Bioluminescence tomography: Source reconstruction and analysis.

This tech note describes how to reconstruct the 3D source from images taken on the IVIS® Spectrum and SpectrumCT instruments using Living Image® software.

Note: This technical note is the last of a 3-part series describing how to perform bioluminescence tomography using the IVIS imaging systems. Bioluminescence tomography is the process of creating a 3D image of a bioluminescent source from several 2D images taken using different emission filters. You must first complete the steps in the previous bioluminescence tomography technical notes: **Setup and Acquisition** and **Topography** before proceeding. In this series, bioluminescence tomography is also referred to as diffuse light imaging tomography (DLIT).

Automated reconstruction (recommended)

If you are running a newer version of Living Image, the reconstruction is completed in a single click (Figure 1). See below for instructions on performing manual reconstructions.

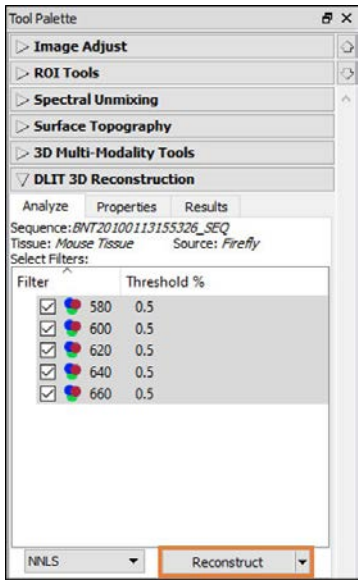

Figure 1: Newer software versions can reconstruct 3D images in a single click.

Manual reconstruction

1. Open **DLIT 3D Reconstruction** tab in Tool Palette.

2. Under the **Analyze** tab, choose the filters to be used for reconstruction (Figure 2). The filters correspond to the images acquired in your sequence in step one of this procedure. **Note:** Living Image will automatically deselect a filter acquisition due to oversaturation or weak signal. For firefly luciferase 580, 600, and 620 nm are essential. Please repeat the acquisition if you do not have sufficient signal (must be at least 600 counts) at these wavelengths.

3. In the **Properties** tab, adjust the **Tissue Properties** to best represent the subject (mouse tissue or phantom) as well as the **Source Spectrum** (firefly, bacteria, etc.) **Note:** If you used the Imaging Wizard for acquisition, these selections will use the input received through the Wizard automatically.

4. Once the filters and properties have been set, click the **Start** button under the **Analyze** tab to review the sequence to be used for reconstruction.

5. The **Data Preview** tab will be displayed in which you can adjust and threshold your data. Thresholds typically default below 5% so most of the signal is included in the analysis. Occasionally a small source will be excluded, you can adjust the threshold **down** slightly to include more signal in the analysis by checking **Select All** and **Data Adjustment** at the bottom of the window. As a general rule, do not decrease thresholds below 0.5% as DLIT artifacts may reconstruct. In most cases, default settings chosen by the software will suffice.

   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 (Figure 3). See the User Guide for more information on using this tool.

6. Once the sequence has been approved and the Data Adjustment (if any) has been set, click the **Reconstruct** button to create the bioluminescent 3D source.

7. When the reconstruction has finished, the **Results** tab will display statistical data about the calculation (Figure 4). Check for an accurate reconstruction by opening the **Photon Density Map** and comparing the **Measured** light diffusion pattern vs. the **Simulated** light diffusion pattern (Figure 5). 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 of radiance and depth of source.

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

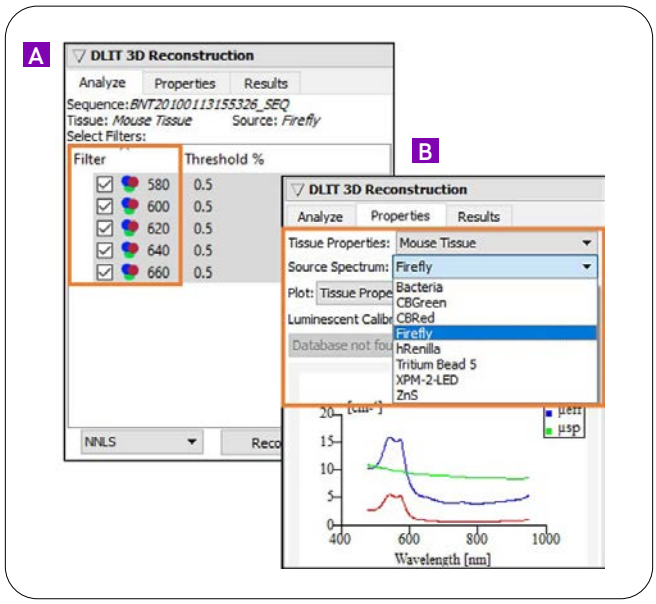

Figure 2: A) Living Image automatically selects the filtered images that have good quality for reconstruction. B) The Properties tab allows you to choose the tissue properties and bioluminescent source.

