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Atomic Force Microscopy (AFM) and Its Uses

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Abstract

The basic principles of atomic force microscope are discussed. The mode of imaging and the mode of operation are described and compared with each other. Finally, a detail analysis on the application of atomic force microscope in the field of nanoscience, biological molecules and surfactants are discussed and the relevant experimental work is summarized.

Keywords:

Contact;
Tapping;
Topography;
Phase;
Force.

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1. Introduction

The invention of atomic force microscopy (AFM) in 1986 [1] is a milestone in the history of nanotechnology [2] that created new opportunities in physics, chemistry, biology and medicine. It is a powerful, multifunctional imaging platform that allows biological samples, from single molecules to living cells, to be visualized and manipulated. Scanning electron microscope suffered from a serious drawback as such the film under study was exposed to vacuum, which not only was liable to a significant change in topography, but also created undue artifacts not present otherwise. Other characterization methods such as scanning tunneling microscopy (STM) required conducting sample. In contrary, atomic force microscope was free from such drawbacks, and could be economically used to get surface images by controlling a number of forces acting between a tiny probe and a very flat solid surface without sample preparation along with information on thickness and height of crevices. Initially started with atomic scale imaging, gradually imaging of non-conductive surfaces in vacuum was obtained [3]. The usefulness of AFM lies herein: (i) It works over a vast temperature range and in almost all environment. [4]. The possibility to operate in liquid environments and at ambient temperature moved AFM towards biology and led to the analysis of biomolecules and cells at (sub-) nanometre resolution [5]. While imaging in buffer solution the native state of the biological system could be maintained, (ii) Signal – to – noise ratio at sub-nanometre resolution, (iii) A variety of probe may be used to locally sense interactions [2].

Other uses of AFM include study of conformation details of polymer chains adsorbed at the solid-liquid interface including their interactions with solid surfaces (crystal dissolution or growth), dynamics of adsorbed chains and changes affected by environmental conditions such as pH, ionic strength, solvent quality etc [6]. Also, a direct 3D visualization of polymer chains was possible. It turned out to be very useful to probe biomolecules (bacteria, protein, DNA) and biological samples from a single molecule to cells, to measure the binding force between ligand-receptor or antigen-receptor pairs [7], to study the mechanical properties of proteins [8] or even to quantify the force needed to extract protein from cellular membranes [7].

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It is also useful to study the nature of hydrophobic interaction in biological interfaces (say blood and polymeric medical devices) [9].

The main challenges that might be across in AFM measurements were unspecific interaction between AFM tip and substrate, aggregation of hydrophobic polymers, adsorption of contaminants present in air or dispersed in solution and the presence of air bubbles [10]. Despite the high resolution that AFM afforded, tip convolution always limits the lateral resolution achieved while scanning single molecules adhered to surfaces, producing images in which the molecules had oversized lateral dimensions. AFM provided morphological images, not chemical information. For chemical imaging, to get an idea of the presence and orientation of specific molecular groups, one need Raman imaging where nanometer resolution was obtained with scanning near field optical microscopy. In spite of these problems, AFM had been vividly used to characterize surface topography.

1.1. History

A major breakthrough occurred in 1982 when Gerd Binnig and Heinrich Rohrer working at IBM in Switzerland invented the Scanning Tunneling microscope (STM) [11], for which they were awarded the Nobel prize for Physics in 1986. The microscope was capable of imaging at the atomic scale without the need of lenses and overcome Rayleigh's criterion,

