

# Atomic Force Microscopy Application in Biological Research: A Review Study

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## Abstract

Atomic force microscopy (AFM) is a three-dimensional topographic technique with a high atomic resolution to measure surface roughness. AFM is a kind of scanning probe microscope, and its near-field technique is based on the interaction between a sharp tip and the atoms of the sample surface. There are several methods and many ways to modify the tip of the AFM to investigate surface properties, including measuring friction, adhesion forces and viscoelastic properties as well as determining the Young modulus and imaging magnetic or electrostatic properties. The AFM technique can analyze any kind of samples such as polymers, adsorbed molecules, films or fibers, and powders in the air whether in a controlled atmosphere or in a liquid medium. In the past decade, the AFM has emerged as a powerful tool to obtain the nanostructural details and biomechanical properties of biological samples, including biomolecules and cells. The AFM applications, techniques, and -in particular- its ability to measure forces, are not still familiar to most clinicians. This paper reviews the literature on the main principles of the AFM modality and highlights the advantages of this technique in biology, medicine, and- especially- dentistry. This literature review was performed through E-resources, including Science Direct, PubMed, Blackwell Synergy, Embase, Elsevier, and Scholar Google for the references published between 1985 and 2010.

Please cite this article as: Vahabi S, Nazemi Salman B, Javanmard A. Atomic Force Microscopy Application in Biological Research: A Review Study. *Iran J Med Sci.* 2013;38(2): 76-83.

**Keywords** • Atomic force microscopy • Scanning tunneling microscopy • Scanning probe microscopy • Dental • Biological

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Received: 7 December 2011

Revised: 2 April 2012

Accepted: 10 June 2012

## Introduction

The atomic force microscope (AFM) is a type of scanning probe microscope (SPM), which uses a fine probe to probe over a surface rather than use electrons or a beam of light (figure 1). This type of microscope yields 3D maps of surfaces. There are some kinds of SPMs aside from the AFM such as the scanning tunneling microscope (STM) and the near-field scanning optical microscope (NSOM).<sup>1</sup> The AFM has a tip which can be modified in many ways to investigate surface properties; it is, therefore, a more developed version of the STM which can image almost any kind of surfaces at nano scales (figure 2).<sup>2</sup>

This review is primarily focused on the AFM and its applications in medicine and dentistry.



Figure 1: AFM for elasticity measurement.

### AFM History and Methods

The AFM is the most commonly used form of the SPM. The origin of the SPM began with the development of the STM in 1982 by Binnig and Rohrer,<sup>3</sup> at the IBM, Zurich. The ability of the STM to resolve the atomic structure on a sample surface earned the inventors the Nobel Prize.<sup>4</sup> However, the STM can only be applied to conductive or semi-conductive specimens. To broaden this type of microscopy so as to study insulators, the AFM was developed in collaboration between the IBM and Stanford University.<sup>5</sup> Commercial AFMs were developed by Stanford researchers in 1998, and the first nanoprobe, called the nanosensor, was developed in 1991.<sup>3</sup>

### Different Types of SPM

1. The STM is widely used in both industrial and fundamental researches to obtain atomic-scale images of surfaces. It provides a 3D profile of the surface, which is very useful to characterize surface roughness, to observe surface defects, and to determine the size and conformation of molecules and aggregates on the surface. The principles of the STM are based on quantum mechanical and also piezoelectric effects. The STM can only image conductive and semi-conductive surfaces, whereas the AFM technique is a more applicable and effective way to image almost any kind of surfaces.<sup>3</sup>

2. The near-field scanning optical microscopy (NSOM) is a type of microscopy where a sub-wavelength light source is used as a scanning probe. The probe is scanned over a surface at a height above the surface of a few nanometers,

