How Cells Are Studied

4

- Looking at the Structure of Cells in the Microscope
- Isolating Cells and Growing Them in Culture
- Fractionation of Cells and Analysis of Their Molecules
- Tracing and Assaying Molecules Inside Cells

Cells are small and complex. It is hard to see their structure, hard to discover their molecular composition, and harder still to find out how their various components function. What we can learn about cells depends on the tools at our disposal, and major advances in cell biology have frequently sprung from the introduction of new techniques. To understand contemporary cell biology, therefore, it is necessary to know something of its methods.

In this chapter we briefly review some of the principal methods used to study cells. We start with techniques for examining the cell as a whole and then proceed to techniques for analyzing its constituent macromolecules. Microscopy will be our starting point, for cell biology began with the light microscope, and this is still an essential tool in the field, along with more recent imaging devices based on beams of electrons and other forms of radiation. From passive observation we move to active intervention: we consider how cells of different types can be separated from tissues and grown outside the body and how cells can be disrupted and their organelles and constituent macromolecules isolated in pure form. Finally, we describe how we can detect, follow, and quantify individual types of molecules and ions within the cell. A revolution in our understanding of cellular function has come from recombinant DNA technology, but because it is a complex subject in itself and depends on an understanding of basic genetic mechanisms, this powerful array of methods will be considered in detail in Chapter 7.

Although methods are of basic importance, it is what we discover with them that makes them interesting. The present chapter, therefore, is meant to be used for reference and to be read in conjunction with the later chapters of the book, rather than as an introduction to them.

Looking at the Structure of Cells in the Microscope [1]

A typical animal cell is 10 to 20 μm in diameter, which is about five times smaller than the smallest particle visible to the naked eye. It was not until good light microscopes became available in the early part of the nineteenth century that all plant and animal tissues were discovered to be aggregates of individual cells. This discovery, proposed as the **cell doctrine** by Schleiden and Schwann in 1838, marks the formal birth of cell biology.

Animal cells are not only tiny, they are also colorless and translucent. Consequently, the discovery of their main internal features depended on the development, in the latter part of the nineteenth century, of a variety of stains that provided sufficient contrast to make those features visible. Likewise, introduction of the far more powerful electron microscope in the early 1940s required the development of new techniques for preserving and staining cells before the full complexities of their internal fine structure could begin to emerge. To this day, microscopy depends as much on techniques for preparing the specimen as on the performance of the microscope itself. In the discussions that follow, we therefore consider both instruments and specimen preparation, beginning with the light microscope.

Figure 4–1 shows the fineness of detail that can be resolved with modern light microscopes, in comparison with electron microscopes. Some of the landmarks in the development of light microscopy are outlined in Table 4–1.

Table 4–1 Some Important Discoveries in the History of Light Microscopy

1611 **Kepler** suggested a way of making a compound microscope.

1655 **Hooke** used a compound microscope to describe small pores in sections of cork that he called "cells."

1674 **Leeuwenhoek** reported his discovery of protozoa. He saw bacteria for the first time nine years later.

1833 **Brown** published his microscopic observations of orchids, clearly describing the cell nucleus.

1838 **Schleiden** and **Schwann** proposed the cell theory, stating that the nucleated cell is the unit of structure and function in plants and animals.

1857 **Kolliker** described mitochondria in muscle cells.

1876 **Abbé** analyzed the effects of diffraction on image formation in the microscope and showed how to optimize microscope design.

1879 **Flemming** described with great clarity chromosome behavior during mitosis in animal cells.

1881 **Retzius** described many animal tissues with a detail that has not been surpassed by any other light microscopist. In the next two decades he, **Cajal**, and other histologists developed staining methods and laid the foundations of microscopic anatomy.

1882 **Koch** used aniline dyes to stain microorganisms and identified the bacteria that cause tuberculosis and cholera. In the following two decades other bacteriologists, such as **Klebs** and **Pasteur**, identified the causative agents of many other diseases by examining stained preparations under the microscope.

1886 **Zeiss** made a series of lenses, to the design of **Abbé**, that enabled microscopists to resolve structures at the theoretical limits of visible light.

1898 **Golgi** first saw and described the Golgi apparatus by staining cells with silver nitrate.

1924 **Lacassagne** and collaborators developed the first autoradiographic method to localize radioactive polonium in biological specimens.

1930 **Lebedeff** designed and built the first interference microscope. In 1932 **Zernicke** invented the phase-contrast microscope. These two developments allowed unstained living cells to be seen in detail for the first time.

1941 **Coons** used antibodies coupled to fluorescent dyes to detect cellular antigens.

1952 **Nomarski** devised and patented the system of differential interference contrast for the light microscope that still bears his name.

1981 **Allen** and **Inoué** perfected video-enhanced-contrast light microscopy.

