Fluorescence Tomography – Setup and Sequence Acquisition

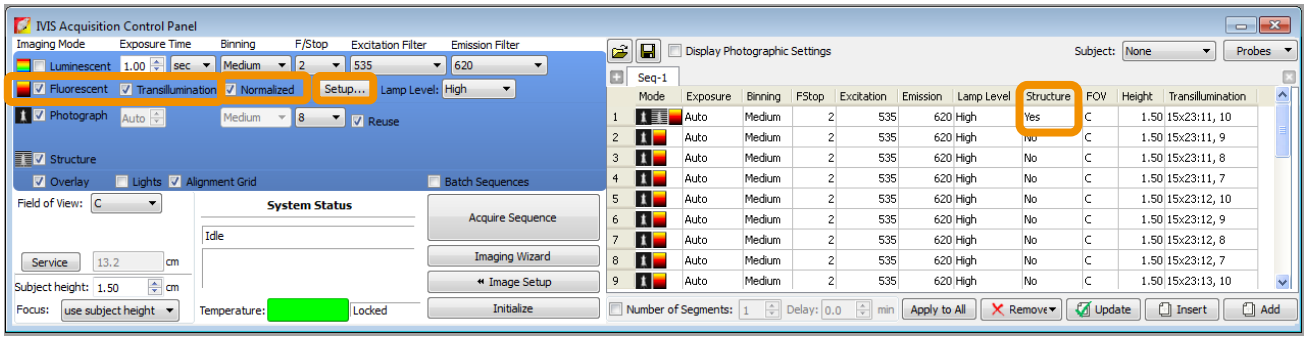
Fluorescence Imaging Tomography (FLIT) utilizes the data obtained from a 2D transillumination fluorescence sequence in combination with a surface topography to reconstruct a fluorescent source in a 3D space. Utilizing FLIT, you can determine the depth of sources in your animal and calculate the absolute intensity of that source at depth.

Note: The Fluorescence Tomography Tech Notes are meant to be used as a series. Please be familiar with the **Fluorescence Tomography – Topography Tech Note 6b** and **Fluorescence Tomography – Source Reconstruction and Analysis Tech Note 6c** before continuing.

Note about Topography: Nude mice result in best FLIT reconstructions. Furred mice, especially symptomatically stressed furred mice, do not create superior topography maps due to the unevenness of the structured light images required to reconstruct the surface topography. Without the reconstructed animal surface, FLIT analysis cannot be performed. It may be necessary to remove the fur from the mouse body, either by shaving or applying a depilatory substance. Furthermore for FLIT, we will be utilizing transillumination points below and recording signal on the surface of the animal. For best results, please shave both sides of the animals that you will be using for these procedures. Additionally, we cannot perform FLIT 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: This guide will walk you through the steps of manually entering your sequences for the FLIT 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 Fluorescence 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. Click **Sequence Setup** in the control panel to operate in sequence acquisition mode and the sequence editor window will open.



2. If necessary, click **Remove** and then select **All** to clear the table.

3. In the control panel, check **Fluorescent** then check **Transillumination** and specify the settings for the fluorescence image (exposure time, binning, F/stop, excitation filter, emission filter).

Notes about Setup: F/Stop should remain consistent for all fluorescent images for optimal 3D results. You have the ability to unselect **Normalized** if the Normalized Transmission Fluorescence (NTF) option is not desired; however it is selected by default and is recommended. NTF is a method for subtracting nonspecific light leakage and allows us to image deeper into the animal with fewer artifacts. Please see the supplemental information in **Transmission Fluorescence – Normalized Tech Note 14c** for more information on the NTF process.

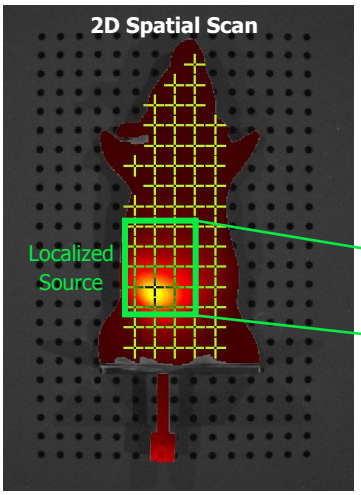
Note: Autoexposure settings are available for LI4 users for information on this feature please see the **Autoexposure Tech Note 2**. It is recommended that you use this feature.

