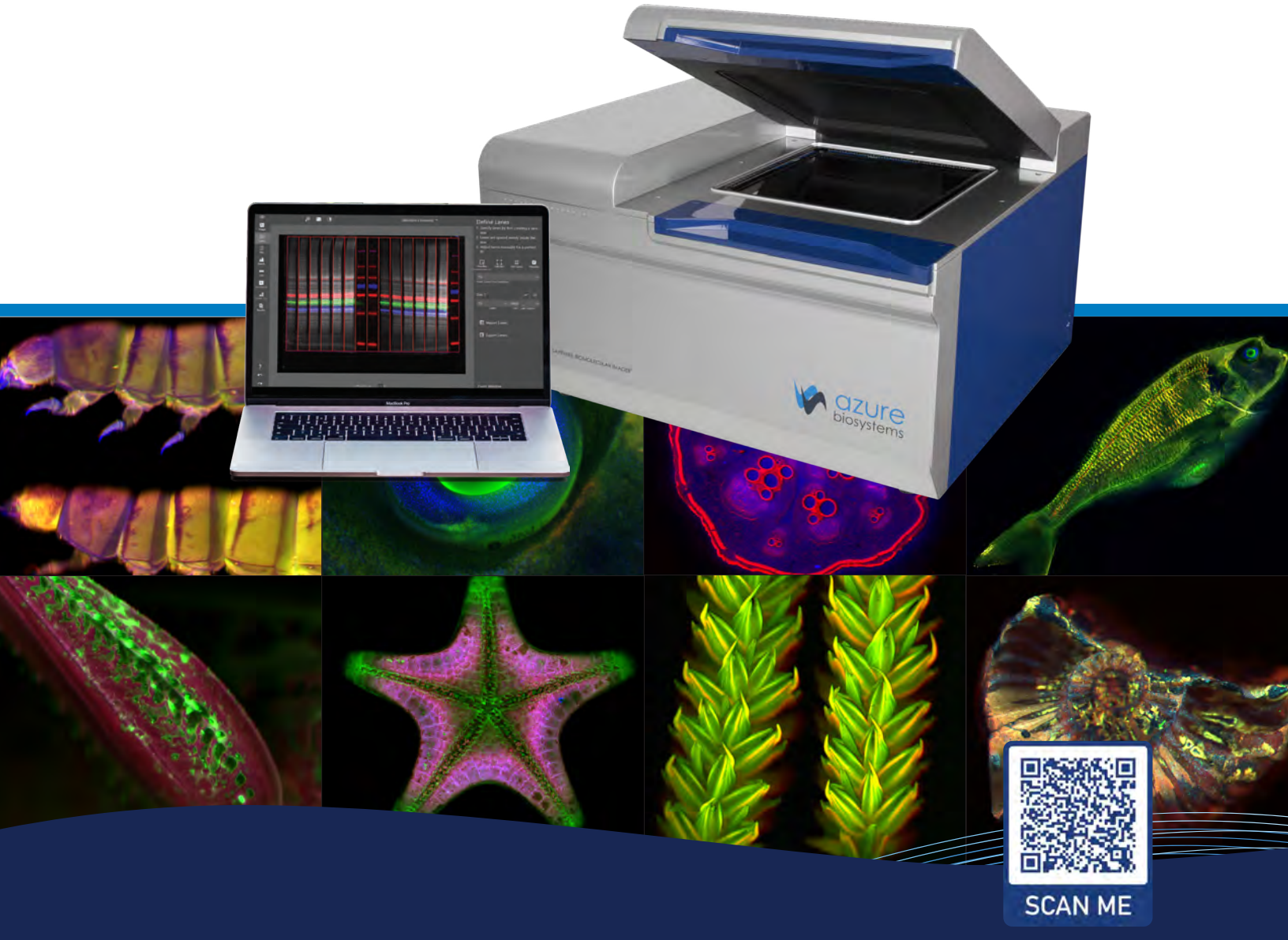
Biozym
SCIENCE IS OUR BUSINESS

azure biosystems

Azure Sapphire™ Biomolecular Imager

APPLICATIONS OVERVIEW

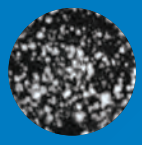


SCAN ME

SEE WHAT YOU CAN ACCOMPLISH WITH A
SAPPHIRE BIOMOLECULAR IMAGER

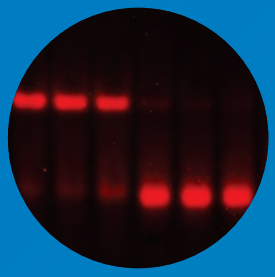
Whatever type of imaging your lab does—whether it's the ubiquitous western blot, Southern blots of 2D DNA gels, visualizing gross morphology of tissues or small model animals, or something more unique—the Sapphire Biomolecular Imager will deliver outstanding, quantitative detection with NIR and RGB fluorescence, chemiluminescence, and phosphorimaging.

Look through this book to see just a few examples of what the Sapphire can do, and then get in touch with us at **info@azurebiosystems.com** to test the Sapphire for yourself.

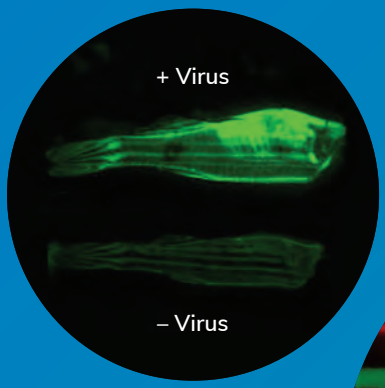


CRYSTAL VIOLET CELL STAIN

2D DNA REPLICATION ASSAY

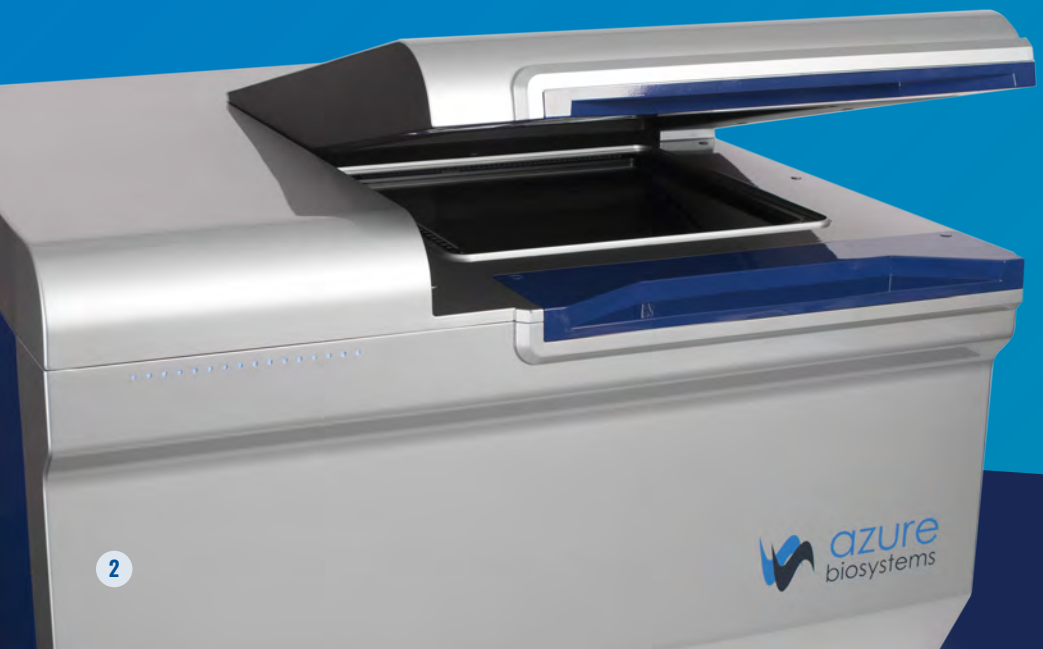


PROTEIN-DNA EMSA



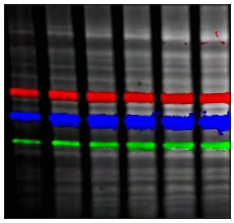
VIRUS-INFECTED ZEBRAFISH

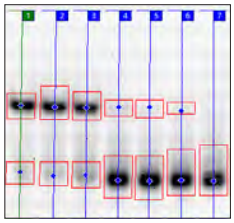
MULTIPLEX WESTERN BLOT + TOTAL PROTEIN STAIN

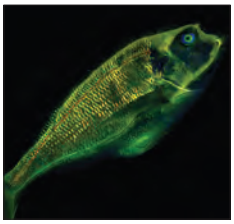


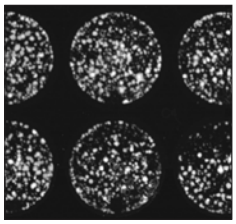
APPLICATIONS

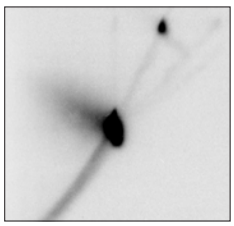
■ Fluorescence Detection ■ Chemiluminescence Detection ■ Phosphorimaging ■ Densitometry





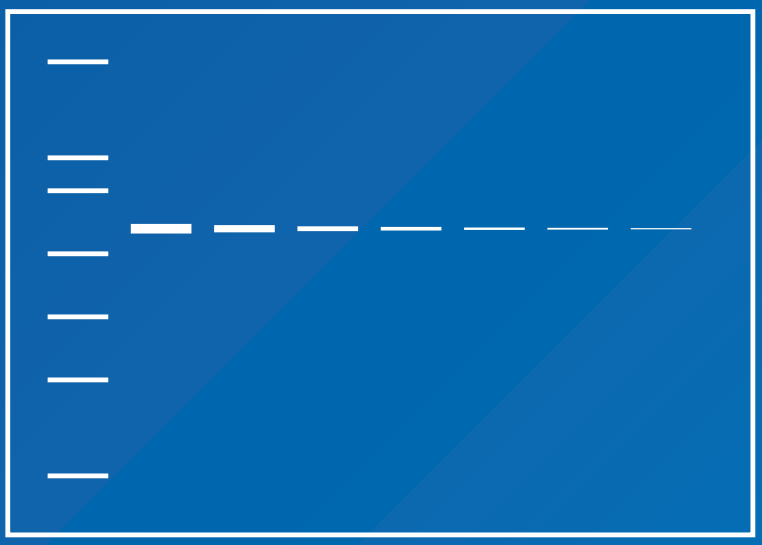






1

BLOTTING IMAGING PART 1— WESTERN BLOTS



What makes the Sapphire so great for sensitive detection and quantitation of chemiluminescent and fluorescent western blots?

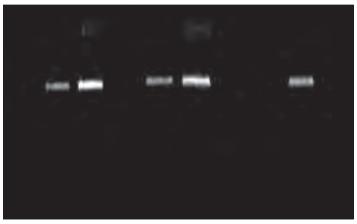
- Four solid-state lasers deliver strong excitation
- Unique, three-detector design maximizes performance by ensuring that the right sensor is used for the type of imaging being done
  1. A sensitive photomultiplier tube (PMT) optimizes blue light detection and phosphorimaging
  2. A high quantum-efficiency avalanche photodiode (APD) enables near infrared (NIR), infrared (IR), red, and green light imaging
  3. A CCD sensor provides chemiluminescent imaging with the same sensitivity as film
- Powerful yet easy-to-use Sapphire Capture and AzureSpot Pro analysis software

FLOURESCENT WESTERNS WITH UP TO FOUR COLOR DETECTION

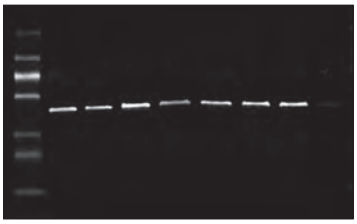
Get faster workflows and more reliable quantitation

Western blotting is a powerful technique useful for characterizing protein-protein interactions, signaling pathways, post-translational modifications, cell surface proteins, RNAi analysis, and more. Quantitative Western blotting aims to measure changes in protein expression in order to make meaningful relative comparisons between treatments or conditions.

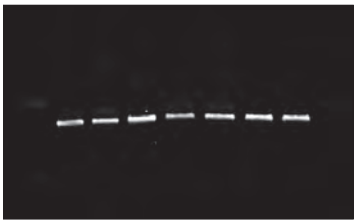
a 490 – transferrin (blue)



b 700 – actin (red)

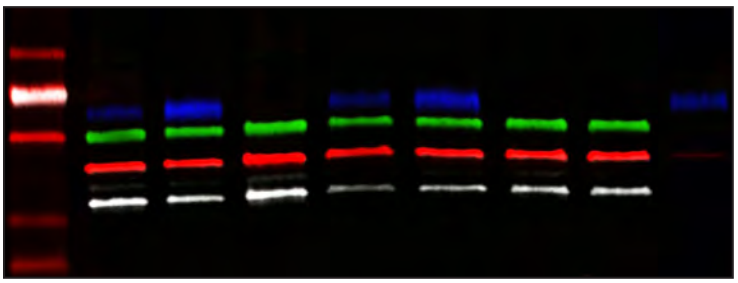


c 550 – tubulin (green)



d 800 – GAPDH (gray)

e



With the use of secondary antibodies labeled with four spectrally distinct fluorophores, the powerful capabilities of the Sapphire enable simultaneous detection of up to four different proteins. Here we show an example where HeLa cell lysates spiked with transferrin were imaged on a western blot that was simultaneously probed with anti-tubulin (550 nm, green), anti ß-actin (700 nm, red), anti-GAPDH (800 nm, gray), and anti-transferrin (490 nm, blue). Sensitive and specific detection of all four proteins can be seen, with no evidence of background auto-fluorescence or bleed-through between channels.

