

CHAPTER 1

AN INTRODUCTION

The atomic force microscope (AFM) is perhaps the most versatile member of a family of microscopes known as scanning probe microscopes (SPMs). These instruments generate images by 'feeling' rather than 'looking' at specimens. This novel mode of imaging results in a magnification range spanning that associated with both the light and the electron microscope, but under the 'natural' imaging conditions normally associated with just the light microscope. The potential to image biological systems in real time, under natural conditions, with molecular, or even submolecular resolution is clearly of interest to biologists. Thus, since their inception, SPMs have had an obvious appeal to biologists and biophysicists. Since these first studies in the early 1980s publications describing biological applications of SPM have grown extremely rapidly. One of the aims of this book is to look at what impact these techniques have made in the biological sciences, and to try to assess their future potential.

Scanning probe microscopy began in the early 1980s when Binnig and Rohrer revolutionised microscopy through the invention of the scanning tunnelling microscope (STM). The importance of this discovery was recognised through the award of the Nobel prize in Physics in 1986. The STM is the first of this large and growing family of probe microscopes, which sense the structure of a surface by scanning it with a sharp probe and measuring some form of interaction between the surface and the probe. The development of the STM arose from an interest in the study of the electrical properties of thin insulating layers. This led to an apparatus in which the probe-surface separation was monitored by measuring electron tunnelling between a conducting surface and a conducting probe. A few years later Binnig and colleagues (1986) announced the birth of the second member of the SPM family - the atomic force microscope, also known as the scanning force microscope (SFM). In the late 1980s commercial STMs became available. Commercial AFMs began to appear in the early 1990s and have evolved through several generations. Refinements and new types of SPMs have appeared and will undoubtedly continue to be developed in the future. Particular developments of importance in biological research are combinations of probe microscopes with light or electron microscopes, cryo AFMs, scanning ion conductance microscopes (SICM) and scanning near field optical microscopes (SNOM).

SPMs are not strictly microscopes: they visualise surfaces by 'feeling' or sensing them with a sharp probe. Conventional (far field) microscopes image by collecting radiation transmitted through, or reflected from the sample. The ultimate resolution is diffraction limited and depends on the wavelength of the radiation.

2 Chapter 1

Thus light microscopes are limited to a resolution of ≈ 200 nm. Higher resolution images of biological materials can be obtained using high energy electrons in the electron microscope (EM). Despite recent advances in the development of environmental EMs molecular resolution still requires that specimens are examined under vacuum or partial vacuum. Electron microscopists have developed many elegant preparative methods to preserve the 'native' structure of biological materials. SPMs image by a different mechanism (Fig. 1.1) and different criteria determine their resolving power.

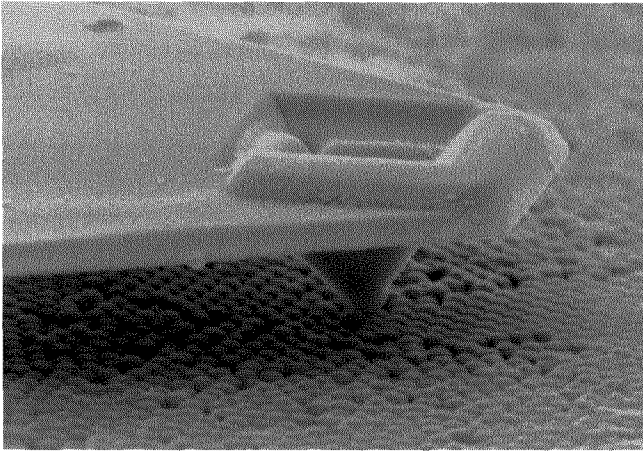


Figure 1.1 Scanning electron microscope image of an AFM tip used to probe the structure of the sample surface. Magnification approximately $\times 10,000$. The probe 'feels' the sample surface. Image courtesy of Paul Gunning.

