

GloMax[®] Galaxy Bioluminescence Imager Operating Manual

Instructions for Use of GM4000 and GM4005



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All technical literature is available at: www.promega.com/protocols/

Visit the website to verify that you are using the most current version of this Technical Manual.

Email Promega Technical Services if you have questions on use of this system: techserv@promega.com

1 Introduction

1.1 Description

The GloMax® Galaxy Bioluminescence Imager is a luminescence microscope that can visualize the output from NanoLuc® Technologies. The combination of this imager and NanoLuc® Technology means you can study functional and dynamic cellular events across a population of cultured cells. These events include protein localization, protein:protein and protein:small molecule interactions, as well as changes in protein abundance via degradation or stabilization. With the GloMax® Galaxy Imager, NanoLuc®-based microplate reader assays (e.g., NanoBiT, NanoBRET or Nano-Glo) can be complemented with luminescence, fluorescence and brightfield microscopy to study and validate cellular model systems prior to and post-screening.

The system is operated by a personal computer (PC) and control software that provide quick and easy navigation through the control options.



Figure 1. The GloMax® Galaxy Bioluminescence Imager.

1.2 Specifications

Instrument Specifications

Imaging Modes	Luminescence, Filtered Luminescence, Fluorescence, Brightfield
Sample Format	Slide, microchamber, 35mm dish, 6-, 12-, 24-, and 96-well plates
Dimensions (W × H × D)	14.8 inches × 18.8 inches × 21.0 inches (37.3cm × 47.7cm × 53.3cm)
Weight	Approximately 62lb (25kg)
Power Requirements	100–240V AC, 50/60Hz
Warranty	1 year parts and labor warranty included
Regulatory Compliance	For Research Use Only. Not for use in Diagnostic Procedures.
CE Compliance	<p>Pollution degree 2</p> <p>Installation category II</p> <p>Indoor use only</p> <p>Main supply voltage fluctuations are not to exceed ±10 percent of the nominal supply voltage</p> <p>This instrument complies with the requirements of the EU Directives for electromagnetic compatibility (EMC Directive) and electrical safety (the LVD). Compliance with the relevant Directives was demonstrated by third-party testing to the appropriate EN and IEC standards. The product bears the CE mark on its label as evidence of compliance, and an EU Declaration of Conformity for the product is available from Promega on request.</p>
Camera	Retiga E7 (CMOS), 4.5 × 4.5µm pixel size, 12-bit (0–4,095 gray scale)
Wavelength Range	400–750nm
Optics	Nikon CFI Plan Apochromat Lambda D 20X/0.75 NA Custom 100mm focal length Tube Lens (~10.35X System Magnification)
Image pixel size	1 pixel = 0.435µm

Fluorescence Excitation Specifications:

Light Source	LED
Excitation Position	Top (trans-) illumination
Wavelength Selection	Interchangeable on-the-fly, with five excitation modules
Excitation Module Included	Blue (Excitation: 480/30nm)
Excitation Modules Available Separately	UV (Excitation: 375/20nm) Green (Excitation: 540/25nm) Green (Excitation: 560/40nm) Amber (Excitation: 620/60nm)
Emission Filters Included	460nm/50BP (band pass), 535nm/40BP, 600nm LP (long pass)
Available Separately	635nm/60BP; 605nm/55BP; 700nm/75BP
Filter Slide	Four position for up to four standard (25mm) filters

Computer Requirements

Operating System	Windows® 10, 64-bit version
RAM	16GB minimum
Disk Space	512GB minimum
Ports	Two (2) USB 3.2 connections for system communication and optional stage top incubator communication
Other Requirements	Microsoft.NET Framework 4.8 or newer (full version)
Monitor	The minimum recommended screen resolution is 2560 × 1440. The recommended screen size is 27 inches.

Additional Computer Recommendations if Using Your Own PC:

- Disable automatic Windows® updates and virus scans
- Disable automatic power off, sleep or hibernation settings
 - Disable “Fast Startup”. Set the power button to Shut Down, not Sleep.
 - Disable any other automatically or manually started software that would consume PC resources during operation.
- Ensure there are no domain policies that would overwrite any of the above settings.

1.3 Product Components

PRODUCT	SIZE	CAT.#
GloMax® Galaxy Bioluminescence Imager System	1 each	GM4000

Includes:

- 1 GloMax® Galaxy Bioluminescence Imager
- 1 PC preloaded with GloMax® Galaxy Control Software and Fiji Image Processing Software
- 1 PC Monitor (27 inches)
- 1 Power Cord for PC
- 1 Power Cord for PC Monitor
- 1 Keyboard for PC
- 1 Mouse for PC
- 1 Blue Fluorescent Excitation Module (Ex: 480nm/30BP)
- 1 Filter Slide containing filters (1 each) 460nm/50BP, 535nm/40BP and 600nm LP filter
- 1 1-Position Slide Holder Insert
- 1 AC Power Adapter with detachable power cord
- 1 USB 3.1 Cable
- 1 Flat-Tip Screwdriver
- 1 Spare XY-Stage Adapter screws
- 1 Side Access Panel with Rubber Insert
- 1 Shipping anchors and thumbscrews set
- 1 Hex wrench for side access panel with extra screws
- 1 Air bulb





PRODUCT	SIZE	CAT.#
GloMax® Galaxy Bioluminescence Imager	1 each	GM4005

Includes:

- 1 GloMax® Galaxy Bioluminescence Imager
- 1 Blue 480/30nm Fluorescence Module, Galaxy
- 1 Filter Slide containing emission filters (1 each) 460nm/50BP, 535nm/40BP and 600nm LP filter
- 1 1-Position Slide Holder Insert
- 1 AC Power Adapter with detachable power cord
- 1 USB 3.1 Cable
- 1 Flat-Tip Screwdriver
- 1 Side Access Panel with Rubber Insert
- 1 Spare XY-Stage Adapter screws
- 1 Shipping anchors and thumbscrews set
- 1 Hex wrench for side access panel with extra screws
- 1 Air bulb

1.4 Precautions and Special Instructions






1.4.1 Safety Symbols and Special Markings

	<p>Warning. Risk of personal injury to the operator or a safety hazard to the equipment or surrounding area.</p>	<p>Avertissement. Risque de préjudice corporel pour l'utilisateur ou risque de danger pour l'appareil ou la zone environnante.</p>
	<p>It is important to understand and follow all laws regarding the safe and proper disposal of electrical instrumentation. Please contact your local Promega Representative for disposal of the instrument and power supply. Please follow your institutional requirements for disposal of the accessories.</p>	<p>Avertissement. Il est important de comprendre et de respecter toutes les lois relatives à la destruction sûre et correcte des appareils électriques. Veuillez contacter votre représentant Promega local concernant la destruction de l'appareil. Veuillez respecter les exigences de votre établissement concernant la destruction des accessoires.</p>
	<p>Warning. UV emitted from this Product. Avoid eye and skin exposure to unshielded product.</p>	<p>Avertissement. UV émis par cet appareil. Evitez toute exposition des yeux et de la peau avec cet appareil ouvert /non protégé.</p>
	<p>Clean up spills.</p>	<p>Nettoyer toutes éclaboussures.</p>

1.4.2 Special Instructions

- Immediately wipe up spills.
- The GloMax® Galaxy Imager contains sensitive optical components and precision-aligned assemblies. Handle with care.
- The objective lens is an air (dry) objective and should never be used with immersion oil. If this happens, lens cleaning paper soaked in petroleum benzine is recommended for cleaning off the oil, followed by ethanol. See Section 4.2.1, Cleaning Instructions.
- Use caution around solvents because they may damage the plastic case of the GloMax® Galaxy Imager.
- Do not submerge the GloMax® Galaxy Imager in water.
- Do not expose the GloMax® Galaxy Imager to temperatures outside the specified range, as damage may occur to the unit that will not be covered under warranty.
- Changes or modifications to this unit not expressly approved by Promega could void the user's authority to operate the equipment.
- Do not use this device in proximity to sources of strong electromagnetic radiation (e.g., microwave oven) because they may interfere with the proper operation.
- Do not use this instrument for anything other than its intended use.
- Always disconnect the power before cleaning or performing routine maintenance.
- Do not disassemble the instrument further than specified in this operating manual for routine maintenance and use.
- If the equipment is used in a manner other than that specified, the protection provided by the equipment may be impaired.
- Do not overfill sample vessel wells because this may lead to spills and/or damage.
- Plug instrument and PC into the same wall socket or outlet strip to assure common grounding.
- Do not leave sample vessel in the instrument after imaging is completed.

1.4.3 Important Safety Instructions—Please save these instructions.

	Power off the GloMax® Galaxy instrument before installing.	Éteignez l'instrument GloMax® Galaxy avant l'installation.
	Close the instrument door when the GloMax® Galaxy is not in use.	Fermez la porte de l'instrument lorsque le GloMax® Galaxy n'est pas utilisé.
	The GloMax® Galaxy is intended for indoor use only.	Le GloMax® Galaxy est destiné à une utilisation en intérieur uniquement.
	Always disconnect the power before cleaning or performing routine maintenance.	Débranchez toujours l'alimentation avant de nettoyer ou d'effectuer un entretien de routine.
	Always disconnect the AC Adapter from the power outlet when not in use.	Débranchez toujours l'adaptateur secteur de la prise de courant lorsqu'il n'est pas utilisé.

1.5 Environmental Requirements

Store and use the GloMax® Galaxy Imager under the following conditions:

Shipping and Storage Conditions	4–50°C, under noncondensing conditions, up to 80% humidity
Operating Conditions	+15°C to +30°C, 20–80% humidity
Operating Altitude Conditions	0 to 2,000 meters above sea level

1.6 Unpacking and Inspecting the GloMax® Galaxy Bioluminescence Imager

Upon receiving the GloMax® Galaxy Imager, inspect it carefully and make certain all accessories are included.

Refer to the checklist shipped with the instrument for order-specific items. Save all packaging materials, if possible, in case the instrument needs to be returned for service. If any item is damaged, contact Promega Technical Services (email: techserv@promega.com).

1.6.1 Unpacking the Instrument from the Shipping Container

1. Remove the straps around the boxes and pallet. Open the solid box containing the PC and monitor and remove them from the box (Figure 2, Panel A).
2. Next, remove the outer sleeve of the bottom box containing the instrument and remove the accessory box and foam insert (Figure 2, Panel B). Carefully lift the box top to expose the instrument which rests on the foam tray.

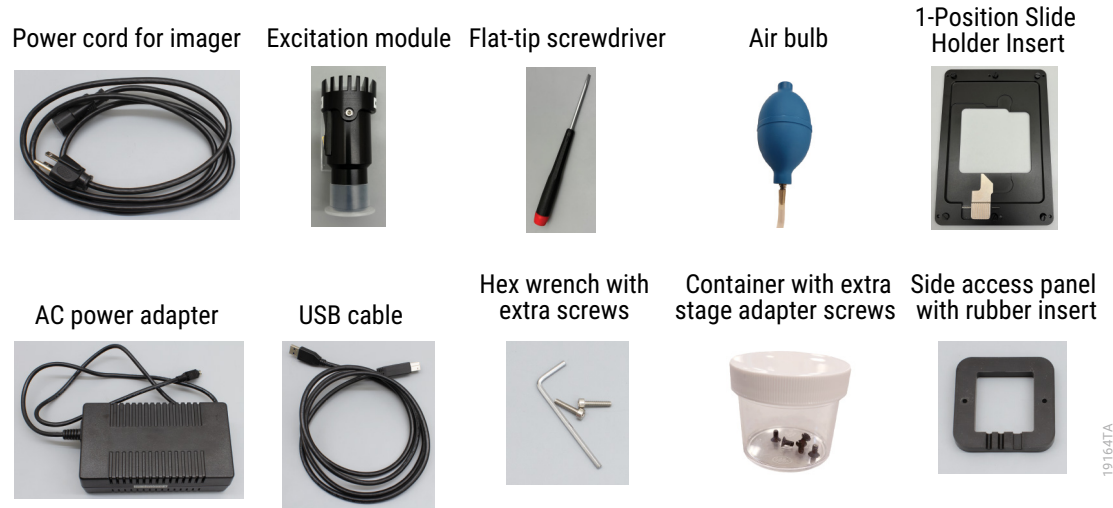
A.**B.**

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Figure 2. Panel A. GloMax® Galaxy shipping pallet. **Panel B.** Foam packaging for the imager.

1.6.2 Unpacking the White Instrument Accessory Box

Figure 3 shows the GloMax® Galaxy Imager accessories that should be present in the accessory box.



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Figure 3. GloMax® Galaxy Imager accessory components. Components shown include: Power cord for imager, Blue Fluorescence Excitation Module, flat-tip screwdriver, air bulb, 1-Position Slide Holder Insert, AC power adapter, USB cable, hex wrench with extra screws, container with spare stage adapter screws, and side access panel with rubber insert (when used with the Stagetop Chamber/Controller accessory). The 1-Position Slide Holder Insert and Fluorescence Filter Slide and Filters are preinstalled on the instrument.

1.6.3 Removing Instrument Packing Brackets

1. Place the GloMax® Galaxy Imager on a flat, level surface.

Note: We recommend that two people lift the instrument into position.

2. Ensure that the instrument is placed in a location that meets the power requirement specifications (100–240V AC, 50/60Hz; Section 1.2).
3. Manually open the top lid of the instrument; remove the foam form from the LED module arm, remove the red thumbscrews of the red stage shipping brackets (Figure 4). Store these in your GloMax® Galaxy Bioluminescence Imager accessory box in case they are needed later to ship the unit for service.

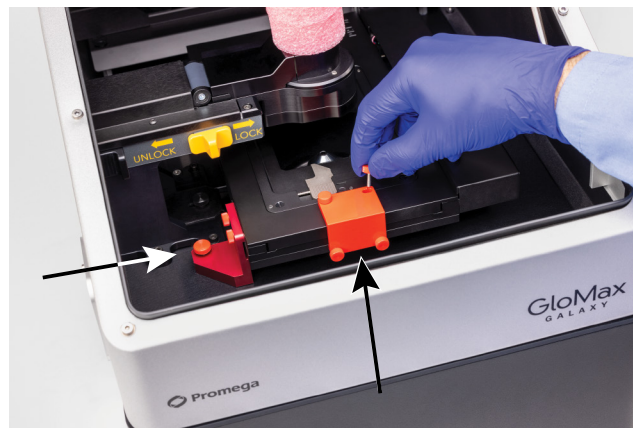


Figure 4. Shipping brackets.

2 Setting Up the GloMax[®] Galaxy Bioluminescence Imager

2.1 Setting Up the GloMax[®] Galaxy Bioluminescence Imager and PC

2.1.1 PC Purchased with the GloMax[®] Galaxy Imager

1. Unpack the PC and Monitor from their shipping boxes.
2. Place the PC and Monitor next to the GloMax[®] Galaxy Imager.
3. Attach the provided PC Power Cord to the back of the computer and to a power outlet.
4. Attach the provided Monitor Power cord to the back of the monitor and to a power outlet.
5. Using the provided monitor to PC cable, connect the monitor to the PC.
6. Using the provided USB cable, connect the PC to the USB port (Type B) on the back of the instrument as shown in Figure 5.

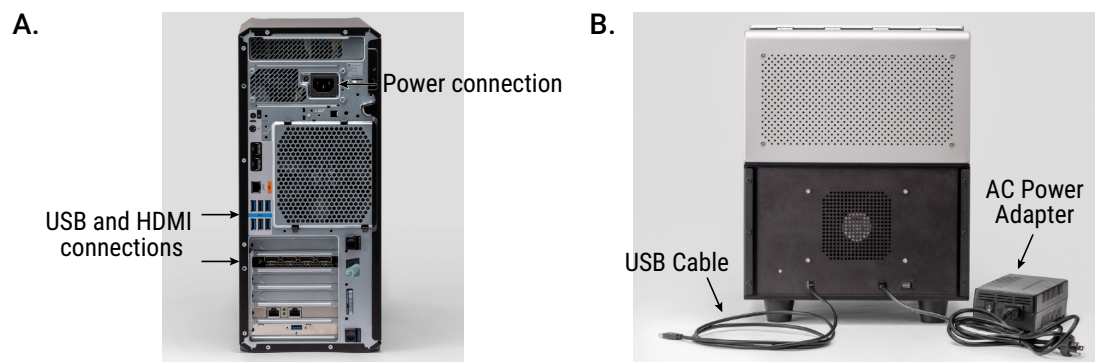


Figure 5. Panel A. Back of the PC. **Panel B.** Back of the GloMax[®] Galaxy Imager.

7. Ensure that the instrument On/Off switch is in the off position.
8. Connect the Instrument AC Power Adapter to the back of the instrument and to a power outlet.

Note: We recommend connecting the instrument to an uninterruptible power supply (~500VA).

2.1.2 User-Provided PC

1. Refer to Section 1.2 for PC and monitor specifications.
2. GloMax® Galaxy Control Software can be downloaded from:
www.promega.com/resources/software-firmware/
3. Copy the software installation package to the target PC and launch the installer.
4. Follow the on-screen instructions to install the GloMax® Galaxy Control Software.
Note: The installer includes Fiji (ImageJ) as an optional application (see Section 8). If desired, check the box to “Install Fiji” on your PC.
5. Using the provided USB cable, connect the PC to the USB port on the back of the instrument (see Figure 5).

2.2 Switching On the GloMax® Galaxy Bioluminescence Imager

1. Switch on the instrument power button on the rear panel of the instrument. Make sure the external power supply On/Off switch is turned on. Ensure the shipping brackets have been removed (see Figure 4, Panel A).
2. Press the power button, left front corner of the instrument, to power on the instrument. An LED in the power button on the front of the instrument will turn yellow, indicating that the instrument is on.
3. Power up the PC by selecting and holding the power button on the front.
4. Power up the monitor by selecting the power button at the front lower right corner.
5. Launch the GloMax® Galaxy Control Software from the Start menu or desktop shortcut. The control software will begin its start-up protocol. After the operating software starts, it will check for a connected and powered instrument and begin the initialization process. A green indicator at the lower right of the control software window will indicate successful connection and initialization (Figure 6), and the LED in the power button on the front of the GloMax® Galaxy Imager will turn green.
6. The GloMax® Galaxy Imager is now ready to use.



Figure 6. GloMax® Galaxy software 'Home' screen.

