

Accurate flexural spring constant calibration of colloid probe cantilevers using scanning laser Doppler vibrometry

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Abstract

Calibration of the flexural spring constant for atomic force microscope (AFM) colloid probe cantilevers provides significant challenges. The presence of a large attached spherical added mass complicates many of the more common calibration techniques such as reference cantilever, Sader, and added mass. Even the most promising option, AFM thermal calibration, can encounter difficulties during the optical lever sensitivity measurement due to strong adhesion and friction between the sphere and a surface. This may cause buckling of the end of the cantilever and hysteresis in the approach–retract curves resulting in increased uncertainty in the calibration. Most recently, a laser Doppler vibrometry thermal method has been used to accurately calibrate the normal spring constant of a wide variety of tipped and tipless commercial cantilevers. This paper describes a variant of the technique, scanning laser Doppler vibrometry, optimized for colloid probe cantilevers and capable of spring constant calibration uncertainties near $\pm 1\%$.

Keywords: calibration, cantilever, colloid probe, spring constant, vibrometry

(Some figures may appear in colour only in the online journal)

1. Introduction

Atomic force microscopy (AFM) is a valuable tool to interrogate the mechanical properties of materials and surfaces. One particular variant of AFM, known as colloid probe AFM, uses cantilevers with a spherical tip in place of the normal sharp tip. The greatly increased radius of curvature (on the order $5\ \mu\text{m}$ instead of $10\ \text{nm}$) of the probe tip allows for more controlled and easily modeled interactions between probe and surface. Spherical particles of a wide range of materials have been attached to cantilevers including silica, silicon nitride, gold, tungsten, and polymers. Often, surfaces are modified chemically to provide higher chemical specificity to the interaction of interest. A comprehensive review of force measurements in AFM that also covers colloid probe techniques has been provided by Butt *et al* [1].

Examples of AFM colloid probes used to measure the physical and chemical interactions between surfaces include measurements of adhesion [2], meniscus forces [3], chemical interactions [4, 5], and Casimir forces [6, 7]. In these applications, the interest is in accurately measuring these forces; therefore, the spring constant of the cantilever must be accurately known.

Although the presence of the sphere attached at the end of the cantilever provides additional measurement capabilities, it also presents additional challenges for spring constant calibration. Three main issues are usually observed. The large physical size of the sphere (sometimes $50\ \mu\text{m}$ or more in diameter) relative to the cantilever size can obscure alignment of the contact point or it may provide a torque on the cantilever because of the long lever arm (the sphere diameter) between the contact point and the cantilever. Any force imposed at the contact point (e.g., by friction during a

load–unload optical lever sensitivity (OLS) measurement) could induce an angle change at the end of the cantilever which may be mis-interpreted as a z deflection [8, 9]. This will introduce an error into the spring constant calibration. The large curvature of the surface of the sphere will also tend to accentuate adhesion and friction issues. Finally, the presence of glue used to attach the sphere to the cantilever can make accurate modeling impractical so techniques that rely on dimensional modeling are problematic. Glue can also cause stiffening of the cantilever so techniques relying on cantilever calibration prior to sphere attachment may not be very accurate.

The most common spring constant calibration techniques all encounter problems that negatively affect the accuracy of applying the technique to colloid probes when compared to probes with standard tips. The reference cantilever calibration method [10] is difficult to apply due to uncertainty in the location of the contact point on the reference cantilever as well as adhesion and friction issues encountered during contact. The Sader calibration method systematically under-values the stiffness of cantilevers when colloid spheres are added as demonstrated by Green *et al* [11] for the ‘Original Sader’ method [12], and Sader *et al* [13] for the ‘General Sader’ method [13]. With very small spheres the errors are only a few percent but becomes increasingly significant with larger spheres such that errors approach 30% when spheres are 2/3 of the cantilever leg width (e.g. a 14 μm diameter sphere on a cantilever with 21 μm wide legs) [13]. Later refinements by Sader *et al* in 2012 [14] simplified the ‘General’ method for determining the hydrodynamic functions for the general plan shapes of a cantilever but suffers from the same underestimation weakness for cantilevers when the hydrodynamic function is determined for the general plan shape and then colloid spheres are added unless the spheres are very small or a new hydrodynamic function is determined for the specific cantilever with the specific sized sphere already attached. The Cleveland added mass calibration technique [15] requires some free flat areas near the end of the cantilever and this is usually taken up by the sphere. The presence of glue precludes the use of even a simple two point added mass calibration estimate since it will modify the intrinsic stiffness of the cantilever in addition to the effect of the added unknown mass from the glue. AFM thermal calibration would seem to be well suited for colloid probes but in this case, the complications occur during the OLS measurement that requires performing an approach–retract cycle to calibrate the voltage–displacement relationship for the setup. This requires that the cantilever sphere actually touch the surface and this introduces adhesion and friction issues [16] that accentuate errors as well as possible contamination of the colloid probe surface. Recently, one AFM manufacturer³ [17] has incorporated the method of Higgins *et al* to develop a

completely non-contact calibration method for both determining cantilever spring constant (k) and OLS of the cantilever in the AFM holder. The technique uses the Sader method to get the k and AFM thermal method to get the OLS. The Sader method is problematic for larger colloid probes; however, if accurate spring constants could be obtained for colloid probe cantilevers via another method, use of the AFM thermal method for determining the OLS would be a reasonable approach to calibrated use of the cantilever in an AFM.

