green Epi	535 nm
Red Epi	635 nm
IRshort Epi	660 nm
IRlong Epi	775 nm

1 Introduction

1.2 The Amersham ImageQuant 800 instrument

Light source	Emission wavelength
White Epi	470 to 635 nm
White Trans	470 to 635 nm

A fully equipped filter turret in the Amersham ImageQuant 800 instrument contains the following emission filters:

Emission filter	Data
Cy2	525BP20
Cy3(UV)	605BP40
Cy5	705BP40
IRshort	715BP30
IRlong	836BP46

Note: The Amersham ImageQuant 800 instrument can be further equipped with custom emission filters, see Amersham ImageQuant 800 Operating Instructions (29372604).

The figure below illustrates how these LED light sources and emission filters cover different wavelength ranges of the light spectrum.



About this chapter

This chapter provides information on how to start and set up the Amersham ImageQuant 800 control software and Amersham ImageQuant CONNECT software.

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 $2.1 \hspace{0.1in} Amersham \,ImageQuant\,800\,control\,software$

2.1 Amersham ImageQuant 800 control software

About this section

This section contains information on how to start the Amersham ImageQuant 800 control software, adjust settings, and manage user accounts.

In this section

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2.1.1 Introduction

System setup

The Amersham ImageQuant 800 is operated through the Amersham ImageQuant 800 control software either via the touchscreen panel or an external monitor. For information on the installation process and how to set up the Amersham ImageQuant 800 system, refer to the Amersham ImageQuant 800 Operating Instructions (29372604).

2.1 Amersham ImageQuant 800 control software

2.1.2 Start the Amersham ImageQuant 800 control software

2.1.2 Start the Amersham ImageQuant 800 control software

Introduction

This section contains information on how to start the Amersham ImageQuant 800 system via Amersham ImageQuant 800 control software. For information on how to set up and install the system, see *Amersham ImageQuant 800 Operating Instructions (29372604)*.

Note: If Amersham ImageQuant 800 GxP license is installed on your system, refer to instructions provided in the Amersham ImageQuant 800 GxP User Manual (29620391).

Start the system

Follow the steps below to start the Amersham ImageQuant 800.

Step	Action
1	Make sure that the instrument is plugged in to a mains power supply.
2	Press the power button on the right side of the instrument to activate it.



Result:

The instrument's self-diagnostic process will initialize.

Note:

The self-diagnostic process will normally take a few minutes.

3

Turn on the computer and log into Windows.

Note:

The Amersham ImageQuant 800 control software will auto-start after Windows login.

Step	Action
4	Tap I Agree to agree to the End User License Agreement .
	Note:
	Uncheck the Show license agreement at startup box to no longer see the End User License Agreement at startup.
5	Enter your Username and Password .
	Note:
	To disable Log in , see the Turn off password protection, on page 22.

6 Tap *Login*.

7 Wait for the **CCD status** to turn green, which shows that the CCD has been cooled to the target temperature.



Result:

Image capturing can begin.

Start the system using an external monitor

It is also possible to start the system via the external PC if an external monitor is being used. To do this, use the following procedure.

Step	Action
1	Make sure that the instrument is plugged in to a mains power supply.

2.1 Amersham ImageQuant 800 control software

2

2.1.2 Start the Amersham ImageQuant 800 control software

Step Action

Press the power button on the right side of the instrument to activate it.



Result:

The instrument's self-diagnostic process will initialize.

Note:

The self-diagnostic process will normally take a few minutes. Connection to a network may take longer.

- 3 Turn on the computer and log into Windows.
- 4 Double click on the Amersham ImageQuant 800 control software icon to connect to the instrument.



5

If Login is turned On, enter your Username and Password.

Note:

To enable or disable **Log in**, refer to Turn off password protection, on page 22.



Image capturing can begin.

Start the instrument from sleep mode

After a set period of inactivity, the instrument enters sleep mode. Tap the touchscreen to wake the instrument from sleep mode. The *Login* screen will display. If password protection has been turned off, the *Home* screen will display.

Note: The default time until the instrument enters sleep mode is 30 minutes. To change this setting, refer to the Amersham ImageQuant 800 Operating Instructions (29372604).

2.1 Amersham ImageQuant 800 control software

2.1.3 System settings

2.1.3 System settings

Introduction

This section describes how to modify the system settings using the Amersham ImageQuant 800 control software **Settings** page. This includes adjusting the camera focus and the screen display, changing fluorescent dye and filter settings, and setting a default save location for image files.

Adjust focus

The focal distance is by default set at a fixed distance. For thick samples however, or when imaging multi-well plates or petri dishes, the focus might need to be adjusted. The image below shows the **Focus view** in the system settings.



To adjust the focus for a specific imaging mode, follow the steps below.

Step	Action
1	Open the imaging mode being used.
	Note:
	In this example, Chemiluminescence imaging mode is used.
2	Open the Settings tab.
3	Select Focus from the left side of the screen.

Step Action

4

Adjust the slider in the *Focus* box (1) to change the focus. The result can be seen in the live view window (2).



Note:

A precise focal distance can also be typed in the Focus (mm) box.

Note:

Clicking on the live view window will zoom in and out of the image.

Adjust the slider in the **Brightness** box (3) to change the brightness of the image.



6

Tap **Save** to save the focus settings and return to the **Chemiluminescence** screen.

Note:

Focus changes will only affect the selected image capture mode. If the user changes to another mode, for example, **Fluorescence**, the focal distance will still be set to the default setting.

5

- 2.1 Amersham ImageQuant 800 control software
- 2.1.3 System settings

Screen display

If an external monitor is being used to control the Amersham ImageQuant 800 instrument, the screen settings can be changed in the **Settings** tab.



Par t	Function	Par t	Function
1	Return to previous window	4	Cancel changes
2	Toggle Full screen on and off	5	Save changes
3	Show or hide mouse cursor	6	Toggle on-screen keyboard on and off

Add a light-filter combination

The Amersham ImageQuant 800 contains an accessible filter turret that can house up to seven different filters, including two custom filters. Depending on your system configuration, these dye settings for each filter are available for selection within the Amersham ImageQuant 800 control software. Additional dye settings can be added in the **Settings** panel. For more information on which filters are supplied with which system configuration, see the Amersham ImageQuant 800 Operating Instructions (29372604).

To add a new light-filter combination for a fluorescent dye to the Amersham ImageQuant 800 control software, use the following steps.

Step	Action
1	Open System settings → Fluor dyes .
2	Tap Add dye .
3	In the Create a dye window, enter the Name of the new dye.

Step	Action	
4	Select a	Light source for the dye.
	Light	▲
	Filter	Rubert
	Focus	uv
5	Selectv	which <i>Filter</i> to use with the dye.
	Filter	<u>ــــــــــــــــــــــــــــــــــــ</u>
	Focus	Cr2-8P
	Correction	G24P G30/0-8P
6	Select v distanc	vhether the Focus is automatic, or manually set a fixed focal e.
	Focus	A
	Correction	Auto

7 Select whether the **Correction** is calculated automatically, or manually apply a set correction file.

Correction	Auto	
	Auto	
	[flat_chemi_lower]	
	[flat_chemi_lower2]	

-0.9 mm

Note:

For more information on correction file, see Image correction files, on page 20.

8 Tap **Save**.

- 2 System Preparation
- 2.1 Amersham ImageQuant 800 control software
- 2.1.3 System settings

Check filter information

The Amersham ImageQuant 800 holds seven filters. These can be either system filters, or custom filters. The installed filters depend on the system configuration. A list of installed filters and their specifications can be seen by tapping the *Filter specs* button.



Note: For more information on preparing custom filters, see the Amersham ImageQuant 800 Operating Instructions (29372604).

Delete a fluorescent dye

To delete a fluorescent dye setting, follow the steps below.

Step	Action
1	In System settings \rightarrow Fluor dyes , select the dye to be deleted by tapping the checkbox.
2	Tap Delete .
3	Тар ОК .

Image correction files

Image correction files are used to correct image non-uniformity in CCD cameras. Correction files are provided for each light source. These are applied automatically to each light source, but can also be set manually by the user when a dye is created, see *Add a light-filter combination, on page 18.*

Note: New custom filter combinations might require generation of new correction files. If new correction files are needed, contact your Cytiva representative for more information.

Default save location

Folder locations for saving images, as well as selection of the default folder, can be changed in the **Settings** menu.

To add a new save location, use the following steps.

