

Chapter 2

Correlation Force Spectroscopy

Correlation Force Spectroscopy: Rationale

In principle, the main advantage of correlation force spectroscopy (CFS) over one-cantilever atomic force microscopy (AFM) is that the thermal noise in the cross-correlation between two cantilevers is much smaller than the thermal noise in the autocorrelation for a single cantilever [43, 47], so here, I examine this prediction. The noise spectrum of a single cantilever and the cross-correlation noise spectrum of two cantilevers are shown in Fig. 2.1. It is clear that the cross-correlation noise spectrum has a much smaller magnitude and is typically about one fourth of the autocorrelation noise spectrum (i.e., thermal noise of a single cantilever) for the same cantilever in the same fluid. This is the first advantage of CFS over AFM: Upon tethering a molecule between the two tips, variation of the smaller cross-correlation noise spectrum due to the molecule force (or molecule mechanical properties: stiffness and friction) is larger when compared to the larger autocorrelation noise spectrum. This means a better sensitivity for single-molecule measurements. It is noted that in single-molecule force spectroscopy, the noise from the solvent coupling of the cantilevers will be the “noise” that sets the limit of thermal force resolution. Thus, by changing from one-cantilever to two-cantilever measurements, a fundamental noise limit in AFM single-molecule force spectroscopy is lowered. The dispersion of noise in the cross-correlation also has the interesting feature that there is a particular frequency near the resonant frequency where the thermal noise is zero (see Fig. 2.1). With respect to Eqs. (1.2) and (1.6), the area under the autocorrelation power spectral density (PSD) is equal to $\langle x^2 \rangle^{1/2}$ and the absolute area under the cross-correlation PSD corresponds to $\max(\sqrt{\langle x_1(t)x_2(0) \rangle})$. It is clear from the figure that CFS has superior force resolution compared to AFM (see Eqs. (1.3) and (1.7)).

Fitted values of γ_c and γ_a (see Fig. 2.2) show that γ_c is about an order of magnitude smaller than γ_a for the distance range $< 1 \mu\text{m}$, which is the applicable range of single-molecule force measurements. From Eq. (1.8), the Brownian force resolution scales with the root of the viscous friction, so the second advantage of CFS over

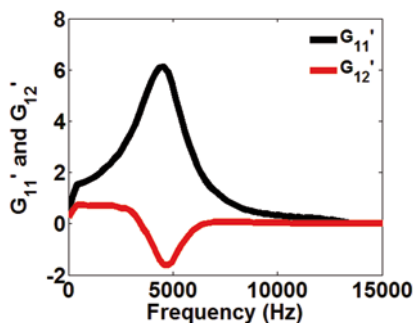


Fig. 2.1 Comparison between the normalized autocorrelation noise spectrum (G'_{11}) and the normalized cross-correlation noise spectrum (G'_{12}) in water at 23 °C. Cantilevers used are ORC8-B: length=200 μm , width=40 μm , $k=0.1 \text{ N m}^{-1}$. The separation between the cantilevers is $d=6.5 \mu\text{m}$ ($s=318 \text{ nm}$) in the vertically offset CFS as shown in Fig. 2.4b. CFS correlation force spectroscopy

AFM is the reduced Brownian noise force owing to fluid coupling against which all other coupling (e.g., that of a straddling molecule) must be measured. Later, I show that the relative changes in coupling are greater for CFS than conventional AFM when a molecule is tethered between the tips. Reduced viscous friction in CFS compared to AFM directly means reduced hydrodynamic interaction which also sets a background noise force.

Having the idea of correlating thermal motions of two cantilevers in mind, different configurations for the two cantilevers can be anticipated. The simplest ones are the antiparallel laterally offset and antiparallel vertically offset configurations. Each of these configurations has its advantages and applications that will be discussed in later sections. So, in the remainder of this chapter, I discuss the analysis of the two cantilever technique, the development of the laterally offset (see Fig. 2.3) and vertically offset (see Fig. 2.4) device, results on rheological measurements, and results on single-molecule force spectroscopy and then future work. Significant portions of this thesis detail equipment development.