FLUORESCENCE IMAGING

Four-color imaging

Pixel size	100 µm
Laser	488 nm (transferrin) 520 nm (tubulin) 658 nm (ß-actin) 784 nm (GAPDH)

Published data

Examples of fluorescent Western blots imaged using a Sapphire:

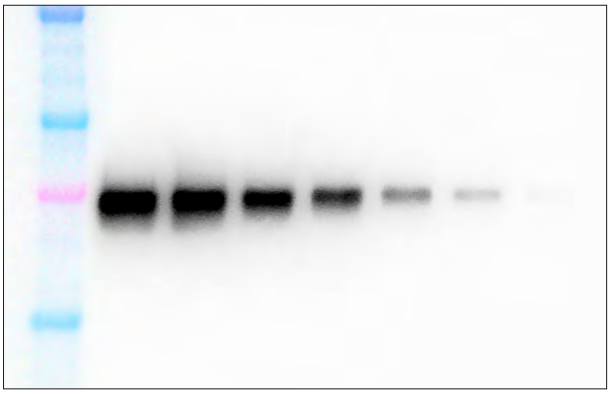
- Rut W, et al. Activity profiling and crystal structures of inhibitor-bound SARS-CoV-2 papain-like protease: A framework for anti-COVID-19 drug design. *Sci Adv.* 2020 Oct 16;6(42):eabd4596. doi: 10.1126/sciadv.abd4596. PMID: 33067239; PMCID: PMC7567588.
- Ha J, Park SB. Callyspongiolide kills cells by inducing mitochondrial dysfunction via cellular iron depletion. *Commun Biol.* 2021 Sep 23;4(1):1123. doi: 10.1038/s42003-021-02643-8. PMID: 34556786; PMCID: PMC8460830.
- Markowitsch SD, et al. Shikonin Inhibits Cell Growth of Sunitinib-Resistant Renal Cell Carcinoma by Activating the Necrosome Complex and Inhibiting the AKT/mTOR Signaling Pathway. *Cancers* (Basel). 2022 Feb 22;14(5):1114. doi: 10.3390/cancers14051114. PMID: 35267423; PMCID: PMC8909272.

SENSITIVE CHEMILUMINESCENT DETECTION

Maximize your Western blot workflow options

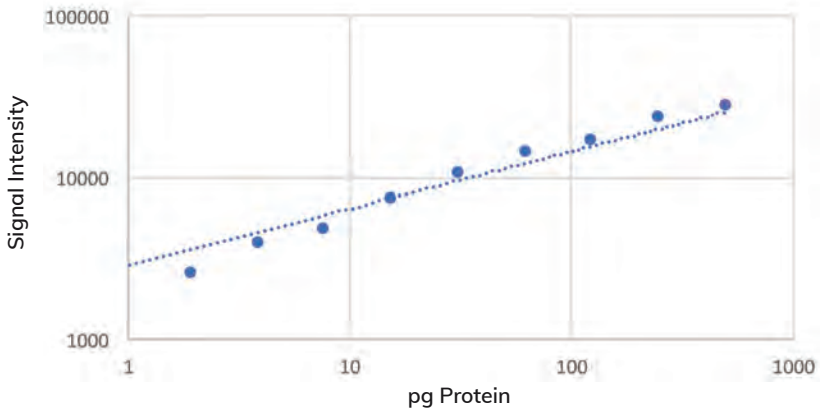
Chemiluminescent Western blotting takes advantage of the enzymatic reaction between horseradish peroxidase (HRP)-labeled secondary antibodies and an enhanced chemiluminescence (ECL) substrate to produce photons of light. The signal enhancement of the enzymatic reaction is useful for detecting small amounts of protein.

Switching to the Sapphire doesn't mean you have to convert all of your familiar and well-validated chemiluminescent protocols to fluorescent ones. Unlike other scanning systems, the Sapphire can deliver chemiluminescent detection with the same sensitivity as film, but with a much broader dynamic range.

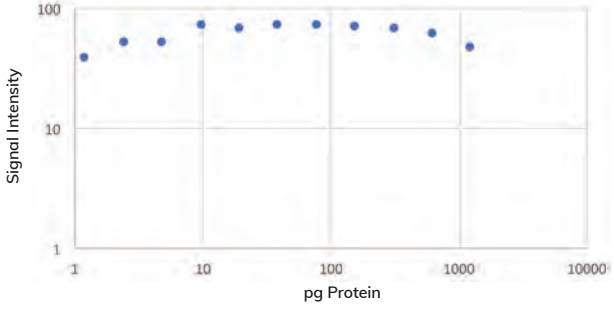


CHEMILUMINESCENCE IMAGING	
Pixel size	1x1 binned image with a resolution of 2688x 2200
Detector	CCD Sensor

SAPPHIRE BIOMOLECULAR IMAGER



FILM

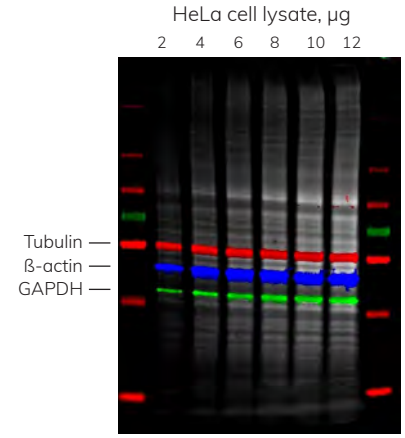


SCAN ME

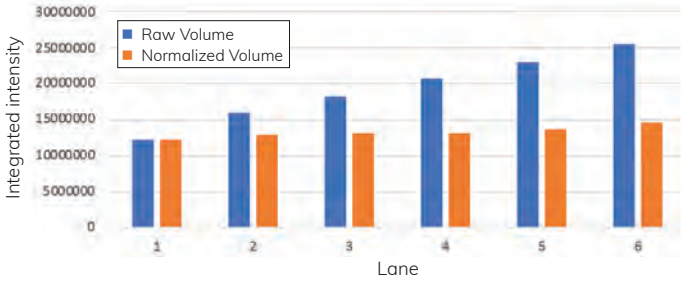
TOTAL PROTEIN NORMALIZATION AND DETECTION OF UP-TO THREE PROTEINS

Generate quantitative western blot data you can count on

Normalization uses an internal loading control or total protein stain in order to correct for variations between lanes and samples. Unless some type of normalization is performed, it is impossible to know if changes in band volume and intensity are caused by biological changes in samples or if they are due to loading or sample inconsistencies or a variance in sample preparation. The technique is used to account for unequal protein concentrations, loading inconsistencies across a gel and transfer variability across a blot and is a must when trying to make meaningful comparisons within Western blots. It gives you a baseline to compare changes in protein expression.



Four-color detection of a blot with increasing amounts of HeLa cell lysate. Tubulin is in red, actin is in blue, GAPDH is in green, and AzureRed/total protein is in white.



Quantitation of the tubulin signal normalized to total protein (orange) shows how TPN can correct for loading differences.

FLUORESCENCE IMAGING

Four-color imaging

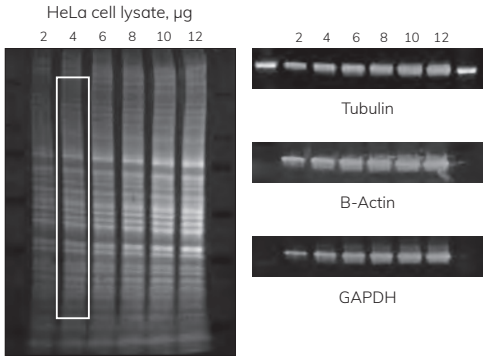
Pixel size	100 µm
Laser	488 nm (ß-actin) 520 nm (total protein stain) 658 nm (tubulin) 784 nm (GAPDH)

AzureRed Total Protein Stain

Easily stain total protein for the most accurate blot normalization.

▸ Azure Catalog Number AC2124

Of the common normalization techniques, total protein stains are gaining preference among major journals because total protein stains are unaffected by experimental conditions. When combined with the AzureRed Fluorescent Protein Stain for total protein normalization, the Sapphire enables simultaneously detection of up to three different proteins and normalization to total protein.



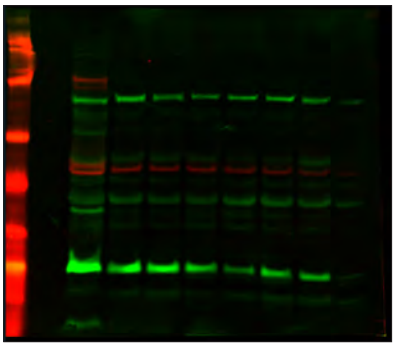
Individual channels of the same blot. To calculate the total protein signal, simply draw a box around the entire lane and normalize your signal-of-interest to the total protein signal as usual.

*Ghosh R, Gilda JE, Gomes AV. The necessity of and strategies for improving confidence in the accuracy of western blots. *Expert Rev Proteomics.* 2014 Oct; 11(5): 549–560. PMCID: PMC4791038.

FLUORESCENT WESTERN BLOTTING TIP

Improve sensitivity by drying your Western blot before imaging

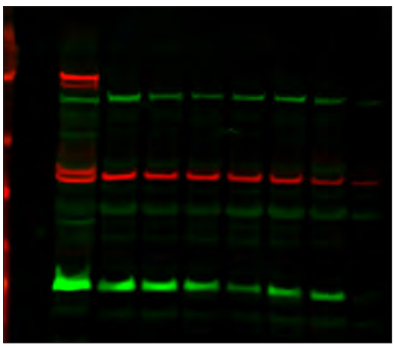
How does imaging wet or dry effect your data? The data below shows the effect of wet and dry imaging with the Sapphire Biomolecular Imager. While scanning a wet membrane does produce detectable signal, drying the membrane results in increased signal intensities, lower background and better signal to noise ratios. Water can attenuate fluorescence and even slight differences in the dryness of different regions of a blot can lead to variable quantitation. Drying your blot prior to imaging can greatly improve sensitivity and the ability to generate reliable quantitative data.



Western blot imaged while wet

FLUORESCENCE IMAGING
Western blot imaged wet

Pixel size	100 µm
Laser	658 nm, 784 nm
Filter	710BP40, 832BP37
Intensity	7 (658 nm), 7 (784 nm)



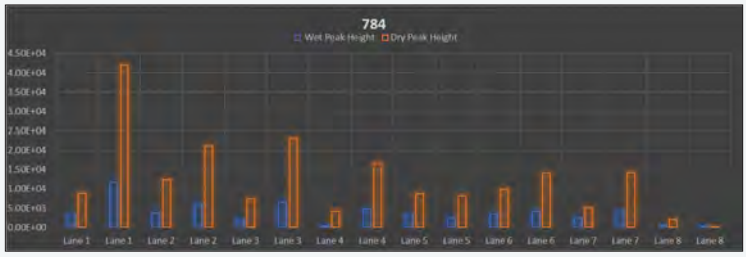
Western blot imaged while dry

FLUORESCENCE IMAGING
Western blot imaged dry

Pixel size	100 µm
Laser	658 nm, 784 nm
Filter	710BP40, 832BP37
Intensity	5 (658 nm), 2 (784 nm)

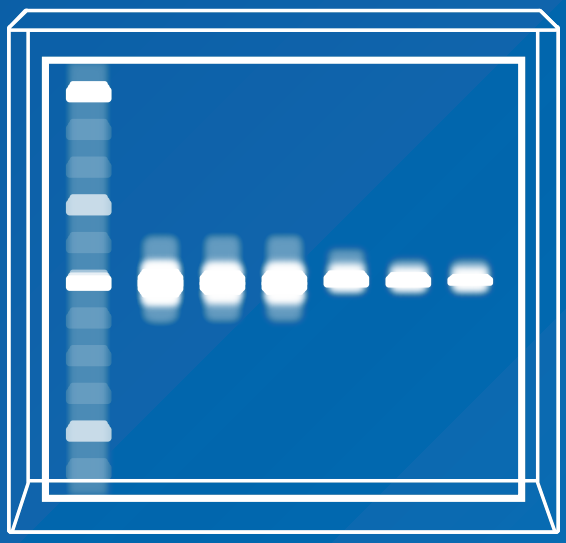
Quantitation comparison

With both excitation wavelengths (658 nm, left; 784 nm, right), signal intensity from the dry blot (orange) is much higher than signal intensity from the wet blot (blue).



2

GEL IMAGING



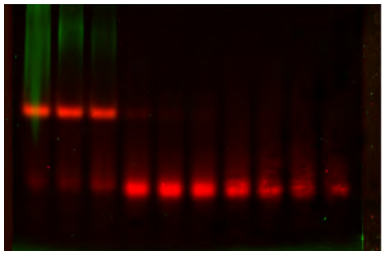
The same three detector technology that makes the Sapphire so great for imaging Western blots is also flexible enough to image a wide-range of gels, whether they are ethidium bromide (EtBr)-stained DNA agarose gels, coomassie-stained protein gels, or even 32P-labeled DNA acrylamide gels and more.

