Atomic Force Microscopy (AFM) is a well-established surface characterization technique for high-resolution visualization and studies of local material properties. Despite a broad acceptance of this technique by researchers in academia and industry, relatively slow scanning rates for collecting images of surface structures are considered as one of major obstacles for its broader and more productive use in applications. The key issues of AFM are the time needed to measure tip-sample interactions when the oscillating probe taps a sample surface, and the time required for a piezo-scanner to adjust the sample-probe distance in response of the obtained data. These demands can be met by using the probes with resonant frequencies above 1 MHz, designing fast scanners with effective resonance frequency of tens and hundreds of kHz, and the implementation of high-speed electronics for microscope control and data collection. The technique of fast imaging were developed by Ando and colleagues [1] with a primary goal of video-rate visualization of biological processes in physiological buffers. Amplitude modulation (AM) mode is the primary technique applied for this purpose. High-speed imaging in liquid media is substantially assisted by over-damping of AFM probe oscillation that reduces a time delay in measurements at single location to the time scale of one or a few micro seconds (μs) with high-frequency probes. Monitoring of fast in-vivo processes or other dynamic surface phenomena is the important but not the only advantage provided by fast imaging. The possibility of quick survey of large and multiple surface areas is a major benefit to any microscopic technique in providing statistically-sound description of sample morphology and structure. Now this has been successfully achieved by AFM.

In this application note we describe fast imaging with Keysight 9500 AFM Quick Scan on multiple samples to demonstrate its capabilities for routine sample measurements with extremely improved productivity, and to provide examples of visualization of several dynamic processes in different vapor environments. The advantage of fast imaging in surface studies with the presence of various organic vapors - the unique feature of Keysight AFM - is also successfully demonstrated.

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Quick Scan Operation with Keysight AFM

As high-frequency AFM probes with resonant frequency above 1 MHz became available, the instrumental efforts in fast scanning began to focused on piezo-scanners and control solutions allowing a precise feedback tracking of surfaces at high scan rates. At Keysight Technologies the unique concert action of a small scale and fast-responding z-piezo stack, which is integrated in the Quick Scan nose cone, and a regular scanner addressing large vertical and lateral displacements has been implemented (Figure 1a).

The proprietary dual-scanner action is regulated by crossover frequency, which is typically around 18-25 kHz. The small nose cone operates at frequencies higher than the crossover one and the large scanner operates at lower frequencies. The agile action of the z-stack is documented by Bode graph (Figure 1b), which demonstrates a practically flat response of the fast scanner up to 60 kHz, i.e. close to the resonant frequencies of the piezo-stack. This bandwidth extension is assisted by controlled depression of the nose cone mechanical resonances. The Keysight AutoDrive software feature allows for optimization of feedback gains for fast response operation.

The scanning rate for Quick Scan operation in AM mode depends strongly on the imaging environment. The probe oscillation is characterized by Q-factor, which is 50-100 larger in air compared to water. Therefore, fast imaging in air is restricted by higher time delays in closed loop cycles at a single sample location thus slower scanning rates. Technically, imaging with 512×512 pixel resolution requires 17.2 sec per frame at 30 Hz rate and 5.1 sec per frame at 100 Hz rate. At lower image resolution of 100×100 pixels, scanning at 150 Hz leads to 0.66 sec per frame. For high-quality imaging with 512×512 pixel resolution at 100 Hz, a probe can spend around 10 µs at one location that is matched by the scanner adjustment with the resonant frequency above 50 kHz. Time delay of 20 µs is larger than one expected for operation in water yet is rather small for measurements in air. Theoretical estimates similar to the ones by Ando and coworkers [1] show that high-quality surface tracking in air requires longer time delays (60-80 µs). Precise imaging of rough surfaces demands more time delay while scanning the steep slopes downwards. At this moment it is rather important to perform a practical evaluation of Quick Scan operation in air to find out the working range of fast imaging AFM applications.
Fast Imaging with Quick Scan

For the illustration of high-speed AFM imaging we have selected self-assemblies of semi-fluorinated alkanes $\text{CF}_3(\text{CF}_3)_{14}(\text{CH}_3)_{20}\text{CH}_3 - \text{F}_{14}\text{H}_{20}$ [2] on graphite, Figure 2a-b. Imaging of two “donut”-shaped self-assemblies, which are 4 nm in height, and several flat ribbons was conducted at scanning rates from 5 Hz to 80 Hz. The related imaging time changed from 102 s to 6.4 s. The self-assembled structures in the images did not change. This is evident also from the height profiles which were taken across two ‘donuts’.

