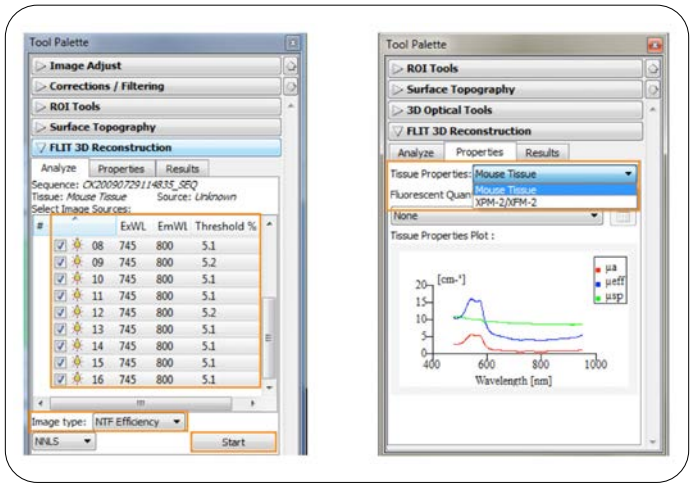
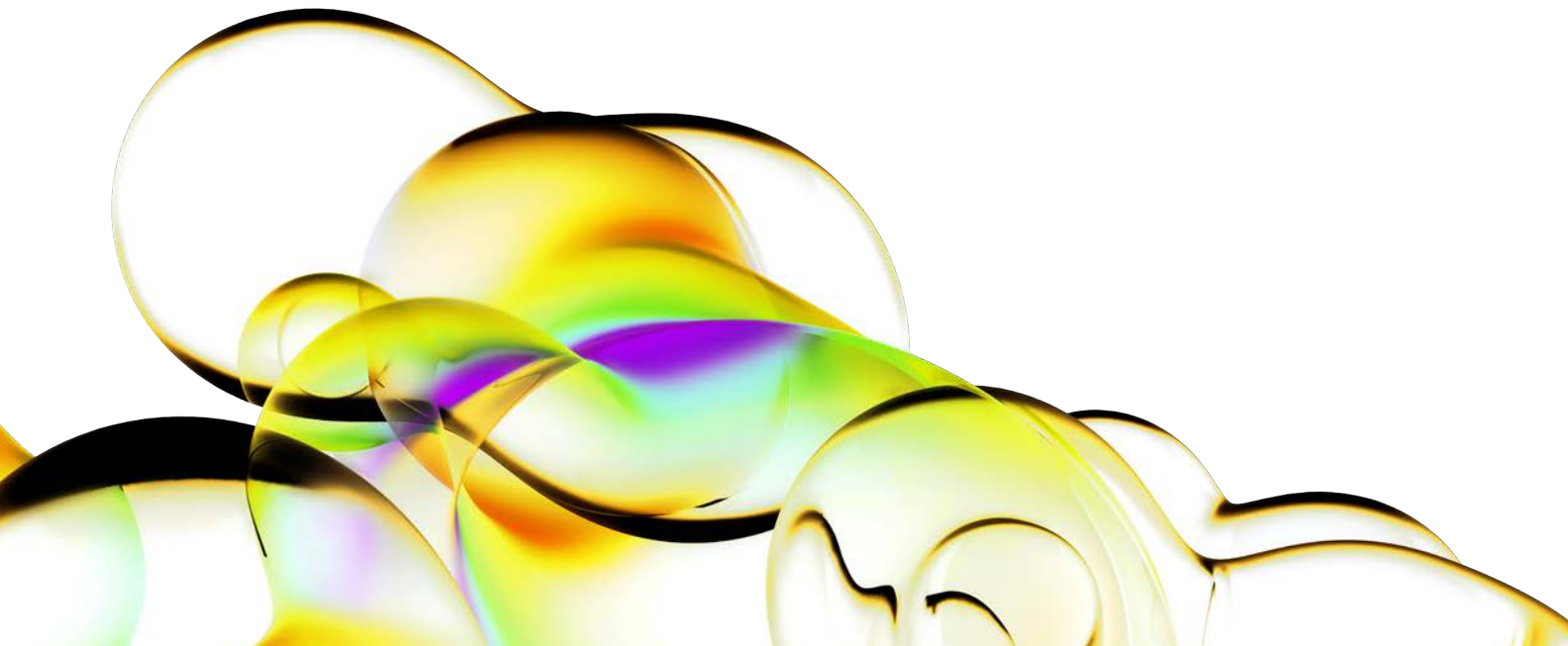
Fluorescence tomography: Source reconstruction and analysis.

