Electronic Supplementary Material (ESI) for Lab on a Chip.

Using Patterned Grating Structure to Create Lipid Bilayer Platforms Insensitive to Air Bubbles

Chung-ta Han and Ling Chao*

Department of Chemical Engineering, National Taiwan University, Taipei, Taiwan

Supplementary Materials

Diffusion coefficients obtained by fluorescence recovery after photobleaching (FRAP). We used fluorescence recovery after photobleaching (FRAP) technique to examine the lipid membrane mobility. The FRAP technique uses laser to bleach fluorescent lipid molecules in a small region of the sample and the diffusion coefficients can be obtained by analyzing the fluorescence recovery with time in this small region.[1-3] The recovery time evolution images of the photobleached spot were recorded by image processing program HCImage (Hamamatsu, Japan). The intensity recovery with time inside the region of interest was then processed and fitted by MATLAB (Mathworks Natick, MA) in order to obtain the diffusion coefficient of the SLB. The fitting algorithm we used for the two dimensional lateral diffusion coefficient was mainly based on the one developed by Axelrod et al.[1] and the details are in the following paragraphs.

The measured fluorescence intensity was normalized by the following equation to subtract the fluorescence background and to make it fit to the modeling equation more easily.

$$f_e(t) = \frac{F_e(t) - F_e(0)}{F_{e,\infty}(t) - F_e(0)}$$

where $F_e(t)$ is the average intensity inside the region of interest which would recover with time, $F_e(0)$ is the average intensity inside the region of interest right after the lipid bilayers photobleached by laser and $F_{e,\infty}(t)$ is the average intensity inside the region of interest which assume to reach after infinite time. Considering the effect of photobleaching of fluorophores and fluctuation of intensity inside the whole observing sight, we took $F_{e,\infty}(t)$ as a function of time in order to eliminate the effect. The variation of $F_e(0)$ was found to be negligible comparing to $F_{e,\infty}(t)$, so we assume the value to be constant here. The calculated recovery percentages $f_e(t)$ would increase from zero to a maximum value, which would depend on the mobile fraction of the lipid bilayers.

The normalized measured fluorescence intensity can be fitted to the theoretical normalized intensity derived from the transport model to obtain the diffusion coefficient parameter. For a bleached spot with its initial profile described by a Gaussian profile, the theoretical intensity with time, $F_K(t)$, can be obtained by solving mass transport equation.

$$F_K(t) = \frac{qP_0C_0}{A}\sum_{n=0}^{\infty}\frac{(-K)^n}{n!\left[1+n\left(1+\frac{2t}{\tau_D}\right)\right]}$$

where q is the fluorophore quantum efficiency, A is the beam attenuation factor, P_0 is the total laser power, K is bleaching parameter, C_0 is the uniform fluorophore concentration before bleaching, and τ_D is the characteristic time of diffusion. The theoretical normalized intensity can be expressed as:

$$f_k(t) = \frac{\mathrm{F_K(t) - F_K(0)}}{\mathrm{F_{K,\infty}(t) - F_K(0)}} = \frac{\sum_{n=0}^{\infty}\frac{(-K)^n}{n!\left[1+n\left(1+\frac{2t}{\tau_D}\right)\right]} - \frac{1-e^{-K}}{K}}{1-\frac{1-e^{-K}}{K}}$$

Note that the bleaching parameter, K, can be obtained by fitting the initial bleaching profile to a Gaussian profile.

$$\mathrm{C(r, t=0)} = C_0\exp[-Kexp\left(-2r^2/_{w^2}\right)]$$

where C(r, t=0) is the fluorophore concentration profile at position r and time t= 0, C_0 is the uniform fluorophore concentration before bleaching. Bleaching parameter, K, and half-width, w, are the fitted parameters to be obtained.

By fitting the experimentally measured $f_e(t)$ to the theoretically obtained $f_k(t)$, we can obtain the fitted parameter τ_D and the diffusion coefficient D can be calculated from the following equation:

$$\mathrm{D} = \frac{w^2}{4\tau_D}$$

References

1. Axelrod, D.; Koppel, D. E.; Schlessinger, J.; Elson, E.; Webb, W. W., Mobility measurement by analysis of fluorescence photobleaching recovery kinetics. *Biophysical Journal* **1976,** *16* (9), 1055-1069.

2. Ratto, T. V.; Longo, M. L., Obstructed Diffusion in Phase-Separated Supported Lipid Bilayers: A Combined Atomic Force Microscopy and Fluorescence Recovery after Photobleaching Approach. *Biophysical Journal* **2002,** *83* (6), 3380-3392.

3. Kang, M.; Day, C. A.; Drake, K.; Kenworthy, A. K.; DiBenedetto, E., A generalization of theory for two-dimensional fluorescence recovery after photobleaching applicable to confocal laser scanning microscopes. *Biophysical Journal* **2009,** *97* (5), 1501-1511.