2.3 Shutting Down the GloMax® Galaxy Bioluminescence Imager

1. Switch off the instrument by holding the power button on the left front corner of the instrument until the LED turns off or by using the On/Off switch on the back of the instrument.
Note: When the instrument is not in use for a prolonged period, turn off the external power supply using its own On/Off switch.
2. If the GloMax® Galaxy Imager is powered off while connected to the Control Software, or the instrument fails to connect and initialize, the indicator at the lower right corner of the Control Software window will be yellow and indicate that the instrument is not connected. You will see one of two different pop-up messages: "Instrument not connected. Reconnect and try again." or "Instrument Disconnected".
3. Close the GloMax® Galaxy software by selecting the **x** in the upper right corner (Figure 6).
4. Shut down the PC using the **Start** button to open the Start menu, select the Power icon on the left side of the Start menu. From the Power drop-down list, select **Shut down**.
Note: When not in use for a prolonged period, the system should be powered off.

2.4 Operating the GloMax® Galaxy Bioluminescence Imager

1. Start a new imaging session by selecting the **Capture** button on the 'Home' screen (Figure 6).
2. The XY-Stage will move to the front of the instrument for access to install the stage adapter and sample vessel.
3. The 'New Session' window will open (Figure 7).

Figure 7. 'New Session' window.

- Customize the Session ID, if desired.
- Select the Vessel type from the drop-down list.

Note: Individual focus offsets that specify the distance of the sample from the objective have been defined for recommended imaging labware to aid in initial focusing. Ensure that the vessel type and manufacturer and part number chosen from the drop-down list corresponds to that in which the sample was prepared. If a new vessel needs to be defined, follow instructions in Section 3.10 for setting a new vessel type definition and corresponding focus distance.

- Supply a text Tag, if desired. Later, the Tag entered can be used to filter the saved Sessions list to more quickly find the desired Session(s).
- Choose your sample type, either Fixed Cells or Live Cells. This selection determines the fine- and extra-fine focus controls, which are user editable. (See the 'Calibration' tab under Preferences in Section 3.9.)
- Check "Use incubator" if the Stagetop Incubator/Controller, GloMax® Galaxy, is installed. (See setup and use instructions at: www.promega.com/products/imaging-devices/glomax-galaxy-bioluminescence-imager/)
- Open the Instrument lid, install the appropriate adapter on the stage, load your sample vessel onto the stage, and close the instrument lid.



Important: Make sure the proper adapter is mounted on the stage before starting a new session! Navigating the stage to the default start position for the selected vessel type can damage the objective if the wrong adapter is installed.

- Select **Start**. The 'Imaging' Screen will open (Figure 8).

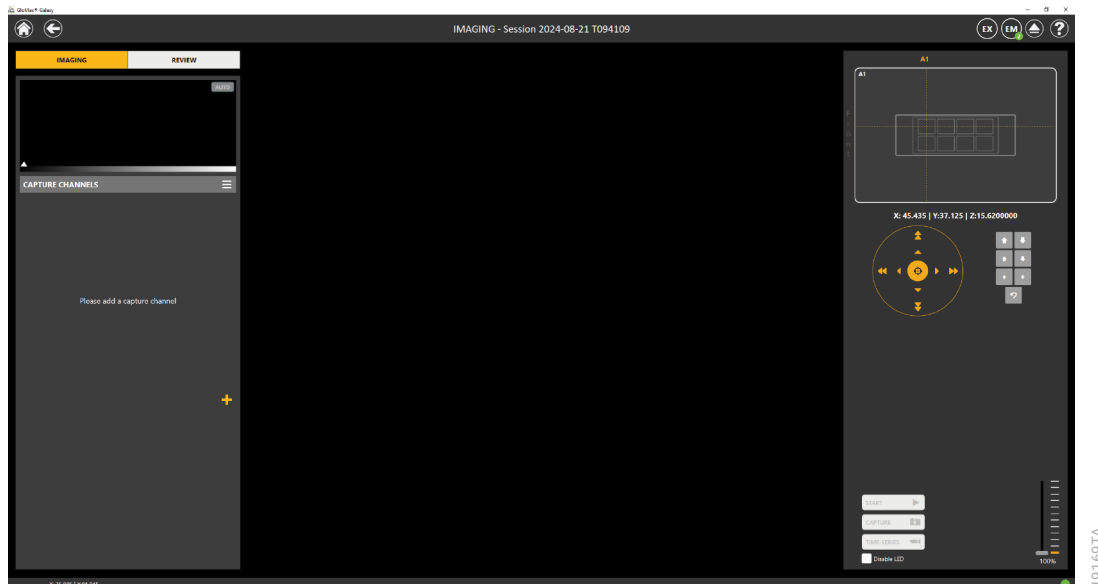


Figure 8. 'Imaging' screen for a new session.

4. The XY-stage will move to the default starting position for the selected vessel type (well A1 of a multiwell plate or center of 35mm dish and slide). The position will be indicated by a dotted cross on the vessel diagram of the 'Imaging' screen (Figure 9).

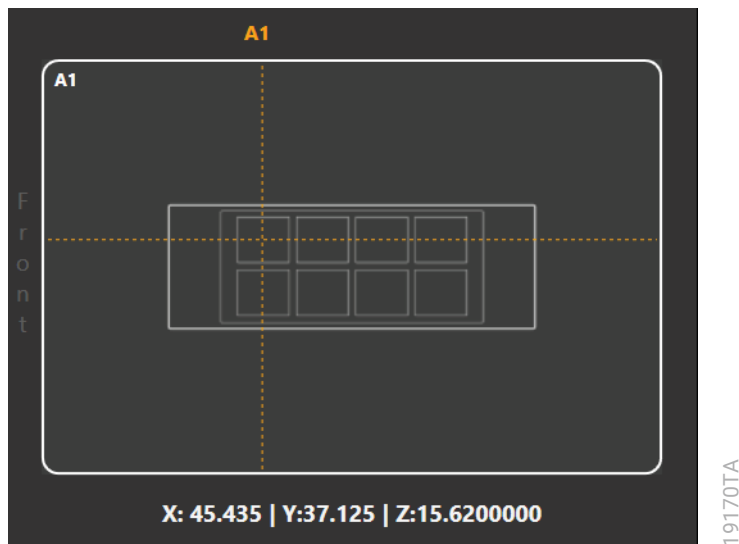


Figure 9. Vessel diagram for the 8-well microchamber slide. Selecting a well position will move the stage to its center. Clicking again within the same well will move the stage to that position within the well. Right-click to create or move to bookmarked positions.

- The objective will move to the default starting position for the selected vessel type. This should be near the focal plane for the recommended vessel. You can create duplicate vessels and adjust the focus distance for imaging in vessels from other manufacturers (see Section 3.11.4, Vessel Default Imaging Position).
- Select the yellow + in the left panel of the screen to add a capture channel. A new Luminescence, Fluorescence or Brightfield channel may be created, or an existing protocol may be selected (Figure 10).

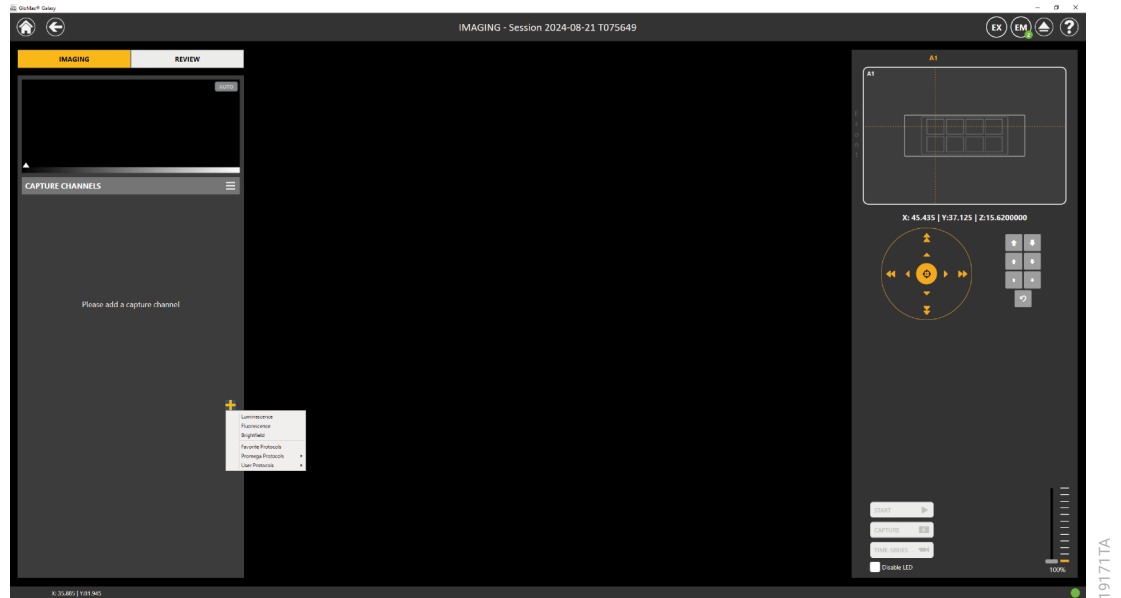


Figure 10. Adding a capture channel.

7. Select the **Start** button to start image acquisition and display a live-view image. The live-view image will be generated using the settings of the selected (highlighted in blue) capture channel (Figure 11).

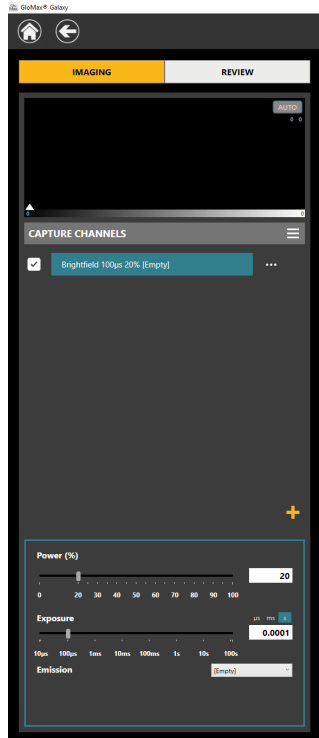



Figure 11. Selected capture channel.

8. Focus using the coarse, fine and extra-fine up and down arrow buttons (hover over these buttons to see the keyboard shortcuts). Select the vessel diagram (upper right in Figure 10) or use navigation buttons (Figure 12) to move the XY-stage to change the field of view. The  button returns the objective to the default starting focal position for a new imaging session.
9. When starting a new imaging session, we suggest establishing the initial focus of the sample using brightfield mode. Focus can then be fine-tuned in either fluorescence or luminescence imaging modes. Use of digital zoom (Section 2.4) can aid in making fine focus adjustments.

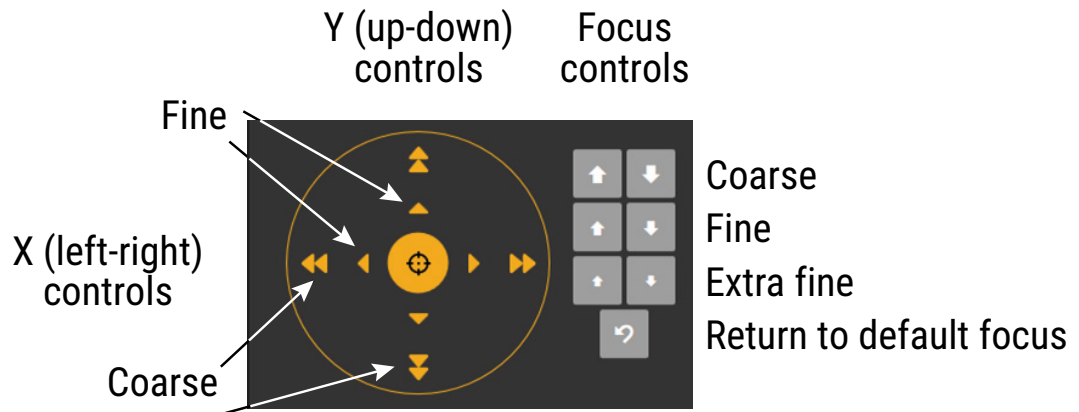


Figure 12. Navigation buttons.

10. A digital zoom feature (Figure 13) enables the user to zoom in on as little 1/10 of the full field of view to see sample detail. The portion of the field of view on screen is highlighted by the yellow box in the live-view thumbnail at the lower right of the 'Imaging' screen. The zoomed region will default to the center of the field of view as the slider is adjusted but may then be dragged to any portion of the image thumbnail. Captures and Time Series initiated with digital zoom active will save a full-field image.

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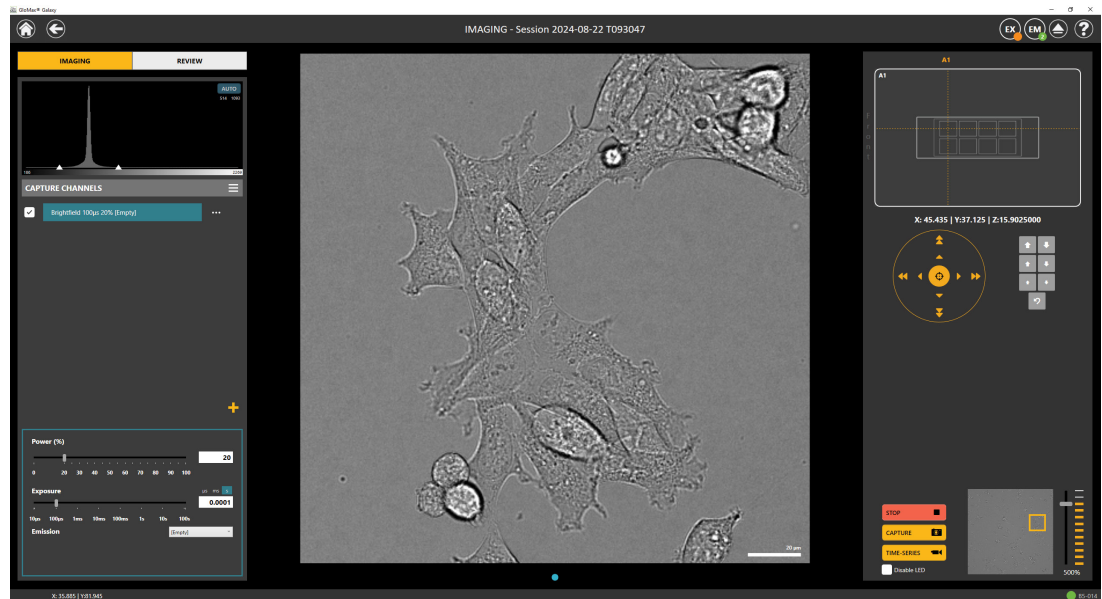


Figure 13. Digital zoom and live-view thumbnail.

11. Use the **Capture** and **Time-Series** buttons to initiate image acquisition.
12. To view images captured during an imaging session, select the 'Review' tab at the upper left of the session window. Captures will be listed on the left panel in the order acquired. The selected capture (highlighted in blue) will be displayed on screen, with the capture channels listed on the right panel. Individual channels may be selected or deselected and adjusted individually. (See 'Review' tab, Section 3.7.)

2.5 Dark Frames

The background signal within luminescence images is composed primarily of camera noise but may also have contributions from light leaks or sources such as phosphorescence from the sample vessel. We suggest capturing images of this background signal to be used in downstream analysis (see Section 8.1). Such images may be referred to “dark frames”.

Dark frames should be captured using the same capture channel settings used for their associated luminescence images. Commonly, dark frames are captured after removing the luminescence sample vessel from the stage, but in some cases, capturing dark frames from a control vessel or well that does not contain luminescence may better match the sample imaging context. If multiple replicate dark frames are captured, they may be stacked, averaged and then subtracted from sample images to remove hot pixels, dead pixels and any noise patterns inherent to the camera sensor.

Note: The GloMax® Galaxy stage is designed to be light impenetrable. Average dark frame signal is typically less than 101 gray units and remains consistent with increasing exposure time. If excessive signal is noted in dark frames, check that the instrument lid is closing tightly and that the lid foam gasket is not damaged. Check the fit and tightness of 2 × 3 inch side panels, the two small panels that cover the side-access ports. If using the Stagetop Incubator/Controller, check that the tubing fits in gasket slots and the side panel is securely attached to the instrument. If increased signal is noted in dark frames as exposure time is increased, there may be a light leak or source of phosphorescence. Phosphorescence occurs when a material becomes “charged” from light exposure and then slowly releases that light energy. For example, some white-walled plates used for luminescence assays are prone to phosphoresce.

3 GloMax[®] Galaxy Control Software

3.1 'Home' Screen

The 'Home' screen (Figure 14) is the main beginning point for interaction with the functionalities built into the GloMax[®] Galaxy Control Software.



Figure 14. GloMax[®] Galaxy software 'Home' screen.

The four 'Home' screen buttons are:

Capture	Initiates a new imaging session to view and image your samples, add capture channels, save protocols and initiate Captures and Time Series. To start a new imaging session, select the vessel type (slide, microchamber, 35mm dish, 6-, 12-, 24-, or 96-well plate), sample type (Live Cells or Fixed Cells) and indicate whether a stage-top incubator is installed or not.
Gallery	Displays a list or table of saved imaging sessions that may be opened and reviewed. List items can be filtered by start and end date, or use a keyword search to find results based on session name or other associated information.
Protocols	Lists all available pre-installed and user-saved protocols. When a protocol is selected, users can view details and use a shortcut from the protocol details panel to initiate a new imaging session (the Capture button).
Settings	Lists instrument information and setup options. You can access Navigation and Camera settings, specify where files are saved on the PC, export log files and view system information.

3.1.1 Home Screen Features

The 'Home' screen and all others contain basic Windows® functions for minimize, maximize and close at upper right corner of the control application window (Figure 15).

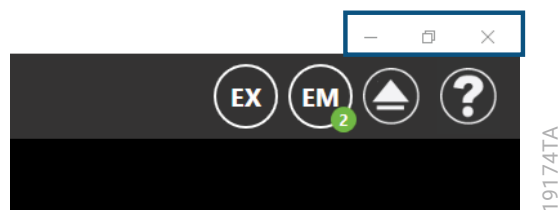









Figure 15. Windows® close, maximize and minimize buttons on the GloMax® Galaxy Control Software window inside the blue box.

