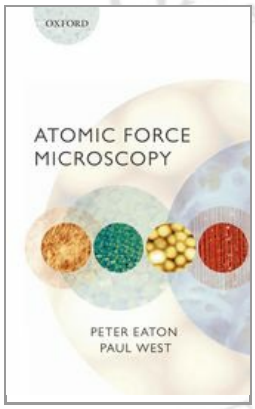

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AFM image artefacts

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[–] Abstract and Keywords

AFM, like any other measurement technique, is prone to artefacts. These can arise due to the AFM probe, the scanner, the instrument electronics, from the laboratory environment, or from many outer sources. Some artefacts are obvious to experienced users, while others are more subtle. Identifying the artefacts so that they can be explained and excluded from analysis is one of the most difficult tasks facing new AFM users. This chapter explains the origins of the artefacts that occur in AFM images, and explains what can be done to avoid them.

Keywords: AFM, artefacts, image artefacts, probe convolution, scanners

All measurements and measurement techniques are prone to artefacts. In AFM imaging, these artefacts are sometimes easy to spot and sometimes very difficult. Some artefacts

can be easily avoided, if the user knows what to look for and knows the source of the error. A few artefacts are unavoidable, but knowing that they exist in an image helps to avoid misinterpreting them as genuine image features. This means that recognizing image artefacts is very important for the AFM user. However, when users begin to use AFM for the first time, it is very difficult to sort the real features from the artefactual. Experienced AFM users as well as novices can benefit from considering the sources of AFM artefacts, as some artefactual features are very subtle, and can only be clearly seen when making particular measurements from an image (for example when measuring line profiles, or Fourier filtering). This chapter contains examples of common AFM artefacts, explains the source of the features, and shows what can be done to avoid them.

6.1 Probe artefacts

Probably the most commonly seen AFM artefacts arise from the probe used to scan the sample. As explained in Chapter 2, all AFM images are a convolution of the topography of the sample with the shape of the tip of the probe (and sometimes with the sides of the probe) [54]. When interpreting AFM images, we often assume that the tip radius is finer than the details imaged, and that the opening angle of the probe is smaller than the angle of the features in the sample. This means that the influence of the tip-shape on the image obtained will be small (but finite). However, even if this is the case, continual use may dull the probe tip or it can break or become contaminated [46]. Often, if the user has many samples to image, the probe will be used until one of these phenomena occurs, and the probe becomes unusable. In either case, the user must know what to look for when the tip degrades, in order to know when to replace the probe.

Common effects seen when imaging with an inadequate probe include:

- The features on a surface appear too large.
- The features, especially holes, appear too small.
- Strangely shaped objects appear.
- Repeating patterns appear in the image.
- The image appears normal on the top of features, but not on their sides.

The best advice if the user is unsure is to use a tip-check sample. This can be any sample that the user is certain of the topography of, and which has relatively fine features, such that the radius of the tip can be determined. In practice, certain types of samples are particularly useful for this operation, and some of the most common ones are described in **(p.122)** Appendix A. In this chapter, images of tip-check samples that were acquired with faulty probes are shown, along with images measured with a new probe, to illustrate the effect that probe damage has on the images obtained.

6.1.1 Blunt probes

Typically, blunt probes will lead to images with features larger than expected, with a flattened profile, due to the effect shown below. Note that holes in a flat surface will show the opposite effect, appearing smaller with blunt probes than with sharp ones (see the lower part of Figure 6.1).

The dilation due to the probe shape as shown in Figure 6.1 is a normal feature of AFM imaging. For example, when measuring globular features with a known diameter of 2 nm it would be normal to find the feature in the AFM image has 2 nm height but 10–20 nm width [279, 375, 376]. However, when it occurs to a large extent it is a problem, because it may significantly alter the apparent size of the features, and can really change their appearance. An example is shown in Figure 6.2. If this effect is noticed, the user should change the probe. If the feature cannot be imaged correctly even with newer probes, then another type of probe (e.g. super-sharp probes or high-aspect-ratio probes) may be required [377]. However, some extremely high-aspect-ratio features can be extremely challenging to image by AFM, no matter which probe is chosen.

The fine details of the BOPP sample when imaged with a sharp probe are seen in the left image in Figure 6.2. When imaged with a blunt, worn probe, as shown in the right image, they disappear, and the sample becomes almost unrecognizable. An example of the effect of pits in a sample becoming smaller with a dull probe is shown in Figure 6.3.

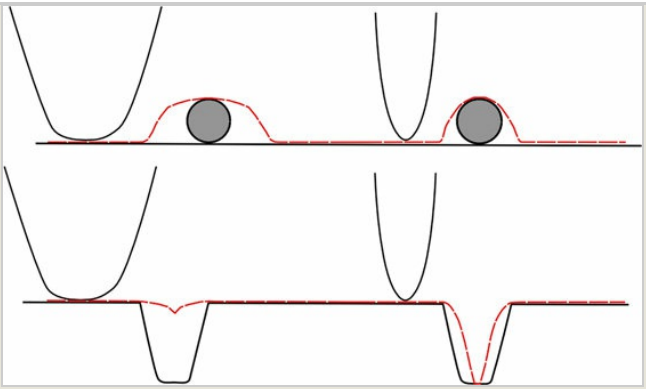

Fig. 6.1. Illustration of probe-based dilation. Convex features such as particles tend to appear wider with blunter probes, although feature height may be accurate. Concave features such as pits tend to appear smaller (both less wide and less deep) with blunter probes.

