

Application Note #142

Imaging of the DNA Double Helix with PeakForce Tapping Mode Atomic Force Microscopy

DNA was one of the first biological molecules visualized by atomic force microscopy (AFM). It continues to be imaged by AFM for studies of DNA structure, topology, dynamics, and interaction with proteins. With a few exceptions, early AFM images showed DNA as a long featureless polymer with no indication of its underlying helical structure. However, with enhanced force control and sharp AFM tips, it has been possible to resolve, in buffer solution, the two oligonucleotide strands of the Watson-Crick double helix for single DNA molecules that were physisorbed on a mica substrate.^{1,2} Recent advances in AFM have made such studies more achievable.³ In particular, Bruker's exclusive PeakForce Tapping® technology has enabled routine high-resolution imaging of the DNA double helix at quantifiable imaging forces, without the need for specialized probes or restrictive AFM designs.

The Introduction of AFM into Biological Research

The introduction of TappingMode™ in the early 90s led to a significant increase in the use of AFM for biological research.⁴⁻¹⁴ In TappingMode, the probe oscillates at its fundamental resonance frequency, and the vertical position of the tip (or sample) is continuously adjusted to maintain a constant amplitude of oscillation as the probe scans across a surface. This constant amplitude is usually set slightly below the amplitude of the freely oscillating probe

at some microns away from the sample surface. The probe oscillation essentially represents a tapping motion, with the probe continuously moving in and out of contact with the surface. The intermittent nature of the tip-sample contact reduced the shear forces associated with the previously used contact mode AFM. This puts less stringent demands on how rigidly the sample of interest is attached to a hard substrate, reducing the need of fixation and thus allowing the sample to be imaged under more physiologically relevant conditions.

Unfortunately, despite the advantages that TappingMode offers for studying the structure of biological samples, it has been criticized for ultimately providing lower-resolution images of biomolecules than contact mode imaging.¹⁵ Key to obtaining high-resolution AFM images is the ability to control the tip-sample interaction forces during imaging. For the setpoint amplitude to be an accurate measurement of the tip-sample forces, the free oscillation amplitude (at some microns above the surface) needs to remain constant. For TappingMode in liquid this is often not the case, since the cantilever amplitude not only depends on the cantilever resonance, but also on its convolution with mechanical resonances of the fluid cell (the so-called 'forest of peaks').¹⁶ As the liquid in the fluid cell changes shape, volume and composition throughout an experiment, these resonances shift. This can result in changes to the

force applied between the tip and the sample, as the free amplitude of the cantilever changes. It can therefore be difficult to accurately determine and control the imaging force during a TappingMode experiment.

PeakForce Tapping Mode for Routine High-Resolution Imaging of Biomolecules

In 2010, Bruker released the PeakForce Tapping AFM imaging mode. In the short time since its introduction, PeakForce Tapping has seen a rapid uptake in its use for the study of biomolecules.¹⁷⁻²⁶ In PeakForce Tapping mode, the tip-sample distance is modulated in a sinusoidal motion at amplitudes that are typically less than 100 nm and at frequencies of 1 or 2 kHz. When the AFM probe is brought into contact with the sample surface, the tip-sample interaction is controlled by maintaining the maximum force, or “peak force,” between the tip and the sample constant (see figure 1a). If one considers the motion of the probe in terms of Z position, we are essentially performing a force curve at every pixel position on the sample surface (see figure 1b). An advantage that PeakForce Tapping has over other force-distance curve based imaging modes is that PeakForce Tapping utilizes a continuous feedback loop to adjust the relative tip-sample position. As such, the imaging force control benefits from the results of the previous tip-sample interactions. PeakForce Tapping also uses sinusoidal ramping rather than linear ramping such that the tip velocity approaches zero as the tip approaches the surface. Together, these features of PeakForce Tapping enable direct and precise control of the tip-sample interaction force, facilitating imaging in fluid environments at forces of 100 pN or less. This helps protect both the AFM probe and the sample from potential damage and is one of the key factors in enabling high-resolution imaging. Additionally, imaging in PeakForce Tapping is considerably quicker than other force-distance curve-based imaging modes. As PeakForce Tapping operates at much higher frequencies (1-2 kHz) it is capable of performing thousands of force curves per second. PeakForce Tapping images can therefore be

acquired at traditional imaging mode scan rates at high pixel resolution ($\geq 512 \times 512$ pixels).

