Azure Sapphire™ FL Biomolecular Imager

User Manual

Safety and Regulatory Compliance

Radiation

Radiation hazard prevention

The Sapphire FL Biomolecular Imager is not equipped with any radioisotope or radiation generating unit, and is therefore not regulated by radiation hazard prevention laws. However, the Sapphire FL is capable of scanning storage phosphor screens, which may be polluted by radioisotopes.

CAUTION: **If radioisotope (RI) pollution occurs, stop use of the instrument immediately and follow the instructions of your radiation administrator.**

Laser Safety

WARNING: Making adjustments or carrying out procedures not specified in this manual can result in harmful exposure to laser radiation.

Azure Sapphire FL Biomolecular Imager is a Class I laser instrument that houses up to three Class IIIB lasers inside the instrument.

Under the specified operating procedures, the instrument does not allow operator exposure to laser light. The lasers, with power of 5–25mW, are accessible in the interior of the instrument.

General Information

Azure Sapphire FL Biomolecular Imagers include a laser illumination system that provides up to three narrow band excitation wavelengths at a time for fluorescent applications. The imaging systems are certified to comply with CE, cTUVus and CB Scheme compliance standards. This laser system is, by its appropriate classification and definition, a non-removable laser system as it is not operable when the optical modules are removed from the system.

Safety Features

Azure Sapphire FL Biomolecular Imagers are designed to prevent direct and collateral human exposure to radiation by means of a safety switch on the front side of the imager. The switch reacts to “lid open” and “lid closed” states and defeats all power to internal light sources when the lid is in the “open” position. Lasers and other internal system light sources will not power on unless the lid is fully closed. If the lid is opened during imaging, all light sources will immediately power off to prevent human exposure to internal illumination sources. In addition, the entire laser system is fully enclosed within the system enclosure and there are no viewing ports, windows, or openings to facilitate viewing of, or exposure to, radiation fields from direct impact, reflection, or leakage.

Maintenance

Azure Sapphire FL Biomolecular Imagers do not require regular, periodic, or preventative maintenance in the form of adjustments, calibrations, or other standard maintenance procedures to maintain optimal performance, thereby removing the need for users or their service technicians to initiate any actions where exposure to laser radiation could occur.

Serviceability

Replacement of faulty optical modules is a manufacturer-only repair action and not a customer-service action. Laser repair or replacement may be performed in the field by Azure Biosystems authorized service technicians, or by return of the faulty optical module(s) and/or the entire system to Azure Biosystems, or its authorized service location(s) for laser repair or replacement. Lasers are deemed to be faulty or defective if users discover images that show evidence of output signal level loss, a significant difference between the output signal levels between optical modules, or complete loss of output signal level in an optical module. Users or their service technicians should make no attempt to determine the cause of faulty laser operation, and should promptly contact Azure Biosystems at support@azurebiosystems.com or their nearest Azure Biosystems authorized service location.

Caution

The safety switch in the Sapphire FL is designed to prevent exposure to laser radiation. If the lid is opened while the scanner is in operation, the laser safety switch will cut power to the lasers. Exposure to laser radiation can be harmful, and viewing laser light directly can cause blindness.

Azure Sapphire FL Biomolecular Imagers contain a defeatable safety switch system. It is not recommended or advised by Azure Biosystems, under any circumstances, for users to defeat the safety system and perform laser imaging, or imaging with any light source, with the lid open. The lid must be fully closed.

Electrical Safety Precautions

Be sure to take proper precautions when handling any electrical equipment. NEVER work on any live circuit, fixture, receptacle, or switch. Safety rules to follow whenever working with any electrical appliance include:

- Always shut off power at the main disconnect before changing a fuse.
- Always shut off power to the circuit before repairing or replacing a switch, receptacle, or fixture.
- Always tape over the main switch, empty fuse socket, or circuit breaker you are working on.
- Always check that the circuit is dead before beginning work on it. Using a circuit tester or voltmeter can help confirm this.
- Always unplug any appliance before repairing it.

Protective earth terminal

The ground terminal, intended for connection to an external protective conductor for protection against electric shock in case of a fault, is located on the inside back panel.

For Research Use Only

This instrument is suitable for research use only. It must be used, therefore, only by personnel who know the health risks associated with the reagents that are normally used with this instrument.

Warranty

Azure Sapphire FL products are warrantied against defects in materials and workmanship for one year unless otherwise outlined on the sales order or www.azurebiosystems.com/warranty. If any defect occurs in the instrument during this warranty period, Azure Biosystems, Inc. will repair or replace the defective parts at its discretion without charge. The following defects, however, are specifically excluded:

- Defects caused by improper operation, including removing or replacing laser modules while the system is powered on (also referred to as “hot swapping”).
- Repair or modification done by anyone other than Azure Biosystems or the company’s authorized agent.
- Use of spare parts supplied by anyone other than Azure Biosystems.
- Damage caused by accident or misuse.
- Damage caused by disaster.
- Corrosion caused by improper solvents or samples.

Voltage Setting Information

Azure Sapphire FL Biomolecular Imager has a power supply that automatically chooses the correct voltage for your country or region.

Certificates of Conformity

CE Conformity

Azure Sapphire FL Biomolecular Imager is in conformity with the provisions of the following CE Directives, including all amendments, and national legislation implementing these directives:

- EMC Directive 2014/35/EU

And that the following harmonized standards have been applied:

- EN61010-1: 2010
- EN60825-1

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Legal

Sapphire FL is protected by one or more U.S. and/or foreign patents listed at www.azurebiosystems.com/patents.

Table of Contents

1. Introduction

The Azure Sapphire FL Biomolecular Imager is a next generation laser scanning system that provides exceptional data quality through extremely sensitive detection, ultra-high resolution, and broad linear dynamic range.

The Sapphire FL allows for advanced flexibility in fluorescent and phosphor imaging. The instrument is capable of hosting up to three optical modules at one time, each of which can be easily swapped by the end user.

1.1—Table of Specifications

Base System	Sapphire FL Biomolecular Imager	Laser based scanning system
IS4000	Scanning area	25cm x 25cm
	Scanning modes	Simultaneous, Sequential, Extended Dynamic Range (EDR)
	Resolution	5µm – 1000µm
	Image output	16 bit Tiff
	EDR output	24 bit data
	Maximum scanning speed	250mm/s
	Animal imaging	Compatible with commercially available anesthesia systems
	Dimensions	593mm(L) x 630mm(W) x 399mm(H)
	Weight	43.5kg (empty of optical modules; each optical module weighs 0.6 kg)
	Power requirements	100 – 240 VAC ± 10%, 50/60 Hz
	Computer options	Windows laptop computer (IS2011) or Windows Desktop computer (IS2012)
	Sample types	Membranes, plates, slides, gels, phosphor screens, small animals, and more

Part number	Name	Laser Diode Emission Wavelength	Emission Filter	Detector (APD or PMT)
Standard Optical Modules				
IS4001	488 Standard Optical Module	488nm	513/17nm	photomultiplier tube (PMT)
IS4002	532 Standard Optical Module	532nm	572/28nm	avalanche photodiode (APD)
IS4003	638 Standard Optical Module	638nm	676/37nm	avalanche photodiode (APD)
IS4004	685 Standard Optical Module	685nm	720/24nm	avalanche photodiode (APD)
IS4005	784 Standard Optical Module	784nm	829/62nm	avalanche photodiode (APD)
IS4006	Phosphor Imaging Standard Optical Module	638nm	424nm/SP	photomultiplier tube (PMT)
Custom Optical Modules				
IS4030	375 Custom Optical Module	375nm	452/45nm	photomultiplier tube (PMT)
IS4031	450 Custom Optical Module	450nm	494/34nm	photomultiplier tube (PMT)
IS4032	488 (YFP) Custom Optical Module	488nm	534/20nm	photomultiplier tube (PMT)
IS4033	532 (Propidium) Custom Optical Module	532nm	624/40nm	avalanche photodiode (ADP)
	Additional custom optical modules available upon request			

Part number	Name	Description
Standalone Laser Options (Does not include emission filter)		
IS4023	Sapphire FL 375nm Laser	375nm Laser Module
IS4024	Sapphire FL 450nm Laser	450nm Laser Module
IS4025	Sapphire FL 488nm Laser	488nm Laser Module
IS4026	Sapphire FL 532nm Laser	532nm Laser Module
IS4027	Sapphire FL 638nm Laser	638nm Laser Module
IS4028	Sapphire FL 685nm Laser	685nm Laser Module
IS4029	Sapphire FL 784nm Laser	784nm Laser Module
	Custom laser options available upon request	
Standalone Emission Filter Options (Does not include laser module)		
IS4008	Sapphire FL 452nm Filter	452/45 nm emission filter
IS4009	Sapphire FL 676nm Filter	676/37nm emission filter
IS4010	Sapphire FL 534nm Filter	534/20nm emission filter
IS4011	Sapphire FL 494nm Filter	494/34 nm emission filter
IS4012	Sapphire FL 513nm Filter	513/17nm emission filter
IS4013	Sapphire FL 624nm Filter	624/40nm emission filter
IS4046	Sapphire FL 720nm Filter	720/24nm emission filter
IS4047	Sapphire FL 829nm Filter	829/62nm emission filter
IS4049	Sapphire FL 572nm Filter	572/28nm emission filter
	Custom emission filter options available upon request	
User-Design Modules		
	Contact Azure Biosystems for user-selected laser and filter wavelengths	
Accessories	Name	Description
IS1015	Sapphire Eraser	Designed to erase signal from phosphor imaging screens

2. Installation and Setup

2.1—System Placement

Warning! Excessive Weight Hazard – Please use two or more people to lift the system. Failure to do so can result in system damage and personal injury.

As with all electrical instruments, the Azure Sapphire FL Biomolecular Imager should be located away from water, solvents, or corrosive materials, on a flat and stable surface with adequate clearance on all sides. The system must remain stationary during operation. Furthermore, the system should be placed away from interfering electrical signals and magnetic fields. If possible, a dedicated electrical outlet should be used to eliminate electrical interference from other instrumentation in your laboratory. The Sapphire FL should be installed at no more than 3000 meters above sea level.

2.2—Connecting to Power

The power entry module is located in the lower corner on the back panel of the system. Connect the power cord to a secure power outlet.

It is important to connect the system to a well-grounded power source.

2.3—Power On/Off the System

To turn on the system

1. Switch on the system master power by flipping the power switch on the back panel of the instrument to the on (|) position.
2. Once the system power is on, wait for the light panels to stop flashing green. When the light panels stop flashing and turn off, the system is ready to turn on.
3. Press and hold the power button on the front left of the instrument for 5 seconds. The light panels will turn solid green, indicating that the system is now ready to use.

