SYNGENE

REDUCING ERRORS IN IMAGING WORKFLOWS

WHITE PAPER

IMPROVING DATA ACCURACY FROM CAPTURE TO QUANTIFICATION

This white paper outlines best practices for reducing errors in gel and blot imaging workflows, supporting accurate, consistent, and reproducible analysis.

Abstract

Accurate image analysis is critical for generating reliable and reproducible data from gel electrophoresis and blot workflows. Variability introduced during image capture, processing, quantification, and reporting can affect both data quality and interpretation. This white paper outlines practical approaches for reducing errors and improving consistency throughout the imaging workflow.

Introduction

Accurate image analysis is critical to generating reliable scientific data. In gel and blot workflows, errors introduced during image capture, processing, and quantification can significantly impact results, leading to poor reproducibility and reduced confidence in findings.

By implementing standardised imaging and analysis workflows—supported by integrated software solutions such as GeneTools and GeneSys from Syngene—laboratories can reduce variability, improve accuracy, and ensure consistent, defensible data.

This paper outlines key stages where errors commonly occur and how they can be minimised through best practices and software-enabled workflows.

1. Image Capture and Preparation

Reliable analysis begins with high-quality image acquisition. Errors introduced at this stage cannot be corrected downstream, making it essential to optimise capture conditions.

For a quick reference, download our one-page guide featuring 5 practical tips for optimal gel and blot imaging.

DOWNLOAD

Image Preparation and Processing

Careful image preparation is essential to ensure accurate and reproducible analysis. Prior to quantification, images should be optimised to improve visibility while preserving the integrity of the original data.

- Apply appropriate noise reduction to minimise background interference. For chemiluminescent blots, features such as HDNR in GeneSys can help improve signal quality without compromising data integrity 

- Ensure the background is as even as possible across the image. This can be achieved by minimising membrane autofluorescence in fluorescent Western blots (e.g. using low-fluorescence PVDF membranes and optimising antibody concentrations), optimising stain concentrations for DNA/RNA gels, and ensuring chemiluminescent reagents are evenly distributed across the membrane
- Adjust contrast and brightness to aid visualisation of faint bands, ensuring weaker signals are clearly visible
- Avoid excessive processing. Over-processing can remove or distort data; however, uniform adjustments to contrast and brightness, as well as cropping to remove irrelevant areas, are generally acceptable
- At Synoptics, analysis is always performed using the raw data. Image processing should support interpretation but must not alter the underlying quantitative information.

Saving and File Formats

Image format plays a key role in preserving data quality and ensuring reliable analysis:

- Use high-bit-depth formats (e.g. TIFF) for quantitative analysis
- Avoid compressed formats (e.g. JPEG) that can result in loss of data
- Maintain consistent file naming and storage practices to support traceability
- Save images in the secure Syngene (.sgd) format, which stores raw data alongside diagnostic information from image capture

The .sgd format ensures that original data is preserved and can be revisited or re-analysed if needed. It is recommended to save images in this native format and export to other file types only when required for reporting or sharing.

Standardised file handling reduces the risk of data loss and supports data integrity throughout the workflow.

2. Quantification, Qualification, and the Role of Band Volume and Background

Understanding band volume and background correction is essential for both quantification and qualification in gel and blot analysis.

Quantification: Measuring Signal Intensity

Quantification measures band intensity using densitometry, where band volume reflects the amount of DNA, RNA, or protein present.

Reliable quantification depends on:

- **Band volume —** the total integrated signal within a band
- **Background correction —** removal of non-specific signal to isolate the true intensity

These factors directly influence the accuracy and precision of the measurement. Accuracy refers to how closely the measured band intensity reflects the true signal, while precision describes the consistency of measurements across replicates. Poor background subtraction, signal saturation, or weak band definition can reduce both accuracy and precision.

