azure biosystems

BOSTON UNIVERSITY

Department of Biology
Proteomics and
Imaging Core Facility

Sapphire™ Biomolecular Imager

User Manual

Rev 20180418

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.

Model	Key Components
Sapphire RGBNIR	488nm LD, 520nm LD, 658nm LD, 784nm LD

	Sapphire RGBNIR
Laser excitation wavelengths	488, 520, 658, 784
Bit depth	16 bit
Scanning area	25 cm X 25 cm
Scanning mode	Simultaneous
Pixel size	10 micron – 1000 micron
Detectors	Photomultiplier Tube, Avalanche Photodiode
Scanning speed	50 cm/s
Filters	Blue 518BP22, Green 565BP24, Red 710BP40, IR 832BP37
Dimensions (W X H X D)	75 cm X 45 cm X 70 cm
Power requirements	100 – 240 VAC ± 10%, 50/60 Hz
Computer	Windows 10 laptop
Imaging area, chemiluminescent	16 cm X 13 cm
Bit depth	16 bit
Resolution	2688 X 2200
Imaging time	0-60 minutes
Visible imaging	Yes
Sample types	Storage phosphor screen (imaging plate)

To turn on the system

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

Push the power button on the front of the system for 5 seconds to put the system in standby mode

3. Proper Usage – Please Read Before Use

DO	DO NOT
Wipe the imaging surface with ultrapure water, 70% ethanol or isopropyl alcohol if available, and a lint free wipe.	Wipe the surface with anything else. Many detergents and cleaning chemicals have fluorescent properties which will interfere with the scanning of fluorescent blots.
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 near by.	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 Sapphire Biomolecular Imager clear.	Put anything on top of the imager. The Sapphire 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. 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.
Close the imaging lid whilst imaging.	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.
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.

