Preparation of mica supported lipid bilayers for high resolution optical microscopy imaging

Matysik, Artur; Kraut, Rachel Susan

2014

Matysik, A., & Kraut, R. S. (2014). Preparation of mica supported lipid bilayers for high resolution optical microscopy imaging. Journal of Visualized Experiments, 88, e52054.

https://hdl.handle.net/10356/93740

https://doi.org/10.3791/52054

Video Article

Preparation of Mica Supported Lipid Bilayers for High Resolution Optical Microscopy Imaging

Artur Matysik[1], Rachel S. Kraut[1]

[1]School of Biological Sciences, Nanyang Technological University

Correspondence to: Artur Matysik at art.matysik@gmail.com

URL: http://www.jove.com/video/52054
DOI: doi:10.3791/52054

Keywords: Bioengineering, Issue 88, mica, bilayer, lipids, TIRFM, imaging, SMT, AFM

Date Published: 6/7/2014

Citation: Matysik, A., Kraut, R.S. Preparation of Mica Supported Lipid Bilayers for High Resolution Optical Microscopy Imaging. *J. Vis. Exp.* (88), e52054, doi:10.3791/52054 (2014).

Abstract

Supported lipid bilayers (SLBs) are widely used as a model for studying membrane properties (phase separation, clustering, dynamics) and its interaction with other compounds, such as drugs or peptides. However SLB characteristics differ depending on the support used.

Commonly used techniques for SLB imaging and measurements are single molecule fluorescence microscopy, FCS and atomic force microscopy (AFM). Because most optical imaging studies are carried out on a glass support, while AFM requires an extremely flat surface (generally mica), results from these techniques cannot be compared directly, since the charge and smoothness properties of these materials strongly influence diffusion. Unfortunately, the high level of manual dexterity required for the cutting and gluing thin slices of mica to the glass slide presents a hurdle to routine use of mica for SLB preparation. Although this would be the method of choice, such prepared mica surfaces often end up being uneven (wavy) and difficult to image, especially with small working distance, high numerical aperture lenses. Here we present a simple and reproducible method for preparing thin, flat mica surfaces for lipid vesicle deposition and SLB preparation. Additionally, our custom made chamber requires only very small volumes of vesicles for SLB formation. The overall procedure results in the efficient, simple and inexpensive production of high quality lipid bilayer surfaces that are directly comparable to those used in AFM studies.

Video Link

The video component of this article can be found at http://www.jove.com/video/52054/

Introduction

The overall goal of the present protocol is to show a method for preparing mica surfaces for high resolution imaging of mica supported lipid bilayers (SLBs) using optical total internal reflection fluorescence microscopy (TIRFM) or confocal microscopy, which could also be combined with atomic force microscopy (AFM).

SLBs are a widely used model for numerous studies of lipid clustering, phase separation, dynamics of bilayer components or their interactions with peptides, proteins or other compounds[1-5]. Different substrates might be used for SLB formation (*i.e.* glass, mica, silicon dioxide, polymers) depending on the nature of the study[4,6-8]. Typical membrane studies rely on microscopy-based imaging techniques, such as TIRFM and AFM. Thus, for TIRFM imaging, a glass surface is a typical choice because glass is transparent. Preparation of glass is relatively easy, and the quality of the results is primarily determined by thorough surface cleaning prior to deposition of lipid vesicles. AFM due to its high axial resolution requires mica surfaces. Mica is a silicate mineral, with close to perfect basal cleavage. Thus, the freshly cleaved mica is atomically flat, allowing observation of membrane height differences even at the sub-nanometer scale[9].

Diffusion studies using methods such as fluorescence correlation spectroscopy (FCS), single molecule tracking (SMT), and fluorescence recovery after photobleaching (FRAP) showed however, that lipid membrane dynamics depend heavily on the type of surface onto which they are deposited, whereby glass and mica can give widely varying results[10,11]. These differences include not only the diffusion coefficients of the membrane probes, but also the detection of separate populations of particles diffusing with different rates, and possibly switching between different states.

