When compared to other common microscopy techniques (optical, SEM, TEM), the atomic force microscope’s (AFM’s) broad potential for nanoscale imaging and characterization of numerous physical surface properties has been somewhat offset by its slow imaging speed. Thus, the AFM has sometimes been seen as a powerful “specialty tool” to use when other suitable techniques are not available. The AFM community has spent considerable effort over the last decade looking for ways to address the speed limitation of AFMs, and through this research many of the fundamental technological challenges have been addressed on an academic scale. Driven by the researcher’s quest for discovery, many of these efforts were aimed at improving the time-resolution of the AFM in order to view dynamic processes on the nanoscale; while some also anticipated the need for the versatility and productivity of a fast general-purpose AFM.

Bruker’s Dimension FastScan™ development team worked with many AFM leaders to understand their research objectives and related enabling technologies, iteratively exploring various design considerations in the pursuit of bringing together the best of all these solutions into one tool. The ultimate result is the creation of an AFM that ideally marries high-resolution performance with rapid imaging. This application note details how the Dimension FastScan AFM accomplishes this ideal.

Applications Requiring Greater AFM Speed

When looking at atomic force microscopy applications that would most benefit from improvements in imaging speed, we found that they could be broadly categorized into three main areas (see figure 1):

1. The efficient exploration of an unknown, heterogeneous sample, to understand the different morphologies that best represent the surface, and to finally capture a representative set of images at publication quality. We call this “survey,” and it represents the largest of the three areas.

2. The quantitative characterization of a surface property (roughness, number of phases, particle size and shape, stiffness, etc.) on a large number of samples of the same class. In this case, the AFM images are only an intermediate; the end product is a graph representing the measured property versus a parameter of sample creation (temperature, concentration, stress). We call this highly applied area “screening.”

3. The observation of sample changes over time, at sufficient speed to time resolve the observed process, where the process can be protein dynamics, aging phenomena, etc. We call this “classic” area of high-speed atomic force microscopy “dynamics.”
interaction force. In an AFM, there is an inherent tradeoff between imaging speed and tip-sample force. Any system can be run incrementally faster when this tradeoff is utilized. However, it is critical to note that the speed comes at the expense of increased interaction-force, and is generally limited by either a) the image quality becoming unacceptable or b) the tip-sample forces becoming destructive to the tip and/or sample.

This is still true when building and using a higher speed AFM. The most general and useful way to describe the speed increase of an AFM is by the characterization of its full system transfer function (FSTF) at constant tip-sample force. Unfortunately, the FSTF is not familiar to many, is difficult to measure without specialized equipment, and is hard to use for absolute comparisons due to its dependency on the force used in the measurement. We therefore find that a more intuitive way to describe a fast AFM’s speed is by the factor of speed improvement it has over a “normal” AFM a) on the same samples, b) at the same image quality, and c) at the same tip-sample force. We call this the “improvement in bandwidth,” as it correlates directly with the more technical interpretation of this term, when looking at a property of the AFM’s entire FSTF (see figure 2a).

The AFM tracks the sample surface using a feedback loop that observes and maintains the interaction of the AFM probe with the sample surface during scanning by adjusting the tip-sample separation. The components involved are the AFM probe, photodiode and electronics, controller, amplifier, and Z actuator. Each component in the feedback loop introduces its own dynamics (e.g., a delay) and the sum of all component delays sets an upper limit for the speed at which the feedback loop can track the sample surface.
surface. This sum of the individual components' behaviors, or transfer functions, is the full system transfer function (FSTF), which determines the AFM's performance. Speed variations between different samples are introduced into the FSTF on the end of cantilever response to the sample, e.g., the best suited probe and imaging mode, allowable tip-sample force, and smallest sample topography to be resolved.

Looking at the FSTFs, one can see that the response is flat for low frequencies, and eventually rolls off for higher frequencies (see figure 2b). What is interesting is that for a standard AFM imaging at 1 line per second and 512 pixels per line, the pixel frequency would be around 1 kilohertz (with trace and retrace). At this modest rate, the FSTF shows that the standard system would already have some difficulty responding to features from pixel to pixel. It is this constant pushing of the speed limit that sometimes makes the force and gain adjustments required to get the best image quality such an art form.

