Customized atomic force microscopy probe by focused-ion-beam-assisted tip transfer

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We present a technique for transferring separately fabricated tips onto tipless atomic force microscopy (AFM) cantilevers, performed using focused ion beam-assisted nanomanipulation. This method addresses the need in scanning probe microscopy for certain tip geometries that cannot be achieved by conventional lithography. For example, in probing complex layered materials or tall biological cells using AFM, a tall tip with a high-aspect-ratio is required to avoid artifacts caused by collisions of the tip's sides with the material being probed. We show experimentally that tall ($18\text{ }\mu\text{m}$) cantilever tips fabricated by this approach reduce squeeze-film damping, which fits predictions from hydrodynamic theory, and results in an increased quality factor (Q) of the fundamental flexural mode. We demonstrate that a customized tip's well-defined geometry, tall tip height, and aspect ratio enable improved measurement of elastic moduli by allowing access to low-lying portions of tall cells (T lymphocytes). This technique can be generally used to attach tips to any micromechanical device when conventional lithography of tips cannot be accomplished. © 2014 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4892075>]

Mapping the elastic moduli of cells or other tall and steep structures by atomic force microscopy (AFM) presents several challenges (Fig. 1). First, to accurately measure the elastic modulus, the shape of the tip must be known to infer the contact geometry during indentation.¹ For this reason, colloidal tips have been widely used due to their well-defined spherical geometry,^{2–4} despite the drawback of their relatively low spatial resolution ($\sim 1\text{ }\mu\text{m}$). On the other hand, while sharp, conical tips, fabricated by wet etching techniques, offer high-resolution measurements, their sides are often poorly defined. Second, precisely measuring low contact forces ($<100\text{ pN}$) on low modulus cells mandates the use of AFM cantilevers with low spring constants (from 0.01 to 0.1 N/m). These soft cantilevers have inherently low quality factors (Q) in liquid (around 1), which are further reduced when in close proximity to the sample due to squeeze-film damping.^{5,6} The low Q reduces the performance of dynamic imaging such as tapping-mode AFM and degrades the signal-to-noise ratio critical for low-force measurements on cells. Third, the topography of cells can sometimes be too tall ($>10\text{ }\mu\text{m}$) or too steep ($>80^\circ$) to be properly imaged. Imaging artifacts often arise because conventional tips are unable to access the lower portions of the cell due to insufficient tip height and aspect ratio.^{7–9} Most commercial AFM probes fail to meet the aforementioned challenges for mapping the elastic moduli of cells.

Here, we demonstrate a focused ion beam (FIB)-based technique to transfer a tall and high-aspect ratio tip onto a silicon nitride cantilever (Figs. 2(a) and 2(b)). Our technique can be used to assemble any two micro/nano structures together and is especially useful when fabrication in a single lithographic process is not feasible or economical.

Various other techniques based on FIB assisted nanomanipulation have been developed to fabricate customized AFM tips, e.g., carbon nanotube tips, nanobit tips, and vapor-deposited metal tips.^{10–12} These other approaches require the construction of specialized equipment such as micro-grippers or nanorobotic micromanipulators. In contrast, our approach uses a standard nanomanipulator found in many FIB systems.

The elastic moduli of biological cells are highly variable (0.1–100 kPa (Ref. 13)) and spatially heterogeneous, owing to nanoscale cytoskeletal networks and dispersed organelles. To study such heterogeneity, AFM is used to map the distribution of local elastic moduli of biological cells in aqueous environments.^{14–16} In the widely used force-volume (FV) mode,¹⁷ force-distance (FD) curves are sequentially acquired at predefined grid positions to form a “map.” Mechanical

FIG. 1. Tall and high-aspect ratio tips are necessary to probe the low parts of cells near steep cellular features and ease near-surface damping due to the squeeze-film effect.

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FIG. 2. Customized tip transfer onto AFM cantilevers. (a) and (b) Tall-tip-bearing silicon nitride cantilevers. (c) and (d) High aspect ratio and sharpness of a customized tip.

models of contact, such as the Hertz or Derjaguin-Muller-Toropov models, which require knowledge of the tip geometry, are then used to extract the local elastic modulus from each FD curve.

