



Technical Working Area 29

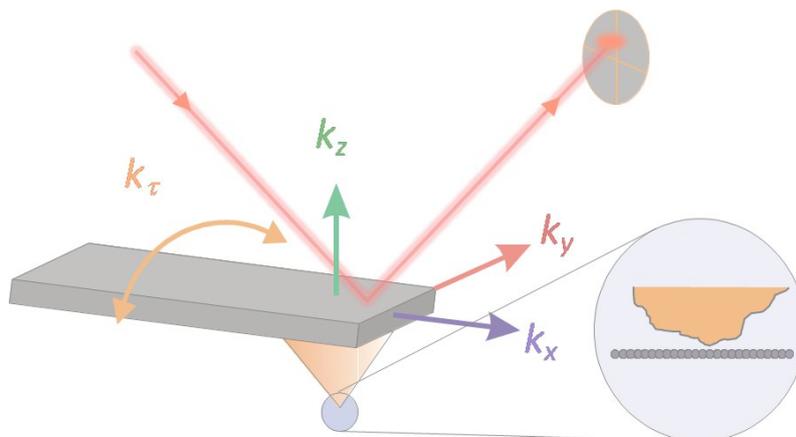
# Nanomechanics Applied to Scanning Probe Microscopy

## *Mini Round Robin on AFM Cantilever Spring Constant Calibration*

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## Summary

A mini round robin (MRR) was conducted among three laboratories in three countries to evaluate testing protocols for determining the flexural spring constants of AFM cantilevers. This MRR was intended as an initial foray into this area in order to experience logistical, handling, and testing issues that might come up in a larger round robin with many different participants. By experiencing and addressing problems in the MRR it was anticipated that a future round robin could be conducted with fewer problems.

A kit comprised of six test cantilevers (silicon nitride, triangular) and a reference cantilever chip, was mailed to each laboratory in sequence. Each laboratory was asked to perform cantilever calibration procedures on the test cantilevers using whatever procedure they were familiar with. Two written drafts of procedures for an added mass method and a reference cantilever method were included with the test kit. The results were collected and the data compared.

All three laboratories performed the reference cantilever method and the statistical analysis of the results of each laboratory indicated good agreement between the results obtained from the three laboratories. One of the laboratories conducted added mass calibrations, and their spring constant values were consistent with those obtained with the reference cantilever method.

Inspection of the reference cantilever after all of the testing revealed that two of the three reference cantilevers on the handle chip (ones not actually used for calibration) had been damaged (broken off) during the MRR. Inspection of the test cantilevers after the MRR revealed significant chipping damage on the edge of the handle chip and significant amounts of debris particles on surfaces of the test cantilevers. The inference is that these two are related and chipping damage produced during handling of the test chips generated the debris particles. To remedy this, it is suggested that future handling procedures specify a particular type of forceps with a flat paddle end that does not produce as high a stress concentration on the sides of the chip during handling. In addition, a method was suggested for removing the test chips from the adhesive gel used for transportation by rocking the chip and peeling it off the gel. This method should reduce the forces needed to extract the chips from the storage case and will ultimately reduce the amount of debris generated during sample handling.

Inspection of the tips of the test cantilevers after the MRR showed tip wear and debris attachment to the end of the cantilever. This was likely due to the contact between the tip and surface during force curve measurements necessary for the reference cantilever method. The data suggest that one potential issue in a wider round robin might be the effect of a changing tip morphology (tip wear) on calibration results. As more participants test the same cantilever, this cumulative damage effect may become more significant. It is also anticipated that sharper Si cantilevers may be more sensitive to this effect therefore procedural limitations (e.g., limiting the amount of force or the stroke length actually applied during testing) should be implemented to limit cumulative damage from a large round robin among many participants.

## **Objective**

The measurement of force in an atomic force microscope (AFM) depends on the spring constant of the cantilevers used in the apparatus. To date, there is no standard method for evaluating the flexural spring constants of AFM cantilevers. It is the goal of the VAMAS technical working area on Nanomechanics Applied to Scanning Probe Microscopy (TWA29) to investigate the use of different procedures for evaluating the flexural spring constants of AFM cantilevers to determine their suitability for performing this task. Before conducting a comprehensive round robin, a mini round robin (MRR) was deemed necessary to serve as a prototype for the larger study and address many of the pitfalls and issues that would arise from such a study. This report describes the execution of and results obtained from this mini round robin study.

## **Aims and Instructions**

The spring constant of AFM cantilevers determines to a large degree the forces that are applied to samples during testing. Manufacturing processes have evolved to the point where variation of material property (silicon nitride) and dimensional control (Silicon) have improved but there is still considerable uncertainty in the actual spring constant of individual cantilevers. The most common strategy employed by users who need a reliable understanding of the spring constant is to calibrate it either just prior to or just after measurement. Since there are no standard methods for calibrating AFM cantilevers, the aim of the technical working area on Nanomechanics Applied to SPM is to evaluate suitable methods for calibrating the spring constant of AFM cantilevers. While the ultimate aim of any standard development effort is a procedure with a high degree of precision and absolute accuracy, the scope of this initial effort lies mainly with examining the precision and applicable ranges of the candidate techniques. The results of these studies can then be turned over to appropriate groups within ISO for a more rigorous refinement of the procedures with regard to accuracy.

This mini round robin (MRR) was designed to address some of the relevant preliminary issues of sample dissemination, sample handling, data formatting and reporting. Each participant was sent the test kit consisting of a set of six identical contact mode silicon nitride cantilevers and a reference cantilever chip consisting of three reference cantilevers. The participants were asked to conduct cantilever calibrations on each test cantilever using whatever calibration procedure they were familiar with. Two draft procedures (Reference Cantilever Method and Added Mass Method) were included with the test kit so that participants could be given some guidance in determining the values in a consistent way. These draft procedures are included in Appendix 1 and 2. Participants were also asked to send back the results of the calibration in a spreadsheet so the results could be compared. When one participant had completed the testing, the kit was mailed to the next participant. Thus each test cantilever was examined by each participant in succession.

After all of the labs had participated, the cantilevers were inspected visually (optical microscope) and in an SEM by NIST to determine if any damage had occurred during handling.

## The Test Kit

The test kit consisted of six contact mode cantilever chips (DNP cantilever, Veeco Metrology, Santa Barbara, CA) and a reference cantilever chip (CLCF cantilever, Veeco Metrology, Santa Barbara, CA) and were supplied by the vendor. The test cantilevers were taken from similar locations within a wafer production batch and were selected for anticipated consistency in spring constant. The cantilever to be tested was the “DNP A” cantilever on each chip. In the terminology of the chip where the cantilever legs are described in relative terms as either “fat” or “thin” and either “short” or “long”, the “DNP A” test cantilever fits the description of “short-fat.” The relative location of the DNP A cantilever on the chip is shown in Figure 1. The nominal spring constant, provided by the manufacturer for this cantilever, is 0.58 N/m. The six chips were placed on a “X4 Gel-Pak” storage box (Gel-Pak, Hayward, CA) using the first six places in row “A” as outlined below. For reporting purposes, these test cantilevers were described as samples 1-6.

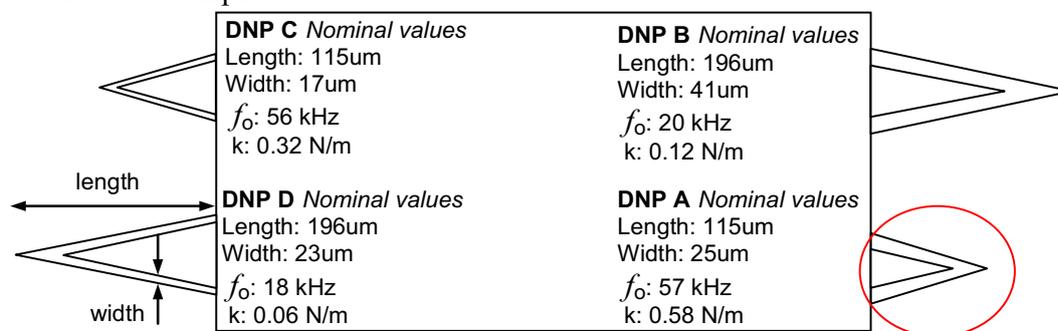


Figure 1. Schematic for test cantilever chips showing locations of cantilevers.

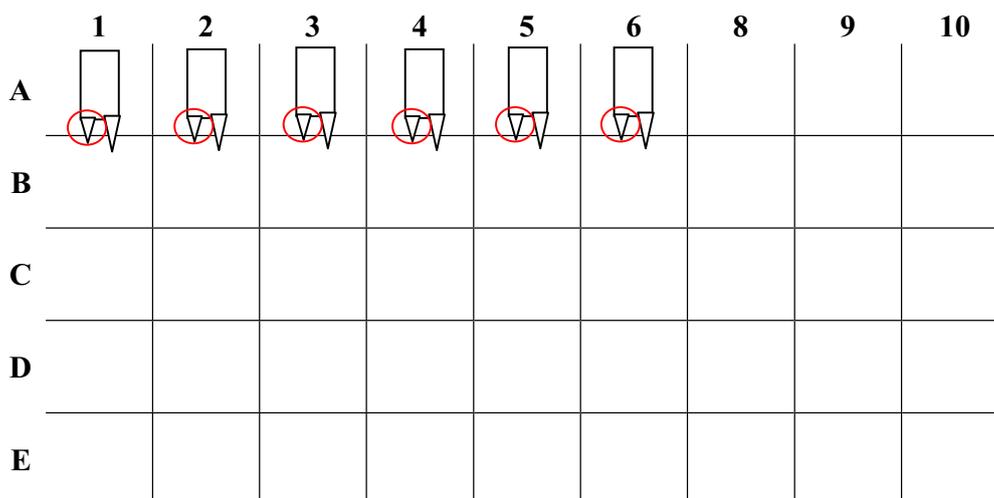


Figure 2. Orientation and placement of the test cantilever chips within the Gel-Pak box.

The storage box allowed safe shipping of the chips during the mini round robin. The chips were oriented so that the cantilever of interest (“A”) was located toward the lower left corner of each chip. The three unused cantilevers on each chip were not intentionally altered in any way.

