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ACCURATE DETERMINATION OF SPRING CONSTANT OF ATOMIC FORCE MICROSCOPE CANTILEVER AND COMPARISON WITH OTHER METHODS

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Abstract: We present an AFM cantilever calibration system: Nano Force Calibrator (NFC), which can provide accurate spring constant calibrations with traceability to SI. Two types of commercial beam-shaped AFM cantilevers (contact and tapping mode) are investigated using the NFC. Uncertainty analysis reveals that the uncertainty of present method is less than 1%. In addition, comparison between other famous calibration methods (dimensional, cantilever-on-cantilever, and Sader method) and the NFC method is performed to assess the uncertainties of other three methods. From the comparison results, we estimate that the uncertainties of dimensional, cantilever-on-cantilever, and Sader method are around 10-15%, 10%, and 15-40%, respectively.

Keywords: atomic force microscopy, calibration, cantilever, spring constant

1. INTRODUCTION

Determination of a spring constant of atomic force microscope cantilever has been one of the major concerns to nano-mechanic and bio-mechanic researchers, who measure very small forces ranging from 1 pN to 100 nN using an atomic force microscope (AFM) for mechanical tests, such as nanoindentation and DNA tensile stress measurement [1, 2]. The measured force in an AFM is simply obtained by multiplying the known spring constant of a cantilever and its deflection. However, manufacturers do not provide a reliable spring constant of each cantilever because they can't control the thickness of it with a tolerance required to give all cantilevers repeatable spring constants. Thus, accurate force measurements require identifying characteristics of each cantilever through a calibration.

Reflecting this trend, over the last 15 years more than a dozen calibration methods have been proposed and recently comparison reports have been published [3,4]. Each method has its merits and disadvantages, and some techniques are commercialized [5, 6]. On the other hand, there are standardization activities in ISO, which has a mission to assess the need for standards, related to the characterization and specification of tips and cantilever properties in scanning probe microscopy. One of the proposed activities of this group is to develop a documentary standard for AFM normal spring constant calibration [7]. However, the common problem is that the calibration uncertainties of currently used methods range from 5 % to 30 % [3, 4]. Moreover, no reference method exists to assess them. The main reason for the high uncertainty is that most methods depend on the dimensions and material properties of the cantilever or use a complex model in order to calculate stiffness. With a growing need for quantified force metrology in AFM community, it is time to investigate the accurate calibration method of AFM cantilevers to provide a standard for other calibration methods that have been popularly used without a traceable link to Système International d'Unités (SI).

Recently, we have developed an AFM cantilever calibration system; we call it *Nano Force Calibrator* (or simply, the NFC) with the goal of providing a traceable and accurate spring constant calibration with the uncertainty ten times lower than the current level of uncertainty. In our previous work [8], we have demonstrated its calibration performance through uncertainty evaluation based on a series of experiments with a commercial piezoresistive cantilever. Calibration principle is simple. A cantilever to be calibrated directly probes a precision balance of the compensating type. The force acting on the tip of the cantilever is measured by the balance while the corresponding deflection is measured as the displacement output of a capacitive sensor integrated into the precision stage that displaces the cantilever. The spring constant of the cantilever is determined by dividing the force into the deflection.

In this paper, we will elaborate on the NFC and examine two different types of commercial beam-shaped AFM cantilevers. The first are MPP-31120 Veeco contact mode cantilevers and the second are TESP Veeco tapping mode cantilevers. Both types of cantilevers will be calibrated using the NFC first, and then calibrated again using three different calibration methods, and the results compared. The results obtained from the NFC are used to verify the accuracy of other calibration methods. Among them, the first method, which is so called geometrical method, uses a famous Euler's formula of a beam-shaped cantilever and requires knowledge of the Young's modulus of the cantilever material and dimensions. The second method had been developed by several authors, which is called cantilever-on-cantilever method [5, 9, and 10] and requires reference cantilevers to be compared. The third method was developed by *Sader, Chon, and Mulvaney* [11] and requires knowledge of the cantilever's resonance frequency, quality factor and plane dimensions.