The work of Chung *et al* [16] is a comprehensive comparison of several techniques for calibrating the spring constant of colloid probe cantilevers and encompasses the reference cantilever (and a variant—the piezoresistive force transducer) as well as the AFM thermal method. In a series of repeat tests on several colloidal probe cantilevers they obtained relative uncertainties⁴ of $\pm 11\%$ (reference cantilever method), $\pm 14\%$ (piezoresistive force transducer method), and $\pm 5\%$ (AFM thermal method). They also introduced a unique electrostatic microelectrode probe technique that eliminated the need to touch the surface during calibration and were able to obtain relative uncertainties near $\pm 10\%$.

Laser Doppler vibrometry (LDV) has been applied to measure the frequency spectra of a wide variety of vibrating structures, from MEMS and hard drives to cantilevers [18] and nanowires [19, 20]. The issue of the absolute accuracy of the LDV measurements for the vibrational amplitude scale required for the thermal calibration method on AFM cantilevers (on the order of 10 pm) has been uncertain until recently because manufacturer’s instrument calibrations are usually associated with an amplitude scale orders of magnitude higher (1–100 nm). Previously, Gates and Pratt [21] demonstrated that the LDV thermal calibration method was capable of calibrating the (static) spring constant of tipless, uniform, rectangular reference cantilevers with very good accuracy and precision by comparing the measurements to an SI traceable technique [22]. Uncertainties were typically near $\pm 1\%$. Later, Gates *et al* [23] showed that accurate LDV thermal calibrations could be extended to a wider variety of commercially available cantilevers useful for AFM by applying the proper mode correction factor (MCF) to the LDV thermal measurements. This included triangular and rectangular cantilevers as well as cantilevers which had a small mass added to the ends (either as tips or small colloid spheres). They demonstrated that MCF’s for most cantilevers varied from 0.95 to 1.0, depending on the type of cantilever and mass added. Uncertainties near $\pm 1\%$ were obtained. It should be noted that other researchers [14, 18] have reported somewhat higher uncertainties (actually precisions) for LDV thermal calibration measurements, nearer 2–4%. A detailed explanation of some of the LDV instrumentation and acquisition variables that might affect measurement uncertainty is provided in the appendix of [21].

³ Certain commercial equipment, instruments, or materials are identified in this paper to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

⁴ Unless otherwise specified, uncertainties expressed in this paper are ± 1 standard deviation and relative uncertainties are ± 1 standard deviation/mean and expressed in % (also termed relative standard deviation—rsd).

This paper describes an adaptation of the LDV thermal calibration method to colloidal probe cantilevers. The technique uses LDV to both determine the MCF for the colloid probe cantilever and calibrate the flexural (normal) spring constant. The calibration procedure is described with particular attention to determining the correct calibration location for the cantilever by optimizing the spectral signal for the LDV. The effect of added mass on the MCF is demonstrated using a set of commercially available colloid probe cantilevers with nominally identical cantilevers but varied sphere sizes. The results are ultimately validated for accuracy by comparing the calibration to an independent calibration using an electrostatic force balance (EFB) [24], which is SI traceable.

2. Equipment

Cantilever thermal vibration spectra were obtained using the NIST Polytec MSA-500 scanning LDV described in more detail elsewhere [21] and capable of measuring frequency up to 2 MHz. An essential capability of the instrumentation is the use of pure digital decoding coupled with high resolution quadrature signal conversion of the displacement data. Using digital demodulation enables these measurements by providing excellent stability and high sensitivity, both of which are critical to accurately measuring the low amplitude (<100 pm) thermal vibrations. This is especially true when measuring higher order eigenmodes at greater frequencies and smaller amplitudes (<10 pm). Most measurements were made using a 20× objective lens which provided a 2.5 μm diameter laser spot size. An additional key system feature of the commercial instrument relevant to colloidal probe calibration is the ability of the instrument to electronically steer the sample laser spot to different locations within the optical field of view. This particular surface ‘scanning’ variant of LDV is referred to in this paper as scanning laser Doppler vibrometry (SLDV). The instrument can also determine the relative intensity of the signal derived from the reflection of the sample and reference laser beams from the surface. The combination of beam steering and signal intensity feedback allowed us to develop an algorithmic optimization of the sample beam position on the cantilever.

Colloid probe cantilevers were either purchased commercially or made in-house. Those fabricated at NIST were made by gluing spheres to the ends of commercial cantilevers. Typically this was accomplished in an AFM by using a two part epoxy and a cantilever mounted in the AFM. By lowering the tip of the cantilever onto a drop of glue on a silicon chip, a small bead of glue could be made to adhere to the end of the cantilever. The end of the cantilever can then be positioned over a single spherical particle on a separate part of the silicon chip, and lowered onto it to attach the sphere. The accuracy of SLDV thermal spring constant calibrations on a colloid probe cantilever was ultimately checked against the NIST EFB [24] which is both absolute and SI traceable. This balance uses traceable electrical calibrations to generate an electrostatic force while simultaneously measuring the

balance displacement with a laser interferometer. A traceable spring constant can be measured by pressing the sphere from the colloidal probe against a flat graphite loading button attached to the balance while simultaneously measuring the balance force and displacement. The entire balance is housed in a vacuum chamber that is evacuated and thermally equilibrated prior to conducting a calibration. The capacitance gradient is calibrated prior to each run and for the current work, the load frame compliance compensation was found to be negligible. Details are provided in [22]. The entire process takes several days for a single sample but it is one of the few force calibration methods that is capable of measuring a cantilever with such a low stiffness (0.05 N m⁻¹) with good precision and an SI traceable accuracy (uncertainty better than ±1%) [23, 25].

