A comparison of atomic force microscopy, confocal fluorescence microscopy and Brewster angle microscopy for characterizing mixed monolayer surfactant films.

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The ability to tailor the structure, composition and mechanical properties of surfactant films at solid-air and liquid-air interfaces is of considerable technological importance. Our group and others have been investigating properties of mixed monolayer films comprised of hydrogenated and perfluorinated surfactants through a variety of microscopy imaging techniques. These monolayer films are often highly-structured at the micrometer and nanometer length scale, and invaluable information about the morphology, composition and underlying molecular-level organization of the films can be determined via atomic force microscopy, confocal fluorescence microscopy and Brewster angle microscopy. Each of these microscopy approaches provides different (and often complementary) information about monolayer films and, when used in combination, can provide a comprehensive assessment of monolayer film properties at both the solid-air and liquid-air interfaces. In this review, we compare these techniques in terms of spatial resolution, mechanism of contrast and potential imaging artifacts for the characterization of several representative mixed monolayer films.

Keywords monolayers; surfactants; atomic force microscope; Brewster angle microscope; confocal fluorescence microscope; phase-separation

1. Introduction

Surface active molecules (surfactants) are used in a diverse range of important technological applications, including oil extraction and recovery, development of drug delivery formulations, flotation of minerals in mining processes and a host of others. Assessing the structure, composition and mechanical properties of adsorbed surfactant films, both at solid-air and liquid-air interfaces, is crucial for optimizing many of these applications and a range of modern microscopy imaging techniques have, either individually or in conjunction, proven to be invaluable in this regard. The objective of this mini-review is to survey, compare and contrast three surface-sensitive microscopy techniques, namely atomic force microscopy, confocal fluorescence microscopy and Brewster angle microscopy for the structural and chemical analysis of mixed surfactant monolayer films.

There has been significant interest in recent years in monolayer surfactant films comprised of mixtures of hydrogenated and perfluorinated surfactants. Several examples of typical surfactant molecules that can form these mixtures, and those used in the model monolayer film systems described in this review, are shown in Figure 1. While many scientists working in surfactant science are broadly aware of the physical properties of hydrogenated surfactants, perfluorinated surfactants are generally less well known. Perfluorinated surfactants such as the fatty acid perfluorotetradecanoic acid and perfluorooctadecanoic acid shown in Figure 1C, D tend to have low surface tension, spread rapidly at the air-water interface, are highly permeable to gaseous oxygen and are often highly lipophobic [1]. Significant concerns relating to the long-term bioaccumulation and potential toxicity of some fluorinated molecules exist (see for example [2]), but there are nonetheless numerous valuable technological applications for these materials.
Fig. 1 Chemical structures of A) arachidic acid (AA), B) 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), C) perfluorotetradecanoic acid (PA) and D) perfluoroocadecanoic acid (C18F).

When combined in monolayer films (referred to as Langmuir or Langmuir-Blodgett (LB) monolayers at the liquid-air and solid-air interfaces, respectively), hydrogenated and perfluorinated surfactants can be thermodynamically miscible, partially miscible or completely immiscible, with the extent of miscibility depending on detailed chemical properties of the constituent molecules such as molecular weight and head group polarity [3]. The physical and chemical properties of mixed monolayer films are a strong function of the chemical identity of the surfactants that comprise them and overall film properties can often be tailored by manipulating film composition.

Under appropriate preparation conditions, mixed monolayer films form complex, diverse morphologies, with structural features ranging from nanometers to micrometers in size and with varying degrees of long-range order. Researchers in this field often wish to correlate film morphology with chemical composition and the underlying molecular-scale organization of surfactant molecules. Structural and compositional characterization of monolayer films is a significant technical challenge because of the nanometer (or smaller) length-scale of the features of interest in conjunction with the extremely small amount of material (one molecular layer) that make up the film. Great care must also be taken when imaging monolayer films composed of organic molecules (and perfluorinated molecules in particular) as they are prone to measurement-induced damage. For example, electron or x-ray based spectromicroscopies, while capable of combined structural and chemical mapping, often cause severe sample damage to mixed hydrocarbon-perfluorocarbon monolayers [4]. Only microscopy techniques that offer extremely high spatial resolution, sensitivity, surface selectivity and that cause minimal sample perturbation can be used to successfully characterize these systems. Further, it is often desirable to measure monolayer films at both the solid-air and liquid-air interfaces, and there is no single microscopy technique that is capable of meeting all of these requirements.