1988 Commercial confocal scanning microscopes came into widespread use.

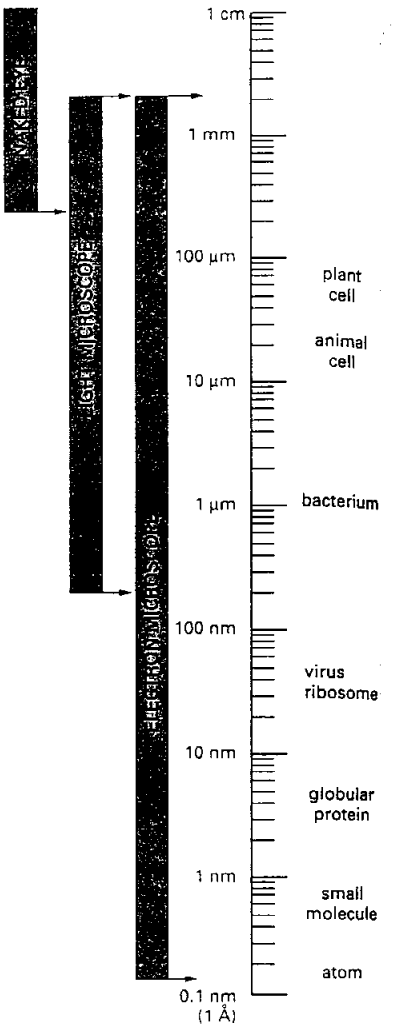


Figure 4–1 Resolving power. Sizes of cells and their components drawn on a logarithmic scale, indicating the range of objects that can be readily resolved by the naked eye and in the light and electron microscopes. The following units of length are commonly employed in microscopy:

µm (micrometer) = 10^{-6} m
nm (nanometer) = 10^{-9} m
Å (Ångström unit) = 10^{-10} m

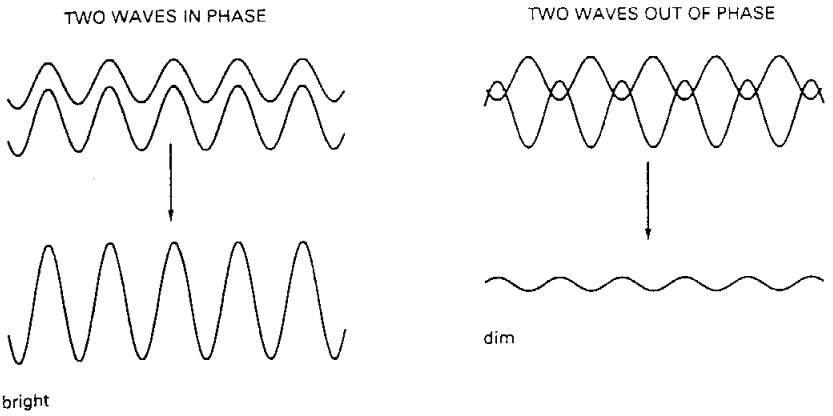


Figure 4–2 Interference between light waves. When two light waves combine *in phase*, the amplitude of the resultant wave is larger and the brightness is increased. Two light waves that are *out of phase* partially cancel each other and produce a wave whose amplitude, and therefore brightness, is decreased.

The Light Microscope Can Resolve Details 0.2 μm Apart [2]

In general, a given type of radiation cannot be used to probe structural details much smaller than its own wavelength. This is a fundamental limitation of all microscopes. The ultimate limit to the resolution of a light microscope, therefore, is set by the wavelength of visible light, which ranges from about 0.4 μm (for violet) to 0.7 μm (for deep red). In practical terms, bacteria and mitochondria, which are about 500 nm (0.5 μm) wide, are generally the smallest objects whose shape can be clearly discerned in the light microscope; details smaller than this are obscured by effects resulting from the wave nature of light. To understand why this occurs, we must follow what happens to a beam of light waves as it passes through the lenses of a microscope.

Because of its wave nature, light does not follow exactly the idealized straight ray paths predicted by geometrical optics. Instead, light waves travel through an optical system by a variety of slightly different routes, so that they interfere with one another and cause *optical diffraction* effects. If two trains of waves reaching the same point by different paths are precisely in phase, with crest matching crest and trough matching trough, they will reinforce each other so as to increase brightness. On the other hand, if the trains of waves are out of phase, they will interfere with each other in such a way as to cancel each other partially or entirely (Figure 4–2). The interaction of light with an object will change the phase relationships of the light waves in a way that produces complex interference effects. At high magnification, for example, the shadow of a straight edge that is evenly illuminated with light of uniform wavelength appears as a set of parallel lines, whereas that of a circular spot appears as a set of concentric rings (Figure 4–3). For the same reason, a single point seen through a microscope appears as a blurred disc, and two point objects close together give overlapping images and may merge into one. No amount of refinement of the lenses can overcome this limitation imposed by the wavelike nature of light.

The limiting separation at which two objects can still be seen as distinct—the so-called **limit of resolution**—depends on both the wavelength of the light and the numerical aperture of the lens system used (Figure 4–4). Under the best conditions, with violet light (wavelength, $\lambda = 0.4$ μm) and a numerical aperture of 1.4, a limit of resolution of just under 0.2 μm can theoretically be obtained in the light microscope. This resolution was achieved by microscope makers at the end of the nineteenth century and is only rarely matched in contemporary, factory-produced microscopes. Although it is possible to *enlarge* an image as much as one wants—for example, by projecting it onto a screen—it is never possible to resolve two objects in the light microscope that are separated by less than about 0.2 μm: such objects will appear as one.

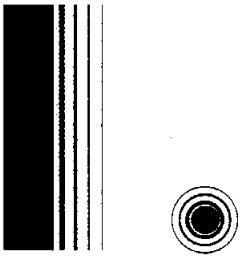


Figure 4–3 Edge effects. The interference effects observed at high magnification when light passes the edges of a solid object placed between the light source and the observer.

