INVESTIGATING STRUCTURES AND MECHANICAL PROPERTIES OF BIOMATERIALS AND LIVING SAMPLES

Large-Area AFM Mapping for Mechanobiology Studies in Cells and Tissues

A. Körnig[1], J.C. Escolano[1], J. Turney[2], A. Dulebo[1] , T. Müller[1] and T.Henze[1]

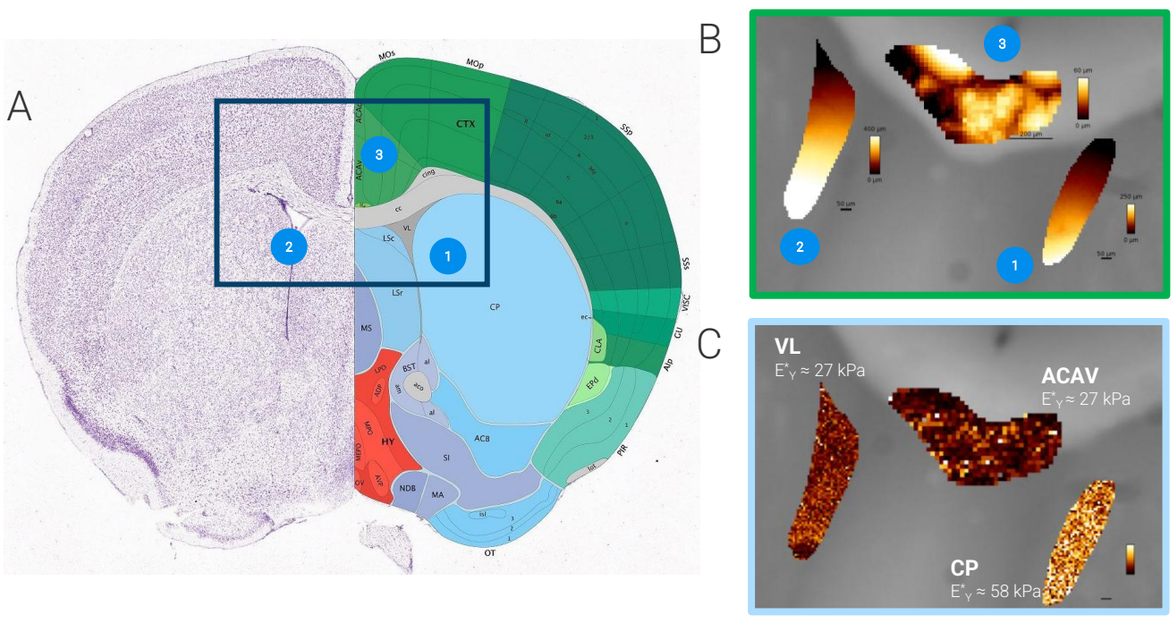
[1]JPK BioAFM, Bruker Nano Surfaces, Am Studio 2D, 12489 Berlin, Germany
[2]Swansea University, Singelton Park, SA2 8PP, Wales, UK

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 large tissue samples

We developed a SmartMapping mode that synchronizes AFM head motors with XYZ-piezo movement, enabling continuous, high-resolution mapping over large areas without user intervention. This enhances both precision and throughput in AFM. The method was validated on mouse brain tissue sections, generating detailed mechanical property maps across multiple, large-scale regions.



#	Murine Brain Region	Expected Brain Structure	Expected Stiffness Ranking	Experim. Stiffness
1	*CP (Caudate Putamen), close to VL*	Subcortical gray matter, densely packed with neurons and interspersed myelinated fiber bundles	Stiffest, a solid subcortical structure with dense gray matter and myelinated fibers.	58.8 Pa
2	*VL (lateral ventricle)*	Fluid-filled ventricle containing CSF, lined by ependyma, with no solid cellular structure.	Soft-to-Intermediate, as it is a CSF-filled cavity with no intrinsic stiffness.	27.4 Pa
3	*ACAV (Anterior Cingulate Area, Ventral Part)*	Layered cortical gray matter, agranular, with high neuropil and some myelinated axons.	Soft-to-Intermediate, a soft cortical region with gray matter and minimal myelination.	20.7 Pa

