Chap. 6 Seeing Small Things

6.1 Introduction

- In micro- and nano-technology, the particle sizes are so small that they cannot be seen with the unaided eyes. -> High-powered microscopes – light microscopes, electron microscopes, and scanning probe microscopes – are needed to see cells, molecules, and atoms.

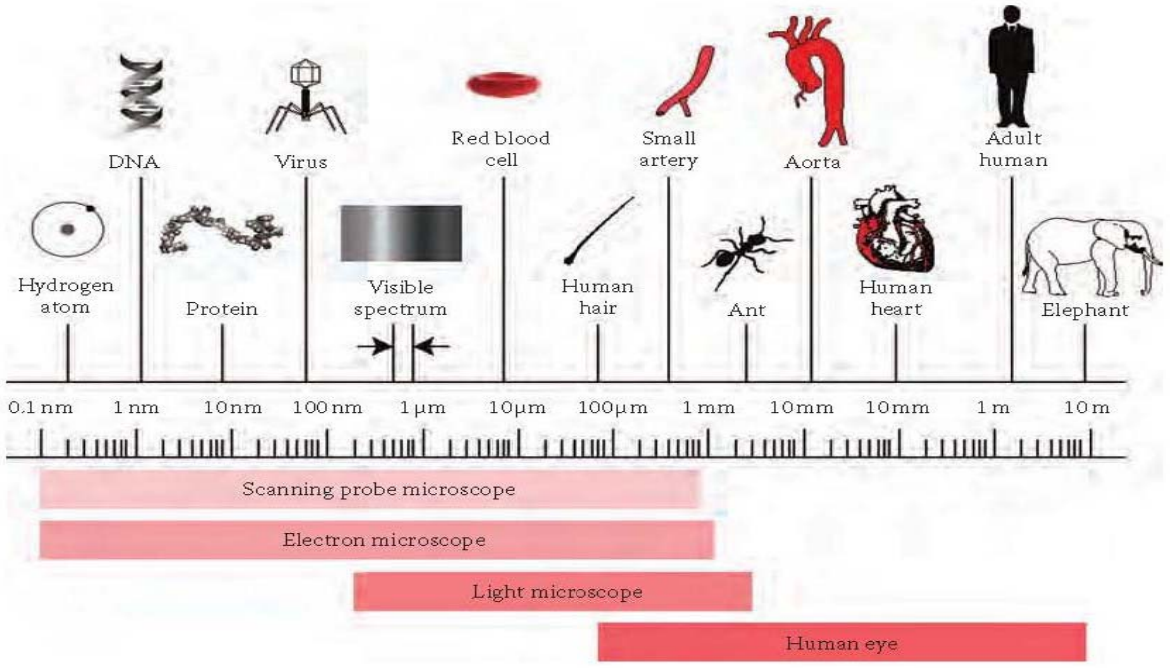

Figure 6.1 Typical size of things and the approximate operating range of various microscopes and the human eye in order to resolve features. The ranges are approximate.

6.2 Human Eye

- The eye has a cornea and lens, iris, retina, and photoreceptors.

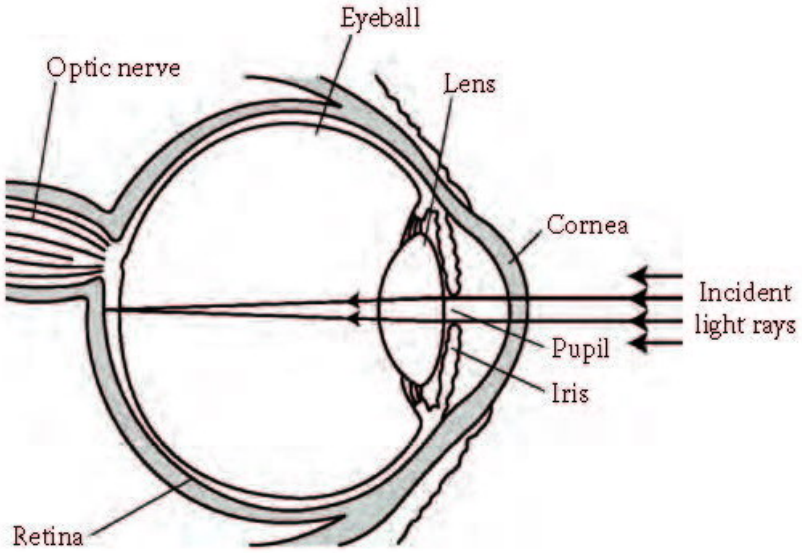

Figure 6.2 Illustration of the human eye anatomy and a light ray that is focused on the retina. National Institutes of Health, Bethesda, MD.]

- The retina contains millions of photoreceptor cells (called cones and rods).

- As light enters the eye, the iris opens and closes to allow a suitable amount of light into pupil, and the iris acts like an aperture in microscopes.

- In the near-sighted (or far-sighted) eye, the image is focused in front of (or behind) the retina.

6.3 Some Microscope Definition

- Wavelength and phase, refractive index, numerical aperture, resolution and resolving power, magnification, depth of field, contrast.

6.3.1 Wavelength and Phase: $\lambda \sim R$

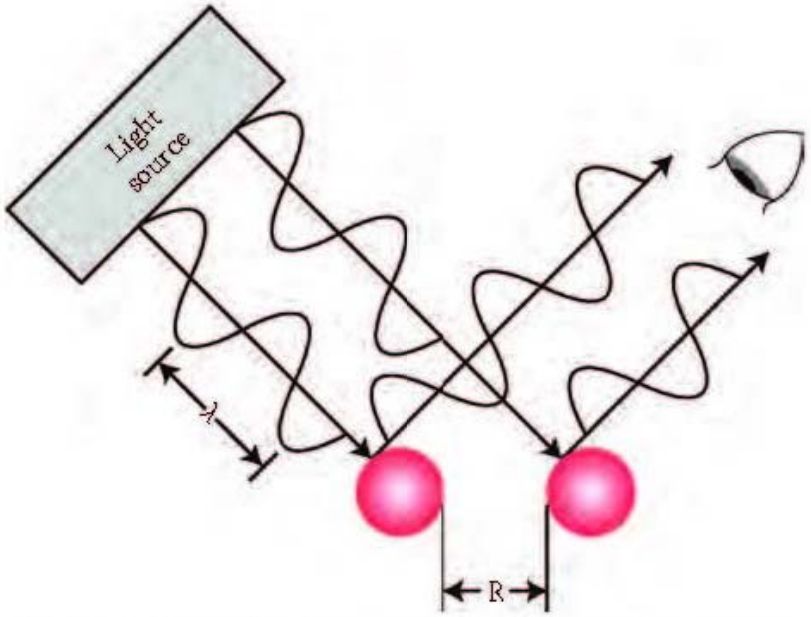

Figure 6.3 Illustration of how a small wavelength is needed to see the separation of two particles. The separation between the particles is the resolution (R). In order to resolve the two particles, the wavelength (λ) must be similar to their spacing (R), or $\lambda \propto R$.