While no individual microscopy technique can fully characterize mixed monolayers, there are a number of modern instrumental approaches that can provide useful partial information. For LB films, scanning-probe microscope techniques, and in particular, the atomic force microscope (AFM), are well-suited for morphological mapping. The AFM, developed in the early 1980s as an offshoot of the Scanning Tunneling Microscope, has subsequently found wide-spread application in the imaging of surface structures at the micrometer to nanometer scale and has proven to be invaluable for imaging of soft, non-conductive materials [5]. At its most basic level, the AFM operates by raster scanning a sharp probe tip (radius of curvature typically tens of nanometers) over a surface of interest and correlating deflections of the probe with the underlying surface morphology. A huge number of variations of AFM imaging have been developed, including intermittent contact mode (“tapping mode”) imaging, mechanical imaging (friction, phase, pulsed force), electronic imaging (conductive, magnetic) and others [6]. AFM imaging provides excellent spatial resolution (typically several nanometers in the lateral direction, limited primarily by convolution of surface features with the finite-sized tip, and less than a nanometer in the vertical direction, generally limited by electronic or vibrational noise), and is often non-destructive. However, AFM images intrinsically lack chemical information and combined morphological - compositional mapping with the microscope can be challenging, even when using more exotic variations of AFM imaging. Further, it must also be noted that AFM imaging is only applicable to solid-supported films, and when characterizing phase-separation and surfactant monolayer film growth, the dynamic surface is the air-water interface. Caution must also be taken that the LB deposition process itself does not alter the surfactant film structure, as has been seen in a number of different surfactant systems (see [7, 8], for example).

Confocal fluorescence microscopy is of great utility for imaging mixed surfactant films, and fluorescence microscopes are both well-developed and commonly available in many research labs. For monolayer analysis, films are
doped with trace amounts (typically <1 mol% of the total surfactant content) of a fluorescent surfactant analogue (commonly a fluorescent fatty acid or phospholipid) prior to deposition onto solid substrates and then imaging in a confocal fluorescence microscope (CFM). Because of the excellent sensitivity of modern fluorescence microscope detectors (typically low dark-count photomultiplier tubes), high numerical aperture (NA) objective lenses and highly-emissive and photosensitive fluorescent probes, confocal fluorescence microscopy can easily detect emission from a single molecular layer of surfactant. Spatial resolution is limited by optical diffraction to ~300 nm, though the advent of super-resolution microscopy techniques (see [9] for a review) offers potential order-of-magnitude improvements on this. Fluorescence microscopy can be used to image monolayers at both the solid-air and liquid-air interfaces, though the instrumentation required for the latter is somewhat specialized and will not be discussed further in this review. The primary shortcoming of confocal fluorescence microscopy for LB film analysis is that the technique is prone to artifacts related to partitioning of the fluorescent probe molecule between different phases (liquid-expanded and liquid-condensed phases) of the film (see for example, [10]). For example, in mixed perfluorocarbon-hydrocarbon films that are labeled with a fluorescent fatty acid analogue, non-fluorescent regions in the sample can be attributed to the presence of perfluorocarbon or the presence of a liquid-condensed region that has “squeezed out” the fluorescent probe (vide infra). This can significantly complicate analysis of CFM images for these systems.

Characterization of monolayer film structure and dynamics at the air-water interface is most commonly performed with a Brewster angle microscope (BAM). With this microscope, image contrast is generated optically by reflecting laser light off a monolayer film-covered surface [11]. When p-polarized light impinges upon a clean water surface with an angle of incidence equal to the so-called Brewster angle, (around 53° for the air-water interface) light is entirely transmitted. However, when a surfactant film with refractive index different from that of water is present, a substantial fraction of the light is reflected at the interface and can be used to generate an image. The approach generates diffraction-limited images of monolayer film structure directly at the air-water interface with video frame rates and does not require the addition of probe molecules to the surfactant film. Image contrast can, in theory, provide information about chemical composition and details of molecular organization within the monolayer, though in practice, extracting this information can be problematic [12-14]. Because of this issue, discussed in detail below, BAM imaging is primarily used as a qualitative tool for measuring gross film morphology and dynamics. An additional shortcoming of the technique is that while the approach offers diffraction-limited imaging, modern BAM instrument optics are typically optimized for large-scale imaging and the diffraction-limited size of domains is typically on the order of several microns, further limiting the technique’s ability to extract molecular-scale information from films.