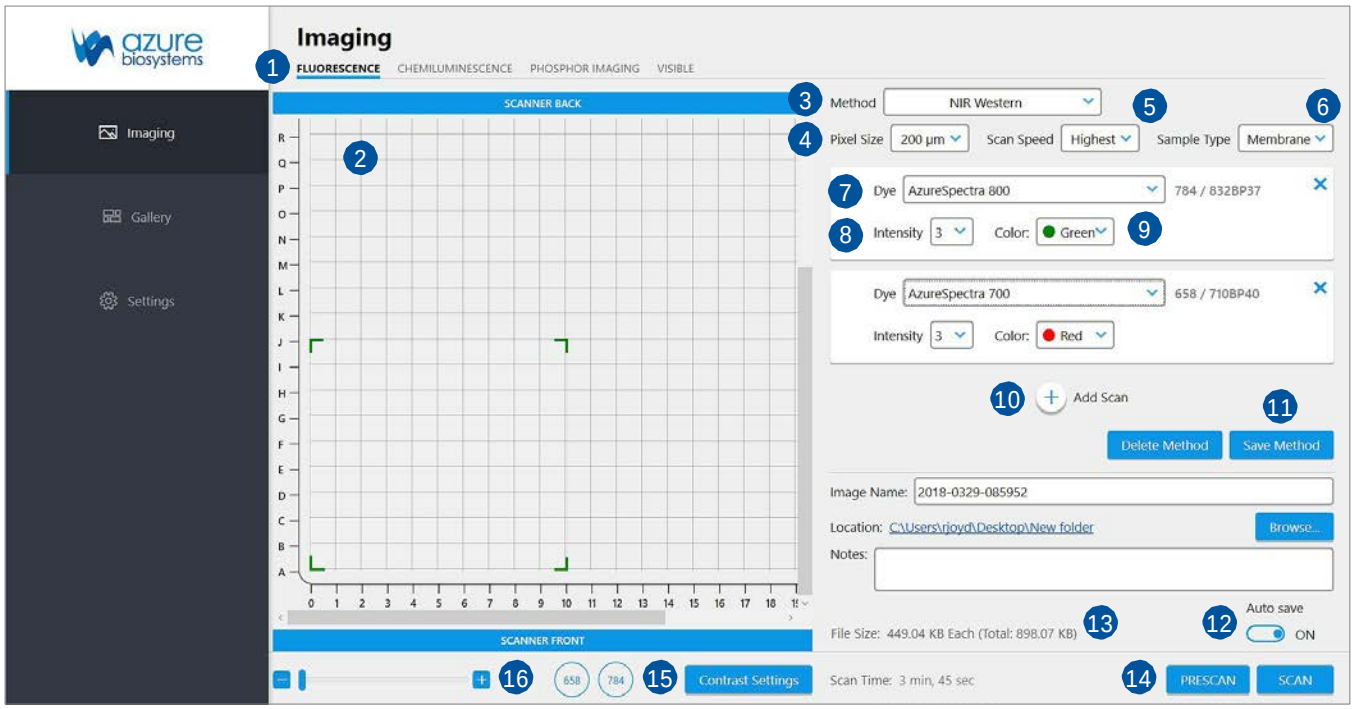
4. Sapphire Capture Software Overview

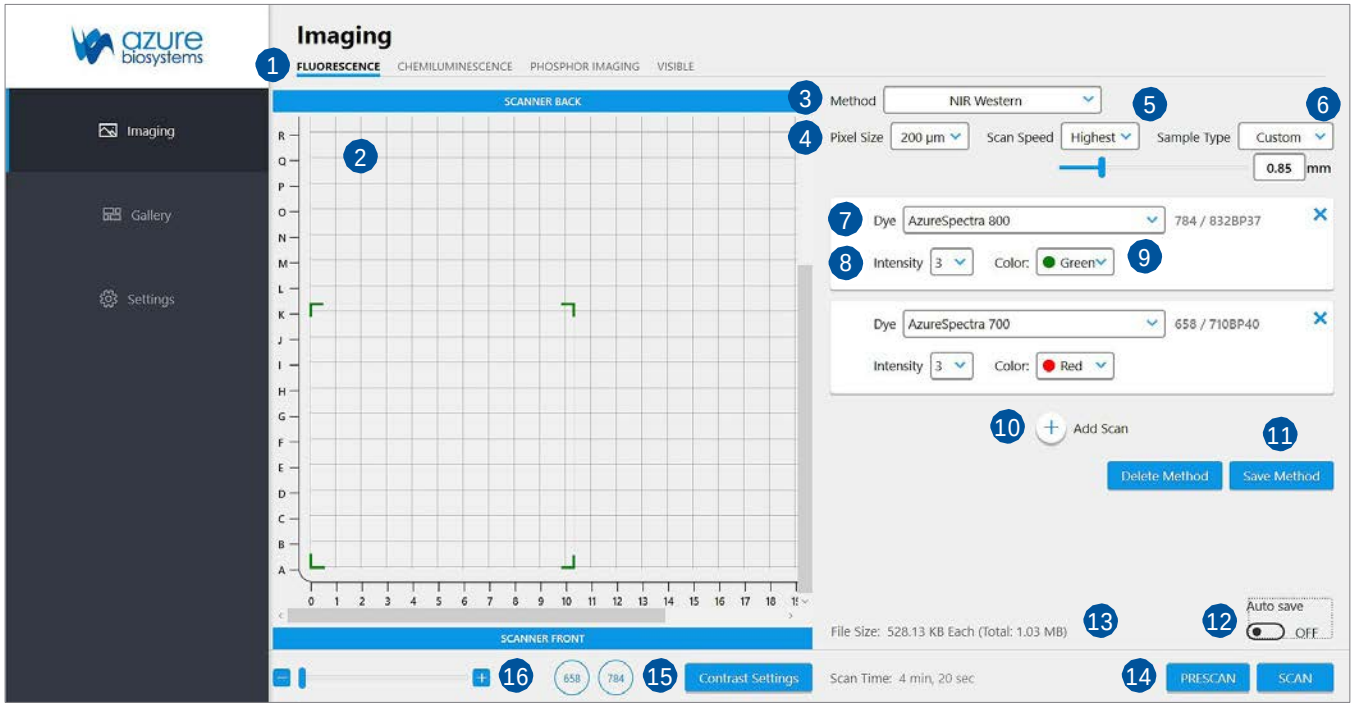
4.1 Launch the Sapphire Capture Software

