Exercise 04

1 Fluorescence microscopy

1.1 Fluorescence recovery experiment

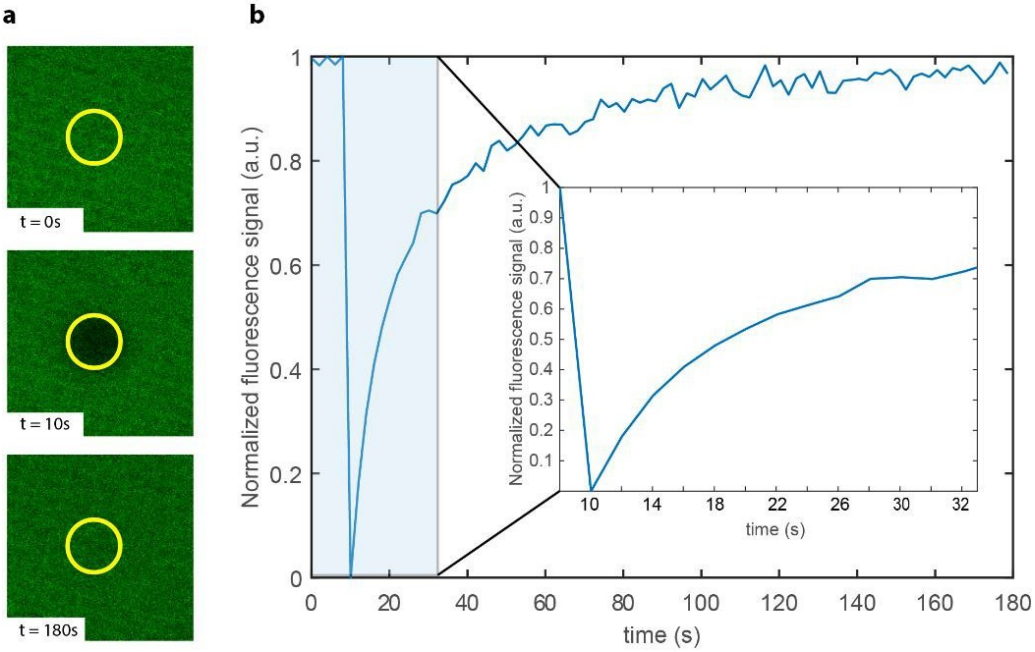

Figure 1: Membrane fluidity. a) FRAP microscopy images of a fluorescently tagged supported lipid bilayer at measurement start ($t = 0\,\mathrm{s}$), directly after bleaching ($t = 10\,\mathrm{s}$) and at $t = 180\,\mathrm{s}$. The bleaching spots have a diameter of $d = 10\,\mu\mathrm{m}$ and are indicated with yellow circles. b). Normalized FRAP experiment results.

To assess the fluidity of the supported lipid bilayers used in a real-time microfluidic experiments, one can perform fluorescence recovery after photobleaching (FRAP) measurements. The FRAP experiments shown in Figure 1 were carried out with a confocal microscope. Estimate the diffusion coefficient of the lipid molecules from the extracted fluorescence recovery curve. Compare the fluidity of the lipid bilayers of this experiment with the one mentioned in Ref [1]. ($D = 0.99\,\mu\mathrm{m}^2\,\mathrm{s}^{-1}$) If we assume that both experiments were done with the same lipid molecules and the same fluorescence labels which one do you expect had a higher temperature of the environment?

1.2 TIRF Microscopy

Total internal reflection fluorescence microscopy (TIRFM) employs evanescent fields induced with total internal reflection (TIR) on glass-water (or glass-buffer) interface. Exponentially decaying evanescent field generates a very thin electromagnetic field region (usually less than 200nm) which can be utilized to excite fluorophores within that very thin surface region, as shown in Figure 2. TIRFM improves signal-to- noise by limiting the excitation volume to the very thin vicinity of the surface.
TIR is observed when a propagating wave strikes a medium boundary with an angle larger than the critical angle. In a case $n_2 > n_1$, the critical angle is defined as the incident angle θ_i when transmission angle θ_t reaches 90°.

