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Minireview

Feeling the forces: atomic force microscopy in cell biology

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This paper is dedicated to Souzan Nicholson on her 21st birthday

Abstract

Atomic force microscopy allows three-dimensional imaging and measurements of unstained and uncoated biological samples in air or fluid. Using this technology it offers resolution on the nanometer scale and detection of temporal changes in the mechanical properties, i.e. surface stiffness or elasticity in live cells and membranes. Various biological processes including ligand-receptor interactions, reorganization, and restructuring of the cytoskeleton associated with cell motility that are governed by intermolecular forces and their mode of detection will be discussed.

Keywords: Atomic force microscopy; Intramolecular forces; Intermolecular forces; Molecular forces; Cellular forces

Introduction

The atomic force microscope (AFM), first described by Binnig et al. [3], has become a powerful tool in biology that can provide three-dimensional images of surface topography of biological specimens in ambient liquid or gas environments. Unlike other techniques, atomic force microscopy can use samples with just minor preparation, e.g. staining, coating etc., over a large range of temperatures and in repetitive studies. The high resolution (in the nanometer range) allows topographical imaging of samples such as DNA molecules [26], protein adsorption or crystal growth [41], and living cells adsorbed on biomaterials [4]. In addition to topographical measurements, AFM is also capable of complementary techniques that provide information on other surface properties, e.g. stiffness, hardness, friction, or elasticity.

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In this minireview we first introduce the principle of AFM and then describe its various application forms before discussing some specific measurements in more detail.

Principle of AFM and imaging methods

Principle of operation

The key element of the AFM is the cantilever (Fig. 1). It consists of one or more beams of silicon or silicon nitride of 100–500 μm in length and 0.5–5 μm in thickness. At the end of the cantilever a sharp tip is mounted to sense the force between the sample and tip. For normal topographic imaging, the tip is brought into continuous or intermittent contact with the sample as it raster-scans over the surface. An optical system is then used to measure the changes of the laser beam reflected from the gold-coated back of the cantilever onto a position-sensitive photodiode (PSPD), which can measure changes in the position of the incident laser as small as 1 nm. The instrument is available in several operating modes that can be chosen depending on the sample, environment, and measurements required. For more detailed reading refer to Schoenenberger et al. [38].

Contact mode

The contact mode is the original AFM imaging mode, which can be implemented in both air and fluid. The AFM probe, end-mounted on a flexible cantilever, is brought into 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 an electronic feedback circuit. Topographic

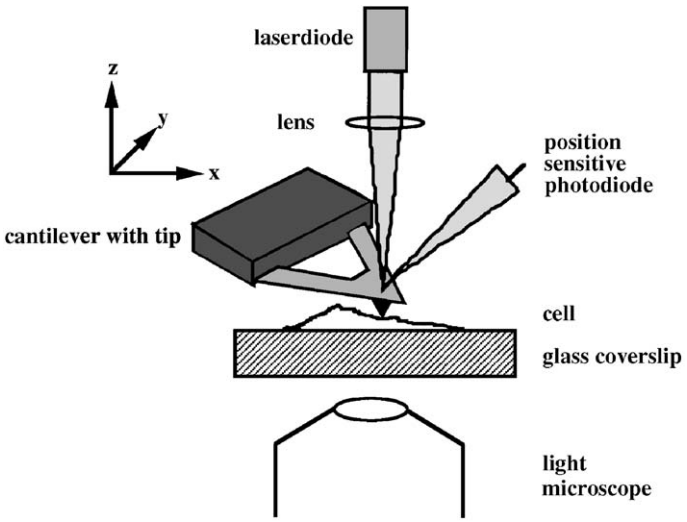

Fig. 1. A schematic representation of an atomic force microscope. The tip is mounted on a soft cantilever spring and the deflection of the cantilever is detected by an optical lever using a laser beam. The cantilever can be positioned in (x, y, z) direction. A light microscope is used for positioning the tip on the sample.

images are generated by mapping the vertical distance the scanner moves as it maintains a constant deflection at every lateral data point. Probe tracking forces—held consistently below 100pN—have been made possible on biological specimens in fluid using this technique.

Tapping mode

The tapping mode is a more recent development in which the imaging probe is vertically oscillated at or near the resonant frequency of the integrated cantilever. An electronic feedback circuit maintains the oscillation at a constant amplitude during scanning. The image is produced by mapping the vertical distance the scanner moves as it maintains a constant oscillation amplitude at each lateral data point. The key advantage of the tapping mode is the elimination of the lateral shear forces present in contact mode, which, for many specimens, can damage the structure being imaged. Tapping mode AFM can be conducted in air or fluid.

Phase imaging

Phase imaging is relatively new and has the advantage of being able to be performed at the same time as topographic imaging with tapping mode, i.e. both topographic and phase images can be obtained in a single scan. Because the interactions between the tip and the surface depend not only on the topography of the sample but also on other characteristics (such as hardness, elasticity, adhesion, or friction), the movements of the cantilever to which the tip is attached depend also on these properties. In phase imaging, the phase of the sinusoidal oscillation of the cantilever is measured relative to the driving signal applied to the cantilever to cause the oscillation. Phase images are produced by recording this phase shift during the tapping mode scan. Phase imaging can detect, for example, different components in polymers related to their stiffness [27] or areas of different hydrophobicity in hydrogels immersed in saline solutions.