Fig. 1 | Large Area Mapping of Mouse Brain Tissue

A. Characteristic murine brain anatomy indicating the analyzed region. B. Topography of mapped regions 1, 2, and 3 (cantilever: SAA-SPH-1 µm, hemispherical tip). C. Corresponding apparent Young's modulus maps. Average stiffness values for the three regions are summarized in the table (right). *Sample courtesy of Prof. Ingolf Sack, Charité-Universitätsmedizin Berlin, Germany*

Mapping the nanomechanics of 3D cell populations

Using SmartMapping, we recorded the nanomechanical properties of 3D spheroids formed by SKOV-3 cells. Their highly corrugated surface, spanning over 100 µm in height, poses a challenge for conventional AFM due to limited Z-piezo range.

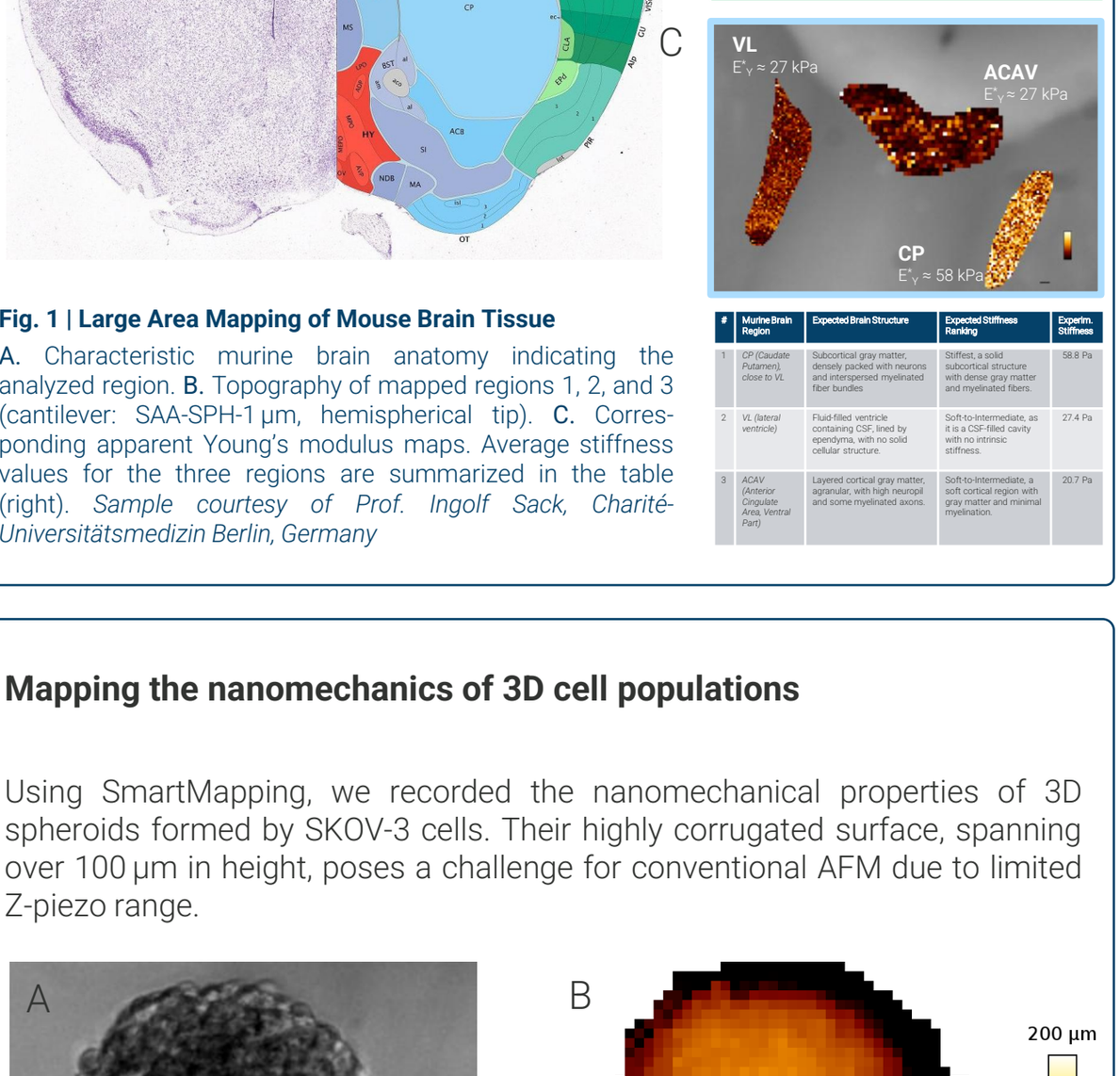


Fig. 2 | SmartMapping of Spheroids

A. Brightfield optical image of a SKOV3 Spheroid B. Topography. C. Young's modulus. D. Adhesion. Spheroids were immobilized on a glass slide and mapped in PBS at ambient conditions.
Sample courtesy of Prof. Lewis W. Francis, Swansea University Medical School, UK

NanoWizard® V and CellWizard™ Stage

NanoWizard® V AFM

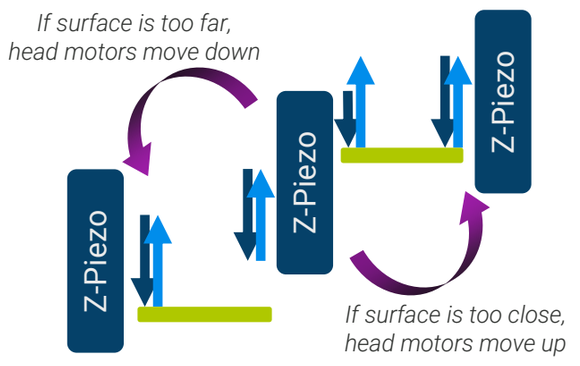
- FD curve-based scanning modes for multiparametric imaging
- Fast imaging to observe dynamic processes
- Perfect optical integration in combination enables large area accessibility
- Dynamic mechanical analysis for viscoelastic samples

CellWizard™ Stage

