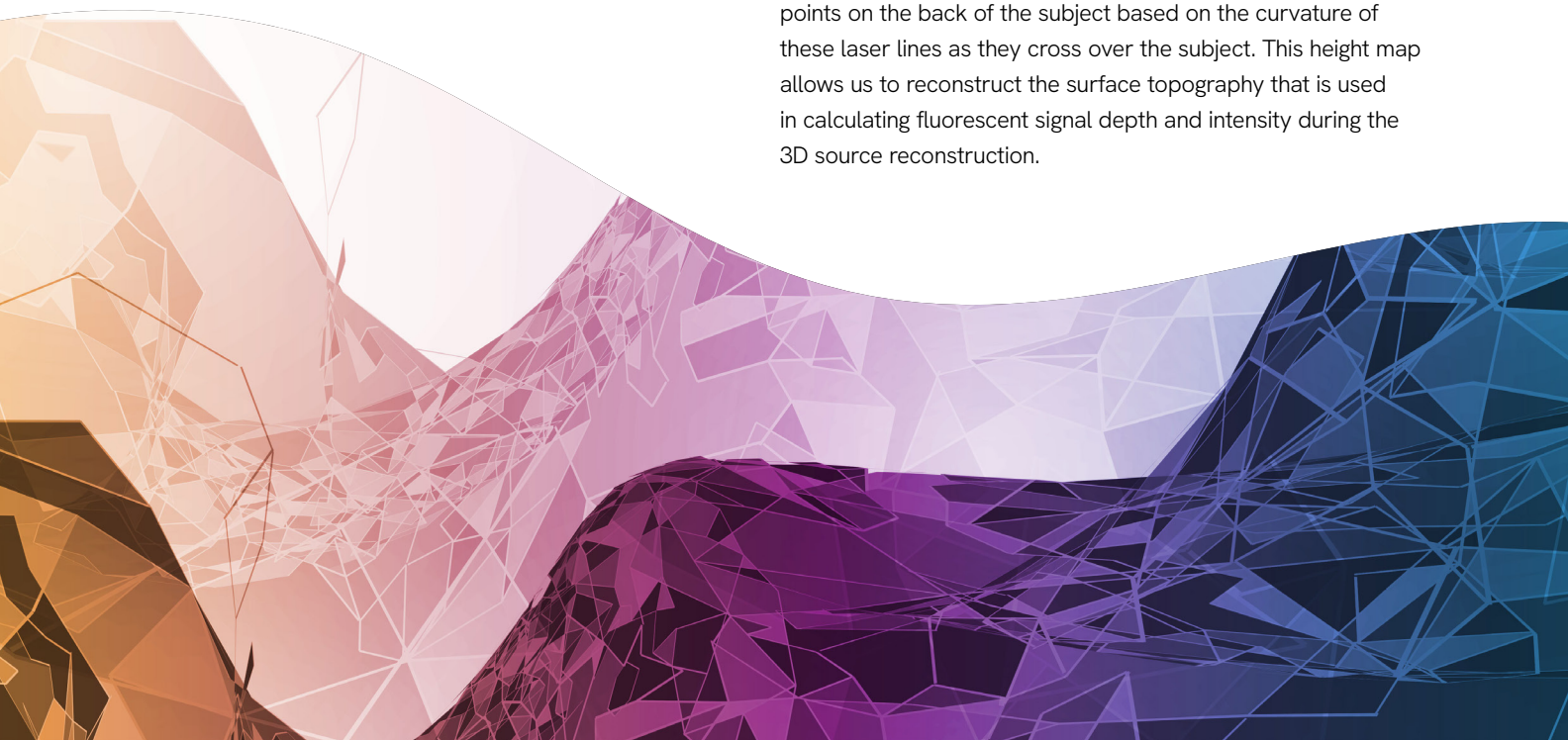
Fluorescence tomography – topography.

This tech note describes how to use Living Image® software to create a surface topography for 3D images obtained on IVIS® imaging systems.

Fluorescence tomography (also referred to as fluorescence imaging tomography - "FLIT") is an imaging technique that takes information from many 2D images to reconstruct a 3D image of a fluorescent source. IVIS instruments that have a transilluminator are capable of doing fluorescence tomography. Some advantages to 3D imaging over 2D imaging are: pinpoint source localization, identification of truly colocalized signals, co-registration with anatomical imaging modalities like MRI and CT, and conversion of optical signal to other meaningful measures like pmol of dye or number of cells.