6.3.2 Refractive Index

- Refractive index is the ratio of the speed of light in vacuum to that in another medium.

TABLE 6.1 Refractive Index for Some Common Media Materials

Media	Application	Refractive Index
Vacuum (~air)	Reference medium	1.00[a]
Water	Immersion lithography (electronics)	1.33[a]
Oil	Immersion lithography (electronics)	1.53[b]
Si (silicon)	Photonics (electronics)	4.24[a]
SiO_2 (fused quartz)	Optical glass fibers (telecommunications)	1.46[a]

[a] Reynolds, J., http://vary.info/topics/t0077.asp
[b] 3D Millennium, http://www.m3corp.com/a/tutorials/refraction.htm

6.3.3 Numerical Aperture

- NA = [RI] x sinϕ with ϕ = the angle which the refracted light ray makes with the incident ray when exiting the lens.

*The closer the objective lens is to the focal plane, the larger the refracted angle ϕ becomes.

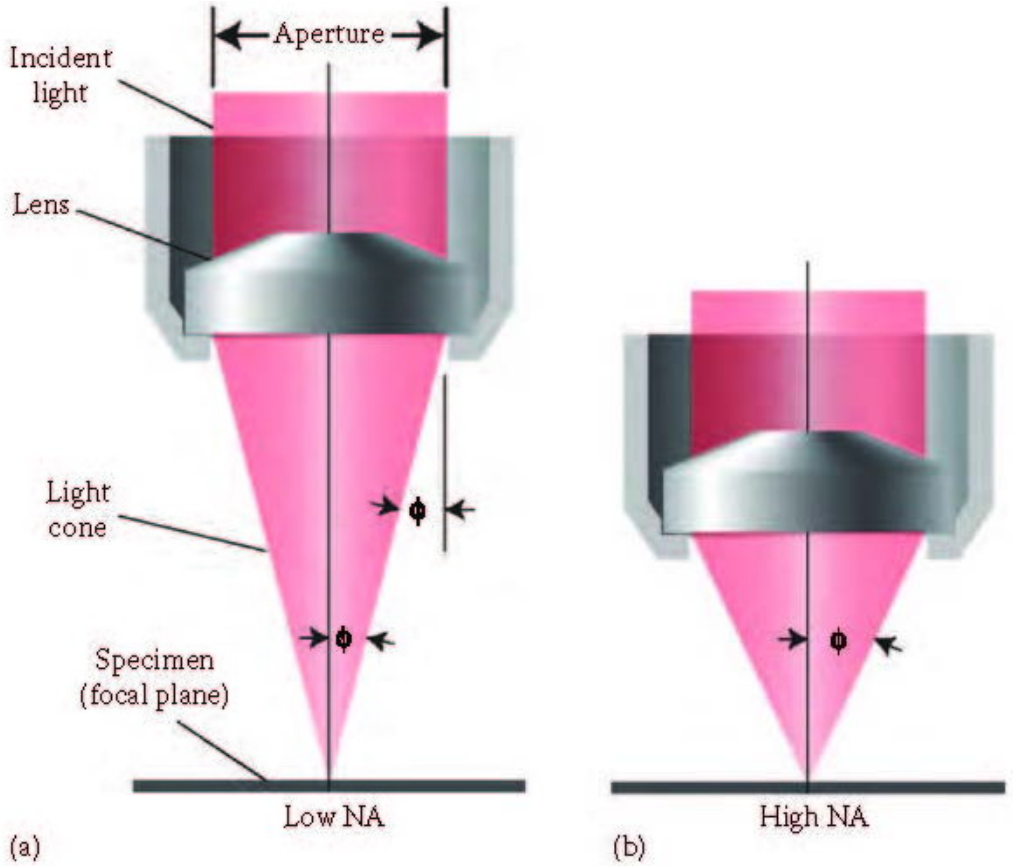

Figure 6.4 Illustrating effect of: (a) lens with low numerical aperture (NA), showing long focal length, small (ϕ) angle, and (b) lens with high numerical aperture (NA), showing shorter focal length and larger (ϕ) angle. [Adapted from Hornyak, G.L. et al., *Introduction to Nanoscience*, p. 118, CRC Press, Boca Raton, FL, 2008.]

6.3.6 Depth of Field

- Depth of field is the range of distance between the object (specimen) and lens (objective lens) where an image of the object is in sharp focus.

*In optical microscopes, higher magnifications of an image have smaller depths of field (2D plane).
*Confocal microscopes and scanning electron microscopes have higher depths of field and display 3D images of objects.

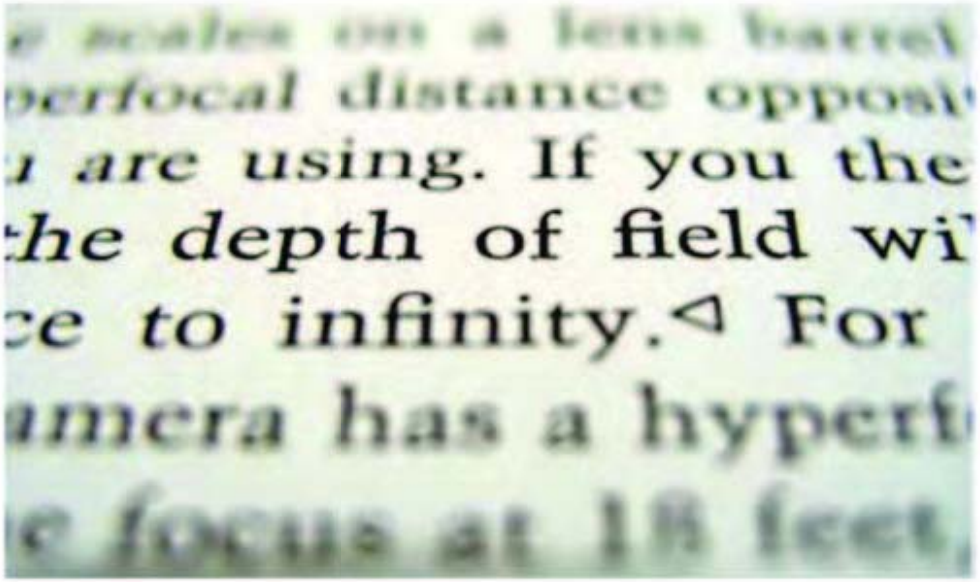

Figure 6.6 Illustration of printed page showing a small depth of field. [Courtesy of Wikimedi

6.4 Light Microscope : Optical, Fluorescent, Confocal

6.4.1 Optical Microscope

- Optical microscopes can provide a maximum magnification of ~ 1500x, resolving bacteria and sizes down to ~ 0.2 um.
- Conventional optical microscopes provide a 2D image with a small depth of field.

