



Fluorescence Tomography – Source Reconstruction and Analysis

Note: This Tech Note is part of a series for Fluorescence Imaging Tomography (FLIT). You must first follow the steps in the preceding **Fluorescence Tomography – Setup and Acquisition Tech Note 6a** and **Fluorescence Tomography – Topography Tech Note 6b** before you can perform the steps detailed below.

1. Open **FLIT 3D Reconstruction** tab in Tool Palette.
2. Under the **Analyze** tab, choose transillumination images to be used for 3D reconstruction

Note: Living Image will automatically deselect points due to oversaturation or weak signal. Use as many points as possible.

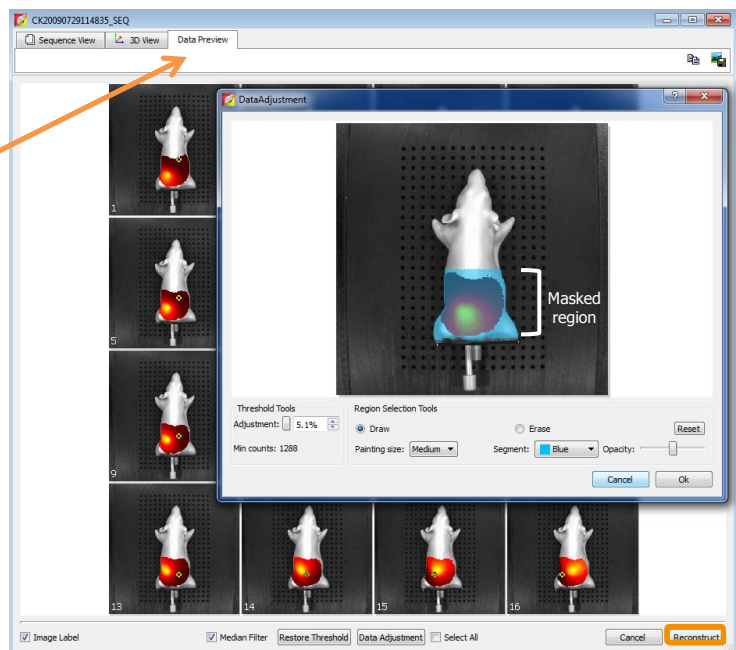
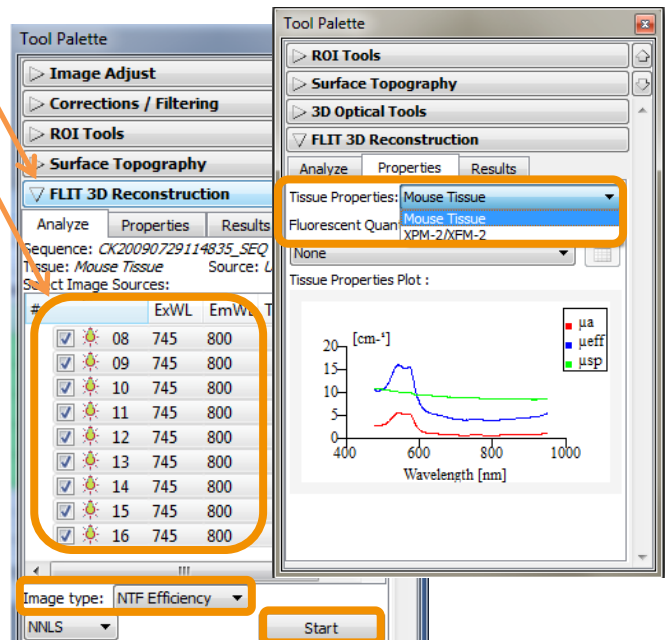
3. **NTF Efficiency** is the recommended default Image Type for all 3D FLIT reconstructions. This selection utilizes NTF to subtract background and facilitate deeper reconstructions. For more information on NTF, please see **Transillumination Fluorescence – Normalized Tech Note 14c**.