The other FSTF was measured on the Dimension FastScan system. The FSTF also rolls off eventually, although at approximately 20x higher frequency. The expected improvement in imaging bandwidth is therefore on the order of 20x.

**Optimizing AFM System Components**

Before we go into examples in the areas of survey, screening and dynamics to demonstrate this, we will take a look at the technology improvements that were made in each AFM system component to achieve the Dimension FastScan's overall gain in bandwidth and to utilize it in a productive work flow.

**Cantilever**

The AFM cantilever is, from a simplified physics perspective, a spring-mass system, with a first resonance frequency of $\sqrt{k/m}$. This is important because (in imaging modes other than contact mode) the cantilever’s first resonance generally needs to come to equilibrium with the sample surface in order to provide the information needed to track the surface. The settling time this takes depends on the cantilever’s first resonance frequency (f), divided by its quality factor (Q). Therefore, to make a cantilever for faster imaging, f must be increased or Q reduced. One can increase f by increasing the spring constant k, or by reducing the mass m. Increasing the cantilever’s spring constant is not desired, because of our stated goal to image faster, but at similar tip-sample force. The mass can be reduced by making the cantilever smaller.

The settling time can be further reduced by reducing Q, i.e., by increasing the dampening of the cantilever. One way to do this is to give the cantilever a wide, closed shape and to reduce tip length, which increases the air dampening between the cantilever and the sample surface. Reducing Q would proportionally increase the tip-sample force per oscillation cycle (because the cantilever comes to equilibrium over fewer cycles and therefore needs to pass more of its kinetic energy to the sample in each cycle). One therefore needs to further reduce the cantilever’s spring constant to offset this. This still pays off as Q contributes to the settling time linearly, while reducing the spring constant k reduces f with a square root dependency.

In the pursuit of high-speed AFMs, smaller cantilevers were recognized as the key to higher imaging speeds from the beginning. However, designing a cantilever that balances all these factors well, while still being able to be manufactured in large quantities and at reasonable cost, within the tighter tolerance requirements imposed by smaller cantilevers, has been one of the biggest obstacles to commercial high-speed AFM technology.

Figure 3 shows Bruker’s Broadband™ A, B and C probes. These probes are smaller than conventional AFM probes, and are designed specifically for the operation in the FastScan system. They were developed and are manufactured by Bruker AFM probes, using Bruker’s proprietary silicon tip on nitride cantilever process, for the best combination of flexibility and sharpness.

**Laser Optics**

Operating smaller cantilevers requires an AFM with smaller laser spot size. The reason all AFMs use a contain very small spot size to make them compatible with all sizes of cantilevers is that small spot size AFMs have reduced optical lever deflection sensitivity. When being used with a shorter cantilever, this effect is cancelled because the mechanical lever deflection sensitivity is increased. Thus, for a given number of nanometer deflection, the angle change of a shorter cantilever is larger.
The reason smaller spot size leads to reduced deflection sensitivity is optical resolution. To make a smaller spot, the numerical aperture, or cone angle, of the incoming laser beam needs to be larger. The cantilever acts as a mirror, so for a larger, more strongly converging incoming beam, (to create a smaller spot), the outgoing beam must be equally divergent. The AFM measures cantilever deflection by the angle change of the reflected laser beam during the movement of the laser spot on a four-quadrant photo detector. The signal generated is proportional to the movement of this projected spot, divided by the size of the projected spot. Therefore, a more diverging beam, from a smaller laser spot, results in reduced optical deflection sensitivity. The consequence is that each cantilever should be operated with an appropriate spot size, i.e., the largest spot size that will not spill off the edge of the cantilever (which would cause interference and increased noise).

The FastScan head has three different spot sizes that can be selected simply by turning a switch. This design accommodates the broadest range of cantilevers, including all “normal sized” cantilevers, Broadband probes, and even smaller (experimental or future commercial) probes, each at their optimal performance.

