

BME2102: Introduction to Biomechanics

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Review: The Buoyant Force



- $F_B = V_d \gamma$
 - V_d = Volume displacement (m^3)
 - γ = Specific Weight of Fluid (N/m^3)

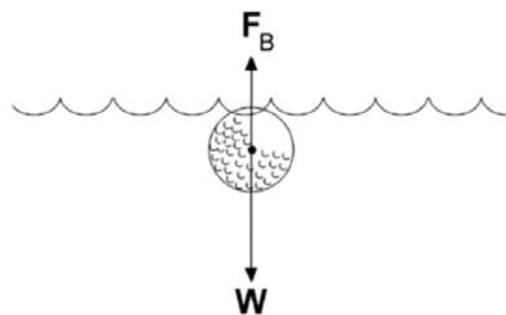


Figure 8.1. The resultant vector of gravity (W) and buoyancy (F_B) will determine if an inanimate object floats. This golf ball will sink to the bottom of the water hazard.

Review: Drag Force

$$F_D = \frac{1}{2} C_D \rho A v^2$$

where

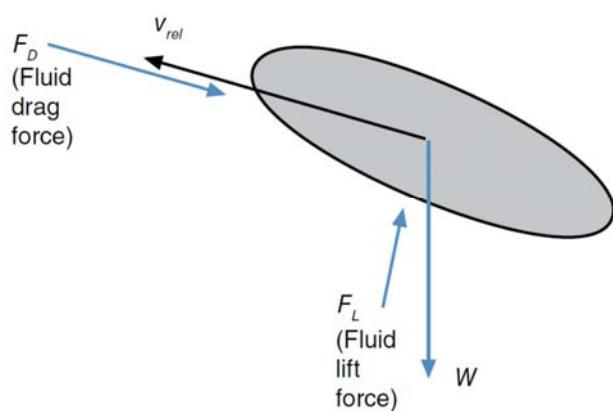
F_D = drag force,

C_D = coefficient of drag,

ρ = fluid density,

A = reference area (usually the cross-sectional area of the object perpendicular to the relative velocity), and

v = relative velocity of the object with respect to the fluid.



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Review: Drag Force

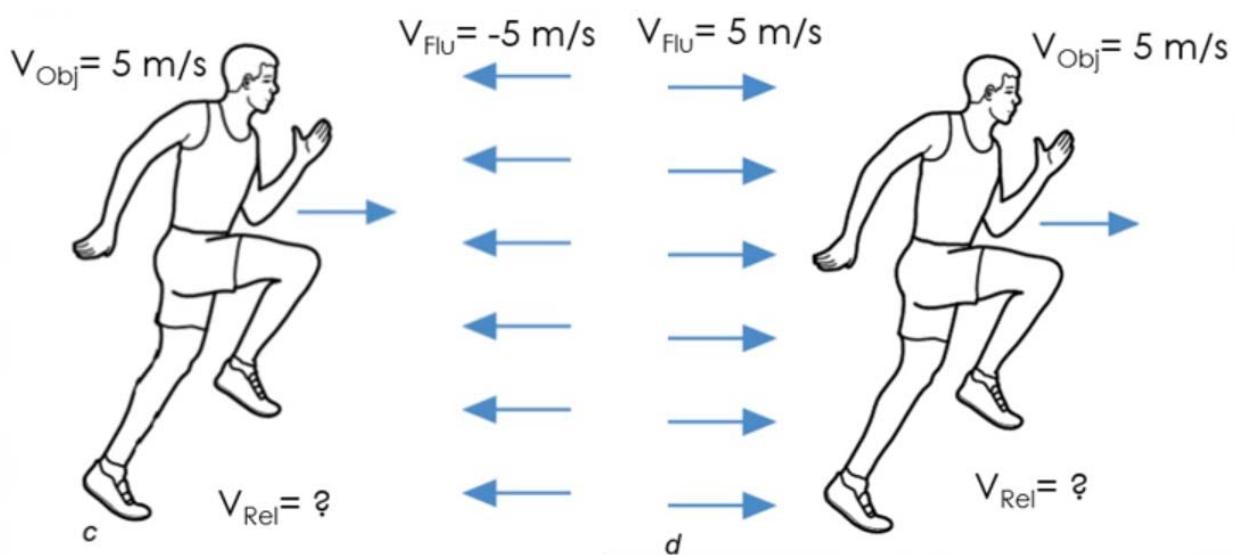


Figure 8.4 Relative velocity between a runner and the wind.

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Review: Lift Force

$$F_L = \frac{1}{2} C_L \rho A v^2$$

where

F_L = lift force,

C_L = coefficient of lift,

ρ = fluid density,

A = reference area (usually the cross-sectional area of the object perpendicular to the relative motion),

v = relative velocity of the object with respect to the fluid.

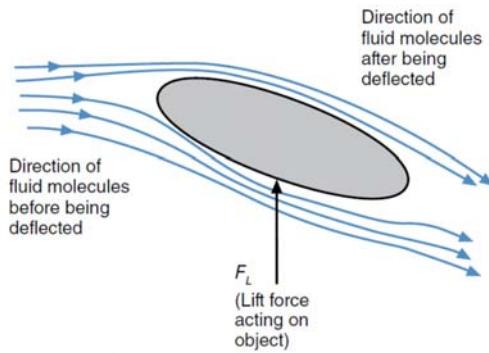


Figure 8.11 The fluid molecules passing by an object are deflected laterally. The change in direction is a lateral acceleration caused by the force exerted by the object. The reaction to this force is the lift force acting on the object.

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Review: Magnus Force

- Created on pressure systems developing on either side of a spinning object.
- One side will be spinning in the same direction as the relative velocity.
 - This creates a low pressure area
- The other side will be spinning in the opposite direction of relative velocity
 - This creates a high pressure area

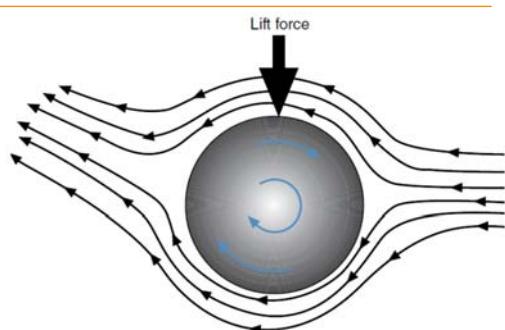


Figure 8.14 A ball with topspin has a lift force that acts downward on the ball.



https://en.wikipedia.org/wiki/Magnus_effect

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Review: Effects of Dynamic Fluid Forces

- Two objects similar in size and shape will experience the same dynamic fluid forces, but the more massive object will experience less acceleration.

$$\sum F = ma$$

where

$$F \propto \rho A v^2$$

$\sum F$ = net force,

$$a = \frac{\sum F}{m} \propto \frac{\rho A v^2}{m}$$

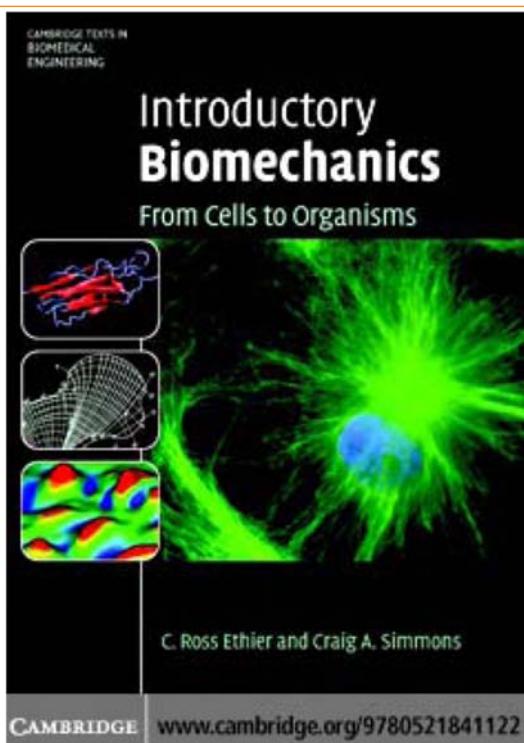
m = mass of the object, and

$$a \propto \frac{\rho A v^2}{m}$$

a = acceleration of the object.

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Textbook



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Introduction to Eukaryotic Cellular Architecture

- Eukaryotic cells contain a number of specialized subsystems, or *organelles*, that cooperate to allow the cell to function.

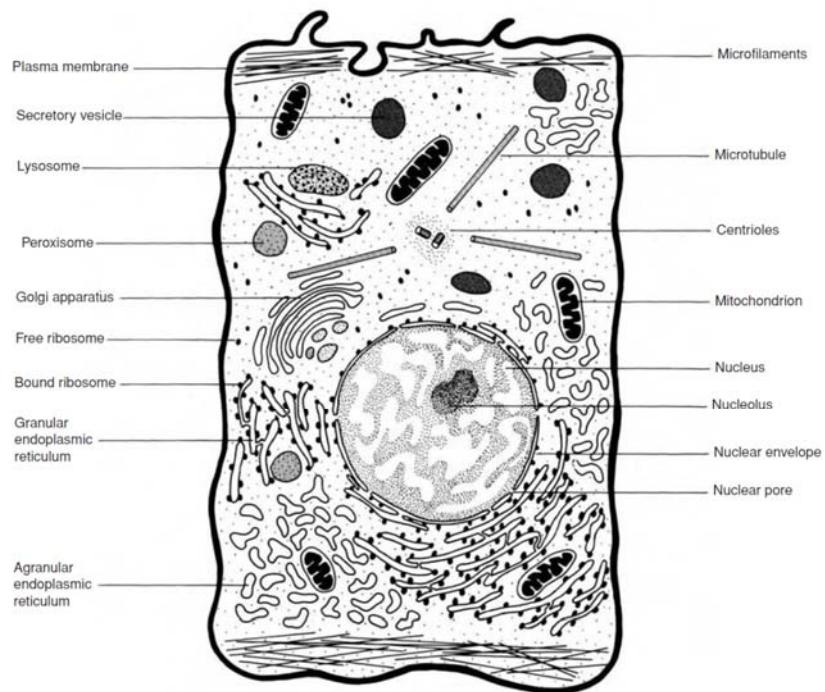


Figure 2.1

Structures and organelles found in most human cells. This diagram is highly schematized but serves to indicate the major features of the cellular organelles. From Vander et al. [3]. Reproduced with kind permission of the McGraw-Hill Companies.

