TechNote #300

Taking Correlative Microscopy on Opaque Samples to the Next Level with BioMAT

Correlative microscopy is increasingly becoming the investigative discipline of choice for life and materials science applications. From the investigation of bacterial growth on metallic surfaces, fluorescent polymers, and coatings to the study of biochips, bionics, and surface chemistry samples, the more advanced the application, the greater the need to combine the capabilities of optical microscopy and atomic force microscopy (AFM).

While AFM enables the characterization of surface properties, the investigation of nanomechanics and structure, and the manipulation of objects on the nanoscale, optical measurements are instrumental in revealing more bulk details and compositional contrast via fluorescence or immunological labelling of molecules and epitopes.

Bruker's BioMAT biomaterials workstation combines both principles and opens new possibilities for correlative microscopy on opaque samples. The main challenge limiting correlative microscopy on non-transparent samples has been in providing both techniques with simultaneous access to the sample. To maximise the capabilities of optical microscopy, objective lenses with extremely short working distances are required, leaving AFM no space to access the sample. The BioMAT workstation (see Figure 1) delivers a solution that integrates the specific advantages of both upright optical microscopy and AFM.

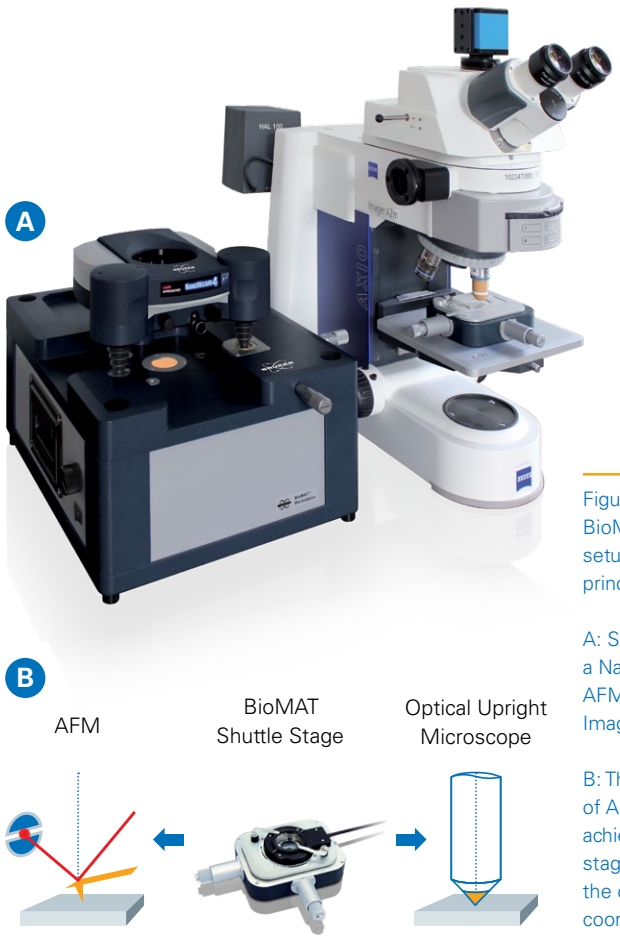

Figure 1:
BioMAT workstation setup and operation principle

A: Setup combining a NanoWizard 4 XP AFM (Bruker) with Axio Imager.A2m (Zeiss).

B: The spatial separation of AFM and optics is achieved via a shuttle stage that enables the correlation of both coordinate systems.

A NanoWizard® AFM is an integral element of the combination, delivering high resolution imaging and unmatched nano-mechanical and nano-electrical characterisation capabilities. The BioMAT workstation enhances these capabilities by enabling the combination of AFM with almost all upright microscopy techniques (DIC, brightfield, fluorescence, confocal laser scanning, FLIM, FRET, FRAP, FCS, IR, Raman).

The BioMAT workstation – flexibility meets precision

Key components of the BioMAT workstation are the BioMAT base with built-in optics and a portable shuttle stage with a kinematic mount for transferring the sample between the AFM and the upright optical microscope. The BioMAT base is a solid mechanical platform that enables high resolution AFM imaging. It comprises an integrated optical system for position calibration, a focusing system, and illumination, all instrumental for attaining the positional accuracy required for AFM alignment and precise and reproducible investigation of the same region of interest with both optics and AFM.

