

# Trans-illumination fluorescence — normalized.

## Note

You have to be using Living Image® (v4.0) software or higher to use this technique. This tech note is designed as a supplement to Transillumination Fluorescence Tech Note. For information about setup of your 2D transillumination fluorescence sequences, please first consult that tech note.

Normalized transmission fluorescence is a technique that allows us to subtract background light leakage through thin tissue from transillumination images utilizing an extra image captured with a neutral density (ND) filter. The ND filter dampens the intensity of the halogen lamp to 1/100th of the source intensity but does not filter out specific wavelengths. Light of all wavelengths is allowed to pass through the animal and the image is collected with the emission filter of your choice.

## Theory

The background transmission image will give us an indication of where light passes through tissues with less absorption such as in areas of the animal where the tissue is thinner essentially giving us an indication of light **transmission** through individual areas of the mouse. We then normalize the image taken with the specific excitation filter for your fluorophore with this **transmission** image - the same emission filter will be used for both images. We can then effectively “subtract” any background signal due purely to tissue optical density and focus on signal that is specifically produced by your probe of interest.

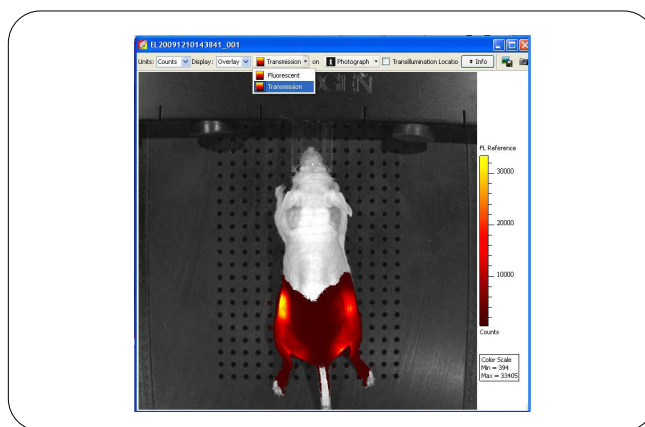


Figure 1: Attenuation map created by excitation Neutral Density (ND2) filter/emission 620 nm

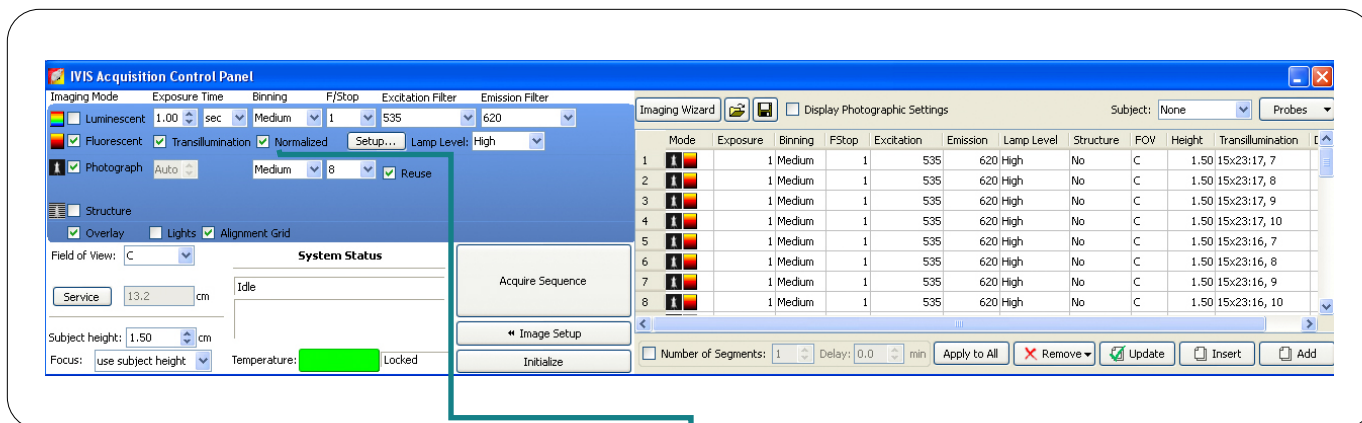


Figure 2: Verify “Normalized” option is selected in Control Panel

1. To pair background images with your transillumination images, simply select the **Normalized** check box either on the **Control Panel** or in the final **Imaging Wizard Screen**.
2. Select the transillumination points that you would like to capture for the image and then select the appropriate excitation and emission filters for your fluorescent probes.
3. As described above, one image will be acquired to determine **transmission** of light through the tissue (termed **Transmission**) and one will be acquired for the specific filter pair (termed **Fluorescent**).