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Introduction to Eukaryotic Cellular Architecture

- Walls (the membranes)**
- A framework (the cytoskeleton)**
- Engines (the mitochondria)**
- A command center (the nucleus)**
- Packaging plants (the Golgi apparatus).**
- A disposal system (the lysosomes).**

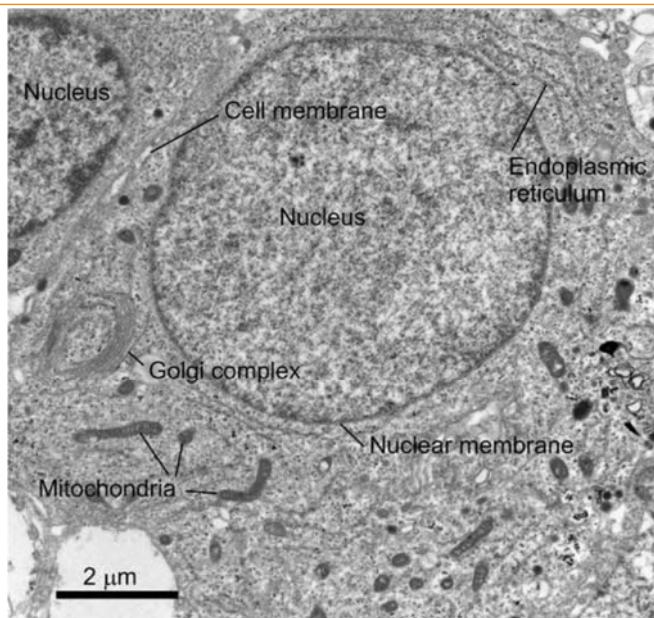


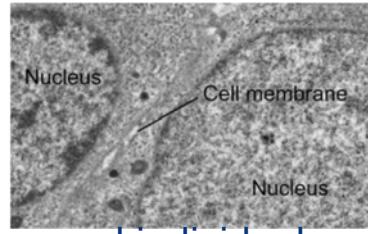
Figure 2.2

Transmission electron micrograph of an insulin-producing pancreatic cell, showing several of the structures depicted schematically in Fig. 2.1. A prominent nucleus delimited by the nuclear envelope (membrane) is present, as are several organelles in the cytoplasm: mitochondria, Golgi complex, and endoplasmic reticulum with associated ribosomes. A second cell is visible at the top left of the image. Sample stained with uranyl acetate and lead citrate. Micrograph courtesy of Mr. Steven Doyle, University of Toronto.

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Walls (*the membranes*)

- These barriers are primarily made up of lipids in a bilayer arrangement, augmented by specialized proteins.
- They serve to enclose the cell, the nucleus, and individual organelles (with the exception of the cytoskeleton, which is distributed throughout the cell). The function of membranes is to create compartments whose internal materials can be segregated from their surroundings. For example, the cell membrane allows the cell's interior to remain at optimum levels of pH, ionic conditions, etc., despite variations in the environment outside the cell. The importance of the cell membrane is shown by the fact that cell death almost invariably ensues if the cell membrane is ruptured to allow extracellular materials into the cell.



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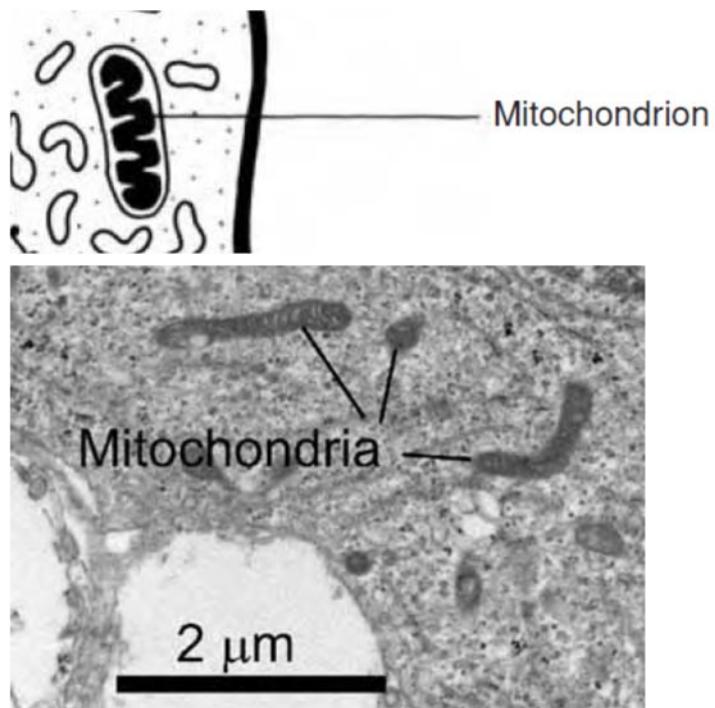
A framework (*the cytoskeleton*)

- This organelle consists of long rod-shaped molecules attached to one another and to other organelles by connecting molecules.
- The cytoskeleton gives the cell form, allows it to move, helps to anchor the cell to its substrate and neighbors, and speeds the transport of materials within certain types of cells. We will consider the cytoskeleton in greater detail later in this chapter.

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Engines (the mitochondria)

- These organelles produce most of the basic energy-containing molecules from certain substrates such as glucose. Then these energy-containing molecules are used by other subsystems within the cell.



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Cells

- Clearly a single cell is a remarkable assortment of complex subsystems.
- It is also a miracle of miniaturization: all of the above systems fit into a neat package having a typical mass of 2×10^{-8} g, and a typical diameter of order 15 μm !

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Cell's energy system and cytoskeleton of the cell

- Now that we have a basic overview of the components of a eukaryotic cell, let us look in more detail at cellular biomechanics and mechanobiology.
- Energy:** We will start (Section 2.2) with some basic ideas about how the cell uses energy, which we will see resembles energy flow in a thermal energy-generating station in some ways.
- Cytoskeleton:** Then we will delve into more detail about the cytoskeleton of the cell, focusing on its mechanical properties and how it helps to anchor the cell to its surroundings (Sections 2.3 and 2.4). The main focus here will be to give the reader enough biological background to understand Sections 2.5 and onwards.

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Mechanical properties of cells

- Mechanical Properties and Models of Cells**
 - When engineers talk about the mechanics of conventional engineering materials, they can refer to handbooks that tabulate the properties of, for example, different types of stainless steel, and describe the internal structure of these steels.
 - Can we do that for cells? Not quite, but a body of data is slowly being accumulated about the “mechanical properties of cells.” In Section 2.5, we will tackle the tricky question of how one measures the mechanical properties of a single cell, while in Section 2.6 we will introduce some engineering models that, in combination with experimental data, teach us something about the cell’s internal mechanics.

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Mechanotransduction

-
- Mechanotransduction:** One of the remarkable things about most cells is how *good* they are at sensing relatively small levels of mechanical stimulation, while living in a constantly changing biomechanical environment. How do they do this? Many details of this process, known as *mechanotransduction*, are unknown, but in Section 2.7 we will discuss, in general terms, current thinking on how adherent cells are able to sense and respond to mechanical stimulation.

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Response of cells to mechanical stimulation

-
- **Response of cells to mechanical stimulation**
 - Finally, we consider the consequences of the response of cells to mechanical stimulation. In Section 2.8, we describe some of the experimental tools that are used to apply mechanical stimuli to tissues or small groups of cells in culture. Using these devices, the effects of mechanical stimulation on several cell types have been determined. In Section 2.9, we present some of the effects on cells from three specific tissues (vascular tissue, cartilage, and bone) and we consider the implications for the whole tissue. Let's get started!

20

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The cell's energy system

- Life requires energy. At the cellular level, energy-consuming tasks include:
 - motion, including both cellular shape changes and locomotion of the cell on its substrate
 - synthesis of compounds
 - transport of ions and other molecules, both within the cell and between the cell and its surroundings

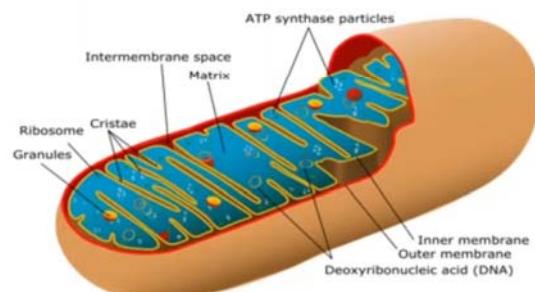
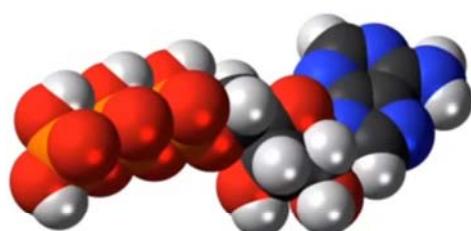
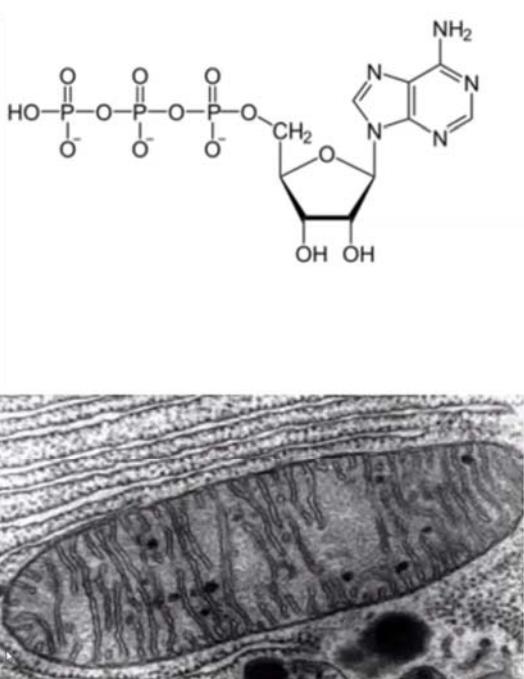
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The cell's energy system



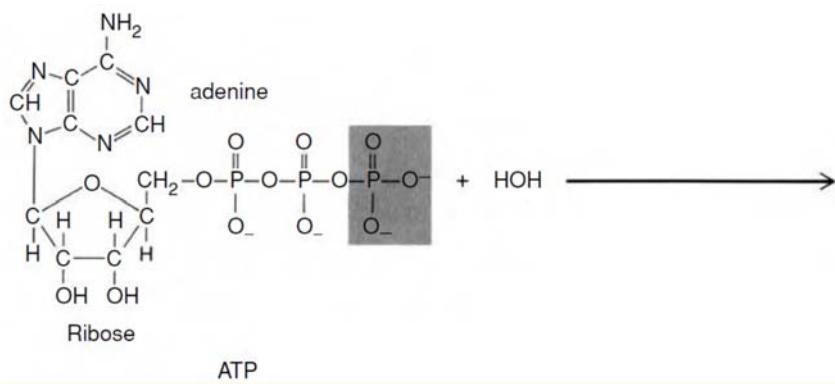
The cell's energy system

- How does the cell utilize food energy? When we eat a meal, the constituent foodstuffs are acted upon by the digestive enzymes and broken down into simpler compounds, transferred into the bloodstream across the intestinal walls, and then transported throughout the body.
- Individual cells are therefore presented with a complex mixture of compounds from which they must obtain energy. The cell solves this problem by having specialized “energy plants” (mitochondria), which are able to use compounds such as glucose and fatty acids to produce a common energy-containing molecule that all cellular organelles can use. This common molecule is *adenosine triphosphate* (ATP), formed from *adenosine diphosphate* (ADP) and phosphate (PO_3^{2-}) in the following reaction:
$$\text{ADP} + \text{PO}_3^{2-} + \text{energy} + 2\text{H}^+ \rightarrow \text{ATP}$$

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The cell's energy system

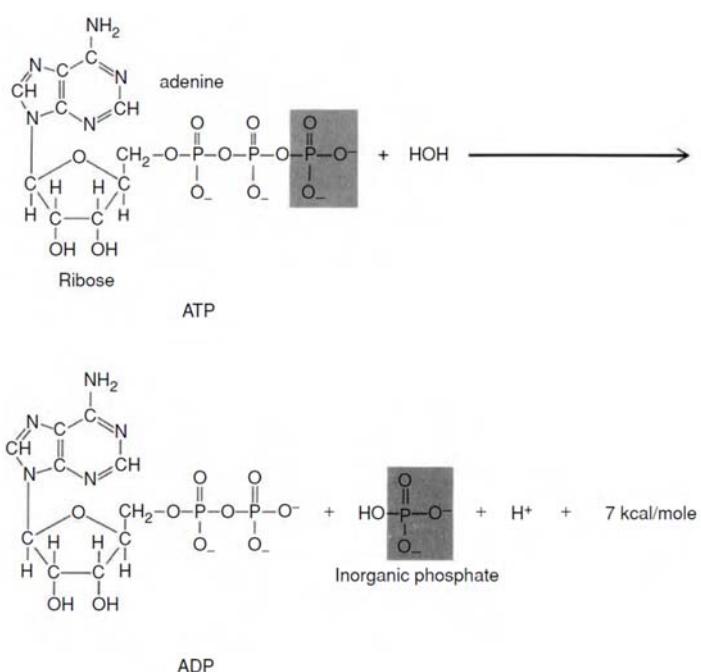
- Energy is stored in the chemical bond between ADP and PO_3^{2-} (Fig. 2.3). A mechanical analogue is a spring, which starts in an uncompressed state (ADP) and is then compressed and held in place by a catch (PO_3^{2-}). The organelles can “release the catch” to produce energy, with by-products ADP and PO_3^{2-} . We say, therefore, that *ATP is the common currency of energy within the cell*.



