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Atomic Force Microscopy: A Source of Investigation in Biomedicine

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Abstract

It is sometimes necessary to monitor biological samples in their native state and in their physiological environments in order to understand the complex biological systems. AFM (atomic force microscopy) analysis, both of fixed cells, and live cells in physiological environments, is set to offer a step change in the research of cellular function. With the ability to map cell topography and morphology, provide structural details of surface proteins and their expression patterns and to detect pico-Newton force interactions, AFM represents an exciting addition to the arsenal of the cell biologist. The use of AFM in the area of biomedical research has been proposed for some time, and is one where a significant impact could be made. Fixed cell analysis provides qualitative and quantitative sub-cellular and surface data capable of revealing new biomarkers in medical pathologies. In recent year, Atomic Force Microscope (AFM) has provided a range of now opportunities for viewing, Manipulating and analyzing bio-molecules in the environments. And will hopefully allow application to be developed for AFM in Medicine and biotechnology. Here, we review the current status of AFM in the field.

1. Introduction

Atomic Force Microscopy: is a very high-resolution type of scanning probe microscopy, with demonstrated resolution on the order of fractions of a nanometer, more than 1000 times better than the optical diffraction limit.[3] Since its invention in 1986, initially it was developed for the characterization of nanometre-scaled semiconductor devices (i.e., AFM was applied almost exclusively to characterize the surfaces of non-biological materials, and even today its major applications are still in

the visualization of microcircuits, material sciences and nanotechnology) but is increasingly used in biological and biophysical research due to its unique analytic capabilities. And after sometimes [5], the atomic force microscope had become one of the most important tools for imaging the surfaces of objects at nanometer scale resolutions. It is considered by many to be a strong competitor to conventional methods for the investigation of structures, such as in electron microscopy and X-ray scattering. [2]The newly developed atomic force microscope is a valuable tool for studying physical and biological structures provides a unique window to the micro world of cells, sub-cellular structures, and bio-molecules. The AFM can image the three-dimensional structure of biological specimens in a physiological environment. This enables real-time biochemical and physiological processes to be monitored at a resolution similar to that obtained for the electron microscope. Atomic force microscopy since the initial reports of considerable progress has been made, but applications in the field of biology are exploring biological structures under conditions in which living organisms exist. The application of the AFM to biological and biomedical research has increased exponentially.In this paper next few sections describes about the Working principle (section 2), modes of operations (section 3), advantages and disadvantages (section 4), applications of AFM in Various Fields (section 5), conclusion(section 6) and its future scope(section 7).

Working principle: The key step for the popularization and commercialization of the atomic force microscope was the introduction of the optical lever for the detection of cantilever movement. The basic configuration of this design is shown in Fig. below.

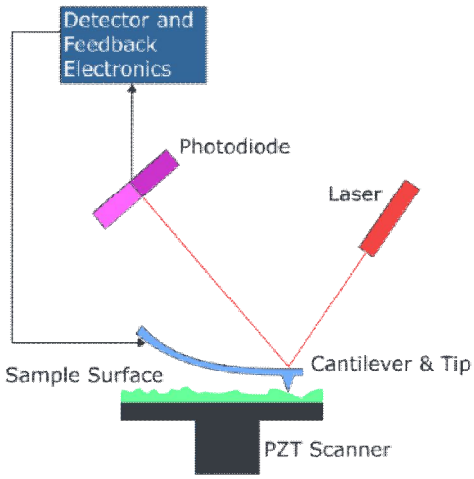

Fig. 1: A typical atomic force microscopy detection scheme.

In this approach [5], the AFM consists of four major parts: a cantilever with a sharp tip as shown in Fig. 2, normally made of silicon or silicon nitride, mounted underneath it; a piezo-scanner that drives the cantilever; a laser diode; and a position sensitive detector.

