# PERFORMANCE WITHOUT COMPROMISE

# Azure Imaging Systems



# Reliable, reproducible imaging for Western blots and more

Azure Biosystems provides a unified Western Blot workflow, from the high-performance imaging system and analysis software to the reagents and consumables. Our imaging systems give you the flexibility you need for your research, while delivering solutions for quantitative Western blot imaging.



### Each Azure Imaging System provides:

- **Flexibility**—High sensitivity and performance for both chemiluminescent and fluorescent imaging
- Quantitative accuracy—Designed for quantitation, our reagents, imaging system, and software work seamlessly together to help you follow best practices for Western blot publication
- Intelligent workflow—Our user interface allows total customization over imaging protocols, while ensuring repeatability from sample to sample. Our systems feature Auto-Focus, Auto-Light Control and Auto-Image Capture. Easily configure with external PC, if required.
- Data integrity—Azure Biosystems meets the standards for publication in all major journals, and additionally offers software to enable 21 CFR Part 11 Compliance

- A. NIR WESTERN BLOT
- B. CHEMI WESTERN BLOT
- C. CHEMI WB WITH COLOR MARKER F. PROTEIN GEL
- D. 3 COLOR FLUORESCENT WB
- E. BIOLUMINESCENT BACTERIA

- G. WESTERN BLOT STAINED WITH AZURERED
- H. DNA GEL
- I. 4 COLOR FLUORESCENT WB



# Choose your system



Azure 600



Azure 500



Azure 400



Azure 300



Azure 200

NIR
RGB
CHEMI
UV
BLUE LIGHT
COLOR

NIR CHEMI

UV

BLUE LIGHT

COLOR

RGB
CHEMI
UV
BLUE LIGHT
COLOR

CHEMI
UV
BLUE LIGHT
COLOR



Upgradable to 600
Upgradeable to
Q module

Upgradable to 600

Upgradable to 400, 500, and 600 Upgradeable to Q module

Upgradable to 300, 400, 500, and 600

#### **LIGHT SOURCES**

The flexibility of Azure Imaging Systems comes from the wide variety of light sources.



# Flexibility for your applications

The Azure Imaging Systems are multichannel, multimodal imagers, with near-infrared, visible light, and UV excitation channels. Detect Cy dyes, Alexa dyes, Safe dyes, Trihalo compound based gels, and more.

#### A SNAPSHOT OF COMPATIBLE DYES\*

- AzureRed
- AzureSpectra 800
- AzureSpectra 700
- AzureSpectra 650
- AzureSpectra 550
- AzureSpectra 488
- Alexa Fluor® 488
- Alexa Fluor 546
- Alexa Fluor 555
- Alexa Fluor 633
- Alexa Fluor 647
- Alexa Fluor 680
- Chemiluminescence
- Coomassie Blue
- Coomassie Fluor™
- Orange
- Cy®2
- Cy®3
- Cy®5

- Deep Purple<sup>™</sup>
- DyLight® 488
- DyLight 550
- DyLight 633
- DyLight 650
- DyLight 680
- DyLight 755
- DyLight 800
- DyLight 600
- ECL Plex<sup>™</sup>
- Ethidium Bromide
- GelStar®
- IRDye® 650
- IRDye 680LT
- IRDye 680RD
- IRDye 700DX
- IRDye 750
- IRDye 800CW
- IRDye 800RS
- Ponceau

- Qdot® 525
- Qdot 565
- Odot 585
- Qdot 605
- Odot 655
- Qdot 705
- Qdot 755
- Revert<sup>™</sup>
- Silver Stain



\*Other dyes are also possible. Compatible dyes depend on your system configuration.

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## 600 | 500 | 400 | 300

# Chemiluminescent imaging

Just as sensitive as film, but easier and quantitative, our Azure Imaging Systems will revolutionize your chemiluminescent workflows and eliminate your darkroom.

#### QUANTITATIVE CHEMILUMINESCENT IMAGING

With Azure Imaging systems, the software notifies you when bands are saturated and are not suitable for quantitaition.