**Z Scanner and Driver**

The second most critical (and challenging) component in the AFM feedback loop that needs to be tailored for high speed is the Z scanner. In traditional AFM tube scanners, the Z scanner was one with the XY scanner. Therefore, it did not have separate dynamics that could be maximized independently. Flexure scanners can resolve this issue. In this case, the (relatively slower) X and Y axis can move a fast, low-mass, low-inertia Z scanner around. The mass difference of the XY scanner and the mass moved by the Z scanner can be sufficient to allow the Z scanner to have its own isolated, fast dynamics. This concept has been used in academic high-speed efforts. The challenge for a general-use system is to maintain usability as well as closed-loop control. From the standpoint of fast dynamics, tip scanning systems are more robust and less limited than sample scanning systems, because the mass of the cantilever chip is small and well defined. Sample scanning systems in general impose restrictions on sample format and mass, and this restriction is multiplied for high-speed systems.

Even for a tip scanning system, the design of a good Z scanner is challenging. The goal is to maintain a long Z range, with straight motion, good linearity and integrated low-noise position sensing, while at the same time allowing easy loading of cantilevers, and waterproofing of the scanner surfaces for fluid operation and cleaning.

Figure 4 shows the FastScan system’s Z scanner. The cantilever chip is held by a light-weight, self-contained clip with sufficient mounting force to ensure good coupling for high-frequency (MHz) tapping excitation. The Z motion is flexure guided. The 3-micron travel is gauged by Bruker’s proven, ultralow-noise strain gauge technology. The Z scanner can be removed from the head, and placed on a load stand, for easy cantilever loading.

The exposed surface materials are glass and titanium, which provide excellent fluid resistance and biocompatibility. The Z scanner can be thoroughly washed and cleaned for optimum, low-background DNA and protein imaging in fluid, using a patent-pending wash station design that protects the Z scanner from the environment while exposing the surfaces to be cleaned. The wash station doubles as a rugged storage/shipping container.

**Electronics**

The remaining components in the feedback loop are electronics. On the controller side, the system is based on Bruker’s NanoScope® V controller, which was designed with...
high speed in mind and performance reserve to support it. An additional high-performance piezo amplifier drives the Z piezo at high bandwidth and at high slew rates. The high slew rate is required to deliver the power to drive high frequencies also at large amplitudes.

**XY Scanner**

XY flexure scanners are widely available, and have nice properties, such as large scan range, flat motion, and good weight carrying capacity. However, they generally are not known to be fast. The XY flexure design is desirable for a fast AFM, as discussed above, to isolate Z scanner dynamics from the rest of the system. The FastScan uses an exclusive, patented XY flexure design invented by the Hansma Lab at UC Santa Barbara, and developed further by Bruker (see figure 5a). The design rigidly couples the X and Y piezo stacks directly to the scan stage via a set of parallel flexures from each piezo axis, while maximizing Z rigidity by directly coupling the scan stage to the frame via a second set of flexures, to provide flat scanning motion at high speeds, and suppress any inertial coupling from the fast Z axis.

The scanner is capable of 30x30-micron motion (see figure 5b), with a 3-nanometer full range flatness (see figure 5c). The axes are monitored by ultralow-noise strain gauge position sensors.

**Vision Optics**

AFMs are typically combined with an optical microscopy capability to find a sample region of interest, and coarse-position the AFM cantilever above it before engaging. To realize the gain in productivity promised by faster AFM, this optical capability must not be compromised and should provide good optical image quality on a range of opaque and transparent samples (see figure 6a). The challenge on a fast AFM is that the space (and numerical aperture) above the cantilever is needed both to have a good resolution optical image, and to project a smaller laser spot. The solution is therefore to fully integrate the microscope into the AFM head, and use the objective lens for both imaging, and laser focusing and return.

In doing this, the integrated optical microscope also serves a second purpose: to position and focus the laser spot on the cantilever (see figure 6b). In the Dimension FastScan AFM, the optical focus, laser position and four-quadrant detector offset are all motorized, and are therefore controllable through the software user interface. The system automatically focuses onto the cantilever or the sample surface, depending on the workflow step, prior to engage, and shows cantilever and sample in focus when the AFM is engaged.