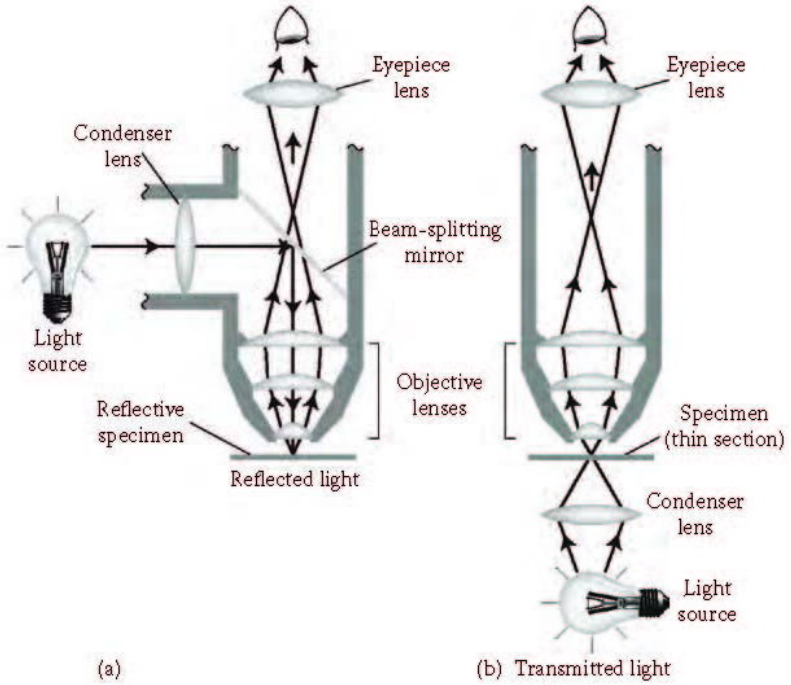

Figure 6.7 Illustration of typical optical microscopes operated under (a) reflected light and (b) transmitted light.

6.4.2 Fluorescent Microscope

- Fluorescent microscope is mainly used in biology to identify biomolecules (like DNA and proteins), organelles, and cells.
- It uses UV light to excite fluorescence of different colored light in order to distinguish different biological features and their location with respect to each other.
- Different fluorescent dyes (called fluorophores) are chemically attached as functional groups to different molecules.
- The fluorophores absorb UV light and re-emit photons at longer wavelengths in visible spectrum.

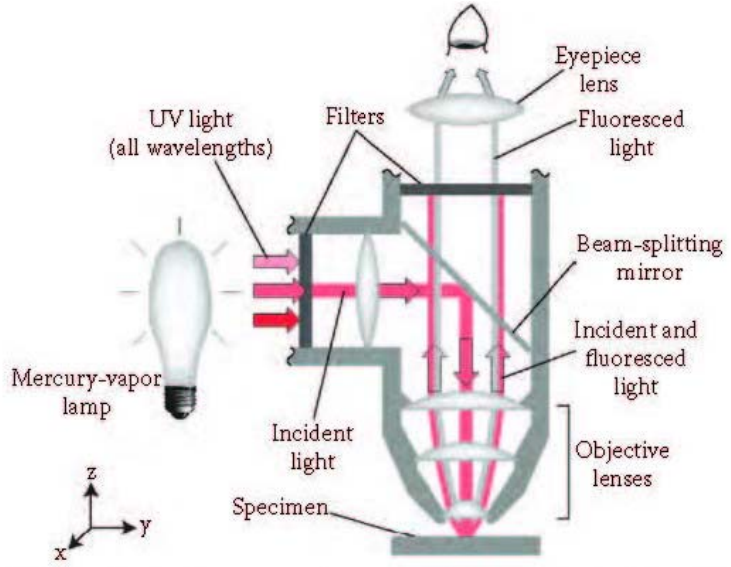

Figure 6.10 Schematic illustration of a fluorescent microscope. The incident light reflected from the specimen is filtered out; so the viewer only sees the fluoresced light. [Adapted from Univ. of Victoria, Epi-fluorescence with the micro-

6.4.3 Confocal Microscope

- Confocal microscope scans the specimen in the *x-y* plane with a laser beam and displays the specimen in a 3D image; laser scanning confocal microscope (LSCM).
- "Confocal": conjugated (or several combined) + focal plane -> several combined focal plane.

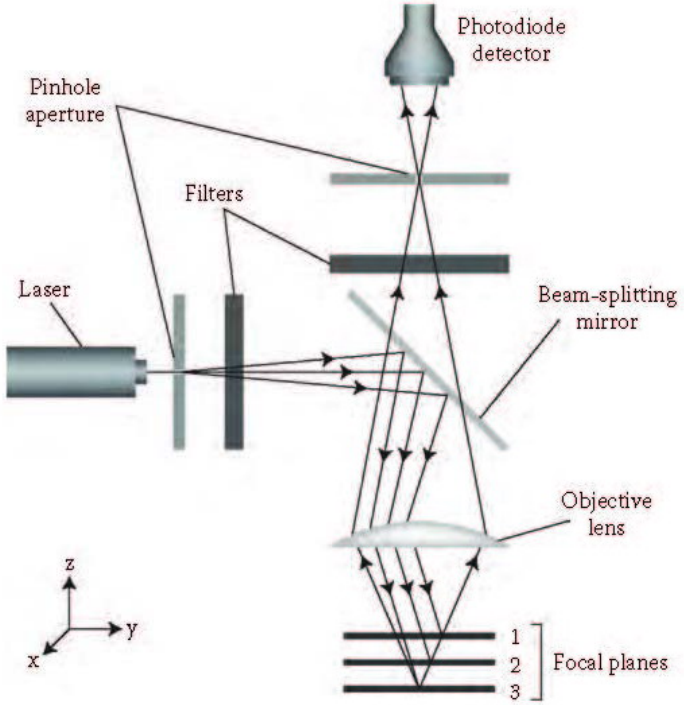

Figure 6.11 Schematic diagram of a confocal microscope and the paths of the incident laser light. Shown are the reflected light rays from three focal planes. Although not shown, the reflected light rays from focal planes 1 and 2 have double reflections as shown from focal plane 3. [Adapted from Olympus, Theory of confocal microscopy;

- A series of “specimen focal planes” that integrated along the *z* axis (*z* axis stacks) -> 3D image.

