Force Resolution in Force Spectroscopy Experiments: Thermal Noise and the Effect of Measurement Bandwidth

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INTRODUCTION

The atomic force microscope (AFM) is increasingly being used for force measurements in the piconewton regime. As attempts are made to measure smaller and smaller forces it becomes more important to understand several factors that influence the force resolution of the technique. The optical lever approach results in extraordinarily sensitive measurements of cantilever deflection. It is routine to obtain sub-Angstrom noise levels. Since cantilevers with spring constants around 10 pN/nm are widely available, this would seem to imply that measurements of sub-piconewton forces should be possible. However, this unfortunately is not the case.

PHYSICAL LIMITS ON AFM FORCE RESOLUTION

The sub-Angstrom deflection noise levels that are used to specify the performance of the optical lever system are measured by reflecting the laser beam off of a very stiff cantilever or the probe substrate itself. When this very stiff surface is replaced by a very soft cantilever the noise in the deflection measurement is no longer dominated by the noise in the optical lever system itself, but rather by the thermal noise of the cantilever. This is the origin of the term "thermally limited" that is often used to describe force measurement performance. Thermal noise is the result of the intrinsic Brownian motion of the cantilever. From the equipartition theorem[1], we can write an expression for the thermal energy, k_bT, relating it to the mean-squared amplitude of the cantilever motion, $\langle x^2 \rangle$, and the spring constant, k, of the cantilever:

$$\frac{1}{2} k\langle x^2 \rangle = \frac{1}{2} k_bT$$

From this, we can derive a simple expression that allows us to estimate the root mean square (RMS) noise for force measurements due to thermal noise:

$$F_{RMS} = k\sqrt{\langle x^2 \rangle} = \sqrt{k_bT \cdot k}$$

If we evaluate this for soft cantilever spring constants in the range of 10–30 pN/nm, we find that the RMS force noise should be in the range of about 6–11 pN. However, this alone does not completely define the force resolution of AFM measurements.

INFLUENCE OF THE MEASUREMENT BANDWIDTH

The thermal noise of the cantilever occurs in a bandwidth near the resonance frequency of the cantilever. Therefore the measurement bandwidth, determined by the sampling rate and any data averaging or other filtering, can have a large effect on the observed noise in force data. In aqueous solutions, where many of these piconewton regime measurements are being made, the resonance frequencies of most cantilevers are quite low, typically less than 10 kHz. This is well within the possible measurement bandwidth of the AFM. For instance, Veeco AFMs running on the NanoScope V controller can capture standard force curves at data rates of up to 40 kHz. Using the High Speed Data Capture feature this can be increased all the way up to 50 MHz.

However, the measurement bandwidth is also determined by any data filtering that occurs on the deflection signal. This can include analog filters, digital filters, and basic data averaging (i.e. "moving" or "boxcar" averaging). Analog filtering on the NanoScope V controller is primarily intended to reduce aliasing effects caused by frequency components in the signal that exceed the Nyquist frequency, which is half the sampling rate. Therefore, the normal "low-speed" deflection signal, sampled at 500 kHz, is filtered at 200 kHz. The "high-speed" deflection signal, which can be sampled at up to 50 MHz, is AC-coupled and low-pass filtered at about 6.5 MHz.

In addition to this analog filtering, a digital filter on the deflection signal can optionally be used. This can be selected using the "LP Deflection" parameter found under the "Other" parameter list. The cutoff frequency for this filter is selectable within the range of 2–20 kHz using the "LP Deflection" parameter found under the "Feedback" parameter list.

Finally, the sampled data can be further filtered by applying a moving average. This can be implemented in two different ways. First, the ramp rate and the number of points per curve ("Number of Samples" parameter) determine an overall data capture rate. For instance, a 1 Hz ramp rate and 19968 points per curve combine to give the maximum data capture rate of about 40 kHz (1 Hz = 0.5 s per direction, which at 19968 points per curve is about 25 µs per point, or about 40 kHz). Using fewer points per curve simply averages more points to downconvert the signal to a lower bandwidth. Second, a moving average can be applied to the data using the "Average Points" parameter found under each "Channel" group in the ramp mode parameter lists. This results in similar filtering of the data but retains more points per curve, which is important when fitting functions to the data and in order to maintain good resolution in the distance axis of the data.