The sample is transferred in the shuttle stage between the AFM and the optical microscope, facilitating precise movement of the sample between the field of view of the optics and the AFM scan range without losing its position. This transfer can be repeated as often as necessary, allowing the sequential measurement with optics and AFM for timelapse studies. Using dipping lenses in the upright microscope, the setup can be operated in a fluid environment.

Biology and engineering: a whole new world of applications

The BioMAT workstation is the ideal solution for investigating samples on non-transparent substrates, such as tissue sections and bacterial biofilms. The combination with optics is advantageous for applications in surface chemistry and polymer sciences or performing nano-electrical measurements on semiconductors or micro-electromechanical (NEMS/MEMS) systems. Fields of application for non-transparent samples that can be analysed using the BioMAT Workstation include, e.g.:

Fields of application
· Biochips, such as DNA or protein chips
· Cell-electronic interfaces, cell chips or patterned substrates for cell growth
· Tissue engineering scaffolds and implants
· Bacterial or yeast studies on non-transparent substrates like metals, or biofouling on plastics
· Bionics
· Plant biology
· Nanostructured surfaces of PDMS or other imprints
· Nano- and microelectromechanical systems (NEMS/MEMS)
· Nanoparticles, powders, foams, paint, or thin films
· Biocompatible surfaces, such as organic coatings on metals, plastics, or silicone substrates

Application examples

The BioMAT workstation can be used to study Chinese hamster ovary (CHO) cells on gold electrodes (see Figure 2). A number of ongoing studies focus on capturing cellular viability in cell culture or bioreactor systems via online sensors at the cell-electronic interface.

Bruker's quantitative imaging (QI) mode is an automated, easy-to-use AFM measurement tool. Force distance curves recorded at each pixel enable the complete online and offline analysis of cell properties and simultaneous characterization of their structure and mechanical properties.

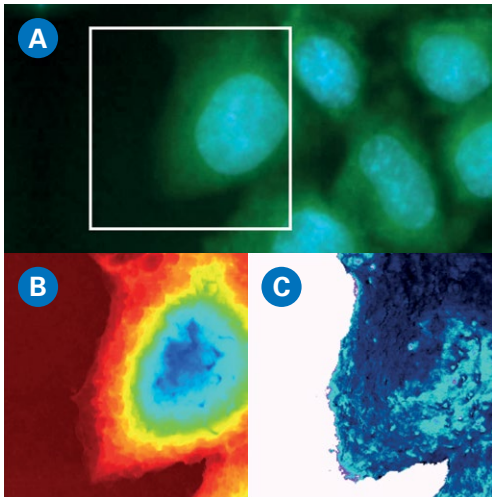

Figure 2:
Living CHO cells

A: Living cells were seeded onto a gold electrode and measured in liquid with a BioMAT PetriDishHeater at 37 °C by means of quantitative imaging. Cells were fluorescently stained with Hoechst (blue nuclei) and fluorescein diacetate (green cytoplasm) before being imaged with 63 × dipping objective.

B: Topography image of the marked region (Z-range: 6 µm).

C: Corresponding Young's modulus image (Z-range: 10 kPa) for a scan range of 30 µm × 30 µm.

A further example is the study of J-aggregates, a type of fluorescent dye with an absorption band that shifts to a longer wavelength with increasing sharpness as a result of a supramolecular self-organization. The characteristic fibre-like structures of J-aggregates on SiO_2 become apparent after correlation of the fluorescent and AFM topography images (see Figure 3).

Bruker's DirectOverlay feature ensures the perfect optical integration depicted in Figures 2 and 3. It synchronizes the upright optical images and AFM measurements and enables the intelligent integration and correlation of complete optical and AFM datasets. Following the automated calibration, optical images can be directly correlated with AFM images, increasing the efficiency of experiments.