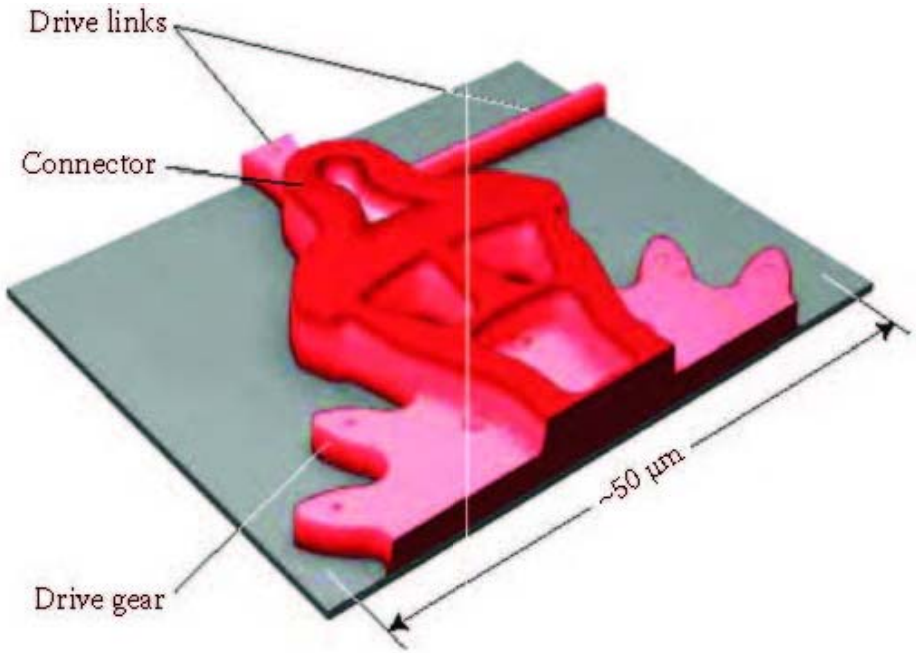

Figure 6.12 Confocal microscope image of micro-gear linkage system (that is shown in Figure 8.14 as SEM and optical microscope images). The drive gear is typically of ~50µm diameter. [Courtesy of Sandia National Laboratories,

6.5 Electron Microscopes : Scanning Electron, Transmission Electron

6.5.1 Scanning Electron Microscope (SEM)

- SEM uses a focused beam of electrons that is scanned in the *x-y* plane of the specimen.
- It exhibits magnification of ~20x - ~200,000x, resolving nano-features greater than ~1-3 nm.
- Modern SEM provides a 3D images of the specimen's surface topography.

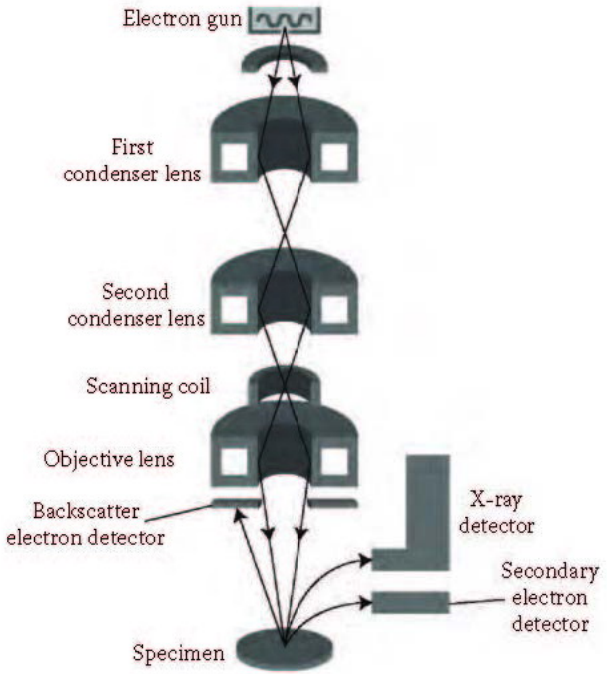

Figure 6.14 Schematic illustration of a SEM. The electron gun, lenses, scanning coil, detectors, and specimen are enclosed in a vacuum chamber (not shown in the illustration).

- The electrons, ejected from a heated (~3000^{0}C) tungsten element in the e-gun, are accelerated under a high voltage (~1 - 30kV).
- When the high energy e-beam collides with the specimen, three common types of radiation are emitted: secondary electrons, back scattered electrons, and x-rays.
  *Secondary electrons are low energy electrons that are knocked off the surface atoms and provide a 3D topographical image of the surface.
  *The x-rays, emitted from the near-surface atoms, provide a microchemical analysis of the elements in the specimen, called energy-dispersive x-ray spectroscopy.

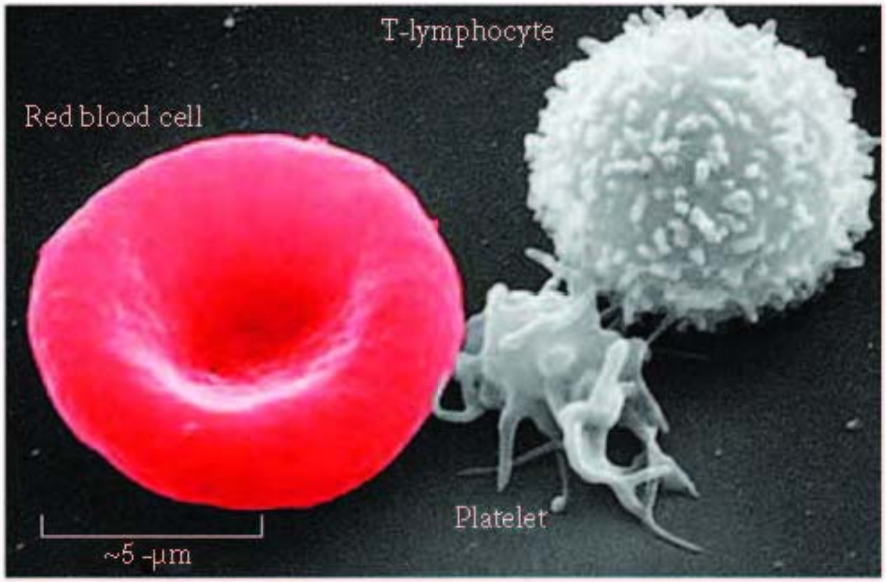

Figure 6.13 SEM image of a human red blood cell (left), platelet (center), and T-lymphocyte (white blood cell, right)

6.5.1 Transmission Electron Microscope (TEM)

- TEM provides higher magnification than SEM and reaches ultimate resolution of ~1,000,000x, resolving atoms at ~0.2 nm.