Force mode

The atomic force microscope can also probe elastic properties or adhesion on a surface by generating force curves. These curves are generated by performing controlled vertical tip-sample interactions, without lateral scanning movement and while recording the cantilever's deflections. Force curves measure nanonewton-range vertical forces applied to the surface, and allow the estimation of the nanomechanical properties of the samples, e.g. the elasticity of living cells [1]. The ability to coat the tip with different molecules (proteins, lipids) has increased the utility of force curves in understanding the specific attraction between a ligand and its receptor [9,40]. This technique can also be used to measure charge densities on surfaces [18], to estimate the folding force of biomolecules like titin [34], and to measure forces associated with polymer elongation [35]. Fig. 2 shows the (elastic) Young's modulus for a wide range of materials.

Other force microscope techniques

Lateral force microscopy (LFM) is useful for mapping frictional properties on surfaces. In this mode, the torsional forces applied to the cantilever are monitored during contact mode scanning. These forces are related to the friction on the surface, and the LFM data can be recorded simultaneously with contact mode topographical data. In force modulation mode, a small vertical oscillating movement is applied to

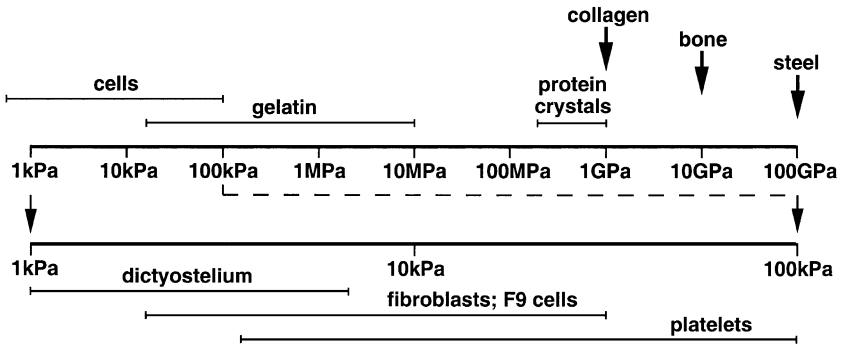

Fig. 2. Young's (elastic) moduli of different materials. The diagram shows a spectrum from very hard to very soft: steel > bone > collagen > protein crystals > gelatin, rubber > cells.

the tip while in contact with the sample. This causes the cantilever to bend, and the measure of the bending amplitude reveals the stiffness or elasticity of the surface, since a stiff area induces a larger cantilever deflection than does a soft area. In nanoindentation a diamond tip is used to indent the surface at a known force. By measuring the depths of indentations, it is possible to evaluate the relative hardness of materials. For more detailed reading, refer to Cappella and Dietler [5].

Imaging of biological samples

The unique capability of AFM to study the dynamic behavior of living and fixed cells has opened up new fields in cell biology. Recent examples of dynamic in vitro analysis demonstrate some of the unique capabilities that AFM provides for cellular analysis. For example, plasma membrane in migrating epithelial cells has been imaged in real time. The dynamic membrane invagination process was observed in the presence of calcium, and when calcium levels were reduced, the process was prevented. Lipidic pore formation of 30 nm could also be resolved during calcium reduction performed on living renal epithelial cells. Cytoskeletal dimples in the plasma membrane and membrane-bound filaments were resolved to fifty nanometers on small patches of plasma membrane [30,31]. More recently, Chasan et al. [6] showed, using AFM to study CFTR-containing liposomes in solution deposited on freshly cleaved mica, that these cystic fibrosis transmembrane conductance regulators (CFTR) directly interact with the actin cytoskeleton.

Another group used AFM to observe platelet activation, showing microfilament structure, granula transport towards the cell cortex, and the redistribution of cellular components during activation [11]. In addition, cell membranes can be labeled with immunogold, opening the door to high-resolution mapping of cell surface antigens [29]. Many of these studies have taken advantage of existing protocols to immobilize samples on suitable substrates such as direct mounting to glass coverslips or polylysine-coated glass. This provides a uniquely simplified environment for cellular imaging free from stains and fixatives.

Other techniques have been developed by taking advantage of the atomic force microscope's ability to micromanipulate different materials. By increasing the scanning force on isolated patches of rat liver membranes, the upper membrane layer can be stripped away from the extracellular surface's hexagonal arrays of gap junctions. These studies were performed in situ under near-physiologic conditions. The surface structure was resolved down to three nanometers with the membranes fully submerged in

phosphate buffered saline (PBS) [20,21]. Results such as these demonstrate just some of AFM's unique capabilities to provide information on cellular structure and organization. Goldmann and Ezzell [14] and Goldmann et al. [15,16], studied the elasticity of both wild-type F9 mouse embryonic carcinoma and vinculin-deficient F9 cell lines using AFM, and rheologic as well as cell indentation techniques. The indentation of the cells by scanning AFM was used to produce a viscoleastic map of the two cell types (Fig. 3). Transfecting the vinculin-deficient cells expressing the head or tail or the head and tail region of vinculin separately showed that only intact vinculin restores the viscoelastic properties [17].

