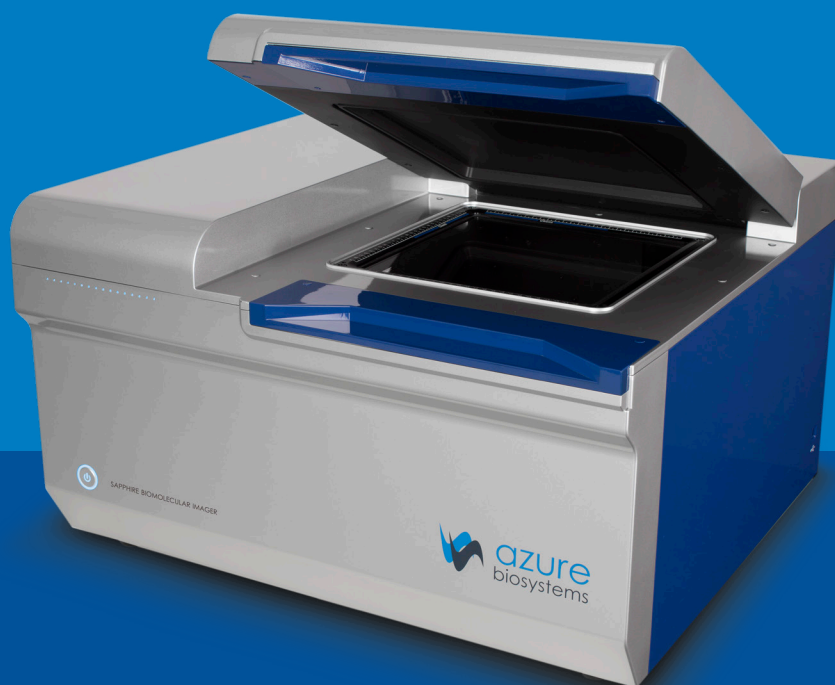




Sapphire™ Biomolecular Imager User Manual



Safety and Regulatory Compliance

Radiation

Radiation hazard prevention

The Sapphire Molecular Imager is not equipped with any radioisotope or radiation generating unit, and is therefore not regulated by radiation hazard prevention laws. However, the Sapphire 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.

The Sapphire Biomolecular Imager is a Class I laser instrument that houses four 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

The Azure Biosystems Sapphire imaging systems include a laser illumination system that provides up to four narrow band excitation wavelengths for fluorescent applications. The imaging systems are certified to comply with CE, cTUVus and CB Scheme compliance standards and consists of two fully enclosed electronic modules located inside of the imager. This laser system is by its appropriate classification and definition a non-removable laser system as it is not operable when the laser modules themselves are removed from within the system.

Safety Features

Azure Biosystems Sapphire Biomolecular Imaging Systems 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 access 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

The Azure Biosystems Sapphire laser systems 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 would occur.

Serviceability

Replacement of faulty laser 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 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 laser modules, or complete loss of output signal level in a laser 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 is designed to prevent you from being exposed to the laser radiation. If you open the sample lid while the scanner is in operation, the laser safety switch cuts power to the lasers. Exposure to laser radiation can be harmful, and viewing laser light directly, can cause blindness.

Azure Biosystems Sapphire systems 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 access lid open. The access 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 you should 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 you determine 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

The Azure Sapphire products are warrantied against defects in materials and workmanship for one year unless otherwise outlined on your 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.
- 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

The Azure Sapphire Biomolecular Imaging System has a power supply that automatically chooses the correct voltage for your country or region.

Certificates of Conformity

CE Conformity

The following Azure™ Sapphire Biomolecular Imager, models: RGB, NIR, RGBNIR, are in conformity with the provisions of the following CE Directives, including all amendments, and national legislation implementing these directives:

- Low Voltage Directive 2006/95/EC
- EMC Directive 2014/30/EU

And that the following harmonized standards have been applied:

- EN61010-1: 2001
- EN61326-1: 1997+A1:1998+A2:2001+A3:2003 Class A
- EN61000-4-2, EN61000-4-3, EN61000-4-4, EN61000-4-5, EN61000-4-6, EN61000-4-11

Protection category: IP20 according IEC 60529

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Legal

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

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1. Introduction

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

The Sapphire family of instruments includes the following products:

- Sapphire NIR
- Sapphire RGB
- Sapphire RGBNIR
- Sapphire PI

All models are compatible with optional chemiluminescence and phosphor imaging modules.

1.1 Key Components and Applications

Model	Key Components	Applications
Sapphire RGB	488nm LD, 520nm LD, 658nm LD	RGB visible fluorescence imaging
Sapphire NIR	685nm LD, 784nm LD	NIR fluorescence imaging
Sapphire RGBNIR	488nm LD, 520nm LD, 658nm LD, 784nm LD	RGB and NIR fluorescence imaging
Sapphire PI	658LD	Phosphor-imaging

1.2 Optional Modules

- Phosphor – Imaging phosphor screens exposed to radioactive samples
- Chemi – Imaging chemiluminescent samples with sensitivity of film
- Q-Module – Add dye flexibility and increase quantitative capability by adding 520 laser channel to Sapphire Biomolecular Imager – NIR
- 488 Laser Module – Add dye flexibility by adding 488 laser channel to Sapphire Biomolecular Imager – NIR

1.3 Optional Modifications

- Cy5 Modification – Modify the red channel of any Sapphire Biomolecular Imager to a 638nm laser and detector
- Cy5.5 Modification – Modify the 685 channel of a Sapphire Biomolecular Imager - NIR to a 658nm laser and detector
- NIR700 Modification – Modify the red channel of any Sapphire Biomolecular Imager to a 685nm laser and detector

1.4 Table of Specifications

	Sapphire NIR	Sapphire RGB	Sapphire RGBNIR	Sapphire PI
Part number	IS1024	IS1025	IS1026	IS1027
Laser excitation wavelengths	685, 784	488, 520, 658	488, 520, 658, 784	658
Bit depth	16 bit	16 bit	16 bit	16 bit
Scanning area	25 cm X 25 cm	25 cm X 25 cm	25 cm X 25 cm	25 cm X 25 cm
Scanning modes	Simultaneous, Sequential	Simultaneous, Sequential	Simultaneous, Sequential	Phosphor-imaging
Pixel size	10 micron – 1000 micron	10 micron – 1000 micron	10 micron – 1000 micron	10 micron – 1000 micron
Detectors	Avalanche Photodiode	Photomultiplier Tube, Avalanche Photodiode	Photomultiplier Tube, Avalanche Photodiode	Photomultiplier Tube
Scanning speed	50 cm/s	50 cm/s	50 cm/s	50 cm/s
Filters	Red 725BP40, IR 832BP37	Blue 518BP22, Green 565BP24, Red 710BP40	Blue 518BP22, Green 565BP24, Red 710BP40, IR 832BP37	Blue 390BP40
Dimensions (W X H X D)	75 cm X 45 cm X 70 cm	75 cm X 45 cm X 70 cm	75 cm X 45 cm X 70 cm	75 cm X 45 cm X 70 cm
Weight	140 lbs	140 lbs	140 lbs	140 lbs
Power requirements	100 – 240 VAC ± 10%, 50/60 Hz	100 – 240 VAC ± 10%, 50/60 Hz	100 – 240 VAC ± 10%, 50/60 Hz	100 – 240 VAC ± 10%, 50/60 Hz
Computer Options	Windows 10 laptop OR Windows 10 Pro desktop with HD monitor	Windows 10 laptop OR Windows 10 Pro desktop with HD monitor	Windows 10 laptop OR Windows 10 Pro desktop with HD monitor	Windows 10 laptop OR Windows 10 Pro desktop with HD monitor
Chemiluminescent Upgrade	Optional	Optional	Optional	Optional
Imaging area, chemiluminescent	16 cm X 13 cm	16 cm X 13 cm	16 cm X 13 cm	16 cm X 13 cm
Bit depth	16 bit	16 bit	16 bit	16 bit
Resolution	2688 X 2200	2688 X 2200	2688 X 2200	2688 X 2200
Imaging time	0-60 minutes	0-60 minutes	0-60 minutes	0-60 minutes
Visible imaging	Yes	Yes	Yes	Yes
Phosphor Imaging Module	Optional	Optional	Optional	N/A
Sample types	Storage phosphor screen (imaging plate)	Storage phosphor screen (imaging plate)	Storage phosphor screen (imaging plate)	Storage phosphor screen (imaging plate)

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 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. Further, 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 Azure Sapphire Biomolecular Imager 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 right 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 right panel of the instrument to the on (|) position.
2. Once the power is on to the system, wait for the light panels to stop changing color, and then 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 system is now ready to use.