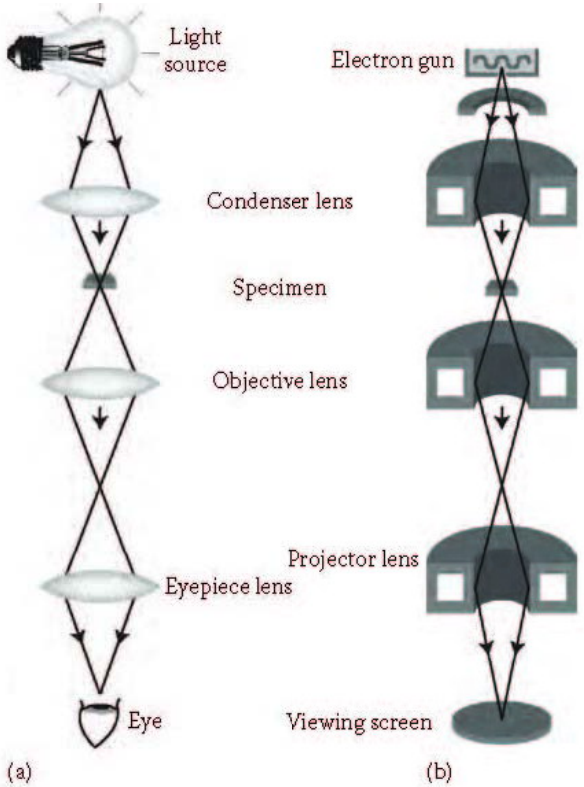

Figure 6.17 Comparison of (a) optical microscope using transmitted light, and (b) TEM. The two microscopes are conceptually designed the same. The optical microscope uses light rays and the TEM uses electrons to resolve features.

- The wavelength of an electron: $\lambda \propto V^{-1/2}$. are It exhibits magnification of ~20x - ~200,000x, *From 10 to 300kV, the wavelength can be reduced from ~12 to ~2 pm (pico-m).
- The practical resolution for TEM is ~0.1 nm, about the bond length between O-H atoms in a water molecule. Silicon has an atomic diameter of ~0.2 nm.
- TEMs typically operate in the range of the acceleration voltage of 50 – 300kV.

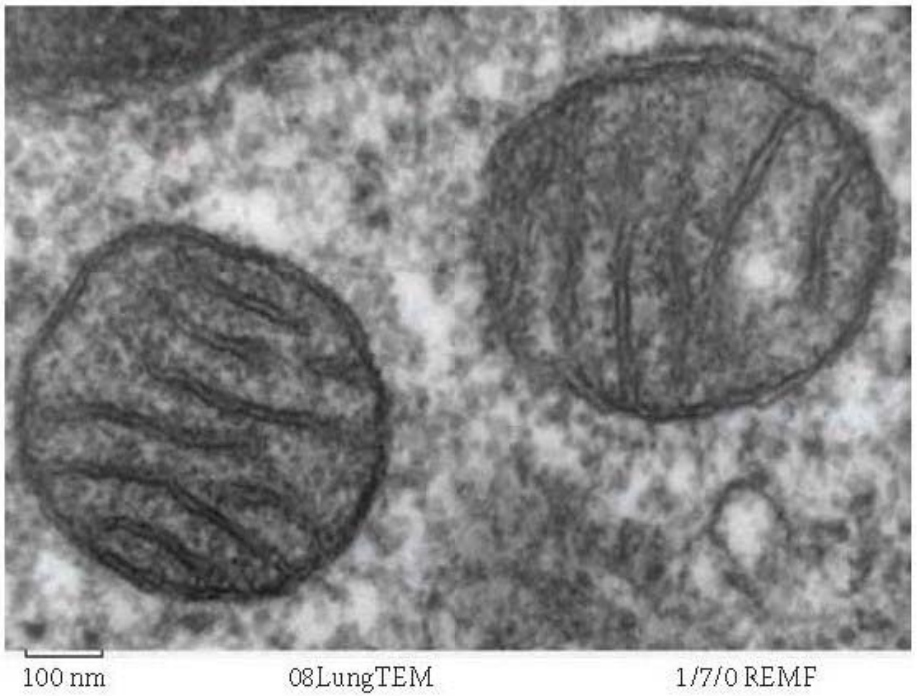

Figure 6.18 Transmission electron micrograph (TEM) of mitochondria from mammalian lung tissue cut through a thin section, showing its matrix and membranes that was cut from a thin section. [Courtesy of Lisa Howard,

6.6 Scanning Probe Microscopes : Scanning Tunneling, Atomic Force

6.6.1 Scanning Tunneling Microscope (STM)

- STM works on the principle of electron tunneling (or flowing) from the surface atoms across a barrier to an atomically sharp probe tip.
  *Probe tips are usually made of tungsten (W), platinum-iridium (Pt-Ir), or gold (Au).
- STM has a resolution of ~0.1 nm and a magnification of ~1,000,000x.
- STM can 1) display the arrangement and topography of surface atoms, 2) move atoms to new locations, and 3) determine wave patterns in the electron structure of atoms.

