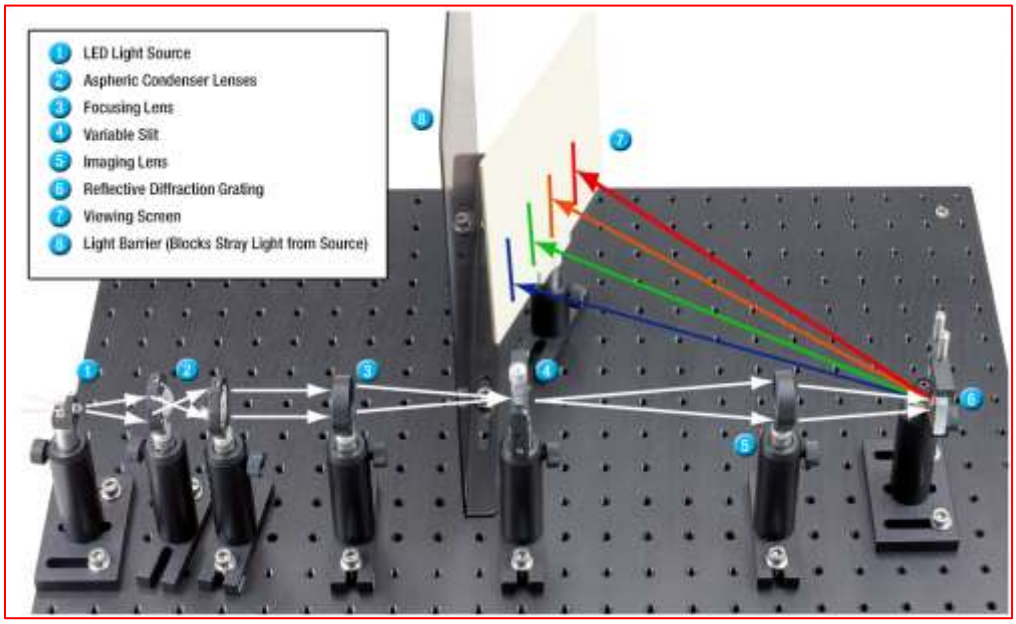
C. Post-Lab Solutions

Show all work. If you use other programs (e.g. MATLAB, Excel, ImageJ, etc.) to perform calculations, explain the logic behind your steps.

1. What is the difference between a spectrometer, and a spectrophotometer? When would you want to use one or the other? (10 pts)

   A spectrometer is a tool to separate the components of light and measures wavelength vs intensity. A spectrophotometer is a system which measures samples of interest, and records wavelength vs intensity after light has passed through the sample. Use a spectrometer to characterize light sources; use a spectrophotometer to perform absorption spectroscopy.

2. Starting from the optical fiber, sketch a rough ray diagram of the spectrometer and illustrate what happens to the light rays as it interacts with the optical components. Be sure to include the effects of **refraction** and **diffraction**. (10 pts)



3. If you increase the lines/mm density of your grating, will this increase, decrease, or not change the resolution of your spectrometer? Explain why. (10 pts)

   The higher the groove density of the grating, the higher the spectral resolution will be. The maxima in the diffraction pattern are found for angles satisfying the relationship:

$$Dsin\theta = n\lambda$$

As "D" is the spacing between grooves, when D is small (higher groove density of the grating), then the angle between each wavelength will increase. The result is less overlap area between different wavelengths in the spectra, thus increasing the spectral resolution of spectrometer.

4. The color webcam we used to capture spectra of light sources contains a Bayer mask. How does that affect the performance of the spectrometer? (10 pts)

   For the Bayer mask over the CCD, each square of four pixels has one filtered red, one blue and two green. The Bayer mask absorbs a high proportion of the light incident on each CCD pixel, which results in a lower quantum efficiency (and therefore lower light sensitivity for a given aperture size).