In addition to protecting delicate samples and tips from damage by maintaining low imaging forces, PeakForce Tapping has also made imaging in fluid easier and effectively more consistent by eliminating the need to tune the cantilever. Unlike TappingMode, PeakForce Tapping does not operate at the resonance frequency of the AFM probe, such that cantilever tuning is simply not needed. PeakForce Tapping technology has also facilitated the self-optimizing ScanAsyst® imaging mode. In ScanAsyst, auto-optimization of the imaging setpoint prevents setpoint drift, which commonly occurs in other AFM operating modes, such as TappingMode and contact mode, due to resonance peak shifting and/or cantilever deflection drift. This auto-optimization of the imaging force at the point of each tip-sample interaction enables PeakForce Tapping to acquire high-resolution images more routinely than contact mode or TappingMode. Together with auto-optimization of other parameters in ScanAsyst mode, such as gain and scan rate, PeakForce Tapping now results in faster, more consistent data, regardless of the user skill level.

The performance of PeakForce Tapping can be illustrated by imaging single virus capsids. In previous AFM studies of virus capsid structure where TappingMode was successfully employed, the virus particles were typically arranged in a two-dimensional (2D) crystal structure.²⁷ The 2D array provides the mechanical stability to the individual virus particles so that they are not distorted or damaged under the force of the AFM probe. As virus capsids are delicate structures, high-resolution images to date have typically been obtained using “jumping mode” AFM.^{28,29} As in PeakForce Tapping mode, jumping mode conducts discrete force curves along the fast scan axis, with topography data being derived from these force curves. However, unlike jumping mode, for each individual force curve in PeakForce Tapping the real-time feedback loop subtracts background artifacts that are typically caused by viscous drag due to the motion of the cantilever in fluid. The removal of this background increases the sensitivity at which the peak force is detected and therefore allows the use of much lower imaging forces. Figure 2 shows a PeakForce Tapping image of a single herpes simplex virus. The arrangement of protein molecules as three-dimensional (3D) subunits on the surface of the virus capsid, also known as capsomeres, is clearly visible. It is important to note that these virus particles were imaged as individual and isolated particles without lateral stabilization.

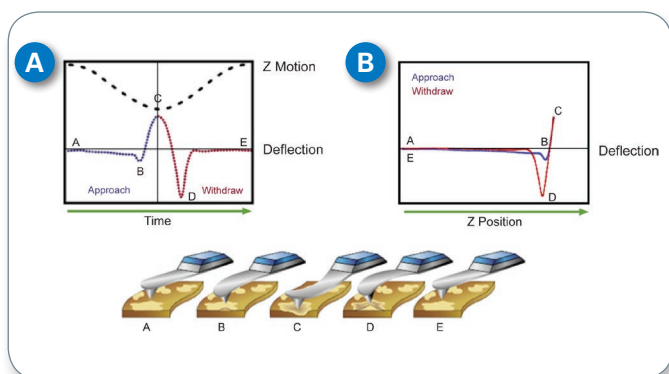


Figure 1. In PeakForce Tapping Mode the AFM probe is modulated at low frequency (1-2 kHz). (A) As the probe is brought into contact with the surface, the feedback signal is the maximum or “peak” force applied to the surface. (B) If the motion of the probe is considered in terms of Z position, one is essentially performing a force curve at every position of the sample surface.

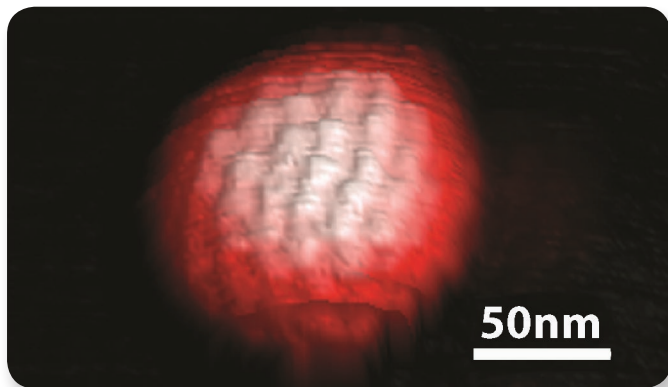


Figure 2. 3D topography image of a single herpes simplex virus obtained in ScanAsyst mode in buffer solution. The spatial arrangement of the individual protein molecules on the surface of the virus capsid, also known as the capsomere, is clearly visible in the AFM image (ScanAsyst Fluid+ probe, $k \sim 0.7$ N/m).