The tip-transfer technique presented here entails two steps conducted in the FIB system: shaping the tip for the specific needs of the experiment, and transferring the tip to a tipless cantilever. We started by milling and sharpening a tip prior to transfer. We prepared a wafer of tall tips using a potassium hydroxide (KOH) etch to create a $2\text{ }\mu\text{m}$ tall pyramidal tip followed by Deep Reactive Ion Etch to create a $4\text{ }\mu\text{m}$ wide $\times$ $\sim 15\text{ }\mu\text{m}$ tall rectangular column. The fabricated tip was then reshaped using ion beam milling to improve the aspect ratio near the tip apex, to better define the tip geometry, and to lower the total mass of the tip. In the final sharpening step, a concentric circle pattern with an inner diameter of 125 nm excluded from milling was used. The resultant tip is $15\text{--}18\text{ }\mu\text{m}$ tall, and has a cone angle of 15° in the last $2\text{ }\mu\text{m}$ from the tip apex, and has a tip radius of $<10\text{ nm}$ (Figs. 2(c) and 2(d)). This tip geometry is especially useful for performing elastography with high spatial resolution in areas of steep topography.

We transferred the tip using a FIB instrument (Helios NanoLab 600i, FEI, Hillsboro, OR) equipped with a tungsten nanomanipulation probe (Omniprobe, Oxford Instruments). First, the probe was brought close to the pre-milled tip (Approach; Figs. 3(a) and 3(b)). A rectangular patch of platinum (Pt) (500 nm thick) was deposited over the gap between

the probe and the tip by ion-beam-assisted deposition using an ion current of 7.7 pA and 30 kV of energy (Welding; Fig. 3(c)). To release the tip, we milled a line pattern at the base of the tip using a high ion current of 80 pA. The sample stage was then lowered and the tip was moved over to the cantilever (Lift out; Fig. 3(d)). We used tipless gold-coated silicon nitride cantilevers with spring constant of 0.06–0.2 N/m (either HYDRA50R or HYDRA100R, AppNano, Mountain View, CA). To minimize contact between the sides of a tip and the surface being scanned, a tip should be positioned perpendicularly to the substrate surface. However, for most AFM systems, the cantilever's chip body is mounted with a $10^\circ\text{--}15^\circ$ tilt from the sample surface. To compensate for this tilt, we employed a homemade holder that holds the cantilever at a tilt of 11° in the FIB system. The tall tip was then carefully lowered by the nanomanipulator onto the edge of the cantilever and the lateral positioning was confirmed with scanning electron microscopy (SEM). Attaching the tip at the very end of the cantilever maximizes the mechanical sensitivity of the lever and allows the tip to be positioned during experiments by optical microscopy from above or below. Platinum deposition by the ion beam was used to attach the tip to the cantilever (Attaching; Figs. 3(e) and 3(g)). To fill up the wedge-shaped space between the bottom of the tip and the cantilever, at least $1\text{ }\mu\text{m}$ of Pt was deposited (same deposition condition as above). Finally, we severed the Pt patch joining the nanomanipulator probe and the tip by milling a line pattern, using the same ion current (Detach; Fig. 3(f)).

A major obstacle to scanning samples in liquid is the damping due to the hydrodynamic flow of liquid that is confined between the cantilever and surface. This so-called squeeze-film effect worsens the dynamic performance and force sensitivity of the cantilever, which affects AFM operation in dynamic (such as tapping mode) or quasi-static (such as FD curves) conditions. To investigate the influence of squeeze-film damping, and to see if utilizing a tall tip can improve performance, we measured the thermal spectrum at different tip heights with respect to a glass substrate using a standard AFM (MFP-3D, Asylum Research, Santa Barbara, CA). We tested silicon nitride cantilevers of two different lengths ($100\text{ }\mu\text{m}$ or $50\text{ }\mu\text{m}$, labeled as “long” and “short,” respectively). To avoid static charging while transferring the tip, the cantilevers were purchased with Au coating on both sides of the cantilevers. Due to the variability in the thickness and film quality of the Au and of the cantilevers themselves, the long cantilevers had a higher resonance frequency and spring constant than the short ones. For each cantilever length, one tipless cantilever and two tip-bearing cantilevers

FIG. 3. Tip-transfer technique.

were measured. Measurements were made in phosphate buffered saline (PBS) at room temperature, and the cantilevers were calibrated using the thermal method in the same buffer. The tip-bearing cantilevers had slightly lower f_0 and Q due to the minimal increase in the mass of the tip and the hydrodynamic damping acting on the tip itself (Fig. 4).