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The cell's energy system

- Structure of ADP and ATP. The breakdown of ATP to ADP and inorganic phosphate yields 7 kcal/mole energy.
From Vander et al. [3]. Reproduced with kind permission of the McGraw-Hill Companies.



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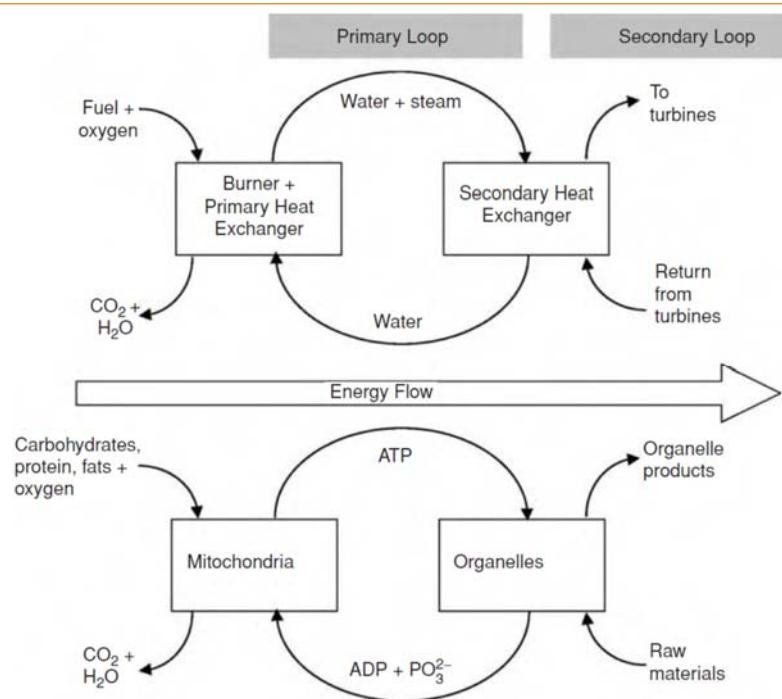
The cell's energy system

- It is important to note that ADP and phosphate are recycled, to be once more combined in the mitochondria to yield ATP. This is similar to the movement of primary loop cooling water in a thermal generating station, and it emphasizes that ATP is merely a transient carrier of energy within the cell (Fig. 2.4).

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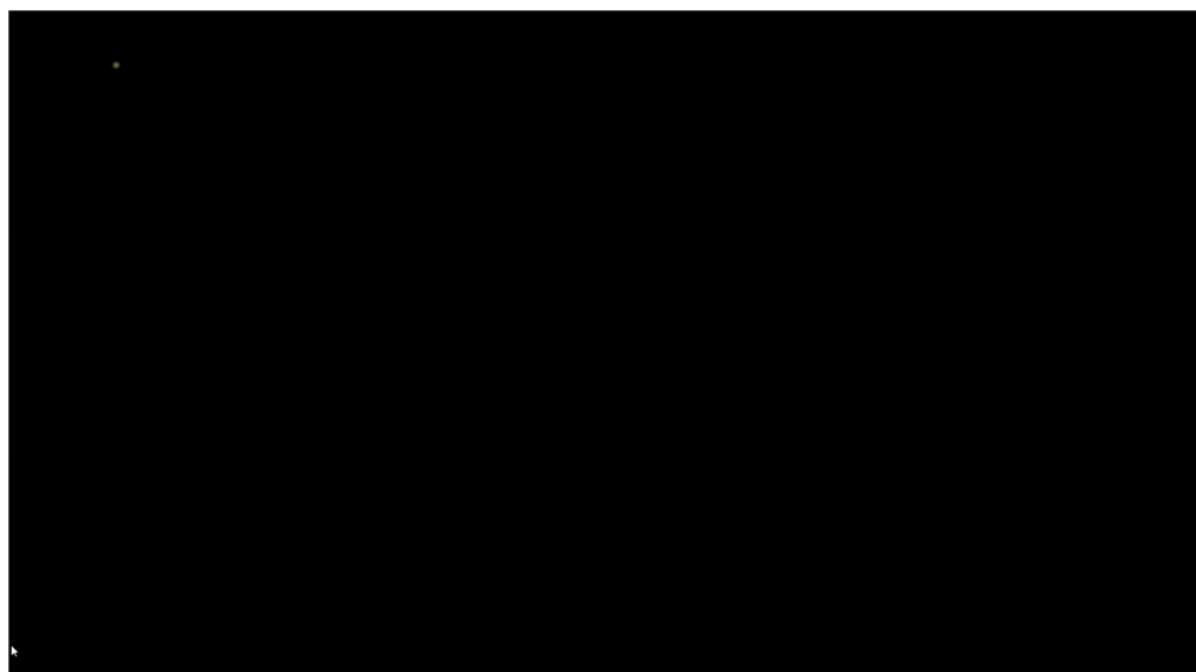
The cell's energy system

- Energy flow in a thermal generating station (top) and a cell (bottom). Note that primary loop cooling water is analogous to ATP in that it is a transient vehicle for energy storage which is recycled.



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Cytoskeleton



Overview of the cytoskeleton

-
- Just as an understanding of a cell's architecture and its energy system is critical to understanding cellular biology, the characteristics of the cytoskeleton are central to understanding a cell's biomechanical behavior. Here we will only give an overview of this fascinating topic. Students are encouraged to consult the references if they wish to learn more about the cytoskeleton.

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The cytoskeleton

-
- The cytoskeleton is an elaborate network of fibrous proteins that can adopt a remarkable range of configurations (Figs. 2.5 and 2.6). It:
 - establishes and maintains the **shape** of the cell
 - allows the cell to **move** (the process of *locomotion*)
 - provides **mechanical strength and integrity** to the cell
 - is central to the **intracellular transport of organelles**, especially in large cells such as axons
 - is essential during **cell division**, where it plays a key role in many processes, including chromosome separation in mitosis and meiosis.

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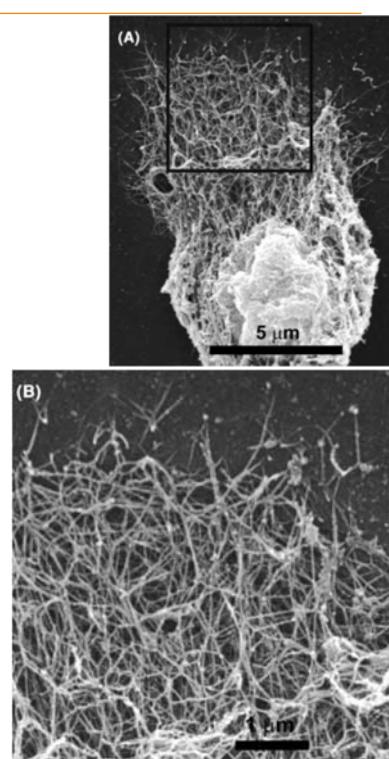
The cytoskeleton

- The cytoskeleton consists of three types of filament, each with a specialized protein composition:
 - *actin filaments* (7–9 nm in diameter), also called *microfilaments* or – in skeletal muscle cells – thin filaments,
 - *intermediate filaments* (10 nm in diameter), and
 - *microtubules* (approximately 24 nm in diameter).
- The interaction between all three filament types helps to determine the cell's mechanical behavior. We will briefly review the function of each of these filament types.

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The actin component of the cytoskeleton

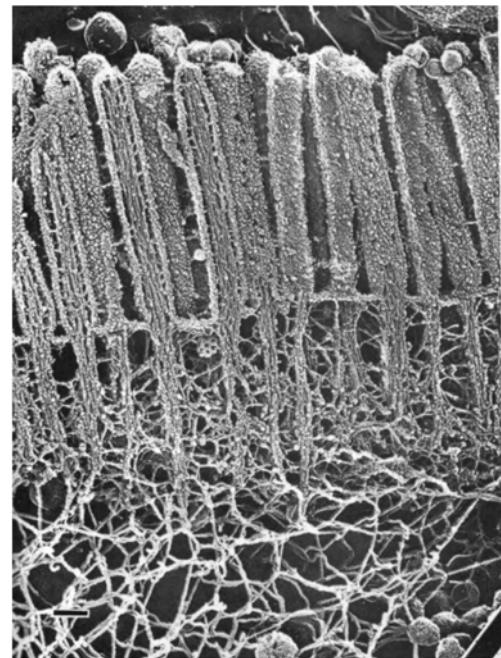
- Scanning electron micrograph of the **actin** component of the cytoskeleton within a rat fibroblast adhering to an N-cadherin-coated glass cover slip (A). A high-magnification inset of the boxed region is shown in (B). The rich, highly interconnected actin network is clearly visible. Cells were extracted with a detergent solution, fixed in glutaraldehyde, post-fixed, and gold sputter coated before visualization. Images courtesy of Dr. Tarek El Sayegh, Faculty of Dentistry, University of Toronto.



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Cytoskeleton of the brush border of intestinal epithelial cells

- Cytoskeleton of the brush border of intestinal epithelial cells. Tight **actin** filaments are evident in the microvilli, the finger-like structures in the top half of the image. The actin extends from microvilli into the cytoplasm of the cell, where it connects with a network of actin, intermediate filaments, myosin, and other cytoskeletal proteins. The scale bar in the lower left corner is 0.1 μm . Reproduced with permission from Bershadsky and Vasiliev [4] and from the *Journal of Cell Biology*, 1982, 94, 425–443 by copyright permission of the Rockefeller University Press.



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Actin filaments

- Actin exists within the cell in two forms, as a globular protein (G-actin) and as a filamentous protein (F-actin). G-actin has a molecular weight of approximately 43 kDa, and consists of a single polypeptide chain. Monomeric G-actin binds one Ca^{2+} and one molecule of ATP. F-actin is formed by the polymerization of G-actin, which causes the bound ATP to be hydrolyzed to ADP and a phosphate ion (Fig. 2.7, color plate). The ADP remains bound to the actin subunit within the F-actin chain.

