# Calculating Camera Bias

Background

When visualizing a fluorescence image, we would expect the intensity value of a pixel to correspond only to the intensity of fluorescence in the sample. However, every camera has a background offset that gives every pixel a non-zero value even in the absence of light. We call this the camera bias.

The bias value is necessary to counteract fluctuating read noise values which might otherwise go below zero. The value of the bias therefore should be above zero and equal across all pixels. The bias value doesn't contain any detected signal so it's important to subtract it from an image before attempting to calculate the signal in photoelectrons.

Method

To calculate the camera bias:

1. Set your camera to a zero millisecond exposure time
2. Prevent any light entering the camera by closing the camera aperture or attaching a lens cap
3. Take 100 frames with these settings
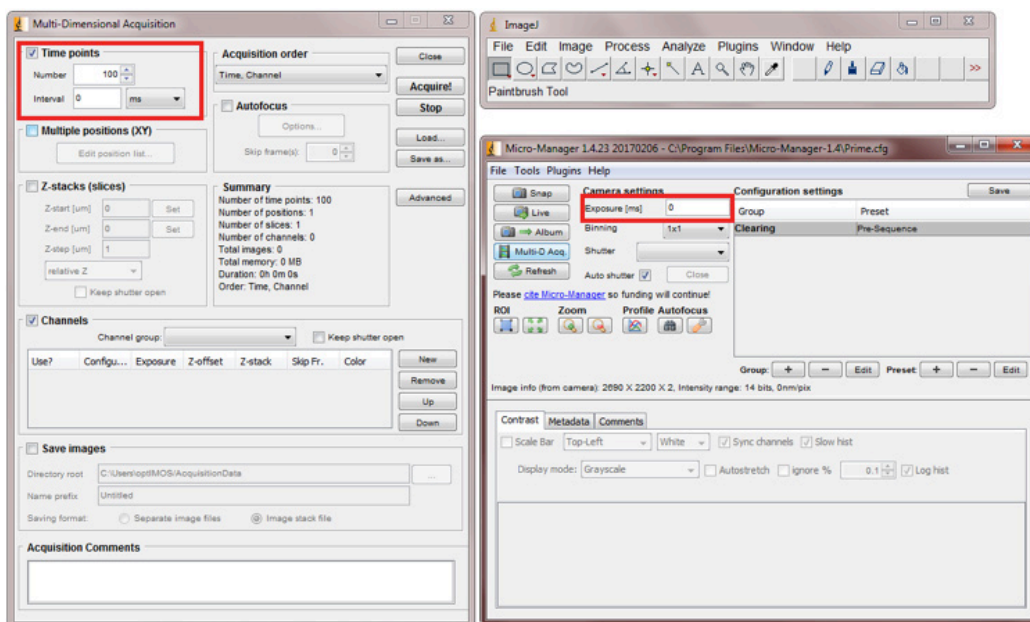
4. Calculate the mean of every frame by selecting Stacks from the Image menu and then clicking on Plot Z-axis profile. This should give you the mean values of every frame in the Results window.
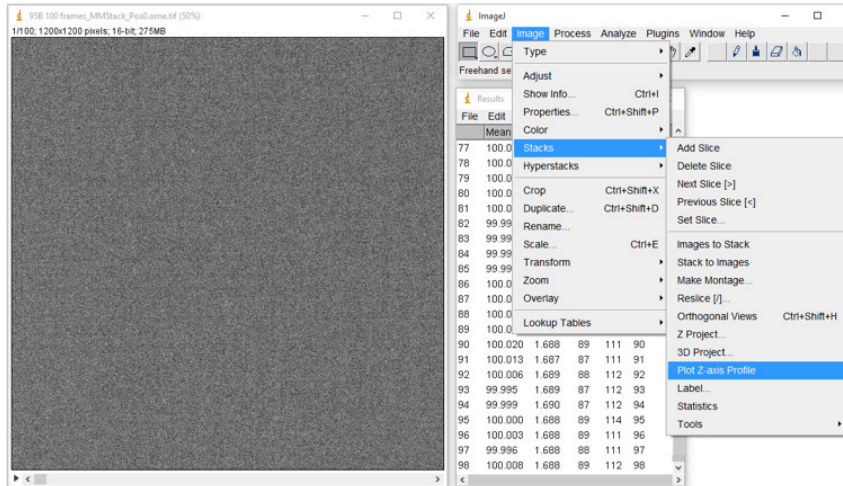
5. Calculate the mean of the 100 frame means by selecting Summarize in the Results menu
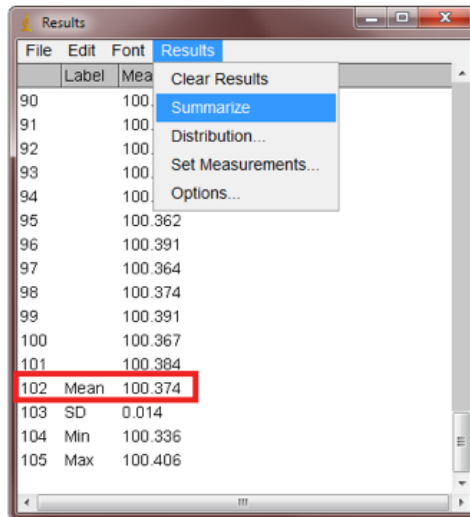
The bias is the mean of a single frame so by plotting the mean values of all 100 frames we calculate a more accurate bias.

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