Thus, the direct comparison of results obtained using TIRFM and AFM techniques is often problematic, unless the same surface (in this case mica) is used. Although there are some studies where TIRFM and AFM bilayer imaging was conducted on the same mica surface[12,13], mica is rarely used for optical microscopy, mostly because of handling problems. Mica preparation requires cutting by hand into thin leaflets, which are then glued to the coverslip using optical adhesive[12]. This method however requires some practice to achieve satisfactory results. Moreover, the surfaces obtained are often wavy and thick, making them difficult to use with low working distance, high numerical aperture lenses.

Mica surfaces prepared as described in this protocol are very thin (~220 µm, including the coverslip thickness of 170 µm) and extremely flat, avoiding “waviness”, which is critical for successful high resolution imaging. They can be used for TIRFM or confocal setups. Moreover, the same samples can be transferred to AFM, and even imaged simultaneously with TIRFM/confocal and AFM. Combining these two techniques allows direct correlation of diffusion behavior with bilayer membrane structure[14]. Because mica surfaces are freshly cleaved, they are clean and

do not require time consuming, poorly reproducible, and potentially dangerous cleaning procedures (glass cleaning protocols usually include chemicals such as Piranha solution, sulfuric acid, sodium/potassium hydroxide). Mounting of a small chamber, also described in the protocol, reduces the volume of vesicles required for effective bilayer formation to less than 50 µl. Finally, the whole process of surface assembly is not time consuming (preparation takes less than 30 min), and does not require a high degree of manual skill, as does conventional mica cleavage and gluing.

Protocol

1. Mica and Slides Preparation

1. Place No. 1½ (0.17 mm) coverslips into staining rack.
2. Sonicate for 30 min in 2% detergent at 60 °C.
3. Wash 20 times with deionized water.
4. Remove slides using forceps and blow dry using compressed air or nitrogen.
5. Cut mica sheet into 10 x 10 mm square pieces using scissors or razor blade.
6. Cut each mica piece into 2-3 thinner leaflets using razor blade.
   NOTE: This step requires use of sharp blade.

2. Mica Assembly and Chamber Mounting

1. Clean microscopic glass slide with ethanol.
2. Glue leaflet of mica cut in step 1.6 to glass slide using optical adhesive. Low viscosity adhesive is advised to better spread the drop and glue the mica.
3. Cure under UV lamp, 10 min.
   NOTE: Adhesive is cured by UV light with maximum absorption in the range of 350 to 380nm and the recommended energy required for full cure is 4.5 J/cm^2. However, different light sources can be used for this step (see materials provided by adhesive supplier).
4. Expose a clean mica surface by removing the first few layers with Scotch tape.
5. Place small drop (~20 µm) of optical adhesive onto the mica surface.
   NOTE: At this step, high viscosity adhesive with low autofluorescence level is advised. Our experience showed that using combination of low (step 2.2) and high viscosity adhesives increases significantly effectiveness of mica splitting (step 2.7).
6. Gently place freshly cleaned coverslip onto the drop of adhesive, avoiding air bubbles, let settle for 1 min.
7. Cure under UV lamp, 10 min.
   NOTE: after this step, the sandwich of glass slide, mica and coverslip can be stored for a relatively long period of time (up to few weeks). Proceed to the next step just before the actual SLB preparation, to make sure the mica surface is fresh.
   NOTE: Adhesive is cured by UV light with maximum absorption in the range of 350 to 380nm and the recommended energy required for full cure is 4.5J/cm^2. However, different light sources can be used for this step (see materials provided by adhesive supplier).
8. Using an exacto knife, gently dismount the coverslip from side glass slide as shown in the video. In most cases, a thin and flat layer of mica will remain attached to the coverslip. Mica attached to glass slide can be reused (repeat from step 2.4).
9. Check the surface quality by naked eye or under a dissecting microscope, to make sure that mica layer is still glued to the coverslip and was not removed completely during splitting in step 2.8. Making a small scratch with a forceps or dissecting needle will help to distinguish between the adhesive which has a detectably different consistency from mica.
10. Remove rubber seal from a 1.5 ml vial cap and glue the cap upside down to the surface using optical adhesive or nail polish, and cure with UV lamp, or let air dry 10 min, respectively.
    NOTE: If the sample is prepared for simultaneous optical (TIRFM or confocal) with AFM imaging, a 1.5 ml vial cap might be too small to mount the AFM head on the microscope stage. In that case, the cap might be substituted by any plastic O-ring with a bigger diameter, suitable for AFM head mounting. In case when the experiment requires removing the cap without damaging the mica surface, silicone grease can be used instead of glue or nail polish.

