S5-1

Atomic Force Microscopy: a Powerful Tool for Biological Engineering on the Micro/Nano Scale

R.W. Lovitt and C.J. Wright

Centre for Complex Fluids Processing, Multidisciplinary Nanotechnology Centre, School of Engineering, University of Wales Swansea, Wales UK.

Abstract

This paper covers the basic principles of the AFM and how these systems may be used to image biological materials and measure particle-surface interactions in process environments. e.g. visualize molecules and structure on surfaces in aqueous environments, measure forces of interaction of proteins and DNA, biosurface and cells. Examples of work include applications biological spore control agents control systems, process materials selection for example appropriate filters for biological processing, mechanical properties and bio-surface engineering.

Introduction

Engineering of biological processes at the micro and nano scale is becoming more viable as our knowledge and new instrumentation make it possible to manipulate and observe these systems. In so doing we will not only make new innovative devices for diagnostics, catalysis etc but also understand more clearly the fundamentals of many important processes mediated by biological systems. The are many drivers to scale down such systems and these include

1. reducing in the amount of materials required to make a sensor or device
2. the ability to manipulate individual components and gain information about these materials
3. the visualization of surfaces, molecular complexs and individual molecules and how they intercat in a real environment
4. the identification and measurement of the processes occurring at the micro/nanoscale

One of the main challenges of biology is to decipher the complex architecture of molecular networks from a "bottom up" or "top down" perspective so as to understand the function of such systems and to manipulate or engineer them into novel function entities. The bottom up strategy requires a quantitative understanding of how molecular components interact with one another and how they are organized into functional assemblages to carry out important tasks within the cell. One of the most important challenges is

how metabolism is organized within the cell. The top down approach involves quantitative measurement and analysis of cellular function. A good example of this may be seen in investigating the processes involved in adhesion of cells to surfaces and the consequences of such behavior in colonization and infection of animals by microbes.

The tools for study micro/nano technologies are now being developed rapidly and both the top down and bottom up approaches to be applied to understand and exploit biological systems. Key to all of these approaches is the ability to probe biochemical and chemical characteristics of molecules and assemblages at very high temporal and special resolution. The AFM is one such tool.

Binnig *et al,* (1986) invented atomic force microscopy (AFM). It is the most successful member of the scanning probe microscopy family mainly due to is versatility and ease of use. AFM can be used to study a range of materials and surfaces in different environments, gaseous or aqueous with very little surface preparation or coating. In addition to the generation of nanometer scale images, AFM can also be used as a force sensor. The potential of the AFM has yet to be fully recognized by the biotechnologist a consequence of the technology's recent emergence and expense. However, advances in AFM instrumentation and its falling costs means that this technology is not longer restricted to the physics laboratory. More operators with prior knowledge of biological systems are beginning to apply AFM to their research and it has proven extremely useful in the study of biological specimens providing powerful insights into surface structure, surface interactions and mechanical properties of biological surfaces and molecules AFM has been a vital technique in the visualization measurement and analysis of key processes with manipulation at these scales the aim of the paper is to outline the principles of AFM and to illustrate how it can be used to understand how biological systems may be engineered more effectively on the micro- and nano-scale.

