



Amersham™ Imager 600 series, imaging & applications

Imagination at work.

Outline

- Introduction to imaging and Amersham Imager™ 600
- 1D/Western blotting & quantitative imaging
- Performance and Applications



Amersham™ Imager 600 & imaging



What is a CCD¹ imager?

A camera specialized for producing digital images of gels and membranes



¹CCD - Charge-Coupled Device: A light-sensitive silicon chip often used as a photodetector in digital camera systems.

What makes a good CCD imager?

- High sensitivity
- Good linearity
- Wide dynamic range
- Minimal crosstalk



What offers a CCD imager?

Automatic image acquisition

No need for multiple exposures

Image archiving

Easy to save, transport and share images

Safety and Cost

Less Environmental Health Safety issues – no waste from fixative and developer etc.

Savings on film costs



Amersham™ Imager 600 series

Confident imaging



Excellent performance for gel and membrane imaging

Consistently delivers

- high-resolution images
- high sensitivity
- broad dynamic range

in all imaging modes

- chemiluminescence
- fluorescence staining/label
- visible color staining



Amersham™ Imager 600 series Design



Amersham™ Imager 600

For maximized confidence

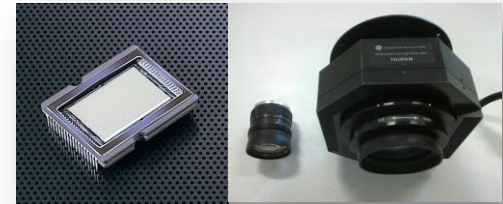
Intuitive

- Intuitive control software & integrated analysis
- Intuitive iPad™ operation
- Immediate data generation
- Minimized training time



Performance

- Superior optics for highest sensitivity & image quality: Super-honeycomb CCD & FUJINON™ lens
- Support all imaging modes
- Improved multiplexing functionality
- Quantitative imaging data



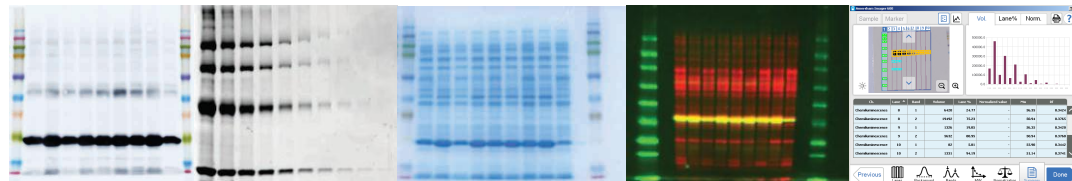
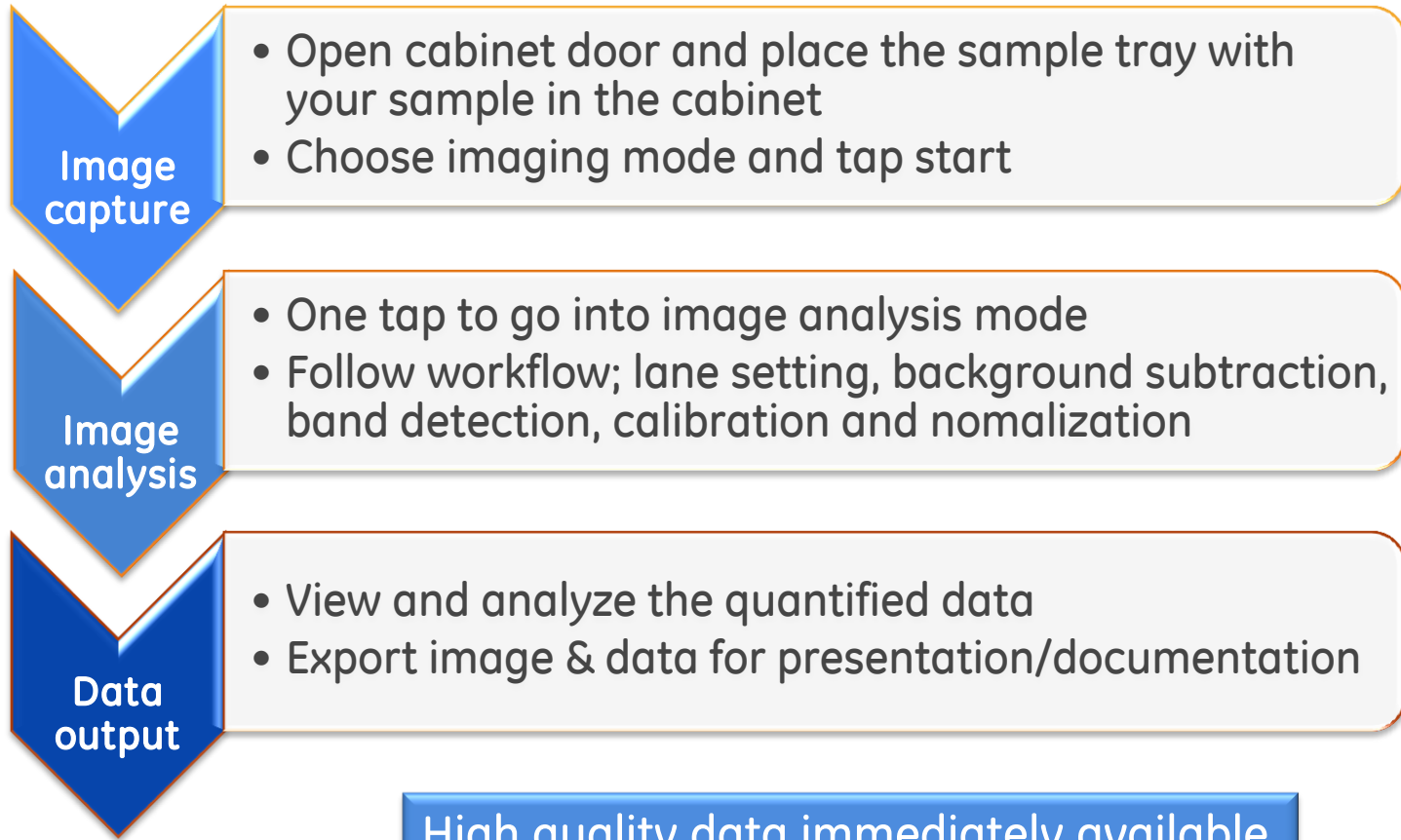
Robust

- Minimal Maintenance
- Proven application expertise
- Over 5,000 installed base system
- Proven Validation support & extensive IQ/OQ documentation



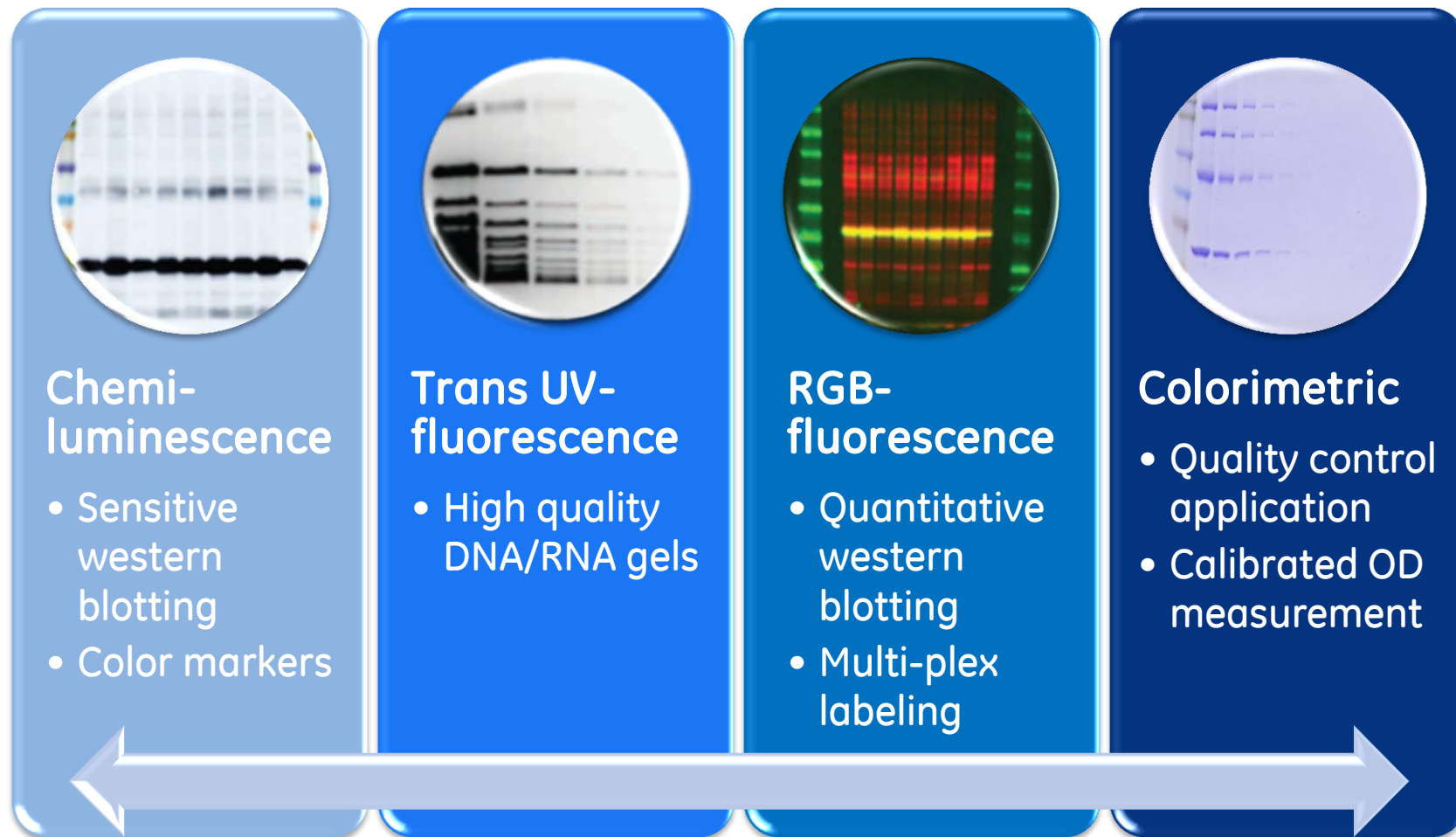
Amersham™ Imager 600

Effortless image acquisition & analysis



Amersham™ Imager 600

Multiple imaging modes



Amersham Imager 600

Flexible interface settings



Standard interface

iPad WiFi connection



Alternative interface 1

Wired touch screen
(DVI+USB+power)
No wireless device



Alternative interface 2

Standard monitor
(DVI+power) and mouse
Optional keyboard
No wireless device



Amersham Imager 600

Pre-configured models



	Amersham Imager 600	Amersham Imager 600 UV	Amersham Imager 600 RGB	Amersham Imager 600 QC
Chemiluminescence & Epi white*	X	X	X	X
UV Fluorescence	O	X	X	X
Calibrated OD measurement	O	O	X	X
RGB Fluorescence	O	O	X	O
Field of view / acquisition area (mm) **	220 x 160 (110 x 80)	220 x 160 (110 x 80)	220 x 160 (110 x 80)	220 x 160 (110 x 80)