(a) Find the expression of critical angle (θ_c) in terms of n_1 and n_2 , by using the Snell's Law:

$$n_i \sin(\theta_i) = n_t \sin(\theta_t)$$

(b) Find the critical angle θ_c for an interface between glass ($n_2 = 1.5$) and air ($n_1 = 1$).

[1] O. Limaj *et al.*, "Infrared Plasmonic Biosensor for Real-Time and Label-Free Monitoring of Lipid Membranes", Nano Lett., vol. 16, no. 2, pp. 1502–1508, Feb. 2016.

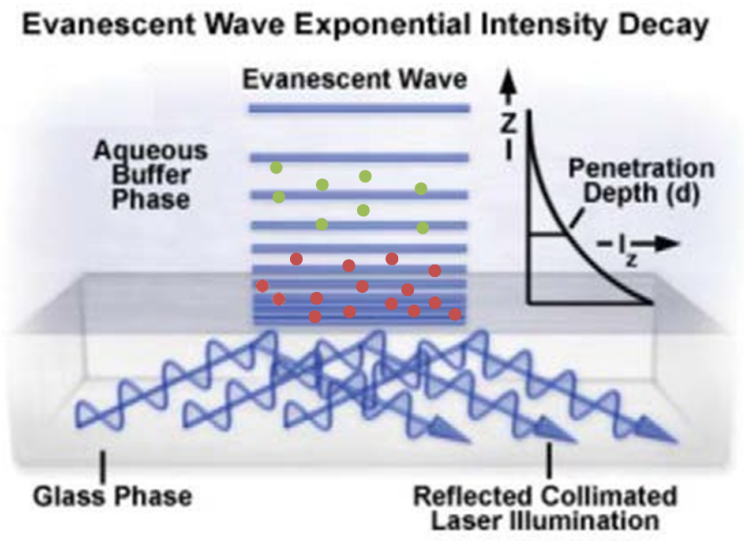

Figure 2: Illustration of evanescent waves and fluorophore excitation within the evanescent field.

For a configuration given in Figure 3, if the incident angle is above the critical angle, electromagnetic wave will be totally reflected into optically dense medium (n_2) and decay evanescently into optically less dense medium (n_1). The penetration depth into medium 1 (where intensity decays by a factor of 1/e) is given by:

$$d_p = \frac{\lambda_0}{4\pi n_1 \sqrt{\left(\frac{n_2}{n_1}\right)^2 \sin^2(\theta_i) - 1}}$$

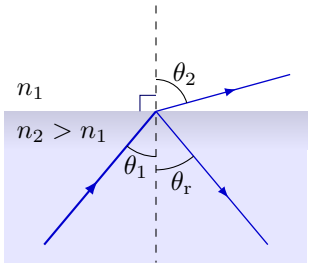

Figure 3: Refraction of light on a border from optically thick to optically thin material.

(c) Assuming that an electromagnetic wave with the free space wavelength of $\lambda = 1550\,\text{nm}$ is incident on the interface between glass (medium 2) and air (medium 1) with an angle of 80° relative to the surface normal. Find the penetration depth of this wave into surrounding air.

(d) Suppose you would like to use the evanescent wave to image an aqueous solution ($n = 1.33$) of the biomolecules with a diameter of $d = 50\,\text{nm}$ on cell membrane. The biomolecules are labeled with the *AlexaFluor488*, which emits light at $\lambda_{\text{em}} = 519\,\text{nm}$ when illuminated with a $\lambda_{\text{ex}} = 488\,\text{nm}$ laser. Assume you can affix the biomolecules on the surface. However, the molecules suspended in the aqueous solution also scatter and emit light that adds noise to your measurement. You have three different glass slides with different indices of refraction: $n_{g_1} = 1.45$, $n_{g_2} = 1.66$ and $n_{g_3} = 1.78$. Which glass slide should you use to obtain a better signal-to-noise ratio for your measurement? Assume you launch your laser beam at a 68° angle from the surface normal.