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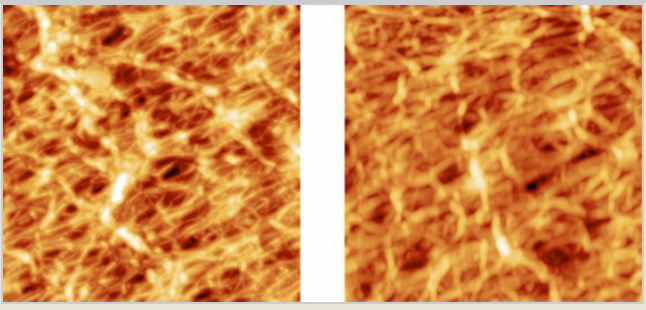

Fig. 6.2. Illustration of the effect of using a blunt probe. These two images are of the same sample, and both are 1.5 μm × 1.5 μm × 40 nm. The image on the left was taken with a sharp probe, the image on the right with a blunt probe. The sample is BOPP, a useful sample to characterize the sharpness of IC-AFM probe tips, see Appendix A9.

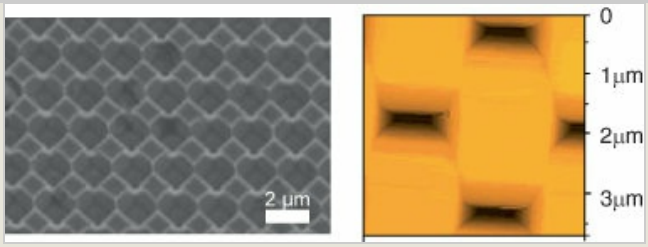

Fig. 6.3. Example of features appearing smaller due to the use of a blunt probe. Left: SEM image of a test pattern of squares (NT-MDT grating TGX, see Appendix A). The sides of the squares are all equal. B: AFM image of the test pattern. Because the probe is not sharp, the test pattern squares appear much smaller than they should, and appear as rectangles instead of squares.

6.1.2 Contaminated or broken probes

Contamination of AFM probes is quite common, and scanning certain samples leads to dirty probes more quickly than others. In particular, biological or other soft samples, or any sample with loose material at the surface, tend to contaminate probe tips quickly, leading to image degradation [378]. On the other hand, breaking of the AFM probe is less common, but still occurs, mainly when the probe accidentally touches the sample outside of feedback control. The reason these two problems are described together is than they can give very similar results. When imaging a sample with a broken or dirty probe, the resulting images often contain features with unexpected shapes, due to convolution of the misshapen tip with the sample features. Examples are shown in Figure 6.4. Any repeating patterns within the images, which are not expected based on what is known of the sample, are likely to be due to a broken or contaminated probe.

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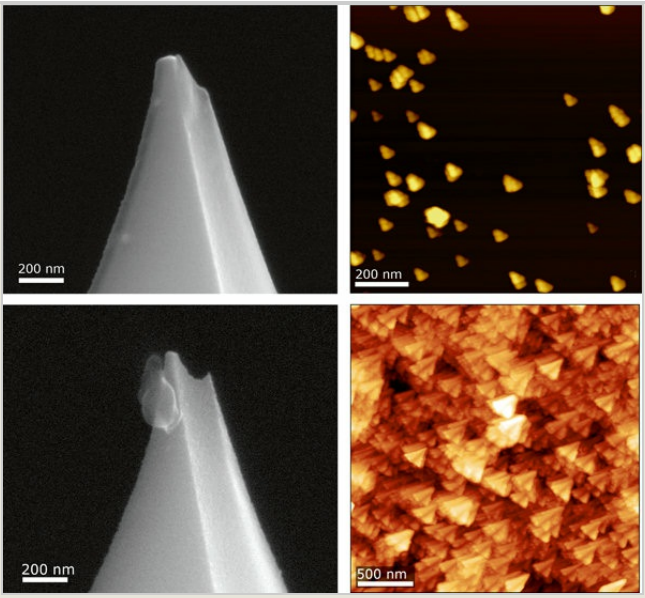

Fig. 6.4. Examples of how images produced with broken or dirty probes show repeating patterns in the images. Left: SEM images of damaged and dirty probes. Right: AFM images produced using the probes shown on the left. Images with repeating patterns like these are usually due to broken or dirty probes.

Double tips

A further example of damage or contamination of tips altering the image is the creation of multiple tips. If the tip breaks such that it has small spikes at the end, or more commonly, has debris attached near the tip, the sample may be imaged both by the true tip, and the debris. This results in multiple copies of each feature appearing in the image [379]. It's not possible to distinguish which image feature is from the 'true' tip, and double, or multiple copies of each feature occur in the image, as shown in Figure 6.5.

When the user determines that the probe is blunt, contaminated, or broken, they must replace the probe. Some procedures for cleaning of AFM probes have been described [380], however, in the authors' experience, it is usually simpler and far more effective to replace the probe than to try to clean it.

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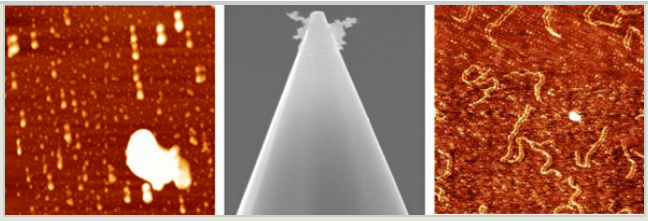

Fig. 6.5. Example of double-tip imaging. Left: an image of vesicles measured with a dirty tip. Right: DNA molecules measured with a broken tip, each molecule has a false 'twin' next to it. Centre: a badly broken and contaminated tip which produced double-tip images like these.

