Applications of Atomic Force Microscopy *for Contact Lens Manufacturing*

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Atomic Force Microscopy (AFM) has advanced biomaterials R&D by offering the unique ability to analyze surface properties non-destructively with nanometer-level resolution in ambient air or liquids. Polymer products used as biomaterials, including intra-occular implants and contact lenses, have represented one of the fastest growing sectors in the medical device industry. Significant increases in manufacturing output of contact lenses, especially in disposable products, are driving demand for better quality control and innovative new designs. Surface characterization is an integral part of contact lens R&D and quality control. Optical and electron microscopy, and stylus and optical profilometry are some of the conventional techniques used for characterizing the lenses and for developing and improving production processes. This application note describes some areas where the AFM provides new capabilities for surface characterizations that help speed product development efforts and, improve product quality, performance and yields.

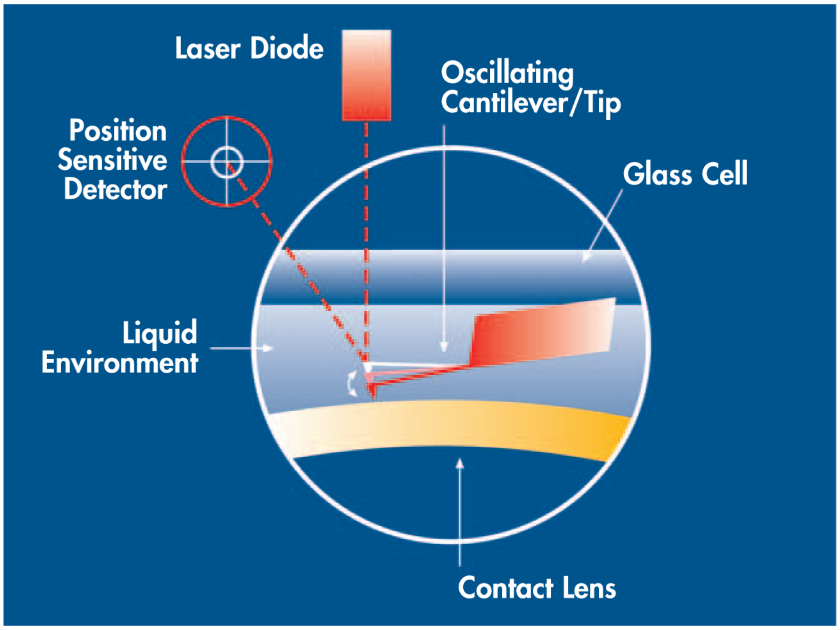


Figure 1. Schematic diagram of TappingMode AFM operation in liquid on a fully hydrated contact lens.

The AFM Techniques

Contact mode is the original AFM imaging mode and can be implemented in both air and liquid. The AFM tip, at the end of a flexible cantilever, is brought into light contact with the sample surface and raster-scanned across the surface by a piezoelectric scanner. Changes in the cantilever deflection during scanning are monitored and kept constant using electro-mechanical feedback. Topographic images are generated by mapping the distance the scanner moves vertically to maintain a constant deflection at every lateral data point.

TappingMode AFM is a more recent development in which the imaging probe is vertically oscillated at or near the resonant frequency of the cantilever (Figure 1). Electro-mechanical feedback maintains the oscillation at a constant amplitude during scanning. The image is produced by mapping the distance the scanner moves vertically, to maintain the constant

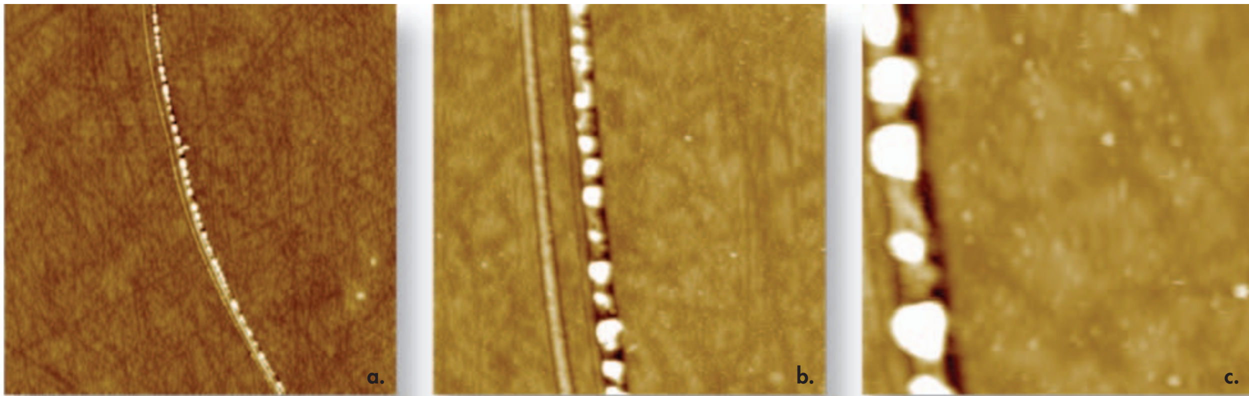


Figure 2. TappingMode in saline solution images of a fresh, out-of-the-box, commercially available contact lens. (a) 47μm, (b) 10μm, (c) 4μm scans.