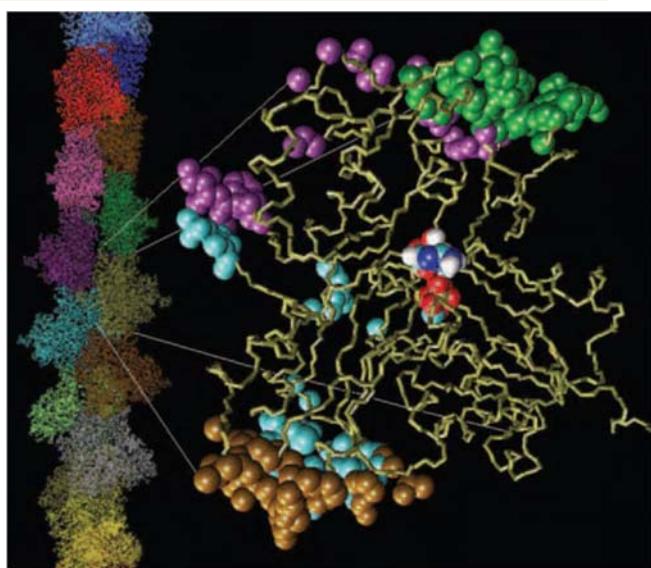
36

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Three-dimensional structure of a single G-actin monomer and an F-actin strand

- Three-dimensional structure of a single G-actin monomer (right) and an F-actin strand (left). Amino acids on the actin monomer are color coded to indicate interactions with adjacent monomers in the F-actin filament. Colored elements in the central portion of the monomer are bound ADP and Ca²⁺.
Reproduced with permission from the University of Illinois [5], based on Lorenz *et al.* [6] and Kabsch *et al.* [7].



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-
- F-actin chains are dynamic structures that grow and break down according to their position within the cell and the activities of the cell at any given instant. F-actin filaments are polarized, having an end where G-actin monomers are preferentially added (the fast-growing “barbed” or “+” end) and an end where the filament is either slowly growing or disassembled (the “pointed” or “−” end). Thus, individual actin monomers move along filaments, tending to be added at the + end and moving to the – end, in a process known as *treadmilling* (Fig. 2.8, color plate).

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Actin filaments

-
- The polymerization and breakdown of F-actin are regulated by several proteins, including actin depolymerizing factor/cofilin, members of the gelsolin/villin protein family, and CapZ. The lifetime of actin filaments, the length of the filaments, the percentage of actin in polymeric form, and the number of barbed ends change as a function of the cell’s activity. For example, in confluent bovine aortic endothelial cells, the mean filament lifetime is approximately 40 min and about 70% of the cell’s total actin is present as F-actin [8]. Confluent cells tend to be relatively quiescent, exhibiting only modest amounts of cellular movement. In contrast, in subconfluent endothelial cells, which are more active, the mean filament lifetime is only approximately 8 min and only approximately 40% of the cell’s actin is present in polymerized form [8].

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- Because of its biomechanical importance, a number of authors have experimentally estimated the Young's modulus of F-actin filaments. Some of these measurements are summarized in Table 2.1, which shows a fairly tight range of values of order 2 GPa, approximately 100 times less than the modulus for steel.

Table 2.1. Summary of Young's modulus values for F-actin measured by various methods. Modified with permission from Janmey *et al.* [13].

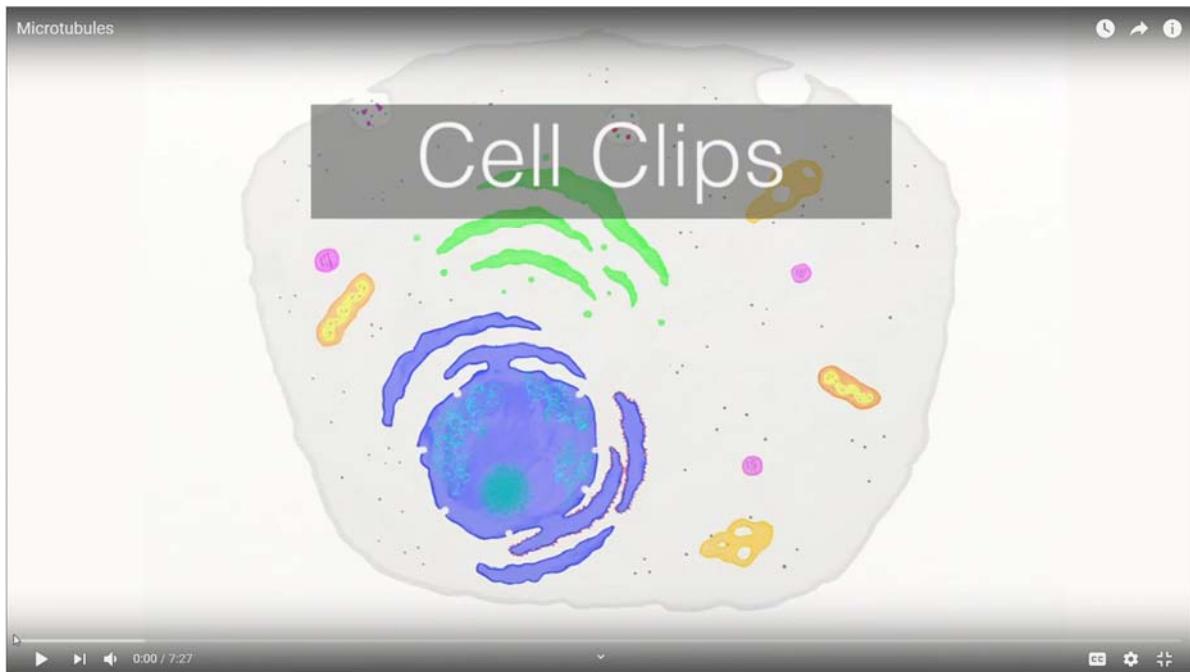
Authors	Method	Estimated Young's modulus (N/m ²)
Kojima <i>et al.</i> [9]	Micro-needle/single filament	1.8×10^9
Huxley <i>et al.</i> [10]	X-ray diffraction frog/muscle	2.5×10^9
Higuchi <i>et al.</i> [11]	Optical diffraction/rabbit skeletal muscle fiber	Not in paper, by comparison: $\sim 2 \times 10^9$
Wakabayashi <i>et al.</i> [12]	X-ray diffraction/frog muscle	Not in paper, by comparison: $\sim 2 \times 10^9$

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Intermediate filaments

- There are currently six known classes of intermediate filament protein. Each class contains multiple members, so that the intermediate filaments together make up a very diverse group of molecules whose expression is cell-type specific [2]. For example, the *keratins* are expressed in epithelial cells, while glial cells express *glial fibrillary acid protein* (GFAP), neurons express various *neurofilaments*, and endothelial cells express *vimentin*.

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Microtubules

- Microtubules are hollow cylindrical structures assembled from dimers of the proteins α -tubulin and β -tubulin (Fig. 2.9, color plate). They are dynamic structures, with the potential to grow or shrink at each end by the addition/removal of dimers. Like F-actin, microtubules are polarized: the + end is always more active than the other end. In vitro under appropriate conditions, tubulin monomers spontaneously polymerize to form a network of filaments (Fig. 2.10). Similar networks are present in cells.

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- Microtubules play a key role in the structure of cilia and flagella, where they provide structural rigidity and assist in generating motion. Microtubules are often associated with molecular motors, which are proteins designed to travel along the microtubule, usually to help to transport something. These molecular motors include:
 - the *kinesins*, which move towards the + end of the tubule
 - the *dyneins*, which move towards the – end of the tubule.
- <https://www.youtube.com/watch?v=ZpEKOH4LBAc>

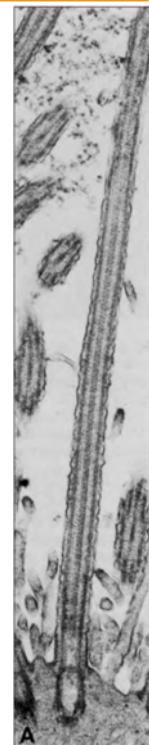
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- Molecular motors can be used for more than just transporting materials along the microtubules. Figure 2.11 shows a micrograph of a cilium, demonstrating the characteristic distribution of nine bundles of two microtubules around the periphery of the cilium and two central microtubules. This micrograph also shows interconnecting filaments between the central and peripheral microtubules. These connecting filaments are motor proteins. Forces generated by these motor proteins, in conjunction with the structural integrity of the microtubules, produce the characteristic back-and-forth motion of the cilium.

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Micrograph of a cilium

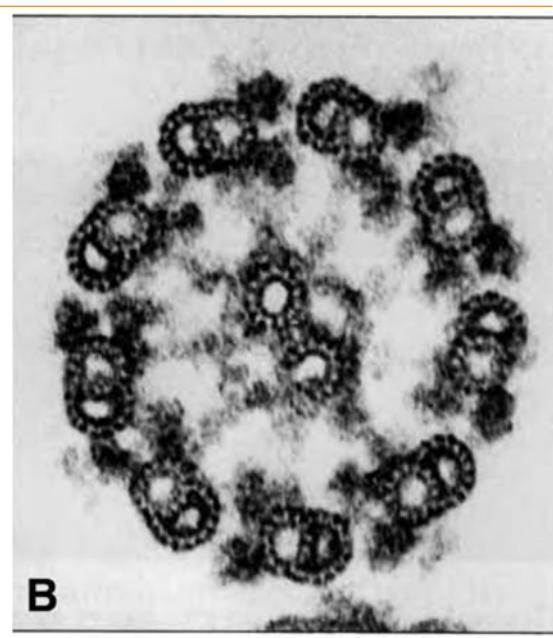
- (A) Electron micrograph of the longitudinal cross-section of a cilium from a human bronchus epithelial lining cell. Reproduced with permission from D. H. Cormack. *Ham's Histology*, 9th ed. (London: Lippincott, 1987).



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Micrograph of a cilium

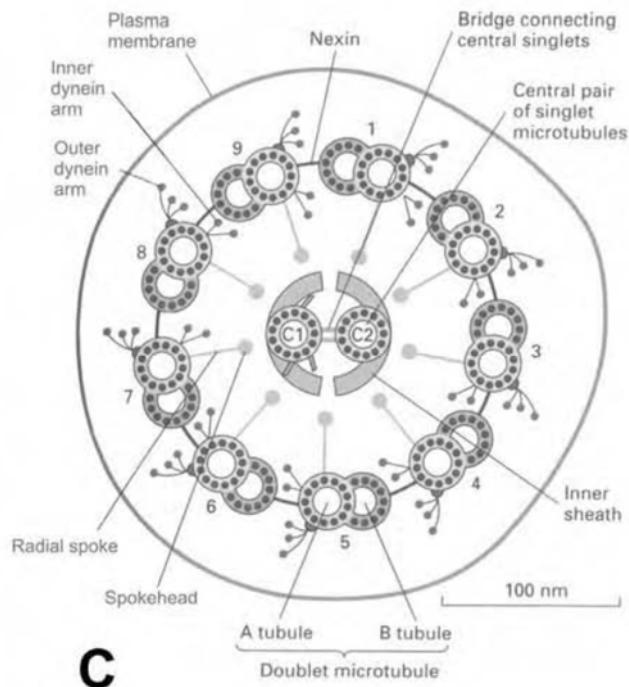
- (B) Electron micrograph of the transverse cross-section of a flagellum of a green alga cell showing the characteristic "9+2" arrangement of microtubules. This microtubule arrangement is found in almost all flagella and cilia from single cell organisms to humans. (B) reproduced with permission from Alberts *et al.* [B. Alberts, A. Johnson, J. Lewis, M. Raff, K. Roberts *et al.* *Molecular Biology of the Cell*, 4th ed. (New York: Garland Science, 2002).].