The combined effect of the "Scan Rate", "Number of Samples", and "Average Points" parameters results in a parameter called "Effective BW," which is found under the ramp channel parameter group. This is calculated by:

$$\textit{Eff. BW} = \frac{2 \cdot \textit{Scan Rate} \cdot \#\textit{Samples}}{\textit{Average Points}}$$

This is an estimate of the measurement bandwidth for that channel of force curve data. Note that the rolloff of the filtering that results from a moving average differs from that of a usual first order low pass filter. That is, the attenuation of the signal begins at frequencies well below the cutoff frequency calculated in Eqn. (3) whereas the attenuation of a normal first order low pass filter would only be –3 dB at the cutoff frequency. The rate of rolloff is increased as more points are used in the average, similar to using a higher order filter. The practical effect of these differences is that the effective bandwidth calculated will be somewhat higher than the actual bandwidth, which means that frequency components near the high end of the bandwidth will be substantially attenuated.

By limiting the bandwidth in any of these ways it is possible to exclude a portion of the thermal noise from force measurements. This is perhaps best illustrated by considering a power spectral density plot of the deflection signal, as shown in Figure 1. This shows the thermal noise (blue points) fit to a simple harmonic oscillator function (red line). Obviously the noise occurs in a peak centered on the resonance frequency of the cantilever. By integrating the area under this peak we can calculate the RMS force noise. If we set the limits of integration to the bandwidth of our measurement we can obtain the theoretical RMS force noise for a given bandwidth. Experimentally, however, it is impossible to achieve a measurement bandwidth that precisely limits the bandwidth to the desired range because the filtering cutoff frequencies are not infinitely sharp.

Experimental RMS force noise measurements were made with the same cantilever used to measure the data in Figure 1, which was the rectangular cantilever on a Veeco MLCT probe[2] with a spring constant 24.2 pN/nm. A series of measurements were made in order to demonstrate the effect of each method of limiting the measurement bandwidth. According to Eqn. (2), a cantilever with this spring constant should have RMS force noise of about 10 pN.

Figure 2A shows the effect of changing the digital low pass filter cutoff frequency while keeping the data capture rate fixed at 40 kHz and without any data averaging. Because the rolloff of the digital filter is not as sharp as that obtained by data averaging, we see that the force noise is only modestly decreased even at the lowest cut off frequency. While effective for reducing high frequency noise, the digital low pass filter is not very well suited for reducing the low frequency thermal noise. Note that the total noise measured in a bandwidth up to 20 kHz, 10.9 pN, agrees well with the predicted value of 10 pN.

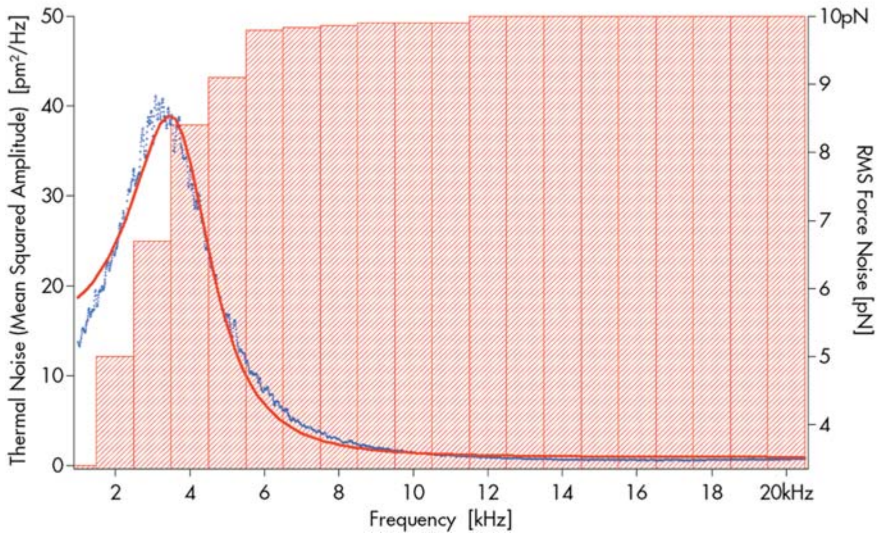

Figure 1: The power spectral density plot of the deflection signal shows the thermal noise occuring at the resonance frequency of the cantilever, here about 4 kHz. The columns show the RMS force noise that should be measured in a bandwidth from DC to the frequency shown.

