

The Olympus Bio-Lever – Small, Low Noise Cantilever for AC in Fluid and Force Measurements for Soft Samples

Single molecule force measurements rely on the ability to measure exquisitely small changes in the position of the force-sensing cantilever. In an optimized instrument such as the MFP-3D™ AFM, positional noise is dominated by Brownian motion of the cantilever. It has been recently shown that one way to reduce the thermal noise of a lever is to simply make it smaller.¹ The Bio-Lever, the first small, low noise cantilever manufactured by Olympus Optical Co., does this by proportionally decreasing both the thickness and the length, without increasing the spring constant of the cantilever.

Table 1 compares three commercially available cantilevers. All three are diving board shaped. The resonant frequency, spring constant and quality factor were all obtained from fits to the first resonant mode in the thermal power spectrum. Until now, the silicon-nitride diving board lever, Microlever B from Thermomicroscopes (now Veeco), has been the

lowest noise lever commercially available for single molecule force measurements. The Bio-Lever A achieves the same spring constant as the Microlever B, but in a cantilever nearly four times shorter and over 2x quieter. The Bio-Lever, pictured in Figure 1, is specifically designed for single molecule force measurements. Because of the excellent noise performance, the levers have many other applications such as soft sample imaging and soft sample cell elasticity measurements. Furthermore, the levers are fabricated in matched pairs. This allows one lever to act as a reference in chemical sensing and other applications.

Noise Comparison

To illustrate the noise comparison of a Microlever vs a Bio-Lever, the force spectral density for the Microlever B and the Bio-Lever A is plotted in Figure 2a. For force measurements, we are typically interested in the cantilever's response at relatively low frequencies.

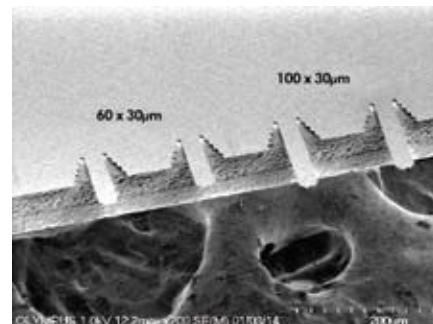


Figure 1: New Olympus Bio-Lever. The levers are 150nm thick, low-stress SiN. The 60µm long lever has a nominal spring constant of 25 pN/nm and 5pN/nm for the 100µm long lever. Their reduced size pushes their resonant frequencies higher while maintaining small spring constants, significantly lowering noise for force measuring and imaging applications.



Unique "V" on the end of the tip allows accurate positioning of the tip. 60µm lever (foreground), 100µm lever (background).

Table 1

Lever	Type	k(N/m)	f ₀ (kHz)	Q	Length (µm)	Width (µm)
1	Microlever Lever B	0.028	11.3	25	200	25
2	Olympus BioLever A	0.026	34.0	52	60	30
3	Olympus BioLever B	0.005	16.0	5	100	30

Equation (1) allows the RMS force in a given bandwidth to be calculated. It is important to define a lower frequency and upper frequency in the integral. The difference between f_2 and f_1 is referred to as the “measurement bandwidth”.

(1)

$$F_{RMS} = \sqrt{\int_{f_1}^{f_2} F^2(f) df}$$

F_{RMS} as a function of the upper integration limit f_2 is plotted in Figure 2b. In Figure 2b, a lower integration limit of $f_1=100\text{Hz}$ was arbitrarily chosen. Figure 2b allows you to estimate the noise in a given bandwidth for each lever. Two examples are illustrated on the graph of the integrated noise (see the vertical dotted lines and the associated circles in Figure 2b). From the figure, if you were to bandpass filter the cantilever noise between 100Hz and 3kHz, the

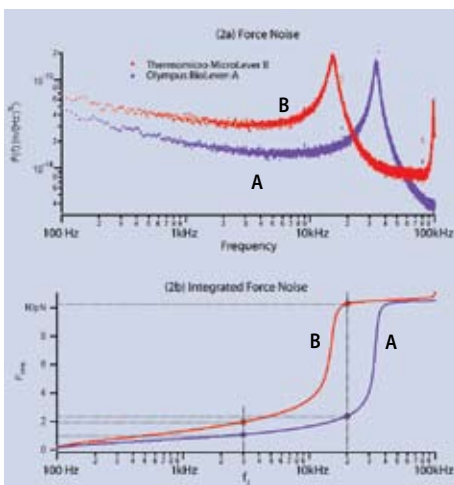


Figure 2: Experimentally measured cantilever noise. The dots in Figure 2a show the experimentally measured force noise spectra (“A” is a Bio-Lever and “B” is a Microlever). Figure 2b shows the integrated force (see equation 1). The dashed lines show how to compare the RMS force noise for different measurement bandwidths. The points at 3kHz indicate that the RMS noise for the Microlever B is roughly 2pN, while Bio-Lever A is closer to 1pN. At 20kHz, the RMS noise for Microlever B has jumped to over 10pN (that frequency range includes to first resonance) while Bio-Lever A is less than 2.5 pN.