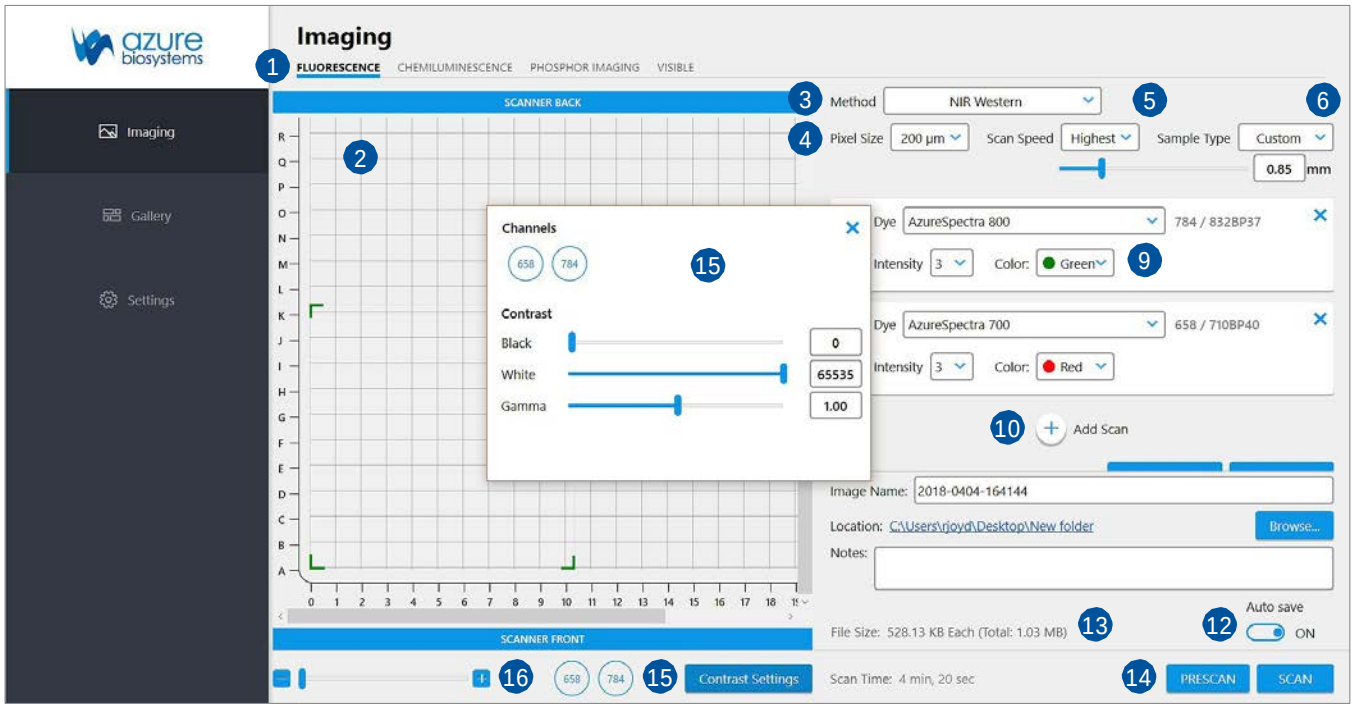
Double click the Sapphire desktop icon to launch the scanner software.

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. **METHOD** – Several scanning protocols are preset within the software for easy imaging set up. Use the Method 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 to 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 METHOD** – Save a scanning method to the Methods 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. **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.

15. **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.

16. **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 screen. 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 Method 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.
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 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.

4.2.2 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 Method from the drop down menu (NIR Western, Visible Fluorescent Western, 4 Channel Western)or select New Method to create a new method.

    Note: Western Methods 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.
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.

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.

4.2.3 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 Method 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.

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.

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.

4.2.4 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 Method 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.

