Azure Imaging Systems

User Manual

for the Azure 200, Azure 280, Azure 300, Azure 300Q, Azure 400, Azure 500, Azure 500Q, Azure 600

Safety and Regulatory Compliance

Important Safety Information

Please read these instructions before operating the Azure Imaging System.

UV Safety Precautions

> ⚠️ **UV** **CAUTION** Because U.V. radiation can cause serious damage to unprotected eyes and skin, we recommend to wear U.V. protection glasses or face shield.

The Azure Imaging System comes with a built-in ultraviolet (UV 200-400nm) Transilluminator. Exposure to UV radiation can cause permanent damage to the eyes and skin. The enclosure confines the radiation within the system and shields the user from exposure. The system is also equipped with a two-way safety interlock switch which automatically cuts off the power to the transilluminator when the door is opened during normal use.

It may become necessary to defeat the safety lock or operate the transilluminator outside the system for service or band excision. In these cases, be sure to use the following safety precautions:

- Always wear UV-protected eyewear that is specified by the safety equipment manufacturer as providing protection at the wavelength(s) used, making sure that the eyewear protects any areas where radiation may come through (UV sunglasses may not prevent UV radiation from coming in through the sides or around the lenses).
- Always cover all skin that may be exposed to UV light, especially the face, neck, hands, and arms.
- Always make sure that any UV protection devices (such as the safety switch on the light cabinet apparatus) are working properly. If not, discontinue use until the device(s) are properly repaired.
- Use only UV lamps in the transilluminator and ensure they are the correct size and voltage.

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 ground 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 of the back panel.

Hot surface warning

Under normal conditions, the temperature of glass surface of UV transilluminator is below 50 °C and safe to touch. However, if the system malfunctions, it is possible that the glass surface temperature may exceed 80°C. Please exercise caution when touching the glass surface if this occurs.

Laser Safety

General Information

The Azure Imaging Systems models 600 and 500 both include a laser illumination system that provides 685nm and 784nm narrow band excitation for NIR applications. The imaging systems are certified to comply with CE, UL and CSA safety standards. The laser modules consist of two fully enclosed electronic modules located on the right and left interior sides of the imaging chamber. 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

The Azure Imaging Systems are designed to prevent direct and collateral human exposure to radiation by means of a safety interlock switch located on the right front side of the imaging chamber. The safety interlock reacts to "door open" and "door closed" states and defeats all power to internal light sources when the door is in the "open position". Lasers and other internal system light sources will not power on unless the door is fully closed. If the access door is opened during imaging, all light sources will immediately power off to prevent human exposure to internal illumination sources. Right and left laser modules located in the 500 and 600 imaging chambers are installed in a highly diffused protective metal enclosure preventing exposure to full laser radiation power. 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. It is possible to override the safety switch, which allows the user to turn on the light sources with the door open, which may be useful for band excision in UV.

Maintenance

The Azure Imaging Systems models 500 and 600 do not require regular, periodic, or preventive maintenance in the form of adjustments, calibrations, cleaning, 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 in either the left or right laser module, a significant difference between the output signal levels between the left and right lasers modules, or complete loss of output signal level in either or both laser modules. 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 Azure Imaging Systems models 500 and 600 contain a defeatable safety interlock system. It is not recommended or advised by Azure Biosystems, under any circumstances, for users to defeat the interlock system and perform laser imaging, or imaging with any 500 or 600 light source with the access door open. The access door must be fully closed.

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 Imaging System products are warranted 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.
- Unauthorized movement of the Azure Imaging System.

Voltage Setting Information

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

CE Conformity

The following Azure Imaging Systems, models: 200, 280, 300, 300Q, 400, 500, 500Q, 600 are in conformity with the provisions of the following EC Directives, including all amendments, and national legislation implementing these directives:

- Low Voltage Directive 2006/95/EC
- EMC Directive 2004/108/EC

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

FCC Standards on Radio Frequency

This device complies with Part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) this device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation.

Note: This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense.

Changes or modifications not expressly approved by the party responsible for compliance could void the user's authority to operate the equipment.

Contact

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Table of Contents

1.   Introduction

The Azure Imaging System offers high performance imaging for a wide variety of applications. The family of instruments includes the following products: 200, 280, 300, 300Q, 400, 500, 500Q, and 600.

Select which one fits your applications now, and learn more about upgrading later when your needs change.

Each Azure imaging workstation can be upgraded in the field to a higher model with a wider range of applications. Contact your Azure representative to find out about upgrade options at info@azurebiosystems.com.

The Azure imaging system includes the following key components:

- **Cabinet** – The cabinet is a light tight image station. It includes the camera and lens, light sources, filter wheel, integrated touchscreen computer, Ethernet, and USB ports for data collection and external computer control.

- **Camera** – High resolution camera, preinstalled in the system.

- **UV Transilluminator** – The UV illuminator provides 302nm and 365nm trans illumination. It is mounted on a pull-out tray.

- **EPI LEDs** – The LED module allows you to take images using Blue Light excitation and true color images.

- **EPI RGB LEDs** – High intensity LED illuminators (Azure 400 and 600).

- **Solid-State Laser** – (Azure 500 and 600).

- **Filter Wheel** – The 7-position filter wheel is automated. It includes the standard Orange filter for most UV fluorescent applications, and additional Red, Green, Blue, and/or IR filters for multichannel systems.

- **Integrated Computer** – The touch screen PC is completely integrated for system control and image acquisition.

- **Black Tray** – Stored in the front door, the Black Tray (also called Blot Tray) is required for imaging all blots. For fluorescence and chemiluminescence applications that do not use the UV Transilluminator, it can be placed on the UV transilluminator, or on the top shelf in the cabinet.

- **Trans White Table** – The Trans White Table can be plugged into the system with the cable behind the UV transilluminator. This table is placed on top of the UV transilluminator and provides an even light source that can be used for imaging of colorimetric gels, silver stain gels, cell-culture plates and other transparent, colorimetric samples. The Trans White Table will only turn on when the system door is closed.

- **USB Ports** – There are 2 USB ports for data transfer. One located on the front panel, and another on the lower right side of the cabinet. A USB A to B port is also available for optional connection to an external computer.