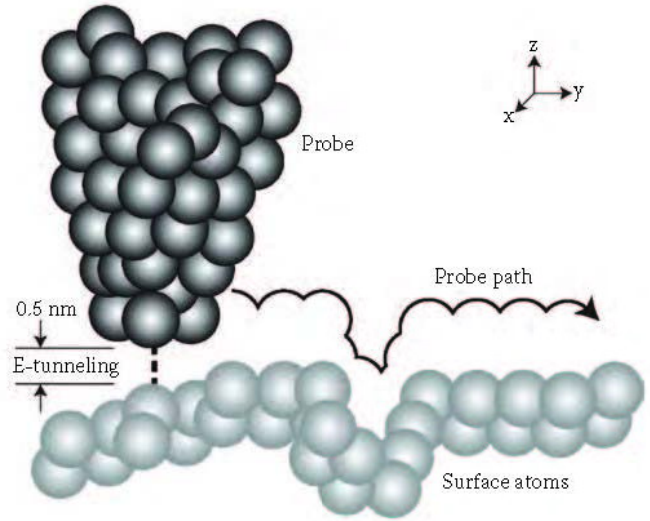

Figure 6.20 Illustration of metal probe of scanning tunneling microscope (STM) moving across a conductive metal surface, showing e-tunneling current (dotted line). There is an applied voltage between the probe and sample surface

- STM has two methods of control: constant current (e-tunneling) and constant height.

  *Constant current: the image appears as light and dark contrast due to the tip height variations. The x-y plane shows the position of atoms and their surface topography. The z-axis shows the vertical contour lines of atoms as they stick out of the surface.

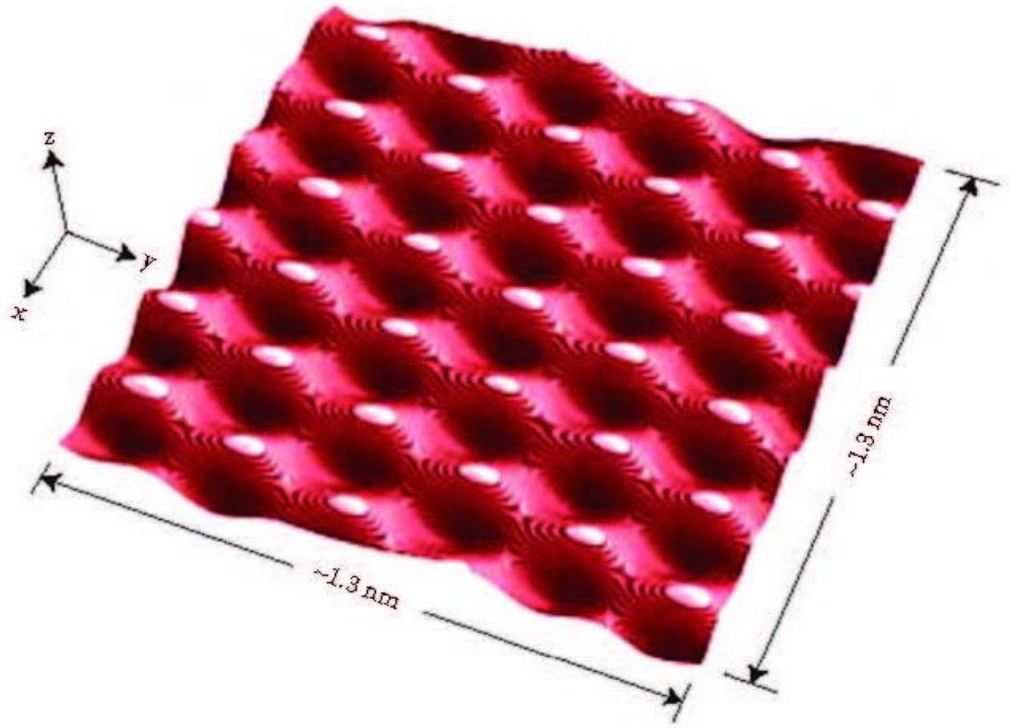

Figure 6.21 STM image showing the carbon atoms in highly-oriented pyrolytic graphite. Scan size: 1.3 nm × 1.3 nm.

*Constant height: the variation in current produces a 3D image of the e-waves
Quantum corral is confining of electrons into an artificial nano-size structure.
*The surface electron states on Cu were confined to a closed structure (corral) that were defined by barriers built from Fe adatoms (adsorbed atoms).

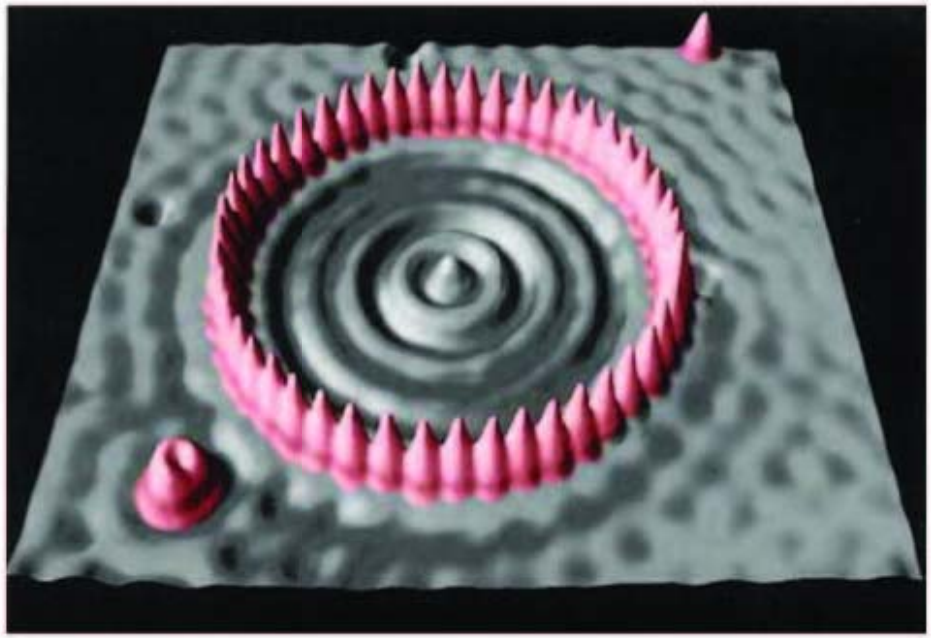

Figure 6.22 STM image of 48 iron atoms in a circular ring on copper surface. [From IBM

[Question] How can STM move an atom from one position to another on a substrate?

- The probe tip must break the atomic bonds at one location, grab the atom, transport it, and then reform the bonds at a new position.