Note: This Technical Note is part of a series for Fluorescence Imaging Tomography (FLIT). The user must first follow the steps in the preceding **Fluorescence Tomography – Setup and Acquisition Technical Note** and **Fluorescence Tomography – Topography Technical Note** before performing 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.

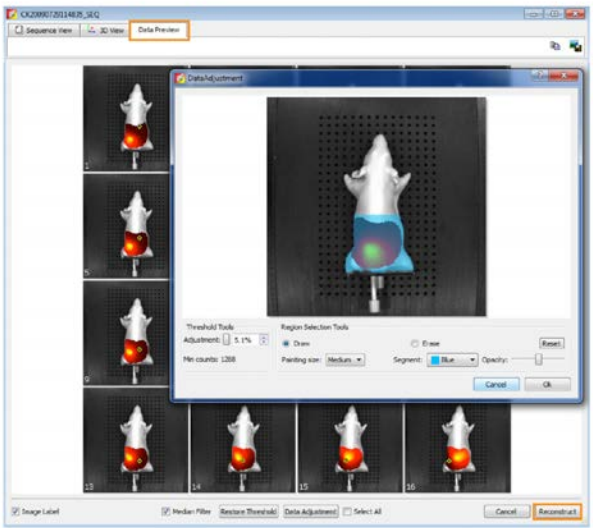




3. **NTF Efficiency** is the recommended default Image Type for all 3D FLIT reconstructions. This selection utilizes NTF to subtract background and facilitate deeper reconstructions. For more information on NTF, please see **Transillumination Fluorescence – Normalized Technical Note.**

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

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



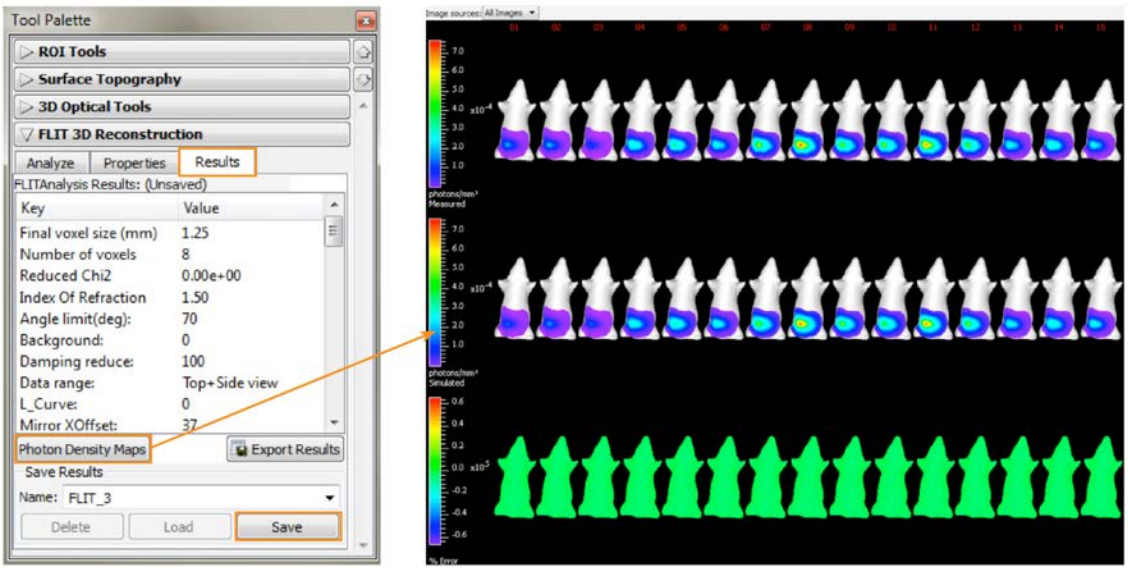
5. Once the images are selected and properties have been set appropriately, click the **Start** button under the **Analyze** tab to review the sequence to be used for reconstruction.