Step	Action
1	In the Settings screen, tap Save locations .
2	Tap Add location .
3	In the file browser, navigate to the desired folder and tap Select .
4	Tap Save .
5	To make the folder the default save location, tap the check box next to the folder name and tap Make default .
	Result:
	The default symbol will appear next to the selected folder.

f

2.1 Amersham ImageQuant 800 control software

2.1.4 Account management

2.1.4 Account management

Introduction

This section describes how to create and manage user accounts with the standard Amersham ImageQuant 800 control software.

Note: If Amersham ImageQuant 800 GxP license is installed on your system, refer to instructions provided in the Amersham ImageQuant 800 GxP User Manual (29620391).

Account creation and changing passwords can be performed by any user. To reset user passwords, delete user accounts, or turn password protection on and off the master password is needed. This is provided to the administrator by a Cytiva representative when the instrument is installed.

Turn off password protection

By default, the Amersham ImageQuant 800 control software is password protected. It is possible to turn off the password requirement for the Amersham ImageQuant 800 by disabling the **System login**. To do this, use the following procedure.

Step Action





2

Next to System login settings, tap System login ON/OFF.

Step Action

3 Enter the master password and tap **OK**.



4

Slide the System user login ON/OFF toggle to OFF.



Close the **System user login ON/OFF** window by tapping the cross in the top right corner.

Result:

Tap **Done**.

The System login settings now shows as OFF.

System login settings	OFF	System login ON/OF

6

5

2.1 Amersham ImageQuant 800 control software

2.1.4 Account management

Step	Action
7	Tap cogwheel and tap Shut down to shut down the Amersham ImageQuant
	800 system.



Note:

The new setting is available after restarting the Amersham ImageQuant 800 control software.

Create a new user

New users can be added in the **Settings** screen. To do this, use the following steps.

Step	Action
1	In the Settings screen, tap User accounts on the left side of the screen.
2	Tap User accounts on the left side of the screen.

2 System Preparation 2.1 Amersham ImageQuant 800 control software 2.1.4 Account management

Step	Action			
3	Tap Add us	ser.		
	Номе	Chemiluminescence Colorimetric	Fluorescence	a a 🔹
	System settings			Help 🕐
	C Focus	Current User settings	💄 user	Change my password
	& Sleep			
	Display	System users	2. Add user	Manage users
	🔶 Filters			
	ති Fluor dyes	System login settings	ON	System login ON/OFF
	Over accounts			
	Save locations			
	X Service		+Back (8	Cancel Save
	CCD Status	💄 John Roland	Tray position Lower	8 Apr 2019 13:47



The Add user screen shows.

	Đ
🌒 cytiva	
Amersham ImageQuant 800	
Cancel	
	Carcel Carcel

- 4 Enter the **Username** for the new user.
- 5 Enter a **Password** for the new user.
- 6 Type the password again in the **Confirm password** space.
- 7 Tap**Add**.
- 8 Tap **Done**.

Delete a user

To delete a user account, use the following steps.

Note: Account deletion can only be performed by an administrator with the master password.

- 2.1 Amersham ImageQuant 800 control software
- 2.1.4 Account management

Step	Action				
1	In the Settings screen, tap User accounts on the left side of the screen.				
2	Next to System users , tap Manage users .				
	System users 2, Add user				
3	Tap the box next to the desired user to select that account.				
4	Tap Delete .				
5	In the Delete user window, tap Yes .				
	Cancel Vs				
6	Enter the <i>master password</i> and tap OK .				
	Entermaster password Cancel OX				
	Note:				
	This action cannot be undone after tapping OK . If an account is deleted in error, an new account can be made. See Create a new user, on page 24.				

Change a password

To change the password of the current user, follow the procedure below.

Step	Action
1	In the Settings screen, tap User accounts on the left side of the screen.

2	Tap Chang	ge my password	Ι.	
	HOME	Chemiluminescence Colorimetric	: Fluorescence	@ # <mark>\$</mark>
	System settings			Help 🕥
	C Focus	Current User settings	Luser	Change my password
	& Sleep			
	🖵 Display	System users	2. Add user	Anage users
	& Filters			
	R Fluor dyes	System login settings	ON	System login ON/OFF
	② User accounts			
	Save locations			
	🛠 Service		de Back	(A) Cancel

Tray position Lower

Result:

CCD Status

The Change password screen shows.

John Roland

3 Enter the *Current password* for the current user.

		Ð
	Change password	
	Hew password Confirm password	
	Cancel Change	
	CCD Status	
4	Enter a New password for the user.	
5	Type the password again in the Confirm password sp	oace.
6	Tap Add .	
7	Tap Done .	

Reset a password

If a user has forgotten their password and is unable to access their account, the administrator can reset their password. This requires use of the master password. To reset a password, use the following steps.

2.1 Amersham ImageQuant 800 control software

1

2.1.4 Account management

Step Action

Tap the cogwheel icon in the top left corner and tap **Settings**.



2 Tap the box next to the desired user to select that account.

	Manage u	isers
-	Select	Users
	-	Username1
		Username2

3 Tap **Reset password**.

4 Enter the *master password* and tap **OK**.



Result:

The user's password is reset to 0123456789.

5 Tap Save.

2.2 Amersham ImageQuant CONNECT

About this chapter

This chapter contains information on how to connect to and manage Amersham ImageQuant 800 instruments via the Amersham ImageQuant CONNECT software package.

In this section

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2.2.2	Connect to the instrument	31

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2.2.1 Introduction

Introduction

It is is possible to access the Amersham ImageQuant 800 remotely via a computer elsewhere on the network using Amersham ImageQuant CONNECT. This allows users to remotely book experiments using the **Scheduler** function, and to manage image files.

The Amersham ImageQuant CONNECT software client is delivered on a DVD. To install Amersham ImageQuant CONNECT, insert the DVD and run the installation file.

ImageQuant CONNECT Home screen



The image below shows the Amersham ImageQuant CONNECT *Home* screen

Part	Function
1	List of available Amersham ImageQuant 800 instruments
2	Name of the active user
3	Sync instruments
4	Options to open Settings , Logout from the software, show software version information, or show the License agreement .

2.2.2 Connect to the instrument

Add an instrument to the ImageQuant CONNECT software

To connect a new instrument to the Amersham ImageQuant CONNECT software, use the following steps.

Step	Action
------	--------

1 Click the options icon in the top right corner and click **Settings**.



2

Click Add instrument.

номе		Instrument 2	v 0	, e ¢
System settings	Instrume	ents		
Host address	Select	Nickname	IP Address	
		Nick name 1	192.168.1.127	
		Nick name 2	192.168.1.126	
		Nick name 3	192.168.1.125	
			Add instrument	D elete
			+ Back 🛞 Cancel	Site
🔮 Busy	💄 🛛 John Rol	and		7 Jun 2019 10:02

Result:

A new line appears on the *Instruments* list.

2.2 Amersham ImageQuant CONNECT

3

2.2.2 Connect to the instrument

Step Action

Enter the *Nickname* of the new instrument in the new line.

System settings	Instrum	ents			
Host address	Select	Nickname	IP Address		
		Nick name 1	192.168.1.127		
		Nick name 2	192.168.1.126		
		Nick name 3	192.168.1.125		
	> 🗆		192.168.1.127		
			⊗ Add i	nstrument	8 Delet

4 Enter the *IP Address* or the network computer name of the new instrument.

Note:

Network settings depend on the local network. Contact your system network administrator to find, or set the IP address or network name of the instrument.



Click Save.

5

Log in to ImageQuant CONNECT

To log in to Amersham ImageQuant CONNECT and access the Amersham ImageQuant 800 from a remote computer, use the following procedure.

Step	Action
1	Double click on the Amersham ImageQuant CONNECT icon.
2	Carefully read the license agreement and click I Agree .

Step Action

3

4

Enter your User name and Password. Click OK.

Result:

The Amersham ImageQuant CONNECT *Home* screen shows.



Note:

To refresh the status of the connected instruments, click **Sync**.

Select the instrument to connect.

Result:

The selected instrument *Image Library* screen shows.



Change instrument

To access another instrument on the network via Amersham ImageQuant CONNECT, either click the *HOME* button in the top left corner to return to the *Home* screen and select another instrument, or use the following steps.

2.2 Amersham ImageQuant CONNECT

1

2.2.2 Connect to the instrument



Click the **Instrument** drop-down menu at the top of the screen.



2 Click the instrument to which you want to connect.

Sync instruments

To refresh the list of connected Amersham ImageQuant 800 instruments, click the refresh button at the top of the screen.



Disconnect an instrument

To disconnect an instrument from ImageQuant CONNECT, use the following steps.

