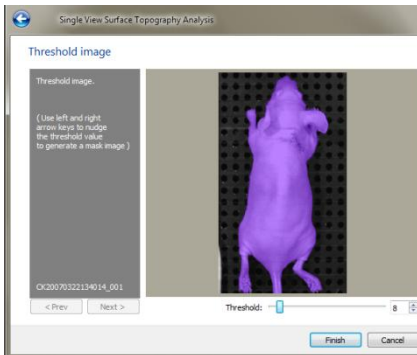
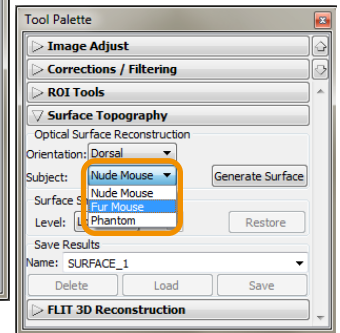
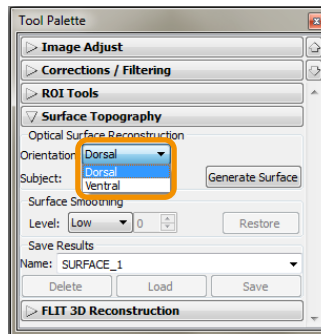


Bioluminescence Tomography - Topography

The IVIS Spectrum has a laser galvanometer that we routinely use to project the FOV onto the surface 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. This height map allows us to reconstruct a shell or isosurface of your animal. This shell is referred to as the Surface Topography and is used in calculating bioluminescent signal depth and intensity during the DLIT 3D source reconstruction.

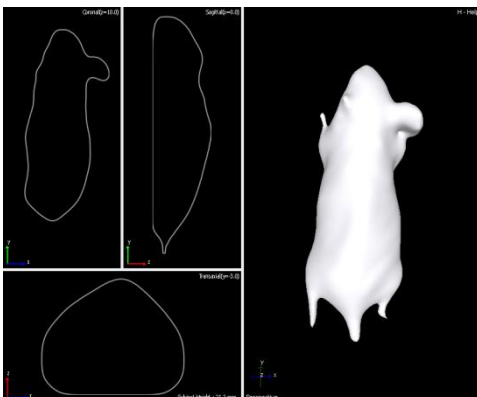
Note: You must first consult and acquire a sequence as suggested in the [Bioluminescence Tomography – Setup and Sequence Acquisition Tech Note 4a](#) before proceeding with the steps in this tech note.

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. 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 tomography analysis box will appear.
5. Draw a crop box that includes the entire subject then click **Next**.



6. The thresholding tool will appear as a purple mask over the subject and defines the area of interest for the surface topography reconstruction. 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 the 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 left.
8. To save the results, enter a Name: then click **Save**.
9. Continue to the [Bioluminescence Tomography – Source Reconstruction and Analysis Tech Note 4c](#) for reconstruction of your bioluminescent source.

Note: We can not perform DLIT analysis on black furred animals even after shaving. The pigmentation in the skin does not allow the algorithms to distinguish between the subject and the stage. This is evidenced by the inability to mask properly around the black mice because they can not be distinguished from the surrounding stage.