Abstract
The remarkable feature of Scanning Probe Microscopes (SPM) is their ability to “view” details at the atomic and molecular level, thus increasing our understanding of how systems work and leading to new discoveries in many fields. These include life science, materials science, electrochemistry, polymer science, biophysics, nanotechnology and biotechnology. This article presents the principles of atomic force microscopy and describes the necessary components of such equipment. The essential property is the interaction force between the tip and the sample, which depends on their distance. At close contact the force is repulsive while at a larger separation the force is attractive. This results in different operation modes which should be chosen according to the characteristics of the sample, since each mode has different advantages. Atomic force microscopy is currently applied to various environments (air, liquid, vacuum) and types of materials such as metal semiconductors, soft biological samples, conductive and non-conductive materials. With this technique size measurements or even manipulations of nano-objects may be performed.

1. Introduction
In all Scanning Probe Microscope (SPM) techniques a tip interacts with the sample surface through a physical phenomenon. Measuring a “local” physical quantity related with the interaction, allows constructing an image of the studied surface. All the data are transferred to a PC, where, with the use of the appropriate software, an image of the surface is created.
The scanning tunneling microscope (STM) is the ancestor of all scanning probe microscopes. It was invented in 1982 by Gerd Binning and Heinrich Rohrer at IBM Zurich. Five years later they were awarded the Nobel Prize in Physics for their invention.

The atomic force microscope (AFM) was also invented by Binning et al. in 1986.

While the STM measures the tunneling current (conducting surface), the AFM measures the forces acting between a fine tip and a sample. The tip is attached to the free end of a cantilever and is brought very close to a surface. Attractive or repulsive forces resulting from interactions between the tip and the surface will cause a positive or negative bending of the cantilever.

The bending is detected by means of a laser beam, which is reflected from the back side of the cantilever. Figure 1 shows the basic concept of STM and AFM.

![Figure 1. Principle of STM (left) and AFM (right).](image)

### 2. Components of the microscope

#### 2.1. Piezocrystals

Piezocrystals are ceramic materials that expand or contract in the presence of voltage gradient and conversely, they develop an electrical potential in response to mechanical pressure. In this way, movements in x, y and z direction are possible.
2.2. Probe
The probe represents a micromachined cantilever with a sharp tip at one end, which is brought into interaction with the sample surface. Each probe has different specifications and shape. V-shaped cantilevers are the most popular (but also there are rectangular), providing low mechanical resistance to vertical deflection, and high resistance to lateral torsion. Cantilevers typically range from 100 to 200 µm in length (l), 10 to 40 µm in width (w), and 0.3 to 2µm in thickness (t).

![Figure 2. Dimensions of the cantilever.](image)

Integrated cantilevers are usually made from silicon (Si) or silicon nitride (Si₃N₄). They are characterized by their force constant and resonant frequency, which have to be chosen according to the sample to be studied. Additionally an optical detection system and electronics for the management of scanning procedures and data acquisition are necessary.

3. Beam Deflection Detection
To detect the displacement of the cantilever, a laser is reflected off the back of the cantilever and collected in a photodiode. The diode is divided into four parts, as seen in Figure 3. When the laser is displaced vertically along the positions top (B-A) and bottom (D-C), there exists a bending due to topography, while if this movement is horizontal left (B-D) and right (A-C), it produces a torsion due to "friction" (lateral force).
3. Forces versus distance curve
A force sensor in an AFM can only work if the probe interacts with the force field associated with a surface. The dependence of the van der Waals force upon the distance between the tip and the sample is shown in Figure 4.

In the contact regime, the cantilever is held less than a few angstroms from the sample surface, and the interatomic force between the cantilever and
the sample is repulsive. In the non-contact regime, the cantilever is held on the order of tens to hundreds of angstroms from the sample surface, and the interatomic force between the cantilever and sample is attractive (largely a result of the long-range Van der Waals interactions).