**Workflow**

Much attention was paid to supporting the increased productivity that results from scanning faster with an efficient operating workflow. A step-by-step process guides the user when exchanging the cantilever.

When starting the process, the system moves the stage into a configuration where it is easy to access and remove the Z-scanner, which is released from the head by the push of a button. The flip-over cantilever clip design and side access grooves make cantilever chip removal and insertion straight-forward even for occasional users. After inserting the new cantilever chip, the cantilever type is selected from an extensive built-in database that contains information about cantilever sensitivity, resonances in different media, recommended spot size, etc. This information enables workflow simplifications throughout the software (standardized gains across different cantilevers and spot sizes, reliable auto tune), and allows the user to work with SI-units (instead of system-internal arbitrary units) for many parameters.

The AFM laser and vision optics are con-and par-focal, i.e., the AFM laser spot is always in the center of the vision...
optics, and focused on the cantilever when the cantilever can visually be seen in focus. So, to position the laser spot on the cantilever, one simply:

1) focuses the cantilever image in the software, using software buttons,
2) clicks on the cantilever (optionally the laser position can be fine tuned by clicking “optimize,” or manually using arrow buttons),
3) clicks “zero detector,”
4) clicks “auto tune” for TappingMode™.

For imaging in air, this is the entire process to load and adjust the new probe. In fluid, using a “thermal tune” (Fourier spectrum of the thermal cantilever oscillations) is the best method for finding the (very broad) resonance peak of the cantilever. A new “Fast Thermal Tune” feature overlays a thermal spectrum over the cantilever sweep in a few seconds.

Once the system’s imaging bandwidth was increased, it became apparent that, especially for measurements on multiple sample sites, the time to engage the probe to the sample became a rate limiting step. In air, the FastScan uses a new fast engage feature that exploits the effect of squeeze-film dampening of the air between cantilever and sample to engage very rapidly (typically in <10 seconds) to within a few microns of the sample. This new feature is also available for sample navigation, using the cantilever

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**Figure 7:** a) HF etched mica (TappingMode, Air, Probe: Broadband-A). 20µm scan size shows a flat surface with individual etch pits. The pits correspond to flaws in the lattice that lead to higher local etch rates. b) Detail from inside one etch pit shows the individual mica layers as “terraces”. The other scan size extreme: Mica lattice (contact mode, Air, Probe: Broadband-B), here imaged at relatively low scan rate and high (1024x1024 pixel) resolution, demonstrating low electrical and mechanical noise, and excellent stability of the automatable, large-sample, tip-scanning Dimension Icon/FastScan platform.

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**Figure 8:** Tip life was measured on a rough, hard, highly abrasive TipCheck sample at 17 lines per second. The tip radius was software estimated from consecutive scans (TappingMode, Broadband-A Probe). The total tip radius increase from 200 TipCheck images is 0.9nm (13%), or 4.5Å/m tip travel.
dampening to sense the proximity of the surface and move it into optical focus. This is particularly useful on very flat, clean, or transparent sample surfaces (glass, mica), where good optical references are sometimes hard to find.

The FastScan system is a tip-scanning design based on Bruker’s high-end Dimension Icon® platform. Supported by the optical microscopy features integrated with the FastScan head, all motorized-stage sample navigation, focus-to-engage, and automation features of the Icon platform are available with the FastScan.

Fast Scanning Quality and Force Control

Figure 7a shows a scan of HF-etched mica, taken at 20 lines per second (i.e., within 12 seconds). The etch pits start with a single flaw in the lattice (induced by natural radioactivity). The 20-micron zoom-out demonstrates the FastScan system’s ability to scan fast over relatively large scan areas (XY scan range = 30 microns), and the flatness of the scan (<3-nanometer bow). The 2-micron zoom-in shows the individual mica steps taken at 48 Hertz, or 5 seconds/image (see figure 7b). The data shown is Z sensor data, illustrating the noise performance of the Z position sensor (0.6 angstroms at 20-Hertz scan rate) on atomic-scale topography. Figure 7c shows an image of the mica lattice. This 20-nanometer image was taken at a high resolution of 1024x1024 pixels, and a relatively low scan rate of 6.5 lines per second. This ultimate-resolution image was achieved without the expense of the mechanical noise and thermal stability expected from a high-end research AFM platform.