3. Supported Lipid Bilayer (SLB) Formation

1. Place freshly prepared liposome solution into chamber with surface. The minimum volume required for SLB formation is ~30 µl.
   NOTE: For more details on the liposome and SLB formation, refer to published protocols, such as such as Bag *et al.*, 2014[15].
2. Proceed with SLB formation using desired protocol. During incubation and imaging, the chamber can be placed in a heat-block or heated microscope stage to maintain temperature required to keep the lipids being used above their melting temperature.

Representative Results

The diffusion behavior of fluorescent lipid probes in SLBs is different depending on the substrate. TIRFM combined with the SMT technique is a valuable method for visualizing particle movements and extracting their diffusion coefficients. Single molecule signals of a Sphingomyelin-ATTO647N probe diffusing in a DOPC (1,2-dioleoyl-*sn*-glycero-3-phosphocholine) bilayer supported on glass and mica are shown on the attached animated figure. The mica surface was prepared according to the protocol presented here. To estimate optical aberrations, full width at half maximum (FWHM) was measured and averaged over the 20 point spread function (PSF) using Mosaic 2D PSF Size ImageJ plugin[16,17]. The measured FWHM for glass and mica were 441nm and 464nm respectively (**Figure 1**). The 22nm difference in resolution between imaging on glass and mica is not significant. In both cases, the PSF centroid of each single fluorescent molecule can be localized in successive frames, and linked into particle trajectories over time with Mosaic Particle Tracker ImageJ plugin[16,17]. **Figure 2** shows sample trajectories of particles diffusing across the bilayer supported on both surfaces. The mean square displacements (MSDs) of the fluorescent probe diffusing in DOPC membrane

supported on glass and mica was plotted on the **Figure 3**. Due to coexistence of multiple populations two population model was used to extract fast and slow diffusion coefficients, according to method described by Schutz (**Figure 4**, **Figure 5**)[18]. The diffusion coefficients and their fractions were extracted using TrackArt software[19] and are summarized in **Table 1**.

The results from this example prove the existence of two separate states of the diffusing probe: fast and slow. The diffusion coefficient of the fast population is roughly 1.5 times higher on mica than on glass. The slow component however, is almost immobile on glass (<0.01 μm^2/sec), compared to a D of only ~1/10 the fast population on mica.

