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4 Optimizing AFM Imaging in PFT for High Resolution AFM Imaging on DNA

In 1 collection

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ABSTRACT

This is part 4 of the "Atomic Force Microscopy of DNA and DNA-Protein Interactions" collection of protocols.

Collection Abstract: Atomic force microscopy (AFM) is a microscopy technique that uses a sharp probe to trace a sample surface at nanometre resolution. For biological applications, one of its key advantages is its ability to visualize substructure of single molecules and molecular complexes in an aqueous environment. Here, we describe the application of AFM to determine the secondary and tertiary structure of surface-bound DNA, and its interactions with proteins.

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COLLECTIONS ⓘ



Atomic Force Microscopy of DNA and DNA-Protein Interactions

KEYWORDS

Atomic force microscopy, AFM, DNA, Supercoiling, Double helix, DNA-protein binding

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GUIDELINES

For high resolution, minimally invasive imaging, it is important to optimise imaging parameters, as the apparent height of the molecule and observable level of detail can change as a function of the applied force (Fig. 4). Samples should be kept hydrated throughout imaging, in particular those containing ionic salts where precipitation can contaminate the sample and compromise imaging (*see* **Note 15**).

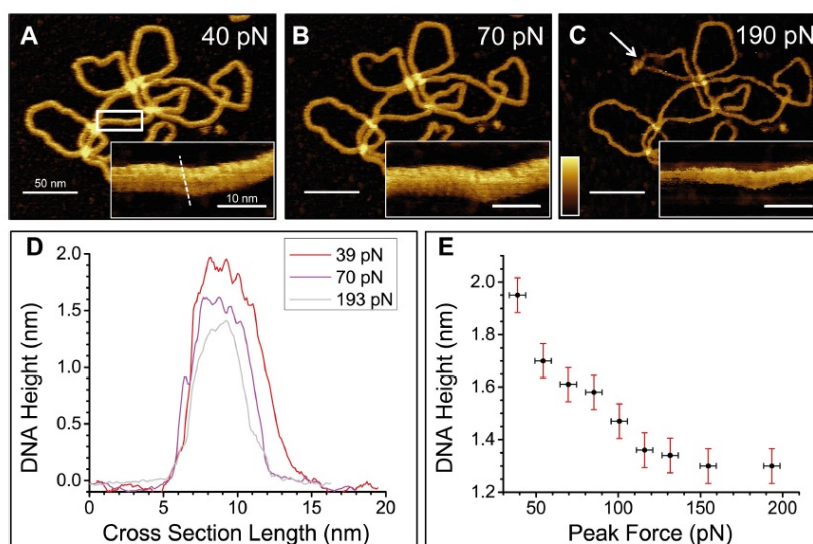


Fig. 4 Double-helix, corrugation and height of a DNA plasmid in AFM topography, with the DNA adsorbed using Ni^{2+} ions ([protocol 2, method 2.1](#)) and the data acquired by PeakForce Tapping (protocol 4.). (**a–c**) A plasmid imaged at maximum forces of 39, 70, and 193 pN, respectively, with the major and minor grooves of the DNA double helix visualized at higher magnification (*insets*). When imaging at higher forces, the AFM tip may displace the DNA laterally (white arrow shown in **c**). Color scales: 3 nm (for low magnification); 2 nm (for the *insets*). (**d**) Height profiles, measured across the DNA, as marked on the *inset* of **b** by a dashed line, for different applied forces. (**e**) Measured height along the same section across the molecule (as **d**), as a function of maximum (peak) force. Adapted from ref. 3, with permission.

Figure 4 shows the effect of altering the applied force on a DNA plasmid imaged in PeakForce Tapping mode. The DNA was immobilized using the divalent cation method and gains were optimized at each force. At low force, the molecule cannot be adequately tracked (Fig. 4a), whereas at high force, the plasmid is significantly compressed, and the DNA starts to be moved laterally by the AFM tip (Fig. 4c). At optimum force, the banded or stranded DNA structure is clearly resolved along the plasmid (Fig. 4b, inset) whilst compression accounts for a ~20% reduction in the expected height of the molecule. The effect of the applied force can be seen as a reduction in the height of the molecule in Fig. 4d. This follows a trend, shown in Fig. 4e.

When using sufficiently sharp AFM tips, PeakForce Tapping can be used for high resolution imaging of the double helix of DNA. Figure 2 shows high resolution scans of DNA plasmids and minicircles, showing the secondary structure of DNA and supercoiling-induced defects.