One question frequently asked is the effect of fast scanning on tip life. Tip abrasion and tip life are a concern when covering more sample surface in a shorter amount of time. A tip life limited to a few images would limit productivity and drive up operating cost. Long tip life is also an indicator of excellent, consistent force control.

Figure 8 shows an image of a TipCheck (Aurora Nanodevices, Inc.) sample, a test structure for assessing tip sharpness, with many sharp edges that make it useful for the characterization and reconstruction of AFM tip shape. These samples are known as “tip-eaters,” i.e., as causing excessive tip wear, which sometimes limits their usefulness for characterizing tips.

The sample was scanned at a rate of 17 lines per second. The plot on the right measures the estimated tip radius, reconstructed from the sample over successive images, using the Blind Tip Estimation feature in the NanoScope software. The graph illustrates that the fast imaging of a very rough, hard sample will wear the tip radius very slowly, (0.9 nanometers over 200 frames). This is a worst-case situation, and the same level of tip abrasion cannot be expected for most “normal” samples. However, it does put a comfortable upper limit on the effect, and illustrates the consistent low-force tracking performance.

Force control is further demonstrated by imaging a fragile sample of known morphology, and intricate, steep topography.

Figure 9 shows an image of a Celgard® polypropylene battery separator membrane. The membrane is a highly ordered “sieve,” consisting of filaments a few nanometers in diameter, and larger perpendicular “linkers” (which also have a sub-structure). The sample is challenging to image because of the combination of nanometer-filaments supported only at their ends, with deep trenches in-between. For good judgment of performance, the filaments should be oriented approximately perpendicular to the scan direction. To image the trenches the tip needs to enter tens of nanometers. To come back up onto the filament the tip-filament interaction must not cause high lateral forces on the filament. The least effect would be a blurring of the filament edges, and permanent damage to the filaments can also occur. These effects can be further judged in detail when looking at the phase image, where they won’t be masked by the overall topography. Loss of tracking (forces too light to stay in contact) would also become apparent in phase. The example in figure 9 displays excellent force control on Celgard®, at a scan rate of 10 lines per second, consistent with a gain in imaging bandwidth of 10–20x when compared to a standard AFM.
Application Examples for Survey, Screening and Dynamics

As mentioned above, the three application areas that benefit most from a higher-bandwidth AFM with the same data quality, force control, operating cost and ease of use as a standard AFM can be categorized under survey, screening, and dynamics. The Dimension FastScan was applied to each of these categories.

Survey

Survey is the understanding of the representative morphologies of a heterogeneous, unknown sample. This is a very common situation when using an AFM (or any microscopy technique) on a new sample. Especially for complex (e.g., biomaterial) samples, the large majority of imaging time is often spent looking at enough sample surface to understand what is important, rather than capturing the final images that represent the sample. The famous parable of the three blind men describing an elephant comes to mind, one describing the tail, one describing the trunk, and one describing the tusks. Covering a larger area of the sample, with sufficient detail and within an acceptable amount of time leads to a better, more balanced view of the parts and their respective roles.

A higher bandwidth AFM can be applied toward this goal in different ways. On a rough sample, more sites can be engaged and imaged in a shorter amount of time. The NanoScope software’s MIRO image overlay capability can be used to keep track of all the scans within one context, and in relation to an overview optical image. On a fairly flat sample, another way to survey the sample is to capture a very large scan area with very high pixel resolution. The data can then be zoomed into and analyzed (even without using further tool time) and representative areas can be magnified and published. A big advantage of this method is that one can decide on the best scale and framing after taking all the data. Faster imaging brings the time for a high pixel resolution image (e.g., 16 megapixels) down from several hours to a few minutes. The data shown in figure 10 consists of one 16-megapixel image of a 20-micron scan range on a PTFE polymer film, acquired in 8 minutes, with data zooms of various interesting morphologies, as well as phase data for two of them. Looking at the overall dataset, one has good confidence of the morphologies that can occur on this surface, while also achieving high-quality images from the high-resolution dataset.