$$d=0.61\,\lambda / \mathrm{Sin}\alpha \quad (1)$$

where d is the minimum distance at which two-point light sources can be resolved and is typically of the same order of magnitude as that of the wavelength of light, λ, and α is the angular aperture of the objective lens. Theoretical resolution limit was ~200nm assuming the lens was perfect and free from aberrations. But the choice of material was partly limited by the requirement of a conducting material so that tunnelling current could be measured. However, the need for imaging insulating materials, such as polymers and biological specimens, was soon realized and in 1986, Gerd Binnig invented the atomic force microscope [1]. Here, there was no requirement for the sample to be conducting. It was the only topographic characterization tool of surfaces at a sub nanometer spatial resolution or resolving force with pico-newton sensitivity at ambient temperature. Frequency modulation AFM was advantageous for molecular resolution even on insulators [12]. Cryo-AFM operating at temperatures below 100K had yielded molecular sub structural images of immunoglobulin [13]. The microscope's ability to operate in a liquid environment, especially physiological buffers, makes it a promising tool for studying the native structure of biological materials.

2. Research Method

2.1. Basic Principles

The principle of AFM relied on the use of a sharp pyramidal tip mounted on a soft cantilever. The spring constant value (usually very low, ~ 0.01 - 40N/m) might be calculated from the cantilever's spring geometry. For a uniform, rectangular cross section, the cantilever's spring constant is given by

$$k_C = Ewt^3/4l^3 \quad (2)$$

where 'w' is the cantilever's width, 'l' is its length, 't' is its thickness, and 'E' is the elastic modulus. If the cantilever spring constant was known, the cantilever deflections might be converted to quantitative force data using Hooke's Law,

$$F=k_C z \quad (3)$$

where F is the magnitude of the force acting between the tip and the sample and 'z' is the cantilever deflection at its free end. Most cantilever probes were rectangular or triangular with a "two-beam" geometry connecting at the tip. Many AFM cantilevers were also coated with one or more layers of metal for reflectivity and other surface modifications. The cantilever was bought into close proximity to the surface where both attractive and repulsive intermolecular forces (van der Waals, electrical, magnetic, short range, capillary, or any other electromagnetic force) acting between the tip and the surface cause the cantilever to undergo a vertical deflection. Images of the sample's surface topography were obtained by recording the cantilever deflections using three most used deflection techniques: the laser reflection method, interferometric method and the piezo resistive method. In the laser reflection method, a laser beam was reflected from the cantilever surface and directed to a two- or four- quadrant photodetector. Any vertical movement of the cantilever produced due to the interaction made the beam to be reflected higher or lower in the detector. The light hitting any part of the detector was transformed into photocurrent. There were four independent light sensors and correspondingly four different currents could be used. The amount of photocurrent from the top part (1+2) minus the amount of current from the bottom part (3+4) is a signal proportional to the vertical motion of the cantilever [14]. Other components, as illustrated in Figure 1 include:

(i) A scanner in three dimensions that made the tip move relatively on the surface. Usually the motion or scanning was done with a piezoscanner. It was a device, made of piezoelectric materials that typically could be extended or contracted by applying suitable voltages. It was made to scan the sample following a

rectangular matrix, with a fast and a slow axis. The scanner also moved the cantilever up or down to obtain the topography of the sample. Piezoelectric scanner performance could be limited due to non-linearity in the scanner, noise, drift in the high voltage supply or thermal drift of AFM apparatus itself.

(ii) A feedback system that senses instantaneous cantilever deflection and adjusts scanner elements to maintain a constant interaction between the tip and sample. The ultimate output of the feedback loop was a representation of the sample topography or height. As shown in Figure 1, initially a signal processing circuit produces a signal. It was then compared to a 'set point', which was actually the cantilever deflection in contact mode or amplitude of the cantilever oscillation in tapping mode (measured by a lock in amplifier). On comparison, it generated an error signal which was the difference of amplitude of oscillation and the set point. The error signal was the input of a feedback calculation. The job of the feedback calculation was to keep the error signal equal to zero. The result of this calculation was then used to control the tip-sample separation through a high voltage amplifier. The resultant voltage was fed to a piezoscanner (discussed above) that ultimately changes the tip-sample separation to image the topography.

(iii) A software that could interpret data points and convert it into pixels for an image.