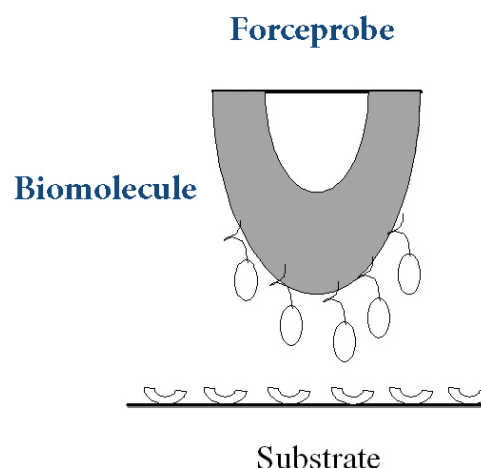


Figure 2: Modified tip of the AFM.

and optical images with a resolution well beyond the usual “diffraction limit” -about 50 nm- can be constructed. Dynamic properties can also be studied at a sub-wavelength scale using this technique.<sup>3</sup>

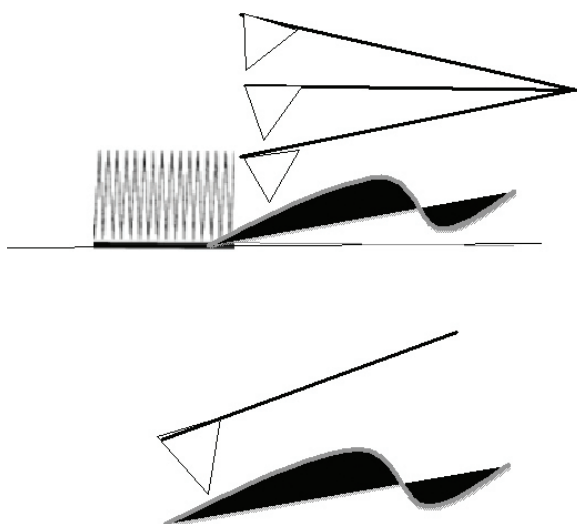
### Principles of AFM

The mechanism of the AFM is based on the detection of forces acting between a sharp probe and the surface of the sample. The probe is known as the AFM tip or the AFM sensor, which is attached to a very flexible cantilever. There are several methods to detect any motion of the cantilever. Nowadays, most AFMs use laser-beam detection, which is an optical system.<sup>1</sup> There are position-sensitive detectors called photo diodes. Laser light is reflected from the cantilever onto the photo-diode, position-sensitive detector. The AFM tips and cantilever are micro fabricated from silica or silicon nitride, which should have contact or near contact with the surface of interest.<sup>2</sup> Very small forces are produced between the probe and the surface by passing through the probe via the surface, and these forces enable the AFM system to record the deflection of the cantilever. The deflection of the cantilever is called “stiffness of cantilever”.<sup>1</sup> This stiffness can be measured by the Hooke law. The stiffness is recorded visually and can be visualized on the computer in real time.<sup>1,2</sup>

### AFM Modes of Operation

1. Contact mode: It is widely used among the different modes of the AFM. The AFM tip is in actual contact with the sample surface in this mode (figure 3a).<sup>6</sup>

2. Lateral force microscopy: The areas of the higher and lower frictional forces are measured by this mode.



**Figure 3 (a and b):** Comparison between the two AFM scanning types: tapping mode (a) and contact mode (b). Usually, the first one can minimize sample deformation.

3. Nanocontact mode: The cantilever is oscillated above the surface of interest at a distance in this mode, which is no longer in the repulsive regime but in the attractive regime of the inter-molecular force curve. The operation of nanocontact imaging is quite difficult in ambient conditions because of the existing thin layer of water on the tip and the surface of interest. As the tip is brought close to the surface of the sample, a small capillary bridge is created between the tip and the sample, causing the tip to “jump-to-contact” (figure 3b).<sup>6,7</sup>

4. Dynamic force\intermittent contact: This is also known as the tapping mode. The AFM tip touches or taps the surface and it is closer to the surface than the nanocontact mode. This mode is known to improve the lateral resolution of soft samples.<sup>2,6</sup>