To turn off the system

1. Press and hold the power button on the front of the system for 5 seconds to put the system in standby mode. The light panel will turn off, but the power button will remain solid green, indicating that the master power is still on.
2. Turn off master power to the system using the power switch on the back of the instrument.

Azure Biosystems recommends leaving the system master power turned on via the switch on the back panel, as this switch may not be easily accessible.

2.4—Software Installation

The Azure Sapphire FL Capture Software is pre-installed. To launch the software, double click on the desktop icon. The system will take a few seconds to initialize.

Once the system initializes, the software opens to Imaging mode when properly connected to the instrument, and is ready to use.

2.5—Connecting Other USB Devices to the System

The external computer is connected to the Sapphire FL system via Ethernet.

You may attach regulatory approved, Windows OS supported USB keyboard, USB mouse, or other USB input devices to any of the unused USB ports that remain on the external PC.

3. Proper Usage – Please Read Before Use

DO	DO NOT
Shut down the instrument power via the power button on the front of the instrument and the Sapphire FL Capture Software prior to removing or replacing laser modules.	Attempt to remove or replace laser modules while the instrument is powered on (also known as “hot swapping”). Doing so can result in irreparable damage to the laser modules and/or the instrument itself, and will void the warranty.
Wipe the imaging surface with a microfiber cloth. For persistent contamination of the glass surface, contact Azure Biosystems Technical Support.	Wipe the surface with anything else. Many detergents and cleaning chemicals have fluorescent properties which will interfere with the scanning of fluorescent blots. Using non-microfiber cloths may also lead to contamination.
Leave the system master power on at the back.	Attempt to access the back switch, unless it is readily accessible.
Keep the system computer connected on a table nearby.	Put the system computer on top of the scanning apparatus. The heat generated by the PC may damage the unit.
Keep the top of the instrument clear.	Put anything on top of the instrument. The Sapphire FL was not designed to withstand forces acting down on top of the imager.
Remove your samples from the imager when finished.	Leave your samples in the imager. Many samples can contain hazardous chemicals, such as ethidium bromide or radiolabeled proteins, that can cause damage or distress to the next samples or users.
Leave your system where it is installed.	Move your Sapphire FL. The Sapphire FL weighs 43.5kg (when empty of optical modules; each optical module weighs 0.6 kg) and is not designed to be moved. Moving the Sapphire FL without the express permission of Azure Biosystems may void warranty or service contracts covering the imager.
Close the lid while imaging.	Leave the lid open while imaging. A safety mechanism disarms the lasers and prevents them from turning on when the lid is not properly shut.
Call Azure Biosystems, or email support@azurebiosystems, in the event of an issue with the instrument.	Attempt to fix the instrument yourself, as some of the components may be hazardous.

3.1—Changing Optical Modules

- Begin by opening the Sapphire FL Capture Software while connected to the instrument.
- Click “Change Laser Modules” in the bottom left corner of the Imaging module in the Capture software.
- The system will issue a prompt indicating that the scan head will be moved to the front of the instrument. Click “Start” to proceed.
- Once the scan head has homed to the front of the instrument, the system will issue a second prompt to initiate closing the software. Click “Close” to proceed.
- Hold down the power button on the front left of the instrument for 5 seconds to power off the instrument.
- **Important: NEVER change optical modules while the system power is on! Doing so will corrupt the optical modules and potentially the entire system.**
- With the system powered off, open the lid. Use the Front Panel Access Key (located in the anesthesia cabinet) to open the front panel and access the optical module ports.
- If all ports are full, remove the desired optical modules from the instrument as follows:
  - Using fingers, unscrew the optical module from its port using the knob located at the top of the laser module.
  - Once unscrewed, slide the optical module out of the port. Store unused optical modules in Optical Module Storage Containers. Be sure to screw the optical module into the storage container the same way it is secured into the port to prevent it from sliding out.
- Once the intended port(s) on the instrument are available, insert the newly selected optical module into a compatible port. Reference the key on the inside front panel to confirm optical module and port compatibility.
- Insert the optical module into the port. Using fingers, screw the optical module into the port using the knob located at the top of the optical module. The connection should be finger-tight.
- Close the front panel.
- Power on the instrument by holding the power button on the front left of the instrument for 5 seconds. The light panels will turn green, indicating that the system is now ready to use.
- Launch the Sapphire FL Capture Software. A prompt will require the user to input the filter information currently associated with any newly loaded optical modules. Click “Ok” when finished selecting filter information.
- The key in the bottom left corner of the software will update to display the newly loaded laser module and filter information. Newly loaded optical modules will now be available for use.

3.2—Changing Filters

- The emission filters in the optical modules are changeable by the user outside of the Sapphire FL system.
- To change an emission filter, grip the filter by the handle and pull straight out from the laser module while outside of the instrument. Note the orientation in which the filter fits into the laser module, as filters only fit into laser modules in one orientation.
- Insert the desired emission filter into the filter slot in the laser module. The filter and slot are orientation-specific and will only fit together one way. Make sure the filter is tightly clicked into place before using the optical module.
- Replace the optical module into the Sapphire FL system as specified above in the Changing Optical Modules section.
- Filters that are not in use may be stored in the back of the Optical Module Storage Containers.

3.3—Imaging Living Animals

- The Sapphire FL is compatible with commercially available small animal anesthesia equipment.
- The anesthesia cabinet is located on the top of the instrument, behind the lid hinges. Push down on the front of the cabinet lid to open the cabinet and access the anesthesia port.
- Attach anesthesia tubing to the anesthesia port by pushing the tubing onto the nozzle.
- The five anesthesia ports are located behind the glass scanning surface and are visible when the lid is open. When not in use, ports are closed with a screw-top cover. To access the anesthesia port nozzles, remove the covers by using the back of the Front Panel Access Key as a screwdriver. Keep ports that are not in use covered.
- Attach anesthesia tubing to the anesthesia port(s) by pushing the tubing onto the nozzle(s).

4. Sapphire Capture Software Overview

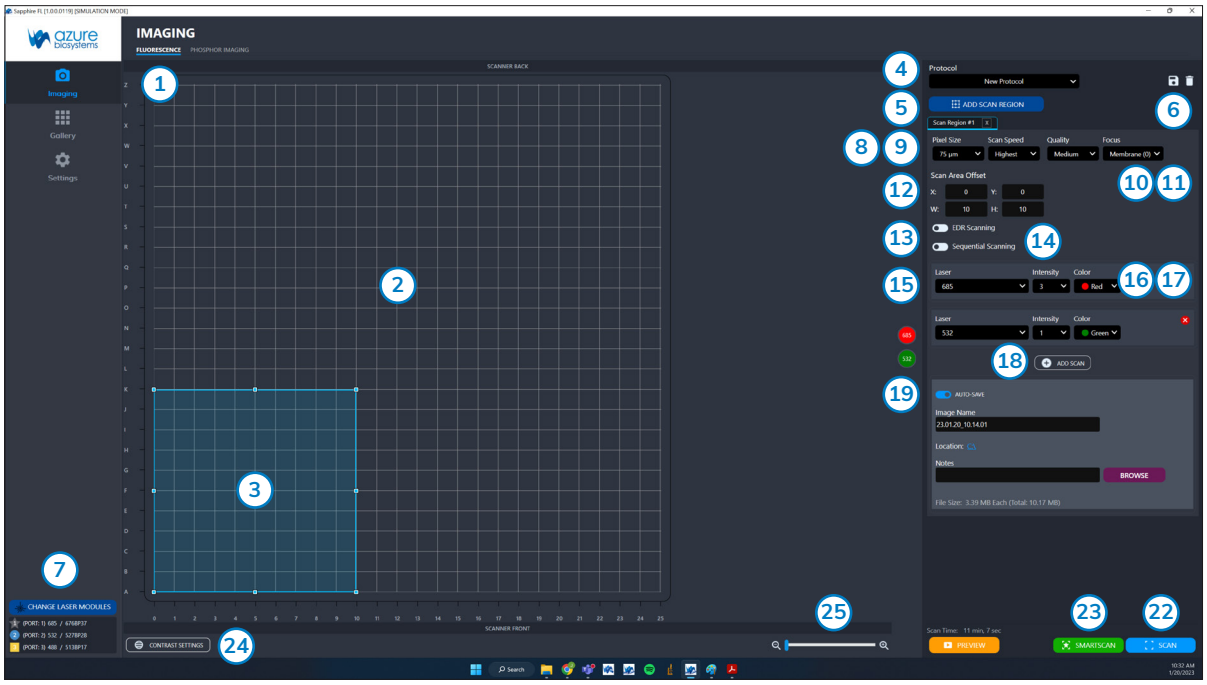
4.1—Launch the Sapphire Capture Software

Double click the Sapphire FL desktop icon to launch the Sapphire FL Capture Software.

When the computer is connected to the Sapphire FL, the software will open in Imaging mode. Opening the software when not connected to the Sapphire FL will prompt a message indicating that the scanner is not connected. Click “OK” to move to Gallery mode. Imaging mode is not accessible when the scanner is disconnected.

4.2—Fluorescence Imaging

The Fluorescence module utilizes lasers and PMT or APD detectors to scan fluorescent samples.



1. **FLUORESCENCE** – Select the FLUORESCENCE tab at the top of the imaging section.
2. **IMAGING AREA** – The Imaging Area represents the glass scanning surface of the Sapphire FL with each square in the Imaging Area grid representing one square centimeter. Use the squares on the scan area outline to select the area of the imaging screen covered by your sample. Note that the letter and number coordinates surrounding the glass scanning surface correspond to the same letters and numbers in the Imaging Area selection grid. Ensure the sample is contained wholly within the designated imaging area.

   Note: Any portions of sample that fall outside the imaging area will not be captured in the final image.

   Note: Scanning time is impacted by increases in the Y-axis (A-Z labeled axis). To decrease scanning time, orient the longest portion of samples along the X-axis and the shortest portion along the Y-axis.
3. **SCAN REGION BOX** – Move and resize the Scan Region box to select the region of the imaging area to be scanned. Each scan region has its own scan region box. Resize scan region boxes by dragging the corners or by entering coordinates in the Scan Area Offset fields (see point 12).
4. **PROTOCOL** – Several scanning protocols are preset within the software for easy imaging set up. Use the Protocol drop down menu to select from these preset methods, select a previously saved imaging protocol, or to set up a new protocol.

