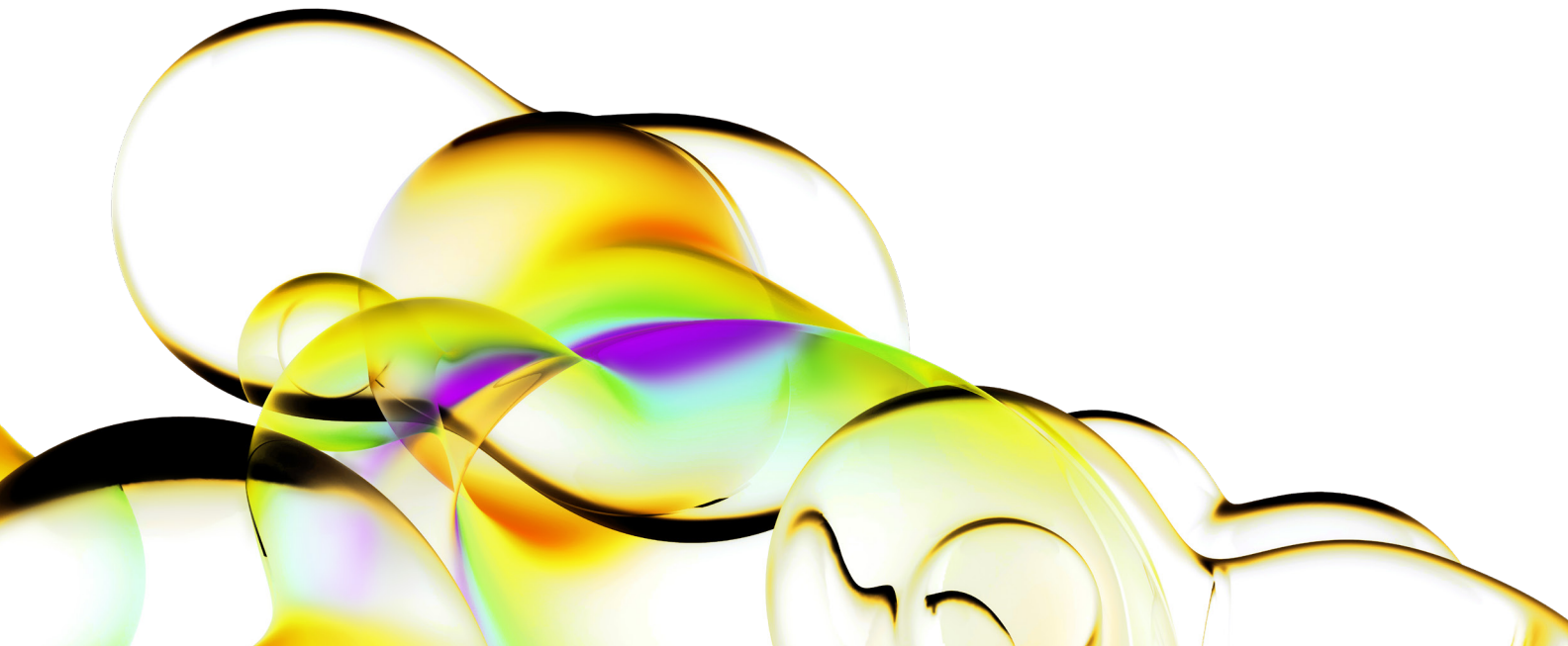
# Transillumination Fluorescence

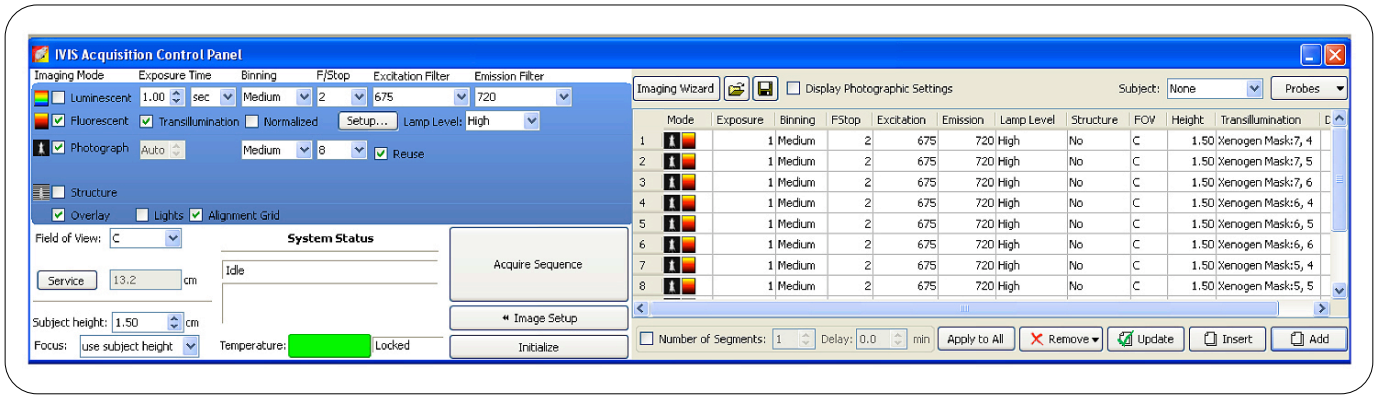
Transillumination is a fluorescence imaging technique that utilizes an excitation light source located below the stage. Transillumination is superior to epi-illumination at detection of red-shifted, deep tissue fluorescent sources due to the transilluminator's concentrated delivery of excitation light into the subject via a 2 mm beam and lower autofluorescence levels attained due to the position of the animal in relation to the excitation light.