3. LDV thermal method

The basic LDV thermal calibration method consists of placing an inverted, horizontal, test cantilever under the lens of the LDV, focusing on the surface, locating the sample and reference beams at appropriate locations and acquiring a spectrum [21]. Typically this is done with the cantilever still secured in its original gel-pack storage box (with the cover opened). The area under the first flexural resonance peak is used to calculate the mean square thermal deflection of the cantilever which can be related to the cantilever static stiffness (k) through the equipartition theorem as:

$$k = \frac{\chi_1 k_B T}{\langle z_1^2 \rangle}, \quad (1)$$

where k_B is the Boltzman constant, T is absolute temperature and $\langle z_1^2 \rangle$ is the mean square amplitude of the first resonance peak of the cantilever at that temperature. The term χ_1 is the MCF for the first resonance peak which relates the dynamic stiffness of the first flexural eigenmode (measured by vibrometry) to the static stiffness useful in the AFM

$$\chi_1 = \frac{k}{k_1^{\text{dynamic}}}. \quad (2)$$

Typically, MCF values for commercial cantilevers vary from 0.93 (some triangular) to 0.97 for rectangular cantilevers but tipped ones can be a bit higher depending on the amount of added mass [23] so significant errors can be introduced if the MCF is ignored. Recently, Sader *et al* [26] used a finite element 2D model for a tipless, solid, equilateral triangle cantilever and estimated that the MCF for this shape was 0.85 so errors of up to 15% may be introduced if this MCF is disregarded.

One important feature of the LDV thermal method is that it measures the cantilever spring constant at the location of the sample laser beam; therefore, placement of this laser spot is critical. For tipped cantilevers it is not possible to get a signal reflecting off the tip itself therefore measurements are made on flat, reflective sections of the cantilever near the tip and length correction adjustments are made to the measured

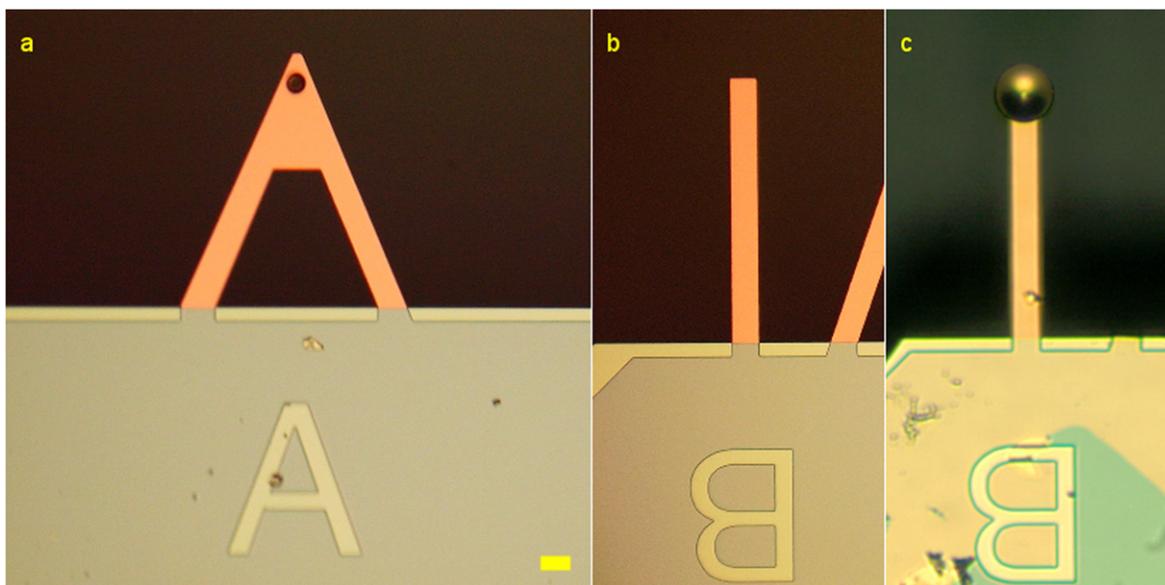


Figure 1. MLCT series silicon nitride cantilever examples. (a) 13 μm diameter sphere on tipless cantilever A, (b) tipless cantilever B, and (c) a 45 μm sphere glued to the end of a tipless cantilever B. The fiducial line in the bottom right corner of figure (a) is 20 μm long.

spring constant assuming a cubic relationship. Tips are usually fairly small with a base of perhaps 5 μm so the length corrections are relatively minor and the uncertainty propagated through these corrections are fairly small. Previously, Gates *et al* [23] demonstrated good accuracy (uncertainty near 1%) could be achieved using the LDV thermal calibration method on tipped triangular cantilevers when experimentally measured MCF's and length corrections are applied.