X

O

*

**

Standard

Optional

Non-quantitative documentation only

Lower tray position (Upper tray position)

Amersham Imager 600 user Interface

Taking usability to the next level

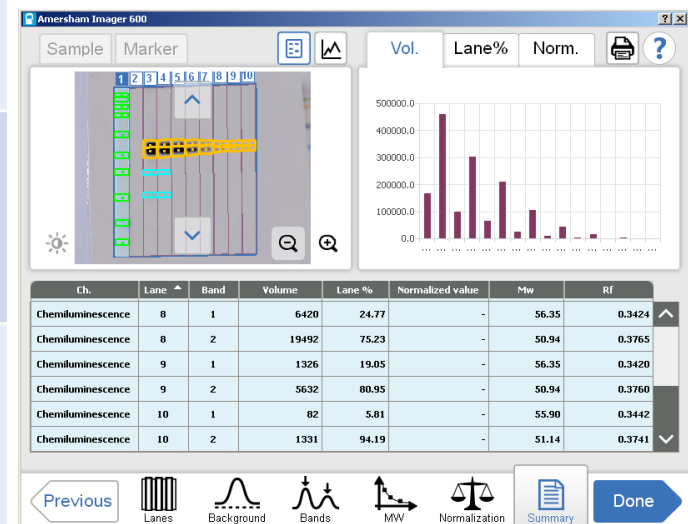
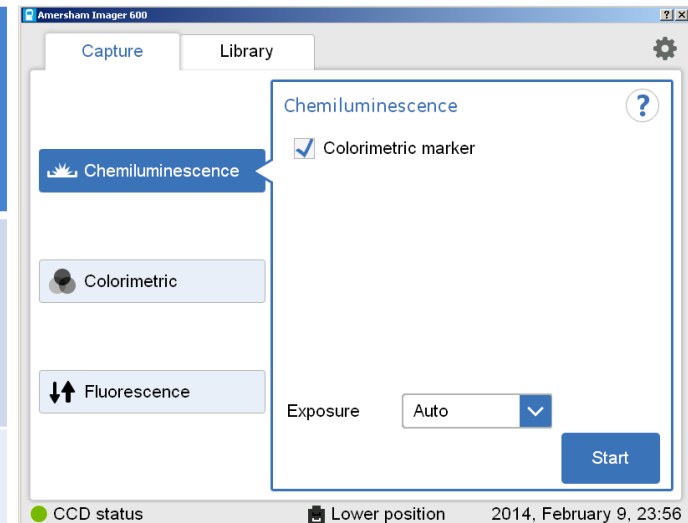
Key features

Intuitive

Fast with minimal user input required

Integrated image capture and analysis

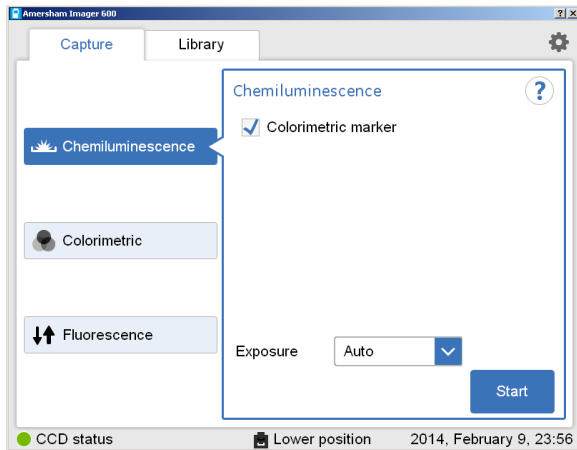
Contemporary Design



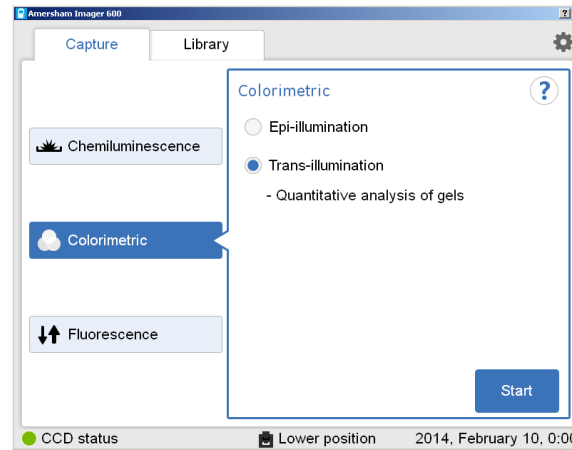
Amersham™ Imager 600

Simple image capture steps

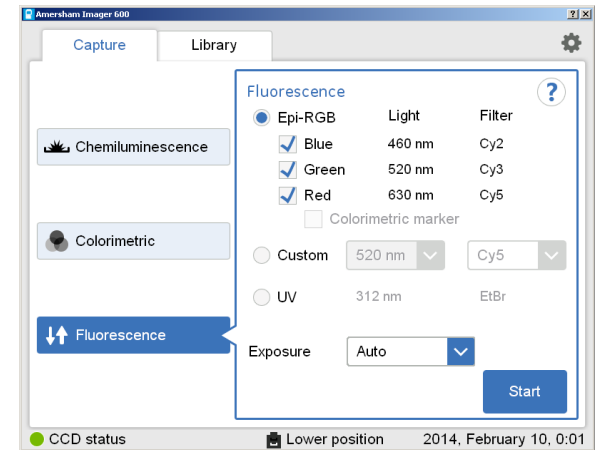
Chemiluminescence



Colorimetric

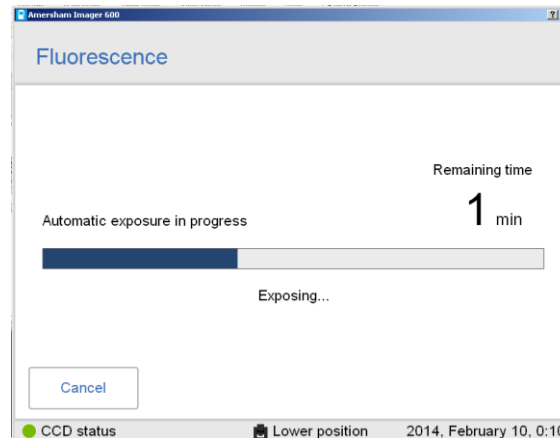
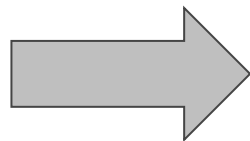


Fluorescence



1-3 taps to capture single channel image

5 taps to capture 3 multiplex images



Amersham™ Imager 600

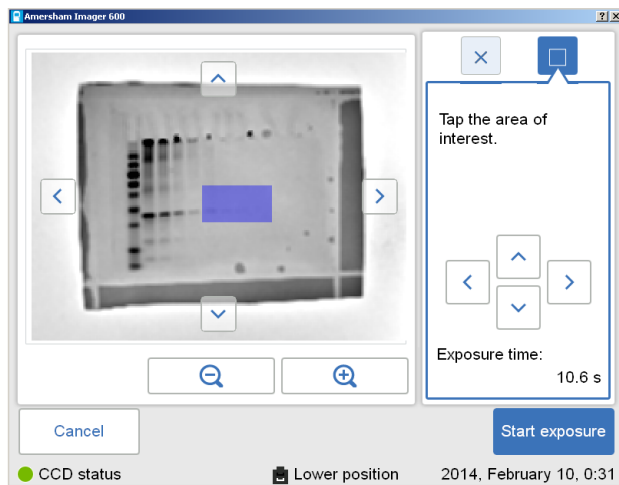
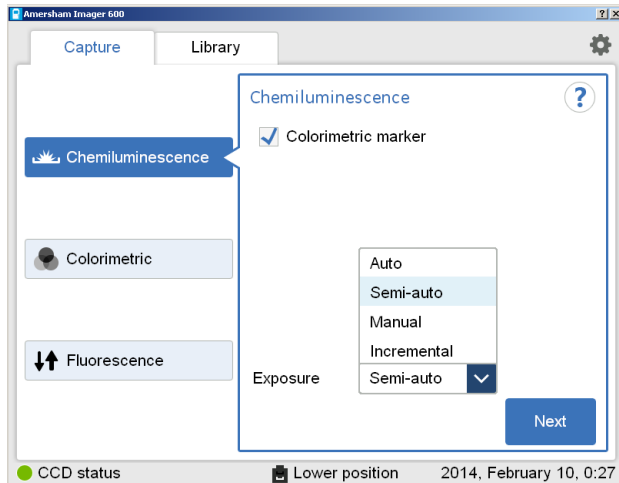
Convenient capturing

Multiple exposure types

- Auto
- Semi-auto
- Manual
- Incremental
- Semi-auto

Semi-auto

- Specify the region of interest and capture with the optimised condition

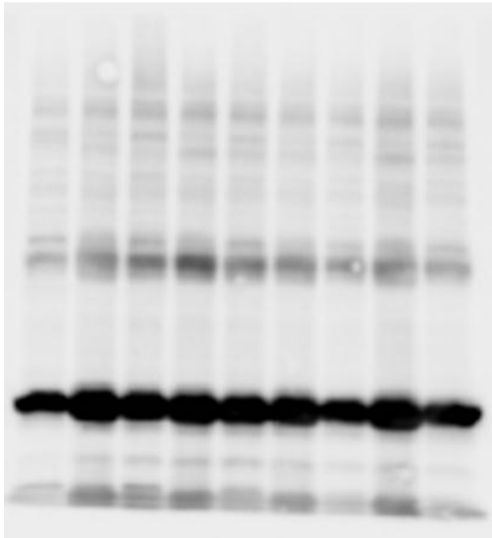


Amersham™ Imager 600

Unique feature - Composite color image

Sample image

Chemiluminescence (tif)



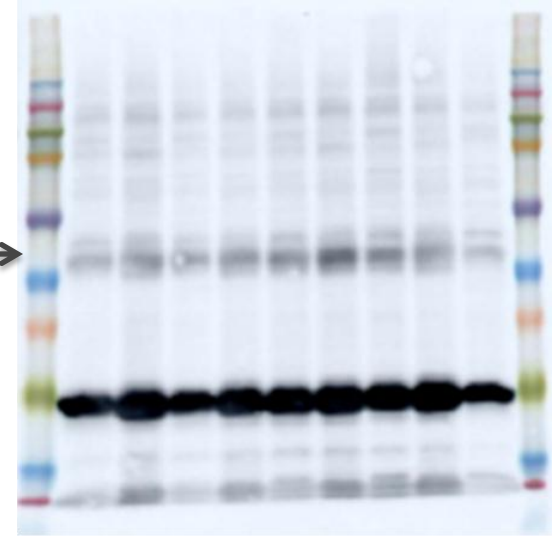
Color marker image

Epi white (jpg/tif)



