The aim of this book is to demystify AFM. When you’ve read this book, you should understand how AFM works, including the main modes of operation, how to make measurements with an AFM, how to optimize your measurements, how to analyse your data, how to spot and to avoid problems with it, and you should have a good idea of what AFM is useful for.

This book was written so that the reader can dip in and out of the book, and that the chapters will be – more or less – readable independently, but the book will make most sense read from start to finish. Certainly if you know nothing about AFM yet, you will get the most out of this book if you read it all the way through, in the right order. But if you already know how the technique works and just want to analyse data, go straight to the chapter on image analysis; it will be perfectly readable without reading the prior sections. We assume no prior knowledge about AFM. This book is designed to be readable to someone with a freshman college-level of education, and an interest in AFM. On the other hand, some of the sections are highly detailed, and we expect that even experienced AFM operators will find a lot of useful information in them.

The first chapter introduces AFM, and places it in the context of the preceding techniques, as well as how it compares with other microscopy techniques. The second chapter describes how modern AFMs are built, and how they work. Even if you are an experienced AFM user there may well be details in Chapter 2 that you are not aware of. Knowledge of how the instrument works can greatly improve your use of it, and we hope that without going into too great technical details, this chapter has all the information an AFM user could need about how AFMs work, and importantly, why they work that way.

The third chapter then describes the major AFM modes in use. We discuss the way the modes work, and what information they can give, as well as the advantages and disadvantages of the different modes available. After describing the modes used to collect sample topography (i.e. imaging modes), modes used to get other information about the sample are described, for example how to use AFM to get thermal, magnetic, and mechanical information about a sample’s surface. AFM can also be used to record information about how individual molecules interact, and even how protein unfolding can be measured. All these modes are extensively referenced, and there are examples of each in the last chapter as well, showing a typical application of these modes in use.

In Chapter 4 we describe how to measure AFM images. If you have already measured images, you might be tempted to skip this chapter, but it may still be worth a look, because almost every user of AFM measures their images in a slightly different way, and you may well find some useful tips here. Particularly, we show examples of how you can use the information in the preceding chapters to understand why your images are good (or not so good). We show how to optimize scanning conditions, for the best resolution, image quality, and accuracy. This information should not be seen as the replacement for your instrument manual, but a complement to it. In combination with the other information in
this book, this chapter should help you to understand more deeply the process of scanning AFM images, so you can get better, more reproducible images.

Even the best data needs the right treatment to get the most useful information out of it, and Chapter 5 is all about how to process, display, and analyse AFM data. This chapter will be particularly useful if you have AFM data provided to you by an instrument operator which you did not collect yourself. Initially AFM analysis software can be very confusing, as there are so many different operations you can carry out, some of which may permanently alter your data. It is important to only apply those operations which are useful for your application, and avoid ‘over-processing’ of your data. This chapter will show how to maintain data integrity, and how to optimize and process the data for best effect.

Chapter 6 shows how to spot common artefacts in AFM images. Like all scientific measurements, AFM is prone to a number of artefacts, and unless you already know your sample very well, they can be quite tricky to spot. After years of usage this becomes second nature, and certain recurring artefacts will be obvious when they occur. But some rare artefacts can be easily missed, and new AFM users have little chance of knowing when an image has something wrong with it. The artefacts can come from the tip, from the environment, or be inherent in the technique itself. In this chapter, we give examples of the common image artefacts, and describe what you can do to avoid them.