Step Action

1

Click the options icon in the top right corner and click **Settings**.

System settings	Instruments		V secongs	
Host address	Select Nickname	IP Address	About	
	Nick name 1	192.168.1.127	Se License	
	Nick name 2	192.168.1.126	2. Logout	
	Nick name 3	192.168.1.125	Ů Exit	
		× .	Add instrument	🗑 Delete
		4. P	0	

Step	Action				
2	Click the box next to the instrument to be deleted.				
	Select Nichname IPAddress				
3	Click Delete .				
4	Click Save .				
	Note:				
	To undo the deletion, click Cancel . This must be done before the Save button is clicked.				

3 Use of methods in ImageQuant 800 control software

About this chapter

This chapter contains information on how to create and run methods using the Amersham ImageQuant 800 control software.

In this chapter

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3.2	General settings	38
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3.5	Colorimetric image capture	62
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3.1 Introduction

Amersham ImageQuant 800 control software methods

A method is the combination of all settings that affect an image capture. This includes imaging mode, exposure, tray position, and any advanced setting such as binning or image area. Methods can be saved and opened at a later time. If an opened method is changed, it needs to be saved again. Image files contain information on the method and image settings used. See help text in the Amersham ImageQuant 800 control software for more information.

Available imaging modes

The Amersham ImageQuant 800 supports three modes of image capture: chemiluminescence, colorimetric, and fluorescence. The main functions and uses of these modes are described in the table below.

Image capture mode	Imaging options	Sample type
Chemiluminescence	Chemiluminescence	Enzymatically labelled membranes (e.g., horse- radish peroxidase)
	With colorimetric marker	Colorimetric markers (e.g., Amersham Rainbow™ Markers)
Colorimetric	Gel documentation	Colorimetric stained gels (e.g., Coomassie and silver staining)
	OD measurement	Quantitative densitom- etry measurement
Fluorescence	Fluorescence	Fluorescent labelled membranes, DNA gels (e.g., ethidium bromide and alternatives staining)
	With colorimetric marker	Colorimetric markers (e.g., Amersham Rainbow Markers)

Note: If Amersham ImageQuant 800 GxP license is installed on your system, refer to instructions provided in the Amersham ImageQuant 800 GxP User Manual (29620391).

3.2 General settings

3.2 General settings

Introduction

This section describes settings that are used across some or all of the imaging methods.

Tray position

Depending on the imaging method being used, samples can be placed in the upper (1) or lower (2) tray position.



The position of the tray is detected automatically by the Amersham ImageQuant 800 control software and displayed at the bottom of the screen. The display is automatically updated if the tray position is changed.

Номе	Chemiluminescence	Colorimetric	Fluorescence	e #	•
🖆 Open method					Help 🕐
1. Select Exposure	2. Basic settings			Advanced settings	
Auto Manual Time series SNOW	E Colorimetric r	narker		Binning 3 x 3 Qb Fluorescence multiplex ID Capture area 107 x 147 mm	Select Select
Save method	• Heartha	84	Trav position	Pre-capture	Start

The *Tray position* indicator will display as *Upper*, *Lower*, *NP lens for plates*, and *None*.



SNOW mode

The Signal to Noise Optimization Watch (SNOW) reduces noise and avoids saturation of the image. Multiple exposures are captured and merged by averaging the results into a single image. This improves the signal-to-noise ratio by removing artefacts and reducing false positive signals. This setting can be used for **Chemiluminescence** and **Fluorescence** imaging methods.

Run a method

To open and run a previously saved method, use the following steps.

Step	Action
1	In the method editor for Chemiluminescence , Colorimetric , or Fluores-
	cence, select Open method.

- 3 Use of methods in ImageQuant 800 control software
- 3.2 General settings

2	In the Metl	hods wind	low, tap to	select	the m	ethod	and ta	p Selec
	Methods						He	lp ᠿ X
	Name	User	Imaging mode	Exposure mode	Colorimetric marker	Fluor.multi. / Dyes	Tray	Binning
	My Method 1	Username	Chemiluminescence	Time-series	ON	None	Upper	4 × 4
	My Method 2	Username	Fluorescence	Manual	OFF	СуЗ	Lower	3 x 3
	My Method 3	Username	Chemiluminescence	Manual	ON	Cy2	Upper	2 × 2
	My Method 4	Username	Colorimetry	Gel Document	ON	None	Lower	1×1
	My Method 5	Username	Fluorescence	Auto	OFF	IRshort	Upper	16×16
	뮰 Delete					8	Cancel	Select

Result:

The image capture method screen is shown.

3 Tap Start.

Help function

For information, tips, and help when using the ImageQuant 800 control software, tap the *Help* button in the top right corner.



3.3 Advanced settings

Introduction

This section contains information on tools found in the *Advanced settings* panel in the method creation screens.

Capture area and Live View

The Amersham ImageQuant 800 has a maximum image capture area of 160×220 mm. This capture area is dependent on which tray position is being used and whether the **NP lens** is being used. The capture area under each setting is detailed in the table below.



Tray position	Capture area
Upper	80 × 110 mm
Lower	160 × 220 mm
	133 × 183 mm
	107 × 147 mm
	80 × 110 mm
NP lens for plates	146 × 161 mm

When using the lower tray position, it is possible to crop the capture area. The option for this is found under *Advanced settings* in the method window for all imaging methods. To change the capture area of the lower tray position, use the following steps.

Step	Action
1	Under Advanced settings , tap Capture area .

- 3 Use of methods in ImageQuant 800 control software
- 3.3 Advanced settings

Step	Action
2	Tap the tabs on the right side to select the desired capture area.
	• 160 × 220 mm
	• 133 × 183 mm

- 107 × 147 mm
- 80 × 110 mm

Result:

The capture area is changed to the selected size.



Note:

Changing the capture area is only possible in the **Lower** tray position. In the **Upper** tray position, it is possible to see a live view of the capture area to position the sample within the default capture area.

3 Tap Select.

Binning

CCD binning is the process of combining adjacent pixels on an image sensor into one larger pixel. Combining pixels in this manner allows the detection of fainter signals leading to a greater sensitivity. Because pixels are grouped together, this also leads to a loss of resolution of the image. The illustration below shows an example of 2 × 2 binning. The four highlighted pixels are grouped into one larger pixel.



The illustration below shows an example of 3×3 binning. The nine highlighted pixels are grouped into one pixel. This allows for detection of a fainter signal in image analysis, but results in a loss of resolution compared to 2×2 binning.



When performing image capture on the Amersham ImageQuant 800, it is possible to adjust the binning settings for either greater sensitivity or greater resolution. Increasing the sensitivity results in a decrease in the exposure time. The option for this is found under *Advanced settings* in the method window for all *Chemilumines-cence* and *Fluorescence* imaging. To change the binning settings, use the following procedure.

3.3 Advanced settings

Step	Action		
1	Under Advanced sett	ings , tap Select binning.	
	Advanced settings		
	Binning	Select	
	3x3		

2 Tap to drag the slider to move the slider towards *High resolution*, or *High sensitivity*.



3 Tap Select.

Tip: To avoid image saturation, either set a lower binning or reduce the exposure time.

3.4 Chemiluminescence image capture

About this section

This section contains information on how to create and run chemiluminescence imaging methods.

In this section

Section	on	See page
3.4.1	Chemiluminescence imaging	46
3.4.2	Automatic chemiluminescence exposure	50
3.4.3	Manual chemiluminescence exposure	53
3.4.4	Time series chemiluminescence exposure	55
3.4.5	SNOW chemiluminescence exposure	58

3.4 Chemiluminescence image capture

3.4.1 Chemiluminescence imaging

3.4.1 Chemiluminescence imaging

Introduction

Chemiluminescence imaging is used to image luminescent samples (e.g., horseradish peroxidase) on membranes and multi-well plates. For membranes with colorimetric markers, such as Amersham Rainbow markers, additional colorimetric image capture automatically produces an overlay image comprising color and chemiluminescence image captures. The table below states which accessories are needed for chemiluminescence imaging.

Imaging mode	
Without colorimetric marker	Black tray
With colorimetric marker	Black tray with White insert

The Chemiluminescence screen

The illustration below shows the main components of the **Chemiluminescence** screen.