5. **ADD SCAN REGION** – Add a second, adjustable scan region box to the imaging area. The new scan region will have its own, dedicated settings (including optical modules, pixel size, focus, etc.). When all settings are set for all scan areas, select Scan to initiate scanning of all scan regions in order. For example, the system will complete Scan Region #1 before beginning Scan Region #2.
6. **SAVE PROTOCOL** – Save a scanning method to the Protocol drop down to easily access frequently used settings. Saved protocols can also be deleted.
7. **PORT KEY** – This non-editable area displays which optical modules are currently loaded in each Port in the scanner. The Port Icons in the Key match the Port Icons on the instrument itself. Filter information is selected at the time of loading the optical module into the system and can be edited by clicking the Laser and Filter Pairing button in the Settings module.
8. **PIXEL SIZE** – Adjust the scanning resolution by selecting the pixel size. A smaller pixel size will result in a higher resolution image. Smaller pixel sizes allow for more detailed capture and analysis but take longer to acquire.
9. **SCAN SPEED** – Selects the speed at which the scan head travels. For most applications, including phosphor imaging, Highest scan speed is recommended.

   Note: Slower scan speeds to do not increase the signal measured.
10. **FOCUS** – Changing the Focus adjusts the focal plane of the scan. Selecting Custom Focus will bring up a slider that allows adjustment of the focal plane from -1.0mm below the glass to 6.0mm above the glass. Once the optimal focal plane of a sample is determined through the Custom function, it is recommended that it be added to the list of available Focus heights in the Settings module so that it can be applied to future scans, if desired.

Focus	Focal Plane
Membrane 0mm	On the glass (0mm)
Gel +0.50mm	0.50mm above the glass
Plate +3.00mm	3.00mm above the glass

Focus	Focal Plane
Slide +1.00mm	1.00mm above the glass
Custom	Adjustable
Z-Scan	Adjustable

11. **Z-SCAN (option in Focus drop down menu)** – Choose multiple focal planes per scan to create a Z stack of images.
12. **SCAN AREA OFFSET** – Displays the coordinates of the selected scan region. Coordinates may be adjusted to specify the scan region.
13. **EDR SCANNING** – Extended Dynamic Range Scanning. Selecting this setting allows for imaging with a broader dynamic range than usual. This setting increases the scan time.
14. **SEQUENTIAL SCAN** – By default, the Sapphire FL scans all channels simultaneously. Turning on Sequential Scanning scans each channel individually rather than simultaneously.

    Note: With Sequential Scanning, the estimated Scan Time is per channel.
15. **LASER** – the Laser menu allows selection of each optical module currently loaded in the instrument for scanning. Note that the standard Phosphor optical module is not accessible in this menu and may only be selected through the PHOSPHOR IMAGING tab. Eligible optical modules will appear in the drop down menu for selection.
16. **INTENSITY** – Adjust the power of each laser by selecting the appropriate Intensity level, with L1 being the lowest power and 10 being the highest. A higher laser intensity will increase the sensitivity and allow for better detection of lower concentrations of sample, but too high of an intensity may cause saturation and increased background and may bleach fluorophores more quickly.
17. **COLOR** – Choose which color to represent each Laser wavelength. Note that coloring does not affect image capture or data.

18. **ADD SCAN** – Add additional channels to the protocol. To remove an unwanted channel, click the X located on the top right corner of the Laser box.
19. **AUTO-SAVE** – Choose whether or not to have images auto-save upon scan completion. Enter a file name in the Image Name field (default name is YY.MM.DD_TT.TT.TT, where time is HH.MM.SS), and select or create a folder for images to be saved in by selecting the Browse button. Clicking on the blue link next to Location will take you to the current active save folder. Any comments entered in the Notes field will save with the file upon scan completion. The save window will minimize when Auto-Save is turned off.
20. **FILE SIZE** – The estimated image file size based on the current selected parameters (Scan Area, Pixel Size, Scan Speed, EDR, and Sequential Scanning all affect File Size) will appear below the Auto-Save window. File Size is listed as the size for each individual channel with the total file size listed in parenthesis.
21. **PREVIEW** – Select PREVIEW to initiate a low resolution, unsaved scan. Preview scans are useful in determining correct sample orientation and positioning, scan region, and intensity settings.
22. **SCAN** – Select SCAN to initiate a scan using the set parameters. The estimated scan completion time for the area and parameters selected will be displayed next to Scan Time.
23. **SMARTSCAN** – SmartScan automatically determines the optimal intensity level for a sample prior to scanning. To use SmartScan, set all scan parameters and scanning area (Intensity does not need to be set.) and click SmartScan. The system will determine and set the proper Intensity level and perform a final scan according to set parameters.
24. **CONTRAST SETTINGS** – Select Contrast Settings to open the PREVIEW CONTRASTING window. The currently engaged lasers are indicated at the top of the window by circle buttons with the laser wavelengths inside. The color of each circle matches the color selected for the corresponding laser. Clicking on these buttons will activate or deactivate the preview image for that channel.

    Use the sliders under Contrast to adjust the contrast of the preview image. Contrast can be adjusted for each individual channel or for the preview image as a whole.

    Note: Adjusting the contrast only adjusts how the preview image is visualized. It does not affect the final data or image.
25. **ZOOM** – Use the slider or the (+) and (-) buttons to zoom in or out on the Imaging Area. This is useful for small area scans and viewing the image preview of specific areas of the scan.

4.2.1—Scanning a Fluorescent Membrane

1. Place your membrane, **sample side down**, on the glass scanning surface. Make sure that the sample is wholly contained within the imaging grid (A 01 – Z 25) and that the glass surface is clean and free of debris.
2. Azure recommends placing the imaging mat black side down on top of your membrane to keep it flush against the glass imaging surface.
3. Click the FLUORESCENCE tab in the capture software to enter the fluorescence imaging module.
4. Select the area you would like to scan by dragging the corners of the scan region box over the area covered by your membrane. The estimated scanning time will appear above the Preview button.
5. Select the desired Protocol from the drop down menu or select New Protocol to create a protocol.
6. Choose the desired resolution and scan speed. Set the Focus to Membrane. This sets the focal plane to the level of the glass scanning surface.
7. If creating a New Protocol, use the drop down menus to select the optical module(s) you are using along with the color you would like each signal to appear in the image. Add additional optical modules to any method by clicking the (+) Add Scan button.

8. Change the laser intensity to your preference for the sample you are imaging. Intensity ranges from L1 (lowest; use this setting to scan strong signals) to 10 (highest; use this setting to scan weak signals).

   Note: *Use the PREVIEW function to initiate a quick, low resolution scan to help determine the best Intensity level to use OR select SMARTSCAN after all parameters are set (except intensity) to have the system determine the laser intensities before automatically performing the final scan. Because SmartScan ignores the selected intensity setting to instead determine the optimal intensity of the scan, the Intensity menu can be set to any number before selecting SmartScan without affecting the actual intensity of the scan.*

9. Select "EDR Scan" (to take an image with Extended Dynamic Range) and/or "Sequential Scan" (to activate the lasers sequentially rather than simultaneously) if desired.

   Note: *Selecting EDR Scan will disable the laser Intensity drop down menu(s), as the software will calculate the required intensities without user input for this setting.*

10. Use the Auto-Save switch to turn Auto-Save ON or OFF. If Auto-Save is ON, use the Browse button to select where to save the auto-saved images.
11. Enter or edit the file name under Image Name. By default, the image will save as YY.MM.DD_TT.TT.TT where time is HH.MM.SS.
12. Select PREVIEW to initiate a quick, low resolution scan that can be useful in determining the correct sample positioning, scanning area, and intensity setting.
13. Select SCAN to initiate scanning at the set parameters, OR select SMARTSCAN to have the system determine the laser intensities and scan at the set parameters.

    Note: *Selecting SMARTSCAN tells the software to choose the appropriate laser intensities. The current intensity settings will be ignored by the software. The other scan parameters (such as resolution, focus, etc.) must still be set by the user.*

14. A preview image will begin to appear in the selected scan region as the scan commences. Use the channel buttons to the right of the capture window to select which channels are visible in the preview image.
15. During scanning, visualization of the preview image may be adjusted by clicking on the Contrast Settings button. This will open a Channels window that will allow you to select which channels to preview and adjust contrast.

    Note: *Adjusting the contrast through this window only affects visualization of the captured image. It does not affect captured data.*

16. Upon completion of the scan, the software will automatically navigate to the Gallery module. Images will appear according to the color chosen for each channel. Multiplexed scans will appear as a single image and can be separated into individual signal channels for analysis, if desired.

4.2.2—Scanning a Fluorescent Gel

1. Place your gel on the glass scanning surface. Make sure that the sample is wholly contained within the imaging grid (A 0 – Z 25).
2. Azure recommends placing a Background Quenching Sheet on top of the gel to create a flat, black background. Using the imaging mat on top of gels is not recommended.
3. Click the FLUORESCENCE tab in the capture software to enter the fluorescence imaging module.
4. Select the area you would like to scan by dragging the corners of the scan region box over the area covered by your gel. The estimated scanning time will appear above the Preview button.
5. Select the desired Protocol from the drop down menu or select New Protocol to create a new protocol.

6. Choose the desired resolution and scan speed. Set the Focus to Gel. This sets the focal plane to 0.5mm above the glass scanning surface. This focal plane is ideal for gels of approximately 1.0mm thickness.

   Note: *For thicker gels or to set a different focal plane, select Custom from the Focus menu and use the slider to set the focal plane. If you are unsure of your gel's thickness, scan your gel at several different focal planes using the Focus setting Z-Scan and setting the Z-Scan Focus Setup parameters as desired. From the resulting images, choose the custom focus height that results in the highest signal and sharpest image.*

7. If creating a New Protocol, use the drop down menus to select the laser module(s) you are using along with the color you would like each signal to appear in the image. Add additional laser modules to any Protocol by clicking the (+) Add Scan button.

8. Change the laser Intensity to your preference for the sample you are imaging. Intensity ranges from L1 (lowest; use this setting to scan strong signals) to 10 (highest; use this setting to scan weak signals).

   Note: *Use the PREVIEW function to initiate a quick, low resolution scan to help determine the best Intensity level to use OR select SMARTSCAN after all parameters are set (except intensity) to have the system determine the laser intensities before automatically performing the final scan. Because SmartScan ignores the selected intensity setting to instead determine the optimal intensity of the scan, the Intensity menu can be set to any number before selecting SmartScan.*

9. Use the Auto-Save switch to turn Auto-Save ON or OFF. If Auto-Save is ON, use the Browse button to select where to save the auto-saved images.

10. Enter or edit the file name under Image Name. By default, the image will save as YY.MM.DD_TT.TT.TT where time is HH.MM.SS.

11. Select PREVIEW to initiate a quick, low resolution scan that can be useful in determining the correct sample positioning, scanning area, and intensity setting.