6.1.3 Probe–sample angle

When scanning large features, artefacts can be introduced by having a large angle between the probe and the sample, as illustrated in Figure 6.6. Ideally, the AFM probe should be perpendicular to the sample surface.

Solving this problem is achieved by adjusting the angle between the probe and the sample so that they are perpendicular. Often, a set of three adjustment screws on the microscope allows the user to adjust this angle. In many microscopes the probe is designed to be at a 12° angle with respect to the sample, and some probes are designed with this angle in mind, i.e. such that when the cantilever substrate is at 12° to the sample, the probe will be perpendicular to it. Some AFMs do not have mechanical adjustments to control the probe–sample angle. In this case, the sample must be adjusted to correct the probe–sample angle.

6.1.4 Side-wall/probe imaging

Certain samples with extremely high-aspect-ratio features are very difficult to image correctly, and they can interact with the probe in such a way that the image contains repeating images of the probe, or of the side-walls of the probe. Examples of features that produce side-wall images are spherical micro-organisms, spherical particles or red blood cells, with their typical doughnut-like shape, images of which often are great on the top of the cell, but it's not possible to image the sides of the cell, and images of the probe side-

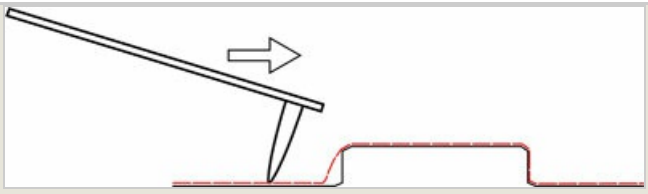

Fig. 6.6. Illustration of probe–sample angle problems. With the probe at an angle to the sample, distortions are introduced, and sample features appear asymmetric.

(p.126)

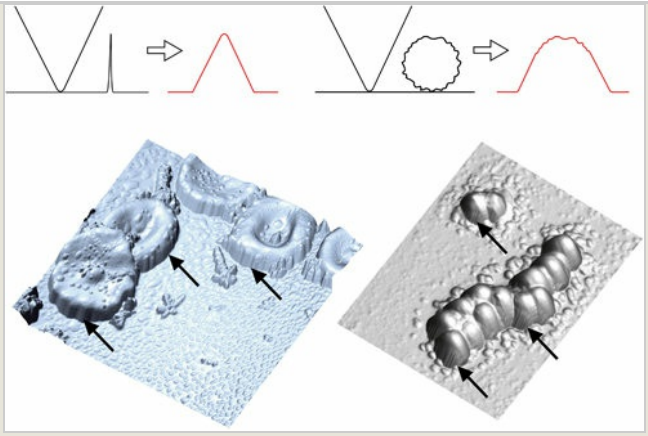

Fig. 6.7. Effect of probe or probe side-wall imaging. Top: illustrations of the effect of imaging a spike – an image of the probe is produced – and imaging a sphere-like feature – only at the top is the sample topography reproduced, and the rest of the image feature shows the probe's side-walls. Bottom: examples of probe–side-wall imaging. Left: red blood cells, right: *S. aureus* bacteria. In both cases, only the upper parts of the cells can be imaged correctly (some examples of probe side-wall imaging highlighted by arrows).

wall appear instead (see Figure 6.7) [381, 382]. Samples with spike-like features (including certain tip-check samples, see Appendix A) lead to repeated copies of the tip in the resulting images [383].

Typically, any image showing square pyramid-shaped features will be showing images of the probe rather than true sample features, so these image features can be discounted. In order to avoid this problem, the user is recommended to use a shaper tip, specifically, one with a higher aspect ratio. Silicon nitride contact-mode probes are very prone to producing images of the probe side-wall, as they typically have much wider opening angles (*ca*. 35–40° versus 15–20° for most intermittent contact-mode probes). If this artefact causes real problems, for example in metrology applications, super-high-aspect-ratio probes are also available (for example, with opening angles <3°) [377]. Example images of such probes are shown in Figure 2.30. However, for spherical samples such as nanoparticles or the cocci shown in Figure 6.7, parts of the sample will always be unavailable to most AFM experiments. Imaging of probe side-walls will tend to increase if there is a mismatch between the angle of the probe and the sample, as described in the previous section.

6.2 Scanner artefacts

As described in Chapter 2, there are a number of different scanner designs available for commercial AFMs. However, by far the most common design in use is the piezoelectric tube scanner. This scanner is used because it is easy to integrate into the instrument, cheap **(p.127)**

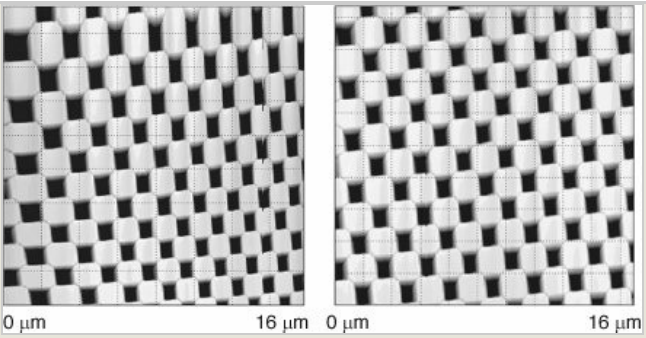

Fig. 6.8. Effect of *x-y* non-linearity in AFM images. Left: example of an AFM image of a test sample (TGX01, see Appendix A) when scanned without correctly linearizing the AFM scanner. Right: linearized AFM image of the same sample. The spacing of the squares at the top, bottom, left and right sides should be all the same distance apart. Images courtesy of Mikromasch.

to produce, and gives rapid and very precise response under most circumstances. However, most AFM scanners do introduce some artefacts into the images obtained, the tube scanner more than most. The artefacts described in this section all occur with piezoelectric tube scanners. Many of them are avoided when using a linearized scanner (see Chapter 2).