The connected Instrument name appears in the title bar of the control application. The following buttons are also displayed within the title bar:

	Home	From any screen other than the 'Home' screen, select this icon to return to the 'Home' screen.
	Exit	Closes the GloMax® Galaxy software.
	Back	When active, selecting the Back button will return the interface to the screen accessed prior to the current screen.
	Excitation Modules	Displays the installed excitation module (Figure 15).
	Emission Filter	Displays the current emission filter configuration (Figure 19) and moves the filter slide to a safe position for removal to change emission filters. Up to four filters can be installed on the GloMax® Galaxy Imager. Filters are manually assigned in the software by selecting the filter at each position from a drop-down list. Filters may be added to the drop-down list using the 'Custom Filters' tab. The excitation filter slide accepts standard 25mm filters, no more than 5mm thick, installed in an Emission Filter Holder/Retainer (Cat.# GM4017).
	Unload Vessel	Moves the instrument stage forward to access the sample vessel. The stage will return to its previous position when the instrument lid is closed and the Unload Vessel button is selected again.
	Help	Opens the GloMax® Galaxy Bioluminescence Imager Operating Manual.

3.2 Exchanging the Excitation Module

Each of the GloMax® Galaxy excitation modules is recognized by the instrument when the module is installed. Select the **EX** button on the Galaxy® Control Software header to view the Excitation Module (Figure 16).

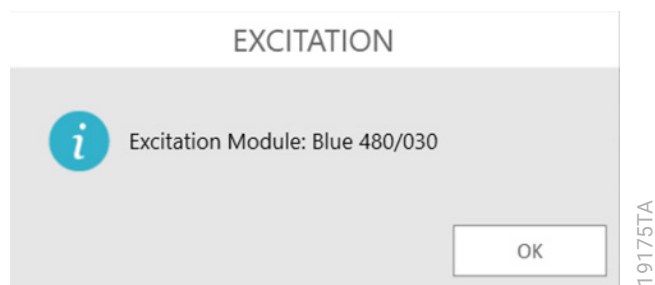


Figure 16. Excitation configuration window.

To install a GloMax® Galaxy excitation module, align the electrical contacts on the module housing with the contact pad inside the module arm and push it down until it clicks into position (Figure 17).

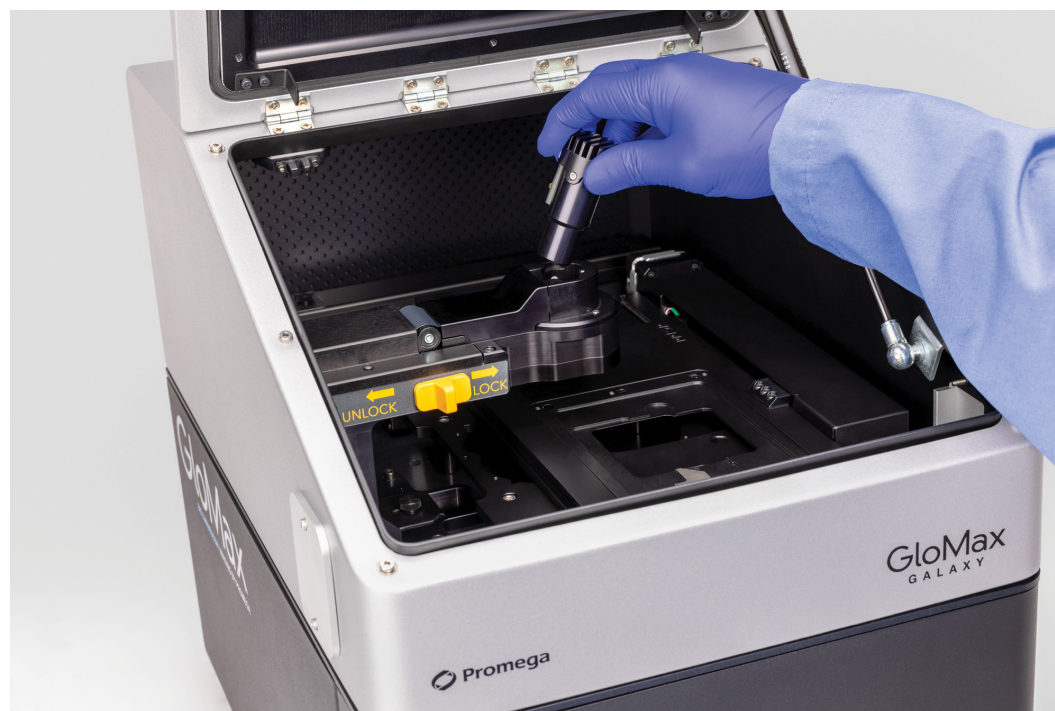


Figure 17. Installing an excitation module.

To remove a GloMax® Galaxy excitation module, make sure the module arm is locked, firmly grip the top of the LED module, and pull it up and out of the module arm.

3.3 Changing the Emission Filter Configuration

1. The GloMax® Galaxy Imager should be on and connected before attempting to remove the filter slide. Select the **EM** button on the Galaxy® Control Software header to open the Emission Filter Configuration (Figure 15).

The filter slide position is displayed on the EM icon in the header. Opening the 'Emission Configuration' window automatically moves the filter slide to a safe position (Position 2) for removal.



Important: Attempting to remove the filter slide when it is at Position 1 or Position 4 may damage the slide and render the system inoperable.

2. Remove the instrument lower front panel by gripping left and right edges and pulling it toward you (Figure 18, Panel A). The panel is held by magnets and will pull free.
3. Identify the filter slide assembly mounted above the camera and completely loosen the four thumb screws that secure it (Figure 18, Panel B).



Figure 18. Panel A. Removing the front panel. **Panel B.** Filter slide panel. **Panel C.** Removing emission filters.

4. Carefully pull the filter slide panel toward you. As it comes off the aligning posts, continue bringing it away from the instrument until the filter slide assembly is clear of the instrument.
5. Emission filters can be removed from the filter slide by turning counterclockwise (Figure 18, Panel C).

6. Thread the emission filter clockwise into an open position in the filter slide until finger tight. Note the emission filter positions printed on the filter slide (numbered 1–4, left to right, while facing the front of the instrument). Custom filters will need to be installed in a retainer and secured with a locking ring (Section 10, Related Products).
7. Reinstall the filter slide panel by carefully directing it into the instrument toward the aligning posts. Gently push it onto both posts until it stops, hold in place, and turn the thumbscrews until finger tight.
8. Update the emission configuration by selecting the filter installed at each position from the drop-down menu (Figure 19, Panel A). If your filter is not listed, enter it on the 'Custom Filters' tab, which will add it to the drop-down list (Figure 19, Panel B).
9. Select **OK** to complete the emission filter update process.

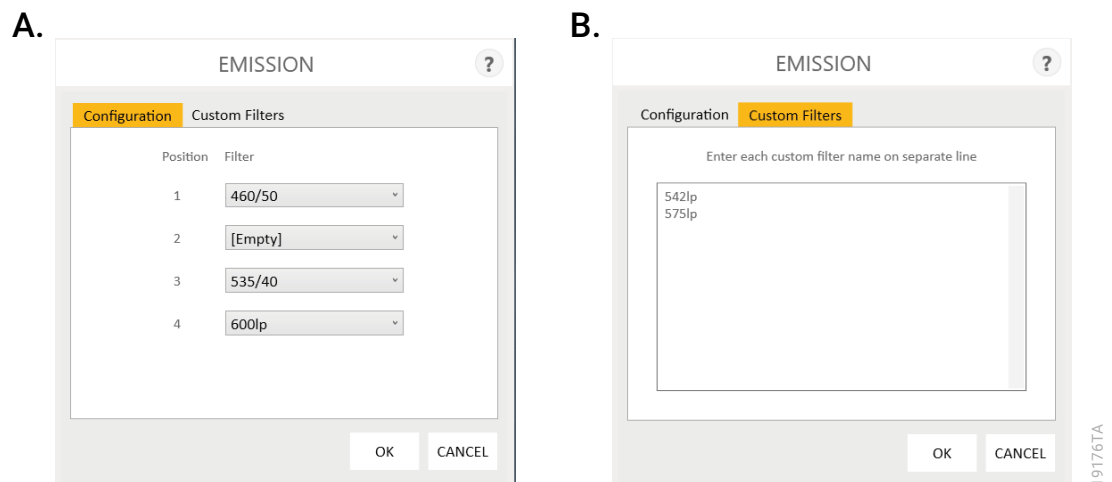


Figure 19. Panel A. Emission filter 'Configuration' tab. **Panel B.** Emission filter 'Custom Filters' tab.

3.4 Creating Capture Channels

Capture Channels are added and configured on the 'Imaging' tab during an active Imaging Session by selecting the **+** in the capture channels list (Figure 10). Options include Luminescence, Fluorescence and Brightfield.

Capture channels will be captured in the order displayed on the 'Imaging' tab (top to bottom; Figure 20, Panel A). To change the order, select the **...** button at the right of the capture channel. Select the options Move Up, Move Down, Move to Top, Move to Bottom, and Delete to edit the capture channels list (Figure 20, Panel B).

The blue-highlighted channel indicates the active channel in the live-view window. Select a different channel to view that channel.

There is a check box at the left of each capture channel in the list. Only the capture channels that are checked will be imaged during a Capture or Time-Series.

If the check box to the left of any capture channel shows an exclamation point [!], the channel is disabled due to mismatch with the current excitation or emission configuration. Check that the correct excitation module is installed and emission filter configuration. Disabled capture channels will be enabled when the instrument configuration is updated to match the channel settings.

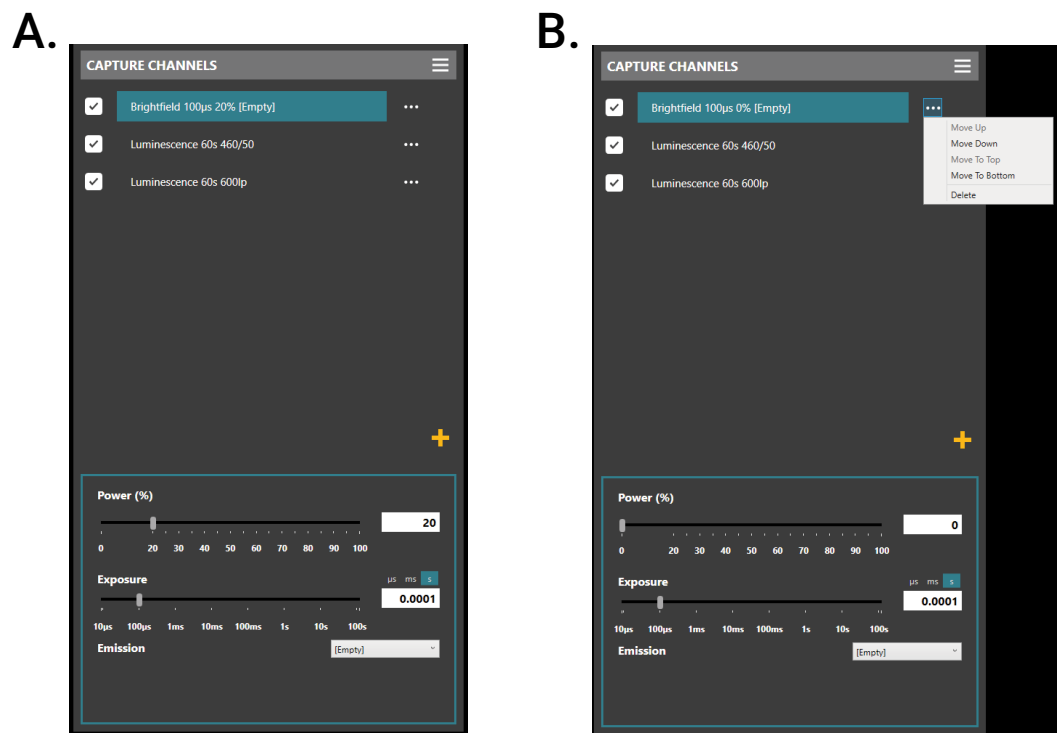


Figure 20. Panel A. Capture channels list. **Panel B.** Capture channel order options.

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3.4.1 Brightfield Capture Channel Settings

The capture channel settings for brightfield imaging include LED Power (%), Exposure (time) and Emission (Figure 21).

- Excitation percent power may be adjusted by dragging the slider, entering a value, or directly selecting the values (20, 30, 40, etc.) on the power scale.
- Exposure times for brightfield imaging will depend on your selected LED module and LED Power settings but will typically range from about 10–200 microseconds.

Note: The LED will turn on for captures when the power (%) is set between 20–100%, even if live view is stopped or the LED is disabled. Use the “Disable LED” check box near the bottom of the right panel to keep the LED off, if desired, while editing capture channels or viewing luminescence in Live View on the ‘Imaging’ screen.

- The Emission selection box enables selection from a list of the installed filters. The emission filter selection will automatically default to “[Empty]” for Brightfield, but any installed emission filter may be selected.

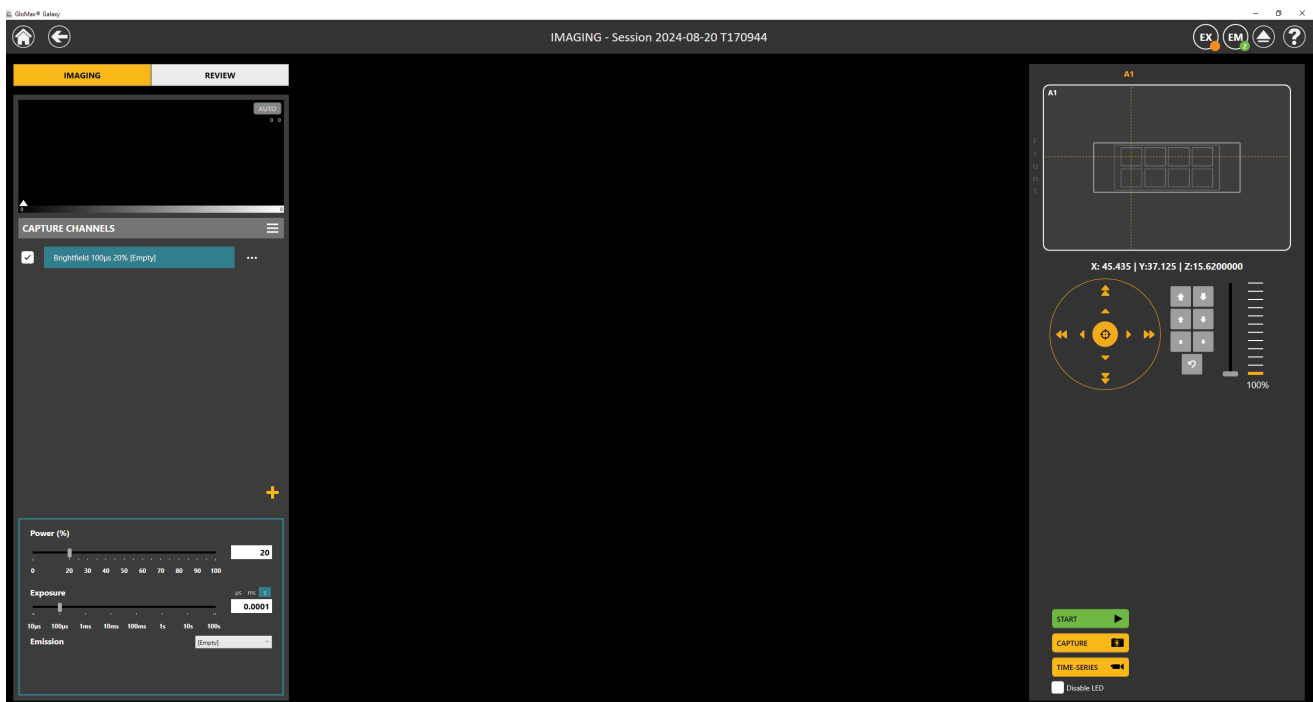


Figure 21. Brightfield capture channel settings.

3.4.2 Luminescence Capture Channel Settings

For luminescence, the capture channel settings include Exposure (time), Emission (Filter) and Binning (Figure 22).

- Imaging unfiltered luminescence will maximize sensitivity for NanoLuc®-tagged targets of interest. Filters are included with the system for imaging NanoBRET® applications including protein:protein interactions and target engagement:

Emission Filter	Luminescence Application
460nm/50BP	BRET (Donor signal)
600LP	BRET (Acceptor signal: HaloTag® NanoBRET® 618 Ligand)

- Exposure times for luminescence will depend on NanoLuc® luciferase expression levels and may range from a few seconds to several minutes to reliably image the sample. The GloMax® Galaxy Imager supports a maximum exposure of 60 minutes (3,600 seconds) for luminescence capture channels.
- Binning includes selections for None, 2x2 or 4x4, and applies only to the Live View image shown on the 'Imaging' screen when the channel is active. All captured images will be saved at full resolution independent of this setting. Binning combines the signal for each 2 × 2 or 4 × 4 square section of pixels, reducing the resolution while increasing sensitivity. When binning is selected, the live view exposure time is reduced by four- or 16-fold, respectively. The faster live-view refresh rate may enable more efficient user optimization of focus and exposure of samples expressing low levels of NanoLuc. For example, a 1-minute exposure with 4x4 binning selected will refresh the image in the live-view window approximately every 3.75 seconds. When captured, however, a full resolution image exposed for 1 minute will be saved.

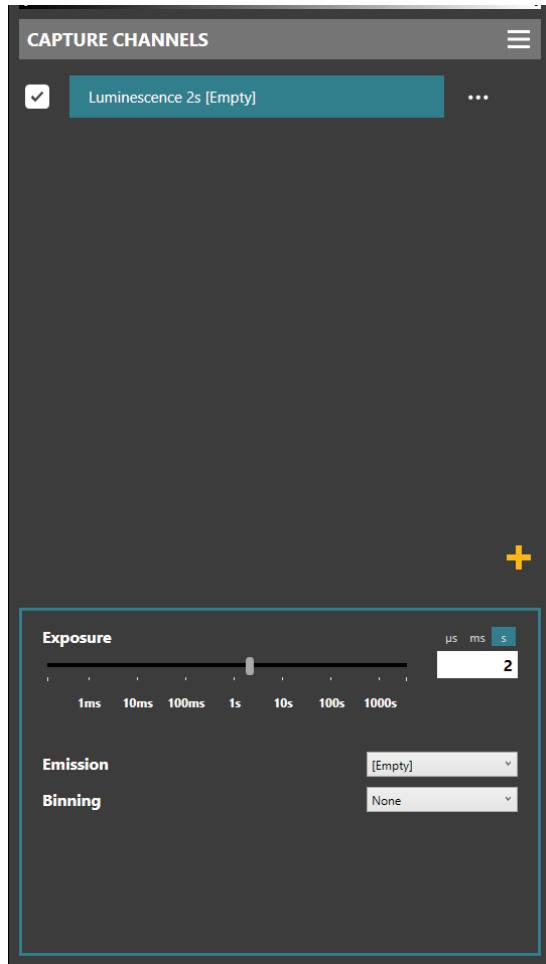


Figure 22. Luminescence capture channel settings.