It is obvious that AFM is not the solution to all scientific and technical problems; it does have some disadvantages, and sometimes other microscopy techniques are more appropriate for a particular problem. However, AFM has been applied with great success to an incredibly wide range of scientific and technological fields, and in the final chapter we present a range of applications that illustrate the breadth and depth of the uses of AFM.
Chapter 1

Introduction

Atomic force microscopy is an amazing technique that allows us to see and measure surface structure with unprecedented resolution and accuracy. An atomic force microscope (AFM) allows us, for example, to get images showing the arrangement of individual atoms in a sample, or to see the structure of individual molecules. By scanning in ultrahigh vacuum at cryogenic temperatures the hopping of individual atoms from a surface has been measured [1]. On the other hand, AFM does not need to be carried out under these extreme conditions, but can be carried out in physiological buffers at 37 °C to monitor biological reactions and even see them occur in real time [2–4]. Very small images only 5 nm in size, showing only 40–50 individual atoms, can be collected to measure the crystallographic structure of materials, or images of 100 micrometres or larger can be measured, showing the shapes of dozens of living cells at the same time [5–9]. AFM has a great advantage in that almost any sample can be imaged, be it very hard, such as the surface of a ceramic material, or a dispersion of metallic nanoparticles, or very soft, such as highly flexible polymers, human cells, or individual molecules of DNA. Furthermore, as well as its use as a microscope, which is to say as an imaging tool, AFM has various ‘spectroscopic’ modes, that measure other properties of the sample at the nanometre scale. Because of this, since its invention in the 1980s, AFM has come to be used in all fields of science, such as chemistry, biology, physics, materials science, nanotechnology, astronomy, medicine, and more. Government, academic and industrial labs all rely on AFM to deliver quantitative high-resolution images, with great flexibility in the samples that can be studied.

An AFM is rather different from other microscopes, because it does not form an image by focusing light or electrons onto a surface, like an optical or electron microscope. An AFM physically ‘feels’ the sample’s surface with a sharp probe, building up a map of the height of the sample’s surface. This is very different from an imaging microscope, which measures a two-dimensional projection of a sample’s surface. Such a two-dimensional image does not have any height information in it, so with a traditional microscope, we must infer such information from the image or rotate the sample to see feature heights. The data from an AFM must be treated to form an image of the sort we expect to see from a microscope. This sounds like a disadvantage, but the treatment is rather simple, and furthermore it’s very flexible, as having collected AFM height data we can generate images which look at the sample from any conceivable angle with simple analysis software. Moreover, the height data makes it very simple to quickly measure the height, length, width or volume of any feature in the image.

The fact that the AFM operates differently from most microscopes, and that the AFM probe physically interacts with the sample, means however that it is not as intuitive to use as optical microscopes. While most people understand the basic principles of light microscope use, i.e. focusing, illumination, depth of field, and so on, the use of AFM
has none of these concepts. There is nothing to focus, there’s no illumination of the sample, and zero depth of field, so operation of an AFM is rather different from many users’ expectations of a microscope. This means that both operation of and understanding the data from an AFM can be initially confusing. However, the principles, which will be explained in the following chapters, are really rather simple and having grasped these, both data analysis and acquisition will become much more intuitive. Like all scientific techniques, atomic force microscopy was a development of previously known methods, but is a technique which led to a revolution in microscopy. The development of AFM from these earlier techniques is discussed in the next section.

1.1 Background to AFM

As mentioned above, the AFM works by scanning a probe over the sample surface, building up a map of the height or topography of the surface as it goes along. It was not the first instrument to work in this way however. The predecessor of the AFM was the stylus profiler, which used a sharp tip on the end of a small bar, to which was dragged along the sample surface, and built up a map, or more often a linear plot, of sample height. An example of an early profiler is shown in Figure 1.1. This profiler, described by Shmalz in 1929, utilized an optical lever to monitor the motion of a sharp probe mounted at the end of a cantilever [10]. A magnified profile of the surface was generated by recording the motion of the stylus on photographic paper. This type of ‘microscope’ generated profile ‘images’ with a magnification of greater than 1000 ×.

A common problem with stylus profilers was the possible bending of the probe from collisions with surface features. Such ‘probe bending’ was a result of horizontal forces on the probe caused when the probe encountered large features on the surface. This problem was first addressed by Becker [11] in 1950. Becker suggested oscillating the probe from an
initial position above the surface to approach contact with the surface. Becker remarked that when using this vibrating profile method for measuring images, the detail of the images would depend on the sharpness of the probe. Stylus profilers are still in use today, and have developed considerably. However, fundamental problems with this sort of instrument persist, notably that the probe touches the surface in an uncontrolled way, which can lead to probe damage in the case of a hard sample, and sample damage in the case of a soft sample. Either of these problems would reduce the fidelity of the image obtained, as well as the resolution achievable.

