JPK BIOAFM **Product Note**

CellMech™ Package

Viscoelastic properties of gels, cells and tissues

Living cells and tissues are complex systems, made up of various structures and compartments, and which are continuously being regenerated and remodelled. Over the past few decades, it has become increasingly clear that the functions of many biological systems are closely related to their mechanical properties and their response to the mechanical environment around them. A crucial aspect of investigating mechanobiology is to go beyond purely elastic models, which do not reflect the complex composition of most biological samples.

Atomic force microscopy (AFM) offers key opportunities to simultaneously measure the morphological and mechanical properties of living biological systems (1). Rheological measurements can also be performed to characterize sample response at different time scales and to measure viscoelastic properties through relaxation (2,3) or modulation experiments (4–6).

The **Bruker Cell Mechanics Package - CellMech** - enables users to perform advanced rheological measurements with JPK BioAFMs. The newly developed MicrorheologyAssistant expands the capabilities of the existing JPK RampDesigner™, a powerful software tool for creating customized force curves in experiments. The MicrorheologyAssistant enables the configuration and automation of measurements across different frequencies, and the collection of a response spectrum at each position. The easy and flexible experiment setup is combined with new data analysis processes for calculating viscoelastic properties and specially developed cantilevers to ensure repeatability.

The NanoWizard® 4 XP BioScience AFM is the perfect system for this type of experiment, providing highest mechanical and thermal stability on inverted optical microscopes during long term experiments. Extending the system with the HybridStage™ or Motorized Precision Stage enable direct access to large sample areas, with motorized movement to selected positions, grids and

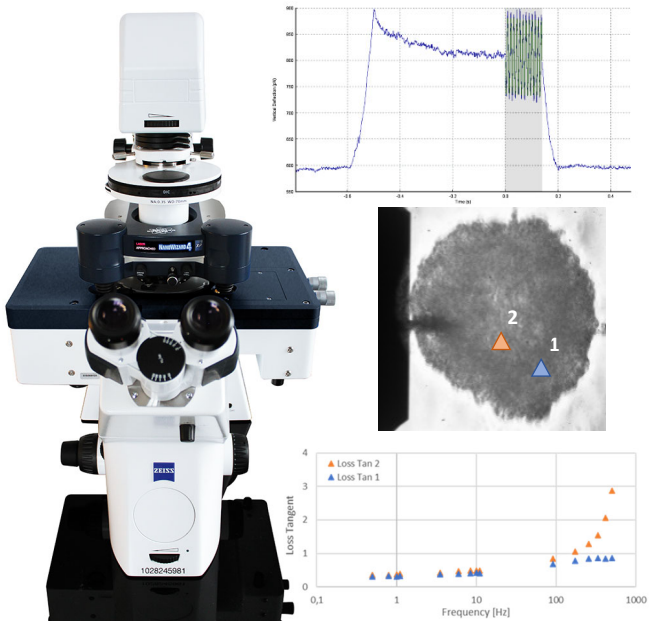

Fig. 1: Measurement Overview: *NanoWizard 4 XP BioScience AFM on a HybridStage together with an overview of Cell Mechanics Package data. Details of the experiment can be found in Figure 3.*

mapping regions. Further options such as ExperimentPlanner™ or ExperimentControl™ increase automation during complex assays and enable synchronized environmental, optical and position control or remote access to the instrument during longer measurements.

MicrorheologyAssistant™

The RampDesigner creates customized force curves and can be used for many different applications, including adhesion, relaxation, or clamp experiments. The flexible segment-based structure allows the user to choose extension or retraction lengths, relaxation pauses on the surface, or any other pre-conditioning routines. The new microrheology feature provides extra sine oscillating segments for probing the sample at clearly defined frequencies.

These measurements can then be repeated at different frequencies to build up a spectrum over several orders of magnitude. The MicrorheologyAssistant controls this process, allowing automatic configuration of user-defined frequency ranges and the probing of a specific set of locations on the sample.

Main features:

- User-defined contact and relaxation phases
- Defined amplitude, frequency, number of periods
- Linear or log spacing of frequency values or user-defined list
- Choice of loop logic through positions and frequencies
- Closed-loop control during sine oscillation
- Automatic adjustment of sample rate or data points

The Data Processing (DP) software derives the viscoelastic properties from each force curve. The sinusoidal signals are fitted to extract the force $F(\omega)$ and indentation $\delta(\omega)$. The phase shift between these two oscillations is used to derive rheological properties such as the elastic storage modulus E′, the viscoelastic loss modulus E″ or their ratio the loss tangent (6). Different tip shapes can be used, making the analysis suitable for different kinds of cantilever. Combined with excellent batch processing capabilities, this allows the easy processing of maps, or the large frequency and location datasets generated by the Microrheology Assistant.