Colloid probe cantilevers pose more of a challenge for LDV since the size of the spheres attached to cantilevers range from a few μm to more than 50 μm in diameter. If small spheres are attached to cantilevers there are usually reflective flat portions of the cantilever near the theoretical contact point of the sphere in the AFM so relatively small length offset corrections could be made, and the contribution of the offset to the spring constant uncertainty should be small. In the example shown in figure 1 for MLCT series cantilevers (Bruker Probes, Camarillo, CA), the small sphere in figure 1(a) is only 13 μm in diameter and the overall length of the cantilever is nominally 175 μm . The LDV sample laser spot could be located within about 9 μm of the center of the sphere, and with placement error and the cubic relationship of the offset correction for the spring constant, this placement choice would increase the uncertainty of the spring constant by about 1.5%. In some cases, like for figure 1(a), reflective areas may be available both (longitudinally) before and beyond the sphere as well as to the sides (no length offset needed) so that reasonably low statistical uncertainties could be achieved (repeatability near $\pm 1\%$). While it may be possible to flip the cantilever over to make the calibration measurement, this increases the uncertainty of the measurement since it would be difficult to determine the exact laser spot placement corresponding to the hidden sphere location.

In the case of a very large colloid sphere an offset correction can be quite large and much larger uncertainties would

be introduced. Figure 1(b) is a photomicrograph of a rectangular cantilever nominally 210 μm long, and 20 μm wide. Figure 1(c) is a similar cantilever onto which a 45 μm diameter sphere has been attached. The distance between the theoretical contact point of the sphere (the middle of the plan outline of the sphere) and a reflective portion of the cantilever where the laser spot could be located is about 24 μm . A length offset correction would be about 36% of the stiffness in this case. In addition, options for measurements beyond and to the side of the colloid sphere are not available, compounding the measurement uncertainty. In cases like this the errors associated with placement uncertainty and the cubic correction could be several percent.

Fortunately, as the large sphere diameter causes one problem it solves another. For large spheres, the convex curvature is large and the very top of the sphere presents a sufficiently flat surface to reflect the laser beam and collect vibrometry spectra provided the surface of the sphere is reflective. Another important feature of the colloid probe cantilever is that one is usually interested in the spring constant at the cantilever length where the sphere would touch the sample surface during use. For a perfectly horizontal cantilever, this coincides with the highest point on the sphere and also where the intensity of the reflected laser should be a maximum. This allows us to use the signal intensity of the reflected laser as feedback to optimize the centering of the laser spot on the center of the sphere, precisely where we want to make the measurement.

It should be noted that the addition of a colloid sphere (no glue) to a cantilever does not fundamentally affect the static stiffness of the cantilever. Its mass, however, affects the MCF, χ . This has been demonstrated in Gates *et al* [23]. One additional point about cantilevers with large colloid spheres attached is that often, significant amounts of glue need to be used to secure the sphere to the cantilever. Any areas of the

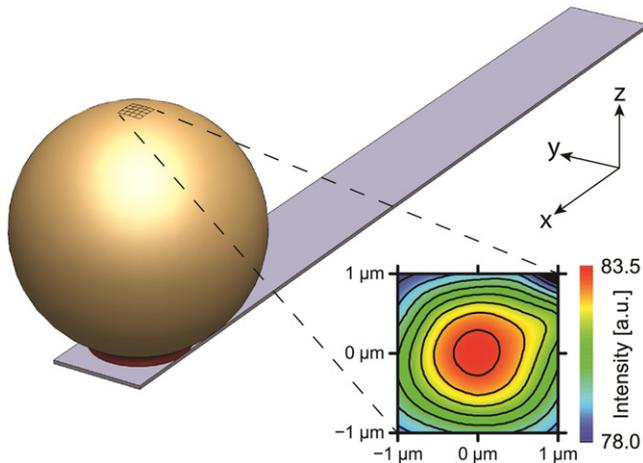


Figure 2. 3D Perspective representation of a large $45\ \mu\text{m}$ diameter sphere attached to a rectangular cantilever showing a 5×5 grid superimposed on the top of the sphere. The contour plot represents the (smoothed) 2D map of reflected laser spot intensity for the grid.

cantilever structure with glue will have different mechanics, potentially resulting in observable changes (increases) to the spring constant of the cantilever-colloid assembly. Attempts to estimate the stiffness of a colloidal probe based solely on the stiffness of the cantilever without the sphere attached will have errors both from the uncertainty in knowing where the sphere will actually be located and the stiffening effect of the glue.

4. LDV signal optimization

The basic method for optimizing the signal for a colloid probe involves focusing the laser spot near the center of the top of the sphere and moving it around slightly in the x - y plane while noting the intensity of the reflected laser signal as feedback. This optimization can be done manually, but to get more consistent results, a computer program was written to systematically step through a 5×5 point square grid of locations around the current laser spot location and re-center the laser spot on the one with the highest signal intensity. Figure 2 is a 3D perspective representation of a colloid probe under the LDV system which contains an inset of an actual signal intensity contour plot (5×5 grid, smoothed) of the steered LDV laser and demonstrates how the laser spot can be steered to various locations of the sphere in order to optimize the signal.

