Scanning force microscopy: new instrumentation and applications
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New instrumentation has led to new ways in which to generate image contrast in scanning force microscopy. Friction and adhesion forces can be used to map out local surface chemistry or biological activity. Gradients of electric or magnetic fields can be used to determine the distribution of liquids, surfactants or polymers adsorbed to surfaces. 'Tapping' the scanning tip against a surface can reveal local viscosity, surface compliance or shape fluctuations in addition to minimizing surface interactions.

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Abbreviations
AFM atomic force microscopy
EFM electric force microscopy
SFM scanning force microscopy

Introduction
The demands of technology keep pushing materials synthesis and characterization further and further into the submicron scale [1**,2,3,4*,5*]. As the dimensions of the structures of interest shrink, there is inevitably an increasing effect of surfaces and interfaces on materials' properties. There is also increasing interest in the structures of soft or self-assembling colloidal materials, including biological membranes and organic thin films. For these nanometer scale structures, it is often difficult to distinguish between the bulk and the surface. Even macroscopic materials are undergoing closer scrutiny as their adhesion, friction, electronic and catalytic properties invariably depend strongly on the first few atomic layers [1**]. It is difficult, however, to determine both the average and local properties of any surface at sufficiently high resolution without resorting to direct visualization, that is, some microscopy technique is required. The most suitable instrument for the usually non-conductive materials of colloid science is the scanning force or atomic force microscope, which was invented by Binnig, Quate and Gerber in 1986 [2]. Recent developments in scanning force microscopy (SFM), especially the refinement and commercialization of 'tapping' [6**,7**,8] and 'lateral force' or friction modes [9,10,11**,12**,13*,14,15**], can provide sufficient contrast to distinguish local chemical or topological features, even on rough surfaces at nanometer resolution. Non-contact modes have been developed that use gradients in electric [16**,17*,18**] and magnetic [19] fields to map out the distribution of charges or magnetic domains on surfaces without deforming or even touching the surfaces. These new microscopies have grown out of earlier work that used scanning probe microscopes to measure van der Waals and hydrogen bonding [20,21*,22,23,24*], and specific ligand-receptor forces [25**,26] as a function of tip-sample separation. Scanning probe microscopes are also capable of controllably modifying surfaces at nanometer scales to pattern surfaces [27,28] and are capable of inducing local chemical changes [29**]. Finally, conventional contact mode SFM can resolve a variety of soft, organic surfaces at sufficient resolution to determine lattice parameters with precision comparable to synchrotron X-ray diffraction [30,31,32**,33*,34–39]. In addition, SFM can resolve even highly deflected or multiple coexisting lattices at molecular resolution [32**,33*]. As a result of this wide range of imaging possibilities, SFM techniques are joining the front line of colloidal research.

This review highlights developments in SFM instrumentation and applications to colloid science over the past two years. Especially important have been applications to the structural or dynamic properties of thin organic films, biological colloids and polymer composites.

Contact mode scanning force microscopy
A conventional scanning force or atomic force microscope traces out contours of constant force [2,3,4*,5*] by rastering a microfabricated cantilevered tip across the sample surface. As the atomic force microscope (AFM) tip is scanned across the surface, the cantilever is deflected by short-range, or contact, forces caused primarily by variations in surface topography. In most commercial instruments, the cantilever deflection is magnified by an optical lever technique [3]. A feedback loop controls the voltage to the vertical piezo element on which the sample is mounted so that the force is held constant as the tip is scanned across the surface of the sample with the horizontal piezo element. An image is built of many scans, each offset from the previous one. This mode of imaging is now commonly called 'contact' mode, in that the SFM tip is in direct contact with the sample during the rastering and imaging process. With contact mode imaging, lattice images at 0.1 nm resolution, which are sufficient to determine lattice constants and symmetry with comparable resolution to synchrotron X-ray diffraction, can be routinely obtained [30,31,32**,33*,34–39,40*]. Moreover, only contact mode SFM provides direct images of grain boundaries and molecular defects in soft organic films [31,32**].
Molecular resolution image and Fourier transforms (FT) of Langmuir–Blodgett films of zinc arachidate (ZnA₂) on mica. (A) 20 nm by 20 nm image of a seven-layer ZnA₂ film showing a defect-free molecular resolution lattice. (B) Two-dimensional FT of (A). The six strongest reflections in the FT form a distorted hexagonal pattern with two distinct lattice repeats of 0.483 nm by 0.557 nm with an angle of 54.7° between them. The resulting interfacial area per molecule is 0.22 nm². The lattice repeats and the measured bilayer thickness of 4.7 nm show that the molecular packing is hexagonal and that the molecules are tilted toward nearest neighbors at an angle of 30°. From the measured interfacial density and tilt, the cross-sectional area perpendicular to the chains in ZnA₂ is about 19 Å², a 5–6% lower density than in CdA₂, in which the chains adopt a closer-packed herringbone lattice [30]. (C) Image (20 nm by 20 nm) of a three-layer film of ZnA₂ showing the three sets of lattice rows. Compare the highly defected structure of this trilayer film to that in (A). (D) Corresponding two-dimensional FT of the image in (C); note the broad, arc-like reflections indicative of long range orientational order, but short range positional order. This heaxatic phase shows that the atomic force microscopy is capable of resolving even highly deflected lattice structures at molecular resolution. The lattice parameters, symmetry and area per molecule are the same as those for the seven layer films.