The other part of the test kit consisted of the reference cantilever chip. To minimize potential damage to the reference cantilever from handling, it was mounted in the middle of a steel puck using double sticky “press tab” adhesive. The reference chip consisted of three reference cantilevers of different length (Figure 3). For the purposes of this study, only the longest cantilever was used. The manufacturers nominal specifications for dimensions (length = 429  $\mu\text{m}$ ) and spring constant (0.711 N/m) were used. The reference cantilever chip was placed into a plastic box to protect it during shipping. A magnet glued to the bottom of the box using pressure sensitive adhesive allowed the steel puck to be secured magnetically within the box for shipping. It could be removed from the box and placed into the stage of the AFM for the calibration step without having to touch the actual silicon chip itself.

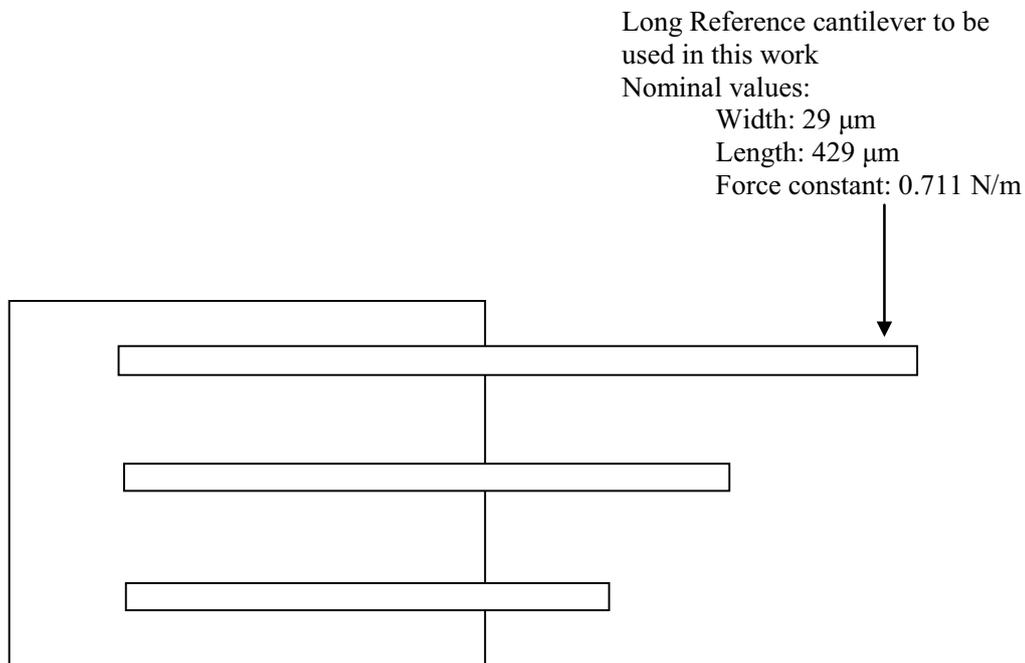


Figure 3 Reference cantilever chip used for this study.

## Participants

Three laboratories from three countries participated in the mini round robin.

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## Equipment

Three different commercial AFM's were used in this study.<sup>1</sup>

Veeco Multimode with a Nanoscope IIIa controller using an open loop scanner

PSIA XE-100 with a closed loop scanner

Veeco Dimension 3000 with an open loop scanner

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<sup>1</sup> Certain commercial equipment, instruments, or materials are identified in this report to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology (NIST), the National Physical Laboratory (NPL), or the National Institute for Materials Science (NIMS) nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

## Results and Discussion

At the conclusion of MRR testing, the samples were received back at NIST and examined for wear and damage. The test cantilevers were all intact but they all had significant amounts of debris and fracture damage over the surface of the chip. The test DNP “A” test cantilever in the optical photomicrograph of Figure 4 is shown in the lower right. An expanded view of the test cantilever is shown in Figure 5 and shows only a single large debris particle on the leg of the cantilever.



Figure 4. Typical Test cantilever chip after the MRR

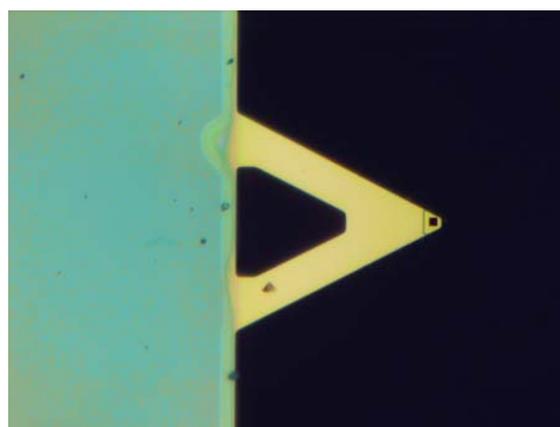


Figure 5. Enlarged view of the DNP "A" cantilever

The fracture damage on the sides of the chip and abundance of large debris particles covering the chip were not present before the start of the MRR and are probably due to chip handling during placement and removal of the cantilevers in the AFM holders. The most likely part of the operation is when the chips are plucked from the Gel-Pak storage and transport tray. Since a significant upward force is required to overcome the adhesive force from the gel, considerable pinching force is often applied to endure a good grip on the chip. If the tweezers used to grasp the chip are pointed, considerable stress concentration can be generated and fracturing of the edges of the chip is likely.

This is one of the most pressing issues associated with conducting a round robin. Sample handling is unavoidable and accumulated damage could potentially influence the results. The more participants involved, the higher the likelihood and level of damage. The fractured edges themselves are mostly a cosmetic issue; however, the debris generation could directly affect the test results. For example, debris on the cantilever does not affect the actual spring constant of the cantilever; however, it can affect the resonance frequency, since mass has been added to the cantilever. Spring constant estimation techniques that utilize resonance frequency, such as added mass and Sader, could therefore be affected. It may also interfere with methods like the reference cantilever method if contact is made with debris particles during calibration and variation in friction and wear are produced.

Practices should be specified to minimize the amount of damage from this issue. Many tweezers use a pointed tip that can generate very high point stresses at the edges of the chip where they contact and fractures can be produced. This is especially problematic when the chip is strongly adhered to the gel and extra pressure is applied to grab the chip to pull it up and off

the surface. It is suggested that participants utilize more specialized tweezers that have a flat blade that applies a firm but more distributed pressure at the edge of the chip. This may help minimize this type of damage. One suggestion is to provide tweezers along with the test kit so that all participants can use the proper handling tool. A second suggestion is offered for the method of freeing the test chip from the gel. In one author's experience (RG), grasping the sides of the chip and gently rocking the chip to one side effectively "peels" the chip from the gel with minimal force and should minimize potential damage to the test chip. In some, more stubborn, cases where the chip is more strongly adhered to the gel it is recommended to combine a small twisting motion (essentially rotating the chip in the plane of the gel surface - approximately 45 degrees) with the rocking motion applied at the end.

Samples from the MRR were also characterized in a field emission scanning electron microscope (FESEM) to determine if more subtle damage had occurred. A low magnification picture of the test chip confirms the gross damage from edge fracture and an abundance of particle debris. Higher magnification of the test cantilever (Figure 6) indicated an occasional large debris particle (in this case almost 10  $\mu\text{m}$ ) and an abundance of fine particulate debris.

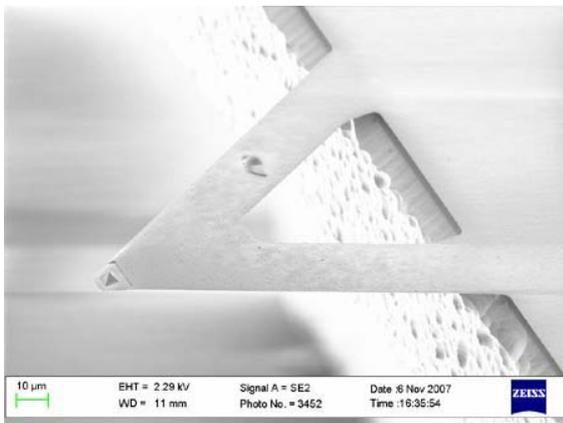


Figure 6. SEM photomicrograph of a test cantilever.

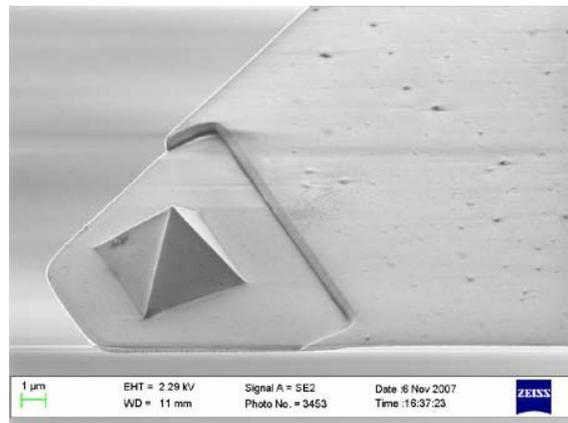


Figure 7. Enlarged portion of cantilever tip.

Higher magnification (Figure 7) showed fine (submicron) debris all over the test cantilever and even on the base of the cantilever tip. Even higher magnification (Figure 8) shows evidence of some apex tip wear and possible buildup of debris on the ridge of the tip. The effect of tip wear could be a possible variant in the estimation of the spring constant in some techniques. Different tips may have different adhesion to the contacting surfaces and may perturb the force curve measurement in less consistent ways. This may affect both the potential precision and accuracy of the intended measurement.

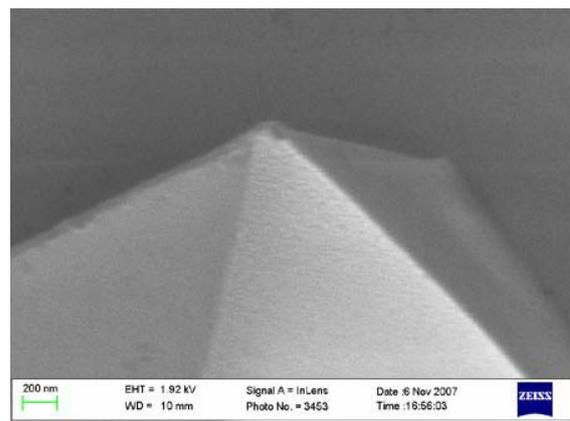


Figure 8. Enlarged portion of the tip apex.