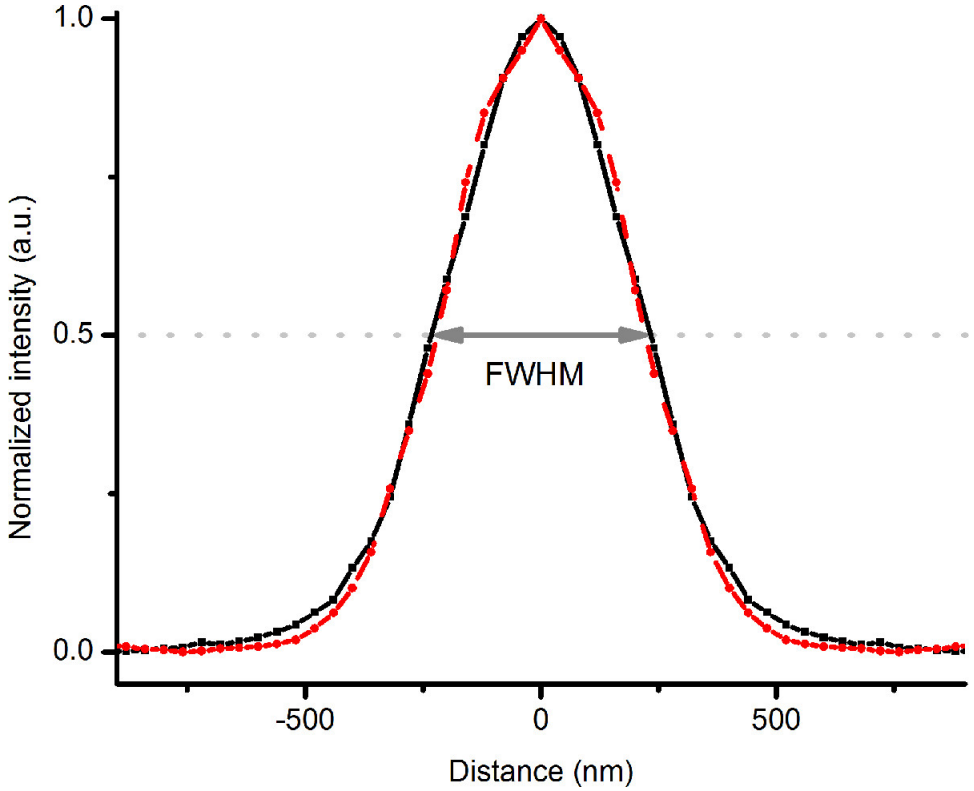

Figure 1. Average PSF size. Average PSF intensity profile for 20 spots measured on glass (black solid line) and mica (red dashed line). The full width at half maximum (FWHM) of the normalized intensity for glass and mica was estimated to be 441 nm and 464 nm, respectively. The 22nm difference indicates that there is no significant drop in imaging resolution between these two surfaces. PSF intensity profile was measured using Mosaic PSF 2D Tool ImageJ plugin[16,17]. Please click here to view a larger version of this figure.

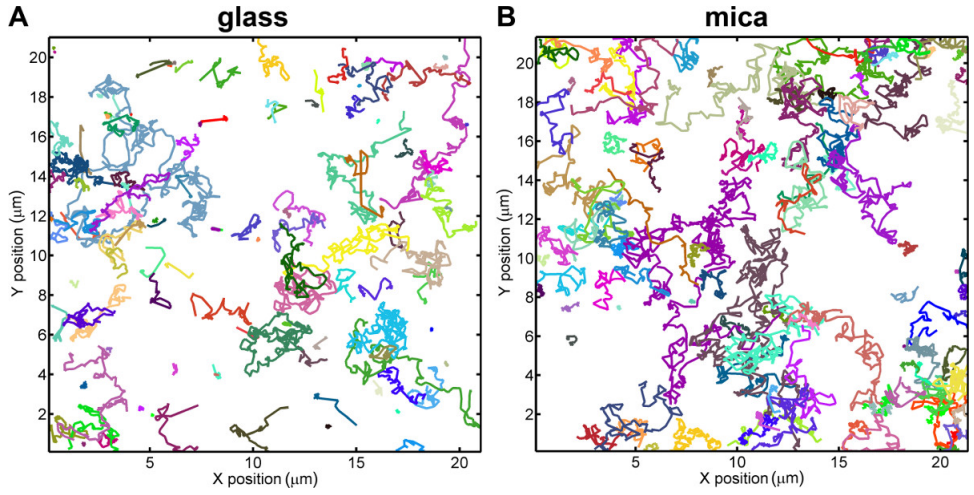

Figure 2. Sample trajectories. Sample trajectories of SM-ATTO647N diffusing in a DOPC lipid bilayer supported on glass (**A**) and mica (**B**). On the glass supported bilayer, the probe is often immobilized on the surface, occasionally switching to the fast diffusing state. Probes diffusing on the mica supported bilayer, in contrast, are rarely immobilized on the surface. Instead, they tend to switch between fast and slow diffusing state. Please click here to view a larger version of this figure.