Contact mode imaging is best suited to chemically homogeneous surfaces that have a local radius of curvature that is large in comparison to the dimensions of the SFM tip [41,42]. Hence, for molecular resolution images, the surface to be examined must have a root mean square roughness of less than a nanometer, as even the best available SFM tips are from 20–50 nm in diameter. As a result, most nanometer resolution SFM images have been from cleavage planes of crystals such as mica, silicon or calcite [3,4,5,23], or from organic films deposited on such surfaces by self-assembly [9,10,11*,12*,13*,14,15*] or Langmuir–Blodgett deposition [30,31,32**,33*,34–39].

The SFM has been used by a number of groups for investigating Langmuir–Blodgett film structure and defects at length scales from 100 μm to 0.1 nm [30,31,32**,33*,34–39]. Prior to SFM, technical applications of these thin films had been limited due to the lack of a non-destructive characterization tool (see Fig. 1). Recent work has shown that even the prototypical organic films made from simple fatty acid and phospholipids exhibit a host of structural features including amorphous [30], hexatic [32**,43], and crystalline [31,32**,37,38] in-plane order; lattice spacings and positional correlations coupled to the type of counter-ion, number of layers and proximity to the substrate [30,32**]; multiple lattices coexisting in the same layer [30,40*]; symmetry breaking of achiral molecules into chiral lattices [35] and periodic defect structures that arise from competition between preferred head and tail lattice spacings [44]. Dislocations and grain boundaries can be seen directly in Langmuir–Blodgett films with SFM, and their effects on film applications assessed [31,32**,43]. Perhaps as a result of our improved understanding of film structure, applications for Langmuir–Blodgett films are increasing in the form of lithographic films [27] and templates for growing or orienting inorganic crystals [45**] or biomolecules such as DNA or proteins [5*,46*,47**] for subsequent imaging by SFM. Further improvements in resolution on soft materials may come from the recent introduction of a low temperature SFM, operated in liquid nitrogen vapor at temperatures ranging from 77–220 K [47**].

'Non-contact' modes of imaging

Contact mode AFM, although ideal for homogeneous films, is less optimal for chemically heterogeneous or rough surfaces. Several new modes of AFM imaging have been developed to exploit chemical and physical hetero-
geneities to create image contrast. The most important new development is what is called ‘tapping mode’ AFM [48]. In tapping mode, the sample is vibrated at high frequency (typically about 20 kHz) so as to gently tap the SFM tip onto the sample. The response of the cantilever to this oscillation depends on the interactions between the tip and the sample. The amplitude of the oscillations is generally set to be from 1–10 nm, depending on the feature heights expected on the surface. The sample is rastered at a much slower speed than it is oscillated, so that lateral forces are minimized. That is, the tip is repeatedly tapped across the sample, rather than being dragged across the sample as in contact mode. In addition to minimizing lateral forces, the normal forces are also reduced by one to two orders of magnitude, from 10–100 nanoNewtons in contact mode to 10–100 picoNewtons in tapping mode, especially under liquids [6*]. With tapping mode, soft protrusions such as membrane- or substrate-bound proteins can be imaged much more readily; Radmacher et al. [7**] have shown that the conformational change of an enzyme can be detected reliably. Tapping mode SFM can also be used to determine dynamic physical properties of the surface such as elasticity and compliance as a function of tapping frequency [8]. Tapping mode AFM will probably be ideal for diffusion and in situ growth studies, as the tapping does not lead to any appreciable force on diffusion markers or nucleating sites.