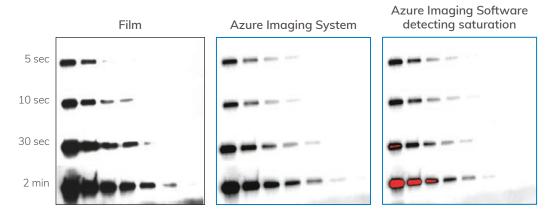


Figure 1. Saturation detection prevents errors in quantitation. The same blot was imaged on both x-ray film and the Azure Imaging System. The Azure system detects when CCD saturation occurs and calculates an auto-exposure time to avoid saturated bands.

#### HIGH RESOLUTION IMAGING

A 9.1MP camera provides high resolution imaging perfect for publications. Change the sample to optics distance using adjustable height shelf for enabled detection. Zoom into the area of interest with ROI imaging to reduce background.

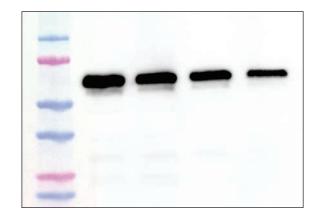
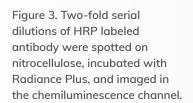
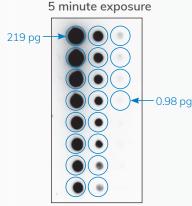


Figure 2. Chemiluminescent Western blot with MW Marker. 2-fold serial dilutions of HeLa lysate were separated by SDS-PAGE and transferred to a nitrocellulose membrane. The blot was blocked with Azure Chemi Blot Blocking Buffer prior to incubation with rabbit anti-hnRNP K primary antibodies. Signal was detected with Radiance ECL substrate.

### **ACCURATE CHEMILUMINESCENT QUANTITATION**

A wide dynamic range is necessary to detect weak bands alongside strong bands. Use multiple binning options, from 1x1 to 8x8 binning modes, to collect more light.





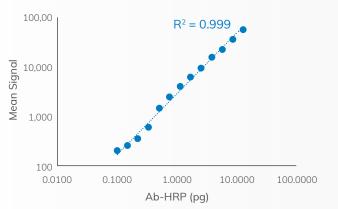


Figure 4. Azure Imaging Systems provide a broad, linear dynamic range to accurately detect strong and weak bands. The accuracy and linearity of the Azure Imaging System and reagents allow you to be confident about differences you see in protein levels.

# Upgrade to the **Q module** for efficient total protein normalization for quantitative Western blots

While many researchers use housekeeping proteins to normalize for load when quantifying bands on a Western blot, the past few years has seen a movement towards using total protein staining instead, for more accurate quantitation.<sup>1</sup>

# AZURE 300 + Q MODULE = CHEMI IMAGING WITH TPS (TOTAL PROTEIN STAIN)

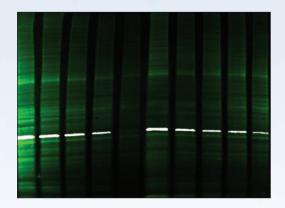


Figure 5. Simultaneous detection of total protein with AzureRed and Chemiluminescent Western. 2-fold serial dilutions of HeLa lysate were separated by SDS-PAGE and transferred to a PVDF membrane. After completion of the semi-dry transfer, the membrane was stained with AzureRed total protein stain. The blot was then blocked with Azure Chemiluminescent Blot Blocking Buffer prior to incubation with mouse anti-GAPDH. The blot was washed 3-times with Azure Blot Washing Buffer then incubated with Azure goat anti-mouse HRP secondary antibody. Chemiluminescent signal was detected with Radiance ECL substrate. After substrate incubation, the blot was imaged to produce an overlay of total protein staining and GAPDH protein. AzureRed is shown in green and GAPDH in gray.

 $<sup>^{\</sup>rm 1}$  Janes KA. An analysis of critical factors for quantitative immunoblotting. Sci Signaling. 2015 Apr 7;8(371):rs2. PMCID: PMC4401487.

# Visible fluorescence imaging

With high resolution, high sensitivity, and low background fluorescence imaging, the Azure Imaging System enables quantitative Western blotting and a whole lot more. Choose the Azure 400 for visible fluorescence, the Azure 500 for NIR fluorescence, or the Azure 600 for both visible and NIR fluorescence.