Part	Function	Part	Function
1	Save image capture settings as a method	12	Toggle full dynamic range
2	SNOW (noise reduction mode)	13	Image library tab

3.4 Chemiluminescence image capture

3.4.1 Chemiluminescence imaging

Part	Function	Part	Function
3	Time series exposure	14	Scheduler tab
4	Manual exposure	15	Settings tab
5	Automatic exposure	16	Help
6	Open saved methods	17	Select binning
7	Return to <i>Home</i> screen	18	Select fluorescence multiplex
8	Chemiluminescence tab	19	Change capture area
9	Colorimetric tab	20	Start pre-capture
10	Fluorescence tab	21	Start capture
11	Toggle colorimetric marker		

Fluorescence multiplex

In **Chemiluminescence** imaging, it is possible to perform sequential chemiluminescence and fluorescence imaging without moving the sample. Up to two fluorescence dyes can be imaged in addition to the chemiluminescence imaging. This makes further downstream processing of the images easier. The option to add multiplex imaging is found in the **Advanced settings** panel when creating a method.

To use the fluorescence multiplex imaging, use the following steps.

Step	Action	
1	Next to Fluorescence m	ultiplex, tap Select.
	Advanced settings	
	Binning 3x3	Select
	육 Fluorescence multiplex	Select
	I드 Capture area 160 x 220 mm	Select

2 Tap Select dye.

- 3 Use of methods in ImageQuant 800 control software
- 3.4 Chemiluminescence image capture
- 3.4.1 Chemiluminescence imaging

Step	Action
3	In the Dye drop-down menu, select the dye to include.

D	VP		
Ĩ	UV	🛊 365 nm	🕹 Cy3(UV)
_	UV	🛊 365 nm	🕹 Cy3(UV)
	Cy2	🙀 460 nm	🕹 Cy2

4 To set the exposure, either: Toggle on the button under **Auto Exposure**. *or*

Enter the exposure time manually under Exposure time

5 To add a second dye, tap **Add dye**.

6 To remove a dye, tap the cross on that dye.

- 11 min sec	00
min sec	
	1/100 see
Exposure time	
min sec	1/100 se
	Exposure time min sec

7 Tap Select.

Note:

To cancel changes and return to the previous screen, tap Cancel.

Image a plate or petri dish

The Amersham ImageQuant 800 is capable of artefact-free chemiluminescence imaging of multi-well plates (e.g., 96-well plates) and petri dishes with the use of the **NP** lens and **NP Tray Guide** accessories.

3 Use of methods in ImageQuant 800 control software 3.4 Chemiluminescence image capture 3.4.1 Chemiluminescence imaging

Note: The NP lens and NP Tray Guide are optional accessories and are only used in chemiluminescence imaging. For more information, see the Amersham ImageQuant 800 Operating Instructions (29372604).

To capture images of multi-well plates and petri dishes, use the following procedure.

Step	Action
1	Place the tray guide inside the black tray .
2	Place the multi-well plate or petri dish into the cutout in the centre of the tray guide .
3	Open the instrument door.
4	Insert the tray into the lower tray position.
5	Insert the NP lens into the lower tray position on top of the tray.
Note:	
	The NP lens must be inserted fully up to the stop position.
6	Adjust the focus if needed, see <i>Adjust focus, on page 16</i> .
7	Close the instrument door.
8	Select capture settings and tap Start .

3.4 Chemiluminescence image capture

3.4.2 Automatic chemiluminescence exposure

3.4.2 Automatic chemiluminescence exposure

Introduction

Automatic exposure uses a short pre-capture exposure to automatically calculate the exposure time. The region of interest used to calculate the exposure time can be manually selected using **Pre-capture** if needed. The calculated exposure time is based on the intensity level of the most intense signal detected in the selected region, or in the entire capture area if no region is predefined. The **Full Dynamic Range** setting can be used to use the full range of intensities to calculate the exposure time. This results in a longer exposure time.

Take an automatic exposure

To take an automatic exposure, use the following steps.

Step	Action	
1	On the <i>Home</i> screen, tap <i>Chemiluminescence</i> image capture.	
2	Under 1.Select Exposure , tap Auto .	
3	If an overlay image of the sample and marker is required, tap the toggle button next to Colorimetric marker .	
	Colorimetric marker	

4 If shorter exposure times are required, tap the toggle button next to **Full dynamic range** to the off position.



Note:

Turning off **Full dynamic range** will reduce the range of intensity levels used in the image.

- 5 Under *Advanced settings*, change the settings for *Binning* and *Capture area* as required. See *Section 3.3 Advanced settings, on page 41* for more information.
- 6 Enter the settings for *Fluorescence multiplex* if required, see *Fluorescence multiplex*, on page 47.
- 7 To save the capture settings for future use, tap **Save method**. In the popup window, enter the **Name** of the method and tap **Save**.

3 Use of methods in ImageQuant 800 control software 3.4 Chemiluminescence image capture 3.4.2 Automatic chemiluminescence exposure

Step	Action
8	Tap Pre-capture .
	Note:
	To take an automatic exposure without predefining a region of interest, tap Start , without tapping Pre-capture .
9	Tap the button to toggle between Point and Area measurement.

Tap at the desired point on the image



- 3 Use of methods in ImageQuant 800 control software
- 3.4 Chemiluminescence image capture
- 3.4.2 Automatic chemiluminescence exposure

Step	Action
10	Either
	Use a point as a frame of reference, toggle to <i>Point</i> and tap the image at the desired location (1).



Or

Use an area as the frame of reference, toggle to **Area**. Tap and drag to select the desired reference area (1).



Note:

The area can be moved without redrawing it by using the arrow buttons (2).

11 Tap Start.

3.4.3 Manual chemiluminescence exposure

Introduction

With manual exposure, the user can enter a specified exposure time.

Take a manual exposure

4

To take a manual exposure, use the following steps.

Step	Action
1	On the <i>Home</i> screen, tap <i>Chemiluminescence</i> image capture.
2	Under 1.Select Exposure , tap Manual .
3	Under Exposure time , tap the box above hour min sec 1/10 sec .



In the **Exposure time** window, tap the box under **hour**, **min**, **sec**, or **1/10 sec** and use either + and - buttons, or the keypad to enter the desired value.



Note:

The maximum time that can be set is 10 hours. The minimum time that can be set is 0.1 sec.

5 Tap **OK** to return to the create method window.

- 3 Use of methods in ImageQuant 800 control software
- 3.4 Chemiluminescence image capture
- 3.4.3 Manual chemiluminescence exposure

Step	Action	
6	If an overlay image of the sample and marker is required, tap the toggle button next to Colorimetric marker .	
	Colorimetric marker	
7	Under Advanced settings , change the settings for Binning and Capture area as required. See <u>Section 3.3 Advanced settings</u> , on page 41 for more information.	
8	Enter the settings for <i>Fluorescence multiplex</i> if required, see <i>Fluorescence multiplex</i> , on page 47.	
9	To save the capture settings for future use, tap Save method . In the popup window, enter the Name of the method and tap Save .	
10	Tap Start .	

3 Use of methods in ImageQuant 800 control software 3.4 Chemiluminescence image capture 3.4.4 Time series chemiluminescence exposure

3.4.4 Time series chemiluminescence exposure

Introduction

A time series exposure takes a series of sequential images.

Time series settings

The *Time series* exposure setting has two additional settings that can be altered. These are described in the table below

Setting	Description
Cumulative	This setting will sum the pixel intensity of all of the previ- ously captured images. For example, the second image will be the sum of the intensity of the first and second image. The third image will be the sum of the first, second, and third images. This setting can be used to determine the optimum exposure time for the sample.
Stop when satu- rated	Image exposure will stop as soon as regions start becoming saturated. In a time series exposure, cumulative imaging will also stop when saturation has been reached. This means that subsequent images will not be taken.

Take a time series exposure

To take a time series chemiluminescence exposure, use the following steps.

Step	Action
1	On the <i>Home</i> screen, tap <i>Chemiluminescence</i> image capture.
2	Under 1.Select Exposure, tap Time series.
3	Under Number of images , use the + and - buttons to select the desired number of image to be taken.
4	Under Exposure time per image tap the white box

4 Under **Exposure time per image**, tap the white box.

- 3 Use of methods in ImageQuant 800 control software
- 3.4 Chemiluminescence image capture

3.4.4 Time series chemiluminescence exposure

Step Action

```
5
```

In the *Exposure time per image* window, tap the box under *min* or *sec* and use either + and - buttons, or the keypad to enter the desired value.



Note:

The exposure time must be set to a minimum of 10 seconds.

6 If image merging is not required, toggle **Cumulative** to the off position.



- 9 Under *Advanced settings*, change the settings for *Binning* and *Capture area* as required. See *Section 3.3 Advanced settings, on page 41* for more information.
- 10 To save the capture settings for future use, tap **Save method**. In the popup window, enter the **Name** of the method and tap **Save**.

