

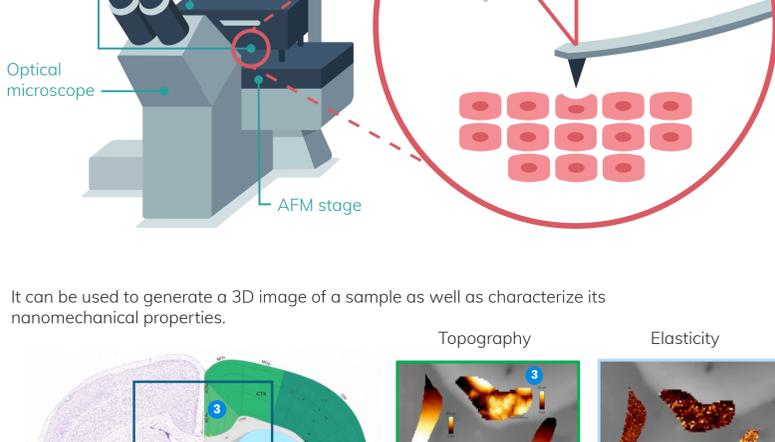
Investigating biomechanics with atomic force microscopy (AFM)

High-speed structural imaging and nanomechanical measurements – such as adhesion and deformation – deliver crucial insights into the relationship between structure, morphology and function at the cellular and molecular level, both in normal and diseased states. These are factors that play important roles in fields such as tissue engineering, developmental biology and nanomedicine.

In this infographic, we dive into how AFM works, the technical features one must consider and how it can be implemented for biomechanical investigation.

How can AFM provide cellular and molecular biomechanical insights?

AFM is a cantilever-based technique that measures the interaction forces between the cantilever probe and sample surface. As it interacts with the sample depending on the 'mode' chosen, the cantilever bends either towards or away from the surface. This movement is recorded by a laser beam that is deflected off the back of the cantilever onto a photodetector.



It can be used to generate a 3D image of a sample as well as characterize its nanomechanical properties.



A complete mouse brain slice sample, courtesy of Ingolf Sack (Charité-Universitätsmedizin, Berlin, Germany).

Technical considerations: operating modes and cantilevers

What you need to know about AFM operating modes

There are three common AFM modes that one can use to investigate a sample:

	Contact mode	Intermittent contact mode	Force spectroscopy
How it works	 The cantilever is pre-set to scan the sample surface without detaching.	 The cantilever is oscillating close to its resonant frequency to tap the sample surface at regular intervals.	 The cantilever is moved up and down on a spot on a sample.
Data collected	Measures the changes in the cantilever's vertical deflection to produce a topographic image of a sample surface.	Measures the changes in the amplitude of the oscillating cantilever to produce a topographic image of a sample surface.	Measures the force applied to a sample as a function of the vertical distance traveled by the cantilever tip, generating 'force-distance curves'. This mode can produce a topographic image of a sample surface as well as characterize mechanical properties.
Pros	 Accurately maintains force applied to the sample	 Reduces forces on the sample, making this mode better for fragile samples	 Provides insight into mechanical properties – such as stiffness and adhesion
Cons	 Prolonged contact exerts relatively high shear forces on the sample during scanning	 More difficult to operate for beginners	 Can create multiparametric images
	 It can't characterize the mechanical properties of a sample	 It can't characterize the mechanical properties of a sample	 Might be slower
When to use this mode	 For studying rigid structures	 The most commonly used AFM imaging mode	 The most versatile mode; it's applicable to all samples, especially delicate and fragile ones

What you need to know about cantilevers

Tip
The stiffness of a cantilever depends on its thickness, which is difficult to control. Therefore, calibrating the cantilever is essential for measuring forces.

Brucker offers pre-calibrated cantilevers.

Imaging protocols	Force spectroscopy experiments
The cantilever selection depends on various factors such as sample stiffness and imaging mode. Soft samples typically require soft tips. However, if the sample is sticky, a stiffer tip may be necessary to overcome adhesion. Additionally, the tip radius needs to be selected accordingly to resolve the sample's topographic features.	To ensure sufficient sensitivity and indentation of the cantilever, it is recommended to select a lever with a stiffness that matches that of the sample. Using a spherical tip is especially recommended for soft samples because it provides a well-defined shape and a selectable diameter for controlling the pressure on the sample.

