Bioluminescence Tomography – Setup and Sequence Acquisition

Bioluminescence Tomography or 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.

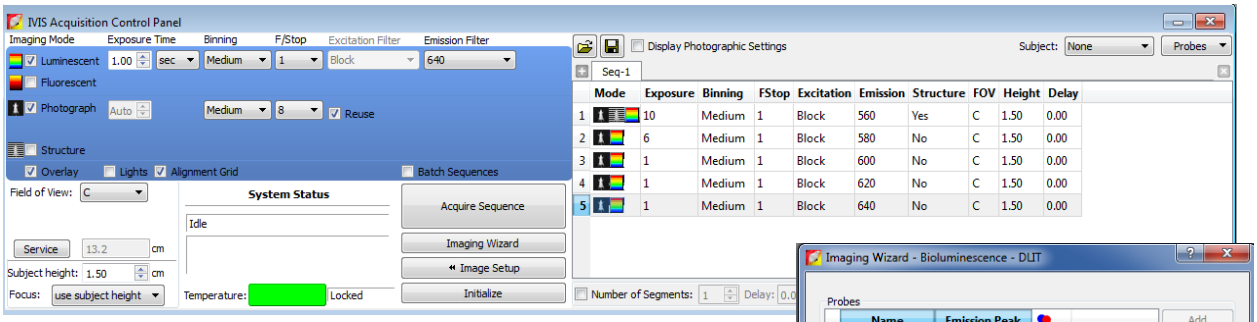
Note: The Bioluminescence Tomography Tech Notes are meant to be used in a series. Please be familiar with the **Bioluminescence Tomography – Topography Tech Note 4b** and **Bioluminescence Tomography – Source Reconstruction and Analysis Tech Note 4c** before beginning your acquisition.

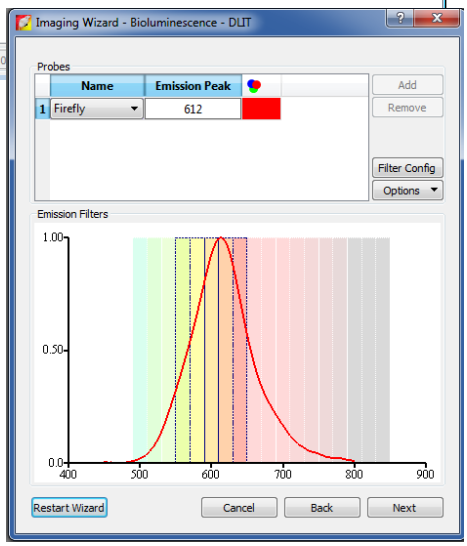
Background: We take advantage of the broad emission spectrum of luciferase as it overlaps the hemoglobin absorption curve (the absorption of visible light by oxyhemoglobin). Beyond 600nm this absorption drops significantly, so if we monitor the amount of light that reaches the surface of the subject over a range of spectral filters from 560 to 640nm (every 20nm) 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.

Note: 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. For more information on determining the kinetics of your model, please see **Kinetic Analysis of Bioluminescent Sources – Biology Tech Note 1**. As mentioned above, sometimes time constraints prohibit the acquisition of six images. In these cases, you can reduce the number to 3 images – 580nm, 600nm, and 620nm 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. Additionally, we cannot perform DLIT analysis on black furred animals even after shaving. 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 the **Autoexposure Tech Note 2** and the **Imaging Wizard Tech Note 11**. These features are specifically designed for ease of use and to streamline sequence setup.



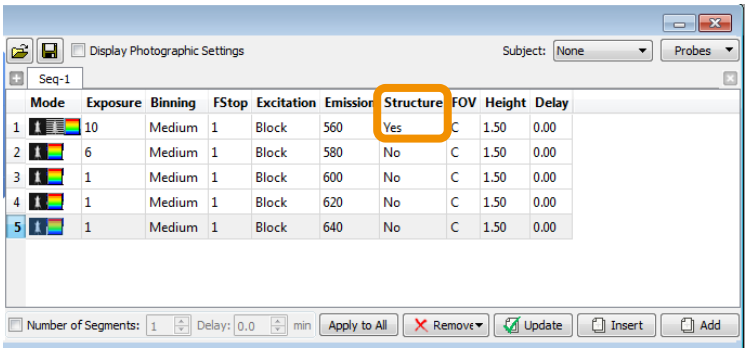


1. In the IVIS System Control Panel, click on **Sequence Setup**.

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 to the right (~612nm), we would optimally select the range from 560nm – 640nm.

