High-Speed Atomic Force Microscopy Enables New Applications

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Introduction

Atomic force microscopy (AFM) [1] is one of the most powerful and dynamic methods for performing nanoscale imaging and materials characterization, enabling scientists and researchers to attain atomic resolution and measure nano-mechanical material properties *in-situ*, all while requiring minimal sample preparation. In spite of these clear advantages, user adoption of AFM has been limited by the technique's slow imaging speed as compared to light microscopy, scanning electron microscopy (SEM), and transmission electron microscopy (TEM). However, recent advances in AFM technology have increased AFM imaging speeds by over an order of magnitude [2–4], opening up a wide range of new applications while greatly improving the user experience.

One of the most beneficial outcomes of higher-speed AFMs is the increase in productivity for everyday nanoscale investigation. Reducing the time to first explore a heterogeneous surface, find the region of interest representing the sample's morphology, and capture a publication-quality image is a clear benefit for scientists. The time reduction allows researchers to reveal nanometer-level characteristics in minutes instead of hours. High-speed AFM also greatly increases productivity in capturing the large number of images needed to understand the synthesis of materials with statistically valid data quantities. Perhaps the most interesting application that benefits from high-speed AFM is the ability to observe dynamic processes with sufficient time resolution to study phenomena such as crystal formation, protein dynamics, or aging processes. This article discusses some examples of these high-speed AFM applications and the new technology that is making them possible.

Sample Exploration

A commonplace use of an AFM involves the exploration of an unknown heterogeneous sample to understand the different morphologies that best represent the surface and to capture a representative set of publication-quality images to document what was found. Especially for complex samples, the majority of imaging time is often spent looking at enough sample surface to understand what is important. On a rough sample, a faster AFM makes it possible to engage and image more sites in a shorter amount of time. A powerful use of high-speed AFM is to capture a large scan area with a high pixel resolution (for example, 5,000 lines with 5,000 points per line). Normally, such an image would take hours to capture; but, with the new generation of high-speed AFMs, a large-area high-resolution image can be acquired in minutes. A single image that contains spatial information ranging three orders of magnitude (from nanometer scales to tens of micrometer scales) can yield whole new levels of understanding. In addition, the data can then be zoomed into, and representative areas can be magnified and published. An advantage of this method is that the user can decide on the best scale and framing after taking the data. By drastically reducing the time investment required to capture a high-resolution image, a high-speed AFM encourages users to explore their samples in this more productive manner (Figure 1).

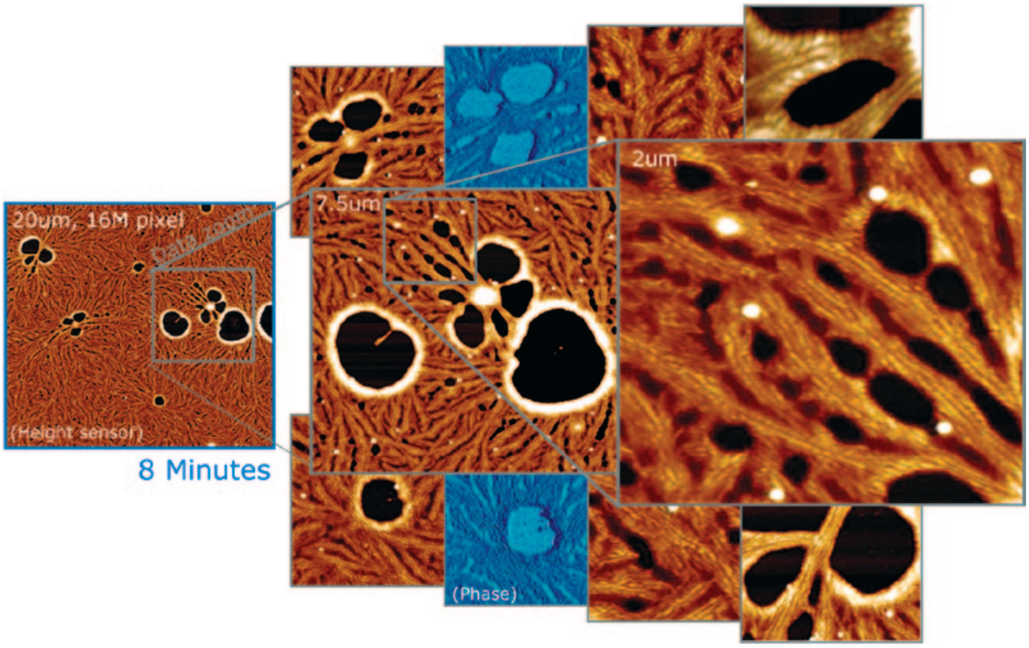

Figure 1: A high-resolution, 16-megapixel image of PTFE polymer film captured by high-speed AFM for later exploration offline.