- **USB Memory Stick** – The Azure Biosystems memory stick contains calibration files relevant to your imaging system.

- **AzureSpot Analysis Software** – Please contact our technical support to receive your electronic license at support@azurebiosystems.com. Be sure to have your system serial number when you reach out for support for the AzureSpot Analysis Software license keys.

1.1 Table of Specifications

Image Resolution	5.4M pixels (200); 6.1M pixels (280); 9.1M pixels (300, 300Q, 400, 500, 500Q, 600)
Epi-illuminations	Blue LED; RGB LED (400, 600); 685nm Solid-State Laser (500, 500Q, 600); 784nm Solid-State Laser (500, 500Q, 600)
Trans-Illumination	302nm/365nm
Maximum Field of View	20.5 cm x 16.5 cm
Image output	16-BIT TIFF, 8-BIT TIFF, JPEG, BMP
Working environment	Ambient temperature/humidity: 0–30°C / 85%
Power requirement	100-240 VAC, 4A
Dimensions (W x D x H)	42 cm x 56 cm x 33 cm
Weight	20.3 kg (44.8 lbs)

Azure 600	Azure 500	Azure 400	Azure 300	Azure 280	Azure 200
NIR FLUOR	NIR FLUOR				
RGB FLUOR		RGB FLUOR			
CHEMI	CHEMI	CHEMI	CHEMI	CHEMI	
UV FLUOR	UV FLUOR	UV FLUOR	UV FLUOR	UV FLUOR	UV FLUOR
BLUE FLUOR	BLUE FLUOR	BLUE FLUOR	BLUE FLUOR	BLUE FLUOR	BLUE FLUOR
TRUE COLOR	TRUE COLOR	TRUE COLOR	TRUE COLOR	TRUE COLOR	TRUE COLOR
TRANS WHITE	TRANS WHITE	TRANS WHITE	TRANS WHITE	TRANS WHITE	TRANS WHITE
	Upgradable to 600	Upgradable to 600	Upgradable to 400, 500, and 600		Upgradable to 300, 400, 500, and 600

- Upgrades available for chemiluminescence, RGB fluorescence and NIR fluorescence.
- Q Module—Upgrade the Azure 500 and Azure 300 to include a green fluorescence Q module to add a dedicated channel for total protein normalization in quantitative Western blots.

1.2 Contacting Technical Support

For questions about installation, setup or general use of the system, please contact support@azurebiosystems.com or call (925) 307-7127, 9am–4pm PST (GMT-8).

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 imaging system 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.

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 Imaging System should be installed at no more than 3000 meters above sea level. The unit should be placed out of direct, bright sunlight or illumination sources to guard against light leaks.

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. Azure Biosystems recommends that you employ a surge protecting power strip to protect against unexpected power surges that may cause damage to the electrical components.


2.3 Turning the System On/Off

The main power switch is located at the back panel, next to the power cord.

1. First, flip the power switch to On (l).
2. To wake the integrated computer, press the power button on the front of the system. The power button on the front of the system will light up green when the system is on.
3. Launch the Azure Imaging System software from the icon on the desktop.

To turn off the system, shut down the built-in computer by:

1. Closing the Azure Imaging System Software from the arrow icon on the bottom left-hand side of the screen, or swiping left to right on the left side of the screen to minimize the software window.
2. Turn off power to the system using the power switch on the back of the instrument.

Azure Biosystems recommends leaving the system on during working hours.
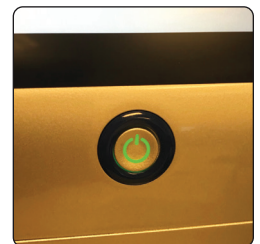
2.4 Software Installation

The Azure Capture Software is pre-installed. When the 13.3-inch touchscreen computer running Windows 10 Enterprise is powered on, launch the Azure Imaging Systems software from the icon on the desktop. If the software has been closed, double click on the desktop icon.

Once the system is done initializing, the software displays the Home screen, and is ready to use.

Please contact Azure technical support at **support@azurebiosystems.com** for questions about upgrading the software.

2.5 Other USB Input Devices

You may attach regulatory approved, Windows OS supported USB keyboard, USB mouse or other USB input devices to either the front or upper USB port on the side of the instrument.

3.   Image Capture Overview

The Azure Imaging System captures high quality images with an intuitive user interface. The icon based software allows you to program multiple applications with the touch of a button. There is no need to adjust the focus on your sample. Simply put your sample in the system, select an exposure time, then hit capture.

3.1 The home page contains the following imaging options:

1. **Chemi Blot** – for samples with luminescent and chemiluminescent signals such as from ECL-HRP. See Section 3.3 for additional information.

2. **Fluorescent Labels** – for samples with fluorescent signals in up to four channels (red, green, blue, and grayscale) for multiplex imaging. See Section 3.4 for additional information.

3. **Nucleic Acid Gels with Dyes** – image fluorescent stained nucleic acid gels. See Section 3.5 for additional details.

4. **Protein Gels with Dyes** – image stained protein gels. See Section 3.6 for additional details.

5. **True Color Imaging** – image colorimetric dyes and colored samples with full color rendering. See section 3.7 for details.

6. **Custom** – customize each imaging specification. Protocols created here can be saved for later use and recalled using the Protocols button. See Section 3.8 for additional details.

Other options include:

7. **Protocol selection** – use to create and recall saved, custom imaging protocols.

8. **Auto Save Functionality** – automatically save any image taken during a session.

On the left-hand side menu there are the following buttons:

9. Reach the home page at any time by clicking ⌂ .

10. Access the Gallery ⊞ to view and edit images. See Section 4.1 for details.

11. Access this User Manual at any time by clicking ⧉ .

12. ⚙ takes you to Settings. See Section 4.3 for details.

13. Click ⏏ to exit the program and return to the desktop.

14. Time and date based on Windows settings.

15. Software version number.

3.2 Key definitions that apply to all imaging types:

1. **Auto Image vs Manually Image**
   - **Auto Image** – determines your optimal exposure time automatically.
   - **Manually Image** – enables you to set each parameter to your preferred specifications.

2. **Pixel Binning** – refers to the pixel binning setting (i.e. how the pixels are grouped). The higher the pixel binning, the lower the image resolution. 1x1 uses the full resolution of the camera, and other possible settings are 2x2, 3x3, 4x4, 6x6, and 8x8. Therefore, choose 1x1 for the highest resolution and widest dynamic range.

