Bioluminescence tomography: Setup and sequence acquisition.

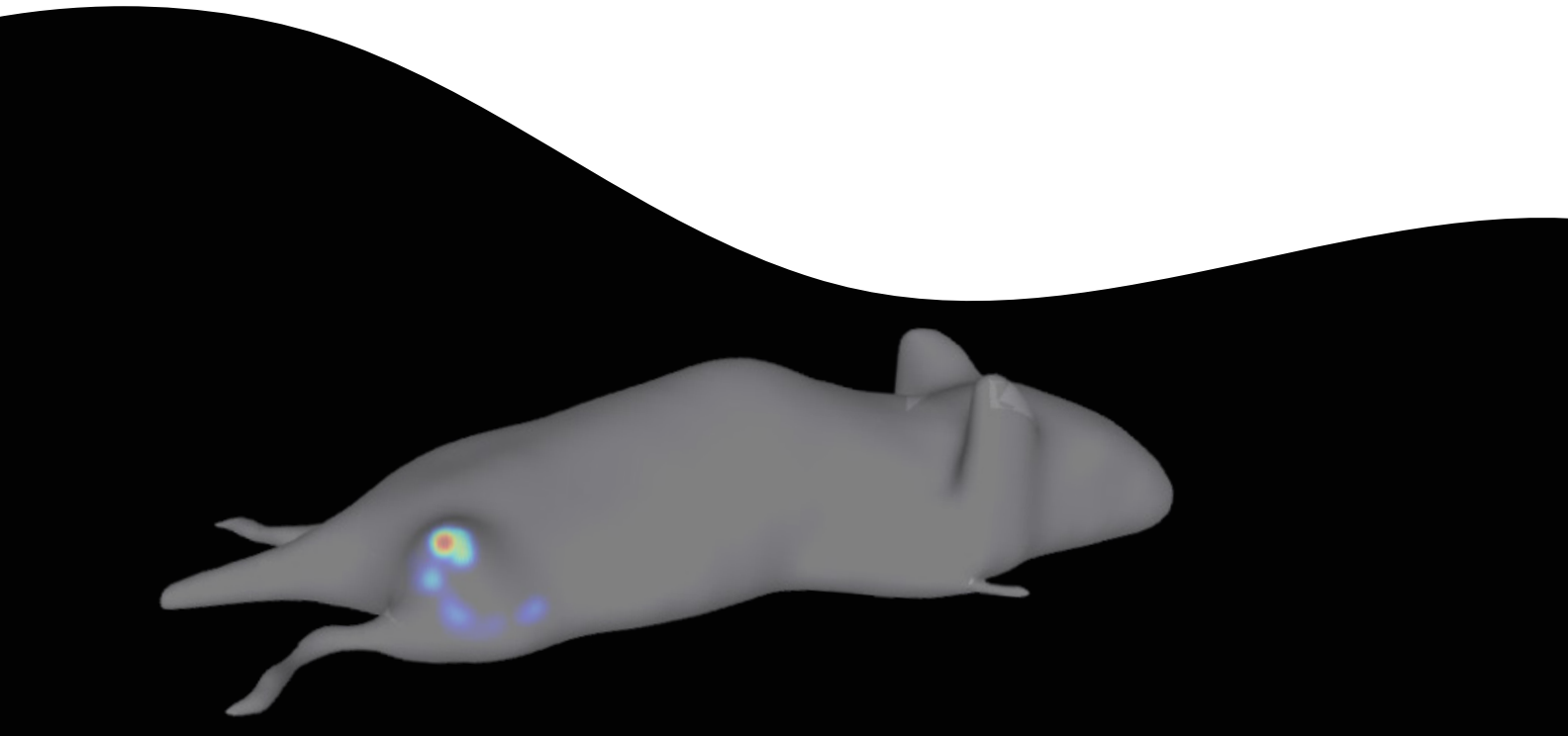
This tech note will discuss how to set up an imaging experiment for bioluminescence tomography

Bioluminescence tomography, also referred to as diffuse light imaging tomography (DLIT), utilizes the data obtained from a filtered 2D bioluminescent sequence in combination with a surface topography to represent the bioluminescent source in a 3D space. Utilizing DLIT, you can determine the depth of sources in your animal and calculate the absolute intensity of that source.

Note: The Bioluminescence Tomography Technical Notes are meant to be used in a series. Please read the following bioluminescence tomography technical notes before beginning your acquisition: **Topography** and **Source Reconstruction and Analysis.**

Background

We take advantage of the broad emission spectrum of luciferase as it overlaps the hemoglobin absorption curve (the absorption of visible light by oxyhemoglobin). Beyond 600 nm this absorption drops significantly, so if we monitor the amount of light that reaches the surface of the subject over a range of spectral filters from 560 to 640 nm (every 20 nm) we can design a set of linear equations which will allow us to reconstruct the depth and determine the total flux of the source(s) of light. This tech note will discuss DLIT using firefly luciferase, as it is the most common reporter for bioluminescent imaging. However, Living Image® also supports several other bioluminescent sources for 3D reconstruction.



