Brewster Angle Microscopy (BAM) for in situ characterization of ultrathin films at air/liquid interfaces

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1. Langmuir monolayers

1.1 Langmuir monolayers: Definition and relevance.

A Langmuir monolayer is defined as a single layer of surface active molecules placed at the air/liquid interface. In most of the cases, the molecules forming the Langmuir monolayers are organic surfactants. The polar headgroups are in contact with the water, thereby being hydrated. On the contrary, the hydrocarbon chains are pointing to the air in order to avoid close contact with the water. See Figure 1 for a schematic picture. This situation is similar to the oil/water interface, although herein we refer exclusively to Langmuir monolayers at the air/water interface. The first report on the adsorption of organic molecules at the air/water interface observed by changes in the surface tension was made by Agnes Pockels.[1] Irving Langmuir developed further the ideas of Agnes Pockels,[2] being awarded for this and other contributions the Nobel Prize in Chemistry in 1932.

The Langmuir technique consists basically in the formation and manipulation of Langmuir monolayers through the following steps. First, a small volume, i.e., microliters, of a surfactant solution using a volatile organic solvent, which is not miscible with water, is gently placed on top of a water surface. The bulk water is named as subphase. The volatile organic solvent spreads on the surface and evaporates quickly. After waiting a few dozen of minutes for complete evaporation and relaxation of the monolayer, compression can be started. The insoluble surfactant molecules are then arranged forming a monomolecular layer at the air/water interface. As commented, the polar heads are pointing to the water subphase and the hydrocarbon chains are pointing to the air. The initial available area per surfactant molecule is determined by the surface of the water subphase and the number of surfactant molecules spread. A movable barrier can be used for the adjustment of the area per molecule. In such a way, the area per surfactant molecule can be finely changed. The continuous recording of the surface tension is performed during compression and decompression of the Langmuir monolayer, leading to the surface pressure-molecular area isotherm.

Figure 1. A) Idealized scheme of a Langmuir monolayer. The surfactant molecules are arranged with polar heads in contact with the water subphase and the hydrocarbon chains pointing to the air. B) Number of published papers containing the keyword “Langmuir monolayer” per year. Source: ISI Web of Knowledge.

The unique advantages of the Langmuir technique include: The fine adjustment of the area per surfactant molecule, the well-defined and simple geometry of the monolayer, and the array of experimental techniques for the characterization of the Langmuir monolayers.
The main focus of the research of the Langmuir monolayers has been the study of the molecular arrangement of surfactants at the air/water interface with a physical chemistry perspective.[3] The use of Langmuir monolayers constituted by phospholipids as model for the cell membranes has been exploited as well in a biophysical perspective. The influence of a number of biologically relevant molecules, i.e., peptides and DNA, on the phospholipid layer structure can be successfully characterized to a large extent.[4] Inorganic/organic hybrids can form non-traditional Langmuir monolayers, as polyoxometalates or nanoparticles.[5,6] The research field of Langmuir monolayers is of current interest, as shown by the increasing number of publications on Langmuir monolayers, see Figure 1.

1.2 Langmuir monolayers: Characterization and instrumental techniques.

Langmuir monolayers can be characterized in situ, at the air/water interface, by using a number of different experimental techniques. Different spectroscopic techniques, e.g., UV-vis, InfraRed, provide structural information. This information can be complemented by synchrotron-based X-ray diffraction and reflectivity.[3] The computational studies of Langmuir monolayers have become relevant in recent years as well.[7]

Concerning the microscopy of Langmuir monolayers, two different approaches have been developed: Fluorescence microscopy and Brewster angle microscopy. Fluorescence microscopy relies on the introduction of a surface active fluorescent probe in the Langmuir monolayer under study.[8] The fluorescent probe is regarded as a controlled contamination of the Langmuir monolayers. Given the certain modification of the physicochemical properties of the surfactant monolayer by the fluorescent probe, the use of fluorescence microscopy for studying the morphology of the Langmuir monolayers is being reduced in the last years. Therefore, most of the researchers working with Langmuir monolayers rely on Brewster angle microscopy.


2.1 Physical basis for Brewster angle microscopy.

The Brewster angle microscope was developed simultaneously by Hénon and Meunier [9] and by Hönig and Möbius.[10] The latter team set up a manufacturing company, being the leading company in producing Brewster angle microscopes.[11] The first pictures of a Langmuir monolayer are shown in Figure 2.