The tipless cantilever can be brought into contact with the surface (i.e., zero cantilever-sample separation). For tip-sample separations less than $5\ \mu\text{m}$, an FD curve triggered at 200 pN was performed to position the tipless cantilever or the tip apex of a tall-tip-bearing cantilever at a given height with respect to the sample by the Z-piezo scanner in closed-loop control. A thermal spectrum was then taken, and the top 50% of the fundamental resonance peak was fitted to a thermal model of simple harmonic oscillation to extract f_0 and Q . For separations greater than $5\ \mu\text{m}$, the $5\ \mu\text{m}$ height was first established by a single FD curve as above and then the cantilever was moved to the desired height by raising the scanner head. Measurements were made three times at each height and averaged (Fig. 4).

We found that for both long and short tip-bearing cantilevers, there was a minuscule decrease in f_0 and Q near the

surface as compared with $10\ \mu\text{m}$ away or further (Fig. 4, X and + patterns). Moving the tip from 10 to $0.2\ \mu\text{m}$ from the surface effectively moved the cantilever itself from 26 to $\sim 16\ \mu\text{m}$. Thus, the cantilevers with tall tips stayed largely outside the region of strong, squeeze-film damping. This relatively stable dynamic performance of the tall-tip-bearing cantilever will be especially beneficial when using dynamic imaging modes to scan tall structures.

In contrast, we found that both f_0 and Q decreased dramatically when the tipless cantilevers were brought within $20\ \mu\text{m}$ of the glass surface. This observation is consistent with previous reports showing that squeeze-film damping becomes appreciable when the cantilever is at a distance less than the cantilever's width from the surface, which was $35\ \mu\text{m}$ here.^{6,18} To gain insight into the changes in dynamic performance, we fitted the experimental data to a model adapted from the dynamic response of a thermally excited cantilever¹⁹ using a hydrodynamic function for situations that are close to the surface.²⁰ In brief, the frequency response function (FRF) of the fundamental flexural mode of the thermally excited cantilever as a function of tip-sample separation H was calculated using

$$\frac{Y(\omega, H + H_0)}{k_B T} = \frac{\pi \rho_f b^2 \omega \Gamma_i(H + H_0) \times 0.3915L}{1.0302k_c - \omega^2 [\rho_c A + 0.25\pi \rho_f b^2 (\Gamma_r(H + H_0) - i\Gamma_i(H + H_0))] \times 0.25L}$$

Y is the cantilever deflection in frequency space, $H + H_0$ is the corrected tip-sample separation (H_0 is explained below), $k_B T$ is the thermal energy, ρ_f and ρ_c are the density of the surrounding liquid and the cantilever, b , A , and L are the width, cross-section area, and length of the cantilever, respectively, k_c is the spring stiffness, and Γ_i and Γ_r are the imaginary and real part of the complex hydrodynamic function from the appendix of Tung *et al.*²⁰ as a function of angular frequency and $H + H_0$. The experimentally determined peak frequency was used as an estimate of f_0 , and the Q factor was determined by the half-power method.²¹

This model assumes that the cantilever is parallel to the substrate surface, but in reality the cantilever base was tilted $\sim 11^\circ$ from the surface. To account for this difference, we added the correction term H_0 to the tip-sample separation and sought the H_0 best fitted to the experimental data. The spring stiffness k_c was the other fitted parameter. We found, by fitting the experimental data, that $H_0 = 5.6\ \mu\text{m}$ and $k_c = 1.79\ \text{N/m}$ for the long cantilever, and $H_0 = 6.0\ \mu\text{m}$ and $k_c = 0.32\ \text{N/m}$ for the short cantilever. Using these parameters, the hydrodynamic model captured the sharp decrease in both resonance frequency and Q factor (dashed lines, Fig. 4) as the tipless cantilevers approached the surface. Interestingly, the correction term H_0 was found to be independent of the cantilever length, suggesting that when the cantilever is tilted, the effect of hydrodynamic damping is concentrated mostly at the portion closest to the surface.

The direct impact of decreasing f_0 and Q was an increased floor of thermal noise. The effect of thermal

fluctuations on force is given by $F_{\text{noise}} = \sqrt{\frac{2k_c k_B T B}{\pi f_0 Q}}$, where B is the detection bandwidth.²² Indeed, we found that proximal to the surface, the squeeze-film effect resulted in 4–5 fold higher noise for the tipless cantilevers as compared to the tip-bearing cantilevers (Fig. 4).