Examination of the reference cantilever chip before (Figure 9) and after (Figure 10) the MRR revealed that two of the three cantilevers from the original series had been broken off during the exercise. Looking at the “after” picture, one can actually see the large piece of rectangular debris on the chip near the bottom cantilever that would appear to be the remnant of the shortest reference cantilever. This suggests that it was broken off by pulling up on it – otherwise it would have been propelled downward and lost to the adhesive holding the chip in place.

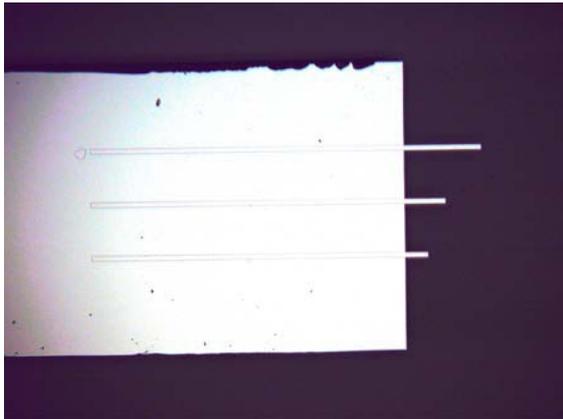


Figure 9. Reference cantilever chip before MRR.

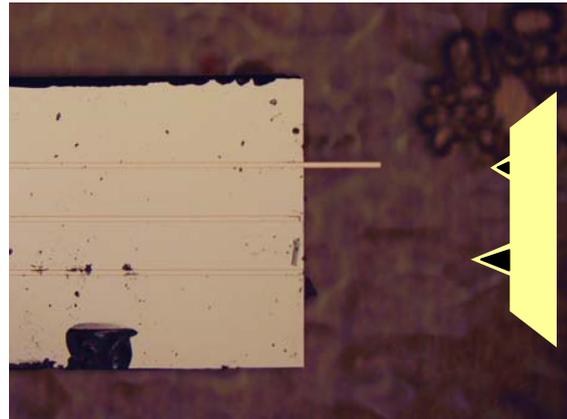


Figure 10. Reference cantilever chip after MRR.

This observation is puzzling until one recalls that the working end of the test cantilever actually consists of the two cantilevers – the shorter one being tested and a longer one offset by several hundred micrometers. This is similar to the offset for the longest and shortest cantilever in the reference chip. If you superimpose the test cantilever outline onto the reference cantilever image on Figure 10 you can see that the long cantilever test actually falls near the shorter reference cantilevers. This information, coupled with the knowledge that the test cantilever is inclined during calibration and suggests a possible explanation for the breakage of the shorter reference cantilevers. When the short test cantilever is pressing on the end of the longest reference cantilever, the longest test cantilever is in front of the shortest reference cantilever but projects a few micrometers below it due to the incline angle of the test cantilever and its increased length (some 80  $\mu\text{m}$  longer). If the short test cantilever is translated while in close proximity to the chip surface, the long test cantilever could “hook” the reference cantilever and pull it upwards and break it off.

This scenario points out one issue with using cantilever chips with multiple tips. Often, these tips are out of range of the optical system (video etc.) monitoring the area of interest. Even the act of conducting a force curve on a flat surface in which the short cantilever has flexed only 500 nm in the z direction can cause the longer test cantilever to flex a few  $\mu\text{m}$  in the z direction. This will certainly damage the tip of the test cantilever but may also create unintended damage to surface structures as well. This imposes certain restrictions of round robins conducted among many users. First, it indicates that only one test cantilever on each chip should be tested. Second, care must be exercised to ensure that cantilevers and features not in view do not cause inadvertent damage to the test system. Thirdly, caution must be taken to safeguard the reference cantilevers so that multiple beams can be used during evaluations. One option is to specify a back-off distance that exceeds the extra projection depth of the longest test cantilever that may be present. Ultimately, the most prudent choice may be to break off any unused test cantilevers

on the test chip to completely avoid this problem. This has the additional advantage of avoiding any confusion about which cantilever to test in case of mis-orientation during handling.

In the case of this MRR we were fortunate that these effects did not damage a needed reference cantilever or interfere with the results of the study. In a larger study involving multiple reference cantilevers it certainly would have.

Results of the calibrations were received from participating laboratories via e-mail and collated. The data format requested (as stated in Appendix 1) was for static table spreadsheets as shown below in Tables 1 and 2. Each participant was responsible for providing the calculations for estimating the test cantilever spring constant using the equations provided in Appendix 1.

$$k_{test} = k_{ref} \left( \frac{S_{rigid}}{S_{cant}} - 1 \right) \cos^2 \varphi \quad \text{where} \quad k_{ref} = k_{end} \left( \frac{L}{L - \Delta L_{tip}} \right)^3$$

This required measuring the back-set of the tip apex on each test cantilever ( $\Delta L_{tip}$ ) and using that parameter to calculate the off-end correction for the point of contact on the reference cantilever. The overlap cantilever alignment procedure provided in Appendix 1 (by RG & MR) was intended to address the critical alignment issue of contact point placement on the reference cantilever that can significantly affect precision of the method. The procedure appears to have been adequate based on the results. No constraints were placed on which force curve (approach or retract) was used for the slope estimation. It was suggested that force curve ramp length start at 500 nm, but it could be adjusted to suit the requirements of the particular experiment. A minimum of six measurement pairs (on a rigid surface and on the reference cantilever) were requested for statistical reasons. It was requested that the raw measurement data be provided for each test cantilever in the table format shown in Table 1.

**Table 1 Example raw data for reference method**

<b>Cantilever ID</b>	<b><math>\Delta L_{tip}</math> um</b>	<b><math>\varphi</math></b>	<b>Ramp size, nm</b>	<b>Test #</b>	<b><math>S_{rigid}</math> V/nm</b>	<b><math>S_{cant}</math> V/nm</b>
DNP 1A	5.0	11°	300		Approach or retract	Approach or retract
				1	0.023	0.014
				2	0.022	0.015
				3	0.022	0.015
				4	0.023	0.014
				5	0.022	0.014
				6	0.023	0.015

A second series of tables was requested summarizing the calculation of the test cantilever spring constant using the data from the six pairs of runs on each test cantilever.

**Table 2 Example calculated data for reference method**

Test #	L um	$\Delta L_{tip}$ um	$k_{ref}$ N/m	$k_{test}$ N/m
	429	5.0	0.736	
1				0.482
2				0.350
3				0.350
4				0.482
5				0.428
6				0.400
			Avg	0.415
			Std Dev.	0.060
			RSD, %	14.4

While the procedures supplied with the test kit provided an approximate structure for providing the data using a static (i.e., no embedded calculations in cells) data format there was considerable difference among the participants on the actual spreadsheet style and content that made collating more difficult and would prove even more daunting for a full round robin with many more participants. It is suggested that a specific template spreadsheet file be provided for all participants so that the chance of errors during data collating be minimized. Utilizing a dynamic cell calculation could also ensure that each participant used the exact same equations to reach the final data forms.

Since all three labs utilized the reference cantilever method, the collated data can be easily compared and are summarized in Figure 11. The error bars in each sample represents the standard deviation of the mean using the six repeat data on each cantilever.

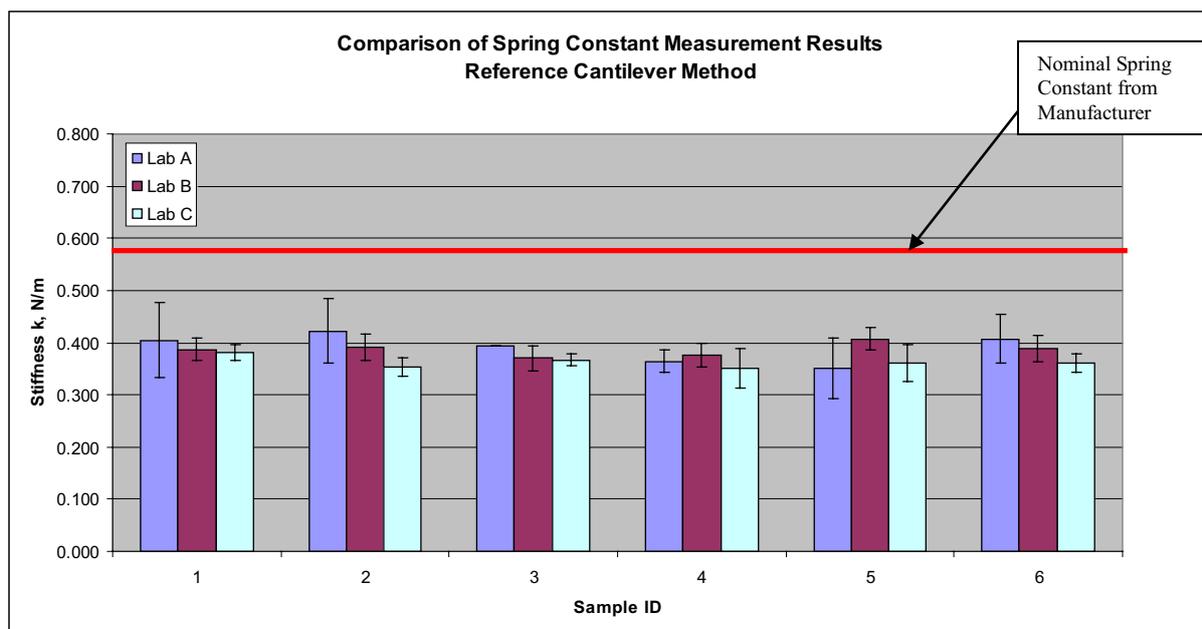


Figure 11. Results of reference cantilever method on the six test cantilevers from three different labs.

The average repeatability (relative standard deviation) of the measurements for all six cantilevers were 11% (Lab “A”) and 6% (Labs “B” and “C”). Three things are apparent from the graph. First, All three labs had statistically similar results. Second, the six test cantilevers were similar in spring constant. Third, the spring constants estimated from the reference cantilever method were all lower than the nominal value assigned by the manufacturer by about 30%. While the average repeatability of the measurements for lab “A” was typical for this type of measurement reported in the literature ( $\pm 10\%$  to  $\pm 30\%$ ), the observation of the variation of error bars for lab “A” suggests a granularity in the data that points to a statistical analysis issue. Test #1 had a repeatability of  $\pm 17\%$  (rsd) while test #3 had perfect repeatability. Looking at the raw data indicated that the problem lay with the number of reported significant digits (two) from that lab. Since the actual numbers reported (0.014) had a small first digit, change of just one digit in the second number is a change of almost 10%. The effect is to blow up small variations in the data. This highlights the need to specify a minimum number of significant figures (in this case three) for the raw data. If spreadsheet tables are included for each participant, then the number of significant figures might be specified in the cell format of the spreadsheet ensuring uniformity of data precision from each participant.

