Subtracting background ROI from a sequence.

This tech note discusses why we recommend subtracting a background region of interest (ROI) from the measurement ROIs when performing fluorescence quantitative analysis.

Fluorescence imaging (FLI) can result in higher background signal compared to bioluminescence imaging (BLI) due to autofluorescent sources, non-specific localization and clearance of injected probe, and leakage of excitation light through the emission filter. For these reasons, we recommend subtracting a background region of interest (ROI) from the measurement ROIs when performing FLI quantitative analysis.

1. Load the images to be examined as a group (see "Load As Group" Technical Note).

2. If epi-illumination was used, remove the instrument background using the Adaptive FL Background Subtraction tool (see Adaptive Fluorescence Background Subtraction Technical Note).

3. Open the specific image to be used for drawing your background ROI. Select **Average Bkg ROI** from the **Type** dropdown menu under **ROI Tools** (Figure 1).
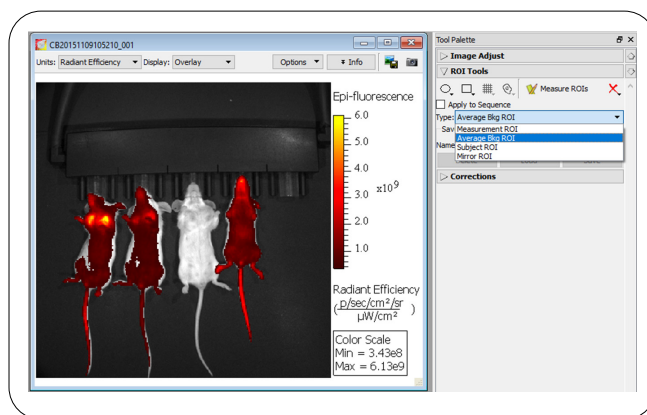

Figure 1. Average Bkg ROI can be selected from the ROI tools tab in the tool palette.

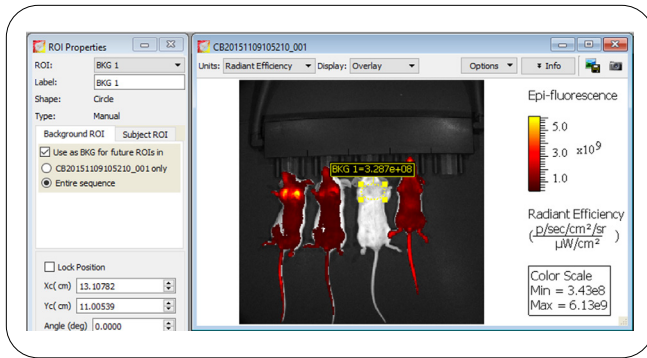4. Draw the background ROI in an area of the animal representative of average background levels.

Figure 2. ROI Properties window. here, you can choose to subtract the selected background from subsequent measurement ROIs.

5. Double click on the **BKG 1** ROI and the **ROI Properties** window will open (Figure 2).

6. Check **Use as BKG for future ROIs** in, then select **Entire Sequence**. Every subsequent measurement ROI drawn on images in that sequence will automatically subtract the average background as calculated from the **BKG 1** ROI.

7. Switch back to **Measurement ROI** in the **Type** dropdown menu in **ROI Tools** and draw the measurement ROIs. The user will notice that each measurement ROI is linked by the presence of **(BKG 1)** in the measurement window and in the **ROI Measurements** table which denotes the specific background ROI has been subtracted correctly (Figure 3).
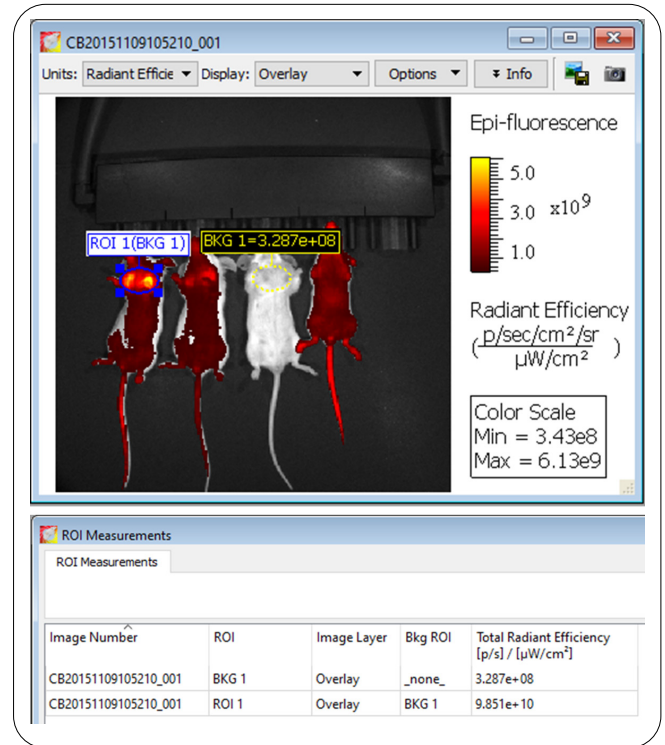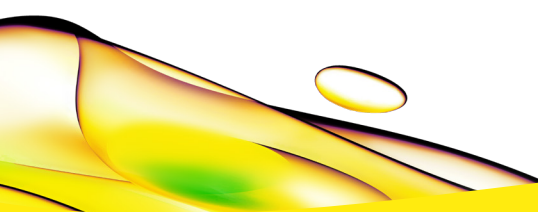

Figure 3. The label in the image and the ROI measurements table reflect the background ROI is being subtracted from the measurement ROI.