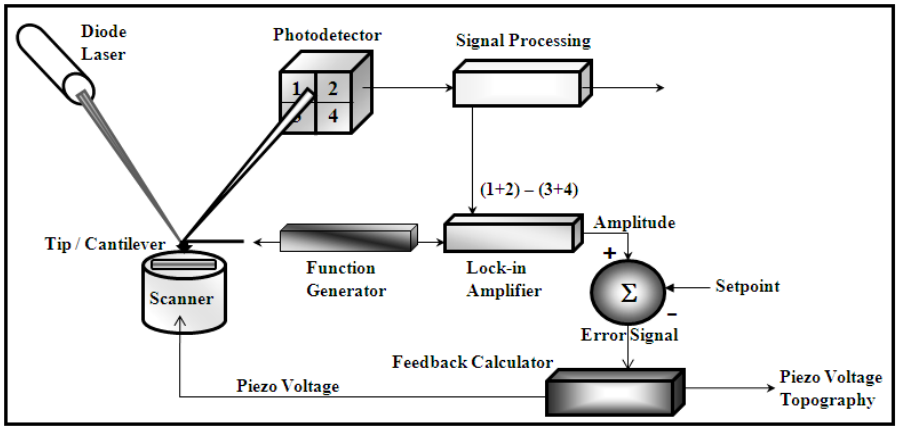

Figure 1. Laser reflection method and the schematics of a feedback loop in a non-contact mode AFM [15].

2.2. Mode of imaging

Many reviews had been published in the past two decades that described the use of certain AFM imaging modes to characterize systems [16-18]. Dufrene and his coworkers outlined the AFM imaging modes applicable for specific biological systems [19]. After the invention of AFM, it was found that in order to maximize the opportunities of AFM imaging particular in biology and in other fields, various technological developments would be required to address certain limitations of the method. This had led to the creation of a range of new imaging modes, which continue to push the capabilities of the technique today. There were two basic modes in AFM in which topography or phase images could be obtained: contact mode and non - contact mode.

2.2.1. Contact mode

The first AFM imaging mode invented was the contact mode in which raster scans a tip over the sample and adjusts pixel-by-pixel the height of the tip so that the force applied to the sample was kept constant (Figure 2a). The resulting height image resembled the sample topography with the resolution depending on the radius of the tip, sample corrugation, physical properties of the sample and how precisely the feedback system contours the tip over the soft biological sample. Here the loading force varied because of the thermally induced drift of the cantilever. This mode operated under a strong repulsive force between the tip and the surface. There were two basic ways to obtain topography in this mode: constant force mode, which was a constant deflection mode, and variable force mode, which was a free deflection mode. AFM could create permanent topographical patterns on a sufficiently soft surface in this mode at relatively high force and thereby "scratching" the surface (Figure 2a) [20]. This mode of AFM could provide topographs of single membrane proteins at lateral and vertical resolution of less than 1 nm and greater than 0.1 nm respectively. Forces greater than 100 pN should be avoided as they could cause reversible or irreversible deformations in soft biological systems [21].

2.2.2. Dynamic or Tapping or Oscillation or Non-contact mode

The advantage of the mode of imaging was: (a) to minimize the friction and the force applied between tip and sample, (b) the lack of lateral forces that pushed aside weakly adsorbed molecules and, (c) the small loading forces. Following were the techniques that were kept in mind while operating samples in

this mode: (a) The cantilever was oscillated close to resonance while scanning, (b) It was a technique in which the tip-surface interaction was in the contact and the non-contact region intermittently [22]. The tip only touched the sample at the very end of its downward movement thus minimizing friction, (c) In close proximity to the sample surface, the interactions between tip and sample changed both the cantilever amplitude (amplitude modulation AFM) and resonance frequency (frequency modulation AFM), allowing them to be used as feedback parameters. When the AFM tip approached the sample, the tip jumped to the sample at a critical distance due to weak van der Waals attractive forces. This mechanical instability set a lower limit to the imaging force that could be used, which was circumvented in this mode. The only limit of the loading force was the accuracy of the electronics and the detection system. However, it did not vary with time as the AC detection method eliminated this problem. Some other forces could be in action, such as electrical and magnetic forces. The main advantage of non-contact mode compared to contact mode was that, as the tip was always in the weak attractive region the sample and tip were not easily damaged (Figure 2b). Imaging in liquid was enhanced by coating the cantilever with a magnetic film and using an oscillating magnetic field covering a wide range of frequencies to drive the cantilever (Magnetic non-contact mode AFM).