3 Use of methods in ImageQuant 800 control software 3.4 Chemiluminescence image capture 3.4.4 Time series chemiluminescence exposure

Step Action

11 Tap **Start**.

Result:

The **View image** screen will show while all of the images are being taken. The current image being taken will show as **Exposing** and images to be taken afterwards as **Pending**.

1/4	0	2/4	0				
				٥			
Exposing		Pending					
3/4	0	1/4	0				
				Q			
Pending		Pending		Q			
				•			
						5	ton

3.4 Chemiluminescence image capture

3.4.5 SNOW chemiluminescence exposure

3.4.5 SNOW chemiluminescence exposure

Introduction

SNOW exposure reduces noise and avoids saturation of the image by taking multiple exposures and merging them into a single image. This improves the signal to noise ratio.

The level of signal to noise improvement at which the SNOW function stops can be calculated automatically using **Auto stop**. Alternatively, the user can input the desired **Target improvement ratio**. For example, if a **Target improvement ratio** of 5.0 is entered, the process will stop when the signal to noise ratio has improved by 5-fold.

Note: In some exposures, the maximum signal to noise ratio might be reached before the manually set **Target improvement ratio** is reached. This is seen when the **S/N improvement number** curve flattens out. The **Target improvement ratio** can be altered during a run or the process can be stopped and the image saved directly if this happens.

Take an image using SNOW

Step	Action
1	On the <i>Home</i> screen, tap <i>Fluorescence</i> image capture.
2	Under 1.Select Exposure , tap SNOW .
3	If an overlay image of the sample and marker is required, tap the toggle button next to Colorimetric marker .
	Colorimetric marker
4	Under Advanced settings , change the settings for Binning and Capture area as required. See <u>Section 3.3 Advanced settings</u> , on page 41 for more information.
5	To save the capture settings for future use, tap Save method . In the popup window, enter the Name of the method and tap Save .
6	Tap Start to begin the image pre-capture.

To capture an image using the SNOW function, use the following steps.

Step Action

7 In the pre-capture screen the entire image is pre-selected as the auto time area. To change this, tap **Select** next to **Step 1: select auto time area** (1) and tap and drag to select the auto time area (2).



Result:

8

The auto time area is selected and appears with a blue border.

In the pre-capture screen, tap **Select** (1) next to **Step 2: Select area of interest**. Tap and drag to select the area of interest (2).



Result:

The area of interest is selected and appears in green.

- 3 Use of methods in ImageQuant 800 control software
- 3.4 Chemiluminescence image capture

3.4.5 SNOW chemiluminescence exposure

9

Step Action

Tap **Select** (1) next to **Step 3: Select background**. Tap and drag to select the background area (2).



Note:

The SNOW algorithm calculates signal and noise from the region of interest and the selected background area. Select a flat background area that is free from bands, stain speckles, or dust. If it is not possible to select such a background area, for example if a post-stained gel exhibits many dye speckles in the gel background, it is recommended to select a smaller area, or an area next to the gel.

Result:

The background area is selected and appears in yellow.

10 To manually enter a target improvement ratio, toggle off *Auto stop* (1), tap the box next to *Target improvement ratio* (2), and enter a new value.



3 Use of methods in ImageQuant 800 control software 3.4 Chemiluminescence image capture 3.4.5 SNOW chemiluminescence exposure

Step	Action
11	Tap Start .
	Result:
	The instrument will begin CAPTURING . After each image capture, the signal to noise improvement ratio will update.
12	To manually enter a target improvement ratio during image capture, toggle off <i>Auto stop</i> , tap the box next to <i>Target improvement ratio</i> , and enter a new value.

Result:

The manual boundary will show in the graph.



3.5 Colorimetric image capture

3.5 Colorimetric image capture

About this section

This section contains information on how to create and run colorimetric imaging methods.

In this section

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3.5.3	Optical Densitometry	66

3 Use of methods in ImageQuant 800 control software
3.5 Colorimetric image capture
3.5.1 Colorimetric imaging

3.5.1 Colorimetric imaging

Introduction

Colorimetric imaging is used to image colorimetric stained gels (e.g., Coomassie and silver staining). This setting can also be used for quantitative densitometry measurements. The table below states which accessories are needed for colorimetric imaging. This depends on whether Epi or Trans illumination is being used. Epi illumination can be performed on the upper or lower tray position. Trans illumination can be performed in the lower tray position only.

Imaging mode	
Epi illumination	Black tray with White insert
Trans illumination	Glass tray

Note: To achieve the best possible accuracy, trans illumination is recommended. This method is only available in certain instrument configurations. For more information on instrument configurations and upgrading the Amersham ImageQuant 800 instrument, see Amersham ImageQuant 800 Operating Instructions (29372604).

The Colorimetric tab

The illustration below shows the main components of the **Colorimetric** screen.



Part	Function	Part	Function
1	Save image capture settings as a method	4	Open method

- 3 Use of methods in ImageQuant 800 control software
- $3.5\ \, {\rm Colorimetric}\, {\rm image}\, {\rm capture}$
- 3.5.1 Colorimetric imaging

Part	Function	Part	Function
2	OD measurement	5	Start capture
3	Gel documentation		

3.5.2 Gel documentation

Introduction

Gel documentation can be used to take images of colorimetric stained protein gels. In this mode, basic settings are predetermined and cannot be changed.

Take a gel image

To take a colorimetric gel image, use the following steps.

Step	Action
1	On the <i>Home</i> screen, tap <i>Colorimetric</i> image capture.
2	Under 1.Select Exposure, tap Gel documentation.
3	Under Advanced settings , change the settings for Capture area as required. See Section 3.3 Advanced settings, on page 41 for more information.
4	To save the capture settings for future use, tap Save method . In the popup window, enter the Name of the method and tap Save .
5	Tap Start .

3.5 Colorimetric image capture

3.5.3 Optical Densitometry

3.5.3 Optical Densitometry

Introduction

For optical density (OD) measurements, pixel data are converted to OD values to obtain a linear correlation between the output signal and the amount of sample. Each instrument is factory calibrated using an OD step tablet. In this mode, basic settings are predetermined and cannot be changed.

Note: OD measurements can only be made in the lower tray position using Trans illumination.

Take an OD image

To take an OD colorimetric image, use the following steps.

Action
On the <i>Home</i> screen, tap <i>Colorimetric</i> image capture.
Under 1.Select Exposure , tap OD measurement .
Under Advanced settings , change the settings for Capture area as required. See Section 3.3 Advanced settings, on page 41 for more information.
To save the capture settings for future use, tap Save method . In the popup window, enter the Name of the method and tap Save .
Tap Start .
Note:
Both color and greyscale images for analysis are saved in the image file folder.

3.6 Fluorescence image capture

About this section

This section contains information on how to create and run fluorescence imaging methods.

In this section

Section		See page
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3.6.3	Manual fluorescence exposure	74
3.6.4	SNOW fluorescence exposure	77

3.6 Fluorescence image capture

3.6.1 Fluorescence imaging

3.6.1 Fluorescence imaging

Introduction

Fluorescence imaging is used to image fluorescent samples, for example in gels, membranes, multi-well plates, and petri dishes. For membranes with colorimetric markers, such as Amersham Rainbow markers, additional colorimetric image capture produces an overlay image comprising color and fluorescence image captures.

Note: For best image quality in **Fluorescence** imaging mode, it is recommended to not use the **White insert** due to auto-fluorescence from the insert. If a colorimetric image is needed, for example from a gel with Colorimetric marker, it is recommend to capture images firstly with the **White insert** for Mw determination and then without the **White insert** for best fluorescence detection of weak bands.

The Fluorescence tab

The illustration below shows the main components of the *Fluorescence* screen.



Part	Function	Part	Function
1	Save image capture settings as a method	8	Select dyes
2	SNOW (noise reduction mode)	9	Select capture area
3	Manual exposure	10	Toggle colorimetric marker
4	Auto exposure	11	Toggle full dynamic range
5	Open method	12	Start pre-capture
6	Help	13	Start image capture

3.6 Fluorescence image capture

3.6.1 Fluorescence imaging

Part	Function	Part	Function
7	Select binning		

Select dyes

Dye	Wavelength (nm)	Filter
UV	365	Cy3(UV)
Cy2	460	Cy2
СуЗ	535	Cy3(UV)
Cy5	653	Cy5
IRshort	660	IRshort
IRlong	775	IRlong

To select which dye or dyes are being used in the sample, use the following steps. A maximum of three dyes can be analyzed at one time.