Viscoelasticity of living cells and spheroids

The **CellMech package** for Bruker's NanoWizard family is designed for the combination of rheological measurements with optical microscopy. This is vital for identifying regions of interest and enables the correlation of results with optical measurements. The sine modulation segments can be integrated with Force Mapping measurements to give a clear spatial resolution at a specific probing frequency. An example of these defined frequency maps is shown in Fig. 2 for the loss modulus E″ of living Vero Cells mapped at 200 Hz.

The characterization of mechanical properties is also vital for larger structures, and for following the progression of individual cells to aggregates towards tissue. Cell spheroids are useful as a 3D in vitro tumour model (7) of several thousand cells. Figure 3 shows measurements on SKOV-3 spheroids. Here, specific positions were set, and multiple frequencies automatically measured as a series of curves. The results from two locations are shown: Position 1 nearer to the edge, and Position 2 nearer to the centre of the structure. The storage and loss moduli are larger for position 1, while the loss tangent (ratio of loss to storage modulus) increases with frequency at position 2, showing a more fluid-like response.

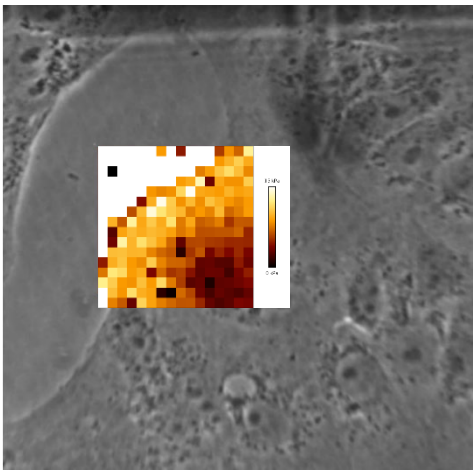

Fig. 2: Rheology Map of Living Cell: *Overlap of optical phase contrast image with a 16 x 16 map of loss modulus E″ at 200 Hz of living Vero Cell*

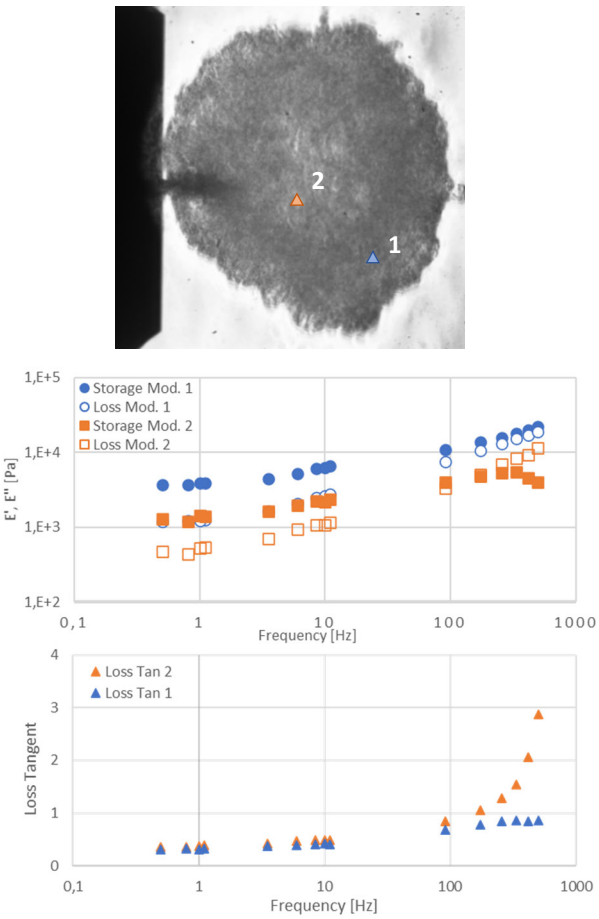

Fig. 3: Rheology on 2 different locations on a SKOV-3 spheroid: *The spectra shows that elastic moduli are different in the centre and on the outside. The centre becomes more "fluid-like" at higher frequencies.*

Novel Bruker probes deliver quantitative results

The probes used to contact the sample are vital for ensuring quantitative results in mechanical measurements. Both the shape of the tip end and the calibration of the spring constant are critical for the analysis. Bruker has extensive experience in the development of highest quality probes for accurate nanomechanical mapping of very soft samples with controlled tip end radius and individually factory-calibrated spring constants.