This technique requires that a surface topography be constructed. The surface topography can also be thought of as a "shell" or isosurface of the animal that defines the boundary between tissue and surrounding air. The software algorithms need a container within which to reconstruct the source in 3D space. The surface topography will be divided into smaller sub-volumes called "voxels." If using the IVIS® SpectrumCT, the software will use a CT scan to create the surface topography. If using the IVIS Spectrum, the instrument will create the surface topography from a structured light image (Figure 1).

Structured light images are produced by casting parallel lines onto the subject using a laser galvanometer and taking a black and white photograph. 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 the surface topography that is used in calculating fluorescent signal depth and intensity during the 3D source reconstruction.



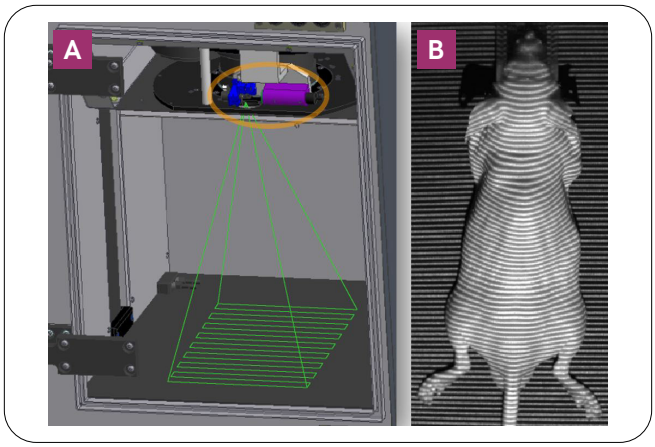


Figure 1: (A) The laser galvanometer projects parallel lines onto the stage of the instrument. (B) The bending of the lines as they pass over curved parts of the mouse is used to determine the height.

SEE ALSO: The **Fluorescence Tomography – Setup and Sequence Acquisition** Technical Note and **Fluorescence Tomography – Source Reconstruction and Analysis** Technical Note in the Fluorescence Tomography series.

Automated surface topography (recommended)

With newer versions of the Living Image software, the surface topography is created automatically either from a micro-CT image or from the structured light image shown in Figure 1. 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 or the automated method did not properly assign the topography). If this is the case, follow the instructions below.

Manual surface topography

1. Open **Surface Topography** tab in Tool Palette (Figure 2).
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 topography analysis box will appear.