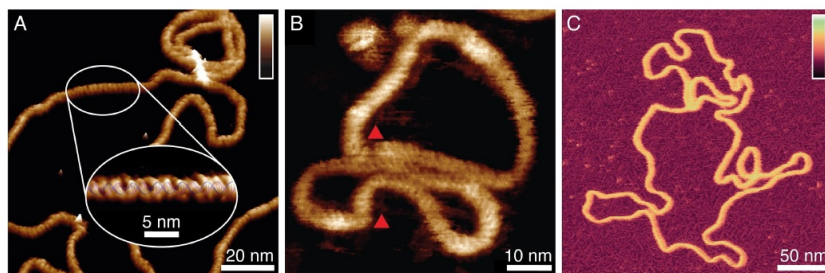


Fig. 2 High-resolution topographic images of DNA acquired by PeakForce Tapping mode (protocol 4.). The divalent cation method ([protocol 2, method 2.1](#)) is used to adsorb (a) DNA plasmids and (b) 339 base-pair DNA minicircles. In a, the two strands of the DNA double-helix are captured. *Inset*: a higher resolution image digitally straightened and overlaid with a cartoon representation of the B-DNA crystal structure. Color scales: 2.5 nm (main), 1.2 nm (*inset*). In b, defects and disruptions in the canonical B-form DNA are observed (red triangles), as a step-change in the angle of the helix. Color scale (scale bar in a): 2.5 nm [ref. 11, with permission]. (c) A DNA plasmid adsorbed onto PLL_{1000–2000}-functionalized mica ([protocol 2, method 2.3](#)) where the chains of poly-L-lysine making up the underlying substrate are resolved. Colour scale: 8 nm [adapted from ref. 31, with permission].

SAFETY WARNINGS

For hazard information and safety warnings, please refer to the SDS (Safety Data Sheet).

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- 1 Once the tip has reached the surface, minimize the setpoint to the point at which the maximum force barely exceeds the force noise (~ 70 pN).
- 2 Optimise *Sync Distance New* such that the marker (a small diamond on the force curve, *see* Fig. 3c) aligns with the peak of the force curve. Set the *Sync Distance QNM* equal to this for imaging and check that the Force-Z curve is 'folded' about the point of maximum force (*see* **Note 21**).
- 3 Reduce the total length of the force curve (*z* length or ramp) to ≤ 10 nm, reducing hydrodynamic drag and maximising sampling near to the sample surface (*see* **Note 22**).
- 4 Adjust the *Lift Height*, such that the baseline of the force curve is flat (Fig. 3c). *Autoconfig* will also reset the background subtraction, but may perturb the sample.
- 5 Begin scanning an area of $\sim 500 \times 500$ nm².
- 6 Locate a DNA molecule of interest.
- 7 Adjust the *Feedback Gain* (if appropriate) to ensure the molecule is adequately tracked (*see* **Note 29**).

- 8 If the molecule cannot be tracked (*see* **Note 23**), increase the force to allow tracking of the molecule. This will also require readjustment of the gains.
- 9 Ensure that the molecule being tracked is stable under imaging by verifying that it does not significantly shift between subsequent scan lines.
- 10 Reduce the *Scan Size* by zooming in on the DNA molecule of interest.
- 11 Increase the number of pixels per line to obtain ~0.5 nm per pixel (*e.g.*, 256 pixels per line for a 120 nm scan).
- 12 Reduce the applied force by reducing the *PeakForce Setpoint* and re-adjust the gains if required (*see* **Note 24**).
- 13 Lateral drift or creep may be visible as the objects appearing to move across the image between subsequent scans (*see* **Note 25**). Under such conditions, higher scan speeds, or allowing the microscope to further equilibrate may improve resolution.
- 14 Align the molecule to the direction along which the scan lines are recorded (the fast scan direction) for highest resolution.
- 15 If the part of the force curve above the baseline appears sinusoidal, as opposed to flat. The background will need to be recalculated by re-adjusting the *Lift Height* (*see* **Note 26**).
- 16 Optimize the applied force and gains by increasing and decreasing the force in the range where the DNA molecule is not overly compressed (*i.e.*, the measured height of the DNA should be ~20% of its known 2 nm diameter, *see* Fig. 4) to maximize resolution.
- 17 Other imaging parameters will vary depending on the antilever. Parameters when using an Fast-Scan D are outlined in Table 1.

PFT Parameters	Typical value
Scan Size [nm]	120 - 250
Pixel density [pixels/line]	256 - 512
Line rate [Hz]	3-5
Imaging PeakForce Setpoint [pN]	~ 70 *
PF acquisition Frequency, <i>see</i> Note 19 [kHz]	8
LP Deflection Bandwidth [kHz]	20
Sync Distance, <i>see</i> Notes 19 and 21 [μs]	70 / 20
PeakForce Amplitude, Note 22 [nm]	5-10 *
Lift Height, <i>see</i> Note 26 [nm]	5-7
Z Range (Z Limit if using MultiMode® 8) [nm]	500-1000
Deflection Limit [V]	≤12.24

Table 1: Typical parameters used for an FastScan-D cantilever on a FastScan Bio™ AFM system, operating in PeakForce Tapping mode

*Parameters may need to be adjusted when imaging on a surface passivated with PLL-*b*-PEG ([protocol 2](#), method 2.3). The *PeakForce Setpoint* and *PeakForce Amplitude* may need to be increased to 130 pN and 20 nm respectively ([protocol 5](#)).