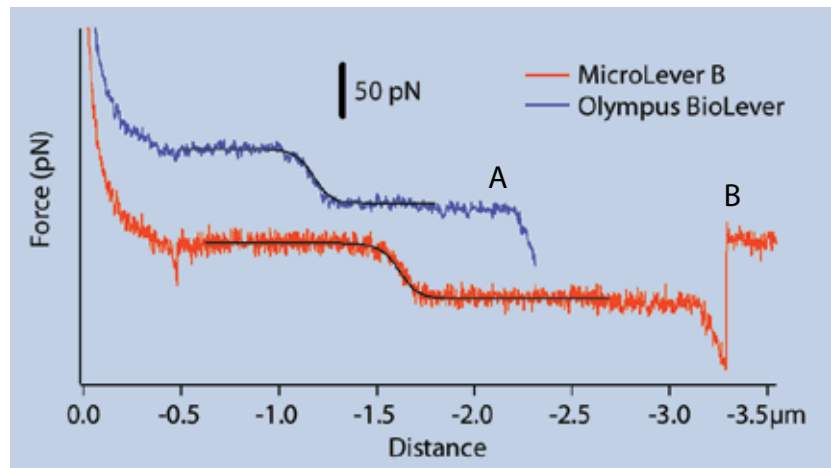


Figure 3: B-S transition in λ -digest DNA curve made with a Bio-Lever, “A”, and Microlever, “B”. Both measurements were close to being thermal noise limited. The background noise for the Bio-Lever A was about 3pN (in a 1Hz-1kHz bandwidth) and about 5pN for the Microlever B. The transition was fitted using a hill function for both transitions and found to be $57\pm 3\text{pN}$ for the Microlever and $55\pm 4\text{pN}$ for the Bio-Lever.

Microlever B would have 2pN RMS of force noise while Bio-Lever A would have 1pN RMS. Similarly, bandpassing the measurement between 100Hz and 20kHz makes the RMS noise for the Microlever B jump to over 10pN while the Bio-Lever is less than 2.5pN. The Microlever B noise has increased so dramatically because the resonant frequency is roughly 11kHz, and that frequency range is included in the integration. For typical values of f_2 , the RMS force noise on Bio-Lever A is roughly a factor of two less than it is for the Microlever B. This is a consequence of the reduced size of the Bio-Lever¹. Data for Figure 2 was taken with the cantilevers in air. Although the

physics is more complicated in water², similar noise reductions occur.

Examples

As a practical application of lower noise measurements to single molecule force measurements, Figure 3 shows a measurement of the B-S transition in λ -digest DNA in PBS solution.³ The red curve “B” was made with the Microlever, and the blue curve, “A”, with the Bio-Lever. Both measurements were close to being thermal noise limited. However, because the thermal noise level was higher for Microlever B, the signal to noise ratio is reduced for that lever. The background noise for the Microlever B was about 5pN and about 3pN for the Bio-Lever. The transition was fitted using a hill function for both transitions and found to be $57\pm 3\text{pN}$ for the blue curve and $55\pm 4\text{pN}$ for the red. The force curves were made over the same sample.

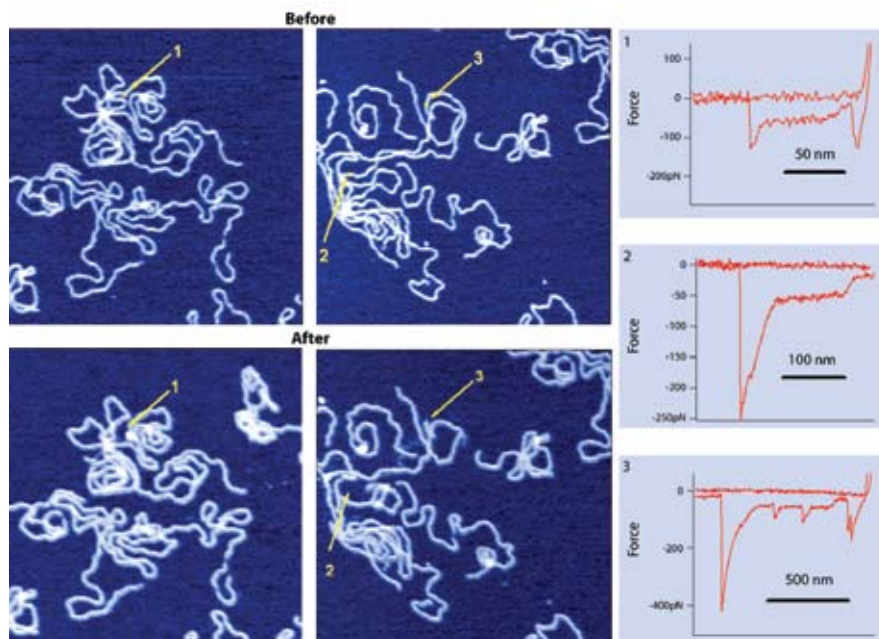
It is apparent from the horizontal axis that the DNA strand length was different for the two measurements. Note that the nominal transition force of 65pN typically reported in the literature is greater than that observed here. Lower forces have been observed in the presence of higher salt concentrations. No effort was made to control the salt concentration in these measurements.