(a) (b)

Figure 2. Schematic drawing of an AFM in operation in (a) contact mode and (b) non-contact mode in liquids. In (a), soft samples were easily distorted by lateral forces [23]. In (b), the cantilever was oscillated sinusoidally with a frequency between 15-35 kHz. Lateral forces were minimized and the loading force could be controlled very precisely.

Other forms of improvisation on AFM include AFM-CW charge writing. It was a technique in which conductive tips were used to inject positive or negative electrical charges (by applying a bias voltage) into an insulating material (such as a polymer), that could trap charges for a sufficient amount of time so that the surface exhibits an electrostatic field [24].

2.3. Modes of Operation

2.3.1. Contouring a surface (structural imaging) or Topography

Particle morphology was obtained from AFM height images. Saturation particle densities (Γ_{max}) and surface coverage (θ_{max}) were estimated from digital analysis counting at different sample's areas. From the height profiles of the AFM images, preferential particle adsorption to the surface in monolayer coverage for $\kappa a < 0.62$, where κ = Debye screening length and a = particle radius and κa = screening parameter were evaluated [25]. The topography feature relied on the complex interaction mechanisms between the AFM tip and sample. Stiffness, roughness, surface charge and chemistry or friction with the sample could change the oscillation of the tip or the contrast of the picture. Thus, while imaging unknown samples, well characterized reference samples must be used [26].

2.3.2. Phase Image

Imaging in the non-contact mode also allowed the phase shift of the cantilever to be detected, which could yield information on the viscoelastic properties of the material that the cantilever was touching. If the tip touched some material, which was soft or sticky, the phase of the cantilever oscillations would lag that of the driving oscillator. This phase information could allow for the detection of biomolecules or surface changes of nanoparticles [27] that might be difficult to image in topographic mode. The interaction between the scanning tip and the sample surface might consist of attractive and repulsive forces, resulting in different phase shifts or phase lags. Tapping mode-AFM phase images were generated based on the phase lag between the oscillation frequency of cantilever and driver. Since the phase lag reflected the interactions between the scanning tip and the sample surface, phase images contained the advantage of demonstrating the surface properties of particle samples. The force applied to the AFM tip could be adjusted for mechanical manipulation, the tip could be functionalized with chemical groups to manipulate specific sample regions. Bar et al. [28] pointed out that the force experienced by the AFM cantilever (or scanning tip) at the lower turning point of oscillation played the most influential role in the resultant phase images. Both physical

properties of sample surfaces and chemical characteristics such as the hydrophilicity / hydrophobicity feature of a sample surface affected the phase shift.

2.3.3. Force-distance curve analysis

The interactions between a tapping mode AFM scanning tip and sample surface consisted of attractive and repulsive forces, resulting in different phase shifts or phase lags. To investigate the force effects on the phase shift, various research groups had described the oscillation behaviors of the AFM scanning tip by measuring dynamic force curves and developing mathematical models. Repulsive forces occurring between the scanning tip and relatively stiff surfaces enhanced the phase shift towards the positive direction, while attractive forces resulted in a negative phase shift [29]. While chemical characteristics such as the hydrophilicity / hydrophobicity feature of a sample surface also appeared to affect the phase shift [30], most studies focused on the effects of the physical properties of sample surfaces on the phase shift. This was a powerful method to probe the mechanical properties of a sample. The growth mechanism of soap free polymerization of styrene [31], gradual transformation of probucol from an amorphous to a crystalline state indicating the stiffness increase in nanoparticles [32], *in vitro* and *in vivo* application to study the force of nanoparticle – cell membrane interactions using a surface modified tip [33] were the various facets of information available from force-distance curve analysis. Biophysical properties were measured by approaching the AFM tip to and retracing it from the biological sample while recording single force–distance (FD) curves. Approach FD curves gave information on height, surface forces and mechanical deformation of the sample, or derived its elastic modulus and energy dissipation. Retraction FD curves allowed adhesion forces to be measured. Force curves contained information about the surface properties such as topography, adhesion, friction, elasticity and interaction between single molecules [34,35].

