A High-Speed Atomic Force Microscope Capable of Video-Rate Imaging

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INTRODUCTION

Over the past 20 years the atomic force microscope (AFM) has revolutionised our ability to generate real space images of surfaces at the nanometre scale. The popularity of the AFM over other scanning probe microscopes is due to its ability to image a material, whether it conducts or not, in a wide range of environments, leading to applications in surface, material and biological sciences. These capabilities also enable the non-invasive preparation of samples and the ability to follow physical processes in situ.

Where the atomic force microscope is limited is in the time taken to collect an image compared to other microscopic techniques. The microscope’s inherent mechanical nature and serial data collection results in an image acquisition time from tens of seconds to several minutes. It has been a long-standing goal to increase the imaging speeds of AFMs, as this would offer many advantages. At present the slow image acquisition time only enables the visualisation of effectively static or very slow processes. Increased image rates would directly provide the ability to follow events and dynamic processes on shorter time scales. Further advantages of high image rates would be: the ability to quickly map large areas with high resolution; allow collection of repeated measurements for statistical confidence; and increase the throughput where many samples require analysis such as process control and inline monitoring.

An additional benefit of high speed imaging, in particular at video rate, is the ability of the operator to interact intuitively with the microscope in real time, offering the ability to use the AFM like an optical microscope but with nanometre resolution.

UNLOCKING THE SPEED OF THE AFM

The key to unlocking the speed of an AFM is to understand the mechanisms that limit the measurement time for each image. The three principle limits, which all result from the serial and mechanical nature of the microscope, are: the speed of the X-Y scanner, the speed of the Z actuator and the response time of the cantilever.

Firstly, to generate the image a probe must be scanned relative to the surface of the sample with sub-nanometre precision. This is typically achieved with a piezoelectric-based actuator which is relatively massive with a resonant frequency of hundreds of Hertz, limiting the scanner’s operation to tens of Hertz so as to avoid resonances and instabilities of the scanner and retain high resolution. Thus for an image with 256 lines, a scan time greater than ten seconds is typical.

Secondly, for the tip to track the surface of the sample with constant force, the interaction must be measured and the position of the tip adjusted to follow the topography of the sample. A further piezoelectric actuator is used to control the Z position of the tip, but once again its frequency response is limited due to inertia.

Figure 1a: Collagen fibres imaged with the VideoAFM. The image was acquired in 67 milliseconds. A sequence of 1800 images was collected... The image shows the 67 nm periodic banding along the fibre. Scale bar = 100 nm.
Thirdly, the cantilever not only positions the tip but also measures the interaction force with the sample. The rate at which the cantilever can respond to changes in the tip-sample interaction force limits the maximum scan speed.

In order to overcome these three limitations and develop a high-speed AFM, a method of implementing a high X-Y scan rate and high speed for both the Z-axis and cantilever response is required. But how fast is high speed? If the images are to be presented to the microscope operator in real time at video rate this would require an increase in speed of approximately one thousand times compared to conventional approaches.

A number of groups have developed high speed AFM by optimising the mechanical performance of the stages and the cantilever. Imaging times below ten seconds have been achieved with actively controlled cantilevers [1,2] and times below 100 ms have been demonstrated [3] although the maximum scan area becomes limited. It is desirable to produce an instrument capable of achieving sub-100 ms frame rates and micrometre imaging areas.

To deliver such an increase in capabilities, the existing speed limitations must be overcome. However, pushing the mechanical resonant frequencies ever higher is not feasible and a completely new approach is required. An instrument based on work by Humphris et al. [4,5] and Infinitesima Ltd utilises a different methodology that overcomes these limitations which are imposed by the conventional approach.

**A NEW HIGH-SPEED AFM**

We have developed a new video-rate atomic force microscope, the VideoAFM, that overcomes these speed limitations by using the following strategies:

1. The conventional scanner was replaced with a microresonant scanner that utilises, rather than avoids, mechanical resonance to achieve high speed X-Y scanning. The use of a mechanical resonance has the added advantage of not suffering from any hysteresis, which is a common problem with conventional scanners.
2. A special cantilever was used that incorporates a passive mechanical method of maintaining a constant tip-sample interaction at high tip velocities and is capable of allowing MHz pixel rates.
3. Dedicated high-speed electronics were incorporated for real-time image capture, image processing and display.

These developments have resulted in an instrument that is capable of imaging 3 x 3 µm square areas at 25 frames per second with a 128 x 128 pixel resolution or at 15 frames per second with 256 x 256 pixel resolution. The high-speed electronics enable the presentation of the images to the operator in real time providing a direct intuitive interaction with the microscope and specimen.

In the following sections we show examples that demonstrate some of the capabilities and abilities of the video-rate AFM to image soft samples, biological and polymer surfaces, map large areas in real time with high resolution and visualise dynamic processes with high time resolution.

**HIGH-SPEED IMAGING OF POLYMER CRYSTALIZATION**

The AFM has been previously applied to the visualisation of dynamic processes by collecting a series of images to generate a movie. To overcome the slow image acquisition time, previous studies have slowed the process down by controlling the conditions, for example temperature, and the assumption was made that the physics of the process remains unaltered. High-speed imaging can be used to avoid these experimental complications and test this assumption.

The high temporal resolution of the video-rate AFM has been utilised to study polymer crystallisation. Figure 2 shows selected images from a sequence of 182 images that captures the dendritic growth of a thin film of polyethylene oxide (PEO). The high temporal and spatial...
tial resolution of the microscope enables the
detection of a change in growth rate of the
two dendrite arms A and B which slow down
as they approach to within 30 nm of each
other (Figures 2 and 3). The authors of this
work drew our attention to the radius of gyra-
tion of the PEO polymer which is 30 nm,
attributing the slowing of the fronts to com-
petition for material between the fronts. The
exceptional feature of this dynamic observa-
tion is that, beyond the continuum effects on
diffusion rates, the molecular size of the poly-
mer in the melt appears to directly influence
the growth when the gap is reduced to the
same scale as the molecules, at which point
they may be crossing the gap between the two
fronts.

MAPPING LARGE AREAS WITH
HIGH RESOLUTION
Rather than re-imaging the same area repeat-
edly to obtain high temporal resolution the
area being imaged can be moved and the
resulting images tiled together, mapping a
large area but retaining the high resolution of
the original images. This technique enables
long features to be correlated with high reso-
lution [8].

Figures 4a and 4b provide two examples
where the surface of polyethylene oxide has
been mapped by titling multiple images. Fig-
ure 4a shows an area of 12×10 micrometres
which has been collected by scanning the
imaging window in a square raster pattern.

Alternatively, Figure 4b was created by the
microscope operator by moving the image
window using the real-time presentation of
the image to decide how to explore the sur-
face. The surface structure over large areas is
clearly visible and the soft polymer has not
been damaged by the high tip speed.

CONCLUSIONS
Here we have introduced a high-speed atomic
force microscope that is one thousand times
faster than a conventional AFM and presented
selected results from its applications to bio-
logical and polymer specimens.

High-speed atomic force microscopy is an
emerging technique capable of giving
researchers an intuitive real-time image of
phenomena at the nanometre scale. By over-
coming the speed limitations of traditional
AFMs, new research areas are possible, such as
the study of dynamic processes with high spa-
tial and temporal resolution. We believe that
this will be an essential tool in the rapidly
developing areas of biotechnology and
nanotechnology.

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