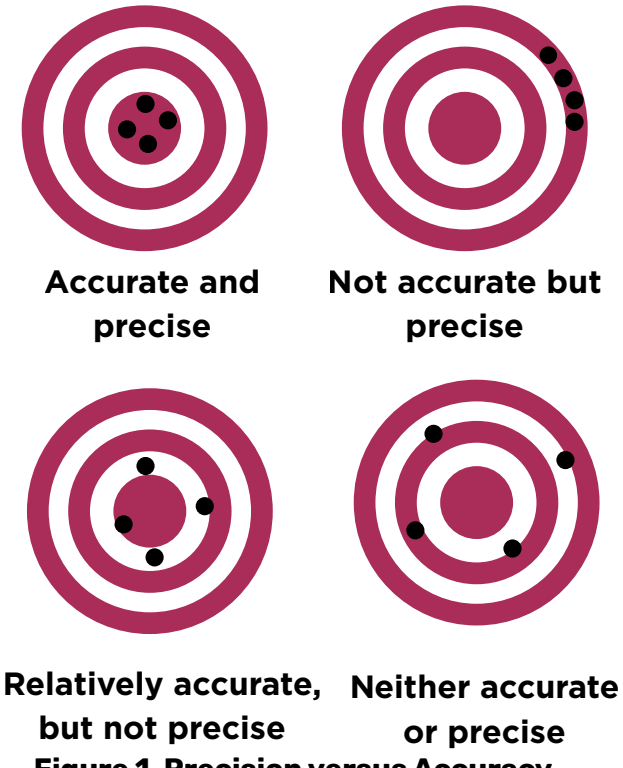

Figure 1-Precision versus Accuracy

Qualification: Confirming Signal Identity

Qualification verifies that the detected signal is correct and biologically relevant.
This includes:

- Confirming band presence or absence
- Verifying expected molecular weight
- Assessing band quality and specificity

High or uneven background and poorly resolved bands can obscure faint signals and make it difficult to distinguish true bands from artefacts or noise

Background Subtraction Methods

There are multiple approaches to background subtraction, and the choice of method can influence both quantification accuracy and data consistency. Common approaches include rolling disc-based corrections, track borders and slope, track borders, lowest slope and manual each suited to different image characteristics.

Further detail on selecting and applying these methods is available in a dedicated application note for GeneTools DOWNLOAD, which outlines how background subtraction can be performed effectively within the software.

Accurate image analysis relies on both correct identification of the target band and precise measurement of its intensity. Careful consideration of band volume and appropriate background correction ensures that results are both meaningful and reproducible.

Band and Lane Detection

Accurate lane and band identification is essential for both reliable quantification and correct qualification.

- Define lanes consistently across the gel to ensure comparable analysis
- Automatically detect bands where possible to reduce user variability
- Use profile views to verify peak detection and confirm true signal

GeneTools supports automated lane and band detection, helping to standardise analysis and improve reproducibility. Where adjustments are required, the software provides flexibility to refine results, including:

- Adjusting lane width
- Moving or tilting lanes to align with the image
- Manually adding or removing bands

This combination of automation and user control ensures that analysis remains both efficient and accurate, particularly when working with complex or variable images.

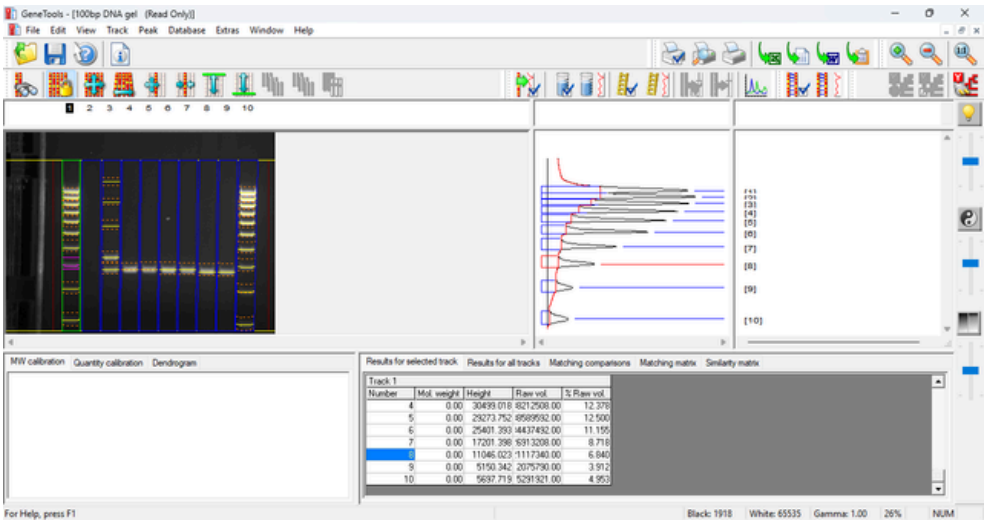

Figure 2-Automatic Band and Lane detection in GeneTools

3. Multi-Image Analysis

Multi-image analysis plays an important role in reducing variability and improving consistency across imaging workflows in molecular biology research. When multiple gels or blots are analysed in parallel, differences in exposure settings, staining intensity, and manual interpretation can introduce variability that impacts data reliability.

Standardising the analysis of multiple images helps ensure that experimental comparisons are made under consistent conditions, supporting more accurate interpretation and reducing user-dependent error. This is particularly important in studies involving batch processing or longitudinal datasets, where reproducibility is critical.

Dedicated analysis platforms, such as GeneTools analysis software (Syngene), support multi-image workflows by enabling users to apply consistent analysis parameters across multiple gels and blots. This helps maintain uniformity in lane definition, background correction, and densitometric evaluation, improving comparability between experiments and strengthening overall data integrity.