2. NANO FORCE CALIBRATOR (NFC)

2.1. Description of calibration system

The NFC used in our experiment is shown in the photo of Fig. 1 and the calibration setup is illustrated schematically in Fig. 2. We use a commercial microbalance (Mettler-Toledo Ltd., UMX5 model) with 5 g capacity and 0.1 µg resolution. Since a local acceleration of gravity where the NFC is installed is measured to be (9.7983 ± 0.001) ms^{-2}, a deadweight produced by a mass of 0.1 µg corresponds to 0.97983 nN; i.e., the force resolution of the NFC. A load button, made of an optical glass (BK7) sphere with 1 mm diameter, is mounted on a protrusion of a weighing pan. The cantilever can probe the load button through a combination of motions of two different positioning devices: one is a manually-operated coarse three-axis stage to globally position the cantilever over the load button, and the other is a single-axis precision stage with an integrated capacitive feedback sensor (PI GmbH, P-621.1CD model) to scan the cantilever automatically along the balance axis with 0.3 nm resolution over maximum 100 µm travel. These two positioning devices are stacked together and this stage assembly is installed on the balance case. The cantilever position is monitored by a video microscope, as shown in the inset of Fig. 1. To reduce the noise from thermal fluctuations, air turbulences and acoustical noise, the NFC is enclosed by an isolation box. The temperature variation inside the box is maintained less than ±1 mK for 20 min.

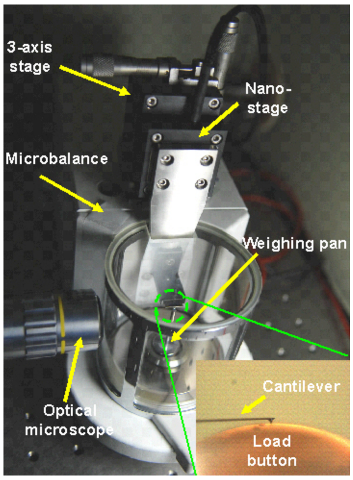

Fig. 1. A photo of the Nano Force Calibrator (NFC)

2.2. Calibration principle

As stated above, the calibration principle is simple; displacing a cantilever in a fixed increment toward the load button so that it presses down the load button and then, the force and displacement are measured and recorded. The spring constant of the cantilever can be determined from the slope of the force vs. displacement curve, as shown in Fig. 2. However, when the cantilever to be calibrated has high stiffness, a correction will be required for the stiffness of the balance.

The balance used in NFC operates at compensation mode; i.e., when a load is applied, the weighing pan firstly moves downward, and then the electromagnetic force compensates the load such that the weighing pan moves back to original position. Thus, the stiffness of the balance (k_b) is theoretically infinite, but actually the balance has finite stiffness. We obtained the stiffness of the balance by applying a known load and measuring the corresponding deflection of the weighing pan using a calibrated video microscope. The measured value was (1585 ± 100) Nm^{-1}. On the other hand, it is not necessary to consider the stiffness of a stage assembly (k_a) consisting of the three-axis stage and the precision stage, which is measured to be approximately 63400 Nm^{-1}; i.e., it is high enough stiffness compared with that of cantilever to be calibrated. The corrected spring constant k_c can be computed from the measured spring constant k_m as

$$\frac{1}{k_c} = \frac{1}{k_m} - \frac{1}{k_b} \qquad (1)$$

From equation (1), when we calibrate an AFM cantilever with a spring constant of 1 Nm^{-1}, the correction due to the balance stiffness is only about 0.06%. In case of a stiffer cantilever such as a tapping-mode cantilever with a spring constant of about 100 Nm^{-1}, the correction is about 6%, which is not negligible for accurate calibration.