The given example of Quick Scan imaging of $\text{F}_{14}\text{H}_{20}$ self-assemblies shows that the time on AFM experiment is reduced more than 10 times compared to the regular imaging that has dominated applications of this technique so far. In following examples we will prove that this increase of productivity is more general as it covers the experiments on larger scales, corrugated samples, and compositional mapping with phase imaging and industrial specimens.
Morphology and lamellar structures of polymer materials are commonly studied with AFM [3] and typical scanning rates are below 1 Hz for images with dimensions of 5 µm and larger. For smaller scales the scanning rates are 1-2 Hz. Using Quick Scan technology one can apply scanning rates of 10 Hz and larger without any loss of image quality and resolution. The height image of melt-crystallized linear low-density polyethylene (LLDPE), which was collected in 1024×1024 pixels format at 10 Hz rate, is shown in Figure 3a. Lamellar structures of semi-crystalline polymers such as LLDPE with glass transition below room temperature are best visualized in phase imaging. The phase contrast in the images in Figure 3b-c, also recorded with 10 Hz rate, clearly differentiate between the stiffer extended lamellar structures and small additive particles (all seen with bright contrast) and amorphous polymer matrix (dark contrast). Fine details of LLDPE lamellar structures are resolved in 1-µm image, Figure 3c. Stacks of tiny overgrown lamellae are distinguished on some bright fibers, which represent the edge-on standing lamellae, and on the wider structures at the top of the image.

Productivity of AFM studies is of a particular concern in industrial applications. The Quick Scan that offers rapid imaging at large scales and profiling of corrugated surfaces is invaluable for broader use of this technique in different industries. One of the mainstream applications of the polymer–polyethylene, which is produced at the largest volume, is the manufacturing of packaging films. Such films should exhibit variety of properties to address the control of adhesion, wear and other characteristics, and AFM can be very helpful in clarifying morphology, structure and composition of polymer films. The images of a commercial film made of low-density polyethylene (LDPE), which were recorded with scanning rates from 10 Hz to 30 Hz, are shown in Figure 4a-d.

Figure 4a-d: Height images of a commercial LDPE film at different scales. All images were obtained at 512×512 pixels with scanning rates at (a) 10 Hz, (b) 20 Hz, and (c)-(d) 30 Hz.
The large-scale morphology of this film in height images, which is presented in Figure 4a-b, is characterized by sets of fine linear features, which are aligned in the inclined direction from the bottom of the images to their top. The individual strands of ~ 300 nm in width (more clearly seen in Figure 4d) are composed of stacks of short extended blocks of 150-200 nm in width, which are oriented perpendicular to the main direction. Such morphology with the orientation along a machining direction is common for polyethylene packaging films. Other component, which is seen on the surface, represents aggregates of layered crystalline structures. The flakes and their aggregates of several microns in dimensions are best resolved in height images of 40 µm in size (Figure 4b) and in the smaller images (Figure 4c-d). These flakes, which modify surface properties of polymer films, are the important material component, and their surface distribution, surface coverage and size of the aggregates can be learned from AFM images taken in various surface locations. With Quick Scan collecting such images at 40 µm (Figure 4b) in 25 seconds, becomes quite time-efficient. Therefore, fast imaging makes AFM most suitable for multi-site studies – the basis of comprehensive surface analysis.

![Figure 5a-e. Height and phase images (512x512 pixels) of PS/LDPE film, which were obtained at 10 Hz rate.](image)
Another example of fast AFM studies deals with compositional mapping of polymer blends, which is based on phase imaging. Ultrathin film of polystyrene (PS) and LDPE exhibits a phase-separated morphology, in which one component has formed a flat matrix and the other has aggregated into elevated domains with sub-micron dimensions, Figure 5a–e. In several locations of the largest-scale image (Figure 5a) the domains have assembled into larger aggregates, which are surrounded by the regions with local film de-wetting. This observation, which was made at a 75–µm area with 10 Hz rate that took less than 1 minute, provides more comprehensive morphology data than 10-µm images (Figure 5b–c), which show only small domains. The latter, which are embedded inside the matrix, have slightly different height as judged by their brightness. In the phase image, they all exhibit darker contrast as the matrix is bright. These contrast variations are consistent with binary composition of the blend. At smaller scale (Figure 5e) the phase contrast has revealed fine structures of the circular domains, which can be assigned to a lamellar population of semi-crystalline LLDPE as compared to the amorphous polymer component - PS. This compositional mapping has been obtained with Quick Scan 10–30 times faster than with the regular AFM.

