Bioluminescence tomography: Topography.

This tech note describes how to create a surface topography for 3D images taken using the IVIS® Spectrum and SpectrumCT instruments using Living Image® Software.

Introduction

Bioluminescence tomography is an imaging technique that uses several 2D images taken with different emission filters to create a 3D rendering of a bioluminescent source. 3D images help us pinpoint the source location and determine true colocalization of two or more signals. The first step in reconstructing the 3D image is to create a surface topography or "shell" of the animal which will be divided into smaller volumes called voxels.

To create the surface topography, the IVIS systems will either use information from a microCT image (IVIS SpectrumCT) or a laser galvonometer (IVIS Spectrum). The laser galvanometer is routinely use to project the FOV onto the stage of the instrument. It produces the green outline you see on the stage when the door is opened. We utilize this laser to project a series of parallel lines across your subject.

We acquire a photographic image (the Structured Light Image) when the lines are projected across the animal and from that image we can calculate the height at points on the back of your subject based on the curvature of these laser lines as they cross over the subject (Figure 1). This height map allows us to reconstruct a shell or isosurface which is used in calculating signal depth and intensity during 3D reconstruction. In this technical note, bioluminescence tomography will also be referred to as diffuse light imaging tomography (DLIT).

NOTE: You must first consult and acquire a sequence as suggested in the technical note: **Bioluminescence Tomography: Setup and Sequence Acquisition** before proceeding with the following steps.

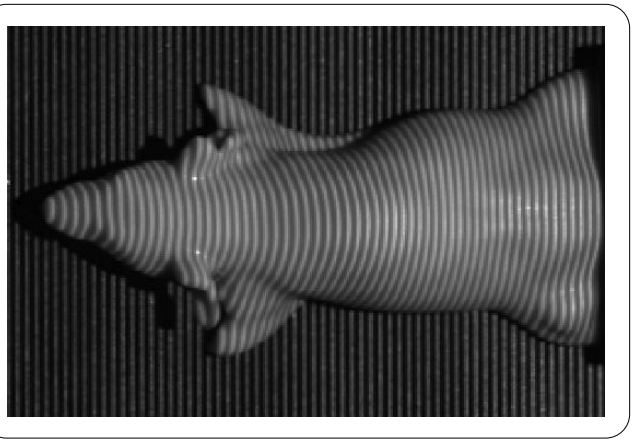


Figure 1. The IVIS Spectrum uses a laser galvonometer to cast parallel lines of light onto the subject to create a structured light image.

Automated creation of surface topography

In newer versions of Living Image, the surface topography will automatically be created based on the microCT or structured light image. This happens when the one-click reconstruction button is used. We recommend that you set up all of your 3D imaging experiments using the Imaging Wizard to ensure that the proper acquisition settings are used.

There may be situations where the surface topography needs to be done manually (e.g., you are running an older version of the software, the automated method did not properly assign the topography, or you have multiple mice in the field of view). If this is the case, follow the instructions below.

Manual creation of surface topography (IVIS spectrum)

1. Open **Surface Topography** tab in Tool Palette.
2. Select the **Orientation** i.e. Dorsal or Ventral and the Subject type from the dropdown menu i.e. Nude/Furred mouse or Phantom (Figure 2). It is recommended to use the **"Furred"** option for all haired mice, regardless of shaving or depilatory use. Use **"Nude"** for hairless nude mice.
3. Surface Smoothing can be applied **after** the reconstruction. Use the lowest possible smoothing level, as overcompensation can cause a loss in the surface volume or height. The default is set to **Low**.
4. Click **Generate Surface** and the topography analysis box will appear.
5. Draw a crop box that includes the entire subject then click **Next** (Figure 3).

