In Vivo Imaging

Working with Image Math

Image Math is a rudimentary but effective method for Spectrum and Lumina users to subtract background from images without performing Spectral Unmixing.

Sequence Setup and Theory

You will need to choose two excitation filters for Image Math. One filter to specifically target autofluorescent background and one filter to specifically target your probe. In the example below, we want to target mCherry in the gut. We chose Ex. 570/Em. 620 as these filters lie close to both peak excitation and emission for mCherry.

To image autofluorescent background, Ex. 500/Em. 620 was utilized. Since the emission filter should remain consistent with this procedure, we certainly cannot shift the excitation filter to the right. Furthermore, autofluorescence drops significantly as you move toward the near infrared end of the spectrum. Therefore, the best filter for imaging autofluorescence will always be shifted towards the shorter wavelengths. As can be seen from the mCherry excitation curve to the left, excitation at 500 nm will not significantly excite the mCherry protein.
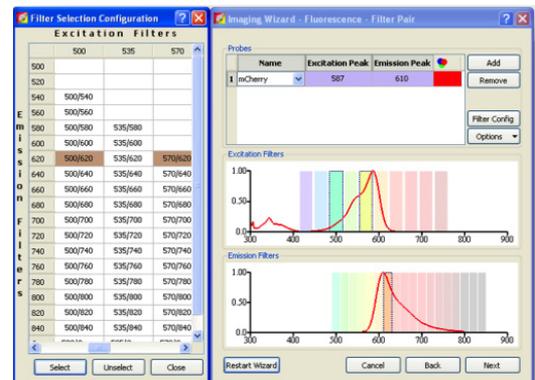
The selection criteria above assume that you are using the IVIS Spectrum, Lumina II, XR, or Kinetic with the numbered filters. If you have the IVIS Lumina, Lumina II, 50, 100 or 200 with the standard filter wheel, simply select the labeled background (BKG) filter that corresponds to the specific filter which you selected. For example, specific filter pair Ex. DsRed/Em. DsRed – autofluorescent background filters Ex. DsRed BKG/Em. DsRed.

Acquisition and Analysis

1. After selecting filter pairs, select **Acquire Sequence**.

2. Change units to Radiant Efficiency and then the resulting sequence can be utilized for Image Math analysis.

3. Draw an ROI on the animal that includes signal which is indicative of background in the image. (**Note:** You only need one ROI on either the background or specific image.)

4. Under **Tools** select **Image Math for** …(your sequence).

5. The **Image Math Window** will open.
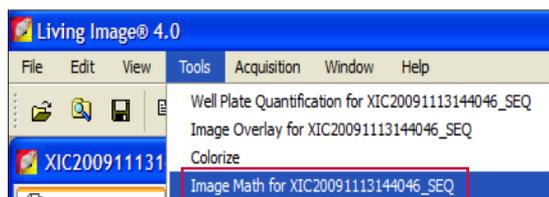
6. Select the image in **A** which corresponds to the specific filter pair.

7. Select the image in **B** that corresponds to autofluorescence.
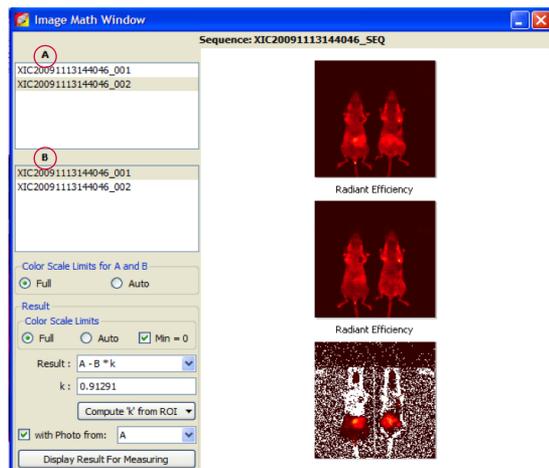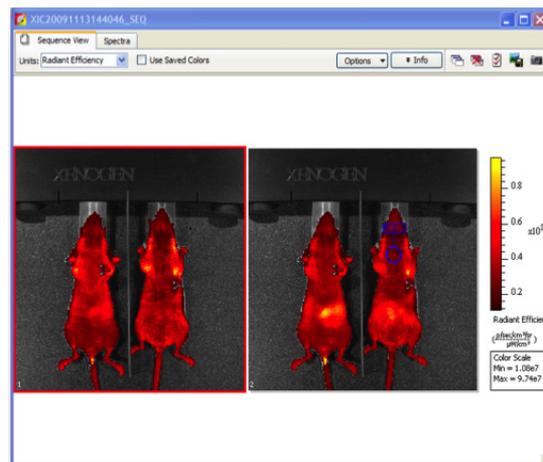
8. We will utilize the ROI that you drew in step 3 to determine the k value. K values tell us how the autofluorescence has changed as a result of excitation at different wavelengths. We determine a ratio and multiply the background image by that ratio. Therefore it is important that you select **Compute 'k' from ROI** and select the ROI that you have drawn.

9. Click **Display Result for Measuring** and the image will appear.

10. At this point the background subtracted image can be used for quantitative analysis of your source via ROI measurements.

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