So far, we have considered fast AFM studies on relatively smooth samples where surface corrugations did not exceed 100–150 nm. The surface analysis of more rough samples brings definite limitations to imaging speeds as it was mentioned earlier. For the minimization of tracking error time delays at locations on steep edges need to be increased. The related example is given by the images of microporous nitrocellulose membrane (Figure 6a–d), which is commonly used for protein blotting because of their high protein-binding affinity. Surface morphology of the membrane is relatively flat with multiple grainy aggregates on top and numerous pores up to a few hundreds of nm in size. The height of the grains can be up to 300 nm above the average level, and the holes are up to 350 nm in depth. Therefore, surface corrugations can be up to 700 nm in size. A precise profiling of the membrane surface at areas up to 40 µm in size has been achieved with 5 Hz rate (Figure 6a–b). At smaller scale (Figure 6c) the scanning rate can be increased to 10 Hz with a reliable profile tracking as judged by a coincidence of the trace and retrace height profiles of 10 µm long, Figure 6d. This has also been proven by comparing the height profiles, which were recorded at 5 and 10 Hz rates (Figure 6e). At 10 Hz rate the imaging of corrugated surface with Quick Scan is at least 10 times faster than studies performed before.
Monitoring of Dynamic Processes

Fast imaging of several examples demonstrated above has pointed out the substantial increase of AFM data throughout for general purpose studies. The use of Quick Scan for monitoring dynamic surface processes offers truly invaluable information, which could not be obtained by other means. We have selected several practical examples to show the benefits of the Quick Scan imaging. AFM images, which are collected at the locations of interest with high-speed up to several frames per second best in generating movies are beyond the topic of this application note. Therefore, we have restricted their demonstration by a selection of individual images that best illustrate major structure transformations.

Humidity-induced Structural Transformations of \(\text{F}_{14}\text{H}_{20}\) self-assemblies

The dissimilar properties and size of fluorinated and hydrogenated parts of semi-fluorinated alkanes \(\text{F}_{14}\text{H}_{20}\) leads to variety of self-assembled structures such as lamellae, flat ribbons, braid-like sequences, donuts, etc. [2]. The structural transitions from one to another self-assembly can be induced by different means: temperature, substrate, chemical vapors as well as the tip-force in AFM measurements. Several transformations were observed during fast and continuous imaging of two \(\text{F}_{14}\text{H}_{20}\) domains on mica surface during a humidity increase in the environmental chamber of Keysight 9500 microscope. Selected images of this series during scanning at 20 Hz rate are shown in Figure 7a-h. A couple of the smaller-scale images in Figure 8a-b, which show fine surface structures, were extracted from images in Figures 7a and 7g.

![Figure 7a-h. Height images of \(\text{F}_{14}\text{H}_{20}\) self-assemblies on mica, taken from a sequence of images (512×512 pixels). The images were recorded during gradual hydration of the sample at 20 Hz rate.](image)
Initially, circular $F_{14}H_{20}$ domains were formed on mica after spin-casting a droplet of diluted solution of this compound in perfluorodecalin, Figure 7a. They are relatively flat in the form of arrays of ribbons (Figure 8a) with an average height in around 2 nm. Only some ribbons exhibit slightly twisted terminals, which are higher than their surroundings. The combined action of the tip-sample interactions during scanning and rising humidity leads to transformation of the central parts of the domains. This change reflects a partial transformation of the ribbons’ terminals to the circular and thicker structures – supposedly the precursors of donuts, which are typically around 4 nm in height, Figure 7b-c. The ribbons at the domains’ borders also undergo transformations into the braid-like sequences. The latter have spread laterally and encircled substrate regions, such as one at the lower right corner of the bottom domain, Figures 7d. At the same time the spreading of whole domains leads to their flattening as the formed donuts in the central parts have settled directly on the substrate. The spreading has continued further with a formation of new encircled regions, particularly, at the periphery of the bottom domain, Figure 7e-f. The conversion of flat ribbons to the braid-like or curled structures that has initiated at the periphery has extended inside of the domains (Figure 7f-g) and more substrate regions became confined by the curled boarder structures. After the humidity reached saturation, the transformations have been practically accomplished, and only minor structural changes can be found in Figures 7g-h. The laterally extended domains are formed by the chains of curled chains and the isolated donuts.