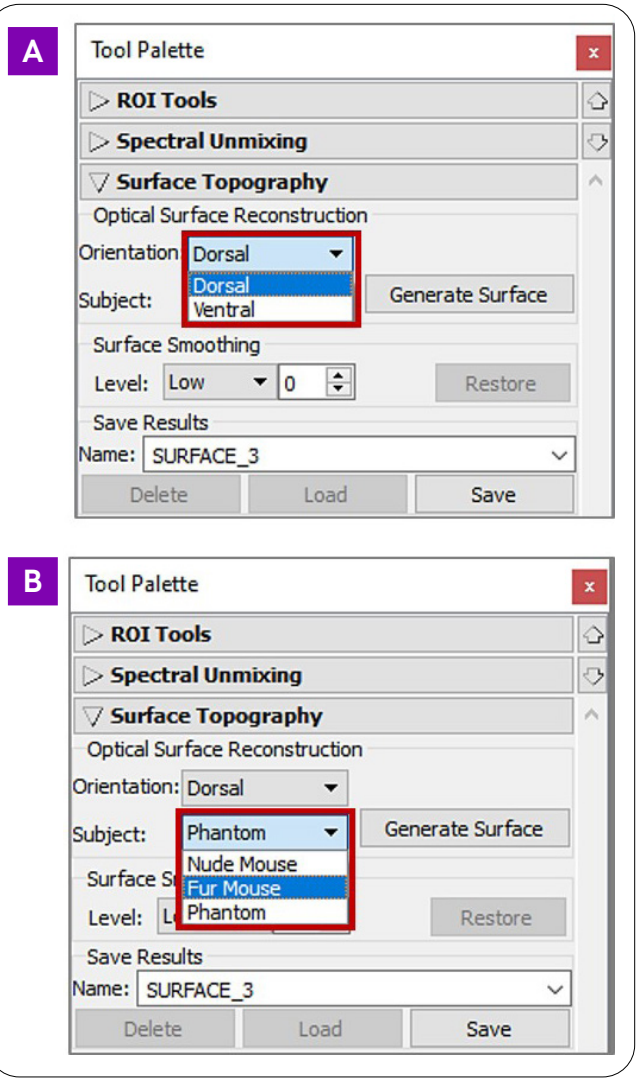


Figure 2. Manual creation of the surface topography requires selection of the (A) subject orientation and (B) subject type in the Tool Palette.

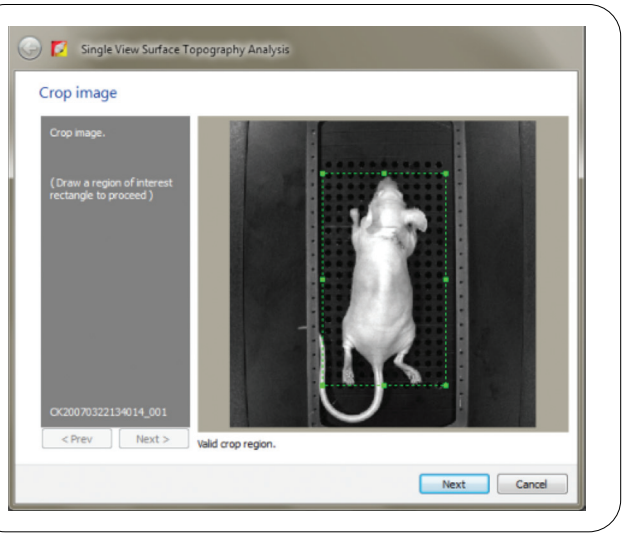


Figure 3. Draw a box around your subject to indicate where the surface topography should be created.

6. The thresholding tool will appear as a purple mask over the subject and defines the area of interest for the surface topography reconstruction (Figure 4). The mask should match the underlying photograph of the subject as closely as possible. If adjustments are necessary, adjust the threshold value so that the mask fits the subject image:
   - Press the left or right arrow keys on keyboard
   - Move the Threshold slider left or right
   - Click the arrows or enter a new value in the box
7. Click **Finish** and the reconstructed mesh will appear in the right panel while slices of coronal, sagittal and transaxial axes are shown to the right (Figure 5).
8. To save the results, enter a Name: then click **Save**.
9. Continue to the technical note: **Bioluminescence Tomography: Source Reconstruction and Analysis** for reconstruction of your bioluminescent source.