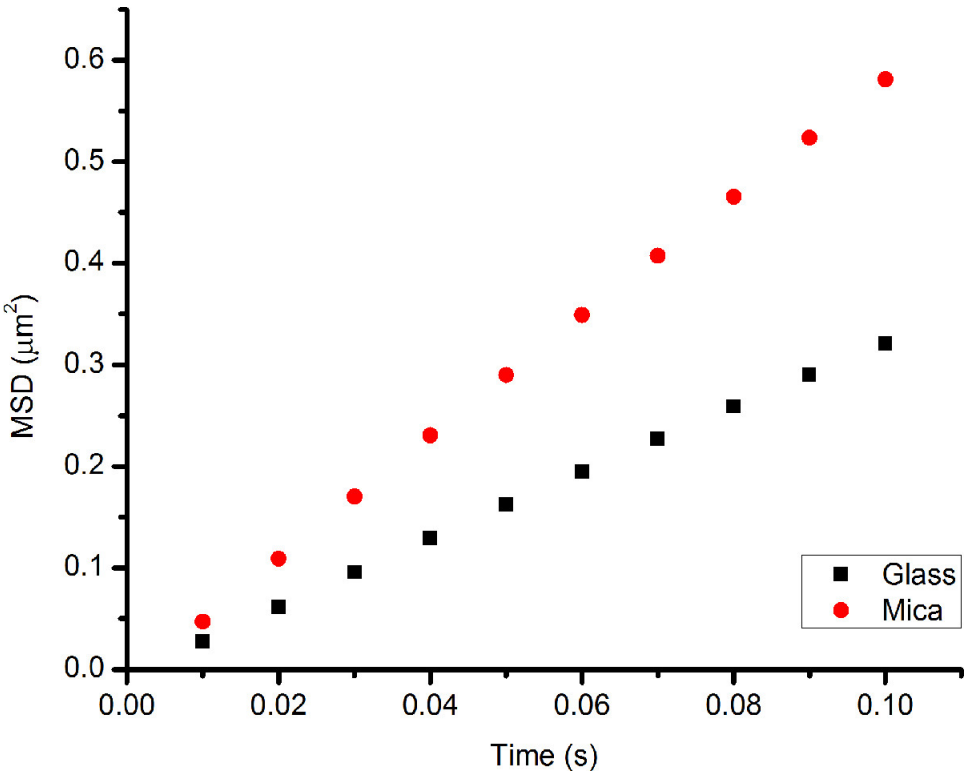

Figure 3. Mean square displacements. Mean square displacements of SM-ATTO647N particles diffusing on a glass- (■) and mica- (●) supported DOPC bilayer. Please click here to view a larger version of this figure.