CFS: Development

CFS consists of a pair of micrometer-scale cantilevers that are closely spaced (see Fig. 2.3). Each cantilever undergoes thermal fluctuations due to interaction with the fluid molecules. However, the thermal fluctuations of the cantilevers are not independent, but coupled through the fluid spanning the gap of the two cantilevers. By studying the correlated fluctuations of the cantilevers, properties of the intervening material can be obtained. Detection of the thermal fluctuations of the cantilevers is done using optical lever technique using individual lasers and individual photodetectors for each cantilever. Precautionary measures should be taken to cancel cross talk between the two lasers in the detection systems, for example, by using appropriate optical filters in front of photodetectors or spatial adjustments of the

Fig. 2.2 Experimental measurements of γ_a , the viscous friction on a single cantilever, and γ_c , the viscous friction between two closely spaced cantilevers, as a function of distance. γ_c is much smaller

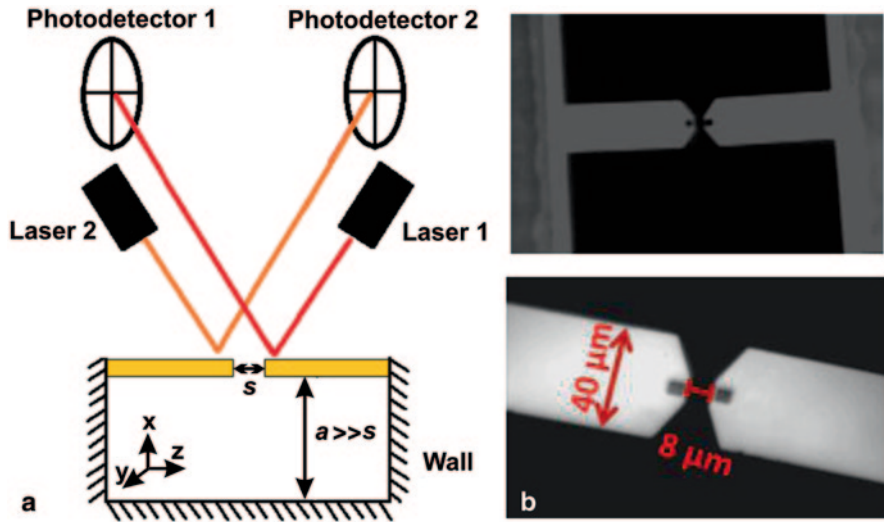
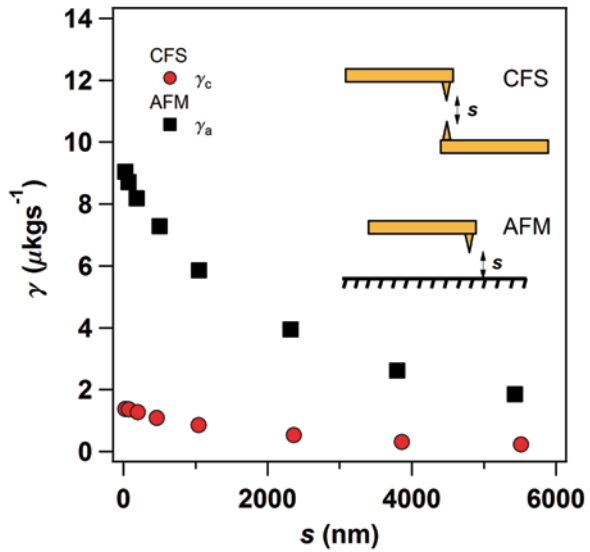


Fig. 2.3 **a** Schematic of the cantilevers and detection system in the antiparallel laterally offset CFS. **b** Light microscope images of closely spaced AFM cantilevers seen from above. The cantilevers are $100 \mu\text{m}$ long and $40 \mu\text{m}$ wide. The lateral separation between the cantilevers is $8 \mu\text{m}$. CFS correlation force spectroscopy, AFM atomic force microscopy

lasers and photodetectors. I use the absence of a cross-correlation at zero time lag as a requirement for no instrumental cross talk between the signals. It is important that the signals are recorded synchronously, because any phase lag between the two detection systems will produce artifacts. This check can be done by shining both lasers on one cantilever and calculating the autocorrelation (of one of the laser signals) and cross-correlation (of the two laser signals). A similar auto- and cross-correlation