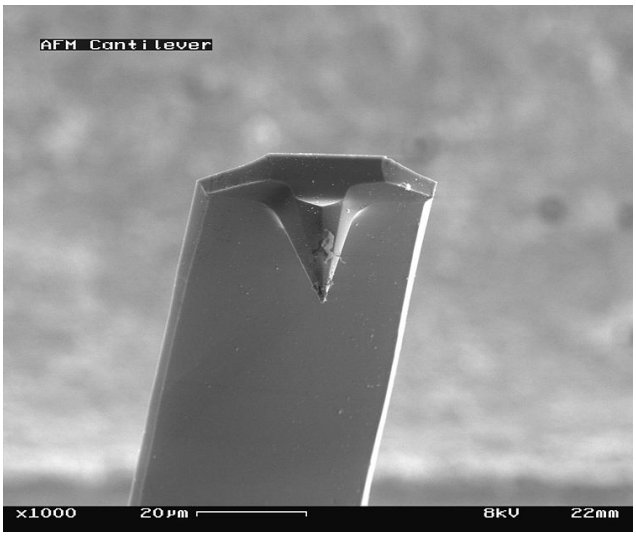

Fig. 2: Electron micrograph of AFM tip.

As the tip scans over the surface, the interactions between the AFM tip and the features on the surface cause displacement of the cantilever. This displacement is measured by detecting the deflection of a weak laser beam, generated by the laser diode, reflecting off the back of the cantilever with the photodiode detector. The atomic force microscope creates topographic images of the surface by plotting the laser beam deflection as its tip scans over the surface. This design greatly improved the sensitivity of the design of the microscope as cantilever displacement can easily be amplified by the light path. Atomic resolution images of a variety of surfaces have been achieved with this design. However, the most important point of this design for biomedical purposes is that it makes operation of the atomic force microscope possible in ambient environments or in aqueous solutions at room temperature or at 37^{o} C. These conditions are required for imaging native biological samples in their functional and physiological environments.

2. Modes of operations

[5]The interatomic interactions between point-like objects or atoms can be repulsive or attractive. This gives options for the atomic force microscope to operate in different modes. Among these modes, the most popular are the contact mode and noncontact mode.

In the contact mode, a force is applied by the AFM on the sample, and therefore the interaction between the AFM probe and the sample is repulsive. As the scanner gently traces the tip across the sample, the contact force causes the cantilever to flex to accommodate changes in the sample's topography. The normal force applied creates a substantial frictional force when the probe scans over the surfaces. Both the normal and the frictional force can damage vulnerable biological samples. To image these kinds of samples, the force applied must be carefully controlled.In the non-contact

mode, the cantilever is oscillated at a distance (normally 5-15 nm) above the sample surface. The attractive force between the tip and the surface, which is largely due to van der Waals forces or Coulomb and dipole interactions, changes depending on distance. These force changes induce alterations in the resonant behaviour of the oscillating cantilever. Frequency shift or phase and amplitude changes can be used to generate images. Real non-contact imaging is extremely difficult to achieve. In order to achieve the highest resolution, the probe has to be brought close enough to the surface to effectively detect the attractive force gradient. Thus, the oscillating probe very often slightly touches the sample surface, and becomes intermittent-contact (or tapping) mode AFM.

3. Advantages and Disadvantages of Atomic Force Microscopy

Just like any other tool, an atomic force microscopy's usefulness has limitations. When determining whether or not analyzing a sample with an AFM is appropriate, there are various advantages and disadvantages that must be considered.

3.1 Advantages

AFM has several advantages over the scanning electron microscope (SEM). Unlike the electron microscope which provides a two-dimensional projection or a two-dimensional image of a sample, the AFM provides a three-dimensional surface profile. Additionally, samples viewed by AFM do not require any special treatments (such as metal/carbon coatings) that would irreversibly change or damage the sample. While an electron microscope needs an expensive vacuum environment for proper operation, most atomic force microscopy modes can work perfectly well in ambient air or even a liquid environment. This makes it possible to study biological macromolecules and even living organisms. In principle, AFM can provide higher resolution than SEM. It has been shown to give true atomic resolution in ultra-high vacuum (UHV) and, more recently, in liquid environments. High resolution AFM is comparable in resolution to scanning tunneling microscopy and transmission electron microscopy.

3.2 Disadvantages

A disadvantage of AFM compared with the scanning electron microscope (SEM) is the single scan image size. In one pass, the SEM can image an area on the order of square millimeters with a depth of field on the order of millimeters. Whereas the AFM can only image a maximum height on the order of 10-20 micrometers and a maximum scanning area of about 150×150 micrometers. One method of improving the scanned area size for AFM is by using parallel probes in a fashion similar to that of millipede data storage.