3.4.3 Fluorescence Capture Channel Settings

For fluorescence, the capture channel settings include LED Power (%), Exposure (time) and Emission (Filter; Figure 23). The Excitation is set to the module installed in the instrument when the capture channel is added. To add a capture channel for a different excitation module, install that module first.

- Excitation Power may be adjusted by dragging the slider, entering a value, or directly selecting the values (20, 30, 40, etc.) on the power scale.
- Exposure times for fluorescence will depend on your target and LED Power (%) settings but will typically range from several hundred milliseconds to several seconds. The GloMax® Galaxy Imager supports exposures up to 120 seconds for fluorescence capture channels.

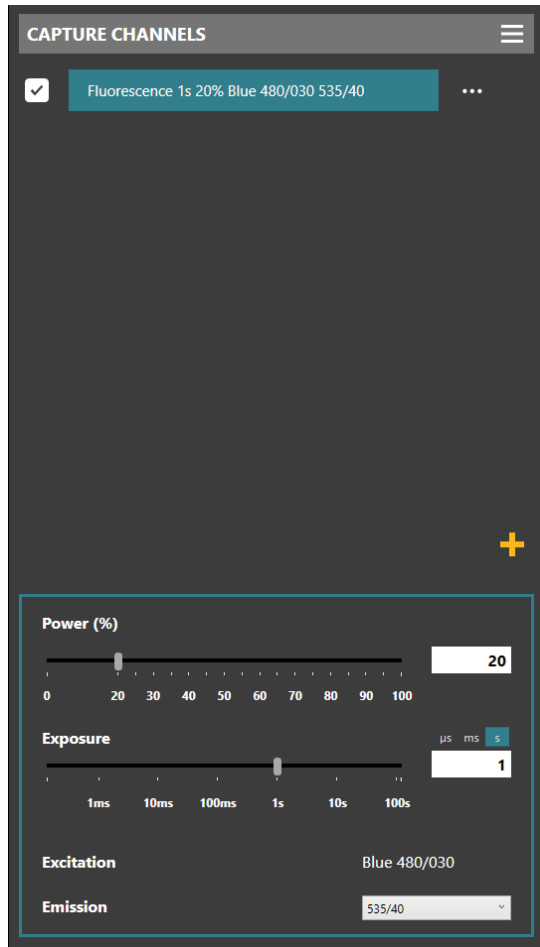
Note: The LED will turn on for captures when the Power (%) is set from 20–100%, even if live view is stopped or the LED is disabled. Use the “Disable LED” check box near the bottom of the right panel to keep the LED off, if desired, while editing capture channels or viewing luminescence in Live View on the ‘Imaging’ screen.

- The Emission selection box enables selection from a list of the installed filters. The emission filter selection will automatically default to the filter selection matching the installed excitation module as listed in Table 1, but users may select any installed filter.

Table 1. GloMax® Galaxy Excitation Module and Emission Filter Pairings for Common Fluorescent Targets.

Excitation Module	Default Emission Filter	Common Fluorescent Targets
UV (Excitation: 375/20nm)	460nm/50BP*	DAPI, Hoechst, Blue fluorescent protein (BFP)
Blue (Excitation: 480/30nm)*	535nm/40BP*	Green fluorescent protein (GFP), Alexa Fluor 488, FITC, SYBR
Green (Excitation: 540/25nm)	605nm/55BP	Cy3, Janelia Fluor 549, DsRed, Alexa Fluor 555, MitoTracker™ Orange
Green (Excitation: 560/40nm)	600LP*	Texas Red, Alexa Fluor 568, Alexa Fluor 594, MitoTracker™ Red, mCherry, Cy3.5
Amber (Excitation: 620/60nm)	700nm/75BP	Cy5, Janelia Fluor 646


*Included with the GloMax® Galaxy Imager.



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Figure 23. Fluorescence capture channel settings.

3.5 Saving Capture Channels

1. New user protocols may be created and saved within an imaging session. To create a new protocol, define the desired capture channels, select  on the right of the Capture Channels header, and select **Save**.
2. You will be prompted to name the protocol. You also can provide a protocol tag. This text entry may be used to filter the saved protocols list later (Figure 24).

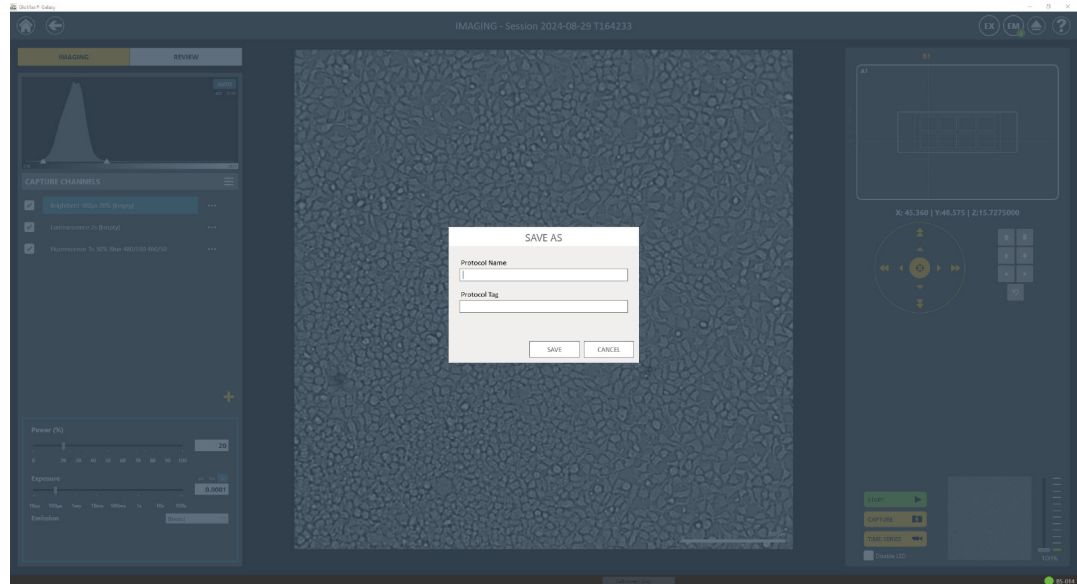


Figure 24. Save Protocol name prompt from the GloMax® Galaxy software.

3. To save the new protocol, select **Save**.

3.6 Other 'Imaging' Screen Features

3.6.1 Live-View Image

The center panel of the 'Imaging' screen displays a real-time image of the current field of view using the active capture channel settings when Live View image acquisition is active. The active live-view image updates regularly based on the exposure setting of the active capture channel. A blue dot at the bottom of the live-view image will flash as the camera acquires images.

Right-clicking on the live-view image provides options to:

- Save the live-view image.
- Access the live-view display options where you can show or hide the scale bar and invert its color (black/white).

3.6.2 Histograms

A histogram of the live-view image is displayed at the upper left corner of the 'Imaging' screen (Figure 25).

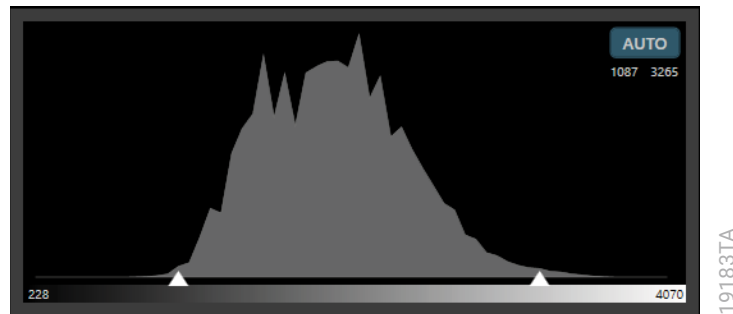


Figure 25. Live-view image histogram.

The histogram x axis adjusts to the minimum and maximum pixel gray value present within the image. The marker positions, shown as white triangles, indicate the gray value settings used for display of the adjusted image. The gray values for the marker positions are noted at the upper right corner of the histogram graph.

3.6.3 Histogram Adjustments

- When the Auto button at the upper right corner is blue, the marker positions are automatically set. Automatic settings may not be effective for all samples and sample types, including small tissue samples or sparsely plated cells.
- The markers may be moved manually by clicking and dragging them along the x axis, which will update the live-view image in real time. Clicking immediately left or right of a marker incrementally nudges the marker position step-by-step for finer adjustments.
- Adjusting either marker will toggle the Auto setting off and change the Auto button to gray. Automatic adjustment of the marker positions can be reactivated by selecting the **Auto** button.

3.6.4 Bookmarks

Positions within the vessel may be bookmarked by right-clicking on the vessel diagram and selecting **Create New Bookmark** from the menu. Each bookmark may be given a custom name, and the XY and focus positions will be saved (Figure 26).

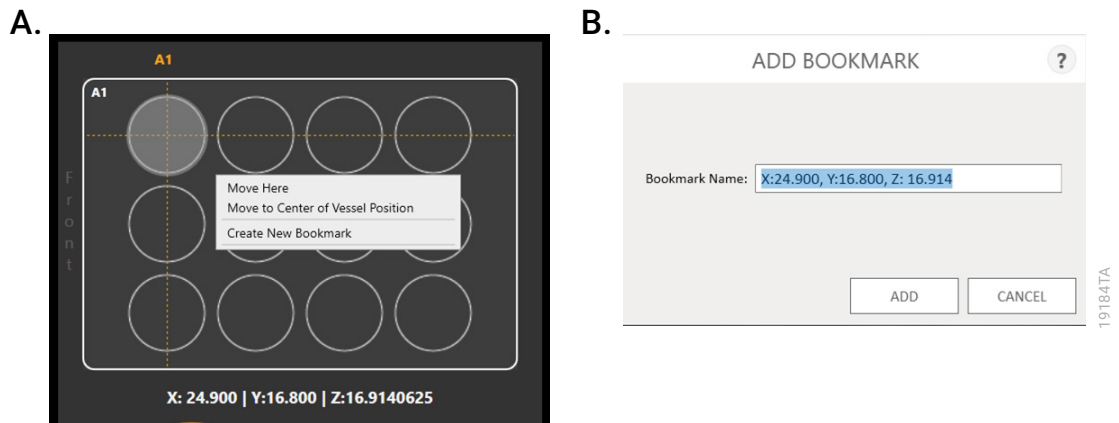


Figure 26. Adding bookmarks. Panel A. Right-click on the vessel diagram and select **Create New Bookmark**. **Panel B.** You can edit the bookmark name in the 'Add Bookmark' window.

The list of saved bookmarks is accessed by right-clicking the vessel diagram. Selecting a bookmark from the list will move the stage to the bookmarked position.

Bookmarked positions are only maintained for the duration of the active session and are cleared when the session is closed.

3.6.5 Disable LED

When checked, the “Disable LED” option turns off the LED module, preventing it from being turned on for active bright field or fluorescence capture channels. You may find this useful for limiting sample exposure to excitation light while configuring capture channels. The “Disable LED” option does not prevent the LED from being turned on during a Capture or Time Series.

3.6.6 Capture

The Capture button initiates capture of selected (check box) capture channels.

1. To start a new capture, select the **Capture** button at the lower right of the ‘Imaging’ screen.
2. The ‘Capture Settings’ window will appear (Figure 27).

The screenshot shows a 'CAPTURE SETTINGS' dialog box. It has a title bar with a question mark icon. The dialog contains four text input fields: 'ID' with the value 'Session 2024-04-25 T081505', 'Name' with the value 'Fluorescence_A1_2024_04_25_08_15_21', 'Comment' which is empty, and 'File Path' with the value 'Y:\integrated\Zeus\Session 2024-04-25 T081505\Fluorescence_A1_2024_04_25_08_15_21.tiff'. At the bottom right of the dialog are two buttons: 'START' and 'CANCEL'. On the right side of the dialog, there is a vertical label '19185TA'.

Figure 27. ‘Capture Settings’ window.

3. Edit the Name field and add a comment, if desired. (The Comment field entry can be viewed in the Session report and image metadata.) The session ID shown was entered at the start of the session and cannot be edited. The File Path is the gallery folder specified in Preferences on the ‘Common Settings’ tab. The default folder location is **C:\Users\Public\Documents\GloMax Galaxy**.

4. During a capture, a pop-up window will show the elapsed and remaining capture time and will display images as they are captured. Select the tabs for different channels on the left side of the window to toggle and view the respective images (Figure 28).

No other system features may be used during a Capture or Time Series. However, image files will be saved as they are captured and can be opened and viewed using Fiji.

While a capture is in progress, the green LED light in the power button will blink, indicating that the system is actively acquiring images.

When the capture is completed, select the **Close** button to return to the 'Imaging' screen.

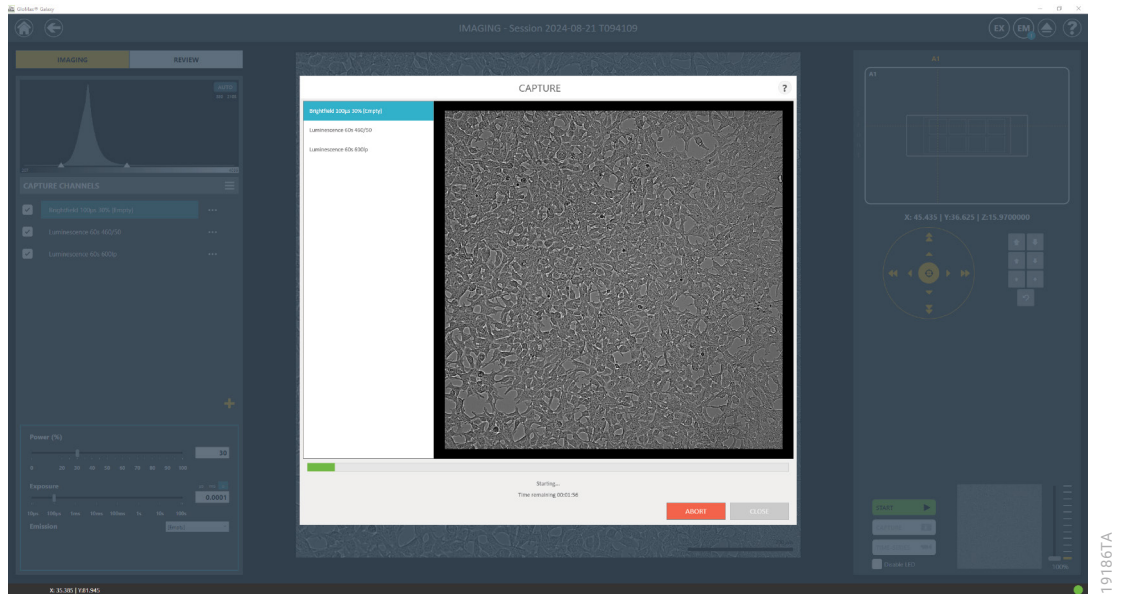


Figure 28. 'Capture' pop-up window.

3.6.7 Time Series

The Time Series function initiates a series of captures to be performed over a defined time frame.

1. To start a new time series capture during an imaging session, select the **Time-Series** button at the lower right of the 'Imaging' screen.
2. The 'Capture Settings' window for the time series will appear (Figure 29).

Figure 29. Time-Series 'Capture Settings' window.

3. Edit the Name field and add to the Comment field, if desired. (The Comment field entry will be viewable in the Session report and image metadata.) The session ID was entered at the start of the session and cannot be edited. The File Path is set in Preferences on the 'Common Settings' tab.
4. Enter the Total Capture Time for the series of captures by using the arrows to adjust hours, minutes and seconds, or manually entering the desired values.
5. Enter the Capture Interval for the series of captures.

Note: The minimum interval will be pre-determined based on the capture channels programmed; entering a value less than this minimum will display a message indicating an invalid entry.
5. The capture number will be calculated and displayed. A capture will be made at the start of the time series and then at each capture interval entered until the total capture time for the series is reached.
6. Select **Start** to begin the time series or **Cancel** to return to the Imaging screen.

7. During a time series, a pop-up window will show the elapsed and remaining time and the images captured from the last timepoint. Select the tabs for different channels on the left side of the window to view the respective images (Figure 30).
8. No other system features may be used during a capture or time series. However, image files will be saved as they are captured and may be opened and viewed as they are saved using Fiji.
9. While a capture is in progress, the green LED light in the power button will blink, indicating that the system is actively acquiring images.
10. When the capture is completed, close the window to return to the 'Imaging' screen.

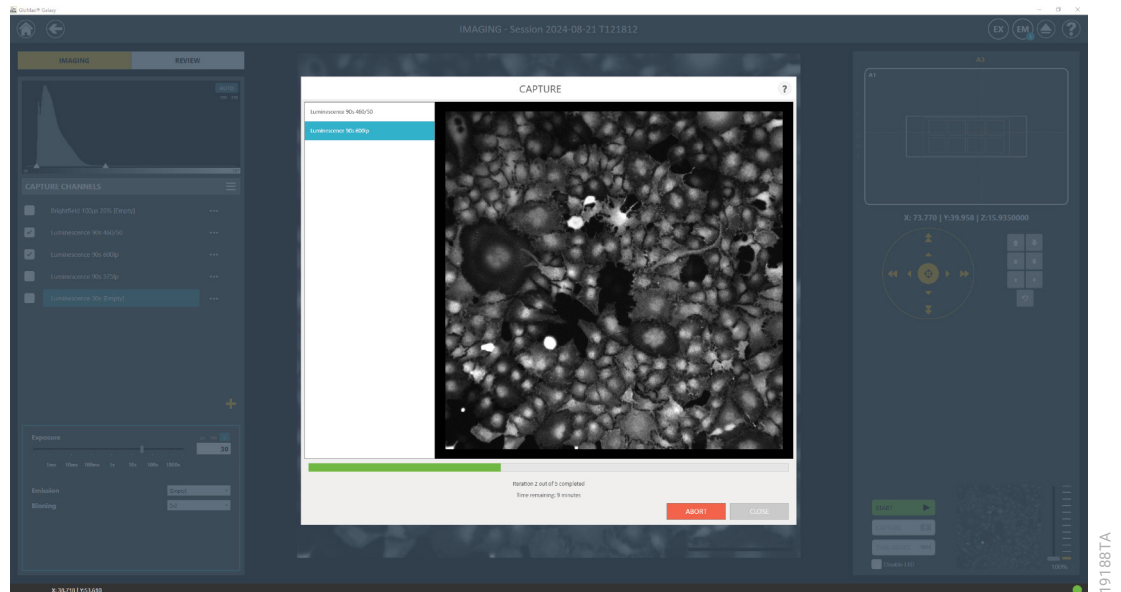


Figure 30. Time-series captures and progress bar.