NOTE: It is very difficult to perform DLIT analysis on black furred animals even after shaving due to the pigmentation of the skin. You may have difficulty masking your subject because the software cannot distinguish the black mice from the surrounding black stage.

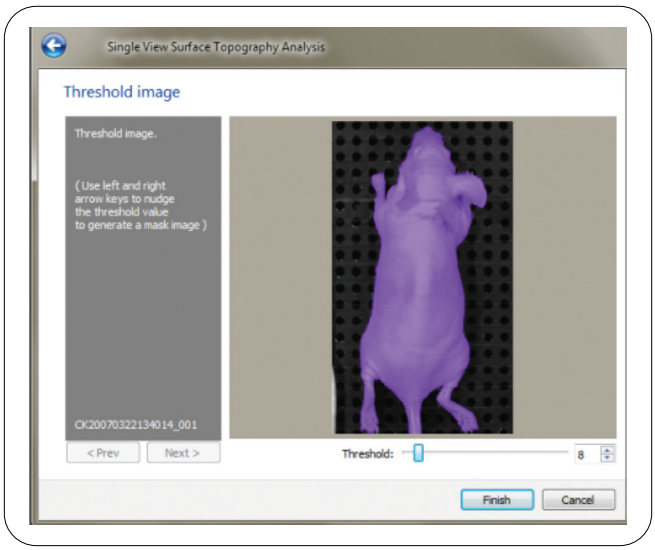


Figure 4. Adjust the threshold so only the subject is highlighted with the purple mask.

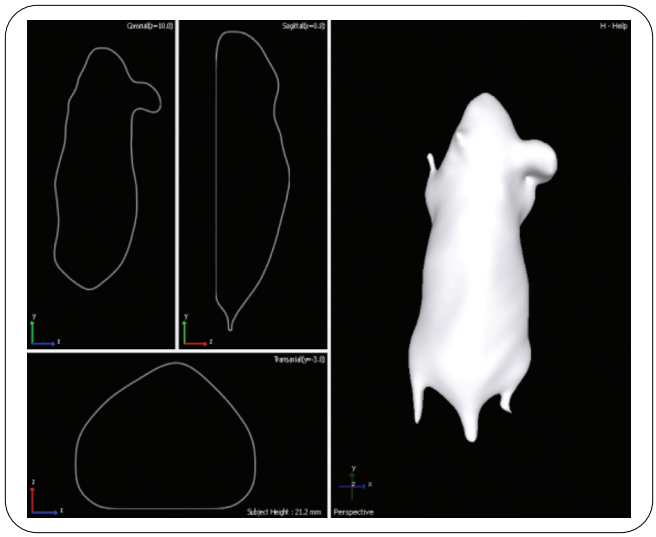
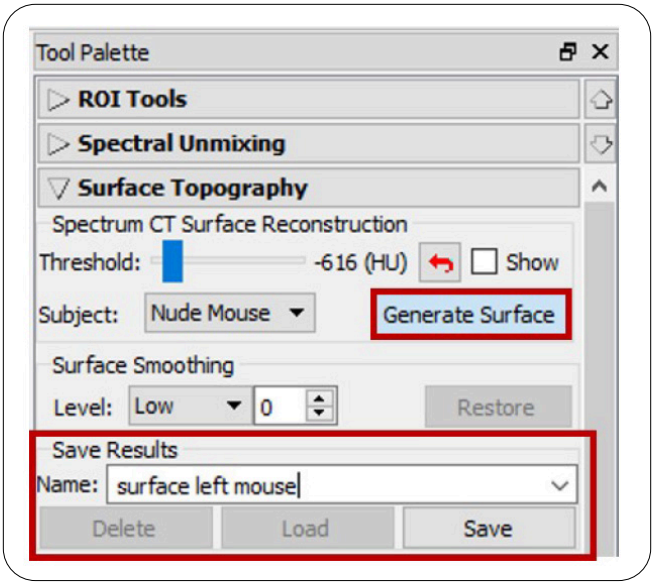


Figure 5. The final surface topography will appear in the 3D viewing screen to the right of the 2D coronal, sagittal, and axial slices.