A second calibration method, the added mass method, was utilized by laboratory “B” and is compared to the reference cantilever method in Figure 12. The method used to acquire and report the added mass data are provided in Appendix 2. The repeatability of the added mass method was estimated at 6% (rsd) by laboratory “B” and is typical for their experience with this method.

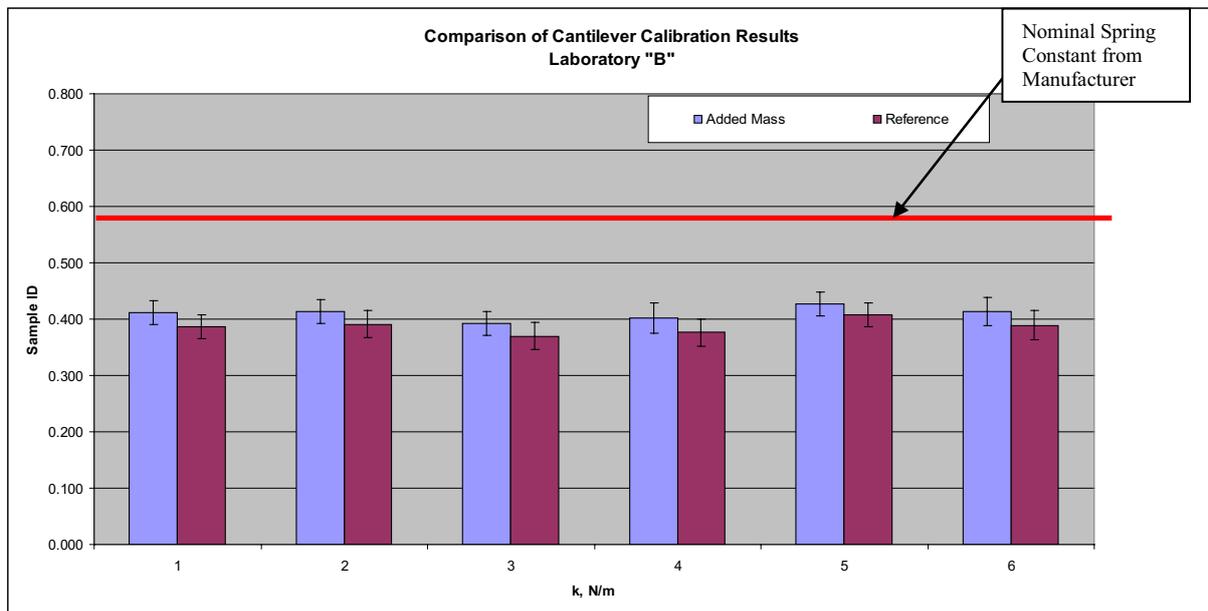


Figure 12. Comparison of added mass and reference cantilever methods for laboratory “B”.

The results of the two methods agree well statistically and reinforce the previous observation that the six test cantilevers are very similar in spring constant and that the measured calibration values are about 30% lower than the nominal values from the manufacturer. While the scope of this MRR is limited to looking at the precision of the calibration methods and not the accuracy,

the numerical agreement of the two methods is a positive sign that these two have similar absolute uncertainties.

### **Conclusions and Recommendations for a Future Round Robin**

Several recommendations for improving the execution of a future round robin have been provided during the discussion portions of this report. They are summarized here in bulleted form.

- Include flat bladed tweezers in test “kit”
- Break off unused cantilevers from test chips
- Use chip rocking/twisting method of removal from storage gel
- Use dynamic spreadsheet template for data entry and reporting
- Report to three or more significant figures

In addition, systematic characterization of the sample and reference cantilevers prior to and after testing may help document the effects of a large number of participants on the validity of round robin results on such methods where small scale changes may have considerable influence. Optical micrographs at several scales and resonance frequency measurements of the cantilevers are suggested.

The number of test cantilevers (six) used in this MRR is, in retrospect, excessive and increased the workload while offering little additional insight. It is suggested that the number of samples be reduced to two or three in future studies with the additional focus being put onto providing a wider range of cantilever types ( $\text{Si}_3\text{N}_4$  & Si, rectangular & triangular, range of spring constants) that cover the needs of the community. While this MRR was conducted without loss of either test or reference cantilevers (at least the ones that counted), it is anticipated that a wider round robin with more participants increases the likelihood of accidental damage to the samples and thought should therefore be given to providing a “backup” specimen (either test cantilever or reference artifact), “just in case” so that participants later in the study are given their chance to contribute to their full potential.

**APPENDIX 1**  
**AFM Cantilever Spring Constant Calibration**  
**DRAFT Reference Cantilever Method**

(Authors: Richard Gates and Mark Reitsma, NIST)

**1. Scope**

This method covers the calibration of the spring constant in the z (vertical) direction for Atomic Force Microscope (AFM) cantilevers using a reference cantilever.

**2. Referenced documents**

- 2.1 Torii, A., Sasaki, M., Hane, K. and Okuma, S. A method for determining the spring constant of cantilevers for Atomic Force Microscopy. *Measurement Science and Technology*. **7**, 179 (1996).
- 2.2 Tortonese, M. and Kirk, M. Characterization of application specific probes for SPMs *SPIE* **3009**, 53 (1997).
- 2.3 Gates, R. and Reitsma, M., Precise atomic force microscope cantilever spring constant calibration using a reference cantilever array. *Rev. Sci. Instr.* **78**, 086101 (2007).

**3. Terminology**

- 3.1 *Test cantilever*: a cantilever to be calibrated.
- 3.2 *Reference cantilever*: a cantilever of known spring constant (supplied for this method, can be purchased from Veeco, for example).
- 3.3 *Reference substrate*: microfabricated chip containing the reference cantilevers (Figures 1 and 3).
- 3.4 *Compliance region of force-displacement curve*: this refers to the elastic deflection portion of an AFM cantilever when pressed against another material (Figure 2).

**4. Significance and Use**

The reference cantilever method is used to calibrate AFM cantilever spring constants. This procedure is for the z (vertical) direction bending spring constant and can be applied to rectangular and triangular cantilevers, whether coated or uncoated, with sharp tips or colloidal probes. The basic assumption for this procedure is that the spring constant of

reference cantilever used for calibration should be in the same range of magnitude with the test cantilever spring constant.

## 5. Summary of Test Method

The method utilizes a reference cantilever of known spring constant. The reference cantilever is placed on the sample holder which was then placed on the sample stage of an AFM. A test cantilever of unknown spring constant is placed in the AFM cantilever holder and aligned with the laser deflection-sensor optics just as it would be for normal imaging operation. The test cantilever is brought into close proximity to the reference cantilever and a series of measurements are made using the force-displacement curve mode of the AFM.

Figure 1 shows the AFM configuration for this method where the reference cantilever mounted on a (z) scanning piezo AFM sample stage (Veeco Multimode<sup>®</sup> AFM used in this study) [it will also work in configurations in which the upper test cantilever is mounted on a (z) scanning piezo holder]. The test cantilever is secured above the reference cantilever in a cantilever holder.

In order to perform the reference calibration method, the test cantilever z deflection must be measured on both the reference cantilever ( $\delta_{cant}$ ) and an infinitely stiff surface ( $\delta_{rigid}$ ) approximated by the reference substrate.

$\delta_{rigid}$ : The test cantilever is placed into contact with the (Silicon) reference substrate, shown in Figure 1. The deflection of the test cantilever on this surface is measured as the substrate is moved vertically by an amount  $\delta_{rigid}$ .

$\delta_{cant}$ : The test cantilever is placed into contact with the free end of the reference cantilever, as illustrated in Figure 1, and the deflection of the cantilever under test,  $\delta_{cant}$ , is measured as the base of the reference cantilever moves vertically by the amount  $\delta_{rigid}$ .

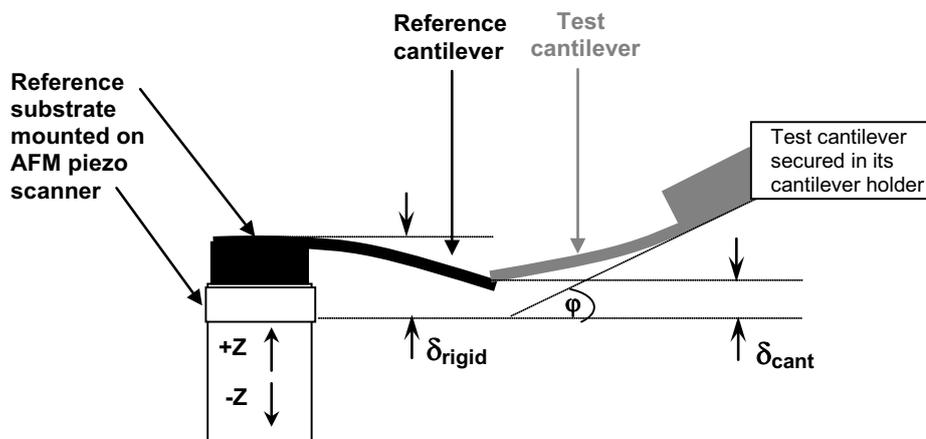


Fig. 1 Schematic diagram of the test cantilever pressed against the reference cantilever

The relationship between the spring constant of a test cantilever, the reference cantilever, and the deflections measured during contact were originally given by Torii et al. (ref 2.1) for horizontal cantilevers and by Tortonese and Kirk (ref 2.2) for an inclined test cantilever. Unfortunately, a small error in the derivation in reference 2.2 placed the  $\cos$  term in the denominator when it belongs in the numerator as a  $\cos^2$  term (ref. 2.3). For an 11 degree incline in the test cantilever this difference is about 6%. The correct form is:

$$k_{test} = k_{ref} \left( \frac{\delta_{rigid} - \delta_{cant}}{\delta_{cant}} \right) \cos^2 \varphi \quad \text{Equation 1}$$

In practical terms, the cantilever calibration is accomplished by recording the force-displacement curves for both (rigid surface and the test cantilever) cases and measuring the slopes of the straight-line (compliance) portions of the data. A typical force-displacement curve is shown in Figure 2.