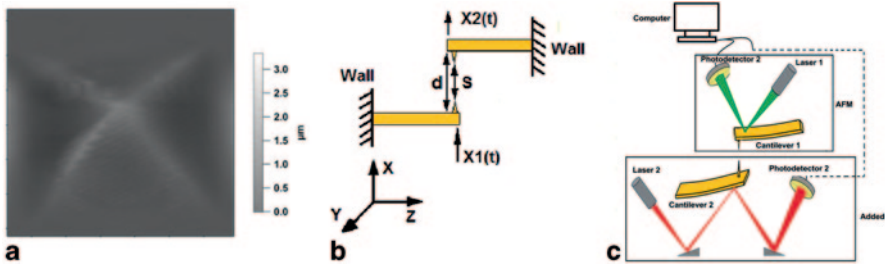


Fig. 2.4 **a** Atomic force microscopy contact mode image in water of the bottom cantilever tip obtained by scanning the bottom cantilever and measuring the height signal. This imaging is used to align the tips. **b** Schematic of antiparallel vertically offset cantilevers. **c** Schematic of the cantilevers and detection system in the antiparallel vertically offset CFS. CFS correlation force spectroscopy

function in this case justifies no phase lag between the two detection signals. Another important parameter is the thermal drift of components due to temperature fluctuations in the laboratory. While there is no way to cancel this effect, it is possible to minimize it by taking appropriate measures in each CFS configuration, which are discussed later. By measurement of the PSD as a function of laser power, I showed that the laser had a negligible effect on the temperature of the cantilever. The other important parameter is the gap of the two cantilevers. By increasing the gap, the two cantilevers are less coupled, thus the correlation in thermal fluctuations is weaker, i.e., the cross-correlation noise spectrum is weaker.

Laterally Offset Configuration

In the laterally offset CFS, a pair of commercial AFM cantilevers is mounted in an antiparallel configuration between two glass slides, as shown schematically in Fig. 2.3a. The cantilevers are positioned under an optical microscope and then glued in place (see Fig. 2.3b).

The thermal drift is minimized in laterally offset CFS because of small distances and material matching: The two cantilevers are joined by the cantilever chip ($\sim 3 \times 1 \text{ mm}^2$ of silicon nitride), a thin layer of glue, a glass slide, and a second cantilever chip. The cantilevers are mounted $> 1 \text{ mm}$ away from the glass slide, so there is minimal fluid coupling between the cantilever and rigid glass wall. In the vertical direction (Fig. 2.3a), all materials are matched for the left and right cantilevers, and in the horizontal direction, the bases are separated by only about $400 \text{ }\mu\text{m}$ of glass, which is a much smaller connection than between the tip and the sample in a commercial AFM. There are no “moving” parts; the only motion is molecular motion of the fluid and the fluctuating deflection of the cantilevers due to interaction with the fluid at equilibrium.

The deflection of each of the cantilevers is measured by two lasers (Schäfer+Kirchhoff GmbH, Hamburg, Germany) reflected by the cantilever onto a position-sensitive diode (Pacific Silicon Sensor, CA). In this case, I use a different

wavelength of laser (635 and 680 nm) for each cantilever, and (in some experiments) use a wavelength filter over each diode and different light paths to prevent cross talk between the signals. The signals are recorded synchronously by an Asylum Research AFM controller (Nyquist frequency, $f_{Ny}=25$ kHz) for type B cantilevers and a National Instrument (Irvine, CA) PCI-6110 Data Acquisition card ($f_{Ny}=500$ kHz) for type A cantilevers.

