High resolution imaging with the NanoWizard® BioAFM

Since its invention, the atomic force microscope (AFM) has been used to image a wide range of different samples. When the AFM was modified such that it could image samples in buffer it became possible to address biological questions under physiological conditions with this technique. The detail in atomic force images is unrivaled by other microscopy techniques that can be used to image samples in fluid, due to the signal to noise ratio of the instrument. In addition, samples dried to preserve structure do not need to be further treated to generate contrast.

The NanoWizard® BioAFM from JPK Instruments has a number of features that enhance the capacity of this technology for the highest resolution imaging of biological samples. Namely, the JPK Nanowizard® is linearized in all three dimensions. That is, there is a closed-loop feedback that ensures precise positioning in the x and y axes as well as in the z axis. Additionally, the Nanowizard® further extends the applicability of atomic force microscopy (AFM) imaging by enabling simultaneous AFM imaging with additional optical microscopic techniques. Both of these features can save the user time and resources when striving for that perfect, high resolution image.

Atomic lattice of mica

Fig. 1 Mica imaged in contact mode. Scan size, 40 x 60 Å

Superior engineering and stability is required for the acquisition of high resolution images. To demonstrate the stability of the JPK Nanowizard® even when installed on an inverted light microscope, freshly cleaved mica was imaged in contact mode in air. The atomic lattice of mica can clearly be seen (Fig 1.)

Hexagonally packed intermediate layer

The hexagonally packed intermediate (HPI) layer of the archaebacteria, Deinococcus radiodurans, has been extensively studied using atomic force microscopy [1, 2].

Fig. 2 (A) HPI layer patch on mica, imaged in closed loop contact mode, in fluid. (B) High resolution image of HPI subunit pores. Red circle - example of a closed pore, blue circle – example of an open pore. Defects in the lattice are also evident, e.g. the missing suunit in the pore marked with a white arrow. Image (B) kindly provided by Dr. Patrick Frederix, University of Basel.

The HPI layer of D. radiodurans forms a surface layer, presumed to act as a kind of molecular sieve to regulate transport of nutrients and metabolites in and out of the cell. Data has been generated on the structure and function of the HPI layer using a variety of different techniques, from biochemistry to electron microscopy. However, AFM imaging of this sample can be carried out in fluid, at high resolution, to follow dynamic changes in protein structure.
The HPI layer is extracted from whole cells with detergent and then adsorbed to a freshly cleaved mica surface. The stable packing of the individual protein elements facilitates the acquisition of high resolution images. The HPI layers form patches on the mica surface, and overview images of these patches already reveal the regular lattice-structure of the HPI layer (Fig 2, A).

After the acquisition of an overview image of an HPI membrane patch, a suitable region can be selected for imaging at higher resolution (Fig 2, B). As the x-y positioning of the JPK Nanowizard® is controlled by a closed-loop feedback system the instrument will “zoom in” to the selected region with high accuracy. This enables the user to take fewer scans, reducing the likelihood of contaminating the tip or damaging the membrane patch.

**Nuclear pore complex**

The eukaryotic cell is organised into compartments called organelles. Controlled transport across the membranes surrounding each organelle allows the cell to compartmentalize specific molecules, a process that underlies cellular function. In the nuclear membrane, the nuclear pore complex (NPC) is responsible for transport of various molecules into and out of the nucleus.

As the life science version of the JPK Nanowizard® is fully integrated into an inverted, light microscope, transmission microscopy can be used to scan the sample for a region that does not contain large amounts of debris, before scanning. In such a way the user can, once again, reduce the time required to find a suitable region for scanning, and decrease the chance of contaminating the tip.

Unlike the HPI layer of *D. radiodurans*, preparations of NPC do not simply contain NPC condensed into a lattice. The samples are prepared from whole nuclei, in this case from *Xenopus laevis*, and can be quite heterogeneous [3].

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**Fig. 3** DIC image of NPC sample. The use of DIC clearly highlights debris.

**Fig. 4** Contact mode images of NPC on a glass coverslip. An overview image shows a mixture of NPC and contaminating material. The JPK Nanowizard® can then accurately zoom in on regions of interest for higher-detail scans.
NPC samples, on a glass coverslip, were imaged using differential interference contrast (DIC) microscopy, clearly highlighting debris that would be impossible to visualise using bright field microscopy (Fig 3). The tip was then positioned over an area with minimal debris and an overview scan acquired (Fig 4). Again, the capacitively controlled feedback then allows precise selection of an area for a higher resolution scan.

**DNA imaging**

Most of the data generated on the structure and function of DNA has come from the field of molecular biology. However, with the signal to noise ratio of AFM this fundamentally important biological molecule can be studied at high resolution in liquid and in air, to elucidate physical structure and the interaction of DNA with DNA-binding molecules. Under appropriate conditions, DNA can be adsorbed to freshly cleaved mica and imaged in buffer. Figure 5 shows Lambda phage DNA (ac mode in fluid).

The interaction of various proteins with DNA is fundamental in the processes of replication and transcription. One example is the association of DNA with histones to form nucleosomes. This condensing of DNA around the nucleosome core (consisting of a histone octamer) plays a role in the regulation of DNA replication, and transcription, as the condensed DNA is not accessible to other DNA-binding proteins.

**Fig. 5** Topographs of Lambda phage DNA, imaged in intermittent contact mode in fluid. Colour scale 0-2 nm for both A and B.

In this case, the linearized, 3 kb plasmid pGEM was incubated with nucleosomes in a ratio of 1 mole of DNA to 20 moles of histone octamers. The pGEM plasmid has 20 putative nucleosome binding sites, however, it can be seen that under the incubation conditions, nucleosomes did not bind at all 20 binding sites (Fig 6).

**Conclusions**

The signal to noise ratio, lack of requirement for staining or pretreating and capability to function in liquid makes AFM imaging an extremely powerful method for describing, at high resolution, the structure of biological samples. The design of the JPK Nanowizard® can facilitate such high resolution studies. For instance, the accurate positioning in x and y (due to closed-loop feedback) reduces the number of scans that are required to “focus” at high resolution on a region of interest. This decreases the likelihood of damaging delicate samples and of contaminating the tip.
For samples prepared on glass, such as the nuclear pore complex described above, contaminating debris can be easily avoided by searching for a suitable area using transmission light microscopy, again saving the user time.

The benefit of using AFM for such imaging studies lies in the capability of AFM to image samples in liquid, under physiological conditions. JPK Instruments manufactures the Biocell™ that can allow the user to modify conditions during scanning (Fig 7), such as controlled temperature changes or the in situ addition of relevant molecules, further enhancing the applicability of the JPK Nanowizard® for the highest resolution imaging of biological samples.

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**Literature**


