

Next-Generation AFM Force Mapping of Challenging Samples with SmartMapping

Atomic force microscopy (AFM) is an invaluable technique for determining the nanoscale mechanical properties of a diverse scope of samples, ranging from hard materials to soft and challenging biological matter. It offers unparalleled high-resolution capabilities, delivering critical insights into material behavior, in particular, in the complex and heterogeneous structures of polymers, composites, and biological samples. AFM generates detailed quantitative maps of sample properties, such as Young's Modulus (stiffness), adhesion force, and viscoelasticity, crucial properties in the analysis of mechanical variability at small scales.¹

AFM is also a key technique for determining the biomechanical properties of living cells, tissues, and biomaterials, significantly enhancing the investigation of cell behavior, disease progression (e.g., in cancer, fibrosis), and the effects of therapeutic agents.² In addition, AFM can be used to measure molecular-level forces, advancing our understanding of protein folding, biomolecular interactions, and DNA mechanics.³ AFM achieves nanometer resolution over micrometer or even millimeter-sized regions, which is particularly relevant for samples like hydrogels, cell layers, and biological tissues. Furthermore, it requires minimum sample preparation and measurements can be performed under near-physiological conditions.⁴

Several challenges arise, however, when performing mechanical mapping over large surface areas, in particular when investigating rough, soft, or highly deformable samples. Even well-prepared samples can exhibit significant height variations when examined over hundreds of micrometers. The Z-piezo of an atomic force microscope, which tracks topography, typically operates within a range of 15 microns, which can easily be exceeded when measuring biological samples. In addition, the lateral scanning capability of an atomic force microscope is generally restricted to approximately 100 microns. Although it is possible to stitch together multiple smaller scans to cover a larger area, this approach inherently compromises the accuracy of the absolute topographical information.⁵

This technical note discusses how Bruker's SmartMapping feature addresses these challenges. It offers additional key functionalities that greatly enhance the performance of atomic force microscopes for biological studies (BioAFMs), simplifying the selection of scanning areas and significantly increasing the efficiency of the measurements.

HEADER IMAGE:
400 μm \times 250 μm
freehand-shaped
SmartMapping map of
sheep muscle tissue with
an optical fluorescence
image in the background.

Sample courtesy of Prof. Dr.
Ansgar Petersen, BIH,
Center for Regenerative
Therapies, Charité
Medical University, Berlin,
Germany.

Advanced Mapping Capabilities for Challenging Samples

SmartMapping marks a significant advancement in the efficiency, flexibility, and ease of performing AFM measurements. It combines five innovative mapping features in one mode to:

- **Map sample topographies larger than the Z-scan range of the BioAFM:**
Ideal for measuring rough and highly corrugated samples
- **Map areas larger than the XY-scan range of the BioAFM:**
Effective for mapping large samples areas
- **Select flexible, freehand drawn areas around a sample for automated mapping:**
Valuable for selecting specific regions of interest, cell types, or characteristics
- **Automated correction of cantilever drift:**
Necessary for optimal cantilever deflection readout during long-term experiments
- **Batch acquisition of multiple maps:**
Essential for automated collection of multiple, predefined maps and streamlined data acquisition

To tackle samples with irregular, rough topographies that exceed the natural Z-scan range of the BioAFM, SmartMapping combines the high-resolution piezo scanner with the head's motorized vertical adjustment, enabling large vertical adjustments for the sample height. This is achieved by analyzing the acquired force curves in real time, and when the limit of the piezo is approached, a motor adjustment is triggered.

For lateral mapping beyond the native XY-scan range of the atomic force microscope, SmartMapping merges motorized stage movements with the high-resolution piezo scanning into a unified map. The acquisition time is also optimized by balancing the fast positioning of the BioAFM head with the generally slower translation of the motorized stage. This coordinated approach ensures efficient coverage of larger areas without compromising spatial resolution.