In the “Approach” portion, the piezo (with attached reference cantilever/substrate) first moves upward in the +Z direction (see Figure 1) toward the test cantilever. The test and reference are out of contact at (a) and no deflection in the test cantilever occurs. Contact between the test cantilever and reference cantilever occurs at (b), sometimes accompanied by a small “snap-on” as the surfaces are brought into such close proximity that surface attractions pull them together.

As the sample continues to translate in the +z direction the test cantilever continues to deflect at the same speed as the surface it is touching. The region along (c) is called the *compliance region*.

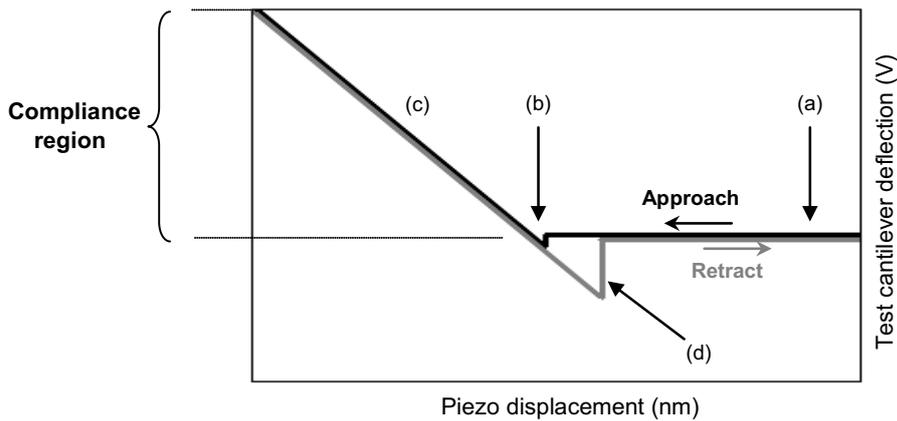


Fig.2. Force displacement curves of the approach and retract portions

For ideal elastic materials free of interferences, the compliance region portion of the trace should be linear. For the “retract” portion of the force curve, the piezo scanning direction is reversed (-Z; see Figure 1), and the compliance region along (c) is traversed again. Often, attractive forces between the test cantilever tip and the surface cause the surfaces to stay together until point (d) when the tip “snaps off” the surface. From then on, the piezo completes its scan with the surfaces out of contact once again along (a).

Calculation of the test cantilever spring constant is performed according to the steps suggested in reference 2.2. The actual units used in the force curve slope estimation do not matter since the results are taken as a ratio, as long as the same units are used in both measurements:

$S_{rigid}$  is the slope of the compliance region when the test cantilever is in contact with the reference substrate; typically, this value is given in volts per nanometer (V/nm) but volts per volt (V/V) is also commonly encountered.

$S_{cant}$  is the slope of the compliance region when the test cantilever is in contact with the free end of the reference cantilever.

If the normal spring constant of the reference cantilever at the actual point of contact of the tip is  $k_{ref}$ , then the normal spring constant of the test cantilever,  $k_{test}$ , can be calculated as

$$k_{test} = k_{ref} \left( \frac{S_{rigid} - S_{cant}}{S_{cant}} \right) \cos^2 \varphi \quad \text{or} \quad k_{test} = k_{ref} \left( \frac{S_{rigid}}{S_{cant}} - 1 \right) \cos^2 \varphi \quad \text{Equation 2}$$

where  $\varphi$  is the angle between the Test cantilever and the horizontal (see Figure 1). This angle value is supplied by the AFM manufacturer.

## 6. Atomic Force Microscope instrumentation

While the procedure can be used on any AFM, the procedure is written based on the instrument used (Veeco Multimode<sup>®</sup> AFM with Nanoscope IIIa controller). The general requirement for the AFM are:

6.1 The AFM must be equipped with an optical microscope capable of viewing a mounted reference cantilever and test cantilever simultaneously in order to align and superimpose them with a reasonable degree of accuracy. Tip placement accuracy should be 5  $\mu\text{m}$  or better.

6.2 AFM instrument must be able to acquire and save force-displacement curve data.

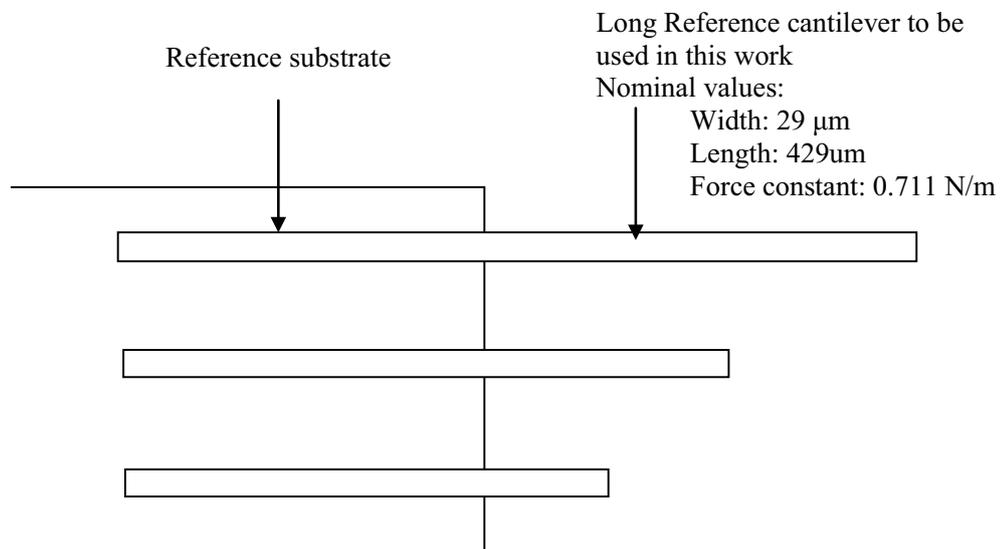
## 7. Materials and preparation

### 7.1 Optics

All optical instrumentation used for length/dimension measurement (including the AFM overhead optics) should be calibrated before use. We have found that one practical way to make rapid alignment measurements using video optics is to translate the cantilever a prescribed amount (e.g. cyclic scanning 10  $\mu\text{m}$ ) and note the extremes of motion on the video screen. A properly sized marker (e.g. 5  $\mu\text{m}$ ) applied to the video screen then provides a fiduciary comparison for estimating the tip location for aligning the test and reference cantilevers.

### 7.2 Reference cantilever

For this procedure, a commercial reference cantilever chip (Veeco® CLFC-NOBO) has been supplied. The chip consists of three reference cantilevers. The reference cantilever to be used is the longest cantilever in the set as illustrated in Figure 3 (nominal values given by the supplier: Width: 29  $\mu\text{m}$ ; Length 429  $\mu\text{m}$ ; Spring constant 0.711 N/m).



**Figure 3**

### 7.3 Measure the position of the Test cantilever tip, $\Delta L_{tip}$

Record an optical image of the integrated tip (cantilever inverted) and measure the distance between the integrated tip apex and the end of the cantilever as illustrated in Figure 4. This distance,  $\Delta L_{tip}$ , must be considered in order to accurately locate the contact point of the tip on the reference cantilever.

In practice, for a triangular test cantilever, this can be accomplished by visually estimating the relative position of the integrated tip with respect to the two “V” portions of the test cantilever. The integrated tip apex relative location will be in the same spot when the cantilever is flipped over as it would be for any top-view optical system.

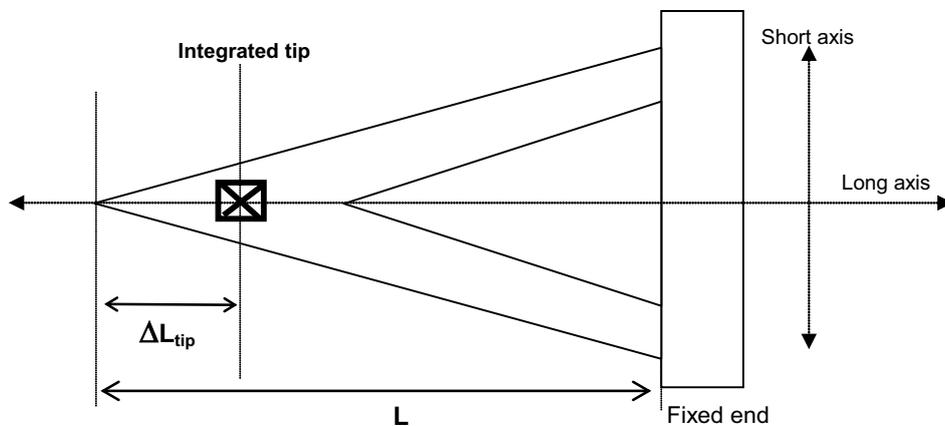


Figure 4

## 8. Procedure

The following procedure was written specifically for the instrumental setup used in our laboratory. The commercial AFM used was a Veeco® Digital Instruments Multimode AFM with a Nanoscope IIIa controller. Variations of this procedure may be required for other experimental setups.

- 8.1 Adjust the xy positioning of the AFM head to make sure it is roughly centered over the sample stage. Ensure the AFM sample stage is well lowered (i.e. “tip up”), and then insert the test cantilever (in holder) into the AFM head and clamp it in place.
- 8.2 Focus the overhead optics onto the test cantilever. It will be pointing to the left as shown in figure 4. Once a clear image of the test cantilever can be seen, adjust the optics xy position to place the cantilever on the right side of the field of view. From this point on, do not adjust the xy positioning for the overhead optics. Remove the test cantilever holder from the AFM head.

