

# 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 (Figure 1).

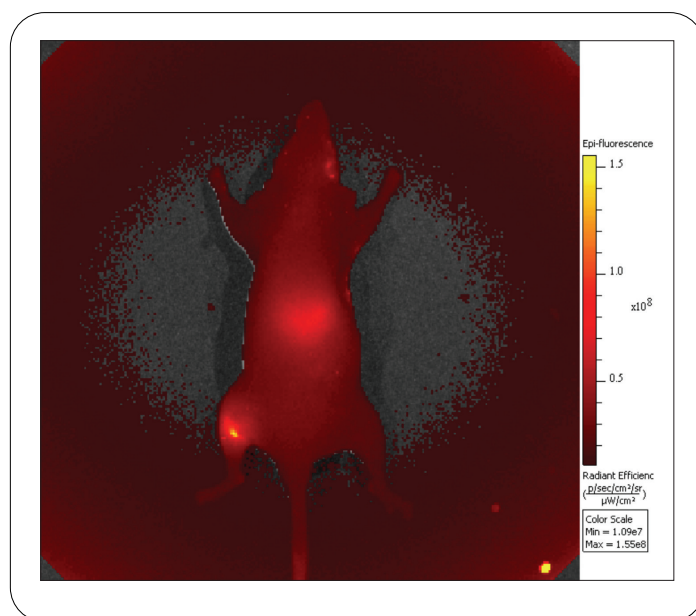
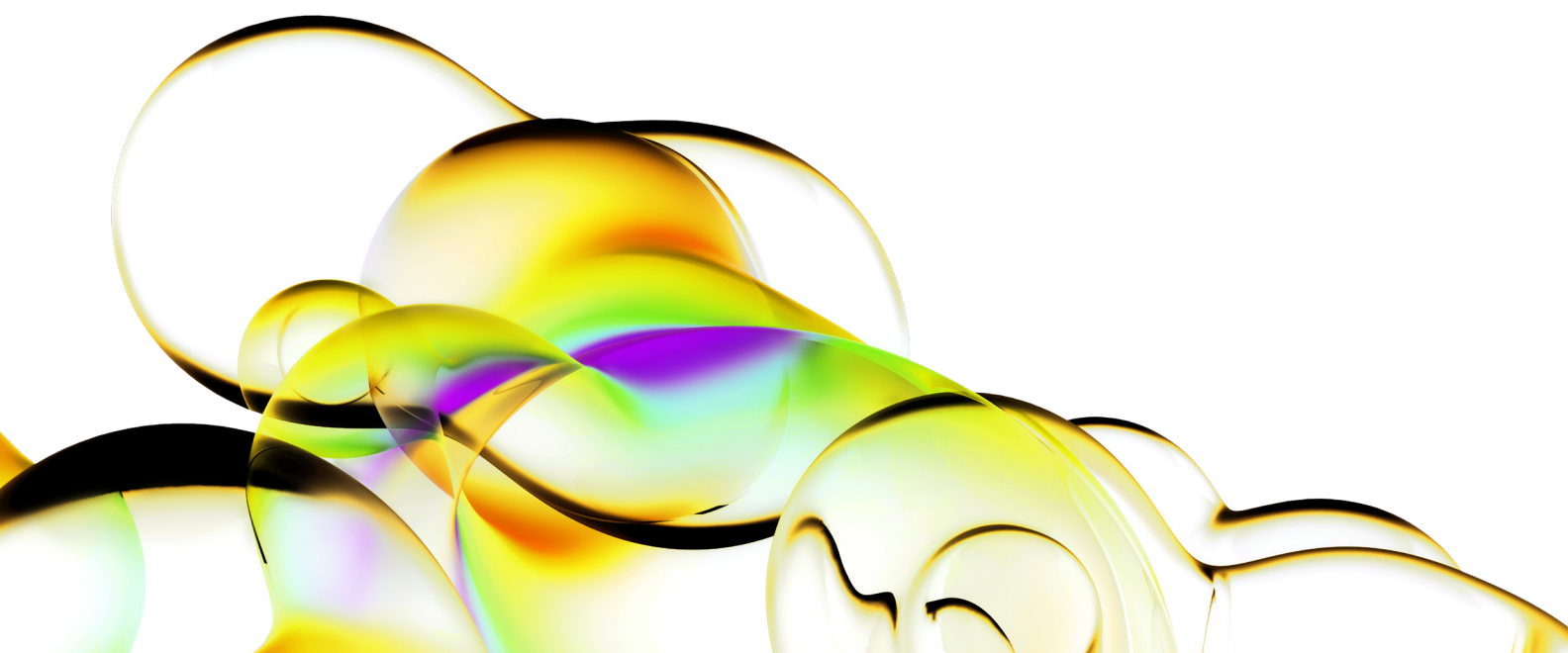