Nanomanipulation can also be done with the Bio-Lever and MicroAngelo™. MicroAngelo is the built-in nanolithography and manipulation feature that is standard with all MFP-3D AFM systems.

Figure 4 shows before and after scans on λ -digest DNA in fluid using the Bio-Lever. Force curves were made at user-selected sites 1, 2 and 3 (“before” image). The DNA appears wider on the “after” images because the tip has picked up contaminants (presumably DNA) while performing the force curves. The force curves show the characteristic B-S transition of stretched DNA.³ The length scales of the B-S plateau correspond to the DNA pulled off the surface (compare the before and after images).

Conclusion

In a real system, it is best to choose the smallest lever available, subject to the constraint that it is thermal noise limited. If it is softer than that, the thermal noise will be larger. If it is stiffer, the other noise sources get multiplied by a larger spring constant, resulting in larger force noise.

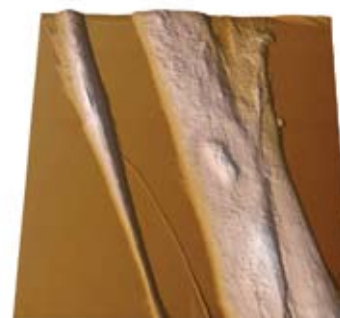
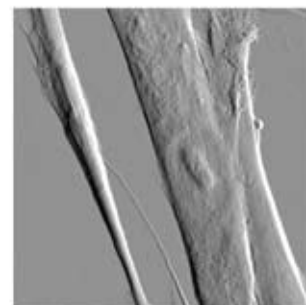


Figures 4: λ -digest DNA imaged using a Bio-Lever, $1\mu\text{m}$ scan, with force curves taken at three different points (right). Different portions of the B-S transition are visible in the force curves.³

For use with MFP AFMs, the quietest commercially available lever is clearly the Olympus Bio-Lever. For more details on noise measurements or other questions, please contact us at support@AsylumResearch.com.

References

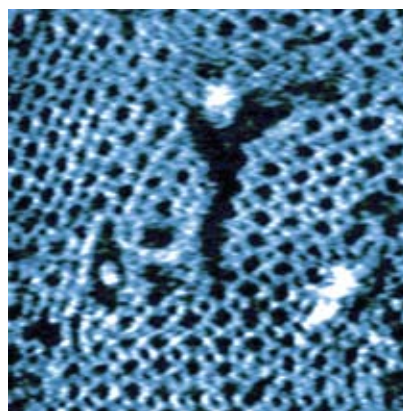
1. Viani, M.B. et al, Journ. App. Phys., Aug. 1999, vol.86, (no.4):2258-62.
2. Sader, J.E., Journ. App. Phys., 1 July 1998, vol.84, (no.1): 64-76.
3. Sequence dependent mechanics of single DNA molecules M. Rief, H. Clausen Schaumann and H. Gaub Nature Structural Biology, vol. 6, 1999 p. 346.



Fixed MRC-5 Fibroblast cells imaged in PBS using AC Mode. $70\mu\text{m}$ scan size.

Bio-Lever Specifications

Tip/lever material	SiN with gold coating	
Lever shape	Rectangular	
Lever thickness	0.16 μ m	
Lever width	30 μ m	
Lever length	60, 100 μ m (two cantilevers per chip)	
Spring constant	60 μ m 100 μ m	27pN/nm 6
Resonant freq.	60 μ m 100 μ m	37kHz 13
Tip shape	"V"	
Tip height	7 μ m	
Tip radius	30nm (<40)	
Tip angle	<90° front <45° side	
Coating	Au, tip side and back	
Packaging/Pricing	Half wafer (210) 70 35 10 Singles	\$5,850 \$1,980 \$1,094 \$350 \$40/lever



RNA network imaged using a Bio-Lever, 250nm scan, sample courtesy of A. Chworos & L. Jaeger, UCSB.

*Note: Specifications are typical and provided by the manufacturer.
Tip pricing is subject to change without notice.*