40 seconds, unbinned

40 seconds, 2X2 bin
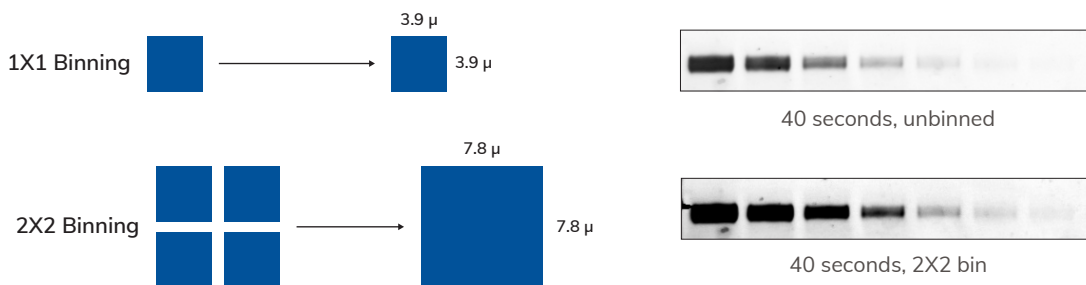
Pixel binning improves image sensitivity. Pixel binning is a powerful technique for digital imaging that increases sensitivity by combining pixels to make a larger "super-pixel." The super-pixel images improve the signal-to-background ratio (SBR) when compared against unbinned images.

3. **Exposure** – choose the exposure time manually or use autoexposure, which will allow the software to capture the image for you using the full dynamic range of the camera. There are four options for Exposure:
   - **Rapid Capture** – software will optimize a short exposure time to the brightest signal on the blot.
   - **Overexpose** – 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. This will saturate part of the image.
   - **Wide Dynamic Range** – 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. Uses the full dynamic range of the camera.
   - **Manual** – select a custom exposure time. The maximum exposure time is 60 minutes for chemiluminescence detection and 5 minutes for fluorescence imaging.

4. **Live Mode** – in Manually Image menu, tap [icon] to enable Live Mode. Click again to close. Live Mode illuminates the imaging area to allow you to see a live image to verify sample placement.
   - **Auto-exposure to region** – set area that you want the autoexpose to focus on.
   - **Capture selected region** – generate an image of the selected region of interest.

3.3 Chemi Blot Imaging Mode

Use this with samples that have luminescent and chemiluminescent signals such as from HRP.

1. Place sample in the center of the Black Tray.

2. Place the tray in one of two positions: on the UV transilluminator or on the upper shelf, in the middle of the imaging system.

   Note that using the upper shelf will block the shelf underneath, so shelves must be used one at a time.

   - Use the position on the UV transilluminator for larger samples.
   - Use the position on the upper shelf for small samples. Using this top shelf increases the resolution of the blot.

3. Close the door to the system.

4. Select **Chemi Blot** from the home screen.

5. The program is set to image chemiluminescent signal in the gray channel.

6. Choose additional imaging channels, such as Blue Ladder for imaging blue-stained ladders using red fluorescence, or TotalStain Q for total protein normalization, if the corresponding channels are available in the system.

   To select an additional dye, hit one of the colored ⊕ buttons to bring up the dye selection menu. Click the name of a dye to select it.

   Note: List of dyes available is customizable in the Settings tab.

7. Select **Color Marker** to create an overlay of the visible molecular weight marker/ladder and the ECL signal. The marker image can be manually overlaid on top of the chemi image, maintaining the positions on the gel or membrane in the gallery, or automatically merged after capture by selecting **Auto Merge** in Settings. The **Color Marker** button will appear green when active.

   Note: The option for color marker imaging will disappear if multiplex imaging the chemi channel with additional fluorescent channels (such as fluorescent ladders and total protein stains).

8. Use **Auto Image** if you want the system to determine the optimal default exposure for each selected channel using the RapidCapture exposure mode.

9. Select **Manually Image** to control all imaging settings.

10. Select the **Pixel Binning** setting based on the desired sensitivity and imaging resolution. See Section 3.2 for more details.

11. Exposure type can be selected for each channel. Select between the different auto exposure modes or choose **Manual Exposure Type** to enter the desired exposure time. See Section 3.2 for more details on the different Auto Exposure options.

12. The CCD Cooled icon will turn green once the CCD has reached optimal cooling.

13. The info button offers recommendations on imaging and directions on whether a tray is required for the imaging type chosen.

14. In **Manually Image** menu, tap ⟨•⟩ to enable **Live Mode**. Click again to close. Live Mode illuminates the imaging area to allow you to see a live image of the position of your sample, and offers the following additional options:

    - **Auto-exposure to region** – set area that you want the autoexpose to focus on.
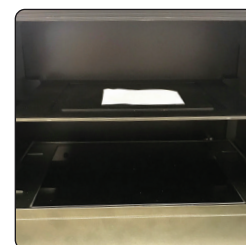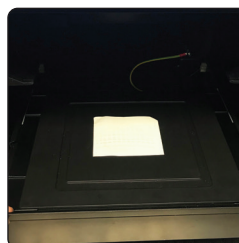
- **Capture selected region** – will generate an image of the selected region.

15. Select the **Capture** button to capture the image according to the set specifications.

16. When imaging a single channel chemiluminescent image with or without color marker, the additional option of Imaging Mode is available. Select between Single image capture, Cumulative image capture and Multiple image capture.

    - **Single** – captures a single image according to the exposure type selected.

    - **Cumulative** – captures multiple images at the user set time interval and cumulates the signal of each subsequent image. 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 (default. Number of exposures adjustable in Settings/General). The first image displayed will be a one minute exposure. The second image displayed, at the two minute interval, will be a two minute exposure. The final, tenth image, will be the sum of ten, 1-minute images. Images appear and can be viewed in the Gallery tab as they are acquired.

    - **Multiple** – select between 1 and 5 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

17. After capture, images will appear in the Gallery automatically. The Gallery can be reached at any time by pressing the Gallery icon.

