Atomic Force Microscopy: Images and Interactions

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INTRODUCTION

Atomic force microscopy is a versatile tool for the study of surfaces and surface material development. AFM’s unique ability to function both as an imaging device and force sensor with nanometre resolution in both gaseous and liquid environments has led to unique insights into surface phenomena. AFM can also act as a platform for emerging techniques that exploit the instrument’s nanoscale positioning, for example near-field optical microscopy (NSOM). This article will, however, limit discussion to AFM imaging of surfaces, direct measurement of forces such as colloidal interactions and protein domain strength, combination of these two abilities and exploitation of AFM in the development of new surfaces.

AFM IMAGING

AFM is an established imaging technique that has related structure to function for a wide range of surfaces. AFM generates a topographical image by systematically moving a sharp tip (2 µm long), held at the apex of a cantilever, across a surface within air or liquid with little sample preparation or modification. The extension and retraction of a piezo ceramic crystal is responsible for the movement of the tip across the surface. As the tip tracks the surface the forces between the tip and the surface cause the cantilever to bend. A device such as an optical lever system measures the deflection of the cantilever. The optical lever of most commercial machines consists of a laser beam reflected from the gold-coated back of the cantilever onto a position sensitive photodiode (PSPD). The PSPD can measure changes in the position of the incident laser beam as small as 1nm, thus giving sub-nanometre resolution of the cantilever deflection.

The standard imaging method is that of contact mode in which the tip is rastered across the surface as it is held in close proximity to the sample by the force of the bent cantilever (imaging force). The alternative non-contact mode allows the imaging of soft samples without bringing the tip into contact with the surface. In this mode the cantilever is vibrated and the vibrational parameters monitored as the tip is brought into proximity with the surface and scanned over an area. The resolution, however, is lower than that achieved in contact mode operation. The predominant choice for imaging soft samples is tapping mode.

but it is in the areas of biological sciences and polymer chemistry where the most recent and exciting advances have been achieved, with an emphasis on surface structure linked to polymer properties or biological function. Figure 1 is an example of an AFM contact image of a polymer surface. The polymer was Solvay PE100, an experimental formulation of pipe-grade polyethylene. The surface had been polished using a series of progressively finer abrasives and etched under controlled acid conditions. The sample’s morphology has clearly defined rings that are 15-20µm in diameter. This is smaller than previously reported. Analysis of the polymer’s surface structure has implications for the prediction of a polymer’s formation and properties. The high resolution of AFM images of soft samples has greatly aided the study of self-assembling monolayers (SAMs), with a large number of studies within the last two years relating structure of SAMs, as imaged by AFM, to the process of formation and molecular organisation. AFM has also been used to achieve subnanometre resolution of biological samples. For example, AFM images of the 729 bacteriophage head-tail connector have suggested that the connector and its movement play an important role in the packing of viral DNA [1].

AFM can study surface phenomena in real time. Once a sample is under the AFM tip and in a controlled environment the system can be maintained or incrementally altered and studied for hours. Recent studies that have exploited this capability include the study of the conformational changes of outer membrane pore protein pores of the bacteria Deinococcus radiodurans and Escherichia coli [2] and the segmental dynamics of DNA [3]. A further refinement of the AFM imaging technique is to functionalise the tip, for example with a hydrophobic coating or with an anti-

Figure 1:
Contact mode AFM image of an experimental polymer surface, Solvay PE-100 (Sample supplied courtesy of Dr D.H.Isaac, M.Eng. UWES).

where the cantilever is vibrated so that the tip intermittently contacts the surface. This intermittent contact serves to reduce the lateral forces incident on the soft sample, reducing surface damage and tip contamination but maintaining resolution. When imaging in liquid, a further control is afforded by the choice of solution. Increased length of ionic double layers in low ionic strength solutions permits the tip to be held at a greater distance away from the surface than in higher ionic strength solutions, lowering the possibility of damaging the sample.

There are other AFM imaging modes that allow the mapping of surface properties. These include: lateral force microscopy and frictional forces; magnetic force microscopy and magnetic fields; scanning electrochemical microscopy and capacitance; and pulsed force microscopy that map surface mechanical properties. Most modern commercial AFM instruments generate maps of these surface properties at the same time as contact mode images.

Atomic force microscopy imaging continues to be exploited by the semiconductor industry, for example in the development of new surfaces.

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Figure 2:
Scanning electron micrograph of an Aspergillus niger spore immobilised at the apex of an AFM tipless cantilever to construct a cell probe.
body to an antigen located at the surface of interest. This technique has been used to map the distribution of proteins and functional groups on surfaces such as ELISA wells, fungal spores and mammalian cells. For a comprehensive review of advances in AFM imaging of polymer surfaces and biological surfaces see [4-6].