In the following article, we discuss these film characterization issues in further detail, drawing from several different examples of mixed perfluorocarbon-hydrocarbon monolayer surfactant systems studied previously in our lab [4, 15-21]. The relative benefits and disadvantages of the three microscopy techniques are discussed, and the merits of combining all three approaches to provide an in-depth characterization of a mixed film system are provided.

2. Materials and Methods

2.1 Monolayer film preparation

Monolayer surfactant films were typically prepared by depositing aliquots of a mixed surfactant solution (see Figure 1 for surfactants used) in a volatile organic solvent onto a clean, aqueous subphase (ultrapure Millipore water, pH 5.5, resistivity of 18.2 MΩ⋅cm⁻¹) supported in a Langmuir trough (KSV) and allowing the organic solvent to evaporate. The surface pressure (π) was measured with a Wilhelmy balance equipped with a roughened platinum or paper Wilhelmy plate. Films were then compressed to the desired surface pressure (see text for specific examples) with typical compression rates of ~ 10 mm⋅min⁻¹. Films would either be deposited onto clean, solid substrates (freshly cleaved muscovite mica for AFM imaging or plasma-cleaned microscope coverglass for CFM imaging) and allowed to dry in air, or measured directly at the air-water interface via BAM imaging. For fluorescence imaging, mixed surfactant solutions were doped with ~2x10⁻³ mol% Bodipy-PC (2-(4,4-difluoro-5-methyl-4-bora-3a,4a-diaza-s-indacene-3-dodecanyl)-1-hexadecanoyl-sn-glycero-3 phosphocholine) prior to deposition.

2.2 Atomic force microscope measurements

AFM imaging was carried out in either contact or tapping mode using a commercial instrument (Dimension Hybrid Nanoscope, Veeco Metrology). Images were typically 20 μm x 20 μm in size, with 512 data points per line. Monolayer films could be imaged repeatedly without causing tip-induced damage, though most of the films under investigation could be entirely removed from the substrate (“scratched” off) if desired by repeatedly scanning the sample at high operating forces with a stiff cantilever.
2.3 Confocal fluorescence microscope measurements

Fluorescence images (generally 100 μm x 100 μm) were collected on a modified Zeiss LSM410 laser-scanning confocal microscope using the 458 nm excitation line from a multiline argon-ion laser. Emission from the sample was filtered through a 500 nm longpass filter before reaching the detector.

2.4 Brewster angle microscope measurements

BAM imaging was performed at the air-water interface using a commercial microscope (UltraBAM, KSV-NIMA) equipped with a 658 nm laser illumination (nominal p-polarization), a 0.25 NA microscope objective lens and a CCD detector (frame capture rate of 20 frames per second). The instrument also had a polarizing optic (analyzer) positioned between the reflected beam and the detector to allow for anisotropy-based reflection measurements.

