1. A $2.5 \times 10^{-3}$ M (25 µM) solution of fluorescein is placed into a cuvette of 1 cm path length. The molar absorptivity of fluorescein is $9.3 \times 10^{4}$ M$^{-1}$ cm$^{-1}$ for light of wavelength 490 nm.

a) The cuvette is placed in a fluorimeter. 490 nm light is passed through the fluorescein sample from left to right. Calculate the fraction of the initial intensity of 490 nm light that exits from the right side of the cuvette.

\[
\frac{I}{I_0} = 10^{-ECL} = 10^{-6.82} = 2.33
\]

So only 0.5% of the 490 nm light exits from the cuvette.

b) Fluorescein, when excited at 490 nm, will emit fluorescence that peaks at 514 nm. Considering your answer to part a), what is the ratio of the brightness of fluorescein fluorescence on the left side of the cuvette to that on the right side?

4 pts. Because the fluorescence emission is directly proportional to the excitation (490 nm) excitation intensity, the ratio of fluorescence emission on the left to that on the right equals the ratio of excitation intensities. Therefore:

\[
\frac{F_L}{F_R} = \frac{I}{I_0} = \frac{1}{0.000047} = 214
\]

c) Considering your answer to part b), would doubling the concentration of fluorescein double the total fluorescence signal from the whole sample that is detected? Explain.

8 pts. No, it wouldn't. Doubling C will increase the value of the absorbance $A = \varepsilon CL$. That will decrease the amount of 490 nm light that makes it to the right hand side of the cuvette (by over 200-fold) so the fluorescence on the right will be less bright than it was for 25 µM fluorescein (twice the amount of dye, but 1/10 the excitation intensity). So the total signal will not double. (This is called the inner filter effect in fluorescence spectroscopy.)
2. The proton magnetic resonance spectrum of partially deuterated methyl ethyl ether (as shown) was measured in a 100 MHz spectrometer.

\[
\text{CHD}_2\text{CH}_2\text{OCHD} \quad \begin{array}{cccc}
   \text{a} & \text{b} & \text{c} \\
   \downarrow & \downarrow & \downarrow \\
   \text{I}_1 & \text{I}_2 & \text{I}_3 & \text{I}_4 & \text{I}_5 & \text{I}_6 & \delta (\text{ppm})
\end{array}
\]

a) Which line or lines (I₁ to I₆) can be assigned to proton a?

\[4\text{pts} \quad \text{I}_4, \text{I}_5 + \text{I}_6 \text{ because the two } b \text{ protons split the a line into a triplet and this proton is far from the e}^-\text{- withdrawing oxygen.}\]

b) Which line or lines (I₁ to I₆) can be assigned to proton c?

\[4\text{pts} \quad \text{I}_1 \text{ because these protons are most strongly affected by the e}^-\text{- withdrawing oxygen (so they have the largest chemical shift) and the oxygen reduces their spin-spin coupling to the } b \text{ protons.}\]

c) If the distance between lines I₂ and I₃ is 0.2 ppm, what is the distance between lines I₄ and I₅?

\[4\text{pts} \quad 0.2 \text{ ppm because both sets of splittings are due to spin-spin coupling between the same set of } b \text{ protons (a and b).}\]

d) For protons in this spectrometer that have a chemical shift of 0, their energy levels are split due to the magnetic field in the spectrometer. If the lower energy level is called \( E_0 \) and the upper one \( E_1 \), what is the ratio of probability of finding the proton in the lower level to that in the upper level (i.e. \( P_0/P_1 \)) when \( T = 300 \text{ K} \)?

\[4\text{pts} \quad \text{This is a 100 MHz spectrometer, so the difference in energy levels } E_1 - E_0 = h\nu = (4.026 \times 10^{-24} \text{ J sec})(100 \times 10^6 \text{ sec}) = 6.026 \times 10^{-22} \text{ J.}\]

\[ P_0 = \frac{g_0 e^{-E_0/kB}}{g_0 e^{-E_0/kB} + g_1 e^{-E_1/kB}} \quad \text{and} \quad P_1 = \frac{g_1 e^{-E_1/kB}}{g_0 e^{-E_0/kB} + g_1 e^{-E_1/kB}} \]

where \( g_0 = g_1 = 1 \) since there is only one site of each energy level.

\[ \frac{P_0}{P_1} = \frac{e^{-E_0/kB}}{e^{-E_1/kB}} = e^{(E_0 - E_1)/kB} = e^{(-6.026 \times 10^{-26} \text{ J})/(1.38 \times 10^{-23} \text{ J/K})(300 \text{ K})} = e^{1.0 \times 10^{-5}} = 1.000016 \]