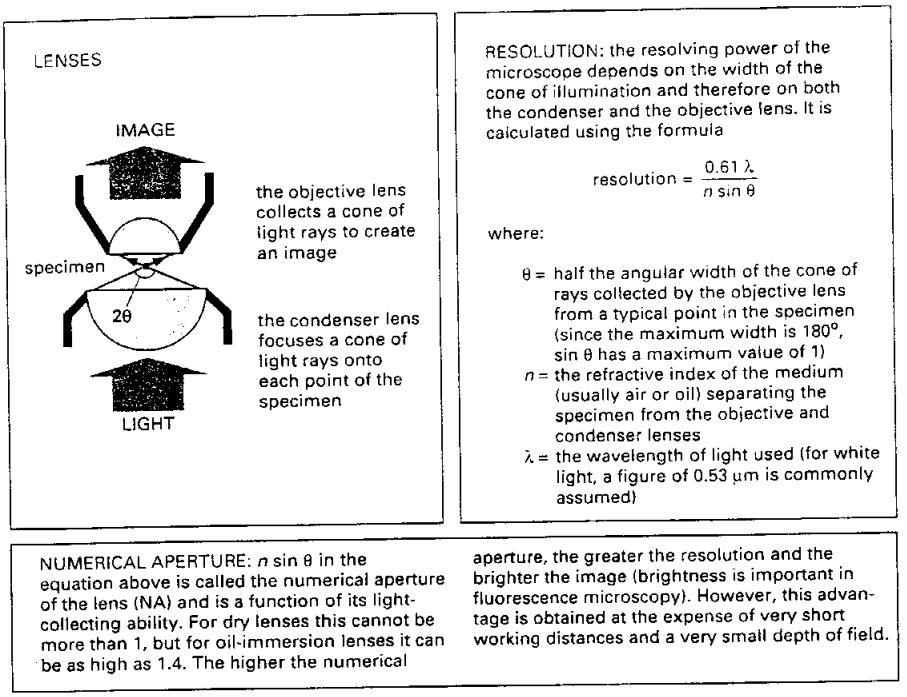


Figure 4–4 Numerical aperture. The path of light rays passing through a transparent specimen in a microscope, illustrating the concept of numerical aperture and its relation to the limit of resolution.

We shall see later how interference and diffraction can be exploited to study unstained cells in the living state. First we discuss how permanent preparations of cells are made for viewing in the light microscope and how chemical stains are used to enhance the visibility of the cell structures in such preparations.

Tissues Are Usually Fixed and Sectioned for Microscopy

To make a permanent preparation that can be stained and viewed at leisure in the microscope, one first must treat cells with a fixative so as to immobilize, kill, and preserve them. In chemical terms, **fixation** makes cells permeable to staining reagents and cross-links their macromolecules so that they are stabilized and locked in position. Some of the earliest fixation procedures involved immersion in acids or in organic solvents, such as alcohol. Current procedures usually include treatment with reactive aldehydes, particularly formaldehyde and glutaraldehyde, which form covalent bonds with the free amino groups of proteins and thereby cross-link adjacent proteins.

Most tissue samples are too thick for their individual cells to be examined directly at high resolution. After fixation, therefore, the tissues are usually cut into very thin slices (**sections**) with a *microtome*, a machine with a sharp metal blade that operates like a meat slicer (Figure 4–5). The sections (typically 1 to 10 μm thick) are then laid flat on the surface of a glass microscope slide.

Tissues are generally soft and fragile, even after fixation, and need to be **embedded** in a supporting medium before sectioning. The usual embedding media are waxes or resins. In liquid form these media will both permeate and surround the fixed tissue; they then can be hardened (by cooling or by polymerization) to a solid block, which is readily sectioned by the microtome.

There is a serious danger that any treatment used for fixation and embedding may alter the structure of the cell or its constituent molecules in undesirable ways. Rapid freezing provides an alternative method of preparation that to some extent avoids this problem by eliminating the need for fixation and embedding. The frozen tissue can be cut directly with a cryostat—a special microtome that is maintained in a cold chamber. Although **frozen sections** produced in this

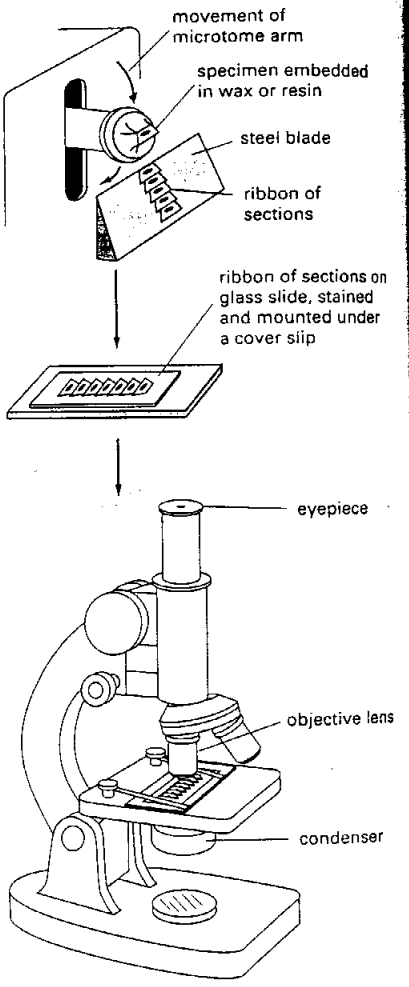


Figure 4–5 Making tissue sections. How an embedded tissue is sectioned with a microtome in preparation for examination in the light microscope.

way avoid some artifacts, they suffer from others: the native structures of individual molecules such as proteins are well preserved, but the fine structure of the cell is often disrupted by ice crystals.