- High precision motorized stage for large area measurements

SmartMapping for rough surfaces

- Automated range check of Z-piezo
- Repositioning of AFM scan-head to recenter the force curve in the z-piezo range
- Adding the motors to the effective vertical scan range



Automatic Navigation

Optical images can be segmented using e.g., CellPose to detect the position of features and define Regions of Interest (ROI).
The ROI are used to create a measurement list of AFM scan regions. This list is processed automatically, to obtain multiparametric AFM data of multiple cells.

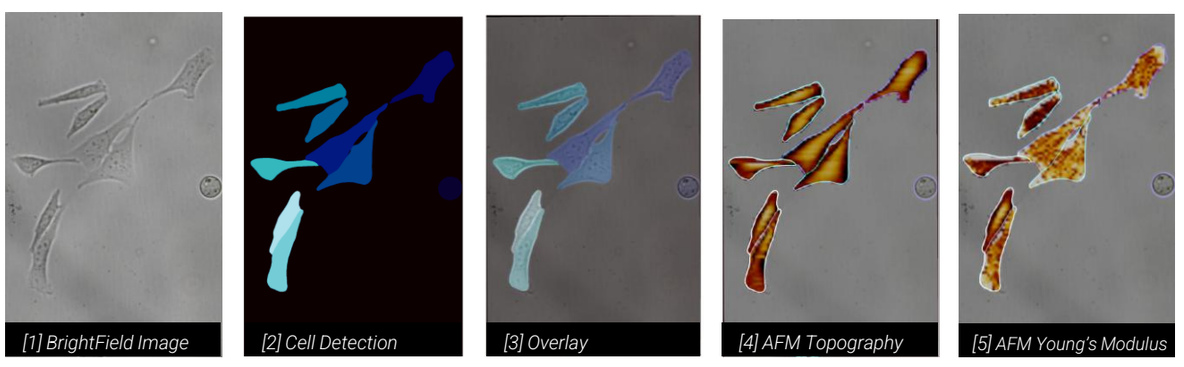


Fig. 3 | Segmentation, Automatic Navigation and MultiScan on living cells

From the bright field image [1] the position of living 3T3 cell was detected using a pretrained CellPose model [2]. The automatically defined ROI were measured with SmartMapping to obtain the Topography [4] and Young´s modulus of the cells [5].
Sample courtesy of Dr. Stefanie Wedepohl, Freie Universität Berlin

Viscoelastic measurements with SmartMapping

Complex force ramps within SmartMapping were used to characterize viscoelastic materials. The probe is brought into contact, and the applied force is modulated at multiple frequencies to extract both storage (elastic) and loss (viscous) moduli (MicroRheology). User-defined cell selection confines measurements to target regions, significantly accelerating data acquisition.