In 1971 Russell Young demonstrated a non-contact type of stylus profiler [12]. In his profiler, called the topografiner, Young used the fact that the electron field emission current between a sharp metal probe and a surface is very dependent on the probe sample distance for electrically conductive samples. In the topografiner (shown in Figure 1.2), the probe was mounted directly on a piezoelectric ceramic element which was used to move the probe in a vertical direction \((z)\) above the surface. Further piezoelectric elements moved the probe in the other axes over the sample.

An electronic feedback circuit monitoring the electron emission was then used to drive the \(z\)-axis piezoelectric element and thus keep the probe–sample distance at a fixed value. Then, with the \(x\) and \(y\) piezoelectric ceramics, the probe was used to scan the surface in the horizontal \((X-Y)\) dimensions. By monitoring the \(X-Y\) and \(Z\) position of the probe, a 3-D image of the surface was constructed. The resolution of Young’s topografiner was limited by the instrument’s vibrations.

In 1981 Binnig and Rohrer, working at IBM, were able to improve the vibration isolation of an instrument similar to the topografiner such that they were able to monitor electron tunnelling instead of field emission between the tip and the sample. This instrument was the first scanning tunnelling microscope (STM) [13–15]. A schematic diagram of the STM is shown in Figure 1.3. The STM works by monitoring the tunnelling current and using the signal, via a feedback loop, to keep the STM tip (a sharp metal wire) very close to the sample surface while it is scanned over the surface in the \(X\) and \(Y\) axes in a
raster pattern. Like the topografiner, the movement of the tip over the surface in $x$, $y$ and $z$ is controlled with three piezoelectric elements (in Figure 1.3, the three elements are integrated together in a tube structure; this is discussed further in Chapter 2). The distance the $z$ piezo has to move up and down to maintain the tunneling current at the same value is equivalent to the sample height, so the computer can build up a map of sample height as the tip scans over the surface. The reason the instrument was so much more successful than the topografiner is that electron tunnelling is much more sensitive to tip–sample distance than field emissions, so the probe could be scanned very close to the surface. In fact, the probability of electron tunnelling is so strongly dependent on distance that effectively only the very last atom of the STM tip can undergo tunnelling. Because it is this last atom which is most sensitive to tunnelling from the surface, the structure of the tip far from the surface is not very important, so atomically sharp tips are easy to produce. For their very first experiments, Binnig and Rohrer levitated the entire instrument magnetically to counter vibrations; however later designs did not require this. The results of these early experiments were astounding; Binnig and Rohrer were able to see individual silicon atoms on a surface, [14, 16]. Without the STM, attaining this kind of resolution required a transmission electron microscope (TEM), which weighs thousands of kilograms, and fills a room. Furthermore, when the STM was invented, atomic structure could only be observed indirectly by diffraction patterns, while the STM could do it directly by imaging individual atoms. That the STM could do this when it was only a small instrument, suspended with springs to counter vibrations, seemed incredible, and Binnig and Rohrer later shared the Nobel Prize for physics in 1986 for the invention of the STM [17].