6. The **Data Preview** tab will be displayed in which the user can adjust and threshold the data. Thresholds typically default at or above 5% with fluorescence due to the presence of autofluorescence that can lead to artifact reconstructions. Use the default settings chosen by the software and threshold the resulting voxels, not the acquired raw data. Please consult with an Advanced Imaging Trainer or Technical Support Specialist if there are issues with artifacts in the FLIT models.

Adjustments can be made to the threshold for individual images by double clicking on them. Within this window is also a masking tool that will allow masking in a region of the mouse for consideration and exclude other areas. See the User Guide for more information on using this tool.

7. Once the thresholding has been approved and Data Adjustments (if any) have been set, select the **Reconstruct** button to complete the calculations.

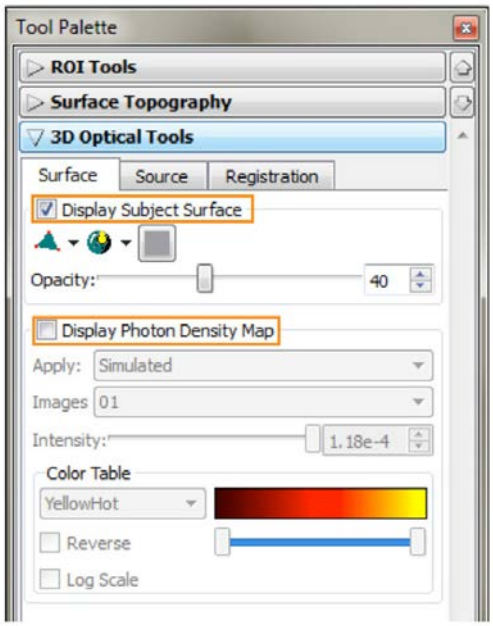
8. When the reconstruction has finished, the **Results** tab will display statistical data about the calculation. Check for an accurate reconstruction by clicking the **Photon Density Map** and comparing the **Measured** light diffusion pattern vs. the **Simulated** light diffusion pattern. The % error between these two maps is illustrated on the bottom row. If the bottom row has a near-zero %



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

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** the FLIT reconstruction with the Save button on the bottom right.

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

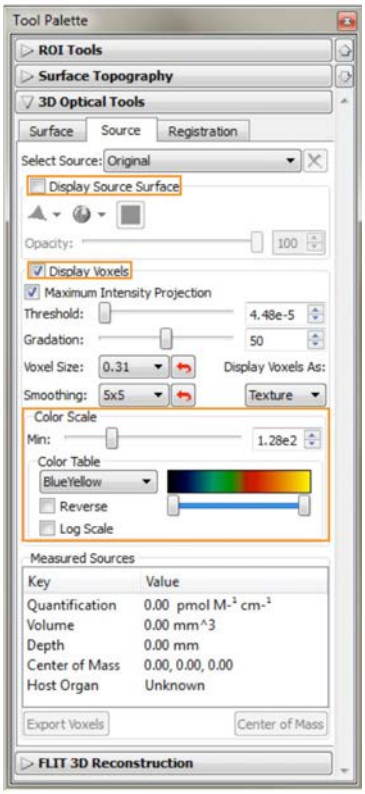


10. Once the reconstruction has been saved, the **3D Optical Tools** section of the Tool Palette will provide access to three sections of the reconstruction: **Surface, Source** and **Registration.** By default, the Subject Surface, or topographic map, will be turned on to allow 3D orientation of the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

Additionally, the 2D light diffusion pattern may be selected to visualize, 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.



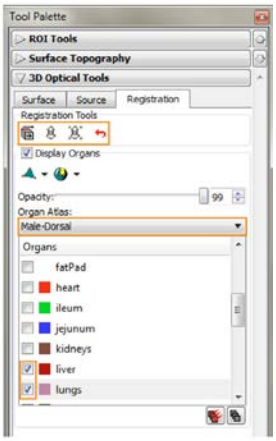
For quantification of the source, the user must select **Display Voxels.** The displayed source represents a 3D diffusion pattern and it can be selected using the **Voxel Selection Tool** and at-source measurements can be recorded (see Step #13 in this Technical Note).

