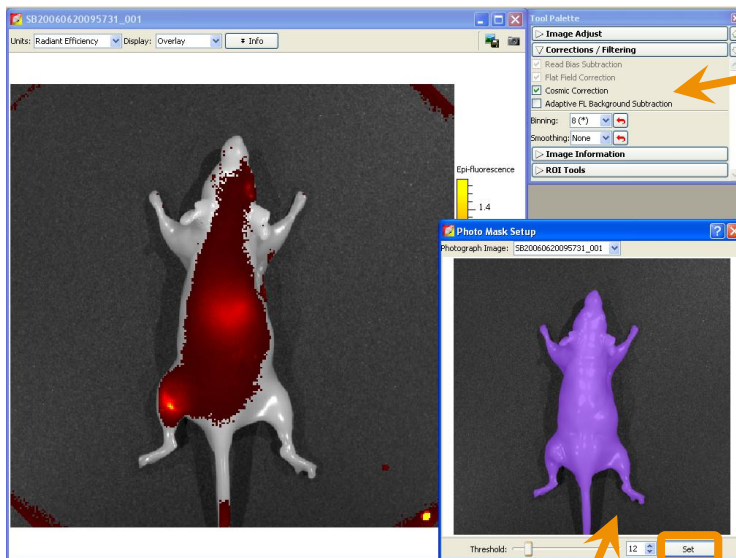
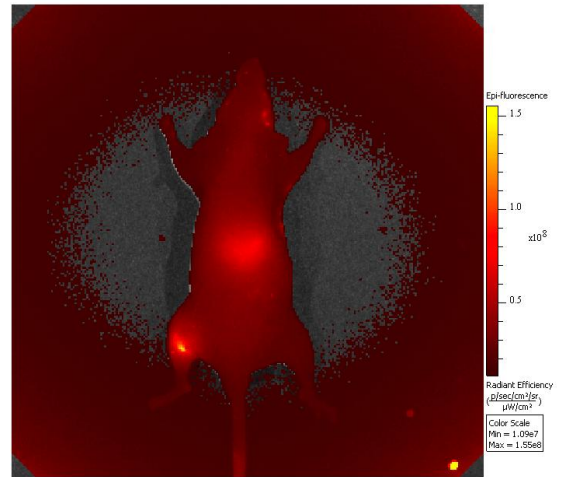




Adaptive Fluorescence Background Subtraction

Instrument background occurs when excitation light leaks through the emission filter. This occurs more frequently when the excitation and emission filters are narrowly separated. The ring you see is a result of non specific light reflecting off of the stage at an incident angle and passing through the filter causing what appears as leakage around the edges.

To combat this problem we have included the adaptive fluorescence background subtraction tool.



You will find this under the **Corrections/Filtering** section of the **Tool Palette** after you have acquired a fluorescence image or sequence of images. After checking this tab, you will be prompted to threshold your image. Threshold works by using the color contrast between your subject and the stage. Use the purple mask in

conjunction with the threshold slider to specifically mask the subject leaving the stage dark. Press **Set**. The background area will be subtracted automatically from the resulting image or sequences of images.

Additionally, a right-click -> Crop Area in the window will allow you to draw a square shape around the mouse to limit the threshold to areas within the defined region. This can be helpful for subjects with little contrast against the black stage (i.e., BL-6 mice). When using the crop feature, be certain to omit as much background as possible by cropping close to the subject.

