

How to determine saturation in your *in vivo* optical imaging studies.



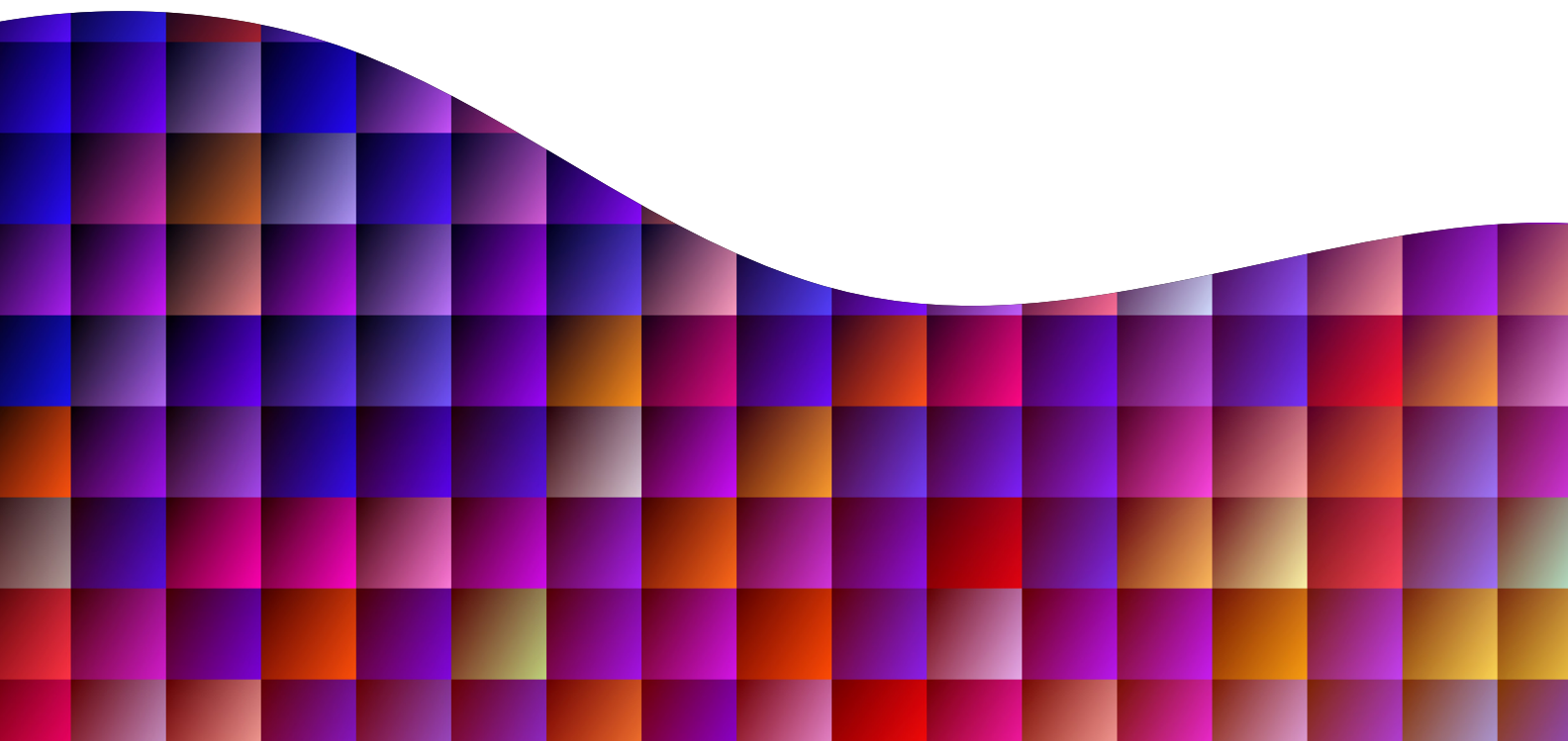
To understand saturation, imagine each pixel as a water glass and a photon as a drop of water. We can measure drops of water falling into each glass until it is full and can no longer contain any additional water. When pixels are full and photons can no longer “fit” into the pixels, this is termed saturation. Any one pixel can only collect 65,535 counts before it saturates.