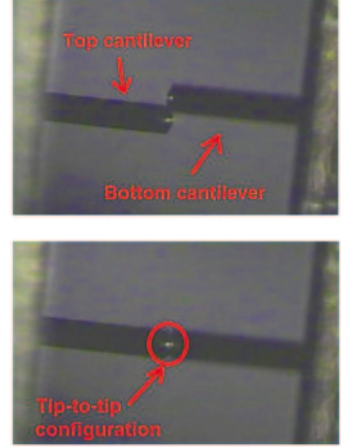
Vertically Offset Configuration

The vertically offset CFS consists of a pair of commercial AFM cantilevers mounted in an antiparallel vertically offset configuration, as shown schematically in Fig. 2.4b. The top cantilever is mounted in a commercial AFM (Asylum Research, MFP3D-bio, CA) that has the full functionality of AFM, such as sub-nanometer resolution in deflection and displacement in three dimensions. A home-built cantilever mount and optical lever cantilever deflection was incorporated into the AFM in place of “the sample” (see also Fig. 2.4c). For this additional cantilever deflection sensor, a laser (Schäfter + Kirchhoff GmbH, Hamburg, Germany, 51nanoFCM) is steered by a mirror onto the cantilever and the reflection off the cantilever falls onto a second mirror which then steers the beam onto a split photodiode (Pacific Silicon Sensor, CA, QP50-6-18u-SD2). A different wavelength (680 nm) of laser was used for the bottom cantilever compared to the top cantilever (superluminescent diode with an 860-nm wavelength) to allow frequency filtering, thereby preventing optical contamination between the two signals. The two laser beams are also physically separated so that they do not contaminate each other. The main danger of unwanted cross-correlation (that does not arise from the fluid) is from light from a single laser that ends up striking both cantilevers and entering a single diode. This could occur if the cantilevers are parallel and if the laser beam is larger than the cantilever or the cantilever allows transmission of light. I try to cover all these possibilities by using standard gold coatings on the cantilevers, by focusing the laser beams to a spot size that is smaller than the cantilever, and by not making the cantilevers perfectly parallel.

The experimental procedure is as follows. A fluid droplet is injected and held in place by capillary force and the system is left to reach equilibrium for about 40 min. Initially, while the cantilevers are at large separation, the PSD of each cantilever is measured and a simple harmonic oscillator fit is applied to PSDs to yield the resonant frequency and quality factor of each cantilever at infinite separation. The PSD in voltage units is given by:

$$\text{PSD}(\omega) = (\text{Inv. OLS})^{-2} \times \left[\left(\frac{4k_B T}{k_c} \frac{\left(\frac{2\pi\omega_r}{Q} \right)}{\left(\left(\frac{\omega}{\omega_r} \right)^2 - 1 \right)^2 + \left(\frac{2\pi\omega}{Q} \right)^2} \right)^{1/2} + C \right]^2, \quad (2.1)$$

Fig. 2.5 Top-view optical microscope images of the cantilever pair. (*Top*) The two cantilevers are brought in proximity of each other. (*Bottom*) The two cantilevers are aligned coarsely using micrometer translation stage and then with nanometer precision using the piezoelectric devices of the AFM. *AFM* atomic force microscopy



where Q is the quality factor, C is the white noise, and Inv. OLS is the inverse optical lever sensitivity (nm/v).

To determine the optical lever sensitivity and thereby the spring constant, the top cantilever is brought into contact with a hard flat surface on the bottom cantilever mount and a single-force extension measurement is performed. This is done by translating the clamped end of the top cantilever downward a known distance with the z-piezo, and measuring the uncalibrated deflection of the cantilever in units of volts. When the tip of the top cantilever is in contact with the flat surface, the deflection in nanometers is equal to the distance travelled by the z-piezo. Inv. OLS of the top cantilever is then the slope of the deflection z-piezo plot in the contact region. Knowing the Inv. OLS of the top cantilever and fitting the PSD of the top cantilever to Eq. (2.1), the spring constant of the top cantilever can be calculated. The calibration of the bottom cantilever can be performed in the same manner by pressing against the bottom surface of the top cantilever chip. Alternatively, for the bottom cantilever, initially the two cantilevers are pressed against each other by translating the clamped end of the top cantilever downward a known distance with the z-piezo. In the contact region, the force between the two cantilevers is the same. Thus, by knowing Inv. OLS and spring constant of the top cantilever, spring constant of the bottom cantilever can be calculated (see Eqs. (2.2) and (2.3)). Finally, by fitting PSD of the bottom cantilever to Eq. (2.1), its Inv. OLS can be calculated:

$$\Delta y = |\Delta x_1| + |\Delta x_2|, \quad (2.2)$$

$$F_1 = F_2 \Rightarrow k_2 = \frac{k_1 \times |\Delta x_1|}{\Delta y - |\Delta x_1|}. \quad (2.3)$$

