SYNGENE

HDNR

WHITE PAPER

OPTIMISING CHEMILUMINESCENCE WESTERN BLOT IMAGING WITH HDNR IN GENESYS

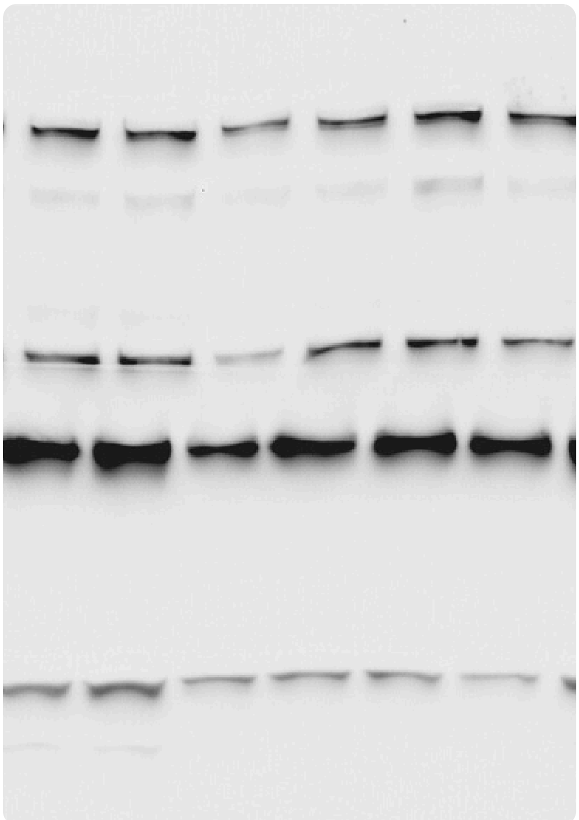
This white paper introduces HDNR, a new feature in GeneSys software, designed to deliver high-resolution, reproducible imaging for enhanced chemiluminescence blot quantification.

Abstract

Accurate image acquisition is critical in western blotting, converting the membrane into a digital image for clear visualisation and reliable analysis of protein molecular weight and expression levels. This white paper introduces HDNR, a new feature in GeneSys image capture software, developed to support researchers in selecting the optimal capture mode for chemiluminescence western blots. HDNR ensures precise, high-resolution imaging to improve data quality, reproducibility, and quantification accuracy.

Introduction

In many western blot experiments, researchers aim to simultaneously detect both low- and high-abundance proteins on the same membrane. This presents a significant imaging challenge: long exposure times, required to visualise faint, low-abundance bands, often result in overexposure or saturation of highly expressed proteins. Conversely, short exposures suitable for abundant proteins may fail to detect weaker signals.



This trade-off highlights the importance of dynamic range—defined as the ratio between the strongest and weakest signals a system can detect within a single image. A limited dynamic range restricts the ability to capture the full spectrum of protein expression, compromising both sensitivity and quantification accuracy.
Understanding the dynamic range capabilities and limitations of your imaging system is critical for generating reliable data and interpreting western blot results with confidence. When a blot requires extended dynamic range imaging, a single exposure cannot adequately capture both strong and weak signals without risking saturation.

To address this, Syngene's GeneSys image capture software includes the HDNR feature which enables researchers to image chemiluminescent western blots with enhanced dynamic range, allowing for the simultaneous detection of high- and low-abundance proteins. This feature helps maximise sensitivity while minimising the risk of signal saturation, ensuring a more accurate and quantifiable representation of protein expression.

How does HDNR work?

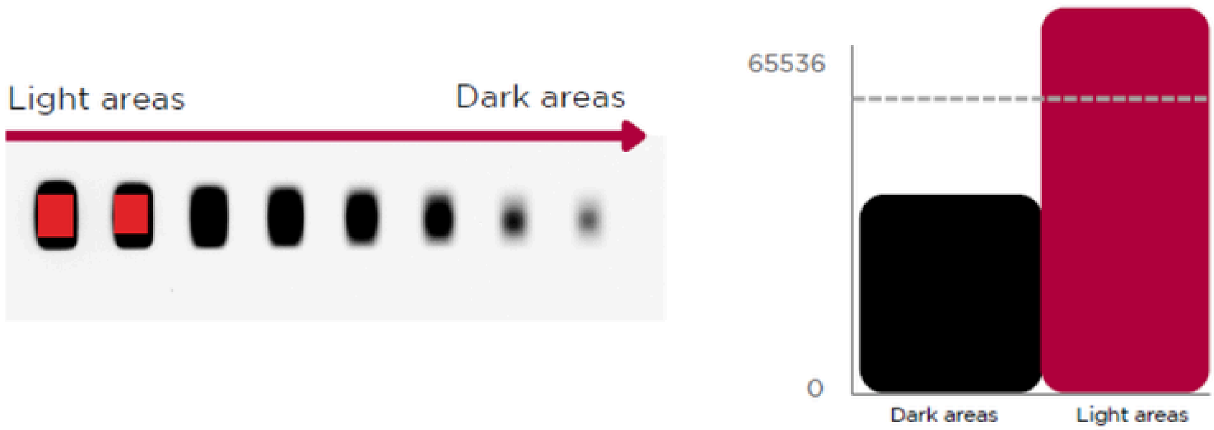
HDNR technology enhances western blot image acquisition by capturing and integrating multiple exposures of the same blot. Specifically, a short exposure is used to accurately record high-intensity signals, while a longer exposure captures low-abundance proteins that produce weaker signals. These exposures are algorithmically combined into a single composite image, enabling simultaneous visualisation of both strong and faint bands.
Crucially, HDNR prevents saturation by reconstructing the brightest signal regions using extrapolated values from the short exposure. This ensures that high-intensity signals are preserved without overexposure, while low-intensity signals benefit from the extended exposure time.
By leveraging the optimal signal from each exposure, HDNR improves the signal-to-noise ratio (SNR) and reduces background interference. This results in clearer visualisation of low-abundance proteins and enhances the overall quantitative accuracy of the blot.