18. Go back one screen at any time by pressing the back icon.
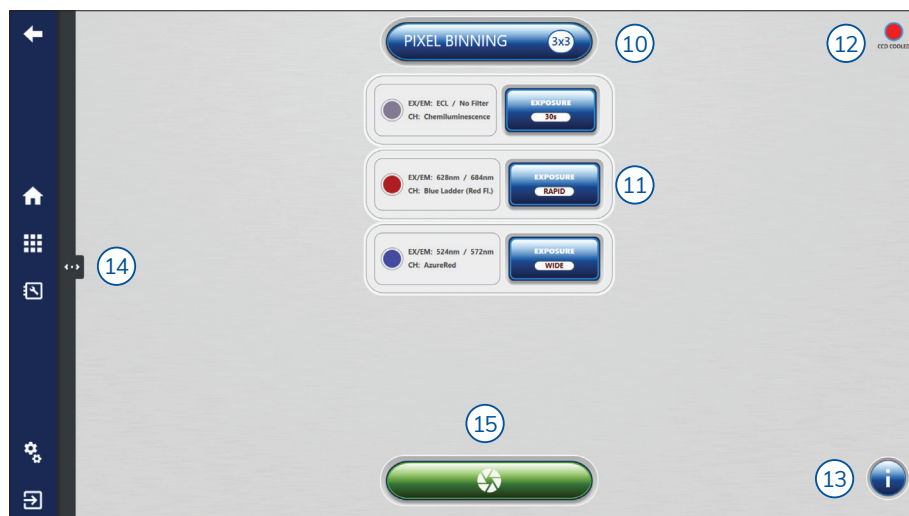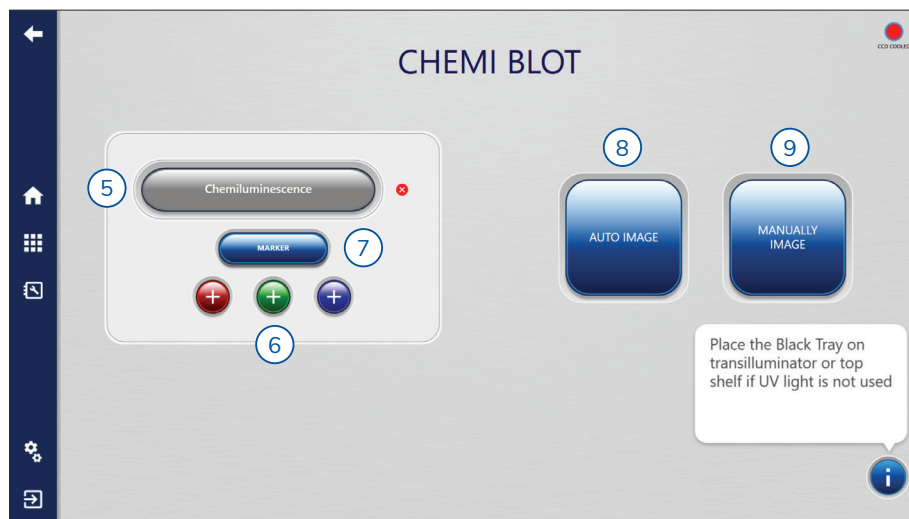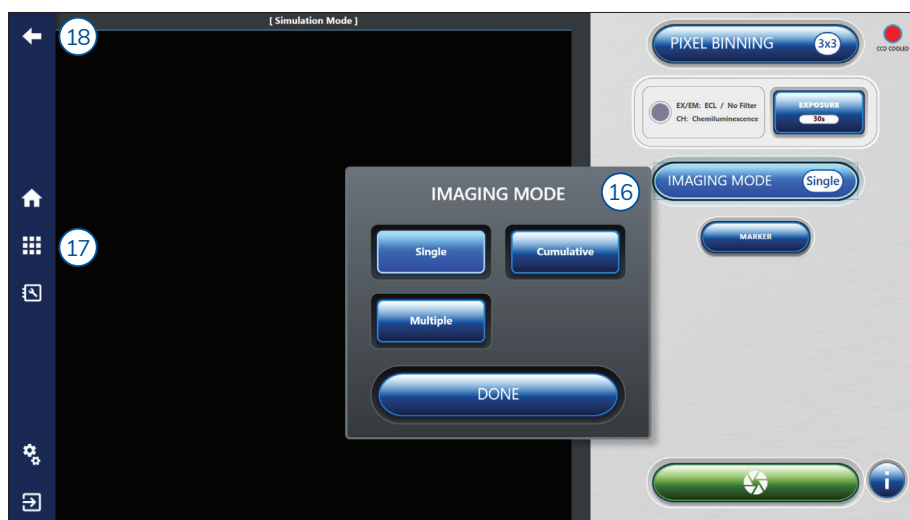
3.4 Fluorescent Labels Imaging Mode

Use this for samples with fluorescent signals in up to four channels (displayed in red, green, blue, and grayscale pseudocolor) for multiplex imaging.

1. Remove the black tray from its position on the inside of the door, and place the fluorescent blot or gel in the center of the tray. Then place the tray and sample on the bottom or top shelf inside the imager.

2. Close the Azure Imaging System door.

3. Select **Fluorescent Labels** from the home screen.

4. Select desired fluorescent labels by clicking on any one of four color channels: red, green, blue, and gray, and selecting the desired dye to assign it to that pseudo-color channel.

   Note: List of dyes available is customizable in the Settings tab.

5. Use **Auto Image** if you want the system to determine the optimal default exposure for each selected channel using the RapidCapture exposure mode.

6. Select **Manually Image** to control all imaging settings.

7. Select the **Pixel Binning** setting based on the desired sensitivity and imaging resolution. See Section 3.2 for more details.

8. Exposure type can be selected for each channel. Select between the different auto exposure modes or choose **Manual Exposure Type** to enter the desired exposure time. See Section 3.2 for more details on the different Auto Exposure options.

9. The info button offers recommendations on imaging and directions on whether a tray is required for the imaging type chosen.

10. In **Manually Image** menu, tap [icon] to enable **Live Mode**. Click again to close. Live Mode illuminates the imaging area to allow you to see a live image and offers the following additional options:

    - **Auto-exposure to region** – set area that you want the autoexpose to focus on.
    - **Capture selected region** – capture an image of a selected region of interest.