To turn off the system

1. Push the power button on the front of the system for 5 seconds to put the system in standby mode
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 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 is done initializing, the software displays the Imaging tab, and is ready to use.

2.5 Connecting Other USB Devices to the System

The external computer is connected to the Sapphire system via a USB 3 connection.

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

Wipe the imaging surface with ultrapure water, 70% ethanol or isopropyl alcohol if available, and a lint free wipe.

Leave the system master power on at the back.

Keep the system computer connected, on a table near by.

Keep the top of the Sapphire Biomolecular Imager clear.

Remove your samples from the imager when finished.

Leave your system where it is installed.

Close the imaging lid whilst imaging.

Call Azure Biosystems, or email support@azurebiosystems, in the event of an issue with the instrument.

DO NOT

Wipe the surface with anything else. Many detergents and cleaning chemicals have fluorescent properties which will interfere with the scanning of fluorescent blots.

Attempt to access the back switch unless it is readily accessible.

Put the system computer on top of the scanning apparatus. The heat generated by the PC may damage the unit.

Put anything on top of the imager. The Sapphire was not designed to withstand forces acting down on top of the imager.

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.

Move your Sapphire. The Sapphire Imager weighs 135lbs (61kg) and is not designed to be moved. Moving the Sapphire Imager, without the express permission of Azure Biosystems, may void warranty or service contracts covering the imager.

Leave the imaging lid open while imaging. A safety mechanism disarms the lasers and prevents them from turning on when the imaging lid is not properly shut.

Attempt to fix the instrument yourself as some of the components may be hazardous.

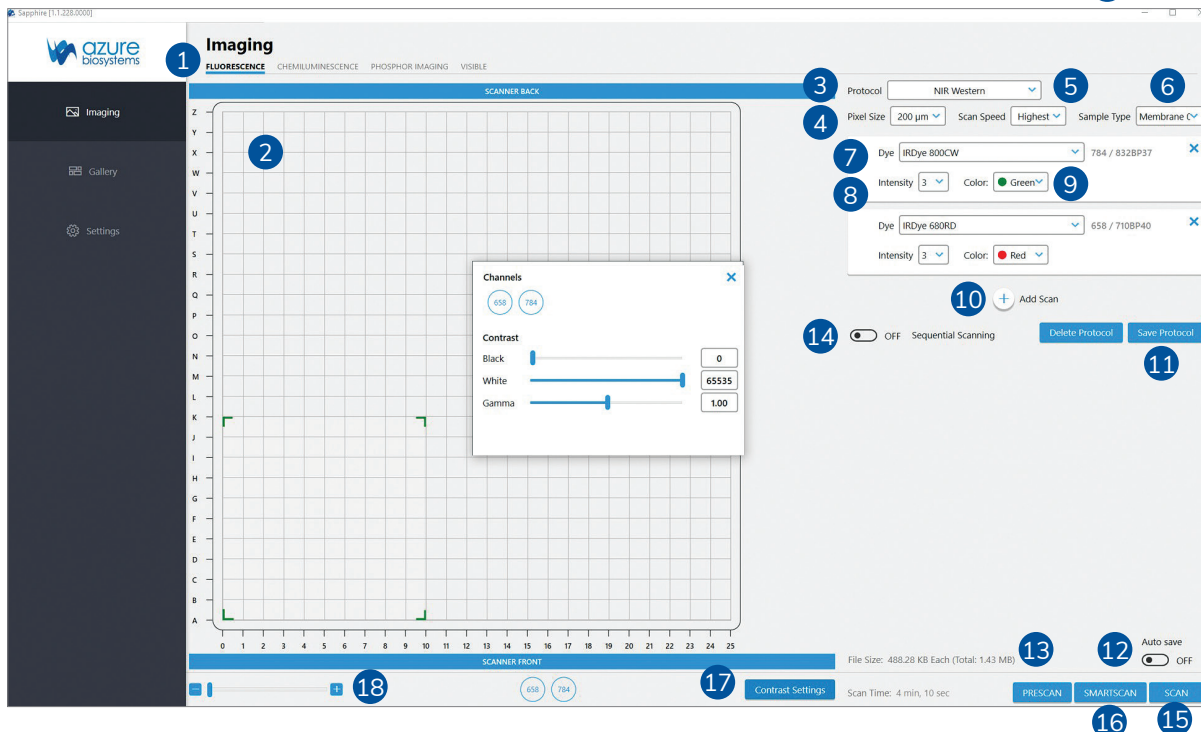
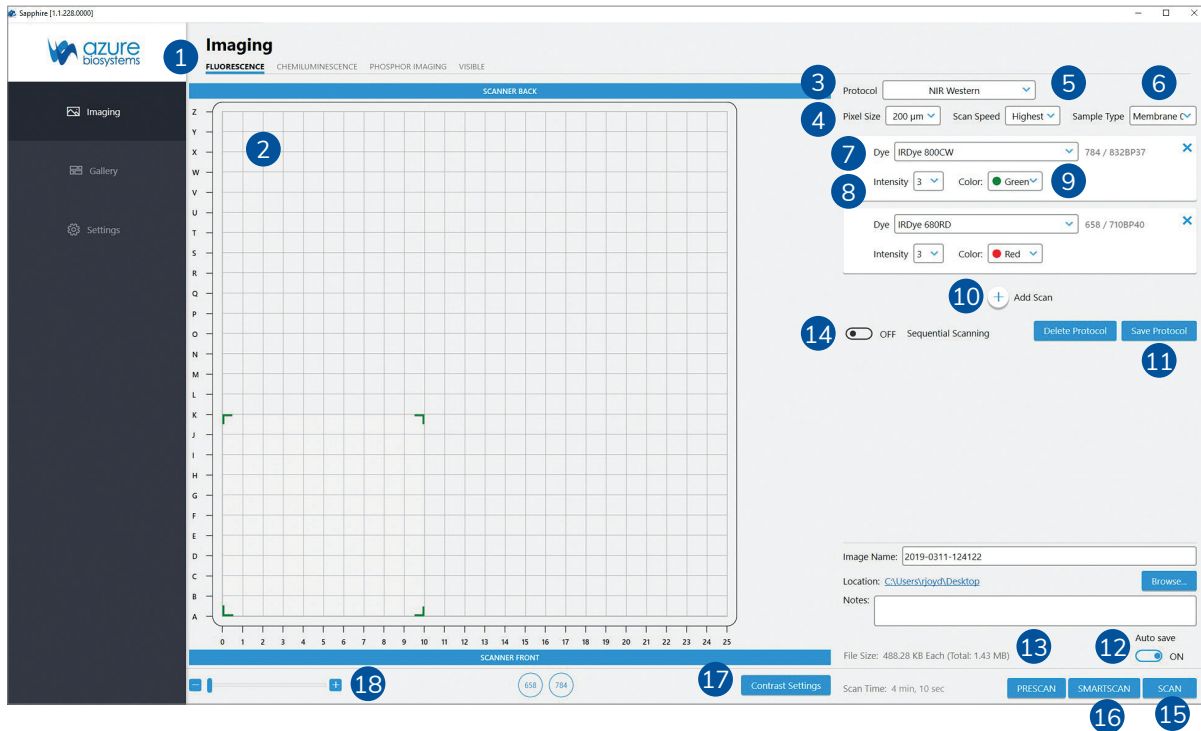
4. Sapphire Capture Software Overview

4.1 Launch the Sapphire Capture Software

Double click the Sapphire desktop icon to launch the scanner software.