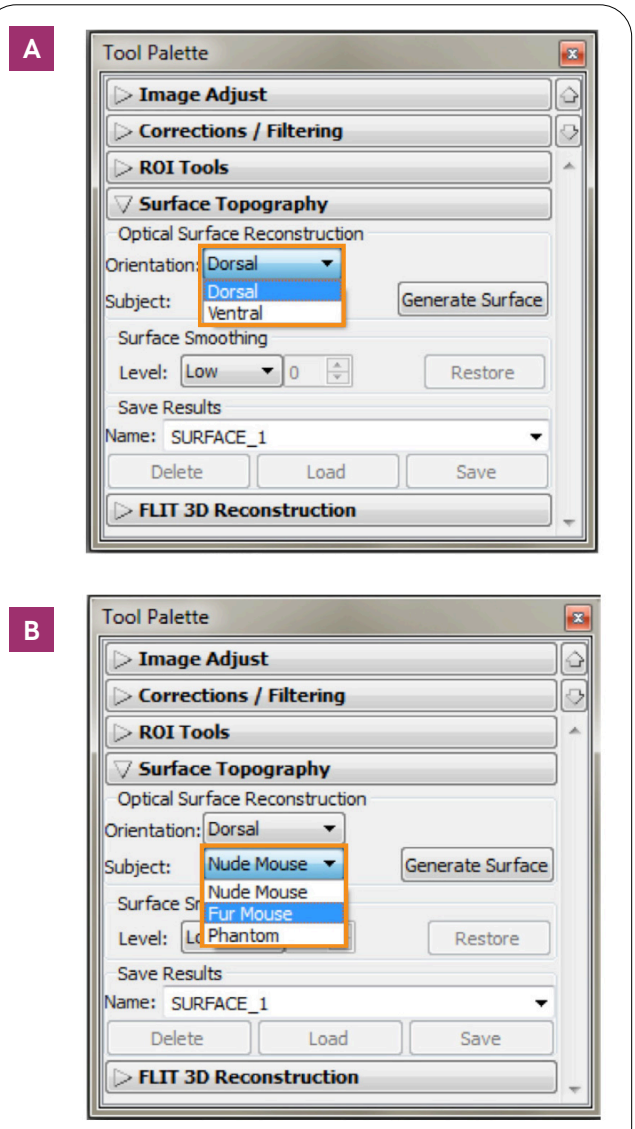


Figure 2: The Surface Topography tab in the Tool Palette allows you to select the (A) orientation and (B) subject type.

5. Draw a crop box that includes the entire subject then click **Next** (Figure 3).

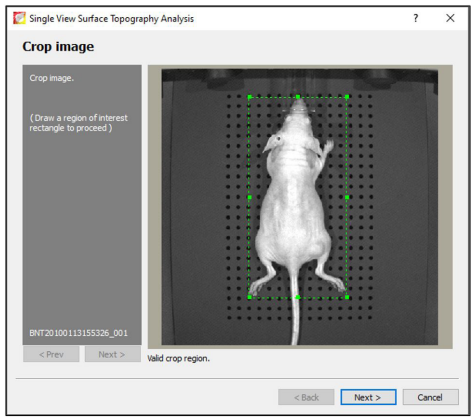


Figure 3: To generate a manual surface topography, fit the crop box to the shape of the animal.

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 the keyboard
   - Move the Threshold slider left or right
   - Click the arrows or enter a new value in the box.

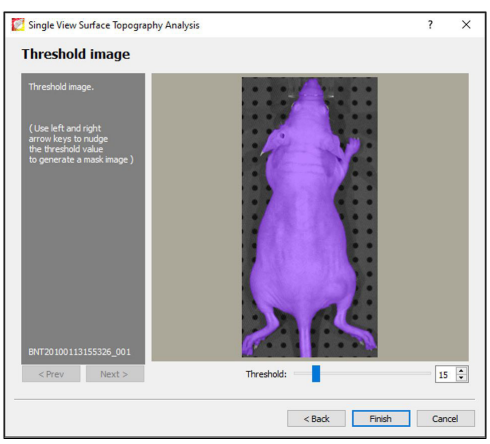


Figure 4: Set the threshold for the surface topography. Adjust the threshold slider bar until the subject is masked purple and the background is blank.

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 (Figure 5).

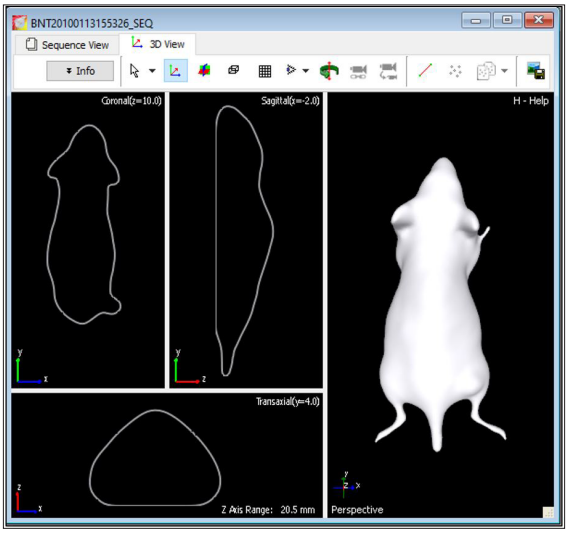


Figure 5: The final 3D surface topography will appear on the right-hand side of the screen. The 2D coronal, sagittal, and transaxial views appear on the left-hand side.

8. To save the results, go to the **Tool Palette** and under the **Surface Topography** tab, enter a Name, then click **Save.**

9. You are now ready for the last step - signal reconstruction. For instructions on how to do this, continue to the next and final technical note for this series: **Fluorescence Tomography – Source Reconstruction and Analysis.**

NOTE: FLIT analysis is very difficult to perform 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.

Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA
(800) 762-4000
www.revvity.com