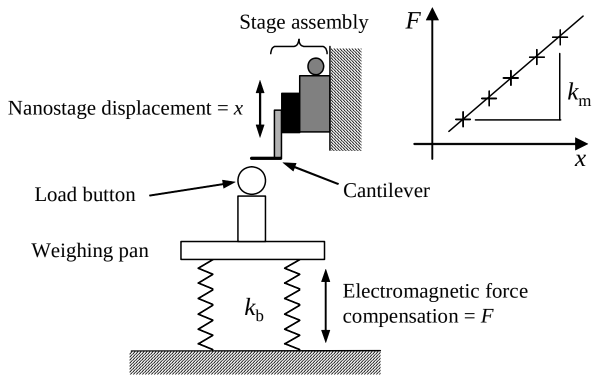

Fig. 2. The principle of cantilever calibration using the NFC

2.3. Performance evaluation

The accuracy of spring constant calibration depends on measurement uncertainties of two major quantities; force and displacement. Thus, we have estimated the uncertainties of force and displacement measurement in a traceable way. For the uncertainty estimation of the force measurement, the balance was tested using E1 class weights traceable to the KRISS national mass standard. Six weights of 1, 2, 5, 10, 20, 50 mg were used because the force measured in the determination of the spring constant ranges from 10 ~ 500 µN. Each weight was loaded 10 times in the center of the weighing pan, and balance indications were recorded. The standard uncertainties and corresponding data are listed in Table 1. The correction *C* was calculated by subtracting the mean of 10 indications from the nominal value of the test load, and the repeatability was taken as the standard deviation. The maximum relative uncertainty for the mass measurement is 5.0×10^{-4} at 1 mg load, of which uncertainty is most significant. On the other hand, the relative uncertainty of the measurement of the acceleration of gravity measurement is negligible, i.e., on the order of 5.0×10^{-6}. Thus, the maximum relative uncertainty of force measurement is 5.0×10^{-4}.

The uncertainty of displacement measurement has been estimated based on the calibration certificate provided by the manufacturer, which proves that the instrument was calibrated with traceability to SI. According to the test document of the precision stage, the stage movements were compared with a calibrated commercial laser interferometer (Zygo ZMI 1000) in steps of approx. 6 μm over a full travel range of 100 μm. The maximum nonlinearity and repeatability of the stage movement were reported to 6 nm and 1.3 nm, respectively. Thus, a conservative estimate of the relative uncertainty due to the precision stage can be calculated to 1.0×10^{-3} assuming that the uncertainty of the laser interferometer is in a relatively insignificant order of 10^{-5}.

Table 1. Uncertainty of the balance at six discrete loads. The uncertainty due to the balance resolution *d*, 0.1 μg, is negligible

Quantity or Influence	Correction or Standard uncertainty in μg					
Indication (mg) ≈	**1**	**2**	**5**	**10**	**20**	**50**
Correction (*C*)	-0.1	-0.6	-0.7	-0.4	-0.6	-0.4
Repeatability	0.2	0.3	0.1	0.2	0.2	0.2
Resolution (d/√12)	negligible					
Test loads	0.5			1.0		
Weight buoyancy	negligible					
Uncertainty of correction u(*C*)	0.5	0.6	0.5	0.5	1.0	1.0

3. OTHER CALIBRATION METHODS

3.1 Dimensional method (Euler's formula)

If the cantilever beams look like a diving board, the spring constant can be analytically obtained using relatively simple mechanics. The cantilever beam is of uniform cross section and carries a load, *F*, at its free end. By assuming a small vertical deflection, the deflection, *z*, at the end of the cantilever can be calculated to be

$$z = \frac{FL^3}{3EI} \tag{2}$$

where *L* is the length of the cantilever, *E* is Young's modulus and *I* is the moment of inertia. For a rectangular cross section cantilever of width, *w* and thickness, *t*, *I* is given by

$$I = \frac{1}{12} wt^3 \tag{3}$$

The spring constant for the rectangular beam is defined by $k = F/z$ and hence is expressed as a simple closed form as follows:

$$k = \frac{Ewt^3}{4L^3} \tag{3}$$

This equation is only applicable to cantilevers where $w << L$ since the bowing of the cantilever across the width is ignored. The uncertainty of dimensional method is mainly attributed to the uncertainties of thickness measurement and Young's modulus.

3.2 Cantilever-on-Cantilever (COC) method

This method needs a 'standard' cantilever, of which the spring constant is known. Two steps are required to complete a calibration. The first step is to obtain a force versus distance curve with 'unknown' cantilever being pushed against a flat and hard surface (assumed to be infinitely hard relative to the cantilever compliance). The slope, S_h, stands for the cantilever deflection when the 'unknown' cantilever is in contact with the hard surface.

The next step is to land the end of the 'unknown' cantilever on the end of the 'standard' cantilever as shown in Fig. 3 and obtain the force-distance curve. The slope, S_c, in contact region will be a measure of the combined spring constants of the two-cantilever system. Since the spring constant, k_s, of the 'standard' cantilever is known, that of the 'unknown', *k*, can be calculated from

$$k = k_s \frac{S_h - S_c}{S_c \cos\theta} \tag{4}$$

where θ is defined in Fig. 3

For accurate calibration, selection of the 'standard' lever is crucial. As k_s is closer to *k*, the smaller uncertainty can be achieved. According to Ref. [9], k_s should be matched to *k* within a dynamic range such that $0.3\ k_s < k < 3.0\ k_s$. The uncertainty of this method is between 10 -30 % [3, 9].