11. Select the **Capture** button to capture the image according to the set specifications.

12. After capture, images will appear in the Gallery automatically. The Gallery can be reached at any time by pressing the Gallery icon.

13. Go back one screen at any time by pressing the back icon.
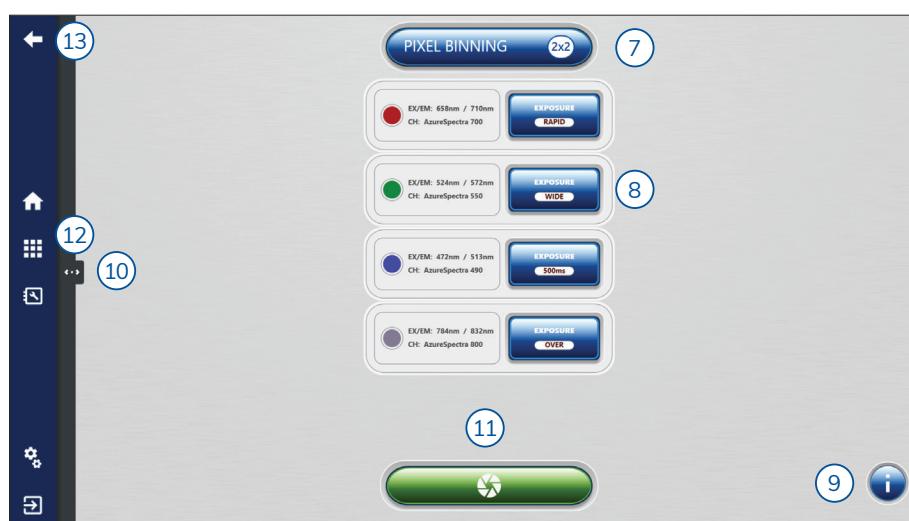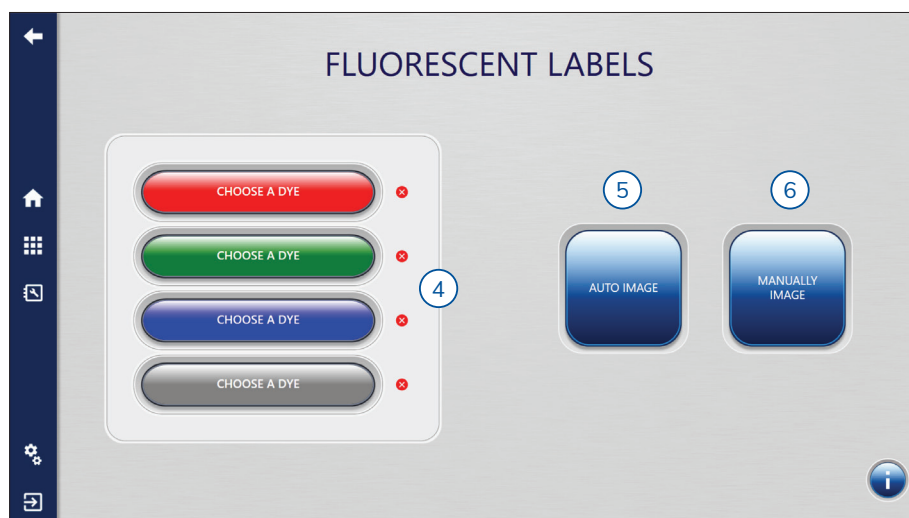
3.5 Nucleic Acid Gels with Dyes Imaging Mode

Use this for nucleic acid samples stained with fluorescent dyes (displayed in red, green, blue, and grayscale pseudocolor).

1. Open door and place sample directly on trans UV illuminator if using UV modes. If using Blue light for the excitation, place sample on the black tray and place in the imager.

2. Close the Azure Imaging System door.

3. Select **Nucleic Acid Gels with Dyes** from the home screen.

4. By default, the software will be set to gray as the primary channel. Click the gray **Choose a Channel** button to select the desired dye for imaging.

   Note: List of dyes available is customizable in the Settings tab.

5. Add additional dyes if desired by clicking on any one of the three additional color channels: red, green, blue, and selecting the desired dye to assign it to that color channel

6. Use **Auto Image** if you want the system to determine the optimal default exposure for each selected channel using the Wide Dynamic Range exposure mode.

7. Select **Manually Image** to control all imaging settings.

8. Select the **Pixel Binning** setting based on the desired sensitivity and imaging resolution. See Section 3.2 for more details.

9. Exposure type can be selected for each channel. Select between the different auto exposure modes or choose **Manual Exposure Type** to enter the desired exposure time. See Section 3.2 for more details on the different Auto Exposure options.

10. In **Manually Image** menu, tap [icon] to enable **Live Mode**. Click again to close. Live Mode illuminates the imaging area to allow you to see a live image and offers the following additional options

    - **Auto-exposure to region** – set area that you want the autoexpose to use to determine exposure time.
    - **Capture selected region** – capture an image of the selected region of interest.

11. **UV Override** is useful for band excision, or pre-warming the UV bulbs. Click the UV Override button to engage.

    - For band excision, click the **Band Excision** button and use the key located on the door of the Azure Imager to manually override the UV safety mechanism in the door lock of the imager. Use the correct protection described in the UV Safety Precautions sections of this manual.

12. Select the **Capture** button to capture the image according to the set specifications.

13. After capture, images will appear in the Gallery automatically. The Gallery can be reached at any time by pressing the Gallery icon.