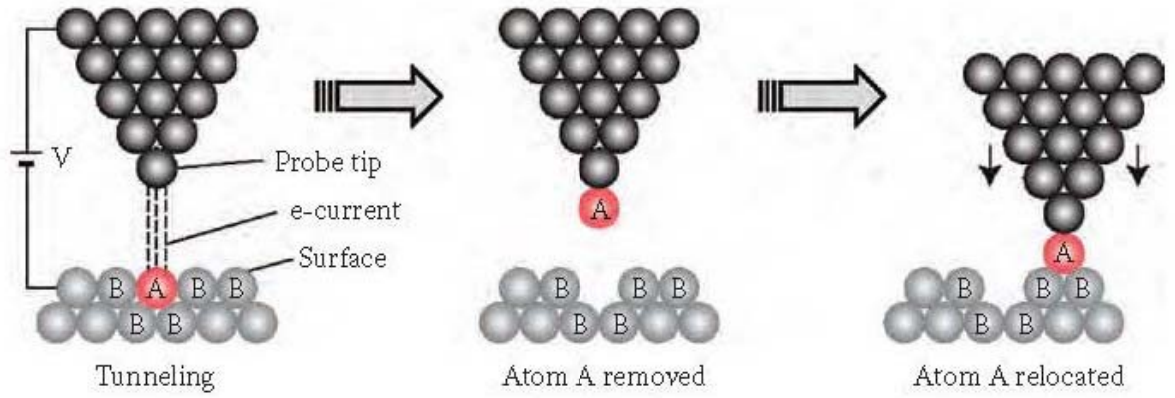

Figure 6.23 Two dimensional illustration of probe tip of STM approaching surface atom A under an applied voltage, enabling tunneling (e-current flow). Atom A is removed and transported to another position on the surface.

6.6.2 Atomic Force Microscope (AFM)

- STM cannot be used to resolve the surface structures of non-conductive specimens like most biological materials, polymers, and ceramics.
- AFM utilizes a flexible cantilever with a sharp probe tip mounted on its end.
  *The probe tip mechanically scans the surfaces of nano-structural and micro-structural features and maps their topography.
- AFM has a resolution of ~0.1 um, similar to the STM and TEM.

*Probe tips come in a variety of different materials – silicon, silicon nitride, diamond, and carbon nanotubes.
*Typical radius of the probe tip is 5 – 15 nm, and the probe height usually varies between 10 and 20 um.
*Light from a laser is reflected off the shinny top surface of a cantilever and photons of light are collected by a photodetector. As the probe tips scans the surface, the hills and valleys of the specimen cause the cantilever to deflect up and down along the z axis.

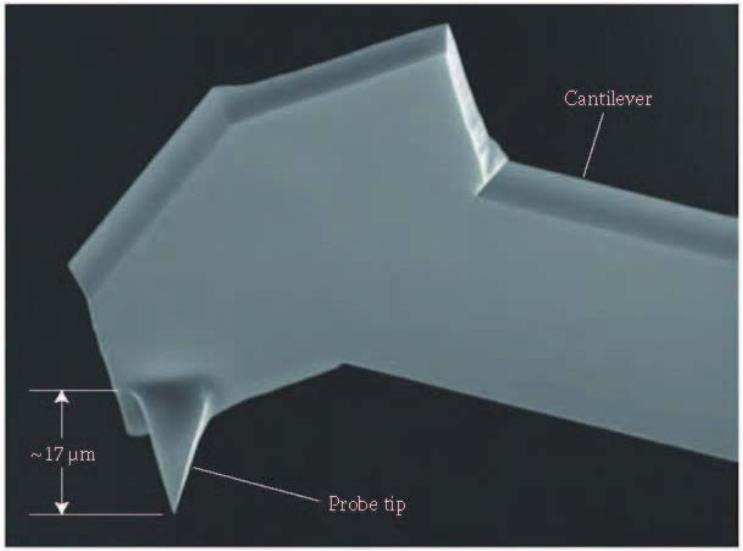

Figure 6.24 SEM image of a torsionally coupled cantilever and probe tip that is used for HarmoniX™ imaging with an atomic force microscope. [Courtesy of Veeco Instruments Inc., Woodbury, NY.]

- Reflected light from the cantilever surface is converted into an electronic image, which is displayed as contrast in the z axis. The tip-specimen interaction force is measured by cantilever deflection.

*The shape of the cancer cell is distinguished by the ARM x-y scan and the topography of the cell is distinguished by the z axis (out-of-paper) contrast.

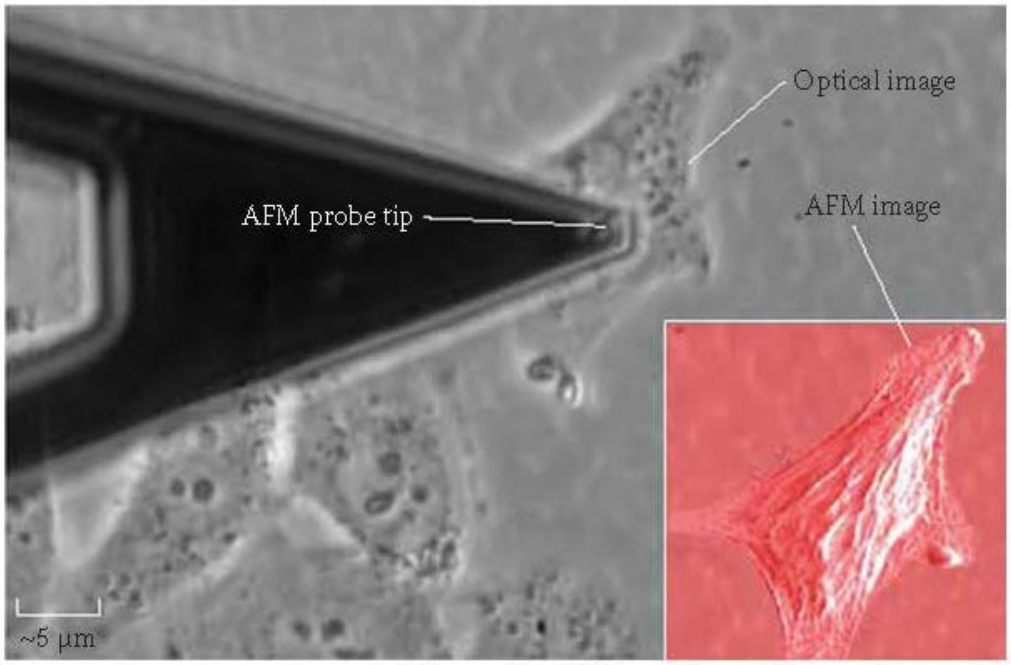

Figure 6.25 Micrograph shows the top-view optical image of an AFM cantilever probe scanning a cancer cell (colored in red). The cancer cell is shown in two images: 2-D optical image and a superimposed 3-D AFM image of the same cancer cell. [Courtesy of Asylum Research Corporation, Goleta, CA.]