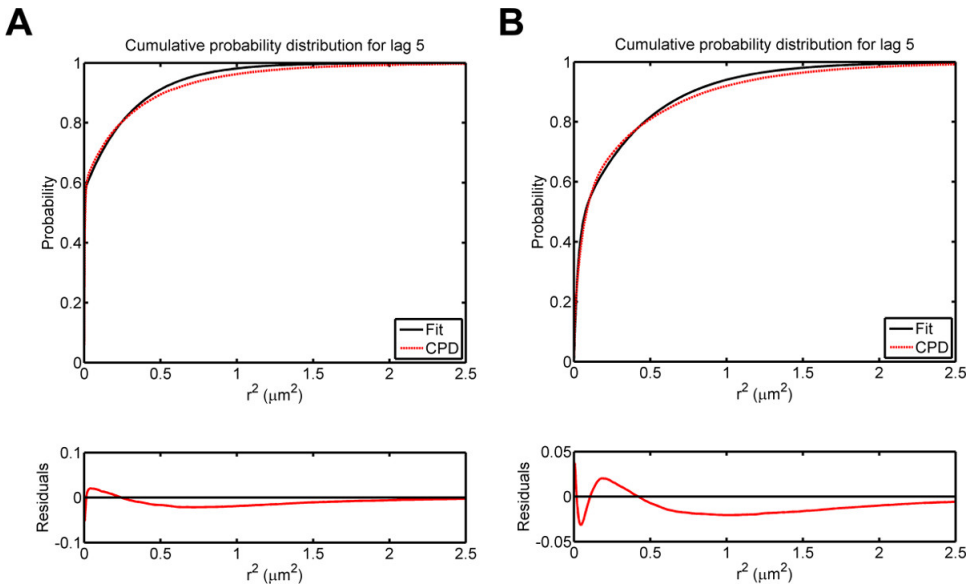

Figure 4. Cumulative probability distribution fits. Cumulative probability distribution (CPD) of square displacements and fits for the bi-exponential (two-population) diffusion model of SM-ATTO647N particles diffusing on glass- (**A**) and mica- (**B**) supported DOPC bilayers. Distributions and fits are presented only for the fifth$^{\text{time-lag}}$ (Δt = 50 msec). Calculations and plots are obtained using TrackArt[19] software. Please click here to view a larger version of this figure.

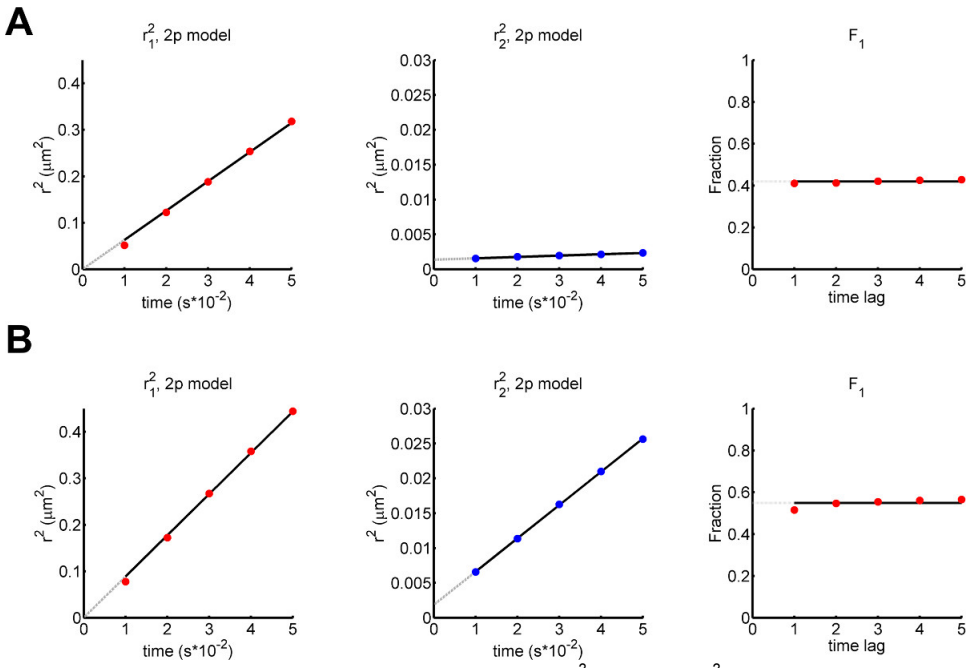

Figure 5. MSD and fraction plots. MSD plots for the fast (r_1^2) and slow (r_2^2) diffusing population and fraction of the fast population (F_1) calculated from the CPD fits. Results are presented separately for SM-ATTO647N particles diffusing on glass- (**A**) and mica- (**B**) supported DOPC bilayers. Calculations and plots were obtained using TrackArt[19] software. Please click here to view a larger version of this figure.