Once sections have been cut, by whatever method, the next step is usually to stain them.

Different Components of the Cell Can Be Selectively Stained [3]

There is little in the contents of most cells (which are 70% water by weight) to impede the passage of light rays. Thus most cells in their natural state, even if fixed and sectioned, are almost invisible in an ordinary light microscope. One way to make them visible is to stain them with dyes.

In the early nineteenth century the demand for dyes to stain textiles led to a fertile period for organic chemistry. Some of the dyes were found to stain biological tissues and, unexpectedly, often showed a preference for particular parts of the cell—the nucleus or mitochondria, for example—making these internal structures clearly visible. Today a rich variety of organic dyes is available, with such colorful names as *Malachite green, Sudan black,* and *Coomassie blue,* each of which has some specific affinity for particular subcellular components. The dye *hematoxylin,* for example, has an affinity for negatively charged molecules and therefore reveals the distribution of DNA, RNA, and acidic proteins in a cell (Figure 4–6). The chemical basis for the specificity of many dyes, however, is not known.

The relative lack of specificity of these dyes at the molecular level has stimulated the design of more rational and selective staining procedures and, in particular, of methods that reveal specific proteins or other macromolecules in cells. It is a problem, however, to achieve adequate sensitivity for this purpose. Since relatively few copies of most macromolecules are present in any given cell, one or two molecules of stain bound to each macromolecule will often be invisible. One way to solve this problem is to increase the number of stain molecules associated with a single macromolecule. Thus some enzymes can be located in cells through their catalytic activity: when supplied with appropriate substrate molecules, each enzyme molecule generates many molecules of a localized, visible reaction product. An alternative and much more generally applicable approach to the problem of sensitivity depends on using dyes that are fluorescent, as we explain next.

Figure 4–6 A stained tissue section. A section of thick human skin, stained with a combination of dyes, hematoxylin and eosin, that is commonly used in histology.

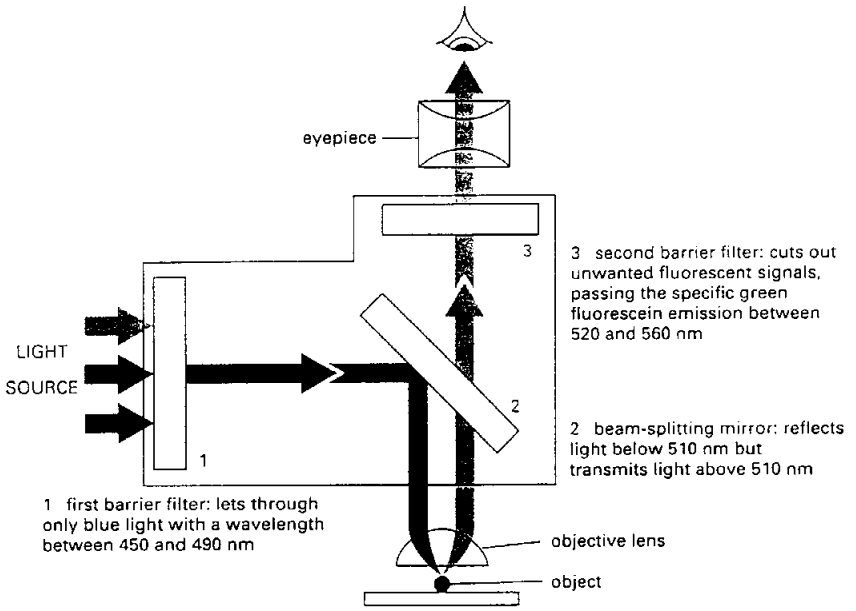


Figure 4–7 The optical system of a modern fluorescence microscope. A filter set consists of two barrier filters (1 and 3) and a dichroic (beam-splitting) mirror (2). In this example the filter set for detection of the fluorescent molecule fluorescein is shown. High-numerical-aperture objective lenses are especially important in this type of microscopy since, for a given magnification, the brightness of the fluorescent image is proportional to the fourth power of the numerical aperture (see also Figure 4–4).

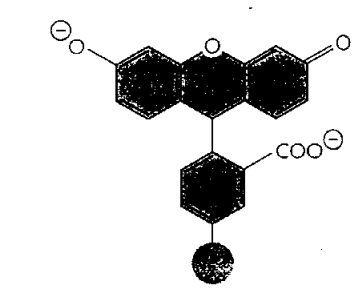
Specific Molecules Can Be Located in Cells by Fluorescence Microscopy [4]

Fluorescent molecules absorb light at one wavelength and emit it at another, longer wavelength. If such a compound is illuminated at its absorbing wavelength and then viewed through a filter that allows only light of the emitted wavelength to pass, it is seen to glow against a dark background. Because the background is dark, even a minute amount of the glowing fluorescent dye can be detected. The same number of molecules of an ordinary stain viewed conventionally would be practically invisible because they would give only the faintest tinge of color to the light transmitted through this stained part of the specimen.