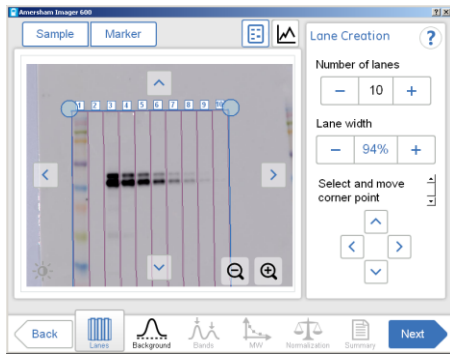
Composite image of sample

and color marker (jpg)

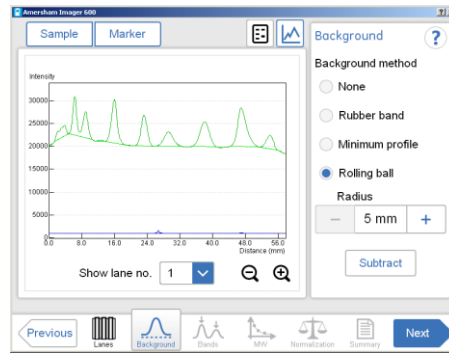


Amersham™ Imager 600

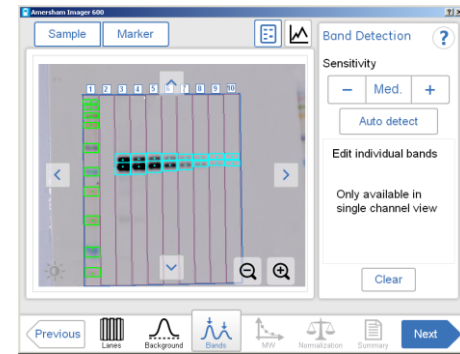
Simple image analysis steps



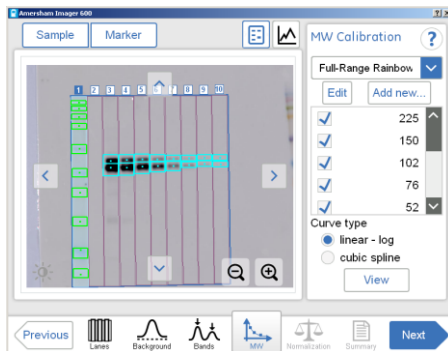
Lane



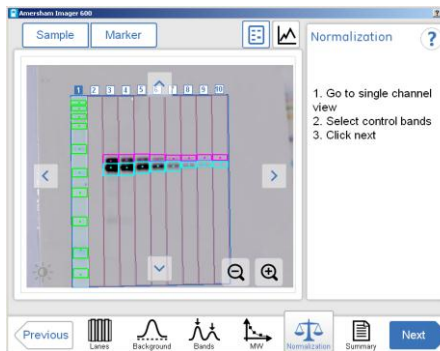
Background



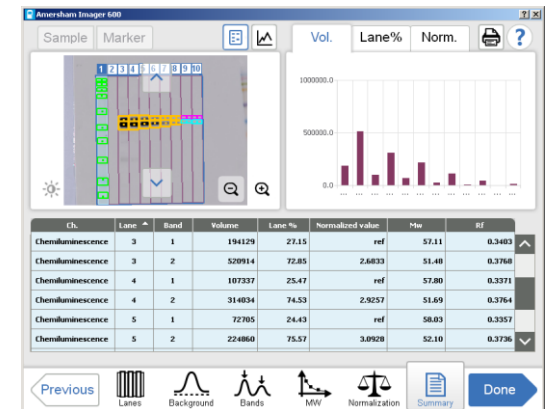
Band



MW marker



Normalization



Summary



Amersham™ Imager 600

Key benefits

- ✓ Superior CCD optics from Fujifilm™ (f/0.85 FUJINON™ lens)
- ✓ Publication Quality Images (317dpi)
- ✓ Integrated user-friendly software with 3 control options including iPad™
- ✓ Built-in auto-calibration for quantitative OD measurement of colorimetric samples (*models QC & RGB)
- ✓ Technical product expertise of our Field Application Scientists
- ✓ Robust instruments requiring minimal maintenance



1D/Western blotting & quantitative imaging



1D Electrophoresis in theory

Separation of proteins in a gel according to the size of the proteins.



Horizontal easy to use
gel running system
Amersham™ ECL™ gel
system



Large proteins

MW Marker

Small proteins

Acrylamide Gel stained with Coomassie™ Blue

Small proteins migrate faster than large proteins

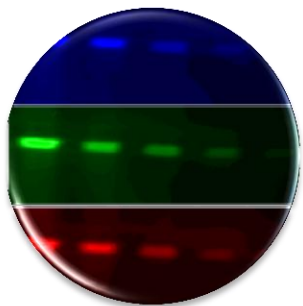


Various options for protein visualization

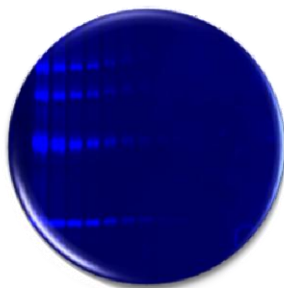
After electrophoretic separation

Pre labeling

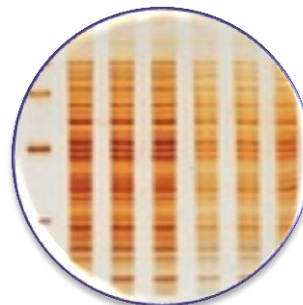
Post staining



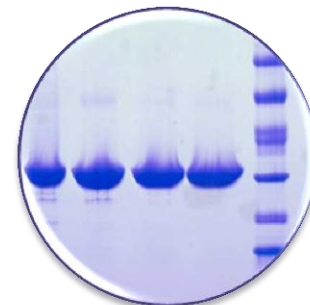
CyDye™



SYPRO™ Ruby



Silver



Coomassie™
Blue

Detection limit

LOD 0.02-0.2ng

LOD 0.52-2ng

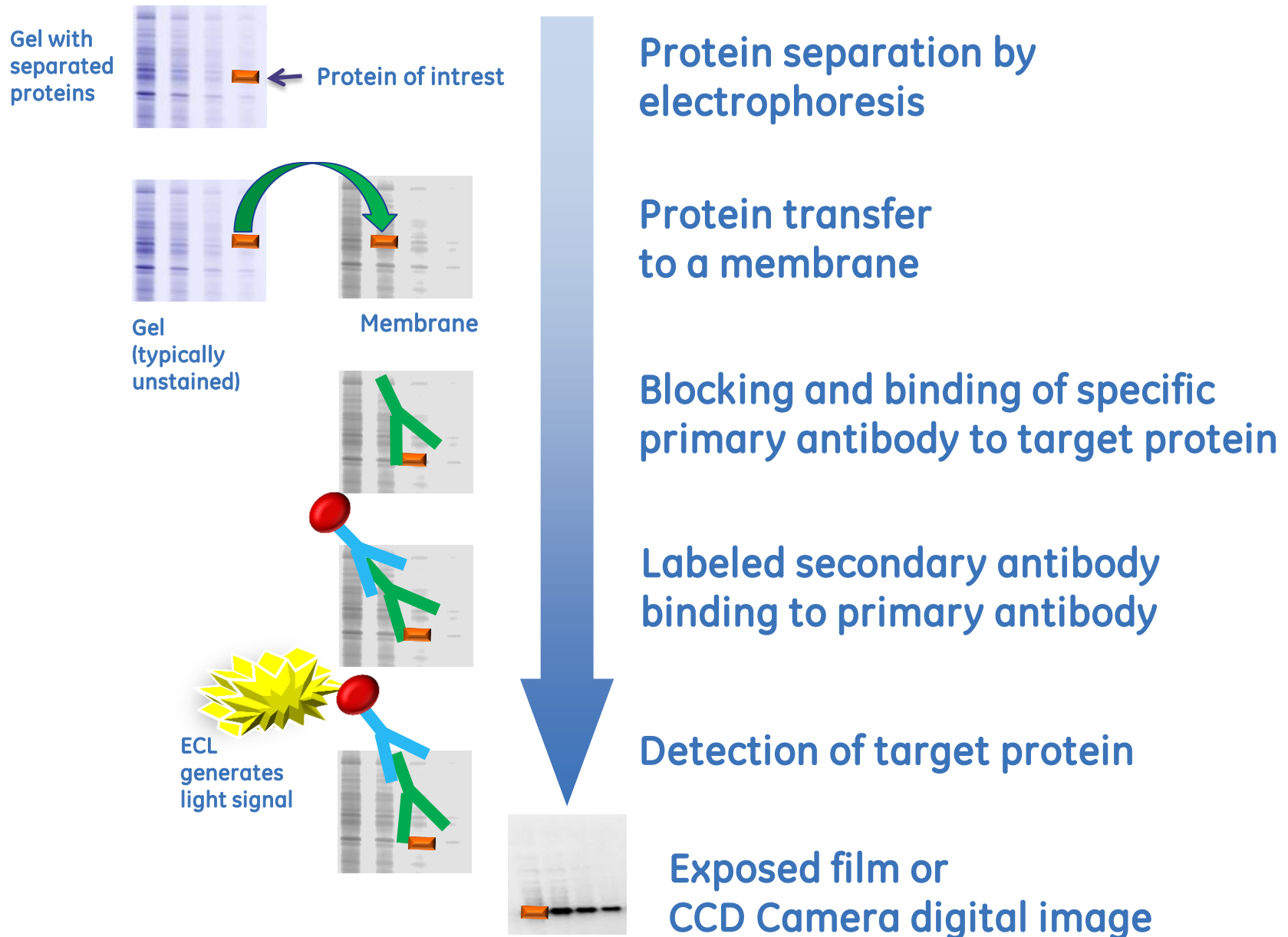
LOD 0.5-2ng

LOD 10ng

Dynamic range



Western blotting workflow



When could Western blotting be used?

