Large scan area high-speed atomic force microscopy using a resonant scanner

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A large scan area high-speed scan stage for atomic force microscopy using the resonant oscillation of a quartz bar has been constructed. The sample scanner can be used for high-speed imaging in both air and liquid environments. The well-defined time-position response of the scan stage due to the use of resonance allows highly linearized images to be obtained with a scan size up to 37.5 μm in 0.7 s. The scanner is demonstrated for imaging highly topographic silicon test samples and a semicrystalline polymer undergoing crystallization in air, while images of a polymer and a living bacteria, *S. aureus*, are obtained in liquid. © *2009 American Institute of Physics.*
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I. INTRODUCTION

The atomic force microscope (AFM) is one of the most widely used tools for imaging and manipulating nanoscale structures in many areas of science and technology including biology, nanotechnology, and material science. Its versatility and wide applicability come from the combination of high spatial resolution, relatively noninvasive nature, ease of sample preparation, and the large number of different measurements of surface properties that can be made with a single instrument.[1] However, a major disadvantage of commercial AFMs is the low rate at which images can be captured, which limits their application for both the study of dynamic processes that occur at high rate, and for rapid surface inspection at nanometer resolution for industrial quality control purposes.

The limitation in imaging speed arises mainly from the requirement for high mechanical stability of the AFM scan system, from the bandwidth of the feedback loop that maintains tip-sample contact, and from the response time of the cantilever. Several approaches have been adopted in the attempt to improve imaging speed. By using a flexure structure to increase the rigidity of the scanner system and small cantilevers to respond fast, Hansma *et al.*[2] have attained fast scan images up to 13 μm at 4 frames/s. The use of complex control algorithms for the scanner motion that learn from and then counteract the scanner's instability have pushed this even further, with line frequencies up to nearly 8 kHz. High-speed imaging by using a cantilever with an integrated piezoelectric actuator has been reported by Sulchek *et al.*,[3] minimizing the mass that must be moved by the z-feedback loop. Ando *et al.*[4] have developed a high-speed AFM system using a rigid scanner and fast electronics with small cantilevers, and obtained a scan area of 240×240 nm^2 with 25 frames/s in dynamic mode. Most research has focused on increasing the scan speed by using smaller/integrated cantilevers, enhancing the scanner system rigidity, and/or increasing the feedback bandwidth by using fast electronics and control methods. Each of these approaches is particularly suitable for a particular application.

Taking a different approach, Humphris *et al.*[5] constructed a high-speed scanning probe microscope making use of the resonant oscillation of the scan stage, and achieved a tip velocity of more than 20 cm/s. A high-speed scanning near-field optical microscope and an AFM have been successfully implemented by this approach.[5,6] The high-speed AFM, termed VideoAFM,[7] significantly enhanced the scanning speed of AFM by using a micromachined tuning fork oscillator as a fast scanner. Through the use of very soft cantilevers and a pinning or "direct" force that maintains tip-sample contact, the probe tip is forced to track the sample surface at the high tip velocities used for imaging. This high tip velocity limits the scan size in VideoAFM systems to 4–5 μm, larger areas being acquired by tiling multiple smaller images.

Although high-speed operation of AFMs has been investigated and realized by several groups, most of the previous instruments sacrifice scan size to ensure a stable and linear image. For many applications, such as following processes occurring at a cellular scale, for surface patterning, or for more general surface inspection, there remains a need for rapid scanning over the scale of tens of micrometers, i.e., over scan areas comparable to those found in most commercial AFMs. Here we present a new sample scanner capable of large area high-speed scanning, and its integration into an atomic force microscope.

II. INSTRUMENTATION

A. Analysis of VideoAFM tuning fork scanners

In previous works,[5,6] the resonance of a quartz crystal tuning fork was utilized to provide the fast scan axis of

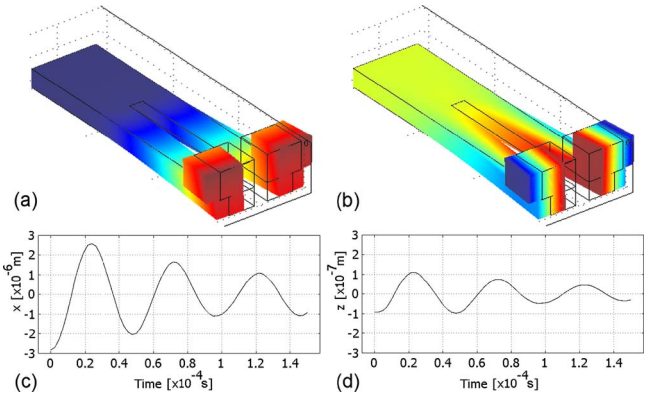

FIG. 1. (Color online) (a) and (b) show a FEM of a tuning fork used for VideoAFM scanning. In (a), the color scale shows the total displacement from rest, while in (b) it shows only the displacement perpendicular to the plane of the fork. (c) shows the total displacement as a function of time from the point at which the displacement is 3 μm, while (d) shows the corresponding vertical displacement.

