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DEPARTMENT OF BIOTECHNOLOGY
FACULTY OF ENGINEERING & TECHNOLOGY

Atomic Force Microscopy

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Atomic Force Microscopy

Atomic force microscopy (AFM) or scanning force microscopy (SFM) is a very high-resolution type of scanning probe microscopy (SPM). An **atomic force microscope** is a type of high resolution scanning probe microscope that has a resolution that you can measure in fractions of a nanometer. It was pioneered in 1986 by Nobel Prize Winner Gerd Binnig along with Calvin Quate and Christoph Gerber.

•Atomic force microscopy (AFM) is used for quantitative and qualitative data based on different properties like morphology,. It is also used for the study of soft and hard synthetic materials regardless of their conductivity and opaqueness. The atomic force microscope has been used to study the interactions between the *E. coli* GroES and GroEL chaperonin proteins, to map plasmids by locating restriction enzymes bound to specific sites, and to follow the behavior of living bacteria and other cell

• In AFM, the force between the sharp probe tip (< 10 nm) and the sample surface, with a 0.2–10 nm probe-sample separation, is measured. The probe is attached with a cantilever, which deflects upon interaction; this deflection is measured by the reflection of a laser beam by the "beam bounce" method.

Atomic Force Microscopy principle

Surface Sensing

An AFM uses a cantilever with a very sharp tip to scan over a sample surface. As the tip approaches the surface, the close-range, attractive force between the surface and the tip cause the cantilever to deflect towards the surface. However, as the cantilever is brought even closer to the surface, such that the tip makes contact with it, increasingly repulsive force takes over and causes the cantilever to deflect away from the surface.

Detection Method

A laser beam is used to detect cantilever deflections towards or away from the surface. By reflecting an incident beam off the flat top of the cantilever, any cantilever deflection will cause slight changes in the direction of the reflected beam. A position-sensitive photo diode (PSPD) can be used to track these changes. Thus, if an AFM tip passes over a raised surface feature, the resulting cantilever deflection (and the subsequent change in direction of reflected beam) is recorded by the PSPD.

Imaging

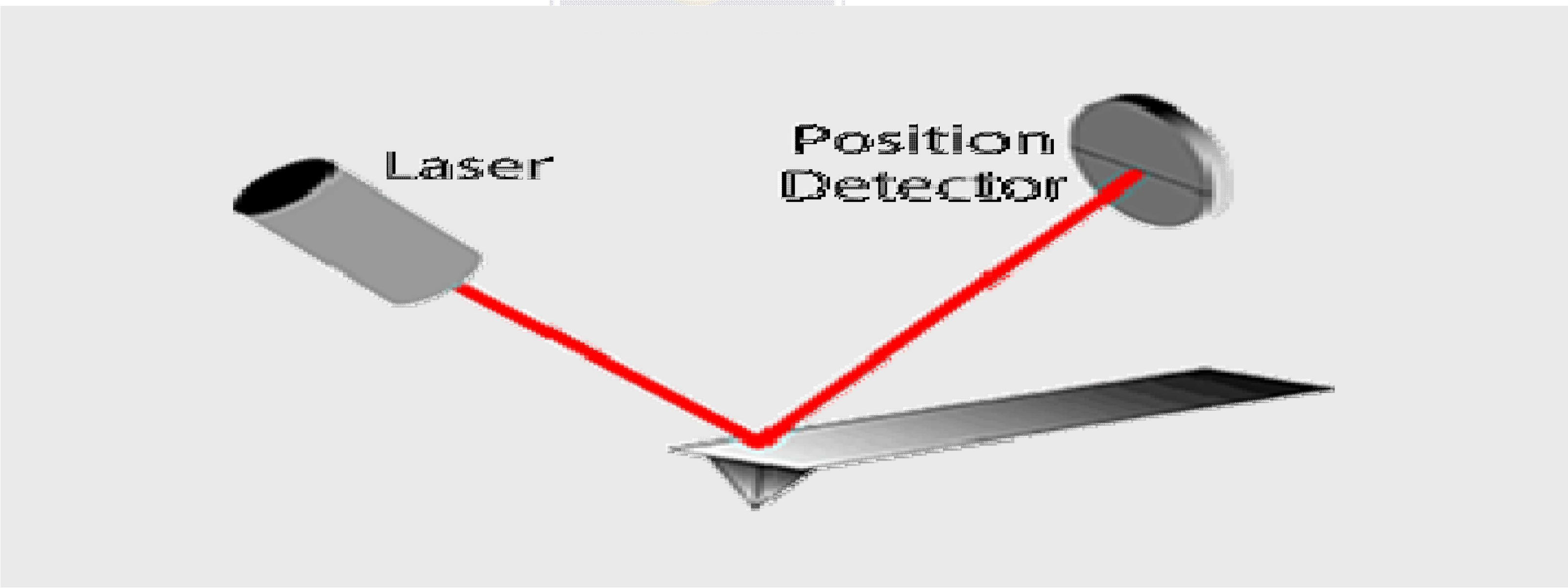
An AFM images the topography of a sample surface by scanning the cantilever over a region of interest. The raised and lowered features on the sample surface influence the deflection of the cantilever, which is monitored by the PSPD. By using a feedback loop to control the height of the tip above the surface—thus maintaining constant laser position—the AFM can generate an accurate topographic map of the surface features.