- 8.3 Place the reference cantilever sample puck onto the center of the AFM sample stage without adjusting the overhead optics in x or y. Focus the overhead optics onto the reference cantilever chip. Carefully move and rotate the reference cantilever puck using tweezers so that the reference cantilever to be used is in the field of view and pointing horizontally and to the right on the viewing screen as shown in figure 3.
- 8.4 Place the test cantilever (in holder) into the AFM head and clamp it into place.
- 8.5 Using the xy positioning for the AFM head, ensure that the position of the Test cantilever can be adjusted such that it can reach both the free end of the reference cantilever to be used, as well as the reference substrate.
- 8.6 Focus and optimize the AFM laser optics (on the Test cantilever) as described in the AFM instrument manufacturer's instruction manual.
- 8.7 Lower the AFM head ("tip down") until the test cantilever is close to the reference cantilever. This can be done by focusing the overhead optics on the reference cantilever and lowering the AFM head in small increments until the test cantilever comes into view, but not quite into focus.
- 8.8 Using the xy positioning of the AFM head, move the test cantilever over to the free end of the reference cantilever to be used. Align the centerline of the long axis of the test cantilever with that of the reference cantilever (see Figure 4). Align both cantilevers such that the end of the test cantilever coincides with the end of the reference cantilever. Using the end of the reference cantilever as the zero point, adjust the AFM head position so that the end of the test cantilever is positioned at  $2x\Delta L_{\text{tip}}$  (see 7.3) from the end of the reference cantilever. This will place the tip of the cantilever at a contact point  $\Delta L_{\text{tip}}$  from the end of the reference cantilever.
- 8.9 As a start, use a force curve ramp size of 500nm and engage the samples as described in the AFM instrument manufacturer's instruction manual. Once the tip has engaged the sample, a force curve can be acquired and saved. Ensure that the constant compliance region (see Figure 2) of the acquired force-separation curve is linear. If bowing is seen, reduce the Z scan size and acquire another force-separation curve. Note that both  $\delta_{\text{rigid}}$  and  $\delta_{\text{cant}}$  should be recorded at the same ramp size. Save the force curve data under an appropriate name (e.g. rigid01).
- 8.10 Before adjusting the xy positioning of the AFM head, make sure the reference and test specimens are well separated in the Z direction (i.e. tip retracted). Position the test cantilever over a clean area of the substrate base of the reference cantilever (see Figure 3).
- 8.11 Lower the AFM head until the test cantilever is a few microns above the reference substrate. This can be done by focusing the overhead optics onto the reference

substrate surface and lowering the AFM head in small increments until the Test cantilever comes into view, but not quite into focus.

8.12 Engage the samples as described in the AFM instrument manufacturer's instruction manual. Acquire and save the force curve data as before. The two force curves (on reference cantilever and substrate) constitute the data pair that is used in calculating the spring constant.

8.13 Repeat 8.6–8.12 to acquire further force curve measurements. At least six (6) measurement pairs should be recorded. Each data set pair should be adequately labeled to reveal the pairing (e.g Cant01, Rigid01 etc.) to facilitate later data analysis. Minimal delay (<3 minutes) should be allowed between the two measurements in a pair of measurements to minimize instrument drift effects.

## **9. Sources of Error**

The largest potential source of error lies in the location of the tip on the reference cantilever. Since for a rectangular cantilever beam the spring constant changes with the length *cubed*, even small errors can affect the final measurement.

## **10. Precautions**

Care should be taken to ensure that the z scan range in the force curve does not exceed the linear range of the photodetector/optical lever system. Excessive force applied between the test cantilever and a surface may also cause damage to the tip and buckling of the end of the cantilever.

## **11. Results reporting and adjustment**

For each force-separation curve, isolate the compliance region portion of the data (shown in Figure 2) and determine the slope of this region. It is recommended that a graphical assessment of the analyzed portion of the data also be made to ensure no artifacts are included in the data analysis.

Please indicate how the compliance slope is determined (e.g. linear regression fit to ASCII data; using a software package) and how much of the compliance curve data is used.

### 11.1 Results reporting table

Table 1 is an example of how the results should be recorded (electronic spreadsheet format is preferable).

Cantilever ID	$\Delta L_{tip}$ um	$\phi$	Ramp size, nm	Test #	$S_{rigid}$ V/nm	$S_{cant}$ V/nm
DNP 1A	5.0	11°	300		Approach or retract	Approach or retract
				1	0.0234	0.0145
				2	0.0221	0.0151
				3	0.0229	0.0157
				4	0.0235	0.0142
				5	0.0226	0.0146
				6	0.0238	0.0153

**Table 1**

### 11.2 Sample calculation

The nominal value given for the spring constant of the reference cantilever is the estimated stiffness at the end of the beam. In this method, however, the load is applied to the reference cantilever at a distance of  $\Delta L_{tip}$  from the end (see 7.3) which will result in a slightly higher stiffness. To correct the calculated data for the distance between the point of contact between the two cantilevers ( $\Delta L_{tip}$ ) and the end of the reference cantilever we need to apply an *off-end loading correction*. Since  $k$  (spring constant) varies as the cube of the length ( $L$ ), the off-end correction is applied as:

$$k_{ref} = k_{end} \left( \frac{L}{L - \Delta L_{tip}} \right)^3 \quad \text{Equation 3}$$

where  $L$  is the length of the reference cantilever from its fixed end to its free end and  $k_{end}$  is the spring constant of the reference cantilever defined at the end of the cantilever. For the reference cantilever used in this work, the nominal value provided by the supplier is  $k_{end} = 0.711$  N/m.

The value of  $k_{ref}$  is then used in Equation 2. Using the value of  $\phi$  for our AFM (11°) in Equation 1, along with the other data shown in Table 1,  $k_{test}$  is calculated for each test as shown in the table 2. The average, standard deviation and relative standard deviation of the six measurements should also be provided in the table.

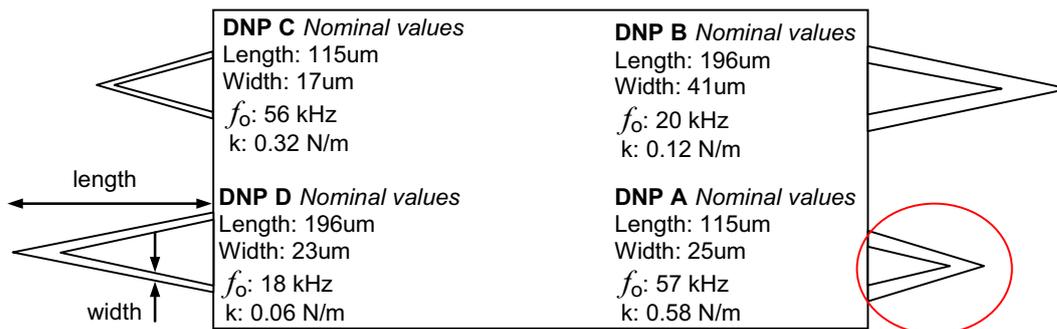
Test #	L um	$\Delta L_{tip}$ um	$k_{ref}$ N/m	$k_{test}$ N/m
	429	5.0	0.736	
1				0.482
2				0.350
3				0.350
4				0.482
5				0.428
6				0.400
			Avg	0.415
			Std Dev.	0.060
			RSD, %	14.4

**Table 2**

## 12. Cantilevers to be calibrated

### 12.1 Cantilever type

The cantilevers to be calibrated in this work are six DNP “A” cantilevers. On the microfabricated chip, DNP A are the short, thicker legged cantilevers as indicated in Figure 5 below. The values listed are the nominal values provided by the manufacturer.



**Figure 5**

### 12.2 Orientation of cantilevers on gel-pack

The DNP A cantilevers to be used are numbered 1-6 and orientated on the supplied gel-pack as shown in Figure 6 below. Care should be taken to ensure that cantilevers are returned to the pack in the correct orientation.

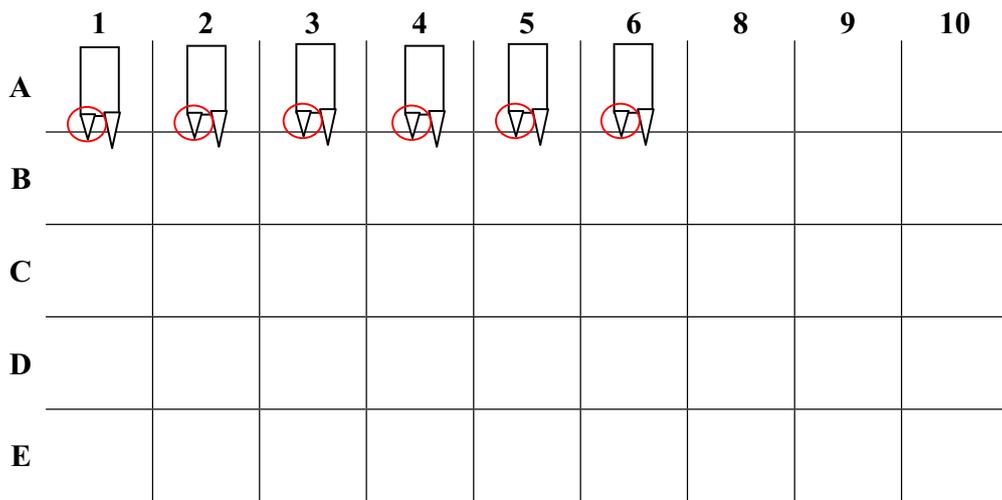


Figure 6

## **AFM Cantilever Spring Constant Calibration DRAFT Added Mass Procedure**

(Authors: Richard Gates and Mark Reitsma, NIST)

### **1. Scope**

This procedure covers the calibration of Atomic Force Microscope (AFM) cantilever spring constants in the z direction (vertical) using the added mass (“Cleveland”) method, modified for off-end corrections.

### **2. Referenced documents**

- 2.1 Cleveland, J.P., Manne, S., Bocek, D., Hansma, P.K. A nondestructive method for determining the spring constant of cantilevers for scanning force microscopy. *Review of Scientific Instruments*. **64**(2) 403 (1993).
- 2.2 Sader, J.E., Mulvaney, P., and White, L.R. Method for the Calibration of Atomic Force Microscope Cantilevers. *Review of Scientific Instruments* **66** (7) 3789 (1995).
- 2.3 Sader, J.E. Parallel beam approximation for V-shaped atomic force microscope cantilevers. *Review of Scientific Instruments* **66** (9) 4583 (1995).

### **3. Terminology**

- 3.1 *AFM*: Atomic Force Microscope
- 3.2 *Resonance frequency*  $f$ : is the first bending mode resonance frequency in the z axis direction, perpendicular to the (x-y) plane of the cantilever.
- 3.3 *Cantilever resonance frequency*:  $f_0$  is the normal (z) resonance frequency of the cantilever without added mass.
- 3.4 *Test cantilever*: cantilever to be calibrated.
- 3.5 *Cantilever holder*: AFM cantilever holder, which is used to mount the test cantilever in AFM.
- 3.6 *Loaded resonance frequency*:  $f_i$  is the normal (z) resonance frequency of the cantilever measured with an added mass ( $m_i$ ).