MEASURE PROTEIN-DNA BINDING USING EMSA

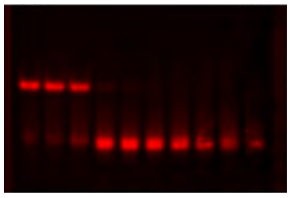
Image delicate gels while still in glass plates

The electrophoretic mobility shift assay (EMSA), a.k.a. gel shift assay, is a great way to monitor any type of stable binding reaction such as protein-protein, protein-ligand, and protein-DNA. The technique can be used to analyze sequence specific interactions as complexes of protein or protein and DNA migrate slower than unbound protein or DNA, causing a "shift" in the bands within a sample.

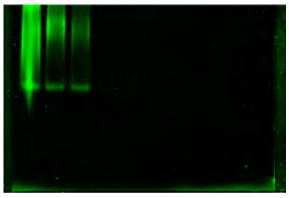
Traditionally, EMSAs are performed with radioactive isotopes, but the technique can also be adapted to use non-hazardous fluorescent dyes, which can decrease assay time by cutting the time required for film or screen exposure.



Overlay



658 nm



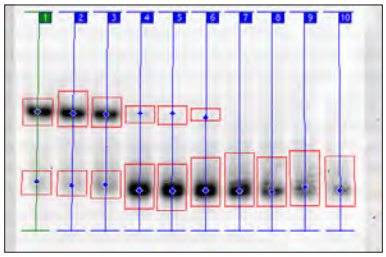
784 nm

FLUORESCENCE IMAGING

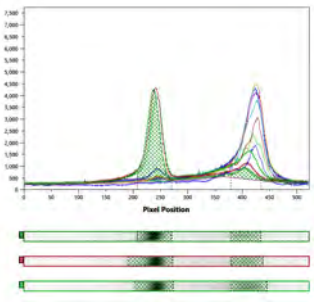
EMSA (Gel shift)—gel imaged while still in glass plates

Pixel size	100 µm
Laser	658 nm, 784 nm
Filter	710BP40, 832BP37
Analysis	AzureSpot 1D module, Normalized volume vs. volume

Here we show the results of a demo testing the Sapphire's ability to image a protein-DNA binding reaction using EMSA (DNA is shown in red and protein in green). The powerful lasers used in the Sapphire enable imaging of the gel directly within the glass plates, reducing the risks of breaking these delicate gels during transfer to blotting paper or while drying.



Visualization of band alignment (658 nm)



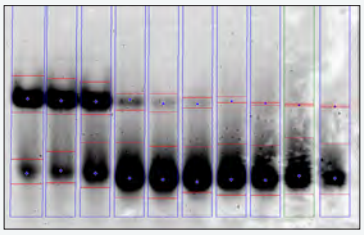
Published data

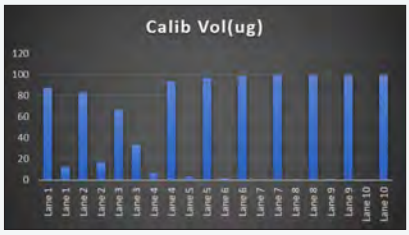
See examples of EMSAs imaged using a Sapphire in:

- H-NS Family Members MvaT and MvaU Regulate the Pseudomonas aeruginosa Type III Secretion System. EAW McMackin, AE Marsden, and TL Yahr. *J Bacteriol.* 2019 Jun 21;201(14). pii: e00054-19.

Quantitation

Measurement of bound and unbound DNA is easily accomplished in the AzureSpot software.

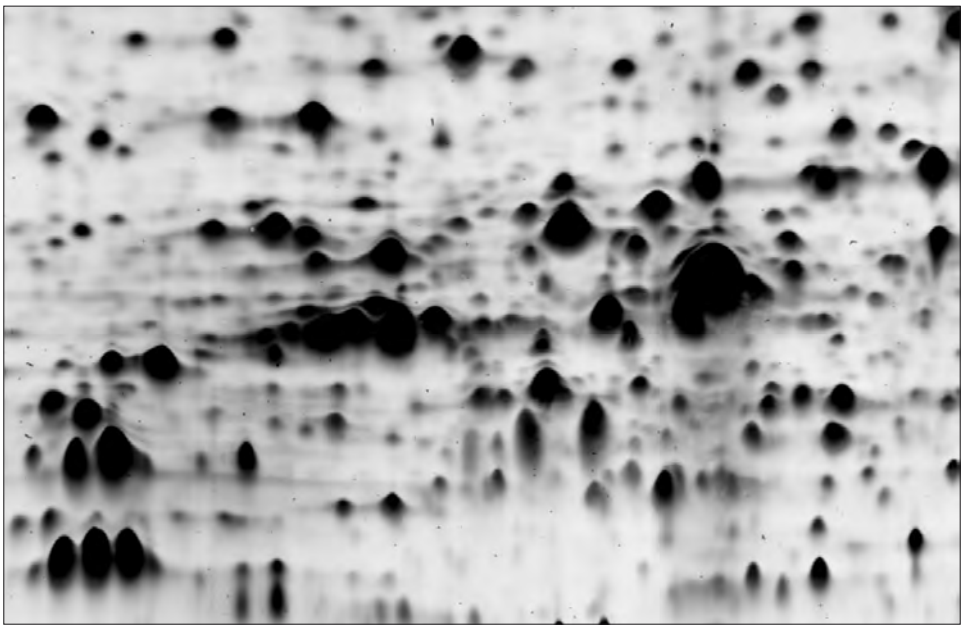




VIEW AND QUANTIFY SYPRO RUBY-STAINED 2D PROTEIN GELS

Analyze proteomics studies with ease

While 1D polyacrylamide gel electrophoresis is great for most applications, many proteomics and other studies benefit from an additional dimension of separation to resolve co-migrating proteins and their isoforms. Here we show a close-up of a 2D protein gel that was stained using Sypro Ruby. With a 50 µm resolution scan, you can easily see the distinct spots, which can also be quantified in the AzureSpot software.



FLUORESCENCE IMAGING

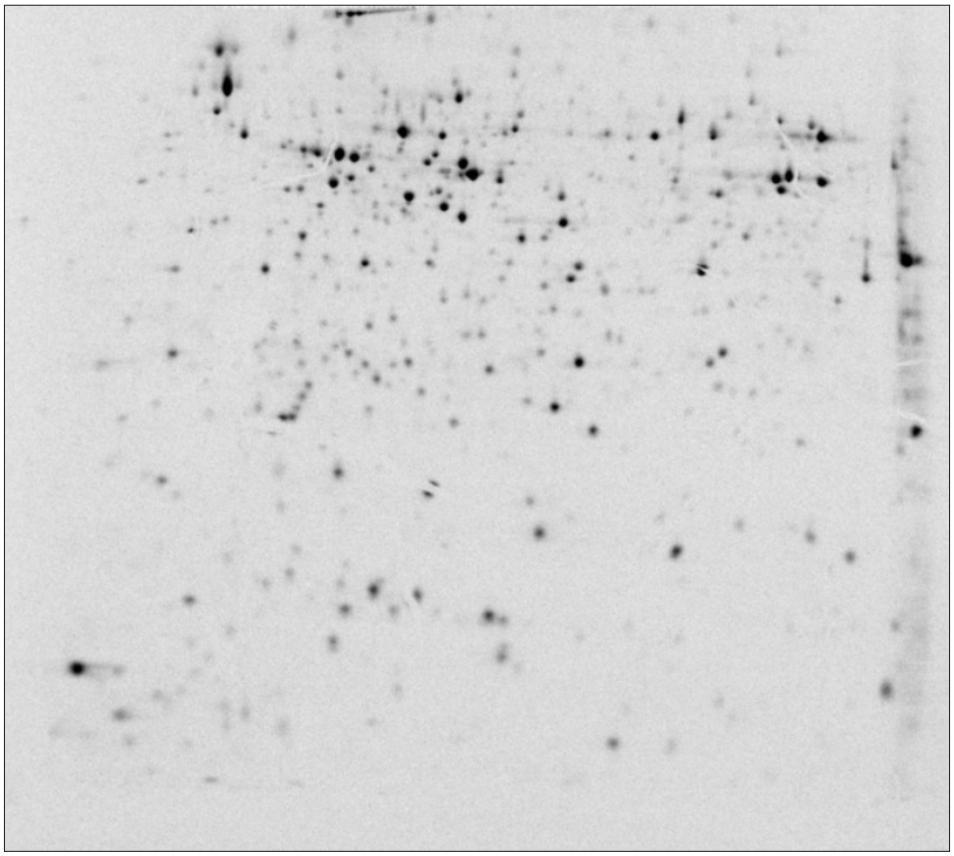
2D protein gel

Pixel size	50 µm
Laser	658 nm

VIEW AND QUANTIFY ^{35}S-LABELED PROTEINS IN 2D GELS

Perform proteomics analysis on metabolically-labeled samples

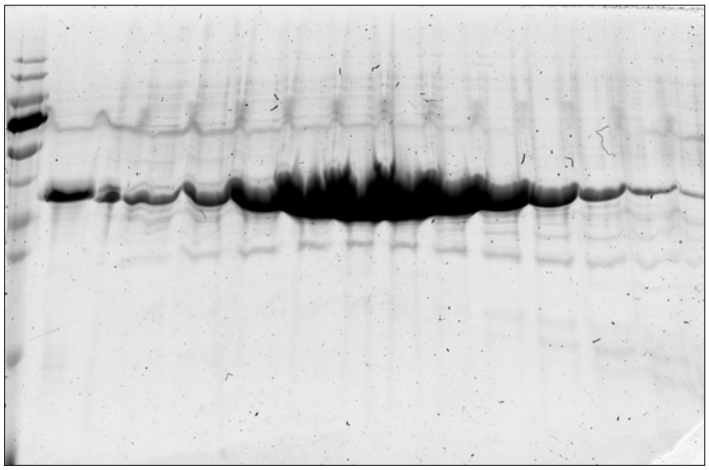
For more sensitive detection, 2D gels can be run with protein samples isolated from cells grown in the presence of 35S-labeled methionine. The radiolabel becomes incorporated into cellular proteins which can be directly detected using the Sapphire's phosphorimaging capabilities. As with the Spyro Ruby-stained gel, the individual spots can be quantified using the AzureSpot software.



PHOSPHORIMAGING	
2D protein gel	
Pixel size	200 µm

IMAGE COOMASSIE- AND SILVER-STAINED PROTEIN GELS

Coomassie and silver stains are common stains for detection and quantitation of proteins within a gel. While the Sapphire is powerful enough for high-resolution scanning applications, it can also be used for both scanning or CCD documentation quick documentation of protein gels. Here we show coomassie- and silver-stained gels. The Sapphire is compatible with a wide range of stains and the large scanning bed can accommodate multiple gels. With the Sapphire, you can choose which detection method best suits your assay – fluorescent detection or CCD imaging.

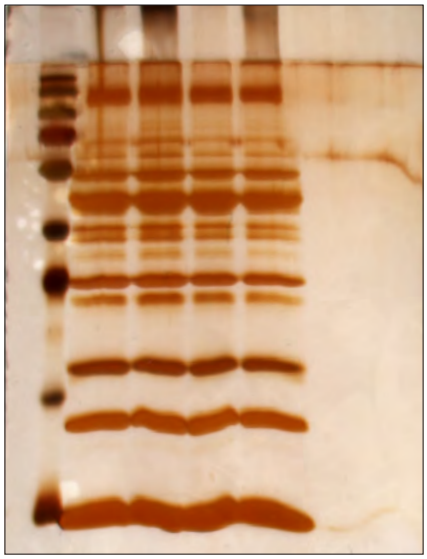


Coomassie-stained protein gel

NIR FLUORESCENCE IMAGING

Coomassie protein gel

Pixel size	100 µm
Laser	658 nm



Silver-stained protein gel

CCD IMAGING

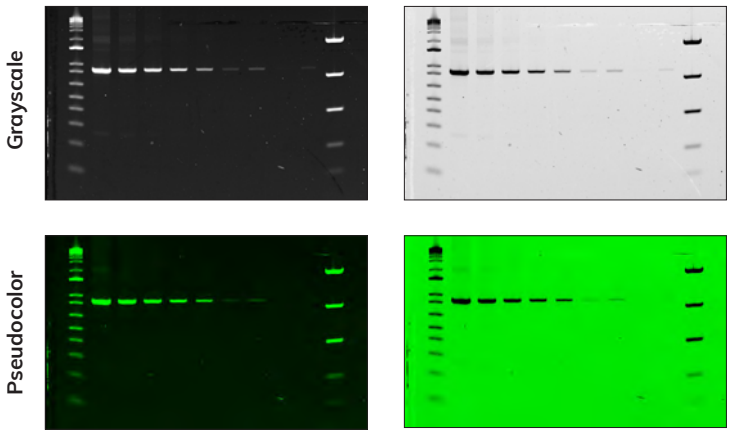
Silver Stained protein gel

Pixel size	1x1 binned image with a resolution of 2688x 2200

Contact us at
info@azurebiosystems.com
if you'd like to find out if a specific stain is supported.

