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Abstract. This article presents the main areas of application of atomic force microscopy in biology and medicine, describes the principle of an atomic force microscope and the main modes of its operation. The works and results of the leading laboratories in the stated topic are considered. There are a number of proposals for generalizing the considered results into a unified knowledge base on diseases and the general state of health of the human body.

1. Introduction

Atomic force microscopy (AFM), which is also known as scanning probe microscopy (SPM), belongs to the class of high-resolution measurement methods for analyzing the microstructure and topographic qualities of materials [1]. In 1982, Gerd Binnig, together with Kelvin Quait and Christopher Gerber, presented a version of the tunneling microscope, which is based on measuring the interaction forces between atoms, so this tunneling microscope was called atomic force (AFM). AFM provides great prospects for obtaining images of the surface of various objects with atomic resolution in liquid and air spaces, as well as in vacuum conditions above the average level. In the conditions of the present time, the main areas of application of AFM include natural sciences, biology and industry. However, in the fields of biology and medicine, until recently, scientists could not detect images of biological and biochemical structures in their physiological aqueous media in real space with a resolution that exceeds the diffraction limit of natural optical microscopy (about 350 Nm).

2. The principle of operation of the AFM

Atomic force microscopy is based on determining the interaction between the surface of the analyzed sample and the probe of the microscope, which is fixed at the end of an elastic cantilever console. The bending of the console occurs due to the forces acting on the probe from the sample side. These are usually Van der Waals forces. The force of interaction of the probe with the surface is controlled by fixing the bending level.

But in reality, the result of the study can be influenced by magnetic and electrostatic forces, elastic and adhesive forces. The radiation of the semiconductor laser is focused on the elastic console of the probe sensor.

The reflected radiation is fixed using a photosensitive element - a photodiode with four sections, which makes it possible to determine the direction and degree of displacement of the probe sensor console [2]. The main characteristics recorded by the optical system are the degree of torsion deformation under the action of tangential components of the interaction forces of the probe with the

surface and the degree of bending deformation of the console under the action of the normal component of the attraction or repulsion forces.

Before starting the analysis the optical system of the microscope should be adjusted so that the reflected radiation falls into the center of the photodetector. In this case the photocurrents from all sections of the photodiode will have the same value. Due to the deformation of the bending of the console under the action of interaction forces the reflected beam is deflected from the central position. The change in the photocurrent in each section is the result of this shift. The degree and direction of displacement of the cantilever console is parameterized by a change in the photocurrent, which is called the difference current. The photocurrent received from 4 sections of the photodiode allows creating a voltage in the feedback unit, which is stored as a surface relief. The distance between the probe of the microscope and the surface of the analyzed sample is maintained at an unchanged level by means of a tubular piezo motor. The voltage that is applied to the tubular piezo motor is the voltage in the feedback circuit [1].

3. Operating modes

The operating modes of the AFM can be divided into two large groups: contact and contactless. In the contact mode, the AFM registers the repulsive forces of the interatomic interaction, in the non-contact mode – the attracting ones. In the contact mode the AFM applies a force to the sample and therefore the interaction between the AFM probe and the sample is repulsive.

When the scanner gently passes the tip over the sample, the contact force causes the cantilever to bend to adapt to changes in the topography of the sample. The normal applied force creates a significant friction force, when the probe scans the surfaces. Both normal and frictional forces can damage vulnerable biological samples. In order to obtain a correct image of these types of samples, the applied force must be carefully controlled.

