Working with the Biomaterial Workstation BioMAT™

Introduction

The atomic force microscope (AFM) is an extremely flexible instrument that can be used for imaging, measuring forces and elastic properties and manipulating a variety of samples, at high resolution. The applicability of AFM is further extended in combination with light microscopy as optics deliver more bulk details and by fluorescence, compositional contrast. To date, the combination of AFM and light microscopy has been limited to samples on transparent substrates, where AFM has top-down access to the sample and an inverted light microscope has bottom-up access to the same area [1,2].

However, there are many areas of research in which the combination of AFM with optical microscopy on opaque samples would be a powerful tool. Topics such as bacterial growth on metallic surfaces, bionics and surface chemistry, fluorescent polymers and coatings, i.e. areas from both life and material science could be addressed with such a combination of techniques.

The main problem limiting the effective combination of these techniques on non-transparent surfaces has been providing access for both techniques at the same spot on the sample surface. To reach the full capabilities of optical microscopy, objective lenses with an extremely short working distance are required, leaving no space for AFM access to the same position. JPK Instruments has developed a solution to allow integration of upright optical microscopy and AFM, named the BioMAT Workstation (Figure 1).

***Fig. 1**: NanoWizard AFM integrated into the BioMAT Workstation*

Combining Optical Upright Microscopy and AFM

The BioMAT Workstation spatially separates the upright optical microscope from the AFM to assure that neither of the two techniques is compromised [3]. The key element of the BioMAT Workstation design is the portable shuttle stage on which the sample is loaded (Figure 2). The transfer of this shuttle stage from the upright optical microscope to AFM and vice versa allows precise positioning of the sample on both microscopes such that the same area is imaged by both systems. This transfer can be repeated as often as necessary, allowing the sequential measurement of optics and AFM.

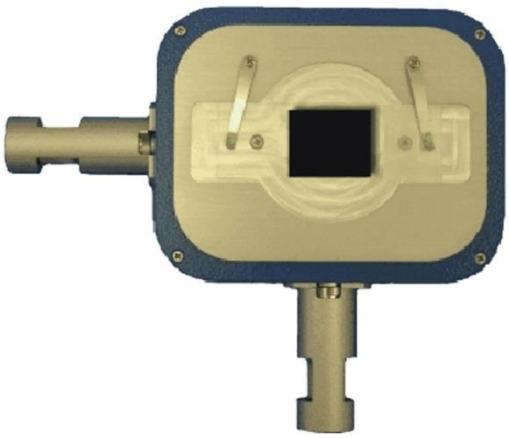

***Fig. 2:** The BioMAT shuttle stage*

For combining upright light microscopy with AFM first the BioMAT Workstation has to be aligned, matching the AFM

scan rage to the field of view of the optical microscope. To do this, a transparent reference sample, a cross-hair structure made of chrome, width of 5 μm, that can imaged with both systems is used. The reference sample is fixed on the shuttle-stage and imaged in the upright optical microscope. The adjustment screws of the shuttle stage are used to center the reference cross relative to the ocular cross of the optics (figure 3). Now the shuttle stage can be carefully transferred to the BioMAT Workstation without touching the adjustment screws.

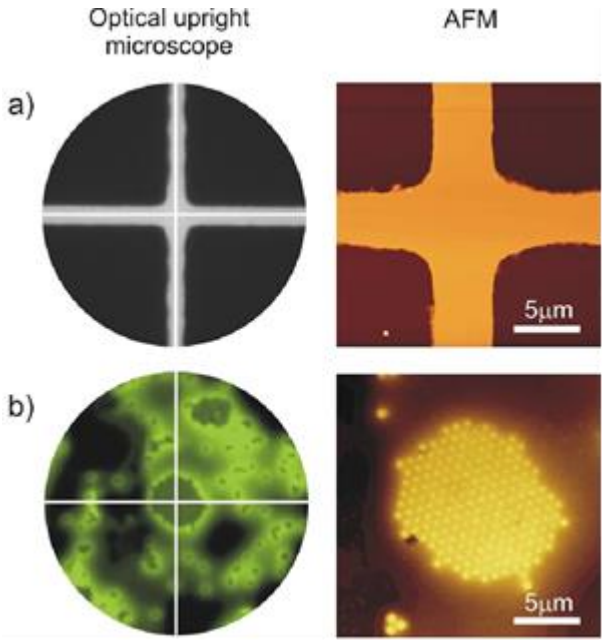

Fig. 3: *a) Alignment procedure on a reference sample (left: 40x optical brightfield, right: corresponding AFM height image). b) To demonstrate imaging of the same area a mixture of 200nm fluorescence PMMA and 1mm silica beads were first imaged in the upright microscope (left) and then with the AFM (right).*

The BioMAT Workstation has integrated inverted optics, such that the AFM tip can be coarsely aligned with respect to the chrome cross on the reference sample (figure 4). This coarse alignment involves adjusting the position of the AFM head on top of the BioMAT Workstation such that the AFM tip position matches the center of the reference cross. An AFM topographic image of the cross is then be taken (figure 3). From this reference image, the coordinates within the 100μmx100μm scan range of the center position of the optical field of view on the sample can be determined. By carefully exchanging the reference sample for the sample of interest without disrupting the adjustment screws, the same area of the sample can be imaged sequentially with the two separate microscopes.