#### **MULTIPLEX DETECTION**

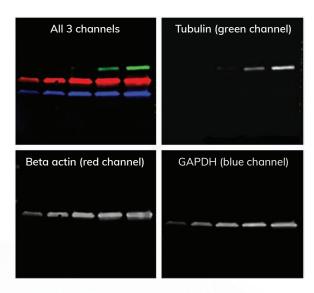


Figure 6. Digital image of 3-color western blot using Azure Biosystems 600 imager. Lanes (from left to right) loaded with 1, 2, 5, 10, 20 µg HeLa cell lysate. Probed for tubulin (top), beta actin (middle) and GAPDH (bottom). The following settings were used: Light sources 6/7/4; Exposure time 1s/13s 204ms/677ms; Filter positions 6/7/4; Aperture 6400; Focus 5000/5250/5000; bin level 1x1.

#### **BEYOND THE BLOT**

What truly sets the Azure Imaging System apart from other comparable systems is the ability to image more than just blots. Sure, in-gel fluorescence (Figure 7) and media plates (Figure 8) are not much of a stretch, but it's the Azure Imaging Systems' unmatched depth-of-field that enables imaging more three-dimensional samples such as mice (Figure 9) and zebrafish (Figure 10).

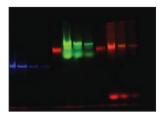


Figure 7. Fluorescent protein in native gel.

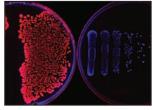


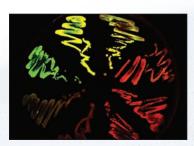
Figure 8. GFP- and mCherry-expressing E. coli.



Figure 9. Mouse with RFP-expressing subcutaneous tumor.



Figure 10. GFP-expressing zebrafish.



#### **FULL-COLOR RGB LEDs**

The Azure Imaging System's full-color multiplex RGB LEDs and filters broaden your imaging capabilities to visible fluorescence wavelengths, increasing flexibility and expanding multiplexing options while keeping system-size compact and value high.

## 600 | 500

# Infrared laser excitation for quantitative Western Blot imaging in the NIR

### **IMPROVE YOUR DATA QUALITY**

The Azure Imaging Systems' laser technology offers two near-infrared (NIR) detection channels enabling a user to study more than one protein in an assay, even if those targets overlap in molecular weight. Easily resolve and quantify co-migrating bands, such asphosphorylated versus pan-protein forms. Imaging with NIR dyes offers signal stability and low background.

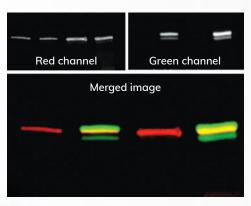


Figure 11. Fluorescent western blot of STAT1 and phospho-STAT1. The blot was probed with anti-phospho-STAT1 and anti-STAT1 followed by fluorescent secondary antibodies, and then imaged on Azure Imaging system. Top right is the green channel, using IR-800; top left is the image of the red channel, using IR-700. Bottom image is both channels merged.

## NIR LASERS KEEP SIGNAL HIGH AND BACKGROUND LOW

Our high-performance multiplex NIR lasers and filters deliver robust excitation energy which maximizes emission strength for optimal sensitivity.



Figure 12. Two-fold serial dilutions of AzureSpectra-800 labeled antibody were spotted on nitrocellulose, and imaged in the 800nm channel for 20 seconds.

Upgrade to the **Q module** for efficient total protein normalization for quantitiative Western blots

# AZURE 500 + Q MODULE = MULTIPLEX IMAGING WITH TPS

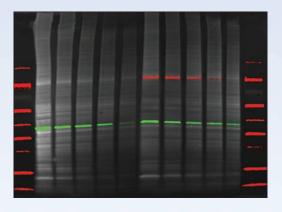


Figure 13. Simultaneous detection of total protein content with AzureRed and two-color NIR Western using AzureSpectra secondary antibodies. 2-fold serial dilutions of HeLa (left) and IFNa-treated HeLa lysate (right) were separated by SDS-PAGE and transferred to a PVDF membrane. After transfer and before blocking, the membrane was stained with AzureRed total protein stain. Then, the membrane was incubated simultaneously with rabbit antiphospho STAT-3 and mouse anti-GAPDH) primary antibodies. After washing, the blot was incubated simultaneously with AzureSpectra goat anti-rabbit IR700 and AzureSpectra goat anti-mouse IR800 secondary antibodies.



biosystems

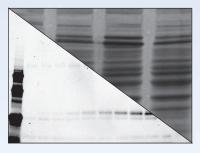




Blue Light Imaging
SYBR® Safe, SYBR® Gold, SYBR® Green

#### "SAFE" DYE DETECTION

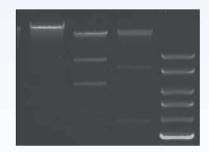
A less toxic alternative to ethidium bromide, lessharmful "Safe" dyes can be imaged with the EPI Blue LEDs standard in all the systems.