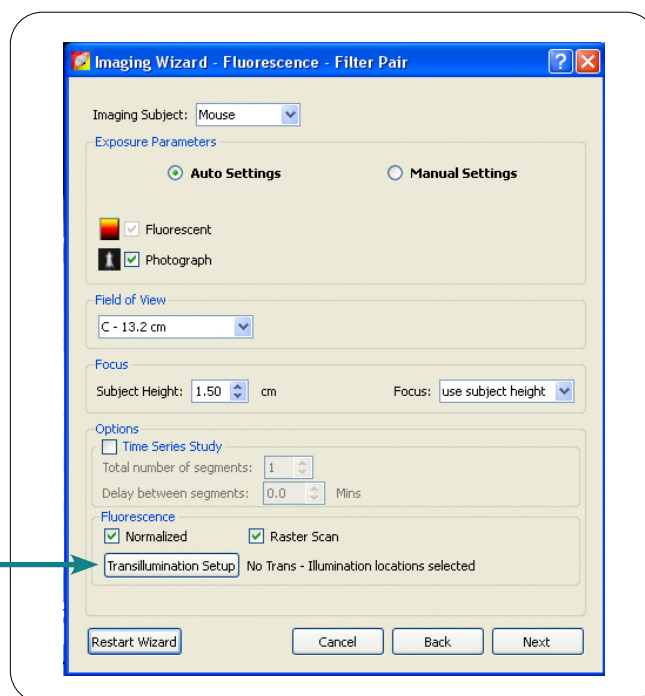


Figure 3: “Normalized” option is set by default in the Imaging Wizard

4. You can switch between these images using the drop-down box at the top of the image.

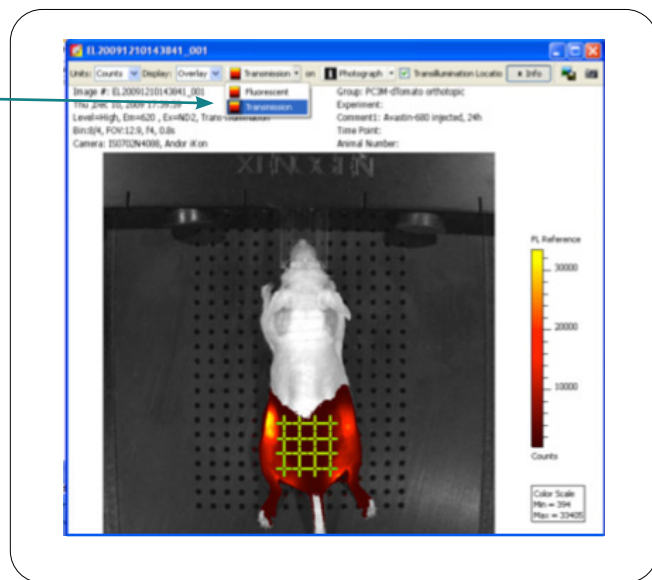
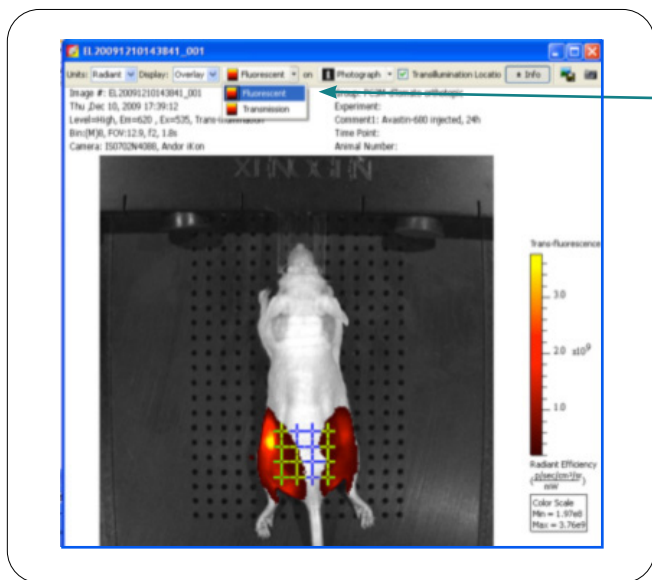


Figure 4: Excitation 535 nm/Emission 620 nm

Figure 5: Excitation ND2/Emission 620 nm

5. Background subtraction/normalization is done automatically after the appropriate unit is selected. To view the normalized image, change the units to **NTF Efficiency**.

6. The normalized image will be displayed and can be analyzed and measured as with any other fluorescent image capture.

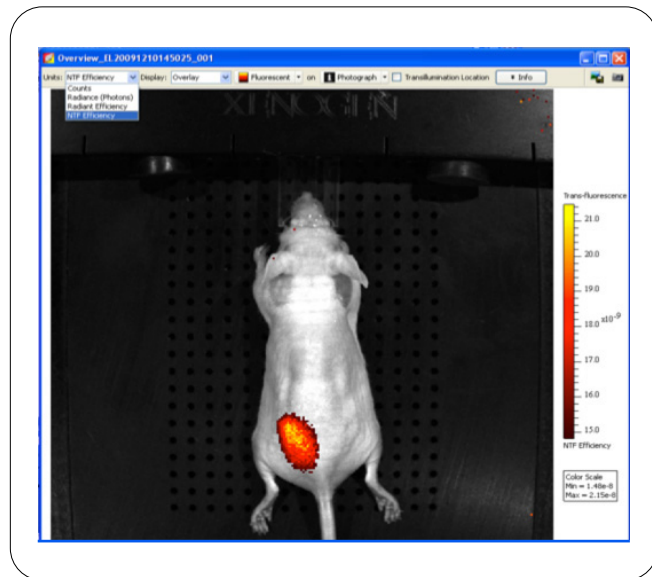


Figure 6: Final image shown with the NTF Efficiency values