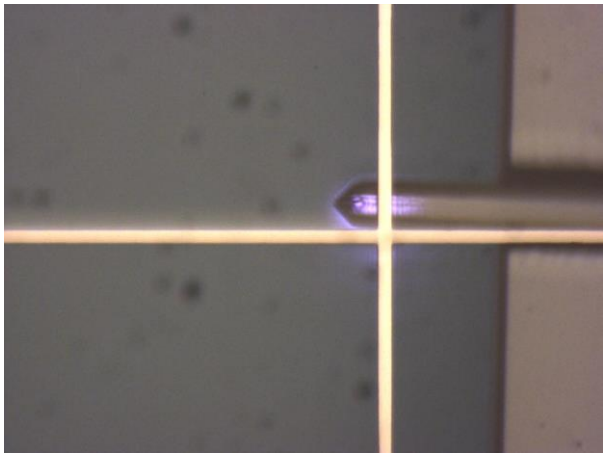

Fig 4*: The cantilever of the AFM can be aligned with the chrome reference cross using the alignment optics integrated into the BioMAT work station. This allows the user to establish common reference points in both AFM and optical space that can be applied to a sample of interest.*

Thiobacteria on Sulfur Surface

One area of research where combining light microscopy and AFM on opaque substrates would be useful is in the investigation of bacteria with metal surfaces. Thiobacteria can leach mineral sulfides from various metals. During this process of bioleaching the bacteria, which mostly belongs to species of Thiobacillus ferrooxidans are in close contact to the mineral sulfides, forming a monolayered biofilm on their opaque substrate [4].

AFM is particularly suitable for investigation such biofilms as it allows the visualization and characterization of biological samples in physiological conditions with high spatial resolution. The option to combine AFM with uncompromised fluorescence microscopy is a powerful means to correctly interpret and validate the topographic images obtained by AFM with the help of corresponding images of fluorescently labelled structures. Since almost all sulfur containing minerals are opaque such samples are the perfect application for the BioMAT Workstation.

Here, Thiobacillus ferrooxidans (courtesy, Prof. Sand, University Duisburg-Essen) was grown on a piece of compressed, elementary sulphur. For fluorescence microscopy the bacterial DNA was stained using DAPI (4',6-diamido2-phenylindol). Fluorescence images were acquired with a Zeiss Axio-Imager.A1m fitted with a 100x Zeiss Acroplan water immersion objective. After imaging on the Axio-Imager, the specialized sample holder was transferred to the BioMAT Workstation and AFM imaging was conduc-

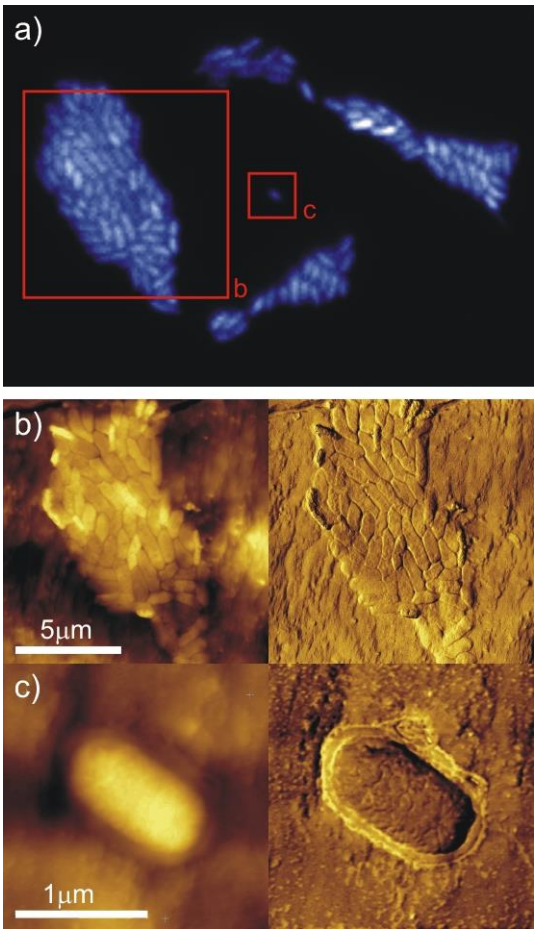

Fig 5: *Imaging of bacteria on an opaque sulphur surface. In (a) DAPI stained bacteria have been imaged using fluorescent microscopy. The marked regions correspond to the AFM images in (b) and (c). While the fluorescent image makes it clear where the bacteria are located, high resolution structural information can only be derived from the corresponding AFM images.*

ted in intermittent contact mode using the NanoWizard AFM. As contrast in AFM is based on structure, the images of bacteria on the surface of the elementary sulfur are complex. One can see steps and structures in the sulphur substrate, as well as groups of bacteria on the surface. By comparing the AFM image with the fluorescence image of the same area it is clear which surface features correspond to bacteria (figure 5). When it is clear exactly which region of the surface contains bacteria the surface properties of the metal and the biofilm can be charecterised in high resolution with the AFM. Here, pili can be seen extending from the bacterium (arrows), across the surface, likely aiding in the adhesion of the bacteria to the surface.

Conclusion

With such a tool many new possibilities for combined imaging of biological samples on opaque surfaces are now possible. The investigation of structural and elastic properties of various types of cells on patterned surfaces can provide valuable information about the interaction of cells with potential implant surfaces. As seen here, the effect of biofilm formation on a metal or crystalline surface can be investigated. Polymer formation on opaque surfaces can also be investigated, using both fluorescence microscopy and AFM. Such access to a sample with the two forms of microscopy further extends the applicability of the AFM for characterization of samples on opaque surfaces.

References

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