Advantages of AFM

- **Highest resolution available:** AFM's lateral resolution allows imaging and measurement of features on the order of a few nanometers; the vertical (height) resolution is <1Å.
- **Quantitative 3-D surface maps:** AFM images can be computer-rendered with any tilt or rotational angle, and provide accurate measurements in all three dimensions on features of interest.
- **Operation in liquid:** AFM can characterize contact lenses in their native liquid environment.
- **Non-destructive:** Whether in air or in liquid, the AFM characterizes the sample without damage.
- **Material properties characterization:** The AFM combines several techniques in a single instrument. On and near the surface, topography, adhesion, viscoelasticity, hardness, friction, and other properties can be revealed – again, with nanometer resolution.

oscillation amplitude at each lateral data point. The key advantage of TappingMode is the elimination of the lateral shear forces present in contact mode, which, on many specimens, can damage the structure being imaged. TappingMode AFM can be conducted in an air or liquid environment. The images and measurements in this application note demonstrate that many contact lenses, even hydrogel contact lenses with high water content, can be imaged with TappingMode AFM in air or liquid. TappingMode also facilitates concurrent "phase imaging," which also provides information on material properties. All images in this article were generated using the Digital Instruments NanoScope® Controllers with MultiMode® and Dimension™ AFMs, offered by Veeco Instruments Inc.

Comparison of AFM with Other Techniques

The fact that AFM senses small chemical or mechanical forces point-by-point by directly contacting the natural sample surface distinguishes it from other surface analysis techniques. TappingMode AFM complements and improves upon other types of microscopy. Three key advantages of AFM over conventional microscopic techniques are, (1) surfaces can be analyzed with nanometer-level resolution in three dimensions, (2) the analysis can be performed in ambient air or in liquids, and (3) sample preparations and imaging environments known to generate artifacts are eliminated (e.g., dehydration, fixation, freezing, staining, coating, etc). Table 1 summarizes the main differences between AFM and other conventional imaging and profiling techniques. Compared to stylus profilometry, AFM provides higher lateral resolution (by two orders of magnitude), without sample damage due to high contact forces (AFM imaging forces are more than three orders of magnitude smaller than those of stylus profilometry). Optical profilometry provides high vertical resolution (0.1nm), but its lateral resolution is relatively poor.

Examples of AFM Applications for Contact Lenses

Characterization of surface finish quality

Figure 2 shows three TappingMode AFM images of a brand new commercial soft contact lens under saline. The prominent linear feature that

	Atomic Force Microscopy	Stylus and Optical Profilometry	Scanning Electron and Confocal Microscopies
Resolution	Lateral resolution down to 1 nm scan range of up to 120μm. Vertical resolution to less than 1Å. Vertical range up to 7μm.	Lateral resolutions sub-nanometer. Vertical resolutions down to 1Å.	Lateral resolution for confocal microscopy down to 170nm; vertical resolution to ~500nm. SEM resolution down to 2nm. Scan range usually on the order of 1mm.
Environment	Many different environmental conditions are permissible during analysis including nearly all transparent liquids and a range of temperatures (-40°C to 220°C), without loss of resolution.	Analysis normally confined to air-dried samples.	Environment usually limited to ambient air or vacuum chamber. Image distortions or loss of resolution can occur in liquids.
Sample Preparation	No sample preparation; true *in-situ* analysis of untreated sample. Dynamic processes can be monitored on single lens.	Little or no sample preparation.	Sample preparations are time consuming and/or cause artifacts (e.g., dehydrations, stainings, and coatings). *Ex-situ* analysis only.
Materials Properties	Chemical and mechanical materials properties including surface adhesion, hardness, and friction. AFM tip can be chemically modified in order to measure tip/sample interactions.	Not useful for characterizing chemical and mechanical surface properties.	Not useful for characterizing chemical and mechanical surface properties.
Sample Damage	AFM, particularly TappingMode technique is non-destructive. Tip forces are finely controlled (<10^{-9} N)	Stylus profilometers exert high forces against sample surface (>10^{-6} N); can cause damage.	Usually non-destructive, but beam artifacts and damage can occur on insulative or hydrated samples with SEM.