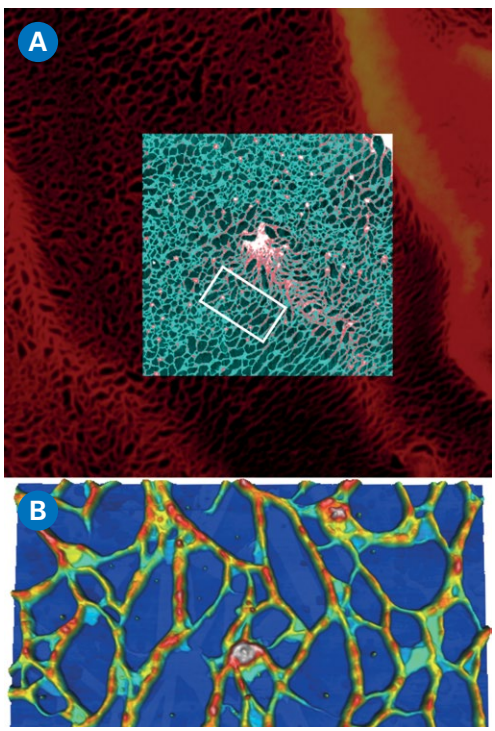

Figure 3:
J-aggregates on SiO2 surfaces

A: Fluorescence image (40 × objective) overlaid with AFM height channel (XYZ-range: 60 µm, 60 µm and 60 nm).

B: Detailed topography image of the J-aggregates fibrillar structure from the location marked above (XYZ-range: 15 µm, 8 µm and 60 nm).

Contemporary research guidelines for high-end correlative microscopy have increased the demand for measurement stability and resolution. This final example demonstrates high-resolution imaging on a membrane patch of bacteriorhodopsin isolated from purple bacteria (see Figure 4). The HyperDrive fluid imaging package was used to image bacteriorhodopsin, providing highest resolution at lowest interaction forces between tip and sample.

Subsequent scans in the bacteriorhodopsin patch reveal the submolecular organization of the 4-5 nm trimers. Here, HyperDrive imaging was combined with fast scanning to demonstrate the potential of the technique and how the BioMAT workstation can be applied for real-time monitoring of dynamic molecular processes with high spatial and temporal resolution.

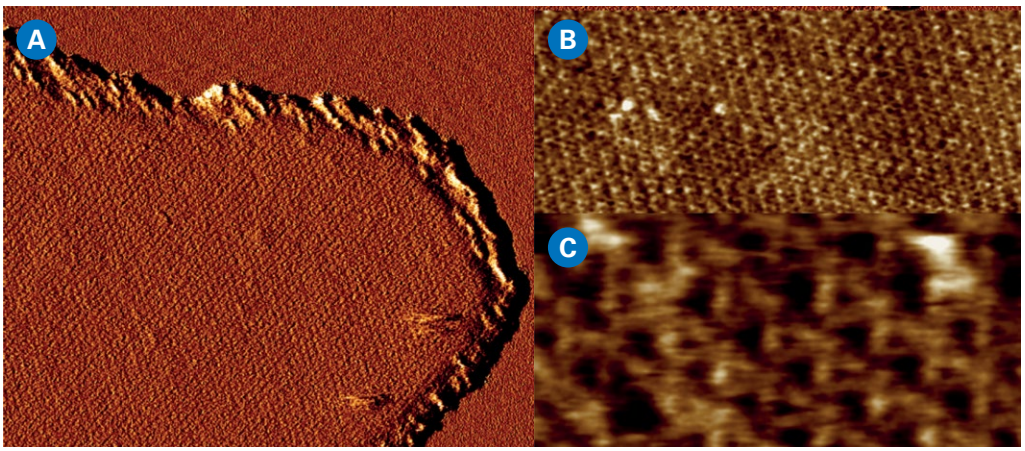

Figure 4:
High-resolution fast imaging of bacteriorhodopsin 2D crystals

A: High-resolution phase channel of bacteriorhodopsin 2D crystal patches imaged with HyperDrive at ambient temperature in buffer.