a scanning probe microscope. The Infinitesima Ltd. VideoAFM uses AFM cantilevers attached to a quartz tuning fork, scanning the tip rather than the sample and hence allowing larger samples to be imaged. More recently, an alternative approach using a brass bar[8] has been used for similar (tens of kilohertz) high-speed scanning. In the current work the aim is not ultimate scan speed, but rather to obtain high scan speed and large area scanning. Previously we have found that tip velocities greater than $\sim$20 cm s^{-1} are difficult to maintain with any except the flattest samples, as the force required to maintain tip-sample contact becomes so large that the surface and/or tip are damaged, so simply driving the existing microtuning forks to very large amplitudes is not appropriate. In liquid, this problem is even more in evidence as it is much more difficult to apply a force directly between the tip and the sample to maintain tip-sample contact, and the tuning forks are inevitably heavily damped from immersion in the liquid.

A secondary issue with the use of tuning fork based scanners comes from the effect of adding mass to the tuning fork legs. The tuning fork legs are finely tuned to act as a pair of coupled oscillators, giving a very high quality factor resonance. However, once masses are added to the legs, even if this is done equally on both legs, the mechanics of the oscillation are changed considerably. To explore this we have developed a finite element model (FEM) of the tuning fork motion with and without masses attached to the legs within COMSOL MULTIPHYSICS.[9] Standard values for the Young's modulus, Poisson's ratio, and density of single crystal quartz from the literature were used, and the dimensions of the tuning fork measured. To approximate the oscillation of the fork during VideoAFM imaging, the legs in the model were displaced from rest to $\sim$10 μm and then released. The data shown is that collected as the oscillation passed through a peak-to-peak distance of 3–5 μm, equivalent to the typical scan size of a VideoAFM image.

Figure 1 shows the resultant deformation of the tuning fork with loads applied to the legs as typically used in sample scanned VideoAFM. It is clear that the resultant oscillation of the tuning fork is no longer largely planar, but rather the lateral vibration is coupled to a rotational oscillation resulting in the surface of the fork experiencing a variation in height of approximately 200 nm. As this variation occurs at a frequency that is considerably greater than the bandwidth of the conventional AFM feedback, it will result in a variation in force over the sample surface of 5–10 nN depending on the exact stiffness of the VideoAFM cantilever used, as well as the formation of a background signal due to variation in cantilever deflection across the image. Such a background is indeed observed in unflattened experimental VideoAFM data, and could not be explained by a FEM that did not include the tuning fork in the model.[9] Such out of plane oscillations are not desirable. For a robust large scan area scanning system that uses resonance, tuning forks are not suitable unless the added mass can be very significantly reduced or somehow counterbalanced.

B. Scanner design

For resonant scanning, any object with a well-defined resonant frequency can be used. Ideally this should have a high quality factor (Q-factor), the resonance to be driven should be well separated in frequency from other resonances of the body, and motion should be largely constrained to a single plane. To avoid the problems with tuning forks outlined above, we have chosen to use a macroscopic quartz bar with rectangular cross-section as illustrated schematically in Fig. 2(a). Quartz has been widely used in mechanics and electronics due to its excellent mechanical stability and generic high Q factor. As variations in resonant frequency will lead to changes in scan area when scanning at resonance, the high thermal stability of quartz is also advantageous.

In the new sample scanner the macroscopic quartz bar is used as a passive resonator, the scanner consisting of a clamp with an integrated mini piezoactuator to excite oscillation of the bar, and a nanopositioning stage with its travel direction oriented parallel to the long axis of the bar, as illustrated schematically in Fig. 2(b). Fast axis scanning is realized by the resonant oscillation of the quartz bar at its natural frequency. The natural resonant frequency of a rigid body is determined by its material property and geometric size. By controlling the length of the effective resonator, we can define resonant frequencies which correspond to different fast scan line rates. Figure 2(c) shows the calculated variation in resonant frequency of a quartz bar such as that in Fig. 2(b). Hence the same "scan stage" can be used to access a wide frequency range, although adjusting this parameter during imaging is not currently possible.