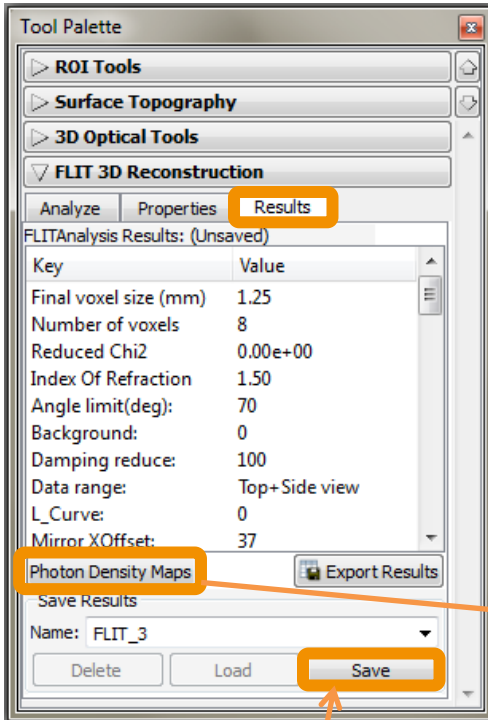
4. In the **Properties** tab, adjust the **Tissue Properties** to best represent the subject (mouse tissue or phantom).

Note: If you used the Imaging Wizard for acquisition, these selections will use the input received through the Wizard automatically.

5. Once the images are selected and properties have been set appropriately, click the **Start** button under the **Analyze** tab to review the sequence to be used for reconstruction.
6. The **Data Preview** tab will be displayed in which you can adjust and threshold your data. Thresholds typically default at or above 5% with fluorescence due to the presence of autofluorescence that can lead to artifact reconstructions. In most cases, default settings chosen by the software will suffice. In models with very high autofluorescence, thresholds can be increased up to 20% to reduce the number of artifacts in the reconstructed source. Please consult with an Advanced Imaging Trainer or Technical Support Specialist if you are having issues with artifacts in your FLIT models.

You can also adjust the threshold for individual images by double clicking on the image you wish to adjust. Within this window is also a masking tool that will allow you to mask in a region of the mouse for consideration and exclude other areas. See the User Guide for more information on using this tool.

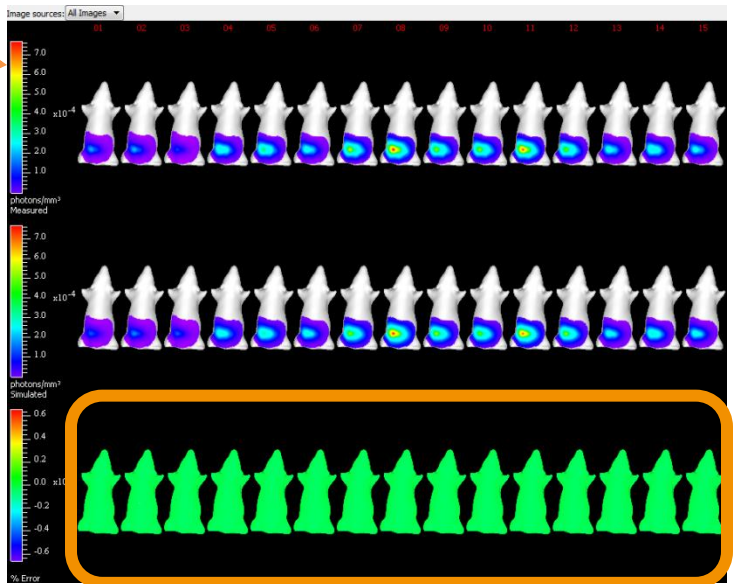




8. When the reconstruction has finished, the **Results** tab will display statistical data about the calculation. Check for an accurate reconstruction by clicking the **Photon Density Map** and comparing the **Measured** light diffusion pattern vs. the **Simulated** light diffusion pattern. The % error between these two maps is illustrated on the bottom row. If the bottom row has a near-zero % difference between Measured and Simulated maps (indicated with a green color), the reconstruction was successful and the image may be used for quantification and spatial measurements. Save your FLIT reconstruction with the **Save** button on the bottom right.