AFM MEASUREMENT OF INTERACTIONS

To generate a force-distance curve the cantilever deflection is recorded as a function of tip-sample separation as the piezo scanner of the instrument brings the tip into contact with the sample. To improve the relevance of force measurement the AFM silicon nitride tip can be coated with materials such as proteins or replaced with a particle of known material and geometry to construct 'colloid probes' (Fig. 4a insert). Such use of colloid probes allows the comparison of the force-distance data with theoretical predictions [7]. A further approach is to immobilise a single biological cell at the apex of a tipless cantilever [8]. Figure 2 is a scanning electron micrograph of such a cell probe; in this case an Aspergillus niger spore was immobilised on the cantilever [9]. Figure 3 is an example of a force-distance curve. In this case, the measured force is presented as a function of piezo displacement when an A. niger spore immobilised at the apex of a cantilever is retracted from an atomically flat mica surface, after momentary contact, and then brought into contact again in an aqueous environment (10^{-2} M NaCl pH 7). The force plot shows characteristic features that can be used to measure interactions such as electrical double layer interactions and mechanical properties on the nanoscale. Between A and B the change in deflection of the cantilever is directly dependent on the retraction of the piezo scanner. This section of the force plot is called the region of constant compliance; the spore remains in contact with the surface, however the incident force from the bent cantilever is decreasing. At C the force of the bent cantilever overcomes the forces holding the spore in contact with the surface. Thus the spore snaps away from the surface. The difference in force between C and D is a quantitative measure of the adhesive force, in this case 5.0 nN distance. From D to E the force is zero and the sample is moving further apart and the deflection of the cantilever is independent of the piezo scanner movement (zero force). At E the movement is reversed and the spore approaches the surface along EDF. As the spore gets nearer to the surface, the cantilever will deflect in a direction according to whether attractive or repulsive forces dominate the spore-surface interaction.

In order to convert the raw data recorded from the PSD into force as a function of true sample-tip separation, four parameters must be measured: zero force, zero distance, cantilever spring constant and cantilever deflection sensitivity (slope of constant compliance region). The spring constant of the cantilever can substantially vary from the manufacturer's specifications. Thus, most laboratories adopt a method of spring constant calibration [10]. The shape of force distance curves is a characteristic of the surfaces that are interacting [11]. The adhesion of a spore to an atomically flat mica surface may be compared to the adhesion measured with AFM for other particles. The detachment of hard inorganic colloid probes occurs over a very small range (1-5 nm) which also demonstrates that the stretching of the glue used to immobilise particles on the cantilever is negligible. A. niger spores became detached from a mica surface typically over a distance of 5 – 20 nm. The detachment of yeast cell probes from a mica surface occurred over a larger distance (200 – 400nm). The shape of the force-distance curve adhesion component of microbiological cells being pulled away from the surface suggests that there are multiple bonds being broken and that there is a degree of cell stretching (B-D) (Fig. 3). The differences of adhesion component shape are indicative of the physical and chemical differences of the types of particles; the spore is a dormant fungal cell with a wall strengthened to resist environmental chemical and physical stresses, the yeast cell is a deformable viable cell that is responsive to its environment, and the adhesion of hard inorganic particles is governed by generic physio-chemical forces.

The cell-probe technique was used to study the interactions of fungal spores in air and liquid. AFM measurements in air demonstrated that capillary forces dominate the interactions of spores with material used in air filters and that humidity is an important determinant of filtration process efficiency [12]. Both facts have been neglected in existing theories of air filtration. The contribution of specific interactions and long range electrostatic forces on the adhesion of A. niger spores was measured by bringing immobilised spores into contact with a freshly deaved mica surface in electrolytes of different concentrations and pH [9]. Specific interactions between the surface and appendages on the spore surface were found to play an important role in adhesion and this must be accounted for in theoretical predictions of spore adhesion.

AFM can act as a nanoscale stress and strain gauge, quantifying the forces involved in the extensions of molecules. This application was first demonstrated when studying the stretching of dextran filaments [13]. The technique was termed single molecule force microscopy. A biotin coated AFM tip was used to pick up the streptavidin-functionalised ends of dextran filaments and then retracted from the surface. The cantilever deflection was used to measure the force that was applied to the filament as the distance from the surface increased. The measured elongation curves showed close agreement with theoretical predictions based on entropy springs with segment elasticity. The theoretical predictions allowed detailed interpretation of the filament deformation.