There has been recent success imaging individual proteins and other small molecules using AFM. Rigid fibrils, such as collagen, can easily be imaged by the microscope. The periodic 70 nm banding pattern and 30 nm subbands are clearly resolved [13]. Smaller molecules that do not have a high affinity for common AFM substrates have been successfully imaged by employing selective affinity-binding procedures. Thiol incorporation at both the 5′ and 3′ ends of short PCR products has been shown to confer a high affinity for ultra-flat gold substrates [19]. Another example of using AFM to image the cell surface is a similar approach that was used to immobilize antibodies (IgG) on treated mica. In this case, the low affinity that IgG molecules have for mica was overcome by cloning a metal-chelating peptide into the carboxy-terminus sequence of the IgG's heavy chain. The recombinant sequence was transfected into cells that expressed the complementary light chain. The purified IgG containing the metal-chelating peptide was shown to bind in a region-specific manner to nickel-treated mica [23]. Covalent binding of biological structures to derivatized glass substrates has enabled high-resolution imaging of some samples that are not stable on untreated glass substrates. Bacteriophage T4 polyheads do not seem to adhere tightly to glass coverslips. However, after derivatization with a photoreactive cross-linker, the tubular polyheads, and the hexagonal capsomere can be resolved [24]. In another example, the lattice structure of the HPI layer of *D. radiodurans* can be clearly imaged using this technique. The atomic force microscope's ability to provide accurate height measurements demonstrated that the native HPI layer was preserved with close correlation to freeze-dried unstained HPI layer data generated by electron microscopy. In this study the HPI layer is imaged in buffer with 1 nm lateral resolution and 0.1 nm vertical resolution [25]. New approaches such as these have provided a solid foundation from which research is expanding into more complexity. Higher-resolution imaging of a variety of small molecules continues to improve at a rapid pace.

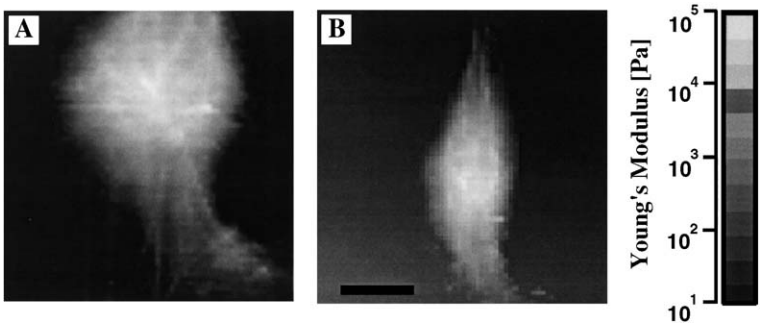

Fig. 3. Elasticity map of a wild-type F9 cell (A) and a vinculin-deficient F9 cell (B). The local elastic or Young's modulus is obtained by taking 64 × 64 force curves while raster-scanning over the cell surface and subsequently analyzing each individual force scan. The vertical gray scale bar indicates the local differences in elastic moduli in (Pa). The overall elastic difference between these species was calculated at ~ 20% [14]. The horizontal black bar equals 10 μm.

In the last few years, AFM has opened even more exciting new avenues in biology and biophysics for probing cells. Imaging the surface topography at (sub) nanometer resolution as well as measuring local physical properties by pulling on macromolecules under physiological conditions has provided new insight into the nano-mechanical properties of e.g. titin, cadherin, ATP synthase, single DNA, elastin [2,7,8,36,39]. Reproducible images of native OmpF porin at ~ 0.5 nm lateral and ~ 0.1 nm vertical resolution—at variable electrolytes to detect the electrostatic potential—could be directly correlated with various structural and functional states [28,33]. Imaging and nano-manipulation by AFM has also included the disruption of antibody-antigen bonds, the dissection of biological membranes, the nano-dissection of protein complexes, as well as the controlled modulation of protein conformations [12]. Recently, Oesterhelt et al. [32] combined AFM with single molecule force spectroscopy and described the unfolding pathway of individual bacteriorhodopsin molecules, and Scheuring et al. [37] generated images of single native membrane proteins at (sub) nanometer resolution.

Future directions

While new techniques for conventional AFM continue to evolve, scientists are investigating the atomic force microscope's ability to measure force in the nanonewton range in order to quantify molecular binding interactions. Using the well-characterized interaction of biotin and streptavidin, AFM is able to differentiate between the binding forces of biotin, desthiobiotin, and iminobiotin [10]. Binding forces can also be quantified when a small number of molecular interactions are taking place, providing piconewton binding measurements for individual molecular pairs [22]. This could have broad applications to a variety of important ligand-receptor interactions. There are many exciting possibilities for scanning probe microscopy and there is no other tool that allows such convenience and flexibility at this level of resolution. Regardless of scientific goals, instruments such as the nanoscope will open the door to a whole new world of molecular science.

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