White Light Imaging Coomassie Blue, Silver Stain

#### **PROTEIN ANALYSIS**

Protein gels stained with Coomassie blue or silver stain can easily be imaged using the white light tray.



UV Imaging Ethidium Bromide

## DNA DETECTION WITH ETHIDIUM BROMIDE

With a dual-wavelength 302 nm and 365 nm UV transilluminator, images of ethidium bromide-stained DNA gels can be captured in a fraction of a second. A safety switch prevents accidental exposure to the light sources when the door is open. For band excision, the imager can be operated with the door open and the UV transilluminator can be pulled out. The switch can be overridden with a custom key.

# AzureSpot Analysis Software

Providing tools for the analysis of gels and blots, AzureSpot makes complex analysis a simple process. Designed to be either fully automated or manual, AzureSpot provides the flexibility and accuracy for your data analysis.

## **WORKFLOWS TO SUIT YOUR ANALYSIS REQUIREMENTS**

### **Automation for High-Throughput Analysis**

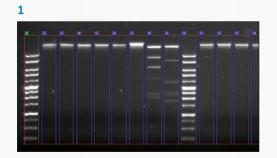
Start with an automatic analysis, or begin by creating lanes. Each step of the workflow is shown below, enabling fine tuning at each step. For 1D gels, highly developed algorithms accurately detect lanes and bands even on distorted gel images.

The user then has full control of the visualization tools and data display – outputting only those data fields that are of importance as well as the images of choice.

#### **TOOLS FOR:**

- 2D DENSITOMETRY
- AUTOMATIC LANE AND BAND DETECTION
- MOLECULAR WEIGHT ANALYSIS
- QUANTITY CALIBRATION
- ANNOTATION
- MULTIPLEX ANALYSIS
- DENDROGRAM
- COLONY COUNTING
- ARRAY
- SIGNAL NORMALIZATION

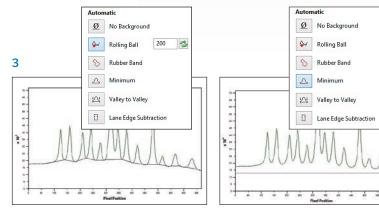
## Fully-Automated or Semi-Automated Option



Lay a lane grid over your sample.



Set threshold values, then detect your bands.



Correct for background, choose from a wide variety of options.











5.4 MP 65,536 grayscale

Specifications		
Camera		
Peltier Cooling		
7 Position Filter Wheel		
UV 302 nm & 365 nm		
Color Imaging/Visible Imaging		
Chemiluminescence		
Visible/RGB Fluorescent Imaging		
NIR Fluorescence Imaging		
Epi Blue Light Imaging		
Q module option		
Field of View		
Footprint (W x H x D)		

600
9.1 MP 16-bit, 65,536 grayscale
-50°C regulated cooling
✓
✓
✓
✓
<b>✓</b>
<b>✓</b>
✓
_
20 x 15 cm
41 x 56 x 33 cm

	500
	9.1 MP 16-bit, 65,536 grayscale
	-50°C regulated cooling
	✓
	✓
	✓
Ser.	✓
	_
	✓
	✓
-	✓
	20 x 15 cm
100	41 x 56 x 33 cm
	H W E 150

400
9.1 MP 16-bit, 65,536 grayscale
-50°C regulated cooling
✓
✓
✓
✓
✓
_
✓
_
20 x 15 cm
41 x 56 x 33 cm

300	200
9.1 MP 16-bit, 65,536 grayscale	5.4 MP 16-bit, 65,536 grays
-50°C regulated cooling	N/A
✓	✓
✓	✓
✓	✓
✓	_
_	_
_	_
✓	✓
✓	_
20 x 15 cm	20 x 15 cm
41 x 56 x 33 cm	41 x 56 x 33 cm



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