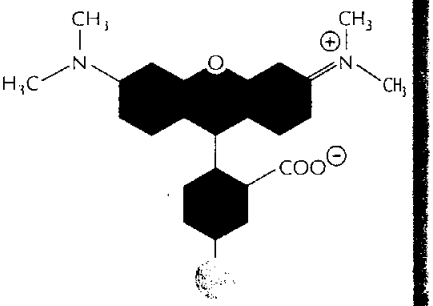
The fluorescent dyes used for staining cells are detected with the help of a **fluorescence microscope.** This microscope is similar to an ordinary light microscope except that the illuminating light, from a very powerful source, is passed through two sets of filters—one to filter the light before it reaches the specimen and one to filter the light obtained from the specimen. The first filter is selected so that it passes only the wavelengths that excite the particular fluorescent dye, while the second filter blocks out this light and passes only those wavelengths emitted when the dye fluoresces (Figure 4–7).

Fluorescence microscopy is most often used to detect specific proteins or other molecules in cells and tissues. A very powerful and widely used technique is to couple fluorescent dyes to antibody molecules, which then serve as highly specific and versatile staining reagents that bind selectively to the particular macromolecules that they recognize in cells or in the extracellular matrix. Two fluorescent dyes that are commonly used for this purpose are *fluorescein,* which emits an intense green fluorescence when excited with blue light, and *rhodamine,* which emits a deep red fluorescence when excited with green-yellow light (Figure 4–8). By coupling one antibody to fluorescein and another to rhodamine, the distributions of different molecules can be compared in the same cell; the two molecules are visualized separately in the microscope by switching back and forth between two sets of filters, each specific for one dye. As shown in Figure 4–9, three fluorescent dyes can be used in the same way to distinguish three types of molecules in the same cell.

Important new methods, to be discussed later, enable fluorescence microscopy to be used to monitor changes in the concentration and location of specific molecules inside *living* cells (see p. 183).



fluorescein (green)



tetramethylrhodamine (red)

Figure 4–8 Fluorescent dyes. The structures of fluorescein and tetramethylrhodamine, two dyes that are commonly used for fluorescence microscopy. Fluorescein emits green light when activated by light of the appropriate wavelength, whereas the rhodamine dye emits red light. The portion of each molecule shown in *orange* denotes the position of a chemically reactive group; at this position a covalent bond is commonly formed between the dye and a protein (or other molecule). Commercially available versions of these dyes with different types of reactive groups allow the dye to be coupled either to an —SH group or to an $-NH_2$ group on a protein.

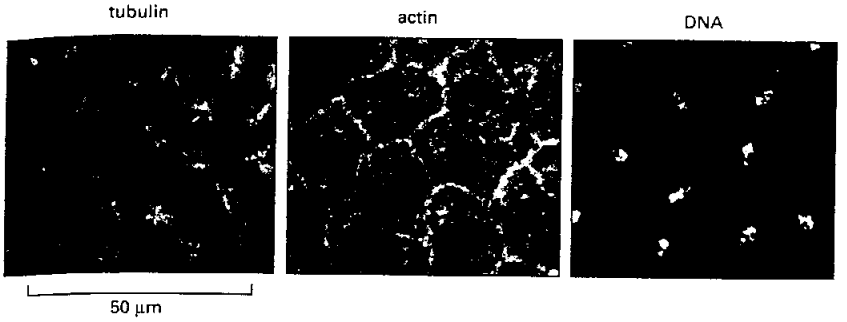


Figure 4–9 Fluorescence microscopy. Micrographs of a portion of the surface of an early *Drosophila* embryo in which the microtubules have been labeled with an antibody coupled to fluorescein (*left panel*) and the actin filaments have been labeled with an antibody coupled to rhodamine (*middle panel*). In addition, the chromosomes have been labeled with a third dye that fluoresces only when it binds to DNA (*right panel*). At this stage, all the nuclei of the embryo share a common cytoplasm, and they are in the metaphase stage of mitosis. The three micrographs were taken of the same region of a fixed embryo using three different filter sets in the fluorescence microscope (see also Figure 4–7). (Courtesy of Tim Karr.)

Living Cells Are Seen Clearly in a Phase-Contrast or a Differential-Interference-Contrast Microscope [2, 5]

The possibility that some components of the cell may be lost or distorted during specimen preparation has always worried microscopists. The only certain way to avoid the problem is to examine cells while they are alive, without fixing or freezing. For this purpose light microscopes with special optical systems are especially useful.

When light passes through a living cell, the phase of the light wave is changed according to the cell's refractive index: light passing through a relatively thick or dense part of the cell, such as the nucleus, is retarded; its phase, consequently, is shifted relative to light that has passed through an adjacent thinner region of the cytoplasm. Both the **phase-contrast microscope** and the **differential-interference-contrast microscope** exploit the interference effects produced when these two sets of waves recombine, thereby creating an image of the cell's structure (Figure 4–10). Both types of light microscopy are widely used to visualize living cells.