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 Biomolecular Imager with each square in the Imaging Area grid representing one square centimeter. Use the green corners to select the area of the imaging screen covered by your sample.

Note 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. To decrease scanning time, place long samples along the X-axis.

- 3. 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.
- 4. 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.
- 5. SCAN SPEED** – Selects the speed at which the scanning head travels. A slower scan speed can produce more accurate signal to noise ratios for low concentration samples by increasing the sampling per pixel but will greatly increase the scanning time. For most applications, Highest scan speed is recommended.

Note: Slower scan speeds do not increase the signal measured.

- 6. SAMPLE TYPE** – Changing the Sample Type adjusts the focal plane of the scan. Selecting Custom Sample Type will bring up a slider that allows adjustment of the focal plane from 0mm to 4.0mm above the glass. Note that Custom focal distances are not saved in Image Info. Once the optimal focal plane of a sample is determined through the Custom function, it is recommended that the specific sample type and focal plane be added in Settings so that it can be recalled in the image info and for future scans.

Sample Type	Focal Plane
Membrane 0mm	On the glass (0mm)
Gel +0.5mm	0.5mm above the glass
Plate +3.0mm	3mm above the glass
Slide +1.0mm	1mm above the glass
Custom	Adjustable

- 7. DYE** – Select scanning wavelengths by using the drop-down menu to select from the available list of dyes. The corresponding excitation and emission wavelengths are noted on the right hand side of the selected dye.

Note: If a specific dye is not listed, choose one with a similar excitation and emission, or add it to the list in Settings.

8. **INTENSITY** – Adjust the power of each laser by selecting the appropriate Intensity level with 1 (one) being the lowest power and 10 (ten) 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 fluorophores more quickly.
9. **COLOR** – Choose which color to represent each dye/wavelength in the preview image during scanning. Note that coloring does not affect image capture or data.
10. **ADD SCAN** – Add additional scanning channels to the protocol. To remove an unwanted channel/dye, press the X located on the top right corner of the channel box.
11. **SAVE PROTOCOL** – Save a scanning method to the Protocol drop down to make it easier to access frequently used settings and protocols. Saved methods and protocols can also be deleted.
12. **SAVE IMAGES** – Choose whether or not to have images Autosave upon scan completion. Enter an file name in the Image Name field (default name is YYYY-MMDD-TTTTTT), and select or create a folder for images to be saved in by pressing 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.
13. **FILE SIZE** – The estimated image file size based on the current selected parameters (Scan Area, Pixel Size, and Scan Speed all effect 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.
14. **SEQUENTIAL SCAN** – By default, the Sapphire Biomolecular Imager scans all channels simultaneously. Turning Sequential Scanning ON scans each channel individually from first channel selected to last instead of scanning simultaneously.

Note: With Sequential Scanning the estimated Scan Time is per channel.

15. **SCAN** – The estimated scan completion time for the area and parameters selected will be displayed next to Scan Time. Select PRESCAN to initiate a low resolution, unsaved scan. Prescans are useful in determining correct sample orientation and positioning, scan area and intensity settings. Select SCAN to initiate a scan using the set parameters.
16. **SMARTSCAN** – SmartScan automatically determines the optimal intensity level of 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.
17. **CONTRAST SETTINGS** – Select Contrast Settings to open the preview contrasting window. The blue circle buttons displayed under Channels correspond to the currently engaged lasers. Clicking on these buttons will activate or inactivate 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.
18. **ZOOM** – Use the slider or the (-) and (+) buttons to zoom in 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, protein side down, 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 the imaging mat black side down on top of your membrane to keep it flush against the glass imaging surface. For best results, also use Azure's Background Quenching Sheets.
3. Click the **FLUORESCENCE** tab in the capture software to enter the fluorescence scanning module.
4. Select the area you would like to scan by dragging the corners of the box over the area covered by your membrane. The estimated scanning time will appear at the bottom of the capture window.
5. Select the desired Protocol from the drop down menu (NIR Western, Visible Fluorescent Western, 4 Channel Western) or select New Method to create a new method.
6. Choose the desired resolution and scan speed. Then set the Sample Type to Membrane. This sets the focal plane to the level of the glass scanning surface.
7. If creating a New Method, use the drop down menus to select the dye you are using along with the color you would like the signal to appear in the preview image. Add additional dyes to any method by clicking the (+) Add Scan button.
8. Change the signal Intensity to your preference for the sample you are imaging. Use Intensity 1 (one) to scan strong signals and Intensity 10 (ten) to detect weaker signals. NOTE: Use the PRESCAN 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 to have the system determine the scan intensity before automatically performing the final scan.
9. Enter or Edit the file name under Image Name. By default, the image will save as YYYY-MMDD-TTTTTT where time is HHmmss.
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. Clicking PRESCAN will initiate a quick, low resolution scan that can be useful in determining the correct membrane positioning, scanning area and intensity setting.
12. If no Prescan is needed, select SCAN to initial scanning at the set parameters, OR select SMARTSCAN to have the system determine the scan intensity and scan at the set parameters.
13. A preview image will appear in the selected scanning area. Use the blue channel buttons beneath 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, 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 Sequential Scanning was chosen, each individual channel will appear as a grayscale image along with the final multiplexed image.

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 using Background Quenching Sheets to create a flat, black background and protect your gel before placing the imaging mat black side down on top of your gel.
3. Click the **FLUORESCENCE** tab in the capture software to enter the fluorescence scanning module.
4. Select the area you would like to scan by dragging the corners of the box over the area covered by your gel. The estimated scanning time will appear at the bottom of the capture window.
5. Select the desired Protocol from the drop down menu (NIR Western, Visible Fluorescent Western, 4 Channel Western) or select New Method to create a new method.

Note: Western Protocols set the sample type to Membrane by default. This sets the focal plane to 0mm (the level of the glass).

6. Choose the desired resolution and scan speed. Then set the Sample Type 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, choose the Sample Type Custom and use the slider to set the focal plane.

7. If creating a New Method, use the drop down menus to select the dye you are using along with the color you would like the signal to appear in the preview image. Add additional dyes to any method by clicking the (+) Add Scan button.
8. Change the signal Intensity to your preference for the sample you are imaging. Use Intensity 1 (one) to scan strong signals and Intensity 10 (ten) to detect weaker signals. **NOTE:** Use the PRESCAN 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 to have the system determine the scan intensity before automatically performing the final scan.
9. Enter or Edit the file name under Image Name. By default, the image will save as YYYY-MMDD-TTTTTT where time is HHmmss.
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. Clicking PRESCAN will initiate a quick, low resolution scan that can be useful in determining the correct gel positioning, scanning area and intensity setting.
12. If no Prescan is needed, 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.
13. A preview image will appear in the selected scanning area. Use the blue channel buttons beneath 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, 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 Sequential Scanning was chosen, each individual channel will appear as a grayscale image along with the final multiplexed image.

4.2.3 Scanning a Fluorescent Slide

1. Place your slide sample side up on the glass scanning surface. Make sure that the sample is wholly contained within the imaging grid (A 0 – Z 25).
2. Azure recommends using a Background Quenching Sheet behind your sample before placing the imaging mat black side down on top of your slide.
3. Click the **FLUORESCENCE** tab in the capture software to enter the fluorescence scanning module.
4. Select the area you would like to scan by dragging the corners of the 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 (NIR, Visible Fluorescent Western, 3 Channel Image) or select New Method to create a new method.
6. Choose the desired resolution and scan speed. Then set the Sample Type to Slide. This sets the focal plane to 1mm above the glass scanning surface.

Note: If you prefer to place your slides with the sample side down, select Membrane as the Sample Type to set the focal plane level with the glass scanning surface, or select Custom and set the focus slider to 0mm.

Note: For slides of a different thickness than 1mm, use the Custom Sample Type to select the appropriate focal plane.