12. Select SCAN to initiate scanning at the set parameters, OR select SMARTSCAN to have the system determine the laser intensities and scan at the set parameters.

    Note: *Selecting SMARTSCAN tells the software to choose the appropriate laser intensities. The current intensity settings will be ignored by the software. The other scan parameters (such as resolution, focus, etc.) must still be set by the user.*

13. A preview image will begin to appear in the selected scan region as the scan commences. Use the channel buttons to the right of the capture window to select which channels are visible in the preview image.

14. During scanning, visualization of the preview image may be adjusted by clicking on the Contrast Settings button. This will open a Channels window that will allow you to select which channels to preview and adjust contrast.

    Note: *Adjusting the contrast through this window only affects visualization of the captured image. It does not affect captured data.*

15. Upon completion of the scan, the software will automatically navigate to the Gallery module. Images will appear according to the color chosen for each channel. Multiplexed scans will appear as a single image and can be separated into individual signal channels for for analysis, if desired.

4.2.3—Scanning a Fluorescent Slide

1. Place your slide sample **face down** (coverslip towards the glass scanning surface) within a slide holder to raise the slide slightly above the glass scanning surface. Make sure that the sample is wholly contained within the imaging grid (A 0 – Z 25).

   Note: *It is important that the slide(s) be placed in a slide holder and not directly on the glass surface. Failure to do so may result in Newton's rings interference in the image. Use the Z-Scan Focus function to determine the optimal scanning height of your slide in the slide holder. Once the optimal Focus is determined, use the Custom option to set this Focus for future scans of the same type.*

2. Azure recommends placing a Background Quenching Sheet on top of the slide to create a flat, black background. Using the imaging mat on top of slides is not recommended.
3. Click the FLUORESCENCE tab in the capture software to enter the fluorescence imaging module.
4. Select the area you would like to scan by dragging the corners of the scan region box over the area covered by your slide. The estimated scanning time will appear above the Preview button.
5. Select the desired Protocol from the drop down menu or select New Protocol to create a new protocol.
6. Choose the desired resolution and scan speed. Set the Focus to Slide. This sets the focal plane to 1mm above the glass scanning surface.

   Note: *For slides of a thickness other than 1mm, use the Custom Focus to select the appropriate focal plane. For best results, measure the height of your slide holder and use Custom Focus to set the correct focal plane. If you are unsure of the correct distance, scan your slide at several different focal planes using the Focus setting Z-Scan and setting the Z-Scan Focus Setup parameters as desired. From the resulting images, choose the custom focus height that results in the highest signal and sharpest image.*
7. If creating a New Protocol, use the drop down menus to select the laser wavelength(s) you are using along with the color you would like each signal to appear in the preview image. Add additional lasers to any Protocol by clicking the (+) Add Scan button.
8. Change the laser Intensity to your preference for the sample you are imaging. Intensity ranges from L1 (lowest; use this setting to scan strong signals) to 10 (highest; use this setting to scan weak signals).

   Note: *Use the PREVIEW function to initiate a quick, low resolution scan to help determine the best Intensity level to use OR select SMARTSCAN after all parameters are set (except intensity) to have the system determine the laser intensities before automatically performing the final scan.*
9. Use the Auto-Save switch to turn Auto-Save ON or OFF. If Auto-Save is ON, use the Browse button to select where to save the auto-saved images.
10. Enter or edit the file name under Image Name. By default, the image will save as YY.MM.DD_TT.TT.TT where time is HH.MM.SS.
11. Select PREVIEW to initiate a quick, low resolution scan that can be useful in determining the correct sample positioning, scanning area, and intensity setting.
12. Select SCAN to initiate scanning at the set parameters, OR select SMARTSCAN to have the system determine the laser intensities and scan at the set parameters.

    Note: *Selecting SMARTSCAN tells the software to choose the appropriate laser intensities. The current intensity settings will be ignored by the software. The other scan parameters (such as resolution, focus, etc.) must still be set by the user.*
13. A preview image will begin to appear in the selected scan region as the scan commences. Use the channel buttons to the right of the capture window to select which channels are visible in the preview image.
14. During scanning, visualization of the preview image may be adjusted by clicking on the Contrast Settings button. This will open a Channels window that will allow you to select which channels to preview and adjust contrast.

    Note: *Adjusting the contrast through this window only affects visualization of the captured image. It does not affect captured data.*
15. Upon completion of the scan, the software will automatically navigate to the Gallery module. Images will appear according to the color chosen for each channel. Multiplexed scans will appear as a single image and can be separated into individual signal channels for analysis, if desired.

4.2.4—Scanning a Fluorescent, Plate-Based Assay

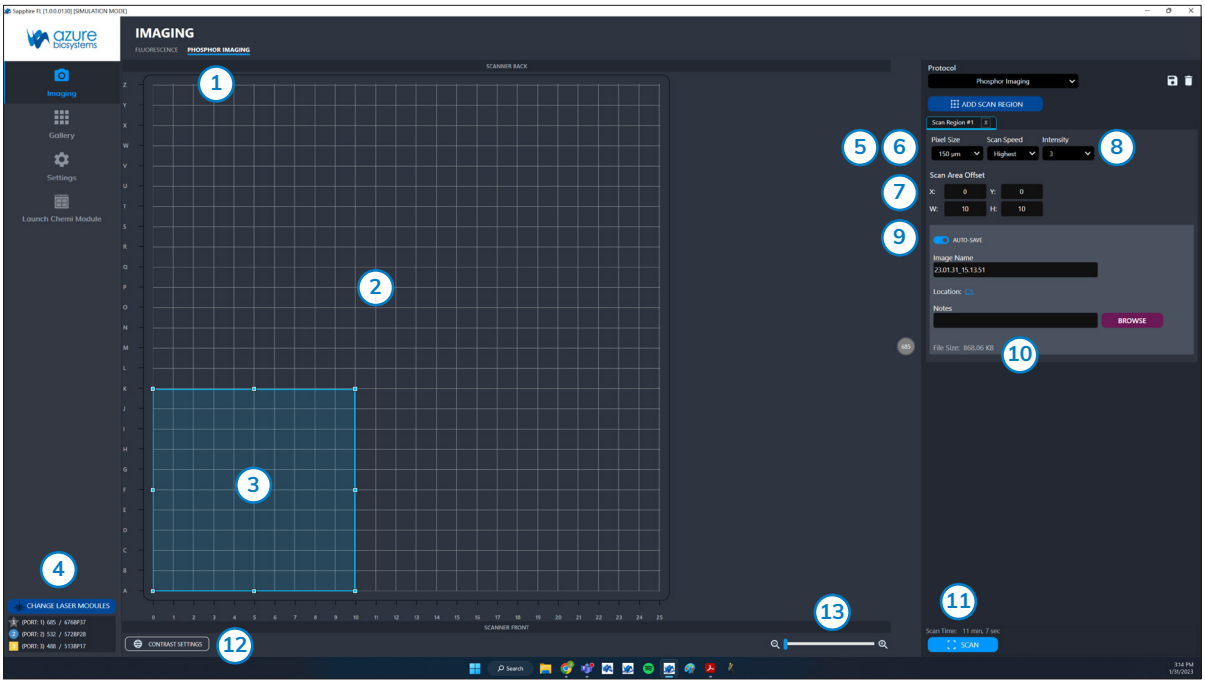
1. Place your plate (with optically transparent bottom) directly on the glass scanning surface with the wells facing up so that the bottom of the plate will be scanned. Make sure that the sample is wholly contained within the imaging grid (A 0 – Z 25).
2. Azure recommends placing a Background Quenching Sheet on top of the plate to create a flat, black background. Using the imaging mat on top of plates is not recommended.
3. Click the FLUORESCENCE tab in the capture software to enter the fluorescence imaging module.
4. Select the area you would like to scan by dragging the corners of the scan region box over the area covered by your slide. The estimated scanning time will appear at the bottom of the capture window.
5. Select the desired Protocol from the drop down menu or select New Protocol to create a protocol.
6. Choose the desired resolution and scan speed. Set the Sample Type to Plate. This sets the focal plane to 3mm above the glass scanning surface.

   Note: *For most standard 96-well plates, 3mm is the distance between the bottom of the skirt and the bottom of the wells. For best results, measure this distance for your individual plate type and use Custom Focus to set the correct focal plane. If you are unsure of the correct distance, scan your plate at several different focal planes using the Focus setting Z-Scan and setting the Z-Scan Focus Setup parameters as desired. From the resulting images, choose the custom focus height that results in the highest signal and sharpest image.*
7. If creating a New Protocol, use the drop down menus to select the dye you are using along with the color you would like each signal to appear in the preview image. Add additional dyes to any method by clicking the (+) Add Scan button.
8. Change the laser intensity to your preference for the sample you are imaging. Intensity ranges from L1 (lowest; use this setting to scan strong signals) to 10 (highest; use this setting to scan weak signals).

   Note: *Use the PREVIEW function to initiate a quick, low resolution scan to help determine the best Intensity level to use OR select SMARTSCAN after all parameters are set (except intensity) to have the system determine the scan intensity before automatically performing the final scan.*
9. Use the Auto-save switch to turn Auto-Save ON or OFF. If Auto-Save is ON, use the Browse button to select where to save the auto-saved images.
10. Enter or edit the file name under Image Name. By default, the image will save as YY.MM.DD_TT.TT.TT where time is HH.MM.SS.
11. Select PREVIEW to initiate a quick, low resolution scan that can be useful in determining the correct sample positioning, scanning area, and intensity setting.
12. Select SCAN to initiate scanning at the set parameters, OR select SMARTSCAN to have the system determine the scan intensity and scan at the set parameters.

    Note: *Selecting SMARTSCAN tells the software to choose the appropriate laser intensities. The current intensity settings will be ignored by the software. The other scan parameters (such as resolution, focus, etc.) must still be set by the user.*
13. A preview image will begin to appear in the selected scan region as the scan commences. Use the channel buttons to the right of the capture window to select which channels are visible in the preview image.
14. During scanning, visualization of the preview image may be adjusted by clicking on the Contrast Settings button. This will open a Channels window that will allow you to select which channels to preview and adjust contrast.

    Note: *Adjusting the contrast through this window only affects visualization of the captured image. It does not affect captured data.*
15. Upon completion of the scan, the software will automatically navigate to the Gallery module. Images will appear according to the color chosen for each channel. Multiplexed scans will appear as a single image and can be separated into individual signal channels for analysis, if desired.



4.3—Phosphor Imaging

The Phosphor Imaging module utilizes a laser and PMT detector to capture the release of energy from storage phosphor screens.