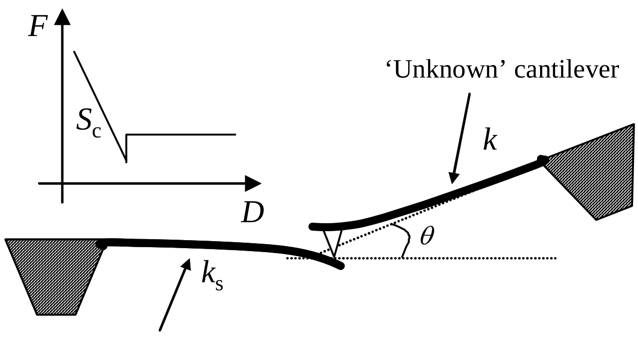

Fig. 3. A schematic of the setup for force-distance measurement of a 'unknown' cantilever relative to a 'standard' cantilever.

3.3 Resonance method (Sader's formula)

This method proposed uses dynamic behaviors of a cantilever. Thus, it is categorized as resonance methods (or dynamic methods) which infer *k* from either resonance frequency or from resonance envelope. The formula is as follows:

$$k = 0.1906 \rho_f w^2 L Q_f \Gamma_i(\omega_f)\omega_f^2 \qquad (5)$$

where ρ_f is the density of the fluid in which the cantilever is immersed (usually air), ω_f is the angular resonance frequency in air, Q_f is the quality factor in air, and Γ_i is the imaginary component of the hydrodynamic function, Γ, which depends on the Reynolds number Re = $\rho_f \omega_f w^2/4\eta$ only, where η is the viscosity of the surrounding fluid.

The equation (5) can only be valid for beam-shaped cantilevers with aspect ratios (*L*/*w*) in the range 3-14 and with the Q-factor >> 1. This method can provide accuracy of the order of 15-20% [12].

4. SPRING CONSTANT CALIBRATION

4.1. Cantilevers

The first type of cantilever studied was the contact mode probe (MPP-31120, Veeco Instruments, USA) made of n-doped silicon. The nominal dimensions provided by a manufacturer for the length, width and thickness of these levers are 450, 35 and 4 µm, respectively. The nominal spring constant is 0.9 Nm^{-1} but actually it can vary between 0.45 ~ 1.8 Nm^{-1}. The second type was also the n-doped silicon cantilever, but it is non-contact (or tapping) mode cantilever (TESP, Veeco Instruments, USA). The nominal dimensions provided by a manufacturer for the length, width and thickness of these levers are 125, 30 and 4 µm, respectively. The manufacturer gives the nominal spring constant of 42 Nm^{-1} and also notes that the spring constant can range between 20 and 80 Nm^{-1} in a datasheet. As shown in Fig. 4, both types of cantilevers have similar geometry, which is close to that of an ideal rectangular beam. However, the shape of the cross section of the beam is a trapezoid rather than a rectangle due to the manufacturing process.

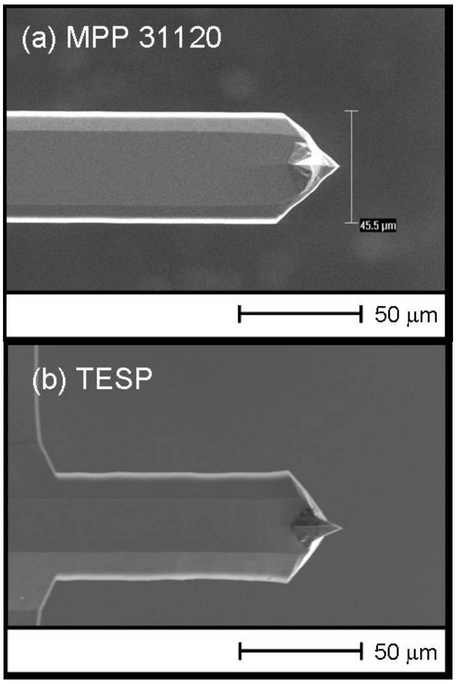

Fig. 4. SEM images of two types of cantilevers to be investigated: (a) MPP 31120 contact mode cantilever (b) TESP tapping mode cantilever