Table 1. Comparison of techniques.

appears in these three images was a surprised finding. The detailed three-dimensional structure is visible in progressively smaller scans, and the features can be measured for their in-plane and out-of-plane (vertical) size.

It is not fully known what size and type of defects or features on the contact lens surfaces are critical in prompting unfavorable responses by the eye.

The adhesion and entrapment of protein and contaminants between the lens and the cornea are believed responsible for promoting the growth of bacteria.

To help understand how and where protein molecules and contaminants adhere to the lens, the topography of new and used lens surfaces can be mapped in great detail with AFM (Figures 2, 3).

Figure 4 shows a TappingMode AFM image of a contact lens in saline solution, which was made using a diamond-turned mold. The diagonal cross-section reveals the short and long range variation in height. The periodicity of the surface grooves is a 1.5μm (the Digital Instruments NanoScope software uses a Fast Fourier Transform (FFT) to make this measurement). The grooves resulted from the manufacturing process. When combined with clinical studies, this type of information can help clarify the effect of different size grooves.

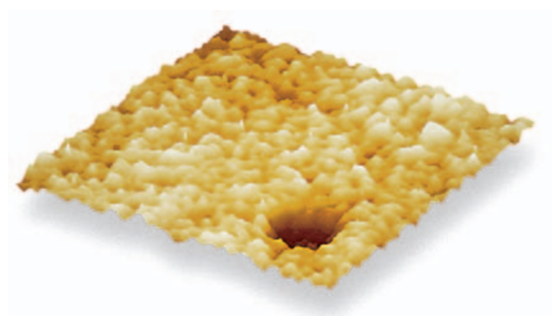


Figure 3. TappingMode image of a 1μm x 1μm area of the same type of contact lens as in Figure 2, also immersed in saline. The RMS roughness is 3.5nm for the area shown. A surface defect or pit, clearly seen in the lower right of the image, measures 170nm in width and 150nm in depth and, therefore, is large enough to trap proteins or contaminants. However, it is too small to be resolved in liquid using conventional techniques.

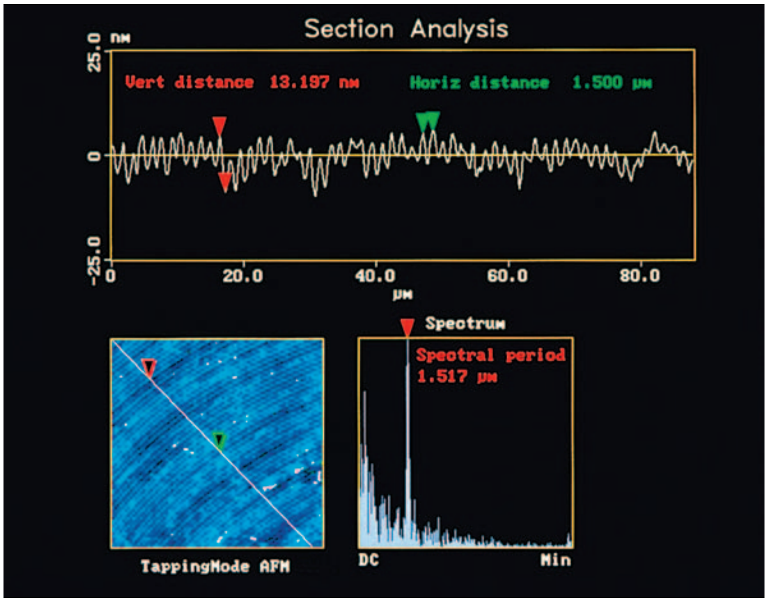


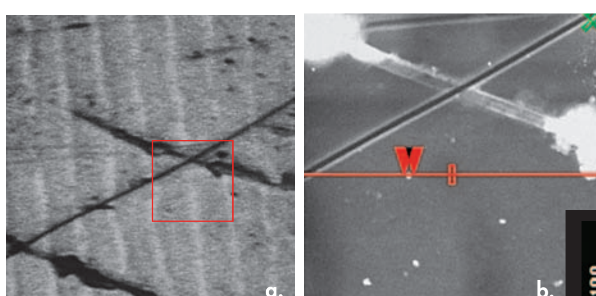
Figure 4. TappingMode AFM measurements on the grooves of a hydrogel lens in saline solution. The grooves originate from the diamond lathed mold. Section Analysis reveals a 1.5µm periodicity (peak in spectrum) and nanometer-sized peak-to-valley heights of the grooves at various locations. The cross-section can be drawn and analyzed as often as desired, and in any direction, because the AFM image, unlike a SEM micrograph, is a digital object that includes 3-dimensional measurement information about the area scanned. 65µm scan.