Expression and purification

- Protein purification
- Recombinant proteins
- Tagged proteins



Cell and molecular biology

- siRNA efficiency
- Stimulation effects
- Posttranslational modifications
- Protein-protein interactions
- Protein regulation



Clinical research

- Proteins in serum
- Antibody detection



Western blotting detection methods

Target protein
Primary antibody

HRP conjugated
Secondary antibody

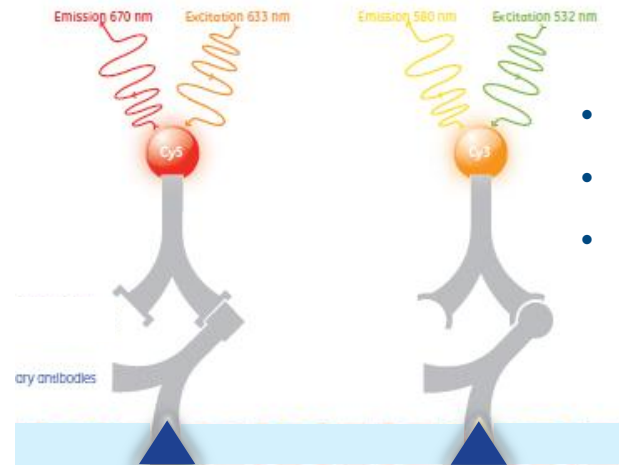
Amersham™ ECL Select™
Amersham ECL Prime
Amersham ECL™

Chemiluminescence

- Indirect detection
- Non stable and declining signal
- Single protein detection

Amersham ECL Plex™
CyDye™ labelled
Secondary antibody

Fluorescence



- Direct detection
- Stable signal
- Multiplex detection

Chemiluminescent detection

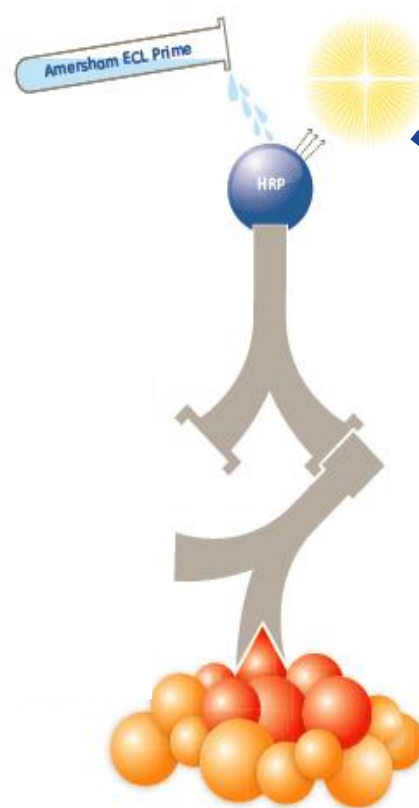
Chemiluminescent detection reagent

Amersham™ECL™
Amersham ECL Prime
Amersham ECL Select™

Secondary HRP linked antibody

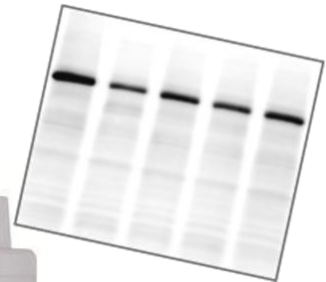
Primary antibody

Target protein



X-ray film

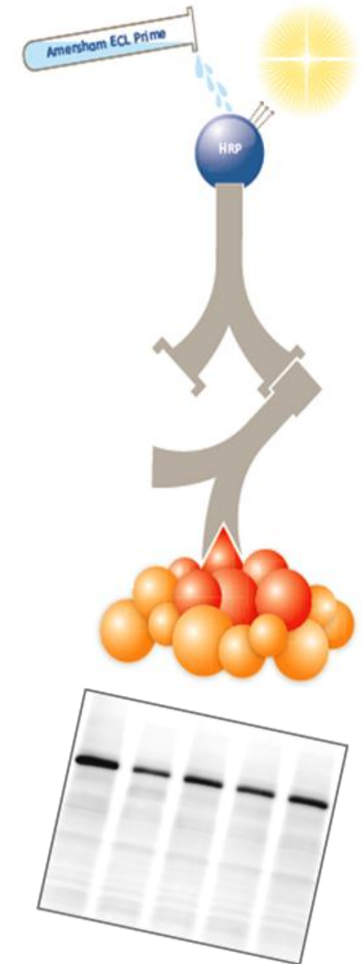
Amersham Hyperfilm™ ECL
Amersham Hyperfilm MP



Amersham Imager 600

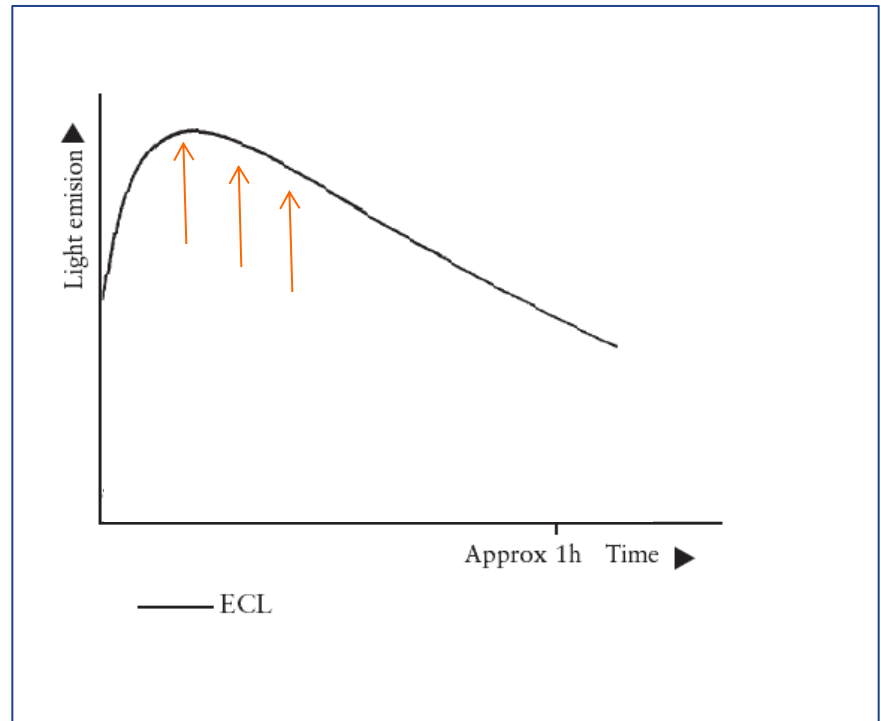
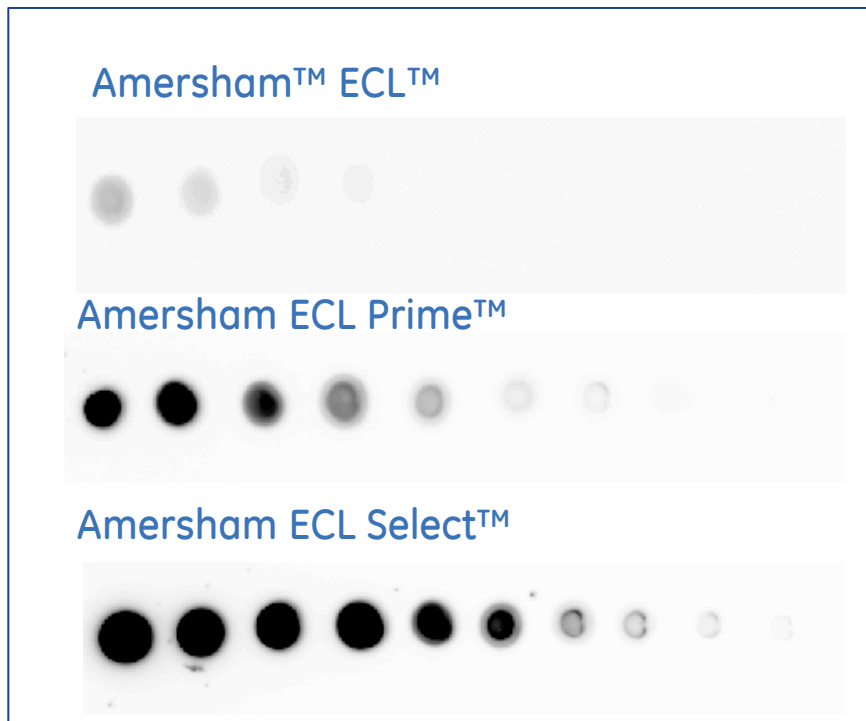
Chemiluminescent Western blotting

Signal	Detection
Indirect signal from a chemical reaction Visible light, unstable	Single protein
Sensitivity	Imaging
From low to high Reagent and imaging dependent	X-ray film CCD imager
<ul style="list-style-type: none">+ Well established method+ Wide range of reagents and HRP antibodies+ Versatile imaging- Variation in signal intensity between blots- Fading signals- Stripping and re-probing required for second protein detection- Strong signals may cause saturation on X-ray film	



Chemiluminescent signal

Intensity and stability variates



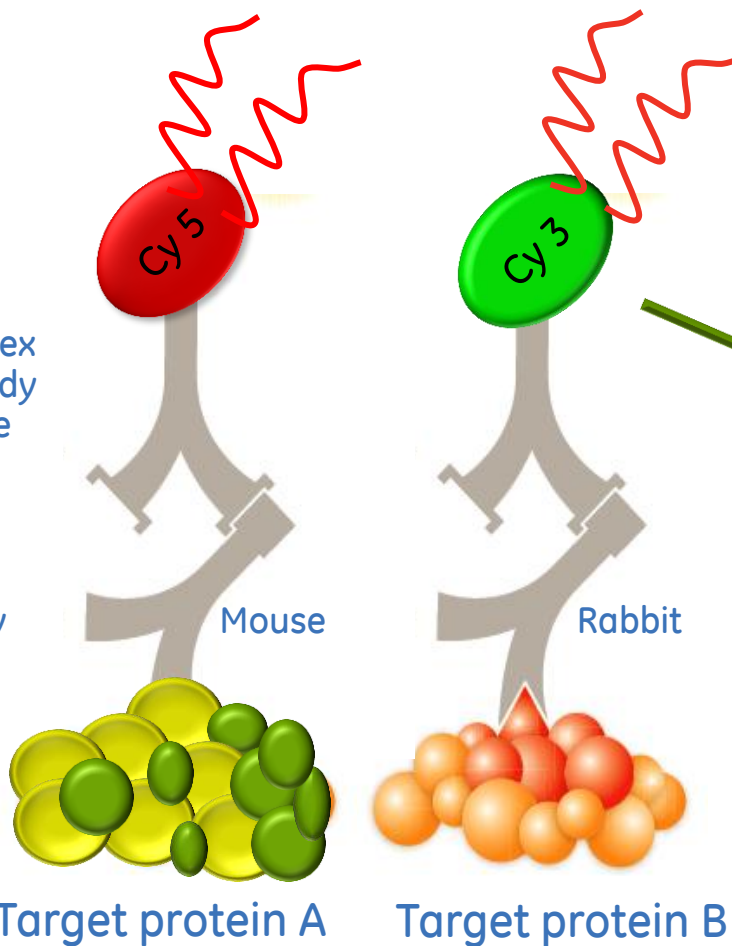
Signal intensity varies between reagent and is time dependent

Fluorescent detection

Amersham ECL Plex™

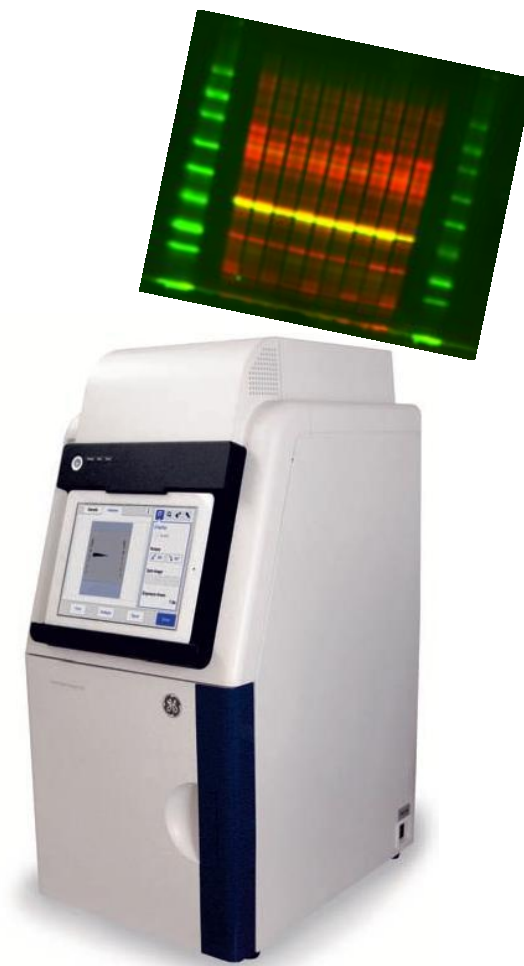
Amersham ECL Plex
secondary antibody
CyDye™ conjugate