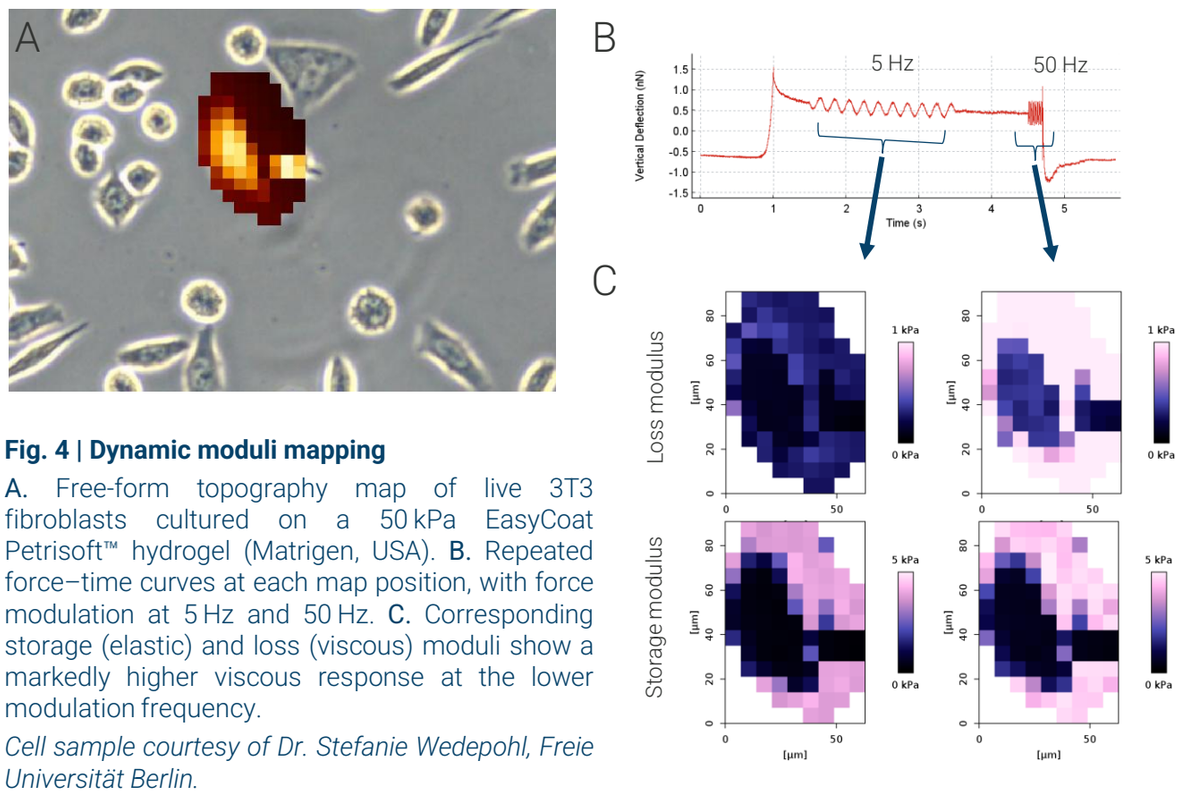


Fig. 4 | Dynamic moduli mapping

A. Free-form topography map of live 3T3 fibroblasts cultured on a 50 kPa EasyCoat Petrisoft™ hydrogel (Matrigen, USA). B. Repeated force–time curves at each map position, with force modulation at 5 Hz and 50 Hz. C. Corresponding storage (elastic) and loss (viscous) moduli show a markedly higher viscous response at the lower modulation frequency.

Cell sample courtesy of Dr. Stefanie Wedepohl, Freie Universität Berlin.

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Innovation with Integrity