6.2.1 X-Y calibration/linearity

All atomic force microscopes must be calibrated in the *X-Y* axis so that the images and measurements obtained are accurate. The motion of the scanners should also be linear so that the distances measured from the images are accurate. Due to the non-linearity of piezoelectric scanners, without correction, the features on an image will typically appear smaller on one side of the image than on the other, see Figure 6.8. Once the scanner is properly linearized, it is also critical that the scanner be calibrated. In other words, it is possible for the scanner to be linear but not calibrated. If the calibration is incorrect, then the *X-Y* values measured from line profiles will be incorrect.

A common method for correcting the problems of *X-Y* non-linearity and calibration is to add calibration sensors to the *X-Y* piezoelectric scanners. These sensors can be used to correct the linearity and the calibration in real time; often, such a system is described as having linearized scanners. If these are not available, and non-linearity is detected in images, then the instrument should be re-linearized according to the manufacturer's instructions. Typically this is carried out with a test grid as illustrated above, and in Appendix A. Note that non-linearity at just one edge of the image could be due to other effects; see the other sections in this chapter.

6.2.2 z calibration and linearity

Height measurements in an AFM require that the piezoelectric ceramics in the *Z* axis of the microscope are also both linear and calibrated. Usually the microscope is calibrated at only **(p.128)**

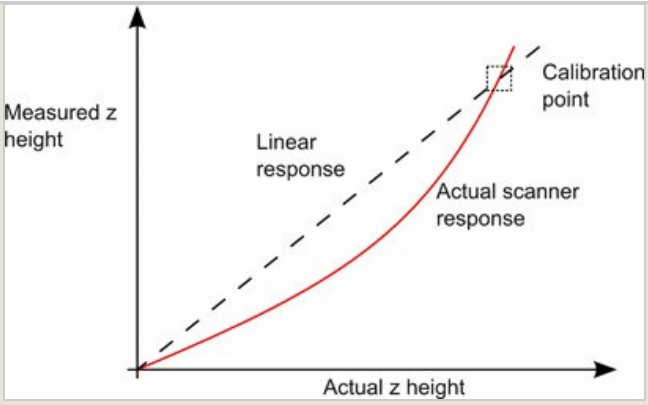

Fig. 6.9. Graph showing the relationship between an actual *z* height and a measured *z* height in an AFM. Usually only one calibration point is measured as shown by the box, and the *z* piezoelectric is assumed to be linear, as shown by the dashed line. However, as is often the case, the piezoelectric actuator is non-linear, as shown by the solid line. In such cases incorrect *z* heights are measured unless the feature being measured has dimensions close to those of the calibration specimen.

one height. However, if the relationship between the measured *z* height and the actual *z* height is not linear, then the height measurements will not be correct, see Figure 6.9.

The only way to ensure absolutely accurate *z* height measurements at a range of heights is to use an instrument with a sensor for the *z* piezoelectric. An alternative, which only works for measurements of features within a particular height range, is to recalibrate the instrument using a calibration specimen of known height, which is similar in size to the features which will be measured. Typically the *z* axes of AFM microscopes are calibrated using semiconductor test samples with features on the order of 100–200 nm in height. So, for example, measurements of small features of 5–10 nm could not be expected to be very accurately measured under these circumstances. In this case, it would be best to recalibrate the instrument using a test sample of known height in the range 5–10 nm. Alternatively, some samples can be used as an internal standard, avoiding the need to recalibrate the AFM [279]. Some widely available *Z*-height calibration standards are described in Appendix A.

6.2.3 Scanner bow

The scanners used in AFM instruments often move the probe in a slightly curved motion over the sample surface. This is typically the case for tube scanners fixed to the microscope body at one end, and free to move at the other – currently the most common design in AFM. As shown in Figure 6.10, this motion gives rise to a curvature or 'bow' as it is most often known, in the resulting images. This tends to give a small variation in *z* height over a relatively large *X*-*Y* area, so it is most obvious with flat samples.

This artefact cannot be avoided with instruments whose design is prone to it, but the effect can be removed in processing. The procedures to carry out this operation are described in Section 5.1.1.

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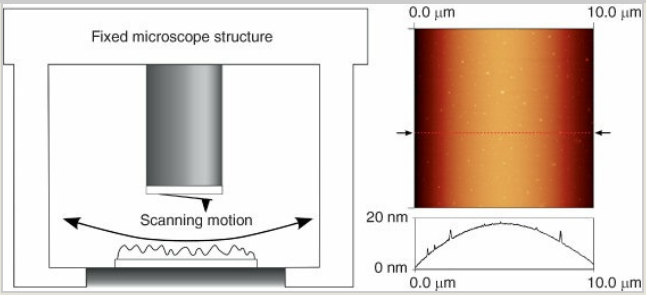

Fig. 6.10. Effect of scanner bow. Left: with tube scanners fixed at one end, the trajectory of the probe is curved. Right: the result is an apparent curvature in the height of measured samples, although the height change is small over a large area.