The Brewster angle microscopy is based on an elegant trick using basic optics. Given two different phases 1 and 2, two different refractive indexes are found, \( n_1 \) and \( n_2 \). In the case of air and water, the refractive indexes are \( n_{\text{air}} = 1.00 \) and \( n_{\text{water}} = 1.33 \). The Brewster angle (\( \theta_{\text{Brewster}} \)) is defined as the incident angle under which p-polarized radiation is not reflected at the interface of media 1 and 2. The value of the Brewster angle can be predicted using the Fresnel equation (1):

\[
\theta_{\text{Brewster}} = \arctan \left( \frac{n_2}{n_1} \right)
\] (1)

For the case of the air/water interface, the value of the Brewster angle is \( \theta_{\text{Brewster}} = 53.1^\circ \). In other words, no reflection occurs for p-polarized radiation with an angle of incidence of 53.1° at the air/water interface. The formation of a Langmuir monolayer on the air/water interface with a different refractive index changes the Brewster angle. In this scenario, a certain fraction of the incoming p-polarized radiation is reflected from the Langmuir monolayer-covered region of the air/water interface. The collection of this reflected radiation with a video camera allows the in situ, real time, visualization of the Langmuir monolayer at the air/water interface. The lateral resolution of the BAM instruments is in the range of one to three micrometers. A laser radiation with a wavelength of 532 nm is usually the source of radiation for most BAM instruments.
2.1 Traditional usage of Brewster angle microscopy.

In the standard usage of the BAM, the most relevant modification of the refractive index arises from the hydrocarbon chains of the surfactant molecules. The contribution of the headgroup might be important in the case of absorption of the incident light, as discussed in the forthcoming section.

The hydrocarbon chains modify the refractive index and different experimental factors, such as thickness of the hydrocarbon chains layer, packing density, and the anisotropy in the plane, lead to different intensities of the reflected light. The largest the changes of the refractive index of the air/water interface by the Langmuir monolayer, the brightest the observed region in the BAM picture.

The packing density and the thickness of the Langmuir monolayer can be effectively analyzed using BAM in different cases. The formation of multilayers from a Langmuir monolayer at the air/water interface is probably one of the most representative cases. Esker et al., studied a silicon-based surfactant at the air water interface, showing the formation of nucleation points within the Langmuir monolayer that lead to formation of multilayer, see Figure 3.[12] In a related study, Camacho et al. studied the reversible collapse of a mixed monolayer composed of a porphyrin and a phospholipid, see Figure 3.[13] The dark background observed in the BAM pictures corresponds to the monolayer region. The multilayer regions are thicker, and therefore brighter. The growth of the multilayer could be monitored in situ, at the air/water interface, by simply observing the widening of the bright regions. Remarkably, in both cases a well-defined trilayer was formed. The layer-by-layer growth can also be realized in Langmuir monolayers, as shown in the work with polysiloxanes of Godovsky et al.[14]

Figure 2. First BAM pictures published in Hönig, D. and Möbius, D., J. Phys. Chem. 1991, 95, 4590. The pictures correspond to a Langmuir monolayer of phospholipid DMPE on air/water interface. Copyright 1991 from American Chemical Society

Figure 3. A) Nucleation points for the trilayer formation as bright spots in a Langmuir monolayer of a polysilsesquioxane. Copyright 2005 from American Chemical Society. B) Mixed Langmuir monolayer of a porphyrin and a phospholipid in the collapse region. The bright regions correspond to the trilayer. Copyright 2009 from American Chemical Society.
The BAM pictures of a Langmuir monolayer can show a number of features that are most useful for the effective characterization of the molecular arrangement in the monolayer. Vollhardt et al., successfully discriminated domains in which the azimuthal angle varied for the different domains, see Figure 4 [15]. The azimuthal angle is defined as the relative orientation of the tilted hydrocarbon chain with respect to the air/water interface. In other words, the value of the tilting angle of the hydrocarbon chains is the same for all the domains observed in the DPPG monolayer, albeit the chains are oriented in different directions for the different domains. This feature is observed in the BAM pictures as different brightness for each domain.

The crystalline structure of the hydrocarbon chains in a Langmuir monolayer is usually studied by synchrotron-based Grazing Incidence X-ray Diffraction (GIXD).[16] However, in certain cases the BAM pictures can offer information in this sense. Goldmann et al. studied a model Langmuir monolayer of docosanoic acid on the air/solution interface containing divalent copper ions.[17] The fracture of the domains leads to clearly well-defined angles. These angles correspond to the lattice plane formed by the hydrocarbon chain. In that study, the authors checked the observed structure by BAM with the GIXD data. The validity of the BAM for quantifying the crystalline structure of the monolayer was assured.