Atomic Force Microscopy principle: Working

In addition to Angstrom-level positioning and feedback loop control, there are 2 components typically included in Atomic Force Microscopy: Deflection and Force Measurement.

AFM Probe Deflection

Traditionally, most Atomic Force Microscopes use a laser beam deflection system where a laser is reflected from the back of the reflective AFM lever and onto a position-sensitive detector. AFM tips and cantilevers are typically micro-fabricated from Si or Si_3N_4. Typical tip radius is from a few to 10s of nm.



Measuring Forces

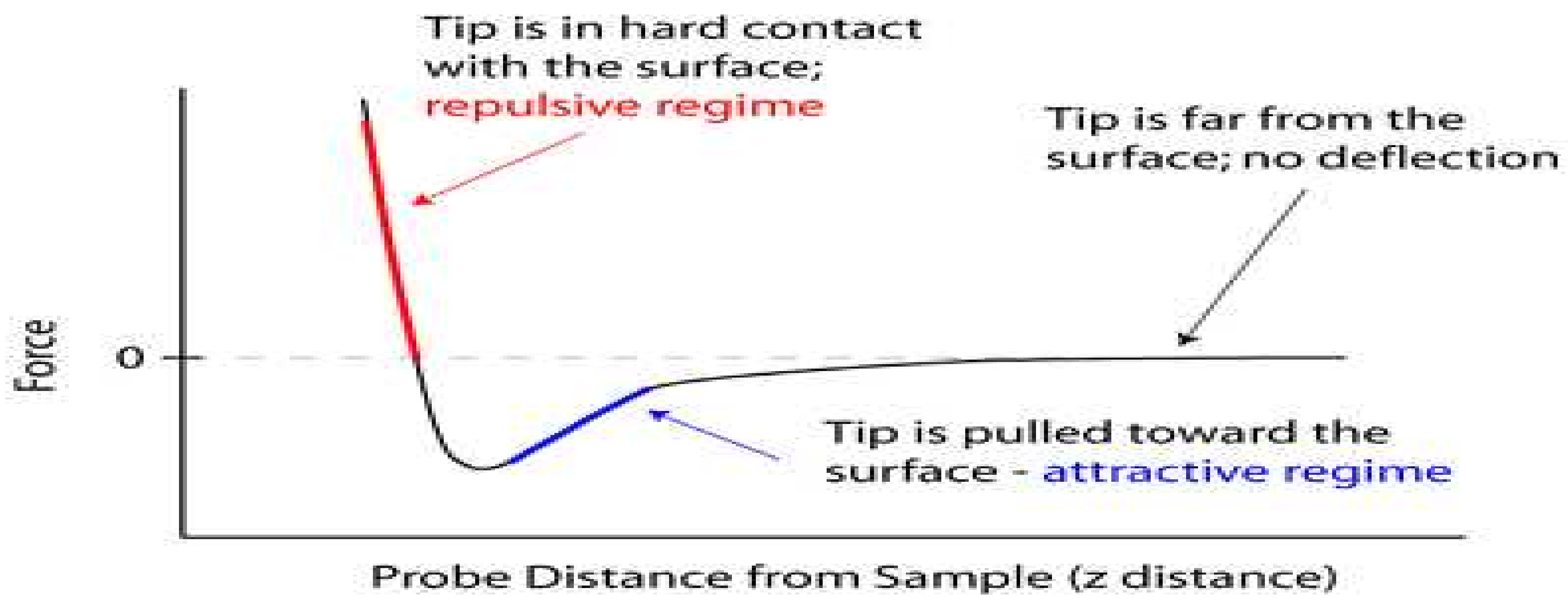
Because the Atomic Force Microscope relies on the forces between the tip and sample, these forces impact AFM imaging. The force is not measured directly, but calculated by measuring the deflection of the lever, knowing the stiffness of the cantilever.