Primary antibody



Target protein A

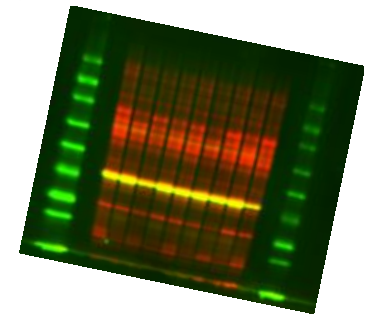
Target protein B



Amersham Imager 600

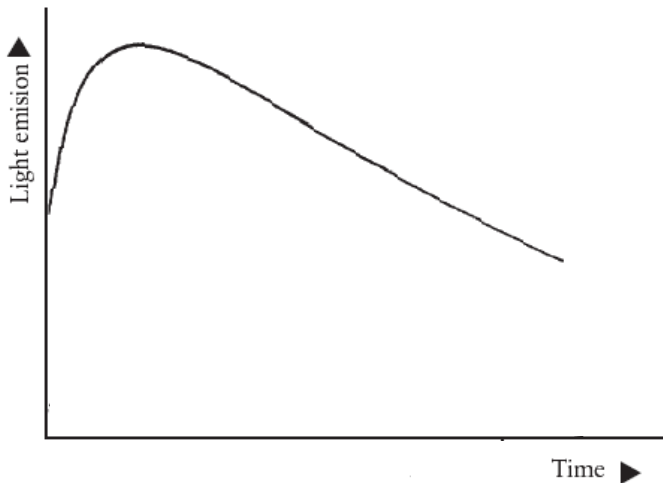
Fluorescent Western blotting

Signal	Detection
Direct, no reagent is required Fluorescence Stable	Single and multiplex
Sensitivity	Imaging
High Broad dynamic range	Laser scanner CCD imager with suitable excitation sources and emission filters
<ul style="list-style-type: none">+ Ability to multiplex detection+ No fading signals, multiple exposures, easy to handle many blots+ More reproducible+ Improved quantitation- Handling to avoid fluorescent artifacts- Limited species of secondary antibodies	



Signal duration

Chemiluminescence vs fluorescence



Chemiluminescent signal declines over time. Intensity varies depending on when signal is captured. Inconsistent visualization.

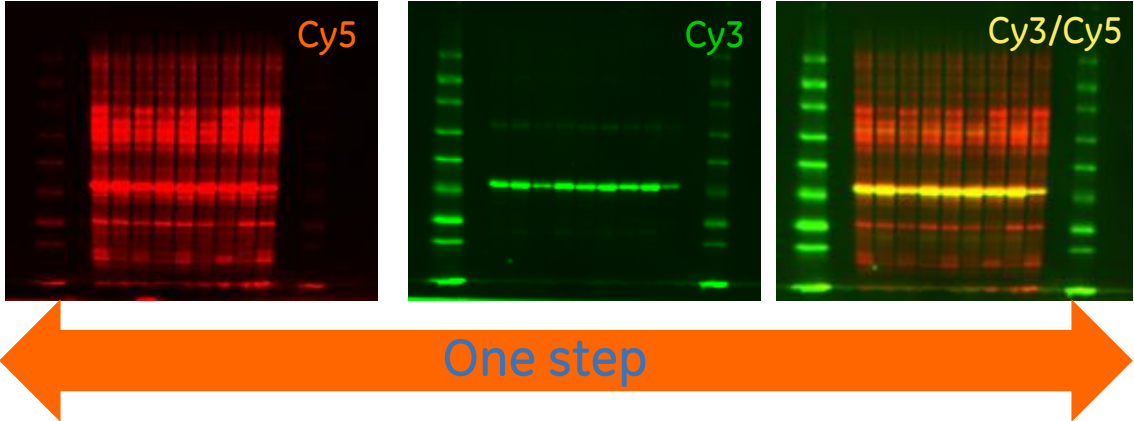


Fluorescent signal is stable over time and does not decline. The intensity is equal all the time. Allows for repeated exposures, convenient handling. More reproducible and consistent visualizations.

Combining 1D and Western blotting- Fluorescent detection

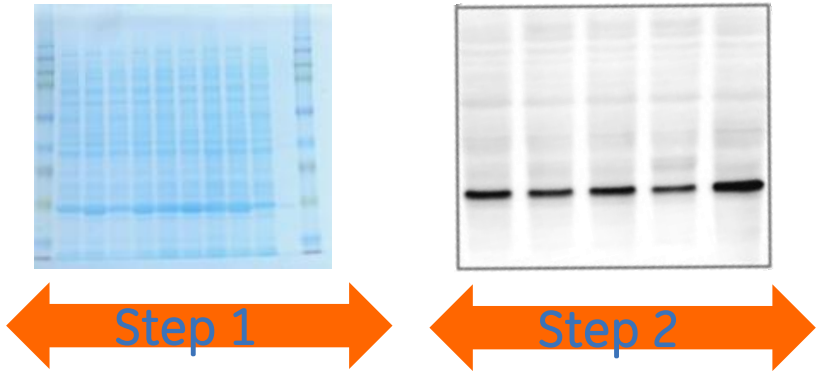
Fluorescent detection

- No post-staining
- Convenient
- Time saving
- Sensitive, Broad dynamic range, Multiplexing
- Quantitative
- Digital images



Traditional detection

- Coomassie and chemiluminescent WB-
- post staining and indirect detection in two steps
- Less sensitive, less dynamic range and no multiplexing
- Digital images



Quantitative Imaging

- **Linearity**

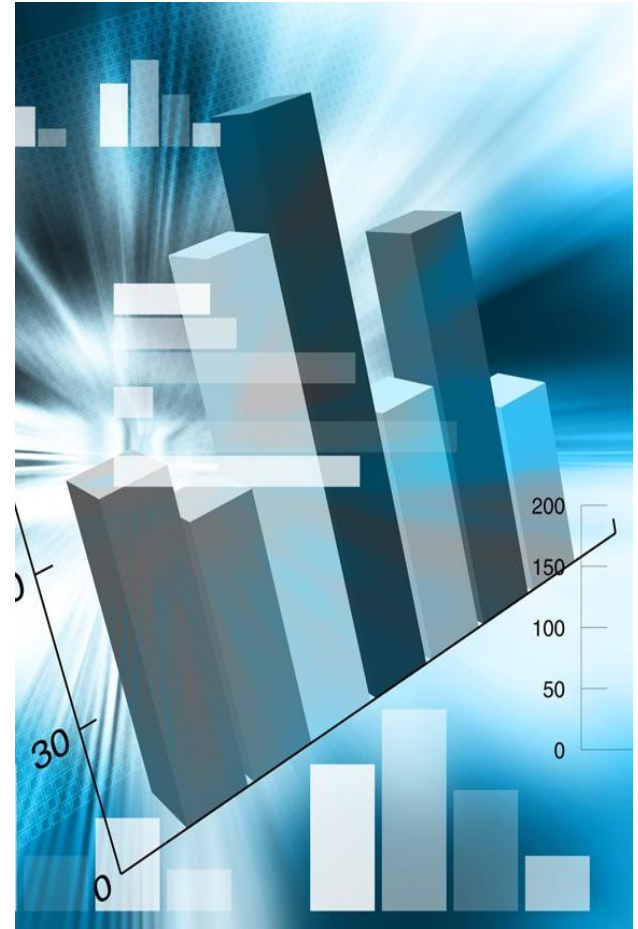
Signal response proportional to protein amount

- **Wide dynamic range**

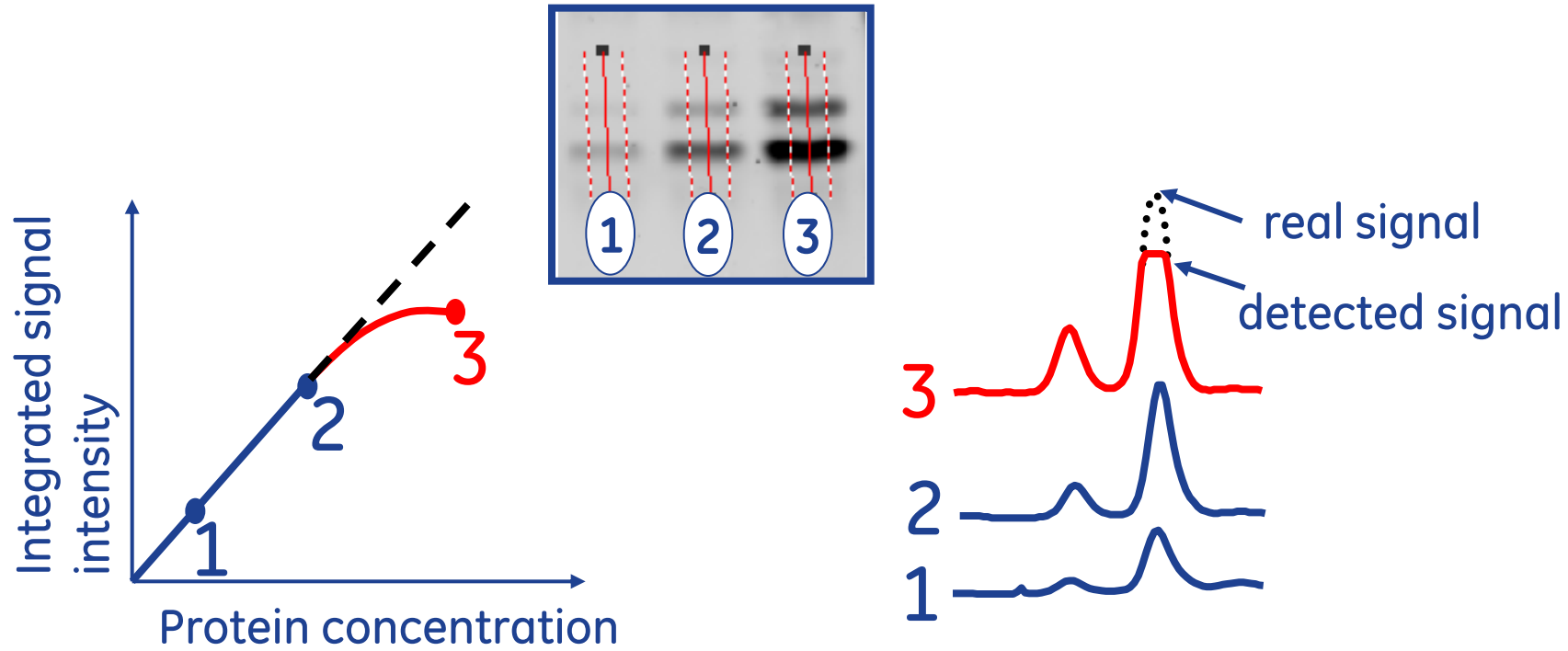
Allows quantitation across a wide range, quantifying both high and low expressed protein levels