6.2.4 Edge overshoot in the Z axis

Hysteresis in the piezoelectric ceramic that moves the cantilever in the perpendicular motion to the surface can cause edge overshoot. Hysteresis is an inherent property of piezoelectric materials, and means that forward and backward movements are not exactly equivalent. The effect in the *Z* axis affects the AFM's ability to trace accurately over step profiles. This problem is most often observed when imaging microfabricated structures such as patterned Si wafers or compact disks, but may be observed in any sample with sharp-edged features. The effect can cause the images to appear visually better because the edges appear sharper. However, a line profile of the image structure shows errors, as shown in Figure 6.11.

Edge overshoot cannot be avoided by the user. It will only occur on microscopes without a *z* axis calibration sensor, however. In cases where this occurs step height measurements should only use the unaffected (flat) portion of the feature profile.

6.2.5 Scanner creep

Creep in piezoelectrics gives rise to the phenomenon that when an instantaneous voltage is applied to the piezoelectric and maintained, the response of the material does not follow exactly the applied voltage, but instead continues to move in the same direction as the initial offset, even when the voltage is no longer changing. This is illustrated in Figure 6.12. The practical effect of this is that when the user translates the scanning position on the sample, moves the probe to the start of a new scan, or zooms into a previous scan (all of which are done by rapidly changing the voltage applied to the piezoelectric), distortion occurs in the image. The duration of this effect is limited, and eventually it disappears. An example of this distortion (‘scanner drift’) is shown in Figure 6.12.

This artefact can be removed by simply waiting for the piezo position to stabilize. One way is to make an initial scan in any new region, before recording a second scan free of

(p.130)

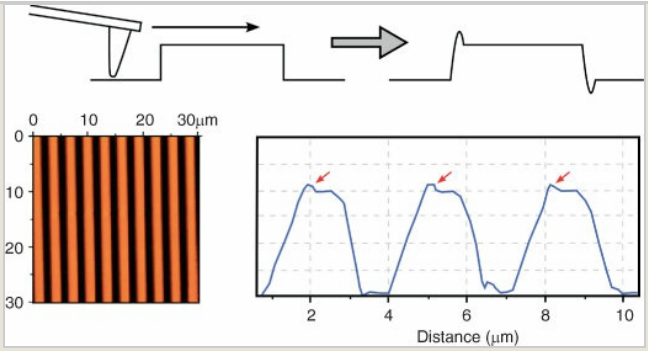

Fig. 6.11. Edge overshoot in the *z* axis. Top: the probe is scanned from left to right across a feature on a surface; overshoot may be observed in the line profile at the leading and trailing edge of the features. Bottom: the AFM image of a test pattern appears to have no artefacts at first glance (left), but a line profile of the test pattern shows overshoot at the top of each of the lines (right, overshoot arrowed).

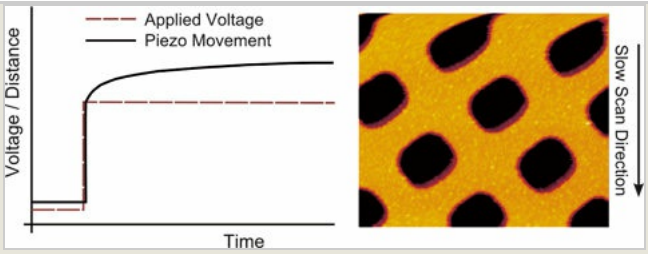

Fig. 6.12. Scanner drift cause and effect. Left: creep in piezoelectric scanners causes the scanner to keep moving even after the applied voltage stops changing. Right: the effect on AFM images is most often seen as a distortion in the beginning of the scan (here, scanning from the top).

distortion. Alternatively, the instrument can be set to do a continuous line scan in the new position. When the user observes that the features in the line scan are no longer changing, the drift has stopped and the image scan should then be begun.

6.2.6 Z angle measurements

Mechanical coupling between the piezoelectric ceramics that move the probe in the *x* or *Y* directions and the *Z* direction can cause substantial errors when trying to measure vertical angles with the AFM. This sort of crosstalk is common in piezoelectric tube scanners, and means that the accuracy of angles in the *Z* axis measured with most AFMs **(p.131)**

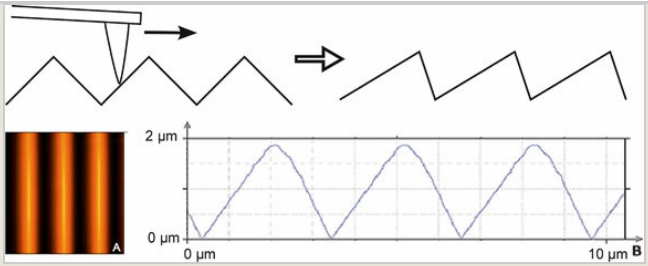

Fig. 6.13. Illustration and example of errors in *Z* angle measurement by AFM caused by crosstalk. Top: illustration of the effect. The sample has a series of repeating triangles at its surface. A line profile of the sample shows that the triangles do not appear symmetric. Bottom: real AFM image of a sample having a triangle pattern at its surface, and a line profile extracted from the AFM image. Although the angles of the two facets are in reality equal, the AFM image suggests that this is not so.

are unreliable. This error can best be measured with a sample that has repeating triangle structures. An example of this is shown in Figure 6.13.