Figure 3: The masking tool allows you to select a specific region for reconstruction.

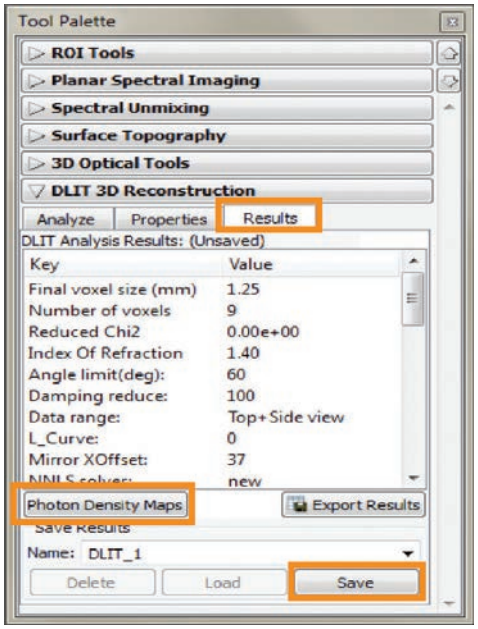

Figure 4: The Results tab shows analysis results for reconstruction which can be saved and loaded again at a later time.

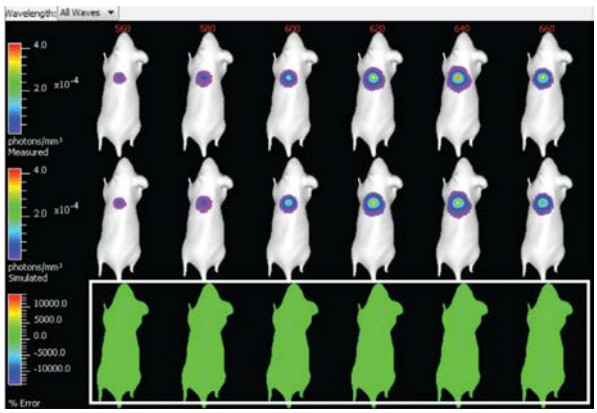

Figure 5: The photon density map shows percent error between measured and simulated light diffusion patterns per image. Green indicates the error is low and the reconstruction was successful.

Image adjustment tools

1. 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 (Figure 6). Additionally, you may select to visualize the 2D light diffusion pattern, either the Simulated or Measured, for each wavelength used in the analysis by clicking the **Display Photon Density Map** box.

2. The **Source** tab contains all the tools necessary to adjust visualization of the reconstructed bioluminescent sources (Figure 7). 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 source volume. Users can adjust the color and geometric rendering of the bioluminescent 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 **Measure Source Tool** and at-source measurements can be recorded (see Figure 8).

   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. If using the Measure Source tool to select an area to quantify, avoid adjusting the size of your source using the Threshold slider as this will change the quantification in addition to the display.

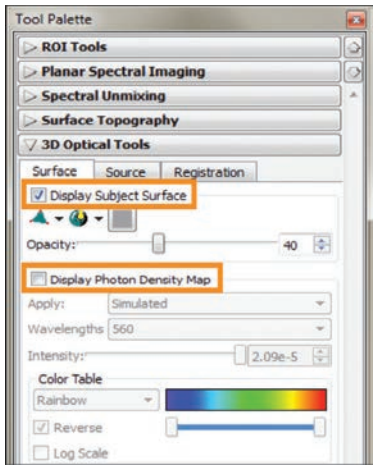

Figure 6: The Surface tab allows you to adjust the display of the surface topography.

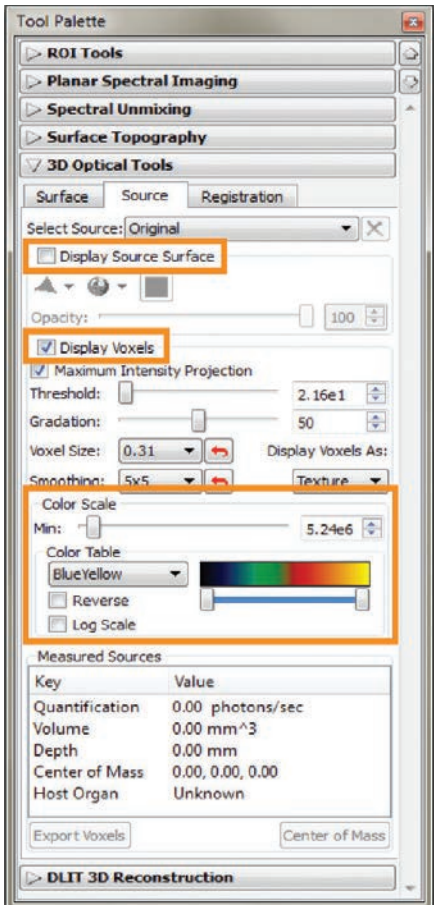

Figure 7: The Source tab allows you to adjust the display of the bioluminescent source voxels.