3. Results and Discussion

Our group has spent considerable effort over the past few years investigating film morphology and domain growth mechanisms in mixed monolayer LB films comprised of arachidic acid (AA; Figure 1A) and perfluorotetradecanoic acid (PA; Figure 1C) [4, 17, 18]. These two surfactants are perfectly immiscible at the air-water interface and their LB films exhibit a characteristic phase-separated polygonal surface morphology. An AFM image of a 2:1 mole ratio AA–PA LB film deposited at a surface pressure of π = 20 mN⋅m⁻¹ is shown in Figure 2A, along with a cross-sectional analysis. The polygonal domains are typically several microns in length and 0.7 nm in height above the matrix, with the former varying strongly as a function of the amount of time the film components are allowed to incubate at the air-water interface (i.e. domain growth period) before deposition. For this mixed system, AFM imaging alone can provide a great deal of information about film structure and when used in conjunction with reasonable inferences, can also be used for molecular-scale chemical mapping. The height difference between the domains and matrix, a parameter to which the AFM is exquisitely sensitive, corresponds extremely well with the difference in nominal length of the AA and PA molecules (2.5 nm and 1.8 nm, respectively). This suggests that the polygonal domains consist of vertically adsorbed AA surrounded by a continuous matrix of vertically adsorbed PA. Of course, additional information is needed to verify this assignment; in the case of AA-PA films, we have done so through a variety of additional AFM experiments, including systematically varying the composition of film and measuring the resulting change in area occupied by the purported AA domains as well as selectively removed the AA via soaking films in a suitable organic solvent prior to imaging and identifying previously occupied regions [18]. Assignment of the polygonal domains to AA was later independently verified through use of x-ray photoelectron emission microscopy [4].
While AFM images intrinsically lack chemical information, in the case of the AA-PA mixture a simple metric (differences in molecular length) that allowed chemical identification was measurable because the component molecules were aligned vertically and the AFM is exquisitely well-suited to measuring small vertical distances. While fortuitous, this convergence of events is a rarity and many mixed perfluorocarbon-hydrocarbon systems are not so amenable to this simple analysis. Further, the act of transferring the film from the liquid-air to solid-air interface must cause minimal perturbations to the film or risk further complications in chemical mapping. To illustrate, consider the morphology of LB monolayer films prepared from a fully-miscible combination of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC; Figure 1B) and perfluoroctadecanoic acid (C18F; Figure 1D) deposited at $\pi = 30 \text{ mN} \cdot \text{m}^{-1}$ [16]. Note, the thermodynamic miscibility of the film components was verified through $\pi$–area isotherm measurements. Figure 2B shows an AFM image and cross-section of a 1:1 mole ratio film prepared on a pure water subphase. Films consisted of elevated thread-like structures (typically 4.5-5 nm in height) on a continuous matrix. For this miscible mixed system, there were no readily identifiable domains that could be assigned to one particular chemical component; the heights of the thread-like structures indicated they were multilayer aggregates of surfactant and there were no other surface features that were readily detectable. Further, the DPPC-C18F monolayer films were highly cohesive (Gibbs excess free energies of mixing were $\sim -2 \text{kJ mol}^{-1}$), and the strong interactions between the DPPC and C18F likely resulted in formation of multimolecular aggregates on the mica substrate instead of a continuous monolayer film. In short, the AFM can provide gross morphological mapping for this system, but cannot easily be used for chemical mapping applications. While highly sensitive to surface structures, the chief failing of the AFM, at least in its simplest mode of operation, is its fundamental lack of chemical information.

Confocal fluorescence microscopy can be well-suited for structural and chemical mapping of mixed perfluorocarbon-hydrocarbon monolayer films, but great care must be taken to avoid artifacts intrinsic to the technique. To illustrate these issues, a CFM image of a 1:1 mole ratio LB film of DPPC-C18F that has been doped with a fluorescent DPPC analogue (Bodipy-PC) is shown in Figure 3A, along with a control sample of a pure DPPC monolayer containing the same fluorescent label in Figure 3B. As noted previously, thermodynamic measurements indicate that the DPPC-C18F system is entirely miscible, and the (somewhat naïve) expectation might be that the images should be uniformly fluorescent, corresponding to uniform distribution of the fluorescent probe throughout the film. However, as seen in Figure 3A, this is clearly not the case. For the mixed film, images consisted of three distinct regions, including a moderately fluorescent continuous matrix (labeled ‘a’), non-fluorescent voids (labeled ‘b’), and occasional highly luminescent aggregates (labeled ‘c’).
One might be tempted to assign the dark voids to the non-fluorescent film component (C18F), the matrix to the fluorescent component (DPPC) and be somewhat baffled by the presence of the highly-fluorescent aggregates. However, this is incorrect. The issue is clarified by a control sample of DPPC doped with fluorescent analogue (Figure 3B), which also shows three distinct regions of different luminescent intensity (moderately fluorescent matrix, voids and occasional aggregates). As noted in the Introduction, fluorescent lipid probes are often preferentially squeezed-out of liquid-condensed regions in phospholipid films, which results in, even for single component phospholipid films, regions that are non-fluorescent. This effect is particularly important in highly-compressed films such as those used in our studies (films are typically compressed to $\pi = 20 - 30$ mN·m$^{-1}$ to ensure stable deposition) and because of this, all fluorescently-labeled films will necessarily contain non-fluorescent void regions. On the basis of results from the control samples in conjunction with the corroborating thermodynamic measurements, the dark voids can be assigned to liquid-condensed regions of the mixed film in which the fluorescent probe has been squeezed out and the continuous matrix to mixed regions that are homogenously loaded with the probe. The composition of the bright aggregates is more difficult to determine, and we have observed them in a wide range of different mixed films labeled with various fluorescent probes. We have tentatively attributed formation of these aggregates to accumulation of fluorescent probe molecules in pinhole defects in the film, or to film drying-related artifacts [15]. Further exploration of this issue is required in order to extract maximum useful information out of fluorescence images of these samples.