Visualising surfaces using ATOMIC forces

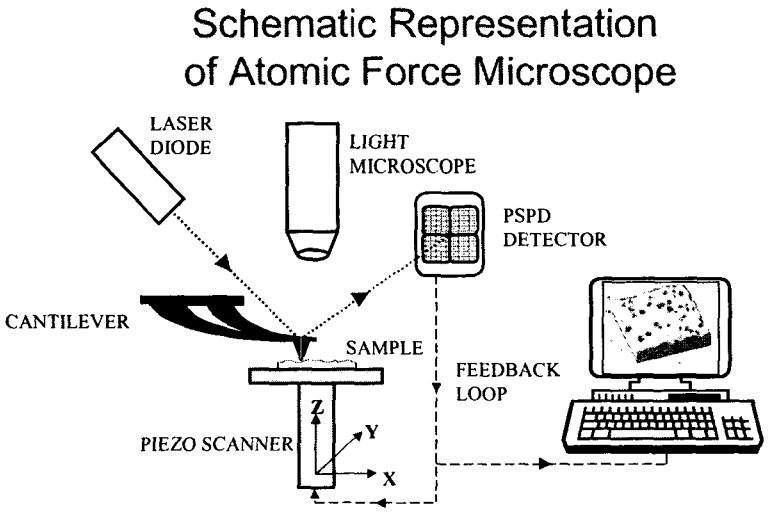

Figure 1. schematic representation of the principle of operation

Principle of operation

Figure 1 is a schematic representation of an AFM instrument. A sharp tip or other probe at the end of a cantilever is systematically scanned across a surface of interest to generate a topographical image. The extension of retraction of the piezo ceramic crystal infers fine position control. The micro-machined cantilever is typically between 200 and 400 μm in length, 20-50 μm width and 0.34 to 3 μm thickness. The stiffness of the cantilever is a function of these dimensions. The cantilever can be rectangular are triangular. The tip sited at the apex of the cantilever is normally about 2 μm long and can be machined into a variety of shapes. Image resolution is improved by the aspect ration of the tip apex and the best quality tips are down to about 10 nm. As the tip tracks the surface the forces between the tip and the surface cause the cantilever to bend. A device, normally an optical lever system using a laser beam is reflected on the gold-coated tip into a positional sensitive diode array (PSPD). In an air or water system, PSPD can measure the changes in the position of the incident laser beam as small at 1 nm.

Instruments have several operating modes that are chosen depending on the sample and environmental condition employed. The simplest is **Contact mode** e surface, but the resolution of the image is often reduced.

Non contact mode, as the name suggests, allows imaging of soft samples with out bring the tip in contact with the surface. In this mode the cantilever is vibrated and the vibrational parameter monitored as the tip is brought into proximity with the surface and scanned over the surface. As the surface forces change and affect the vibrating cantilever. The feed back loop operated to maintain a constant via vibrational amplitude. The feed back signal is then used to generate the topographic image. The resolution of this technique is not as good as contact mode. The best general method using intermittent contact or **tapping mode** where the cantilever tip comes into contact intermittently this reduced damage of soft samples while maintaining high resolution.

Also able to use **lateral force** (frictional forces) here the frictional forces between the surface and the tip are mapped. Another mode can be employed to map surface properties is that of **force volume mode**. Thus, the information that can be derived from the force curve such as adhesion properties and mechanical properties can be mapped. However this method can be demanding on both instrument time and computer power. In addition care has be taken to negate errors when measuring the force distance curve.

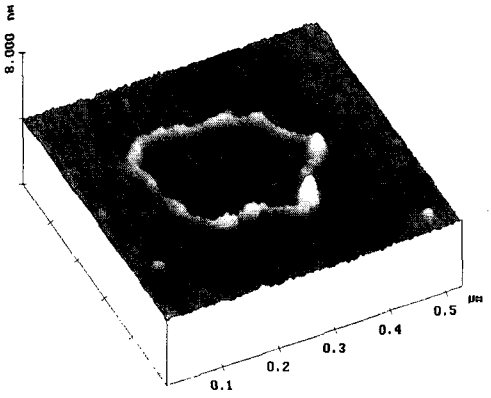

Figure 2. Plasmid DNA from E coli perfomed in tapping mode.

Using these techniques it is possible to image down to the molecular level and lipids, proteins DNA can be visualized. Figure 3 shows an image of plasmid DNA from *E coli.* using tapping mode from our lab.

Analysis of forces to measure surface interactions

Apart from visualizing forces at surfaces and making images and the other main application is to make measurements of force between surfaces and the tip in environments that a relevant to function. The force distance capability of AFM has been shown provide detailed information about biological systems. To generate a force distance curve the cantilever deflection is recorded as a function of the tip sample separation as the tip is brought into contact with surface. Figure 3 below illustrates the force distance profile as the tip is brought to and retracted from the surface. Note, the distance between F and G represents the adhesive force, while G to A measures the snap away from the surface also within this part of the curve the give information about the nature of the release such as elasticity of the sample and the probe. The shape of the approach curves BCD gives information of the about forces acting between the probes and the surface e.g. attractive or repulsive and is a function of solution chemistry as well as the surface chemistries. The nature of the solid may also be probes for its softness.

