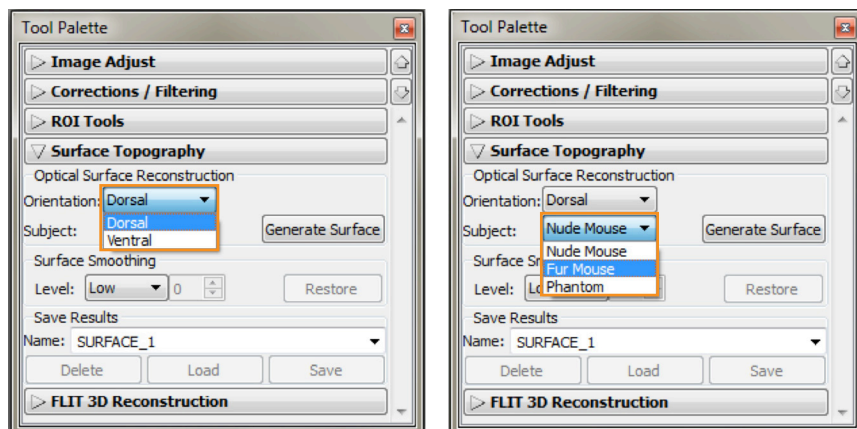


# Fluorescence Tomography – Topography

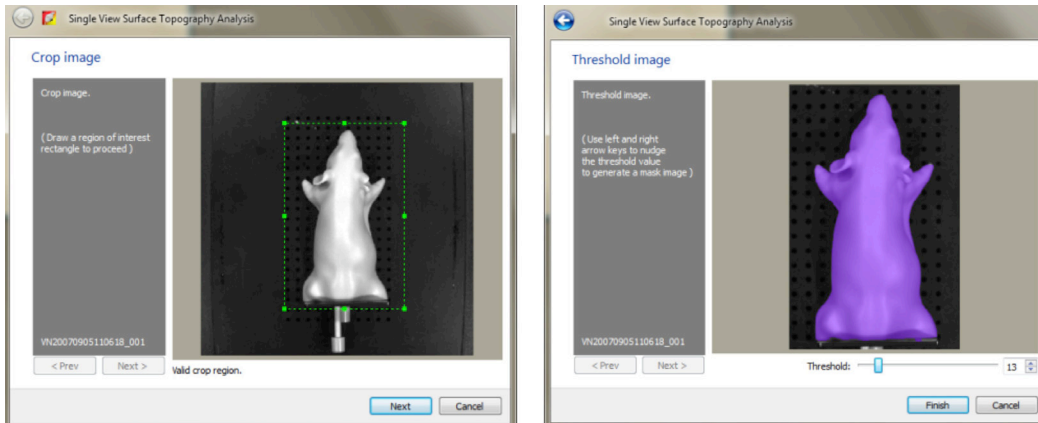
The IVIS® Spectrum has a laser galvanometer that is routinely used to project the FOV onto the surface of the instrument. It produces the green outline seen on the stage when the door is opened. This laser is utilized to project a series of parallel lines across the subject. Photographic images are acquired (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 the 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 the animal. This shell is referred to as the Surface Topography and is used in calculating fluorescent signal depth and intensity during the DLIT 3D source reconstruction.

**See also:** Please review the [Fluorescence Tomography – Setup and Sequence Acquisition Technical Note 6a](#) and [Fluorescence Tomography – Source Reconstruction and Analysis Technical Note 6c](#) in the Fluorescence Tomography series.

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**.



- Click **Generate Surface** and the tomography analysis box will appear.
- 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 quick reference guide for **Fluorescence Tomography – Source Reconstruction and Analysis Technical Note 6c** for further assistance.

**Note:** FLIT analysis cannot be performed 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.