In SPM images are obtained by measuring changes in the magnitude of the interaction between the probe and the specimen surface as the surface is scanned beneath the probe. Hence the resolution will depend on the sharpness, or apparent sharpness of the probe tip, and the accuracy with which the sample can be positioned relative to the probe. SPMs are capable of 'atomic' resolution on flat surfaces and such resolution can be achieved in gaseous or liquid environments. For macromolecules atomic resolution is only possible for simple molecules in which each atom is in intimate contact with the surface of a flat substrate. However SPMs do allow sub-molecular resolution on most biopolymers under 'natural' conditions. Thus the attractive potential of SPMs for biologists is the ability to visualise molecular processes under natural or physiological conditions. They offer the resolution of most commercial electron microscopes but under the experimental conditions familiar to the light microscopist.

The first biological studies were made using STM. The tunnelling current decays exponentially with increasing separation between the surface and the probe. A change in probe-sample separation of atomic dimensions will lead to an order of magnitude change in tunnelling current. This means that tunnelling effectively occurs from the atom on the tip nearest the surface, and the probes behave as if they are atomically sharp. Thus STMs offer the highest resolution obtainable by SPM. However, the rapid decay in the tunnelling current basically restricts investigations to the study of thin interfaces, or individual biopolymers. For larger biological systems the probe-sample separation would become too large and any tunnelling current would be expected to be too small to detect. Furthermore, the sample surface needs to be conducting and this usually means coating the biological sample, offsetting the main advantages of the SPM method. With the AFM there are no such restrictions on the size of the specimen that can be examined, and biological samples ranging in size from individual molecules to cells or tissues can be, and have been, imaged in their native state. AFMs, and refinements such as cryo-AFM, have become the preferred SPM methods in biology. It was originally believed that SPMs were non-invasive techniques. In practice sample damage and displacement plagued the early uses of STM and AFM. Understanding and overcoming these problems has led to reliable and reproducible methods of imaging. Emphasis has passed from validation of the microscopes to their use to study biological problems.

Other types of SPM have and will continue to become important in biology. A likely candidate, now available commercially, is the scanning ion conductance microscope (SICM). The basic principles of SICM and appropriate biological applications will be discussed. There is a growing appreciation of the value of combining AFM with conventional optical or confocal microscopes, or of using them in conjunction with surface techniques such as surface plasmon resonance, and the basis of some of these combined microscopes will also be described. The early generation of combined microscopes suffered in resolution when compared to stand alone AFMs. However, the latest generation of combined instrument offer the resolving power of a stand alone AFM with the versatility of combined optical microscope for locating and characterizing biological samples. It is still difficult to obtain high-resolution images on soft biological samples such as cells, and the newer technique of scanning ion conductance microscopy and advances in both far-field and near-field optical microscopy are worthy competitors. As probe microscopy has matured the emphasis has moved from just obtaining images to a more general use of the technique to solve problems in biology. Increasingly probe microscopes are seen as tool kits for studying biological systems: applications range from generalized mapping of parameters such as elasticity, friction or charge, specialized affinity mapping of surfaces and

the use of probe microscopes to modify and manipulate biological systems. Increasingly the ability to measure forces between molecules at the single molecule level under natural conditions is rivalling imaging applications of AFM: applications range from the characterization of individual molecules, studies of molecular interactions and their roles in friction and adhesion at both molecular and cellular levels. This book will concentrate on biological applications of AFM. The advantages and limitations of the technique will be assessed. The literature in this area is vast and increasing rapidly. Thus it is not possible to reference all of the published papers on a particular topic. Rather we have tried to cite books, recent reviews and selected research papers. The papers have been chosen to emphasise a point, or to provide a route to the literature in this area. The choice is not meant to imply priority and omission of papers is purely the result of the limitations of space and time and is not necessarily a reflection on the quality of the publication.

What do we wish to achieve in writing this book? One aim is to introduce the AFM, to describe the type of apparatus available and how it is used. A second aim is to look at the types of biological samples which have been studied, to look at how successful these studies have been, and to assess whether the use of AFM has generated new knowledge or understanding in these areas. In general terms we wish to look at what can and what cannot be done, if it is possible to do it then how is it done, and at what has been done and where things may go in the future. Who do we hope will benefit from reading this book? We hope that the book will provide a good resource base for literature on biological applications of AFM. The information presented should allow the reader to critically evaluate published present and future AFM data on biological systems, to decide whether AFM is likely to be useful in their areas of interest and, for new recruits to AFM, provide a basis for deciding what sort of technique to invest in, its inherent limitations, how to avoid and recognize artifacts and thus how to optimise its use to solve problems.