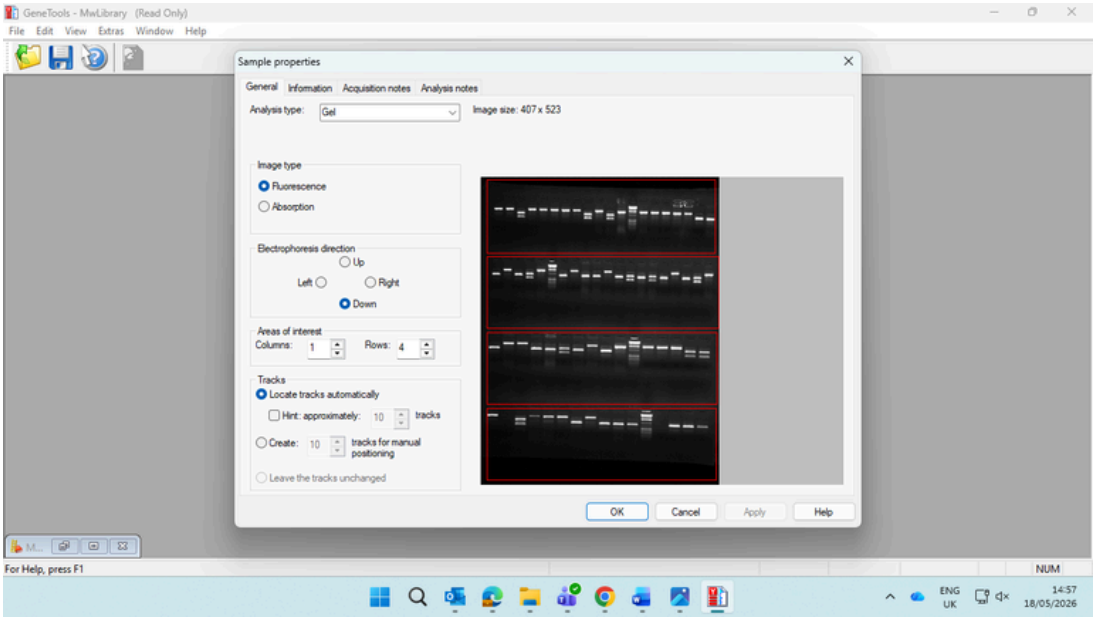

Figure 2-Multi-gel analysis in GeneTools

4. Reporting and Data Export

Clear and consistent reporting is essential for maintaining data integrity and ensuring transparency in imaging-based workflows. Standardised reporting formats help reduce transcription errors, improve traceability, and support reproducibility across experiments and research groups.

Effective data export functionality enables researchers to transfer analysed results directly into commonly used formats for further statistical evaluation, record keeping, or publication. Maintaining a consistent structure in exported data also helps minimise manual handling errors and ensures that key metadata and quantification outputs are preserved accurately.

Analysis platforms such as GeneTools analysis software (Syngene) support streamlined reporting and data export by allowing users to generate structured reports directly from analysed gel and blot images. This includes exporting densitometric data, molecular weight assignments, and annotated images in consistent formats suitable for downstream use. By reducing manual intervention in data transfer, this approach helps minimise errors and supports standardised, reproducible reporting across laboratory workflows.

5. Data Integrity and Compliance Considerations

Maintaining data integrity is a critical requirement in regulated laboratory environments, particularly where imaging data contributes to validated workflows and decision-making. Ensuring that results are accurate, complete, and consistently recorded supports both scientific reliability and regulatory compliance.

In accordance with principles aligned to 21 CFR Part 11, electronic records and data handling processes must be secure, traceable, and reproducible. While audit trail functionality is a key component of many fully regulated systems, laboratories can still strengthen compliance by adopting standardised workflows that minimise manual intervention, reduce transcription errors, and ensure consistent application of analysis parameters.

A structured approach to image acquisition, analysis, and reporting helps support data integrity by limiting variability introduced through user-dependent processes. Consistency in software-driven analysis and controlled export of results further contributes to maintaining reliable electronic records suitable for review and verification.

GeneTools analysis software (Syngene) supports these objectives by providing a controlled environment for gel and blot image analysis, enabling consistent application of molecular weight determination and densitometric quantification methods. By standardising analytical workflows and reducing manual handling of data, it helps laboratories operate in a manner consistent with good data management practices in regulated settings.

Conclusion

Errors in imaging workflows can arise at multiple stages—from capture to quantification and reporting. Without standardised processes, these errors can compromise data accuracy and reproducibility.

By implementing structured workflows supported by integrated tools such as GeneSys and GeneTools, laboratories can:

- Improve consistency in image capture
- Reduce subjectivity in analysis
- Enhance accuracy in quantification
- Streamline reporting and data handling

Ultimately, reliable results depend on controlling variability at every stage of the workflow.

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Explore how integrated solutions from Syngene can support your lab in generating data you can trust.

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