In cases of spheres with a large radius of curvature and smooth, reflective surfaces, the signal can saturate at 100% for more than one point on the grid. In these cases, the average of all the 100% locations is selected as the maximum signal point where the beam re-centers. The beam positioning software program can be run iteratively so that if the initial grid sweep improves the maximum signal by more than one step increment in either x or y , it can be run again to further improve it and 'home in' on the optimal signal for that colloid probe. Eventually, it will keep re-centering on the same point,

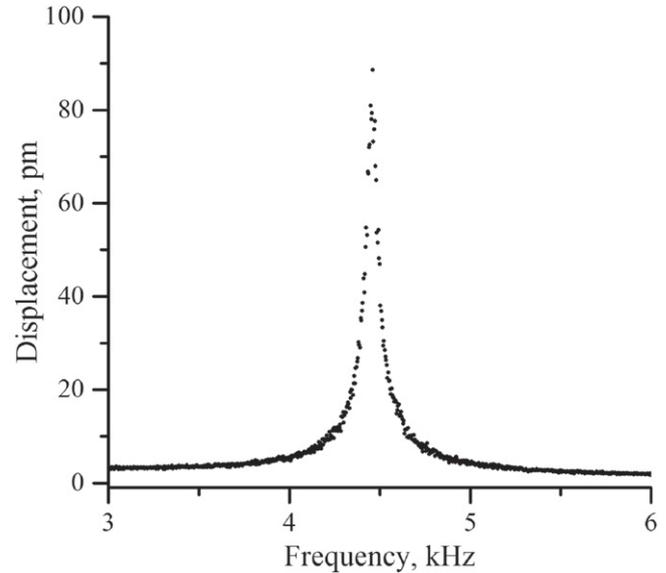


Figure 3. First flexural mode resonance peak for colloid probe CP1.

signifying that no further improvement is gained and the thermal spectrum can then be acquired. The step size can be varied but is generally based on the size of the laser spot (i.e. the power of the lens) and each step is $1/5$ the spot diameter. For example, a $20\times$ lens has a $2.5\ \mu\text{m}$ diameter spot size and the step size would typically be $0.5\ \mu\text{m}$. This technique offers a significant improvement over the location uncertainty that would be present for a manual placement and offset measurement.

5. LDV thermal calibration measurements

Once the laser signal is optimized, obtaining spring constants for colloid probe cantilevers is a two stage process. Initially, the MCF needs to be measured for the particular combination of cantilever type and size of sphere attached. Once the MCF is determined, the flexural spring constant of the cantilever can then be calibrated.

The MCF for a colloid probe cantilever is determined using the experimental method of Gates *et al* [23] which uses the relative areas under the peaks of the eigenmodes (power spectral density versus frequency plot). Adding a mass to the end of the cantilever decreases the peak areas of the higher order flexural modes relative to the first flexural mode, thus increasing the MCF. In the case of adding a $45\ \mu\text{m}$ polystyrene sphere to the end of a MLCT-B cantilever, the peak area of the second flexural eigenmode is reduced to three orders of magnitude below that of the first flexural resonance mode. As a result, the experimentally measured MCF is 1.00 which is 3% higher than the typical MCF of a tipless rectangular cantilever.

An LDV thermal frequency spectrum for the first flexural mode resonance peak for an MLCT-B colloid probe cantilever is shown in figure 3. The original resonance frequency for a tipless cantilever of this type is close to 15 kHz so the

Table 1. Summary colloid probe LDV thermal calibration data, parsed by different signal optimization methods.

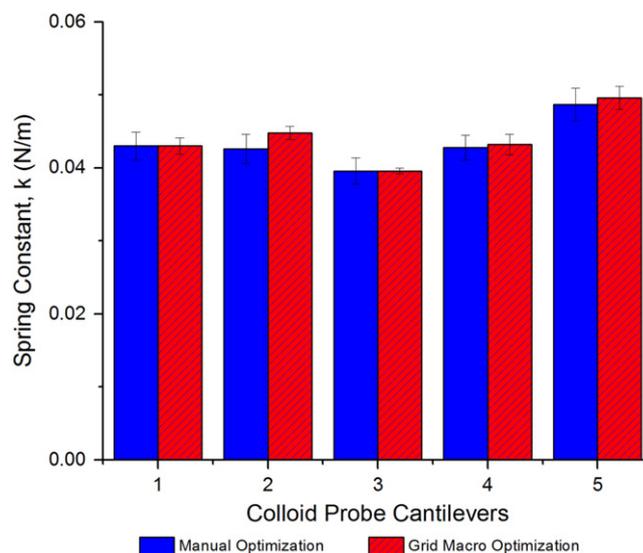
Cantilever	Manual			Automated grid		
	# of runs	k (N m ⁻¹)	k std dev (%)	# of runs	k (N m ⁻¹)	k std dev (%)
CP1	4	0.0430 ± 0.0019	4.4	7	0.0430 ± 0.0013	3.1
CP2	6	0.0426 ± 0.0020	4.7	5	0.0448 ± 0.0009	2.0
CP3	6	0.0396 ± 0.0018	4.5	5	0.0396 ± 0.0004	1.1
CP4	5	0.0428 ± 0.0017	4.0	5	0.0432 ± 0.0014	3.2
CP5	3	0.0487 ± 0.0022	4.5	15	0.0496 ± 0.0016	3.2

sphere addition has reduced the frequency of the peak (now 4.5 kHz) by about a factor of three. This particular single measurement estimated the spring constant of the cantilever at 0.0434 N m⁻¹ with an uncertainty in the area fit measurement of 1.4%.