A second major development is to use contact mode AFM, but record the lateral force, or friction between the tip and sample in addition to the topography. Variations in the friction between the SFM tip and a chemically heterogeneous surface can be readily detected by using a four quadrant photo detector to monitor the twist of the cantilever in addition to its vertical motion [9,12**,13*,14,15**]. Friction force microscopy has been very successful in revealing two-dimensional phase separation in self-assembled monolayers [9,12**,14,15**] or polymer films [13] that is impossible to detect with contact mode AFM. Although it does not appear to be absolutely necessary for simple images, the SFM tip can also be functionalized to provide a complementary or antagonistic interaction with the surface to be imaged [10,11**]. These chemically well characterized tips are ideal for high resolution studies of adhesion and lubrication as well as for imaging chemical heterogeneities at nanometer resolution [11**]. Functionalizing the tip with biologically active molecules may make it possible to localize antigens or specific recognition sites on cells, or in a biosensor [25**,26].

It is also possible to use long-range forces to deflect the AFM cantilever without actually touching the sample at all. Long-range electrostatic forces, rather than contact forces, [16**,49,50**] can be used to map out a surface. Manner and Gaub [16**] have imaged very soft, hemicylindrical surfactant micelles loosely adsorbed onto graphite using double layer forces that were generated by the charged surfactants in solution. The resolution was sufficient to determine the shape of the micelles and their epitaxial arrangement on a graphite or mica substrate (see Fig. 2). This type of imaging should prove useful for characterizing the adsorption of surfactants, proteins, metals, and so on to various substrates in solution. As the AFM tip never actually comes into contact with the surfaces being imaged, there is no possibility of deformation. Localized force curves can also be made with the AFM to complement those obtained with the surface forces apparatus [16**,20,21*,49,50**].

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**Figure 2**

Aggregates of tetradecyl trimethylammonium bromide (C\(_{14}\)TAB) adsorbed onto a hydrophobic graphite substrate from a 70 mM solution (twice the critical micelle concentration). Long range electrostatic forces in aqueous solution were used for imaging. Adsorbates were imaged by choosing a force setpoint in the precontact region, using deflection mode with low integral gain. The aggregates were aligned perpendicularly to one of the graphite lattice directions, indicating that the C\(_{14}\)TAB molecules are epitaxially arranged on the surface. The thickness of the aggregates and their alignment suggest that the aggregates are hemicylindrical micelles [16**].

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One of the newest variants of AFM is called electric force microscopy (EFM), and it is especially useful in determining conductive pathways in samples. EFM measures electric field gradients near the surface of a sample by using a sharp conductive tip. The grounded tip measures these gradients by being oscillated near its resonant frequency. The effective spring constant of the tip is altered as it encounters a force gradient from the electric field, changing the cantilever’s resonant frequency. This change is monitored, producing a map of the strength of the electric field gradients. The EFM mode produces two images, one of topography and the other of electric field gradients. First, a line is scanned to give topography; a second pass is made over the same line for a prescribed distance above the topography to image the electric force gradients. This ensures that topography does not influence
(A) A 20×20 μm area of a carbon-black polyethylene composite with a carbon-black volume fraction of 0.1978. Tapping mode SFM image. The image is relatively featureless with some grooves from the mold being visible. (B) Electric force microscope image of the same area, taken simultaneously. Note the large degree of contrast between the conductive regions (black) and the insulating regions (gray). The feature size and shape of the conductive regions confirm that they are the carbon-black aggregates. From images such as these, it is possible to determine the electric percolation threshold and scaling behavior of the composite material [18**].

the electric field images. Viswanathan and Heaney [18**] have used EFM to determine the percolation behavior in a conductor–insulator composite. As can be seen in Figure 3a, the tapping mode image of the sample topography is relatively featureless, whereas the EFM image in Figure 3b clearly shows the location and distribution of the conducting carbon-black phase in the polyethylene insulator background. A similar electric field gradient technique was used to image the condensation and evaporation of thin layers of water on mica surfaces [17*].