Figure 5. TappingMode AFM image of a hydrogel lens in saline. The grooves of the lens surface are faintly visible and run from lower left to upper right in the image. Periodic defects, running from top left to lower right in the image, are clearly detected. Such defects originate on the diamond-turned mold during the lathing process and are subsequently transferred to the lens surface. 20µm scan.

Figure 5 is an AFM image of another hydrogel lens that was made with diamond-turned molds. The lathe grooves run diagonally from bottom left to top right. The periodic occurrence of a defect on the lens surface, approximately every fourth groove, strongly suggests that the origin of these types of defects are traceable back to the mold lathing process.

One way to improve the production yield of contact lenses is to detect and characterize defects early in the production cycle, i.e., on the production molds. AFM is a fast and easy-to-use tool for imaging the molds and accurately measuring feature dimensions. Figure 6a shows a light microscope image of an area on a polystyrene contact lens mold, and Figure 6b is an AFM image taken within the same area. Unlike optical microscopy, AFM provides additional quantitative information about the nature and size of the surface features. Figure 6c shows an AFM image cross-section and measurements of height, width and angle on some of the features.

Figure 7 is a TappingMode AFM image of the central area of a Poly(methyl-methacrylate) (PMMA) button, or blank, used in the manufacture of some rigid gas permeable (RGP) lenses. RGP lens manufacture begins with the formation of such buttons by diamond lathing PMMA or other polymers. The RMS surface roughness for this 50µm scan was 32nm. The pattern of the spiral can be used to determine the degree of uniformity of the lathing. Combined with knowledge of the "speed and feed", the dimensions and spacing of the radial grooves, and the direction of folding of the lathed material, AFM can shed light on the direction and the strength of the shear forces between the diamond tool and the polymer surface.



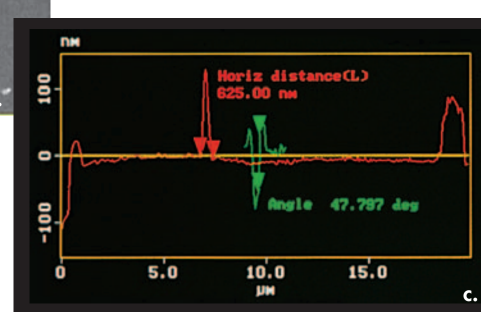


Figure 6. (a) Optical micrograph of a contact lens mold. Approximately 90 x 90µm. (b) AFM top-view image of the same area as box in Figure (a). 20µm scan. (c) AFM cross-sectional plots allow accurate measure-ments of surface feature height, width, and angle and can be made on any cross-section of the AFM image.

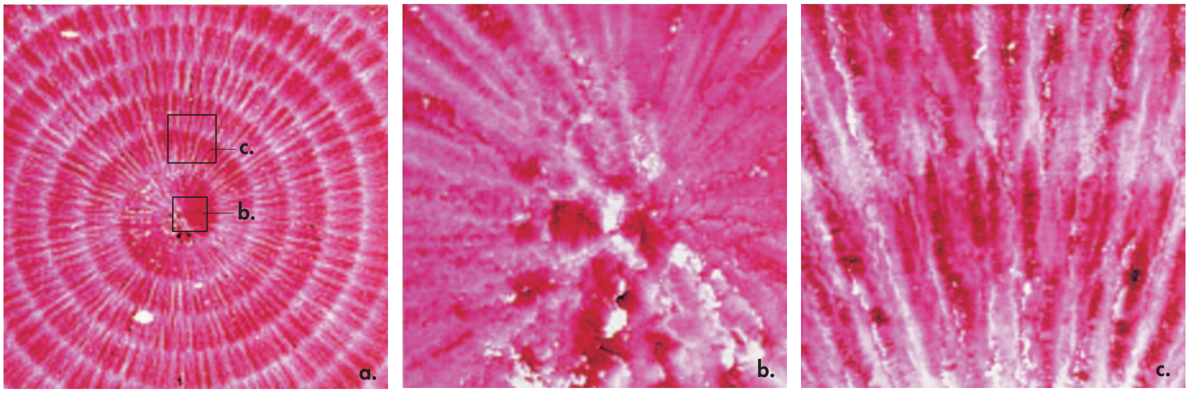


Figure 7. TappingMode AFM image of central area on a diamond-lathed PMMA button (blank). The RMS roughness is 32nm. 50µm scans. Boxed areas in (a) are zoomed and shown in (b) and (c) respectively. Brighter color represents higher features in topography images like these.