PeakForce Tapping Imaging of the DNA Double Helix

DNA is another highly suitable sample benchmark for PeakForce Tapping. It has been extensively imaged by AFM and was one of the first samples used to demonstrate the potential of TappingMode for imaging biomolecules.³⁰⁻³³ DNA is made up of two polynucleotide strands that form a double helix. B-DNA, the “Watson-Crick” form of DNA, exhibits a right-handed helix with a helical repeat (pitch) of ~ 3.6 nm, with major and minor grooves of widths ~ 2.2 nm and ~ 1.2 nm, respectively. The vast majority of DNA images in the AFM literature display DNA molecules as featureless strands. Recent developments in AFM technology, however, have facilitated the visualization of the DNA double helix as a tilted, double-banded structure repeating along the molecule using specialized instruments.^{1,2} Here we will show a method by which the secondary structure of DNA can be imaged using PeakForce Tapping and standard cantilevers.³

As with all AFM studies conducted in fluid environments, sample preparation is central to successfully imaging the DNA double helix. As such, the DNA plasmid must first be adsorbed on a suitable surface. One of the most commonly used substrates for AFM imaging is mica: Its planar structure can be readily cleaved using sticky tape, revealing an atomically flat and clean surface. However, at neutral pH, mica has an overall negative surface charge, which does not favor adsorption of the also negatively charged DNA. Several methods have been developed to overcome this, all of which essentially act to functionalize the mica to create a positive interface to which the DNA can attach.

As early as 1995, Mou et al. resolved the pitch of B-DNA by AFM as a periodic modulation of 3.4 ± 0.4 nm.³⁴ In their study, DNA was adsorbed onto the surface of a cationic supported lipid bilayer, deposited on a mica substrate. Interestingly, the pitch of the DNA was only observed when the DNA strands were densely and uniformly packed on the bilayer surface, and not where bilayers

were populated by individual isolated DNA strands. The researchers concluded that this close packing limited the movement of the molecules, supported by the knowledge that DNA is a highly dynamic molecule, having both translational and rotational movement.³⁵ The resolution obtained on DNA thus depends on the degree of adhesion and immobilization of the DNA molecules on the substrate. Providing an alternative for cationic lipid surfaces, mica can be chemically modified with 3-aminopropyltriethoxysilane (APTES) or 1-3-aminopropylsilatrane (APS), to give a positive interface with which the DNA can interact.³⁶

Divalent cations provide an alternative way to adsorb DNA to mica, where the adhesion can, to some extent, be tuned by the cationic concentration in the solution,³⁷ with Ni^{2+} being a convenient and effective option. The Ni^{2+} ions are used to bridge the negative charge of the mica and mediate adsorption of the DNA strands. Typically, higher Ni^{2+} concentrations lead to a stronger binding of adsorbed DNA molecules to the mica, with the caveat that they also increase the surface contamination by aggregated salt.³ In 2012, Leung et al. were the first to successfully image the major and minor grooves of a single DNA molecule, using a 1-5 mM concentration of NiCl_2 to adsorb the DNA onto a mica surface.¹ While this low concentration helps to minimize any adverse structural effects on the DNA strands, and reduce surface contamination, it also leaves the DNA loosely bound to the mica surface and as such creates a greater challenge for high-resolution imaging. Leung et al. attributed their success to improved force sensitivity, which in this case was achieved by use of a homebuilt interferometry-based AFM in addition to customized small cantilever probes. Others subsequently resolved the DNA double helix by using imaging solutions containing very high concentrations of Ni^{2+} (~ 50 mM).²

