Assignment#4, Fundamentals in BioPhotonics
2021

1. (20%) Sketch the optical path for a confocal and a widefield microscope. Include the necessary optical elements in the excitation and collection paths!
    a. What are advantages and disadvantages of these microscopes? What are the typical use cases?
    b. What is the difference in the power density between these two configurations, when the same laser is used in both cases (488 nm, 100 mW)? What are the consequences of these differences? How can one mitigate them?
    c. How can one implement TIRF in the same setup? What would be the typical use cases for this imaging modality?
2. (10%) Compare two proteins of masses 27kDa and 54kDa. By how much does their diffusion coefficient differ (in the same buffer solution)?
3. (20%) For a confocal volume with width w_{xy} = 250nm and aspect ratio g = 6, which concentration do you have to choose to achieve 1, 10 and 50 particles in the focus (on average)?
4. (10%) Estimate the retention time of particles with diffusion coefficients of D = 10 $\mu m^2/s$, D = 50 $\mu m^2/s$ and D = 500 $\mu m^2/s$ inside the above-mentioned laser focus at a temperature of 37 C.
5. (30%) Look at the two FCS autocorrelation curves in Fig. 1 *(No photophysical effects are incorporated!)*. Estimate for each graph how many particles N have been observed, how many different components there were, what their diffusion time t_D was and possibly the number of particles in each species! Also estimate the diffusion coefficients D of the different particles. Assume the same laser parameters as above. Assume equal quantum yields for all species.

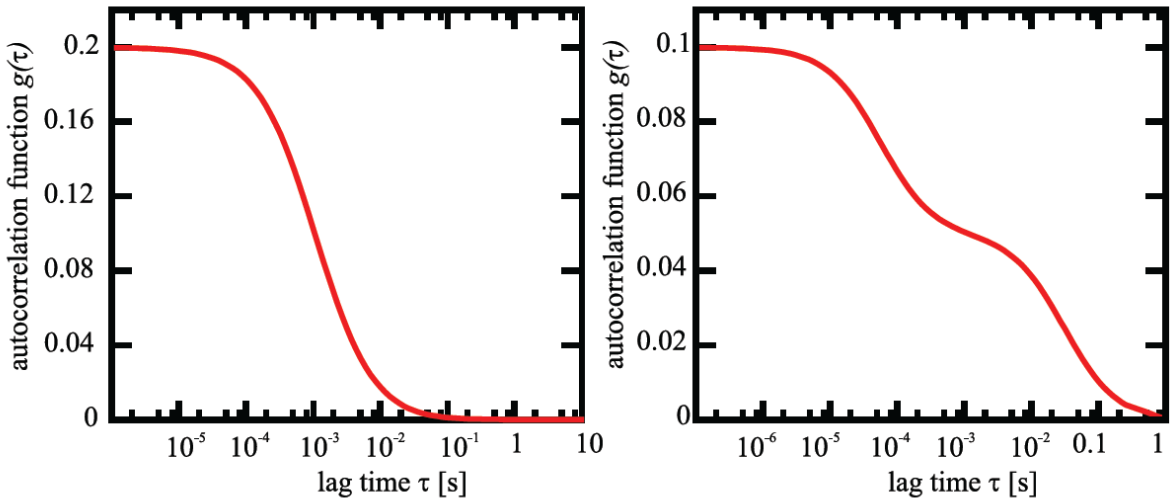

Figure 1 Example FCS autocorrelation curves. No photophysical effects are considered.