Step Action

1

To select the dyes used, under **2.Basic settings**, tap **Select.**

Ֆ	Select dyes	Select
	UV	

- 3 Use of methods in ImageQuant 800 control software
- 3.6 Fluorescence image capture
- 3.6.1 Fluorescence imaging

Step	Action
2	In the <i>Fluorescence dyes</i> window, tap the drop-down arrow and select the dye being used.

Dye			Exposure tim	e	
UV	🛊 365 nm	💠 Cy3(UV) 🔺		11	00
UV	🛊 365 nm	♣ cy3(0V)	min	sec	1/100
Cy2	🌞 460 nm	🕹 Cy2			
Cy3	🛊 535 nm	💠 Cy3(UV)			
	÷	1			

- 3 To add a another dye, tap *Add dye* and select the new dye from the dropdown menu.
- 4 Tap **Select** to return to the method screen.

Sample positioning for IRlong

IRlong imaging can be susceptible to background gradient effects.

Position the sample in the center of the tray for IRlong image capture.

3.6.2 Automatic fluorescence exposure

Introduction

Automatic exposure uses a short pre-exposure to automatically calculate the exposure time. The area of interest is used to calculate the exposure time can be manually selected using **Pre-capture**. The calculated exposure time is based on the intensity level of the most intense signal detected in the selected region, or in the entire capture area if no region is predefined. The **Full Dynamic Range** setting can be used to use the full range of intensities to calculate the exposure time. This results in a longer exposure time.

Take an automatic exposure

To take an automatic exposure, use the following steps.

Step	Action
1	On the <i>Home</i> screen, tap <i>Fluorescence</i> image capture.
2	Under <i>1.Select Exposure</i> , tap <i>Auto</i> .
3	Under Select dyes , select the dye or dyes being used in the image.
4	Tap Select to return to the method screen.

5 If an overlay image of the sample and marker is required, tap the toggle button next to **Colorimetric marker**.



6 If shorter exposure times are required, tap the toggle button next to **Full dynamic range** to the off position.



Note:

Turning off **Full dynamic range** will reduce the range of intensity levels used in the image.

3.6 Fluorescence image capture

3.6.2 Automatic fluorescence exposure

Step	Action
7	Under <i>Advanced settings</i> , change the settings for <i>Binning</i> and <i>Capture area</i> as required. See <i>Section 3.3 Advanced settings, on page 41</i> for more information.
	Tip:
	When using IRlong, it is recommended to use a smaller, central capture area for best quantitation.
8	To save the capture settings for future use, tap Save method . In the popup window, enter the Name of the method and tap Save .
9	Tap Pre-capture .
	Note:
	To take an automatic exposure without predefining a region of interest, tap Start , without tapping Pre-capture .
10	Tap the button to toggle between Point and Area measurement.

Tap at the desired point on the image


3 Use of methods in ImageQuant 800 control software 3.6 Fluorescence image capture 3.6.2 Automatic fluorescence exposure

Step Action 11 Either use a point as a frame of reference, toggle to **Point** and tap the image at the desired location (1).



Or

Use an area as the frame of reference, toggle to **Area**. Tap and drag to select the desired reference area (1).



Note:

The area can be moved without redrawing it by using the arrow buttons (2).

12 Tap Start.

3 Use of methods in ImageQuant 800 control software

3.6 Fluorescence image capture

3.6.3 Manual fluorescence exposure

3.6.3 Manual fluorescence exposure

Introduction

With manual exposure the user can enter a specified exposure time.

Take a manual exposure

To take a manual exposure, use the following steps.

Step	Action		
1	On the <i>Home</i> screen, tap <i>Fluoresc</i>	e nce image capture.	
2	Under 1.Select Exposure , tap Mar	ual.	
3	To select the dyes used, tap Select .		
	ୟୁ Select dyes	Select	

 	Select
UV	

4 In the *Fluorescence dyes* window, tap the drop-down arrow and select the dye being used.

ye			Exposure tim	e	
UV	🗰 365 nm	🕹 Cy3(UV) 🔺	- 4	11	00
UV	🐞 365 nm	🕹 cy3(UV)	min	sec	1/100 se
Cy2	🌞 460 nm	🕹 Cy2			
Cy3	🌞 535 nm	🕹 Cy3(UV)			
	de ere	1			

5

Under **Exposure time**, tap the white box.

Step Action



7

In the **Exposure time** window, tap the box under **min**, **sec**, or **1/100 sec** and use either **+** and **-** buttons, or the keypad to enter the desired value.

Exposu	ure tim	e					\times
	min	sec	1/100 sec				
	+	+	+	1	2	3	
	00	11	00	4	5	6	
	•			7	8	9	
Cancel	Re	set	ок	←	0	→	

Note:

The maximum time that can be set is 10 min. The minimum time that can be set is 0.01 sec.

To add a another dye, tap **Add dye** and select the new dye from the dropdown menu.

Result:

A new dye selection row appears.



- 8 Enter an *Exposure time* for the new dye.
- 9 If needed, add a third dye.
- 10 If an overlay image of the sample and marker is required, tap the toggle button next to **Colorimetric marker**.



3 Use of methods in ImageQuant 800 control software

3.6 Fluorescence image capture

3.6.3 Manual fluorescence exposure

Step	Action
11	Under Advanced settings , change the settings for Binning and Capture area as required. See <u>Section 3.3 Advanced settings</u> , on page 41 for more information.
12	To save the capture settings for future use, tap Save method . In the popup window, enter the Name of the method and tap Save .
13	Tap Start .

3.6.4 SNOW fluorescence exposure

Introduction

SNOW exposure reduces noise and avoids saturation of the image by taking multiple exposures and merging them into a single image. This improves the signal to noise ratio.

The level of signal to noise improvement at which the SNOW function stops can be calculated automatically using *Auto stop*. Alternatively, the user can input the desired *Target improvement ratio*. For example, if a *Target improvement ratio* of 5.0 is entered, the process will stop when the signal to noise ratio has improved by 5-fold.

Note: In some exposures, the maximum signal to noise ratio might be reached before the manually set **Target improvement ratio** is reached. This is seen when the **S/N improvement number** curve flattens out. The **Target improvement ratio** can be altered during a run or the process can be stopped and the image saved directly if this happens.

Take an image using SNOW

Step	Action
1	On the <i>Home</i> screen, tap <i>Fluorescence</i> image capture.
2	Under <i>1.Select Exposure</i> , tap <i>SNOW</i> .
3	If an overlay image of the sample and marker is required, tap the toggle button next to Colorimetric marker .





- 4 Under *Advanced settings*, change the settings for *Binning* and *Capture area* as required. See *Section 3.3 Advanced settings*, *on page 41* for more information.
- 5 To save the capture settings for future use, tap **Save method**. In the popup window, enter the **Name** of the method and tap **Save**.
- 6 Tap **Start** to begin the image pre-capture.

3 Use of methods in ImageQuant 800 control software

3.6 Fluorescence image capture

3.6.4 SNOW fluorescence exposure

7

Step Action

In the pre-capture screen the entire image is pre-selected as the auto time area. To change this, tap **Select** next to **Step 1: select auto time area** (1) and tap and drag to select the auto time area (2).



Result:

The auto time area is selected and appears with a blue border.

8

In the pre-capture screen, tap **Select** (1) next to **Step 2: Select area of interest**. Tap and drag to select the area of interest (2).



Result:

The area of interest is selected and appears in green.

Step Action

9

Tap **Select** (1) next to **Step 3: Select background**. Tap and drag to select the background area (2).



Note:

The SNOW algorithm calculates signal and noise from the region of interest and the selected background area. Select a flat background area that is free from bands, stain speckles, or dust. If it is not possible to select such a background area, for example if a post-stained gel exhibits many dye speckles in the gel background, it is recommended to select a smaller area, or an area next to the gel.

Result:

The background area is selected and appears in yellow.

10 To manually enter a target improvement ratio, toggle off *Auto stop* (1), tap the box next to *Target improvement ratio* (2), and enter a new value.



3 Use of methods in ImageQuant 800 control software

3.6 Fluorescence image capture

3.6.4 SNOW fluorescence exposure

Step	Action
11	Tap Start .
	Result:
	The instrument will begin CAPTURING . After each image capture, the signal to noise improvement ratio will update.

12 To manually enter a target improvement ratio during image capture, toggle off *Auto stop*, tap the box next to *Target improvement ratio*, and enter a new value.

Result:

The manual boundary will show in the graph.



4 Manage images in ImageQuant 800 control software

About this chapter

This chapter contains information on how to view and manage images in ImageQuant 800 control software.