Figure 4. A) Langmuir monolayer of DPPG anionic phospholipid at the air/water interface. Copyright 2000 from American Chemical Society. B) Langmuir monolayer of docosanoic acid at the air/solution interface. Copper ions were present in the bulk subphase. Copyright 2010 from American Chemical Society.

The azimuthal angle of the hydrocarbon chains might vary for the different domains, as shown before. Moreover, the azimuthal angle within a single domain can be different. In such a case, a given domain shows inner textures, with different regions corresponding to different values of azimuthal angle. Langmuir monolayers of arachidic acid with a certain content of cholesterol, i.e., 5-20%, were studied by Johann et al.[18] The domains observed by BAM display inner textures, as shown in Figure 5. The different brightness, as in the previous case, indicates a different value of the azimuthal angle of the hydrocarbon chains. The main difference is that in this case, the different orientations are observed within the same domain. Therefore, the existence of different regions within one domain can be directly observed by BAM. The boundaries between those regions, as noted by the arrows in Figure 5, are a matter of current research.

Figure 5. BAM pictures of the Langmuir monolayer of arachidic acid doped with 5-20% cholesterol after a compression and decompression process. Arrows indicate the boundary region between the inner textures. Copyright 2000 from American Chemical Society.

3.1 A non-standard usage of Brewster angle microscopy.

A new view on the BAM experiments has been developed recently according to experimental findings and subsequent theoretical considerations. The reflection of incoming radiation at the air/water interface is enhanced by the absorption of the radiation from molecules at the interface.[19] Therefore, the amount of reflected radiation during a typical BAM experiment will be enhanced to a certain extent by the presence of molecules that absorb the laser radiation, i.e., 532 nm. In other words, the existence of dyes in the Langmuir monolayer will determine the observed BAM pictures. A greater detail in the theoretical framework for this observed phenomenon has been recently reviewed.[20]

Camacho et al. studied mixed Langmuir monolayers of a phospholipid with a water soluble dye methylene blue.[21] Methylene blue absorbs visible radiation, therefore displaying the enhancement of reflection of the incoming radiation during a BAM experiment. See Figure 6 for representative BAM pictures. In this case, a clear contrast between the different inner textures within the domains is observed. By simulating the inner textures by the Fresnel equations, the molecular arrangement of the methylene blue could be quantitatively assessed. Thus, the different inner textures within the domains correspond to different guiding directions of well-defined aggregates of methylene blue.

![Figure 6](image1.png)

**Figure 6** BAM pictures of the mixed Langmuir monolayer methylene blue:DMPA in equimolar ratio, 1:1. Left: Surface pressure of 5 mN/m. Right: Surface pressure of 10 mN/m. Copyright 2009 from American Chemical Society.

The visualization of the molecular arrangement of the chromophore groups of the dye molecules rather than the hydrocarbon chains of a given Langmuir monolayer offers a unique possibility of studying the headgroup region. The molecular arrangement of the dye molecules at the air/water interface can be engineered for different purposes. In a recent example, a chiral supramolecular structure of a surface-active dye in combination with a phospholipid was built.[22] The chiral shape of the inner textures within circular domains is shown in Figure 7. The chirality of the supramolecular aggregates of the dye molecules was assessed as well by Circular Dichroism measurements.

![Figure 7](image2.png)

**Fig. 7** BAM picture of the mixed monolayer formed by the surface active dye HSP in combination with the phospholipid DMPA. The surface pressure is ca. 15 mN/m. Copyright 2011 from American Chemical Society.

The Brewster Angle Microscopy (BAM) is a current tool for the effective visualization of Langmuir monolayers at the air/liquid interface. The information achievable by BAM experiments is highly useful for obtaining insight into the molecular arrangement of the surfactant molecules in the monolayer as well as in nucleation processes during the first-order transition from a liquid to a condensed phase. The experimental conditions and the chemical nature of the amphiphilic molecules determine the relevance of the hydrophobic and the hydrophilic regions in the visualization of the BAM images. The BAM technique is therefore a powerful tool in interfacial analysis with different perspectives, from physical chemistry to biophysics.

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References