INVESTIGATING STRUCTURES AND MECHANICAL PROPERTIES OF BIOMATERIALS AND LIVING SAMPLES

Enhancing Large-Scale BioAFM Mechanical Characterization with SmartMapping

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Introduction

Atomic force microscopy (AFM) is crucial for nanoscale mechanical property mapping, offering high-resolution characterization of stiffness, adhesion, and viscoelasticity. This capability is essential for understanding material behavior in complex structures like living cells, tissues, and biomaterials, thereby driving forward studies of cell behavior, disease progression, and drug treatments. Nevertheless, challenges such as sample roughness and limited lateral scanning range often hinder large-scale mechanical mapping, particularly for complex and heterogeneous specimens like biopolymers, hydrogels, and tissues.

Mapping of 2D cell populations

We have developed a new SmartMapping mode that addresses these challenges by coordinating AFM head motors and XYZ-piezo movement. This innovation enables continuous, high-resolution mapping over extensive areas without user intervention, enhancing the precision and efficiency of AFM. We successfully tested this feature for comprehensive analysis of hydrogels with different stiffnesses (1 kPa and 50 kPa), analyzing centimeter-scale areas and creating detailed mechanical property maps.

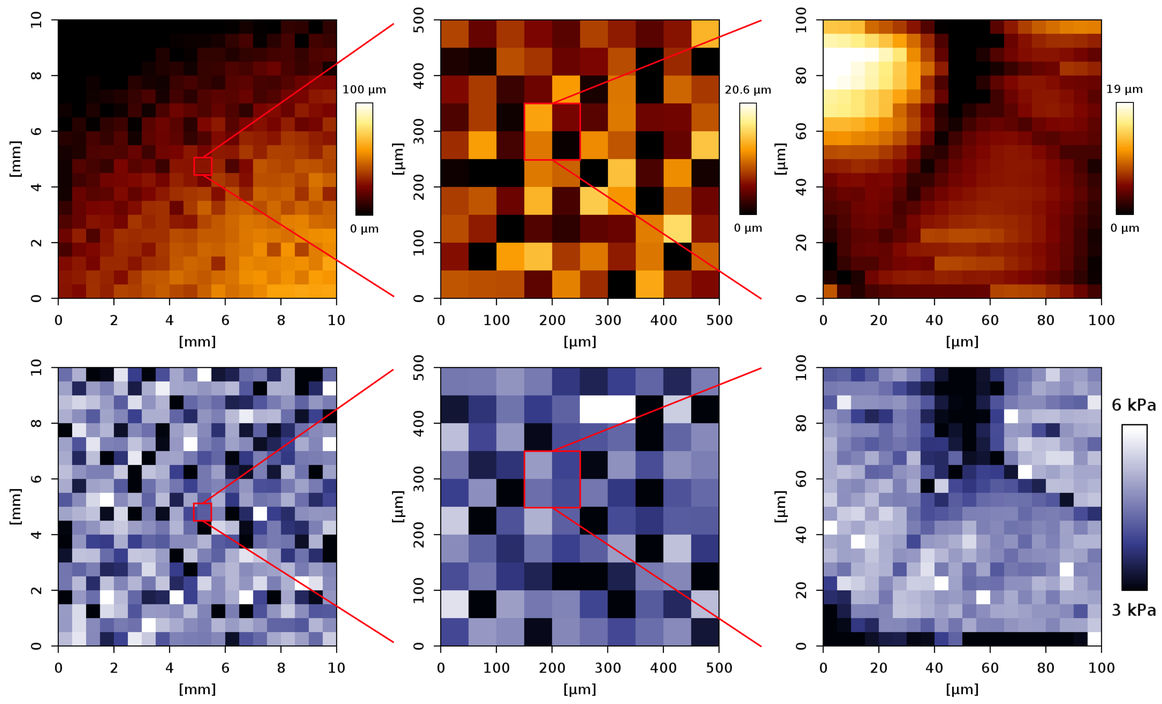


Fig. 1 | Large Area Mapping of Hydrogel

Top: Topography of commercial 1kPa EasyCoat Petrisoft™ hydrogel mapped using the SmartMapping feature. **Bottom:** Young's modulus of the above area.

Mapping the nanomechanics of 3D cell populations

With SmartMapping we recorded the nanomechanics of 3D spheroids formed by SKOV-3 cells. These have a highly corrugated surface spanning more than 100 µm in height.

