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Breaking the speed limit with atomic force microscopy

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Abstract
High-speed atomic force microscopy (AFM) is important for following processes that occur on sub-second timescales for studies both in biology and materials science, and also for the ability to examine large areas of a specimen at high resolution in a practical length of time. Further developments of the previously reported high-speed contact-mode AFM are described. Two instruments are presented: (i) a high-speed flexure stage arrangement capable of imaging at a video rate of 30 fps, and (ii) an ultra-high speed instrument using a combined tuning fork and flexure-stage scanning system capable of ultra-high-speed imaging in excess of 1000 fps. Results of imaging collagen fibres under ambient conditions at rates of up to 1300 frames s^{-1} are presented. Despite tip–specimen relative velocities of up to 200 mm s^{-1}, no significant damage to the collagen specimen was observed even after tens of thousands of frames were acquired in the same area of the specimen.

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1. Introduction

Atomic force microscopy (AFM) (Binnig *et al* 1986) is the most important characterization technique in nanoscience and nanotechnology, being capable of imaging at the nanoscale in both ambient and liquid environments as well as in vacuum. The most serious disadvantage of conventional AFM is its relatively low imaging rate. Conventional AFM typically requires more than one minute to acquire a topographic image, depending on image area and specimen roughness. This drawback limits the processes that can be followed in real time by AFM to those occurring on a timescale of minutes. Also, with no low magnification mode in AFM, scouting over a large area is time consuming. High-speed imaging could alleviate both of these problems.

The speed limitation in AFM arises because it is an essentially mechanical microscope: mechanical scanning of the probe tip relative to the sample, the measurement of the tip–specimen force interaction through the bending of a micro cantilever, and maintenance of a constant tip–specimen force in contact mode or rms amplitude in tapping mode while scanning over the specimen through the vertical repositioning of the specimen relative to the tip. Over the past decade, several AFMs have been developed with the aim of increasing imaging rates. AFMs with multiple cantilevers (Minne *et al* 1998) allow adjacent areas of the specimen to be scanned simultaneously and a composite image of the specimen to be constructed. Although this method allows more rapid scanning of large areas of a specimen it does not allow processes to be followed on shorter timescales. For repetitive processes, a stroboscopic AFM technique (Anwar and Rousso 2005) allows the process to be followed with high temporal resolution. In this technique, a series of AFM scans is collected such that each scan is shifted in phase with respect to the periodic process being studied such that one cycle of the process can be reconstructed with high temporal detail. Since most processes are not periodic, this method has a limited field of application. The most generally applicable technique so far involves the shifting of the response time of the AFM to shorter timescales principally by using small cantilevers of high resonant frequency (>1 MHz in air) in tapping mode with corresponding improvements in the response time of the

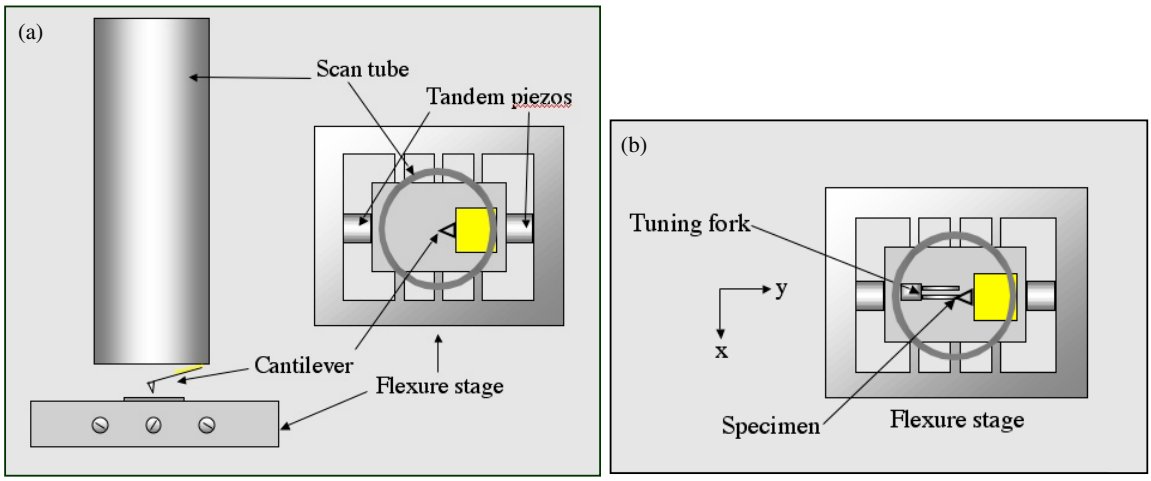

Figure 1. (a) Position of the high-speed flexure scanning stage in relation to a micro cantilever mounted on the scan tube, and, (b), for kilohertz frame rates a high speed tuning fork scanner was mounted on the flexure stage that provided the slow scan direction.
(This figure is in colour only in the electronic version)