4.2. Calibration using the NFC

The first step in the calibration sequence was to manually position the cantilever to be calibrated approximately 10 µm over the top of the balance load button, using the three-axis stage while watching an optical micrograph to observe the tip position. When the manual positioning was complete, the rest of the calibration sequence was fully automated. Several precise motion steps were executed using the single-axis precision stage, pushing the cantilever against the balance load button. At each point, we wait 30 ~ 70 s for achieving balance stability, then sample the balance readings and displacement outputs from the precision stage simultaneously and average for 10 s. The cantilever length sets the limit of maximum deflection to less than its one-twentieth, in order to ensure the linearity of a force-displacement curve.

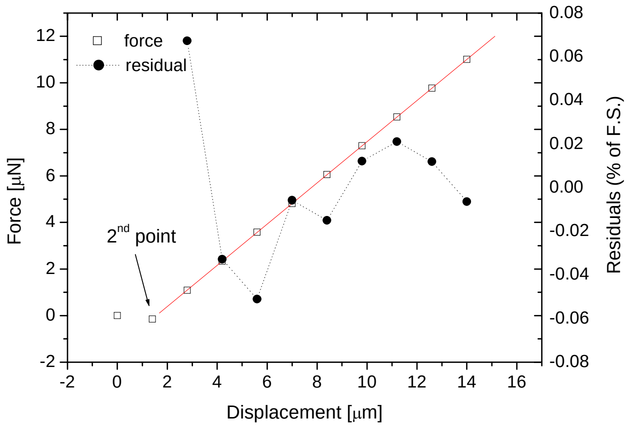

Fig. 5. Force versus displacement curve showing the linear bending property of the cantilever

Fig. 5 shows a force-displacement curve of a MPP31120 cantilever. The maximum deflection was approximately 12 µm, which corresponds to about one-fortieth of the cantilever length. As you can see in Fig. 5, at second point, a minus (i.e. attractive) force of 150 nN was measured. This would be a meniscus force between the tip of the cantilever and the load button due to moisture on the surfaces. The physical contact begins at third point. The maximum contact force is approximately 11 µN at the last point. From point 3 to 11, a linear polynomial fit was performed.

Observing the magnitude and structure of the residuals, we found that this cantilever is sufficiently characterized by a linear polynomial fit, of which the slope determines a spring constant of cantilever. The residuals from the linear fit were less than were less than 0.1% of full load. Since the cantilever shows a linear characteristic over 12 µm deflection, it is possible to determine the slope by using not only multiple steps but also a single big step. It is advantageous to use a single step in order to reduce calibration time and noises due to drift effect. Moreover, a larger force increment can reduce the uncertainty on force measurement. Thus, we used a single step method to determine spring constants of these cantilevers.

In Fig. 6 the variation of the spring constant of the MPP31120 cantilever during a long-term period of several days is displayed. Every two hours, the cantilever was probed and the slope of the step was calculated. The relative deviations from the average value were consistently less than 0.7% with no observable drift, suggesting that our calibration setup is reproducible. The relative standard deviation was about 0.3% of the average value.

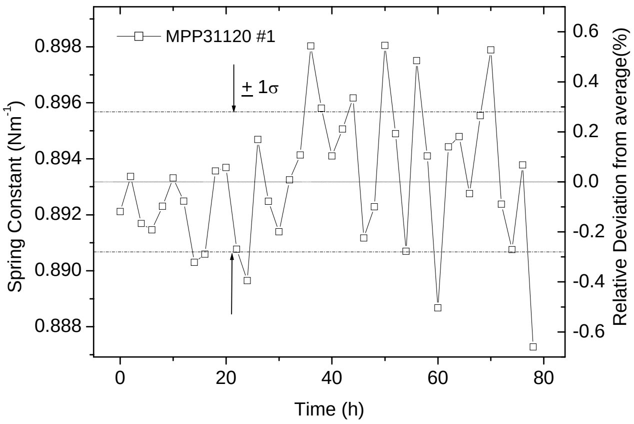

Fig. 6. Scatter of the spring constant of the MPP31120 cantilever during a period of three days

Table 2 summarizes the combined standard uncertainty of the spring constant of the MPP31120 cantilever, and related uncertainty sources. You can find details for each uncertainty source in Ref. [8]. Root-squared sum of all relevant uncertainty sources produces the relative combined uncertainty (u_c), which is determined to be less than about 0.4%. However, we expect that the relative combined uncertainty would be around 0.5% including 'unknown' uncertainty sources, such as irregular contact phenomenon between the tip and the load button. Other two MPP31120 cantilevers produced in the same wafer were calibrated in a same way and results are shown in Table 3.

