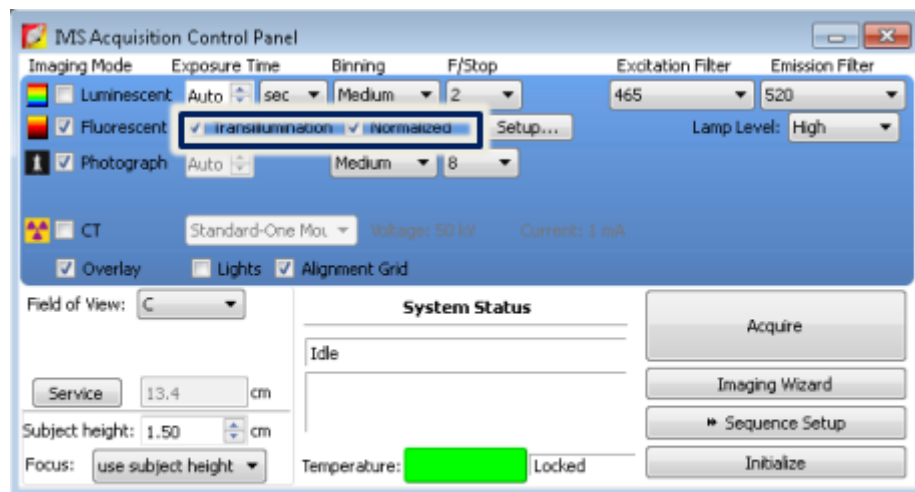# 3D Transillumination Fluorescence (IVIS Spectrum)

Transillumination (excitation light source located below the stage) is recommended if the fluorescent source is deep relative to the imaged side of the animal. It is also required for 3D fluorescence imaging. Use only the single mouse anesthesia manifold when imaging with transillumination.

## Quick 2D Spatial Scan (to determine location of fluorescence)

1. Initialize Living Image software.

2. Wait until camera temperature box turns green (~ 7 min). The default temperature is 37°C

3. Block off all but one port in the IVIS anesthesia manifold.

4. Replace standard base plate with transillumination plate (black plate containing a grid of holes).

5. In the control panel, specify the settings for the fluorescence image (exposure time, binning, F/stop, excitation filter, emission filter).

Control Panel showing setup of parameters for fluorescence imaging via transillumination

6. Set up your transillumination sequence by selecting the points you would like to image in the animal. Click Sequence Setup in the control panel.

7. In the sequence editor, specify the settings for the photographic image and make sure the Reuse box is checked.

8. In the control panel, select the Transillumination option. The Normalized option is selected as default.

9. Click Setup (located next to the Normalized option. The Transillumination Setup window will appear.
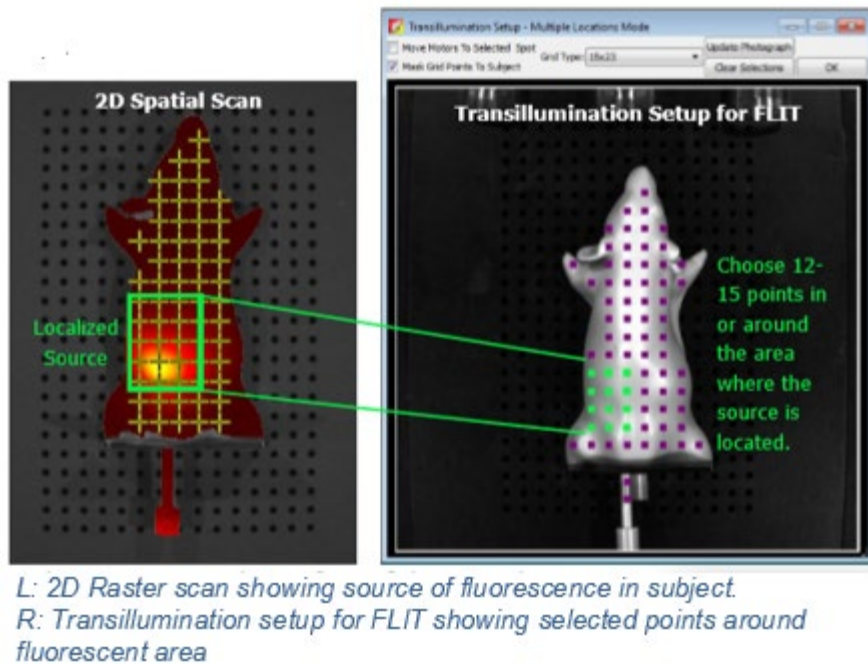
10. Click Yes to update image.

11. Check **Raster Scan** on the top left corner of the Transillumination Setup window.

12. Click the purple dots (specifies all possible locations for transillumination and image acquisition) corresponding to the location of interest. Selected dots will turn green.

13. In the sequence editor, click Add and the acquisition parameters will be added to the Sequence Editor.

14. Click Acquire Sequence in the control panel to start imaging.
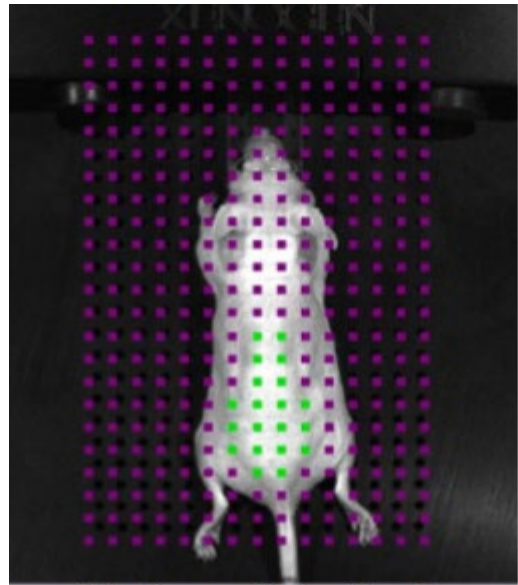
Selecting possible locations for fluorescence image acquisition

3D Image Acquisition

1. Use the Imaging Wizard to set up FLIT (Fluorescence Imaging Tomography). Restart wizard if necessary.

   a. Image option: Fluorescence

   b. Sequence option: FLIT

   c. Choose fluorophore in the dropdown menu

   d. Imaging subject: mouse (Do Not check MIS!)

   e. Choose field of view by referring to green alignment grid in chamber.

   f. Set everything else as default.

2. Click the Transillumination Setup button.

3. The Transillumination Setup window will appear. The software will prompt you to update the image, click Yes.

4. Choose **Mask Grid Points to Subject** on the top left corner of the Transillumination Setup window.

5. In the Transillumination Setup window, choose between 12 to 15 purple points (max) corresponding to the location of interest. Selected dots will turn green.

L: 2D Raster scan showing source of fluorescence in subject.
R: Transillumination setup for FLIT showing selected points around fluorescent area

6. Acquire Sequence.

7. Once image acquisition is complete, confirm that the signal is within proper limits – between 600-60,000 counts.

8. The transillumination locations can be shown/hidden on the images in the sequence by checking the tab at the top of the image.

9. Switch to calibrated units – NTF Efficiency - in the Units dropdown at the top of the image.

10. Save data for later analysis/reconstruction.

When done

1. Copy/transfer data to fileshare.

2. Exit Living Image program. Log off computer.

3. Take off transillumination plate and replace with base plate. Clean chamber with disinfectant wipes and follow shut down procedure for anesthesia (if applicable). **Do not spray alcohol inside chamber!**