electronic and mechanical feedback system, and an increase in scanning frequency (see for example: Viani *et al* 1999, Sakamoto *et al* 2000, Viani *et al* 2000, Ando *et al* 2006). As well as the increases in response speed of the cantilever and the scanning system, the feedback control electronics and feedback algorithms have also undergone considerable improvements for high-speed SPM (Schitter *et al* 2004, Fantner *et al* 2005, Rost *et al* 2005).

An alternative method based on contact-mode imaging has been developed (Humphris *et al* 2005), in which the tip is scanned at high speed relative to the specimen and a conventional AFM feedback system maintains a constant force averaged over the timescale of approximately one frame. Topographic information is obtained through the instantaneous deflection of the cantilever. In the original implementation, a resonant scanning system was used similar to that used in the high-speed scanning near-field optical microscope (Humphris *et al* 2003). In the work presented here, both resonant and non-resonant scanning systems were utilized and for ultra-high speed imaging ($>$1000 frames s^{-1}) a combined resonant and non-resonant scanning system was used.

2. Experimental details

2.1. AFM instrumentation

Two experimental set-ups were used: (i) a high-speed scanning system based on a flexure stage was used for video rate imaging and (ii) a combined tuning fork and flexure stage for ultra-high speed ($>$1000 frames s^{-1}) AFM imaging. Both systems were mounted on a Veeco Dimension 3100 such that conventional AFM could also be performed. In conventional and high-speed AFM, the fast scan direction is usually set as the horizontal direction in the image and the slow scan as the vertical direction. Often, these directions are labelled the x and y directions, respectively.

2.1.1. Video-rate AFM. High-speed scanning (x-direction) was provided by a flexure stage constructed in-house and slow (frame) scanning (y-direction) was provided by a conventional piezo electric scan tube, driven with a rounded triangular waveform, on which the cantilever was mounted (figure 1(a)). This tube also provided the z-direction control in order to maintain on average a constant cantilever deflection. The flexure stage drove a specimen stage of about 1 cm in diameter. The cantilevers used were supplied by Infinitesima Ltd (Oxford). Cantilever bending was measured by the optical lever method (Alexander *et al* 1989, Meyer 1990). Deflection data were captured and image construction performed in real time using Infinitesima electronics (Infinitesima Ltd, Oxford, UK; http://www.infinitesima.com).

2.1.2. Kilohertz imaging rate AFM. For ultra-high speed imaging, the fast scan was provided by tuning forks with resonant frequencies of about 32 kHz and 100 kHz (microscanners supplied by Infinitesima Ltd). The specimen was mounted on one of the tines of the fork. The tuning fork was mounted on the flexure stage that provided the slow frame scan, as this is now too high a frequency ($>$1 kHz) to be performed by the conventional scan tube (figure 1(b)).

2.2. Collagen specimens

The type I collagen samples were obtained from rat tail tendon by dissection (sacrificed animals) and stored in saline at 4 °C until use. Extracts of fibrils were obtained from the bulk tendon by a subsequent dissection, which was followed by rinsing in high-purity water. The extracts were then drawn onto plain glass slides before being dried in a gentle stream of dry nitrogen gas.

3. Results and discussion

High-speed AFM imaging of collagen fibres in ambient conditions was successfully performed with both experimental set ups. The flexure stage was capable of up to 40 kHz oscillations with a maximum scan size of 2 μm. Since this was a non-resonant stage, the actual line rate could be arbitrarily