In the non-contact mode, the cantilever oscillates at a distance (usually 5-15 nm) from the sample surface. To generate images you can use a frequency shift or a change in phase and amplitude. A real non-contact image is extremely difficult to obtain. To achieve the highest resolution, the probe must be brought close enough to the surface to effectively detect the gravity gradient. Thus the oscillating probe very often slightly touches the surface of the sample and becomes a semi-contact AFM mode. Despite the fact that the non-contact AFM mode has the advantage of reducing the contact and shear forces between the tip and the samples, reducing the possibility of damage to soft biological samples, it still has many disadvantages that make it less popular than the contact mode. First, the acting force of the non-contact AFM mode is located in the attracting regions of the force-distance curve, which includes many different forces, therefore the behavior of the force between the probe and the sample is not easy to determine and calibrate [3]. Secondly, the resolution and contrast of the non-contact AFM mode in a liquid are not as good as in air, since the damping of the liquid significantly reduces the sensitivity of the oscillating probe [4]. Thirdly, in some cases it was found that soft structures on surfaces can still be deformed by the oscillating tip of an atomic force microscope [5].

4. Application of AFM in biology and medicine

AFM is one of the most important methods of studying biological systems. The main difference between the AFM method and other methods of scanning probe microscopy is its versatility. The ability to build images in different environments, using different modes of operation, allowed us to study the structure of living cells, to measure the level of interaction with the abiotic surfaces of other cells. AFM was used to study deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), proteins, viruses, bacteria, various tissues and organs [6]. And since the atomic force microscope uses a physical scanning method to obtain images of samples, the preparation of samples for AFM imaging is relatively simple, and does not require freezing, metal coating, vacuum or dye. For samples of proteins and DNA or even bacteria, solutions or suspensions of samples can simply be deposited on perfectly flat surfaces such as mica, graphite and silicon nitrite, the sample can be adsorbed on the surface by weak forces such as Van der Waal interactions. Most commercial atomic force microscopes sold today have the ability to display samples in liquids, since they all use the same optical detection method to control the displacement of

the probe. The samples can simply be coated with water or a buffer solution, and then the probe can be placed directly into the liquid to scan the samples in it. The laser beam can still penetrate the liquid to detect changes in the probe of the atomic force microscope. Manufacturers have also developed various state-controlled containers for visualizing biological samples in liquid. Some of them allow changing the buffer using syringes or pumps, and some of them have precise temperature control functions, which is considered very important for the visualization of living cells [7].

5. Deoxyribonucleic acid (DNA) and Ribonucleic acid (RNA) complexes

DNA and RNA are present in all cells of the body. DNA and RNA are biological polymers that ensure the execution of a genetic program (DNA is responsible for this) and carry out the program synthesis of proteins (RNA is responsible for this). Currently, the study of DNA and RNA is considered the main task of genetic engineering. It was found that the average size of a human cell is about 10 microns, the thickness of the DNA strand is 2 nm, and the length reaches 3 m. The study of DNA is of particular importance, as it can give an idea of the "packing density" of genetic information.

The use of AFM allows us to extract data on the structure and topology of DNA and RNA [8].

The method of physical matching using tags is one of the ways to partially decipher the code written in DNA and RNA. Ligands are used as labels. Ligands are molecules or ions in chemical complexes that are directly connected to a central atom (a complexing agent). Their main property is the recognition of a certain nucleotide sequence and binding to it. The process of "tagging" is as follows: certain ligands (for example, certain protein molecules) are added to a solution containing nucleic acids, that bind to their specific sites of the nucleic acid molecule. As a result, a system is formed that consists of a nucleic acid molecule and a protein molecule attached to it in a certain place. AFM is used to partially decipher the code of a nucleic acid molecule as a method for visualizing nucleic acid molecules and identifying ligands-proteins associated with it. In comparison with classical electron microscopy AFM allows determining the molecular weight of a protein and clearly distinguishing monomers and dimers of protein molecules [9].

AFM is used to study the metaphase chromosome. In addition, structural models of chromatin fiber were proposed based on the AFM results [10]. These studies have proved the usefulness of AFM for obtaining three-dimensional surface topography both in the environment and in physiological liquid conditions. AFM is capable of obtaining images of G-banded chromosomes with a much higher resolution than conventional optical microscopic methods. Thus, the AFM can become a new method of karyotyping.