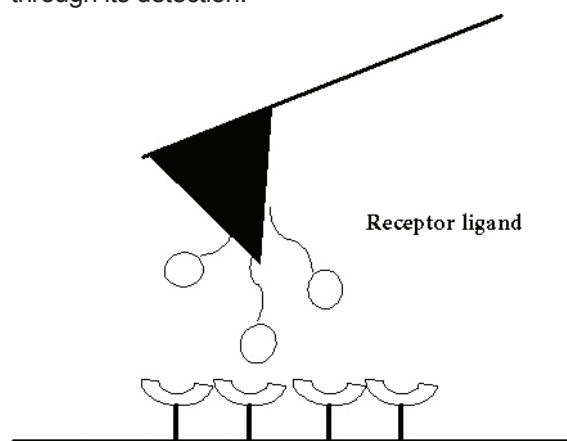
5. Force modulation: In this mode, the slope of the force-distance curve is measured, which is related to the elasticity of the sample.

6. Phase imaging: The phase shift of the oscillating cantilever relative to the driving signal is measured in this mode. This phase shift can be correlated with specific material properties that influence the tip/sample interaction. The phase shift can be used to differentiate areas on a sample with such differing properties as friction, adhesion, viscosity, and elasticity.<sup>6-8</sup>

### Advantages of AFM in Biology and Genetics

In the past decade, the AFM has emerged as a powerful tool to obtain nanostructural details and biomechanical properties of biological samples, including biomolecules and cells.<sup>9-12</sup> It can measure the changes in the mechanical property of the cell

membrane,<sup>10</sup> cell stiffness,<sup>11</sup> and cell viscoelasticity.<sup>12</sup> The AFM-based force spectroscopy is also particularly well-suited to assess cell adhesion,<sup>13</sup> and can stretch researching of cells, thereby allowing measurements of their rheological properties (figure 4).<sup>8</sup> The most important advantage of the AFM technique in biology is studying biological samples directly in their natural environment, especially in buffer solutions in vitro, in situ, and even in vivo without any sample preparation, which was once a very time-consuming task.<sup>3,14</sup> It can also detect the surface of living cells up to the single molecular forces in the field of cell biology.<sup>15-19</sup> Furthermore, there is no limitation in the choice of the type of medium either aqueous or non-aqueous, sample temperature, or chemical composition of the sample. The AFM modality has a limitation only for some transparent mediums that could pass the laser light through its detection.<sup>3</sup>



**Figure 4:** Molecular interaction by AFM Tip.

The AFM has demonstrated some success in studying nano scale, in situ DNA structures, which can lead to the development of more effective gene delivery vehicles. Researchers are utilizing the many benefits of the AFM, namely high resolution, simplified sample preparation, real-time investigation, and non-destructive imaging as well as the ability to perform in liquids and to investigate DNA condensation mechanisms and various gene-packaging materials.<sup>20,21</sup>

Ohara et al.<sup>22</sup> and Osada et al.<sup>23</sup> used the AFM to determine living cells and tissue conditions with their mRNA expression. Many methods of determining mRNA expression require total RNA extraction or cell fixation, which creates difficulties in examining mRNA expression in living cells without causing cell death. Using the AFM technique to extract mRNA prevents cell death.<sup>24</sup> Lymphocytes are defensive body cells. The analysis of the nanostructure and nanomechanics of lymphocytes using the AFM technique from resting and activated to apoptosis

helps researchers with their immunological studies.<sup>25</sup>

### Medical and Pharmacological Applications of AFM Technique

The AFM modality is a novel technique for the detection of the properties of biological membranes, which have been widely employed in biological researches over the last decade. The ability of the AFM to scan the interaction between SLBs (supported lipid bi-layers) and drug is a special advantage of the AFM technique.<sup>26,27</sup> Leclercq et al.<sup>28</sup> imaged the interaction between Azithromycin (as an antibiotic) and SLBs, supported on mica using the AFM, and Guangyong et al.<sup>29</sup> studied membrane proteins, which play a special role in the cell membrane. Probing membrane proteins using the AFM has opened a new research area to study the interactions between molecules at the molecular level (figure 4).<sup>29,30</sup>

Enzyme hydrolysis visualization can be done by the phase imaging mode of the AFM; however, in a research done by Liu et al.<sup>31</sup> hydrolysis of cellulose was determined with the AFM.