Although the STM was considered a fundamental advancement for scientific research, it had limited applications, because it worked only on electrically conductive samples. Despite these limits, STM remains a very useful technique, and is used widely in particular in physics and materials science to characterize the atomic structure of metals and semiconductors, and for fundamental studies of electronic effects at metal surfaces. Figure 1.4 shows an STM image, illustrating the atomic resolution routinely obtained in STM.
Despite the amazing results obtained with STM, the limitation to conducting samples led the inventors to immediately think about a new instrument that would be able to image insulating samples. In 1986 Binnig, Quate and Gerber published a paper entitled ‘Atomic Force Microscope’ [18, 19]. In that paper they described how they replaced the wire of a tunnelling probe from the STM with a lever made by carefully gluing a tiny diamond onto the end of a spring made of a thin strip of gold. This was the cantilever of the first AFM. Although the first instrument was used only for a few experiments, the results produced had such great impact that the first instrument now resides in the science museum in London. The movement of the cantilever was monitored by measuring the tunnelling current between the gold spring and a wire suspended above it. This set-up was highly sensitive to the movement of the probe as it scanned along the sample, again moved by piezoelectric elements. In their paper, Binnig et al. proposed that the AFM could be improved by vibrating the cantilever above the surface [20]. Thankfully nowadays we don’t have to glue tiny diamonds onto gold levers to carry out AFM, but this first instrument led to the whole field of AFM. The instrument, and the first image recorded in AFM, are shown in Figure 1.5.

The AFM caused a revolution. Suddenly, with a relatively cheap and simple instrument, extremely high-resolution images of nearly any sample were possible. While initial images, such as that shown in Figure 1.5, did not have as high resolution as STM, atomic-resolution images were soon reported [21]. Soon after the invention of the AFM, the gold leaf/diamond combination was replaced by much more reproducible cantilever manufacture by silicon lithography, which enables the production of more than 400 cantilevers on a single 7-inch wafer [22]. Furthermore, it was quickly realized that simpler methods than the STM could be used to detect the motion of the cantilever. Nowadays, most AFMs use a light lever to sensitively detect the motion of the cantilever, this method is considerably simpler than the STM set-up, allows for larger cantilever motions, and is
still sensitive to sub-angstrom motions of the cantilever [23, 24]. Furthermore, as suggested by Binnig et al., oscillating modes have further increased the range of samples that AFMs can scan, and reduced the chance of sample damage as well.

Due to the high interest in AFM, commercial instruments were soon being produced, the first available from 1988. Together, AFM and STM are often referred to as scanning probe microscopy, or SPM. A further explanation of terminology in the SPM field is given in Chapter 3. Since AFM and STM instruments share several components in common, it is relatively simple to build an instrument capable of carrying out both kinds of microscopy. Since together they are referred to as SPM, and because some instruments perform both STM and AFM, the techniques are often seen as being very similar. However, since its development, AFM has been modified to measure a huge number of different properties, and perform lots of additional (non-imaging experiments), and combined with the techniques’ greater flexibility in terms of types of samples scanned, means AFM is today much more widely used than STM. This book concentrates on AFM, and will not discuss STM further. For the reader interested in further details of STM, the works [25, 26] are recommended.

### 1.2 AFM today

The AFM can be compared to traditional microscopes such as the optical or scanning electron microscopes for measuring dimensions in the horizontal axis. However, it can also be compared to mechanical profilers for making measurements in the vertical axis to a surface. One of the great advantages of the AFM is the ability to magnify in the X, Y and Z axes. Figure 1.6 shows a comparison between several types of microscopes and profilometers. As shown in Figure 1.6, one of the limiting characteristics of the AFM is that it is
not practical to make measurements on areas greater than about 100 μm. This is because the AFM requires mechanically scanning the probe over a surface, and scanning such large areas would generally mean scanning very slowly. Exceptions to this include parallel AFM that measure small areas but with many probes to build up a large dataset, or ‘fast-scanning’ AFMs, which are discussed in Chapter 2.

When compared to a profiler, the AFM has a greater $X-Y$ resolution because in the AFM the probe is sharper. The fine control of probe–surface forces enabled by this feedback mechanism enables the use of lower loading forces, which allows the use of much sharper probes, resulting in much higher $X-Y$ resolution. The difference in applied force is very high, whileprofilometers will typically apply $ca. 10^{-6}$ N to the surface, AFMs can image with $10^{-9}$ N or less. Profilers can have high vertical resolutions, as low as 0.5 Å. However, much greater bandwidth in the AFM experiments means that practically, the AFM height resolution is far greater than that of the profilometers. This is because the bandwidth limits on profilometers mean that to achieve high height resolution scanning must occur very slowly.