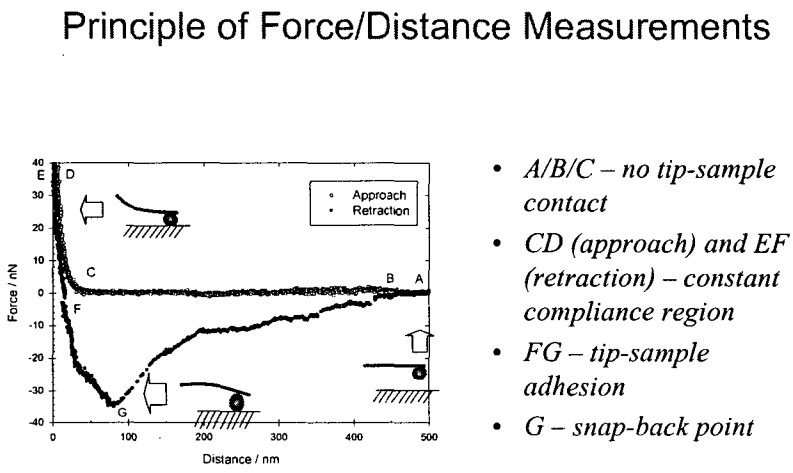

Figure 3. A profile of a Force-distance curve.

Measuring interactions between surfaces and biomolecules.

From the basic principles described above, it is not surprising that AFM tips coated with biological materials to study their interactions with surfaces. An extension of this technique is to add larger objects to the tip to make colloid probes, here the tip is replaced with a sphere of known dimensions and material composition. Figure 4 is an example of one such probe a cellulose sphere is glued to the apex of the cantilever. Using such probes coated with materials such as proteins or carbohydrates interactions can be investigated. The adhesion of cells to surfaces has been studied with AFM. Bowen *et al.*, (1998, 1999, 2000) have studied the adhesion of unfixed yeast cells to different surfaces. An individual yeast cell was immobilized to the apex of the AFM cantilever. We have studied such interactions by placing the yeast cell on the surface and measuring the adhesive properties of the yeast cell as a function of time. Using this method we have studied the choice of appropriate membrane materials for low fouling filtration or for

immobilization of yeast. Similar, other surfaces involving and their interactions with proteins, spores and bacteria can be measured. For example, we have investigated the fouling of medical implants, such as bilary stents, by protein and bacteria. Also we have studied the adhesion of fungal and bacterial spores to filtration materials and the influence of environmental conditions on such phenomena. We are also are studying the adhesion of entemopathogenic fungi such as *Metarhizium flavoviride*. These are used as biological control agents and we have been able to show that the adhesiveness of the spores to insect cuticle, a primary factor in pathonogenicity, is a function of the physiology of spore production as well as the environmental conditions. These measurements have direct relevance to the spore production systems to obtain the most potent (pathogenic) spores and the most economically production system. Bacterial spores are the cause of several problems within food processing particular those associated with milk processes such as *Bacillus cereus*. We are now investigation the significance of our AFM adhesion force measurements and the use of force applied through fluid flow over the surfaces.

We have also studied Cellulose Binding Domains (CBD) hydrid proteins to determine their interaction cellulose and other polymer surfaces. CBD hydrids are being developed in Europe as modifiers of cellulose fibers used in paper and textiles. Our studies are aimed at investigation the nature of CBD cellulose interaction optimize the application to surfaces and their uses. Figure 4 B shows a cellulose probe coated with CBD, we measure the interactions between the probe and flat cellulose surfaces.

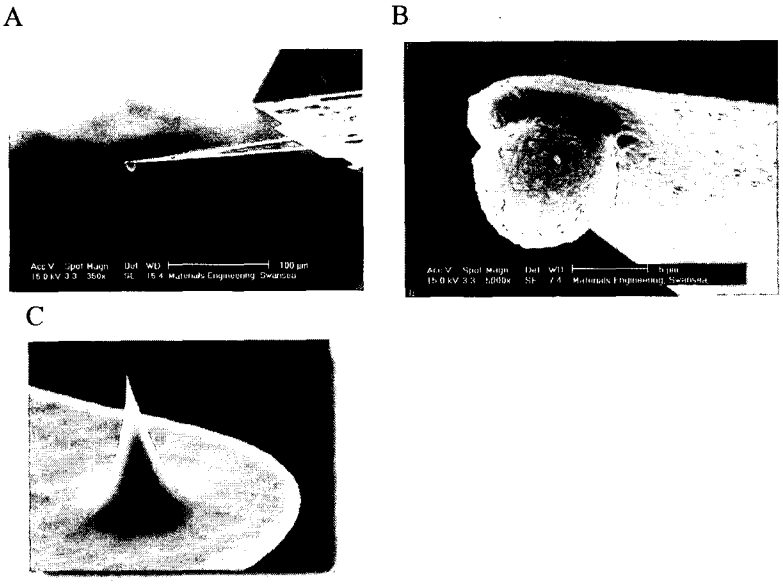

Figure 4. Electron micrographs of cantilever with cellulose sphere attached (A). A CBD -cellulose probe on the cantilever tip (B) used for measuring probe surface interactions. A micro machined tip (C) used for imaging.