In this chapter

Secti	on	See page
4.1	View the image after capture	82
4.2	The Image library screen	88

- 4 Manage images in ImageQuant 800 control software
- 4.1 View the image after capture

4.1 View the image after capture

After image capture, the image will be displayed on the **View image** screen. Here, it is possible to perform some minor edits to the image, such as cropping and rotation.

The View image screen



Part	Function	Part	Function
1	Image	6	Save image
2	Toggle image overlays	7	Return to image capture screen
3	Image information	8	Print image
4	Select editing tool	9	Zoom in and out of image
5	Editing tools		

Image overlays

Depending on the imaging type used, the image overlays tabs will be different.

Imaging method	Display options
Chemiluminescence with colorimetric marker	Toggle Marker on and off

Imaging method	Display options
Chemiluminescence with fluorescence multiplex	Toggle Chemi and fluorescence dyes on and off
Colorimetric	Toggle between Sample (brightfield view) and Color
Fluorescence	Toggle fluorescence dyes on and off

Edit the image

ImageQuant 800 control software provides basic tools for editing captured images before saving them.

🖋 Edit	Contrast	🖍 Intensity	
Invert		-	
Crop	rop Half crop Bord	ler crop Quarter crop	
Rotate		-90 +90 -	
Exposure tim Chemi Color	ie	seconds seconds	

Part	Function			
1	Invert. Toggle on to invert the image colors.			
2	Crop . Set image crops if multiple samples have been used.			
	Note:			
	Using Half crop and Quarter crop will separate the image into 2 or 4 image files when saved.			
3	Rotate image.			
4	Exposure time for each image type.			

- 4 Manage images in ImageQuant 800 control software
- 4.1 View the image after capture



Part	Function
1	Histogram
2	Increase or decrease lower boundary
3	Increase or decrease upper boundary
4	Automatically set the image contrast

Measure the signal intensity

The *Intensity* function can be used to measure the signal intensity of either a single point or a defined area of the image. When an area has been created, it can be moved around the image using the arrows. This function can be used to check if the exposure time was suitable and do a first estimate of signal levels in different positions on the image.

To measure the signal intensity, use the following procedure.

Note: Signal intensity measurements are not reliable in saturated images. Saturated parts of an image are highlighted in purple.



Part	Function
1	Non-saturated band
2	Saturated band

Tip: To avoid image saturation, either set a lower binning or reduce the exposure time.

Step	Action
1	In the <i>Image view</i> screen, tap <i>Intensity</i> .
2	Tap and drag to select the area of interest.



3

Read the intensity signals in the right table.

	Chemi	Cy 2	Cy 3
Maximum	56334	56334	56334
Average	7500	7500	7500
Maximum	4800	4800	4800

Note:

If multiple images are being viewed, the intensities of all dyes will be displayed.

- 4 Manage images in ImageQuant 800 control software
- 4.1 View the image after capture



Time-series images

When taking a *Time series* exposure, different options are available on the *View image* screen.



Part	Function	Part	Function
1	Select all images	6	Individual image information
2	Image number and exposure	7	Scroll up and down through images
3	Select which individual image to save	8	Display selected image on full screen
4	Toggle colorimetric Marker on and off	9	Zoom in all images
5	Selected image for full screen (indicated by blue border)	10	Zoom out all images

4 Manage images in ImageQuant 800 control software

4.2 The Image library screen

4.2 The Image library screen

Illustration of the *Image library* screen

The illustration below shows the main components of the *Image library* screen.



Part	Function	Part	Function
1	Toggle between list and grid (thumbnail) view	7	Selected image information
2	Image thumbnails from selected folder	8	Open selected image
3	Select image folder	9	Copy/move image
4	Select imaging method	10	Delete image
5	Sort images	11	Select all images
6	Search images		

Open an image

To open an image in the *Image library* screen, use the following steps.

1

Tap the *Image library* icon.

Step	Action
2	Tap Folder name and either select one of the default save locations or tap Browse to navigate to the save folder using the system browser.
	Result:
	The images in the save folder will show in the image view panel.
3	Tap to select the desired image.
	Result:
	The image properties will display in the image information panel.
	Note:
	Multiple images can be selected at once.
4	Tap Open to open the image file.
	Result:
	The image or images are opened in the <i>Image view</i> screen.
	Note:
	If multiple images have been selected, the option to Open is not available. The option to Copy/Move this images is available for multiple images.
	The option to Copy/Move this images is available for multiple images

Move image

Image files can be copied and moved in Amersham ImageQuant 800 control software.

Step	Action
1	In the <i>Image library</i> screen, tap to select the image or images to be moved.
2	Tap Copy/Move .

3 On the left side, tap to select the image or images to be moved.

Copy/Move images		3
Image1 20190508_084354_Ch		
20190507_145944_FI		
	Destination	
	c/images	▼ ≦

4.2 The Image library screen

Step	Action			
4	Under Destination select the destination folder or tap the folder icon to browse to a new location.			
	Destination			
	c:/images	•	1	
	c:/images			
	c:/tmp			
	C:/Users/503026659/Documents			

5 Tap **Copy** to copy the image to the new location.

Note:

Tap **Move** to move the image to the new location and remove it from the current location.

Print image

Amersham ImageQuant 800 control software can print images directly to a printer connected on the network. To do this, use the following steps.

Step	Action
1	In the View image screen, tap Print .
2	Tap to select the printer in the Print window.
3	Tap Print .

Save locations

Save locations can be added and the default save location can be chosen in the **Settings** screen, see *Default save location, on page 20*.

5 ImageQuant CONNECT operation

About this chapter

This chapter describes the ImageQuant CONNECT software and provides information on how to use the different functions.

In this chapter

Section		See page
5.1	Introduction	92
5.2	Use the scheduler tool	93
5.3	Manage images	95

5.1 Introduction

5.1 Introduction

The Amersham ImageQuant CONNECT software enables the user to connect remotely to any Amersham ImageQuant 800 instrument on the network. Using this package, it is possible to monitor runs, book a time slot for using the machine, as well as view, and download image files from the instrument.

The image below shows a schematic representation of the Amersham ImageQuant 800 system network.



Part	Function
1	Amersham ImageQuant 800 instrument
2	External PC connected to Amersham ImageQuant 800
3	Local network
4	Computers connected to local network

5.2 Use the scheduler tool

Introduction

The ImageQuant CONNECT scheduler tool can be used to remotely book or edit a time slot on the Amersham ImageQuant 800 instrument remotely.

The Scheduler screen



Part	Function	Part	Function
1	Instrument status	5	Scheduler tab
2	Selected time slot	6	Help
3	Refresh calendar	7	Create new booking
4	Select week	8	Delete booking

Create a new booking

To create a new booking, use the following steps.

Step Action

1 Open the **Scheduler** screen.

5 ImageQuant CONNECT operation

5.2 Use the scheduler tool

Step	Action					
2	Use the	Use the arrows to navigate to the desired week.				
	 A 	AUGUST 4 th - 10 ^t	^h , 2019 🕨			
3	Click on	the calendar	to select the d	lesired time and day.		
		Sunday	Monday			
		4	5			
	7 ^{AM}					
	8 ^{AM}					
4	Click Ne	w booking				
5	In the N next to b	ew booking User and Con	window, additi nment .	ional details can be added in the text bo		

6 Enter the desired end time for the time slot by clicking in the drop-down menu.

From	7:30 am	•	То	7:45 am	
				7:45 am	
			8:0 am	8:0 am	
				8:15 am	

Note:

By default, time slots are set to 15 minutes.

7 Click Save.

Edit a booking

To edit a booking, use the following steps.

Step	Action
1	In the Scheduler screen, select the booking to be edited.
2	Click Edit booking .
3	In the <i>Edit booking</i> window, enter the new information.
4	Click Save .

To delete a booking, select the booking slot and click **Delete**.

5.3 Manage images

The Images tab



Part	Function	Part	Function
1	Image window	6	Image information
2	Select folder	7	Open image
3	Filter by method	8	Copyimage
4	Sort images by date or alpha- betically	9	Select all images in the folder
5	Search images	10	Toggle between list and thumbnail image view

5 ImageQuant CONNECT operation

5.3 Manage images

Open an image

To open and edit an image in Amersham ImageQuant CONNECT, use the following steps.

Step Action

1

Click the **Folder name** drop-down menu and either select one of the listed folders, or click **Browse** to navigate to a folder on the network.



Result:

The image file in the selected folder will display in the image window

2 Click on the desired image or images.

Note:

The **Method**, **Sort by**, and **Search...** tools can be used to help find specific images.