Monitoring of manufacturing processes

Figure 8 shows how TappingMode AFM images in saline solution revealed unexpected information about a novel lens material being developed – information which necessitated changing the manufacturing process itself. The figures show 8µm square areas on two samples of this material at different stages in the manufacturing process. The material is a hydrophobic polymer which is coated with a hydrophilic film and then further processed. The pits (darker areas) on the uncoated hydrophobic lens (Figure 8a) were an unexpected finding for the lens manufacturer. Close inspection of the hydrophilic-coated lens (Figure 8b) reveals that the distribution of the sub-micron light-colored spots on the coated lens is similar to the distribution of the pits (dark areas) on the uncoated hydrophobic starting material (Figure 8a). One possible explanation for this is that the hydrophilic coating filled in the pits and, upon exposure to water, swelled to form the sub-micron spots. The manufacturing process was modified to eliminate the pits in the hydrophobic substrate.

Evaluating performance of eye care liquids

For evaluating the performance of eye care products, a single lens can be repeatedly analyzed with the AFM under different conditions. Figure 9 shows AFM images in liquid of the same area on a RGP lens front-side curve. The RGP lens was worn by a 30 year-old female for an extended period of normal use and storage. Figure 9a shows the RGP lens before cleaning, imaged in a commercial saline solution. Notice that most of the particulate adsorbates line up along the polishing scratches on the lens surface. A commercial RGP cleaning solution was then injected into the saline bath for 10 minutes and then exchanged with several washes of saline. The lens was re-imaged (Figure 9b) and only a portion of the adsorbates had been removed by the cleaning solution. The lens was then manually rubbed with a latex glove while in the cleaning solution and then rinsed with more saline. As shown in Figure 9c, nearly all of the adsorbates were cleaned off the surface by this treatment. Based on these results it is clear that AFM can be used to evaluate and understand the performance differences between various eye care solutions.

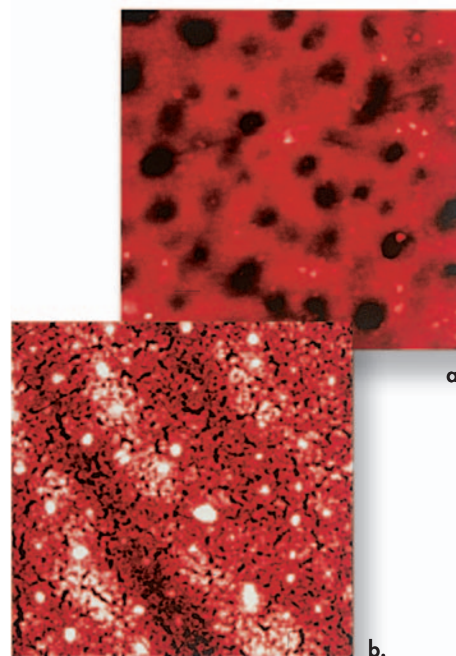


Figure 8. TappingMode AFM images of a (a) hydrophobic silicone hydrogel lens and (b) the same lens material after hydrophilic coating and further processing. Darker areas in (a) are defects that AFM images revealed, and were subsequently eliminated by modifying certain processing steps. 8µm scans.

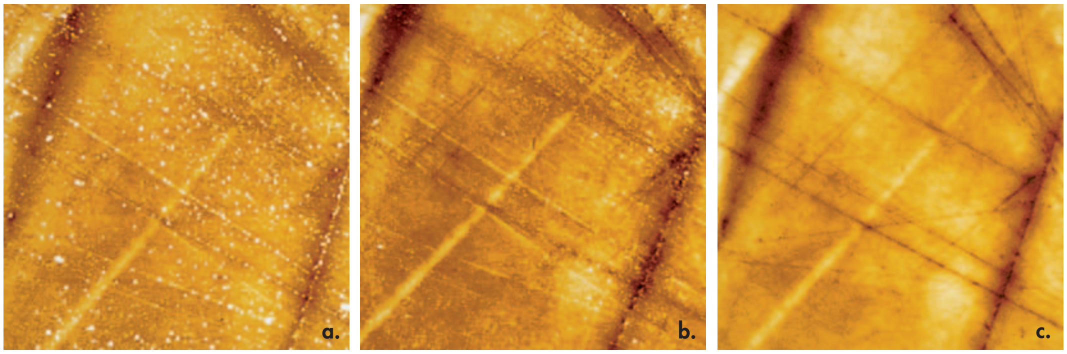


Figure 9. Series of AFM images of the same region on a used RGP lens in saline, (a) before cleaning, (b) after soaking in commercial cleanser, and (c) after soaking in cleanser and rubbing with latex glove. 30μm scans.