   Note: See notes above and use the **Imaging Wizard** for assistance in selecting the appropriate filters for your reporter.

   I. 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.



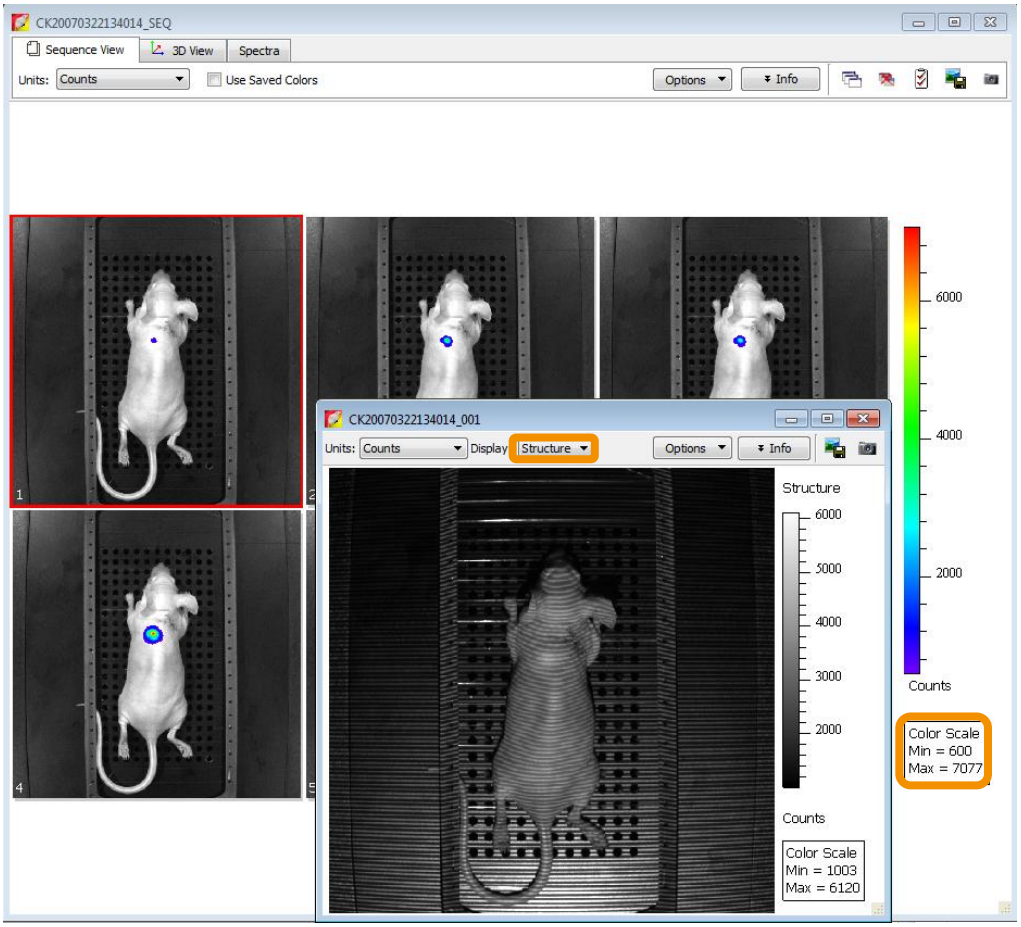
   Important Note: For optimal results, F/stop should remain consistent for all luminescent images acquired. So if you are using autoexposure, be sure to adjust your settings accordingly.

   Note: 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 tech note – **Bioluminescent Tomography – Topography Tech Note 4b**. Only one structured light image is required.

   II. 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.

3. Press **Acquire Sequence** to start acquiring data.

4. 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**.



Color scale can be adjusted to determine if the source intensity is >600 counts

5. 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 **Bioluminescent Tomography – Topography Tech Note 4b**.

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 **Well Plate Quantification Tech Note 15** for more information.