2.3.4. Non - Topographic Measurements

Other non-topographic measurements include:

(a) Mechanical properties such as friction and stiffness,
(b) Electrical properties such as surface potential, electric polarization and surface charge,
(c) Electromechanical properties such as piezoelectricity and electrostriction,
(d) Magnetic properties of materials,
(e) In situ chemical reaction, such as corrosion in an electrochemical cell.

3. Results and Analysis

3.1. AFM in Nanoparticles

SEM & TEM were generally applied techniques to observe the morphology of nanoparticles. However, neither of these techniques could be used to evaluate the properties of nanoparticles in an aqueous environment because the sample should be completely dried before capturing sample's image. Cryogenic SEM and cryogenic TEM were employed for observing samples in a suspended state. But the rapid freezing method might negatively affect the molecular state of the sample. AFM was a promising tool for observing suspended samples providing information on the 3D topography of the sample in the nanoscale range. This method was applied under both ambient and fluidic conditions [36-39]. AFM tips functionalized with a carbon nanotube (CNT) had attracted considerable attention due to the high Young's modulus of the CNTs and their excellent aspect ratio [40]. CNT AFM tips were useful for their unique mechanical, chemical and electronic properties and high resolution can be used to image fine structures such as biological materials like DNA or protein, etc [41]. It was also possible to characterize at atomic scale top and lateral facets or defects of nanometer sized metallic gold particles by AFM under ultra- high vacuum (UHV) in contact mode. We have also imaged linear defects as atomic steps and cluster edges [42].

3.2. Topography of Surfactants

In situ AFM appeared in the 1990s was a powerful technique for imaging micelles adsorbed at the solid-liquid interface, thanks to the pioneering works of Manne et al. [43]. Adsorbed layer structure of cationic [44], gemini [45] or mixed cationic-anionic [46] surfactants on negatively charged mica, graphite, glass, gold or quartz had been well documented in literature [47, 48]. Here the interaction of the surfactants with the surface were rather strong, leading to self-assembled layers (~ 9nm thick) in which each surfactant molecule undergoes strong interaction with other surfactant molecules and/or with the underlying surface (repulsive at short separation distance due to high steric repulsion produced by surfactant layer on the surface) and depending on the surfactant concentration [49]. The surface micelle morphology was frequently different from the micelle shape predicted or observed in the bulk solution. The interaction between the substrate and the surfactant thus played a dominant role in the immobilization of the micellar structure at the surface and determining its interfacial morphology. Self-assembled monolayers of adsorbed molecules were of much interest because they had a dense and stable structure on an atomic level and had potential

application to corrosion prevention, wear protection etc. Aggregate morphologies varied from unstructured bilayer, mesh, cylindrical, hemicylinders to globular, and both electrostatic and hydrophobic interaction play a key role in a well-ordered molecular rearrangement. Self-assembly into surface micelles of non-ionic block copolymer at the air-water interface of diverse morphology (from spherical to rod like, lamellar or vesicular and finally to large compound vesicles) were well characterized by AFM [50]. Aromatic counterions, salts, alcohols and neutral molecules had been found to strongly affect the curvature of surfactant aggregates at the interface [51]. Surface topographies of submonolayers and multilayers of a single crystal of pentacene evaporated on SiO_2 in air have also been investigated by the tapping mode [52]. Very recently, Zhao et al. [53] worked out how to find the molecular volume of a single surfactant molecule from AFM measurements.