14. Go back one screen at any time by pressing the back icon.
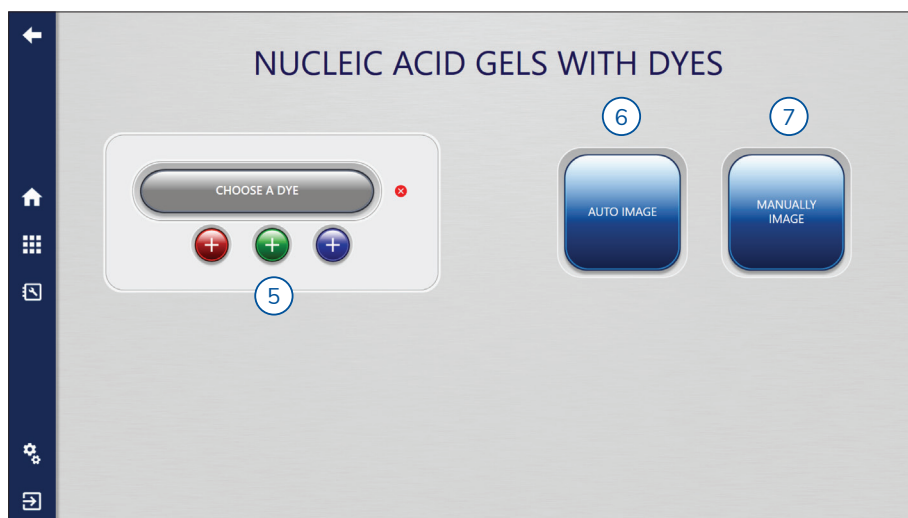
3.6 Protein Gels with Dyes Imaging Mode

Use this for protein gel applications. There are different imaging modalities depending on the dye:

- Fluorescent dyes excited with UV light. Sample placed on the transilluminator (e.g.: light-induced crosslinked UV dyes).
- Fluorescent dyes excited with EPI light (RGB, NIR700, NIR800). Sample placed on top of the Black Tray (e.g.: SYPRO Ruby, Coomassie Blue fluorescence).
- Colorimetric dyes visualized with visible light. Sample placed on top of the Orange Tray or Trans White Table (Silver Stain, Coomassie Blue).

1. Place sample in the center of the field of view (UV Transilluminator or Black Tray, depending on the detection mode).
2. Close the Azure Imaging System door.
3. Select **Protein Gels with Dyes** from the home screen.
4. By default, the software will be set to gray as the primary channel. Click the gray **Choose a Channel** button to select the desired dye for imaging.

   Note: *List of dyes available is customizable in the Settings tab.*
5. Add additional dyes if desired by clicking on any one of the three additional color channels: red, green, blue, and selecting the desired dye to assign it to that color channel.
6. Use **Auto Image** if you want the system to determine the optimal default exposure for each selected channel using the Wide Dynamic Range exposure mode.
7. Select **Manually Image** to control all imaging settings.
8. Select the **Pixel Binning** setting based on the desired sensitivity and imaging resolution. See Section 3.2 for more details.
9. Exposure type can be selected for each channel. Select between the different auto exposure modes or choose **Manual Exposure Type** to enter the desired exposure time. See Section 3.2 for more details on the different Auto Exposure options.
10. In **Manually Image** menu, tap ⟨•⟩ to enable **Live Mode**. Click again to close. Live Mode illuminates the imaging area to allow you to see a live image and offers the following additional options.

    - **Auto-exposure to region** – set area that you want the autoexpose to use to determine exposure time.
    - **Capture selected region** – capture an image of the selected region of interest.
11. **UV Override** is useful for pre-warming the UV bulbs or for light-induced cross-linking of UV-activated for total protein staining. Click the UV Override button to engage.

    - Select **Light On 5 Minutes** for UV dye activation. Check the box for automatic image acquisition if you would like the imager to acquire an image immediately after the 5 minutes.
12. Select the **Capture** button to capture the image according to the set specifications
13. After capture, images will appear in the Gallery automatically. The Gallery can be reached at any time by pressing the Gallery icon.
14. Go back one screen at any time by pressing the back icon.
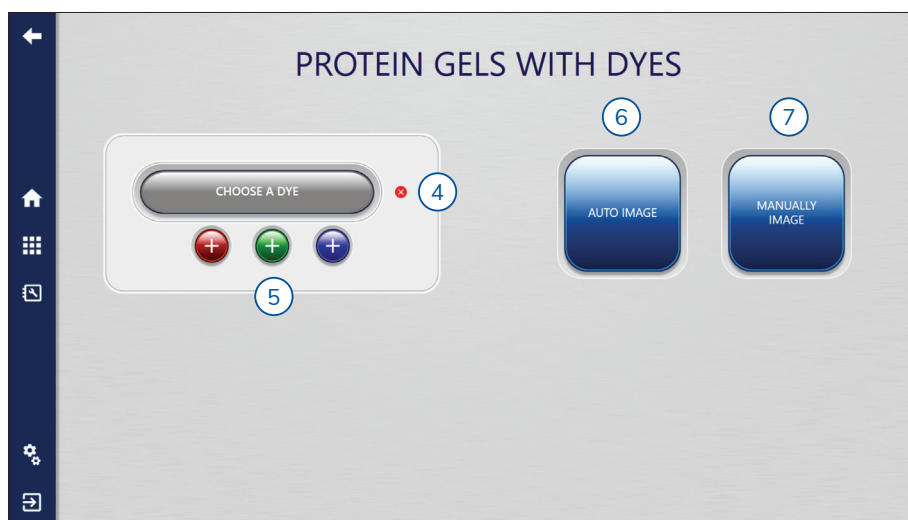
3.7 True Color Imaging Mode

Use this mode to capture the true color of opaque or translucent colorimetric samples.

1. Place sample in the center of the field of view using the tray/table indicated by each imaging option.
2. Close the Azure Imaging System door.
3. Select **True Color Imaging** from the home screen.
4. Choose the imaging type based on sample type
    - Use **Opaque Sample Epi** for opaque colorimetric samples such as colorimetric western blots or TLC plates using the Black Tray.
    - Use **Translucid Sample Epi** for imaging translucent samples such as Coomassie gels using the White Tray.
    - Use **Translucid Sample Trans** for imaging translucent samples such as Coomassie gels using the Trans White Table

      Note: This feature is only available in the Azure 400 and 600 imagers.
5. Click **Auto Image** to capture image. The system will determine the optimal exposure using the Wide Dynamic Range exposure mode.
6. After capture, images will appear in the Gallery automatically. The Gallery can be reached at any time by pressing the Gallery icon.
7. Go back one screen at any time by pressing the back icon.