Employing the same DNA immobilization strategy as Leung et al., our goal was to resolve the helical structure of loosely bound DNA using the low and precisely controlled imaging forces enabled by PeakForce Tapping mode, as achieved by Pyne et al.³ To demonstrate that this type of spatial resolution is not specific to a particular AFM system or probe, we carried out PeakForce Tapping experiments on the MultiMode 8, Dimension FastScan Bio, and BioScope Resolve™ atomic force microscopes (see figure 3) using ScanAsyst Fluid+, MSNLF, FastScan-D, and ScanAsyst Fluid-HR probes, which all have standard silicon tips. PeakForce Tapping imaging on the MultiMode 8 in 10 mM HEPES, 1 mM NiCl_2 , pH 7.4 revealed corrugations along the DNA strand that correspond to the major and minor grooves of the DNA double helix (see figure 4A). A high-resolution image obtained on the BioScope Resolve (inset of figure 4A) was obtained under the same imaging conditions while operating on an inverted light microscope and using ScanAsyst Fluid-HR probes. This image illustrates the widths of the alternating major and minor grooves, at 2.2 nm and 1.2 nm, respectively. To analyze the mobility of the surface-bound DNA, continuous

high-speed TappingMode imaging was performed on the plasmid DNA immobilized on the mica surface in 1mM NiCl₂ (see figure 4B), using the FastScan Bio atomic force microscope and FastScan-D probes that have a small cantilever but a standard silicon tip. The time series of high-speed images illustrates that while some parts of the DNA strand remain immobile under continuous imaging, other parts move over the surface. Therefore, while the Ni²⁺ immobilizes the DNA sufficiently to enable high-resolution imaging of

the helical structure, it also allows for some degree of rotational and translational movement of the individual strands. Height variations were also observed in the topography along the length of the DNA, possibly indicating twisting of the DNA strand (see figure 5A). This would also suggest that the low concentration of Ni²⁺ allows the DNA to maintain a more physiologically relevant structure on the mica surface.

As stated earlier, one of the keys to obtaining high-resolution



Figure 3. Using PeakForce Tapping and standard AFM probes, imaging of the DNA double helix was demonstrated on all of Bruker's high-performance BioAFM systems: (left) Dimension FastScan Bio AFM, (middle) MultiMode 8 AFM, (right) BioScope Resolve AFM.