With the help of AFM we learned to directly observe the clustering of damage to irradiated DNA. This method allows not only to estimate the frequency, but also to analyze the complexity of cluster DNA damage. In addition, in principle, it can be used to analyze damage to chromosomal DNA isolated from irradiated cells [11].

6. Proteins and polypeptides

Proteins (proteins, polypeptides) are high-molecular organic substances consisting of alpha-amino acids bound into a single chain by a peptide bond. It is established that 10-20% of the genome of living organisms is encoded by proteins. AFM is one of the most powerful tools for studying proteins and polypeptides, since in the contact mode it provides a lateral resolution of 0.5-1 nm and a vertical resolution of 0.1-0.02 nm. This is quite enough to study the structure, functioning and structure of the protein. However, to reduce damage and reduce deformations of the studied proteins, it is more reasonable to use a semi-contact mode, which provides horizontal resolution in the range of 1.1-1.5 nm and vertical resolution of about 0.1 nm [12]. AFM makes it possible to visualize proteins and protein structures deposited on the surface under physiological conditions, which is an advantage over ultrahigh vacuum methods such as electron microscopy. The AFM can also provide additional information from the tapping phase or from the AFM functional modes. Sample preparation, probe selection, and imaging conditions are crucial for successful protein imaging [13]. AFM can be used not only for visualization of protein complexes, but also for direct manipulations with the geometric dimensions of the polypeptide chain.

7. Viruses and bacteria

Viruses are small agents that infect all kinds of organisms, from animals to plants and bacteria. Viruses are also among the most serious pathogens in the history of mankind. Understanding the molecular basis of viruses has the potential to develop new therapeutic treatment strategies. The prevalence and variety of forms make viruses one of the most important objects of research. The size of viruses varies from 20 to 250-400 Nm, which is beyond the resolution range of conventional optical microscopy. Thanks to AFM images of viruses were obtained in nanometer resolution [3].

Bacteria are single-celled organisms that have the shape of a rod, ball or spiral. The size of bacteria varies in a wide range from 1 to 750 microns. Therefore, AFM is suitable for a detailed study of the surfaces of bacteria. Currently, about ten thousand species of bacteria have been studied, but their estimated number exceeds a million, which requires further study. To do this, you can use 3 modes of AFM operation: contact, semi-contact and magnetic. In the magnetic mode, magnetic forces have a significant effect on the surface. The AFM uses a special probe to build maps of the distribution of magnetic fields. The magnetic mode is a kind of semi-contact technique, but it has a number of advantages when working in liquid media. Image quality improvement is achieved by eliminating the resonance that occurs in the liquid when working in semi-contact mode. Using an integrated approach and combining the methods of AFM, electron microscopy and biophysics, it is possible to study the processes of interaction between bacteria. [1].

The most common application of AFM in bacteriology is the study of changes on bacterial surfaces caused by a change in condition or treatment with antimicrobial agents. Using this technique, the influence of cultivation conditions on the formation of biofilms of various bacteria was studied. Work was also carried out in the field of research on the antimicrobial effect of antibiotics and antimicrobial peptides. AFM was used to study the antibacterial mechanism of nanophotocatalysts sensitive to visible light [5]. The results revealed a predominant damage to the apical end of rod-shaped bacterial cells by photocatalysis, which was not observed by other methods, such as electron microscopy.

AFM is able to give images of bacteria in the smallest detail, so you can get valuable information about the mode of action of antimicrobial agents. This may lead to improved antimicrobial drug design to combat the problems that arise as a result of increasing the resistance of bacteria to antibiotics.

8. Tissues and organs

The ability of AFM to create three-dimensional images with nanometer resolution allows using this method to study various diseases of the visual organs, such as cataracts. Thus, using AFM in contact and magnetic modes, the structural and physiological properties of the eye tissues of various animals were studied. The analysis of the obtained images allows us to track changes in the physical properties of the lens as it becomes cloudy [14]. In the future, the method of AFM can be used to study various eye tissues and concomitant diseases. The capabilities of AFM for visualization of semiconductors and dielectrics in various environments made it possible to study the dentin of a human tooth and minimize the influence of various artifacts caused by dehydration. The use of AFM is mainly useful in the study of the collagen network of dentin and its changes caused by thermal stress. It is established that changes in the inter-tubular and peritubular dentin are caused by the action of phosphoric acid, self-etching primers, rinses and other components of adhesive restoration materials used in modern dentistry.