The tips are then brought near each other using the course z-control in the AFM (see Fig. 2.5), and then imaging the bottom cantilever with the top cantilever to find the bottom tip. Note that the entire detection system of the bottom cantilever (cantilever, laser, and diode) is scanned to maintain the alignment of the detection system on the cantilever. The Asylum Research AFM has a convenient “go there” function where

the sample can be moved automatically to a location within a previously collected image; this function is used to move the top AFM tip to the apex of the bottom tip and into alignment. Periodically, a new image can be taken to realign the horizontal position of the tips and reestablish the vertical separation zero position between the two tips. This is necessary to account for any drift in position. Once this is done, the z-piezo can be used to establish the required vertical separation. If used in distance clamp mode where the separation is kept constant, from this point and during data collection, there are no “moving” parts; the only motion is molecular motion of the fluid and the fluctuating deflection of the cantilevers. However, if used in force clamp mode where the force on the top cantilever is kept constant, in addition to the molecular motion of the fluid and the fluctuating deflection of the cantilevers, z-piezo can also move to keep the force constant. Force clamp mode can only be used when a molecule is clamped between the two cantilevers. Otherwise, if used in the force extension mode, the top cantilever is retracted from the bottom cantilever at a constant velocity while fluctuations of both cantilevers are measured.

The signals from the top cantilever (part of the commercial AFM) and the bottom cantilever are recorded synchronously by an Asylum Research AFM controller ($f_{Ny} = 25$ kHz) or a National Instrument (Irvine, CA) PCI-6110 Data Acquisition card ($f_{Ny} = 500$ kHz). Alternatively, the vertical offset experiments can be performed in a closed fluid cell with active temperature and solution control. The details of the apparatus are similar to those explained above. In this case, the bottom cantilever is glued on the glass disk of the fluid cell which provides a path for the laser light to enter and the reflection to exit. The reflection is then steered on to a different photodiode using lens and mirror. The bottom detection system is mounted underneath the AFM (Nikon TE2000-U, Japan).

There are a few differences between the laterally offset CFS and the vertically offset CFS: (1) thermal drift is much lower in the former due to material matching and the way that the two levers are mounted, so that drifts are in the same direction, while thermal drift in vertical direction in the vertically offset CFS is a major drawback of the system; (2) the lateral offset device shears tethering molecules, whereas the vertical offset places them under tension–compression. The latter is usually of interest in single-molecule force spectroscopy experiments; (3) the analysis of deflection data requires calibration of the sensitivity of the optical lever, which is usually done by pressing against a solid flat plate. The OLS depends on the alignment of the laser and the calibration must be done in each experiment, and sometimes must be redone during an experiment, if the alignment changes. The difficulty with the lateral offset experiment is that there is no piezo in the system to press the cantilevers against a flat plate. Thus, deflection data are normalized by the area under PSD. While the same approach can be adopted for vertically offset experiments, the presence of z-piezo and flat surfaces makes possible direct measurement of OLS.

Analysis of Correlations Between Two Cantilevers

The implementation of correlation measurements requires a method of analysis to obtain properties of the polymer molecule from the spectrum of deflection fluctuations. As it turns out, these fluctuations are very simply related to the ringdown of