3.7 'Review' Screen

The 'Review' tab can be selected during an imaging session to review captured images (Figure 31).

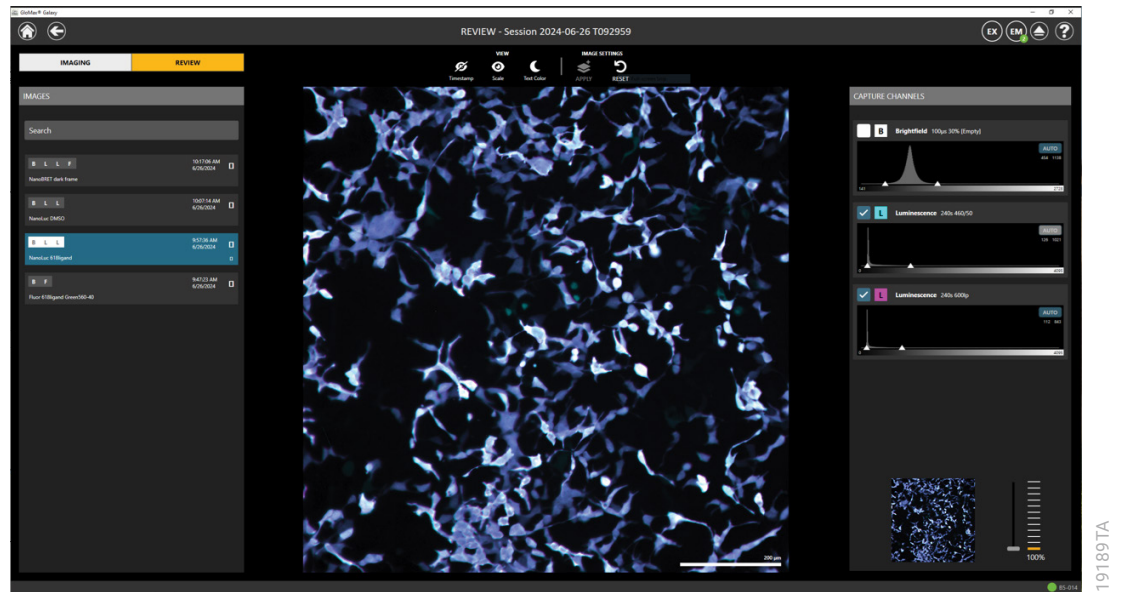


Figure 31. Image session 'Review' screen. The 'Review' screen provides functions to view, adjust and overlay captured images.

3.7.1 'Review' Screen Features

Images List

- Images captured during the current session will be listed in the left panel in the order they were acquired, with the most recent captures at the top.
- The folder icon at the right of each capture name is a link to the folder location where the image files are saved. Image files are saved as .tiff files. The folder location also contains a Report.txt file that summarizes the captures made during the imaging session, including the LED module, LED power setting, emission filter and exposure time used for each image.

Capture Channels List

- The capture channels for the selected capture (highlighted blue in Figure 31) will be listed on the right panel and may be individually viewed, assigned color and adjusted. Changes may be reverted using the Reset button, which resets all changes made during the review.
- When more than one capture channel is selected (checked), they are displayed together.
- A histogram is present and can be adjusted independently for each capture channel. (See Section 3.6.2 Histograms for additional details.)
- To assign a display color for a capture channel, left-click on the letter (**L**uminescence, **F**luorescence or **B**rightfield channel) next to the channel description and select from the drop-down list.

Image View

- The center panel displays the selected image. If multiple capture channels are checked, they will be displayed together.
- The image view has the option of displaying a Timestamp and Scale, which can be toggled on or off by selecting the **Timestamp** and **Scale** buttons above the image view. The **Text Color** button will toggle the color between white or black.
- Digital Zoom control at the bottom right of the 'Review' screen allows up to 1,000% (10X) zoom on-screen with ability to select the portion of the image that is displayed.

3.8 Protocols

Protocols consist of one or more capture channels that can be opened and used during an imaging session. When a protocol is opened during an imaging session, you can clear existing channels or append the capture channels list.

3.8.1 Promega Protocols

Several protocols are preinstalled for specific Promega assays. Promega Protocols may be used to start a new imaging session but may not be overwritten or deleted.

3.8.2 Viewing Protocols

Select **Protocols** from the 'Home' screen to access the list of protocols currently present in the software (Figure 32).

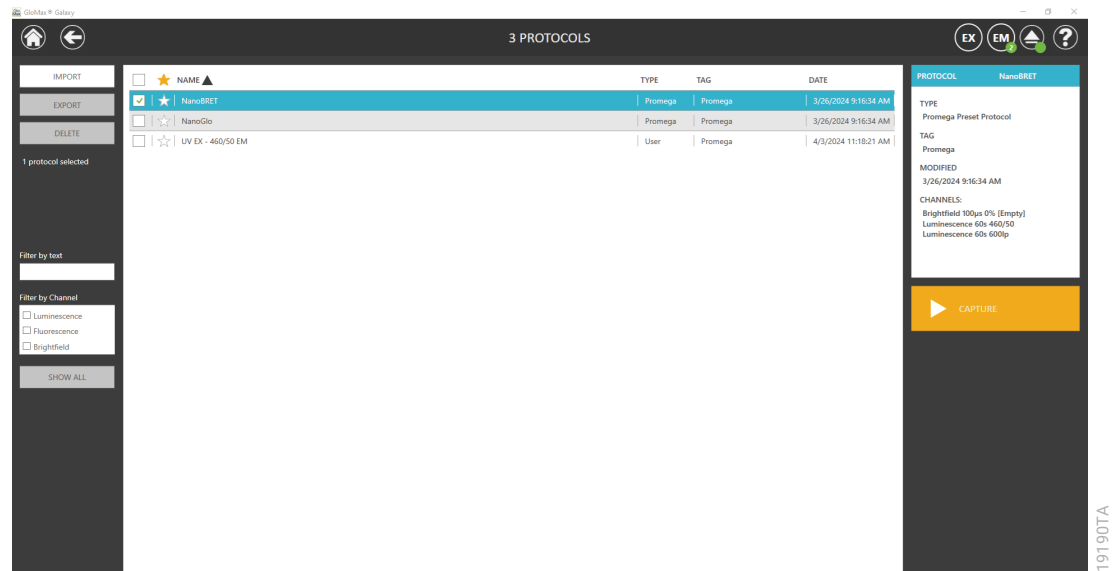





Figure 32. GloMax® Galaxy 'Protocols' screen.

3.8.3 Protocols Screen

Protocols are displayed in a table format with several columns of information. Select any of the column titles at the top of the screen to sort the list of protocols in increasing or decreasing order by that column's contents.

Protocols can be marked as favorites by selecting the star on the left side of the protocol entry.

Favorite protocols are automatically shown at the top of the protocols list the next time you access the 'Protocols' screen and Promega Protocols drop-down menu. There is no limit to the number of protocols that may be selected as favorites.

Name	The Name of the protocol.
Type	Promega (preinstalled) or User (saved by users).
Tag	Additional identifying information entered by a user.
Date	The date and time that the protocol was created.
	Protocol marked as favorite.
	Select protocol to either export or delete it.
	Reverse protocol order.

Protocol Selection Check Box

When performing actions (e.g., Export or Delete), use the check box at the left of each protocol to select the protocol(s) to which the action will apply. The check box in the table header can be used to toggle all of the check boxes on or off for listed protocols.

3.8.4 Filtering the Protocol List

Use the buttons at the middle-left side of the screen to filter the list of protocols by text. This keyword is searched within all fields. All protocols that contain the keyword in any field are displayed.

Protocols can be filtered by channel by checking each channel type (luminescence, fluorescence or brightfield). Only protocols containing capture channels that are checked will be displayed.

Any combination of text and channel selection filters may be used.

Select the **Show All** button to clear all filters.

3.8.5 Viewing Protocol Details

1. To view details of an existing protocol, from the 'Protocols' screen, select a single protocol of interest.

Note: If more than one protocol is selected, no details will be displayed.

2. The protocol details will appear in the panel on the right (Figure 33).
3. To start a new imaging session with the capture channels preloaded, select the **Capture** button below the protocol details on the right.

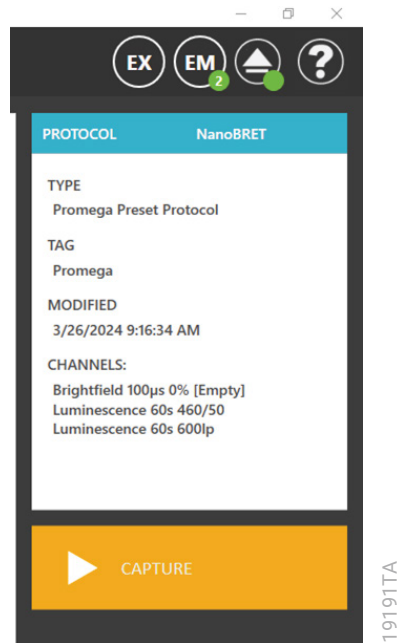


Figure 33. Protocol details. After selecting a protocol, the capture channels can be viewed. Selecting the **Capture** button will initiate a new imaging session with the selected protocol preloaded.

3.8.6 Deleting Protocols

Only user-defined protocols may be removed from the GloMax® Galaxy Software.

1. Select one or more protocols to be removed from the software using the check boxes to the left of each protocol entry. When one or more user-defined protocols is selected, the Delete button becomes active.
2. Press the **Delete** button to remove all selected user protocols from the software. (Deleted protocols cannot be recovered unless they were previously exported.)

3.8.7 Exporting Protocols

User protocols may be shared between GloMax® Galaxy Imagers by exporting from one installation of the GloMax® Galaxy Software and imported onto others. Promega preinstalled protocols for NanoBRET and Nano-Glo cannot be exported, but they can be saved as a user protocol, which can then be exported.

1. Select one or more user protocols to be exported from the software using the check boxes to the left of each protocol entry. When one or more user-defined protocols is selected, the Export button becomes active.
2. Press the **Export** button to export all selected user protocols from the software. A file browser is displayed (Figure 34). You can select the folder location where the exported protocol(s) will be saved. The current location is highlighted. Navigate to the desired folder location. Once a path has been defined, select the **OK** button to export the protocol(s) to the specified path, or select **Cancel** to return to the 'Protocols' screen without exporting.

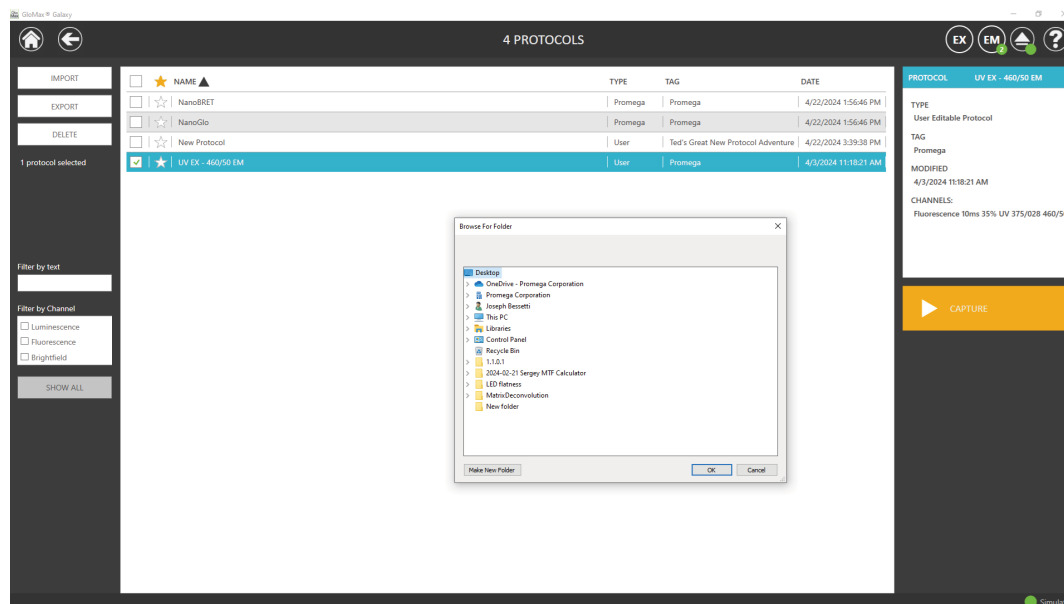



Figure 34. Export protocol 'Browse for Folder' window. When exporting protocol(s), select a folder location to save the exported protocol(s).

3.8.9 Editing a User or Promega Protocol

1. Load the capture channels from an existing user or Promega protocol into a new imaging session.
2. Add, remove, reorder or edit capture channel settings as desired.
3. Select  at the right of the Capture Channels header and select **Save** to overwrite an existing user protocol or **Save As** to create a new user protocol. For Promega protocols, choose **Save As** to save the protocol with a new name.

3.9 'Sessions' Screen

From the 'Home' screen, the Gallery button will open a table listing the previous imaging sessions saved on the system (Figure 35).

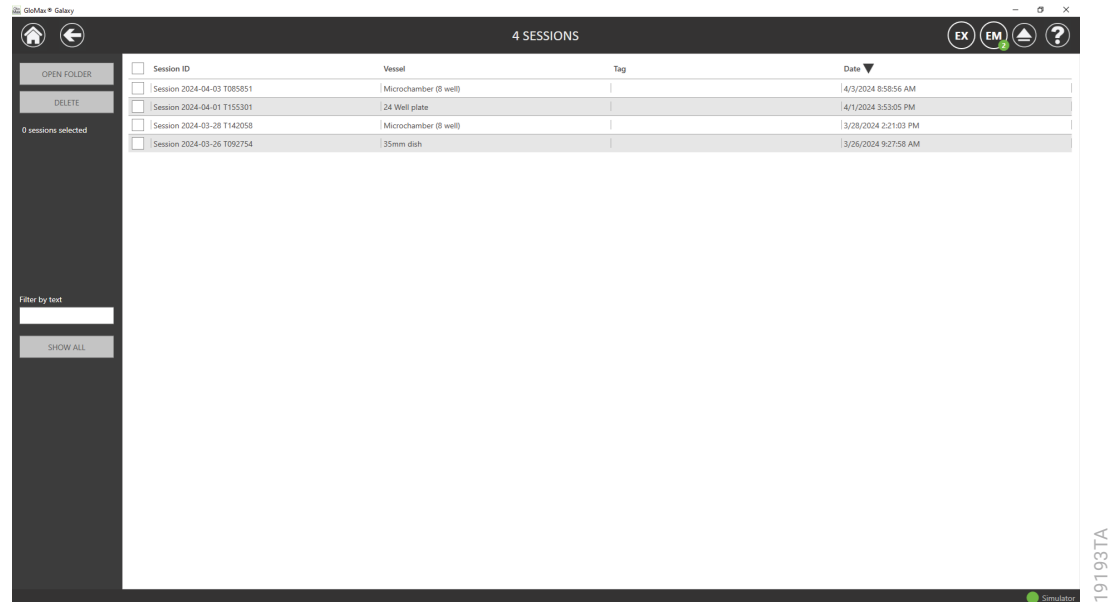


Figure 35. GloMax® Galaxy gallery screen. Saved sessions may be viewed on the 'Review' screen, deleted or the folder location of the stored image files opened.

3.9.1 Managing Saved Sessions

- Select a session ID from the list to open the session in the 'Review' screen to view captured images. (The 'Imaging' tab will be deactivated. To acquire more images, initiate a new imaging session.)
- Check the box to the left of a single session and select the **Open Folder** button to open the folder location of the image files and session report in Windows® Explorer.

3.9.2 Data Management

Checking the box to the left of one or more sessions and selecting the **Delete** button will permanently delete all image files for the selected session from the system.

Note: Once sessions are deleted, they cannot be retrieved. Ensure image files are saved or archived elsewhere prior to deleting them from the GloMax® software.

3.10 Settings

Selecting **Settings** from the 'Home' screen provides access to navigation and camera settings, specify where files are saved, export log files and view system information (Figure 36).

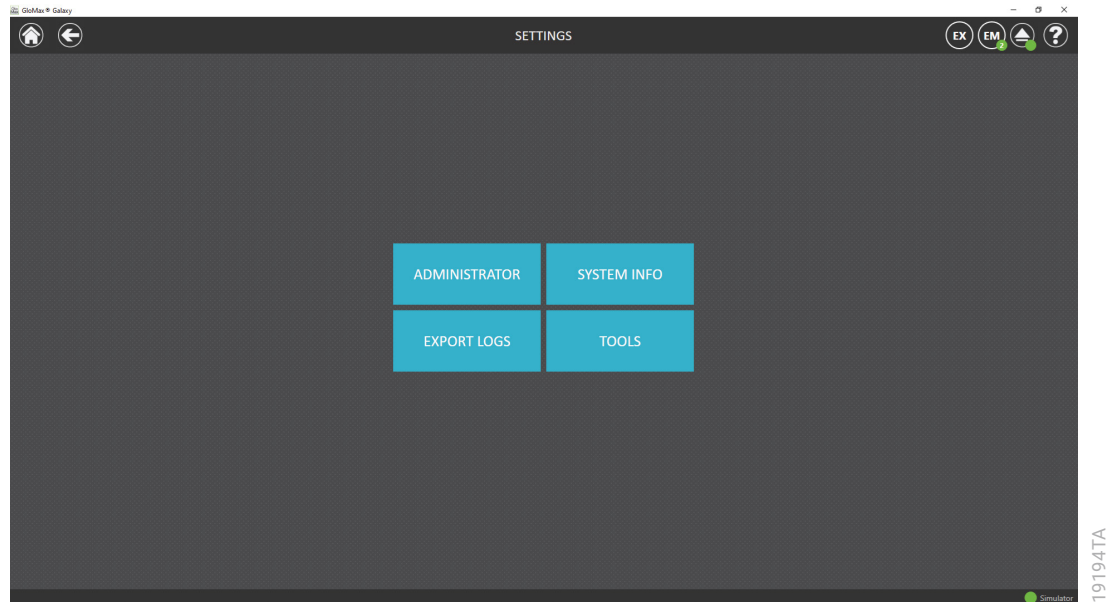


Figure 36. 'Settings' screen.