Screening Applications

In screening applications, the types of expected surface phenomena may be known. The challenge is to determine the relationships between one or more independent variables and one or more responses or dependent variables. Quantifying these relationships requires efficient imaging of multiple sites on multiple samples and analysis of the responses. Imaging speed is obviously critical to accomplishing this goal, but multi-sample loading and automation, consistent operation

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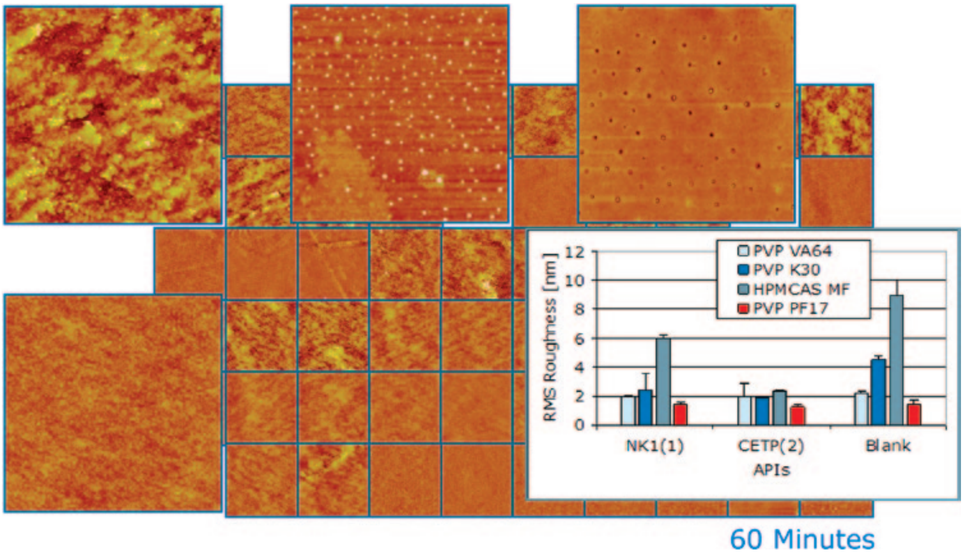

Figure 2: Two Active Pharmaceutical Ingredients (APIs), NK1(1) and CETP(2), were dissolved into four excipients and tested for mixture stability via high-speed AFM. Using a tip-scanning AFM with an automated sample stage, the multiple images were captured quickly and autonomously, enabling the easy collection of large quantities of data from several samples. The tight error bars in the plot of average surface roughness for each sample formulation demonstrates the imaging reliability of high-speed AFM. (Samples provided by researchers at F. Hoffman-La Roche Ltd.)

without user intervention, data management, and batch image analysis also play important roles.

In a typical example, a high-speed AFM was used to determine where an active pharmaceutical ingredient (API) was combined with an inactive excipient to form an amorphous solid with the goal of maximizing the API's solubility after ingestion. The amorphous formulation was solid at room temperature but would otherwise separate into two phases. Microscopic (~100 nanometers) separation and recrystallization of the API must occur to observe the possible phase separation. Researchers at F. Hoffman–La Roche Ltd. and the University of Basel used the latest generation of high-speed AFMs to detect the indicators of instability on a much smaller size scale and at a point earlier in the development process than was possible in the past (Figure 2), thereby accelerating the formulation development cycle. The AFM provided data on multiple sites of 100 or more samples per day [5], enabling the researchers to quickly and easily collect statistically significant data sets.

Dynamic Processes

The time-resolved study of dynamic processes is the application that has provided the greatest impetus to develop faster AFMs. As an example of work in this field, Bruker scientists examined a sample from the group of Yuri L. Lyubchenko at the University of Nebraska Medical Center, with the goal of replicating their methods to loosely bind biomolecules to a substrate for dynamic AFM observation. Lyubchenko et al. use these methods to investigate the structure and dynamics of nucleosomes [6]. In their studies, the samples were prepared by the deposition of nucleosome core particles (NCPs) without the fixation with glutaraldehyde that is typically required for electron microscopy, demonstrating that AFM is gentle enough to image the DNA-NCP complex in its active state (Figure 3). Time-lapse experiments were performed by scanning in liquid over an area of about 800 × 800 nanometers to follow the dynamics of NCPs. The frames show that, over time, DNA spontaneously unwraps from the histone core, and the core disassociates and leaves the area of observation.