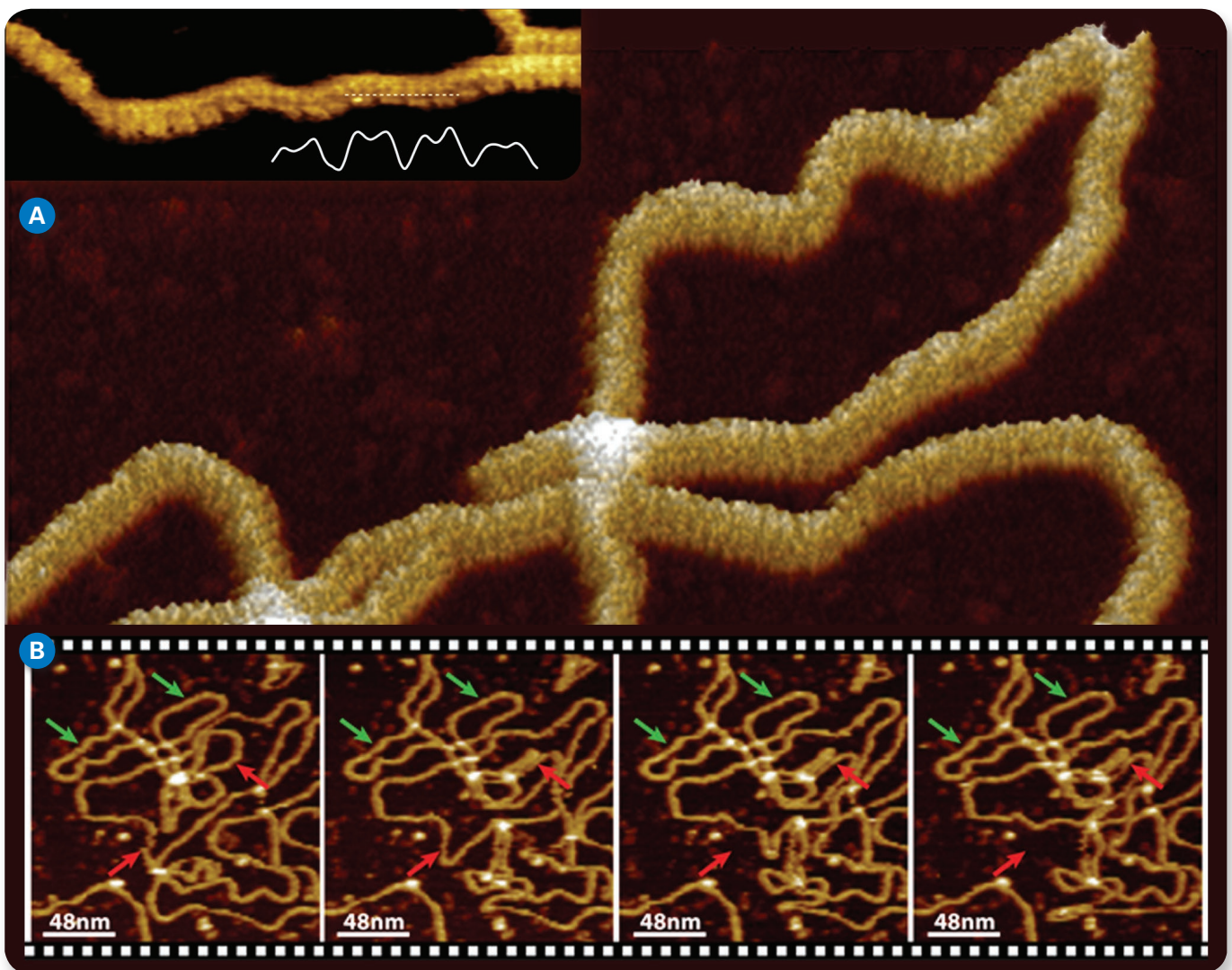


Figure 4. (A) PeakForce Tapping image of a DNA plasmid taken in buffer solution using the Multimode 8 and MSNL-F probes ($k \sim 0.6$ N/m) showing corrugation corresponding to the major and minor grooves of the DNA double helix. The inset is a high-resolution image of a DNA plasmid obtained using the BioScope Resolve operated on an inverted optical microscope and ScanAsyst Fluid-HR probes ($k \sim 0.05$ N/m). The cross section taken along the strand, as indicated by the dotted line, shows the widths of the alternating major and minor grooves at 2.2 nm and 1.2 nm, respectively. (B) Time series of high-speed AFM images of the same type of plasmid DNA obtained in TappingMode showing that at low NiCl₂ concentration some parts of the DNA remain immobile under continuous imaging (green arrows) while other parts of the same strand show a high degree of movement (red arrows). High-speed imaging was conducted on the FastScan Bio AFM using FastScan-D probes ($k \sim 0.2$ N/m).

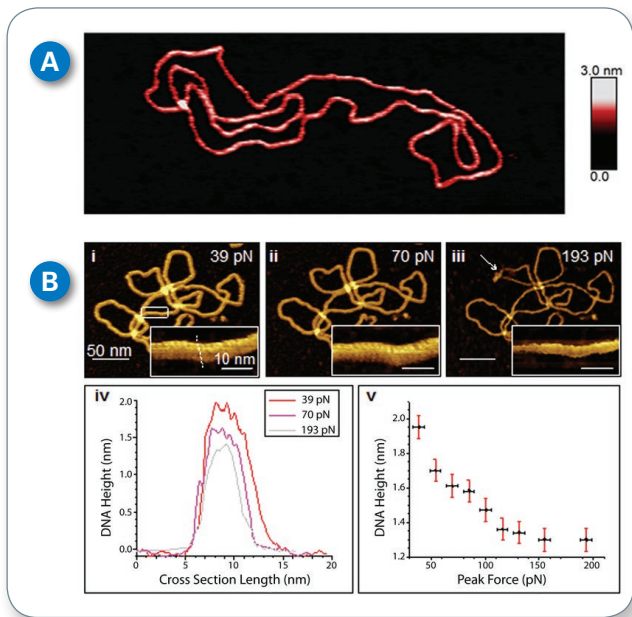


Figure 5. (A) Topography image of a DNA plasmid captured in PeakForce Tapping mode in buffer solution. Local height variations are visible along the molecule as changes in color (white to red). (B) (i-iii) A DNA plasmid imaged at peak forces of 39, 70, and 193 pN, respectively, with the major and minor grooves of the DNA double helix visualized at higher magnification (insets). Color scales: 3 nm (for low magnification); 2 nm (for the insets). (iv) Height profiles measured across the DNA, as indicated by the dashed line in the inset of B, for different peak forces. (v) Measured height along the same section across the molecule (as iv) as a function of peak force. Figure 5(B) is reproduced with permission from Pyne et al.³