Direct observation of enzyme activity with the AFM is possible. In one study, height fluctuations on top of the protein lysozyme adsorbed on mica were measured locally with the AFM, operated in the tapping mode in liquid. Height fluctuations of an apparent size of 1 nanometer, which lasted for about 50 milliseconds, were observed over lysozyme molecules when a substrate (e.g. polyglycosides) was present. In the presence of the inhibitor (chitobiose), these height fluctuations decreased to the level without the polyglycoside. The most straightforward interpretation of these results is that the height fluctuations correspond to the conformational changes of lysozyme during hydrolysis.<sup>32</sup> The interaction between microbes and subsequent development of biofilms at surfaces has far-reaching consequences in medicine and dentistry. Detection or characterization of microbial surfaces and direct measurement of molecular forces and physical properties are other medical applications of the AFM. The microbial surface has been the focus of wide scientific investigation; nonetheless, technology has permitted the quantitative study of the molecular interactions recently. Therefore, the AFM permits not only a high resolution imaging of microbial surfaces but also a direct measurement of molecular forces and physical properties found at the microbial surface of interest.<sup>33</sup> The AFM can be used in genetics courtesy of its nanoprobe to detect mRNA in single living cells. Ohnesorge et al.<sup>34</sup> studied Pox viruses, living cells, and their core's mRNA using the AFM in dynamic form.

Dunlap et al.<sup>35</sup> studied the dynamic processes of the formation mechanism of DNA condensation to make a better overview on the kinetics of this process, which could lead to a significant overview in gene delivery (figure 5).

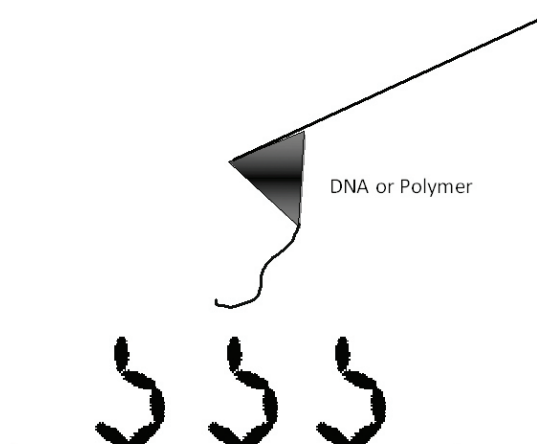


Figure 5: DNA study by AFM.

Another application of the AFM technique is in cardiology. Aging increases the stiffness of cardiac myocytes, and this can be measured with the nano indentation of the AFM. Samuel et al.<sup>36</sup> used the AFM to determine cellular mechanical property changes at a nano-scale resolution in myocytes. Scanning the renal epithelium with the AFM can be helpful for an early detection of renal diseases as well.<sup>37</sup> Moreover, the AFM can be utilized in the field of orthopedics, and changes in the surface topologies of chondrocytes subjected to mechanical forces can be evaluate by it. The cartilage is composed of chondrocytes embedded in a matrix of collagen fibrils interspersed within a network of proteoglycans and is constantly exposed to biomechanical forces during normal joint movement. Characterization of the surface morphology, cytoskeletal structure, adherence, and elastic properties of these mechanosensitive cells are crucial in understanding the effects of mechanical forces around a cell and how a cell responds to changes in its physical environment. The AFM data have shown that there are distinct changes in cell-surface topology and cytoskeleton arrangement in cells following treatment with mechanical forces. The AFM imaging and dynamic tensile forces may help overcome the effect of inflammatory factors on chondrocyte response.<sup>38</sup>

### Dental Application of AFM

Microscopes have opened a new window on the field of dental sciences. Among them, the AFM modality is a novel technique which can image not

only non-living surfaces but also living cells and dynamic environments.