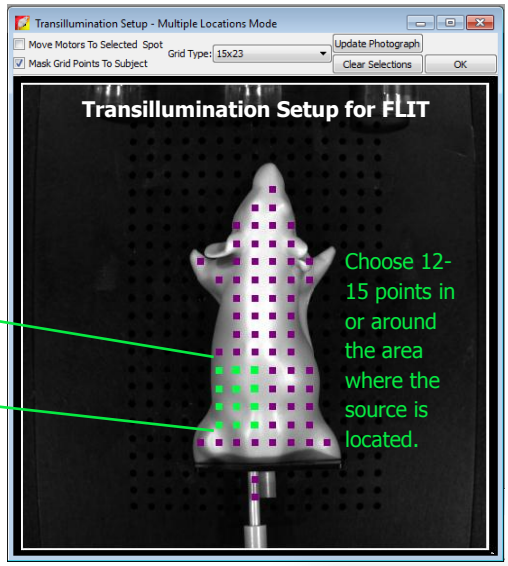
4. Specify the settings for the photographic image and make sure the **Reuse** box is checked.

5. In the Control Panel, click **Setup** and the **Transillumination Setup** window will appear.

6. The software will prompt you to update the photographic image, click **Yes** to acquire a new photograph.

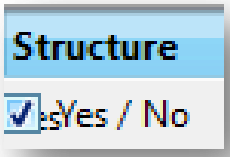
7. In the **Transillumination Setup** window, select 12-15 points immediately surrounding the source of interest (for more information on Transillumination Sequence Setup see **Transillumination Fluorescence - Tech Note 14a**). To choose these points with the most precision, the first step is to determine where your sources are located in the subject. This is accomplished with a 2D transillumination spatial scan of the animal (**Tech Note 14a** will direct you on how to set this up). **Raster scanning cannot be used in conjunction with FLIT as we require spatial information that is only provided when we acquire images using multiple excitation points underneath the animal.** Therefore, scan times will increase dramatically as an image will be acquired at each point selected. If this were not the case, we could simply image using every point in the animal as with a 2D transillumination spatial scan but this would be very time consuming. Use the acquired spatial scan to select points in or around the immediate area where the source is located.

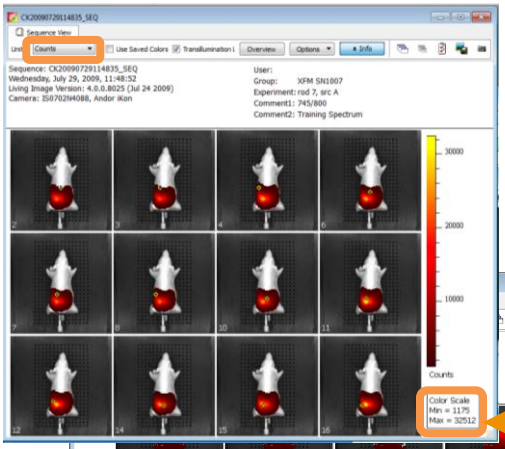




8. In the sequence editor, click **Add** while the Transillumination Setup window is open and the acquisition parameters will be added to the table. Each row will represent one transillumination point selected in the setup window. An image will be acquired at each point selected.

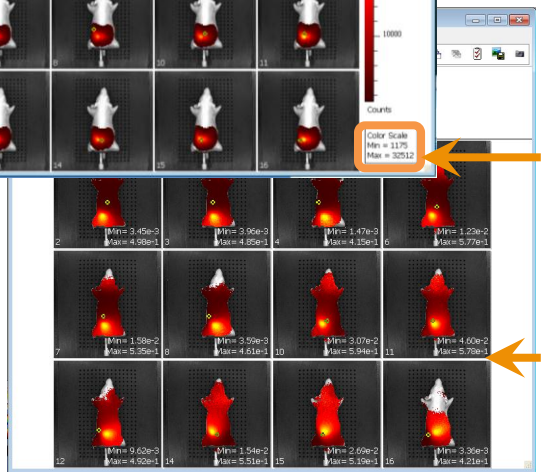
9. One structured light image is required to reconstruct the surface of the animal. In the sequence editor window under the column labeled as **Structure**, click in the cell corresponding to row one and a Yes/No box will appear. Check this box to acquire your structured light image with image one in the sequence.





Note: The structured light image will automatically be assigned when using the **Imaging Wizard**.

