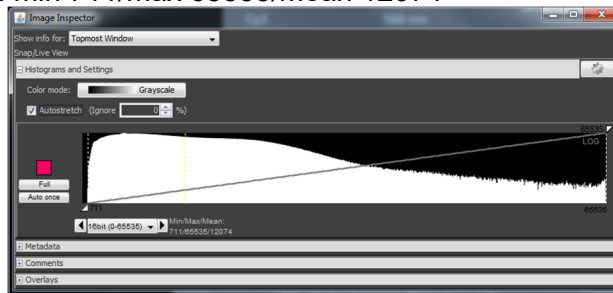


**Biophysics 210: Biological Light Microscopy**  
**Discussion Section 8: Digital Images and Image Analysis**  
**Tuesdays 1-2:30pm**  
**Location: Genentech Hall Rm N114**

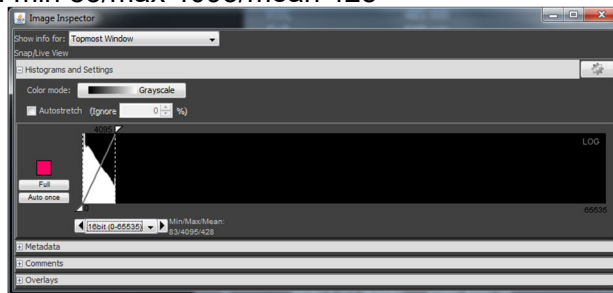
1. You have the following list of file format choices in your acquisition software when you go to save your image. From the list identify the best choices, acceptable formats, and formats to avoid and identify why.
  - Proprietary file format from software (i.e. nd2, czi, lif)
  - OME-TIFF
  - TIFF
  - JPG
  - BMP
  - PNG

2. Below are example histograms from images. Which of the histograms indicate a problem? Why?

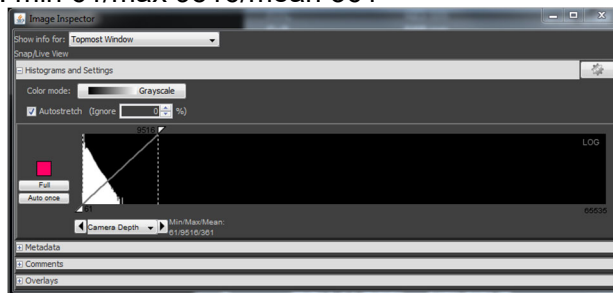
a. Histogram A: min 711/max 65535/mean 12074



b. Histogram B: min 83/max 4095/mean 428



c. Histogram C: min 61/max 9516/mean 361



3. Name 3 sources of variability that arise in imaging data. What steps should be followed to take variability into account and/or limit it?

4. For the following list of z-projection methods explain how your imaging data is transformed. List the pros or cons for each technique and when you would use it.
  - a. Maximum intensity projection
  - b. Average intensity projection
  - c. Median intensity projection
  - d. Sum Slices
  
5. Thresholding is a common technique that is used for data analysis.
  - a. What is the purpose of thresholding in image analysis?
  - b. What is the danger in manually setting your global threshold values? How do you avoid biasing your thresholding?
  - c. What is the purpose of doing erosion/dilation after thresholding?
  
6. Many times we are using filtering techniques as part of a data analysis pipeline to help enhance features. For the following example images we have utilized 3 different filters; Gaussian Blur, Median, and Unsharp Mask. The filters are the same for both sets of images. Match the filter with the resulting image below and explain the purpose of the filter.