7. If creating a New Method, use the drop down menus to select the dye you are using along with the color you would like the signal to appear in the preview image. Add additional dyes to any method by clicking the (+) Add Scan button.
8. Change the signal Intensity to your preference for the sample you are imaging. Use Intensity 1 (one) to scan strong signals and Intensity 10 (ten) to detect weaker signals. **NOTE:** Use the PRESCAN 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 to have the system determine the scan intensity before automatically performing the final scan.
9. Enter or Edit the file name under Image Name. By default, the image will save as YYYY-MMDD-TTTTTT where time is HHmmss.
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. Clicking PRESCAN will initiate a quick, low resolution scan that can be useful in determining the correct slide positioning, scanning area and intensity setting.

12. If no Prescan is needed, 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.
13. A preview image will appear in the selected scanning area. Use the blue channel buttons beneath 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, 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 Sequential Scanning was chosen, each individual channel will appear as a grayscale image along with the final multiplexed image.

4.2.4 Scanning a Fluorescent, Plate-Based Assay

1. Place your plate on the glass scanning surface with the wells facing up so that you are scanning the bottom of the plate. Make sure that the sample is wholly contained within the imaging grid (A 0 – Z 25).
2. If possible, Azure recommends placing a black Background Quenching Sheet or the imaging mat black side down over the plate.
3. Click the **FLUORESCENCE** tab in the capture software to enter the fluorescence scanning module.
4. Select the area you would like to scan by dragging the corners of the 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 (NIR, Visible Fluorescent Western, 3 Channel Image) or select New Method to create a new method.

Note: Western Methods set the Sample Type to Membrane by default, which sets the focal plane to 0mm (the level of the glass).

6. Choose the desired resolution and scan speed. Then 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 to the bottom of the wells. For best results, measure this distance for your individual plate type and use the Custom Sample Type to set the correct focal plane. If you are unsure of the correct distance, scan your plate at several different focal planes and choose the one that results in the highest signal and sharpest image.

7. If creating a New Method, use the drop down menus to select the dye you are using along with the color you would like the signal to appear in the preview image. Add additional dyes to any method by clicking the (+) Add Scan button.
8. Change the signal Intensity to your preference for the sample you are imaging. Use Intensity 1 (one) to scan strong signals and Intensity 10 (ten) to detect weaker signals. **NOTE:** Use the **PRESCAN** function to initiate a quick, low resolution scan to help determine the best Intensity Level to use.

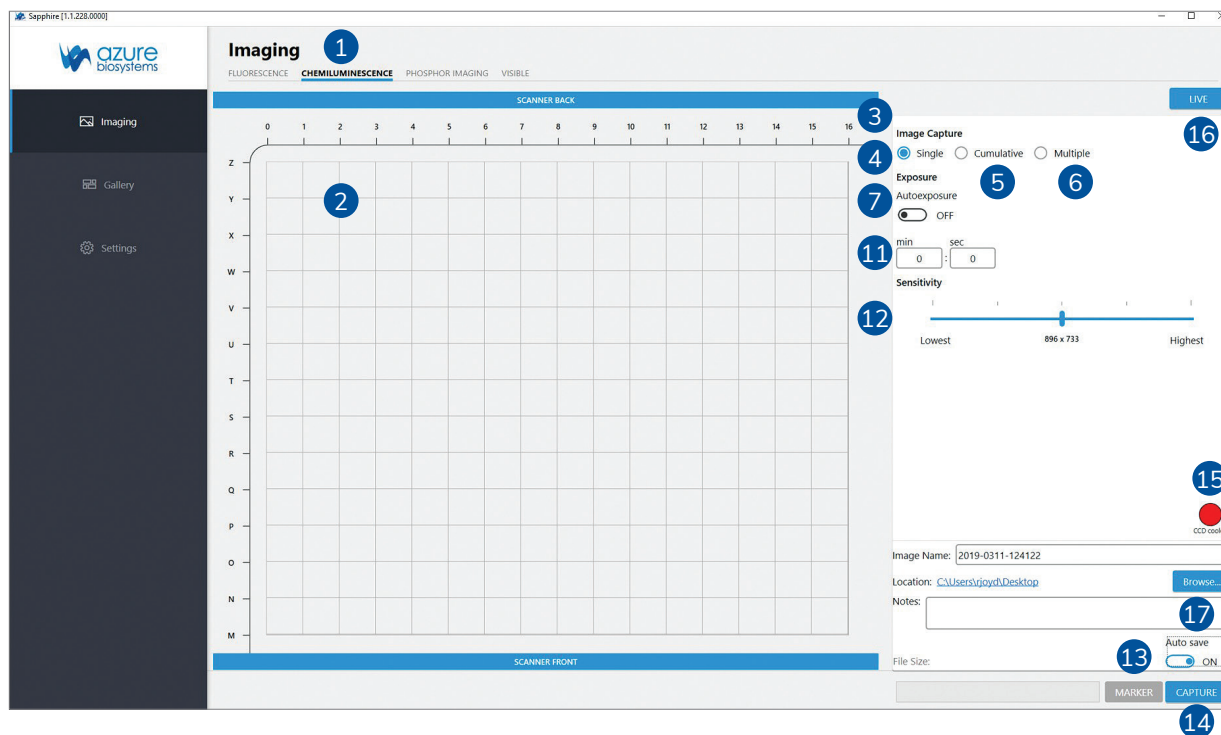
9. Enter or Edit the file name under Image Name. By default, the image will save as YYYY-MMDD-TTTTTT where time is HHmmss.
10. Use the Auto Save switch to turn Auto Save ON or OFF. If Auto Save is ON, use the Browse button to select where to save the auto saved images.
11. Clicking PRESCAN will initiate a quick, low resolution scan that can be useful in determining the correct sample positioning, scanning area and intensity setting.
12. If no Prescan is needed, select SCAN to initiate scanning at the set parameters.
13. A preview image will appear in the selected scanning area. Use the blue channel buttons beneath 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, 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.

4.3 Chemiluminescence Imaging

The Chemiluminescence Module utilizes a cooled CCD camera to image luminescent samples.



- 1. CHEMILUMINESCENCE** – If your Azure Sapphire configuration includes the optional chemiluminescence module, you will first need to select this option in the “Imaging” section.
- 2. IMAGING AREA** – The Chemi Imaging Area is designated by blue markings on the top left corner of the imaging glass. Ensure that chemi samples are wholly contained within the markings. Any sample outside of the marked area will not be imaged by the camera.
- 3. IMAGE CAPTURE** – Select between Single, Cumulative and Multiple Image Capture modes.
- 4. SINGLE** – Take a single chemiluminescent image with a predetermined exposure time, or by using the Autoexpose function.
- 5. CUMULATIVE** – Selecting Cumulative will tell the software to take up to 10 images at the time interval you selected for exposure times.

For example: if you set an exposure time of 1 minute, the system will capture and display one image every minute cumulating each exposure, up to ten exposures. The first image displayed will be a 1 minute exposure. The second image displayed, at the 2 minute interval, will be a two minute exposure. The final, tenth image, will be the sum of 10, 1 minute images. Images appear and can be viewed in the Gallery tab as they are acquired.

- 6. MULTIPLE** – Select between 1 and 10 images. The exposure time for each individual image can be set independently. The images are not stacked. Use the arrow buttons to move between the frames when setting the exposure times.

- 7. AUTO EXPOSURE** – If you are unsure of the required exposure time for your chemiluminescent sample, select Autoexposure to let the software calculate one for you. When Auto exposure is turned on, three different exposure options appear.
- 8. OVEREXPOSURE** – The software will calculate an exposure time that saturates the brightest bands in the blot to potentially capture weaker signals. This is useful for blots where the brightest bands may not be the bands of interest.
- 9. WIDE DYNAMIC RANGE** – The software will calculate an exposure time that is long enough to capture the widest range of signal while avoiding saturation. This autoexposure method is well suited to most blots.
- 10. UNDEREXPOSURE** – The software will optimize a short exposure time to the brightest signal on the blot. Weaker signals may not be visualized in an underexposed image.
- 11. EXPOSURE TIME** – If you know your preferred exposure time, enter it here. For capturing in the multiple image mode, select the time for each exposure. In the cumulative imaging mode, select the time interval for each image to be taken.
- 12. SENSITIVITY** – Changing the sensitivity settings changes both the sensitivity and the resolution of the camera and image. A lower sensitivity setting results in a higher resolution image, while a higher sensitivity setting results in a lower resolution image due to the binning of the pixels. The resolution is displayed under the sensitivity settings.
- 13. MARKER** – If Marker is selected, the system will acquire a white light image of your protein marker/ladder after capture of the chemi image. This can be overlaid on top of the chemi image, maintaining the positions on the gel or membrane.