	Population 1 (fast)		Population 2 (slow)	
	D_1 (µm²/sec)	Fraction (%)	D_2 (µm²/sec)	Fraction (%)
Glass	1.840 ± 0.031	65.19 ± 0.56	0.006 ± 0.001	34.81 ± 0.56
Mica	2.440 ± 0.023	53.88 ± 0.26	0.176 ± 0.002	46.12 ± 0.26

Table 1. Diffusion coefficients. Summary of diffusion statistics. Diffusion coefficients for slow and fast populations and their fractions. Calculations were performed in TrackArt software using a two population model. Trajectories were recognized and linked using Mosaic Particle Tracker ImageJ plugin.

Animated Figure: Single molecules diffusion. Timelapse TIRFM movie of Sphingomyelin-ATTO647N diffusing in a DOPC bilayer supported on glass (left) and mica (right). Scale bar is 5 µm.

Discussion

This protocol describes a method for preparing smooth and thin mica surfaces for lipid bilayer deposition and high resolution imaging. The technique requires minimal manual skills, limited mostly to the careful disassembly of the glass-mica-glass sandwich (step 2.8), which is critical for obtaining a high quality mica surface. Inspection of the freshly cleaved mica is always required at this point, since it is possible for the mica to detach from the optical adhesive without cleaving, leaving exposed areas of optical adhesive. That might result in unwanted deposition of the bilayer on adhesive instead of on the mica. The mica surface prepared using the method described is with few exceptions parallel to the coverslip surface, judging by the uniformly high optical imaging quality we obtained; we therefore did not see a need for additional verification.

The final step in mounting the chamber can be customized. The Video tutorial shows a 1.5 ml glass vial plastic cap being used as a chamber, however this can be substituted with any object of similar shape and desired dimensions, *e.g.* for simultaneous AFM-TIRFM/confocal imaging, where the sample with its holder has to fit into the AFM head. Mounting of a custom chamber can be skipped, if imaging is to be performed using a standard, 35 mm metal cell chamber. In this case however, a 25 mm round cover glass has to be used, and a much bigger volume of liposome solution would be required for SLB formation.

The results presented here show that single molecule imaging and tracking can be easily done on extremely flat mica surfaces thin enough to be amenable to TIRFM imaging, according to the protocol for surface preparation described. The same preparation can be applied to other techniques, including high resolution optical microscopy, such as total internal reflection fluorescence correlation spectroscopy (TIR-FCS). Importantly, SLBs prepared in the same way (or even the exact same samples) can be used in both experimental setups, an essential criterion for direct comparison of results obtained using different methods, *e.g.* AFM, SMT, FCS, and FRAP.

Disclosures

The authors have nothing to disclose.

Acknowledgements

The authors have no acknowledgements.