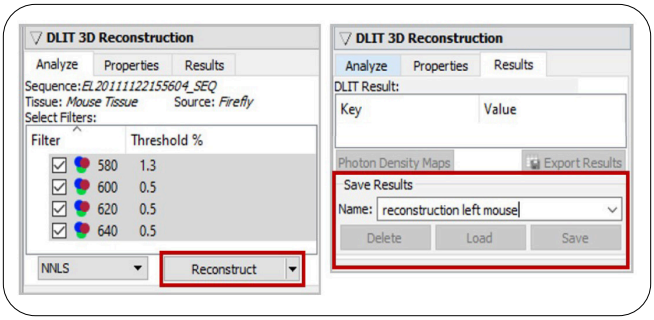
Manual creation of surface topography (IVIS SpectrumCT)

If you acquire a DLIT image on a IVIS SpectrumCT instrument, the CT image will be used to create the surface topography. There are two possible holders that can be used to image the mice: the 1-mouse perforated holder or the 2-mouse holder (Figure 6). If using the latter, the surface topography must be done manually for each mouse individually. To do this, follow the instructions below.

1. Open Living Image and load the DLIT scan.
2. Navigate to the Tool Palette. Under 3D Multi-Modality Tools > Volume, click on the crop tool (Figure 7).
3. Click and drag on the outer boundary so that only the left mouse is visible and the right mouse has been cropped out.
4. Go to Tool Palette > Surface Topography and click “Generate Surface.” Give this surface topography a name (e.g. “surface left mouse”) and click “Save.



5. Go to Tool Palette > DLIT 3D Reconstruction > Analyze and click “Reconstruct.” Save the reconstruction by going to the Results tab, giving the reconstruction a name (e.g., “reconstruction left mouse”) and click “Save.”



6. Repeat this process by cropping to the right mouse, generating and saving a new surface, and generating and saving a new reconstruction.

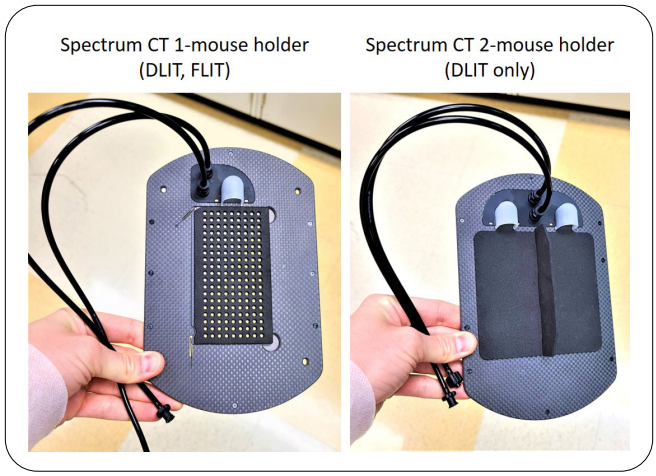


Figure 6. Holders used for 3D imaging on the IVIS SpectrumCT.

Figure 7. If you use the 2-mouse DLIT holder on the IVIS SpectrumCT, you have to create the surface topography for each mouse separately. Use the cropping tool to isolate the left mouse, create the surface topography and reconstruct. Then, crop to isolate the right mouse and repeat the process.

Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA

(800) 762-4000
www.revvity.com

For a complete listing of our global offices, visit www.revvity.com