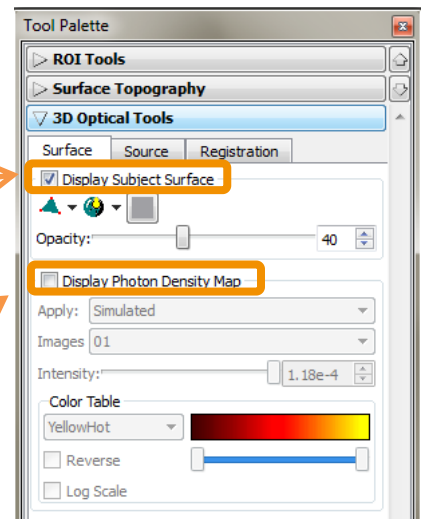
9. Name and Save your FLIT reconstruction with the **Save** button on the bottom right corner of the Results tab.

% Error between Measured and Simulated diffusion patterns per transillumination point. **'Green' means 'go!'**



10. Once the reconstruction has been saved, the **3D Optical Tools** section of the Tool Palette will provide access to three sections of the reconstruction: **Surface**, **Source** and **Registration**. By default, the Subject Surface, or topographic map, will be turned on to allow 3D orientation of your source inside the subject. This Subject Surface can be altered for color, opacity and geometrical rendering using the options in the **Surface** tab.

Additionally, you may select to visualize the 2D light diffusion pattern, either the Simulated or Measured, for each transillumination point used in the analysis by clicking the **Display Photon Density Map** box.

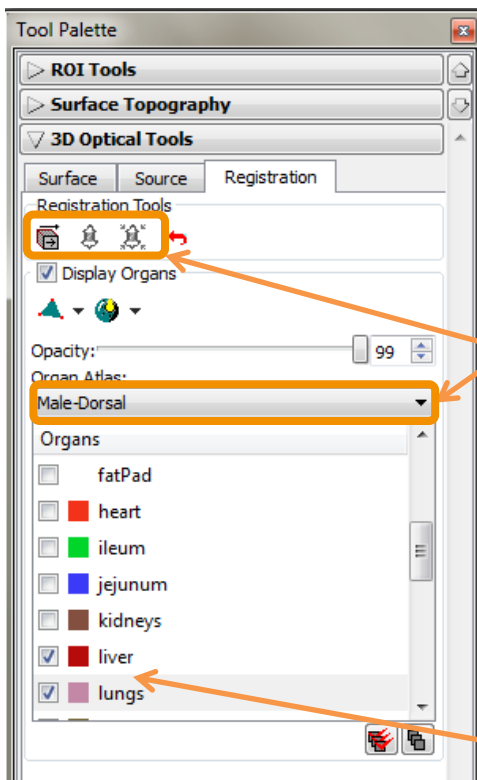
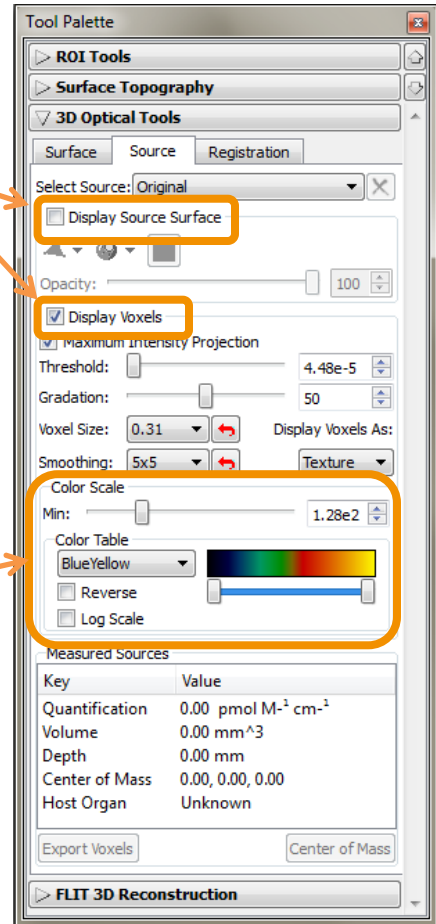


11. . The **Source** tab contains all the tools necessary to adjust visualization of the reconstructed fluorescent sources. Sources consist of multiple **Voxels** (3D pixels) representing relative intensity at that spatial location within the subject.