3.7 *Cantilever tip*: the actual tip apex (point of contact) that is made with the surface when an AFM cantilever is used. The length of the cantilever from the fixed base to the tip is designated as  $L_t$ .

3.8 *Cantilever end*: the free end of the cantilever. The length of the cantilever from the fixed base to the free end is designated as  $L_e$ .

#### 4. Significance and Use

The added mass method is used to calibrate AFM cantilevers in the z (vertical) direction. It can be applied to rectangular, triangular, coated or uncoated cantilevers with sharp tips or colloidal probes. The key requirement is that the locations and the mass of the spheres added for frequency measurements can be measured accurately.

#### 5. Summary of Test Method

5.1 The z resonance frequency,  $f_0$ , of the Test cantilever is measured.

5.2 A tungsten or gold sphere is placed at the free end of the cantilever.

5.3 The size and position of the sphere on the cantilever is measured (e.g. using a suitable calibrated microscope).

5.4 The resonance frequency of the cantilever with attached sphere is measured. The sphere is then removed.

5.5 Steps 5.2 – 5.4 are repeated for at least 2 spheres (3 point plot) with 5 spheres (6 point plot) desirable. The range of sphere size depends on the spring constant but in general 5  $\mu\text{m}$  to 15  $\mu\text{m}$  size spheres are used.

5.6 The mass of each spherical mass added ( $m_i$ ) is calculated from the measured diameter and known density of the material.

5.7 The general relationship between added mass,  $m_i$ , and resonant frequency,  $f_i$ , is

$$m_i = k \left( \frac{1}{2\pi f_i} \right)^2 - m^* \quad \text{Equation 4}$$

where  $k$  is the spring constant of the cantilever. The quantity  $m^*$  is called the ‘effective mass’ of the cantilever. If several known masses are added to the end of a cantilever and resonance frequencies are measured for each added mass, a linear plot of added mass  $m_i$  versus  $(2\pi f_i)^{-2}$  will give a straight line with slope of  $k$  and an ordinate intercept of  $-m^*$ .

## 6. Atomic Force Microscope instrumentation

This method requires an Atomic Force Microscope instrument with hardware and software suitable for cantilever resonance frequency measurement. For this procedure, a Veeco® Digital Instruments Multimode AFM with Nanoscope IIIa controller was used.

## 7. Materials and preparation

This method uses a sharp tungsten wire to pick up, maneuver and attach spherical particles to the cantilever. It relies on attractive meniscus forces to pick up the spheres; therefore it may be sensitive to changes in relative humidity. The relative humidity of the laboratory used for this work was 45% ±5%.

### 7.1 *Spheres*

Powder consisting of spherical gold or tungsten spheres. For this purpose, 325 mesh spherical gold powder can be used (Alpha Aesar®; stock #43900, lot#G11N23, 99.9% purity on a metals basis). Tungsten spherical particles donated by Asylum Research (Santa Barbara, CA) are also available to participants.

### 7.2 *Tungsten wire (for suggested sphere mounting apparatus in 7.5)*

For this work, 0.25mm diameter (Alpha Aesar® #10408, 99.95% purity) tungsten wire was used to create a sharp micromanipulator probe tip to manipulate the spherical particles. Other wire materials that can be sharpened to a fine point may also be suitable.

### 7.3 *Electrochemical etching of a tungsten wire*

There are a number of different techniques available for tungsten wire etching. It is left to the user to decide the best method by which to electrochemically etch the end of a tungsten wire down to a fine point (ca 100nm radius of curvature). Note that too fine a point is not desirable since one wants a large enough area of contact for the sphere to adhere to the tip when the meniscus forms between the contacting surfaces.

### 7.4 *Optical Microscope*

All optical instrumentation used for length/dimension measurement must be calibrated before use.

### 7.5 *Suggested sphere mounting apparatus*

Figure 1 shows the sphere mounting apparatus used in our laboratory. You are welcome to use other setups but please provide details on the apparatus and procedure used. The following are the major components of our apparatus.

7.5.1 *Optical microscope:* A stereo microscope with a long working distance was used to allow simultaneous observation and micromanipulation. A field of view of 500 um or less is necessary to allow location, pickup, and placement of small spheres onto the cantilevers with sufficient control.

- 7.5.2 *Sphere slide*: Tungsten or gold spheres are placed onto a clean glass microscope slide in such a way as to provide a large number of isolated spheres that can be picked up with the tungsten probe.
- 7.5.3 *Probe translation stage*: An xyz translation stage capable of at least 10 mm travel in each direction is used to manipulate the tungsten probe above the surfaces. Micrometers on each axis provide translation adjustment of each axis.
- 7.5.4 *Etched tungsten wire*: An electrochemically etched tungsten wire attached to a rigid rod was used to maneuver the spheres (See Figure 1). An example of such a rod would be a tool steel rod approximately 3mm in diameter and 150 mm long.
- 7.5.5 Pickup and deposition of the spheres is best accomplished with a combination of orthogonal (xyz) mechanical translation axes and haptic (tactile feedback) controls as shown in Figure 1. The translation stage is used for coarse adjustment of the probe to a location just above the surface. By exerting gentle pressure on the rigid rod with one's fingers the operator can cause the tungsten probe tip to smoothly approach the surface in the proximity of a sphere (*ca.* 10-20 $\mu$ m travel).

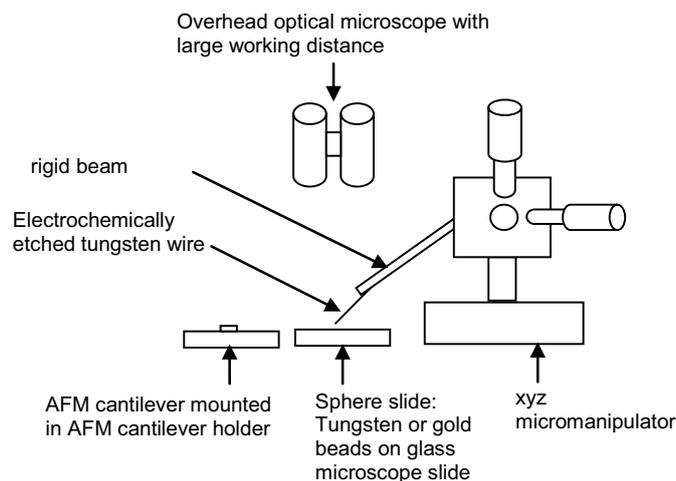


Fig. 1 Schematic diagram of pick up and deposition of spheres

## 8. Procedure

Using ‘non-critical’ cantilevers for practice, it is recommended that the individual user decide the micromanipulation method most suitable to them for mounting and removing spheres. It is also recommended that each user be well rehearsed in their chosen technique before proceeding to calibrate the VAMAS cantilevers. The following steps are based on the suggested sphere mounting apparatus described in 7.5 above.

- 8.1 Ensure the AFM head is raised with sufficient clearance above the sample and place the cantilever holder (containing the test cantilever) into the AFM head. Lock it into place. Focus and optimize the AFM laser optics onto the test cantilever and perform the resonance frequency analysis on the cantilever as described in the AFM instrument manufacturer’s instruction manual. Sometimes this is referred to as “tuning” the cantilever. Once the resonance frequency for the test cantilever has been recorded, remove the holder from the AFM instrument and transport it to the sphere mounting apparatus under the stereomicroscope (Figure 1).
- 8.2 Ensure the surface of the sphere slide is slightly higher (z axis) than the cantilever in the cantilever holder (see Figure 1). Focus the overhead optics onto the sphere slide and select a uniform, symmetric sphere.
- 8.3 Lower the tungsten tip to within several micrometers of the target sphere and use gentle, fine motion to establish contact between the tip and sphere. If performing fine motion by hand, a small force is applied to the semi-rigid beam to traverse the final distance and establish contact with the sphere. By controlling the (finger) pressure to the beam, spheres can be contacted and picked up in a single, smooth, down-up motion. Furthermore, since spheres can move around slightly on the slide before they stick to the tungsten tip, it is found that finger control offers more freedom of movement and can thus be a more effective technique for pick up than using the micromanipulator adjustment micrometers alone.
- 8.4 After the target sphere has been picked up, make sure the tungsten tip is raised enough before removing the sphere slide and replacing it with the cantilever holder. Focus the overhead optics such that both the tip and the cantilever below it can be seen.
- 8.5 Focus the overhead optics onto the cantilever and then carefully lower the tungsten tip down to within several micrometers of the cantilever. Using the same technique as described in 8.3, place the sphere on the end of the cantilever (avoid contact with the integrated tip of the cantilever). Place the sphere close to the centerline of the long axis of the cantilever near the integrated tip (see Figure 2).

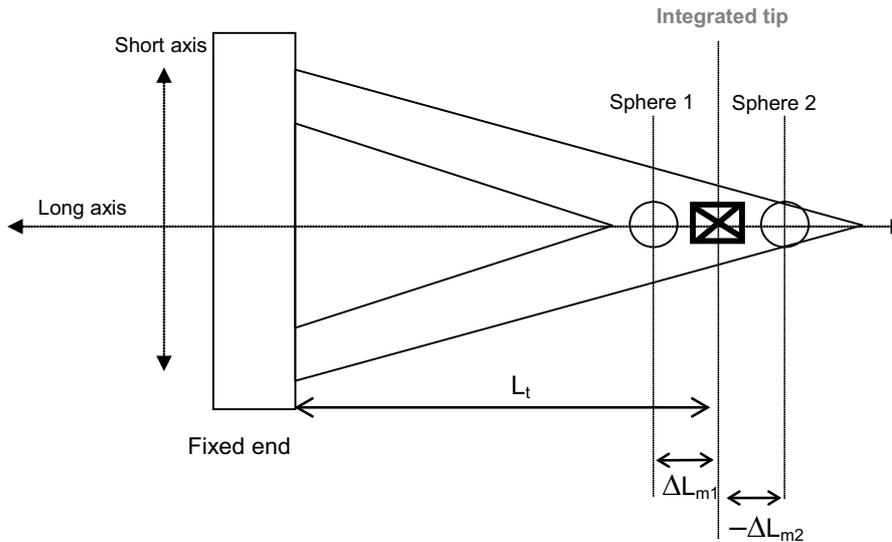


Fig. 2. Placement of the sphere on the center line of the cantilever

- 8.6 Once the sphere has been placed onto the cantilever, raise the tungsten tip clear. Record an image of the sphere on the cantilever. Ensure that two measurements can be made from the image(s): (1) The diameter of the sphere, and (2) the position of the (center of) sphere relative to the integrated tip.
- 8.7 Place the holder (containing the test cantilever) into the AFM head and lock it into place. Focus and optimize the AFM laser optics (on the test cantilever) and perform a resonance frequency analysis on the cantilever as described in 8.1. Once the resonance frequency for the test cantilever has been recorded, remove the holder from the AFM instrument and transport it to the sphere mounting apparatus.
- 8.8 In the same fashion as described in 8.3, remove the sphere from the cantilever.