1. **PHOSPHOR IMAGING** – Select the PHOSPHOR IMAGING tab at the top of the imaging section.
2. **IMAGING AREA** – The Imaging Area represents the glass scanning surface of the Sapphire FL with each square in the Imaging Area grid representing one square centimeter. Use the squares on the scan area outline to select the area of the imaging grid covered by your sample. Note that the letter and number coordinates surrounding the glass scanning surface correspond to the same letters and numbers in the Imaging Area selection grid. Ensure the sample is contained wholly within the designated imaging area.

   Note: Any portions of sample that fall outside the imaging area will not be captured in the final image.

   Note: Scanning time is impacted by increases in the Y-axis (A-Z labeled axis). To decrease scanning time, orient the longest portion of samples along the X-axis and the shortest portion along the Y-axis.
3. **SCAN REGION BOX** – Move and resize the Scan Region box to select the region of the imaging area to be scanned. Each scan region has its own scan region box. Resize scan region boxes by dragging the corners or by entering coordinates in the Scan Area Offset fields (see point 7).
4. **PORT KEY** – This displays which optical modules are currently loaded in each Port in the scanner. The Port Icons in the Key match the Port Icons on the instrument itself. Filter information is selected at the time of loading the optical module into the system and can be edited by clicking the Laser and Filter Pairing button in the Settings module.
5. **PIXEL SIZE** – Adjust the scanning resolution by selecting the pixel size. A smaller pixel size will result in a higher resolution image. Smaller pixel sizes allow for more detailed capture and analysis but will take a longer time to acquire.
6. **SCAN SPEED** – Selects the speed at which the scanning head travels. For most applications, including phosphor imaging, Highest scan speed is recommended.

   Note: Slower scan speeds to do not increase the signal measured.
7. **SCAN AREA OFFSET** – Displays the coordinates of the selected scan region. Coordinates may be adjusted to specify the scan area.

8. **INTENSITY** – Adjust the power of each laser by selecting the appropriate Intensity level with L1 being the lowest power and 10 being the highest. A higher laser intensity will increase the sensitivity and allow for better detection of lower concentrations of sample, but too high of an intensity may cause saturation and increased background and may bleach your sample more quickly.
9. **AUTO-SAVE** – Choose whether or not to have images auto-save upon scan completion. Enter an file name in the Image Name field (default name is YY.MM.DD_TT.TT.TT where time is HH.MM.SS), and select or create a folder for images to be saved in by selecting the Browse button. Clicking on the blue link next to Location will take you to the current active save folder. Any comments entered in the Notes field will save with the file upon scanning completion. The save window will minimize when Auto-Save is turned off.
10. **FILE SIZE** – The estimated image file size based on the current selected parameters (Scan Area, Pixel Size, and Scan Speed all affect File Size) will appear below the Auto-Save window.
11. **SCAN** – Select SCAN to initiate a scan using the set parameters. The estimated scan completion time for the area and parameters selected will be displayed next to Scan Time.
12. **CONTRAST SETTINGS** – Select Contrast Settings to open the PREVIEW CONTRASTING window. The currently engaged laser is indicated at the top of the window by a gray circle button with the laser wavelength inside. Clicking on this button will activate or inactivate the preview image for that channel. Use the sliders under Contrast to adjust the contrast of the preview image.

    Note: *Adjusting the contrast only adjusts how the preview image is visualized. It does not affect the final data or image.*
13. **ZOOM** – Use the slider or the (+) and (-) buttons to zoom in or out on the Imaging Area. This is useful for small area scans and viewing the image preview of specific areas of the scan.

4.3.1—Scanning a Storage Phosphor Screen

1. Place your unmounted phosphor screen, signal side down, on the glass scanning surface. Make sure that the sample is wholly contained within the imaging grid (A 0 – Z 25).
2. Click the PHOSPHOR IMAGING tab in the capture software to enter the phosphor imaging module.
3. Choose the desired resolution and scan speed. Set the Focus to Membrane. This sets the focal plane to the level of the glass scanning surface.

    Note: *Because scanning of a storage phosphor-screen entails the release of energy and thus signal from the screen, it is recommended to scan at the Highest Scan Speed.*
4. Change the laser intensity to your preference for the sample you are imaging. Intensity ranges from 1 (lowest; use this setting to scan strong signals) to 5 (highest; use this setting to scan weak signals).
5. Use the Auto-Save switch to turn Auto-Save ON or OFF. If Auto-Save is ON, use the Browse button to select where to save the auto saved images.
6. Enter or edit the file name under Image Name. By default, the image will save as YY.MM.DD_TT.TT.TT where time is HH.MM.SS.
7. Select Scan to initiate scanning at the selected parameters.
8. A preview image will begin to appear in the selected scan region as the scan commences. Use the channel buttons to the right of the capture window to select which channels are visible in the preview image.
9. During scanning, visualization of the preview image may be adjusted by clicking on the Contrast Settings button. This will open a Channels window that will allow you to preview and adjust contrast.

    Note: *Adjusting the contrast through this window only affects visualization of the captured image. It does not affect captured data.*
10. Upon completion of the scan, the software will automatically navigate to the Gallery module.

4.4—The Image Gallery

As images are acquired, they open in the Gallery module. The Gallery module is designed for opening, closing, saving, and/or printing your images. It also allows you to perform basic image editing on open images.

1. Select the Gallery module on the left side of the screen.

   If no image is open, use File > Open to select an image to open and view. Multiple images may be open simultaneously.

2. Use the Black, White and Gamma sliders to adjust the contrast of your selected image.

3. Images can also be Inverted, Rotated and Resized. See the guide below for a complete description of each option.

1. **FILE** – Here you can save your captured images, open previous images, or print images.

   1.1. **OPEN** – Use this icon to open an image that is stored locally, on a USB, or on the network (for systems that are networked).

   1.2. **CLOSE** – This will close the currently selected image in the Gallery. Be sure to save images before closing.

   1.3. **CLOSE ALL** – This will close all currently open images in the Gallery. Be sure to save desired images before closing.

   1.4. **SAVE** – Allows you to save your image to a USB, to the computer, or to a network drive. Images can be saved as a TIFF or JPEG file. File names are automatically generated with a date and time stamp, but you can override the automatically generated name by clicking the displayed textbox.

   1.5. **SAVE AS** – Allows you to save a copy of an image with an alternate name, file type, or to an alternate file location.

   1.6. **SAVE ALL** – Allows you to save all unsaved images currently open in the Gallery tab.

   Note: Any previously saved images will be skipped by the Save All function. To re-save a previously saved imaged, select Save As.

   1.7. **PRINT** – Allows you to print to a printer connected to the computer (if applicable), or to a printer on the network.

1.8. **PRINT REPORT** – Print an image report including the currently selected image along with its Image Info.

2. **TOOLS MENU** – Here you can find tools to use on your open images.

2.1. **SCALE BAR** - Selecting Scale Bar will open the Scale Bar window. Select the parameters you would like to use for your scale bar, then click "OK" to add the scale bar to your currently selected image.

2.2. **ANIMATED GIF** - Selecting Animated GIF will open the GIF Animation window. Select Source Images for a GIF from images that are currently open in the Gallery. Click "Create Animated GIF" after setting the desired parameters to create a GIF of the selected source images.

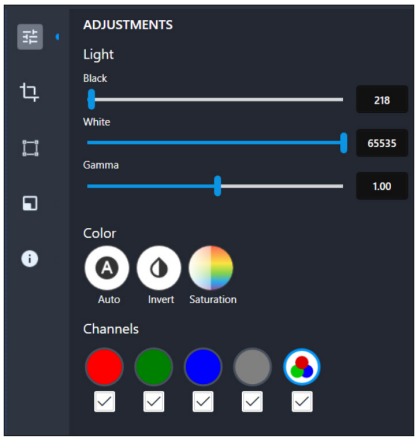
2.3. **CREATE Z-STACK IMAGE**- Selecting Create Z-Stack Image will open the Z Stacking window. Select Source Images for the desired composite image from images that are currently open in the Gallery. Images must be of the same resolution and size to be combined into a Z-Stack Image. Only single channel images may be combined into a Z-Stack Image. Select the desired Projection Type from the pull-down menu, then click Create Z-Stack Image to create the composite image.

3. **CREATE MULTIPLEX** – Overlay multiple, compatible single channel images to create a single, multiplex image. Selecting the Create Multiplex button opens the Merge Channels window. Select the image you would like to assign to each color from the drop-down lists. If merging fewer than four images, leave unused colors blank or select None as the source image. Check Keep Source Images to keep source images in the Gallery after images are merged.

4. **CREATE SIGNAL CHANNEL** – Separate out the different channels of a multichannel image to look at the data from each channel individually, in greyscale. Images will be tagged with the appropriate channel in the file name.

5. **ZOOM** – Use the (+) and (-) buttons to zoom in and out on the selected image.

6. **PIXEL VALUES** – Hover of an area of the selected image to view pixel positions and intensity values.



7. **ADJUSTMENTS MENU**

7.1. **BLACK, WHITE, AND GAMMA SLIDERS** - Use these sliders to manually change the contrast of the currently selected image.

Note: Adjusting these settings will not affect the raw data in your image when saved as a TIFF image. Only images saved as JPEG keep the contrast settings. Azure recommends using TIFF if the image needs to be quantified.

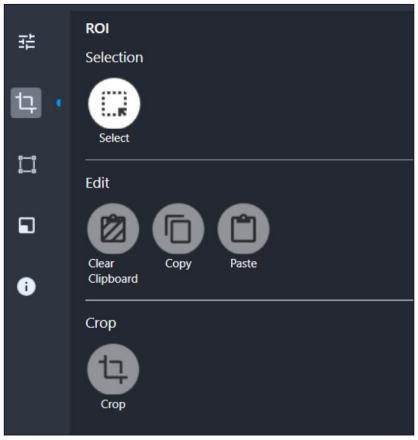
7.2. **AUTO** – Auto contrast will contrast your image to see the most features in the image.

Note: For best results, Azure recommends cropping the image to the area of the sample before auto contrasting.

7.3. **INVERT** – Invert will display your image with dark and light pixels reversed. Invert also inverts the data values, so when an image is opened in another image editing application such as Photoshop or PowerPoint, the image appears the same as in the Sapphire FL Capture Software. Other than inverting the display, this does not change the data in any way.

7.4. **SATURATION** – Selecting Saturation will highlight pixels with intensities beyond the dynamic range of the detector. In colored images, saturated pixels will appear pale pink. In greyscale images, saturated pixels will appear red.