3. The **Registration** tab of 3D Optical Tools allows for an overlay of your Surface/Source reconstruction with a generic Mouse Organ Atlas (Figure 9). Find the appropriate sex and orientation via the drop down 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 (Figure 9). 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.

Selection tools

1. Using the **Measure Source Tool**, the bioluminescent 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 photons/sec 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 bioluminescent source physical volume**. If an Organ Atlas is used, the Host Organ will display which component is nearest to the selected voxels. The preferred method for selecting regions of interest (ROIs) to quantify, is to go to the ROI Tools tab in the Tool Palette and draw a cube around your source (Figure 10). For more details, see the **Drawing ROIs** technical note.

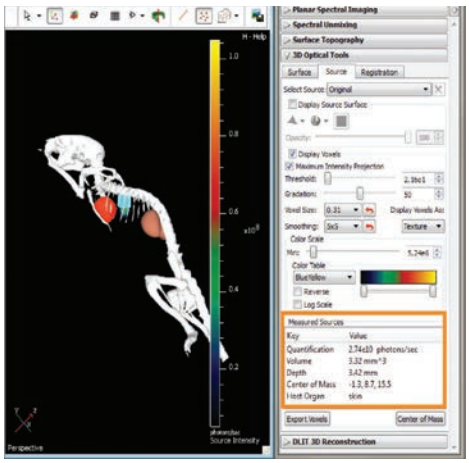

Figure 8: The Measure Source tool allows you to select a region of interest and can provide you with the depth and host organ of the center of mass for the bioluminescent source.

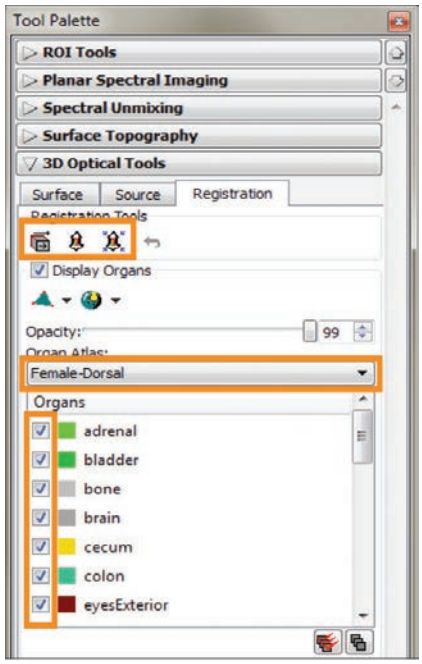

Figure 9: The Registration tab allows you to coregister a digital organ atlas with your subject.

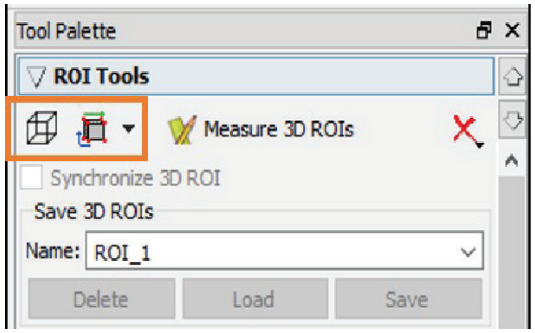

Figure 10: Draw regions of interest in your 3D image using the cube ROI in the Tool Palette.

2. Depth of your source can be determined using the **Measurement Cursor,** left of the **Measure Source Tool**. With your source selected, click **Center of Mass** on the bottom right of the Source tab in the Tool Palette. 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.

3. 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 (Figure 11).

Note: To make an animation of your reconstructed 3D source, see the **3D Movies** tech note.

To make adjustments to the CT component of a 3D scan, see the **3D Multimodality Tools** tech note.

To convert the light intensity in photons/sec obtained from a DLIT scan to number of cells, see the **Well Plate Quantification** tech note.

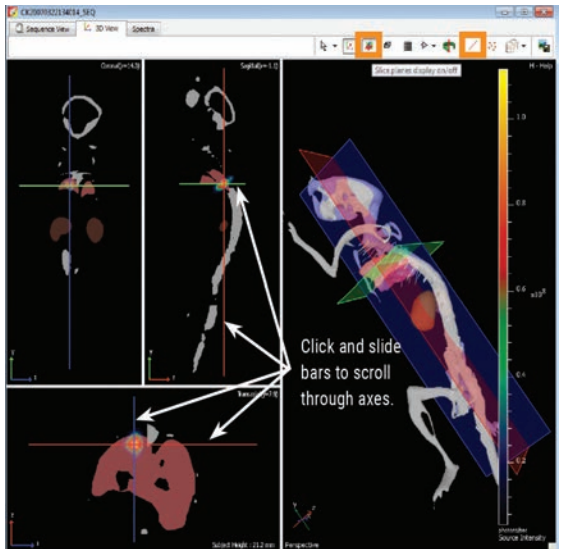

Figure 11: The Slice Planes Tool allows you to scroll through the x-, y-, and z-axes.

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