TRUE COLOR IMAGING
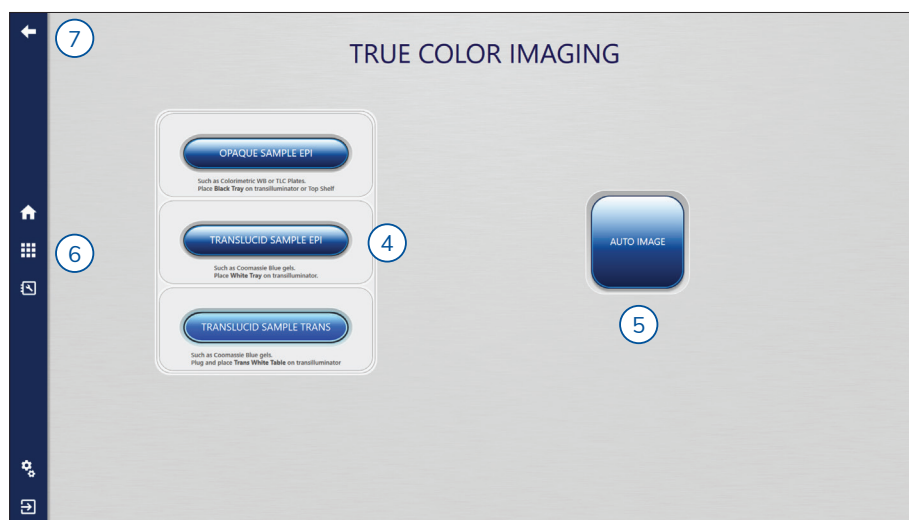
3.8 Custom Imaging Mode

Use this imaging mode to select every aspect of your imaging protocol.

1. Place sample in the imager using the black tray if necessary and close the Azure Imaging System door. If the Black Tray is used, make sure that the tray is in the desired position (i.e.: on the top shelf or on the transilluminator) before selecting Custom Imaging on the Home Screen. The position of the Black Tray will select the default focal plane for imaging.

2. Select **Custom** Imaging from the home screen.

3. Click on the **Protocols** button. Select an existing protocol to use as a base or select New Protocol to create a new protocol from scratch.

4. Select the **Pixel Binning** setting based on the desired sensitivity and imaging resolution. See Section 3.2 for more details.

5. Select the appropriate **Light Source** and **Emission Filter** and assign the desired pseudo color to the channel.

6. Adjust the aperture according to sample type or as desired. We recommend a wider aperture for chemiluminescence and a mid-level aperture for RGB and NIR. The most narrow aperture is best for UV applications.

7. Select to adjust the camera focus. The focus value in custom defaults to the focus value of the selected filter and tray position. If desired, the focus can be manually adjusted by moving the slider.

   Note: For Azure 200, the focus does not need to be adjusted for the UV table or black tray.

8. Select to adjust the exposure type for each channel. Select between the different auto exposure modes or choose **Manual Exposure Type** to enter the desired exposure time. See Section 3.2 for more details on the different Auto Exposure options

9. Click to add additional dyes to the capture protocol. Repeat steps 5 through 8 for each dye added (e.g. Excitation/emission/channel, aperture, focus, and exposure times). Up to four individual dyes may be captured per image (displayed in red, green, blue, and grayscale pseudocolor).

10. Select to display a preview image using the selected settings.

11. Click to enter **Live Mode** to visualize the position of the sample.

12. **Save Protocol** to recall the protocol as set after imaging, or **Delete Protocols** as needed.

13. Select the **Capture** button to capture the image according to the set specifications.

14. After capture, images will appear in the Gallery automatically. The Gallery can be reached at any time by pressing the Gallery icon.

15. Go back one screen at any time by pressing the back icon.
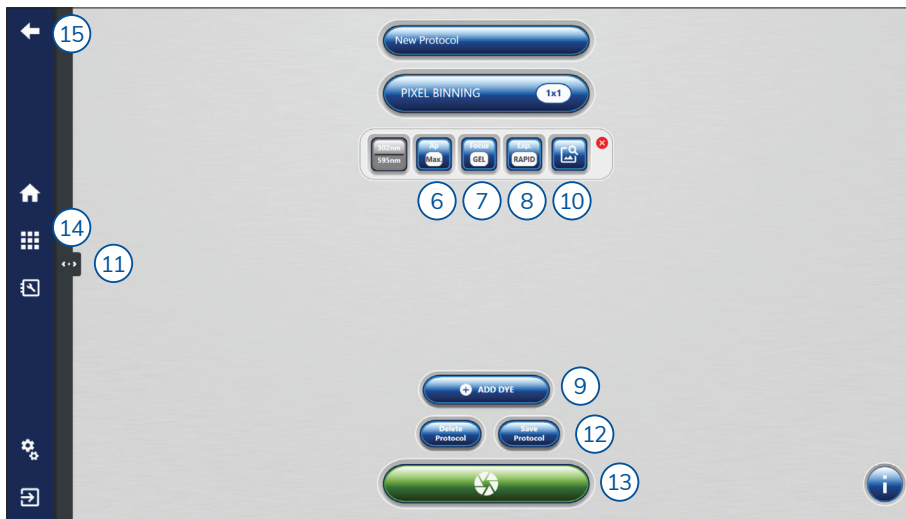
4.   Image Gallery Overview

This section discusses the ways in which you can visually analyze images.

4.1 Gallery

Reach the gallery at any time by clicking ▦ .

1. The following options are available within the File dropdown menu:

    - **Open** – open an image that is stored locally, on a USB, or on the network.
    - **Close** – close the image that you're currently viewing.
    - **Close All** – close all images currently open in Gallery.
    - **Save** – save an image to a USB, the integrated computer, or to a network drive. Images can be saved as a TIFF, Publication TIFF, JPEG or BMP. File names are automatically generated with the date and time stamp. You can overwrite this name by editing it in the displayed textbox.
    - **Save As** – save a copy of a previously saved image with an alternate name, file type, or to an alternate file location.
    - **Save All** – saves any unsaved images open in the gallery tab. The file names will be automatically generated with the date and time stamp.
    - **Export to AzureSpot** – export files and multiplex images as files ready for AzureSpot analysis.
    - **Exit** – exit the software. Program will prompt you to save any unsaved images. If you leave the gallery by the home button, the image(s) will still be there when you return to the gallery.

2. From the Print dropdown menu, you can:

    - **Print** – print to a local printer connected to the system, or to a network printer.
    - **Print Report** – print the image you're viewing as well as data about the image. These include image name, date, protocol type, capture type, binning, exposure time, exposure type, channel name, light sources, filter, aperture, tray type, focus, calibration, software version, and serial number. The images must be the same size and resolution.