A simpler way to see some of the features of a living cell is to observe the light that is scattered by its various components. In the **dark-field microscope** the illuminating rays of light are directed from the side so that only scattered light enters the microscope lenses. Consequently, the cell appears as an illuminated object against a black background. Images of the same cell obtained by four kinds of light microscopy are shown in Figure 4–11.

One of the great advantages of phase-contrast, differential-interference-contrast, and dark-field microscopy is that each makes it possible to watch the movements involved in such processes as mitosis and cell migration. Since many cel-

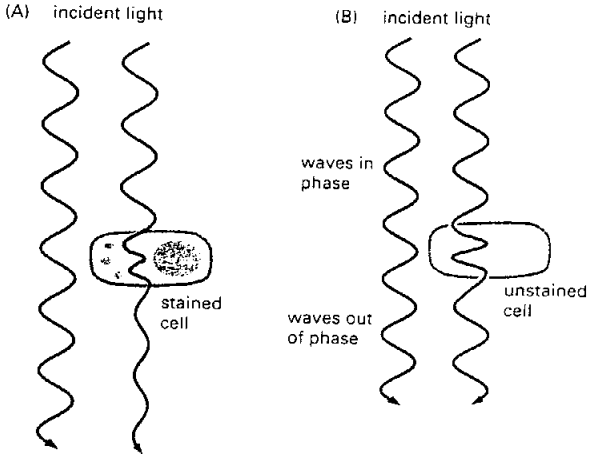


Figure 4–10 Two ways to obtain contrast in light microscopy. The stained portions of the cell in (A) reduce the amplitude of light waves of particular wavelengths passing through them. A colored image of the cell is thereby obtained that is visible in the ordinary way. Light passing through the unstained, living cell (B) undergoes very little change in amplitude, and the structural details cannot be seen even if the image is highly magnified. The *phase* of the light, however, is altered by its passage through the cell, and small phase differences can be made visible by exploiting interference effects using a phase-contrast or a differential-interference-contrast microscope.

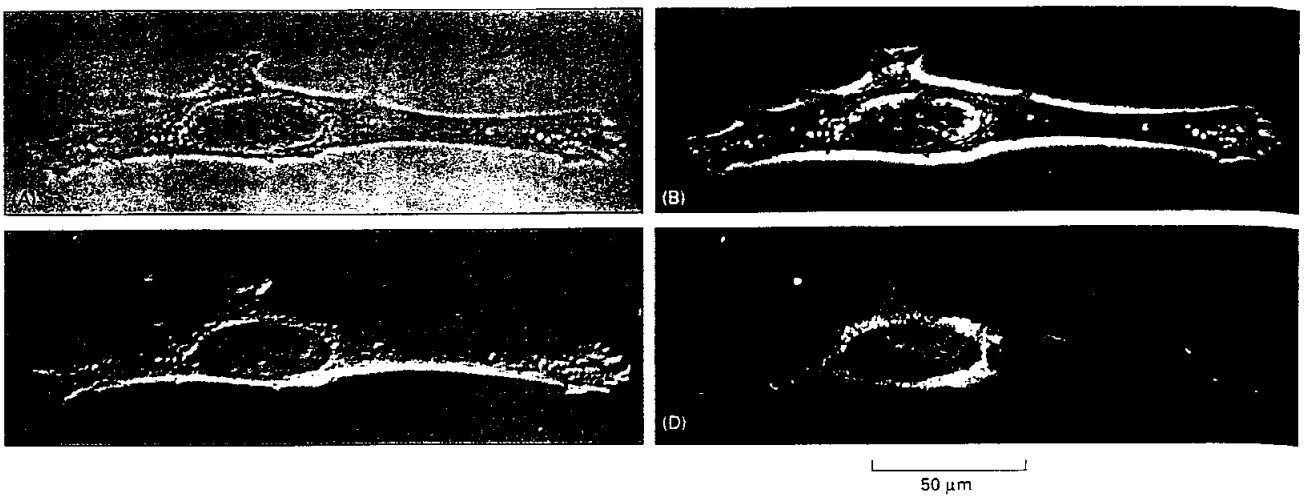


Figure 4–11 Four types of light microscopy. (A) The image of a fibroblast in culture obtained by the simple transmission of light through the cell, a technique known as *bright-field microscopy*. The other images were obtained by techniques discussed in the text: (B) phase-contrast microscopy, (C) Nomarski differential-interference-contrast microscopy, and (D) dark-field microscopy. All four types of image can be obtained with most modern microscopes simply by interchanging optical components.

lular motions are too slow to be seen in real time, it is often helpful to take time-lapse motion pictures (*microcinematography*) or video recordings. Here, successive frames separated by a short time delay are recorded, so that when the resulting film or videotape is projected or played at normal speed, events appear greatly speeded up.

Images Can Be Enhanced and Analyzed by Electronic Techniques [6]

In recent years **electronic imaging systems** and the associated technology of **image processing** have had a major impact on light microscopy. They have enabled certain practical limitations of microscopes (due to imperfections in the optical system) to be largely overcome. They have also circumvented two fundamental limitations of the human eye: the eye cannot see well in extremely dim light, and it cannot perceive small differences in light intensity against a bright background. The first limitation can be overcome by attaching highly light-sensitive video cameras (of the kind used in night surveillance) to a microscope. It is then possible to observe cells for long periods at very low light levels, thereby avoiding the damaging effects of prolonged bright light (and heat). Such *image-intensification systems* are especially important for viewing fluorescent molecules in living cells.