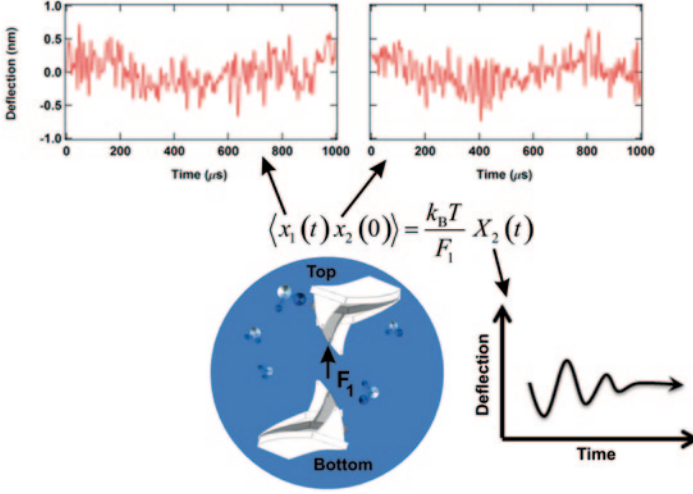


Fig. 2.6 Schematic representation of Eq. (2.5). x_1 and x_2 are the thermal fluctuations of the top and bottom cantilever respectively and X_2 is the deterministic deflection of the bottom cantilever after removal of force F_1 from the top cantilever

a cantilever from an initial displacement, which is itself rather straightforward to model. The simplicity of this modeling is an advantage of CFS. The fluctuation–dissipation theorem [48] states that the manner in which a system returns from a linear macroscopic perturbation is related to time correlations of equilibrium microscopic fluctuations. Paul and Cross (2004) showed that the *stochastic* dynamics of pairs of small cantilevers in fluid could be analyzed in terms of *deterministic* behavior of a pair of cantilevers when cantilever 1 is subject to removal of a force, F_1 [42]:

$$\langle x_1(0)x_1(t) \rangle = \frac{k_B T}{F_1} X_1(t), \quad (2.4)$$

$$\langle x_1(0)x_2(t) \rangle = \frac{k_B T}{F_1} X_2(t). \quad (2.5)$$

Figure 2.6 shows schematic representation of Eq. (2.5). Equation (2.4) relates the autocorrelation of stochastic fluctuations of a single cantilever, $\langle x_1(0)x_1(t) \rangle$, to its deterministic ringdown, $X_1(t)$, after removal of a step force, F_1 , at time zero. t is the time and x is the deflection, which changes with time due to fluctuations. Equation (2.4) is the fluctuation–dissipation theory for a single cantilever. Equation (2.5) relates the cross-correlation of stochastic fluctuations between the two cantilevers, $\langle x_1(0)x_2(t) \rangle$, to the deterministic response of the bottom cantilever, $X_2(t)$, as a result of ringdown of the top cantilever after removal of a step force F_1 ,

at time zero. In practice, the correlations, $\langle x_1(0)x_1(t) \rangle$ and $\langle x_1(0)x_2(t) \rangle$, are measured experimentally, and $X_1(t)$ and $X_2(t)$ are modeled either from finite element (FE) analysis [43] or from an equation of motion for the entire cantilever, such as a simple harmonic oscillator model.

Validation of Fluctuation–Dissipation Theorem for One Cantilever

This thesis work requires the use of Eqs. (2.4) and (2.5), which had not been validated experimentally. So I have measured the left and right sides of Eq. (2.4) experimentally in the same system to test its validity: that is, the ringdown of a cantilever subject to a step force, and the fluctuations of the same cantilever are both measured in water. The step force was applied to the cantilever by first putting epoxy glue on a glass surface, then touching the epoxy with an AFM tip and then retracting the tip. The adhesion between the tip and the glue keeps the tip in contact with the glue, but when the adhesion force is exceeded by the spring force, the cantilever breaks free. The very short range of the adhesion force (< 1 nm) means that when we pull the tip from the solid, the force drops very quickly. This is close to the situation in which a step force is removed at time zero, as required by the theory. The fluctuation data were collected at approximately the same separation that the tip jumps to after separation from the glue.

The comparison is shown in Fig. 2.7. The similarity between the ringdown and the autocorrelation in thermal fluctuations validates the use of Eq. (2.4). Since the same assumptions were made in the derivation of Eq. (2.5), it strongly suggests that Eq. (2.5) is also valid for dual cantilever system. Note that the actual deflection in the ringdown experiment is rather large (nanometer), much larger than the fluctuations in a normal experiment, so the theory should also be valid for small deflections.

The significance of this result is that the fluctuations of two cantilevers can be measured experimentally, which is an easy measurement and nonintrusive. The modeling of the system can be performed analytically as the ringdown of cantilevers, which is an “easy” system to model.