The **CellMech Package** includes two different types of cantilevers from lines specifically optimized for dynamic mechanical measurements. SAA-SPH takes advantage of a newly patented Bruker technique for producing a colloid-like spherical tip for micron-scale averaging, which can be vital for achieving reproducible results on heterogeneous materials. PFQNM-LC is an accurately controlled semi-sharp tip, which combines a specified 70 nm tip radius with a narrow aspect ratio for higher local resolution. Both types feature an extended tip height, which has the double benefit of enabling access to rough topographies while reducing hydrodynamic effects from the liquid layer squeezed between the cantilever arm and the sample during dynamic measurements.

Both types are available as pre-calibrated cantilevers with individually calibrated spring constants, measured using laser Doppler vibrometry (10% guarantee). The latest SPM control software includes specific support for pre-calibrated cantilevers, automatically extracting the sensitivity from thermal vibrations.

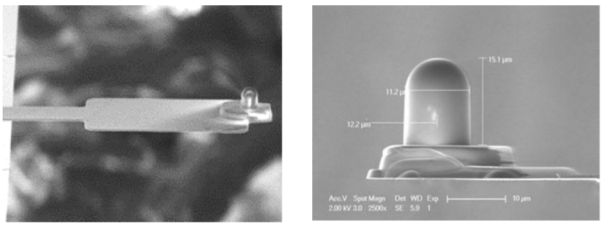

Fig. 4: Bruker SAA-SPH colloid-like probe. *The probe has a well-defined end radius (surface roughness below 4nm), an extended shape and certified calibration details.*

Summary

The new **CellMech Package** opens new possibilities for investigating the viscoelastic properties of biological materials such as cells and tissues.

- Sine modulation measurements at defined frequencies can be freely combined with customized force curves or force mapping
- MicrorheologyAssistant for setting up modulation experiments with defined frequencies over large ranges (0.5 - 500 Hz)
- One-click calibration of sensitivity and spring constant for all kinds of cantilever
- Specifically designed probes with defined radii and pre-calibrated spring constant
- Precise optical navigation and correlative experiments using the NanoWizard BioAFM family with optical microscopy and in combination with optional DirectOverlay™ or DirectTiling™ features

Visit also www.jpk.com/accessories to find out more applications and modes of Bruker's versatile BioAFMs.

Acknowledgements

Bruker BioAFM would like to thank the CEAT project and group of Dr Lewis Francis, School of Medicine, Swansea University, UK, for fruitful discussions, and Dr Lydia Powell, in particular, for providing the SKOV-3 spheroids. Vero cell samples were provided by Prof. A. Hermann/Dr. Thomas Korte, Humboldt University, Berlin, Germany.

References

1. Krieg M, Fläschner G, Alsteens D, Gaub BM, Roos WH, Wuite GJL, et al. Atomic force microscopy-based mechanobiology. Nat Rev Phys. 2019;1(1):41–57. DOI: 10.1038/s42254-018-0001-7

2. Yango A, Schäpe J, Rianna C, Doschke H, Radmacher M, Rotsch C, et al. Measuring the viscoelastic creep of soft samples by step response AFM. Soft Matter. 2016;12(40):8297–306. DOI: 10.1039/C6SM00801A

3. Moeendarbary E, Valon L, Fritzsche M, Harris AR, Moulding DA, Thrasher AJ, et al. The cytoplasm of living cells behaves as a poroelastic material. Nat Mater. 2013;12(3):253–61. DOI: 10.1038/nmat3517

4. Abidine Y, Constantinescu A, Laurent VM, Sundar Rajan V, Michel R, Laplaud V, et al. Mechanosensitivity of Cancer Cells in Contact with Soft Substrates Using AFM. Biophys J. 2018;114(5):1165–75. DOI: 10.1016/j.bpj.2018.01.005

5. Rother J, Nöding H, Mey I, Janshoff A. Atomic force microscopy-based microrheology reveals significant differences in the viscoelastic response between malign and benign cell lines. Open Biol. 2014;4(5):140046. DOI: 10.1098/rsob.140046

6. Alcaraz J, Buscemi L, Grabulosa M, Trepat X, Fabry B, Farré R, et al. Microrheology of human lung epithelial cells measured by atomic force microscopy. Biophys J. 2003;84(3):2071–9. DOI: 10.1016/S0006-3495(03)75014-0

7. Healey GD, Pan-Castillo B, Garcia-Parra J, et al. Antibody drug conjugates against the receptor for advanced glycation end products (RAGE), a novel therapeutic target in endometrial cancer. J Immunother Cancer. 2019;7(1):280. DOI:10.1186/s40425-019-0765-z

JPK BioAFM Business
Bruker Nano GmbH
Am Studio 2D, 12489 Berlin, Germany
productinfo@bruker.com
www.bruker.com/bioafm · www.jpk.com