Traditional force maps are typically limited to quadratic shapes, which can lead to the collection of irrelevant information and inefficient data acquisition, in particular when investigating non-quadratic samples, such as cells. SmartMapping addresses this limitation by allowing the user to select arbitrarily shaped mapping areas directly on the optical image and thereby focus on specific regions of interest on the sample (Figure 1).

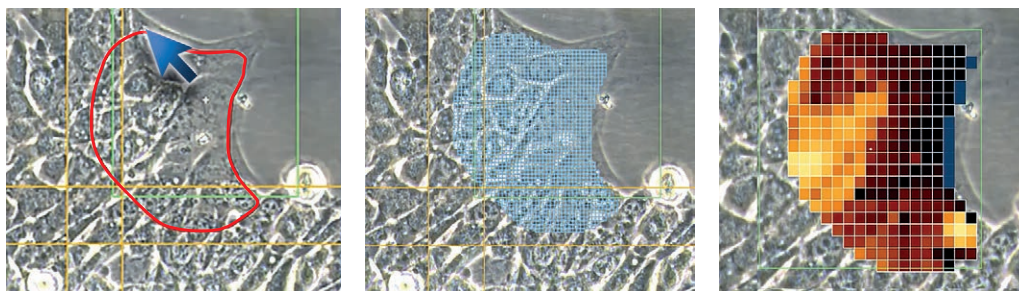


FIGURE 1.
From left to right:
Freehand drawn selection
of area to be mapped.
Definition of mapping
points.
AFM map of selected area.
Size of the green rectangle
in all three images is
100 μm \times 100 μm .

On compatible BioAFM heads, SmartMapping automatically adjusts the cantilever deflection system, compensating for signal offsets that may arise from factors such as temperature-induced bending of the cantilever. In addition, users can define multiple distinct scan regions around the sample, which are then mapped sequentially in an automated process. Together, these capabilities support fully autonomous long-term experiments, minimizing the need for user intervention.

Application Examples

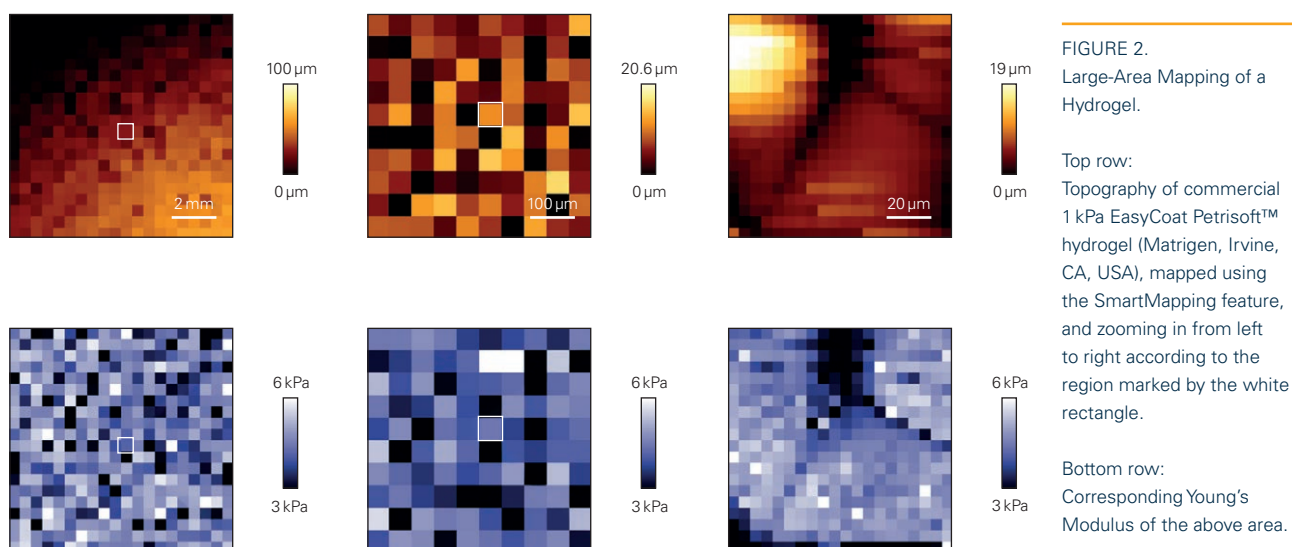
The following examples demonstrate the versatility of SmartMapping in BioAFM measurements on a range of soft, large-area, and topographically complex samples, such as hydrogels, cells, tissues, and spheroids. As SmartMapping is highly adaptable, it is also well-suited for a broad range of non-biological materials.