GET ACCURATE DNA QUANTITATION FROM ETBR-STAINED AGAROSE GELS

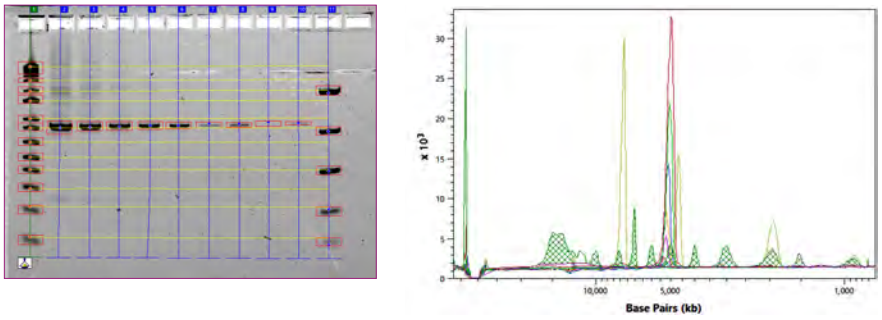
DNA agarose gel electrophoresis is one of the most basic and widespread molecular biology techniques, used to separate DNA according to molecular weight. The Sapphire® Biomolecular Imager uses the fluorescent properties of common DNA dyes, including EtBr, to easily image agarose gels and provide accurate DNA quantitation of stained gels without the use of damaging UV light.



Positive Images **Negative Images**

FLUORESCENCE IMAGING
EtBr-stained DNA agarose gel

Pixel size	100 µm
Laser	520 nm
Filter	565BP24
Analysis	AzureSpot 1D Gel/ Western Blot Quantity Calibration; AzureSpot Toolbox Percentage

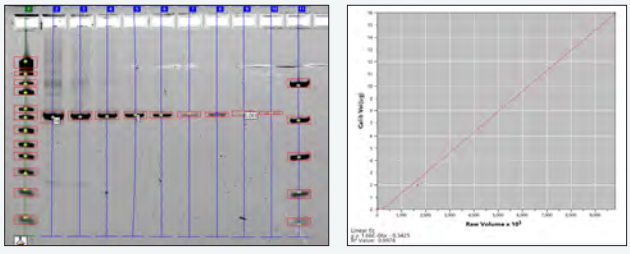


Analysis of gel and plot to show alignment of bands on the gel.

Quantitation comparison

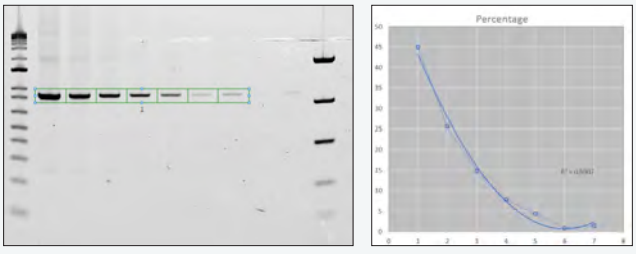
AzureSpot Software comes with a variety of tools for quantitation. Both the Quantity Calibration and the Toolbox Percentage functions provide accurate quantitation

Quantity Calibration



R2 = 0.9978

Toolbox Percentage

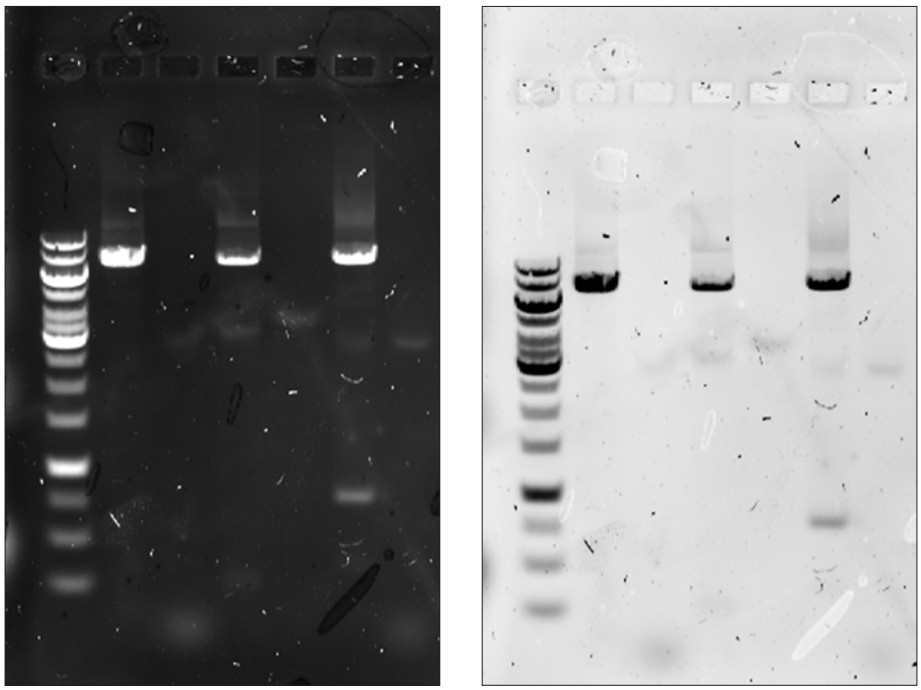


R2 = 0.9907

IMAGE MIDORI GREEN-STAINED DNA AGAROSE GELS

With the ability to visualize a range of dyes, the Sapphire can document and quantify more than just EtBr-stained DNA agarose gels. Here we show an example of a Midori Green-stained DNA gel.

Midori Green-stained DNA gel



Positive **Negative**

RGB FLUORESCENCE IMAGING

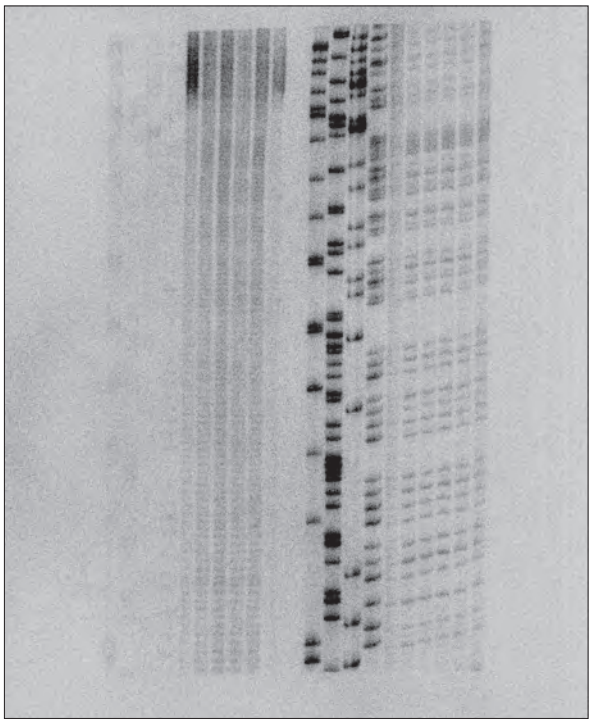
Midori Green-stained DNA gel

Pixel size	100 µm
Laser	520 nm

Contact us at
info@azurebiosystems.com
if you'd like to find out if a specific stain is supported.

DIRECTLY DETECT DNA FOR SANGER SEQUENCING AND FOOTPRINTING

While next generation sequencing has revolutionized how we acquire DNA sequence information, there are still a few key applications where you need to run a DNA sequencing gel, such as DNA footprinting, studying transcription initiation, and mutation analysis. Whether you are using fluorescent dyes or ^{32}P, the Sapphire can image the gel and support your analysis.



PHOSPHORIMAGING
DNA gel

Pixel size	200 µm
Laser	658nm
Filter	390BP40

Published data

See how the Sapphire is used to study how DNA structure affects viral integration in:

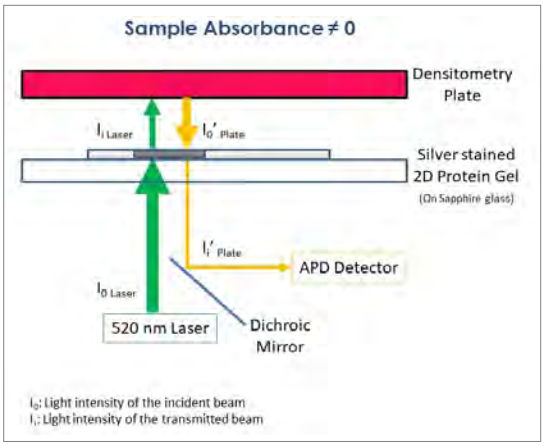
- Nucleosome DNA unwrapping does not affect prototype foamy virus integration efficiency or site selection. Randi M. Mackler, et al. *PLoS One.* 2019 Mar 13;14(3):e0212764.

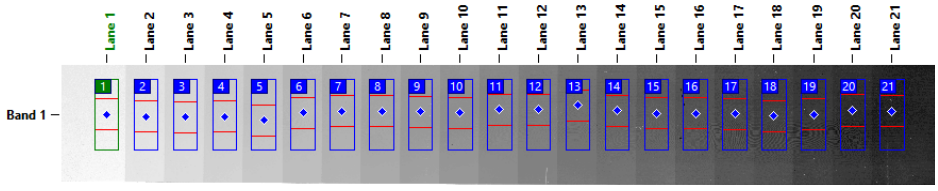
DENSITOMETRY

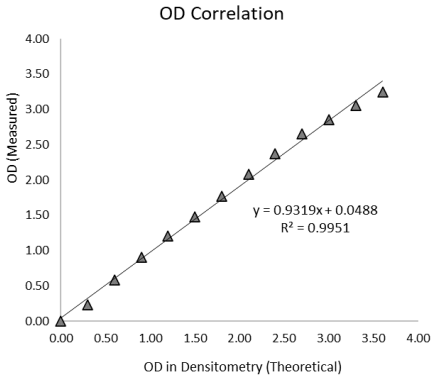
Densitometry is a powerful technique for quantifying and identifying proteins separated by gel electrophoresis and stained with colorimetric dyes. By quantifying the visible gel signal using a calibrated standard, the amounts of loaded proteins can be calculated. However, proper quantification requires high resolution images, linear signal detection, and uniform illumination in order for accurate results.

The Sapphire paired with the Azure DensitoMetrics package provides offers down to 10 micron image resolution, direct signal proportionality over a range of 3.6 OD, and uniform flatbed laser scanners.

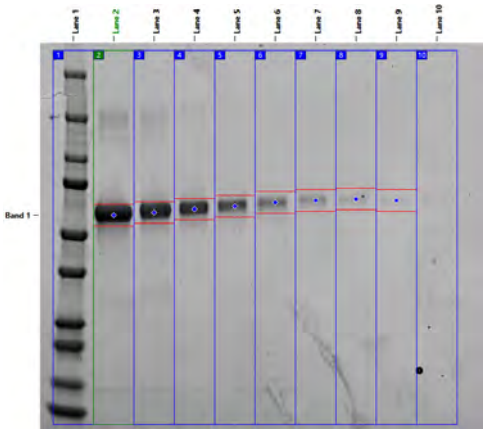
In addition, it accommodates for gels of different sizes and thicknesses with an adjustable focal plane and imaging area of up to 25 x 25cm. Densitometric analysis uses colorimetric gel dyes like Coomassie blue or different silver staining formulations, which are effectively captured by the Sapphire's green channel (520nm laser; 565/24nm emission filter).



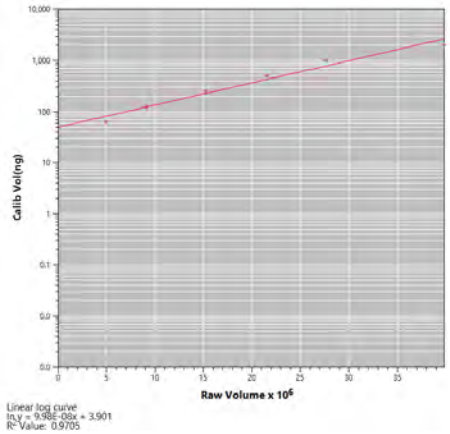




21-Step Tablet analyzed in AzureSpot Analysis Software and scatter plot for the ODs.
For Step OD = 0.30 → Theoretical OD_{Densit} = 0.60
Measurements:
$T_{0.60}$ = 8,763,452 / 33,632,900 = 0.26
$OD_{0.60}$ = – Log (8,763,452 / 33,362,900) = 0.58



Coomassie blue stain of a 1D SDS-PAGE.
Two-fold dilutions starting at 2pg of BSA.
Intensity L1 in 520nm laser channel.

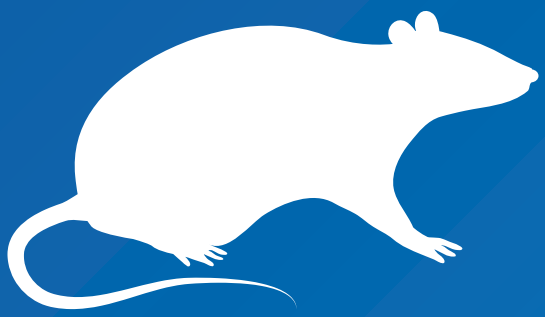


Linear-log scatter plot of a Coomassie blue gel (image on left).

SCAN ME

3

TISSUE & SMALL ANIMAL MODEL IMAGING



With the capability to image down to 10 µm resolution and a 25 cm x 25 cm scanning bed, the Sapphire can go from scanning blots to scanning tissues and small animal models like mice, rats, small plants, and zebrafish. Quickly capture—and quantify—gross anatomy, morphology, protein localization and more.