Table 2. Uncertainty sources and their contributions to the overall uncertainty of the spring constant of MPP31120 cantilevers

Quantity or Influence (Q)		Relative standard uncertainty (u=dQ/Q)	Uncertainty type
Measured value (Nm^{-1})		0.893	
Repeatability (u_s)		3.0×10^{-3}	A
Force	Correction (C_f)	-1.0×10^{-4}	
	u_f	5.0×10^{-4}	B
Precision stage (u_d)		1.0×10^{-3}	B
Balance stiffness	Correction (C_b)	5.7×10^{-4}	
	u_b	3.6×10^{-5}	B
Non-linearity (u_n)		1.0×10^{-4}	B
Orientation (u_o)		2.0×10^{-3}	B
Combined relative standard uncertainty (u_c)		3.8×10^{-3}	
Corrected value with combined standard uncertainty (Nm^{-1})		0.893 ± 0.003	

Similarly, the TESP cantilevers were calibrated, but force and deflection used for calibration are different. The maximum force was about 150 μN when the deflection was about 5 μm, which corresponds to about one-twenty-fifth of the cantilever length. Due to such a large force increment, the relative standard uncertainty due to force measurement has been reduced to 5.0×10^{-5}, but scatter of the spring constant of the TESP cantilever is slightly bigger than that of the MPP31120 cantilever as shown in Fig. 7. The relative standard deviation was about 0.4%. The combined relative uncertainty was calculated to be less than 0.5%, though the expected combined uncertainty would be around 0.6%. Other two TESP cantilevers produced in the same wafer were also calibrated and results are shown in Table 3.

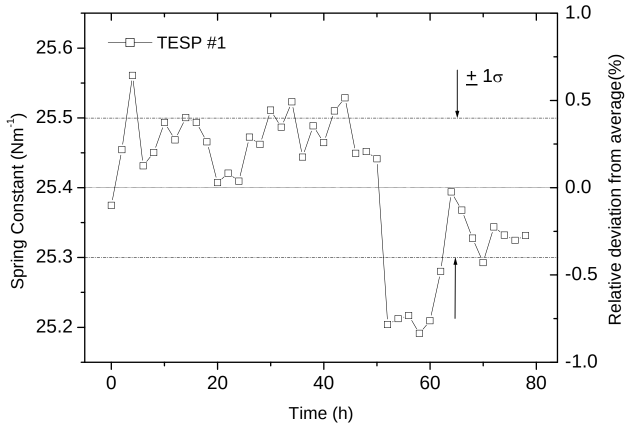

Fig. 7. Scatter of the spring constant of the TESP cantilever during a period of three days

4.3. Calibration using the dimensional method

For both types of cantilevers plan view dimensions of length and width were measured using both of calibrated optical microscope and scanning electron microscopy (SEM). The length was taken as the perpendicular distance from the base to the tip of the cantilever. Since the cross section for both types of cantilevers is a trapezoid, top and bottom widths for each cantilever were measured. The error using an optical microscope is approximately less than ± 1 μm, which leads to 2-3% contribution to the uncertainty. The thickness measurements were performed solely using scanning electron microscopy (SEM). The uncertainty in thickness is more crucial. We estimated it to be about 3%, which contributes about 10% to the uncertainty in the dimensional method. We used Young's modulus of n-doped silicon, 169 GPa, provided by the manufacturer for both types of cantilevers.

For more accurate estimation, we have calculated analytically the moment of inertia of a trapezoid so as to consider the effect of non-rectangular cross section rather than used the equation (3). Results are shown in Table 3.

4.4. Calibration using the COC method

The standard cantilever used in our experiment is 'Force Calibration Cantilever' provided by Veeco Instrument. This cantilever is fabricated using special micromaching process to define dimensions, width, length, and thickness, precisely [5]. Each chip has three cantilevers with different spring constants of 0.71, 4.65, and 26.1 Nm^{-1}. We selected the 0.71 Nm^{-1} for MPP31120 cantilevers and the 26.1 Nm^{-1} for TESP cantilevers to match k_s to k within the dynamic range mentioned in subsection 3.2.

