Troubleshooting BLI & FLI: A Guide

Reliable results start with the right approach

Introduction

In vivo optical imaging (i.e., bioluminescent imaging (BLI) and fluorescence imaging (FLI)) is a non-invasive technique for measuring biological processes. While the Newton 7.0 product line by Vilber delivers highly reliable and consistent results, this guide was created to help users troubleshoot issues that can arise.

Imaging Best Practices

1. Substrate and Probe Handling
    - Substrate viability: use fresh substrate whenever possible; store at manufacturer recommended temperatures.
    - Fluorescence probe functionality: prevent photobleaching by avoiding light exposure.

2. Verify Biological Setup
    - Check gene/protein expression: validate reporter expression using qPCR or western blotting to confirm expression.
    - Assess cell viability: compromised cell viability can lead to reduced/no signal. Consult a cell viability assay.

3. Animal Preparation
    - White fur will absorb less emitted light than dark fur; if possible, shave fur from region of interest.
    - Reduce motion artifacts by properly sedating the animal (i.e., using gas inhalation isoflurane (e.g., 2-3% induction, 1-1.5% maintenance anesthesia)).
    - Ensure heated stage is functioning as intended to prevent cold-induced signal loss in BLI. Adjust animal pad temperature from 20°C to 37°C in the Settings dialog box.

4. Imaging Setup Checklist
    - Animal is correctly positioned: ROI centered in the FOV, focus adjusted to attain highest resolution.
    - Imaging surface is free from fur, fecal matter, urine, etc., to prevent background contamination and uneven lighting.
    - Correct filters are selected (FLI only): match excitation/emission specifications. Contact Scintica if you are unsure based on your selected applications.
    - Exposure time is optimized based on signal strength, FOV, and binning settings.
    - The acquisition software will notify the user if the signal is oversaturated.
    - Binning settings: Use higher binning for low-signal samples (from 2 x 2 pixel binning up to 16 x 16).
    - Substrate incubation time (based on manufacturer instructions: e.g., wait 10-15 minutes after injections before acquiring BLI images).

Other Pro Tips:

Stay consistent throughout longitudinal studies: Maintain acquisition and analysis settings across animals and groups. This includes the plane of anesthesia, substrate dosing in BLI, timing of probe injection, camera settings, and ROI analysis settings (i.e., ROI area and background subtraction).

For weaker signal: Increase binning mode. If signal remains undetected, consider increasing exposure time. Bioluminescence imaging timing: Find the optimal window post-injection for the most consistent peak signal using the Serial acquisition mode.

Troubleshooting Table

Problem	Possible Causes	Solution	Pro Tip
Low/No BLI signal	Substrate expired, erroneous reporter gene expression, inappropriate camera settings.	Use fresh D-luciferin, verify biological setup, and adjust camera settings.**validated commercial FLI dyes according to the manufacturer's instructions.**	Store at -20oC and avoid multiple freeze-thaw cycles. Find optimal time post-administration using the Serial acquisition mode. Increase lens aperture (f/0.7) and binning.
Low/No FLI signal	Mismatch in fluorophore excitation and emission filter being used.	Confirm fluorophore matches the system filters. Use validated commercial FLI dyes according to the manufacturer's instructions.	Use excitation/emission spectra lookup chart (an applications table is available upon request).
High Autofluorescence	Autofluorescence from various biological sources (e.g., chlorophyll, collagen, NADPH, etc.).	Carefully select fluorescent probes that circumvent background fluorescence. Clean off any residual fur from the platform and dry urine from your subject.	Check to ensure that diet does not induce fluorescence (e.g., by chlorophyll in alfalfa).
Signal crosstalk between channels	Overlapping emission spectra.	Select fluorophores with non-overlapping spectra (minimum 100 nm difference is recommended).	Check for using the right emission filters.
Uneven illumination	Poor animal positioning or dirty optics.	Ensure that the animal is positioned within the FOV. Clean the camera lens with a dry Kimwipe prior to imaging.	Check animal position and lens cleanliness using the live Focus window view.
Unexpected signal location	Fluorophore/substrate biodistribution issue.	Check injection route. Confirm gene/protein expression by qPCR or Western Blot.	Administration via IV leads to rapid systemic delivery.

If you have any questions on which method best meets your research needs, feel free to contact us to discuss your model. We have many resources available, from scientist webinars to journal citations, to help point you in the right direction.

For more information please visit our website (www.scintica.com) or feel free to reach out to us via email at info@scintica.com or by phone at 832-548-0895 and we would be glad to assist you.