Note: This Transillumination Fluorescence Tech Note was designed as a series to be used in combination with the Transillumination Fluorescence – Raster Scan Tech Note and Transillumination Fluorescence – Normalized Transmission Fluorescence Tech Note. These two supplemental notes provide additional information and details about these setup options.

Note: This guide will walk you through the steps of manually entering your settings for a transillumination sequence. The Living Image® 4 software versions include an Autoexposure setting and an Imaging Wizard and it is recommended that you use these features to simplify the setup. For detailed questions on how to use these two features, please see the respective tech note associated with each feature.



Important: Before beginning this procedure, please insert the provided transillumination plate into the slot on the surface of the instrument. The plate is easily identifiable and has a grid of holes stamped into it. Place the writing face down into the slot and the end of the plate labeled “Back” to the back of the instrument.




1. Click Sequence Setup in the control panel to operate in sequence acquisition mode. The sequence editor will appear.
2. If necessary, click Remove and then select All to clear the table.
3. In the control panel, specify the settings for the fluorescence image (exposure time, binning, F/stop, excitation filter, emission filter).
   - Note: Autoexposure settings are available for LI4 users for information on this feature please see the Auto-Exposure 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, select the Transillumination option. 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.
6. Next, click Setup. The Transillumination Setup window will appear.
7. The software will prompt you to update the image, click Yes.
8. Raster Scan will be checked on the top left corner of the Transillumination Setup window. This setup option is selected as a default and it is recommended that you use it. With raster scanning, the excitation light is continuously delivered as the transilluminator moves from bottom to top along a column and then from right to left. This will significantly reduce the amount of time required to capture your images (see the Transillumination Fluorescence – Raster Scan Tech Note 6b for more information).
9. In the Transillumination Setup window, click the purple dots (specifies all possible locations for transillumination and image acquisition) corresponding to the location of interest. Selected dots will turn green. To select multiple points left click and drag the mouse to select a grid. To select multiple individual points hold down the control [Ctrl] key and click on the corresponding dots on the setup.

## When choosing transillumination points, follow these tips.

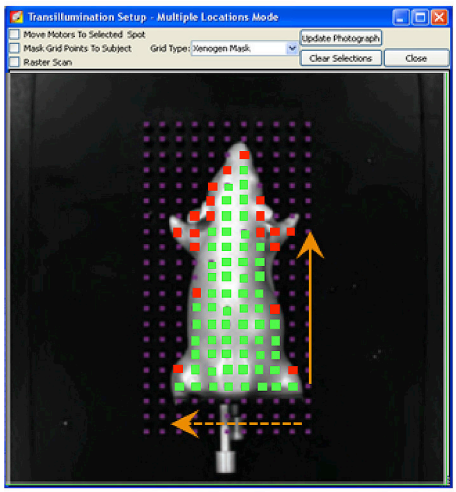
For a spatial scan to determine the location of an unknown source:

Use the grid tool and select the entire mouse. Only select points inside the animal. Checking the Mask Grid Points To Subject check box at the top of the window will delete all points outside of the animal when there is contrast between the mouse and stage. The points in green to the right would show a recommended setup. Red points indicate areas one would avoid.

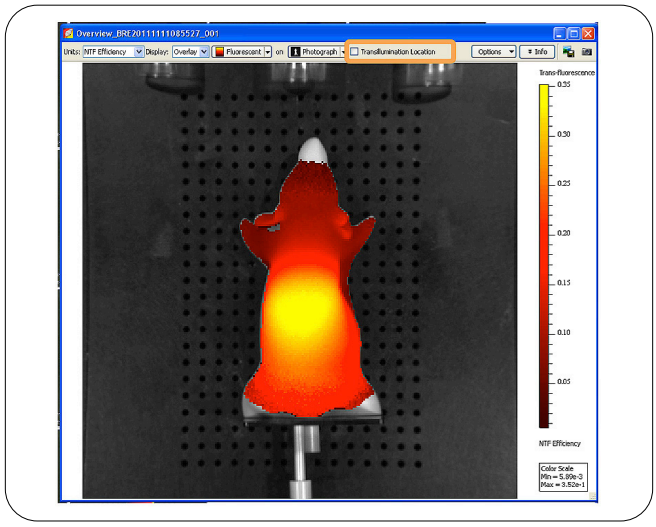
Press Ctrl on the keyboard and deselect according to the following rules:

- Any points outside of the animal
- Any points that correspond to thinner or translucent tissue on the edges of the animal
- Isolated points that are not continuous in a column due to the movement of the transilluminator in Raster Scan mode. It moves from bottom to top and right to left as indicated by the arrows and will not shut off. If we do not deselect discontinuous points, then there will be saturation outside of the animal.

For a scan at a known location (ex. Lung Mets, Orthotopic Tumor, Localized Bacteria): Simply select points corresponding to the area you would like to scan. Keep the number of points consistent from time point to time point.




10. In the sequence editor, click Add and the acquisition parameters will be added to the Sequence Editor.
11. 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.
12. Once image acquisition is complete, confirm that the signal is within proper limits – between 600-60,000 counts.
13. The transillumination locations (green crosshairs) can be hidden/shown on the image by checking the Transillumination Location box at the top of the image.




14. Switch to NTF Efficiency in the Units dropdown at the top of the image to apply the normalization and proper calibration. See Transillumination Fluorescence – Normalized Tech Note 6c for more information about NTF Efficiency.

Note: You can utilize transillumination in combination with spectral unmixing if required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