Attractive forces near the surface are caused by a nanoscopic layer of contamination that is present on all surfaces in ambient air. The amount of contamination depends on the environment in which the microscope is being operated. Repulsive forces increase as the probe begins to contact the surface.

The repulsive forces in the AFM tend to cause the cantilever to bend up. In figure 5 an experimental force vs. distance curve is shown. It corresponds to one cycle of the tip approaching to, getting into contact and separating from the sample.

At the right side of the curve, the scanner is fully retracted and the cantilever is undeflected since the tip is not touching the sample (region I). As the scanner extends, the cantilever remains undeflected until it comes close enough to the sample surface for the tip to experience the attractive van der Waals force.

In the point II, the cantilever suddenly bends slightly towards the surface. As the scanner continues to extend, the cantilever deflects away from the surface, approximately linearly (region III, red color).
After full extension, at the extreme left of the plot (region III, black color), the scanner begins to retract. The cantilever deflection retraces the same curve. In the point (IV), the scanner retracts enough that the tip springs free.

The resolution for AFM instruments is: a) 0.1 nm on sample plane (x, y) for hard and flat surfaces and 0.7-5 nm for soft materials (polymers and biological samples), b) 0.01 nm for z axis.

4. Modes of operation
4.1. Contact Mode
In the so-called contact-AFM mode, the tip makes soft “physical contact” with the surface of the sample.
The deflection of the cantilever Dx is proportional to the force acting on the tip, via Hook’s law, F=-k. x, where k is the spring constant of the cantilever.

In contact-mode the tip either scans at a constant small height above the surface or under the conditions of a constant force. In the constant height mode the height of the tip is fixed, whereas in the constant-force mode the deflection of the cantilever is fixed and the motion of the scanner in z-direction is recorded. By using contact-mode AFM, even “atomic resolution” images are obtained.

For contact mode AFM imaging, it is necessary to have a cantilever which is soft enough to be deflected by very small forces and has a high enough resonant frequency to not be susceptible to vibrational instabilities. Silicon Nitride tips are used for contact mode. In these tips, there are 4 cantilever with different geometries attached to each substrate, resulting in 4 different spring constants

To avoid problems caused by capillary forces which are generated by a liquid contamination layer usually present on surfaces in air, the sample can be studied while immersed in a liquid. This procedure is especially beneficial for biological samples.

4.2. Non Contact Mode
In this mode, the probe operates in the attractive force region and the tip-sample interaction is minimized. The use of non-contact mode allowed scanning without influencing the shape of the sample by the tip-sample forces. In most cases, the cantilever of choice for this mode is the one having high spring constant of 20- 100 N/m so that it does not stick to the sample surface at small amplitudes. The tips mainly used for this mode are silicon probes.
4.3. Tapping Mode (intermittent contact Mode)

The force measured by AFM can be classified into long-range forces and short-range forces. The first class dominates when we scan at large distances from the surface and they can be Van der Waals force, capillary forces (due to the water layer often present in an ambient environment). When the scanning is in contact with the surface the short range forces are very important, in particular the quantum mechanical forces (Pauli Exclusion Principle forces).

In tapping mode-AFM the cantilever is oscillating close to its resonance frequency. An electronic feedback loop ensures that the oscillation amplitude remains constant, such that a constant tip-sample interaction is maintained during scanning.

Forces that act between the sample and the tip will not only cause a change in the oscillation amplitude, but also change in the resonant frequency and phase of the cantilever. The amplitude is used for the feedback and the vertical adjustments of the piezoscanner are recorded as a height image. Simultaneously, the phase changes are presented in the phase image (topography).

The advantages of the tapping mode are the elimination of a large part of permanent shearing forces and the causing of less damage to the sample surface, even with stiffer probes. Different components of the sample which exhibit difference adhesive and mechanical properties will show a phase contrast and therefore even allow a compositional analysis. For a good phase contrast, larger tip forces are of advantage, while minimization of this force reduces the contact area and facilitates high-resolution imaging. So in applications it is necessary to choose the right values matching the objectives. Silicon probes are used primarily for Tapping Mode applications.