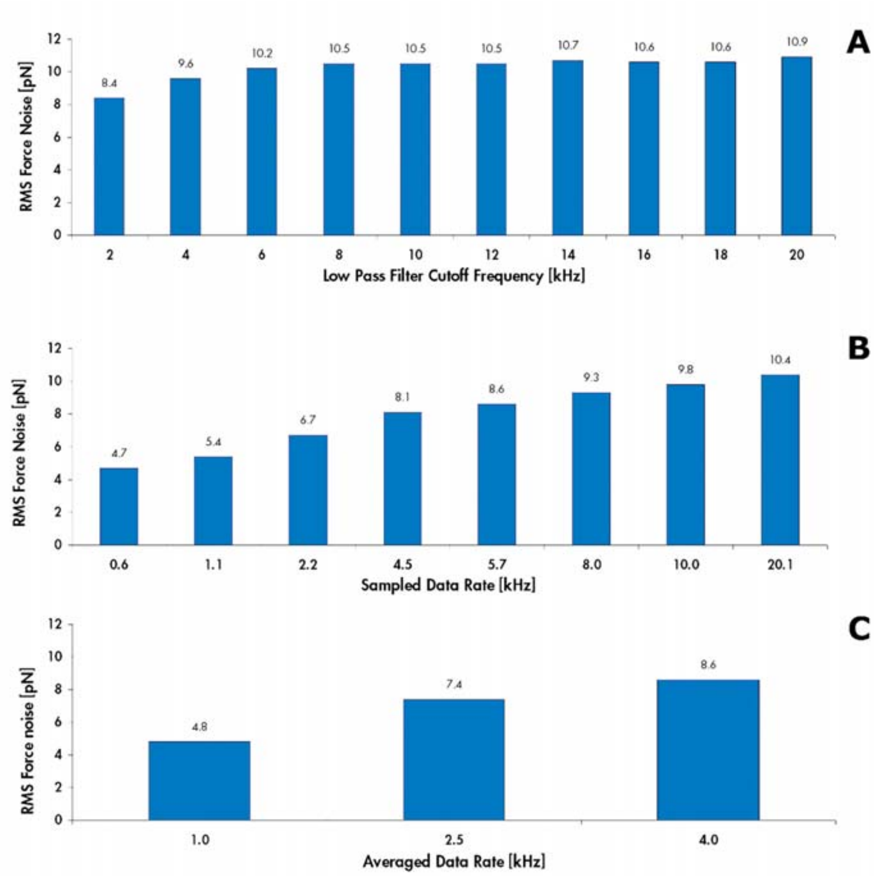

Figure 2: Experimental measurements of RMS force noise under different measurement conditions. (A) 40 kHz data capture rate, no averaging, digital low pass filter enabled (B) No averaging, 20 kHz digital low pass filter, different data capture rates determined by number of points per curve (C) 20 kHz data capture rate, 20 kHz digital low pass filter, different bandwidths determined by averaging points.

Figure 2B shows the effect of changing the data capture rate by adjusting the number of points per curve. Recall that this results in data averaging to reduce the number of data points. We see that for data capture rates beyond the resonance frequency that there is little variation in the force noise. However, at rates at or below the resonance frequency the noise levels begin to drop substantially, ultimately to less than half the original value.

Finally, Figure 2C shows the effect of using the "Average Points" parameter to reduce the measurement bandwidth while holding the data capture rate and digital low pass filter fixed at 20 kHz. The resulting RMS noise values are very similar to those obtained for equivalent bandwidths in Figure 2B, as they should be since they are essentially the same type of filtering. However, this method offers the advantage of keeping the total number of points per curve constant even as the bandwidth is changed. As previously noted, this is often advantageous in order to maintain sufficient distance resolution in the force curves and to provide more points for curve fitting operations (e.g. worm-like chain fits to extension data). We find that this is the most generally useful method of reducing the measured thermal noise in force spectroscopy data

INFLUENCE OF CANTILEVER SELECTION

The measured thermal noise can be further reduced by selecting a cantilever with a resonance frequency beyond the measurement bandwidth. This concept has been exploited by groups working on "small cantilevers"[3,4]. These cantilevers have much higher resonance frequencies and lower viscous damping, which reduces the measured force noise in the measurement bandwidth compared to conventional cantilevers.

Though true "small cantilevers" and compatible hardware are not currently commercially available, some current cantilevers do have considerably higher resonance frequencies, even in fluid. In particular, Veeco's OBL series

Biolevers[2] offer somewhat higher resonance frequencies. Compared to other cantilevers, lower noise can be achieved with the same bandwidth or a higher bandwidth can be used while still obtaining an equivalent noise level.

SUMMARY

The noise level in AFM force measurements is fundamentally limited by the intrinsic thermal noise of the cantilevers. However, the measured thermal noise can be reduced by judicious selection of parameters that control the data sampling rate and averaging of the sampled data. For general force spectroscopy usage, we recommend using the "Average Points" parameter to help reduce the observed thermal noise while still maintaining sufficient data points in each force curve.

REFERENCES

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