The spring constants measured for five different colloid probe cantilevers derived from tipless MLCT-B starting cantilevers are shown in table 1. This summary is parsed into two groups—calibrations that were run using manual signal optimization, and ones that utilized the software-driven automated grid pattern macro optimization method. The data is also plotted in bar chart format in figure 4.

The data demonstrate that both techniques provide similar answers (statistically, each data set is indistinguishable) but optimization of the signal using the macro reduces the statistical uncertainty by about a factor of two. Manual optimization had a relative standard deviation of about 4.4%. Using the automated (software program) grid optimization procedure improves that to 2.4%. The variation among different colloid probe cantilevers in this set is about 20%, confirming the wisdom of individually calibrating each cantilever. When the signal is optimized and a statistically significant number of runs are made, the best precision is achieved. Use of the experimentally determined MCF ensures that the best accuracy is achieved.

One advantage of the dual beam instrument used in this study is that it automatically removes spurious background vibrations that might otherwise obscure important features of the vibrometry spectrum. It requires, however, that the reference and sample beams be simultaneously focused or the signal intensity will suffer, resulting in a lower signal to noise ratio. The fact that the cantilever and top of the sphere lie on different focal planes introduces the need to compromise both the SLDV objective lens selection and the focal plane. High magnification lenses yield small spot sizes and thus small placement uncertainties, but also have a shallow depth of field that degrades signal quality when the sample and reference focal plane are separated. Conversely, low magnification lenses yield larger spot sizes but give larger depths of field, increasing placement uncertainty but improving signal quality from vertically separated sample and reference locations. Ultimately, the best compromise for objective lens selection depends on the relative dimensions of the cantilever and colloid diameter. In terms of focus, it is most important to have the sample beam in better focus than the reference beam since the curved surface of the sphere amplifies any

**Figure 4.** Summary LDV thermal calibrations for 5 different colloid probe cantilevers (CP1–CP5) using manual and automated grid optimized positioning of the sample laser spot on the surface of the sphere.

misalignment and the signal intensity will drop off more quickly when the sample beam is out of focus. For the 45 μ m diameter sphere on cantilever shown in figure 1(c), a 20 \times lens is the maximum magnification that can provide adequate signal intensity for such a large depth of field from the top of the sphere to the cantilever.

Additional experiments on commercial etched silicon colloid probe ‘CP-FM’ cantilevers (SQUBE CP-FM-Au-C5, Bickenbach, Germany) with nominal 7 μ m diameter gold spheres verified that even spheres as small as 3.5 μ m could be interrogated using a 2.5 μ m diameter laser spot size. This suggests that the central core of the spot is sufficient to obtain an adequate LDV thermal resonance frequency spectrum. This sensitivity was previously noted by Biedermann *et al* [20] who used LDV to interrogate nanowires of only 200 nm diameter and obtain thermal spectra from which dynamic stiffness could be obtained. Since the set of commercial CP-FM cantilevers had a wide range of attached Au sphere diameters (from 3.5 to 8.6 μ m) this data set offered the opportunity to demonstrate the effect of added mass on MCF’s within a single cantilever type. Five different batches of four or five cantilevers each were analyzed, resulting in a total of twenty four samples. One of the spheres had detached from one of the samples providing an opportunity to measure a

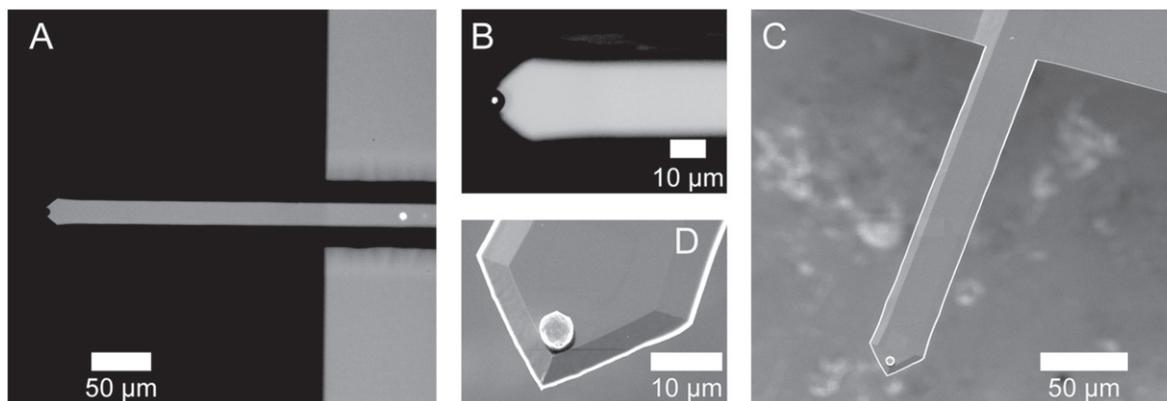


Figure 5. Optical (A) and (B) and SEM (C) and (D) photomicrographs of a typical commercial CP-FM etched silicon colloid probe cantilever used for the SLDV thermal calibration method study.

cantilever with no added mass. No glue residue was readily apparent on this sample and the point at the end of the triangular end of the cantilever where the sphere was typically placed was chosen for the measurement.