B, C: Subsequent higher-resolution height images reveal the substructure of the bacteriorhodopsin trimers. Line rates used for recording the channels top to bottom were 15, 10 and 40 Hz respectively. XZ-scales in A, B and C panels are 400 nm, 150 nm, 50 nm, and 1 deg, 0.4 nm, 1.5 nm respectively.

Key components and specifications of the BioMAT workstation

NanoWizard AFM system	· Recommended Products: NanoWizard 4 XP, NanoWizard V, NanoWizard ULTRA Speed 2, NanoWizard Sense+
BioMAT base with built-in optics	· Solid mechanical base for highest resolution AFM operation · Integrated optical system for position calibration, a focusing system, and illumination · Precision positioning for AFM alignment
BioMAT shuttle stage with sample mount	· Easy handling and transport · 10 mm manual XY travel range for samples · Kinematic mount enables < 5 µm (typically 3 µm) repeatability of AFM and optical microscope co-localization · Fully liquid-proof design · Options: a) Precision sample clamp for a large variety of samples b) PetriDishHeater
Upright optical microscope	· Compatible with a large variety of research grade upright microscopes, e.g., Zeiss AxioImager series, Axio Scope.A1, Axioscope 5; Olympus BX53, BXFM, OLS 4100 LEXT; Nikon Eclipse LV150/150A, Eclipse E600; Leica DMI 4000/5000, DM 6 (after adaptation of manufacturer hardware) · No restrictions on white light and fluorescence reflection microscopy imaging · Advanced optical modes such as FCS, FRET, FLIM, Raman, etc. are also supported (subject to verification of actual optical hardware configuration) · Fully compatible with confocal laser scanning microscopes (CLSM) · Use of water dipping objectives for measurements in fluid · Optical microscope can be located apart from the AFM system, sample transfer is performed via shuttle stage · Modified sample stage with kinematic mount

Summary

The BioMAT workstation, a fully integrated solution, enables the combined use of AFM and upright optical microscopy for the study of non-transparent samples. Its innovative design and outstanding co-localization of regions of interest acquired by both techniques makes BioMAT the ideal solution for the specialized investigation of opaque samples in the life and material sciences. A broad range of environmental control options facilitate its use in applications ranging from biochips, cell chips and patterned substrates to cell adhesion, microbiology, and tissue engineering.

Acknowledgements

We would like to thank Andreas Herrmann and Thomas Korte (Humboldt University, Berlin, Germany) for kindly providing the samples used for the images in Figure 2.

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Recent Publications with BioMAT

1. Liu X, Li Y, Cao J, Zeng Z, Liu X, Zhang R, Li Q, Sand W (2022) Bioleaching of Chalcopyrite Waste Rock in the Presence of the Copper Solvent Extractant LIX984N, *Front Microbiol* 13, 820052.

2. Özsoylu D, Isık T, Demir MM, Schöning MJ, Wagner T (2021) Cryopreservation of a cell-based biosensor chip modified with elastic polymer fibers enabling ready-to-use on-site applications, *Biosensors and Bioelectronics* 177, 112983.

3. Li Q, Zhu J, Li S, Zhang R, Xiao T, Sand W (2020) Interactions Between Cells of Sulfobacillus thermosulfidooxidans and Leptospirillum ferriphilum During Pyrite Bioleaching, *Front Microbiol* 11, 44.

4. Li Q, Becker T, Zhang R, Xiao T, Sand W (2019) Investigation on adhesion of Sulfobacillus thermosulfidooxidans via atomic force microscopy equipped with mineral probes, *Colloids and Surfaces B: Biointerfaces* 173, 639–646.

5. Mougin J, Yesylevskyy SO, Bourgaux C, Chapron D, Michel J-P, Dosio F, Stella B, Ramseyer C, Couvreur P (2019) Stacking as a Key Property for Creating Nanoparticles with Tunable Shape: The Case of Squalenoyl-Doxorubicin, *ACS Nano* 13 (11), 12870–12879. [Using BioMAT as a standalone AFM setup for imaging]

6. Pawolski D, Heintze C, Mey I, Steinem C, Kröger N (2018) Reconstituting the formation of hierarchically porous silica patterns using diatom biomolecules, *Journal of Structural Biology* 204 (1), 64–74.

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