Deep atomic force microscopy

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The Atomic Force Microscope (AFM) possesses several desirable imaging features including the ability to produce height profiles as well as two-dimensional images, in fluid or air, at high resolution. AFM has been used to study a vast selection of samples on the scale of angstroms to micrometers. However, current AFMs cannot access samples with vertical topography of the order of 100 μm or greater. Research efforts have produced AFM scanners capable of vertical motion greater than 100 μm , but commercially available probe tip lengths are still typically less than 10 μm high. Even the longest probe tips are below 100 μm and even at this range are problematic. In this paper, we present a method to hand-fabricate “Deep AFM” probes with tips of the order of 100 μm and longer so that AFM can be used to image samples with large scale vertical topography, such as fractured bone samples. © 2013 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4821145>]

I. INTRODUCTION

Recently, there has been interest in increasing the scan range of Atomic Force Microscopes (AFMs) for biological study^{1–4} and metrological applications.^{5–9} We are interested in using long range AFM to study fractured bone, a sample with rough topography and deep features exceeding the actuation range of conventional AFM. Since the development of the Reference Point Indenter (RPI),^{10–15} an instrument which directly quantifies bone fracture resistance by creating an indent in bone and measuring the depth of the indent produced, we have been eager to image these indents with long range AFM. Previously, we developed a long range AFM based on voice coil actuation with a range of the order of 1 mm in each axis.¹⁶ This range was more than sufficient for imaging our bone fracture samples; however, AFM probes are not commercially available with tip lengths large enough to allow imaging over such a large Z range.

As depicted in Fig. 1(a), a typical cantilever has a small tip to lever length ratio which will cause the lever to contact the side of a deep feature, preventing the tip from touching the sample surface and therefore preventing imaging of deep features. A naive solution to this problem would be to increase the length of the tip, as shown in Fig. 1(b). This would indeed allow the tip to remain in contact with the sample surface, but would introduce tip torsion. As the AFM is not controlled with the model of both a flexing tip and cantilever, this will introduce significant error to the images.

In order to image large scale features such as RPI indents, we have designed a new “Deep AFM” probe, as shown in Fig. 1(c). The Deep AFM probe has a lengthened tip preventing the lever from ever hitting the sample surface, and is supported by two lever arms to minimize torsional effects on the lengthened tip. For reference, simulations of both the Deep AFM probe and single cantilever, each with 200 μm long tips undergoing a 100 μN lateral force, showed the topmost cantilever of the Deep AFM probe to have approximately 47 times less rotation as compared to the lengthened simple cantilever. The large rotation imposed on the single cantilever design makes it impractical for use in imaging deep features.

Here we report a hand fabrication method for manufacturing Deep AFM probes capable of imaging features of the order of 100 μm in depth.

II. EXPERIMENTAL PROCEDURE

A. Probe fabrication

1. Etch several tips using the standard “drop-off” method for electrochemical etching.¹⁷ Prepare tips from 99.9% pure 25 μm tungsten wire. Immerse the wire in a drop of 2M NaOH held in a fine ring of platinum wire. Apply a voltage of 5 V between the tip and ring such that the tip is the anode and the ring is the cathode. Retain both sides of the etched wire.
2. Glue two commercial cantilevers together. We used Bruker NCHV-W probes and Devcon 2 Ton Epoxy. Align the probe under a high magnification microscope such that the cantilevers are in line but offset to compensate for the 12° tilt of most AFMs. Allow approximately 2 h of drying time. All remaining steps also require a high magnification microscope.
3. We found greater success building our probes with the use of a foundational piece of wire of larger diameter than the probe wire first glued between the cantilevers. This wire is easier to manipulate and glue to the cantilevers, and once glued, takes advantage of capillary forces to assist placement of the probe wire. Cut wire of larger diameter to a length of slightly more than the distance between the glued cantilevers. We used 25 μm tungsten wire and a ratio of 5:4 between the wire length and distance between the cantilevers.
4. Using additional 2 ton epoxy and a small wire held in forceps as an applicator, place a small drop of epoxy about 50 μm in diameter on the tip of each cantilever. Be sure to minimize the amount of glue that wicks away from the cantilever tip. It is also important to avoid gluing the reflective side of the cantilever which will be the uppermost cantilever when placed in the AFM.

FIG. 1. Schematic depicting the advantage of Deep AFM cantilevers over current cantilever designs.

5. Wipe the applicator dry, then use it to pick up the larger wire and place it against the glue and on the two cantilevers. There should be enough residual glue on the applicator to stick to the wire and maneuver it onto the cantilevers. Be sure that the foundational piece is vertical relative to the cantilevers. Allow approximately 2 h of drying time.
6. Examine etched tips for sharpness and structure. Keep in mind that if the aspect ratio of the tip is too small, the tip will likely deform and “bend over” during fabrication or use. Select a tip.
7. Place the selected tip onto the sticky side of a regular Scotch tape, with a larger portion of the tape on the side with the sharp end of the tip. Measure and mark a cutting spot on the tape. We cut ours to approximately $400\ \mu\text{m}$. Cut slowly at the mark with sharp scissors.
8. Use a sharp knife to remove the tip from the tape and place it next to the probe.
9. Use the applicator to cover the foundational wire with 2 ton epoxy. Again, be sure that the glue is not wicking onto the cantilevers.
10. Wipe the applicator, and again use its residual glue to pick up the cut tip and bring it into contact with the foundational probe covered in epoxy. Capillary forces will help bring the cut tip into contact, and will tend to align the tip with the foundational wire. Use the applicator to gently push the tip toward this alignment, so that it eventually appears as shown in Fig. 2(a), with the tip parallel to the foundational wire and hanging a desired length below the lower cantilever, defining the usable tip length. We have built our probes with a usable tip length of approximately $200\ \mu\text{m}$. Allow approximately 2 h of drying time.