7.5. **CHANNELS** – When a multicolor image is displayed in the Gallery, you will have the option of viewing single channels, or up to four channels at once. By default, all channels are displayed. Contrasting the image will contrast all channels. To view and contrast a single channel independent of the other channels, select the button that corresponds to the channel you wish to view. To go back to the multichannel image, select the button with the three overlapping colored circles. Alternatively, deselect the checkbox associated with a channel to keep that channel in view but disable it from changes made to contrast.



8. ROI MENU

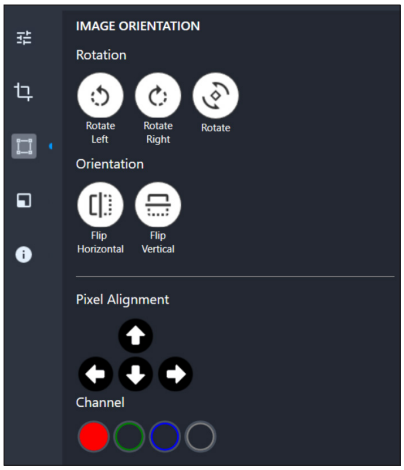
8.1. **SELECT** - Generates a box that you can resize to select an area of the captured image to copy or crop.

8.2. **CLEAR CLIPBOARD** – removes temporarily saved images from the clipboard.

8.3. **COPY** – Copy the whole image, or a selected area of the image, such as a marker or ladder, to the clipboard.

8.4. **PASTE** – Paste the selected part of the image onto another image, creating a third, new image.

8.5. **CROP** – Crop an image to get rid of excess boarders and background, crop a molecular marker or ladder, or to take a closer look at relevant parts of the image.



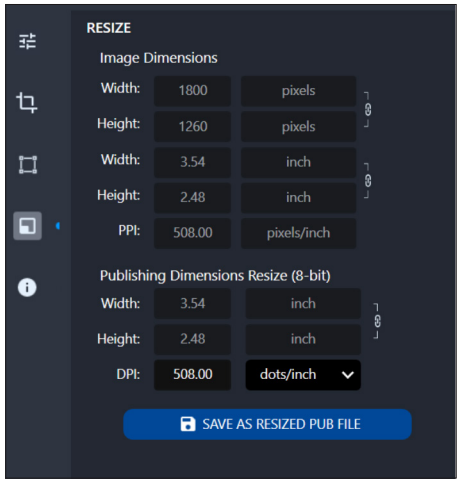
9. IMAGE ORIENTATION MENU

9.1. **ROTATE LEFT, ROTATE RIGHT** - Selecting either of these buttons will rotate the selected image 90° in the designated direction.

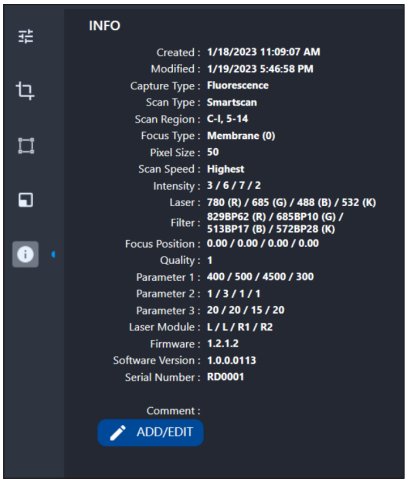
9.2. **ROTATE** – Use the slider to manually rotate the selected image to the desired orientation. Click APPLY to apply the rotation settings to the image.

9.3. **FLIP HORIZONTAL, FLIP VERTICAL** – selecting either of these buttons will flip the selected image in the designated direction.

9.4. **PIXEL ALIGNMENT** – Use the channel buttons to select the single channel you want to align in a multichannel image. Use the arrow buttons to manually adjust the alignment of the selected channel relative to the others.



10. RESIZE MENU – Selecting the resize icon will generate additional editing options. Use this feature to adjust the DPI of captured images without altering the raw data.



11. INFO MENU – Metadata for the selected image is displayed under this menu. Scan parameters used to acquire the image, including Scan Type, Focus, Pixel Size, Scan Speed, Optical Modules, and Intensity settings, are displayed here.

5. Chemiluminescent Imaging and the Sapphire FL Chemiluminescence Module

The Sapphire FL can be equipped with a Chemiluminescence Module. Each Sapphire FL Chemiluminescence Module includes the following key components:

- Body – The body is a light-tight imaging station, containing the camera and lens, mirrors, electrical components, Ethernet, Wi-Fi emitter, and USB ports for data collection and external computer control.
- Stage – The imaging stage is a user-accessible, transparent platform for sample placement just beneath the inner lid, which contains the LED light sources.
- Camera – High resolution 6.29MPx camera, preinstalled in the system.
- LEDs – The LED module allows for visible imaging, utilized for chemi marker images and visible stained gel images in both color or grayscale.
- USB Ports – There are two USB ports for data transfer, both located at the back of the instrument.

5.1—Table of Specifications

Image Resolution	6.29M pixels
Maximum Field of View	10.0 x 15.0cm (4.0 x 5.9in)
Image Output	16-bit tiff, 8-bit jpg
Working Environment	Ambient temperature: 10–30 °C Humidity: up to 80%
Power Requirement	100–240VAC, 1A
Dimensions (W x H x D)	29.2 x 22.2 x 43.2cm (11.5 x 8.75 x 17.0in)
Weight	9.1kg (20.0lbs)

5.2—Chemiluminescence Module Software

The Sapphire FL Chemiluminescence Module uses a unique, web-based capture software that connects through a Wi-Fi signal to the Sapphire FL and is accessible through the main Sapphire FL Capture Software. Click the “Launch Chemi Module” button on the main Imaging tab to launch the web-based software.

Wi-Fi Connection

If your system is equipped with the Sapphire FL Chemiluminescence Module, the module will be connected to the Sapphire FL via wifi during system installation.

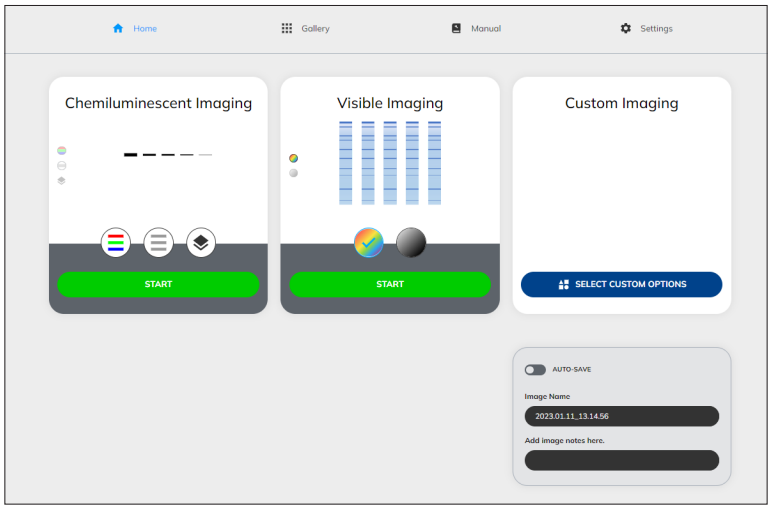
5.3—Image Capture Overview

The Sapphire FL Chemiluminescence Module captures high quality images with an intuitive user interface. Select “Launch Chemi Module” from the left menu of the Sapphire FL Capture Software to launch the Chemiluminescence Module web-based capture interface. The Home Screen expedites the imaging process by providing quick access to the most commonly-used functions. For users that require more specific settings, the Custom Imaging menu also allows for a comprehensive breakdown of the Sapphire FL Chemiluminescence Module’s functionalities.

5.3.1—Home Screen Overview

The Home Screen contains the following imaging options:

- **Chemiluminescent Imaging** – for samples with luminescent and chemiluminescent signals, such as ECL-HRP. See section 5.3.2 for additional information.
- **Visible Imaging** – for samples with visible stains and signals such as Coomassie dye in protein gels, film, or colorimetric-dyed blots. See section 5.3.3 for additional information.
- **Custom Imaging** – for full customization of imaging options. See section 5.3.4 for additional information.
- **Auto-Save** – automatically save images once they are taken. See section 5.3.5 for additional information.

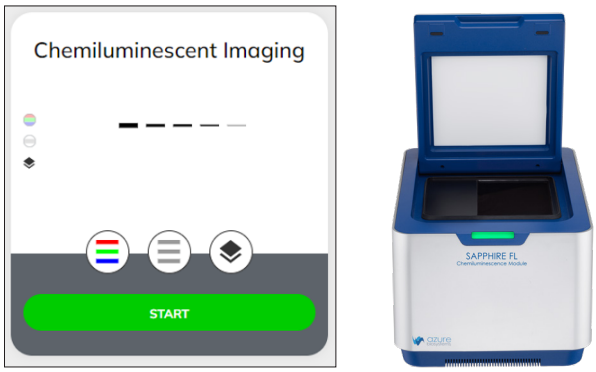


Along the top, the Navigation Bar displays access to the following options:

Button	Button Name	Function
Home	Home	The Home button can be selected at any time to return back to the Home Screen.
Gallery	Gallery	Access the Gallery to view and edit images. See Section 5.4 for details.
Manual	Manual	Instructions to access this User Manual.
Settings	Settings	Access the Settings menu to adjust Sapphire FL Chemiluminescence Module settings. See Section 5.5 for details.

5.3.2—Chemiluminescent Imaging

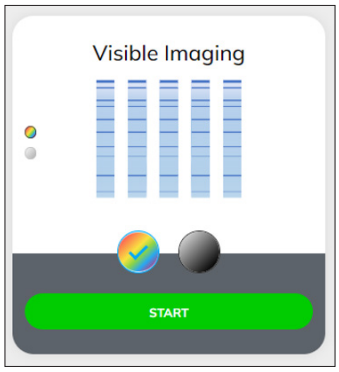
Use this function with samples that have luminescent and chemiluminescent signals such as ECL-HRP.



1. Place sample face-down on the imaging stage.
2. Fully close the Sapphire FL Chemiluminescence Module lid.
3. From the Home Screen, select the desired quick access option(s) in the Chemiluminescent Imaging section. If none of the following options are selected, the Sapphire FL Chemiluminescence Module will take a single chemi image with no marker when START is selected:
    a. **Color Marker** – Select the Color Marker icon to take a true color image in addition to a chemiluminescent image. This function cannot be used at the same time as the Grayscale Marker function.
    b. **Grayscale Marker** – Select the Grayscale Marker icon to take a grayscale image in addition to a chemiluminescent image. This function cannot be used at the same time as the Color Marker function.
    c. **Cumulative Imaging** – Select Cumulative Imaging to take a series of cumulative chemiluminescent images. The number of images can be specified in the General tab of Settings under Chemi Image Mode, with the default setting being two images. This function can be used with either marker function active or with neither active.
4. Select START to begin chemiluminescent image acquisition with an automatically-determined exposure time and 3x3 pixel binning.
    a. If different settings are desired, refer to the Custom Options menu in Section 5.3.4.
5. After capture, the image(s) will appear in the Gallery automatically. The Gallery can also be reached by selecting the Gallery icon.