Imaging timing

Normally, light from bioluminescent sources is collected with an open filter slot, however now we will have to split that source into distinct spectral regions. Therefore each image will only have a fraction of the intensity of the open filter image. You may need longer exposure times or larger binning to obtain the desired 600 counts per image. When setting the luminescent exposure times, please consider the luciferin kinetic profile. The post-luciferin injection imaging onset time and end time should be carefully determined and rationed between emission filters to optimize the signal. Currently, the DLIT **algorithm assumes a flat kinetic profile** with respect to time. Therefore after the luciferin injection, the imaging onset and end time should be during the flattest region of the luciferin kinetic profile (Figure 1). For more information on determining the kinetics of your model, please see Technical Note: **Kinetic Analysis of Bioluminescent Sources.**

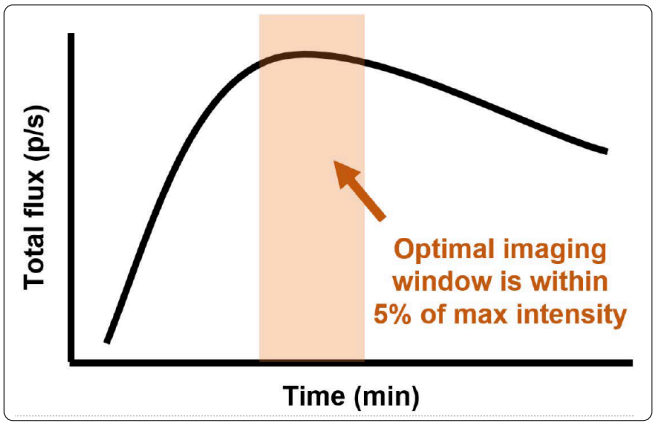


Figure 1. Sample bioluminescence kinetics curve. Inject luciferin and wait to acquire the image until the total flux intensity has plateaued.

As mentioned above, sometimes time constraints prohibit the acquisition of six images. In these cases, you can reduce the number to three images – 580 nm, 600 nm, and 620 nm are the most critical for our analysis. As a rule of thumb if you can achieve sufficient signal (> 600 counts) in under a minute imaging with an open filter configuration by adjusting either the f/stop or binning, you should have sufficient signal to perform a 3D analysis.

Notes about topography: Nude mice result in the best surface topography reconstructions. Furred mice, especially symptomatically stressed furred mice, do not reflect the structured light lines well. The structured light images are required to reconstruct the surface topography. Without the reconstructed animal surface, DLIT analysis cannot be performed. It may be necessary to remove the fur from the mouse body, either by shaving or applying a depilatory substance. Obtaining a surface topography from black furred mice, even after shaving, is extremely difficult due to lack of photo contrast as well as optical signal absorption. The pigmentation in the skin does not allow the algorithms to distinguish between the subject and the stage.

Notes about setup: DLIT is optimized for fields of view B or C. This guide will walk you through the steps of manually entering your sequences for the DLIT sequence. The Living Image 4 software versions include an **Autoexposure** setting and an **Imaging Wizard**. It is highly recommended that the Imaging Wizard be used for Bioluminescence Tomography setup. For questions on how to use these two features please see technical notes: **Autoexposure** and the **Imaging Wizard**. These features are specifically designed for ease of use and to streamline sequence setup.

Recommended

How to set up a DLIT scan: Imaging wizard

1. Click on the Imaging Wizard in the acquisition control panel.
2. Select Bioluminescence, then DLIT.
3. Select your reporter. The software automatically chooses a series of emission filters to use (Figure 2).
4. Select your acquisition settings (Autosettings recommended).
5. The sequence of images to be taken should now be seen in the control panel.
6. Click Acquire Sequence.

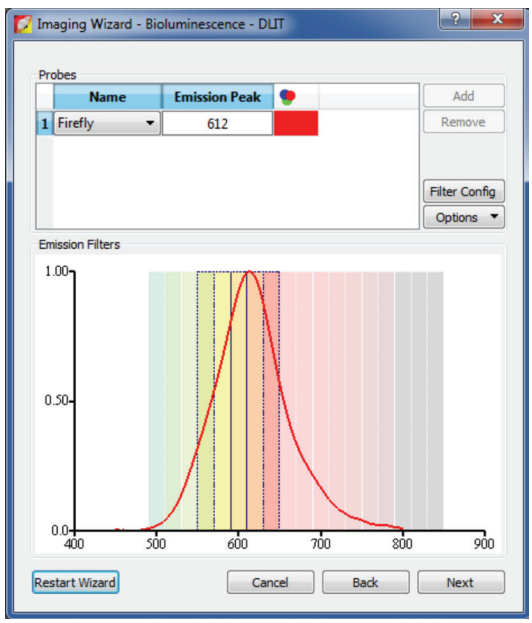


Figure 2. DLIT scan setup using the Imaging Wizard. The software will choose the appropriate filters for your specified reporter (Firefly Luciferase shown here).