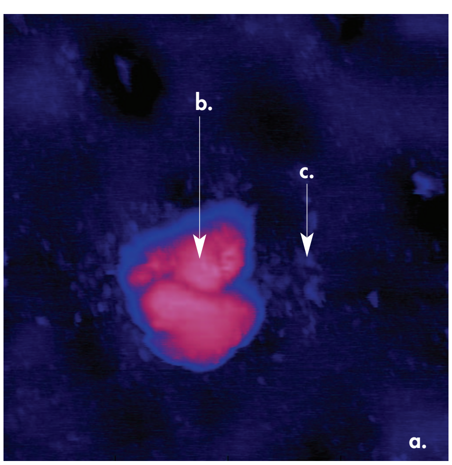
Characterizing Material Properties with AFM

Force vs. Distance Measurements

With force vs. distance curves, AFM can distinguish surface regions of different hardness and adhesion characteristics. Briefly, forces applied on the surface are measured by the deflection of the cantilever while approaching and retracting from the surface. On the left side of the force vs. distance curve (Figures 10b,c), the steeper the slope, the harder the sample. An inverted peak, seen in the middle of the force vs. distance curve, during retraction of the cantilever (white curve) indicates some adhesion between the tip and the sample. For example, Figures 10a-c, representing an area on a soft contact lens in saline solution, reveal that the pronounced surface feature (center of image in 10a) has a greater stiffness and is more adhesive to the AFM probe than the surrounding lens area (10b vs. 10c). The force vs. distance curves not only show the difference qualitatively, but also permit quantitative measurement of the adhesion force (in this case, 5nN). It is also possible to control the tip-sample interactions by functionalizing the tip with a selected protein or chemical group in order to measure specific chemical interactions between the tip and sample.

Phase Imaging

Phase imaging gives yet another way to distinguish between domains with different surface properties on lenses made of heterogeneous polymers or on chemically-modified surfaces. The phase image is generated by monitoring the phase angle of the oscillating probe relative to the phase angle of the signal that drives the probe in TappingMode. Differences in phase shifts indicate differences in material properties of the lens. A topographic AFM image of the same area is also displayed simultaneously with the phase image. The usefulness of phase imaging is illustrated in Figure 11. These images are from identical areas on the surface



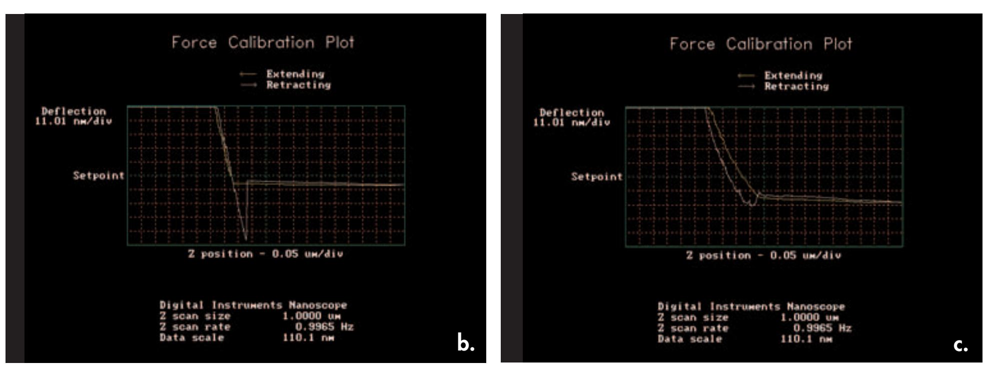


Figure 10. Force vs. distance curves allow characterization of stiffness and adhesion properties of lens surface. (a) TappingMode AFM image in saline of a surface feature (center of image) on a contact lens, 4μm scan. (b) Force vs. distance curve taken on the surface of the central feature. (c) Force vs. distance curve taken at a region adjacent to the central feature. The force vs. distance curves reveal that the central feature has a greater stiffness and is more adhesive to the AFM probe than the surrounding surface material. The surrounding region (c) compiles to the AFM probe as the probe pushes into the sample (yellow line), and very little adhesion is detected when pulling the probe away from the surface (white line).