- **In-lane lane Normalization**

Allows normalization towards house keeping (corrects for uneven sample load)

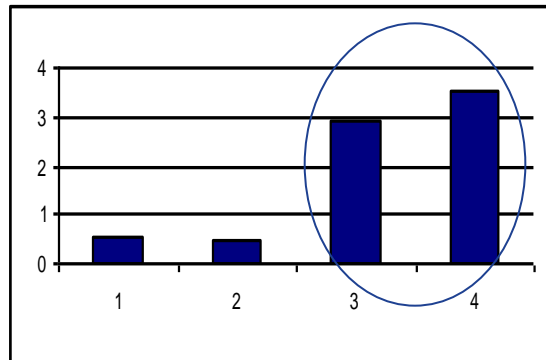
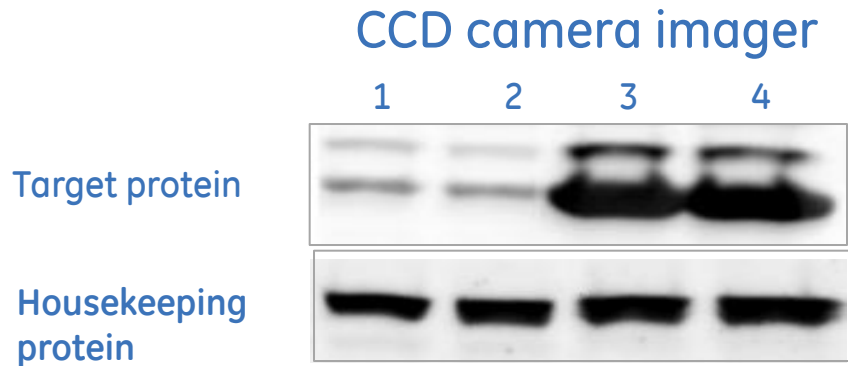


Saturation – Why it is important to avoid

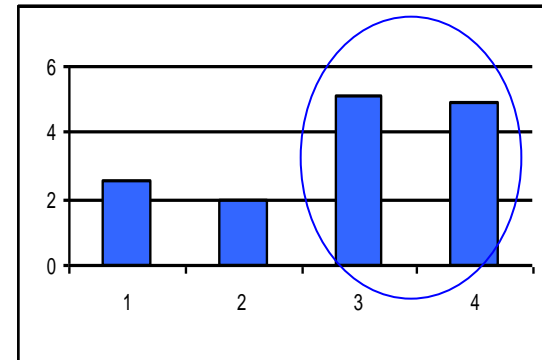
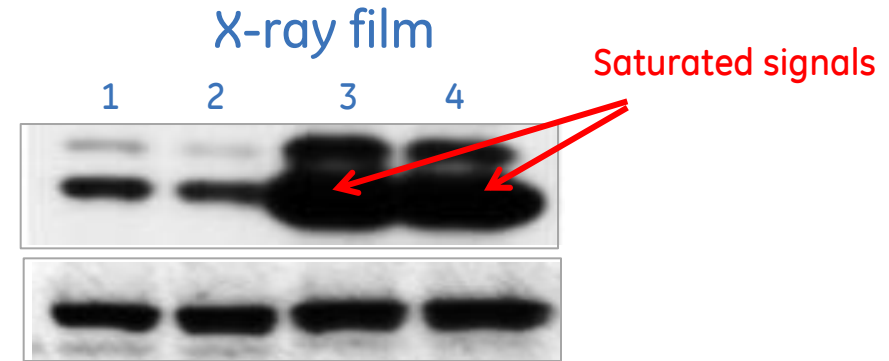


Saturation - signal not proportional to protein concentration

Saturation results in inaccurate quantitation



Accurate quantitation with non saturated signals shows a 6 fold increase of sample 3 and 4



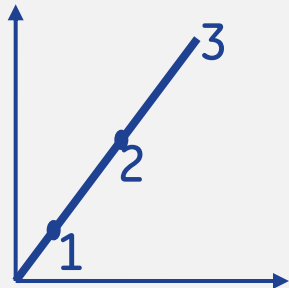
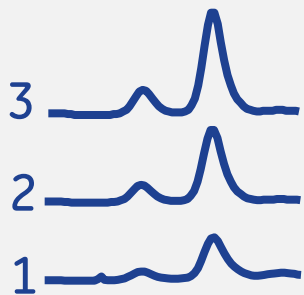
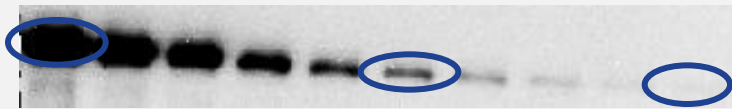
Saturated signals cause inaccurate quantitation and shows only 2.5 fold increase of sample 3 and 4

Why broad linear dynamic range?

CCD camera imager

Linear dynamic range

5 ng ← → 9.8 pg

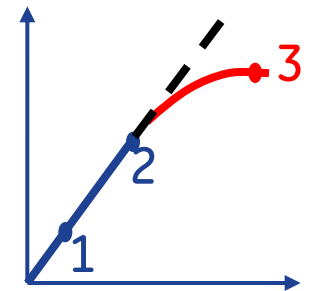
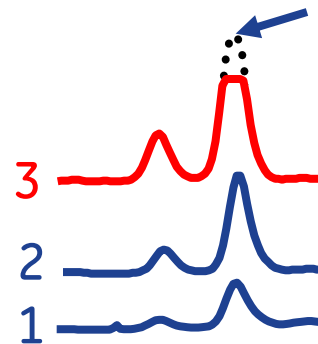
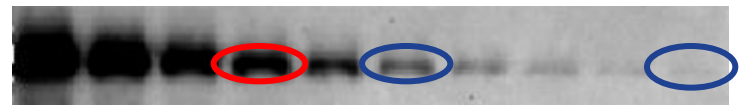


X-ray film

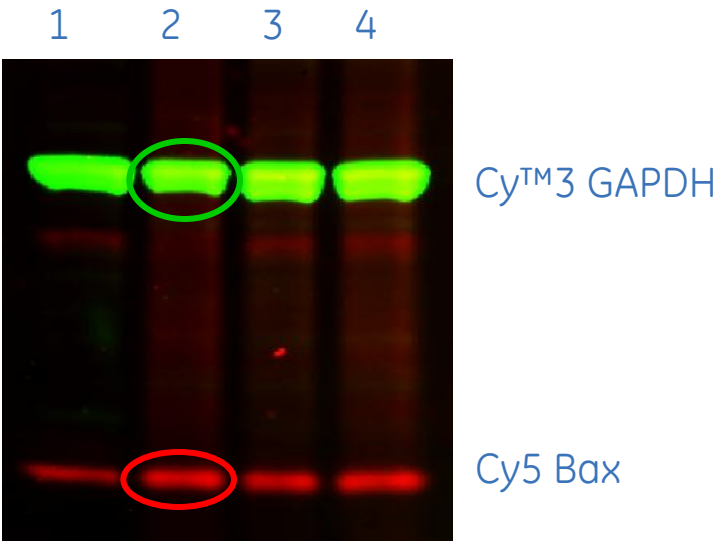
Saturated signals
No linearity

Linear dynamic range

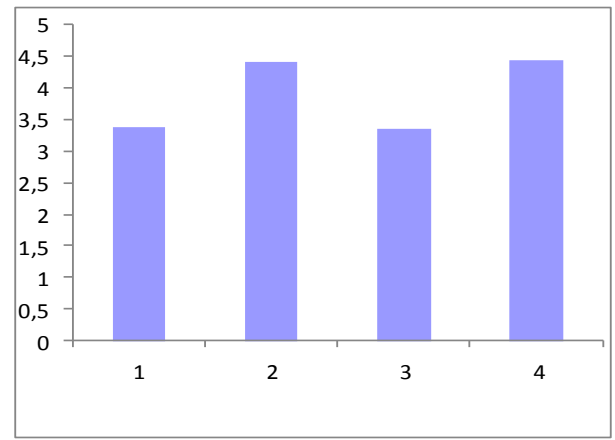
5 ng ← → 9.8 pg



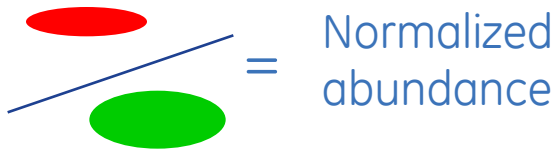
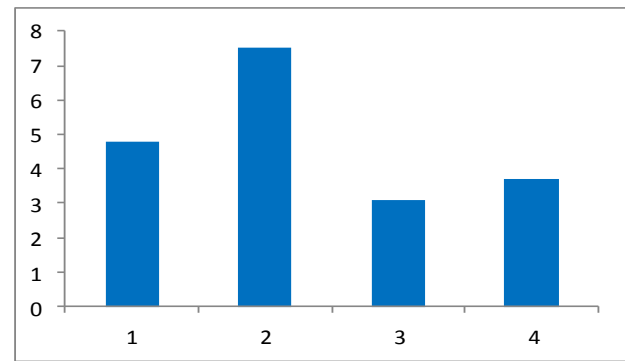
In-lane normalization to correct for uneven sample load



Quantitation of Bax without normalization



Quantitation of Bax with normalization



GE Healthcare's offering

Complete solution for better Western blotting data

Sample preparation

Electrophoresis

Blotting

Probing

Detection and Imaging



SDS-PAGE

Amersham™ ECL™ Gel box
Amersham Rainbow markers



Membranes

PVDF

Amersham Hybond™ P
Amersham Hybond LFP
Amersham Hybond Seq

Nitrocellulose

Amersham Protran
Amersham Protran Premium
Amersham Protran Supported

Blotting Papers

3MM Chr Paper



Secondary antibodies

Chemiluminescence

Amersham ECL HRP α-mouse
Amersham ECL HRP α-rabbit

Fluorescence

Amersham ECL Plex™ Cy™2 α-mouse
Amersham ECL Plex Cy2 α-rabbit
Amersham ECL Plex Cy3 α-mouse
Amersham ECL Plex Cy3 α-rabbit
Amersham ECL Plex Cy5 α-mouse
Amersham ECL Plex Cy5 α-rabbit



Detection reagents

Amersham ECL
Amersham ECL Prime
Amersham ECL Select™

X-ray film

Amersham Hyperfilm™ ECL
Amersham Hyperfilm MP

Imagers

ImageQuant™ LAS 500
Amersham Imager 600

GE Healthcare Imaging products



Versatility



Amersham™ Imager 600

- the versatile CCD camera system for sensitive and quantitative imaging and analysis of gels and blots