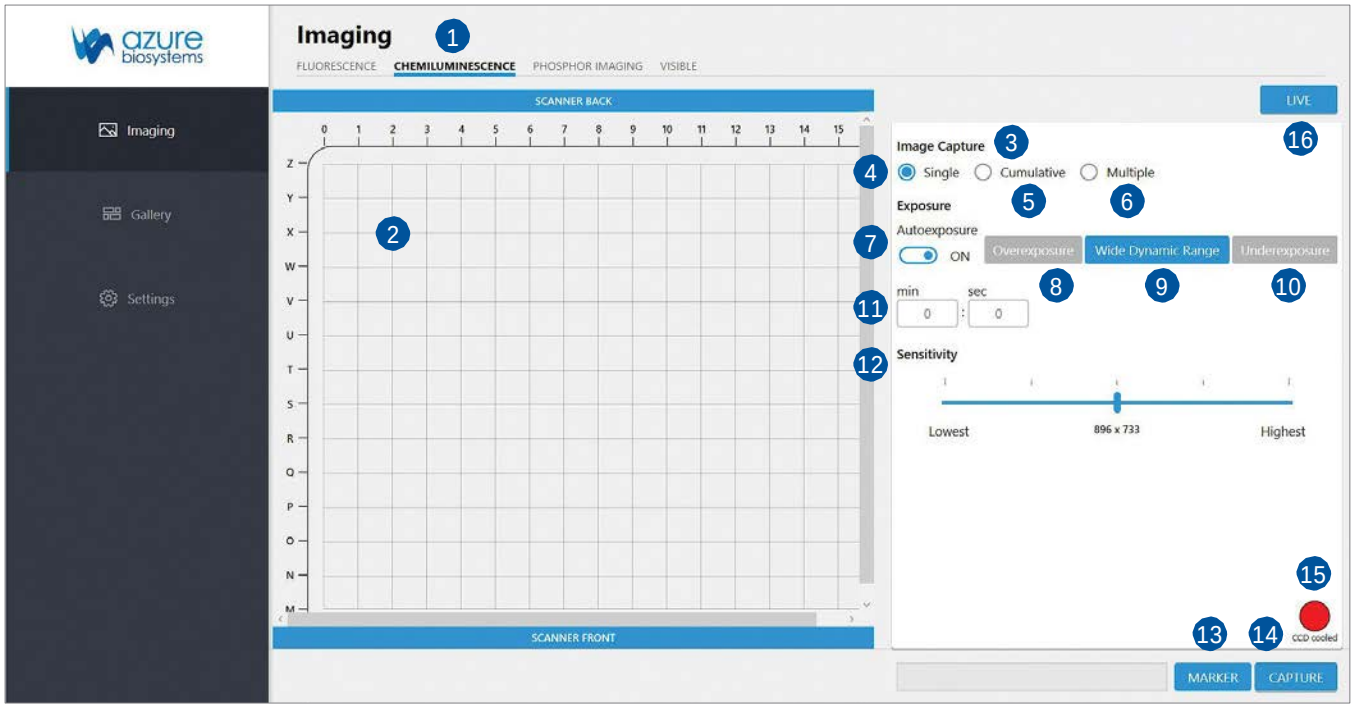
14. During scanning, visualization of the preview image may be adjusted by clicking on the Contrast Settings button. This will open a Channels window that will allow you to select which channels to preview and adjust contrast.

    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 you 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.

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.

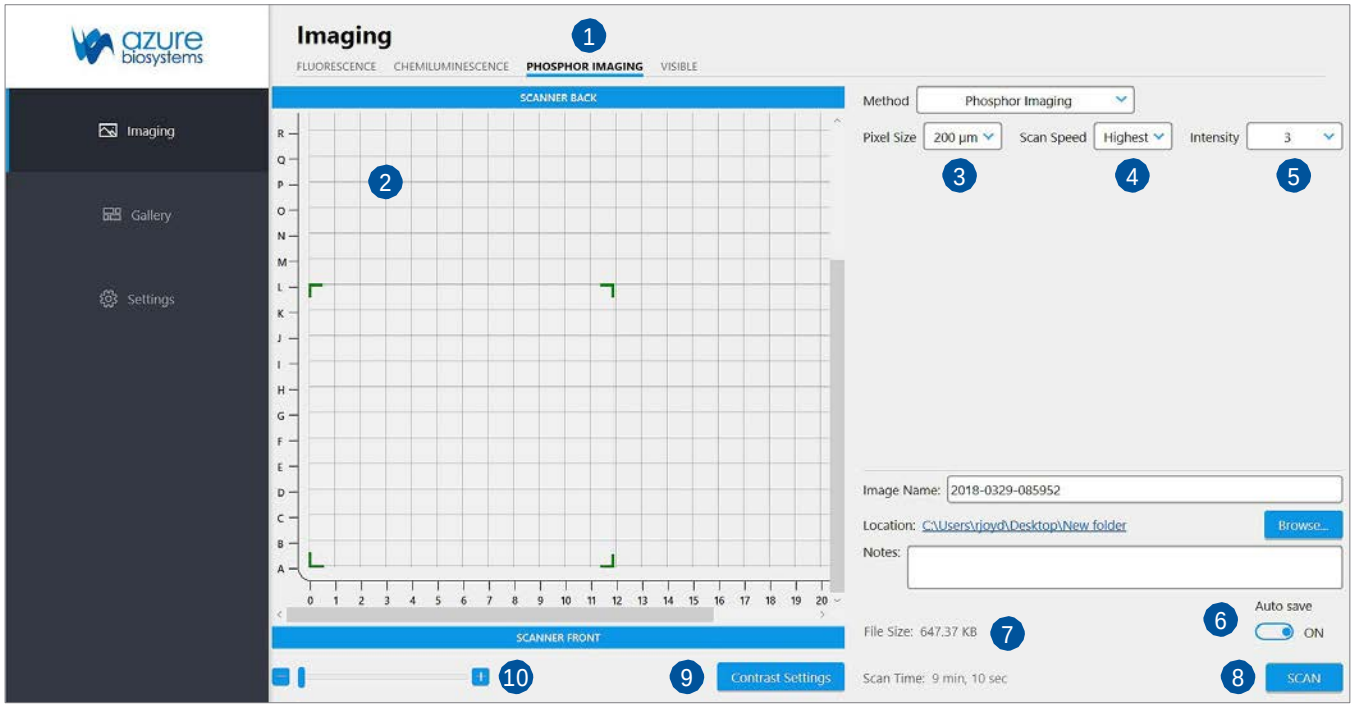
8. Select Marker to take a separate, white light image of your protein ladder, which can later be overlaid on your chemiblot image.