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Micrograph of a cilium

- (C) Diagram of the cross-section of a flagellum, showing the complex internal structure, which is very similar to that of a cilium. Reproduced with permission from Lodish *et al.* [H. Lodish, A. Berk, S. L. Zipursky, P. Matsudaira, D. Baltimore *et al.* *Molecular Cell Biology*, 4th ed. (New York: W. H. Freeman, 2000).], with permission of W. H. Freeman.



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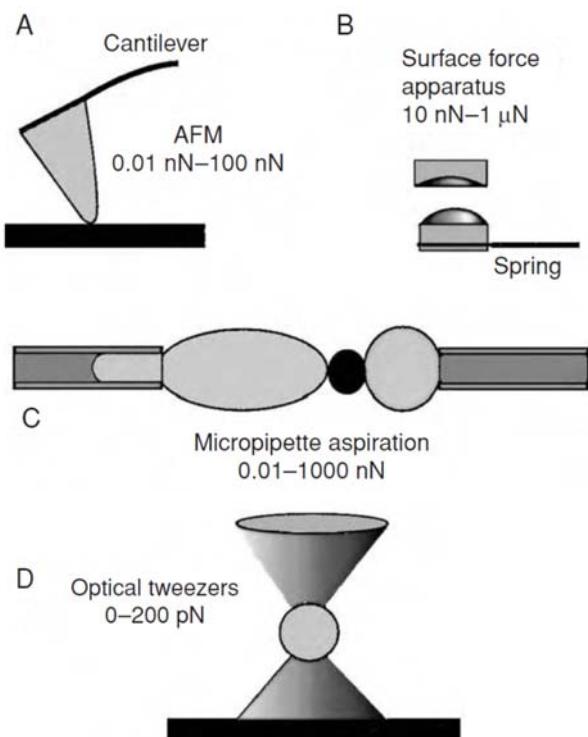
Typical magnitudes of quantities measured on the cellular scale (last column)

Quantity	SI units	“Micro SI” units (suitable for cells)
Distance	m	μm
Force	N	$\text{pN} (= 10^{-12} \text{ N})$ to $\text{nN} (= 10^{-9} \text{ N})^a$
Pressure, stress	$\text{Pa} (= \text{N/m}^2)$	$\text{pN}/\mu\text{m}^2 (= 1 \text{ Pa})$ to $\text{nN}/\mu\text{m}^2 (= 1 \text{ kPa})$

^a Forces of molecular bonds and those exerted by “soft” cells are in the picoNewton range. “Stiff” cells can exert forces in the nanoNewton range.

Methods to measure the mechanical properties of cells and biomolecules

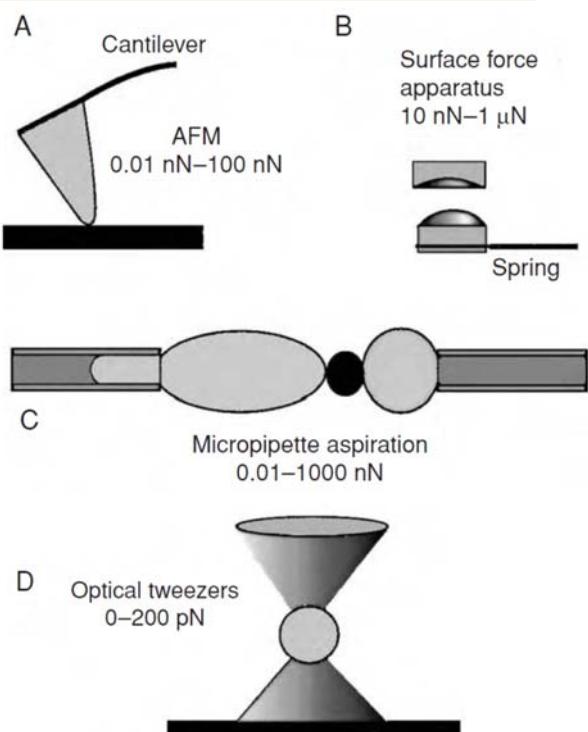
- Force probes used to measure the mechanical properties of single cells and protein interaction forces.
- (A) An atomic force microscope (AFM) shows the probe tip, attached to the cantilever force transducer, coming in contact with a substrate.
- (B) A surface force apparatus, showing the crossed cylinders of the apparatus and the force-transducing spring.



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Methods to measure the mechanical properties of cells and biomolecules

- (C) The bioforce probe, consisting of a cell partially aspirated into a micropipette. A bead (center black sphere) is attached to one cell (left), and a force between the bead and a second cell or glass bead (right) is exerted by aspirating the (left) cell into the pipette.
- (D) Optical tweezers. A bead is held in the optical trap, such that radiation pressure exerted on the bead applies force to adhesive contacts between materials on the bead surface and the substrate.



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Van der Waals Forces

- In physical chemistry, the van der Waals force (or van der Waals interaction), named after Dutch scientist Johannes Diderik van der Waals, is the sum of the attractive or repulsive forces between molecules (or between parts of the same molecule) other than those due to covalent bonds, the hydrogen bonds, or the electrostatic interaction of ions with one another or with neutral molecules or charged molecules.



Gecko climbing glass (Wiki)

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Van der Waals Forces

- The generalized interaction between molecules is given by the Mie pair potential

$$E(r) = -\frac{A}{r^n} + \frac{B}{r^m}$$

attractive repulsive



- A specific case of the Mie potential is the Lennard-Jones potential

$$E(r) = -\frac{A}{r^6} + \frac{B}{r^{12}}$$

where A and B are constants, e.g., for solid argon, $A = 8.0 \times 10^{-77} \text{ Jm}^6$ and $B = 1.12 \times 10^{-133} \text{ Jm}^{12}$.

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- The net van der Waals force is given by

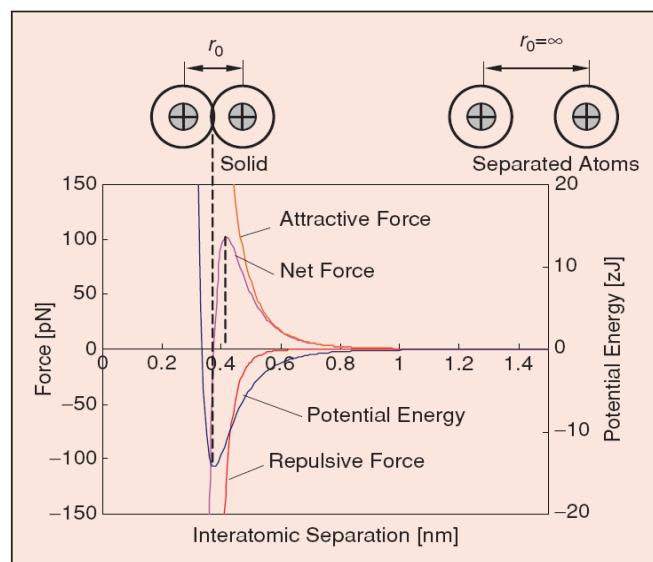
$$F_{\text{vdW}} = -\frac{dE}{dr}$$

- If r is scaled as $\sim L$, the attractive force scales as $\sim L^{-7}$, and thus its importance dramatically increases at the nanoscale. The repulsive force scales as $\sim L^{-13}$, which is important only at subnanometer scales.

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Intermolecular and Interatomic Forces

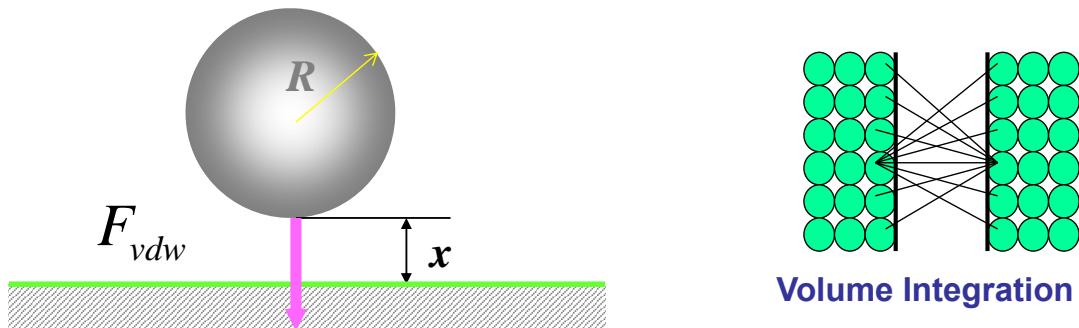
- The bond energy of van der Waals induced dipoles (such as argon solid shown here) is much smaller than electrostatic interaction based intramolecular ionic bonds (e.g., 3.2 eV for NaCl rock salt), metallic bonds (e.g., 3.1 eV for metal Cu) or covalent bonds (e.g., 4 eV for Si and 7.4 eV for C (diamond)), which scale as $\sim L^{-2}$.



The maximum value of F_{vdW} is obtained when $d^2E/dr^2 = 0$, or $r = (26B/7A)^{1/6} = 0.416$ nm, as $F_{\text{vdW}, \max} = 102$ pN. Potential energy: 0.09 eV.

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Van der Waals Forces



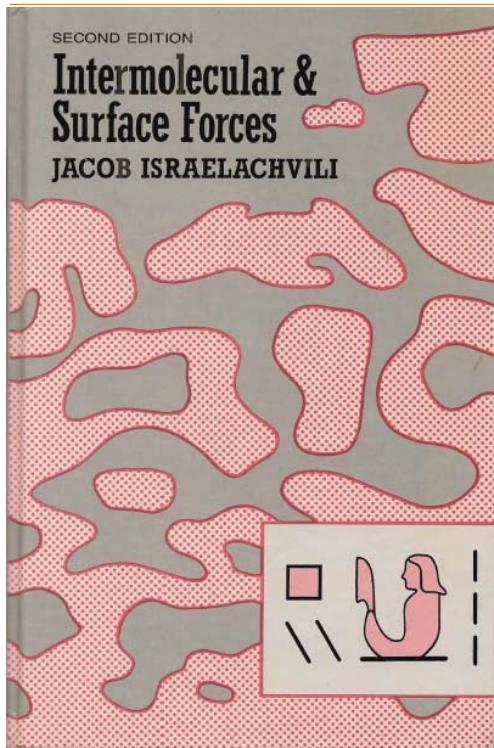
$$F_{vdw} = \frac{HR}{12x^2}$$

$$F_{vdW} \sim x^{-2}$$

$$F_{vdW} \sim R^1$$

H: Hamaker Constant x: Distance R: Radius of Bead

Van der Waals Forces



VAN DER WAALS FORCES BETWEEN SURFACES		177
Two atoms	Two spheres	
$W = -C/r^6$	$W = -A/6D \cdot \frac{R_1 R_2}{(R_1 + R_2)}$	
Atom-surface	Sphere-surface	
$W = -\pi C \rho / 6D^3$	$W = -AR / 6D$	
Two parallel chain molecules	Two cylinders	
$W = -3\pi CL / 8\sigma^2 r^5$	$W = \frac{AL}{12\sqrt{2} D^{3/2}} \left(\frac{R_1 R_2}{R_1 + R_2} \right)^{1/2}$	
Two crossed cylinders	Two surfaces	
$W = -A\sqrt{R_1 R_2} / 6D$	$W = -A / 12\pi D^2 \text{ per unit area}$	

Fig. 11.1. Non-retarded van der Waals interaction free energies between bodies of different geometries calculated on the basis of pairwise additivity (Hamaker summation method). The Hamaker constant A is defined as $A = \pi^2 C \rho_1 \rho_2$ where ρ_1 and ρ_2 are the number of atoms per unit volume in the two bodies and C is the coefficient in the atom-atom pair potential (top left). A more rigorous method of calculating the Hamaker constant in terms of the macroscopic properties of the media is given in Section 11.3. The forces are obtained by differentiating the energies with respect to distance.