The structural changes, which were induced by a raise of humidity, most likely, have been stimulated by a formation of water layer on hydrophilic mica surface. The height contrast of many confined locations inside the spread domains is intermediate between the contrast of the substrate outside the domains and the contrast of the domains’ structures. It is possible that the confined locations are filled with adsorbed water. The competition between the surface structures and co-adsorbed molecules of different chemical vapors will be discussed below in fast imaging study of dynamics of brush polyacrylate macromolecules. High-humidity spreading of $F_{14}H_{20}$ self-assemblies and the brush polymer chains on mica is likely related to high surface tension of water molecules [4].
Phase-Separation Morphology of PS/PVAC Blends

Visualization of selective swelling of the components of heterogeneous polymer materials, which contain hydrophilic and hydrophobic constituents, helps their assign to particular features of sample morphology. This approach has been successfully realized in AFM studies of PS/PVAC (PVAC – poly(vinyl acetate)) blends in methanol vapor and in humid air [5]. Dynamics of swelling of hydrophilic materials becomes accessible with the use of Quick Scan as we demonstrate by experiments on PS/PVAC blends with different weight composition of the components.

Figure 9a-b. Height images of PS/PVAC blend in dry air (a) and in methanol vapor (b), which were taken from a sequence of images (1024 × 1024 pixels), and recorded at 20 Hz rate.

The large-scale AFM images of PS/PVAC blend with equal ratio of the components were continuously recorded as methanol vapor was introduced into Keysight 9500 microscope chamber. The images (1024 × 1024 pixels) were collected at a 30-µm area at 20 Hz, i.e. one image per min. The initial image and the one after the swelling completion are shown in Figure 9a-b. Surface morphology of this blend is characterized by multiple hemi-spherical domains, which densely populate a flat matrix. The higher contrast of the domains in the swollen state hints on their hydrophilic nature allowing their assignment to PVAC. This assignment was further supported by local dielectric measurements [5].

Figure 10a-d. Height images of PS/PVAC blend, which were extracted from a sequence of 30-µm images and recorded at 20 Hz rate during the sample swelling in methanol vapor. The height profiles, taken along the direction marked with a white-dashed line, are shown underneath the images.
The gradual swelling of individual hemi-spherical domains, which are several hundreds of nm in diameter, is presented in series of 8-μm images, Figure 10a–d. The height profiles across three domains (a direction is marked with a white-dashed line in Figure 10a) are placed under the images. The images were extracted from high-density 30-μm images. In dry air, the hemi-spherical PVAC domains separated from the flat matrix by rims, emphasize the phase segregation of the components. As swelling has progressed (Figure 10b–d), the domains’ height has increased 60–70%, and lateral expansion has smoothen the rims. The colored 3D presentation of the swelling process is given on the cover page of this document. Besides the hemi-spherical domains, the round-shaped holes were scattered on the flat matrix (Figure 10a). These locations have grown to the peaks, presumably, by swelling PVAC material inside the holes.

Swelling of PS/PVAC blends with different weight ratio of the components (1:2 and 2:1) is presented in Figure 11a–f. In excess of PVAC, the blend morphology is characterized by the disk-like perforated structure of several µm in diameter, which is spread over a flat matrix. Numerous circular inclusions of different height are seen on the surface of such disks. There are also a few extended domains with the raised circular structures on their surface. One of these domains is shown at the low left corner of Figure 11a. Gradual swelling of 1PS/2PVAC blends has accompanied by two major transformations, Figure 11b–c. The circular inclusions of the disks have enlarged in height, independent on their original level. The matrix regions around the disks have also increased in height that makes them looks like the hills surrounding the lower-lying disks. Therefore, the assignments of the matrix and the inclusions on the surface of disks to PVAC and the disks to PS are obvious.
The phase-separation at different scales was also observed in AFM images of 2PS/1PVAC blend, Figure 11d. The large round-shaped “dimples”, like the one in the center of this image, are surrounded by multiple circular domains embedded into a matrix. Majority of these domains are slightly elevated (up to 15 nm in height) compared to the matrix, and there are few below the matrix level. Selective swelling of this blend in methanol vapor has completely changed the sample topography, Figures 11e-f. The “dimple” location (30 nm in depth) has converted to a hill of 90 nm in height. All surrounding circular locations have risen to 40-60 nm in height. These locations are definitely enriched in PVAC whereas the matrix can be assigned to PS.