The scanning speed of an AFM is also a limitation. Traditionally, an AFM cannot scan images as fast as a SEM, requiring several minutes for a typical scan, while a SEM is capable of scanning at near real-time, although at relatively low quality. The relatively slow rate of scanning during AFM imaging often leads to thermal drift in the image making the AFM microscope less suited for measuring accurate distances

between topographical features on the image. However, several fast-acting designs were suggested to increase microscope scanning productivity including what is being termed videoAFM (reasonable quality images are being obtained with videoAFM at video rate: faster than the average SEM). To eliminate image distortions induced by thermal drift, several methods have been introduced.

AFM images can also be affected by hysteresis of the piezoelectric material and cross-talk between the *x*, *y*, *z* axes that may require software enhancement and filtering. Such filtering could "flatten" out real topographical features. However, newer AFMs utilize closed-loop scanners which practically eliminate these problems. Some AFMs also use separated orthogonal scanners (as opposed to a single tube) which also serve to eliminate part of the cross-talk problems.

As with any other imaging technique, there is the possibility of image artifacts, which could be induced by an unsuitable tip, a poor operating environment, or even by the sample itself. These image artifacts are unavoidable however, their occurrence and effect on results can be reduced through various methods.

Due to the nature of AFM probes, they cannot normally measure steep walls or overhangs. Specially made cantilevers and AFMs can be used to modulate the probe sideways as well as up and down (as with dynamic contact and non-contact modes) to measure sidewalls, at the cost of more expensive cantilevers, lower lateral resolution and additional artifacts.

4. Applications of AFM in Various Fields

4.1 Application in Microbiology

[2]The AFM has been used to viewing and analyzing the ultra structure of microbial cell surface studies and it is used to investigated the property of structure include to analyzing structure of native membrane proteins at sub-nanometre resolution, Function-related conformational changes in single proteins, Surface ultra structure of living cells, Cell surface dynamics, and Morphology of bio-films.

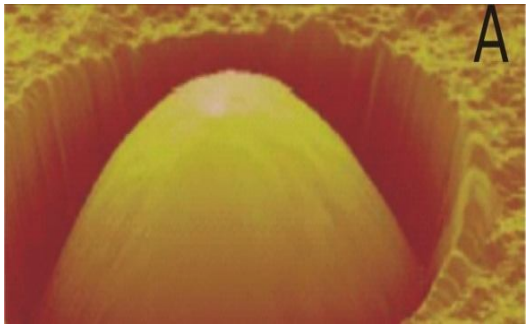

Fig. A: Three-dimensional AFM image of Saccharomyces cerevisiae Yeast cell immobilized in a porous membrane.

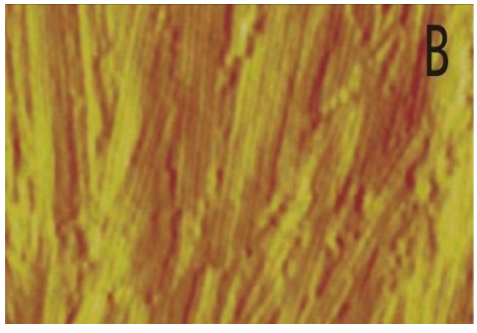

Fig. B: High-resolution deflection image of the surface of Phanerochaete chrysosporium Fungal Spores.

The physical properties and bio-molecular interactions such as Stiffness of cell walls, Local surface charge and hydro-phobicity, Elasticity and conformational properties of single molecules, Mechanical stability of supra-molecular assemblies, Unfolding pathways of membrane proteins, Molecular forces determining cell adhesion and cell aggregation also analyzed.

4.2 Application in Virology

[4]Viruses are parasitic entities composed of the two previously described constituents: nucleic acids (DNA or RNA) and proteins. The most complex viruses are surrounded by a lipid bi-layer and a glycoprotein envelope. Since they cannot multiply without a host organism and they lack a metabolic apparatus, viruses are not considered to be alive. It is well known that viruses can infect bacteria and plants as well as humans. Viral diseases varying in severity from pox to the common cold have plagued mankind since the beginning of history. Their structure can be readily studied using electron microscopy as well as AFM. The AFM resolution on these types of samples is, for the moment, more or less comparable with electron microscopy and has permitted the study of virus substructures. However, because of its ability to operate in liquids, the AFM can bring some new insights in the multiplication cycle of the viruses. While scanning an infected cell, followed the dynamics of the virus expulsion process from an infected cell, as depicted in Fig. C. This type of measurement is inaccessible to any other instrument at such high spatial resolution.