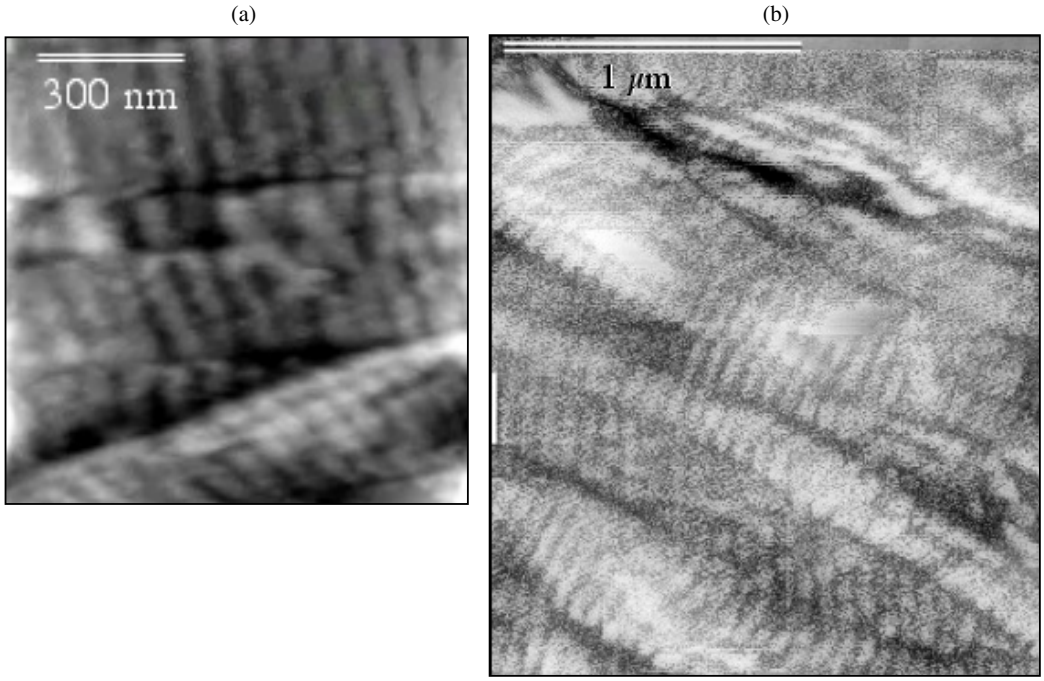

Figure 2. (a) Frame from movie of collagen fibres recorded at 30 fps. (b) Composite image constructed from 12 frames recorded at 30 fps while translating the imaging area over the specimen surface (see figure S2 available at stacks.iop.org/Nano/18/044030).

selected. With the slow scan being implemented with the conventional scan tube, a frame rate of 30 frames s^{-1} (fps) was routinely achieved. This video rate imaging is particularly useful when adjusting the imaging conditions whose effects can be immediately evaluated. Images and movies of the collagen specimen were obtained in air and a single frame is shown in figure 2(a). Several collagen fibres are visible in this image with the banding pattern (spacing 67 nm) clearly visible. A movie showing translation over the specimen was also recorded. From such a sequence of images, a composite image covering a larger area of the specimen can be constructed, and an example is shown in figure 2(b) in which twelve images of adjacent areas were used to produce a composite image of a larger specimen area. The total imaging time for this composite image is 0.4 s.

In order to achieve ultra-high imaging rates, a second set up was implemented in which the fast line scan was produced by a tuning fork oscillating at its resonant frequency of about 32 kHz and the 'slow' scan was produced using the flexure stage. The tuning fork is used at resonance and so produces particularly stable scanning since its Q factor is of the order of 10 000. Resonance also allows higher amplitudes of oscillation to be generated and thus larger areas to be scanned. While the motion is sinusoidal in time, resulting in the probe spending greater time at each end of the scan and less in the centre of this scan, this is readily linearized even in real time.

Using this system, the maximum frame rate of 1300 fps was achieved. Collagen was observed in air and its characteristic banding at 67 nm was clearly observed. Figure 3(a) shows a sequence of consecutive images with each acquired in less than 1 ms and the whole sequence in about 5 ms. The collagen specimen was translated with respect to the probe and figure 3(b) shows a sequence of images in which every 14th image is shown. The translation can now be discerned. From a similar sequence of ultra-high speed images, a linear composite image was generated and is shown in figure 4. Several overlapping fibres can be seen.

By using a tuning fork with the higher resonant frequency of 99.5 kHz, it was possible to achieve higher resolution images (100 × 100 pixels) than with the 32 kHz tuning fork while maintaining the 1000 fps. Figure 5 shows a single frame from a movie (available at stacks.iop.org/Nano/18/044030) acquired using this tuning fork as the specimen stage providing the high speed line scan. The 'slow' scan was again achieved with the flexure stage. With this tuning fork, the maximum x-direction scan size was about 500 nm. The specimen is again type I collagen fibres.