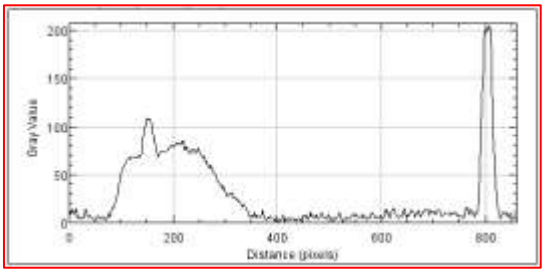
5. Plot the pixel vs intensity curves from the 0^{th} order to the 1^{st} order diffractions for all light sources measured using the spectrometer. Do you think this "white" LED was constructed by combining RGB sources, or is it a blue LED with a yellow phosphor coating? Explain how you know using your data as evidence. (10 pts)

   ImageJ is a freely available program developed by the National Institutes of Health which you may find useful for this question (and in the future). See the short tutorial below:

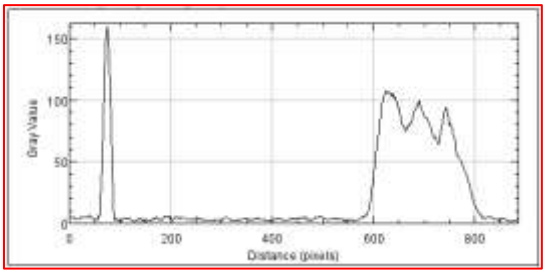
   - Download and launch ImageJ
   - Open the image taken using File > Open
   - Select the line tool from the toolbar

   - Draw a line over the area of the image that you would like to see the intensities of
   - Go to Analyze > Plot Profile:

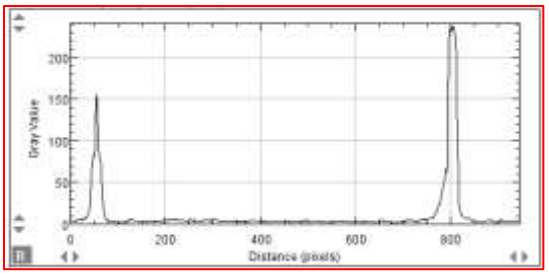
Broadband lamp:



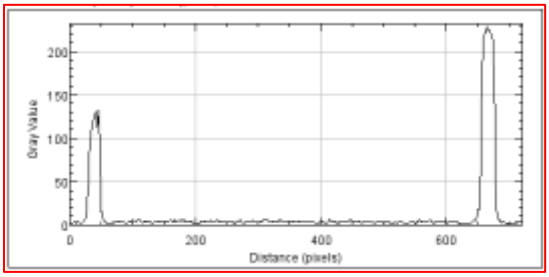
White LED:



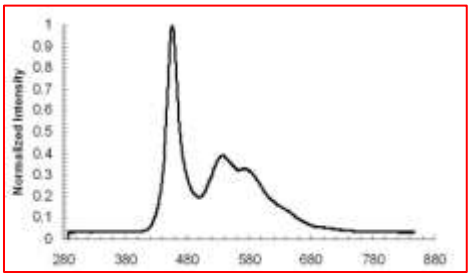
Red laser:



Green laser:



From the intensity profile of the white LED, three distinct peaks can be seen. This LED is most likely a combination of RGB leds in order to produce “apparent” white. A blue LED would have a profile that looks like this:



6. Given that the center wavelength for the red laser was 638 nm, and 532 nm for the green laser, calculate the resolution of the spectrometer. Show your calculations, and round to the nearest nm/pixel. (10 pts)

