Homework is due at the *beginning* of Lecture (8:15AM) on Thursday, December 17th, 2020.

Diffusion

1. A membrane protein has a translational diffusion coefficient of $5 \cdot 10^{-11}$ cm²/s and a rotational diffusion coefficient of 10^4 rad²s⁻¹
   a) How long does it take the protein to diffuse through the length of an E.*coli* cell (2 μm)?
   b) How many rotations does the protein undergo in this time?

FCS

2. Calculate the γ factor in FCS experiments for the following probe volumes:
   a) A cube with side a with intensity: $I = I_0$ for inside cube and on edges
                                                                     $I = 0$ for outside the cube
   b) A sphere with intensity: $I = I_0$ for $r \leq R$
                                                          $I = 0$ for $r > R$
   c) A 3-D Gaussian

3. Assuming a 3-D Gaussian probe volume with $w_r = 200$ nm, $w_z = 850$ nm, calculate:
   a) The hydrodynamic radius of a molecule having a diffusion time (τ_D) of 185 ms in water at room temperature (η (H$_2$O) = 0.8904 centipoise at 25 °C). Use the Stokes-Einstein relationship:
   $$D = \frac{k_b T}{6 \pi \eta r}.$$
   b) Using the γ factor for a 3-D Gaussian, $\gamma = 2^{-\frac{3}{2}}$, calculate the average number of molecules in the probe volume at $G(0) = 2.7$. What is the average molar concentration of the molecule in solution?

4. In fluorescence correlation spectroscopy, any correlated fluctuations in fluorescence intensity will be visible in the autocorrelation function (ACF). For example, The ACF for a unimolecular reaction $A \overset{k_+}{\underset{k_-}{\Leftrightarrow}} B$,where the brightness of the two states varies, is given by:

   $$G(\tau) = G_D(\tau, N_A + N_B, \tau_D)\left[1 + K\left(\Im_A - \frac{\Im_B}{K}\right)^2 e^{-\lambda \tau}\right]$$

   where $K = k_+/k_-, \lambda = k_+ + k_-, and \Im$ *is the fractional intensity of state A or B*, which is in turn defined as:

   $$\Im_i = \varepsilon_i \langle N_i \rangle / (\varepsilon_A \langle N_A \rangle + \varepsilon_B \langle N_B \rangle)$$
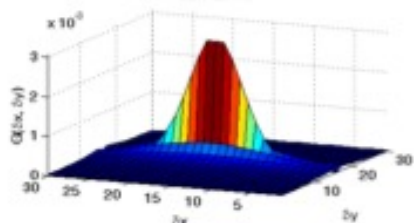
   Show that the relaxation term $K\left(\Im_A - \frac{\Im_B}{K}\right)^2 e^{-\lambda \tau}$ disappears when states A and B have the same molecular brightness.
   (Hint: in equilibrium, $= \frac{k_+}{k_-} = \frac{\langle N_B \rangle}{\langle N_A \rangle}$)
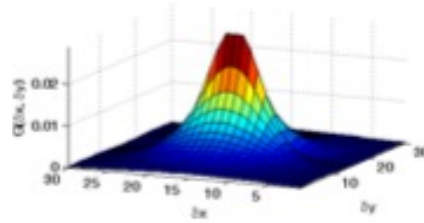
ICS

5. a) The figure below shows the RICS autocorrelation functions for cells expressing GFP and GAG-GFP respectively.
   What properties can we extract from the autocorrelation functions?

| GFP | GAG-GFP |

b) Which of the following image correlation methods are performed on a frame-by-frame basis and which ones are performed between frames?

ICS   TICS   STICS   RICS

Protein Stability and Folding

6. The absorbance cross-section of a molecule is defined as: $\sigma = P\,\pi\,r^2$
   where P is the probability that light impinging on a molecule is absorbed.

   a) Derive an equation for the extinction coefficient where ε is a function of σ.
   b) Consider a chromophore with an absorbance cross-section $\sigma = 6.537 \cdot 10^{-14}\ \text{cm}^2$. Determine the chromophore's extinction coefficient and the absorbance A of a 9 μM solution measured in a 1 cm long cuvette.

   Hint: The fraction of light absorbed can be written as $\dfrac{dI}{I} = \dfrac{-P\cdot\pi\cdot r^2\cdot c\cdot N_A}{1000}\cdot dl$.

7. An RNA polymerase ternary complex consists of DNA, RNA, and RNA polymerase. For single molecule experiments, the DNA has been labeled with Cy3 and the RNA has been labeled with Cy5. The absorption coefficients are:

   RNA Polymerase Ternary Complex: 250,000 M^{-1}cm^{-1} at 280 nm
   Cy3:   12,000 M^{-1}cm^{-1} at 280 nm and
              150,000 M^{-1}cm^{-1} at 552 nm
   Cy5:   12,500 M^{-1}cm^{-1} at 280 nm and
              250,000 M^{-1}cm^{-1} at 647 nm

   The absorption spectrum of the complex measured in a 1 cm long cuvette is shown below:
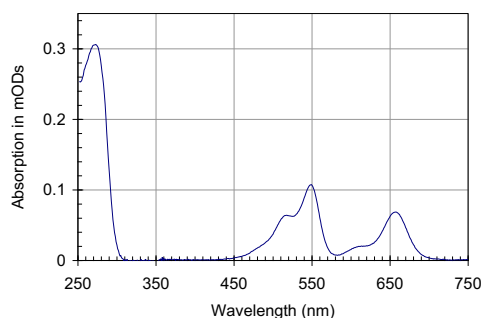
Assuming no free DNA, RNA, RNA polymerase, or fluorescent dye in the sample, answer the following questions:

a) What is the concentration of ternary complex?
b) What is the labeling efficiency of Cy3?
c) What is the labeling efficiency of Cy5?