TRACK PROTEIN MOVEMENT THROUGH TISSUE IN SMALL ANIMALS

Lymphatic antigen tracking in mouse hindpaw

The Sapphire is useful for imaging more than just gels and blots. You can image whole small animal models using fluorescence, chemiluminescence, and phosphorimaging detection. The scan below shows a fluorescently labeled antigen injected subcutaneously into a mouse hindpaw. The animal was euthanized, and fluorescence from the draining popliteal and sciatic lymph nodes was measured.

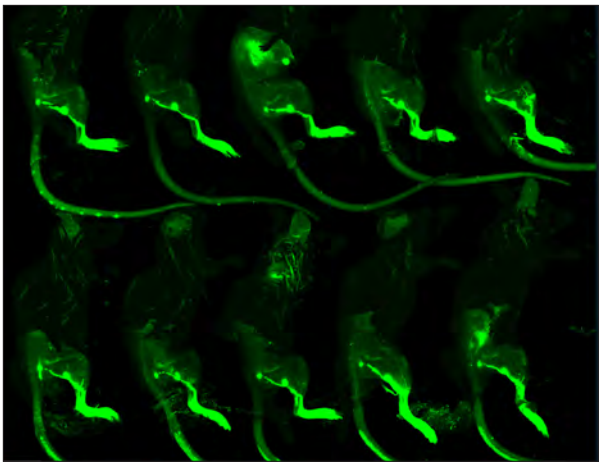
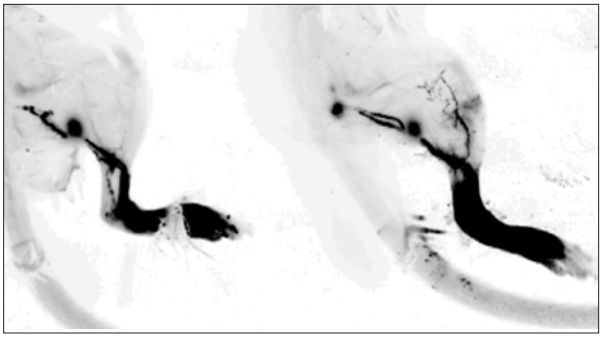


Image multiple animals/samples

FLUORESCENCE IMAGING

Fluorescent Tag in Mouse Model

Pixel size	100 µm
Laser	658 nm, 784 nm
Filter	710BP40, 832BP37
Analysis	AzureSpot 1D module, Normalized volume vs. volume



Negative Image

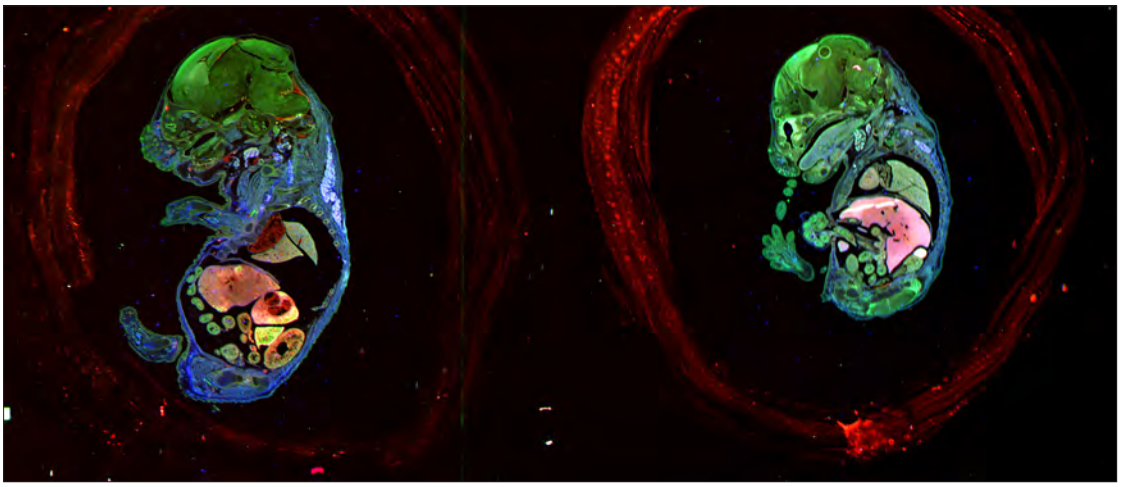
Quantitation

Numbered circles indicate areas with signal to be measured.

TISSUE IN SMALL ANIMALS

Mouse embryos

We used 10micron here and it is an multiplex images of our 488nm, 520nm and 784nm light source. Below are two images of mouse embryos where intricate details, such as the backbone and adipose tissue are visible.



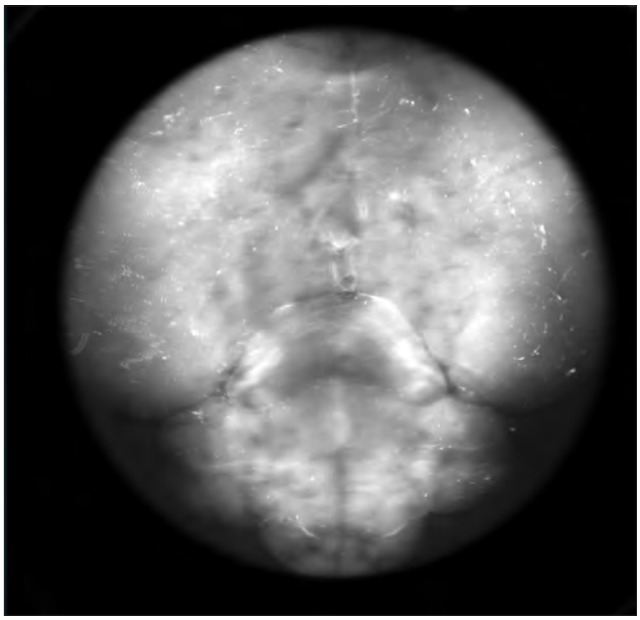
FLUORESCENCE IMAGING
Mouse Embryos

Pixel size	10 microns
Laser	488nm, 520nm, 784nm
Filter	TBD
Analysis	TBD

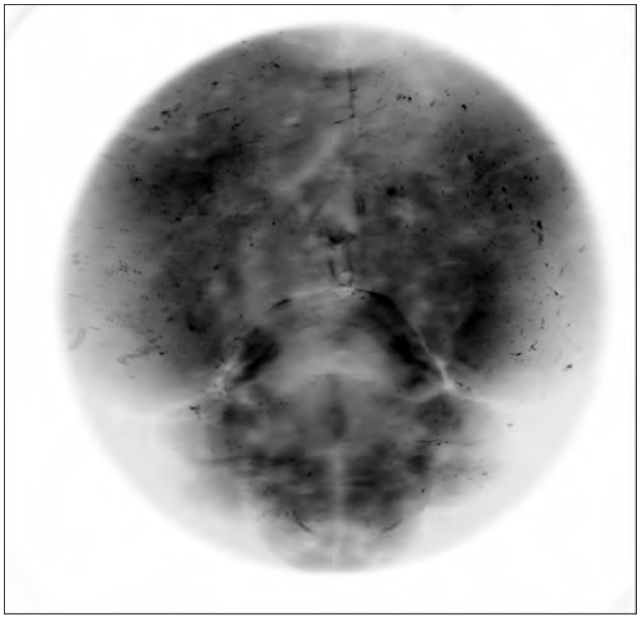
GET INFORMATION ON TISSUE STRUCTURE

Using CLARITY for whole brain imaging

Studying morphology and neural connectivity in the brain has been greatly enhanced with the development of CLARITY, a method for making brain tissue transparent for fluorescence and other imaging modalities. With a resolution down to 10 µm, the Sapphire can be used to image CLARITY-prepared brains from small animal models.



Positive Image



Negative Image

FLUORESCENCE IMAGING
CLARITY-prepared mouse brains

Pixel size	10 µm
Laser	488 nm
Filter	518P22
Intensity	10
Scan speed	Highest

What is CLARITY?

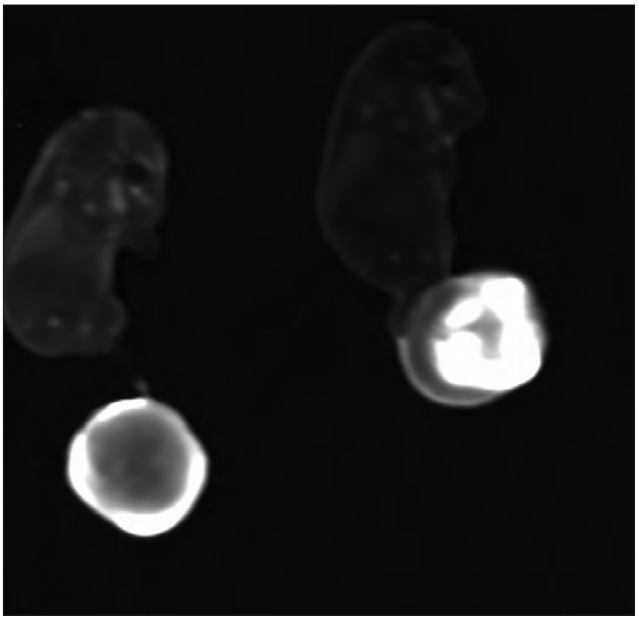
Developed to help neuroscientists better image entire brains, the CLARITY technique is a way to optically clear brain tissue while preserving biologically important molecules like protein and DNA in the context of larger brain structures. In a manner similar to fossilization, lipid bilayers are replaced by a sturdier yet porous and clear hydrogel mesh. Labeled macromolecules lying deeper within the brain can now be imaged. With the flexible and powerful focusing power of the Sapphire, you can obtain wide-field imaging of CLARITY-prepared brains from small animal models.

MEASURE PROTEIN LOCALIZATION IN TISSUE

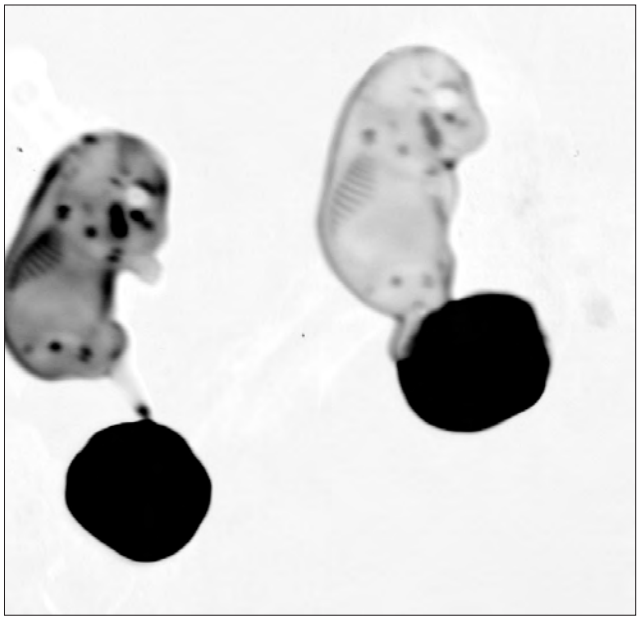
Studying the permeability of embryo/placenta barrier

In another example of tracking protein distribution in different tissues, this demo shows administration of an IR dye conjugated to an antibody that cannot cross the placental barrier (embryo on the right) versus conjugation to an antibody that can cross the placental barrier (embryo on the left).

By imaging with the Sapphire rather than a camera-and-filter setup, you can quickly observe protein localization across distal tissues and easily quantify relative protein distribution.



Positive Image



Negative Image

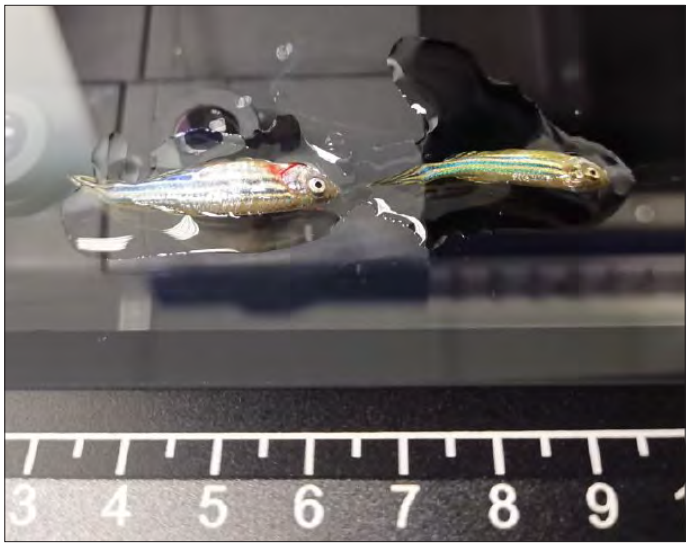
FLUORESCENCE IMAGING

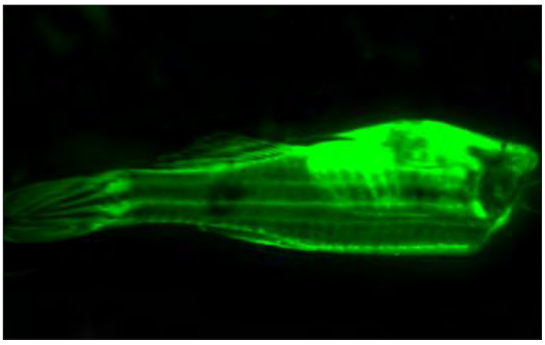
Fluorescently-labeled antibody

Pixel size	50 µm
Laser	784 nm
Filter	832BP37
Intensity	10
Scan speed	Highest

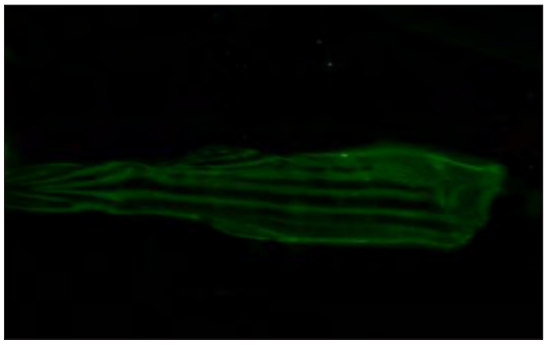
TRACK VIRAL INFECTION AND QUANTIFY VIRAL LOAD

The Sapphire can be used to track localization of more than just protein. In this demo, FITC-labeled virus is used to infect a zebrafish, which is then placed directly onto the Sapphire for imaging.