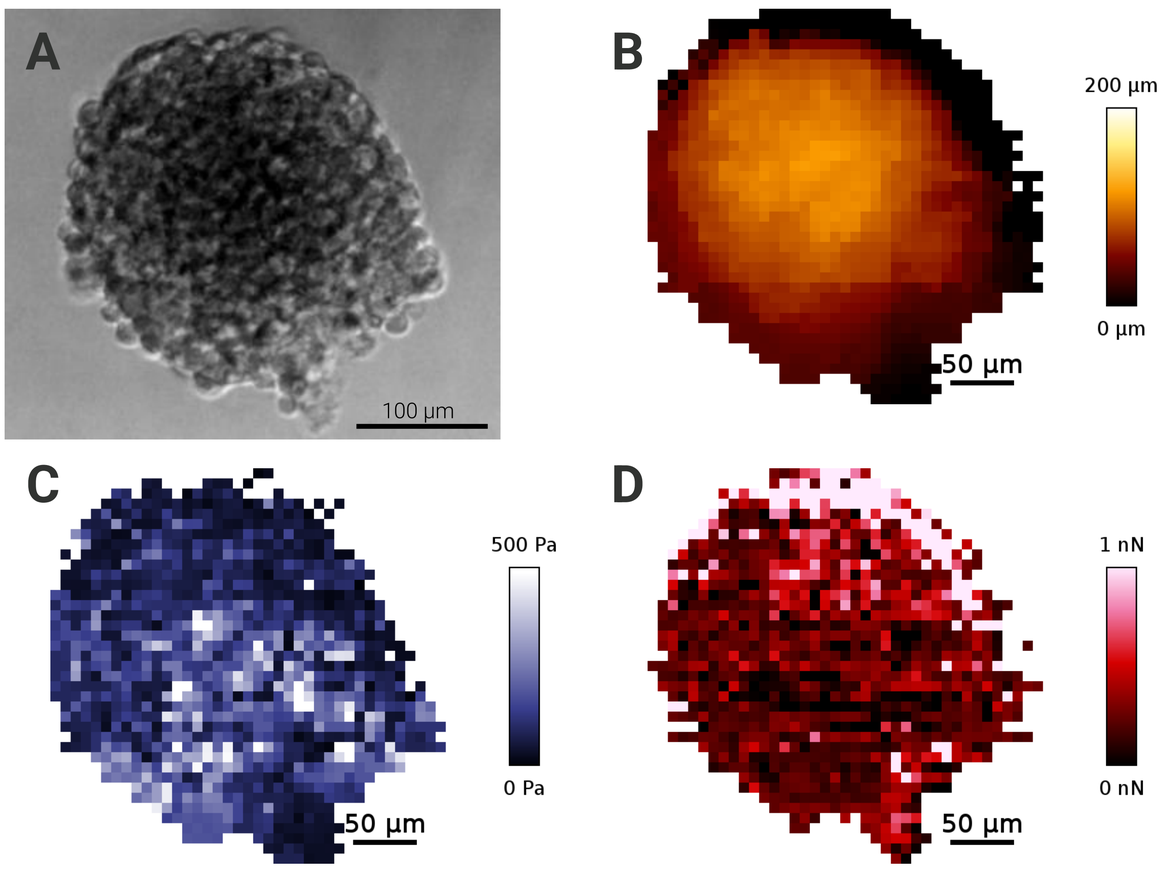


Fig. 2 | SmartMapping of Spheroids

A. Brightfield optical image of SKOV3 Spheroid B. Topography. C. Young's modulus. D. Adhesion. Spheroid was immobilized on a glass slide, and mapped in PBS at ambient conditions.

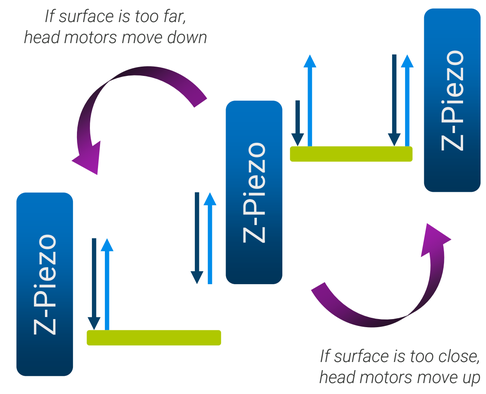
NanoWizard® V and CellHesion300

- FD curve-based scanning modes for multiparametric imaging
- Fast imaging to observe dynamic processes
- Perfect optical integration in combination enables large area accessibility
- Dynamic mechanical analysis for viscoelastic samples
- Automatization for high throughput



SmartMapping for rough surfaces

In the new SmartMapping mode, for each force curve the NanoWizard automatically checks whether it is within the movement range of the z-Piezo. Reaching the upper or lower limit, it repositions the scan head to re-center the force curve in the Piezo range, thus adding the range of the motors to the effective vertical scan range.



Mapping of 2D cell populations

We evaluated the applicability of the new feature for analyzing 2D cell populations seeded on hydrogels. Cells can be precisely selected with individually drawn shapes. Time spent measuring the background is reduced a lot.

