Biological Applications of Scanning Probe Microscopy

Biological Applications of Scanning Probe Microscopy. Scanning probe microscopes (SPMs) have now become established as the foremost tools in the imaging of surfaces and surface-confined structures at up to angstrom resolution. Whilst spectroscopic methods such as Raman, ellipsometry and surface plasmon resonance continue to provide useful information on interfaces, they remain tools of restricted spatial resolution and are accordingly limited to measurements of bulk averages. In contrast, proximal probe methods offer unrivalled imaging of surfaces and with it the ability to address individual molecular species. As scanning probe technology has developed there has been significant progress in the analysis of biological samples, varying in scale from individual biological polymers to complex cellular systems. More recently, the application of scanning probe microscopes has developed beyond imaging into areas such as nanoscale electrical measurement and direct molecular delivery and manipulation.

Atomic Force Microscopy
The atomic force microscope (AFM) is the most commonly used of the scanning probe family, due in part to its ease of use and versatility. The AFM produces a three dimensional image of a surface by scanning an atomically sharp tip, typically made from etched silicon, across a surface. The tip is attached to a cantilever which deflects as the tip experiences forces (both repulsive and attractive) induced by the topography of the surface. The cantilever deflection is recorded by means of a laser beam reflected off the back of the cantilever. Typically, laser movements of less than 0.5 Å can be detected and translated into extremely precise height measurements. Image resolution in AFM is dependent on the contact area between tip and sample (tens to hundreds of atoms). In turn, this contact area is dependent on the sharpness of the point, quoted as a radius and typically 1–10 nm for commercially available tips. Efforts have been made to produce sharper tips by, for example, attaching carbon nanotubes to the apex [1]. Alternative approaches to image quality enhancement include a number of computational deconvolution procedures, where information about the shape of the tip can be extracted from the raw data and used to generate a more accurate picture of the sample [2].
Scanning Tunnelling Microscopy

The scanning tunnelling microscope (STM) was the original proximal probe imaging method and earned its inventors, Binnig and Rohrer, the Nobel Prize for Physics in 1986.

An STM works by applying a voltage (typically several hundred mV) between the probe (for example fine platinum wire) and the substrate surface. These two electrodes are then brought carefully together until, at a sub nanometre separation, an electron tunnelling current can be recorded between them. The tip and sample are then scanned precisely relative to one another and the current is measured as a function of position. STMs offer – in theory – a higher resolution than AFMs due to the lower tip contact area with the sample. However, since the tip-sample position is controlled only by means of the tunnelling current, there is the risk that electrically insulating materials (for example bio-macromolecules with typical resistivities of 1015–1018 Ωm) will be crushed and deformed as the microscope obtains the set-point current. The means by which biomolecules in particular are imaged by STM is the subject of some debate. In normal operating conditions, electrons are assumed to tunnel from tip to substrate or vice versa and not accumulate on the molecules in the gap or interact significantly with molecular states. However, since direct tunnelling through an adsorbed protein would typically involve distances of 30–50 Å, this poses theoretical problems. It has been suggested that adsorbate induced modulations in surface work function and/or density of states have a part to play, but there remains no single mechanism to explain the image contrast produced.

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Both AFM and STM offer a considerable advantage over electron microscopy in the imaging of biological samples in that they can be performed under solution and in an environment closely approximating physiological conditions. This contrasts with diffraction and spectroscopic techniques (including NMR) where molecular
characteristics are recorded as gross averages. A further development of the AFM in the imaging of labile biological samples is the use of an intermittent contact mode. Lateral shear forces are dramatically reduced by “tapping” the surface with the tip at high frequency such that the tip makes only transient contact with the sample at the bottom of each oscillation. The oscillation amplitude changes as energy is dissipated between the tip and the surface as they contact and can be used as a feedback signal to generate topographic images. Additionally, oscillation phase changes, as induced by adhesion, friction and the viscoelastic properties of the sample can be recorded and used in image formation. With AFM it has become possible to monitor dynamic processes as they occur in real time, in near physiological conditions, and crucially this can be done at a truly molecular level. With its clear applicability to biological imaging, the AFM has become an important tool for researchers in medical fields with countless examples of its use. For instance, the environmentally stressed aggregation of native protein into fibrils (and ultimately amyloid plaques) can be monitored (Fig. 1). Additionally, the AFM can successfully image cellular systems and reveal some internal cell structure in addition to surface features (Fig 2). In order to image with STM in solution, and prevent the small tunnelling currents being swamped by capacitative ionic flow, the probe needs to be effectively insulated, often achieved by immersion into molten wax. Despite the technical difficulties involved, it is possible to obtain 3D tunnelling profiles of even small biomolecules at molecular or submolecular resolution under water [3–5] as illustrated in Fig. 3.

**Further Applications of Scanning Probe Methods**

Complimentary to the high resolution images obtainable with tunnelling junctions is the use of a conductive-probe AFM (CAFM). Here, a metal coated AFM tip is held in mechanical contact with the sample. In contrast to an STM experiment, where the location of the tunnelling tip with respect to the molecule of interest is essentially unknown, with C-AFM the position of the tip and the force it exerts on the sample can be controlled. This technique (Fig. 4) has allowed the direct measurement of the electronic properties of a metalloprotein under controllable mechanical modulation [6]. In recent years the AFM has developed additional applications including its use as a nanolithographic device, either by etching a surface or by the delivery of molecules via a water meniscus formed at the tip-surface interface [7]. Advances continue to be made in SPM techniques, but the future perhaps lies in the combination of topographic/electronic structure imaging with simultaneous spectroscopic measurement such that data sets can be obtained, not only on the same system, but on the same molecule. Optical microscopy, including the latest confocal and internal reflection fluorescence methods, have been applied to biological and biomolecular analyses with great success. Though molecular scale characterization with these methods remains difficult, it is now possible to perform
them in situ with AFM using combined apparatus. This has allowed the simultaneous measurement of topology and function via fluorescent markers over nanometre scale areas [8].

SPM methods have developed from the production of high resolution images of surfaces to now become experimental tools in their own right. In understanding and harnessing the means by which the image is produced (tunnelling current in the case of STM and nano-newton scale force with AFM), it is possible to perform a myriad of novel and exciting experiments. This, combined with their unparalleled spatial resolution, will ensure that SPMs will remain at the forefront of work across a great many scientific fields.

References:

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Jason Davis studied Chemistry at Kings College London, where he was awarded numerous prizes and a first class honours degree in 1993. He moved to the Inorganic Chemistry Laboratory at the University of Oxford and, after obtaining a PhD with work on carbon nanotubes, electroanalysis and scanning probe microscopy, he was elected to an Extraordinary Junior Research Fellowship at The Queens College in 1998. A Royal Society University Research Fellowship followed in 1999 and a Lectureship in Chemistry at Jesus College, Oxford, in 2001. He was made a University Lecturer and Official Student and Tutor in Chemistry at Christ Church in 2003. His work has focused on the molecular and nanometre-scale construction and analysis of bioinorganic, sensory, electronic and optical systems. He has published over 50 papers on carbon nanotubes, nanoparticles, biological and molecular sensing, biomolecular electronics and nanotechnology.

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