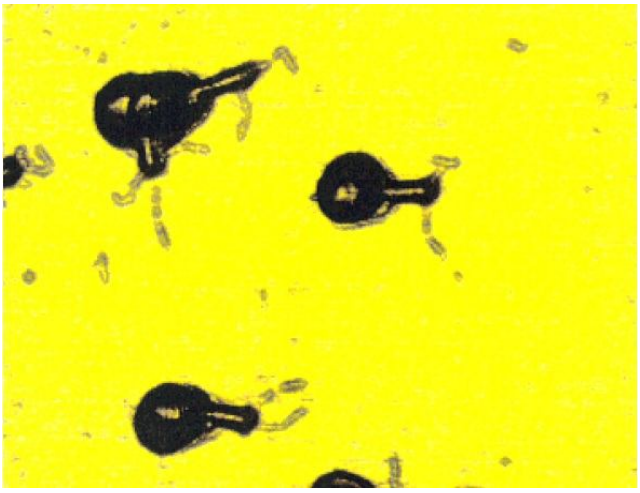

Fig. C: AFM image of a bacteriophage T4 virus. The head, tail and tail fibers of the virus are resolved by the AFM

4.3 Application in DNA and chromosome studies

[5]Transcription is one of the central biochemical processes in gene expression, but it is still not fully understood. Scientists have applied AFM to investigate the mechanism by which transcription is initiated in Escherichia coli by imaging the real-time interactions between DNA and RNA polymerase, providing new insights into the

common mechanisms of all DNA-RNA transcriptions. AFM can also be used to directly measure the folding force between RNA strands at pico-Newton levels with modified tips and a force spectroscopy technique. The atomic force microscope has also been used to study chromatin and metaphase chromosome structures. The results of these studies revealed that the chromatid arm has ridges and grooves along its length that are related to the G/Q-positive and G/Q-negative bands, respectively. The chromatid could also be produced by compaction of highly twisted chromatin fiber loops, and its compaction tended to be stronger in the ridged regions than the grooved regions of the chromosomes. In addition, structural models for the chromatin fiber were proposed based on AFM results. These studies proved the usefulness of AFM in obtaining three-dimensional surface topography in both ambient and physiological liquid conditions. The atomic force microscope is able to obtain much greater resolution images of G-banded chromosomes than conventional optical microscopic methods. With this high resolution, small defects on chromosomes may now be resolved by ATM. The atomic force microscope may therefore have the potential to become a new method in karyotyping

Fig. D: AFM image of a flagellated E. coli cell. The E. coli cell is in its late log phase, and was twice washed with PBS to remove the culture medium and air -dried on mica surfaces for imaging.

5. Future Scope

The paper here presents merely few applications of the Atomic force microscopy. It is currently being utilised in a large number of field. Although the study and its application till now enable us to determine the surface/structure of micro-species, it can even be extended to the prediction of various other properties of the specimen such as the binding specificities and nonspecific DNA bindings, which can somehow be helpful in DNA repairing.

6. Conclusion

The major disadvantage of the atomic force microscope is that it can only obtain surface information from samples. As a result, AFM cannot replace the valuable functions of optical microscopy, scanning fluorescence microscopy or transmission electron microscopy. However, AFM has a nanometer-scale resolution and the ability to operate in liquid environments, which are key requirements in biological imaging. The operation range of the atomic force microscope is suitable for characterizing structures from the molecular to the cellular scale. In addition, AFM has the unique ability to measure molecular forces with high sensitivity. These applications have been exploited to reveal structural details and define the molecular forces involved in a variety of biological systems. Measurements of electrostatic characteristics are also among the emerging advances that can facilitate the analysis of biological and biomedical samples. The need for detailed imaging at the molecular level and for monitoring dynamic biological processes will continue. AFM is therefore likely to play an important and enduring role in biological and biomedical research.

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