10. To acquire the images, click **Acquire Sequence** in the control panel.



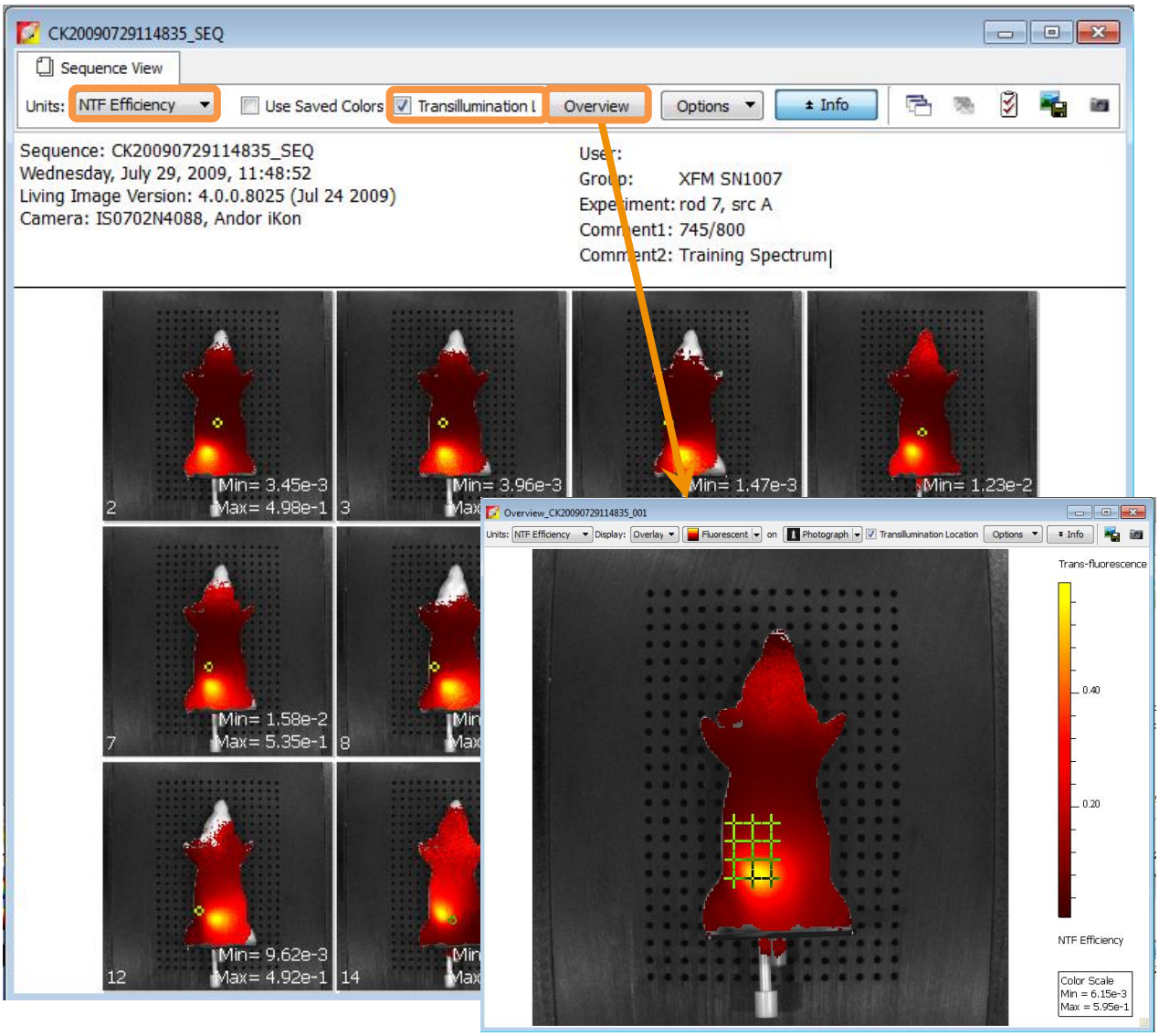
Note: During image acquisition, the **Acquire Sequence** button becomes a **Stop** button. To cancel the acquisition, click **Stop**.

11. Once image acquisition is complete, confirm that the signal is within proper limits – between 600-60,000 counts.

12. The transillumination locations can be shown/hidden on the images in the sequence by checking the tab at the top of the image.

13. Switch to calibrated units – **NTF Efficiency** - in the **Units** dropdown at the top of the image.

14. The **Overview** button can be used to show a cumulative view of the diffusion pattern acquired during the sequence.



13. At this point, the data can be saved until a later point in time or you can continue the procedure by following the steps in the **Fluorescence Tomography – Topography Tech Note 6b**.