In both the video-rate AFM and ultra-high speed kilohertz AFM imaging, no discernible damage to the collagen specimen occurred after tens of thousands of frames. This is clearly a significant phenomenon for not only high-speed AFM but also for nanoscale mechanical systems operating in a high-shear-rate regime. Evidence from preliminary investigations of this phenomenon point to a super lubricity effect associated with thin layers of water confined between the scanning probe and the specimen. These studies will be presented in a separate publication.

4. Conclusions

The relatively soft biological specimen of type I collagen fibres could be imaged by contact-mode AFM at both high-speed video rate (30 fps) and kilohertz frame rates of up to 1300 fps such that each AFM image was acquired in about 750 μs with no significant damage to the specimen even after minutes

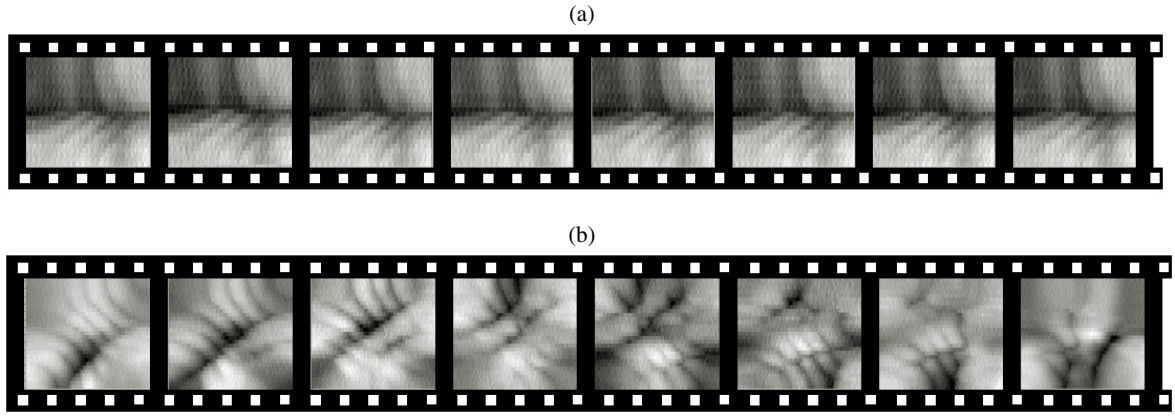

Figure 3. (a) Sequence of images of collagen with banding from movie in which frames were recorded at 750 μs intervals. (b) Sequence showing every 14th image from movie recorded at 1280 fps while translating over the specimen surface (available at stacks.iop.org/Nano/18/044030).

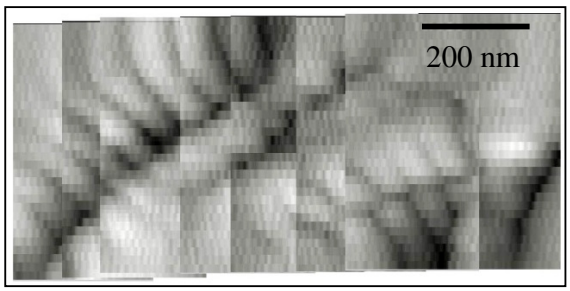

Figure 4. Composite image of collagen fibres constructed from movie sequence of figure 3(b) (available at stacks.iop.org/Nano/18/044030).

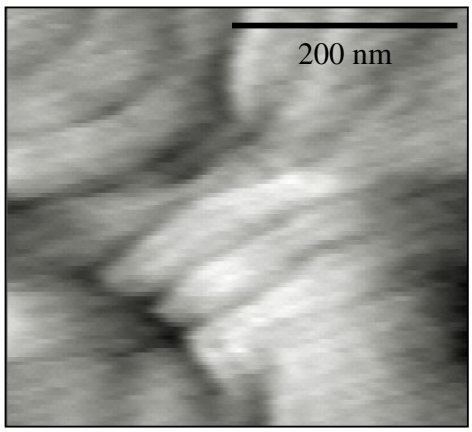

Figure 5. Collagen specimen imaged in air with a line frequency of 100 kHz and a frame rate of 1000 fps (available at stacks.iop.org/Nano/18/044030).

of imaging. This very surprising effect is being investigated and appears to involve a lubrication effect associated with the water confined between the tip and specimen. This high-speed AFM method opens up the possibility of following processes occurring on a timescale of a few milliseconds, and thus would be of particular importance for biomolecular processes.

Acknowledgments

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