**CAUTION:** This is often the most difficult and potentially damaging part of the procedure. If contacting the sphere with the tungsten tip proves unsuccessful, there are several alternatives that can be tried. Switching to a less sharp tungsten tip (stronger meniscus forces) that can more easily pick up the sphere usually helps but the sharper tip must be switched back for the next sphere placement. Spheres can often be removed by very carefully ‘flicking’ (oscillating) the end of the cantilever with the tungsten tip. IMPORTANT: extra care is required to avoid catching the edge of the cantilever with the tungsten probe if this later technique is needed. Alternatively, spheres can often be detached by driving the resonance externally with a moderately high amplitude in the AFM. The sphere has been detached when the resonance peak jumps back to the initial (higher) resonance frequency determined in step 8.2 above.

Care must be taken to ensure that the sphere has actually been removed and has not merely moved to the underside of the cantilever. A resonance frequency measurement can confirm this (the frequency should return to the original resonance frequency ( $f_o$ )).

8.9 Repeat steps 8.2-8.8. It is recommended that a minimum of three sphere measurements are recorded, with a difference of >20% in diameter for each new sphere added. This will be combined with the unloaded (no added mass) resonance frequency measurement to yield a four point plot. A five sphere (six total data point) plot is considered optimal.

8.10 Measure the unloaded resonance frequency of the test cantilever once again as a final step (the value should be within 0.5% of that acquired before calibration).

## 9. Sources of Error

The largest potential for error lies in the sizing of the spheres and the estimates for the sphere placement on the cantilever. For this reason, a calibrated optical microscope with digital image capture capabilities is desirable.

## 10. Precautions

Care needs to be taken in placing the test cantilevers into the AFM holder to avoid breakage. Placement of the sphere onto the cantilever and removal of the spheres is potentially damaging to the cantilever and therefore requires caution.

**THIS PROCEDURE SHOULD ONLY BE ATTEMPTED ON THE VAMAS CANTILEVERS BY TRAINED AND EXPERIENCED PERSONNEL.**

## 11. Results reporting

### 11.1 Sphere positioning and off-tip correction

The spring constant of the cantilever should be determined at the integrated tip position. Since spheres should be placed along the long axis and they cannot be placed in the same position as the tip, it is important to note the position of the sphere relative to the integrated tip of the cantilever. In principle, measuring the resonance frequency of a test cantilever with a mass added on the free end means that the added mass probes the spring constant of the cantilever from its fixed end to the position of the mass. In order to make this correction later on, the position of the sphere relative to the integrated tip needs to be recorded. That is, the distance from the integrated tip apex to the center of the sphere.

Record your sphere positions according to the convention shown in Figure 2. These offsets are then used to correct the added mass using equation 2 to provide the effective masses added ( $m_{ie}$ ). That is, spheres placed between the integrated tip and the free end of the cantilever (e.g. sphere 2) are negative values ( $-\Delta L_m$ ) and will have the effect of increasing the effective added mass relative to the tip apex. Spheres placed between the tip and the fixed end (e.g. sphere 1) are positive values ( $+\Delta L_m$ ) and will have the effect of decreasing the effective added mass.

$$m_{ie} = m_i \left( \frac{L_t - \Delta L}{L_t} \right)^3 \quad \text{Equation 5}$$

Note that for this procedure, we are not taking off-axis loading into account (i.e. sphere placement away from the tip along the short axis). More information about the correction technique to be performed can be found in reference 2.2.

### 11.2 Results reporting table

Below is an example of how the results should be recorded. Electronic (spreadsheet) format is preferable.

Cantilever ID	L um	Sphere material	Sphere number	Sphere diameter, um	Resonance Frequency, kHz	$\Delta L_m$ um
DNP 6A	108	Gold	-	0	62.8	-
		19300	1	3.8	56.0	+1.9
		Kgm <sup>-3</sup>	2	5.7	46.8	-1.4
			3	9.5	31.2	-1.1
			-	0	62.8	-

**Table 1**

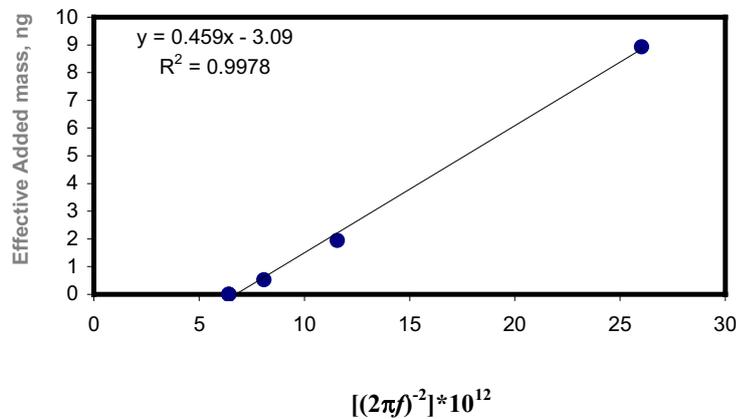
### 11.3 Sample calculation

The following is a sample calculation for the results shown in Table 1 above. The calculation includes the so-called Sader off-end correction (see reference 2.2), which corrects for sphere placements at some distance,  $\Delta L_m$ , away from the desired position of spring constant determination. For this method we want to determine the spring constant at the position of the integrated tip, since this is the point along an AFM cantilever at which loading normally takes place. For each single added mass, use  $\rho = 19300 \text{ kgm}^{-3}$  (density of gold), and  $\rho(4/3)\pi r^3$  to determine the mass,  $m_i$ , of each sphere added. If tungsten is used, the density should be  $19250 \text{ kgm}^{-3}$ . Then apply the off-end correction

(equation 2) to give the effective mass ( $m_{ie}$ ). The general relationship between added mass, resonance frequency and spring constant (equation 1) becomes more specific when  $k$  and  $m$  are defined for the actual location of the integrated tip:

$$m_{ie} = k_e \left( \frac{1}{2\pi f_i} \right)^2 - m^* \quad \text{Equation 6}$$

A plot of measured resonance frequency  $(2\pi f_i)^{-2}$  versus effective added masses ( $m_{ie}$ ) should yield a straight line of slope  $k_e$  (the effective spring constant at the point of the integrated tip) as shown in Figure 3.



**Figure 3**

Regression analysis should be used to calculate the slope, intercept and uncertainty in the slope estimation (standard error of slope estimate). All of these values can be reported on an extended version of table 1.

## 12. Cantilevers to be calibrated

### 12.1 Cantilever type

The cantilevers to be calibrated in this work are six DNP “A” cantilevers. On the microfabricated chip, DNP A are the short, thicker legged cantilevers as indicated in Figure 4 below. The numbers listed are nominal values provided by the manufacturer.

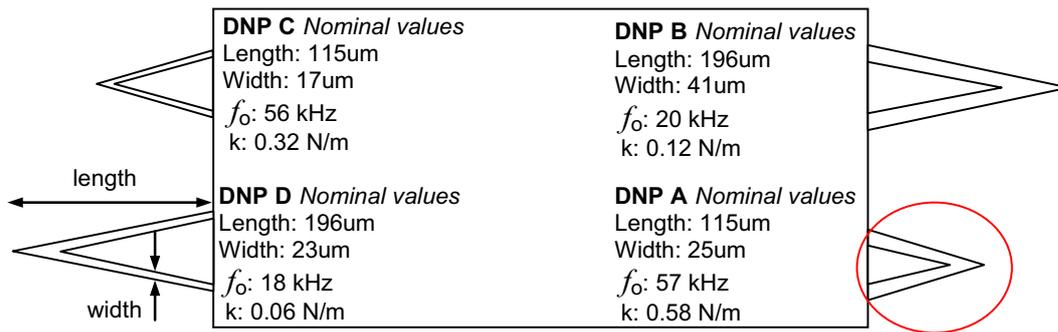


Figure 4

### 12.2 Orientation of cantilevers on gel-pack

The DNP A cantilevers to be used are numbered 1-6 and orientated on the supplied gel-pack as shown in Figure 5 below.

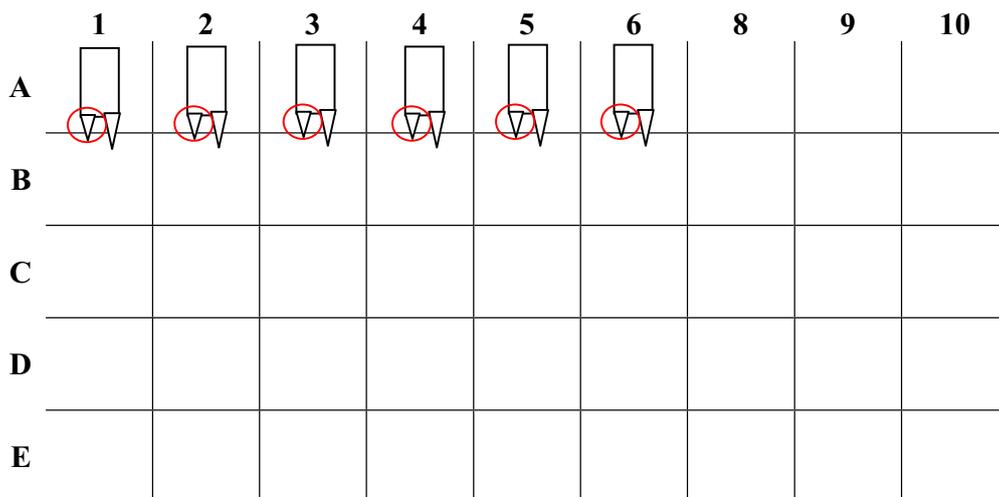


Figure 5