Note: When the Marker function is selected and turned on, the button will appear blue. When the Marker function is off, the button will appear gray.
- 14. CAPTURE** – Click the capture icon to capture the image according to the set parameters.
- 15. CCD COOLED INDICATOR** – The Sapphire is equipped with a CCD capable of cooling down to 50 degrees C below ambient temperature. The CCD Cooled indicator will turn green when the CCD has reached optimal cooling. A cooled CCD is useful for noise reduction in long exposures. It is not necessary to wait for the CCD to reach optimal cooling when expecting exposures to remain under a minute.
- 16. LIVE** – Selecting this view will allow you to see a live view through the ccd camera enabling you to check the alignment of your blot in the field of view.
- 17. SAVE IMAGES** – Choose whether or not to have images Autosave upon image capture. Enter an file name in the Image Name field (default name is YYYY-MMDD-TTTTTT), and select or create a folder for images to be saved in by pressing 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.

4.3.1 Taking a Chemi Image

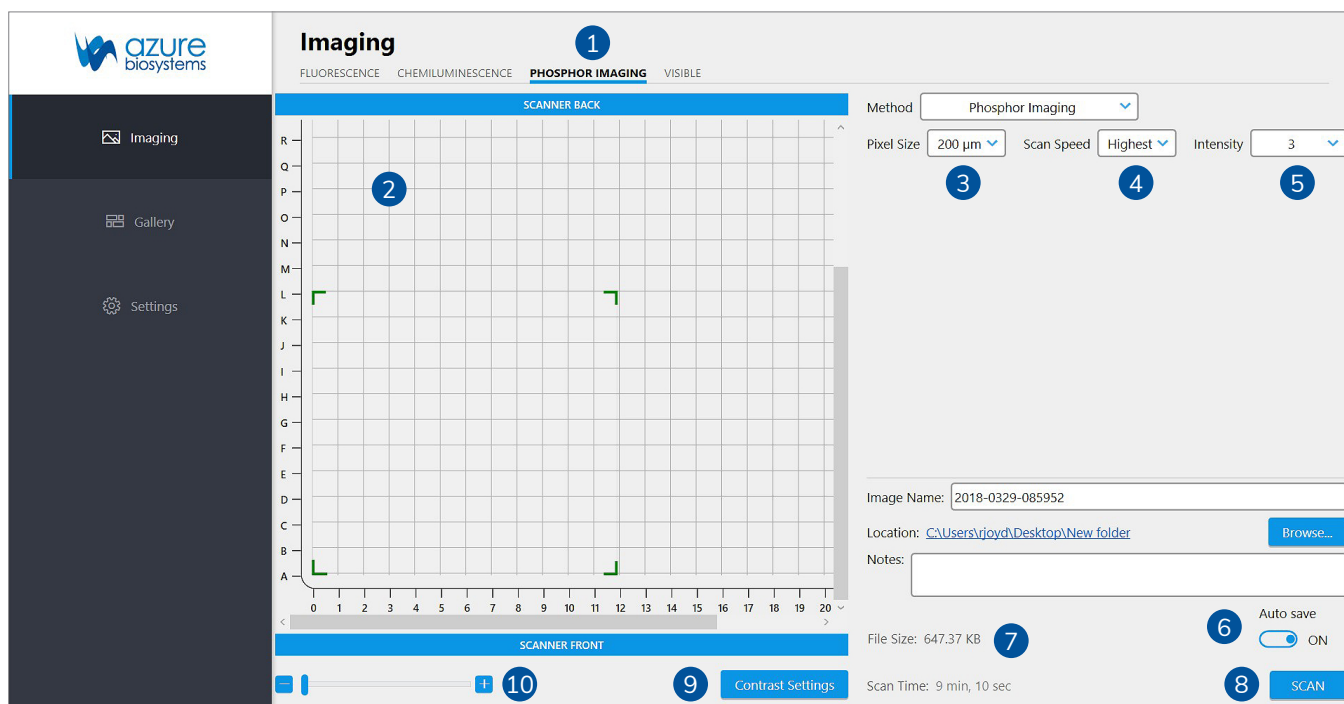
1. Place your blot protein side down, on the imaging surface. Make sure that the sample is wholly contained within the Chemi Imaging Area designated by the blue markings.
2. Azure recommends placing the imaging mat white side down on top of your membrane to keep it flush against the glass imaging screen.

Note: The imaging mat must be used white side down when taking marker images to ensure correct white balance of the image.

3. Select the CHEMILUMINESCENCE tab from the imaging options.
4. Choose your detection mode (single, cumulative, or multiple).
5. Choose your exposure time if known, or choose Autoexposure. When Autoexposure is selected, options for Overexposure, Underexposure and Wide Dynamic Range appear. Refer to the icon guide above if unsure of which option to choose.
6. Choose your sensitivity. High sensitivity comes at the expense of resolution. For best resolution, use low sensitivity.
7. Enter or Edit the file name under Image Name. By default, the image will save as YYYY-MMDD-TTTTTT where time is HHmmss.
8. 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.
9. Select Marker to take a separate, white light image of your protein ladder, which can later be overlaid on your chemi blot image.
10. Select CAPTURE.
11. Your images will appear in the gallery tab.

4.4 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** – If your Azure Sapphire configuration includes the optional phosphor imaging module, you will first need to select this option in the “Imaging” section.
- 2. IMAGING AREA** – The Imaging Area represents the glass scanning surface of the Sapphire Biomolecular Imager with each square in the Imaging Area grid representing one square centimeter. Use the green corners 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. To decrease scanning time, place long samples along the X-axis.
- 3. 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.
- 4. SCAN SPEED** – Selects the speed at which the scanning head travels. A slower scan speed can increase the signal/noise ratio of low concentration samples by reducing the background noise but will greatly increase the scanning time. For most applications, Highest scan speed is recommended.
- 5. INTENSITY** – Adjust the power of each laser by selecting the appropriate Intensity level with 1 (one) being the lowest power and 10 (ten) 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 fluorophores more quickly.

6. **SAVE IMAGES** – Choose whether or not to have images Autosave upon scan completion. Enter an file name in the Image Name field (default name is YYYY-MMDD-TTTTTT), and select or create a folder for images to be saved in by pressing 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.
7. **FILE SIZE** – The estimated image file size based on the current selected parameters (Scan Area, Pixel Size, and Scan Speed all effect 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.
8. **SCAN** – The estimated scan completion time for the area and parameters selected will be displayed next to Scan Time. Select SCAN to initiate a scan using the set parameters.
9. **CONTRAST SETTINGS** – Select Contrast Settings to open the preview contrasting window. The blue circle buttons displayed under Channels correspond to the currently engaged lasers. Clicking on these buttons will activate or inactivate 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.
10. **ZOOM** – Use the slider or the (-) and (+) buttons to zoom in on the Imaging Area. This is useful for small area scans and viewing the image preview of specific areas of the scan.

4.4.1 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. Select the PHOSPHOR IMAGING tab in the Imaging Screen.
3. Choose your desired pixel size and scan speed.

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.