Endodontic diseases are very common currently. In the field of obturation, appropriate fitting of gutta-percha within the canal walls is extremely important. The AFM can be employed to study the gutta-percha cone topography and offer a powerful new tool to evaluate the characterization of the gutta-percha cone surfaces directly. Caroline et al.<sup>39</sup> studied the topography of the apical portion of four different types of gutta-percha, using the lateral force mode of the AFM.

Dental caries are a very common disease nowadays. Dental caries are due to biofilm formation and colonization of bacteria, mostly *Streptococci Mutans*, in dental plaques. Sara et al.<sup>40</sup> studied the surface-function of the biofilm-forming *Streptococcus Mutans*, which is the primary etiological agent in human dental caries.

The AFM is a powerful microscope for a high-resolution examination of the salivary pellicle surface structure in its native (hydrated) state. It avoids artifacts due to fixing and dehydration which occur with scanning electron microscopic analyses. Hanging et al.<sup>41</sup> designed an AFM study to examine the surface of the adsorbed layer of salivary proteins (salivary pellicle) formed in vivo on dental enamel and glass surfaces.

Acid-etching is a technique used to make micro porosities in enamel and dentine surfaces for micromechanical adhesion of composites. In a new study by Sanchesea et al.<sup>42</sup> characterization of bovine enamel and dentine after acid-etching was imaged with the AFM.

El Feninata et al.<sup>43</sup> used the tapping mode of the AFM in order to study collapse and denaturation in dentinal collagen. Demineralization of human enamel will cause formation of dental caries. Mechanical properties of in situ demineralized human enamel can be measured with the nanoindentation of the AFM.<sup>44</sup> Fluoride therapy is a protective technique to avoid dental caries in children. Effects of fluoride treatment on phosphoric acid-etching in primary teeth were studied by Chot et al.<sup>45</sup> via the contact mode of the AFM.

One of the most important fields of modern dentistry is implantology. The AFM can be drawn upon as a tool for testing the biocompatibility of implant materials by investigating the adhesion behavior of osteoblast cells in vitro. This technique allows the investigation of the cytomorphology and cytomechanical properties of living cells on a nano scale. Domek et al.<sup>46</sup> investigated the application of different substances in implantology and the elasticity of living cells.

Yongkah et al.<sup>47</sup> made use of a special kind of

the AFM probe, which was based on the surface of plasmon resonance of gold nanoparticles, for an early diagnosis of squamous cell carcinoma. The resonance of nanoparticles can detect morphological and mechanical changes at the very early stages. A comparison of the mechanics of normal and tumor cells can reveal new information about the mechanisms of malignant transformation. It can also help to understand how cells "mechanically" invade a normal tissue. At the present time, there are only a few studies conducted on this issue.

Osteosarcoma and chondrosarcoma are two fatal cancers, but if diagnosed at early stages, they may be curable. The results of the Docheva D et al. study,<sup>48</sup> showed a direct comparison between the morphometric and biophysical features of different human cell types derived from normal and pathological bones.

## Conclusion and Future Applications

The AFM technique is a 3D topographical modality with a high atomic resolution for roughness measurement. Not only can the AFM provide new information about the surface of the cell properties such as friction and adhesion force measurements and viscoelastic properties but it can also determine the Young modulus and image magnetic or electrostatic properties. In addition, the AFM can analyze any kind of sample in any medium. Indeed, the salient characteristic of the AFM is its ability to study any kind of sample directly in its natural environment without any sample preparation, which is time-consuming and may change the surface properties. The AFM is a unique tool for the assessment of living and dead cell surfaces in nano scales and has, thus, opened a new window on the study of different fields such as cell biology, surface single molecule detecting, dental material quality, molecular interaction, preventive dental therapies, and implant biocompatibility. Further development of the AFM technique will clarify cellular and molecular interaction, mechanism, and mechanics secrets even in single organelle stages.

**Conflict of Interest:** None declared.

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