Almost all commercially available cantilevers are subjected to some squeeze-film damping and operate sub-optimally (grey regions, Fig. 4). When tall tips are fabricated solely by wet etch techniques, the resulting pyramid has excessive mass and is subject to undesirable hydrodynamic damping due to the sidewalls. By increasing the aspect ratio through focused ion beam etching, both the mass and hydrodynamic damping can be decreased. However, high aspect ratio tips are fragile and may slip laterally across a sample. Therefore, the combination of tip height and aspect ratio should be optimized for each application.

Finally, we demonstrated an application for the tall-tip-bearing cantilever by performing force-volume mapping on tall cells in aqueous buffer. Murine CD4^+ T lymphocytes were activated for five days and then cultured on a coverslip coated with Intercellular Adhesion Molecule-1-Fc (R&D Systems) for 1 h, before fixation with 4% paraformaldehyde for 15 min. AFM was performed in PBS. To map topography, we used a short silicon nitride cantilever ($50\ \mu\text{m}$ long) affixed with a $16\ \mu\text{m}$ tall tip that was fabricated using the tip-transfer method. For each FD curve, we used these parameters: force distance of $2\ \mu\text{m}$, loading rate of $1\ \mu\text{m/s}$, and force trigger of 200 pN. Using this cantilever, we measured the topography of a tall cell with a force map of 30×30 pixels and $15 \times 15\ \mu\text{m}$ (Fig. 5). The topography image was free of artifacts.

FIG. 4. Dynamic performance of tall tips close to the sample surface. Resonance frequency in liquid (f_0) and quality factor (Q) of the fundamental flexural mode and the thermal force scaled to the thermal force far away from the substrate (F_{noise}) were graphed as a function of tip-sample separation. Cantilevers with two lengths (100 μm in green and 50 μm in blue) were used here. The dashed lines above the markers of tipless cantilevers were from fitting to the hydrodynamic damping model. The grey region represents the tip heights of most commercially available AFM probes.

FIG. 5. Force volume mapping of a tall T cell with steep topography.

FIG. 6. Force mapping of a tall T cell. (a) Topography (μm) and (b) Stiffness (MPa). The scale bar indicates 2 μm . (c) Six representative force-distance curves on the glass substrate (shades of blue) and the cell body (shades of orange).

The tallest portion of the T cell was 7.2 μm above the glass surface, which is taller than the tips of most commercially available cantilevers. The tall tip we employed successfully probed portions of the cell's surface that would be inaccessible to short or low-aspect-ratio tips, such as points near the steep side surface with a slope of over 80° (single arrow) and the low-lying portion of the cell (double arrows).

We mapped the elastic moduli of a T cell using a tall, tip-transferred cantilever. The force map spanned $12 \times 12 \mu\text{m}$ and consisted of 70×70 pixels. Each FD curve was fitted to a Hertz model of a cone-shaped indenter to calculate the local modulus (E , reduced Young's modulus) as follows: $F = \frac{2E}{\pi(1-\nu^2)\tan\theta} d^2$, where the Poisson ratio ν was 0.5 and the contact angle $\theta = 90^\circ - 15^\circ/2 = 82.5^\circ$. The map shows that stiffness features are decoupled from the topography, such as in patches of soft regions on the cell body (Figs. 6(a) and 6(b)). Such varied measurements for a cell were not unexpected, and highlight the importance of making many measurements across a single cell instead of determining the elastic modulus at a single point. Representative FD curves (three on glass and three on cells, Fig. 6(c)) show the low thermal noise (~ 20 pN r.m.s) of these soft cantilevers and the high heterogeneity of elastic moduli across different portions of the cell.

In conclusion, we have demonstrated a FIB-assisted technique to fabricate customized AFM probes. By transferring tall and high-aspect-ratio tips onto soft silicon nitride cantilevers, we improved the dynamic performance of these probes by reducing both squeeze-film damping and thermal noise, and enabled artifact-free force mapping of tall cells with steep topography. This technique can be implemented in any FIB system with chemical vapor deposition and simple nanomanipulation capability. For new Micro- or Nanoelectromechanical systems (MEMS or NEMS) devices, this tip-transfer technique can help to simplify the fabrication process and facilitate proof-of-principle development by adding the tip to devices post fabrication.

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