# Exercise 04 - Solutions

## 1 Fluorescence microscopy

### 1.1 Fluorescence recovery experiment

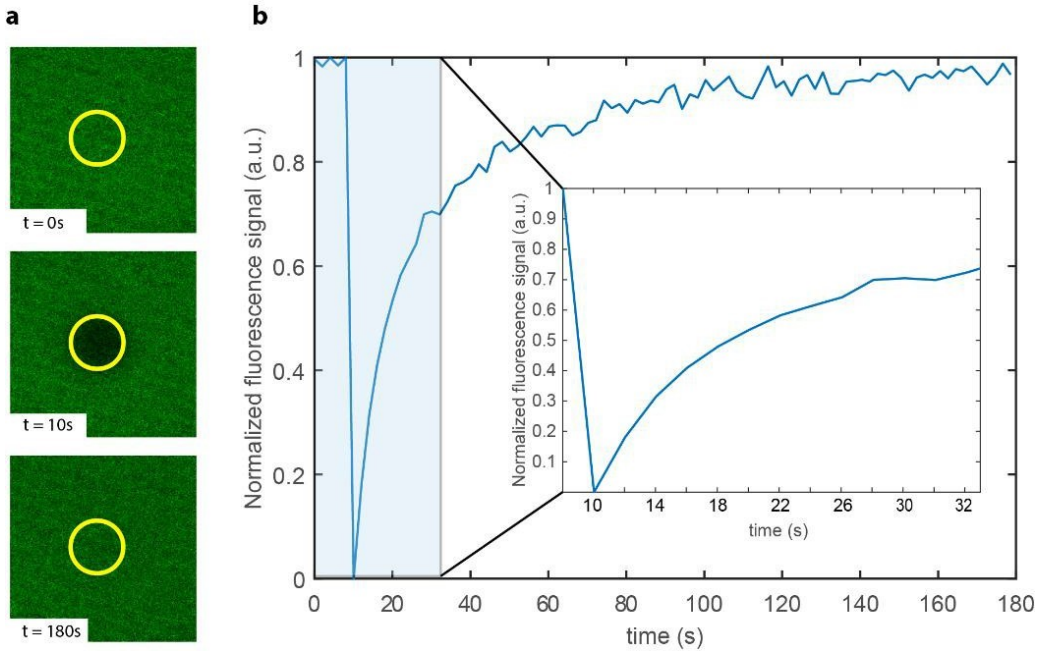


Figure 1: Membrane fluidity. a) FRAP microscopy images of a fluorescently tagged supported lipid bilayer at measurement start ($t = 0\,\text{s}$), directly after bleaching ($t = 10\,\text{s}$) and at $t = 180\,\text{s}$. The bleaching spots have a diameter of $d = 10\,\mu\text{m}$ and are indicated with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

To calculate the diffusion coefficient we need to know the radius of the laser spot size and the fluorescence recovery time ($\tau_{1/2}$). The diameter of the spot size is given in the figure caption, which is $d = 10\,\mu\text{m}$. The fluorescence recovery time can be calculated from the fluorescence recovery curve:

$$\tau_{1/2} = t_1 - t_0 = 18.1\,\text{s} - 10\,\text{s} = 8.1\,\text{s}$$

$$D = \frac{w^2}{4\tau_{1/2}} = \frac{d^2}{16\tau_{1/2}} = 0.77\,\mu\text{m}^2/\text{s}$$

To compare the liquidity of the lipid molecules with the one mentioned in the Ref. [1] we look at the diffusion parameter values $0.99\,\mu\text{m}^2/\text{s} > 0.77\,\mu\text{m}^2/\text{s}$. Therefore, the lipid molecules of this experiment are less mobile than the ones mentioned in the Ref. 1. If we assume that both experiments were done using the same lipid molecules we can conclude that the temperature of the environment in the Ref 1 is higher, because the temperature is directly linked to the kinetic energy of the molecules, therefore the higher the energy (temperature) the higher the mobility of the molecules.

### 1.2 TIRF Microscopy

(a) The critical angle ($\theta_c$) is observed when $_t = 90°$. Therefore, by using the Snell's Law:

$$\theta_c = \arcsin \frac{n_t}{n_i}$$

(b) For the glass ($n_2 = 1.5$) and air ($n_1 = 1$) interface:

$$\theta_c = \arcsin \frac{1}{1.5} \cong 42°$$

[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.

(c) For an electromagnetic wave with the free space wavelength of $\lambda = 1550\,\text{nm}$ is incident on the interface between glass ($n_2 = 1.5$) and air ($n_1 = 1$) with an angle of 80° relative to the surface normal, the penetration depth can be calculated with the given formula:

$$d_p = \frac{\lambda}{4\pi n_1 \sqrt{\left(\frac{n_2}{n_1}\right)^2 \sin^2(\theta_i) - 1}} \cong 113.4\,\text{nm}$$

(d) For the given solution, refractive index ($n = 1.33$), excitation wavelength ($\lambda = 488\,\text{nm}$) and incidence angle ($\theta_i = 68°$), the penetration depth of each glass slides can be calculated:

$$n_{g_1} = 1.45 \rightarrow d_{p_1} = 197.7\,\text{nm}$$
$$n_{g_2} = 1.66 \rightarrow d_{p_2} = 50.1\,\text{nm}$$
$$n_{g_3} = 1.78 \rightarrow d_{p_3} = 39.7\,\text{nm}$$

For the labelled biomolecules with the size of 50 nm, the glass slide with $n_{g_2} = 1.66$ will be the best choice, in terms of signal-to-noise ratio. The glass slide with $n_{g_3} = 1.78$ will give less signal due to short penetration depth ($d_{p_3} = 39.7\,\text{nm}$), and the glass slide with $n_{g_1} = 1.45$ will have higher background noise due to longer penetration depth ($d_{p_1} = 197.7\,\text{nm}$).