The data presented here were acquired using a NanoWizard® V BioAFM equipped with a motorized stage and a Bruker SAA-SPH-5UM hemispherical probe. To maintain a temperature of 37°C, a PetriDishHeater was used during the mapping of living cells.

Large-Area Mapping of Hydrogels

Hydrogels play a pivotal role in biomedical research owing to their soft nature, high water content, and biocompatibility. Accurate measurement of their mechanical properties, in particular stiffness, is essential, as their functionality is closely tied to their elastic and viscoelastic properties.

In this example, a commercial 1 kPa polyacrylamide hydrogel with an area of 1 cm × 1 cm was mapped automatically (Figure 2). While AFM is typically regarded as a nanoscale technique, this example highlights the capability of the SmartMapping feature to extend measurements to centimeter-scale areas. Topography, adhesion, and apparent stiffness maps were generated in real-time, and Young's Modulus was batch-calculated using the native data processing software and Hertzian model for a spherical indenter.



Mapping the Stiffness of 2D Cell Populations on Hydrogels

In this example, the stiffness of living cells, seeded on a 50 kPa polyacrylamide hydrogel, was analyzed. Due to the irregular and varied shapes of the cells, using a squared mapping area would include regions on the substrate that are not of interest, which increases the time required to acquire and process the data. While single-point measurements could be an alternative, it is more practical to select an area that conforms to the shape of individual cells (Figure 3).

SmartMapping's freehand selection feature provides this flexibility, allowing the combination of freehand drawn shapes with quadratic boxes, lines, and single points as needed. The resolution can also be adjusted to improve the capture of specific areas of interest (see Figure 3 inset).