Optical and SEM photomicrographs of a typical CP-FM cantilever are shown in figure 5. The low magnification optical image ((A), left) shows the location of the reference laser spot at the base of the cantilever. The bright spot in the middle of the sphere (enlarged optical image (B)) is actually the reflection of the microscope coaxial illumination from the central (approximately 20%) of the sphere and gives an indication of the region which will reflect the laser spot back up through the objective lens to either the video camera or the vibrometer detector. The SEM images (C) and (D) show the placement of the sphere (5 μm diameter) at the pointed end of the etched silicon cantilever. For an SLDV thermal calibration, the sample laser spot was focused on the sphere and adjusted within the x - y plane using the automated optimization software program. The MCF for each colloid probe cantilever was then experimentally measured at the sphere location [23] using the first three flexural modes of the acquired spectrum. The statistical uncertainty in this measurement was observed to be very low: 0.9806 ± 0.0002 ($\pm 0.02\%$) as demonstrated for a set of 7 repeat measurements over two days on a single CP-FM colloid probe cantilever. This MCF was then used along with the first resonance peak fit data to determine the cantilever stiffness. At least three repeat calibrations were run on each cantilever, relocating and re-optimizing the sample spot position each time. The statistical uncertainty in the spring constant calibration was typically better than $\pm 1\%$. The MCF data, summarized in figure 6 qualitatively demonstrate the influence of added mass on MCF for similar cantilevers of a single type. Error bars for the mass addition (x axis) are based on a rough approximation for the uncertainty in optically determining the sphere diameter (about $\pm 0.25 \mu\text{m}$). The $\pm 0.02\%$ statistical uncertainty in determining the MCF (y axis) is smaller than the symbol size in this plot. It should be noted that the absolute value of the MCF is affected by mass location as well as size and the data in figure 6 reflect both influences so the main unaccounted-for uncertainties in the MCF value is probably due to variation in

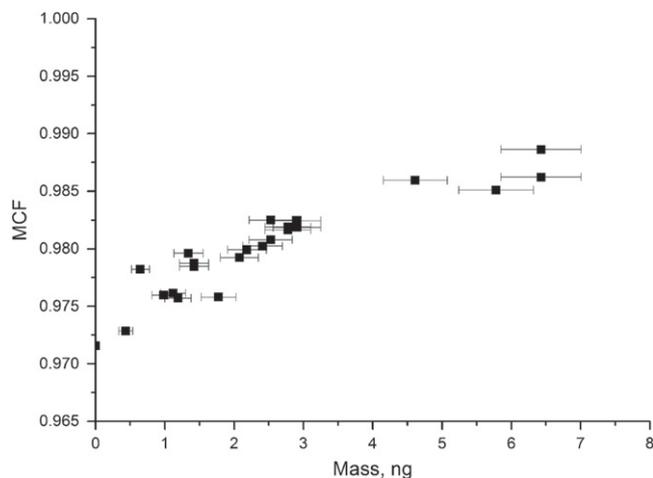


Figure 6. Relationship between MCF and added Au colloid sphere mass for a single cantilever type (CP-FM).

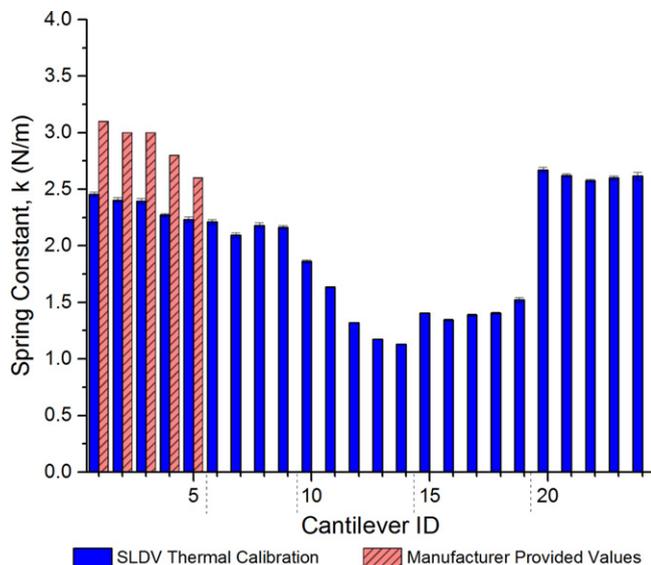
locations of the colloid spheres along the length of the cantilever.

The MCF values vary from a low of 0.972 (for a cantilever that had lost the added mass and was close to the theoretical ideal rectangular MCF value of 0.971) to 0.989 which was obtained for the largest added mass. The effect appears to taper off with increasing mass added and one would expect the MCF to asymptotically approach 1.00 at infinite mass. Analytically we calculated that for a uniform cross section cantilever with an added mass located at 95% of the length, the mode correction factor at the end of the cantilever would exceed 0.99 when the added mass exceeds 30% of the cantilever mass. The SLDV thermal calibrated spring constants obtained for these cantilevers are summarized graphically in figure 7.