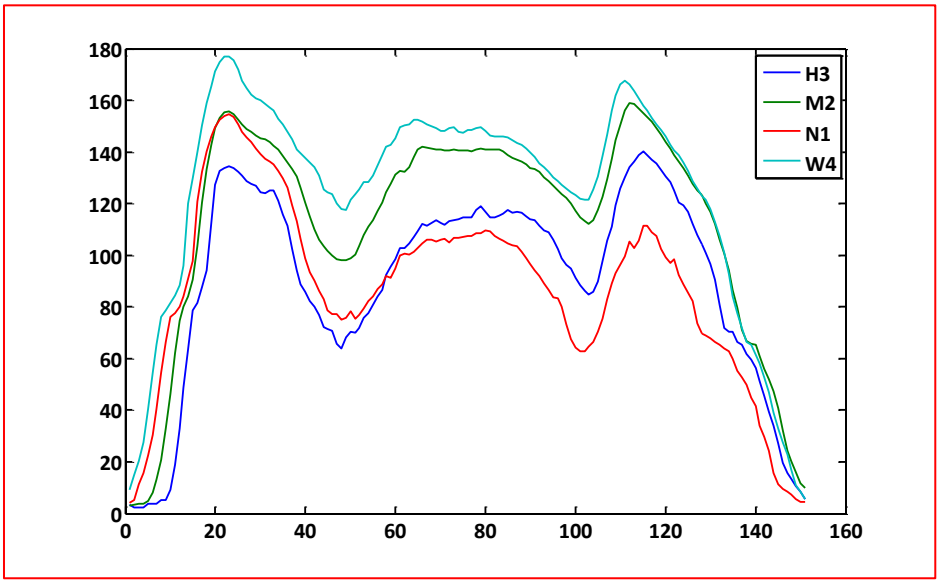
   - For the number of pixels from the 0th order to the 1st order diffraction peaks for each laser.
   - From the images I took:
     - There are 623 pixels between the 0th and 1st order peaks for the **green laser**
     - There are 748 pixels between the 0th and 1st order peaks for the **red laser**
     - Calculation: $\frac{638nm-532nm}{748px-623px} = \sim 1\ nm/pixel$

7. In this lab, four different cuvettes containing various samples were measured. Absorbance is a dimensionless number related to the attenuation of radiant power through a material, and can be defined as: (20pts)

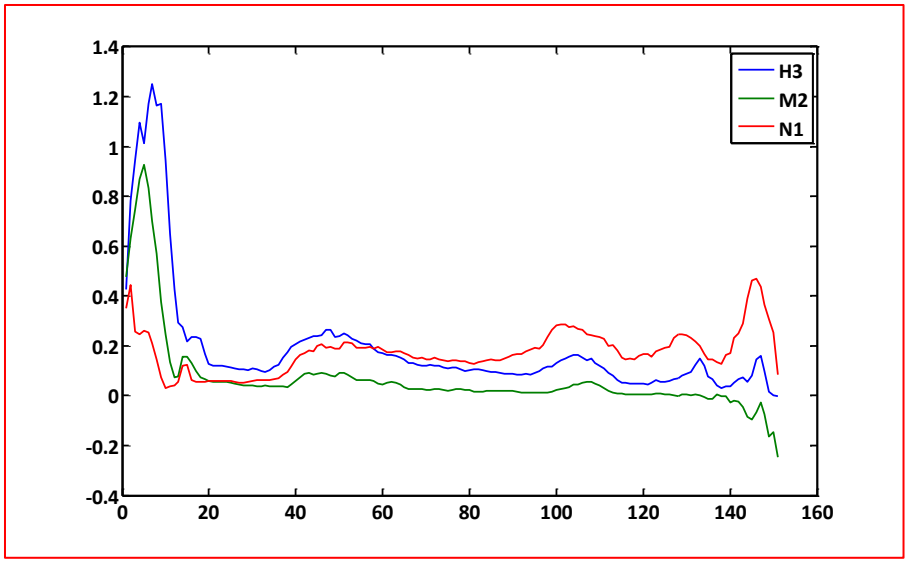
$$A = -log_{10}\left(\frac{P}{P_0}\right)$$

A is absorbance, $\mathbf{P_0}$ is the incident intensity, and **P** is the transmitted intensity

I. Plot the raw intensities for all the samples on one plot. Include a legend.



II. Plot the absorbance for all samples relative to W4 (water). Include a legend.



8. The total absorbance of the sample is related to the sum of absorbance from unique species in the sample:

$$A_{total} = \sum_{i=1}^{n} C_i A_i$$

M3 contained a mixture of two species: a water-soluble dye known as "Nigrosin", and bovine hemoglobin. If N1 contained 20 mg/L of Nigrosin, and H3 contained 800 mg/L of bovine hemoglobin, solve for the amount of Nigrosin and hemoglobin in M3. Justify the method you use to solve this problem. (20 pts)

The method used was non-negative, linear least squares fitting ("lsqnonneg" in MATLAB, or any equivalent method in any program is acceptable). This method can be used because the total absorbance is simply a linear combination of the absorbance of each species present.

Cuvette	Nigrosin (mg/L)	Hemoglobin (mg/L)
N1	20	0
M2	8	410
H3	0	800
W4	0	0