ImageQuant LAS 500

- for high quality chemiluminescence data with compact convenience

CCD imagers



Typhoon™ FLA 9500

- The highly versatile laser scanner with cutting edge performance



Typhoon FLA 7000

- The robust and fast laser scanner for standard fluorescence application and phosphor imaging

Laser scanners

Chemiluminescence

Fluorescence

Radioisotope

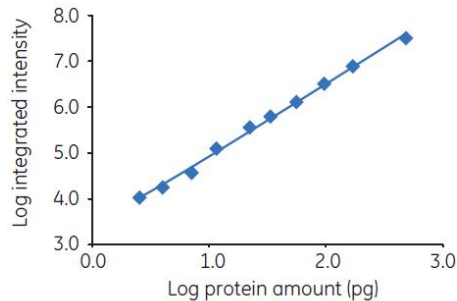
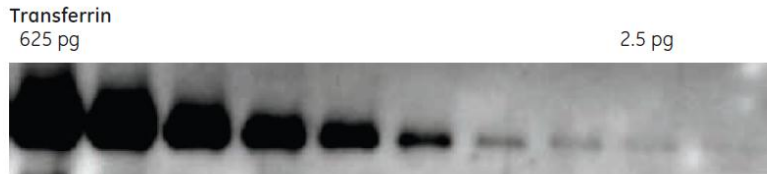


Performance and Applications



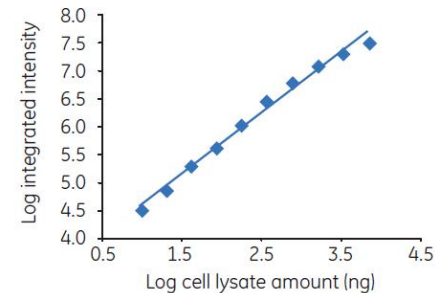
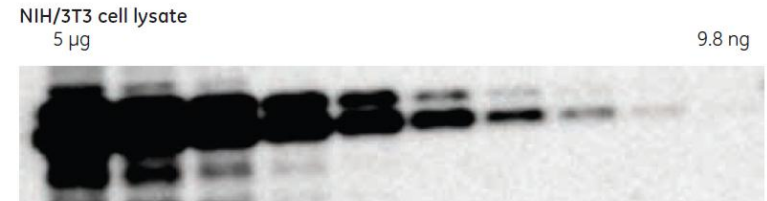
Chemiluminescent detection with Amersham™ Imager 600

High sensitivity – picogram levels



Sample: Two-fold dilution series of transferrin from 625 pg to 2.5 pg
Membrane: Amersham Hybond™ P
Blocking: 3% BSA in PBS-T
Primary antibody: Rabbit anti-transferrin 1:1000
Secondary antibody: ECL™ Anti-rabbit IgG horseradish peroxidase 1:75 000
Detection: Amersham ECL Select
Imaging: Amersham Imager 600
Imaging method: Chemiluminescence
Limit of detection (LOD): 2.5 pg

Broad dynamic range

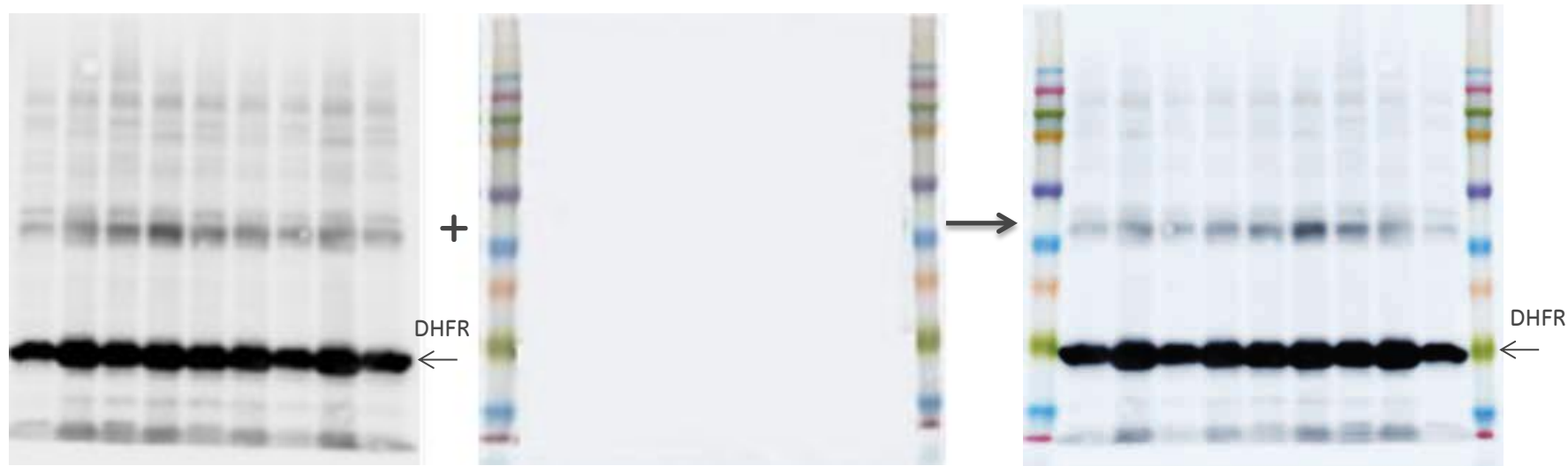


Sample: NIH/3T3 cell lysate two-fold dilution series starting at 5 µg
Membrane: Amersham Hybond P
Blocking: Amersham ECL Prime blocking agent 2% in PBS-T
Primary antibody: Rabbit anti-ERK1/2 1:10 000
Secondary antibody: ECL Anti-rabbit IgG horseradish peroxidase 1:100 000
Detection: Amersham ECL Select
Imaging: Amersham Imager 600
Imaging method: Chemiluminescence
Dynamic range: 2.7 orders of magnitude



Chemiluminescent detection with Amersham™ Imager 600

Composite image of chemiluminescent sample and color marker



Sample: *E. coli* lysate

Membrane: Amersham Hybond™ ECL™

Blocking: 3% BSA in PBS-T

Marker: Full range ECL Plex™ Fluorescent Rainbow™ Marker

Primary antibody: Rabbit anti DHFR C-terminal 1:1000

Secondary antibody: ECL Anti-rabbit IgG horseradish peroxidase 1:100 000

Detection: Amersham ECL Select™

Imaging: Amersham Imager 600

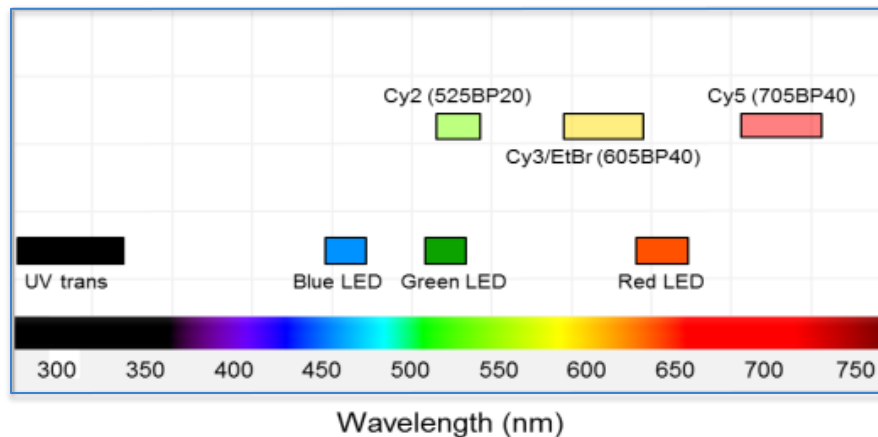
Imaging method: Chemiluminescence



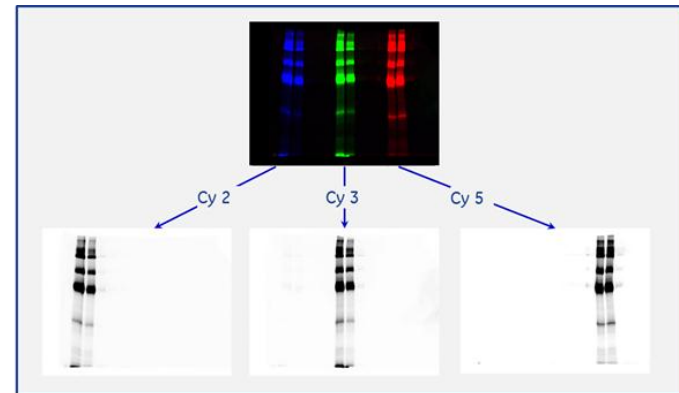
Fluorescent detection with Amersham™ Imager 600

Minimal crosstalk - Spectrally resolved excitation sources and emission filters

Excitation sources and filters



Crosstalk < 1%



Cross-talk was measured using mini-gels with proteins labeled with Cy2 (lane 1 and 2), Cy3 (lane 5 and 6) and Cy5 (lane 9 and 10). The cross-talk levels were very low with detectable cross-talk only for Cy2 in the Cy3 channel (<1%)

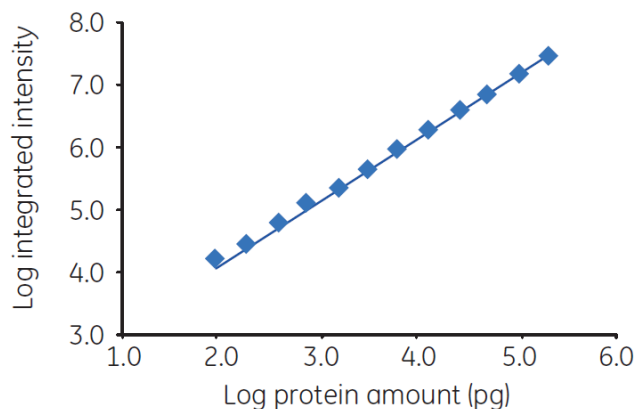


Fluorescent detection with Amersham™ Imager 600

Broad linear dynamic range and high sensitivity

Phosphorylase b
200 ng

98 pg



Sample: Two-fold dilution series of LMW marker with Phosphorylase b, starting at 200 ng

Pre-labeling: CyTM5

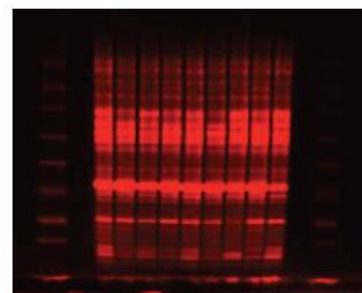
Imaging: Amersham Imager 600

Imaging method: Fluorescence red

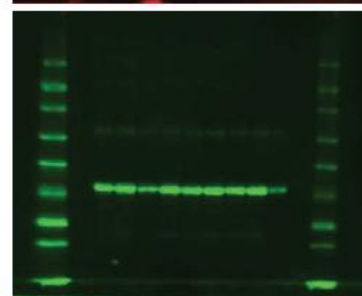
Limit of detection: 98 pg phosphorylase b

