

Quantitating western data etc using alpha innotech set up.

For image quantitation it is important that the image is not overexposed, which will lead to pixel saturation. The alpha innotech camera is 16 bit, so the the max pixel value is $2^{16} = 65536$, ie 65536 grey images can be collected. If your image has pixels with the values approaching this maximum then you need a shorter exposure.

How do I know if my image is saturated?

When acquiring images using the 'focus' or 'expose preview' buttons you can click on 'show saturation' to show saturated pixels in red. Unfortunately this is no use for faint chemiluminescent images that need a long exposure.

Although there is a 'show saturation' option for analyzing saved images this does not work properly. For some reason, even when an image is massively overexposed, the max pixel value in saved images about 64000, and not the maximum value that causes red highlighting. So the image shows as non saturated when it clearly is overexposed. There does not seem to be a way to modify the software to correct this problem.

Suggested work around

If you move the mouse over the darkest (assuming you have inverted you image) bands look at the pixel values - if they are around 64000 you need a shorter exposure. Alternatively you can open the image with image j and looks at the distribution of pixel values using Analyze>Histogram (if necessary using the ROI tool to look at specific bands) - assume that any pixels around 64000 are saturated.

QuantitatingWesterns