This process allows production of Deep AFM probes like the one shown in Fig. 2 with approximately $200\ \mu\text{m}$ usable tip length and tip radii of the order of $100\ \text{nm}$.

B. Bone sample preparation

Bone samples for imaging were chosen from two female 83-year-old cadavers, one with type II diabetes and one with no known bone diseases. Each sample was kept hydrated

FIG. 2. (a) SEM image of a fabricated Deep AFM probe. (b) Higher magnification image of the electrochemically etched tungsten probe tip, with a tip radius of the order of $100\ \text{nm}$.

while polished with progressively finer sandpaper to a final grit of $0.3\ \mu\text{m}$ to remove surface tissue, indented several times with a RPI following the method described by Bridges *et al.*,¹⁵ and cut with a band saw to a size of $12\ \text{mm} \times 6\ \text{mm} \times 1.5\ \text{mm}$. Next, the samples were fixed with 2% glutaraldehyde, and then dehydrated with a series of ethanol baths. Samples were rinsed with deionized water and air-dried before imaging.

C. Imaging setup

We used a Dimension 3100 AFM coupled with a custom purchased nPoint NPXY400Z100-117 piezo stage with extended actuation range in all 3 dimensions. A schematic of the Dimension AFM in position over the nPoint stage is shown in Fig. 3. Use of the nPoint stage was integral to demonstrating the capability of the Deep AFM probe, as it allowed us a range of $440\ \mu\text{m}$ in the X and Y dimensions and $190\ \mu\text{m}$ in the Z dimension. The Dimension AFM and nPoint stage were controlled by a Bruker Nanoscope V controller. The nPoint stage and Dimension AFM were placed on a MinusK 250BM-1 vibration isolation stage to minimize the effect of ambient noise on our imaging.

AFM imaging was performed in tapping mode. Fabricated cantilevers operated at resonant frequencies between $30\text{--}40\ \text{kHz}$. A scan rate of $0.1\ \text{Hz}$, proportional gain of 0.4 ,

FIG. 3. Schematic of imaging setup used in this research. (1) Dimension 3100 AFM. (2) Deep AFM probe. (3) Sample. (4) nPoint long range XYZ positioning stage.

FIG. 4. (a) AFM image, obtained in air with the fabricated Deep AFM probe, of an indent in bone of a female 83 year-old cadaver with no known bone diseases. (b) SEM image of the indent imaged in (a) for comparison.

and integral gain of 0.37 produced the best images at a scan range of $440 \times 440 \mu\text{m}^2$. Images were produced at 256×256 resolution.

III. RESULTS

The two panels of Fig. 4 depict a single RPI indent in the non-diseased bone. This indent was imaged both with a Deep AFM probe in Fig. 4(a) and with a Scanning Electron Microscope (SEM) in Fig. 4(b). The AFM image shows the indent's maximum depth of approximately $120 \mu\text{m}$. It also shows that the outer area of the indent underwent small plastic deformation, returning partially to its original height after indentation, and the central area underwent larger plastic deformation and fracture.

Fig. 5 depicts a single indentation in the bone affected by type 2 diabetes. As with the previous figure, Fig. 5(a) was imaged with a Deep AFM probe and Fig. 5(b) was imaged with SEM. Fig. 5(a) clearly shows the diabetic bone deformed to a greater extent than the non-diseased bone, to a maximum depth of approximately $160 \mu\text{m}$. A small portion of the outer ring underwent plastic deformation; the center failed and collapsed, falling steeply below the outer ring of the indent.

While in both Figs. 4 and 5 the SEM images provide greater resolution, the AFM images contain quantitative depth profiles. The SEM alone does not give quantitative depth profiles. Fig. 6 compares depth profiles from the AFM images in Figs. 4 and 5 at the deepest point of each indent. These depth profiles give quantitative interpretation about the deformation and fracture characteristics of each indent.

FIG. 5. (a) AFM tapping mode image, obtained in air with the fabricated Deep AFM probe, of an indent in bone of a female 83 year-old cadaver with type II diabetes. (b) SEM image of the indent imaged in (a) for comparison.

FIG. 6. Deep AFM line scans comparing the depth of indents inbrk Figs. 4 and 5.

IV. SUMMARY

Deep AFM probes have been successfully hand fabricated from commercially available probes with usable tip length of the order of $200 \mu\text{m}$ and tip radius of the order of 100 nm . The fabrication method we have introduced produces probes with the ability to image samples with larger height variability than have ever been imaged with AFM. We have demonstrated the probes' capacity to image features over $100 \mu\text{m}$ in depth. Deep AFM probes produce images of sufficient quality to correlate with SEM images while supplying depth information which is not apparent from SEM images, providing quantification of depth profile dimensions at each scan line. In the future, we hope to refine our fabrication method to produce probes with increased resolution through integration of current-controlled electrochemical etching for tips of much smaller radii.

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