Linear dynamic range: 3.3 orders of magnitude

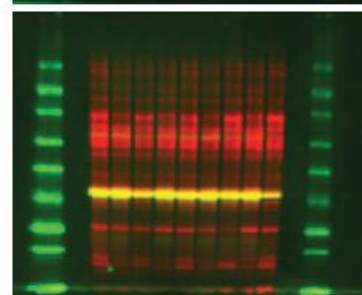
Multiplex detection of total protein and target protein



Prelabeling Cy5
Total protein



WB: DHFR Cy3



Overlay

Sample: E. coli lysates

Blocking: 3% BSA in PBS-T

Primary antibody: Rabbit anti DHFR C-terminal 1:1000

Secondary antibody: ECL™ Plex Goat anti rabbit-Cy3 IgG 1:2500

Imaging: Amersham Imager 600

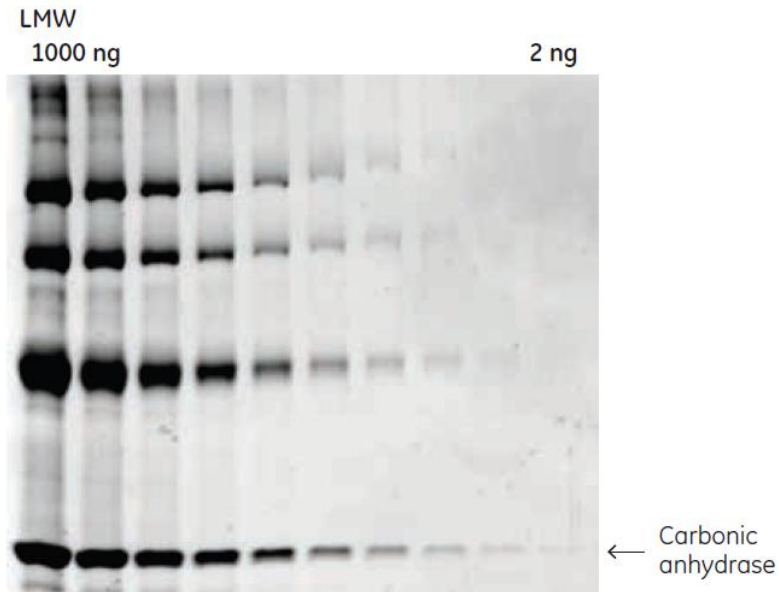
Imaging method: Fluorescence green, red

1 2 3 4 5 6 7 8 9



Fluorescent detection with Amersham™ Imager 600

SYPRO Ruby post staining



Sample: Two fold dilution series of LMW marker starting at 1000 ng

Post staining: Sypro™ Ruby

Imaging: Amersham Imager 600

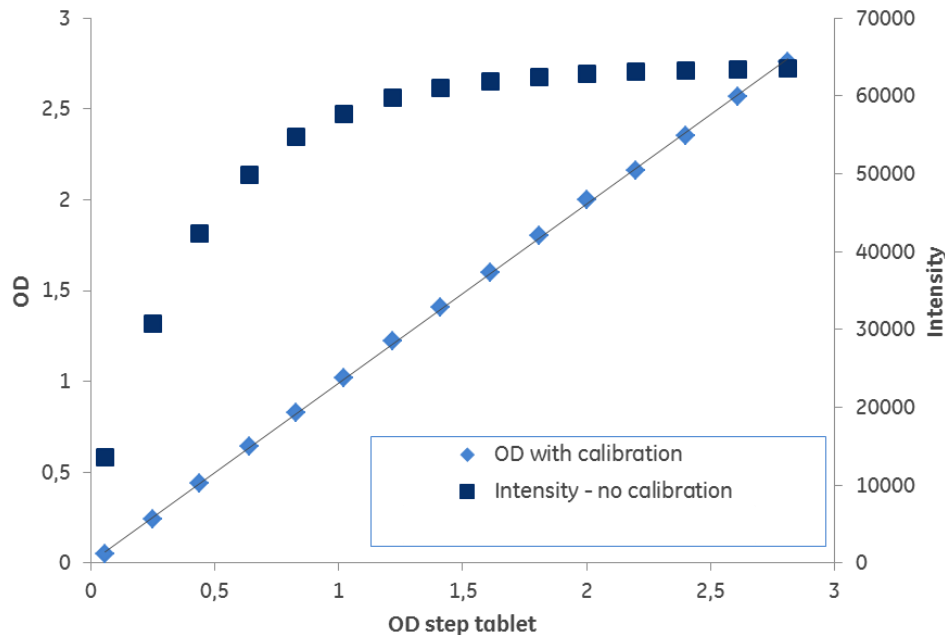
Imaging method: Blue LED excitation

Limit of detection: 2 ng of carbonic anhydrase



Colorimetric detection with Amersham™ Imager 600

Calibrated densitometry in trans-illumination mode
enables quantitative measurements

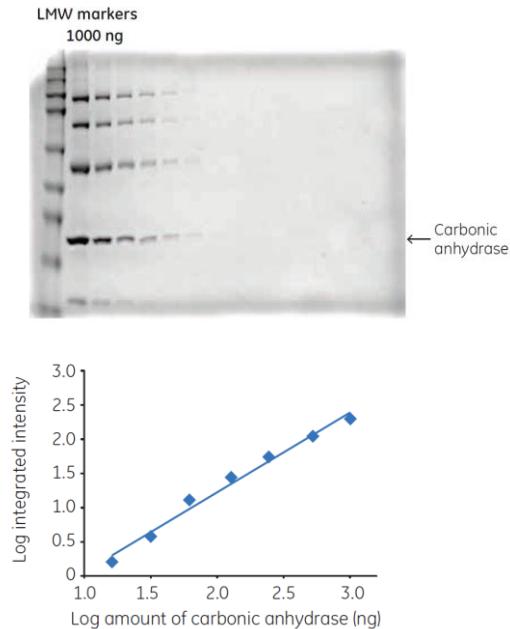


Amersham Imager 600
converts intensity data to
Optical Density (OD values)



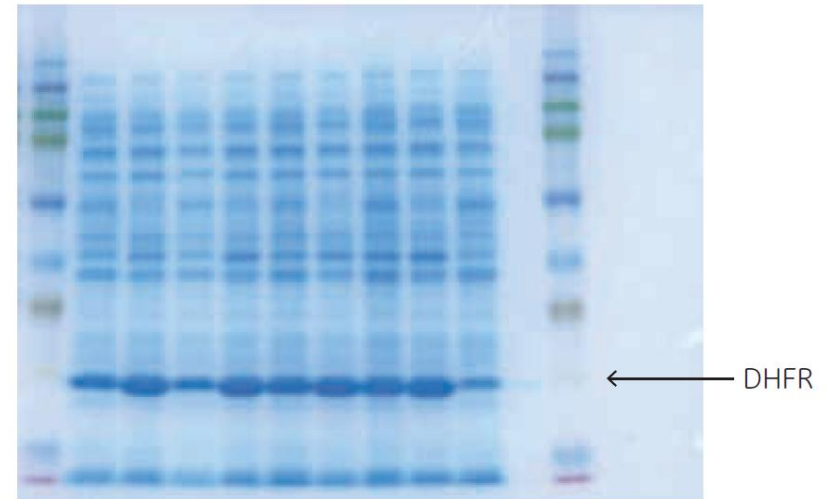
Colorimetric detection with Amersham™ Imager 600

Coomassie Brilliant Blue staining – linear dynamic range



Sample: Two fold dilution series of LMW marker
Post staining: Coomassie™ Brilliant Blue
Imaging: Amersham Imager 600
Imaging method: Colorimetric white transillumination
Limit of detection: 16 ng of carbonic anhydrase
Linear dynamic range: 1.8 orders of magnitude

Coomassie Brilliant Blue staining – color image



Sample: *E. coli* lysates
Marker: Full range ECL™ Plex Fluorescent Rainbow Marker
Post staining: Coomassie Brilliant Blue
Imaging: Amersham Imager 600
Imaging method: Colorimetric, white light Epi-illumination



More information for Amersham Imager™ 600 series

GE Healthcare
Life Sciences

Data file 29-0981-07 AA

Imaging systems, software, and accessories

Amersham™ Imager 600

Amersham Imager 600 series is a new range of sensitive and robust imagers for the capture and analysis of high resolution digital images of protein and DNA samples in gels and membranes. These multipurpose imagers bring high performance imaging to chemiluminescence, fluorescence, and colorimetric applications. The design of Amersham Imager 600 combines our Western Blotting application expertise with optimized CCD technology and novel optics from Fujifilm™. The system has an integrated analysis software and intuitive workflow, which you can operate from an iPad™ or alternative touch screen device, to generate and analyze data quickly and easily.

Amersham Imager 600 delivers:

- **Intuitive operation:** You can operate the instrument from a tablet computer with an intuitive design and easy-to-use image analysis software. You do not need prior imager experience or training to obtain high-quality results. Use the automatic capture mode for proper exposure
- **Excellent performance:** The system uses a super-honeycomb CCD and a large aperture f/0.85 FUJIFILM™ lens, which consistently delivers high-resolution images, high sensitivity, broad dynamic range, and minimal cross-talk
- **Robustness:** Requires minimal maintenance plus our proven expertise in Western blotting and electrophoresis applications, gives you an optimal instrument for multiuser labs. Amersham Imager 600 is an upgradable series of imagers that can grow with your imaging needs

Description

Amersham Imager 600 series is equipped with a dark sample cabinet, a camera system, filter wheel, light sources, and a built-in computer with control and analysis software. Network connection and USB ports are standard (Fig 2).



Fig 1. Amersham Imager 600 series is a range of robust and easy-to-use systems for chemiluminescent, colorimetric, and fluorescent image capture.

Settings such as focus, filter, illuminator, and exposure type are automatically controlled by the integrated software. You would achieve high resolution images and precise quantitation of low signals with the multipurpose 16-bit 3.2 megapixel camera fitted with a large aperture lens. The detector is cooled to reduce noise levels for high sensitivity and wide dynamic range. Rapid cooling leads to a short startup time, which makes the instrument ready to use in less than 5 min.

You can place the sample tray at one of two different heights in the sample compartment to produce image-acquisition areas of 220 x 160 mm and 110 x 80 mm, respectively.



Data file

The screenshot shows the GE Healthcare Life Sciences website. At the top, there is a navigation bar with links for Home, Western Blotting, Products, and Contact Us. The main heading is "Western blotting". Below this, a large banner reads "Announcing the NEW Amersham Imager 600 series" with a "NEW!" badge. The banner lists three key features: "Intuitive", "Excellent Performance", and "Robust". A button below the banner says "Register to keep up to date". At the bottom of the banner, there are three sub-sections: "Western blotting products" (By bringing together the trusted Hybond and...), "Western blotting data quality" (Stable signals, convenient methods, and...), and "How to run a Western blot?" (Need help? Browse our 'how-to' films).

www.gelifesciences.com/amershamwesternblotting

Web page



Videos





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