Van der Waals Forces

<p>Two atoms</p> $w = -C/r^6$	<p>Two spheres</p> $W = \frac{-A}{6D} \frac{R_1 R_2}{(R_1 + R_2)}$
<p>Atom–surface</p> $w = -\pi Cp/6D^3$	<p>Sphere–surface</p> $W = -AR/6D$

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Van der Waals Forces

<p>Two parallel chain molecules</p> $W = -3\pi CL/8\sigma^2 r^5$	<p>Two cylinders</p> $W = \frac{AL}{12\sqrt{2} D^{3/2}} \left(\frac{R_1 R_2}{R_1 + R_2} \right)^{1/2}$
<p>Two crossed cylinders</p> $W = -A\sqrt{R_1 R_2}/6D$	<p>Two surfaces</p> $W = -A/12\pi D^2 \text{ per unit area}$

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Van der Waals Forces

Fig. 11.1. Non-retarded van der Waals interaction free energies between bodies of different geometries calculated on the basis of pairwise additivity (Hamaker summation method). The Hamaker constant A is defined as $A = \pi^2 C \rho_1 \rho_2$ where ρ_1 and ρ_2 are the number of atoms per unit volume in the two bodies and C is the coefficient in the atom–atom pair potential (top left). A more rigorous method of calculating the Hamaker constant in terms of the macroscopic properties of the media is given in Section 11.3. The forces are obtained by differentiating the energies with respect to distance.

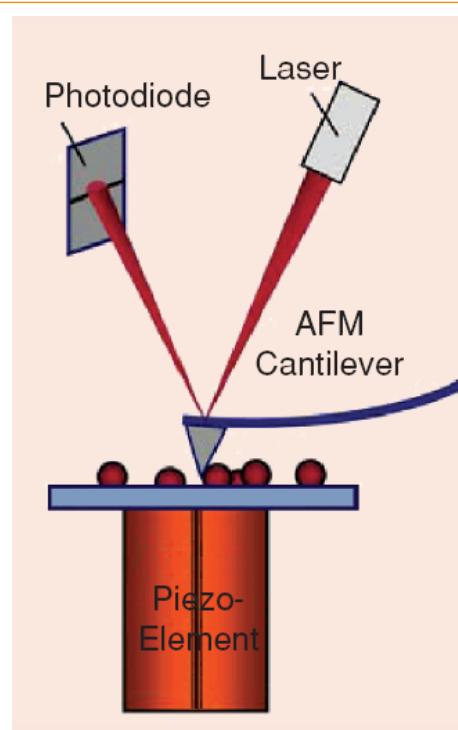
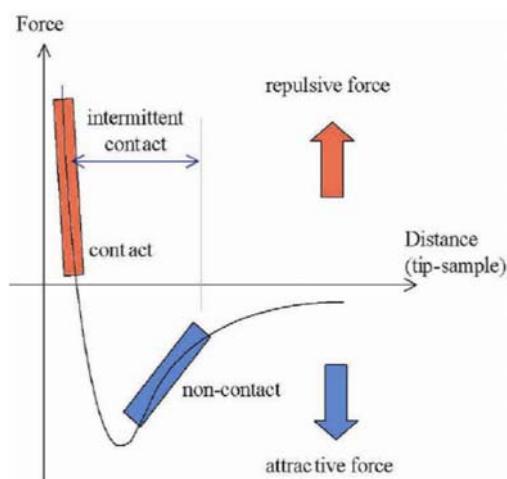
TABLE 11.1 Hamaker constants determined from pairwise additivity, Eq. (11.1).

Medium	C (10^{-79} J m^6)	ρ (10^{28} m^{-3})	A (10^{-19} J)
Hydrocarbon	50	3.3	0.5
CCl_4	1500	0.6	0.5
H_2O	140	3.3	1.5

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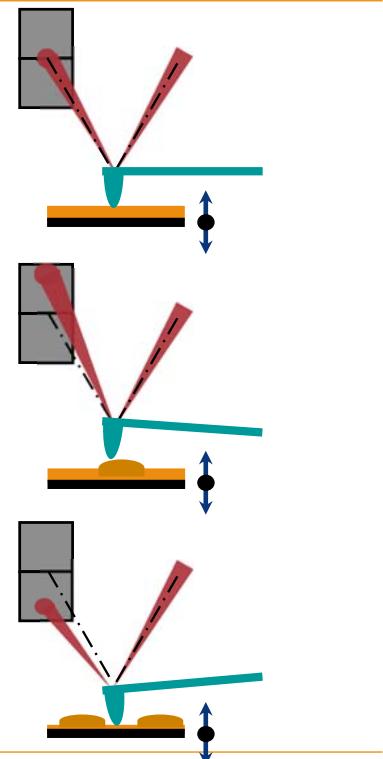
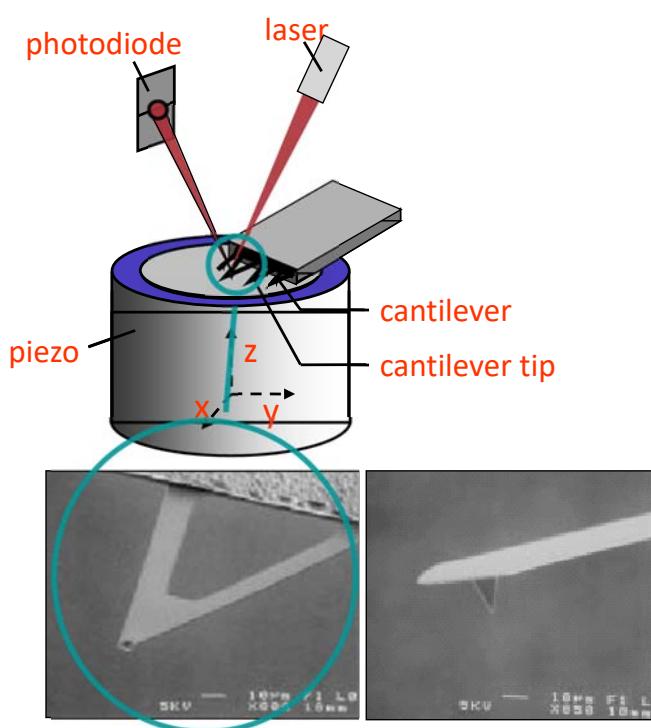
Atomic Force Microscopy (AFM)

- Contact regime (a few hundreds of picometers)
 - Repulsive force (coulomb interaction)
- Non-contact regime (1-10 nanometers)
 - Attractive force (Van der Waals force)



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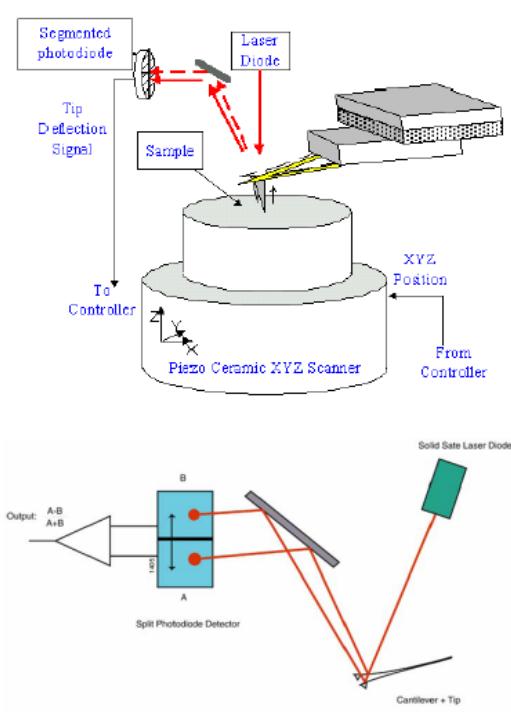
Atomic Force Microscopy



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Atomic Force Microscopy

- Light lever used for contact mode, non-contact mode, and tapping mode.
- Long beam path (several cm) amplifies changes in beam angle.
- Laser light from a solid-state diode is reflected off the back of the cantilever and collected by a position sensitive detector (PSD).
- Angular displacement of cantilever results in one photodiode collecting more light than the other photodiode, producing an output signal which is proportional to the deflection of the cantilever.
- Detects cantilever deflections $< 1\text{ \AA}$ (thermal noise limited).



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Lateral Force Microscopy (Friction)