5.3.3—Visible Imaging

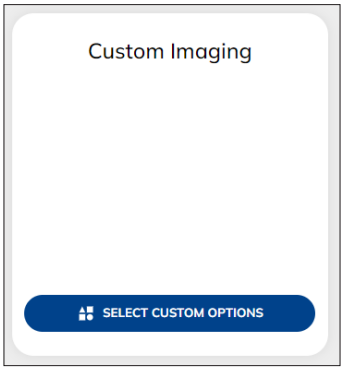
Use this function for samples with visible stains and signals such as Coomassie-dyed protein gels, film, or colorimetric-dyed blots.

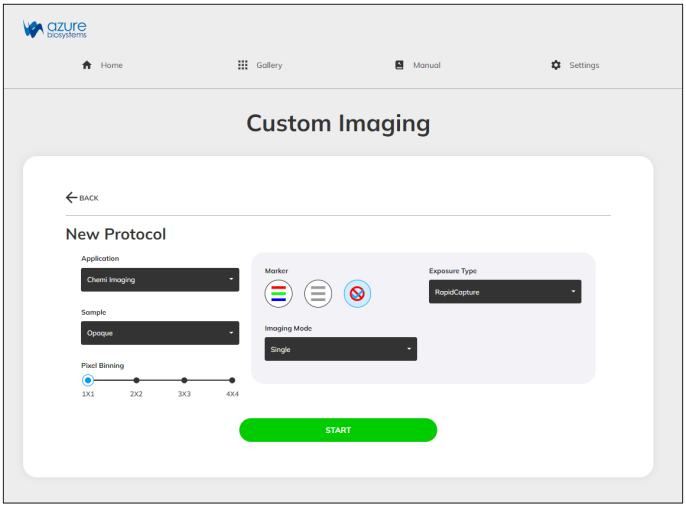


1. Place sample face-down on the imaging stage.
2. Fully close the Sapphire FL Chemiluminescence Module lid.
3. From the Home Screen, select either:
    a. **Color Imaging** – The Sapphire FL Chemiluminescence Module will merge a composite image using individual red, blue, and green images for a full color image.
    b. **Grayscale Imaging** – The Sapphire FL Chemiluminescence Module will take a single image using white light for a black-and-white image.
4. Select START to begin visible image acquisition with automatically-determined exposure time(s) and 1x1 pixel binning.
    a. If different settings are desired, refer to the Custom Options menu in Section 5.3.4.
5. After capture, the image will appear in the Gallery automatically. The Gallery can also be reached at any time by selecting the Gallery icon.

5.3.4—Custom Imaging

The Custom Imaging menu gives access to the Custom Options menu, which allows for full user customization of image acquisition.



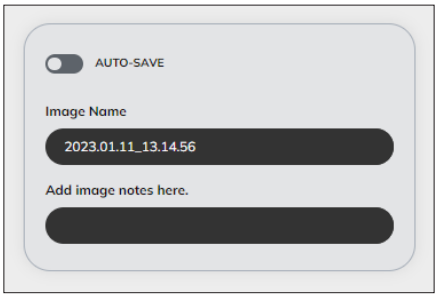


1. Place sample face-down on the imaging stage.
2. Fully close the Sapphire FL Chemiluminescence Module lid.
3. From the Home Screen, select the “Select Custom Options” button to access the Custom Imaging menu.

4. Specify the desired settings in each of the following fields:
    a. **Application** – Choose between Chemi Imaging, True Color Imaging, or Grayscale Imaging
    b. **Sample** – Specify whether the sample is Opaque, Translucent, or select Auto-Detect for the Sapphire FL Chemiluminescence Module to determine sample opacity.
    c. **Pixel Binning** – Select the desired pixel binning between 1x1 (unbinned), 2x2, 3x3, or 4x4 binning.
5. If Chemi Imaging was selected under Application, specify the following Chemi settings as well:
    a. **Marker** – Choose between True Color Marker, Grayscale Marker, or No Marker.
    b. **Imaging Mode** – Choose between Single, Cumulative, or Multiple. These only apply to the chemiluminescent image; if a marker is selected, it will only be taken once.
    c. **Exposure Type** – Choose between RapidCapture, Extended Dynamic Range, Overexposure, or Manual.
    d. **Manual Exposure Time** – If Manual is selected under Exposure Type, input a specific exposure time
6. Select START to begin image acquisition according to the specified settings.
7. After capture, the image(s) will appear in the Gallery automatically. The Gallery can also be reached by selecting the Gallery icon.

5.3.5—Auto-Save

When Auto-Save is enabled, images will be automatically saved in a zip file as both 16-bit tiffs and 8-bit jpgs with the user-specified names and notes.



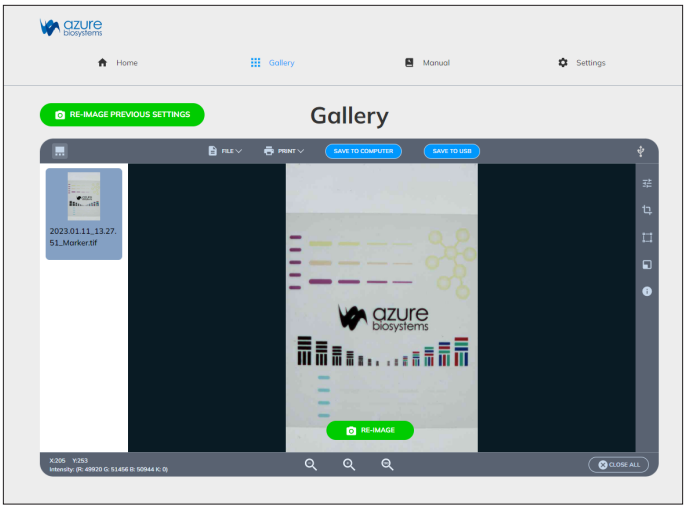
1. Enable auto-save by toggling it on the Home Screen.
2. *Optional: Specify the image name and image notes that will be automatically saved with the next image.*
3. Take an image as usual (see Sections 5.3.2, 5.3.3, and 5.3.4).
4. Each captured image will be automatically downloaded in a zip file containing the image as both a 16-bit tiff and an 8-bit jpg.

5.4—Image Gallery Overview

The Gallery allows for image viewing, editing, and information.

5.4.1—Gallery

Reach the Gallery by clicking the Gallery icon.

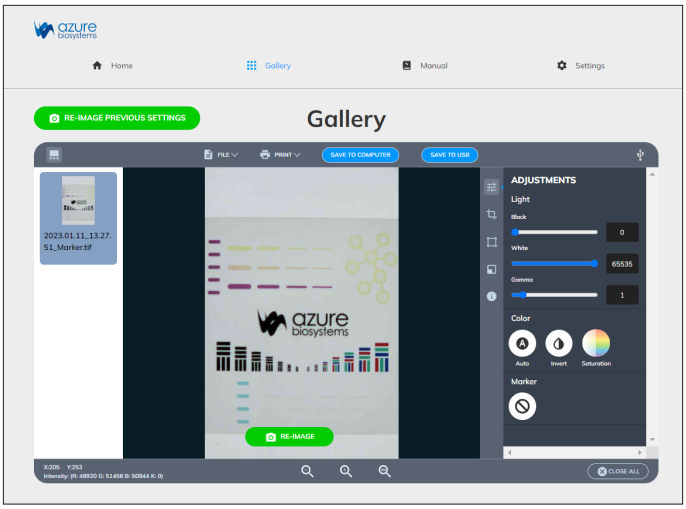


1. The following options are available within the File dropdown menu:
    a. **Open from Sapphire FL Chemiluminescence Module** – Open an image stored on the Sapphire FL Chemiluminescence Module's internal memory.
    b. **Close** – Close the currently selected image.
    c. **Close All** – Close all images currently open in the Gallery.
    d. **Save** – Save the current image directly to the connected device. It will save through the Download pathway of the browser being used.
2. From the Print dropdown menu, you can:
    a. **Print** – Print to a local printer connected to the system, or to a network printer.
    b. **Print Report** – Print the image you're viewing as well as data about the image. These include Exposure type, Bin Level, Gain, Filter, Calibration, Software Version, and System SN.
3. Select the Save to Device button to bring up the following popup:

    a. Select whether to save your image(s) as a 16-bit tiff, 8-bit jpg, or both.
    b. Click SAVE to save the currently-selected image in the specified formats or click SAVE ALL to save all images open in the Sapphire FL Chemiluminescence Module Gallery in the specified formats.

4. Select the Save to USB button to bring up the following popup:
    a. Select whether to save your image(s) as a 16-bit tiff, 8-bit jpg, or both.
    b. Click SAVE to save the currently-selected image in the specified formats or click" SAVE ALL to save all images open in the Sapphire FL Chemiluminescence Module Gallery in the specified formats.
5. Select the Eject USB button to safely eject the connected USB memory drive from the Sapphire FL Chemiluminescence Module.

5.4.2—Image Adjustment Functions



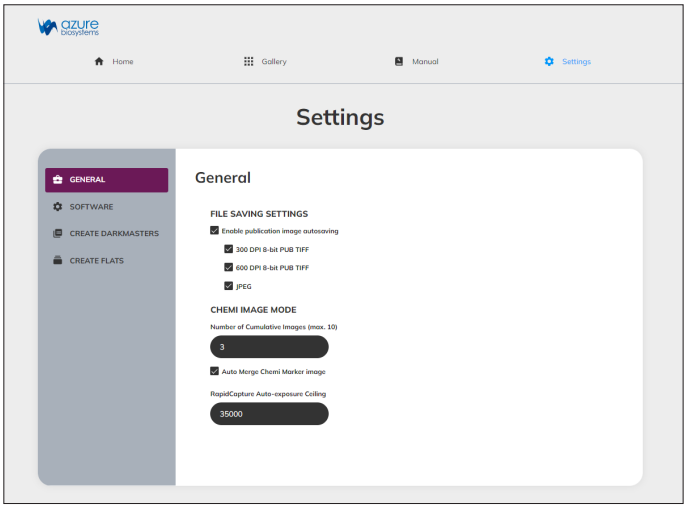
1. **Adjustments**
    a. Adjust the black, white and gamma levels of the image.
    b. Clicking Auto will automatically adjust levels.
    c. Click Invert to invert the colors of the signal and background.
    d. Saturation will highlight in pink which pixels are beyond the detection limit of the camera.
    e. For a chemi image with either color or gray marker, select the Hide Marker button to remove the marker from the display. Deselect the same button to display the marker again.
    f. **Note:** *Adjusting these settings will not affect the raw data when saved as a .tiff file. Only images saved as a .jpg will display with the adjusted contrast settings, as the .jpg file format alters the raw data. Azure recommends using .tiff formats for images that need to be quantified, and saving a copy as a .jpg with contrast adjustments for publication purposes.*
2. **ROI** – Select the region of interest for further actions including Crop and Copy.
    a. Select:
        i. Crop to isolate a particular area of the image. A new tab will open with an image of the selected area.
        ii. Copy to copy a region of interest on a single channel image. After selecting another image of the same size, use the paste option to overlay the copied image.
        iii. Clear clipboard to erase the saved image after cropping or copying so you can select a new region.