BAM imaging is ideally suited for characterizing monolayer film structure and dynamical processes directly at the air-water interface because of its surface sensitivity, high temporal resolution and a mechanism of image contrast that does not require addition of a fluorescent probe molecule to samples. Figure 4A shows a typical BAM image of the AA-PA mixed monolayer system taken at the air-water interface (surface pressure of $\pi = 1$ mN·m$^{-1}$) and a series of images at different analyzer settings. BAM image consisted of a series of highly-reflective domains surrounded by a continuous matrix, similar to what was observed in the AFM images (Figure 2A).
Fig. 4 A) BAM image of a 2:1 mole ratio AA:PA mixed film at the air-water interface ($\pi = 1 \text{ mN} \cdot \text{m}^{-1}$, analyser setting of $0^\circ$), B), C) and D) are BAM images with different analyser settings of $-15^\circ$, $-10^\circ$ and $0^\circ$ respectively. E) Illustration of molecular tilt angle ($\theta$) and molecular tilt azimuth ($\phi$). F) Illustration of a domain comprised of molecules with identical molecular tilt angles but different molecular tilt azimuths.

In general, the signal intensity (reflectivity) in BAM images is related to the refractive index at the interface, the roughness of the interface, the thickness of the interface and any molecular-scale anisotropy of the surfactant film [11]. Because of the complex inter-relationship between these variables, as well as experimental fluctuations in illumination intensity and background scattering, reflectivity values from BAM images of monolayer films are rarely used in a quantitative fashion. However, it is worth considering the images in Figure 4 in the context of these parameters to provide additional insight into monolayer film structure. In terms of the refractive index effects, a complication unique to BAM imaging of mixed perfluorocarbon-hydrocarbon systems is that the refractive index for perfluorocarbons is similar to water ($n_{\text{water}} = 1.33$, $n_{\text{fluoroalkanes}} = 1.27 – 1.29$ [22]), and because of this, perfluorinated surfactants are effectively undetectable. Hence, the bright domains in Figure 4A correspond to regions of AA, whereas the dark regions are either water subphase or the perfluorocarbon. Interfacial roughness is caused by thermal fluctuations of the water subphase and is generally believed to give rise to deviations in film thicknesses of $\sim 3\AA$ which causes some considerable limitations on potential quantitative analysis, should it be attempted.

However, it is the latter two issues noted above, film thickness and molecular orientation, that are particularly important in BAM image analysis of ordered monolayer films. In general, polarized light reflectivity is a function of film thickness as well as molecular tilt angle ($\theta$) and molecular tilt azimuth ($\phi$) (Figure 4E). Consider, now, a monolayer that consists of two distinct domains, in which the domains differ only in terms of molecular tilt azimuth. It is possible for domain to have an identical thickness and molecular tilt angle, yet still generate different reflectivity values because of differences in molecular tilt azimuth (Figure 4F). To extract this information, the analyzer, which lies between the reflected beam and the detector, can be rotated, which in turn provides molecular orientation information. For the AA-PA system studied here, we note that there are considerable differences in contrast within a domain as a function of analyzer angle (Figure 4B-D), which suggests that the domains contain further sub-domains with different azimuthal tilt angles. AFM imaging of samples prepared under similar conditions (data not shown) did not show any substantial variation in film thicknesses within the domains, providing supporting evidence that there are subtle variations in molecular tilt azimuths for these systems.