Table 1 is a summary of the main characteristics of the three modes explained before. In these modes we can work in different environments: air, liquid and vacuum. In contact mode the tip touches the sample surface, which leads to a high force and allows manipulation of the sample. The disadvantage is that the AFM tip may be contaminated by the sample. The opposite happens in the noncontact mode, where the tip stays at a distance above the sample. In tapping mode the tip touches the surface periodically therefore manipulation of the sample, as well as contamination of the tip is possible.
<table>
<thead>
<tr>
<th>Operation mode</th>
<th>Contact Mode</th>
<th>Non-contact mode</th>
<th>Tapping Mode</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tip loading force</td>
<td>low → high</td>
<td>Low</td>
<td>low</td>
</tr>
<tr>
<td>Contact with sample</td>
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<td>No</td>
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<tr>
<td>sample surface</td>
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<tr>
<td>Manipulation of Sample</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Contamination of AFM Tip</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Table 1. Properties of the different operation modes in AFM.

4.4. Advantages and Disadvantages of AFM Modes

Contact Mode AFM
Advantages:
- High scan speeds.
- “Atomic resolution” is possible.
- Easier scanning of rough samples with extreme changes in vertical topography.

Disadvantages:
- Lateral forces can distort the image.
- Capillary forces from a fluid layer can cause large forces normal to the tip sample interaction.
- Combination of these forces reduces spatial resolution and can cause damage to soft samples.

Non-contact Mode AFM
Advantage:
- Low force is exerted on the sample surface and no damage is caused to soft samples

Disadvantages:
- Lower lateral resolution, limited by tip-sample separation.
- Slower scan speed to avoid contact with fluid layer.
- Usually only applicable in extremely hydrophobic samples with a minimal fluid layer.

Tapping Mode AFM
Advantages:
- Higher lateral resolution (1 nm to 5 nm).
- Lower forces and less damage to soft samples in air.
- Almost no lateral forces.

Disadvantage:
- Slower scan speed than in contact mode.
5. AFM images
The tip of the AFM is used:
- for imaging.
- for measuring forces (and mechanical properties) at the nanoscale.
- as a nanoscale tool, i.e. for bending, cutting and extracting soft materials (such as Polymers, DNA, and nanotubes), at the submicron scale under high-resolution image control.

The figures below show different examples of AFM images taken by the modes explained before. The samples can be various materials, like semiconductors, biological material or polymer films.

Figure 7. Three-dimensional AFM images from three different types of Pd samples, grown on 6H-SiC.

Figure 8. Tapping Mode image of biological sample, in this case nucleosomal DNA.
6. Applications
The number of applications for AFM has exploded since it was invented in 1986 and nowadays this technique is involved in many fields of nanoscience and nanotechnology. The remarkable feature of STM and AFM instruments is their ability to examine samples not only in an ultrahigh vacuum but also at ambient conditions or even in liquids.

One of the advantages of AFM is that it can image the non-conducting surfaces, and therefore it is very suitable for biological systems. The AFM is capable of measuring nanometer scale images of insulating surfaces with little or no sample preparation as well as measuring three dimensional images of surfaces and studying the topography.

Some possible applications of AFM are:
- Substrate roughness analysis.
- Step formation in thin film epitaxial deposition.
- Pin-holes formation or other defects in oxides growth.
- Grain size analysis.
- Phase mode is very sensitive to variations in material properties, including surface stiffness, elasticity and adhesion.
- Comparing the tip-samples forces curves for materials to study the ratio of Young’s Modulus (graphite as a reference for measure of the indentation).
- Obtaining information of what is happening under indentation at very small loads.
- By In situ AFM analysis with changes in temperature we can study changes in the structure.

7. References
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