9. Select **CAPTURE**.

10. 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.

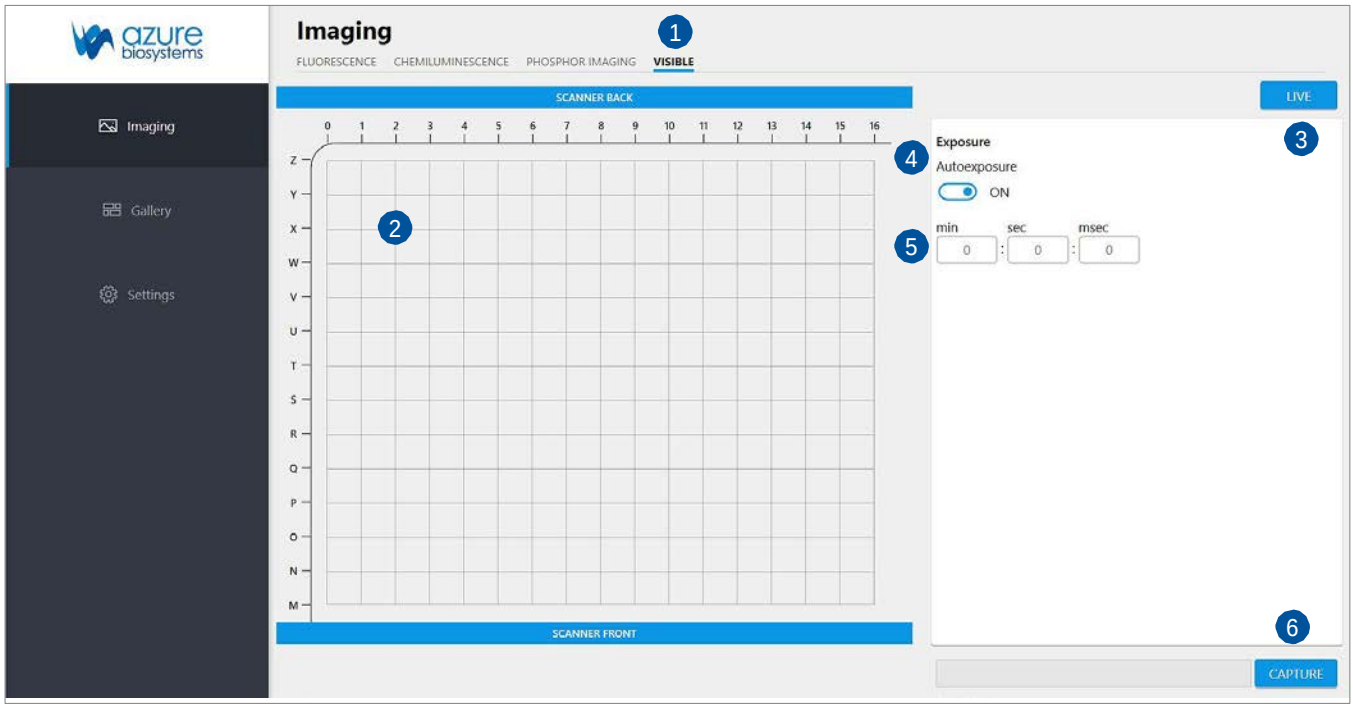
4. Change the signal Intensity to your preference for the sample you are imaging. Use Intensity 1 (one) to scan strong signals and Intensity 5 (five) to detect weaker signals.
5. Enter or Edit the file name under Image Name. By default, the image will save as YYYY-MMDD-TTTTTT where time is HHmmss.
6. 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.