+FITC-labeled virus



-FITC-labeled virus

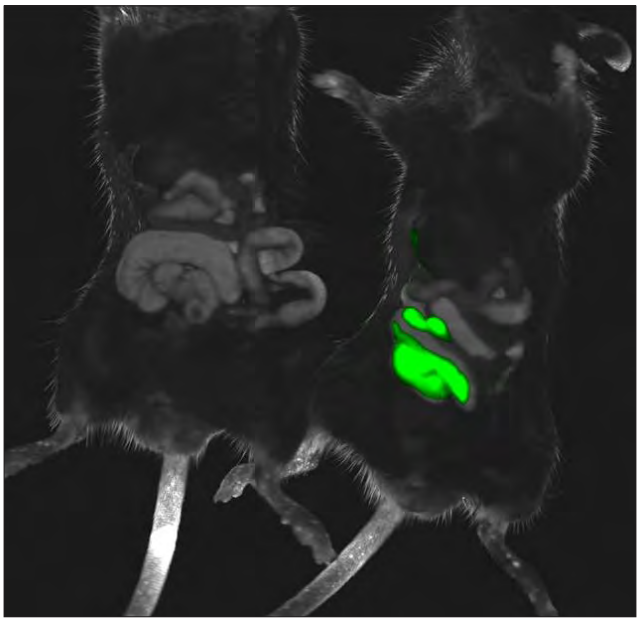
FLUORESCENCE IMAGING

Virus infection in zebrafish

Pixel size	10 µm
Laser	488 nm
Intensity	10

VISUALIZE ANATOMICAL STRUCTURE

The large scanning bed of the Sapphire can accommodate many of the most common small animal models used in today's research labs. Here we show visualization of stained intestine in a rat.



FLUORESCENCE IMAGING
Fluorescently-labeled antibody

Pixel size	50 µm
Laser	784 nm
Filter	832BP37
Intensity	10
Scan speed	Highest

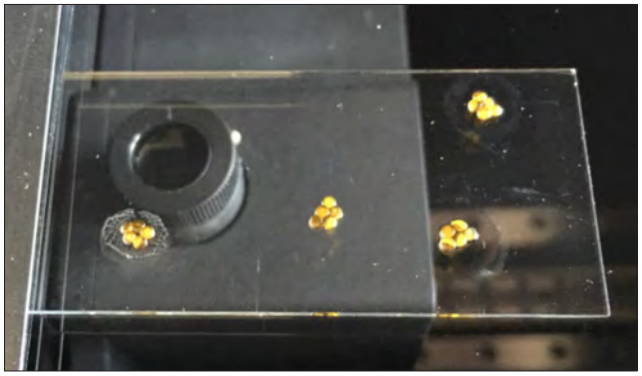
Published protocol

The Sapphire enables a range of tissue visualization, including in plants:

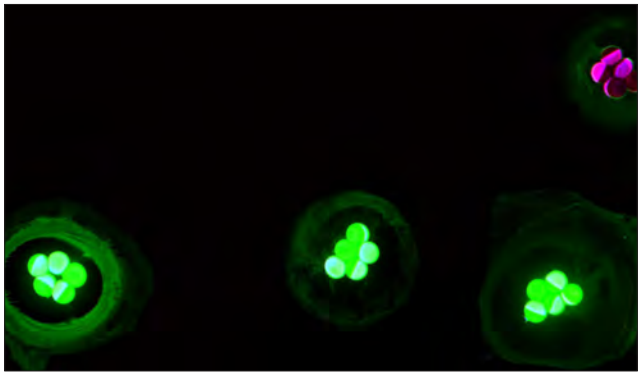
- Detecting Rapid Changes in Carbon Transport and Partitioning with Carbon-11 (11C). Benjamin A. Babst, Richard Ferrieri, and Michael Schueller. *Methods Mol Biol.* 2019;2014:163-176.

IMAGE XENOPUS OOCYTES AND TRACK PROTEIN LOCALIZATION

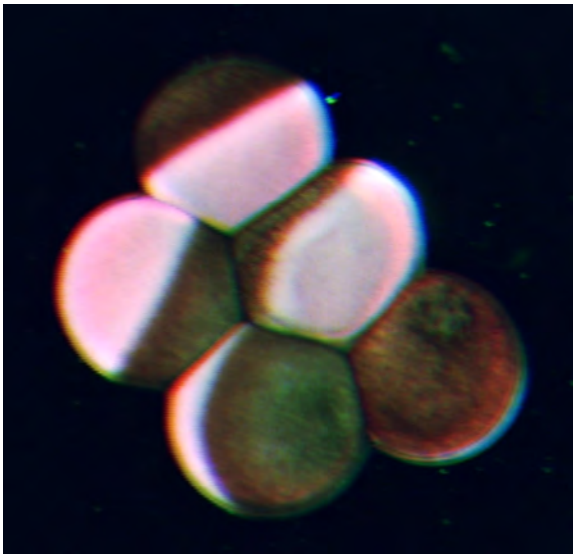
The Sapphire's 10 µm resolution facilitates imaging samples such as Xenopus oocytes and embryos. Here we show oocytes on a slide placed direclty on the scanning bed and imaged. With fluorescently-labeled protein samples, researchers can easily observe localization to specific regions of the oocyte.

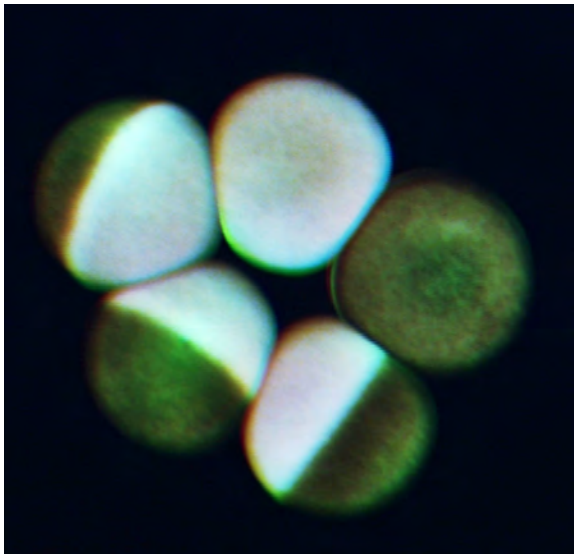


Oocytes on the scanner



Oocytes on the scanner





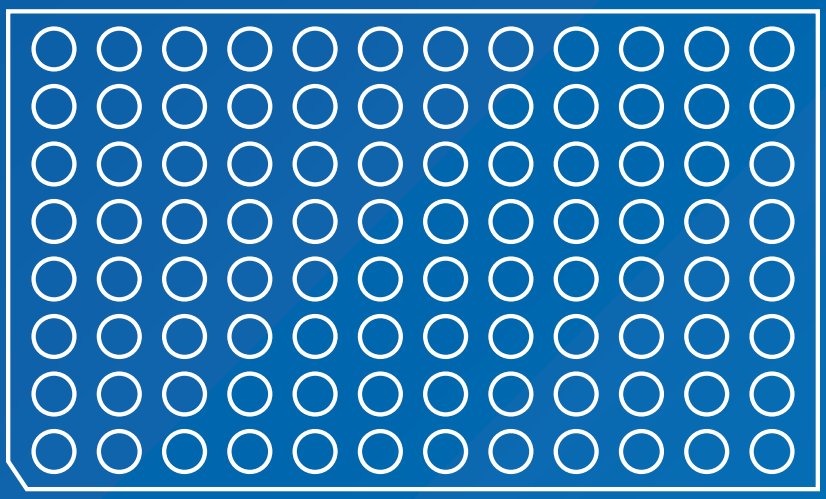
FLUORESCENCE IMAGING

Fluorescently-labeled protein

Pixel size	10 µm
Laser	488 nm, 520 nm, 658 nm, 784 nm

4

96-WELL PLATE IMAGING

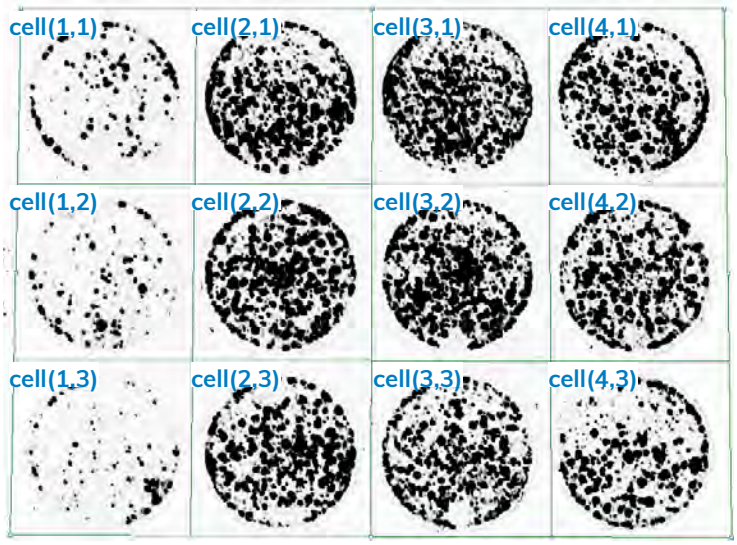


The Sapphire's 10 µm resolution also means you can image and quantify cells within multi-well plates. Use fluorescence detection for a range of quantitative, cell-based assays.

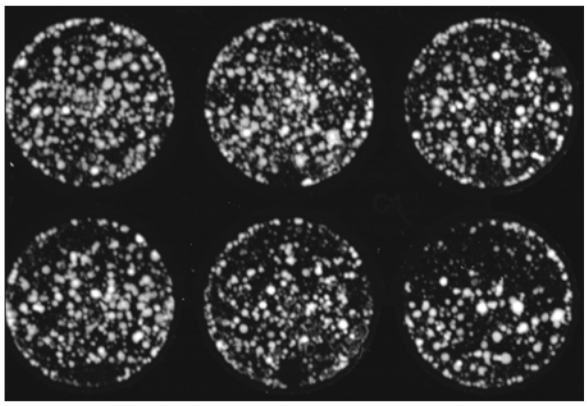
IMAGE CELLS IN MULTI-WELL PLATES

Measuring cell viability using crystal violet

Crystal violet is used to measure cell viability of adherent cells. During the assay, dead cells are washed away and the remaining cells are visualized with the crystal violet dye, which absorbs at 595 nm. The Sapphire enables imaging and quantitation of several multi-well plates at a time, and the abosrbance of each well easily measured.