The study of crystal formation is another important application of AFM involving dynamic processes. AFMs have been used to study polyhydroxybutyrate-co-valerate (PHBV), a biodegradable thermoplastic polymer produced by microbial fermentation [7]. The formation of the crystalline lamella structure of PHBV may determine the mechanical properties of the bulk material. The sample crystallizes from various nucleation points so quickly that by the time conventional AFMs have taken a single scan, the crystal is already formed. Fast scanning is needed to capture the dynamic processes of crystallization. Using a PBHV sample courtesy of Jamie Hobbs at the University of Sheffield, Bruker researchers employed a high-bandwidth AFM to take a 1 μm × 1 μm, 256-line image with a frame time of 2.5 seconds. The resulting movie shows the lamella forming as the crystallization front moves across the frame. It also shows two fronts joining, passing over defects, sometimes incorporating them, and sometimes not. Lower magnification images of this process are shown in Figure 4. These results provide important insights into the growth rates and dynamics of the crystalline lamella.

AFM Technology Advances that Affect Increased Bandwidth

AFM measures the sample by probing the sample surface with an ultra-sharp tip that is attached to the end of a cantilever. Interaction forces between the tip and sample can be measured by detecting the displacement of the cantilever, for example via the laser deflection method [8]. When scanning a sample, the AFM employs a feedback loop, which monitors the cantilever displacement and actuates the AFM *Z*-scanner to maintain a

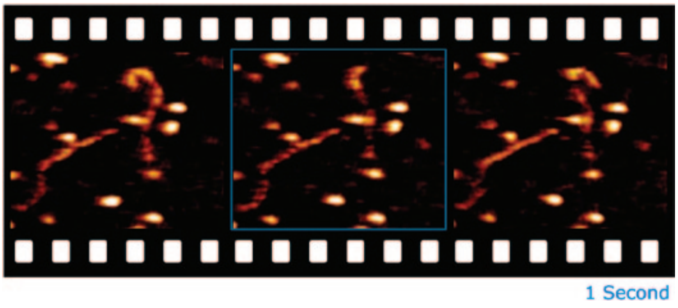

Figure 3: High-speed, 1-frame-per-second AFM images of DNA strands, obtained using the Dimension FastScan™ system, indicate that force control on loosely bound biomolecules can be achieved at high frame rates. Three of 2,100 AFM frames are shown for DNA-NCP in various active states. (APS mica samples courtesy of Yuri L. Lyubchenko of the University of Nebraska Medical Center.)

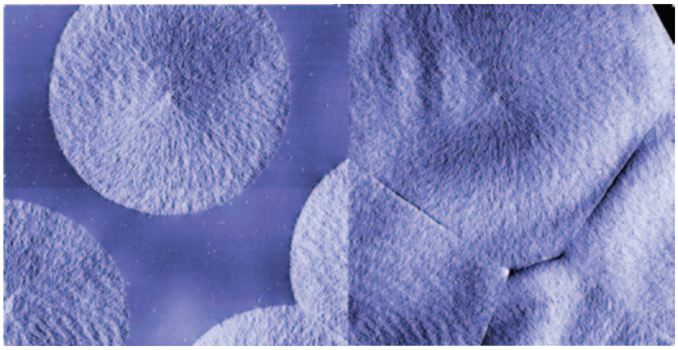

Figure 4: Two high-resolution 20 × 20 µm AFM scan frames show PHBV crystal growth from amorphous phase after quenching. The image on the left shows one AFM image frame of spherulite growth. The image on the right shows a single AFM image frame of the dominant lamellae slowing their growth to a stop. Each of these 13-megapixel images was captured in minutes, enabling researchers to further explore the sample's micro- and nano-scale features offline.

constant tip-sample interaction (commonly call the setpoint). When attempting to scan faster, the AFM *Z*-scanner feedback loop must respond correspondingly faster or the forces between the tip and the sample will deviate from the setpoint, resulting in image distortion that can degrade the AFM tip's resolution and possibly damage soft or delicate samples.

Because feedback loops are only as fast as their slowest component, producing a high-speed AFM that is free of image distortion, resolution degradation, or sample damage requires that the speed (or bandwidth) of every component in the AFM feedback loop be improved significantly. The most challenging components to develop in the feedback loop are the *Z* scanner bandwidth and the AFM cantilever dynamics.