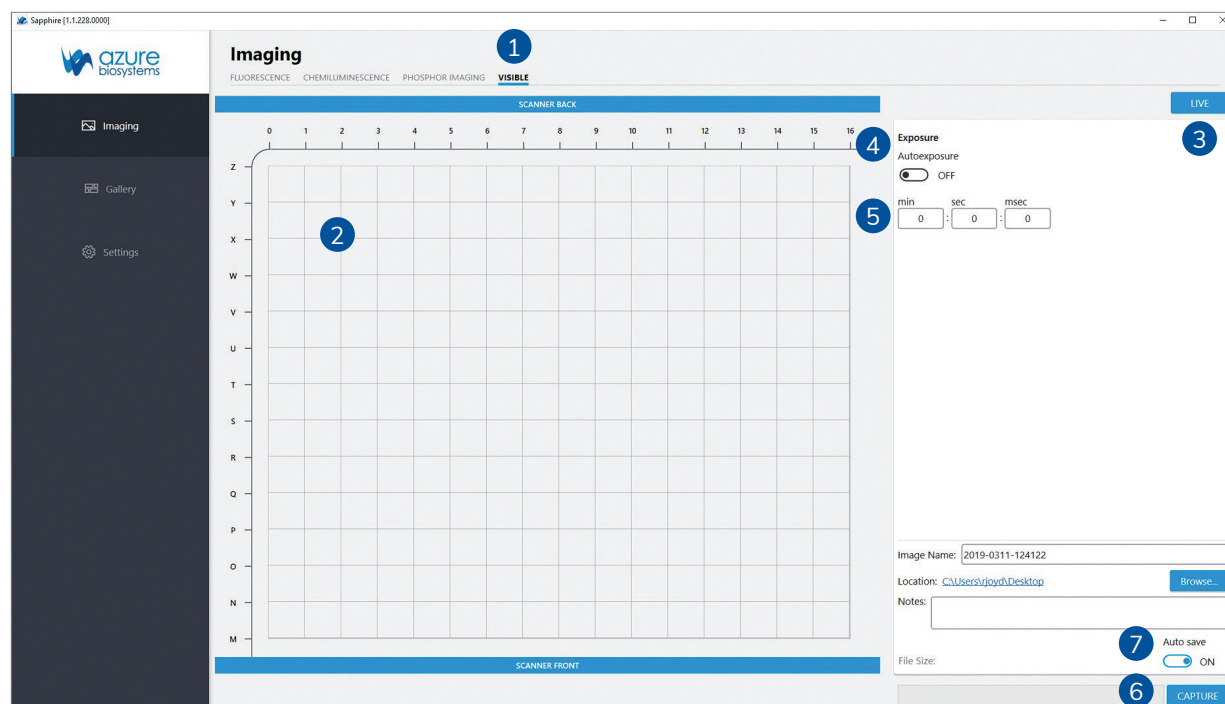
4. Change the signal Intensity to your preference for the sample you are imaging. Use Intensity 1 (one) to scan strong signals and Intensity 5 (five) to detect weaker signals.
5. Enter or Edit the file name under Image Name. By default, the image will save as YYYY-MMDD-TTTTTT where time is HHmmss.
6. Select **SCAN** to initiate scanning at the selected parameters.
7. A preview image will appear in the selected scanning area.
8. 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.

9. The acquired image will appear in the Gallery Tab once scanning is complete.

4.5 Visible Imaging

The Visible Imaging module utilizes white light to generate color images of colorimetric samples.

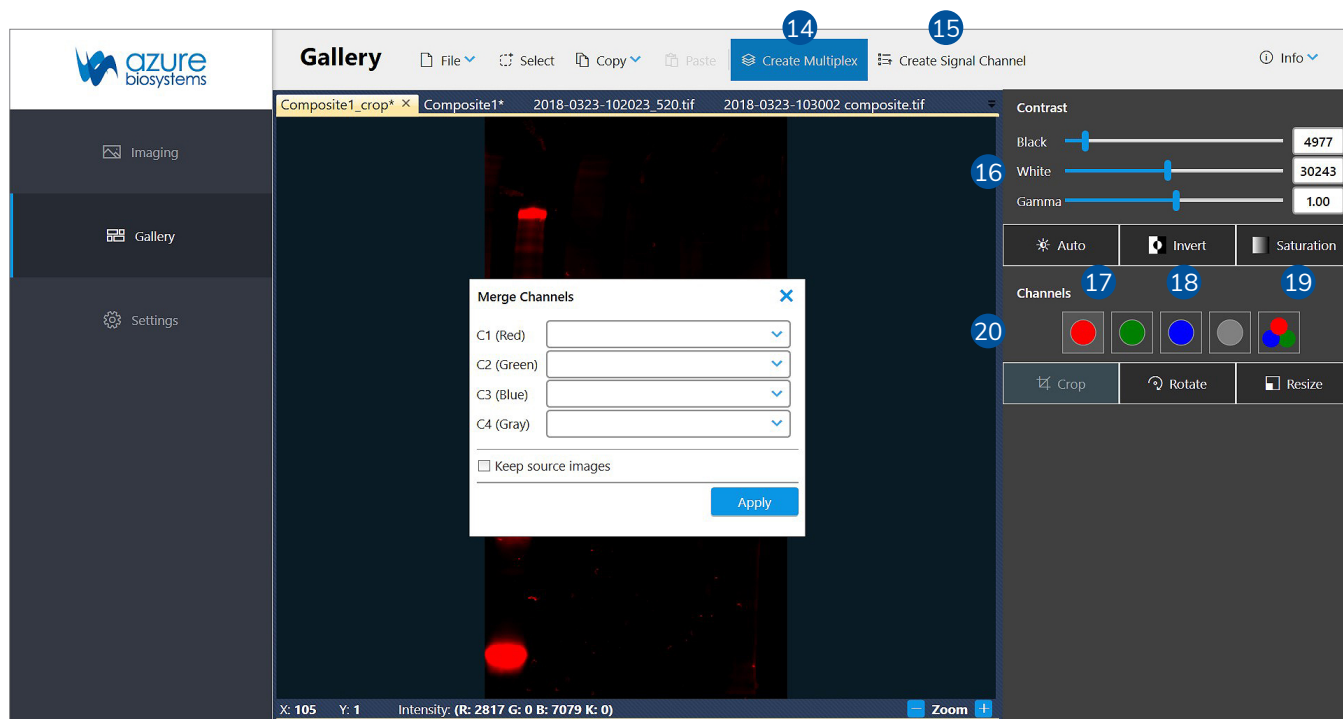
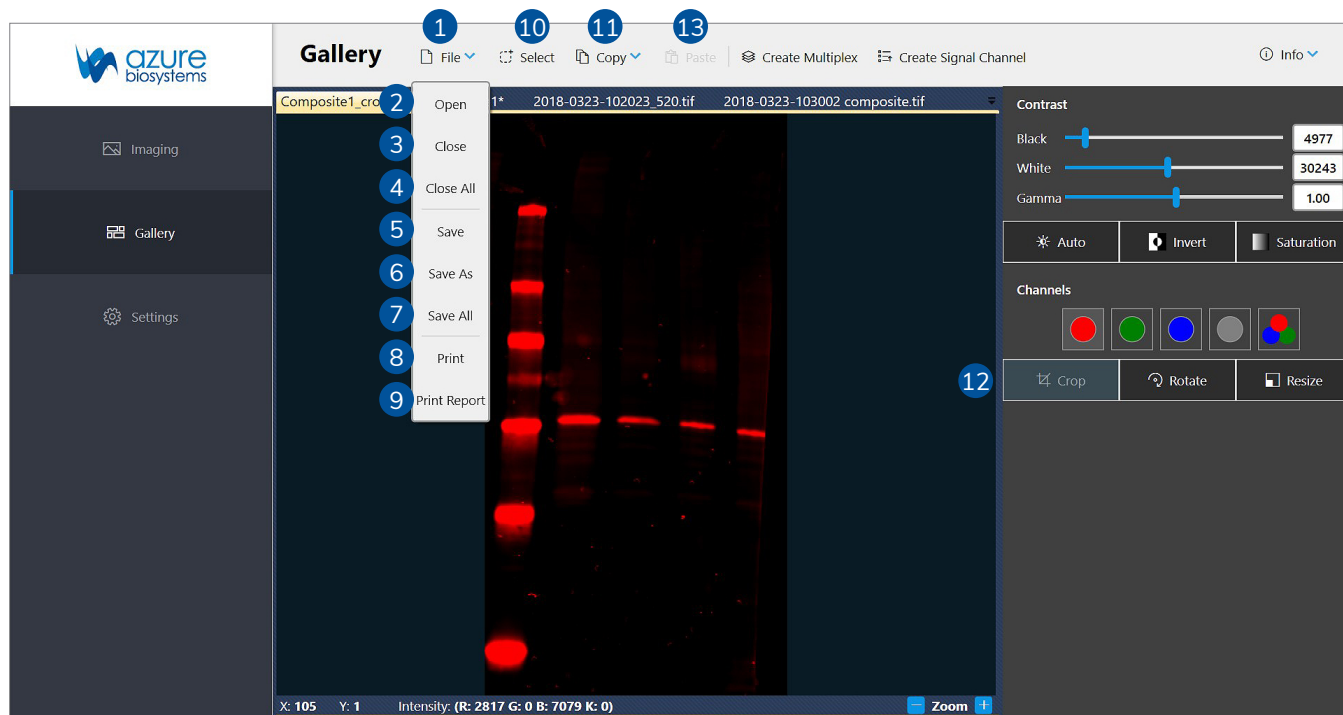