3.10.1 System Info Button

Selecting the **System Info** button will display system details including the Software Version and Build, Instrument Name and Serial Number, Firmware Version, Camera Serial Number and Firmware Version (Figure 37).



Figure 37. GloMax® Galaxy system information window.

3.10.2 Export Logs Button

The software records all actions performed by the instrument. If there are error messages, export the log and contact Promega Technical Services (techserv@promega.com) for further instructions. The log file is useful for troubleshooting events.

After selecting the **Export Logs** button, a 'Browse For Folder' window opens and you can select the location at which logs will be exported (Figure 38). Navigate to the folder location where logs should be exported. Once you have selected the desired path, select the **OK** button to export log files.

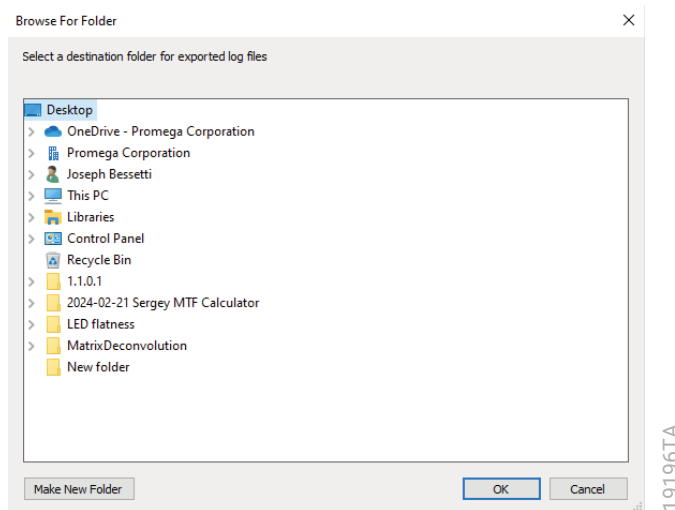


Figure 38. Export logs 'Browse For Folder' window. Select a folder to which logs will be exported. Choose **OK** to export log files.

3.10.3 Tools Button

Selecting **Tools** displays the button that prepares the instrument for shipment. Choosing this button will home the Z-stage and move the XY-stage into position for securing the shipping anchors (Figure 39). If additional adjustment is necessary, the knobs located at the right- and left-rear of the stage may be rotated to manually adjust the stage position. An image of the XY-Stage secured with red shipping anchors is shown in Figure 4, Panel A.

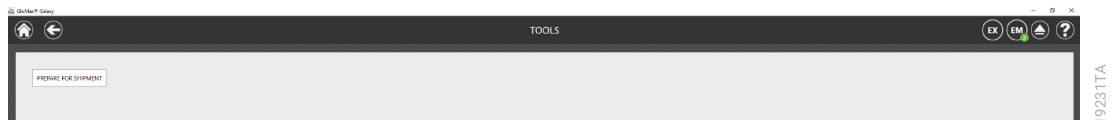


Figure 39. Tools button.

3.10.4 Administrator Button

The Administrator button is only displayed in the GloMax® Galaxy software when users with Administrator-level access view the 'Settings' screen. By default, all users will have Administrator-level access when the software is installed. To limit administrator-level access, the GloMax® software uses a Windows® login user access as a security tool. See Section 5.1, Assigning User Accounts to Promega Groups.

3.11 Administrator Settings

Administrator settings are only available to users with administrator-level access to the GloMax® software. These settings allow the administrator to set software preferences, view and create vessel definitions, update GloMax® Galaxy Imager firmware, and access software Audit Records. To access administrator settings:

1. From the 'Home' screen, select the **Settings** button.
2. On the 'Settings' screen, select the **Administrator** button to open the 'Administrator' screen (Figure 40).

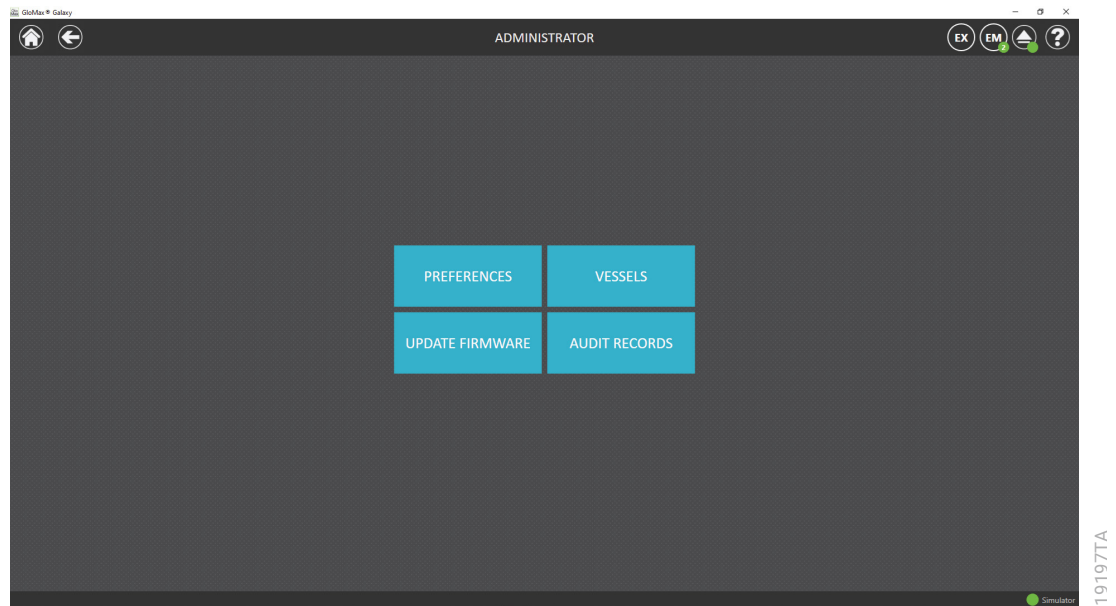


Figure 40. 'Administrator' screen.

3.11.1 Audit Records Button

Administrators can view a chronological sequence of actions performed with the GloMax® Galaxy Imager by selecting the **Audit Records** button within the 'Administrator' screen. An example 'Audit Records' screen is shown in Figure 41. Detailed audit records are automatically generated and stored with the GloMax® Galaxy software and cannot be deleted or edited.

Records may be exported by selecting the **Export Records** button on the upper left corner of the screen.

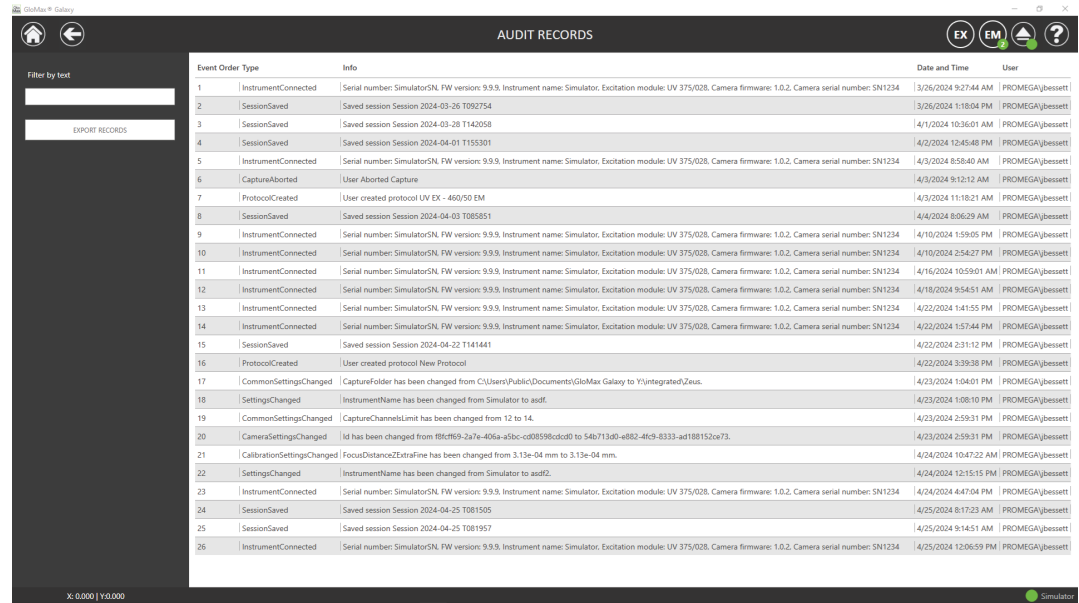


Figure 41. 'Audit Records' screen.

3.11.2 Update Firmware Button

The 'Update Firmware' screen allows administrators to apply firmware updates received from Promega to the system.

3.11.3 Vessels Button

The GloMax® Galaxy software uses stored vessel definitions to enable XY-stage and Z-(Focus) navigation during an imaging session. Supported vessel types are listed in Table 2.

Table 2. List of Recommended Sample Vessels.

Vessel Type	Manufacturer	Cat.#	Dimensions	Stage Adapter
8-well chamber slide (glass-bottom)	Promega IBIDI	GM4061 (80807 IBIDI)	75.4 × 25.6 × 10.8mm (h)	Slide Adapter
Slide (75mm × 25mm)	(various)	N/A	75 × 25mm	Slide Adapter
35mm Petri dish (μ-DISH, optical plastic-bottom)	IBIDI	81218	34.7mm (dia) × 11.7mm (h)	35mm Dish Adapter
96-well microplate (CELLView, glass bottom)	Greiner	655981	85.6 × 127.8 × 17.4mm (h)	96-Well Plate Adapter
24-well microplate (SensoPlate, glass-bottom)	Greiner	662892	85.6 × 127.8 mm × 21.8mm (h)	96-Well Plate Adapter
12-well microplate (glass-bottom)	CELLVIS	P12-1.5H-N	85.4 × 127.4 × 22.4mm (h)	96-Well Plate Adapter
6-well microplate (glass-bottom)	CELLVIS	P06-1.5H-N	85.4 × 127.4 × 21.9mm (h)	96-Well Plate Adapter

h = height; dia = diameter

Except for slides, all recommended vessels have 0.17mm glass bottoms for optimal sample imaging. Glass slides are suitable for imaging fixed samples, and you will obtain optimal image quality using 0.17mm cover glass mounted over the sample and the slide inverted on the stage with the cover glass and sample facing the objective.

3.11.4 Vessel Default Imaging Position

Vessels other than those listed in Table 2 may be suitable for use on the GloMax® Galaxy Imager but may require adjusting the default imaging position. This is the position that the objective moves to at the start of a new imaging session. **If this position is incorrect, the objective may hit the bottom of the vessel or you may have difficulty finding the sample imaging plane.**

Note: If using the Stagetop Incubator and Controller, GloMax® Galaxy (Cat.# GM4010), the focusing steps in Section 3.11.5 should be performed with the stagetop chamber installed.

3.11.5 Evaluating an Alternative Vessel and Setting the Focus Distance

1. Start a new imaging session and select the vessel type that matches the format of your desired vessel.
2. Install the proper adapter on the stage, but do not load the vessel at this point.
3. Select **OK** to start the new session. The xy-stage and objective will move to the initial imaging position for the vessel type that was selected.
4. Open the instrument lid, unlock the LED arm and rotate the LED up away from the stage.
5. Carefully position the desired vessel in the adapter, using one of the two options below:
If the bottom of the vessel contacts the objective lens, decrease the focus distance as follows:
 - a. Remove the vessel, lower the LED arm, close the lid, and use the coarse z focus down button to lower the objective.
 - b. Lower the objective in 1–2mm increments using the coarse z-focus adjustment.
 - c. Insert the vessel. Check if the vessel contacts the objective. If yes, repeat Steps a–c. If not, continue to Step d.
 - d. Focus on the imaging surface in brightfield; cells or a line drawn with a marker in well A1 are useful for finding the proper imaging plane.
 - d. When the sample is in focus, note the z position displayed under the vessel diagram. This value will be used in Step 8.**If the bottom of the vessel does not contact the objective lens:**
 - a. Secure the Vessel, lower the LED arm and close the instrument lid.
 - b. Use the z-focus adjustment buttons to focus on the imaging surface in brightfield. Cells or a line drawn with a marker in well A1 are useful for finding the proper imaging plane.
 - c. When the sample is in focus, note the z position displayed under the vessel diagram. This value will be used in Step 8.
6. After the focus position is determined, close the imaging session, and navigate to Settings → Administrator → Vessels to display the table of vessel types saved in the software.
7. Select the vessel type that matches the format of your desired vessel and select the **Create From** button at the upper left of the screen.
8. Enter a name for the new vessel and set the Focus Distance (mm) to the z position identified in Step 5. Include the vendor and catalog number for the new vessel.
9. Select **Save** to add the new vessel to the database.
10. Return to the 'Home' Screen and initiate a new imaging session by selecting **Capture**. Select the new vessel type and start the session.
11. Verify that the initial focus distance brings the imaging surface of the vessel into view without substantially adjusting the z height.

3.11.6 Preferences

The 'Preferences' screen is where administrators can specify the gallery folder location, set a limit for the number of capture channels that may be defined within a session, define camera crop width and height, define coarse and fine stage motion distances, and update the instrument name. The settings present on each tab on the 'Preferences' screen are described below.

'Common Settings' Tab

On the 'Common Settings' tab (Figure 42), an administrator can change the Capture Channels Limit and define the Gallery Folder location where image files are saved on the PC.

Note: While a network drive may be selected for saving image files, if your network is not reliable or communication slows, there may be data loss.

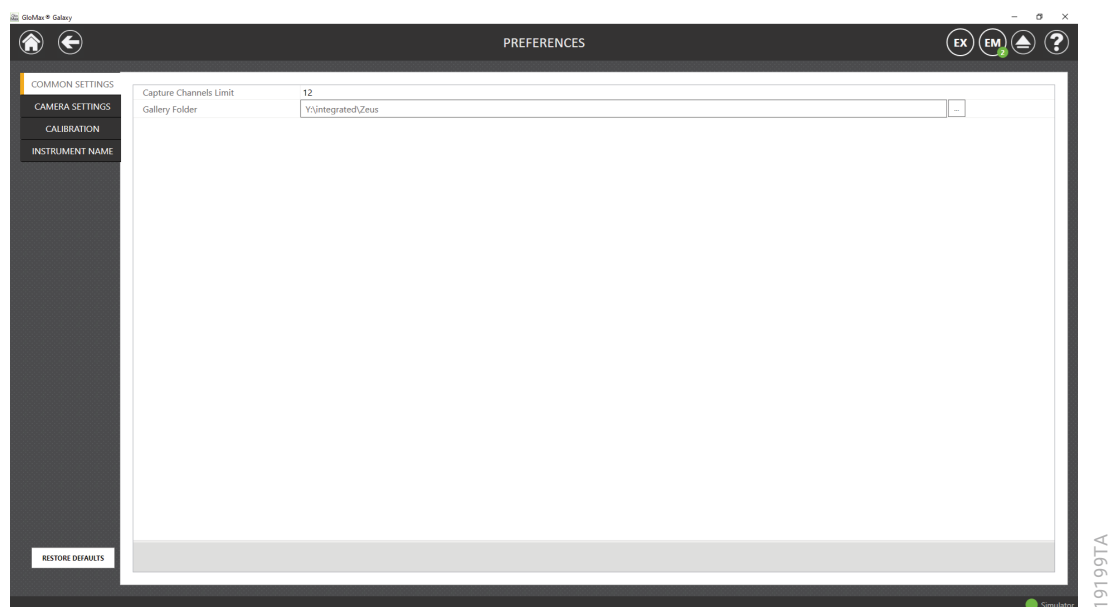


Figure 42. 'Preferences' screen showing the 'Common Settings' tab.

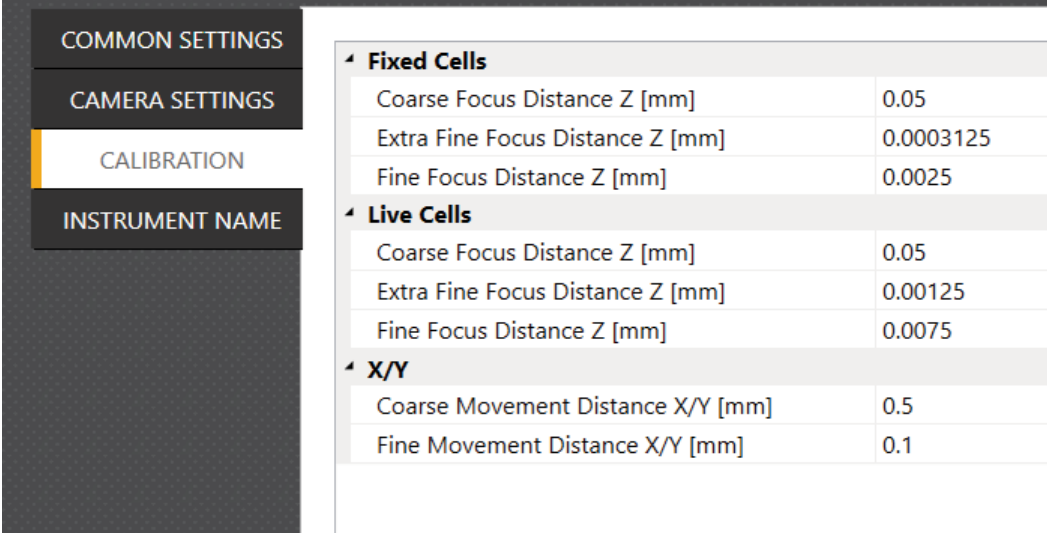
'Camera Settings' Tab

On the 'Camera Settings' tab, an administrator can adjust the crop width and crop height (in pixels) for saved images. The default crop size is 2,200 × 2,200 pixels. The maximum crop width setting is 3,200 pixels. The maximum crop height setting is 2,200 pixels.

Note: If the crop width and crop height are set significantly smaller than 2,200 pixels, consider adjusting the XY coarse and fine motion settings on the 'Calibration' tab to better match the smaller field of view.

'Calibration' Tab

On the 'Calibration' tab (Figure 43), an administrator can adjust coarse and fine motion distances for the XY-stage and for focus (Z). In addition to coarse and fine distances, an extra fine distance is also defined for focus (Z). The minimum setting for Z extra fine is 0.0003125mm, which will provide optimal focus adjustment for fixed samples. Separate focus settings are provided for Live Cell and Fixed Cell imaging.