- Tip-specimen interaction forces and operation modes of AFM – contact, tapping, non-contact.

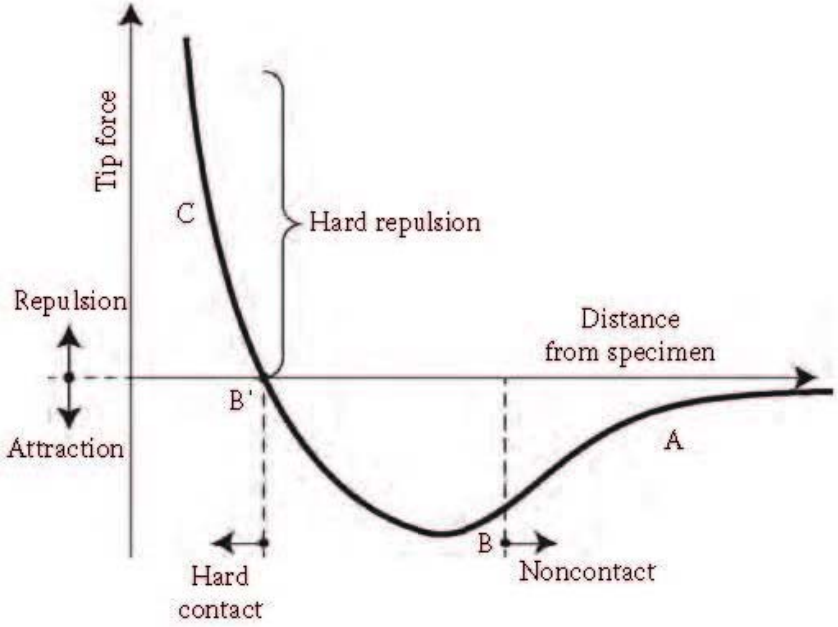

Figure 6.26 Illustration of AFM probe tip force versus tip distance from specimen in contact mode. Contact occurs between B and B′. Hard contact occurs at distances less than B′. Noncontact occurs for distances greater than B.

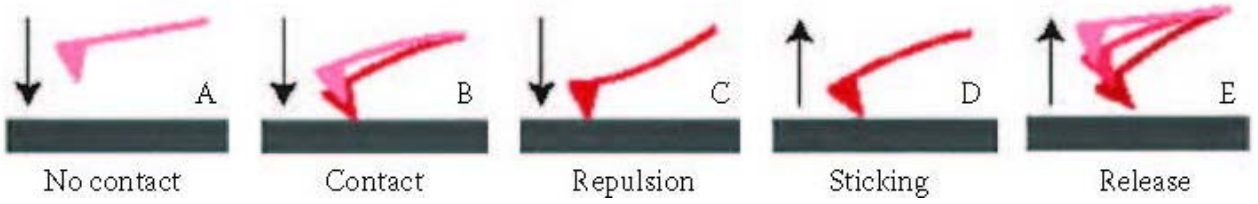

TABLE 6.2 AFM Mode of Operation, Imaging, and Examples of Use

Mode of Operation	AFM Imaging	Examples of Use
Contact	Repulsive regime; hard contact. Imaging by the z-axis cantilever deflection	Solid surfaces of insulators, conductors, semiconductors
Tapping	Intermittent contact and noncontact. Amplitude imaging and phase imaging	Soft or weakly bonded specimens (DNA, proteins)
Noncontact	Attractive regime—no tip–specimen contact. Imaging by cantilever deflection	Soft specimens in aqueous solution

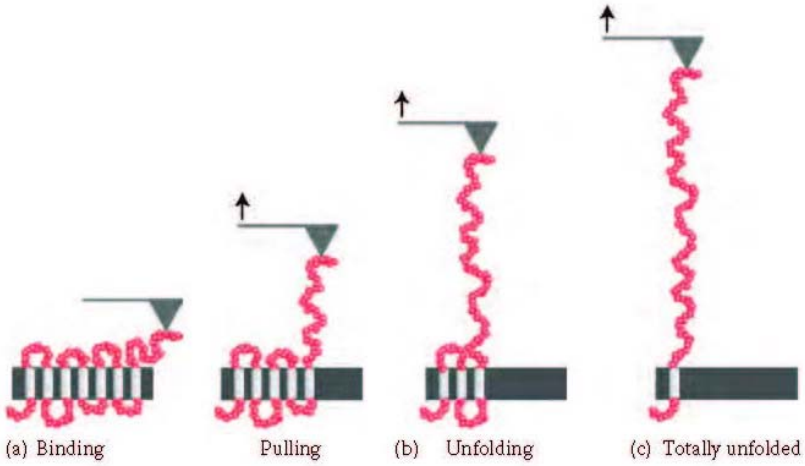

Figure 6.29 Illustration of coiled protein (inside scaffold) being unfolded by AFM probe tip. Image shows (a) binding probe tip to protein, (b) exerting force to pull protein, and protein starts to unfold. (c) Protein is totally unfolded. [Courtesy of Veeco Instruments Inc., Woodbury, NY.]

6.7 Key Concepts Scanning Probe Microscopes : Scanning Tunneling, Atomic Force

- Differences and similarities among the microscopes

TABLE 6.3 Summary Comparison of the Different Microscopes

Microscope	Imaging Method	Magnification (Ultimate)	Resolution (Smallest)	Image	Advantage(s)
Light	Light reflection or transmission	~1,500×	0.2–0.5-μm	2-D surface or thin section	Fast, ease of use and interpretation
Fluorescent	Arc-lamp emitting light from dyes	~1,500× (max)	0.2–0.5 μm	2-D secondary fluorescence	2-D identification of features
Confocal	Laser fluorescence	~1,500× (max)	~0.2 μm	3-D secondary fluorescence	3-D identification of features
SEM	Electrons from surface and layers	~200,000×	~2 nm	3-D surface topography	High depth of field, ease of use
TEM	E-transmission through thin sections	~1,000,000×	~0.1 nm	2-D thin section of material	High magnification of thin layers
STM	E-tunneling from surface atoms	~1,000,000×	~0.1 nm	3-D surface and e-waves	Viewing metals, moving atoms
AFM	Deflection of cantilever	~1,000,000×	~0.1 nm	3-D surface topography	Viewing non-metals, molecules