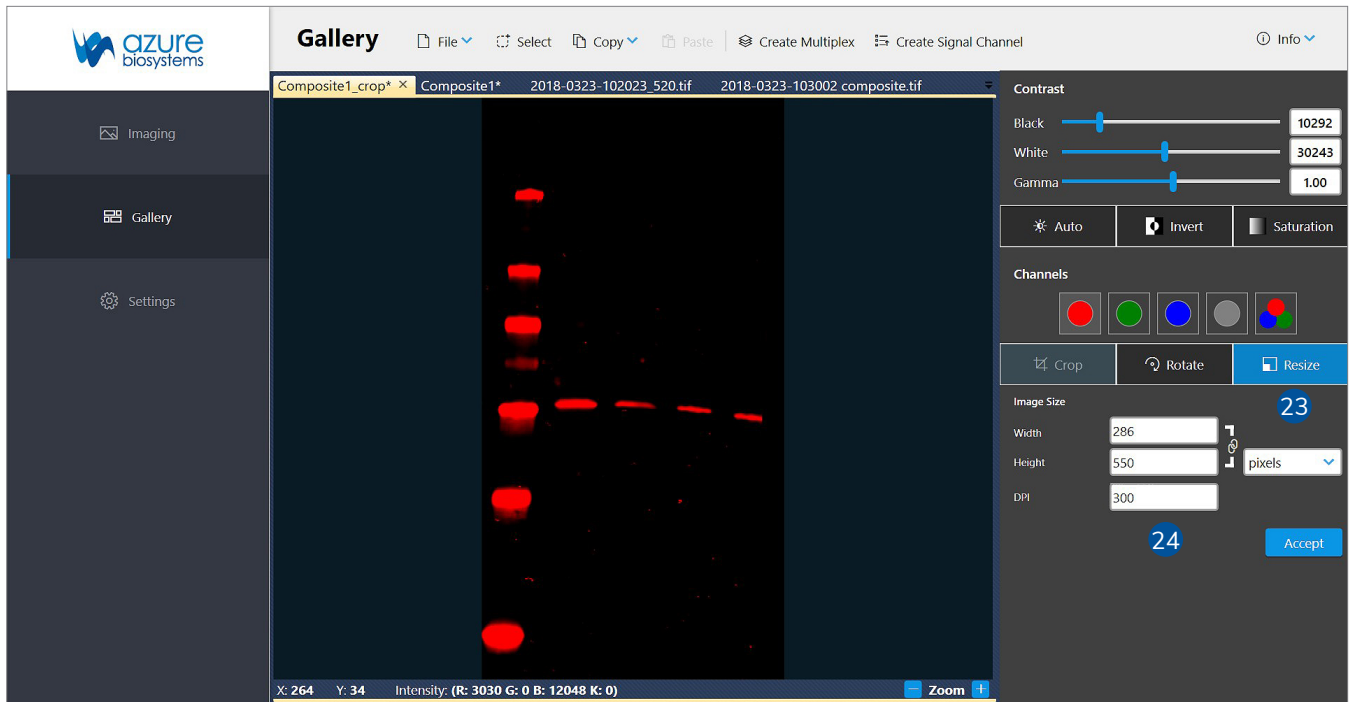
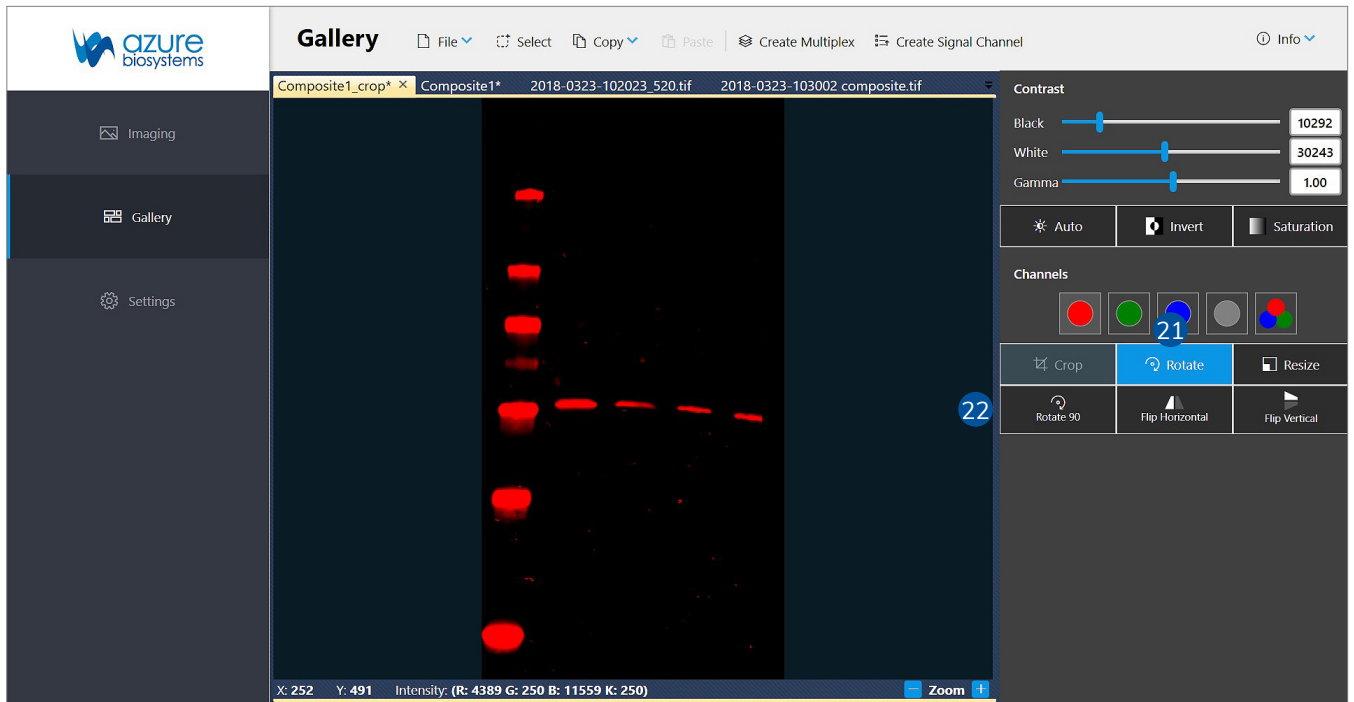
- 1. VISIBLE** – If your Azure Sapphire configuration includes the optional chemiluminescence module, you will also have the ability to take white light images using the Visible tab in the “Imaging” section.
- 2. IMAGING AREA** – The Chemi and Visible Imaging Area is designated by blue markings on the top left corner of the imaging glass. Ensure that colorimetric samples are wholly contained within the markings. Any sample outside of the marked area will not be imaged by the camera.
- 3. LIVE** – Selecting this view will allow you to see a live view through the ccd camera enabling you to check the alignment of your sample in the field of view.
- 4. AUTO EXPOSURE** – If you are unsure of the required exposure time for your sample, selecting Autoexposure will allow the software to calculate one based on your sample. Autoexposure is recommended for most samples.
- 5. EXPOSURE TIME** – If you know your preferred exposure time, enter it here.
- 6. CAPTURE** – Click the capture icon to capture the image.
- 7. SAVE IMAGES** – Choose whether or not to have images Autosave upon image capture. Enter an file name in the Image Name field (default name is YYYY-MMDD-TTTTTT), and select or create a folder for images to be saved in by pressing 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.