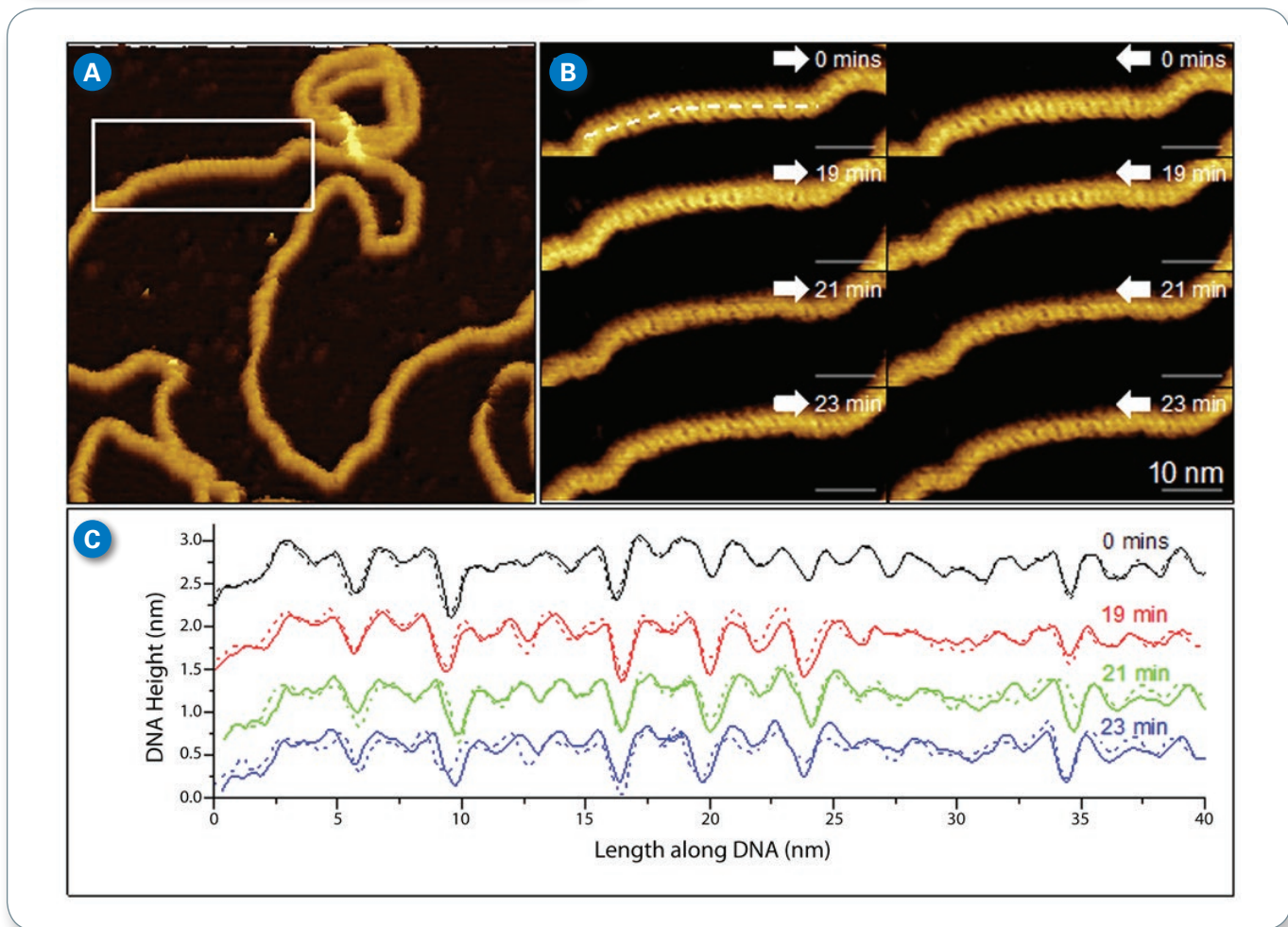


Figure 6. PeakForce Tapping image of groove depth variations in the DNA plasmid topography obtained using the FastScan Bio AFM and FastScan-D probes (small cantilever and standard silicon tip). (A) Low-magnification AFM topography image of a plasmid showing corrugation. The white rectangle indicates the area imaged in B. (B) Higher-magnification trace (white arrow to right) and retrace (white arrow to left) images of this area showing corrugation consistent with the B form of DNA, for consecutive images. (C) Trace (solid) and retrace (dashed) height profiles taken along straight lines as indicated in B, closely following the backbone of the four plasmid scans and averaged over a 5-pixel (~0.5) width. The height profiles confirm the observed corrugation to be the alternating major and minor grooves of double helix structure and that these grooves vary in depth along the DNA strand. The height profiles have been offset by multiples of 0.6 nm for clarity. Color scales: 3.5 nm (A), 1.1 nm (B). Reproduced with permission from Pyne et al.³

images of the DNA double helix is precise and continuous control of the force applied to the sample. PeakForce Tapping has the unique advantage over other intermittent contact modes in that the imaging force is easily quantified at all times. Figure 5B(i-iii) shows the effect of force on AFM topography using PeakForce Tapping mode on the Multimode 8 with MSNL-F probes. To best illustrate how the DNA is compressed with increasing tip-sample force, the height scale is kept the same for all images. At the minimum possible applied peak force of 39 pN, the measured height of the plasmid is close to the 2 nm diameter of DNA, as derived from its crystal structure (corresponding AFM height profiles shown in figure 5B(iv)). There is, however, very little corrugation visible along the length of the DNA strand in corresponding high-resolution images, shown in the inset of figure 5B(i), which may be due to difficulties in tracking the molecule at these low forces. At 70 pN of applied force, a 20% compression of the molecule occurs, reducing the measured height of the plasmid to ~1.6 nm. At this force the corrugation is most visible, as shown in the inset of figure 5B(ii). Beyond 100 pN, the major and minor grooves become less clear (figure 5B(iii)) and the measured heights reduce to <1.5 nm, similar to earlier TappingMode AFM experiments in liquid.