Fixed Cells	
Coarse Focus Distance Z [mm]	0.05
Extra Fine Focus Distance Z [mm]	0.0003125
Fine Focus Distance Z [mm]	0.0025
Live Cells	
Coarse Focus Distance Z [mm]	0.05
Extra Fine Focus Distance Z [mm]	0.00125
Fine Focus Distance Z [mm]	0.0075
X/Y	
Coarse Movement Distance X/Y [mm]	0.5
Fine Movement Distance X/Y [mm]	0.1

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Figure 43. 'Preferences' screen showing the 'Calibration' tab.

Changes to Common Settings, Camera Settings and Calibration will automatically be saved when the user navigates away from the 'Preferences' screen. Select the **Restore Defaults** button at the lower left of the 'Preferences' screen to restore default settings for the displayed tab.

'Instrument Name' Tab

Enter the desired name of the instrument. This feature is typically used if a laboratory has more than one GloMax® Galaxy Imager. After changing the name, select the **Update** button to save the change. The instrument name will be noted in the image file metadata and the session report (Figure 44).

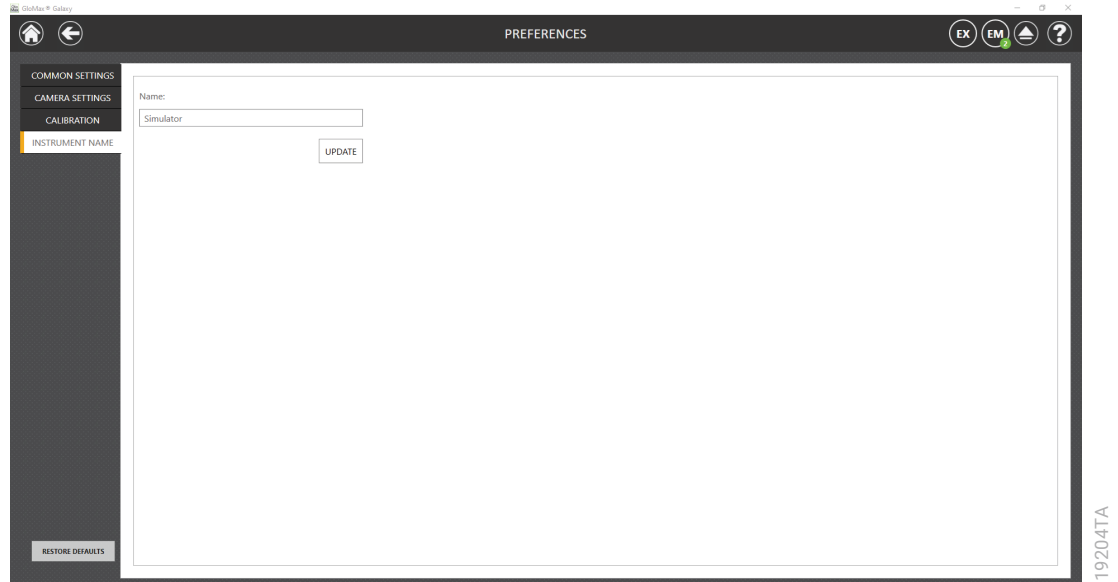


Figure 44. 'Preferences' screen showing the 'Instrument Name' tab.

Select the **Restore Defaults** button at the lower left of the 'Preferences' screen to restore default settings for the displayed tab.

4 Cleaning and Maintenance

4.1 General Imager Care

Turn off the GloMax[®] Galaxy Imager and disconnect the AC power adapter whenever the interior of the instrument is open for cleaning and maintenance.

Residue from reagent spills can inhibit proper stage movement. Immediately clean up any spills. We recommend thoroughly cleaning the interior of the instrument every 30 days as follows:

Clean the stage using a Kimwipes[®] tissue dampened with 70% ethanol. Do not allow excess solution to run off onto other electrical components, because this can cause damage to the instrument and its electronics. Do not use solvents or abrasive cleaners.

Use a cloth dampened with deionized water to periodically clean the exterior of the GloMax[®] Galaxy Imager. Do not use solvents or abrasive cleaners.

4.2 Cleaning Imager Optics

Always wear new gloves when handling or cleaning optical components. Once your gloves are on, cleaning with an alcohol wipe will remove any trace contaminants that may be present on them. Avoid touching your hair, face or any uncleaned surfaces when handling optics.

4.2.1 Cleaning the Objective

- Dust, fingerprints or other contaminants on the objective lens can adversely affect image quality. To ensure maximum performance of the GloMax® Galaxy Imager, we recommend regular cleaning of the objective.
- To access the objective for cleaning, navigate to **Settings** and then to **Tools**. Select the button, **Prepare for Shipment**. This will center the objective in the stage. Temporarily remove the stage top incubator, if installed.
- Use the provided air bulb to remove dust or other particulates from the objective lens. A lens-cleaning brush or other soft brush can be used to remove any remaining dust.
- Fingerprints or grease should be removed using lens cleaning paper or a soft cotton cloth soaked in absolute ethanol. The lens should be wiped in a circular motion from center to edge. A cleaning stick wrapped in lens paper can be used to clean harder-to-reach edges of the objective or tube lens.
- The objective lens is an air (dry) objective and should never be used with immersion oil. If this happens, lens cleaning paper soaked in petroleum benzine is recommended for cleaning off the oil, followed by ethanol as above.

4.2.2 Cleaning the Emission Filters

Optical filters are delicate and must be handled with care. Always handle filters by the edges and place them on clean, soft surfaces if you need to set them down.

Routine cleaning is not recommended. If the emission filters look clean, do not clean them. Unnecessary cleaning can introduce contaminants or cause performance degradation.

If a filter needs to be cleaned, start by using the provided air bulb to remove any loose dust or debris.

Wiping should only be performed if necessary. If required, gently wipe the surface using a lint-free wipe or swab wetted with anhydrous reagent-grade alcohol or acetone. Avoid oversaturating the wipe or swab. Use a slow and steady drag across the surface, applying minimal force.

5 Troubleshooting

For questions not addressed here, contact your local Promega Branch Office or Distributor.
Contact information available at: www.promega.com. Email: techserv@promega.com

5.1 Error Messages

Error Message	Description	Resolution
Instrument Disconnected	Instrument is not on.	Turn on instrument, indicated by the green LED on/off button
Failed to connect: USB communication failure, device turned off or disconnected?	The software has failed to connect to and communicate with the instrument.	Check that the instrument is powered on and connected to the computer via the USB cable. Select the message at the bottom right of the software screen "Instrument Offline – Click to Connect". If the error persists, contact Promega Technical Services.
Failed to connect: Could not detect or initialize camera.	The software has failed to connect to and initialize the camera.	Remove the front panel to check that the camera power cable is connected and that the camera is on. Confirm that the camera communication cable is securely connected.
Camera not detected. Check power and USB connection to camera and try again.		
Camera failure. Please restart the instrument.		

Error Message	Description	Resolution
Axis X not initialized.	The stage is obstructed and unable to initialize.	Ensure the shipping anchors have been removed. Check for other obstructions that may prevent the stage from moving.
Instrument not connected. Reconnect and try again.	The instrument-to-PC connection has failed.	Check that the instrument-to-PC cable is securely connected. Make sure the cable is minimum USB 3.1 and has not been damaged. Confirm that the PC USB port is working properly.
Failed to [camera command]. Camera error detected. Please restart the instrument.	The connected camera failed to respond to a command.	Check connections to camera. Restart the instrument by powering it off then back on. Reconnect to the control software by selecting Instrument Offline-Click to Connect at the bottom right of the control software screen.
Initialization failed on startup due to the lid being open. Close the lid and reconnect.	The lid was open during initialization	Close the instrument lid. Reconnect by selecting Instrument Offline-Click to Connect at the bottom right of the control software screen.
Filter slide communication error: Please reinstall the filter slide panel and press Retry	The system has failed to communicate with the filter slide.	Check that the filter slide panel is installed. Remove and reinstall the filter slide panel and secure using all four thumb screws.
Filter slide timeout. Reinstall filter slide panel and secure thumbscrews		
Failed to capture image: Failed to move slide: Missing or invalid filter slide reply received	The system has failed to communicate with the filter slide.	Check that the filter slide panel is installed. Remove and reinstall the filter slide panel and secure using all four thumb screws.

Error Message	Description	Resolution
Please make sure slide adapter is installed	The system has failed to communicate with the filter slide.	Check that the filter slide panel is installed. Remove and reinstall the filter slide panel and secure using all four thumb screws.
Move aborted. Close the lid to initialize the stage.	The door of the instrument was opened during operation of the stage. This causes the stage motion to be halted.	The system will reinitialize automatically when the door is closed and the error cleared.
Lens may make contact with vessel	The user attempted to adjust focus up with the objective near the edge of the stage adapter hardware.	For nonsupported vessels, check focal distance
Cannot move beyond vessel envelope	The user attempted to navigate outside of the allowed range for the installed adapter and vessel.	Check the current XY-stage position in the vessel diagram to make sure you are not at or beyond the edge of the imaging surface.
Cannot move stage: []-axis out of range	The user attempted to navigate outside of the allowed range for the installed adapter and vessel.	Check the current XY-stage position in the vessel diagram to make sure you are not at or beyond the edge of the imaging surface.
Cannot move objective beyond adapter limit	The user attempted to adjust focus up with the objective near the edge of the stage adapter hardware.	Check the current XY-stage position in the vessel diagram to make sure you are not at or beyond the edge of the imaging surface. Navigate to the center of the vessel or a well and try again.
This instrument has not been calibrated.	The instrument does not have, or has lost, calibration values.	Call Promega Technical Services.
Lid Opened	The door of the instrument door has been opened during the imaging session.	This message will appear during routine access of the stage and sample and will disappear automatically when the door is closed.

Error Message	Description	Resolution
Please close the lid	Appears when the instrument door has been opened. Closes automatically when the door is closed.	Not applicable.
Disk space is low, continue anyway?	Not enough space to save images	Need to create space on the C: drive for more images.
Error Activating Brightfield	Filter slide did not move	Reinstall filter slide.

5.2 Common Problems

Symptom	Causes and Comments
Power failure during a run	In the event of a power failure, turn off the instrument and PC. When power is resumed, turn on the instrument and PC. The results that were generated during the run before the power failure will be saved.

Symptom	Causes and Comments
<p>Dim or low luminescence signal in images</p>	<p>Low luminescent signal or low activity in sample.</p> <ul style="list-style-type: none"> • Increase exposure time. Due to the lower photon flux of luminescence compared to fluorescence, a longer exposure time is necessary to image luminescence. The length of exposure time will depend on the protein expression level. For reference, a GloMax® Discover System reader value of 1×10^5 may require a 3–5 minute exposure setting to acquire a suitable image using GloMax® Galaxy imager. • Optimize treatment conditions <ul style="list-style-type: none"> If possible, use growth medium without phenol red and limit serum to 2% to minimize auto luminescence. When not using the Stagetop Incubator/Controller, use buffered medium (e.g., Opti-MEM) or CO₂-independent medium. • Optimize transfection conditions. Avoid reverse transfection as it can result in poor cell adhesion. Plate cells at a density of 1×10^5 cells/ml and culture for 24 hours before transfection. • Try using an overexpressing NanoLuc® model system with high signal. Live-cell NanoLuc® substrates such as Vivazine™, Endurazine™ and Nano-Glo® Live Cell Assay System must follow the recommended assay collection durations. For example, the half-life of the Nano-Glo® Live Cell Assay is much shorter (approximately 5 minutes) compared to Vivazine™ or Endurazine™ substrates. Due to the extended exposure time needed for luminescence, image only a few samples at a time to maintain the assay signal half-life integrity. • Collect a dark frame image and use it to subtract during imaging processing. • If cell type requires a treated surface for a microplate reader assay, ensure the vessel used for imaging contains a similar treated surface. If using cells with low adhesion strength, apply a coating such as 0.1% gelatin to the imaging vessel surface. • Using 2x2 or 4x4 binning can help increase signal intensity, thereby shortening the time necessary to determine the optimal z focus.

Symptom	Causes and Comments
Luminescence images out of focus or best focus is difficult to find.	Long capture times can make focusing on luminescence more challenging. Increase exposure, use 2x2 or 4x4 binning, or incorporate a live-cell fluorescent stain, such as Hoechst 33342, and fluorescence capture channel to use for optimizing focus.
No image detected in brightfield.	<ul style="list-style-type: none"> • Check that the LED power is on (minimum 20%). • Start live-view display. Check that the image is not saturated (reduce exposure and/or LED power). • Check filter configuration in filter slide holder. • Sample out of focus. Adjust focus. If using a vessel from a manufacturer other than those predefined in the software, refer to Section 3.11.5 to evaluate and set initial focus position.
Image detected in brightfield but not luminescence or BRET	<ul style="list-style-type: none"> • Add Detection Substrate. • Check that the correct emission filters are installed in the filter slide and are correctly identified in the GloMax® Galaxy software. • Increase exposure time. Due to the lower photon flux of luminescence compared to fluorescence, a longer exposure time is necessary to image luminescence. The length of exposure time will depend on the protein expression level. For reference, a GloMax® Discover System reader value of 1×10^5 may require a 3–5 minute exposure setting to acquire a suitable image using GloMax® Galaxy imager. • Optimize treatment conditions If possible, use growth medium without phenol red and limit serum to 2% to minimize autoluminescence. When not using the Stagetop Incubator/Controller, use buffered medium (e.g., Opti-MEM) or CO₂-independent medium. • Optimize transfection conditions. Avoid reverse transfection as it can result in poor cell adhesion. Plate cells at a density of 1×10^5 cells/ml and culture for 24 hours before transfection. • Try using an overexpressing NanoLuc® model system with high signal. • If using cells with low adhesion strength, apply a coating such as 0.1% gelatin to the imaging vessel surface.

Symptom	Causes and Comments
Image detected in brightfield but not fluorescence	<ul style="list-style-type: none"> • Check emission filters installed in the filter slide are correctly identified in the GloMax® Galaxy software. • Check that the excitation module is correct for the fluorescent target. • Check that the LED power is on (minimum 20%). Increase LED power and/or exposure time. • Light exposure can cause photobleaching of the fluorescent dye.
Difficulty finding sample and establishing initial focus	Use of nonsupported labware. See Section 3.11.4, Vessel Default Imaging Position.
No image displayed in live-view window	<ul style="list-style-type: none"> • Ensure live view is activated by selecting the Start button. • Ensure LED power (for brightfield and fluorescence) is set to a minimum value of 20% and that the “Disable LED” check box is unchecked. • Adjust image histogram by manually moving the triangles or selecting the Auto button. • Adjust LED power and/or exposure to increase or decrease signal in the image. If all image pixels are saturated, the live-view window will be blank, and no histogram displayed. Decrease LED power and/or exposure to adjust intensity within the camera dynamic range.

6

Configuring the PC

The GloMax® Galaxy PC allows you to select from a series of options.

Your IT department or site administrator should configure the PC according to the IT rules and procedures pertinent to your institution. The following is a set of guidelines for your IT personnel or site administrator. Depending on your institution's IT policy, you may or may not be able to configure or change the settings for the PC. You can install the full version of Microsoft® Office if you desire. We do not recommend loading other programs onto the PC because these may interfere with the instrument operation.

Note: Some institutions require antivirus software installed on PCs within the institution. If installing an antivirus program, ensure it is set to manual, not automatic, update. Antivirus software will slow the performance of the PC and affect the performance of the GloMax® Galaxy Imager while it is acquiring images.

Additional recommendations if using your own PC:

- Disable automatic Windows® updates and virus scans
- Disable automatic power off, sleep, or hibernation settings
- Disable "Fast Startup". Set the power button to Shut Down not Sleep.
- Disable any other automatically or manually started software that would consume PC resources during operation.
- Ensure there are no domain policies that would overwrite any of the above settings.

Note: The PC that is provided with the GloMax® Galaxy Bioluminescence Imager System (Cat.# GM4000) has already been configured for the settings listed above.

The first time you use the GloMax® Galaxy Imager and PC, check the configuration of the following options. Settings can be changed later if required by following the instructions again.

1. Date and Time

The date and time set on the PC are used for the instrument run log to indicate when a protocol was run on the GloMax® Galaxy Imager, as well as during kinetic runs to mark the time during each read.

2. User Password and Setup

GloMax® software supports the following user roles.

PromegaAdministrator: Promega Administrators have access to all GloMax® Galaxy Control Software features needed to operate and manage the GloMax® Galaxy Imager.

6.1 Assigning User Accounts to Promega Groups

The GloMax® software employs the Windows® login user access levels as a security tool for adding approved users and associated passwords. New user accounts should be created based on policies governing your institution. Only someone with an Administrator account can assign users to Promega groups. You must close the GloMax® software to create user accounts.

1. Follow the instructions for your PC operating system.
2. If you want to add Administrators, select **PromegaAdministrators**. A 'PromegaAdministrator' window will open, which can be used to add existing users to the PromegaAdministrators group.
3. Select the **Add...** button to add users to the PromegaAdministrators group. In the "Enter the object names to select" area of the window, enter the username of the user you wish to add to this group. Select **OK** on this window and select **OK** on the 'PromegaAdministrator Properties' screen to add users to the PromegaAdministrators group.
4. Close the 'Computer Management' screen.

6.2 Removing User Accounts

The GloMax® Software employs the Windows® login user access levels as a security tool for adding approved users and associated passwords. Removing existing users can only be performed by someone with Administrator privileges.

1. Follow the instructions for your PC operating system.
2. Select **Local Users and Groups** and then choose **Groups**.
3. If you want to remove Administrators, select **PromegaAdministrators**. A 'PromegaAdministrator' screen will open, which can be used to remove existing users from the PromegaAdministrators group.
4. Select the user you want to remove and select **OK** to remove selected user from the PromegaAdministrators group. Close the 'Computer Management' screen.

7

Warranty and Service

7.1 Warranty

The GloMax® Galaxy Bioluminescence Imager comes with a one-year warranty from Promega. Additional warranty and service agreements are available. For more information, contact Promega Technical Services. Contact information is available at: **www.promega.com**. Email: **techserv@promega.com**

To obtain service during the warranty period, please take the following steps:

1. Contact Promega Technical Services.
2. Carry out minor adjustments or tests as suggested by your Technical Services contact.
3. If the instrument should be returned for repair, Promega Technical Services will arrange for service by an authorized GloMax® Service Agent. You will be issued a Promega return authorization number. You must obtain a Promega return authorization number (RMA number) before returning an instrument for service.
4. Before returning the instrument, you will be responsible for cleaning it and providing a Certificate of Decontamination (see Section 9). If the instrument has been exposed to any chemical, biological or radioactive hazards, contact Promega Technical Services for decontamination instructions before shipping.