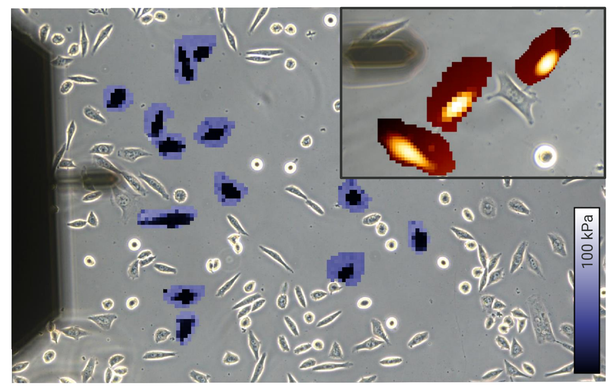


Fig. 3 | Multicellular Stiffness Mapping

Free-form stiffness maps of living 3T3 fibroblast cells cultured on a commercial 50 kPa EasyCoat Petrisoft™ hydrogel (Matrigen, USA). A phase contrast image (1100 x 695 µm) in the background was used to draw free-form regions around the cells of interest. **Inset:** Topography maps of various resolutions. *Cell sample courtesy of Dr. Stefanie Wedepohl, Freie Universität Berlin.*

Mapping of large living tissue samples

We assessed 600 µm thick vibratome-sectioned neuroblastoma tumors with very low stiffness (50-100 Pa) embedded in low-melting agarose gels. We demonstrated that despite their rough surface, here with a vertical range of 220 µm, such tissue samples can be measured over mm-sized areas.

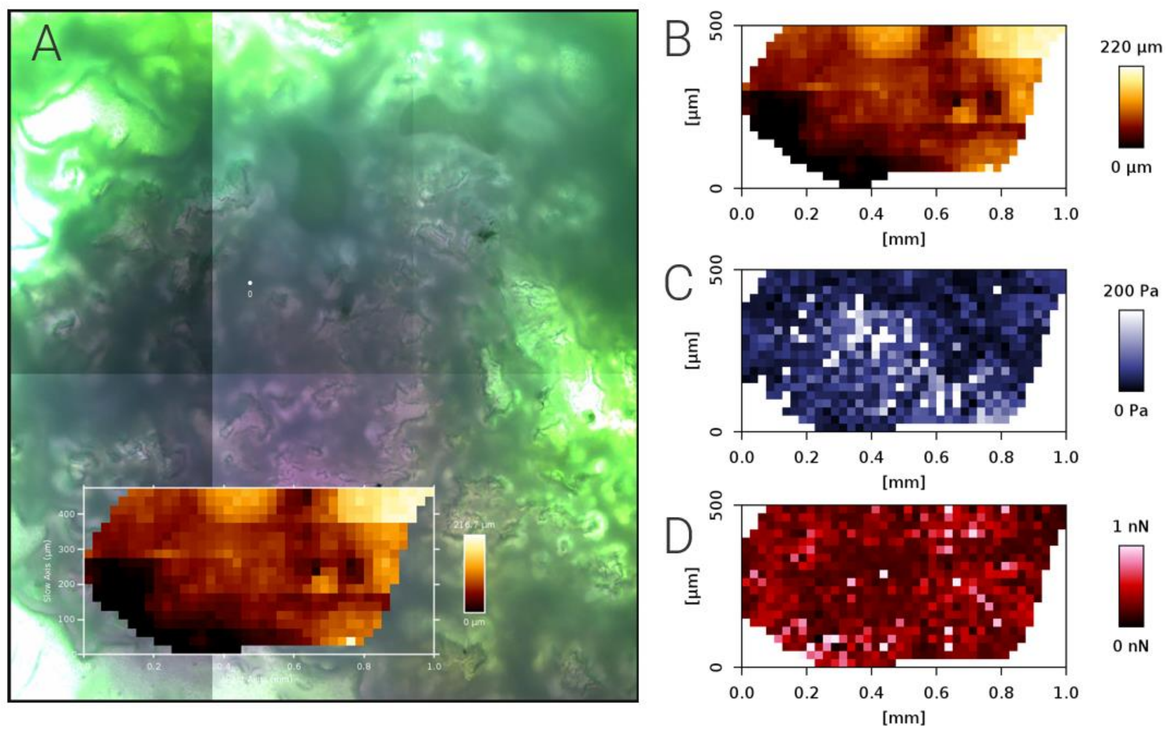


Fig. 4 | Living Tissue Mapping

A. SmartMapping topography data overlayed on brightfield optical image of a vibratome-cut containing a neuroblastoma tumor of a one-year-old transgenic zebrafish cancer tissue. B. Topography. C. Young's modulus. D. Adhesion. *Sample courtesy of Prof. Dr. Ingolf Sack, Charité, Berlin.*

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