Because images produced by video cameras are in electronic form, they can be readily digitized, fed to a computer, and processed in various ways to extract latent information. Such image processing makes it possible to compensate for various optical faults in microscopes in order to attain the theoretical limit of resolution. Moreover, by using video systems linked to image processors, contrast can be greatly enhanced so that the eye's limitations in detecting small differences in light intensity are overcome. Although this processing also enhances the effects of random background irregularities in the optical system, this "noise" can be removed by electronically subtracting an image of a blank area of the field. Small transparent objects then become visible that were previously impossible to distinguish from the background.

The high contrast attainable by computer-assisted, differential-interference-contrast microscopy makes it possible to see even very small objects such as single microtubules (Figure 4–12), which have a diameter of 0.025 μm, less than one-tenth the wavelength of light. Individual microtubules can also be seen in a fluorescence microscope if they are fluorescently labeled (see Figure 4–62). In both cases, however, the unavoidable diffraction effects badly blur the image so that the microtubules appear at least 0.2 μm wide, making it impossible to distinguish a single microtubule from a bundle of several microtubules.

Imaging of Complex Three-dimensional Objects Is Possible with the Confocal Scanning Microscope [7]

For ordinary light microscopy, as we have seen, a tissue has to be sliced into thin sections in order to be examined; the thinner the section, the crisper the image. In the process of sectioning, information about the third dimension is lost. How then can one get a picture of the three-dimensional architecture of a cell or tissue, and how can one view the microscopic structure of a specimen that, for one reason or another, cannot first be sliced into sections? If a thick specimen is viewed with a conventional light microscope, the image obtained by focusing at any one level is degraded by blurred, out-of-focus information from the parts of the specimen that lie above and below the plane of focus. Although this problem can be overcome by complex computer-based image processing applied to a series of images in different focal planes, the method is slow and costly in computing power. The **confocal scanning microscope** provides another, more direct way of achieving the same end result: electronic-imaging methods make it possible to focus on a chosen plane in a thick specimen while rejecting the light that comes from out-of-focus regions above and below that plane. Thus one sees a crisp, thin *optical section*. From a series of such optical sections taken at different depths and stored in a computer, it is easy to reconstruct a three-dimensional image. The confocal scanning microscope does for the microscopist what the CAT scanner does (by different means) for the radiologist investigating a human body: both machines give detailed sectional views of the interior of an intact structure.

The optical details of the confocal scanning microscope are complex, but the basic idea is simple, as illustrated in Figure 4–13. The microscope is generally used with fluorescence optics (see Figure 4–7), but instead of illuminating the whole specimen at once, in the usual way, the optical system at any instant focuses a spotlight onto a single point at a specific depth in the specimen. A very bright source of pinpoint illumination is required; this is usually supplied by a laser whose light has been passed through a pinhole. The fluorescence emitted from the illuminated material is collected and brought to an image at the entry port of a suitable light detector. A pinhole aperture is placed at the detector, at the site that is *confocal* with the illuminating pinhole—that is, precisely where the rays emitted from the illuminated point in the specimen come to a focus. Thus the light from this point in the specimen converges on this aperture and enters the detector. By contrast, the light from regions out of the plane of focus of the

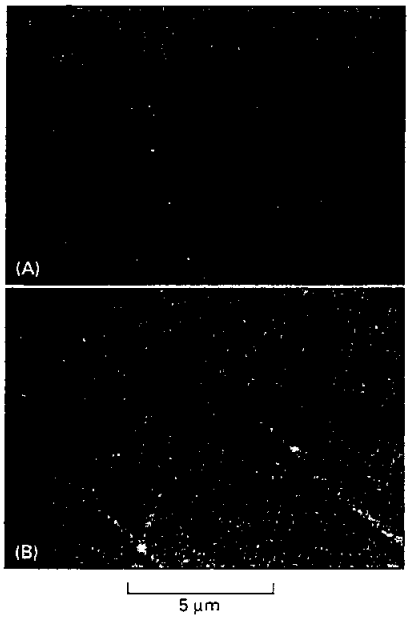


Figure 4–12 Extending the limits of detection. Light-microscope images of unstained microtubules that have been visualized by differential-interference-contrast microscopy followed by electronic image processing. (A) The original unprocessed image. (B) The final result of an electronic process that greatly enhances contrast and reduces "noise." Microtubules are only 0.025 μm in diameter and therefore in this image should appear only 0.1 mm wide. Instead, they appear much wider because of diffraction effects. (Courtesy of Bruce Schnapp.)