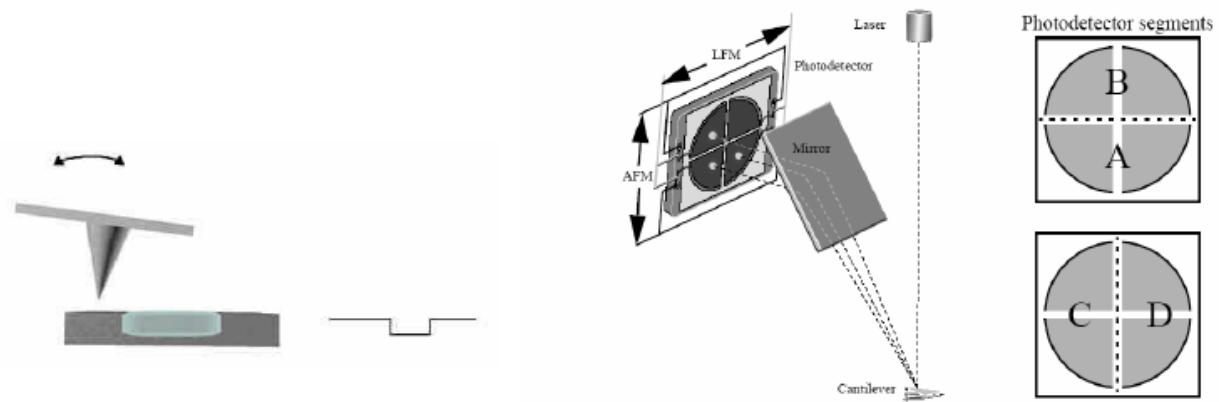


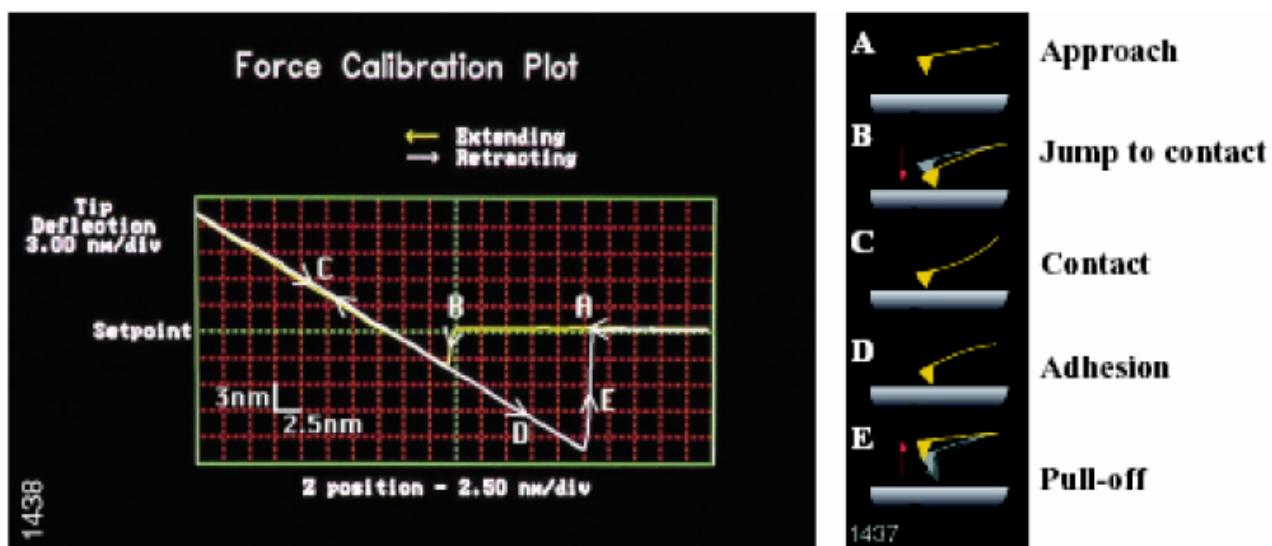
Figure 2.4. Quad photodetector arrangement. Different segments of the photodetector are used for generating AFM and LFM signals.

- The amount of torsion of the cantilever is controlled by changes in topography as well as changes in surface chemical properties.
- Possible to detect / image changes in material properties.

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Force Curves

- Force curves are commonly used to set the imaging force in contact mode and to study attractive, repulsive, and adhesive interactions between the tip and the sample.



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- To understand the operation of the AFM it is important to delve into the operation of the cantilever arm in more detail. If the arm undergoes only small deflection in the vertical direction, then it can be treated as a linear spring with stiffness k_c . In this case, the force F needed to create a vertical deflection z can be written as

$$F = k_c z$$

- Typical values for k_c are about 0.02 to 5 Newton per meter (N/m).

Noise of force measurement

- Remember that the probe tip will typically be in an aqueous environment and will, therefore, be subject to random collisions from water molecules undergoing thermal motion.
- The average energy of a molecule at absolute temperature T is given by $1/2k_B T$, where k_B is Boltzmann's constant, 1.3807×10^{-23} J/K.
- The energy stored in a spring of stiffness k_c as it deflects by an amount z is $1/2k_c z^2$. Equating these two energies allows us to solve for the fluctuations, z , that will occur as a result of random collisions with water molecules as:

$$z = \sqrt{\frac{k_B T}{k_c}}$$

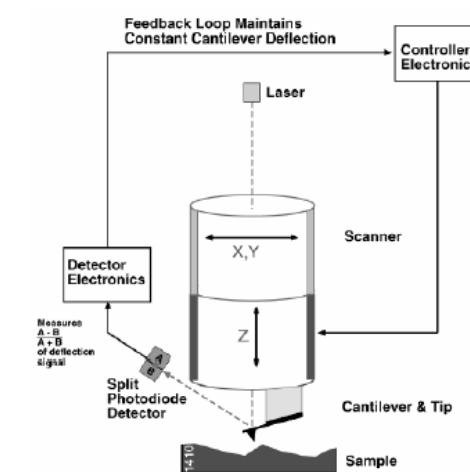
- This corresponds to a force

$$F = \sqrt{k_c k_B T}$$

- which represents the thermal “noise” that will be continually measured by the AFM tip.
- Forces appreciably smaller than this are not resolvable. For a sample at 37 °C (310 K), and an arm stiffness of 0.05 N/m, this corresponds to approximately 15 pN.

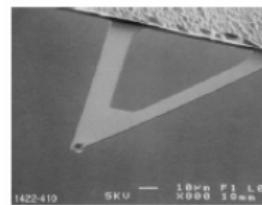
Contact Mode

- Operates by scanning a tip across the sample surface while monitoring the change in cantilever deflection.
- A feedback loop maintains a constant deflection between the cantilever and the sample by vertically moving the scanner at each (x,y) data point.
- Force constants usually range from 0.01 to 1.0 N/m, resulting in forces ranging from nN to μ N.
- The distance the scanner moves vertically at each (x,y) data point is stored to form the topographic image of the sample surface.
- Operation can take place in ambient and liquid environments.

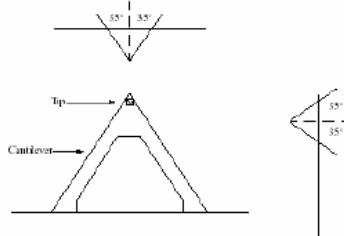


Contact Mode Tips / Cantilevers

- Silicon nitride probes consist of a cantilever integrated with a sharp tip on the end.
- It is necessary to have a cantilever which is **soft** enough to be deflected by very small forces and has a **high enough resonant frequency** not to be susceptible to vibrational instabilities.
- This is accomplished by making the cantilever short to provide a high resonant frequency, and thin to provide a small force constant.



Tip Sidewall Angles of Silicon Nitride Probes



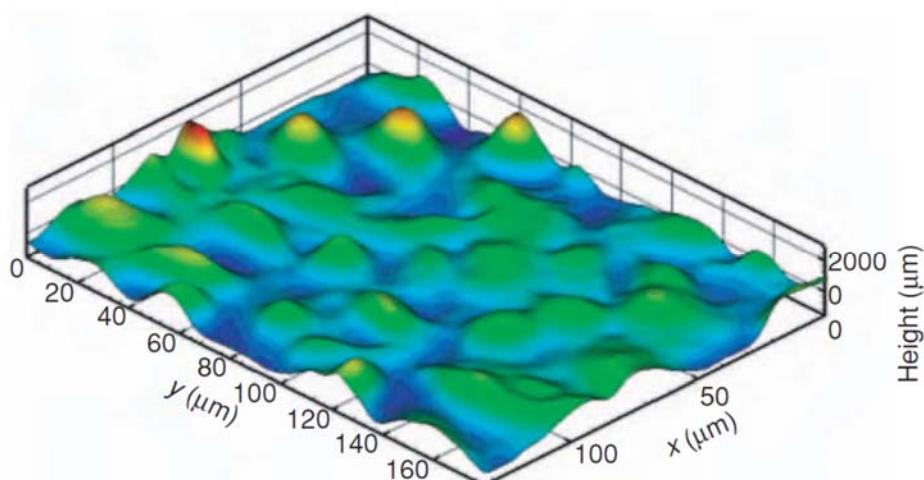
Silicon Nitride Probe Characteristics

Spring Constant (k)	0.58, 0.32, 0.12, 0.06 N/m ^a
Nominal Tip Radius of Curvature	20 - 60 nm
Cantilever Lengths	100 & 200 μm
Cantilever Configuration	V-shaped
Reflective Coating	Gold
Sidewall angles	35° on all 4 sides

a. Calculated spring constant values are based on the 0.6μm silicon nitride thickness; however, this value can actually vary from 0.4μm to 0.7μm. Thickness is cubed in the spring constant calculation, thus, actual values can vary substantially.

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AFM map of cellular topography

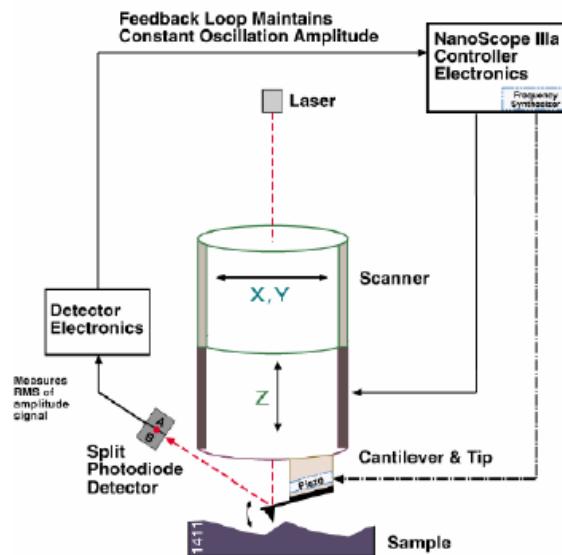


- Atomic force microscopic map of cellular topography for confluent porcine aortic endothelial cells. The vertical scale has been exaggerated. Data courtesy of Dr. Shigeo Wada (Sendai University, Japan), and Mr. James Shaw and Dr. Chris Yip (University of Toronto).

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Tapping Mode

- Tapping Mode AFM operates by scanning a tip on an oscillating cantilever across the sample surface.
- The cantilever is oscillated at or near its resonance frequency (amplitude typically from 20 to 100 nm).
- The tip lightly “taps” on the sample surface during scanning.
- The feedback loop maintains a constant oscillation amplitude by maintaining a constant RMS of the oscillation signal acquired by the split photodiode detector.
- Operation can take place in ambient and liquid environments.



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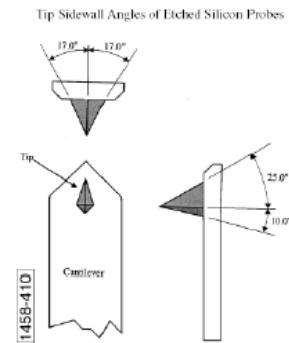
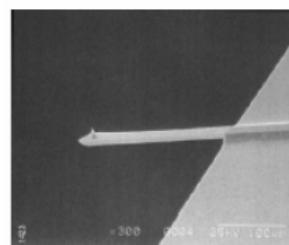
Tapping mode of AFM

- The cantilever arm is acoustically or magnetically driven so that it vibrates at or near its resonant frequency, and it is then moved vertically so that it approaches the cell surface.
- Far from the cell, the arm undergoes oscillations whose amplitude is determined by the magnitude of the driving signal.
- However, as the probe tip approaches the cell it begins to interact with the cell, which changes the magnitude of the arm's vibrations.
- The position where this change in magnitude occurs is a measure of the topography of the cell at that lateral location. Because the probe tip just taps the specimen, rather than being dragged along it, this produces smaller lateral forces on the cell and less potential for damage.

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Tapping Mode Tips / Cantilevers

- Silicon probes are used primarily for Tapping Mode applications.
- The tip and cantilever are an integrated assembly of single crystal silicon, produced by etching techniques.
- These probes can be **much stiffer than the silicon nitride probes**, resulting in larger force constants and resonant frequencies.