Hooke's law gives:

F = -kz

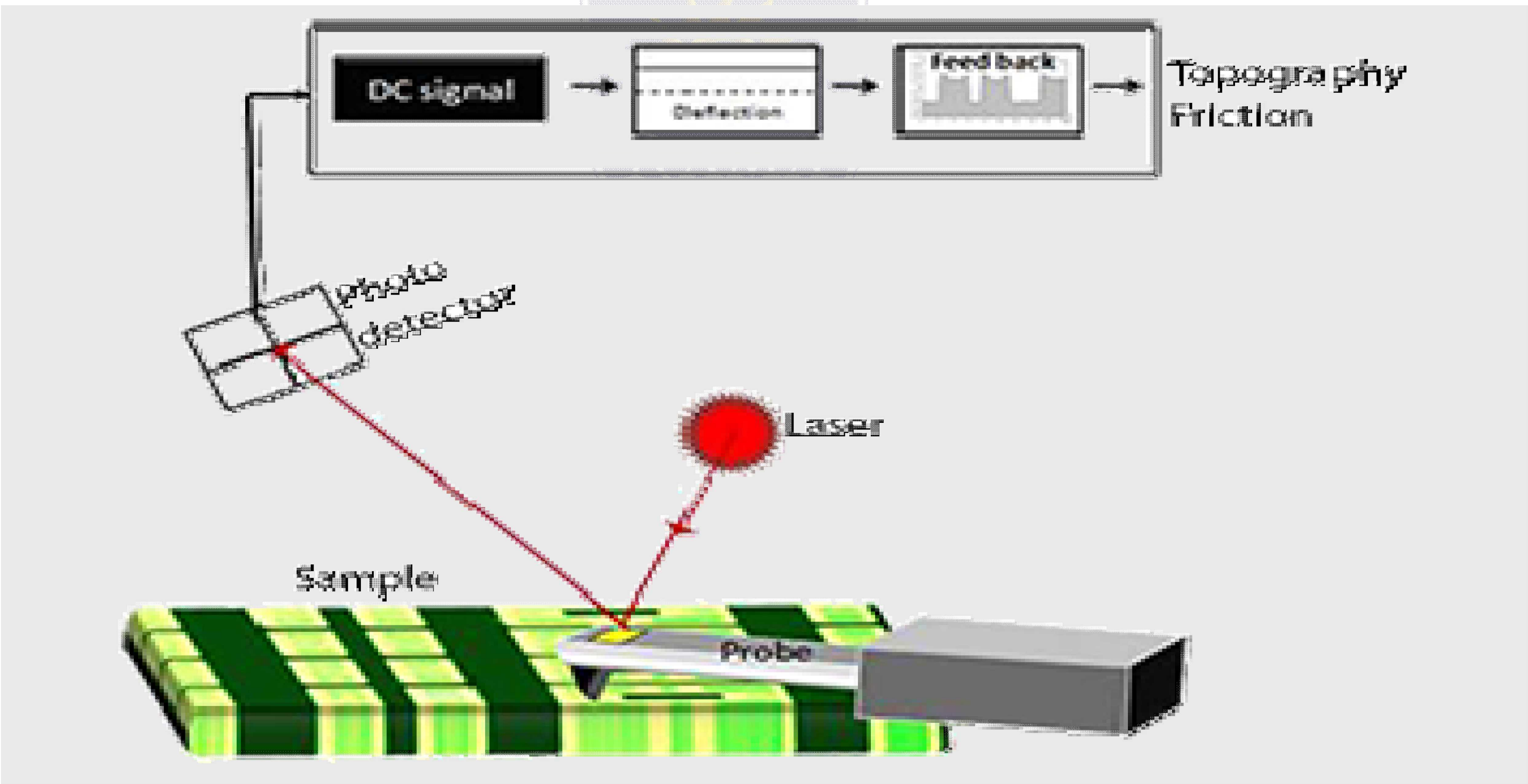
where F is the force, k is the stiffness of the lever, and z is the distance the lever is bent.