How to set up a DLIT scan: Manual setup

1. In the IVIS® System Control Panel, click on Sequence Setup (Figure 3).

2. Compile a sequence of photographic and luminescent images using multiple emission filters that cover the spectral emission curve of the reporter used. For firefly luciferase emission shown in figure 2 (max ~612 nm), we would optimally select the range from 560 nm to 640 nm. Note: For assistance in selecting the appropriate filters for your reporter, use the recommended Imaging Wizard setup method above.

Confirm that Photograph is checked, specify the settings for the Luminescent image (exposure time, binning, f/stop, 1st emission filter – 560 nm in this example) and check Structure to acquire the structured light image (Figure 4). Taking the structured light image allows us to reconstruct the surface of the animal; you will be instructed how to do this in the next topography technical note: **Bioluminescent Tomography**: Topography. Only one structured light image is required.

Note: For optimal results, f/stop should remain consistent for all luminescent images acquired. So if you are using autoexposure, be sure to adjust your settings accordingly.

3. Repeat this step until the sequence is complete with the following modifications:
   a. Deselect the Structure box (only 1 required).
   b. Image using the next emission filter in your chosen range – 580 nm in this example.

4. Press Acquire Sequence to start acquiring data.

5. Make sure each image has between 600-60,000 counts. The structured light image can be seen if you open the first image in the sequence and Display the Structure.

At this point, the data can be saved until a later point in time or you can continue the procedure directly by following the steps in the topography technical note: **Bioluminescent Tomography**.

Note: The value of light intensity for stably transfected cell lines obtained from DLIT can be converted to number of cells after creation of a database for the cell line used in your study. You will need to plate a serial dilution of your cells in a black well plate and image the plated cells. Please read the technical note: **Well Plate Quantification** for more information.

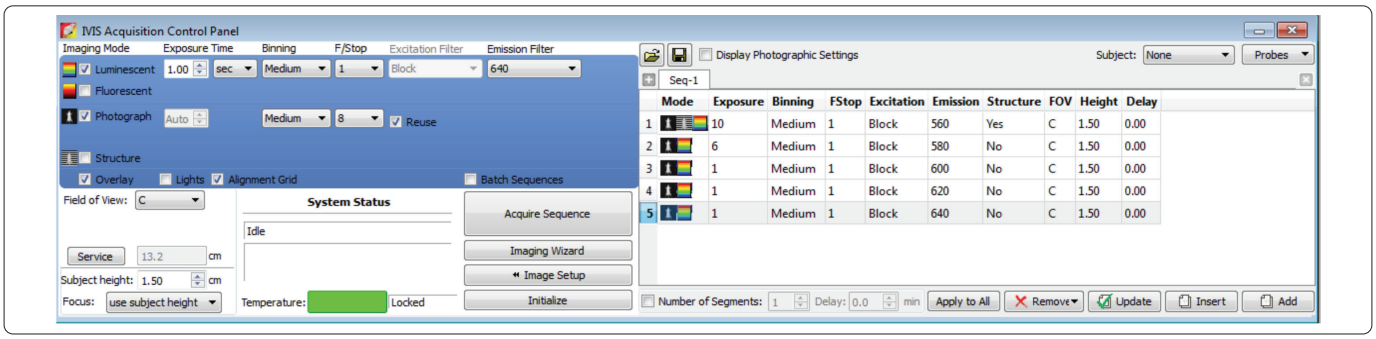


Figure 3. Sequence Setup on the acquisition control panel. To switch between a sequence or single image setup, click the highlighted button. If Sequence Setup is chosen, the control panel will expand and display the series of images to be acquired on the right-hand side. Click the button again to go back to a single Image Setup.

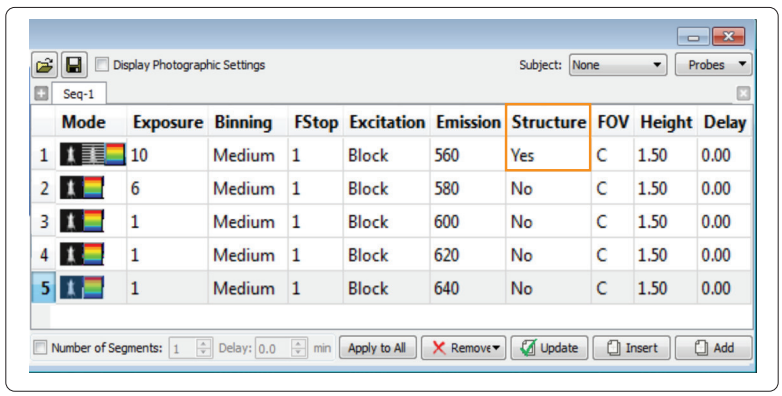


Figure 4. The sequence setup is showing that the photograph taken will also include a structured light image which is necessary for the 3D reconstruction.

Figure 5. The structured light image is shown on top of the individual emission filter images. The color scale can be adjusted to determine if the source intensity is greater than 600 counts.

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