The slow scan axis is performed by the nanopositioning stage (Physik Instrumente) under the clamp along the direction orthogonal to the quartz bar's oscillation. The fast scan piezo and the nanopositioning stage are driven by the control signals from the VideoAFM controller, provided by Infinitesima Ltd., after amplification through two piezoamplifier modules (Physik Instrumente). Both drive signals are sinusoidal. The sample scanner was inserted into a Veeco D3100 AFM with either a Nanoscope IV controller or Extended Nanoscope IIIa controller, working in "contact mode." The sample is attached to a 1 cm diameter metal sample disk, which is held onto the scanner with a small rare earth magnet

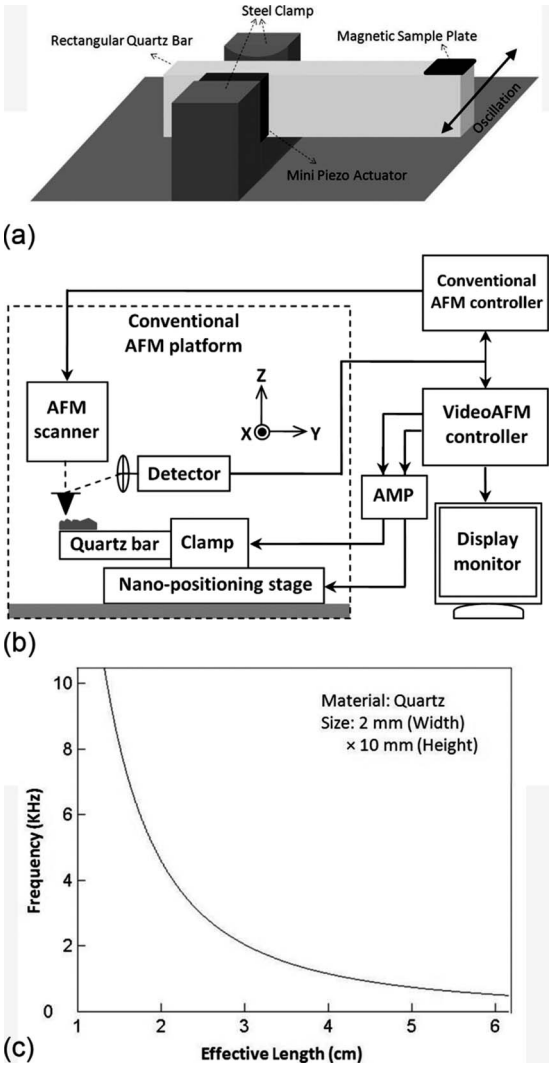

FIG. 2. (a) A schematic diagram showing the geometry of the quartz bar resonator and the clamp. (b) A schematic diagram of the new high-speed sample scanner setup. AMP: amplifier. X: fast scan axis; Y: slow scan axis. (c) The theoretical variation in resonant frequency with effective length for the resonance of a 2-mm-thick, 10-mm-deep quartz bar.

glued (with superglue) to the end of the quartz bar. The deflection signal from the AFM detection system is fed into the VideoAFM controller for data processing and image visualization. The data are corrected in real-time for the sinusoidal tip velocity that results from the use of resonance, and for the sinusoidal velocity of the slow scanner.

For imaging in liquid a small drop of water is placed onto the sample and the D3100 AFM cantilever holder for liquid is used. The fast scan axis (i.e., the axis of oscillation of the quartz bar) is oriented parallel to the long axis of the cantilever. This will be discussed further below.

III. SAMPLE PREPARATION

To test the scan system a number of different samples have been used. To test for linearity and stability of the method, standard silicon calibration grids have been imaged—a 10 μm pitch square grid of holes, depth 200 nm, from Veeco, Santa Barbara, and a 660 nm pitch grid of approximately round holes, depth 60 nm, from Nanosurf. Imaging such silicon structures is also of interest for possible in-line quality control applications of high-speed scanning. Another possible application of high-speed imaging is for following dynamic processes, and the growth of a polymer crystal [polyhydroxybutyrate (PHB)] has been imaged *in situ* at room temperature. The polymer was melted at 200 °C on a glass coverslip, thinned using a razor blade, held in the melt for 2 min, and then quenched to room temperature. The resultant coverslip with molten film was glued with superglue to a metal sample stub, and placed on the quartz scanner for *in situ* imaging during growth. For biological processes imaging in liquid is often essential. Images have been obtained of a semicrystalline polymer film (polyhydroxybutyrate) immersed in water to show the high frequency information obtainable in liquid. A dividing bacterial cell of *S. aureus* has also been imaged in growth medium (brain heart infusion). To prevent the AFM tip from dislodging the bacteria from the surface, and hence to allow stable imaging, a specially prepared substrate containing holes to accommodate the bacteria as described in Ref. 10 has been utilized.