3. Create a multichannel image by merging between two and four channels.

4. Create **Single Channel** separates multiplex images into individual, single-channel images.
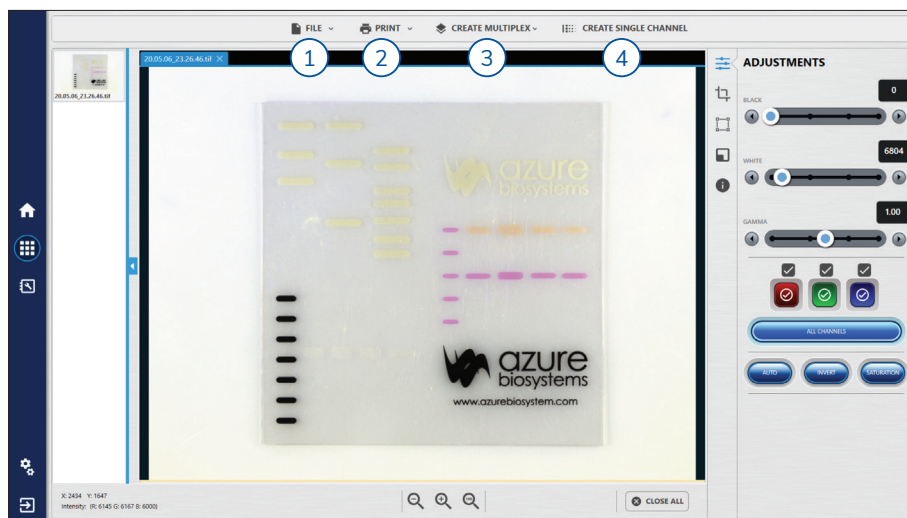
4.2 Image Adjustment Functions

1. **Adjustments**
   - Adjust the black, white and gamma levels of the image.
   - For multi-channel images, use the colored box icons to select which channels are active for viewing and adjusting. Check the box within the color icon to make a channel active for viewing. Check the box above the color icon to make a channel active for adjustments. Click All Channels to activate All Channels for viewing and adjusting.
   - Clicking Auto will automatically adjust levels of the channels selected for adjustment.
   - Click Invert to invert the colors of the signal and background.
   - Saturation will highlight in pink which pixels are beyond the detection limit of the camera.

     Note: Adjusting these settings will not affect the raw data when saved as a .tiff file (16-Bit image). Only images saved as a .jpg, BMP, and PUB tiff (8-Bit images) will display with the adjusted contrast settings. Azure recommends using .tiff formats for images that need to be quantified, and saving a copy in 8-Bit format with contrast adjustments for publication purposes.

2. **ROI** – select of region of interest for further actions including crop and copy.
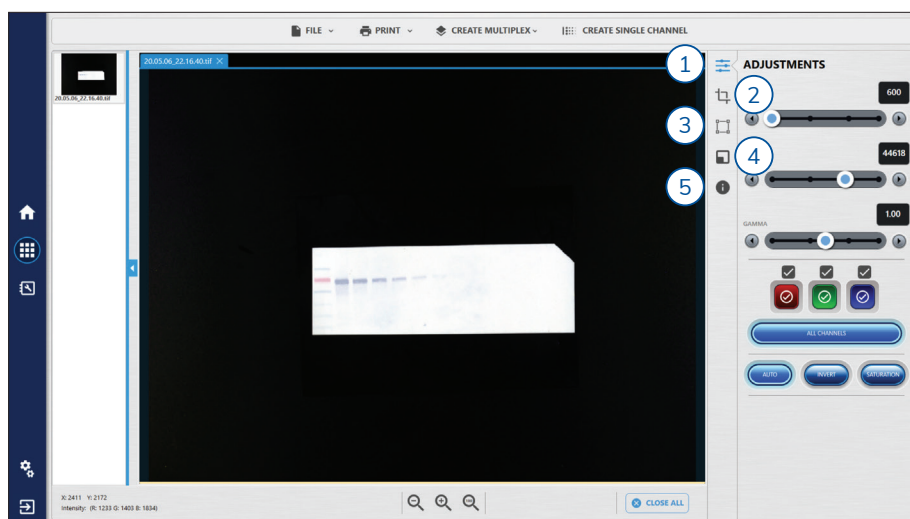   - Select:
     - Crop in order to isolate a particular area of the image. A new tab will open with an image of the selected area.
     - Copy a region of interest on a single channel image. After selecting another image of the same size, use the paste option to overlay the copied image.
   - Clear clipboard erases the saved image done with cropping or copying so you can select a new region.

     Note: If a Marker image was taken alongside a chemi image and the Auto Merge function was not enabled, the Marker and chemi images can be merged using the ROI function. Use ROI to select the marker area from the Marker image, copy, and paste onto the chemi image to merge.

3. **Image Orientation** – rotate or flip images using the options provided. The free rotate function is helpful for images samples that were not imaged straight.

4. **Resize** – customize width, height, and dots per inch (DPI) for 8-Bit publication images.

5. **Info** – gives detailed information about the active image. Displays the parameters for image acquisition including date, protocol type, capture type, protocol, author, binning, exposure time, exposure type, channel name, light sources, filter, aperture, tray type, focus, calibration, software version, serial number, and comments.

5. Settings Overview

You can reach the Settings at any time by clicking 	⚙ .

5.1 General

- **Dye List** – shows a full list of all dyes currently programmed into the system. Add dyes to the list by entering a Channel Name, selecting the Light Source and Emission Filter, selecting the Sample Type(s) and pressing Add.

- **File Saving** – settings allows you to enable the automatic saving of 8-Bit publication images.

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

- **Chemi Imaging Mode** – allows you to select the number of images in cumulative. The default is 10.

- **Check Auto Merge Chemi and Marker Image** – image to enable automatic merging of chemi and color marker images upon capture.

- **Auto-Exposure**

  - **Rapid capture** – will calculate an optimal exposure time based on the signal ceiling entered set here. The ceiling range is 0 to 65,535.

- **Simulation Mode** – allows you to run the software even if not connected to an Azure Imaging System.

5.2 Create Darkmasters, Focus Calibration, Create Flats

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