Positive Image



Negative Image

Name	Volume	Background	Background Level	Background Type	Average Intensity
1 cell(1, 1)	4.24E+06	2.54E+06	38.06	Local Average	101.52
1 cell(1, 2)	2.91E+06	2.61E+06	38.33	Local Average	81.16
1 cell(1, 3)	2.59E+06	2.50E+06	36.66	Local Average	74.72
1 cell(2, 1)	2.25E+07	2.63E+06	38.92	Local Average	372.07
1 cell(2, 2)	2.34E+07	2.67E+06	39.37	Local Average	385.47
1 cell(2, 3)	2.14E+07	2.68E+06	39.25	Local Average	353.36
1 cell(3, 1)	2.00E+07	2.63E+06	38.95	Local Average	334.77
1 cell(3, 2)	2.56E+07	2.76E+06	40.38	Local Average	415.12
1 cell(3, 3)	1.77E+07	2.75E+06	40.2	Local Average	298.88
1 cell(4, 1)	2.28E+07	2.57E+06	38.09	Local Average	375.39
1 cell(4, 2)	2.23E+07	2.63E+06	38.53	Local Average	364.32
1 cell(4, 3)	1.73E+07	2.57E+06	37.31	Local Average	288.56

FLUORESCENCE IMAGING

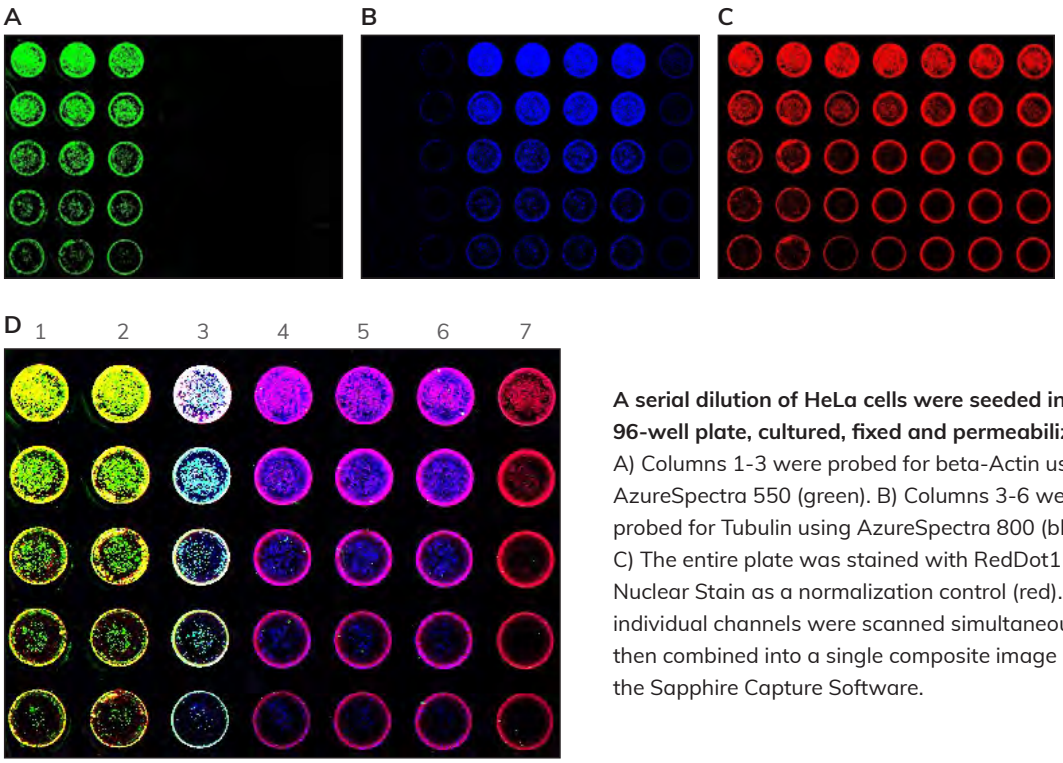
Crystal violet adherent cell viability assay

Pixel size	100 µm
Laser	658 nm
Filter	710BP40
Intensity	5
Analysis	AzureSpot Analysis Toolbox, Grid Shape

IMPROVE EFFICIENCY WITH IN-CELL WESTERN BLOTTING

Accurately quantify intracellular proteins with the repeatability, speed, and throughput of an ELISA

While western blotting has been a lab standard for decades, the high performance of the Sapphire enables time- and labor-saving extensions of the western blot would have been hard to imagine when the technique was introduced. One such extension is in-cell western blotting, where plate-grown cells are fixed, permeabilized, and then probed with antibody in situ. The result is accurate measurement of intracellular protein expression while the cells are still in the plate, which provides a high throughput method for assessing multiple stimulations, end-points, proteins of interest and replicates on a single plate. By using NIR antibodies and the Azure Biosystems Sapphire™ Biomolecular Scanner the potential for in well multiplex analysis also exists offering further improvements to throughput.



A serial dilution of HeLa cells were seeded into a 96-well plate, cultured, fixed and permeabilized. A) Columns 1-3 were probed for beta-Actin using AzureSpectra 550 (green). B) Columns 3-6 were probed for Tubulin using AzureSpectra 800 (blue). C) The entire plate was stained with RedDot1 Nuclear Stain as a normalization control (red). D) The individual channels were scanned simultaneously then combined into a single composite image using the Sapphire Capture Software.

FLUORESCENCE IMAGING

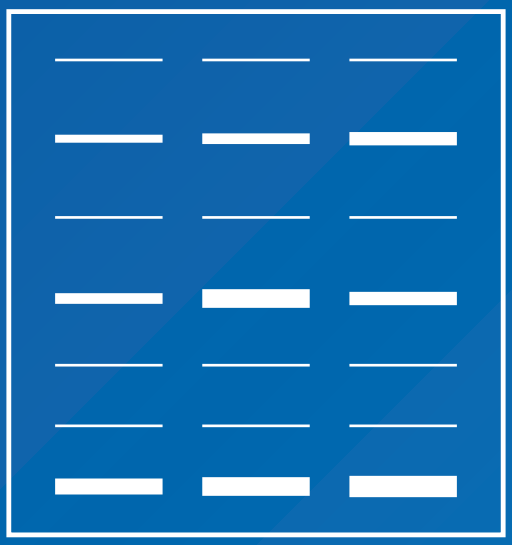
In-cell western blotting

Pixel size	100 µm
Laser	520 nm, 784 nm
Analysis	AzureSpot Analysis

SCAN ME

5

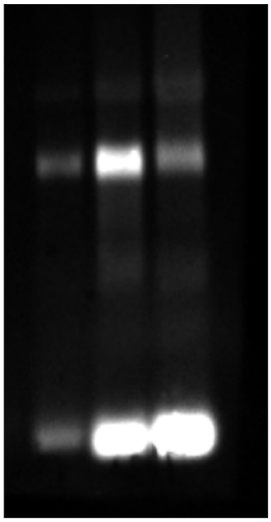
BLOTTING IMAGING PART 1 - SOUTHERN BLOTS



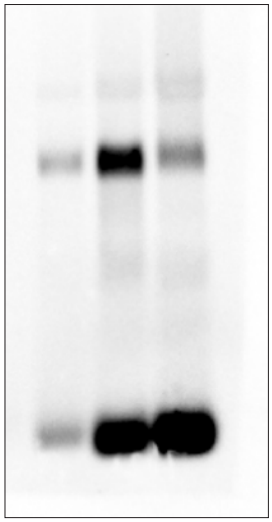
With the ability to image radiolabeled, fluorescently-labeled, and even chemiluminescently-labeled molecules, the Sapphire places an array of southern blot detection technologies at your fingertips.

MEASURING PLASMID ABUNDANCE WITH BOTH PHOSPHORIMAGING AND CHEMILUMINESCENCE

Southern blotting is an excellent method for detecting specific DNA sequences, but it can also provide quantitative information on DNA abundance. In this study, we show a comparison of the linearity of detection of the same plasmid using a P32-labeled probe versus a chemiluminescent detection system. Both methods show similar sensitivity and both can be used for measuring plasmid abundance—R^2 = 0.9742 for P^{32}; R^2 = 0.9599 for chemiluminescence.



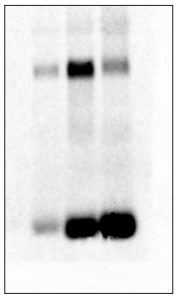
Positive Image



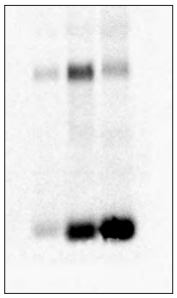
Negative Image

CHEMILUMINESCENCE
Southern blot

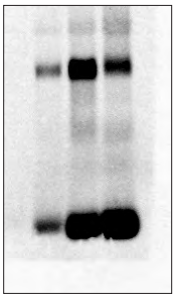
Exposure	90 sec (single mode)
Bin level	3 x 3
Gain	3
Analysis	AzureSpot Analysis Toolbox



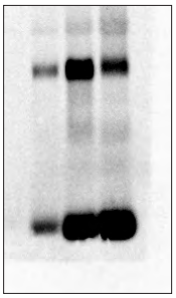
10 sec



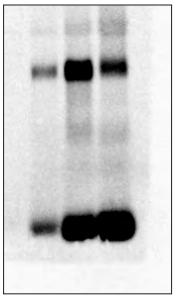
20 sec



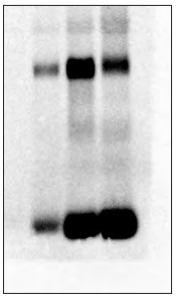
30 sec



40 sec



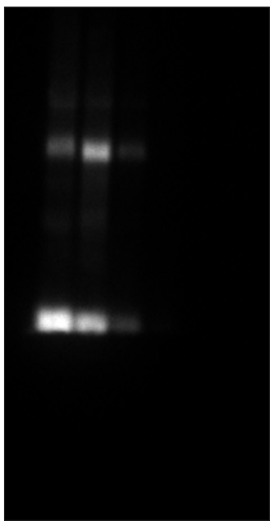
50 sec



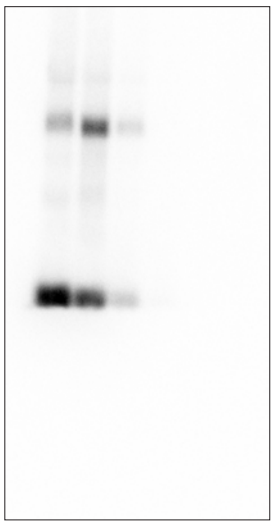
60 sec

CHEMILUMINESCENCE
Southern blot

Exposure	90 sec (single mode)
Bin level	3 x 3
Gain	3
Analysis	AzureSpot Analysis Toolbox



Positive Image



Negative Image

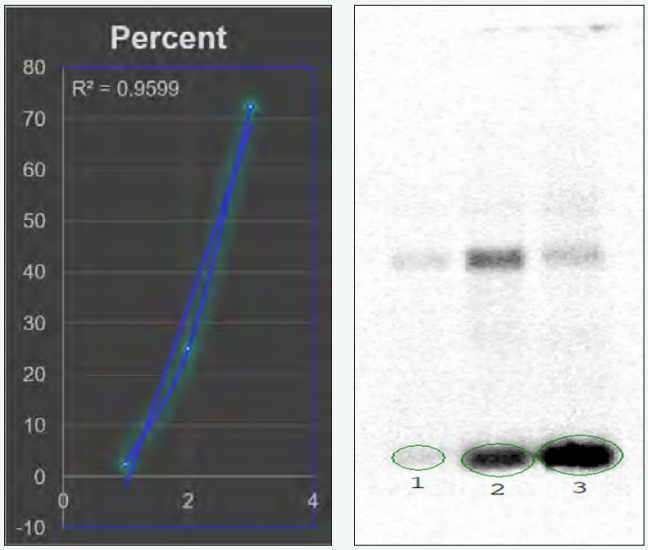
PHOSPHORIMAGING

Southern blot with P^{32}-labeled probe

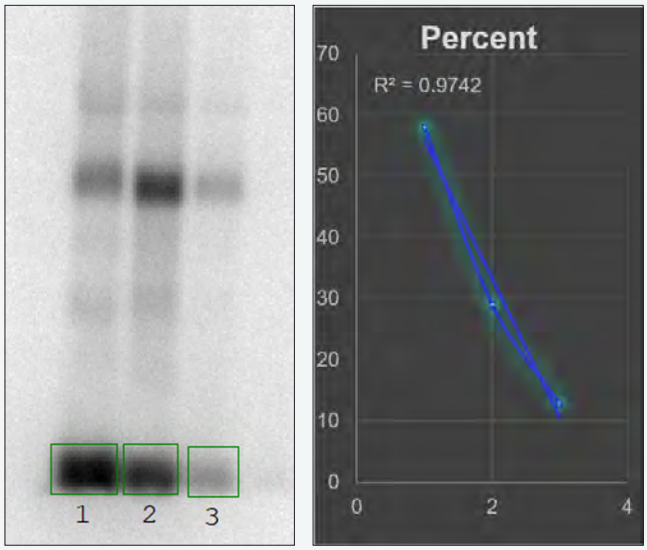
Pixel size	200 µm
Intensity	5
Analysis	AzureSpot Analysis Toolbox

Quantitation comparison

Chemiluminescence



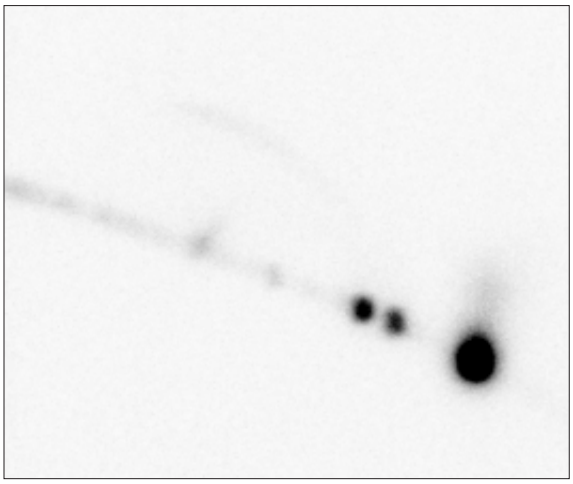
Phosphorimaging



SENSITIVE, QUANTITATIVE DNA DETECTION WITH A ^{32}P-LABELED PROBE

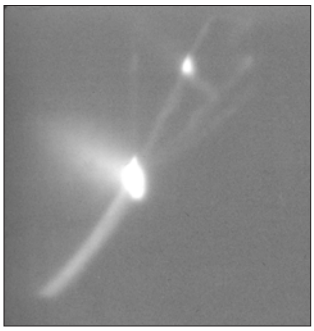
Determining DNA structure with 2D agarose gel electrophoresis

2D agarose gel electrophoresis is an essential technique for understanding DNA structure during replication and recombination, and can differentiate between bubbles, forks, simple Ys, and double Ys. Because these structures can represent only a small fraction of the total DNA loaded on the gel, sensitive detection is a must. Here we show detection and quantitation of 2D agarose gels by Southern blotting with a ^{32}P-labeled probe.