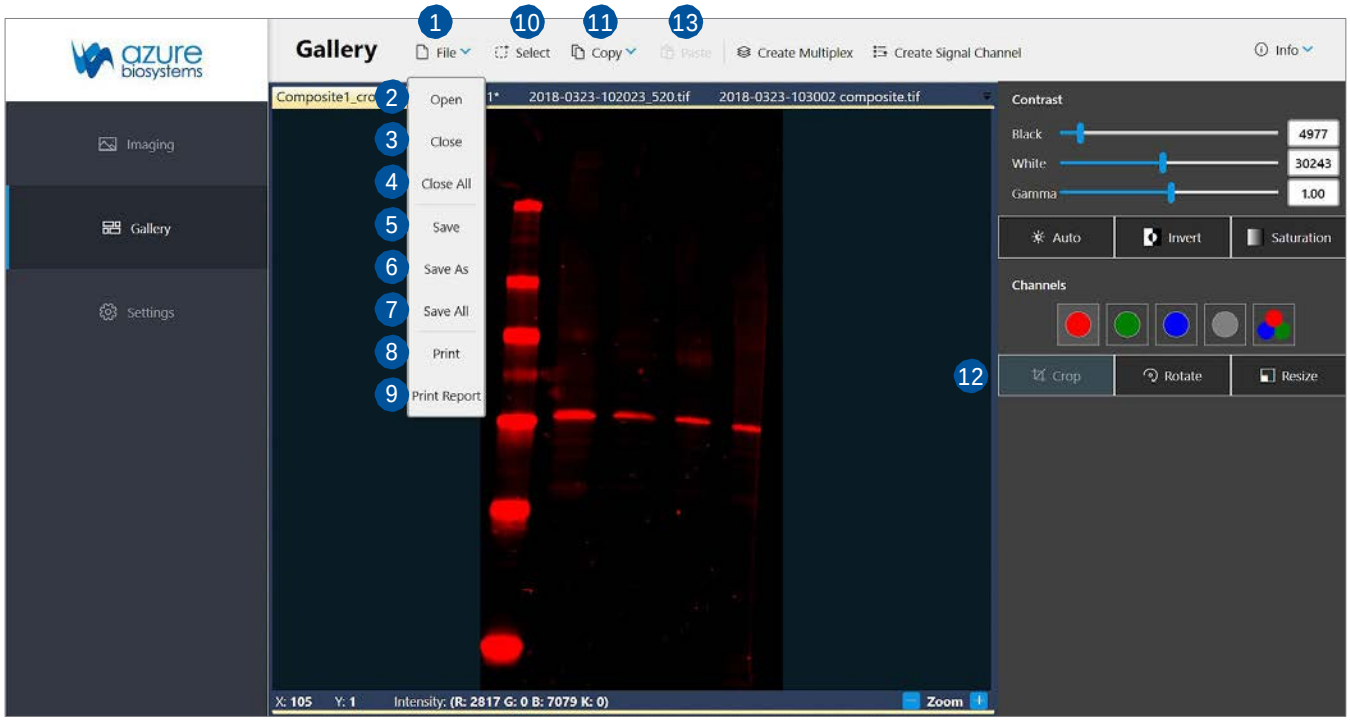


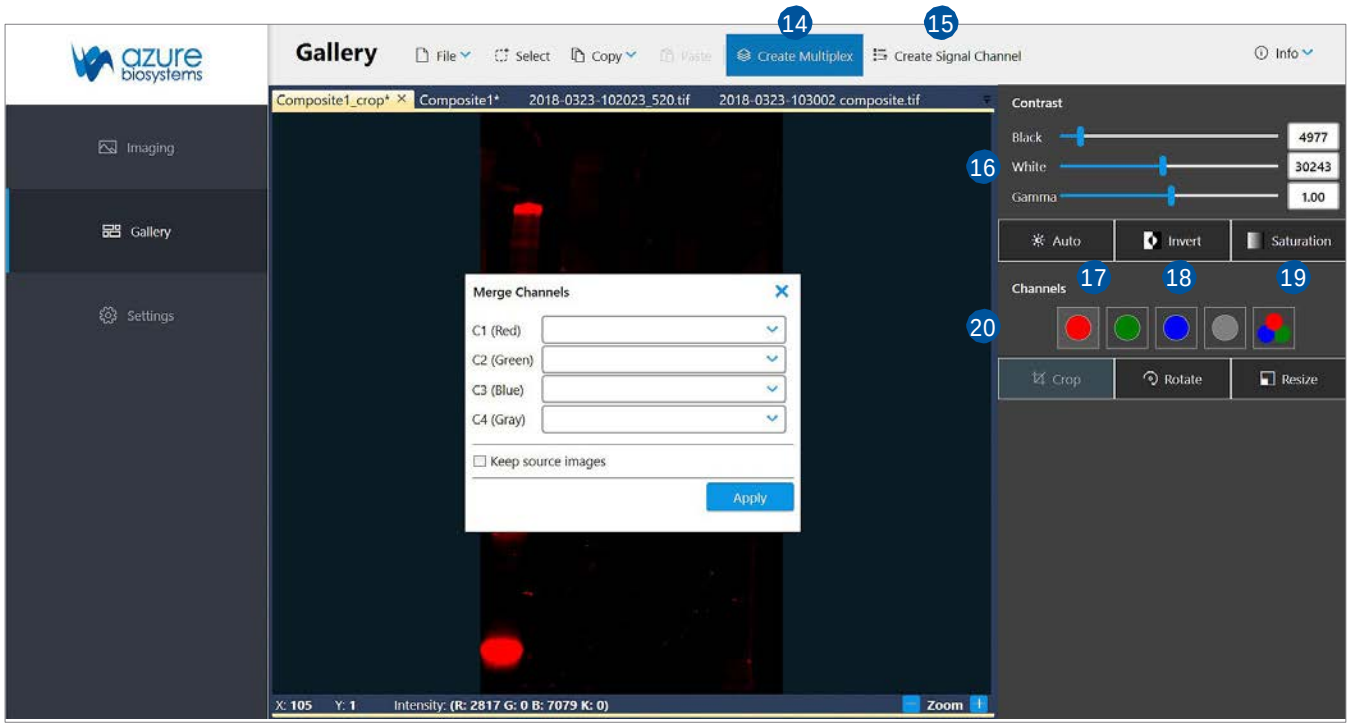
1. **VISIBLE** – If your Azure Sapphire configuration includes the optional chemiluminescence module, you will also have the ability to take white light images using the Visible tab in the "Imaging" section.
2. **IMAGING AREA** – The Chemi and Visible Imaging Area is designated by blue markings on the 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.