3. **Image Orientation** – Rotate or flip images using the options provided. The free rotate function is helpful for samples that were not imaged straight.
4. **Resize** – Customize width, height, and dots per inch (DPI).
5. **Info** – Gives detailed information about the active image. Displays the parameters for image acquisition including date & time, capture type, bin level, software version, and comments.

5.5—Settings Overview

The Settings menu allows for specific customization of the Sapphire FL Chemiluminescence Module imaging and operation settings. It can be reached by clicking the Settings option on the top ribbon.

5.5.1—General



1. **File Saving** – These settings allow you to enable the automatic saving of publication images.

   Note: Publication images are for publication and printing purposes only and should not be used for data analysis.

2. **Chemi Image Mode**
   a. The Number of Cumulative Images setting changes the number of images taken during a Cumulative capture. The default is two images and cannot exceed 10 images.
   b. Check Auto Merge Chemi Marker Image to enable automatic merging of chemi and marker images upon capture.
   c. RapidCapture Auto-exposure Ceiling will calculate an optimal exposure time based on the signal ceiling entered here. The ceiling range is 0 to 65,535 and the default setting is 35,000.

5.5.2—Software

1. **Image Memory** – Show the image storage of the Sapphire FL Chemiluminescence Module's internal memory.
   a. The memory bar visualizes the amount of the Sapphire FL Chemiluminescence Module's internal memory taken up by previously-taken images.

      Note: It is highly recommended to always save images to an external memory source instead of relying on the Sapphire FL Chemiluminescence Module's internal memory.

   b. When the Sapphire FL Chemiluminescence Module's internal memory is around or over 75% full, use the Clear Images button to delete all saved images from unit's internal memory.

c. A prompt will appear to confirm all desired images have been saved to another location. Select Continue to clear all images from the Sapphire FL Chemiluminescence Module's memory or Cancel to prevent the memory clear.

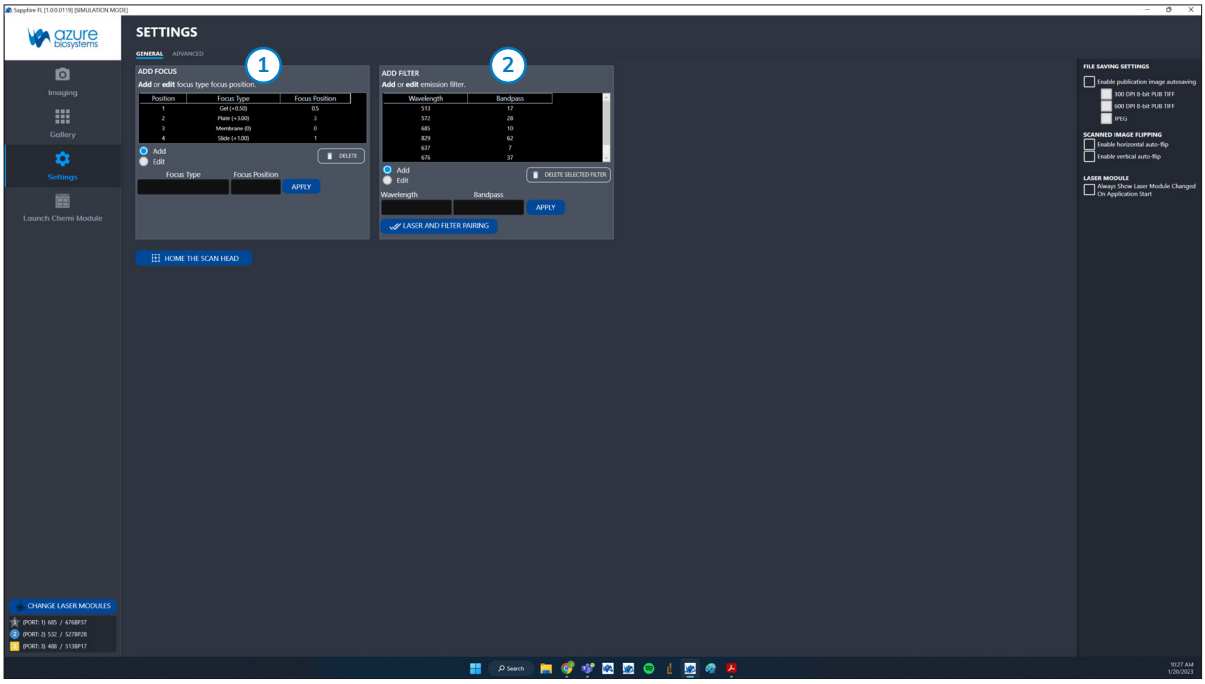
2. **Software Upgrade** – Update the Sapphire FL Chemiluminescence Module's software to a new version.
   a. Download the desired Sapphire FL Chemiluminescence Module software upgrade zip file to your external device (computer recommended) before connecting the device to the Sapphire FL Chemiluminescence Module.
   b. Use the Browse button under the Software Upgrade section to select the Sapphire FL Chemiluminescence Module upgrade zip file **without** unzipping the contents.
   c. Once the upgrade zip file is selected, click Submit to update the Sapphire FL Chemiluminescence Module's internal software.
   d. When the upgrade is complete, the Sapphire FL Chemiluminescence Module will automatically restart itself, indicated by the blue, white, and green light pattern on the unit's front.
   e. Following the restart, reconnect to the Sapphire FL Chemiluminescence Module, open the page, and perform a hard refresh on the web browser by pressing Ctrl+F5 on PC.

      Note: *Once the unit's software has been updated, the Sapphire FL Chemiluminescence Module upgrade file can be removed from the connected device as the Sapphire FL Chemiluminescence Module's software will have been independently updated.*

5.5.3—Create Darkmasters, Create Flats

Create Darkmasters, Create Flats – Darkmasters and Flats are set at the time of manufacture. If you require assistance with any of these settings, please contact Azure Biosystems Technical Support at support@azurebiosystems.com.

6. Settings

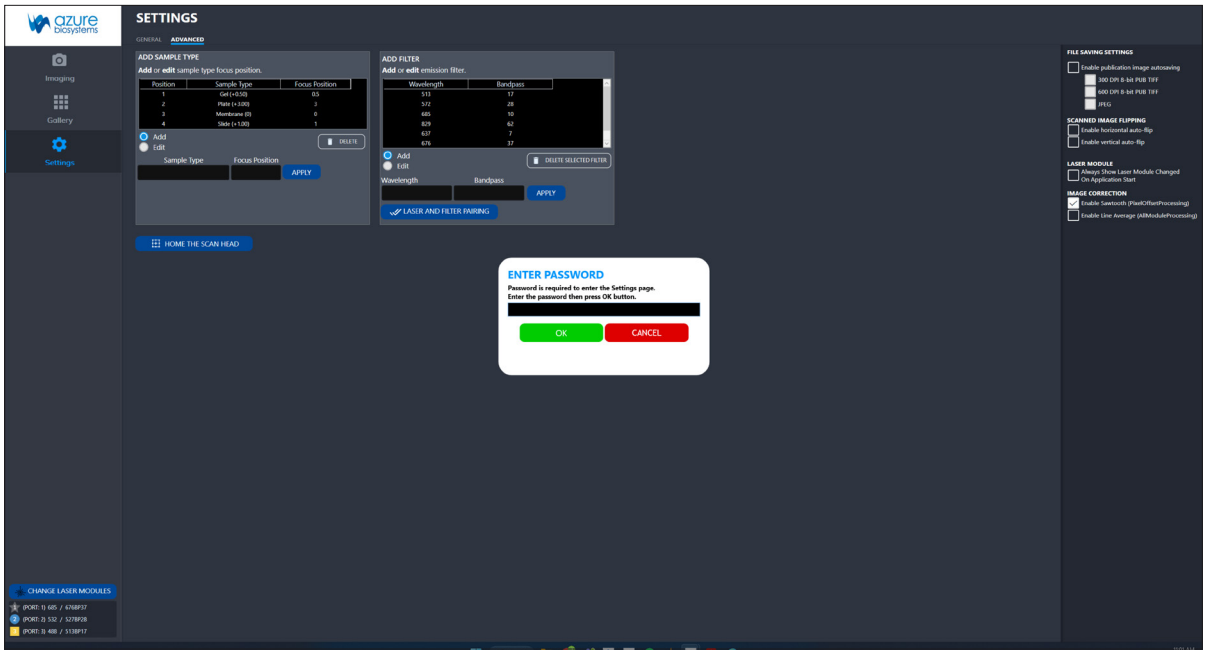


6.1—General Settings

1. **ADD FOCUS** – Use this module to add or edit focus types and to save focus positions determined to be optimal using the Custom Focus setting. To add a Focus Type, select ADD, name the focus type and enter a focus position, then click APPLY. Values indicate mm above or below the glass scanning surface, with 0mm being directly on the glass scanning surface. For example, to add a culture plate with a focus position of 2.75mm above the glass, type in Culture Plate and 2.75 in the appropriate fields. Select APPLY to add the focus type to the drop down list.

   When a Focus that is linked to a Protocol is deleted, the Protocol will default to Custom Focus until a new Focus is assigned to the Protocol.

2. **ADD FILTER** – Use this module to add and delete emission filters from the selection list. To add a filter, type the wavelength and bandpass of the filter into the appropriate fields, then click APPLY. To edit which filter is currently paired with a laser module (as indicated by the CHANGE LASER MODULES Key in the bottom left corner of the software), click LASER AND FILTER PAIRING. Use the menu to reassign currently paired laser modules and filters.



6.2—Advanced Settings

Accessing the Advanced Settings tab will trigger a window prompting you to enter a password. Azure Biosystems does not recommend changing any settings within these tabs, as this can result in software instability or suboptimal imaging parameters. For assistance, please contact support@azurebiosystems.com.

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