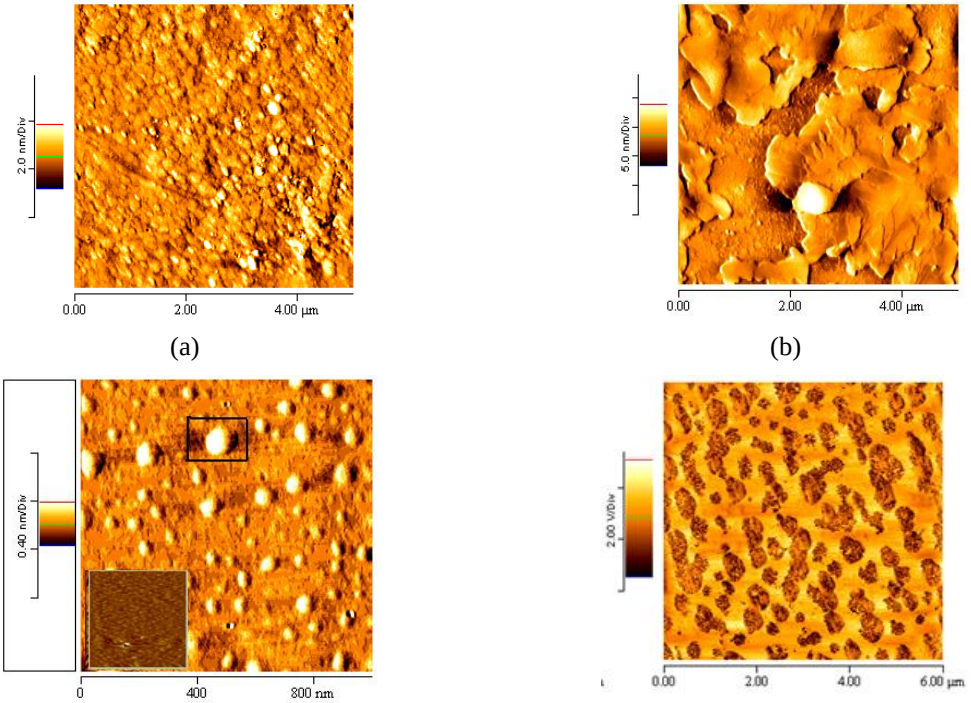

Figure 3. AFM images of gelatin – cetyltrimethylammonium bromide (CTAB) surfactant in boric acid – borax buffer at pH 9 [63]. (a) adsorbed film of pure CTAB surfactant in pre micellar region, (b) adsorbed film of pure CTAB surfactant in post micellar region, (c) adsorbed film of pure gelatin immobilized on silicon substrate, (d) adsorbed film during the interaction of gelatin – CTAB before precipitation.

3.3. Topography of Biomacromolecules

Proteins were best studied under buffer as their structures might be distorted by drying and the biological relevance of dry samples was questionable. However, while under buffer, a single protein was often not immobilized sufficiently well to image with contact mode AFM because they might be swept away or distorted by the scanning of the AFM tip. Non-contact mode AFM was generally more useful for imaging individual proteins under buffer. DNA condensation in presence of cations [54], or globular, filamentous, nodular shaped protein [23] was imaged with clarity. Shape and size of the biomacromolecule depended on the nature of surface (hydrophobicity/hydrophilicity) on which it was adsorbed [55-58]. It had also been employed to characterize the morphologies [59], growth mechanisms and surface energy of protein crystals [60]. Protein adsorption was found to be controlled by the nature and charge of the substrate only.