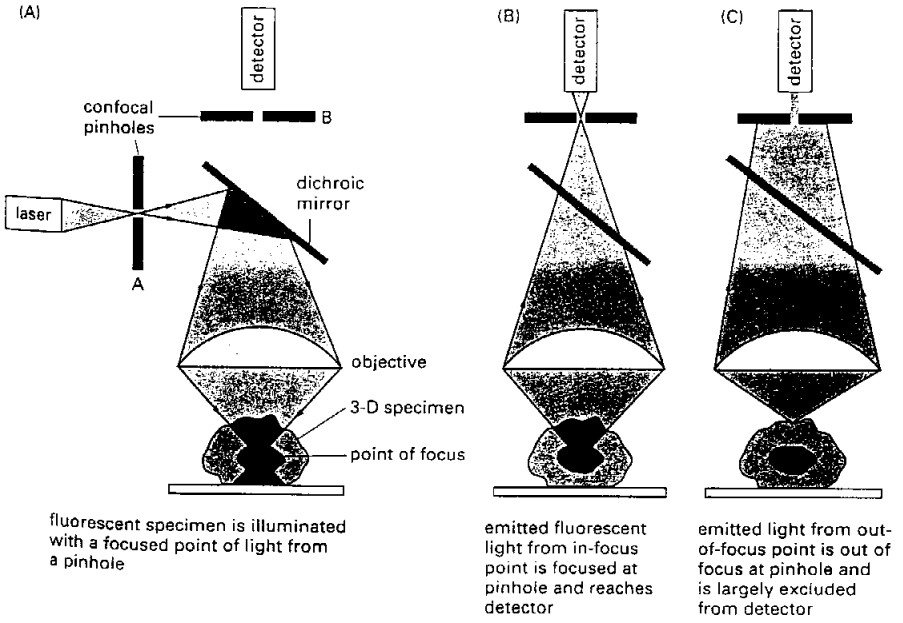


Figure 4–13 The confocal scanning fluorescence microscope. This simplified diagram shows that the basic arrangement of optical components is similar to that of the standard fluorescence microscope shown in Figure 4–7 except that a laser is used to illuminate a small pinhole whose image is focused at a single point in the specimen (A). Emitted fluorescence from this focal point in the specimen is focused at a second (confocal) pinhole (B). Emitted light from elsewhere in the specimen is not focused here and therefore does not contribute to the final image (C). By scanning the beam of light across the specimen, a very sharp two-dimensional image of the exact plane of focus is built up that is not significantly degraded by light from other regions of the specimen.

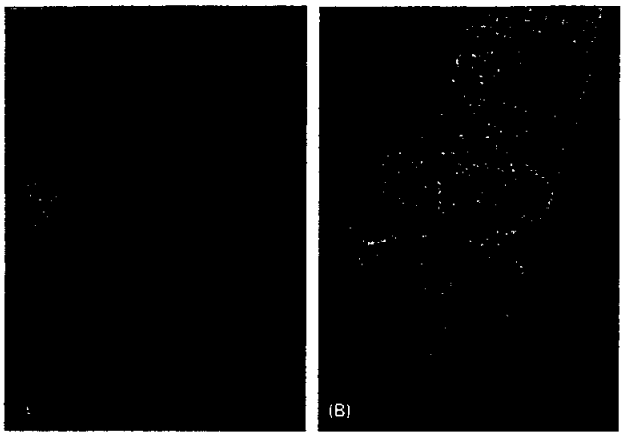


Figure 4–14 Comparison of conventional and confocal fluorescence microscopy. These two micrographs are of the same intact gastrula-stage *Drosophila* embryo that has been stained with a fluorescent probe for actin filaments. The conventional, unprocessed image (A) is blurred by the presence of fluorescent structures above and below the plane of focus. In the confocal image (B), this out-of-focus information is removed, which results in a crisp optical section of the cell in the embryo. (Courtesy of Richard Warn and Peter Shaw.)

spotlight is also out of focus at the pinhole aperture and is therefore largely excluded from the detector (Figure 4–14). To build up a two-dimensional image, data from each point in the plane of focus are collected sequentially by scanning across the field in a raster pattern (as on a television screen) and are displayed on a video screen. Although not shown in Figure 4–13, the scanning is done by deflecting the beam with an oscillating mirror placed between the dichroic mirror and the objective lens in such a way that the illuminating spotlight and the confocal pinhole at the detector remain strictly in register.

The confocal scanning microscope has been used to resolve the structure of numerous complex three-dimensional objects (Figure 4–15), including the networks of cytoskeletal fibers in the cytoplasm and the arrangements of chromosomes and genes in the nucleus.

The Electron Microscope Resolves the Fine Structure of the Cell [8]

The relationship between the limit of resolution and the wavelength of the illuminating radiation (see Figure 4–4) holds true for any form of radiation, whether it is a beam of light or a beam of electrons. With electrons, however, the limit of resolution can be made very small. The wavelength of an electron decreases as its velocity increases. In an electron microscope with an accelerating voltage of 100,000 V, the wavelength of an electron is 0.004 nm. In theory the resolution of such a microscope should be about 0.002 nm, which is 10,000 times greater than that of the light microscope. Because the aberrations of an electron lens are considerably harder to correct than those of a glass lens, however, the practical resolving power of most modern electron microscopes is, at best, 0.1 nm (1 Å) (Figure 4–16). Furthermore, problems of specimen preparation, contrast, and radiation damage effectively limit the normal resolution for biological objects to 2 nm (20 Å). This is nonetheless about 100 times better than the resolution of the

Figure 4–15 Three-dimensional reconstruction from confocal scanning microscope images. Pollen grains, in this case from a passion flower, have a complex sculptured cell wall that contains fluorescent compounds. Images obtained at different depths through the grain, using a confocal scanning microscope, can be recombined to give a three-dimensional view of the whole grain, shown on the right. Three selected individual optical sections from the full set of 30, each of which shows little contribution from its neighbors, are shown on the left. (Courtesy of John White.)