References

1. Giocondi, M.-C. *et al.* Surface topography of membrane domains. *Biochimica et Biophysica Acta (BBA) - Biomembranes.* **1798**, 703-718, doi:10.1016/j.bbamem.2009.09.015 (2010).
2. Garcia-Saez, A. J., & Schwille, P. Surface analysis of membrane dynamics. *Biochim Biophys Acta.* **1798**, 766-776, doi:10.1016/j.bbamem.2009.09.016 (2010).
3. Plochberger, B. *et al.* Cholesterol slows down the lateral mobility of an oxidized phospholipid in a supported lipid bilayer. *Langmuir.* **26**, 17322-17329, doi:10.1021/la1026202 (2010).
4. El Kirat, K., Morandat, S., & Dufrêne, Y. F. Nanoscale analysis of supported lipid bilayers using atomic force microscopy. *Biochimica et Biophysica Acta (BBA) - Biomembranes.* **1798**, 750-765, doi:10.1016/j.bbamem.2009.07.026 (2010).
5. Szmodis, A. W., Blanchette, C. D., Longo, M. L., Orme, C. A., & Parikh, A. N. Thermally induced phase separation in supported bilayers of glycosphingolipid and phospholipid mixtures. *Biointerphases.* **5**, 120-130, doi:10.1116/1.3524295 (2010).
6. Strulson, M. K., & Maurer, J. A. Microcontact printing for creation of patterned lipid bilayers on tetraethylene glycol self-assembled monolayers. *Langmuir.* **27**, 12052-12057, doi:10.1021/la201839w (2011).
7. Satriano, C. *et al.* Plasma oxidized polyhydroxymethylsiloxane--a new smooth surface for supported lipid bilayer formation. *Langmuir.* **26**, 5715-5725, doi:10.1021/la903826d (2010).
8. Bag, N., Sankaran, J., Paul, A., Kraut, R. S., & Wohland, T. Calibration and limits of camera-based fluorescence correlation spectroscopy: a supported lipid bilayer study. *Chemphyschem: a European Journal of Chemical Physics and Physical Chemistry.* **13**, 2784-2794, doi:10.1002/cphc.201200032 (2012).
9. Singh, S., & Keller, D. J. Atomic force microscopy of supported planar membrane bilayers. *Biophysical Journal.* **60**, 1401-1410, doi:10.1016/S0006-3495(91)82177-4 (1991).
10. Przybylo, M. *et al.* Lipid Diffusion in Giant Unilamellar Vesicles Is More than 2 Times Faster than in Supported Phospholipid Bilayers under Identical Conditions. *Langmuir.* **22**, 9096-9099, doi:10.1021/la061934p (2006).
11. Scomparin, C., Lecuyer, S., Ferreira, M., Charitat, T., & Tinland, B. Diffusion in supported lipid bilayers: influence of substrate and preparation technique on the internal dynamics. *Eur Phys J E Soft Matter.* **28**, 211-220, doi:10.1140/epje/i2008-10407-3 (2009).
12. Oreopoulos, J., & Yip, C. M. Combined scanning probe and total internal reflection fluorescence microscopy. *Methods.* **46**, 2-10, doi:10.1016/j.ymeth.2008.05.011 (2008).
13. Shaw, J. E., Slade, A., & Yip, C. M. Simultaneous in situ total internal reflectance fluorescence/atomic force microscopy studies of DPPC/dPOPC microdomains in supported planar lipid bilayers. *J Am Chem Soc.* **125**, 11838-11839, doi:10.1021/ja0370894 (2003).
14. Skaug, M. J., Faller, R., & Longo, M. L. Correlating anomalous diffusion with lipid bilayer membrane structure using single molecule tracking and atomic force microscopy. *J Chem Phys.* **134**, 215101, doi:10.1063/1.3596377 (2011).
15. Bag, N., Yap, D. H., & Wohland, T. Temperature dependence of diffusion in model and live cell membranes characterized by imaging fluorescence correlation spectroscopy. *Biochimica et Biophysica Acta.* doi:10.1016/j.bbamem.2013.10.009 (2013).
16. Sbalzarini, I. F., & Koumoutsakos, P. Feature point tracking and trajectory analysis for video imaging in cell biology. *J Struct Biol.* **151**, 182-195, doi:10.1016/j.jsb.2005.06.002 (2005).
17. Linkert, M. *et al.* Metadata matters: access to image data in the real world. *J Cell Biol.* **189**, 777-782, doi:10.1083/jcb.201004104 (2010).
18. Schutz, G. J., Schindler, H., & Schmidt, T. Single-molecule microscopy on model membranes reveals anomalous diffusion. *Biophys J.* **73**, 1073-1080, doi:10.1016/S0006-3495(97)78139-6 (1997).
19. Matysik, A., & Kraut, R. TrackArt: the user friendly interface for single molecule tracking data analysis and simulation applied to complex diffusion in mica supported lipid bilayers. *BMC Research Notes.* **7**, doi:10.1186/1756-0500-7-274 (2014).