Screening

In screening applications, the space of possible phenomena is typically well understood. However, the dependency between an input parameter (such as an ingredient concentration), or a process parameter, and a nanoscale morphology or property (roughness, number of phases, domain size, defect rate, or mechanical property) must be understood and quantified. For this, it is necessary to image

Figure 10: 20µm, 16MPixel image of PTFE polymer film (left), acquired in 8 minutes. Right: Multiple data zooms showing detail and phase data. Surveying a sample means to explore and understand it’s representative morphologies, and document them in publication quality images. On sufficiently flat samples, one survey method is to take a large, high-resolution scan that can be explored offline for representative morphologies, which can then be magnified and published.
Figure 11 shows an example of AFM screening from the pharmaceutical industry. In this case, the active pharmaceutical ingredient (API) is combined (formulated) with an (inactive) excipient to form an amorphous solid, with the goal of maximizing the API’s solubility after ingestion. The amorphous formulation is solid (frozen) at room temperature but would otherwise phase separate. To observe the possible phase separation with bulk techniques, relatively macroscopic (~100nm) separation and re-crystallization of the API must first occur. The Dimension FastScan AFM can catch the indicators of instability on a much smaller size scale, much earlier. For routine use, the AFM needs to give data on multiple sites of 100 or more samples per day. This throughput is enabled by the combination of >10x faster scan speeds, fast engage, multi-sample automation, robustness of measurement, and ultimate data quality.

Dynamics

The “classic” discipline for high-speed AFM is the time-resolved study of dynamic processes on the scale of proteins and DNA. This application has driven much of the
initial understanding of how to make AFMs faster, while maintaining non-destructive tip-sample forces. What was found is that the main bottleneck was cantilever dynamics, and that to make cantilevers faster without sacrificing force control, cantilevers must be made smaller. As soon as that is done, a range of further requirements arise; to enable the use of smaller cantilevers, to scan faster, and to capture data faster. In this hunt for speed, it was found that the achievable frame rate scales roughly with the dimensions of the cantilevers. It also scales with the data quality, with the number of lines, and with the acceptable pixel blur caused by loose tracking (parachuting). Achieving frame rates >1 frame per second is typically achieved by increasing imaging bandwidth, and by trading image quality for speed. For movie data, this can be quite acceptable to the human eye. In an analogy, a frozen TV picture typically doesn’t look that great individually, but the movie looks good. For the FastScan to be more than a single-purpose movie machine, it was important to have full AFM performance at increased bandwidth, but to be able to further trade off resolution for speed in the way of other high-speed AFMs, and to maintain excellent control of tip-sample forces at high scan rates.

Figure 12 shows three frames from a time sequence of 2100 frames, captured at a rate of 1 frame per second, of DNA in buffer solution, loosely bound to and diffusing on an APS-treated mica substrate.9-11 In the movie, different motions of the DNA can be seen, including a “sliding” motion of the DNA along its contour, and approximately perpendicular to the scan direction.12 This indicates that the DNA’s binding to the substrate is loose enough to allow it to move, and diffusion is not dominated by the back-and-forth scan motion of the AFM tip. This should lay a good foundation for the observation of more complex sample systems, such as DNA-protein complexes, ATP-driven systems, etc.

The Future of Rapid AFM Imaging

The idealistic notion of faster AFM imaging is almost as old as the AFM itself. A number of implementations for specific applications have demonstrated that great increases in AFM imaging speed are possible. We have approached higher speed AFM not as a certain set of applications, by certain fields of research and on certain samples, but with the belief that one would rather always image faster, however not at the expense of quality, sample size or delicacy, usability, or operating cost. That said, we do expect that a faster AFM will open up new areas of investigation over the full range of applications, from routine industrial to molecular biophysics. Most importantly, it will also allow researchers to quickly and efficiently look at and understand a sample at the nanoscale, using the breadth and content richness of the AFM technique.

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Authors

Johannes H. Kindt, Ngh Phan, Bede Pittenger, Adam Mednick, Andrea Slade, Lin Huang, Weije Wang, Natalia Erina, James E. Shaw, and Stephen C. Minne (Bruker Nano Surfaces Division)

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