3.4. Topography of Polymer or Protein-Surfactant Complex etc.

Studies on protein-surfactant complex co-adsorption to explore the nature of colloidal adhesive force (attractive or repulsive) were in use in recent times [61]. Also using very soft cantilever and working with very low forces, the binding and rupture energy of a number of molecules in various environment could be accurately estimated [62]. AFM played a decisive role in studying the nature of polymer – surfactant and protein - surfactant interaction complexes at various stages of interaction (Figure 3). Interaction of protein (gelatin) with a cationic surfactant at various stages in the interaction profile produced images that corroborated well with SEM images [63]. Distinct variations in film organization of albumin – inhibited bovine lipid extract surfactant with and without surfactant protein A (SP - A) were revealed from AFM study and thus it played a vital role in exploring the root cause behind acute respiratory distress syndrome [64, 65].

Interaction between a lung surfactant protein D (attached to the surface) and various carbohydrate ligands (attached to the tip of AFM cantilever) imaged the lateral structure of different lipid bilayers and their morphological changes as a function of time. When binding between the protein and ligand occurred, the cantilever was retracted from the surface and an increasing force on the bond was gradually building up (observed from force – distance curve) [66].

3.5. Topography in other systems

Langmuir-Blodgett (LB) films of ytterbium bisphthalocyanine ($YbPc_2$) mixed with stearic acid (SA) had been probed using atomic force microscopy (AFM). Smaller and more homogeneous distributions of aggregates were observed in LB films containing more percentage of $YbPc_2$ [67, 68]

3.6. Surface Reactions

AFM was well suited for investigation of surface reactions such as weathering of glass. Corrosion phenomena of potash-lime silica glass were studied under the ambient atmosphere, dry nitrogen, and nitrogen with a relative humidity of 50%, as well as under liquids. During the in situ investigations, both contact and non contact mode AFM measurements influenced the corrosion phenomena observed as well as the corrosion process. Contact mode AFM introduced relatively high lateral forces leading to plastic deformations of soft domains whereas tapping mode AFM activated surface processes by the oscillating tip transferring energy to the sample surface. These artifacts introduced by the different techniques were discussed critically by Schmitz and his co workers [69]. To study surface reactions in situ with chemical specificity, from the nanometer perspective it could not be achieved by conventional AFM friction or force measurements due to insufficient resolution, and instrumental or thermal drift, respectively. Schonherr and his co-workers [70] addressed these problems by a novel approach, which was termed as “inverted” chemical force microscopy (ICFM). Here chemical reactions, which took place at the surface of the tip coated with reactants, were probed in situ by force-distance measurements on a scale of less than 100 molecules. The pull-off forces of different monolayers vary with the extent of the reaction and were thus monitored by AFM. Tapping mode AFM was advantageous for imaging small spatial structures like pits or heights in the lower nanometer range. It was more appropriate because it did not alter the surface by introducing shear forces, whereas contact mode AFM scratched away the weakly bound glass materials by its tip.

4. Conclusion

Two types of AFM imaging modes have been developed to provide multifunctional characterization of chemical and biological systems. However, minute improvisation in cantilever like coating it with a magnetic film or conducting material or functionalized with chemical groups specific for sample regions allows more and more systems to be studied. Contact mode can cause reversible or irreversible deformations in soft systems if forces are greater than 100pN. Surface reaction studies prefer tapping mode AFM over contact mode as it does not introduce undue scratches on the system. For topographic images, well characterized reference samples must be used. Phase image provides viscoelastic information of the system. Force – distance curve is a powerful method to probe the mechanical properties of sample. Images of nanoparticles for facets or defects in the nanorange, self - assembly of surfactants at the air – water interface, protein structural change in the buffer environment are the many areas in which AFM analysis provides new insights. Interaction between the substrate and the surfactant played a dominant role in immobilization and determining morphology. Topography of biomacromolecules was preferably done in non-contact mode AFM where surface on which it was adsorbed as it plays a vital role in controlling the image. AFM imaging at various stages of interaction for a polymer or protein - surfactant complex helps to elaborate the structural details of the complex.

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