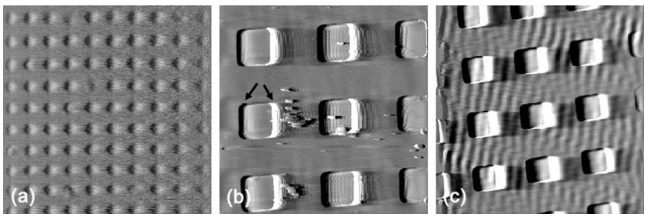

FIG. 3. Fast scan AFM images captured in ambient air using the new sample scanner. (a) An area of 7×7 μm^2 collected at an average speed of 14 mm/s; (b) An area of 25×25 μm^2 collected at an average speed of 51.5 mm/s. (c): An area of 37.5×37.5 μm^2 collected at an average speed of 57.9 mm/s. The sample used in (a) is a silicon calibration grid consisting of 60 nm deep etched pits with a 660×660 nm^2 period. The sample used in (b) and (c) is a silicon calibration grid consisting of 200-nm-deep etched pits with a 10×10 μm^2 period.

IV. RESULTS AND DISCUSSION

Figure 3 shows a series of images of calibration grids with different scan sizes captured with the sample scanner in air. All the images were captured with constant tip-sample contact at 256×256 pixels. Data were collected at 1 frame/s in Figs. 3(a) and 3(b) at a line/resonant frequency of 1.03 kHz, and 0.7 frame/s for Fig. 3(c) at a line/resonant frequency of 772 Hz, with each frame collected in 0.5 or 0.7 s, respectively, as only one direction of motion along the slow scan axis is saved (i.e., only the up or the down scan). The images represent the raw cantilever deflection obtained from the conventional AFM. The scan size in Fig. 3(c) is 37.5 μm. Even larger scan sizes can be obtained but the process is currently limited by the ability of the tip to track the surface. At the extremes of Fig. 3(c) some degradation in the image quality can be seen. This is due to the overall tilt of the sample plane relative to the oscillation of the scanner. As the *z*-feedback is unable to respond, the sample slope causes bending of the cantilever until the diode signal reaches the limit for the VideoAFM input.

As reported previously,[7,9] rapid changes in topography lead to the over bending of the cantilever during feedback

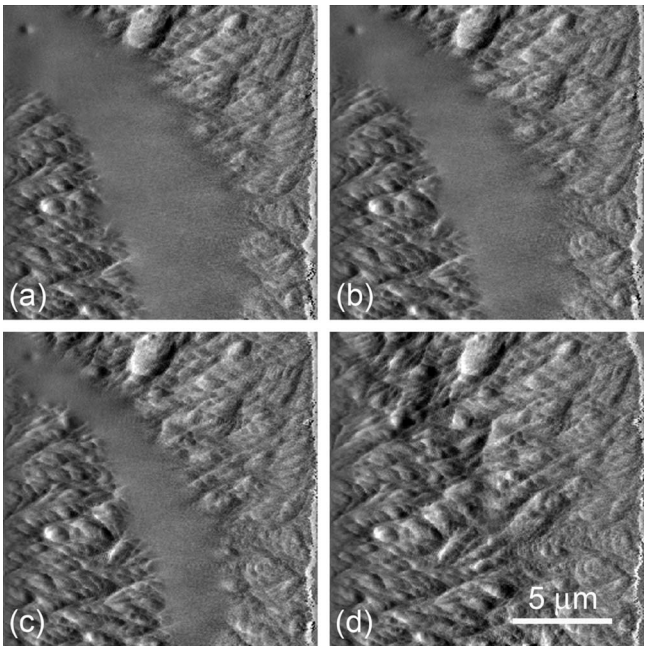

FIG. 4. A series of images showing the crystallization of PHB from the melt, collected using the quartz bar scanner at a rate of 1 frame/s. Part of a series of consecutive images, individual frames collected at (a) $t=0$ s, (b) $t=23$ s, (c) $t=55$ s, and (d) $t=122$ s.

free high-speed scanning, causing relaxation artifacts after each high frequency structure in the image [as arrowed in Fig. 3(b)]. The periodical background pattern in Fig. 3(c) is an optical interference artifact due to overspill of the detection laser from the cantilever and the reflective sample used. In air, it was not possible to use VideoAFM cantilevers at these relatively low line frequencies as excitation of the first constrained mode of the cantilever caused degradation of the image ("ringing" artifacts after rapid changes in slope). Instead, somewhat stiffer (manufacturer's nominal $k=0.5$ N/m) cantilevers were used to provide stable imaging.