Analysis of Thermal Fluctuations to obtain Correlations

In each experiment, fluctuations of the two cantilevers were measured simultaneously: $x_1(t)$ and $x_2(t)$ of cantilevers 1 and 2, respectively. In some measurements, Asylum Research MFP3D controller was used to collect the data at 50 kHz for 50 s or National Instrument (Irvine, CA) PCI-6110 Data Acquisition card was used to collect data at 1 MHz for either 5 or 16 s. This results in N number of samples for each cantilever. Subsequent data processing consists of:

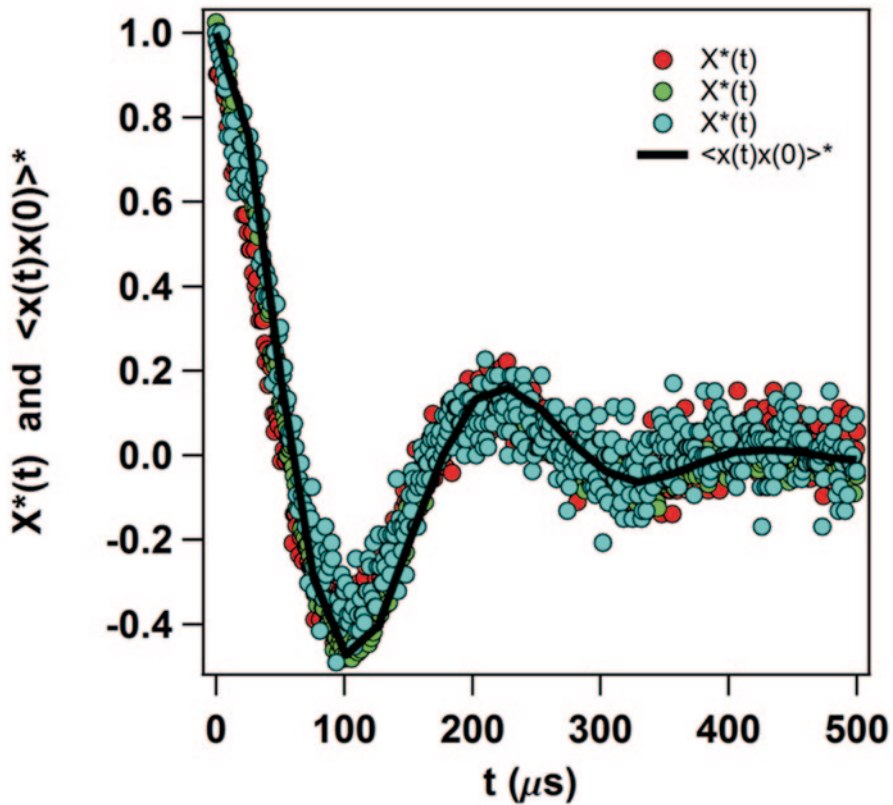


Fig. 2.7 Comparison of the left and right sides of Eq. (2.4). The autocorrelation (normalized by $k_B T / k_c$) and the deflection (normalized by its value at time zero) after release of a step force are plotted on the same axis. Both data sets were collected at 1 MHz with ORC8-B-type cantilever with spring constant 0.1 N/m and resonant frequency 5 kHz in water

1. Dividing the whole data points into N_{ave} bins where each bin has $N_{\text{bin}} = N / N_{\text{ave}}$ number of points
2. Multiplying each bin by a Hanning window¹ and then subtracting a linear curve fit from each bin to remove drift in the signal (this removes the deflection of the AFM cantilevers that arises from small temperature changes). The theory requires that the system is in a stationary state, i.e., the average deflection of the cantilever is invariant for all bins.
3. Taking a Fourier transform of each bin and calculating the raw PSD for each cantilever averaged over all bins:

¹ In signal processing, Hanning window is a window function that helps proper Fourier transforming of time domain fluctuations' data to frequency space using FFT (mentioned in data processing step (3) by zeroing the values of data points at the edges of each bin, so as to keep the periodicity of data required by the FFT.

Correlation Force Spectroscopy for Single Molecule
Measurements

Radiom, M.

2015, XXVII, 117 p. 48 illus., 31 illus. in color., Hardcover

ISBN: 978-3-319-14047-6