^{34,38} At this point, the sample is also at significant risk of being dislocated from the mica surface, demonstrated by the movement of the molecule as indicated by the white arrow. Figure 5B(v) shows that the measured height agrees with the diameter of the DNA for applied forces of around 50 pN or less, while slightly more force may need to be applied to accurately resolve the secondary structure, as evident in the PeakForce Tapping images.

Figure 6A shows a high-resolution image of a DNA plasmid imaged by PeakForce Tapping on the FastScan Bio using FastScan-D probes at low force. This image shows corrugation corresponding to the double helix. To further investigate this structure, the scan size was reduced to image the smaller area highlighted by the white box. High-resolution images of this smaller scan area are shown in figure 6B in which the major and minor grooves of the strand are clearly shown. The double helix structure is clearly visible in both the trace and retrace images, with the scan direction indicated by the white arrows, as well as in a number of subsequent scans that are shown in time order. Interestingly, the major and minor grooves show variations in depth along the strand, which are reproduced between trace and retrace scans and in subsequent images (see figure 6C). This demonstrates that not only can PeakForce Tapping resolve the submolecular features of the DNA double helix but that it is also able to reproducibly image variations in this helical structure.

Conclusion

PeakForce Tapping mode provides precise force control and easy quantification of the tip-sample interaction force, enabling imaging at forces of less than 100pN to obtain high-resolution images of soft biological samples in fluid

environments. The high-resolution imaging capability of PeakForce Tapping mode is demonstrated by resolving the major and minor grooves of the DNA double helix on individual plasmids using Bruker's MultiMode 8, Dimension FastScan Bio, and BioScope Resolve atomic force microscopes. The ability to reliably achieve this type of submolecular resolution consistently, without the need for specialized probes or dedicated AFM designs, is helping to redefine the high-resolution imaging performance of atomic force microscopes for biological samples.

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