Two visualization choices are available: **Display Source Surface** and **Display Voxels**. Choosing Display Source Surface displays the isosurface of the reconstructed volume. Users can adjust the color and geometric rendering of the fluorescent source. It is useful for screenshots, movies, and other visualizations. Quantitative signal intensity and depth measurements are not possible when this option is chosen.

For quantification of your source, you must select **Display Voxels**. The displayed source represents a 3D diffusion pattern and it can be selected using the **Voxel Selection Tool** and at-source measurements can be recorded (see Step #13 in this Tech Note).

You can adjust the look of the reconstructed source with the **Color Table** drop down menu and the **Color Scale** slider to adjust minimum visualized values. Avoid adjusting the size of your source using the Threshold slider as this removes voxels from consideration when measuring for quantitative output.



12. The **Registration** tab of 3D Optical Tools allows for an overlay of your Surface/Source reconstruction with a generic Mouse Organ Atlas.

Find the appropriate sex and orientation via the **dropdown menu** and select one of the three **Registration Tools: Manual, Linear Automatic and Non-linear Automatic**.

Linear and Non-linear automatic use the Surface reconstruction to perform a best fit for the Organ Atlas.

Manual co-registration provides panning, shrink/grow and rotational position options to overlay the Organ Atlas to the Surface shape. When Manual is selected, use the 'Tab' button to cycle through the three tool sets. Once the co-registration is set, organs may be removed or selected to appear in the 3D image along with the bioluminescent source by checking/unchecking the box next to the organ name. The colors of the organs cannot be changed but the transparency of the organs can be adjusted with the provided Opacity slider bar.

13. Using the **Voxel Selection Tool** highlighted below, the fluorescent source can be measured for absolute light intensity and relative position. Draw a tight box around the voxels to be quantified. The voxels selected will turn a blue color. It is important to carefully select only the area needed for quantification.

The light intensity of the voxels in Total Fluorescent Yield (pmol/M cm) will appear in the **Measured Sources** box in the Source tab. **A volume measurement is also given, though this is a volume of light produced generating the 3D diffusion pattern – not a tumor or fluorescent source physical volume.** If an Organ Atlas is used, the Host Organ will display which component is nearest to the selected voxels.

Key	Value
Quantification	1.65e5 pmol M ⁻³ cm ⁻¹
Volume	5.11 mm ³
Depth	7.77 mm
Center of Mass	-4.9, -16.8, 10.0
Host Organ	skin

Click and slide bars to scroll through axes

14. Depth of your source can be determined using the **Measurement Cursor**, left of the **Voxel Selection Tool**. With your source selected, click **Center of Mass** on the bottom right of the Source tab. Coronal, sagittal and transaxial planes will be centered at your selected source and a line extending from the center of mass of your source to the nearest surface of the mouse will be drawn. You can move the cursors to measure from any surface of the animal.

15. The **Slice Planes Tool** will allow you to scroll through a chosen axis of the animal and display exact details about localization of your source in relation to organs in the axis windows on the left side of the 3D display window. Simply move the bars to scroll through the axis.

16. Animations of your 3D source can be produced and shown in .avi, .mov, .mpg or .mp4 format. This tool is found under **Tools -> 3D Animations**. For detailed instructions, please see the User Guide.

Note: The value of light intensity in Total Fluorescent Yield obtained from FLIT for your fluorescent source, either resulting from stably transfected cell lines or fluorescent molecules, can be converted to number of cells or molecules after creation of a database for the reporter used in your study. Please read the **Well Plate Quantification Tech Note 15** for more information.