The AFM has already provided important information about proteins that cannot be obtained by other methods. For example, AFM experiments revealed differences in length between phosphorylated and nonphosphorylated myosin monomers that were not detected by electron microscopy [15].

In addition to visualization, AFM can also be used to measure strength where scanning is not always required. The AFM is capable of applying forces to the sample surfaces at a level of only 10-11 N. Some long-range forces between the tips of atomic force microscopes and samples within tens of nanometers above the surface, such as electrostatic interactions, were measured using the approximation curves of the tips of AFM at certain points of surfaces in aqueous media. AFM is also used to measure the opening force of membrane proteins using modified tips for pulling membrane proteins out of membranes [16].

9. Manipulation of biological objects at the nanoscale using AFM

AFM can be used not only for visualization and measurement of local viscoelastic parameters of the surface of various biological objects, but also for directed force action. Thus, AFM can be used to "cut out" sections of the chromosome. To do this, a separate chromosome is first visualized at low probe pressure or in intermittent contact mode (the resolution in this case can reach 30-50 nm). Then the pressure force of the tip increases to values sufficient for the physical "cutting off" of the selected region of the chromosome, and scanning is performed at this point of the chromosome. The" cut off " fragment of the chromosome sticks to the tip of the probe and can then be separated and used for further genetic analysis.

AFM is increasingly used in the study of biomedical objects. Its advantages over other types of microscopy make this research method a leader in modern biology and medicine [9].

10. Development prospects

In the future, the images obtained by atomic force microscopes can be analyzed automatically by collecting a large database. To date, there are already works in the direction of automated diagnostics based on the analysis of AFM images. There is a fully automated technology for analyzing DNA samples obtained using atomic force microscope systems that take samples on long-chain biopolymers, such as string-like DNA strands.

The considered unique capabilities of AFM-based technologies for imaging, sensing, parametrization and manipulation of late biointerfaces prove the huge potential of atomic force microscopy in the future. In some of these applications, especially in the characterization of complex biosystems, it is advisable to supplement AFM with optical microscopy and spectroscopy. It is the addition of atomic force microscopy with other methods that can be useful for attracting chemical, biophysical, cellular and molecular biology laboratories to work, which expands the capabilities of AFM in biology and medicine. The combination of two or more AFM-based modalities to characterize several parameters of complex biointerfaces, one of which is a promising example of AFM based on rapid detection, increases the variety and volume of data that can be obtained in the experiment. This combination allows, for example, to correlate the events of ligand binding with the topography of protein complexes or living organisms. It seems certain that the AFM-based methods that have revolutionized nanotechnology will have a similar impact on how we consider and use biointerfaces [17].

In the most long-term perspective, atomic force microscopy can become a bridge to the construction of personalized medicine. At the moment, with the help of AFM, it is already possible to diagnose many diseases and trace the predisposition to others, which will undoubtedly help to collect an individual map of human diseases.

11. Conclusion

AFM has a nanometer resolution and the ability to work in liquid media, which is a key requirement for biological imaging. The operating range of the atomic force microscope is suitable for the characterization of structures from the molecular to the cellular scale. In addition, AFM has a unique ability to measure molecular forces with high sensitivity. These applications have been used to identify structural details and determine the molecular forces involved in various biological systems. Measurements of electrostatic characteristics are also among the new advances that can facilitate the analysis of biological and biomedical samples. The need for detailed visualization at the molecular level and for monitoring dynamic biological processes will continue. Thus, AFM is likely to play an important and sustainable role in biological and biomedical research.

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