The SLDV calibration values (solid bars) ranged from a low of $1.130 \pm 0.002 \text{ N m}^{-1}$ to a high of $2.671 \pm 0.021 \text{ N m}^{-1}$ with statistical uncertainties typically below a percent (average of 0.7%). The dashed dividing lines along the bottom of the x axis define each sample set (gel-pack box) obtained from the manufacturer. The overall, factor of two, range of spring constants obtained for these nominally identical cantilevers confirms the necessity for calibrating each individual

Table 2. Comparison of spring constant calibration of colloid probe cantilever CP1 using both SLDV thermal and EFB techniques.

Cantilever	EFB (N m^{-1})	SLDV (N m^{-1})	Discrepancy (%)
CP1	0.0428 ± 0.0014	0.0430 ± 0.0013	0.47

**Figure 7.** SLDV thermal spring constant calibrations for commercial colloid probe cantilevers of a single type.

cantilever for cases where accuracy is desired. Even variation within a single box can be significant (range of almost 50% of the average value for the third set). The shaded bars represent the spring constant calibration provided by the manufacturer (unknown method and uncertainty) for one of the sets (of five) cantilevers. The manufacturer's spring constant values are consistently higher than the SLDV thermal calibration values (average of 23%) but trending in a similar manner so the issue of absolute accuracy needs to be verified.

6. Accuracy validation

The absolute accuracy of the SLDV thermal method for colloid probe cantilevers was confirmed by comparing the SLDV thermal calibration results to independent calibration using another, SI traceable, method. This was done on cantilever CP1 using the NIST EFB which is SI traceable. The results, summarized in table 2 indicate that both techniques provide the same spring constant (discrepancy 0.47%) with about the same uncertainty.

7. Discussion

One key aspect of the successful implementation of the colloid probe SLDV thermal calibration method for determining the spring constants of these cantilevers was the extremely small observed statistical uncertainty in the measurement for small colloid spheres which was significantly smaller than

typical measurements on more ideal cantilevers (long, tipless, uniform, rectangular) [21]. We suspect that the reason is due to the unique arrangement of curvature of the colloid sphere and laser spot size which combine to isolate the spatial variation (and drift) of the laser spot usually present on most surfaces during signal acquisition. The LDV thermal method provides the spring constant calibration at the exact point on the cantilever where the sample laser beam reflects from the surface. The spatial uncertainty in the laser spot location axially along a cantilever is a very strong contribution to calibration uncertainty since spring constant typically varies inversely with length cubed. In a case where the nominal laser spot size is significantly larger than the expected reflection area at the top of a colloid sphere, the sampled laser signal comes from the reflected area (which is spatially invariant relative to the length of the cantilever) instead of the whole laser spot size (which can have a small drift with time). As a result, the small amount of spatial drift that can arise during the course of data acquisition (about 90 s) is eliminated and repeatability of the measurement is enhanced.

The ability of the SLDV to accurately and precisely locate this center of the sphere within the x - y plane and reduce drift issues suggests that placement of small spheres on cantilevers (e.g., reference cantilevers) may provide a means of improving the utility of these devices. For example, a well characterized colloidal probe cantilever, with a defined spring constant could serve as a more precise standard reference artifact to compare spring constant calibration measurements on different SLDV instruments to make sure they achieve the correct accuracy. NIST is currently fabricating standard reference cantilever arrays [22] to be used as reference artifacts for calibrating cantilevers using the reference cantilever method. With a slight modification, colloidal spheres could be attached to the ends of these cantilevers to serve as SLDV thermal calibration artifacts as well.

It should also be noted that the LDV (and SLDV) thermal method described in this paper calibrates the static spring constant perpendicular to the long axis of the cantilever. For use in an AFM, this stiffness must be modified for both the inclined angle of the cantilever and the effect of the size of the colloidal sphere as in the correction of Heim *et al* [27].

The use of a calibrated cantilever is only one of two necessary steps for accurate quantitative force measurements in an AFM. The other necessary step is determining the OLS which is needed to convert voltage (measured by the detector) to vertical displacement of the surface (piezo) and through cantilever stiffness, end up with force. To avoid actually touching the surface (usually required for an OLS determination) one can perform an AFM thermal calibration measurement to back it out. This can be done 'manually' on any AFM equipped with thermal calibration capability by

manually adjust the OLS parameter until the proper (calibrated) k is obtained. Eventually all AFM's could incorporate more automated versions of their software to be able to determine the OLS from the AFM thermal measurement given a known calibrated stiffness for the cantilever.

8. Conclusion

Spring constants of colloid probe cantilevers can now be nondestructively obtained, precisely, and accurately, utilizing SLDV. The technique uses a laser beam reflected from a sample surface and does not require resorting to physically touching the cantilever. Sample handling is minimal and the method can even be applied to cantilevers as received from the manufacturer, mounted in their gel-pack storage cases. With proper selection of objective lens and using a software program to optimize the placement of the sample laser spot by scanning in a grid pattern in the x - y plane, statistical measurement uncertainties of $\pm 1\%$ or better can be achieved. Accuracy requires using the SLDV thermal method to experimentally measure the proper MCF for each cantilever, which for colloid probe cantilevers with large masses attached is close to 1.00. Failure to apply the correct MCF can introduce systematic (bias) errors in excess of 5%, depending on the cantilever used and will limit the accuracy of the calibration. The accuracy of the overall calibration technique was verified by direct comparison between the SLDV thermal calibration colloid probe technique and the NIST EFB calibration method (SI traceable) using a representative colloid probe. Discrepancy between the two calibration measurements was less than 1%.

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