Lab 3. Acquiring Digital Images – Reducing Noise

Objective: You will learn about various sources of noise and reduce it where possible. This will be done by quantifying signal averages and standard deviations.

Sources of noise include, thermal noise, **read-out noise**, and **shot noise**. They each manifest themselves in different ways, and can be eliminated in different ways.

Methods to reduce noise: **cooling**, **integration**, pixel **binning**, **averaging** of frames, but each of these can have drawbacks in terms of spatial and temporal resolution that might be needed for a particular experiment. The proper noise reduction methods are essential to maximize image contrast, resolution and quantify data. Make sure you understand the basics at this point; they should be applied to most digital imaging.

Samples: Kim wipe, lens paper, business card, or thin, weakly fluorescent material.

Procedure: The following procedures are required for this exercise:
A) acquire digital fluorescent images, **B)** measure and **compare the signals** (average, standard deviation, S/N, min & max values), **C)** change the exposure conditions and repeat the measurements, **D)** average signals from successive images, and **E)** bin signals from adjacent pixels.

Use the "Measure Region Statistics" tool for acquiring data values. Turn auto-scale off and set the display scale from 0-4095.

1. Dark noise – **close off light to the camera (no sample)**, integrate for different periods of time, e.g., 0.5s, 2s, 8s, etc. "Measure" -> "region statistics" for each different exposure and compare values between different exposures.

a. How will this "dark signal" influence your sample signal?
b. What is contributing to this "dark signal" background?
c. How can you remove this "background"?
d. Subtract the background and repeat the measurements. How does this make a difference?

Remember to subtract the background each time the exposure condition is changed for all subsequent quantitative measurements.

Put the sample on the stage, focus with a low mag lens, blue excitation with green emission filter.

2. Shot Noise – **While using fluorescence excitation**, acquire an image with different exposure times (use 4-fold differences). Measure the mean and standard deviation of the signal. Shot noise varies as the square root of the signal, so if you change the exposure by 4-fold the mean signal should increase 4-fold, but the standard deviation will increase 2-fold. Look at the image histograms and "region statistics" for each different exposure.

a. Why might the noise be increasing faster than the square root of the signal?
b. What can be done to minimize the noise?
c. What is the minimum possible noise?

3. Averaging – Acquire images with 2 frames averaged and 10 frames averaged.

a. What are the advantages and disadvantages of frame averaging?
b. How would averaging effect a time series depicting a biological event?

4. Pixel Binning – Acquire an image with 1 pixel/bin and 4X pixels/bin; ***reduce exposure 4-fold. Why?***

a. What are the advantages and disadvantages of pixel binning?
b. How would binning effect resolution?