Conclusions
New modes of SFM imaging should help colloid scientists investigate a number of phenomena, including adsorption, percolation, diffusion, phase separation and pattern formation at length scales impossible to probe by any other technique. Although many of these new imaging modes cannot reach molecular resolution, they are especially valuable in the colloidal size regime from about 1 nm to 10 microns. The best aspect of these microscopy techniques is that both averaged and local information can be obtained in realistic environmental conditions. This avoids the difficult preparative steps often required in electron microscopy while maintaining the real space images that are so much easier to interpret than scattering data, especially in phase separated or other heterogeneous systems. Almost any physical property of interest can be 'imaged' via a scanning probe microscope; all that seems to be required is a sufficiently small sensor and a good imagination. These new imaging techniques and
instruments are certain to challenge conventional colloid
science with a host of new data over the next few years.

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All the micrographs in this review are original.

References and recommended reading

Papers of particular interest, published within the annual period of review,
have been highlighted as:
- of special interest
- of outstanding interest

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AFM imaging directly, it is a useful point of departure for thinking about the
nature of what is really meant by "contact" in AFM.

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salts and how the interfacial area determined by the counter-ions determines the
packing of the hydrophobic alkane chains. This paper shows the ultimate
resolution of AFM on organic surfaces.

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under water or other liquids. This is one of the first papers to demonstrate
tapping mode AFM in liquids and points out how to minimize forces while
maximizing resolution.

observation of enzyme activity with the atomic force
Although not directly relevant to colloid science, this paper shows that it is
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resolution. The authors placed a tapping AFM tip over an enzyme and de-
termined conformational and shape changes in real time.

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especially important for phase separation in self-assembled and biological
thin films. See also [12*].

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organosilane monolayers by Langmuir-Blodgett deposition.
This paper also shows lateral force microscopy images of phase separated
domains of self-assembled monolayer films. In contrast to [11*], the tip was
not functionalized, but contrast was developed depending on the frictional
forces on different monolayers.

imaging of polymer surfaces with chemical sensitivity.
This paper uses lateral force microscopy to determine variations in surface
chemistry in a bulk polymer surface.

immobilized organosilane monolayer studied by a scanning

15. Wilbur JL, Biebuyck HA, MacDonald JC, Whitesides GM:
Scanning force microscopies can image patterned self-
This paper is very similar to [12*][13][14], but has a nice explanation of lateral force microscopy as well as nice images of chemically heterogenous
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collodial interest. The authors use electrostatic repulsion between the AFM
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generate an image of micelles adsorbed to the surfaces.

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coated AFM tip were monitored.

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Scanning Probe Methods. Edited by Guntheroth WJ. Netherlands:
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the lateral force imaging of heterogeneous surfaces discussed in [9,10,11*,12*,13*,14*,15*].

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A ligand-functionalized AFM tip is brought into contact with a surface coated
with the specific receptor for that ligand. The force of adhesion of single
receptor-ligand contacts is measured and was shown to be consistent with
measured binding constants for the ligand–receptor pair.


microscopy of the adhesion of surfactant monolayers at
This paper shows how lateral force microscopy can be coupled with a func-
tionalized tip to provide chemical information about surfaces. This work is
especially important for phase separation in self-assembled and biological
thin films. See also [12*].


This paper takes AFM lithography a step further in that instead of scratching an organic film off a substrate with the AFM, a chemically modified AFM tip catalytically etched an organic monolayer to pattern the surface.


This paper shows that AFM is a local probe with molecular resolution, even on highly defected lattices. This paper deals with imaging hexatic order in Langmuir–Blodgett films of zinc arachidate to show that the films have long-range orientational ordering, but short-range structural disorder.


This paper presents high-resolution contact-mode AFM images of phospholipid films under water or buffer as a function of pH and lipid composition. Monolayers have different resistances to the contact force depending on lipid composition and solution pH. The results were consistent with lipid packing arguments used to describe phase transitions.


This paper is an up-to-date review of STM and AFM analysis of Langmuir–Blodgett films.


The authors develop a geometric formalism to describe the convolution of the shape of the AFM tip with a rough surface. They provide a way of improving the surface resolution by a detailed consideration of the tip shape.


This paper is an excellent review of the use of colloidal chemical approaches to creating small particles or thin films of ceramics, semiconductors, or organics using wet chemical approaches.


This paper shows how DNA condenses on a cationic lipid bilayer to make imaging with AFM much simpler.


A low temperature AFM design is presented that appears to be ideal for the colloidal scale size. Low temperature can be used to eliminate Brownian motion, solidify soft materials, and so on.


This is one of the first papers to discuss the use of electrostatic interactions to image surfaces.