7.2 Warranty and Service Agreement Options

GloMax® Galaxy Premier Warranty Upgrade Cat.# SA1484

The Premier Warranty Upgrade includes all parts, labor and on-site service visits by a factory-trained service technician within 3 business days (where available) during the first year of warranty. Additionally, it includes one annual on-site Preventive Maintenance visit by a service technician. Additional Preventive Maintenance visits are available separately.

GloMax® Galaxy Premier Service Agreement

The Premier Service Agreement includes all parts, labor and on-site visits. The system will be repaired and returned to original factory specifications. A service technician will be on-site within 3 business days for repairs. Additionally, it includes one annual on-site Preventive Maintenance visit by a service technician. Additional Preventive Maintenance visits are available separately.

Different options for the GloMax® Galaxy Premier Service Agreement are available as shown below.

Product Name	Cat.#
GloMax® Galaxy Premier Service Agreement, 1 YR	SA1511
GloMax® Galaxy Premier Service Agreement, 2 YR	SA1521
GloMax® Galaxy Premier Service Agreement, 3 YR	SA1531

GloMax® Galaxy Standard Service Agreement

The Standard Service Agreement covers all parts, labor and on-site visits. The system will be repaired and returned to original factory specifications. Additional Preventive Maintenance is available separately.

Different options for the GloMax® Galaxy Standard Service Agreement are available as shown below.

Product Name	Cat.#
GloMax® Galaxy Standard Service Agreement, 1 YR	SA1541
GloMax® Galaxy Standard Service Agreement, 2 YR	SA1551
GloMax® Galaxy Standard Service Agreement, 3 YR	SA1561

GloMax® Galaxy Preventative Maintenance Cat.# SA1488

To keep the system operating at peak performance, we recommend that GloMax® Galaxy Bioluminescence Imagers receive a Preventive Maintenance check at least every 12 months of use. During this procedure, our qualified service personnel test the instrument, check parts for wear and replace them as needed. Additionally, the system is aligned and functionality is verified. Documentation for your files is provided.

GloMax® Galaxy Installation Qualification and Operational Qualification

Product Name	Cat.#
GloMax® Galaxy Operational Qualification	SA1501
GloMax® Galaxy Installation Qualification/Operational Qualification	SA1490

The Installation Qualification service product includes a series of formal instrument checks, delivers written documentation of instrument functionality, and demonstrates that everything ordered with the instrument is supplied and installed at the customer's laboratory. This service product must be delivered by a Promega representative who is certified to perform the Installation Qualification. The service product involves a site visit to perform:

- Installation by qualified Promega personnel
- Inspection of shipping containers, instrument and accessories
- Comparison of items received vs. items on purchase order
- Inspection of laboratory conditions
- Review of all hazards and precautions with users
- Confirmation/installation of correct firmware version
- Testing of instrument run
- Recording and documenting installation and actions

The Operational Qualification service product demonstrates that the instrument functions according to its operational specifications. This service product must be delivered by a Promega representative who is certified to perform the Operational Qualification. The service product involves a site visit to perform:

- Running of operational verification tests
- Documenting of all calibration and test results
- Training of customer(s) to operate the instrument
- Training of customer(s) to use the log book
- Completion of customer-specific log book, instrument sticker and OQ documentation

Stagetop Incubator/Controller Standard Loaner Agreement

For customers who have also purchased the Stagetop Incubator/Controller for GloMax® Galaxy (Cat.# GM4010), Standard Loaner Agreements are available should your Stagetop Incubator/Controller ever need service.

Different options for the Stagetop Incubator/Controller Standard Loaner Agreement are available as shown below.

Product Name	Cat.#
Stagetop Incubator/Controller Standard Loaner Agreement, 1 YR	SA1541
Stagetop Incubator/Controller Standard Loaner Agreement, 2 YR	SA1551
Stagetop Incubator/Controller Standard Loaner Agreement, 3 YR	SA1561

8

Viewing and Processing Images in FIJI (ImageJ)

Images collected on the GloMax® Galaxy Bioluminescence Imager are saved as .tiff files and therefore compatible with a variety of image analysis software. ImageJ is an open-source image processing and analysis program with vast functionality enabled by user-contributed plugins. FIJI is a distribution of ImageJ that provides convenient access to available plugins via an updater. More information about ImageJ and FIJI in addition to a comprehensive user guide is available here: <https://imagej.net/learn/>. Promega distributes Fiji Image Processing Software; it is preinstalled on the PC and included when purchasing Cat.# GM4000. Fiji ImageJ is installed on the included computer in `C:/Users/Administrator/Fiji.app`. When installed on a customer-provided PC, the application will be located in `C:/Users/[username]/Fiji.app`.

A few commonly used functions for general image visualization are below. Learn more at: www.promega.com/products/imaging-devices/glomax-galaxy-bioluminescence-imager/

Intensity adjustments:	Image → Adjust → Brightness/Contrast
Applying a color scale or Lookup Table (LUT)	Image → Lookup Tables
Merge channels into RGB image	Image → Color → Merge Channels
Creating or manipulating image stacks	Image → Stacks
Adding a scale bar to an image	Analyze → Set Scale <ul style="list-style-type: none">• Enter 1 in the Distance in pixels field.• Enter 0.435 in the Known distance field.• Enter μm in the Unit of length field.• Check the box next to "Global" to apply to all opened images. Analyze → Tools → Scale Bar
Pixel binning	Image → Transform → Bin

For importing many images from a time series into a stack in chronological order, use File → Import → Image Sequence. This function indexes images in the session folder by defining the first image of the sequence (Start), the total number of images to import (Count), and the increment (Step). Select **Browse** to select the folder containing the time series images, and ensure the "Sort names numerically" box is checked. Selecting **Use virtual stack** will enable faster import if computer RAM is limiting.

8.1 Background Subtraction and Dark Frame Collection

For luminescence images, you can perform a background subtraction to maximize image contrast, particularly for dim samples, which can also be useful for certain image quantitation. Background subtraction is best performed by collection of one or more dark frames using the same exposure settings as the sample, but with no sample in the instrument. Background pixel grey values are typically around 100 and can be subtracted from sample images in one of two ways:

1. If one dark frame is collected, calculate the mean of all pixel values in the image by selecting Analyze → Measure. With the sample image selected, go to Process → Math → Subtract, and enter this mean pixel value.
2. If multiple dark frames are collected, first create a stack (Image → Stacks → Images to Stack). Then generate an image of averaged pixel values by selecting Image → Stacks → Z Project, and choose **Average Intensity** from the drop-down menu. Use Process → Image Calculator to perform a pixel by pixel subtraction of the dark frame from the sample frame, and select **32-bit (float)** if desired.

9

Certificate of Decontamination

Disinfection and decontamination are required prior to shipping the instrument and accessories for repair. Returned Instruments must be accompanied by a signed and dated Certificate of Decontamination, which must be attached to the accessories box inside the instrument packaging.

To disinfect and decontaminate: Wipe off the inside platform, and inside and outside surfaces using a cloth dampened with 70% ethanol. Follow immediately with a cloth dampened with deionized water. Repeat the procedure as many times as required to effectively disinfect and decontaminate the instrument.

Failure to follow these decontamination guidelines, sign and return the Decontamination Form will result in decontamination charges before the instrument will be serviced.

Select either (A) or (B):

- A. I confirm that the returned items have not been contaminated by body fluids or by toxic, carcinogenic, radioactive, or other hazardous materials.
- B. I confirm that the returned items have been decontaminated and can be handled without exposing personnel to health hazards.

Select the type of material used in the instrument:

- Chemical Biological
- Radioactive**

Briefly describe the decontamination procedure performed:

Date: _____

Place: _____

Signature: _____

Name (block capital letters): _____

** The signature of a Radiation Safety Officer is also required if the instrument was used with radioactive materials.

This instrument is certified by the undersigned to be free of radioactive contamination.

Date: _____

Place: _____

Signature: _____

Name (block capital letters): _____

Title: _____

10 Repacking the GloMax[®] Galaxy Bioluminescence Imager

10.1 Preparing the GloMax[®] Galaxy Imager

1. Position the stage by pressing the **Prepare for Shipping** button within the software (Settings).
2. Turn off the instrument and decontaminate the instrument using 70% alcohol and soft cloth.
3. Unplug the GloMax[®] Galaxy Imager and open the top lid.
4. Remove the excitation module (Figure 45, Panel A) and insert foam into position (Figure 45, Panel B).

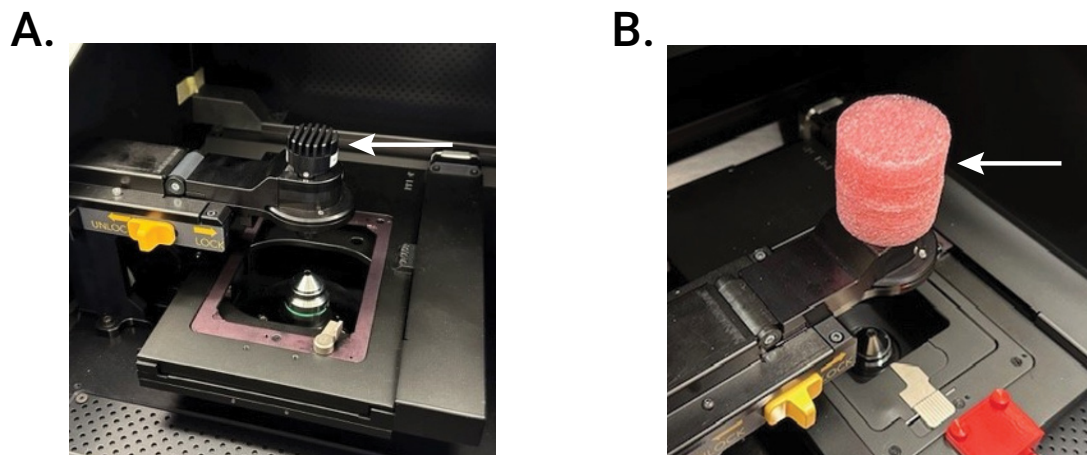
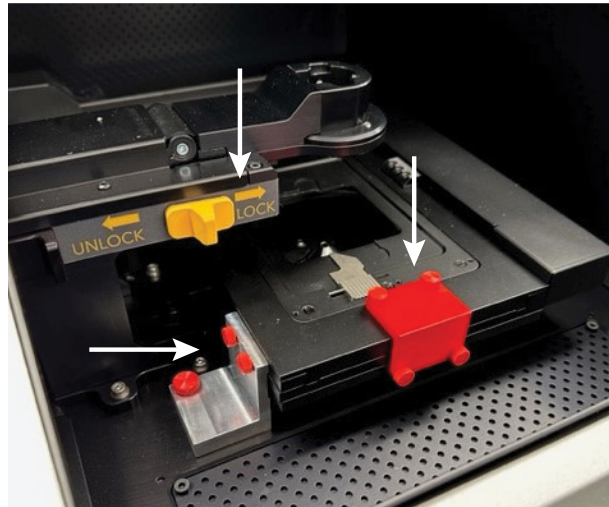


Figure 45. Panel A. Removing the excitation module. **Panel B.** Adding foam.

5. Ensure the arm is in the Lock position. Insert the red and silver Packing Brackets into position (Figure 46). Use the red thumb screws to tighten the locking screws. Failure to complete this step will result in imager damage.



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Figure 46. Lock the arm and fasten the packing brackets.

6. Disconnect AC Power Adapter on the back of the GloMax® Galaxy Imager.
7. Place the GloMax® Galaxy Imager inside the provided plastic bag.

10.2 Packaging the PC and Accessories

1. Power off the PC, then disconnect the USB and power cords from the PC.
2. Disconnect the USB and power cords from the monitor.
3. Pack the PC and keyboard into their shippingbox.
4. Pack the monitor into its box.

10.3 Packing the GloMax® Galaxy Imager Accessories

Please check to ensure all accessories are included. Pack the accessories into the white accessory box.

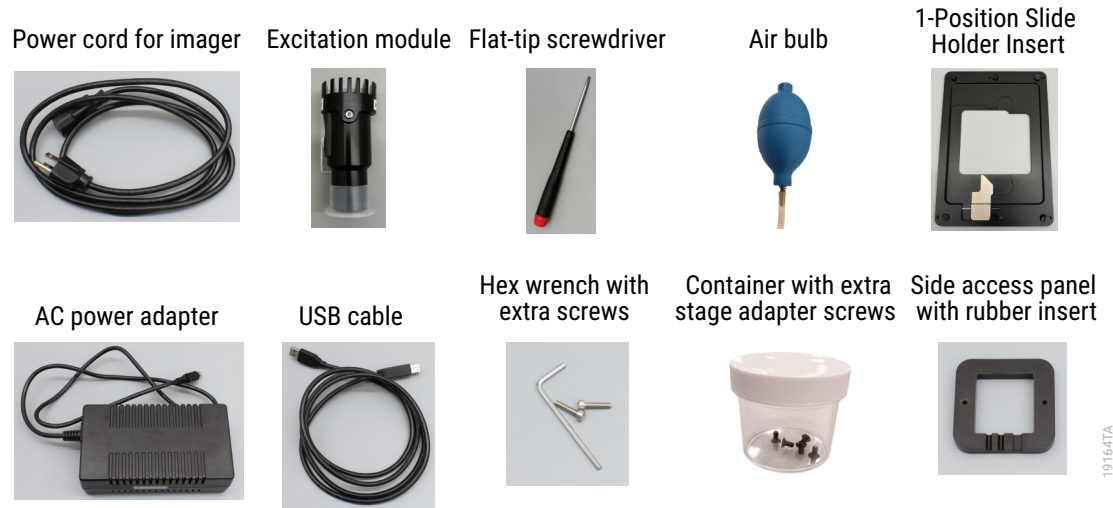


Figure 47. GloMax® Galaxy Imager accessory components.

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10.4 Assemble the GloMax® Galaxy Imager Packaging

1. Place the instrument into the bottom layer of packaging foam. Assemble the packaging foam around the instrument (Figure 48, Panel A).
2. Place the white accessory box onto the packaging foam. Insert the four corner supports (Figure 48, Panel B).

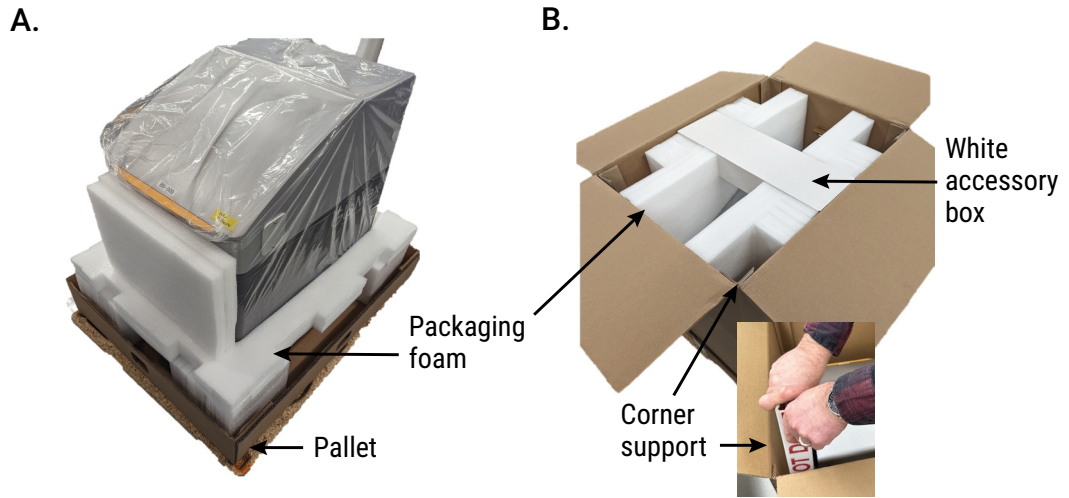


Figure 48. Panel A. Packaging the imager. **Panel B.** Adding accessory box and corner supports.

3. Replace the upper box sleeve to the imager box.
4. Add the imager and PC/monitor boxes onto the pallet and secure with the provided tether cords (Figure 49).



Figure 49. Securing the packaged imager, PC and monitor on the pallet.

11 Related Products

PRODUCT	SIZE	CAT.#
PC, keyboard and mouse, GloMax® Galaxy	1 each	GM4001
Monitor, GloMax® Galaxy	1 each	GM4002
Stagetop Incubator/Controller, GloMax® Galaxy	1 each	GM4010
Microplate Holder Insert, GloMax® Galaxy	1 each	GM4020
Petri Dish Holder Insert, GloMax® Galaxy	1 each	GM4021
1-Position Slide Holder Insert, GloMax® Galaxy	1 each	GM4022
UV 375/20nm Fluorescence Module, GloMax® Galaxy	1 each	GM4030
Green 540/25nm Fluorescence Module, GloMax® Galaxy	1 each	GM4034
Green 560/40nm Fluorescence Module, GloMax® Galaxy	1 each	GM4032
Amber 620/60nm Fluorescence Module, GloMax® Galaxy	1 each	GM4033
Blue 480/30nm Fluorescence Module, GloMax® Galaxy	1 each	GM4031
Filter, 460nm/50bp, 25mm, Galaxy	1 each	GM4011
Filter, 535nm/40bp, 25mm, Galaxy	1 each	GM4012
Filter, 635nm/60bp, 25mm, Galaxy	1 each	GM4013
Filter, 605nm/55bp, 25mm, Galaxy	1 each	GM4014
Filter, 700nm/75bp, 25mm, Galaxy	1 each	GM4015
Filter, 600lp, 25mm	1 each	GM4016
Filter Holder with Retaining Ring, Empty	1 each	GM4017
Side Access Port with Rubber Insert	1 each	GM4018
8-Well Micro-Chamber Vessels	15/pack	GM4061

Custom emission filters for luminescence, BRET, FRET and fluorescence may be obtained from Semrock (www.semrock.com) or Chroma Technology Corporation (www.chroma.com). Filters must be a maximum of 5mm thick and have a diameter of 25mm. Consult the manufacturer's documentation for filter orientation.

Note: Installing custom emission filters requires a separate emission Filter Holder with Retaining Ring (Cat.# GM4017), and Thorlabs Spanner Wrench for SM1-Threaded Retaining Rings (Thorlabs Cat.# SPW602).

Contact Promega Technical Services (techserv@promega.com) to inquire about custom excitation modules.

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All prices and specifications are subject to change without notice.

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