It is worth noting that fast monitoring of gradual swelling of PS/PVAC blends in methanol vapor was straightforward. The reversed process, which was initiated by opening the microscope chamber to air, was extremely quick. For all blends, it has led practically to the same morphology that was prior observed in dry air. Following the reversed structural change was possible after methanol vapor was progressively removed from the chamber by an external pump.
Aggregation and Spreading of Brush Macromolecules

For a number of years, AFM visualization of structural and conformational changes of macromolecules of biological and synthetic origin was demonstrated on numerous examples [6-8]. In previous evaluation of capabilities of Keysight 9500 microscope we have examined the behavior of brush macromolecules in air and in vapors of different organic solvents [8]. These macromolecules (e.g. poly(methacrylate)-graph-poly(n-butyl acrylate – PMA-g-PnBA) are the rigid cylindrical polymer chains with large diameters due to densely grafted side groups. Below, we will show how these studies can be further improved by conducting fast visualization of structural transformations of assemblies of brush macromolecules with side chains of 33 polymer units. Particularly, we have examined an aggregation of single macromolecules in methanol vapor (Figure 12a-h) and the reversed process of macromolecules spreading after the environment was changed to humid air (Figure 13a-f).

The changes induced by methanol vapor have led to gradual aggregation of the macromolecules, and the structural and conformational rearrangements of individual macromolecules can be followed during this process. Two references (a blue oval and four red dashes) were placed on the figures to facilitate such observations. As methanol vapor has spread through the chamber to the sample location, the initial transformations were confined to some reduction of surface density of macromolecules and minor conformational changes in limited number of macromolecules, Figure 12a-b. In the next steps local voids have appeared in several places (Figure 12c), and their number and size have steadily increased (Figure 12d). Only limited number of macromolecules had retained their conformations at this stage. The macromolecule, which was marked with red dashes, is one of them. This did not last long, and it has combined with traces of nearby material (Figure 12e), and later has formed a triangular structure with neighboring macromolecules (Figure 12f). This structure has exhibited some changes in following images (Figure 12g-h), as the number of aggregates has been reduced and their dimensions increased. The multi-macromolecular aggregates became up to 7 nm in height – much larger than height of individual macromolecules (1 nm - 1.5 nm). In the analysis of multiple images, which are recorded during structural transitions, we see features and structural details that were not observed before. For example, in Figure 12f-h we have noticed ultrathin patches of a surface layer (µ1.5 nm in height) under and near some aggregates. As the patches have changed their size and positions, we suspect that they are formed by mobile methanol molecules co-adsorbed on the sample. These patches have disappeared as the methanol vapor was substituted by dry air, Figure 13a.
The methanol-induced aggregation of brush macromolecules has reversible character and their spreading initiated by humid air is visualized in Figure 13a-f. The image in Figure 13a was obtained in dry air after the aggregation. A raise of humidity initiated unfolding of macromolecules from the aggregates and their extension to nearby surface regions. The initial events are seen at the bottom of the image in Figure 13b, which was recorded in the “up” scanning direction. A few aggregates are still seen at the lower half of this image, and individual macromolecules became visible at the locations that were imaged afterwards. The images in Figure 13b-d show the structural changes with interval of 17 seconds. The individual macromolecules have laterally moved to occupy the substrate in a dense manner, Figure 13d-e. The image in Figure 13e was recorded in dry air, and the spreading of macromolecules has preserved. The reversible character of the described structural transformations of brush macromolecules is further supported by image in Figure 13f, which was recorded after methanol vapor was again admitted to the microscope chamber. The dark-contrast locations have appeared in some places, which were less occupied by macromolecules. The latter were pushed nearby, presumably, by co-adsorbed methanol molecules. This observation as many others that can be retrieved from the series of the fastly recorded images partially represented in Figures 12 and 13 will improve understanding of the competition between molecular adsorbates in formation of surface structures.
Conclusions

Practical realization of AFM fast imaging, which has been achieved with Keysight 9500 AFM Quick Scan technology, offers outstanding capabilities for further progress of this surface characterization technique. This conclusion has been substantially proven by studies of variety of industrial and research samples reported in this note. A major advantage is the leap in AFM productivity that has been verified by visualization of structures on flat and corrugated surfaces, and at small and large scales up to 100 µm. Routine recording of high-quality images at large scales in a fraction of minute is the basics of previously unseen efficiency of AFM analysis that for years has limited a penetration of this technique into a wide spectrum of technologically important areas. New horizons are opened with Quick Scan for examination of dynamic processes as, so far, such information was completely inaccessible. As compared to video-rate imaging that has been achieved in limited cases for visualizing dynamics of small biomolecules in liquid, this brought in a different area of AFM fast imaging application, and the progress in this study has direction will further enhance the value of scanning probe microscopy. A wealth amount of information delivered by fast imaging imposes new challenges for efficient data analysis and presentation of the results that need to be addressed in the immediate future.

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