The user cannot control the appearance of this artefact. It occurs with non-linearized tube scanner-based AFMs, and independent *X*-*Y* and *Z* scanners are required for the measurement of correct *Z* angles.

6.3 Image processing artefacts

Some image processing is usually necessary before viewing or analysing any AFM image. As described in Chapter 5, there are a large number of processing operations that can be applied to AFM images. The correct procedures were described in Chapter 5, so here only examples of the artefacts that might be introduced are shown.

6.3.1 Levelling artefacts

Levelling changes the entire AFM image, so the resulting image is different from the raw data. However, it is very often a necessary procedure before useful information can be extracted from an image. Commonly, levelling artefacts are introduced by polynomial fitting routines; Figure 6.14 shows an example of this.

This error is easily avoided by excluding parts of the image from the fit. This was described in Section 5.1.1. Despite the ease with which this artefact is avoided, it is commonly seen in published AFM images.

6.3.2 Filtering artefacts

Image filtering, by definition, alters the data in the image and therefore always introduces some sort of artefact. When presenting AFM data, it is important to specify what filters, if

(p.132)

Fig. 6.14. Examples of line-by-line (polynomial fitting) based levelling artefacts. The left image of nanoparticles is unlevelled. The middle image shows an artefact caused by polynomial line-by-line levelling – the particles seem to be sitting in lowered 'trenches' in the background. The correctly levelled image is shown on the right.

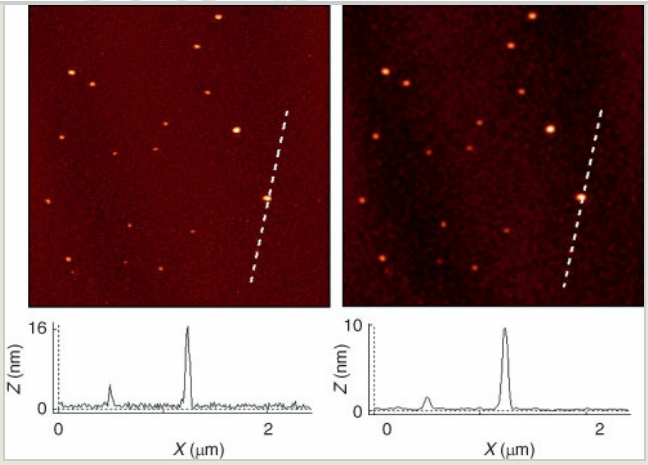

Fig. 6.15. Example of image distortion by filtering. The image of nanoparticles on the left shows considerable noise. Low-pass filtering (smoothing) produced the image on the right. The line profile shows that noise was reduced, but the shapes of the two particles in the line profile were also changed.

any, were applied to the data, because the results from filtered images can be very misleading. For example, low-pass (or smoothing) filters tend to greatly reduce noise in AFM images, but can also introduce artefacts such as changing the shape of features, and increasing the apparent sharpness of steps. An example of filtering artefacts is shown in Figure 6.15.

(p.133) In addition to matrix filters, as illustrated above, Fourier transform-based filtering can also introduce artefacts into an image. This was described in Section 5.3.4, and shown in Figure 5.12.

6.4 Vibration noise

Environmental vibrations in the room where the AFM is located can cause the probe in the microscope to vibrate and make artefacts in an image. Typically, the artefacts appear as oscillations in the image. Both acoustic and floor vibrations can excite vibrational modes in an AFM and cause artefacts.

6.4.1 Floor vibrations

Often, the floor in a building can vibrate up and down by as much as several microns,

typically at frequencies below 5 Hz. The floor vibrations, if not properly filtered, can cause periodic structure in an image. Because it has low amplitude, this type of artefact is most often noticed when imaging very flat samples. Sometimes the vibrations can be started by an external event such as machinery in motion, a train going by, or even people walking outside the AFM laboratory. However, it is often rather difficult to diagnose this type of noise.

6.4.2 Acoustic vibrations

Sound waves (acoustic vibration) can cause artefacts in AFM images. The source of the sound could be from an airplane going over a building or from the tones in a person's voice. The noise of cooling fans from other instruments, or even from the AFM electronics, can also be registered by the AFM. Figure 6.16 is an image that shows the noise derived from a person talking in the same room as the microscope. Diagnosing this type of interference is rather easy; the user must isolate the AFM from the sources of noise or remove them, and look for a change in the signals registered.

The solution to this noise problem, like that from floor vibrations, is isolation from the noise source. Solutions for this were discussed in Section 2.6. Briefly, building vibrations are generally countered by mounting the AFM on a suspended stage that is isolated from the floor. On the other hand, acoustic isolation is accomplished by enclosing the AFM in a cabinet with acoustic shielding on the inside. Alternatively, the noise sources can be removed, and the AFM placed in a location less prone to building vibrations. For this, a room in the basement of the building with little traffic usually serves best.

6.5 Noise from other sources

Floor and acoustic noise are the most common troublesome noise sources in AFM, however, other sources of noise such as electronic noise, which occurs rarely, or noise from a vacuum leak, which is limited only to those instruments that use a vacuum sample mounting system, can sometimes cause problems. The results of poor feedback settings can also appear to give rise to noise in AFM images, when the PID settings are too high.