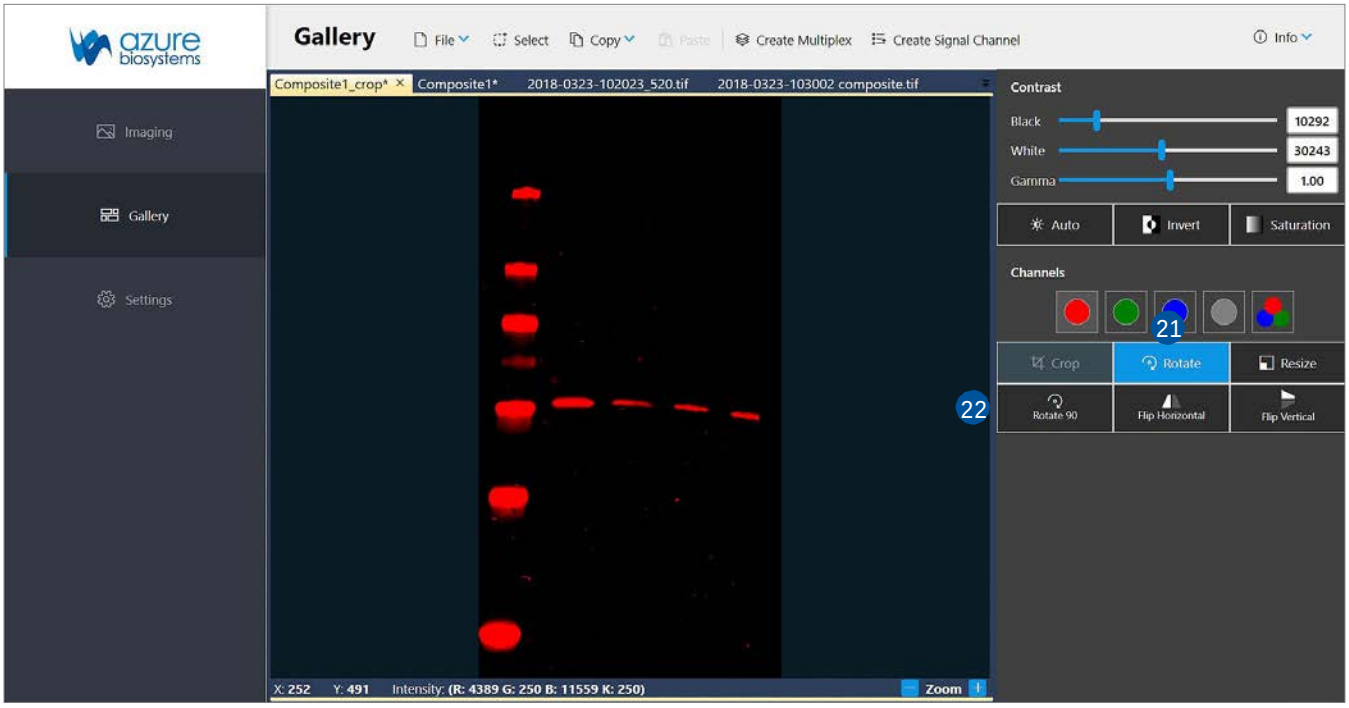
4.6 The Image Gallery

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 had 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.