High-bandwidth *Z* scanner design. The *Z*-axis scanner represents a significant design challenge for high-speed AFMs (Figure 5). In recent years academic researchers have demonstrated high-bandwidth *Z* scanners that traded off speed increases for greatly reduced *Z* range and accuracy. To maximize the value of high-speed AFM to its users, the AFM *Z* scanner must demonstrate at least one order of magnitude increase in bandwidth yet have enough range to allow a broad range of samples, while maintaining ultra-low-noise *Z* position-sensor capabilities to enable accurate surface metrology. In addition, the design should facilitate easy AFM probe exchange and waterproofing of the scanner surfaces for liquid operation and cleaning. The latest generation of high-speed AFMs addresses these needs.

Attaining fast cantilever dynamics. The most common mode of AFM operation is TappingMode™ (also called "dynamic mode" or "intermittent contact mode"), in which the cantilever is oscillated at or near its resonant frequency. This resonant frequency is defined by the cantilever effective mass and spring constant (expressed in N/m), which are in turn defined largely by the cantilever material and geometry. As the tip is brought into proximity of the surface, the oscillation amplitude is reduced by tip-sample forces. The AFM feedback loop maintains a constant oscillation amplitude by actuating the *Z* scanner as the tip is scanned over the sample surface. Maintaining sample measurement accuracy requires that the cantilever amplitude be allowed to return to its equilibrium value, but this process takes finite time and thus is a bottleneck toward attainment of higher scan speeds.

The speed at which the cantilever returns to equilibrium depends primarily on three factors: the cantilever's oscillation setpoint, resonant frequency, and quality factor (*Q*). Cantilever speed increases with decreasing oscillation setpoint, and thus decreasing the oscillation setpoint is a common tactic to improve scan speed. However, doing so increases imaging force, which can damage delicate samples and degrade tip sharpness unless the cantilever spring constant is also decreased. Cantilever speed also increases with increasing resonant frequency and with decreasing *Q*.

The obvious way to increase cantilever resonant frequency is to increase its thickness, but doing so greatly increases the spring constant and therefore the imaging force. Thus, to image faster while maintaining low force, it is necessary to decrease all cantilever dimensions. By properly balancing all the dimensional and materials constraints, it is possible to design a cantilever that enables over one order of magnitude increase in AFM scan speed while maintaining low tip-sample force. Balancing these design factors with scalable, economic manufacturing techniques is critical to the development of commercial AFMs that meet the challenges of emerging high-bandwidth applications.

Improved Laser Optics

Cantilever deflection is measured as the angle change of the reflected laser beam induces movement of the laser spot on a four-quadrant photo detector. The signal generated is proportional to the movement of the projected spot divided by the size of the spot. The cantilever acts as a mirror, so the incoming beam must be more convergent to produce a smaller spot, and this beam, when reflected, is equally divergent.

Thus, a smaller laser spot on the back of the cantilever results in a larger laser spot on the photo detector, resulting in reduced optical deflection sensitivity. This detrimental characteristic is offset by the increase in deflection sensitivity with decreasing cantilever length. Thus, high-speed AFMs that use small cantilevers can still attain atomic resolution. However, to maximize optical deflection sensitivity for a broad range of cantilever lengths, the high-speed AFM needs to allow fast and easy adjustment of the laser spot size to enable the user to select an appropriate spot size, namely, the largest spot size that will not spill off the edge of the cantilever and generate interference and noise. The new generation of high-bandwidth AFMs provide a selection of spot sizes that accommodates different cantilevers, including a small spot size required for the smaller cantilevers used for high-bandwidth scanning.

Tip Scanning Versus Sample Scanning

AFM scanners can be configured as "tip scanning," where the sample is stationary and the tip is moved in three dimensions as it scans over the surface, or as "sample scanning," where the tip is stationary and the sample is moved in three dimensions as it is scanned under the tip. High-speed sample scanning systems are inherently simpler to design and manufacture but are highly restrictive for the user. Sample size is limited to what can fit on the sample scanning stage (usually ~10 mm in diameter by a few mm thick), and the sample's mass negatively impacts the AFM scanner dynamics. In contrast, a high-speed tip scanning AFM can accept very large samples,

