Homework 4

1. A form of the diffraction grating equation is written below:

$$\boldsymbol{Dsin\theta = n\lambda}$$

a) What are some advantages and disadvantages of a grating-based spectrometer compared to a prism-based spectrometer? Be sure to include comments on throughput and spectral resolution. (10 pts)

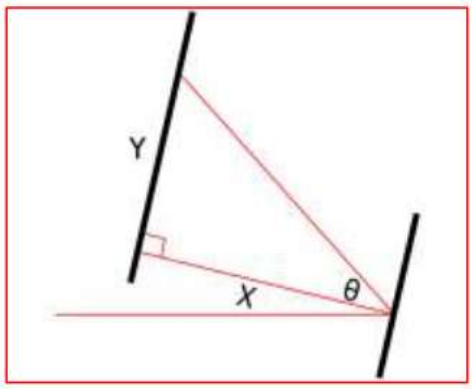
A prism has higher throughput, as light is not lost in the form of higher order diffractions. However, a grating can produce a larger separation distance between wavelengths, allowing you to make higher resolution spectrometers. Furthermore, the wavelength vs separation distance of a prism is non-linear.

b) What is the main advantage of a dual-beam spectrometer versus a single-beam spectrometer? What type of application would a dual-beam spectrometer be ideal for? (10 pts)

Dual-beam spectrometers can acquire the reference signal rapidly without having to replace the sample cuvette. This makes it more stable and advantageous for performing highly precise and dynamic measurements.

c) You construct a benchtop spectrometer using a grating (600 lines/mm), place a viewing screen 50 mm away from the grating, and shine a laser through the system. If you measure the spectral peak of the 2nd order diffraction to be 55.4 mm away relative to the 0th order diffraction peak, and the 1st order diffraction to be 35.4 mm away relative to the 2nd order diffraction peak, what wavelength does this spectral peak belong to? Assume the viewing screen and grating surface is parallel.

i. Sketch a simple diagram of the setup. (5 pts)



ii. Solve for the wavelength of the laser. (15 pts)

We want to use the grating equation, but need to solve for a few variables first. We'll choose to use the 1st order diffraction to solve for the wavelength of this laser. First solve for the distance of the 1st order diffraction peak from the 0th order: $y = 55.4 - 35.4$

Using x = 50 mm: $\tan^{-1}\frac{20}{50} = 21.8°$

Solving for variable: $D = \frac{1\ mm}{600\ line}$

Now apply the grating equation using the first order diffraction (n=1):

$\frac{1\ mm}{600\ line} \times \sin(21.8) = n\lambda = 620\ nm$

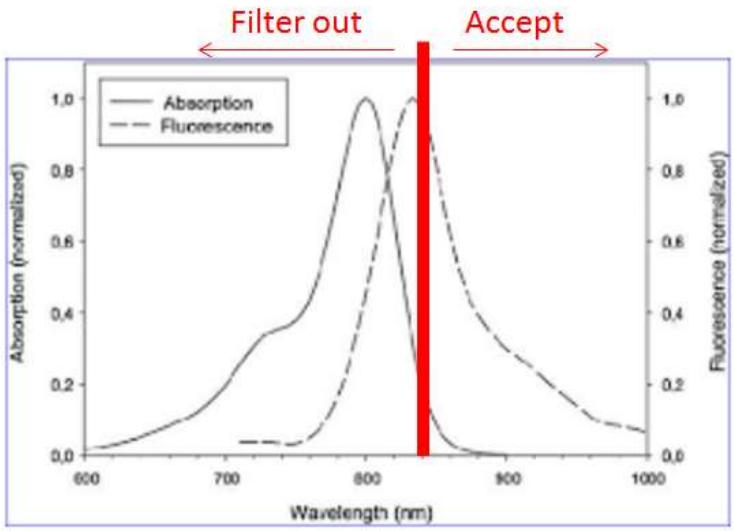
2. You are measuring the autofluorescence signal on several tissue sites which may or may not contain cancerous masses.

a) Explain why you lose autofluorescence signal in cancerous tissue versus precancerous tissue. Give four reasons, and identify the primary contributor. (15 pts)

Tissue restructuring, angiogenesis, nuclear morphology, structural integrity. Structural integrity will have the biggest impact, as it degrades your primary source of signal (collagen).

b) If you use Indocyanine Green dye in your sample, what kind of filter, and what wavelength range should the filter have in order to maximize your signal, while minimizing your background. (10 pts)

From the slides, choose a dichroic, longpass, or bandpass ~850nm in order to block out the excitation wavelength, and still capture the fluorescence signal. Can also use a notch filter to mitigate the excitation light.



3. You are performing an experiment where you insert fluorescently tagged cells several mm below the surface of an ex-vivo sample and using a laser as your excitation source.

a) You perform a measurement on the sample and calculate the total attenuation coefficient (μ_T) to be 1.21 mm^{-1}. Calculate the absorption and reduced scattering coefficients of your

sample if the absorption length is 100 mm. You may assume other light-tissue interactions besides absorption and scattering are negligible. Round your answer to the nearest mm^{-1}. (10 pts)

Use $\mu_a = \frac{1}{l_{abs}} = 0.01\ mm^{-1}$

Since $\mu_T = \mu_a + \mu_s'$, then $\mu_s' = \mu_T - \mu_a = 1.2\ mm^{-1}$

b) Explain what the absorption and scattering length means. If a material has a very long scattering length at all wavelengths, does this mean it is relatively turbid, or relatively transparent? (10 pts)

Absorption length is the average distance between absorption events, and scattering length is the average distance between scattering events. A sample with a long scattering length means scattering occurs rarely. This means light pretty much travels straight through it, thus is relatively transparent.

c) You perform chemical analysis on your sample and discover that your sample is composed of water, lipid, almost no vasculature, and only trace amounts (negligible) of hemoglobin. In order to obtain data quickly, you want to use a 700 nm laser that you already have in the lab.

i. How much absorption would you expect from your sample at 700 nm, and why? (5 pts)

Looking at the absorption spectra of water, lipid, and blood, the main absorber is blood at 700 nm. Since there is a negligible amount of blood, and water and lipid absorb weakly at 700 nm, I would expect minimal absorption.

ii. Your coworker says since your sample has virtually no absorption at 700 nm, your measured signal should be very close to a pure fluorescence signal. Explain if you agree or disagree. (5 pts)

Disagree, scattering will still distort the signal.

iii. If you change your excitation light source to an 810 nm laser, would you expect the amount of distortion in your fluorescence signal to be more, less, or about the same? Explain why. (5 pts)

Although absorption is relatively constant, the amount of scattering goes down following a power law as wavelength increases. The probability of scattering at 810 nm is much less than at 700 nm, thus your signal will be less distorted.