3

Click on the desired image to display the image information in the right panel.

Note:

If more than one image is selected, no information will be displayed.

Step Action

4 Click **Open**.

Result:

The image is opened in the image editing screen.



5

Use the arrow keys (1) to reposition the upper (pink) and lower (blue) boundaries of the histogram (2) to manually adjust the contrast of the image. Click **Auto** to automatically set the contrast, and click **Reset** to return to the original levels.



Note:

Increasing the lower (blue) boundary will lighten the image and decreasing the upper (pink) boundary will darken the image.

- 6 To return to the *Images* tab, click *Close*.
- 7 To save a local copy of the image, click **Download**.

5.3 Manage images

Step	Action
8	Navigate to the local save folder in the browser and click Select folder .
	Result:
	A new folder will be created at the selected location containing a .jpg and .tiff version of the file.
	Information
	Copy is complete.
	ок

Measure the signal intensity

The *Intensity* function can be used to measure the signal intensity of either a single point or a defined area of the image. to use this function, follow the instructions in *Measure the signal intensity, on page 84*.

6 Troubleshooting

About this chapter

This chapter provides information to assist users and service personnel to identify and correct problems that may occur when operating the product.

If the suggested actions in this guide do not solve the problem, or if the problem is not covered by this guide, contact your Cytiva representative for advice.

In this chapter

Section		See page
6.1	Instrument problems	100
6.2	Image problems	102
6.3	Software problems	104
6.4	Error messages	105

6.1 Instrument problems

General troubleshooting procedure

If an error occurs, follow the procedure below to restart the system.

Step	Action
1	Make a note of the error code and error message on the monitor.
2	Shut down the Amersham ImageQuant 800 control software using the usual procedure, see the Amersham ImageQuant 800 Operating Instruc- tions (29372604).
3	If the shut down procedure does not work, use the task manager to force the Amersham ImageQuant 800 control software to exit. Minimize the Amersham ImageQuant 800 control software window to access the Windows desktop and open the task manager.
4	If the software does not shut down, turn off the instrument by pressing the power button on the right side of the instrument.
5	Wait for approximately 10 seconds and turn the system on again.
6	If the error persists, refer to this troubleshooting guide for more information.
7	If the error cannot be resolved using this troubleshooting guide, contact your Cytiva representative.

General problems

This section describes possible causes and corrective actions for various problems with the instrument. If the error persists after taking the suggested corrective actions, contact your Cytiva representative.

Error symptom	Possible cause	Corrective action
Atypical noise is heard	If noise comes from the camera head then a camera head failure has occurred	Turn off the power switch immediately and contact your Cytiva representative
Atypical odor is emitted	If odors are emitted from the camera head then a camera head failure has occurred	Turn off the power switch immediately and contact your Cytiva representative
	If odors are emitted then an instrument failure has occurred	Turn off the power switch immediately and contact your Cytiva representative

Error symptom	Possible cause	Corrective action
Smoke is emitted	If smoke is emitted from the camera head then a camera head failure has occurred	Turn off the power switch immediately and contact your Cytiva representative
	Smoke is emitted from the instrument then an instru- ment failure has occurred	Turn off the power switch immediately and contact your Cytiva representative
The Indicator is illumi- nated red	A hardware error has occurred	If an error code is displayed, make a note of it and contact your Cytiva representa-tive
	The door is open during exposure	Close the door and repeat the exposure
Operation is not stopped when the door is opened	The light source is lit or the motor is running when the door to the instrument is open, an interlock failure has occurred	Turn off the instrument's Power switch immediately and contact your Cytiva representative
	The door to the instru- ment cannot be opened or closed or the door cannot be locked because a foreign object is present in the locking section or the door sensor	Remove the foreign object
	The door to the instru- ment cannot be opened or closed, or the door cannot be locked and no foreign object is present	The locking section is damaged. Contact your Cytiva representative
The instrument cannot be cooled down properly	The ambient air tempera- ture is too high	Lower the room temperature to 28°C or lower
The instrument does not appear to respond to soft- ware control	The instrument is not connected to the external computer	Unplug the instrument and check that all connections between the instrument and external computer are secure

6.2 Image problems

Error symptom	Possible cause	Corrective action
An appropriate image does not appear	An image is overexposed and saturated	Set a lower binning or reduce the expo- sure time
	An image is underexposed	Prolong the exposure time
	The focus is not correctly adjusted	Adjust the focus temporarily. See <i>Adjust focus, on page 16</i>
		If the problem persists contact your Cytiva representative
	The tray, or window covering the light source are dirty	Clean the tray or the window covering the light source
	The wrong sample tray is used	Change to the correct sample tray, see Chapter 3 Use of methods in ImageQuant 800 control software, on page 36
	The wrong light source is selected	Select the correct light source, see Chapter 3 Use of methods in ImageQuant 800 control software, on page 36
	The size of the object exposed does not coincide with the exposed area	Place the sample correctly on the sample tray and place the tray in the correct tray position
Light leaks on the image	The door to the instru- ment is not completely closed	Close the door to the instrument and expose the image again
	The instrument is exposed to direct sunlight	Move the instrument to a place that is not in direct sunlight
The image appears misty	Moisture is condensing in the optical system	Turn off the instrument and wait until the ambient environments meets the specifications
Unevenness appears on the image	The LED is deteriorated from age	Turn off the instrument and contact your Cytiva representative
Shadow bands appear when using White Epi imaging in the upper tray position	Position of White Epi light source relative to the gel lanes	Rotate the gel sample 90° positioning the gel so that the lanes of the gel are parallel to the long side of the tray

Error symptom	Possible cause	Corrective action
Shadow bands appear when using White Epi imaging in the lower tray position	Position of White Epi light source relative to the gel lanes	Rotate the gel sample 90° positioning the gel so that the lanes of the gel are parallel to the short side of the tray
Saturation artefacts in image (blooming)	Maximum charge transfer capacity of the CCD has been reached	Set a lower binning or shorter exposure time to reduce image saturation
Weak background images from previous images are visible	Ghost images are visible due to multiple exposures being taken in quick succession	Turn of the Amersham ImageQuant 800 instrument and wait three minutes before restarting the system
Gradient background in IRlong images of membrane samples	Membrane is placed outside of central imaging area (80 × 110 cm)	Place a single membrane in a central position in the imaging area. Use local background correction to reduce the gradient background, see <i>Sample positioning for IRlong, on page 70</i>
Weak fluorescence bands when using White insert	Auto-fluorescence of White insert	Capture additional fluorescence images for analysis without using the White insert
No improvement of S/N ratio in SNOW mode. Captures are too quickly auto stopped	Appropriate background area was not selected	Select a flat background area which is free from bands, stain speckles, or dust and restart. If there is no such flat back- ground area, select a background area next to the membrane or gel

6.3 Software problems

Error symptom	Possible cause	Corrective action
After exposure, no image is displayed on the screen	The light source does not function properly	Contact your Cytiva representative
	The sample is not in posi- tion	Check the sample position
The software is non- responsive	Transferring large amounts of files can impair the system's performance	Avoid file transfer during operation
Instrument shows as not available in Amersham ImageQuant CONNECT	Network connection problem	Click sync to refresh the list of instru- ments
		Check the that instrument is connected to the network. See <i>Amersham</i> <i>ImageQuant 800 Operating Instructions</i> (29372604)
Instrument is not visible in the instrument drop-down menu in Amersham ImageQuant CONNECT	Instrument connection problem	Click sync to refresh the list of instru- ments
		Return to <i>Home</i> screen and check if instrument is connected
	Instrument is switched off	Make sure that the Amersham ImageQuant 800 is switched on
Booking made in Amersham ImageQuant CONNECT is not showing on host instrument	Network connection problem	Click sync and re-enter the booking
		Check the that instrument is connected to the network. See <i>Amersham</i> <i>ImageQuant 800 Operating Instructions</i> (29372604)
Unable to log in	Forgotten password	Reset the user's password, see <i>Reset a password, on page 27</i>

6.4 Error messages

Error messages and suggested corrective actions

The following table lists error messages that may appear.

For error situations that are not fatal, the error message generally provides an option to continue or abort the run.



6 Troubleshooting

6.4 Error messages

Description	Image	Corrective action
Set interval time to 10 s or longer	Image: Contract of the state of the stat	Tap OK and set a longer exposure time in the method settings.
Save prompt.		The images captured in this session have not been saved. To return to the software and save the image, tap No . To exit the software without saving and new images or methods, tap Yes .

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29383813 AB V:3 11/2020