To test the stability of the scan stage, we left it imaging continuously for 1 h with a 25 μm scan size (a total of 2520 consecutive images). No image distortion along the fast scan axis or appreciable change in scan width was observed. That is, the axis driven at resonance was unaffected within the accuracy of the measurement (1 pixel, or 0.4%). This mechanical stability is a feature of the use of resonance, as a drive voltage of only 30 V to the resonator drive piezo is required for a scan size of 25 μm, minimizing the heating and distortion effects usually experienced by driving piezoelectric actuators over large range for long times.

Figure 4 shows a series of images of the growth of a number of polymer crystalline aggregates (spherulites) directly from a thin layer of amorphous polymer. The growth rate at this temperature is ~ 20 nm s^{-1}, and the images, each collected in 0.5 s (1 frame/s), were taken over a period of 122 s. The resolution of the images is limited only by the pixel size as the smallest features are only 1 pixel wide. The fine features at the growth front (the interface between the smooth and "rough" appearing areas) are individual polymer lamellar crystals, which are ~5-nm-thick in this material. The technique has sufficient spatial resolution and stability to follow processes such as this in real-time.

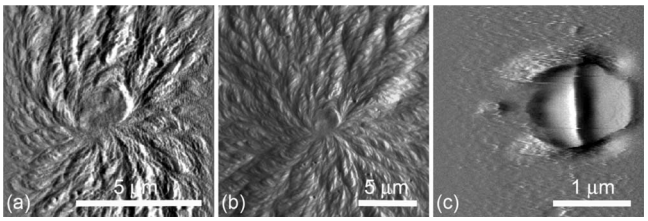

FIG. 5. (a) An image collected under water using the quartz bar scanner, showing the surface of a PHB spherulite, collected in 0.5 s. (b) a conventional AFM contact mode deflection image showing the same area as in (a). (c) An image collected under growth medium using the quartz bar scanner, showing a dividing *S. aureus* bacterial cell.

One of the major targets of developing high-speed AFM is to perform surface investigations under liquid environment for biological applications. Figure 5(a) is an image of a PHB semicrystalline polymer surface in water using the quartz bar sample scanner. As the cantilevers are heavily damped by the liquid, soft ($k=0.03$ N/m) Si_3N_4 cantilevers could be used without exciting excessive oscillation at the cantilever's first constrained mode (as detailed in Ref. 9, it is the constrained modes of the cantilever that are excited during scanning when tip-sample contact is maintained). By mounting the cantilever's long axis parallel to the fast scan axis, the direct force maintaining high frequency tip-sample contact was provided by the hydrodynamic interaction between the cantilever beam and the water that was moving relative to it: a vertical force (driving the tip away from the surface) on the retrace line, and a normal force (driving the tip into the surface, i.e., providing the tip pinning force) on the trace line. As this force is proportional to the tip velocity, it behaves in exactly the way necessary to maximize the efficiency of the surface tracking mechanism, being maximum when the force required maintaining tip-surface contact is greatest as the tip velocity is maximum. High quality images in liquid can therefore be obtained.

Figure 5(c) shows an image of a dividing bacterial cell, *S. aureus*, imaged in growth medium. Here the bacterium appears as a smooth, largely featureless object with a dark cleft in the middle. This cleft is the line of cell division (the septum) of the bacterium. Contact mode images of bacteria at this scale typically appear similarly featureless when imaged in liquid.[10] The tip-sample forces were sufficiently small to avoid damaging the cell, despite its significant topography (the cell protrudes approximately 200 nm from the trapping grid). However, after several minutes of imaging (i.e., several hundred images) the cell was dislodged from the hole and removed by the motion of the tip. For future studies, it may be necessary to develop methods for more rigidly restraining the cells.

V. CONCLUSIONS

We have shown that the use of resonance to perform the fast scan in an AFM can provide scan areas comparable to conventional AFMs but with frame-a-second scan rates. The use of a macroscopic resonator provides a remarkably simple method for large area, high-speed scanning with high stability. The resonant scan stage has potential applications in

microscale/nanoscale quality control, surface patterning, and the investigation of processes occurring under physiological conditions.

ACKNOWLEDGMENTS

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