For the sake of completeness, we note an additional important limitation of BAM imaging for characterizing monolayer films. Since BAM relies upon optical contrast, image resolution is ultimately diffraction-limited. Using the Rayleigh criterion (smallest resolvable distance between two points), this limits spatial resolution to $\sim 2 \mu \text{m}$ for the microscope used in our experiments. This is intrinsically a different length-scale of operation than the AFM and the confocal fluorescence microscope (spatial resolutions on the order of nanometers and several hundred nanometers, respectively) and as such the BAM is more appropriately used for assessing micron-scale film structures rather than nanometer-scale structures. This also tends to limit the range of surface pressures over which BAM experiments are useful. As monolayer films are compressed, the separation between surface structures is decreased and once the spacing between structures reaches the resolution limit of the instrument, the images become uniform in intensity. For the AA-PA systems studied here, this occurs at pressures above $\pi = 1 \text{ mN} \cdot \text{m}^{-1}$. This is a fundamental limitation of the technique, and puts significant restrictions on the range of experimental conditions that can be used for monolayer film studies.
While each of the techniques described above offers its own unique advantages and disadvantages, it is apparent that using all three approaches in combination provides the best possible method for fully-assessing morphology, composition and molecular-level organization of mixed perfluorocarbon-hydrocarbon monolayer films. To illustrate this, we show the combined results of AFM, CFM and BAM characterization of a mixed DPPC-C18F film, prepared under slightly different conditions to those described in the previous section (now using a 1:1 mole ratio mixture of DPPC-C18F prepared on a pH 7.4, 150 mM NaCl aqueous subphase). Results are shown in Figure 5A-C.

AFM images of films deposited at $\pi = 2$ mN-m$^{-1}$ consisted of a series of well-separated, discontinuous domains, typically 1-2 µm in diameter and 2.0 - 2.5 nm in height above a surrounding continuous matrix. Height differences alone do not provide any immediate information on film composition; the two film components have comparable lengths (2.8 nm for DPPC and 2.5 nm for C18F) and the fortuitous circumstances that allowed chemical assignment in the AA-PA system does not apply here. However, there is useful information contained in the images. Clearly there is well-defined domain structure for these samples, suggesting phase-separation, and if the two regions are fully phase-separated DPPC and C18F, the AFM results suggest that one of the film components may be oriented with a significant molecular tilt angle with respect to the other. The CFM results support this, and consisted of a uniform bright background, with a series of discontinuous dark patches (1-2 µm in size). The general film structure and lateral domain sizes (well within the spatial resolution of the CFM) are in good agreement with the AFM data, and taken in combination, suggest the bright continuous matrix consists primarily of liquid-expanded phase DPPC and the dark patches consist of C18F. We note that at the relatively low deposition pressures used to prepare these samples ($\pi = 2$ mN-m$^{-1}$), there will be minimal formation of liquid-condensed DPPC regions, and the dark patches do not consist of liquid-condensed regions from which Bodipy-PC has been squeezed out. Furthermore, liquid-expanded DPPC is unlikely to adsorb normal to the underlying substrate, but rather lie flat (or with a considerable tilt angle to normal), which is consistent with the proposed interpretation of the AFM images. Finally, BAM imaging also provides useful corroborating evidence of this interpretation. Similar to the CFM image, the samples consisted of a bright continuous matrix and numerous dark patches, which must correspond to the DPPC and the C18F, respectively, thanks to the difference in refractive indices of the two samples. The final structural-compositional model for this mixed film is shown in Figure 6. In short, the cumulative information provided by all three imaging approaches allows a complete assignment of the film structure, chemical composition and molecular-scale orientation in the mixed perfluorocarbon-hydrocarbon monolayer.
Fig. 6 Schematic illustration of interfacial organization of a 1:1 mole ratio DPPC-C18F mixed monolayer film using a subphase with 150mM NaCl (pH 7.4) at the solid-air or liquid-air interface.

4. Summary

While there are a number of excellent modern microscopy techniques available for the morphological, compositional and molecular-level orientation mapping of mixed perfluorocarbon-hydrocarbon monolayers, no individual technique can provide all of the necessary information to thoroughly characterize these surfactant systems. Atomic force microscopy, while providing exquisite spatial sensitivity, intrinsically lacks chemical information; confocal fluorescence microscopy can provide both spatial resolution and chemical sensitivity but is prone to labeling artifacts; and the Brewster angle microscope can provide chemical sensitivity and information about the air-water interface but provides limited spatial resolution and is insensitive to perfluorocarbons in Langmuir films. Taken in combination, these three microscopy techniques can provide thorough and comprehensive mapping of fundamental properties of important mixed monolayer systems.

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5. References