(p.134)

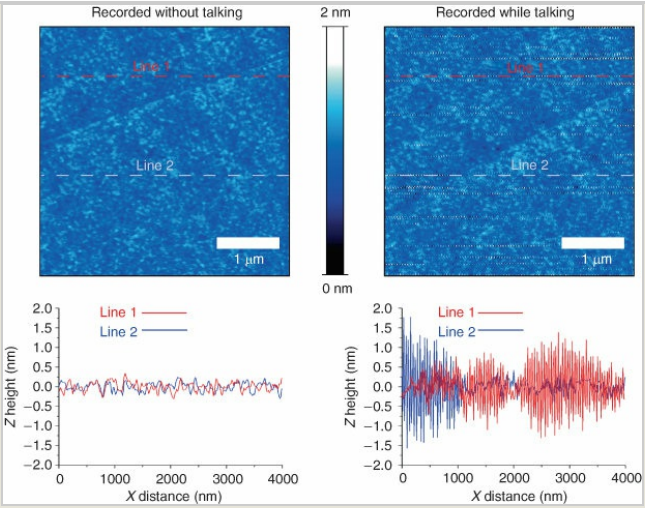

Fig. 6.16. Effect of acoustic noise. This high-z resolution image of a silicon wafer shows the effect of acoustic noise on an image. Right: image and line profiles measured while acoustic noise was present in the room. The acoustic vibrations from a person speaking while the image was acquired are clearly visible in the line scans and the image. Left: image that was measured without the acoustic noise. (A colour version of this illustration can be found in the plate section.)

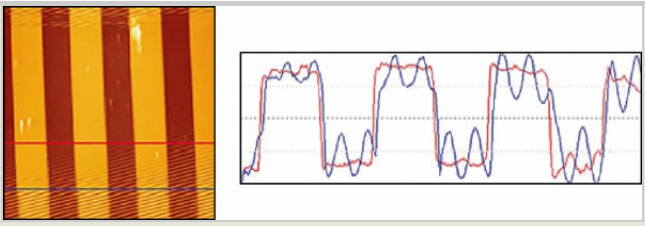

Fig. 6.17. Example of electronic noise in an AFM image. This image of a test pattern has electronic noise at the top and bottom of the scan. The electronic noise in this case was a result of not having a ground wire attached to the stage. The artefact was identified by the oscillation frequency. (A colour version of this illustration can be found in the plate section.)

(p.135) 6.5.1 Electronic noise

Image artefacts can appear in AFM images because of faulty electronics, or accidental electric connections to a part of the AFM. Artefacts from electronics most often appear as regular oscillations or unexplainable repeating patterns in an image, see Figure 6.17. Electronic ground loops and broken components are usually the source of electronic noise.

6.5.2 Vacuum leaks

Atomic force microscopes that are designed for imaging wafers and disks often use a vacuum chuck to hold the wafer/disk while scanning images. A leak in the vacuum between the specimen holder and the specimen can cause image artefacts. The artefact causes a loss of resolution in the image. Cleaning the vacuum chuck and sample and

remounting the sample in the stage often eliminates this problem.

6.6 Other artefacts

In this section we gather some other effects that give rise to problems in AFM. Some of these, such as sample drift and surface contamination are the sort of issues encountered in all high-resolution microscopy techniques.

6.6.1 Feedback settings and scan rate

If the feedback (PID) settings used while scanning are not optimized, then it's very likely that the resulting image will show considerable artefacts. This is because the probe is not tracking the surface, and the cantilever is bending to pass over surface features. The correct settings for the PID circuits are also dependent on the scan rate – higher scan rates may require higher PID settings. This artefact can be identified easily by monitoring the error signal. If the error signal is large, then the probe is not correctly tracking the surface. An example of this is shown in Figure 6.18, but see also Chapter 4 for further discussion of feedback parameter optimization. If the PID settings are too high, 'feedback oscillation' can occur, which looks like high-frequency noise in the image.

6.6.2 Surface contamination

As explained in Section 4.1, suitable sample preparation is vital for reproducible, artefact-free AFM imaging. Substantial contamination at the surface of a sample such as a fingerprint or oil film can cause AFM image artefacts. Such artefacts may appear as streaks on the image especially in locations where there are 'sharp' features and edges on the sample's surface. Often the streaking can be reduced or eliminated by cleaning the sample with a high-purity solvent. An example of this effect is shown in Figure 6.19.

6.6.3 Laser interference patterns

Interference patterns can be created by the laser used to detect the bending of the probe cantilever. The interference appears as low-frequency background oscillations in images and typically has a period that is similar to the wavelength of the laser light being used in **(p.136)**

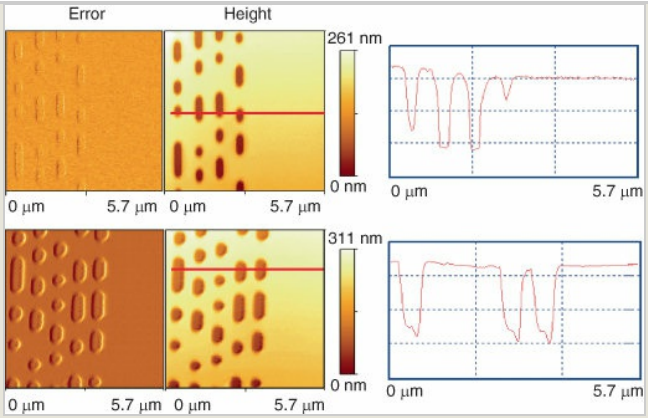

Fig. 6.18. Example of an artefact created by not having the feedback (PID) parameters fully optimized while scanning. In the upper image parameters are optimized, in the lower image parameter are not optimized and the error signal is large. This also leads to less accurate height image, see the line profiles.

