

## Lab 4. Acquiring Digital Images – Dynamic Range, Threshold

**Objective:** Practice setting the [dynamic range](#) and [threshold](#).

**Samples:** Slide provided by instructor

**Procedure:** This lab assumes skills learned in labs 2 and 3. You will learn how to use the imaging software to optimize the dynamic range and set threshold values for analysis.

### Part I: Dynamic Range and Thresholding

Use the [histogram tool](#) and the [measure region statistics](#) tool for acquiring data values. Turn [auto-scaling](#) off and set the [display scale](#) to maximum, e.g. 0-4095.

1. Acquire an initial image of sample 1
2. Examine the dynamic range by using the image histogram

The histogram measures intensity frequencies; the y-axis depicts the number of pixels for each corresponding intensity value on the x-axis.

3. Is the image histogram centered in the dynamic range? Adjust the following parameters to center the histogram peak within the provided range.
  - a. [Adjust the exposure](#)
  - b. [Adjust Pixel binning](#)
  - c. [Adjust Frame Averaging](#)
  - d. [What happens to the histogram after subtracting a background?](#)
4. Adjust the [gain](#) (this adjustment may not be available on all cameras) and examine the image histogram. The gain amplifies the electrical signal produced by the light.
5. Adjust the [offset](#) and [examine the image histogram](#); there may be an offset knob or you can use the [data-to-display scaling function](#). The offset sets the “zero” level; it is the signal level that is defined as the minimum or baseline value (it’s not necessarily zero signal), e.g., it is the lowest data value that you define to be “black” and could include noise or reject real signal.
6. Apply a [green pseudo-color look up table \(LUT\)](#). An LUT assigns various intensities of **R**ed, **G**reen, **B**lue **monitor signal** to digital image values. The green LUT assigns a range of **G** intensities (R,B are turned off) in proportion to the digital image values (1-255 for an 8-bit image), e.g., small values are displayed as low-level green and high values as intense green. [Discriminate between display intensities and image values.](#)
7. Set an “Inclusive [Threshold](#) to select bright objects. A [threshold selectively targets specific intensity ranges](#). Inclusive threshold selects only image pixels that fall within the threshold range. An exclusive threshold selects pixels that fall outside the threshold range. Thresholds are essential for most image-processing (IP) and required for proper quantitative analysis.
8. Select regions-of-interest (ROI's). This requires use of region selection tools.
9. Calculate the average intensity for each region. The average intensity is the sum of all pixels (selected by the threshold) divided by the number of pixels within the ROI.

[Does Adjusting Brightness and Contrast alter the image histogram? Why or why not?](#)  
[What is integrated intensity, how is it different than average intensity?](#)

10. Circle 3 individual ROI's with different intensities.
11. Calculate the average pixel intensity of each region and compare.

[Can you quantify the relative intensities of the objects in the specimen?](#)