The user 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 the source using the Threshold slider as this removes voxels from consideration when measuring for quantitative output.

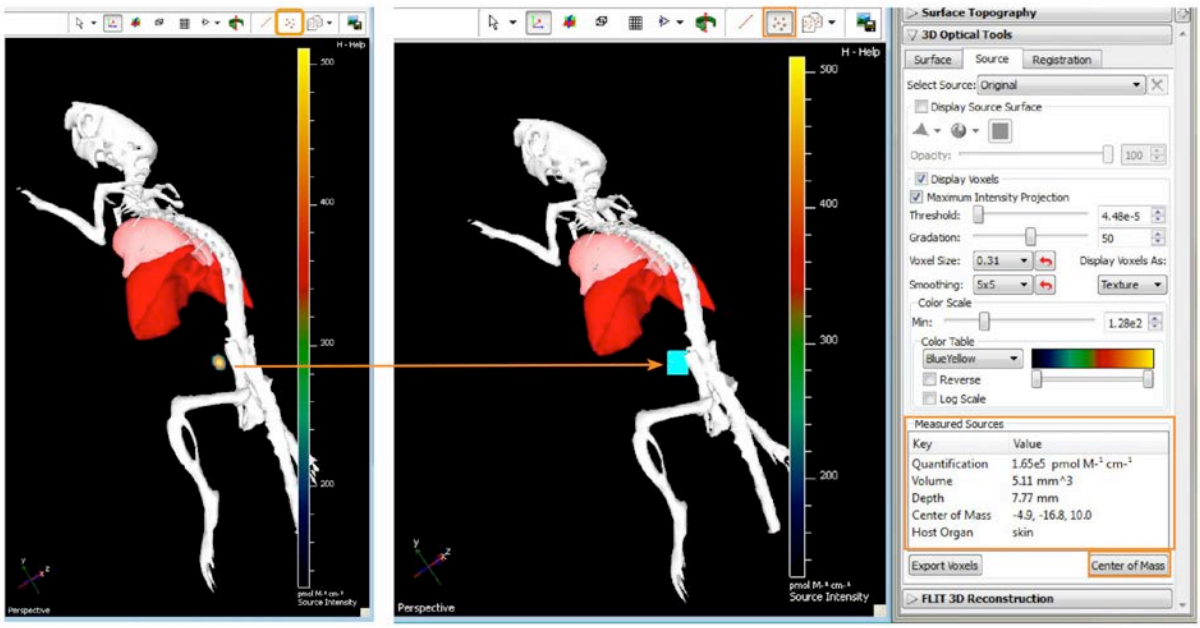
12. The **Registration** tab of 3D Optical Tools allows for an overlay of the Surface/Source reconstruction with a generic Mouse Organ Atlas.

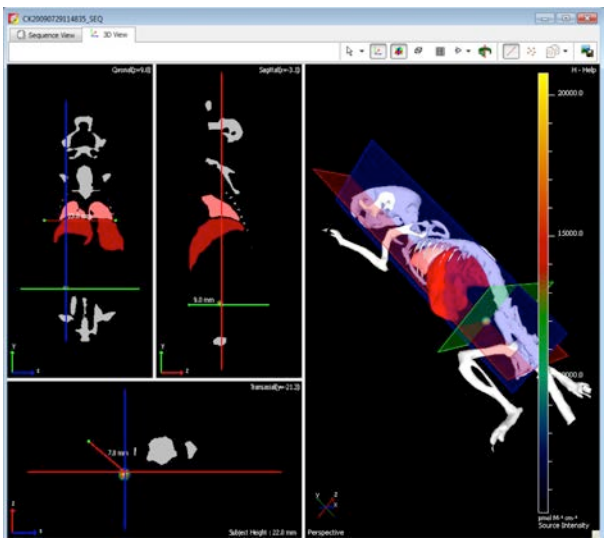
Find the appropriate sex and orientation via the **dropdown menu** and select one of the three **Registration Tools: Manual, Linear Automatic** and **Non-linear Automatic.**

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.

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

15. The **Slice Planes Tool** will allow the user to scroll through a chosen axis of the animal and display exact details about localization of the 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 the 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 the 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 the study.

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