azure biosystems
Gallery
File
Select
Copy
Paste
Create Multiplex
Create Signal Channel
Info
Imaging
Gallery
Settings
Composite1_crop*
Composite1*
2018-0323-102023_520.tif
2018-0323-103002 composite.tif
Contrast
Black
10292
White
30243
Gamma
1.00
Auto
Invert
Saturation
Channels
21
Crop
Rotate
Resize
22
Rotate 90
Flip Horizontal
Flip Vertical
X: 252
Y: 491
Intensity: (R: 4389 G: 250 B: 11559 K: 250)
Zoom


azure biosystems
Gallery
File
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Create Multiplex
Create Signal Channel
Info
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Composite1_crop*
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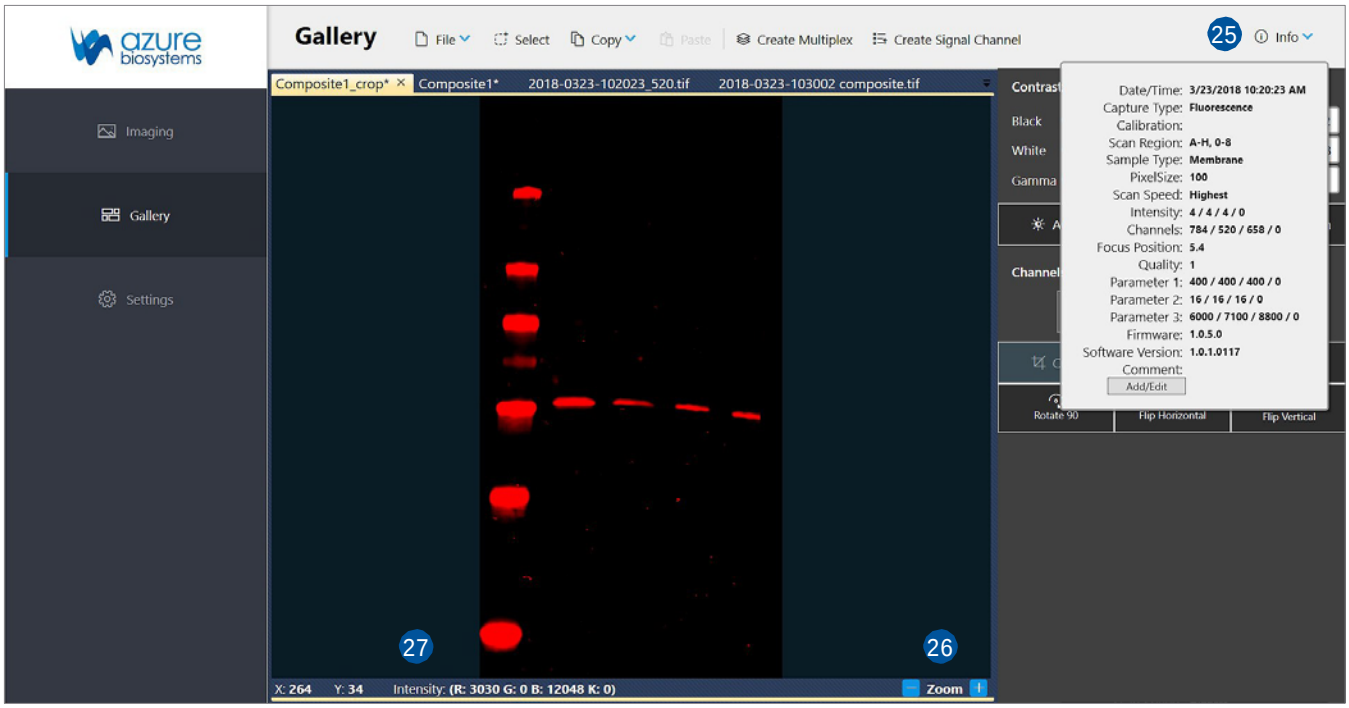
2018-0323-102023_520.tif
2018-0323-103002 composite.tif
Contrast
Black
10292
White
30243
Gamma
1.00
Auto
Invert
Saturation
Channels
Crop
Rotate
Resize
23
Image Size
Width
286
Height
550
pixels
DPI
300
24
Accept
X: 264
Y: 34
Intensity: (R: 3030 G: 0 B: 12048 K: 0)
Zoom



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 boarders 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.