The length-scale of an optical microscope overlaps nicely with an AFM. Thus, an AFM is often combined with an optical microscope and with this combination it is possible to have a combined field of view with a dynamic range from mm to nm. In practice, a simplified optical microscope, known as an inspection scope, is usually used for selecting the location for AFM scanning. However, a combination of high-resolution optical microscopes, often with fluorescence microscopy integration, with AFM also has great advantages, especially in biology. This is discussed further in Chapter 2 and in Section 7.3. The combination of AFM with other microscopes or instruments is made simple by the AFM’s small size.

The AFM is most often compared with the electron beam techniques such as the Scanning Electron Microscope (SEM) or Transmission Electron Microscope (TEM). As may be seen in Figure 1.6, the dimensional range of these techniques is rather similar, with SEM (usually) having a somewhat lower resolution to AFM, while the ultimate resolution of TEM is quite similar to that of AFM. Table 1.1 contains a list of some of the major factors in comparison of AFM with SEM and TEM.

In general, it is easier to learn to use an AFM than an electron microscope because there is minimal sample preparation required with an AFM, and nearly any sample can be
measured. With an AFM, if the probe is good, a good image is measured. Because TEM and SEM usually operate in a vacuum, and require a conductive sample (so non-conductive samples are usually coated with a metallic layer before imaging), AFM has the advantage of being able to image the sample with no prior treatment, in an ambient atmosphere. This makes scanning quicker, and can also mean fewer artefacts are introduced by the vacuum drying, or the coating procedure. On the other hand, AFM image recording is usually slower than an SEM, so if a large number of features on one sample are required, AFM may be considerably slower than SEM for the same sample.

As we will see in the following chapters, AFM can be used for much more than measuring images, however. One of the unique advantages of SPM techniques is the highly accurate positioning of the probe on or close to the sample surface. This has become an enabling technology for the measurement and manipulation of samples on the nanoscale. AFM’s other key advantages are its very high sensitivity, and the fact that the smaller the instrument, the more sensitive it can be. This is the opposite of all previous tools, and means that AFM integration with other techniques is very simple.

## Table 1.1. Comparison of AFM with SEM and TEM.

<table>
<thead>
<tr>
<th>Sample preparation</th>
<th>AFM</th>
<th>SEM</th>
<th>TEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample preparation</td>
<td>little or none</td>
<td>from little to a lot</td>
<td>from little to a lot</td>
</tr>
<tr>
<td>Resolution</td>
<td>0.1 nm</td>
<td>5 nm</td>
<td>0.1 nm</td>
</tr>
<tr>
<td>Relative cost</td>
<td>low</td>
<td>medium</td>
<td>high</td>
</tr>
<tr>
<td>Sample environment</td>
<td>any</td>
<td>vacuum(SEM) or gas (environmental SEM)</td>
<td>vacuum</td>
</tr>
<tr>
<td>Depth of field</td>
<td>poor</td>
<td>good</td>
<td>poor</td>
</tr>
<tr>
<td>Sample type</td>
<td>Conductive or insulating</td>
<td>conductive</td>
<td>conductive</td>
</tr>
<tr>
<td>Time for image</td>
<td>2–5 minutes</td>
<td>0.1–1 minute</td>
<td>0.1–1 minute</td>
</tr>
<tr>
<td>Maximum field of view</td>
<td>100 μm</td>
<td>1 mm</td>
<td>100 nm</td>
</tr>
<tr>
<td>Maximum sample size</td>
<td>unlimited</td>
<td>30 mm</td>
<td>2 mm</td>
</tr>
<tr>
<td>Measurements</td>
<td>3 dimensional</td>
<td>2 dimensional</td>
<td>2 dimensional</td>
</tr>
</tbody>
</table>