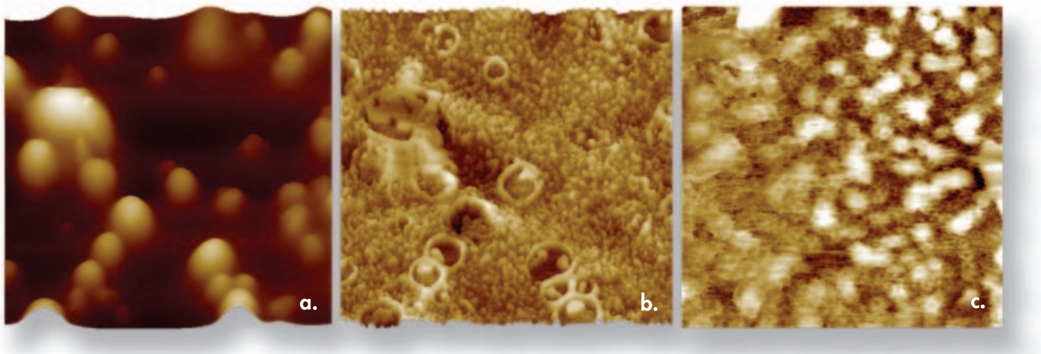


Figure 11. In addition to topographic imaging with TappingMode, phase imaging provides material property information. Shown are simultaneously acquired (a) TappingMode topographic and (b) phase AFM images of hydrogel lens with both hydrophilic and hydrophobic domains, and (c) detail of phase image reveals sub-micron features, including ring-like nanostructures. The phase image reveals different types of patterns that go undetected in the topographic image (e.g., the fine structure of circular domains as small as 10nm. Figures (a) and (b), 4µm scans, Figure (c), 1µm scan.

of a soft contact lens made from a hydrogel polymer with domains of different hydrophilicity. Notice the striking differences between the topographic (Figure 11a) and phase (Figure 11b) images. In the topographic image, randomly dispersed, nodular features of varying sizes are seen on a fairly smooth background. However, in the phase image (Figure 11b), a few of these features appear with some internal structure while others are not seen at all. In addition, the background appears finely speckled only in the phase image. Such differences between topography and phase images are due to material property differences on the lens surface.

Figure 12 is another pair of topography/phase images captured on an experimental hydrogel material in saline solution. This hydrophobic surface, while covered with a mask, underwent a surface modification treatment that created a hydrophilic pattern. The central "cross-like" region (seen clearly in Figure 12b), corresponds to hydrophobic regions, while the four outer areas correspond to hydrophilic regions. The topographic images (Figure 12a,c) show no significant height difference between the masked and unmasked regions of