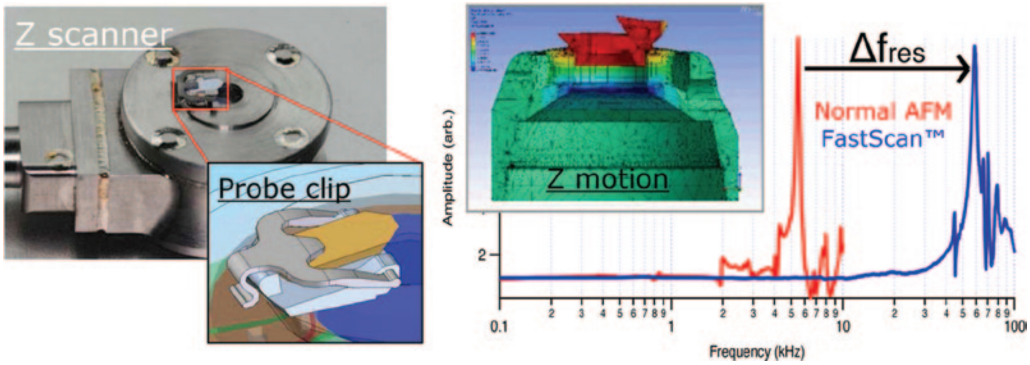

Figure 5: The challenge in *Z* scanner design is to increase resonant frequency while maintaining *Z* range, linearity/orthogonality, and easy probe loading. The Z scanner is waterproof, enabling easy fluid imaging, and the measured frequency response is over 10× faster than older designs. A finite-element-model illustration of the motion of the *Z* scanner is shown.

can move between multiple samples via a motorized sample stage, and exhibits AFM performance unaffected by sample mass. As a result, tip-scanning high-speed AFMs enable a significant increase in user productivity.

Other Requirements for High-Speed AFM

Additional properties for a high-bandwidth AFM include both a high-speed servo controller and a high-performance piezo amplifier that drives the *Z* piezo at high bandwidth and high-slew rates. The *XY* flexure scanner should offer flat scanning motion at high speeds while suppressing any inertial coupling from the fast *Z* axis. AFMs are typically combined with a light-microscopy capability to find a sample region of interest and position the AFM cantilever above it before engaging. Finally, the overall data acquisition workflow including tip loading and setup, sample loading, navigation, engaging, capturing data, final analysis, and image processing need to be streamlined to improve ease of use and to maximize user productivity.

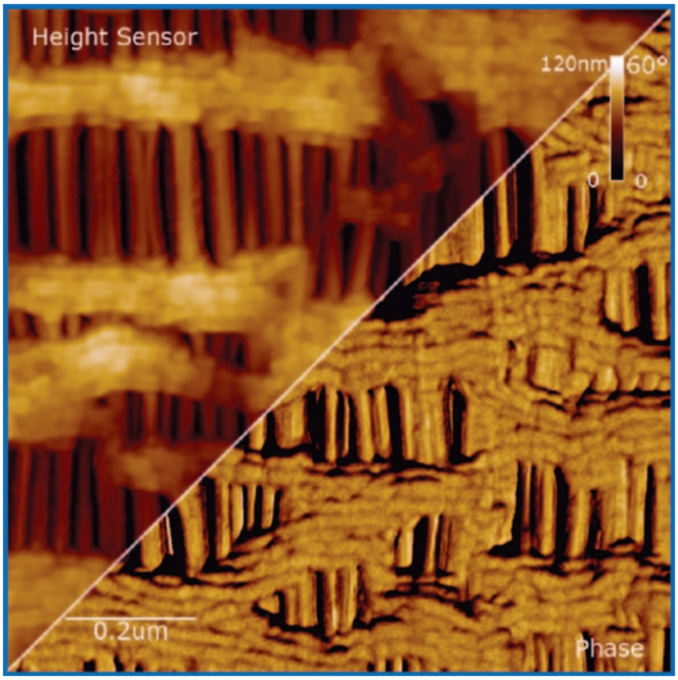

Figure 6: High-speed AFM image of Celgard® polypropylene battery separator membrane. These data were collected simultaneously for topography (upper left) and phase (lower right). The sharp, undisturbed structures of the topography image show excellent tracking qualities of the AFM, and the phase image structure clarity is evidence of the high bandwidth to support high scan rates of the AFM system.

High-Speed AFM Example

An example of the results that can be achieved with the new generation of scanners is an image of a Celgard® polypropylene battery separator membrane (Figure 6). The sample is challenging to image because of the combination of nanometer filaments supported only at their ends with deep trenches in between. The high-speed AFM that was used to capture the image in Figure 6 exhibits good force control at a scan rate of over 20 scan lines per second (~22 seconds per 512-line image), which is consistent with a gain in imaging bandwidth of 10–20× when compared to a standard AFM.

Conclusion

The AFM community has spent considerable effort over the past decade to address the speed and productivity limitations of AFM. Although many fundamental technological challenges have been addressed in academic settings, the latest generation of commercially available high-speed AFMs is the first to marry large scan sizes, large sample capacity, and enhanced productivity with high scan speed, while simultaneously preserving data quality, force control, and operating costs. This new generation of instruments allows researchers to quickly and efficiently perform nanoscale imaging using the full breadth and content richness of the AFM technique, thus enabling both increased productivity and new applications.

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