The slopes of force-distance curves were measured using a commercial AFM instrument (XE-150, PSIA Corp.) with force-distance software. The angle, θ between the 'standard' cantilever and 'unknown' cantilever was 12°. Results are shown in Table 3.

4.5. Calibration using the Sader method

This method requires the plan view dimensions and resonance properties of the cantilever. The width, *w*, was taken as the average between the top and bottom widths. The resonance frequencies and quality factors were obtained from thermal noise spectrum for the MPP31120 cantilevers and from forced oscillation near resonance for TESP cantilevers.

The imaginary parts of the hydrodynamic function, which is found in equations (18), (20), and (21) of Ref. [13] were calculated using Mathematica™ software. The density and viscosity of the air used in this calibration were 1.18 kgm^{-3} and 1.86× 10^{-5} $kgm^{-1}s^{-1}$, respectively. Results are shown in Table 3.

4.6. Results and discussion

Table 3 shows the results for the four different calibration methods for MPP31120 and TESP cantilevers. Also the relative deviations of other methods from the NFC results are shown in Table 4.

Table 3. Comparison of spring constants for MPP31120 and TESP cantilevers using the NFC, the dimensional, the COC and Sader method. The nominal spring constants for MPP31120 and TESP levers, quoted by the manufacturer, are 0.9 and 42 Nm^{-1}, respectively.

Cantilevers		Methods			
		NFC (Nm^{-1})	Dimension (Nm^{-1})	COC (Nm^{-1})	Sader (Nm^{-1})
MPP31120	1	0.893	1.00	0.881	0.585
	2	0.794	0.798	0.870	0.490
	3	0.793	0.822	0.769	0.495
TESP	1	25.85	28.65	25.73	22.22
	2	26.56	28.34	24.23	21.46
	3	24.60	27.41	24.75	20.84

Table 4. Relative deviations from the NFC results

Cantilevers		Relative deviations from the NFC results (%)		
		Dimension	COC	Sader
MPP31120	1	12.6	-1.4	-34.5
	2	0.4	9.5	-38.3
	3	3.7	-3.0	-37.6
TESP	1	10.8	-0.5	-14.1
	2	6.7	-8.8	-19.2
	3	10.26	0.6	-15.3

From these results, it is obvious that Sader method underestimates for both types of cantilevers. We suspect that the underestimation of Sader method is mainly due to non-rectangle cross section of the cantilevers. In case of TESP cantilevers, the relative deviations of Sader method relatively coincide with the claimed uncertainty of the order of 15-20%, but when it comes to MPP31120 cantilevers, situations are different. We are still investigating the results of Sader method for MPP31120 cantilevers.

The COC method estimates the spring constants to be closest values of the NFC method for both types of cantilevers. We think that the almost exact match between 'standard' cantilevers and 'unknown' cantilevers preserves the accuracy of this method. In addition, we have found that the relatively large deviations of 'Number 2' cantilevers for both types are due to slippage of the tip of the 'unknown' cantilever along the upper surface of the 'standard' cantilever. From these results, we can estimate the uncertainty of the COC method to be around 10% when matched 'standard' cantilevers are used. Our estimation of the uncertainty of this method agrees with the uncertainties published by several authors [5, 9].

The relative deviations of the dimensional method are not far from our expectation. The main uncertainty source is the thickness measurements as mentioned above. We can estimate the uncertainty of the dimensional method to be around 10 – 15%.

5. CONCLUSION

We have been developed the AFM cantilever calibration system, which we call 'Nano Force Calibrator (NFC)', with the aim of providing accurate and traceable spring constant calibration for quantified force metrology in AFM. Two types of commercial beam-shaped AFM cantilevers have been investigated. Using the NFC, both cantilevers were calibrated with the uncertainty of less than 1%, which is traceable to SI. The level of uncertainty in spring constant calibration is the smallest uncertainty yet attained by any method to our knowledge. Same cantilevers were calibrated again using three different methods: dimensional, cantilever-on-cantilever, and Sader method. Comparison reveals that the uncertainties of methods relatively coincide with the values estimated by various authors, though more research are needed for Sader method, which shows big relative deviations from the NFC results for MPP31120 cantilevers.

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