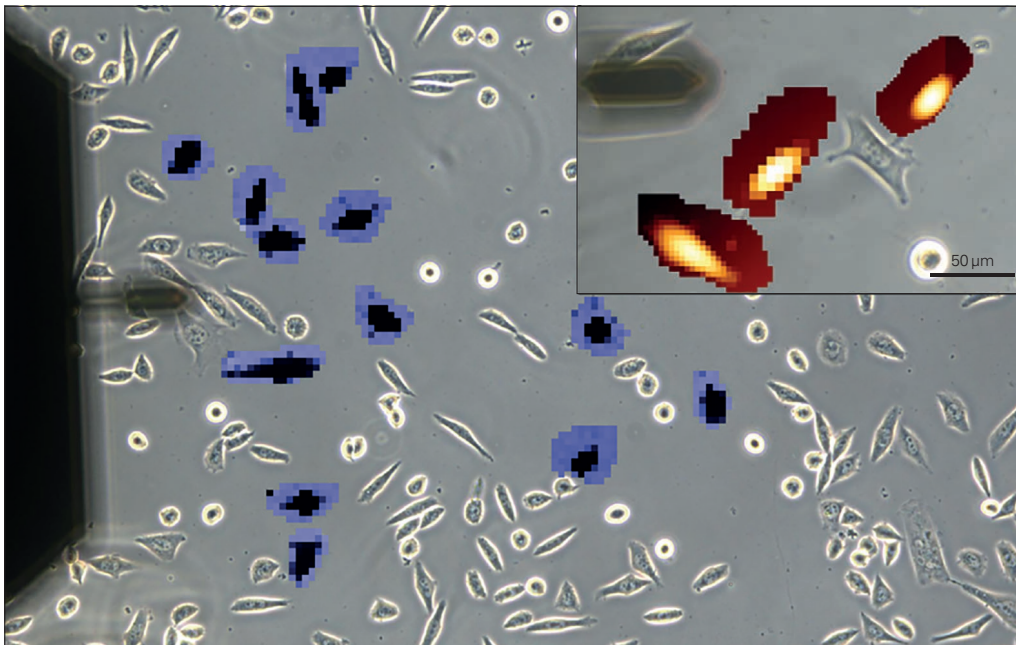


FIGURE 3. Mapping the Stiffness of 2D Cell Populations. Stiffness maps of freehand selected areas generated using SmartMapping on living 3T3 fibroblast cells, cultured on a commercial 50 kPa EasyCoat Petrisoft hydrogel (Matrigen, Irvine, CA, USA). A phase contrast image (1100 μm \times 695 μm) in the background was used to select the cells of interest.

Inset:
Topography maps of varying resolutions, created using SmartMapping on living cells on the hydrogel. A phase contrast image (265 μm \times 165 μm) in the background was used to select the cells of interest.

Cell sample courtesy of Dr. Stefanie Wedepohl, The Free University of Berlin, Germany.

Mapping of Spheroids

Cell-derived spheroids, or three-dimensional (3D) clusters of cells, are attracting significant attention due to their ability to mimic cellular behavior in a way that more realistically reflects a physiologically relevant environment than traditional 2D cell cultures. Spheroids are utilized in various applications, including tumor modeling in cancer research, drug testing in toxicology, tissue engineering in regenerative medicine, and the study of cell-cell and cell-matrix interactions.

Probing the stiffness of spheroids is important, but can be challenging with conventional atomic force microscopy, as spheroids typically have diameters greater than 100 micrometers, exceeding the typical Z-range of AFM instruments. SmartMapping, however, is an ideal tool for working with these structures. Figure 4 illustrates a fully automated map of the topography, adhesion, and Young's Modulus of a spheroid, obtained using SmartMapping.

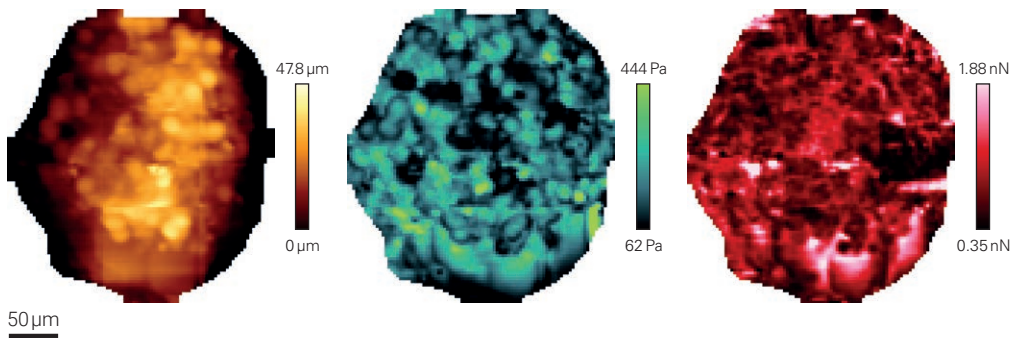


FIGURE 4. SmartMapping of SKOV3 Spheroids.

From left to right: AFM Topography, Young's Modulus, and Adhesion images.

Spheroids were immobilized on a glass slide, mapping was performed in PBS under ambient conditions. Sample and data courtesy of Jordan Turney (Swansea University, UK).