Dynamic range-short exposure

Increasing the exposure time would give the additional detail in the dark areas but at the expense of saturation in the light areas

A second image is captured which has a longer exposure time.

Dynamic range-long exposure



With a long exposure we now have more detail in the dark areas (faint bands) however, we have saturation in the light areas (bright bands).

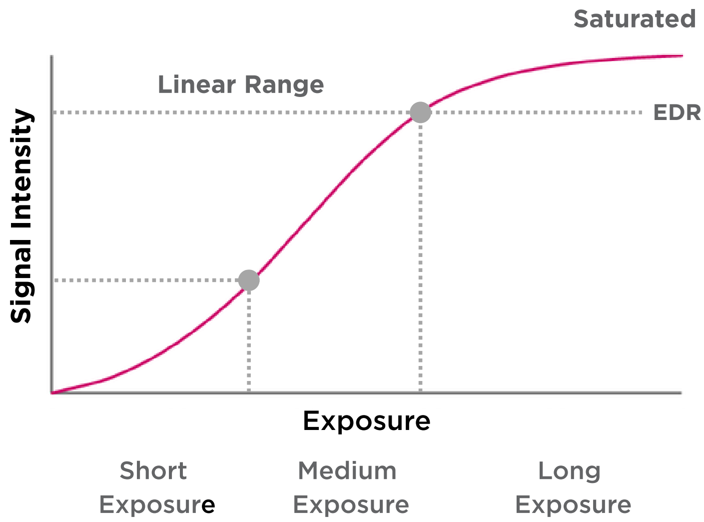
The software now combines these images into a single composite image allowing both bright and weak signals to be observed at the same time.

Dynamic range-HDNR image

By taking a series of images with increasing exposure time we can extract from each one the best detail for each light level.
Tracking the levels of saturation as it occurs and extrapolating the grey level value, the predicted value can be given.

How Does HDNR Retain Linearity?

For reliable quantitation in western blot analysis, it is essential that the signal intensity of protein bands falls within the linear dynamic range of the imaging system. Within this range, there is a direct, proportional relationship between the recorded signal intensity and the amount of target protein present. Signals that fall outside this range may lead to inaccurate quantitation—weak signals may be obscured by background noise, while overly strong signals can saturate the detector, preventing the system from accurately capturing incremental increases in intensity.
HDNR software feature preserves linearity by **c**apturing multiple exposures, ensuring that no individual image is saturated. The software evaluates each pixel across exposures, selecting the most accurate, unsaturated value within the linear range—discarding both overexposed and noise-obscured underexposed data. To maintain quantitative accuracy, intensities are scaled appropriately across exposures, so that longer exposures are scaled down and shorter exposures are scaled up. This approach ensures that the final composite image reflects a true linear relationship between signal intensity and protein amount.



Conclusion

Accurate and reproducible quantitation in western blotting relies heavily on the ability to capture a broad dynamic range of protein signals. Traditional single-exposure imaging methods often force a compromise between visualising low-abundance proteins and avoiding saturation of highly expressed ones.
The HDNR feature in Syngene's GeneSys software offers a powerful solution to this challenge. By capturing multiple exposures and intelligently integrating pixel data from the most linear, unsaturated regions, HDNR generates high-quality images with superior dynamic range. This ensures both faint and intense protein bands are accurately represented—enhancing sensitivity, preserving quantitation linearity, and reducing the risk of image saturation or data loss.
Incorporating HDNR into chemiluminescence western blot imaging enables researchers to more confidently interpret complex protein expression patterns, particularly when detecting targets of varying abundance on a single blot. The result is a significant improvement in data integrity, reproducibility, and overall experimental confidence.

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