Figure 1. Epi-illumination fluorescence image showing instrument background on the stage.



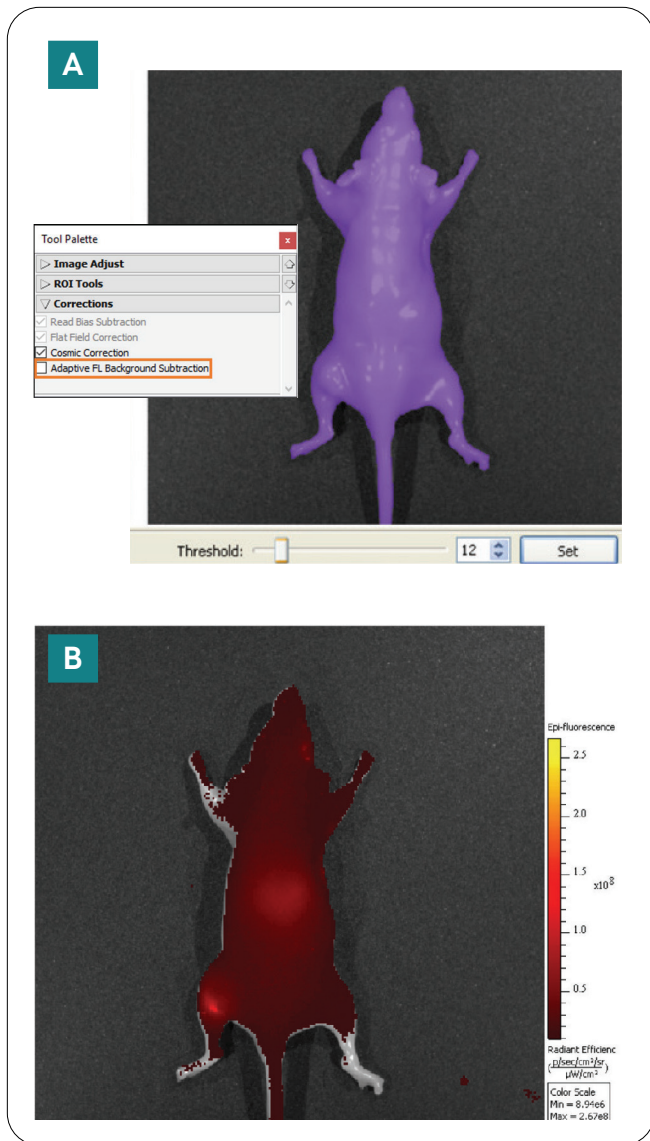


Figure 2. Adaptive FL Background Subtraction tool. A) Access this tool by going to Tool Palette > Corrections. Create a mask over the subject using the threshold slider bar. B) The resulting image has eliminated the instrument background.

## Removing instrument background

To combat this problem, we have included the Adaptive Fluorescence Background Subtraction tool. You will find this under the **Corrections** 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 (Figure 2). You may need to double-click and open single image to access this tool.

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) and for ex vivo organ imaging. When using the crop feature, be certain to omit as much background as possible by cropping close to the subject.

## Negative ROIs

Sometimes you will note negative ROIs after the Adaptive FL Background Subtraction tool has been applied. This is because the background pixels (not highlighted in purple) will be averaged and subtracted from the entire image. If the intensity in part of your subject is lower than the background, you will see a negative ROI value. To avoid this, be sure that your subject is on a black matte surface with very low background signal. We recommend using the black Lexan plastic sheet with the shiny side facing down.