Apart from general adhesion measurements the nature of interactions in solution mean that several other forces may be exploited and investigated and these include Capillary force, Polymer extension, Binding, van der Vaals, Electrostatic, Elastic and Brush surfaces and aimed a the investigation of a specific problem. We are particulary keen to investigate the electrochemical properties of biological materials and surfaces.

Investigating single molecule force microscopy.

Biological molecules can function as structural molecules or as molecules responsible for motion as in muscles, organelle transport and vesicle movement and expulsion. In these cases the mechanical properties of the molecule will influence its behavior. However little is known about the underlying physical properties underlying these functions. The AFM can act as nanoscale stress gauge to quantify the forces involved in extensions of molecules (Fisher *et al.*, 1999). This application was first demonstrated by the stretching of dextran filaments of different length (Rief *et al.*, 1999a). The biotin coated AFM tip was used to pick up steptavidin functionalized ends and then retracted from the surface. The cantilever deflection was used to measure as the distance between the surface and the tip increase. These elongation measurements confirmed the theoretical predictions based upon the entropy springs with segment elasticity. So at low and intermediate forces the deformation of the dextran was governed by entropic forces and twisting of bond angles within the structure while at high forces accompanied by stiffening of the filaments bending of bonds

The investigation of titin molecule deformation has also been investigated and further validated the technique (Rief *et al.*, 1999b). In this case the protein was absorbed fresh gold surface and a tip was ten left in contact with the surface for several seconds to allow a fraction of the molecule to absorb onto the tip. As the tip was withdrawn form the surface a characteristic saw tooth profile was obtained as the titin molecule was stretched. The periodicity of the peaks was between 25-38 nm with the maximum force between 150 and 300 pN and the peaks were though to be the unraveling of the protein domains. This was shown to be true when recombinant titin fragments were constructed and used in the experiments gave the correct number of peaks for the protein domains present. Whent the tension was removed the molecule refolded.

Conclusion and future prospects

As this brief introduction has shown the AFM offer a powerful tool to investigate and manipulate biological materials on the nano/micro-scale. It can be applied fundamentally to obtain basic knowledge of biological surfaces materials and molecules. It is possible to observed and measure interactions of single molecules. However, this is just one important tool and in the future these techniques need to combined with others to enhance and validate such measurements. For example we are combining it with high-speed photography to assess the physical properties of cells. We are investigating the relevance of AFM data with other approaches for example the correlation between applied force through fluid flow and that of forces applied by the AFM.

There are many other instruments that complement this information and include: Plasmon resonance spectroscopy for binding; Optical tweezers for micro-manipulation; quartz crystal micro-balance for measuring surface interaction and confocal microscopy for visualizing surface structure. Finally, the instrumentation is only as good as the software and major advances in data analysis are revolutionizing the potential of this equipment.

References

Binnig G, Quate CF, and Gerber G (1986) Atomic force microscopy. *Phys Rev Lett* **56** 930-933

Bowen WR, Hillal N, Lovitt RW, Wright CJ (1998) Direct measurement of the of the force of the cell using and an atomic force microscope. *J Colloid and Surfaces A Physicochem Asp* **136**, 231-234

Bowen WR, Hillal N, Lovitt RW, Wright CJ (1999) Characterisation of membranes surfaces: direct measurment of biological adhesion using an atomic force microscope *J Mem Sci* **154** 205 212

Bowen WR, Lovitt RW, Wright CJ (2000) Application of atomic force microscopy to the study of micromechanical properties of biological materials. *Biotechnol Lett* **22** 893-903

Fisher TE, Oberhuasen AF, Carron-Vazquez M, Marszalek PE, Fernandez JM(1999) The study of protein mechanics with the atomic force microscope. *Trends in Biochem Sci* **24** 239-384

Reif M, Oestenhelt F, Heymann B, Gaub HE (1997a) Single molecule force microscopy on polysacharide by atomic force microscopy. *Science* **275** 1295-1297

Reif M, Gautel M, Oestenhelt F, Fernandez JM, Gaub HE (1997b) Reverable unfolding of individual titin immunoglobulin domains by AFM. *Science* **276** 1109-1112