Mapping Living Tissue

In this final example, a neuroblastoma tumor sample taken from an adult zebrafish was examined (Figure 5). After dissection, the specimen was embedded in 3% low-melting agarose, cut with a vibratome into 600 micrometer thick slices, affixed to a petri dish, and measured in PBS using SmartMapping.

In addition to being extremely soft and sticky, nervous tissue samples can also exhibit significant surface roughness, even when sectioned correctly. This roughness can result from the attachment process itself, as tissue-specific adhesives can affect the sample's topography. These features can hinder the acquisition of accurate mechanical data and present a challenge when trying to successfully examine large sample areas. To overcome this, additional features like the automated adjustment of the Z-position and laser alignment have been implemented in SmartMapping that facilitate accurate large-area force mapping.

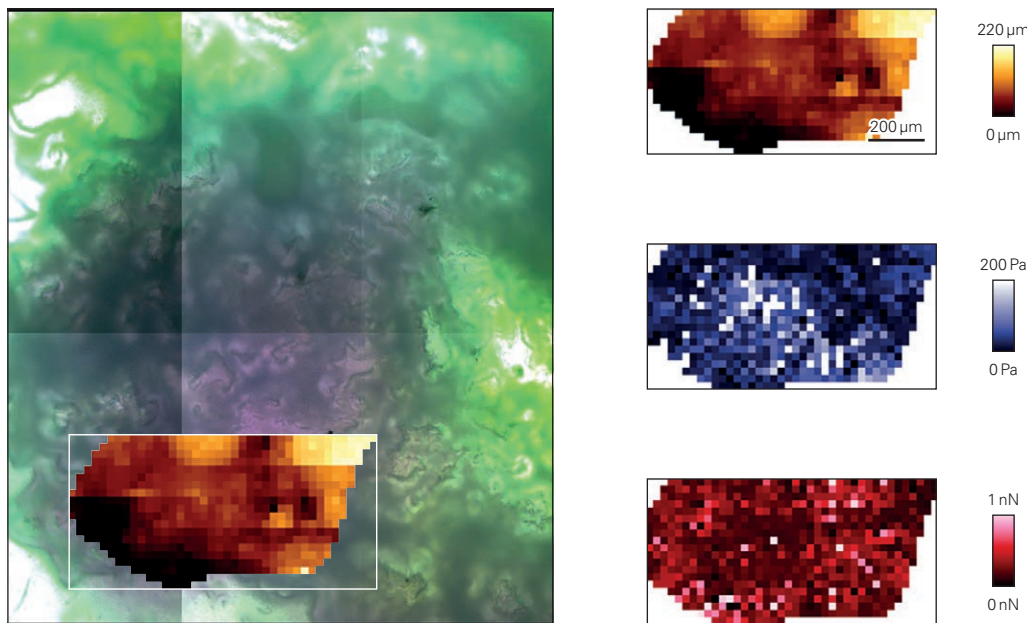


FIGURE 5. Mapping of a living tissue.

Image left: SmartMapping topography data overlay on a brightfield optical image of a vibratome-cut neuroblastoma tumor obtained from a one-year-old transgenic zebrafish.

Image right top: AFM Topography.

Image right middle: AFM Young's Modulus.

Image right bottom: AFM Adhesion.

Sample courtesy of Prof. Dr. Ingolf Sack, Charite, Berlin, Germany.

Outlook

The use of AFM in the characterization of the mechanical properties of biological samples has long been a cornerstone of biomedical and biomaterial research. The introduction of the SmartMapping feature marks a significant advancement in this field. By seamlessly integrating multiple mapping options with automated Z-piezo adjustments, SmartMapping enhances the precision and efficiency of sample analysis.

SmartMapping opens new avenues for studying samples once considered challenging or even impossible to analyze with AFM, paving the way for more comprehensive and insightful research outcomes. In addition, advanced automation features and batch data processing capabilities significantly increase the speed and ease of AFM measurements, maximizing throughput and the generation of statistically relevant datasets that are essential in biomedical and clinical research.

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