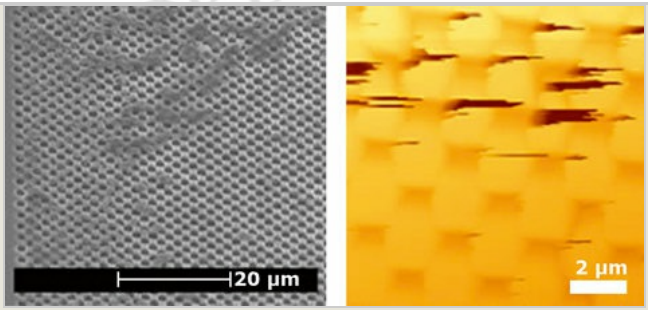

Fig. 6.19. The effect of surface contamination. Left: SEM image of a heavily contaminated calibration grid sample. Right: the contamination causes streaking and prevents the probe from properly following the surface topography in the AFM image.

the AFM scanner (typically 0.5–1.5 microns). This interference originates from laser light spilling over the cantilever, or passing through it, reflecting from the sample surface, and interfering with the light reflected directly from the cantilever. A similar effect can also be seen in force–distance curves, where the interference appears as waviness in the baseline of the force–distance curve, with the same period. This is illustrated along with a typical image showing the artefact in Figure 6.20.

(p.137)

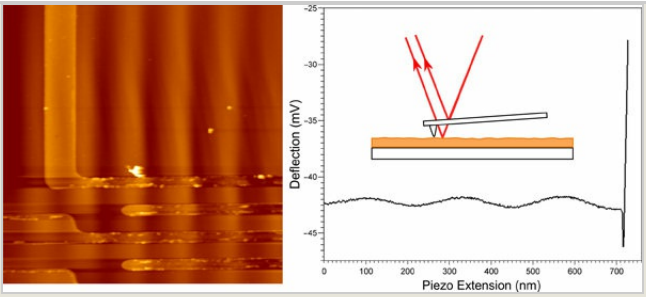

Fig. 6.20. Examples of the effect of laser interference on AFM images and force–distance curves. Left: an image of a reflective sample, showing typical laser interference fringes. Right: the effect on a force curve; the baseline shows similar oscillations. Inset: the artefact originates from interference between the laser beams reflected by the cantilever and the sample.

This effect is reduced in AFM instruments with low coherence lasers, which are fitted in newer instruments. It is also more common with patterned or reflective samples. If the user encounters this problem, it can sometimes be reduced by adjustment of the optical alignment of the AFM. The user should try to ensure the laser is positioned directly in the centre of the cantilever beam, and not too close to the end. See Section 4.2.1 for a laser spot positioning protocol.

6.6.4 Sample drift

A common problem in high-resolution microscopies is sample movement. In general, AFM samples must be well fixed down in order to enable high-resolution imaging. At low resolutions (scans of size larger than 5 μm), some samples do not need to be fixed to the microscope, provided they have a stable substrate. At smaller scan sizes, the sample should be glued to a sample support, which is held (usually magnetically) in the microscope. Even when firmly fixed down some samples can appear to be 'moving' in the microscope. The reason for this is thermal expansion of the sample; this can be exacerbated by sources of heat in the microscope (e.g. the laser or heat from the electronics), leading to samples moving by expansion at hundreds of nanometres per minute, which totally precludes high-resolution imaging. Some samples (e.g. metals) are more prone than others to this effect due to high thermal expansion coefficients.

As shown in Figure 6.21, scanning the sample with the slow scan axis in opposite directions can help to diagnose this problem. Another major problem associated with sample drift is that if the sample drifts in the *Z*-axis, it can prevent scanning altogether. This can be due to expansion in the *Z* axis or expansion laterally, which effectively moves the sample in *Z*, due to sample tilt. Although the feedback system can take account of small **(p.138)**

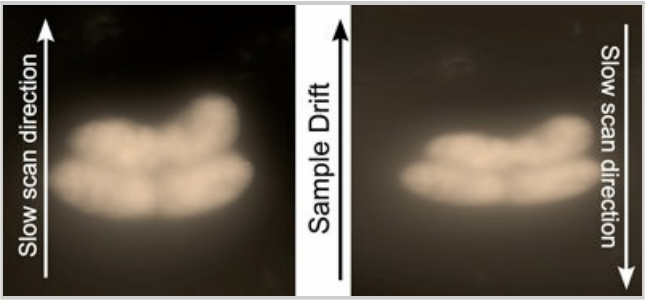

Fig. 6.21. Example of the effect of sample drift on AFM images. The two images of a cluster of *E. coli* bacteria were measured with the slow scan axis in opposite directions. The difference between them indicates that the sample was drifting while scanning. When the sample drifts in the same direction as the slow scan axis, the sample will appear stretched (image on the left); if it drifts in the opposite direction it will be compressed (right image). Scanning in two directions can help to determine the cause of image distortion.

drifts in Z, this effect will eventually cause problems in scanning due to the limited Z scan range of many scanners. If the user determines the sample is drifting, they should attempt to fix the sample down more firmly, and remove possible sources of heat, for example the white light used to illuminate the sample. Sometimes the only solution is to wait for thermal equilibrium.