TappingMode Etched Silicon Probe (TESP) Characteristics

Spring Constant (k)	20 - 100 N/m
Resonant Frequency	200 - 400 kHz
Nominal Tip Radius of Curvature	5 - 10 nm
Cantilever Length	125 μm
Cantilever Configuration	Single Beam
Reflective Coating	Uncoated, Optional Al Coating

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Force mapping mode of AFM

- Other interesting measurements can be made with the AFM on cells. For example, the probe tip can be vertically traversed towards the sample while the cantilever arm deflection is measured. This is known as “force mapping mode” and produces a curve of applied force versus surface deflection (a “force curve”). To understand this curve, we must remember that the probe tip is much harder than the relatively soft biological specimens, so that we effectively have a rigid cone penetrating into the cell, which we will treat as being locally planar and linearly elastic.

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Force mapping mode of AFM

- The key result is that the deflection of the cell at the center of the probe tip, δ , due to an applied force F is

$$\delta^2 = \frac{\pi}{2} \frac{F(1 - \nu^2)}{E \tan \alpha}$$

- where E and ν are the modulus and Poisson ratio for the cell and α is the known cone half-angle. A generalization of this solution that is useful for our purposes is:

$$z - z_0 = \frac{F}{k_c} + \sqrt{\frac{\pi}{2} \frac{F(1 - \nu^2)}{E \tan \alpha}}$$

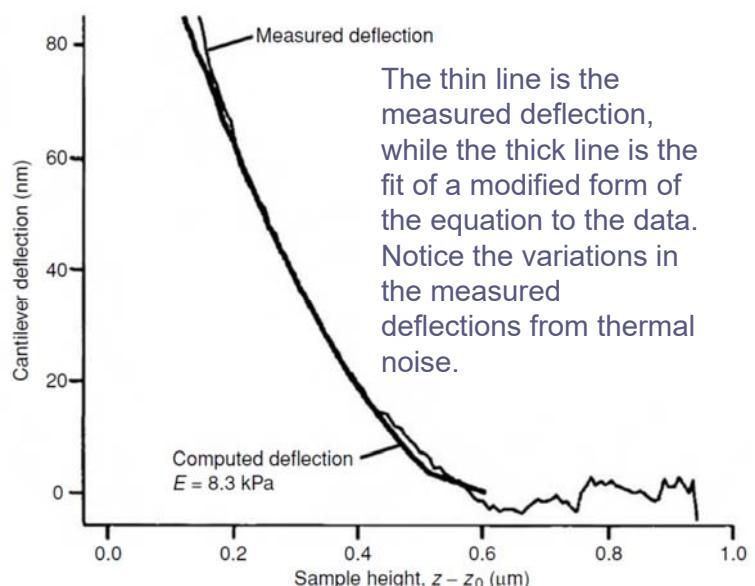
- Here z_0 is the probe height at which the applied force becomes non-zero, and the first term on the right-hand side accounts for the deflection of cantilever arm.

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A force curve

- The vertical axis is proportional to the force applied to the cell by the probe tip. The horizontal axis is the vertical offset of the base of the cantilever arm applied by the piezoelectric actuator, which we denoted by $z - z_0$ in equation

$$z - z_0 = \frac{F}{k_c} + \sqrt{\frac{\pi}{2} \frac{F(1 - \nu^2)}{E \tan \alpha}}$$

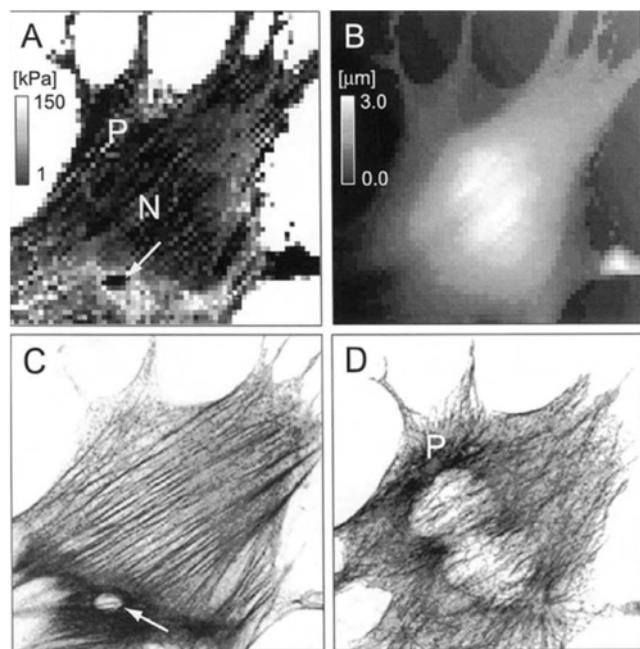


Example force curve for measurement of cellular stiffness of an activated human platelet using the AFM.

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Elasticity and corresponding topography map

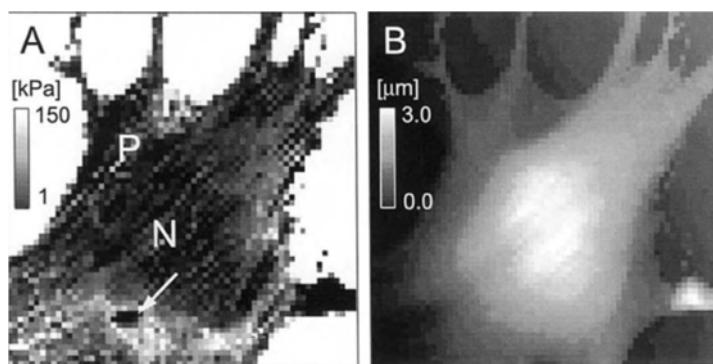
- By assuming a value for ν (usually 0.5), the measured force–displacement data can be fit by Equation (2.5) to obtain z_0 and E . It will be appreciated that the value of E so obtained reflects a local Young's modulus and can be expected to vary over the surface of the cell. For example, the local stiffness has been related to the local cytoskeletal structure in fibroblasts.



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Elasticity and corresponding topography map

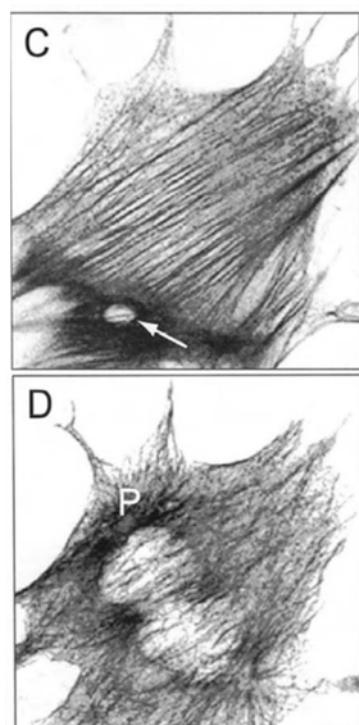
- Elasticity map (A) and corresponding topography map (B) of a living NIH3T3 fibroblast. The nuclear portion (N) is the softest, with a stiffness of approximately 4 kPa. A small softer “island” was observed in the perinuclear region (marked by arrow).



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Elasticity and corresponding topography map

- (C,D) Immunofluorescence images of actin filaments (C) and microtubules (D) for the same cell shown in A and B. A bi-lobed nucleus is visible in the microtubule image. The small open area observed in the actin image (arrow in C) corresponds to the soft “island” observed in the elasticity map. Part of the cell with low actin density and high density of microtubules shows a low Young’s modulus (marked P in A and D). Each image size is 80 μm square.
Reprinted from Haga *et al.* [35], with permission from Elsevier.



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Maximum spatial resolution

- What sort of maximum spatial resolution can we expect with the AFM?
- A typical probe tip radius is 10–50 nm, but unfortunately this lateral resolution is not achievable when measuring cellular stiffness. That is because the probe sinks into the relatively soft cell and consequently measures over a larger area than just the tip. The magnitude of this effect depends on the applied force and the cell stiffness; typical values for lateral resolution are in the range of tens to several hundreds of nanometers. This is small enough to give reasonable resolution when mapping stiffness over a cell.

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Approximate Young's modulus for various materials

Material	GPa	Mpsi
Rubber (small strain)	0.01–0.1 ^[4]	1.45–14.5 × 10 ⁻³
Low-density polyethylene ^[5]	0.11–0.86	1.6–6.5 × 10 ⁻²
Diatom frustules (largely silicic acid) ^[6]	0.35–2.77	0.05–0.4
PTFE (Teflon)	0.5 ^[4]	0.075
HDPE	0.8	0.116
Bacteriophage capsids ^[7]	1–3	0.15–0.435
Polypropylene	1.5–2 ^[4]	0.22–0.29
Polycarbonate	2–2.4	0.29–0.36
Polyethylene terephthalate (PET)	2–2.7 ^[4]	0.29–0.39
Nylon	2–4	0.29–0.58
Polystyrene, solid	3–3.5 ^[4]	0.44–0.51
Polystyrene, foam ^[8]	0.0025–0.007	0.00036–0.00102
Medium-density fiberboard (MDF) ^[9]	4	0.58
Wood (along grain)	11 ^[4]	1.60
Human Cortical Bone ^[10]	14	2.03
Glass-reinforced polyester matrix ^[11]	17.2	2.49
Aromatic peptide nanotubes ^{[12][13]}	19–27	2.76–3.92
High-strength concrete	30 ^[4]	4.35
Amino-acid molecular crystals ^[14]	21–44	3.04–6.38
Carbon fiber reinforced plastic (50/50 fibre/matrix, biaxial fabric)	30–50 ^[15]	4.35–7.25
Hemp fiber ^[16]	35	5.08
Magnesium metal (Mg)	45 ^[4]	6.53
Glass (see chart)	50–90 ^[4]	7.25–13.1
Flax fiber ^[17]	58	8.41
Aluminum	69 ^[4]	10

https://en.wikipedia.org/wiki/Young%27s_modulus

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Mother-of-pearl (nacre, largely calcium carbonate) ^[18]	70	10.2
Aramid ^[19]	70.5–112.4	10.2–16.3
Tooth enamel (largely calcium phosphate) ^[20]	83	12
Stinging nettle fiber ^[21]	87	12.6
Bronze	96–120 ^[4]	13.9–17.4
Brass	100–125 ^[4]	14.5–18.1
Titanium (Ti)	110.3	16 ^[4]
Titanium alloys	105–120 ^[4]	15–17.5
Copper (Cu)	117	17
Carbon fiber reinforced plastic (70/30 fibre/matrix, unidirectional, along fibre) ^[22]	181	26.3
Silicon Single crystal, different directions ^{[23][24]}	130–185	18.9–26.8
Wrought iron	190–210 ^[4]	27.6–30.5
Steel (ASTM-A36)	200 ^[4]	30
polycrystalline Yttrium iron garnet (YIG) ^[25]	193	28
single-crystal Yttrium iron garnet (YIG) ^[26]	200	29
Cobalt-chrome (CoCr) ^[27]	220–258	29
Aromatic peptide nanospheres ^[28]	230–275	33.4–40
Beryllium (Be) ^[29]	287	41.6
Molybdenum (Mo)	329–330 ^{[4][30][31]}	47.7–47.9
Tungsten (W)	400–410 ^[4]	58–59
Silicon carbide (SiC)	450 ^[4]	65
Tungsten carbide (WC)	450–650 ^[4]	65–94
Osmium (Os)	525–562 ^[32]	76.1–81.5
Single-walled carbon nanotube	1,000 ^{+[33][34]}	150+
Graphene (C)	1050 ^[35]	152
Diamond (C)	1050–1210 ^[36]	152–175
Carbyne (C) ^[37]	32100 ^[38]	4,660

https://en.wikipedia.org/wiki/Young%27s_modulus

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