Force-distance curve for Atomic Force Microscopes



Feedback Loop for Atomic Force Microscopy

Atomic Force Microscopy has a feedback loop using the laser deflection to control the force and tip position. As shown, a laser is reflected from the back of a cantilever that includes the AFM tip. As the tip interacts with the surface, the laser position on the photodetector is used in the feedback loop to track the surface for imaging and measuring.



Mode of operation

Contact mode

In this method, the cantilever scans across a sample surface. Because the cantilever is in contact with the surface, strong repulsive force causes the cantilever to deflect as it passes over topographical features. Biological samples are not suitable for contact mode operation because direct contact between probe tip and sample can disrupt fragile biological structure.

Tapping mode

The *tapping* or AM-mode is the most common dynamic mode used in atomic force microscopy. In dynamic mode AFM the cantilever is oscillated with (or near) its resonance frequency near the sample surface. Using a feedback electronic the cantilever sample distance is controlled by keeping either the amplitude or the phase of the oscillating cantilever constant. Since lateral tip–sample forces are avoided by this technique the resolution is typically higher compared to the classical contact mode AFM where tip and sample are in direct mechanical contact.

AFM in biological research

•The AFM has emerged as a powerful tool to obtain nanostructural details and biomechanical properties of biological samples, including biomolecules and cells.

•Studying nano scale, in situ DNA structures, which can lead to the development of more effective gene delivery vehicles

•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

•The analysis of the nanostructure and nanomechanics of lymphocytes using the AFM technique from resting and activated to apoptosis

Test Your Understanding

AFM tips and cantilevers are typically micro-fabricated from

a. Aluminium

b. Quartz

c. Si_3N_4

d. Steel

Atomic Force Microscopy uses one of the following law for measuring deflection forces

a. F = -k.X

b. F = m.a

c. F = (m1. m2)/ r2

d. F = (q1.q2)/r2

Atomic force microscopy (AFM) can be operated in

a. Contact Mode

b. Tapping Mode

c. Flight mode

d. Both (a) and (b)

Atomic Force Microscopy can be used for following applications

a. nanostructural details and biomechanical properties of biological samples

b. Studying nano scale, in situ DNA structures

c. AFM to determine living cells and tissue conditions with their mRNA expression

d. All of the above

References & Further reading

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