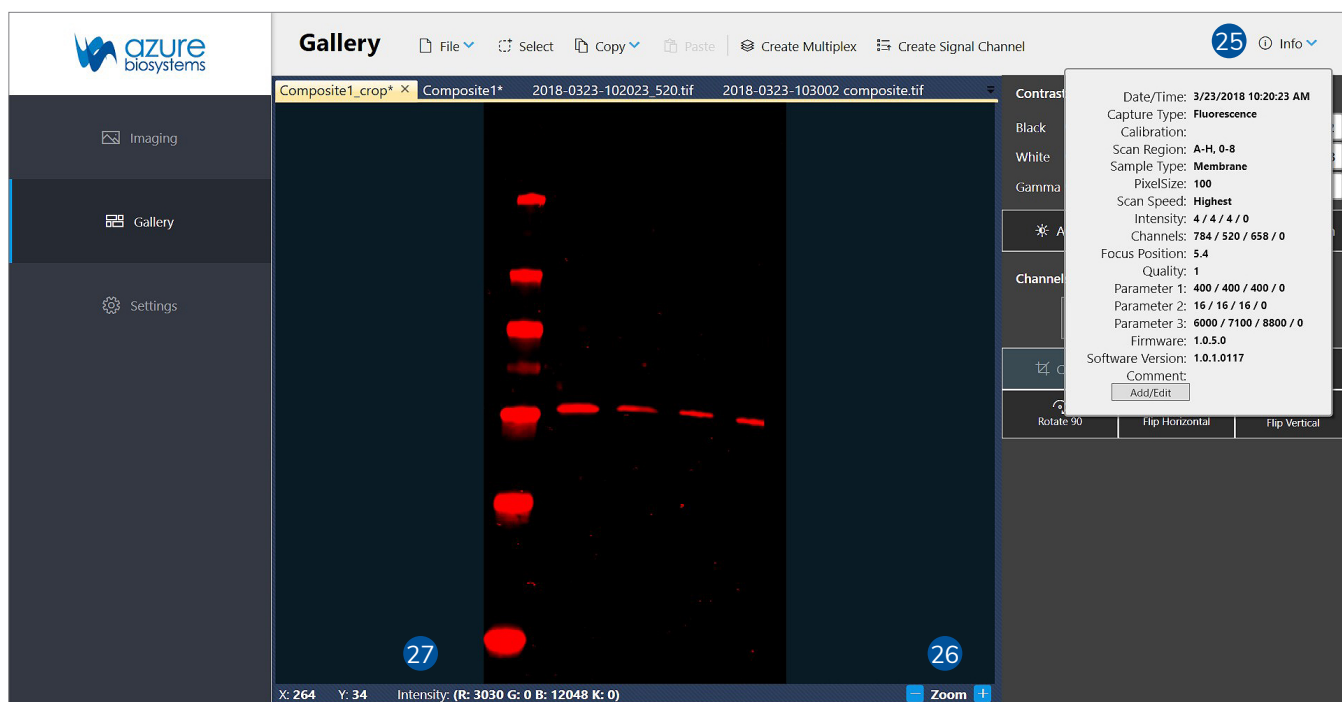
4.6 The Image Gallery

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

1. Click on the GALLERY tab on the left hand side of the screen.
2. Use the Black, White and Gamma sliders to adjust the contrast of your image.
3. Images can also be Inverted, Rotated and Resized. See the Icon Guide below for a complete description of each option.







1. **FILE** – Here you can save your captured images, open previous images, or print images.
2. **OPEN** – Use this icon to open an image that is stored locally, on a USB, or on the network (for systems that are networked).
3. **CLOSE** – This will close the currently selected image in the gallery tab. Be sure to save images before closing.
4. **CLOSE ALL** – This will close all currently open images in the gallery tab. Be sure to save desired images before closing.
5. **SAVE** – Allows you to save you 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. All images are saved as 300 DPI.
6. **SAVE AS** – Allows you to save a copy of an image with an alternate name, file type, or to an alternate file location.
7. **SAVE ALL** – Allows you to save all unsaved images open in 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.
8. **PRINT** – Allows you to print to a printer connected to the system (if applicable), or to a printer on the network.
9. **PRINT REPORT** – Print an image report including the acquired image along with Image Info.
10. **SELECT** – Generates a box that you can resize to select an area of the captured image to copy or crop.
11. **COPY** – Copy the whole image, or a selected area of the image, such as a marker or ladder, to the clipboard.

- 12. CROP** – Crop an image to get rid of excess borders and background, crop a molecular marker or ladder, or to take a closer look at relevant parts of the image.
- 13. PASTE** – Paste the selected part of the image onto another image – creating a third, new image.
- 14. CREATE MULTIPLEX** – Overlay multiple 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.
- 15. CREATE SIGNAL CHANNEL** – Separate out the different channels of a color image to look at the data from each channel individually, in greyscale. Images will be tagged with the appropriate channel in the file name.
- 16. CONTRAST** – Manually adjust the black, white and gamma settings for the displayed 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 would keep the contrast settings. Azure recommends to use TIFF if the image needs to be quantified.
- 17. AUTO** – Auto contrast will contrast your image so as 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.
- 18. 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 software. Other than inverting, this does not change the data in any way.
- 19. 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.
- 20. 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, the image is displayed as a multichannel image. At this point, 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.
- 21. ROTATE** – Selecting the rotate icon will generate additional image editing options.
- 22. ROTATE 90 DEGREES, FLIP HORIZONTAL, FLIP VERTICAL** – By select one or more of these icons, you will shift the alignment of your image.
- 23. RESIZE** – Selecting the resize icon will generate additional editing options.
- 24. RESIZE** – Use this feature to adjust the DPI on capture images without altering the raw data.
- 25. IMAGE INFO** – Select Image Info to show review image scanning parameters.
- 26. ZOOM** – Use the (+) and (-) buttons to zoom in and out of a capture imaged.
- 27. PIXEL VALUES** – Hover of an area to view pixel positions and intensity values.

5. Settings

Settings

GENERAL ADVANCED CREATE DARKMASTERS CREATE FLATS

ADD SAMPLE TYPE
Add or edit sample type focus position.

Position	Sample Type	Focus Position
1	Gel +0.5mm	0.5
2	Plate +3.0mm	3
3	Membrane 0mm	0
4	Slide +1.0mm	1

Add Edit

Sample Type Focus Position

ADD DYE

Position	Dye	Wavelength
1	Alexa 488	488 / 518BP22
2	Alexa 546	520 / 565BP24
3	Alexa 790	784 / 832BP37
4	Cy2	488 / 518BP22
5	Cy3	520 / 565BP24
6	Cy5	658 / 710BP40

Dye Laser Channel

5.1 General Settings

- 1. ADD SAMPLE TYPE** – Use this module to add or edit sample types and to save focus positions determined to be optimal using the Custom Sample Type. To add a Sample Type, select ADD, name the Sample Type and enter a focus position. Values indicate mm above the glass. 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 Sample Type to the drop down list.

When a Sample Type that is linked to a Method is deleted, the Method will default to Custom Sample Type until a new Sample Type is assigned to the Method.

- 2. ADD DYE** – Use this module to add and delete dyes from the selection list. To add a dye, type the name in the field, select the appropriate laser/filter combination and press ADD.

5.2 Advanced Settings, Create Darkmasters, Create Flats

Accessing these settings 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.