This is why your images should always stay within the range of 600 to 60,000 counts per pixel; the minimum being high enough to be statistically quantifiable over background while the maximum is well below the saturation point.

You will receive three warnings when you saturate an image. The first will be a warning window which you will have to close by clicking “OK” (Figure 1).

The second will be a white stripe across the top of the image which will indicate that the image contains saturated pixels. You cannot remove this. The stripe is not visible when images are loaded as a sequence, but will appear when opened into their own image window.

The third warning will launch the Activity Window, a panel along the bottom of the screen that describes all events happening during an acquisition. A yellow warning triangle with a Saturated Pixels message will be displayed.



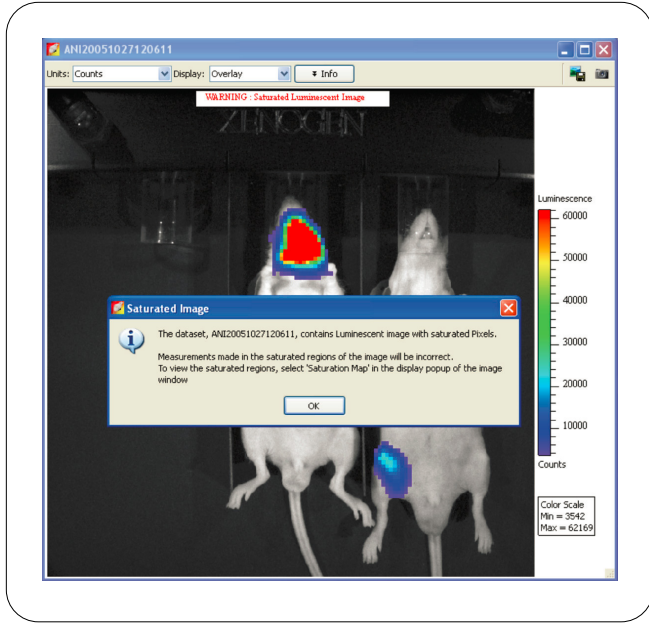


Figure 1. Saturation warnings are shown as a pop-up window and as a banner across the top of the image.

In order to determine exactly which pixels are saturated, select the **Display** dropdown menu and select **Saturation Map** (Figure 2). This will show you in red the pixels which have saturated in an image.

If you have two sources in one image and one source does not contain saturated pixels, you can still measure the unsaturated source. In the example below, signal from the mouse on the right could still be quantified because it does not contain saturated pixels.

However, sources that contain saturated pixels are not quantifiable. You must readjust your sensitivity settings and acquire another image to eliminate the areas of saturation before you can measure.

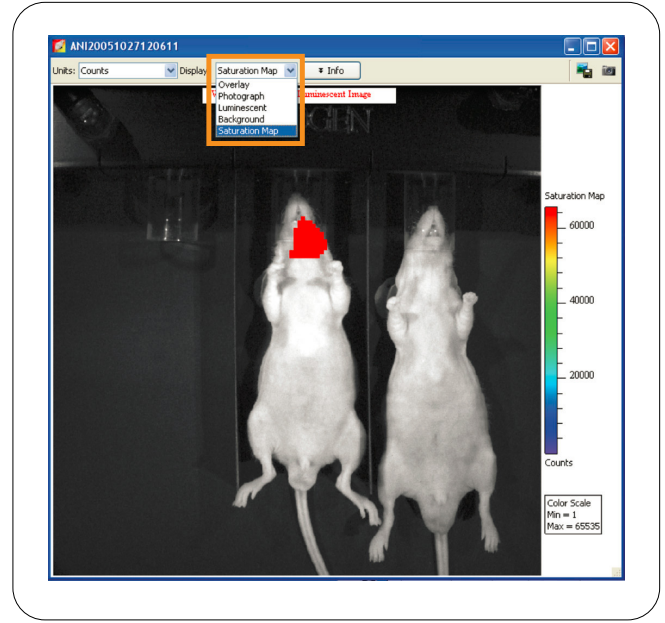


Figure 2. Display of saturated pixels. For images that contain saturated pixels, a saturation map can be opened from the Display menu. It will show only the pixels that have been saturated.