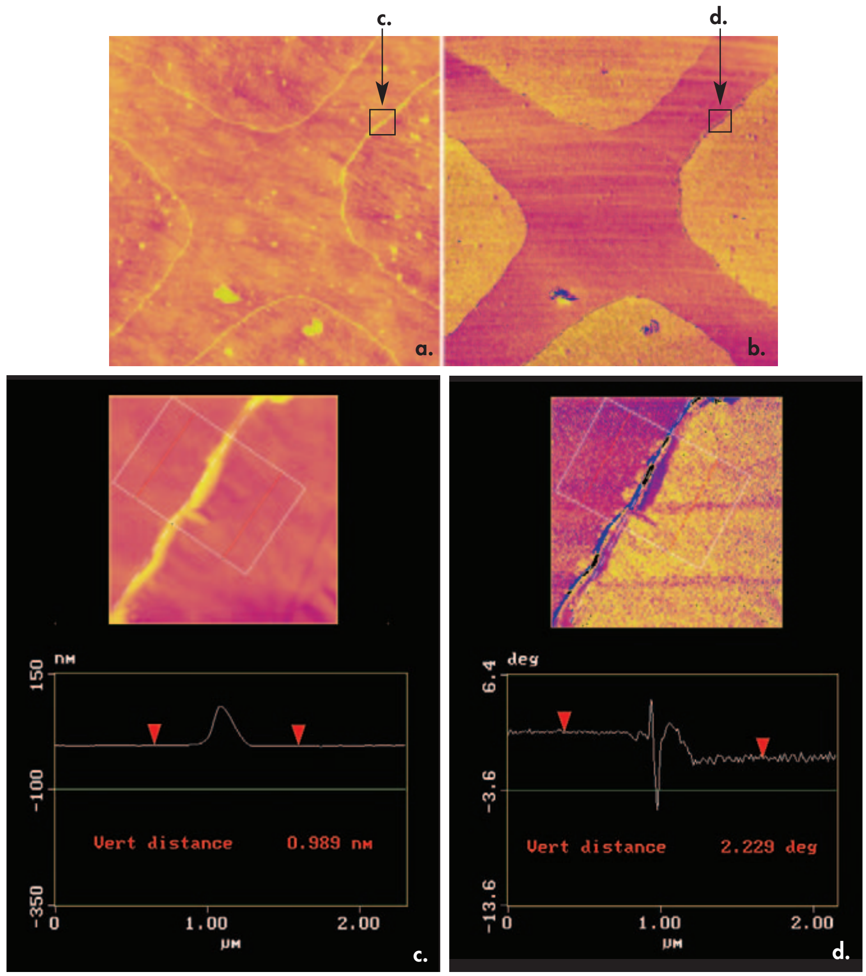


Figure 12. Simultaneously acquired (a) topography and (b) phase AFM images of silicone hydrogel in saline solution. The four outer areas were exposed to a sequence of chemical processing steps. The central cross-like region was masked and so protected from the processing steps and hence retained its hydrophobicity. The intention of the processing was to selectively alter the hydrophilicity of some parts, but not all of, the sample surface. In the phase images (b,d), a marked phase shift is clearly seen across the boundaries. However, the hydrophillic and hydrophobic region show no topographic contrast (a,c). The phase image is clearly providing material property contrast on this well-defined experimental hydrogel surface. 50µm scans.

the lens. In other words, the topography images show the boundaries, but do not provide a clue to material property difference across the boundaries. However, the phase images (Figure 12b,d) show sharp contrast between the masked and unmasked regions, revealing a difference in the material properties of these two regions. AFM phase imaging (in liquid) was the only technique that could distinguish between the regions of different surface properties. The detail of the images and the cross-sections through the images make the point more clearly (Figure 12c,d).

Figure 13 shows yet another example of the usefulness of phase imaging. The TappingMode topography image (a), shows the high and low areas of a contact lens imaged in air. The phase image (b) shows the presence of crystalline-like structures, which are clearly different in their material properties from the area surrounding them. These features are also present in the topography image (a), but the topography image does not tell us if these features are made of the same, or a different material from the rest of the imaged area of the lens. A likely source of the contrast in the phase image is salt crystals formed from the saline solution in which the lens was packaged before being imaged by the AFM.

Other AFM extensions

Surface hardness and wear testing may be done with AFM nanoindentation. This technique uses the AFM tip to indent at known force load and then image the indented surface. The hardness of the sample can be evaluated from the indenting force and the surface area of the resulting indent.

Force Modulation imaging visualizes areas of different stiffness or elasticity. With this technique, the probe is given a small vertical oscillation while it is pushed against the sample. Soft sample regions can be deformed by the probe resulting in a decrease in oscillation amplitude compared to stiff sample regions where sample deformation is less (see Veeco application note entitled "Force Modulation Imaging with Atomic Force Microscopy" for more information).

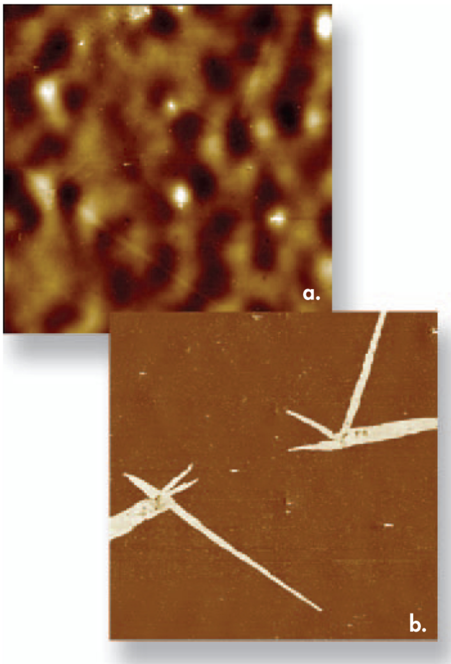


Figure 13. TappingMode topography (a) and phase images (b) of a contact lens in air. Crystalline-like structures in the phase image are possibly salt crystals left after water evaporated from the saline solution. 10μm scans.

Lateral Force Microscopy (LFM) is used to identify surface friction characteristics. With this contact technique, the torsion or lateral twist of the cantilever is detected. Torsion of the cantilever will depend on the frictional properties of the surface (greater torsion corresponds to greater friction). Also, a new technique called Torsional Resonance Mode (TRmode™) — a patented technology, offered exclusively by Veeco Instruments Inc. — can map in-plane anisotropy with nanometer-scale resolution. For more details regarding these implementations of AFM, see the related Applications Notes section of www.veeco.com.

Summary

AFM is a useful new tool for the contact lens industry, as well as for biomaterials R&D in general. AFM measurements help in evaluating surface finish quality, manufacturing processes, protein adsorption and build-up, lens cleaner efficiency, and materials properties – in air or liquids. These types of measurements on lenses and/or molds are very useful for greatly enhancing quality control capabilities. Furthermore, this type of information will help to speed the development of superior polymers and coatings, and new or improved manufacturing processes. It is also demonstrated that AFM can be useful in clinical studies to identify with more confidence the underlying causes of contact lens related discomfort.