AFM IMAGING AND INTERACTIONS

An important advantage of AFM in the study of surfaces is the ability to quantify surface morphology and surface interactions in a single instrument. AFM has allowed for the first time the direct quantification of surface inter-
actions combined with analysis of local topographical variation. Figure 4a is an AFM image of an AFC99 ultrafiltration membrane (PCI Membranes), scanned using a silica-colloid probe instead of a tip (Fig. 4a, insert). This allowed exact positioning of the colloid probe on the surface so that subsequent force-distance curves could be measured at features of interest on the surface. Silica was chosen as a type of foulant occurring in water treatment. The AFC99 polyamide membrane has a substantially greater roughness than most other types of polymer surfaces (P-V = 166 nm, RMS = 21 nm). The measurements of electrical double layer interactions between the silica-colloid probe and the membrane were different depending on surface location. The electrical double layer repulsion at peaks was much reduced in range and magnitude compared to that in the valleys (Fig. 4b). This demonstrates that theories of colloidal interactions with surfaces must account for roughness properties [14].

APPLICATION TO MATERIAL DEVELOPMENT

AFM offers great potential as a versatile tool that assesses the development of surfaces with specific properties. In our laboratory we have used the colloid probe, coated-colloid probe and cell-probe techniques to assess the adhesive characteristics of synthetic ultrafiltration membranes used in industry [15,16]. The approach correctly identified membranes with low fouling properties and introduced the concept to bioprocess engineering of using AFM in the development of novel surfaces prior to costly pilot plant procedures. AFM was also used to investigate the effect of adding sulphonated polyether ether ketone (SPEEK) to the polymer mix used to make polysulphone ultrafiltration/nanofiltration membranes (Fig. 5a) [17]. SPEEK is a water-soluble negatively charged polymer and was expected to greatly affect membrane surface structure due to its low compatibility with polysulphone. Figure 5b shows typical plots of the force as a function of separation distance as a silica-colloid probe approached membranes constructed from different polymer mixtures. Considering first the polysulphone/pyrrolidinone membrane, on approach to the membrane surface the particle jumps into contact, ‘snaps in’, due to long range attraction between the particle and membrane. This type of attraction produces membrane fouling. In clear contrast, the particle does not snap-in to the polysulphone/SPEEK membrane surface, as any attractive interactions are overcome by electrostatic double layer repulsion between the negatively charged membrane and particle surfaces.

This trend was also observed in the retraction of the particle from the two different membranes. A 38 times greater force was required to detach the particle from a polysulphone/pyrrolidinone membrane. AFM imaging and comparison of force-distance curves between silica colloid probes and membranes with different polymer blends suggested that membranes with 5% wt SPEEK had a very promising combination of properties. These were very high permeability, very high rejection of 4 kDa dextran, high rejection of NaCl and low adhesion [17].

Choice of surface finish for a particular purpose is often driven by technical, aesthetic and economic concerns. AFM can be used to quantify surface finish in terms of roughness and assess its impact on properties of concern to the function of the surface. We have used AFM to study commercial stainless steel samples of widely differing surface finishes [18]. Analysis of the AFM images allowed quantification of surface roughness over different area scales: 50x50 µm, 10x10 µm and 1x1 µm. The AFM colloid probe technique was also used to measure directly the adhesion of single polymer latex particles to the surfaces in solution. It was found that the adhesion increased with decreasing roughness, except for the smoothest surface which exhibited very regular surface features on the area scale most relevant to adhesion of the particle (1x1 µm). There was a good correlation between the variability of adhesion over each surface and the corresponding variability in surface roughness. As the colloid probe has dimensions comparable to those of bacteria and yeast cells, such measurements should be of value in the selection of surface finish likely to minimise bioadhesion.

DISCUSSION

AFM instrumentation is constantly improving and its ability to position at the nanoscale means that it is an ideal method to be combined with other analytical techniques. The design of probes and tips held at the end of the AFM cantilever will continue to be refined. The replacement of the tip with flexible, strong carbon nano tubes promises to enhance the study of single molecules or colloids. More research will focus on analysing the material under the AFM tip on the surface either by direct sampling or interpreting the interaction parameters of the tip and the surface. For example, acoustic methods that exploit the influence of different surface materials on the vibration parameters of an AFM tip in contact with the surface material are being developed. The NSOM field promises to develop so that individual atoms on surfaces could be imaged and analysed with light. The AFM scanning speed will increase with several types of probes analysing the surface simultaneously, thus increasing the scope of real-time imaging applications. In conclusion, atomic force microscopy is an essential surface analytical tool that has an extremely promising future.

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REFERENCES