Choosing the right AFM microscope for your research: introducing BioAFM

BioAFM is an atomic force microscope specifically adapted for studying soft, fragile and challenging biological samples in fluid without damaging their structures.

For which samples can a BioAFM be used?



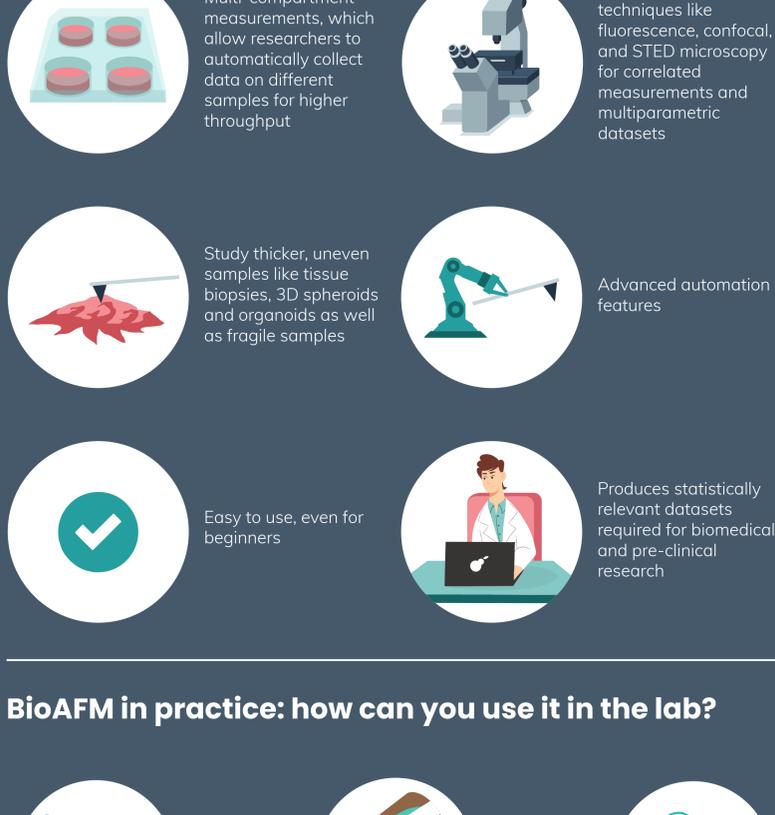
Characterize nanomechanical and viscoelastic properties

Visualize dynamic cellular and molecular processes with high-speed imaging

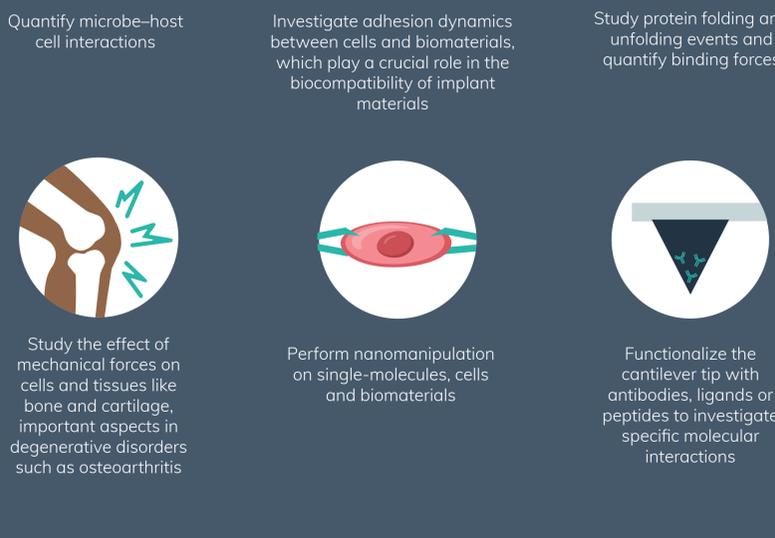
Quantify cell-cell, cell-tissue and cell-substrate interactions

Perform single-cell and single-molecule force

What are the benefits of using a BioAFM?



BioAFM in practice: how can you use it in the lab?



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