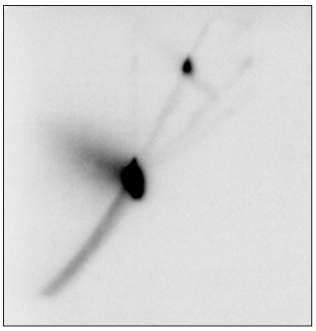


PHOSPHORIMAGING
Southern blot with P^{32}-labeled probe

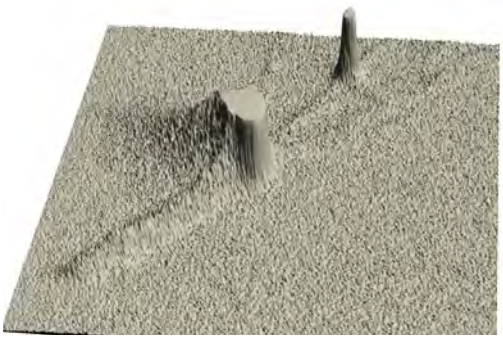
Pixel size	50 µm
Scan speed	Highest
Intensity	5
Focus position	5
Quality	1
Analysis	AzureSpot Analysis Toolbox



Positive Image



Negative Image



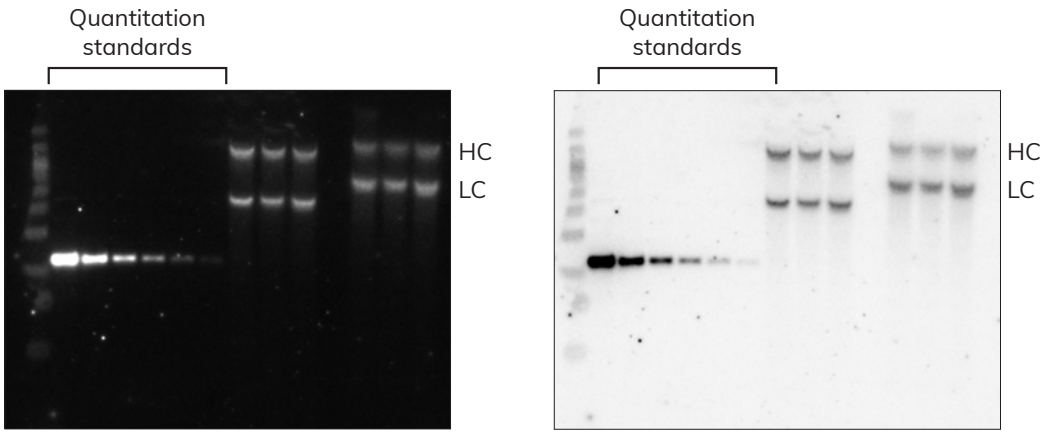
PHOSPHORIMAGING
Southern blot with P^{32}-labeled probe

Pixel size	200 µm
Intensity	5
Analysis	AzureSpot Analysis Toolbox, 3D Viewer

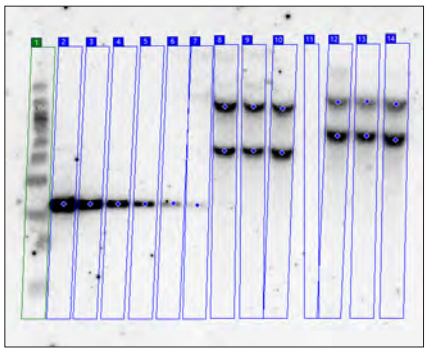
SENSITIVE, QUANTITATIVE DNA DETECTION WITH A ^{32}P-LABELED PROBE

Measuring light chain-heavy chain DNA ratios for recombinant antibody expression

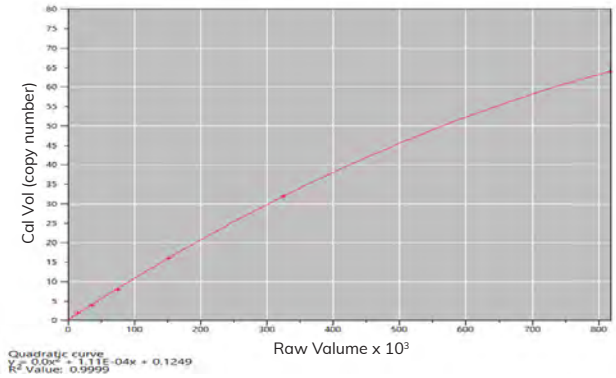
A common step during recombinant antibody production is the measurement of light chain (LC) DNA to heavy chain (HC) DNA ratio. Here the Sapphire was used in this application to detect samples of interest alongside a DNA standard for quantity calibration. The images produced by the Sapphire show data that is not only linear (R^2=0.99) but also highly sensitive with detection down to 2 copies.



Positive Image **Negative Image**



Quantitation



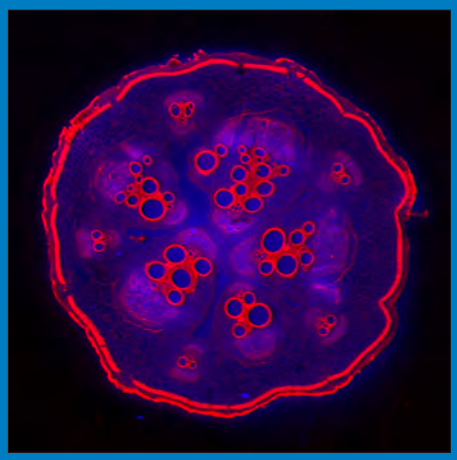
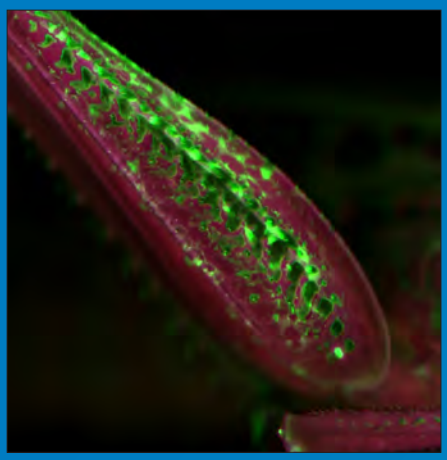
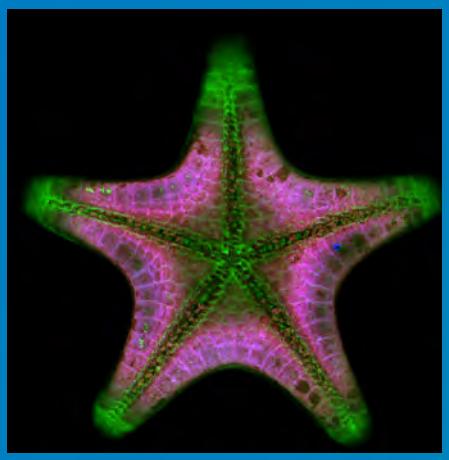
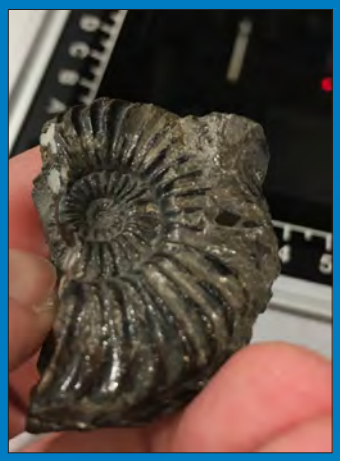
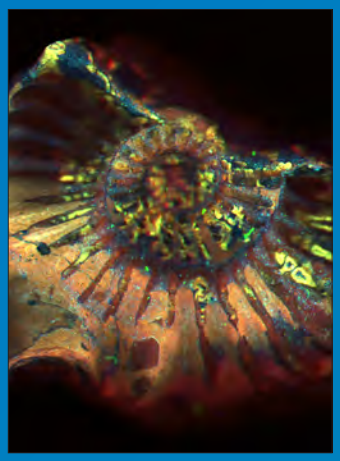
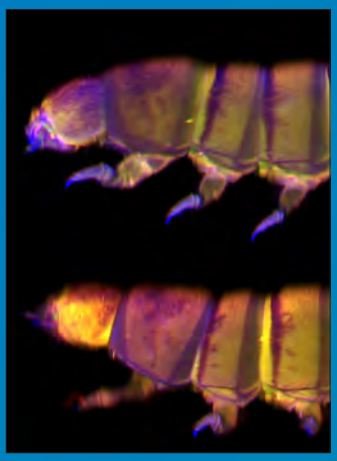
PHOSPHORIMAGING

Southern blot with P^{32}-labeled probe

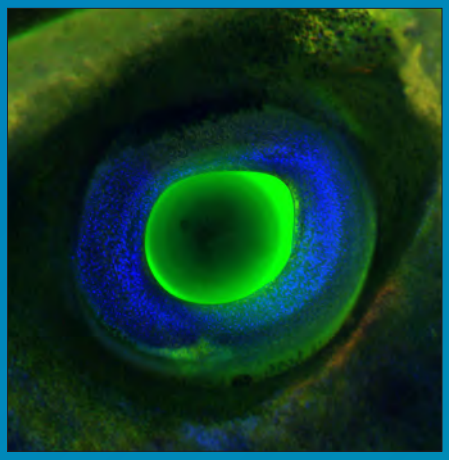
Exposure	1 Day
Pixel size	50 µm
Intensity	3
Analysis	AzureSpot 1D module Graph calibration volume

GENERAL IMAGE CAPTURE

Easily scan & image samples for record-keeping, quantitation, visual inspection, and more...





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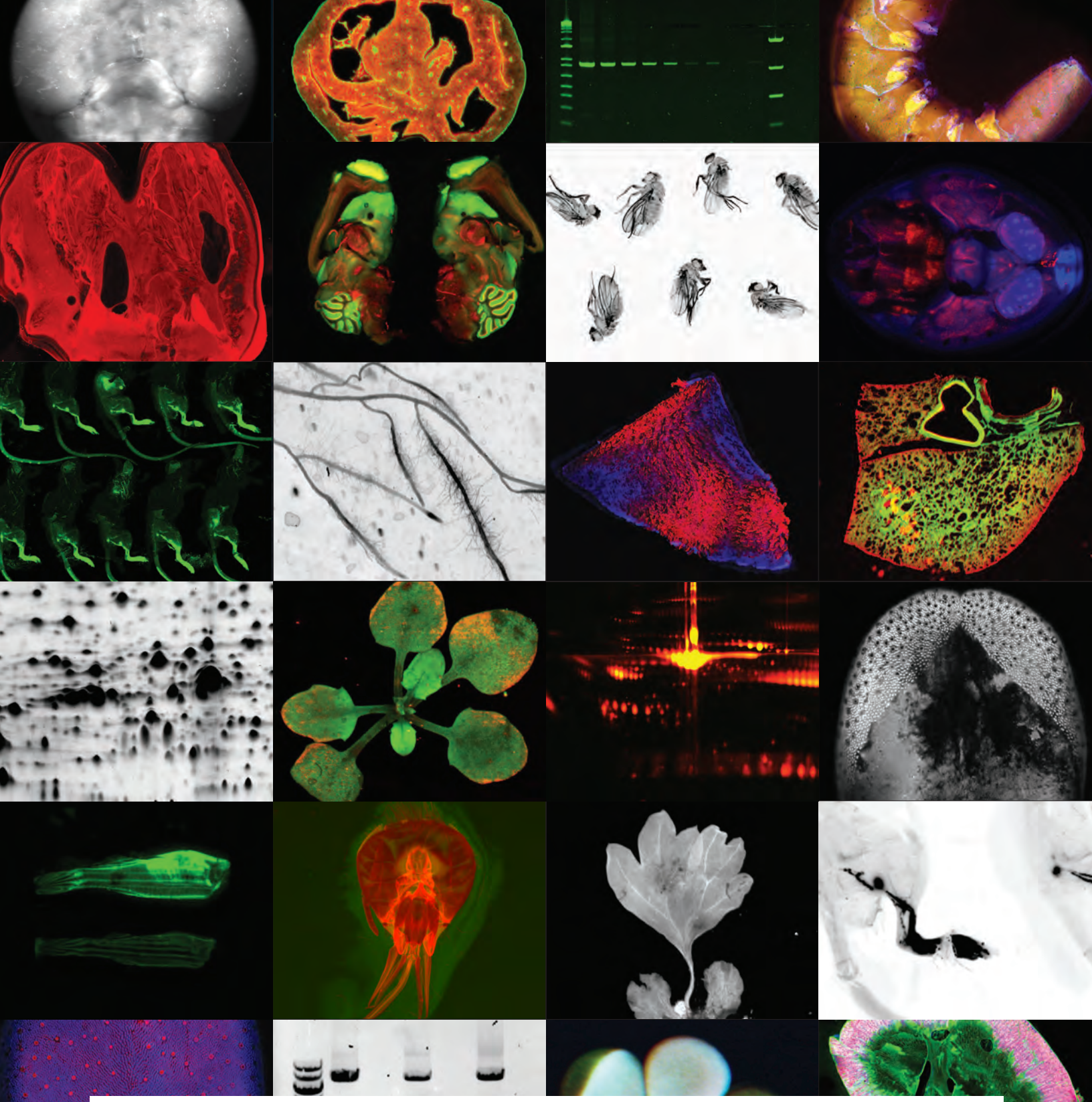
SCAN ME



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