

## Probing the topography and mechanical properties of biomaterials with atomic force microscopy

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The composition, topography, adhesiveness and nanomechanical properties of biomaterials are all factors that affect biological processes, e.g., cell differentiation, morphogenesis and tissue formation [1]–[4]. Atomic Force Microscopy (AFM) is a highly versatile tool, ideal for the characterization of these properties, from single molecules to whole cells and tissues, on the nm scale.

The latest generation of JPK NanoWizard BioAFMs enable fast imaging of challenging biological samples and the visualization of dynamic processes with high spatio-temporal resolution under near physiological conditions. The kinetics of collagen type I fibrillogenesis was imaged in situ revealing the formation of the 67 nm D-banding hallmark.

Our distinctive Quantitative Imaging mode (QI™) measures various sample properties such as topography, nanomechanics and adhesion on the nanometer scale. Complex data like contact point, Young's modulus or recognition images can also be extracted at the same resolution. To demonstrate the capability and flexibility of QI™ mode, different biological samples like living cells have been investigated for their topographical and mechanical properties.

Investigating large, sticky and rough samples such as tissues and hydrogels using AFM has always been a challenge due to the limited z-axis of the AFM. The HybridStage™, equipped with an extended xyz scanner unit up to 300x300x300  $\mu\text{m}^3$ , an additional motorized unit for large sample movements in the mm range and optical tiling, is ideal for investigating such samples. This combination enables multi-region AFM probing over a large, rough sample area and provides additional correlative optical data sets.

A crucial aspect of investigating biomaterials and cell mechanics is to go beyond purely elastic models, which do not reflect their complex composition. The non-elastic properties of such samples can be analyzed via time-dependent creep compliance measurements or microrheology [5]. We have therefore performed rheological measurements combined with optical microscopy to characterise sample response at different time scales and measure viscoelastic properties in living mammalian cells, spheroids, and hydrogels over a large frequency range (0-500 Hz). These measurements derive rheological properties such as the elastic storage modulus  $E'$  and viscoelastic loss modulus  $E''$ .

Adhesion dynamics between cells and biomaterials play a crucial role in, e.g. the applicability of potential implant materials. The AFM based Single Cell Force Spectroscopy platform enables quantitative measurement of the interactions between individual cells and any substrate.

We will demonstrate the capabilities of unique AFM modes and instrumentation to yield complementary information on a variety of biomaterial samples.

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