2.3: Instruments of Microscopy

Skills to develop

• Identify and describe the parts of a brightfield microscope
• Calculate total magnification for a compound microscope
• Describe the distinguishing features and typical uses for various types of light microscopes, electron microscopes, and scanning probe microscopes

The early pioneers of microscopy opened a window into the invisible world of microorganisms. But microscopy continued to advance in the centuries that followed. In 1830, Joseph Jackson Lister created an essentially modern light microscope. The 20th century saw the development of microscopes that leveraged nonvisible light, such as fluorescence microscopy, which uses an ultraviolet light source, and electron microscopy, which uses short-wavelength electron beams. These advances led to major improvements in magnification, resolution, and contrast. By comparison, the relatively rudimentary microscopes of van Leeuwenhoek and his contemporaries were far less powerful than even the most basic microscopes in use today. In this section, we will survey the broad range of modern microscopic technology and common applications for each type of microscope.

Light Microscopy

Many types of microscopes fall under the category of light microscopes, which use light to visualize images. Examples of light microscopes include brightfield microscopes, darkfield microscopes, phase-contrast microscopes, differential interference contrast microscopes, fluorescence microscopes, confocal scanning laser microscopes, and two-photon microscopes. These various types of light microscopes can be used to complement each other in diagnostics and research.
Brightfield Microscopes

The brightfield microscope, perhaps the most commonly used type of microscope, is a compound microscope with two or more lenses that produce a dark image on a bright background. Some brightfield microscopes are monocular (having a single eyepiece), though most newer brightfield microscopes are binocular (having two eyepieces), like the one shown in Figure \(\PageIndex{1}\); in either case, each eyepiece contains a lens called an ocular lens. The ocular lenses typically magnify images 10 times (10x). At the other end of the body tube are a set of objective lenses on a rotating nosepiece. The magnification of these objective lenses typically ranges from 4x to 100x, with the magnification for each lens designated on the metal casing of the lens. The ocular and objective lenses work together to create a magnified image. The total magnification is the product of the ocular magnification times the objective magnification:

\[
\text{ocular magnification} \times \text{objective magnification} = \text{total magnification}
\]

For example, if a 40x objective lens is selected and the ocular lens is 10x, the total magnification would be

\[(40\times)(10\times)=400\times\]

![Components of a typical brightfield microscope.](https://bio.libretexts.org/Bookshelves/Microbiology/Book%3A_Microbiology_(OpenStax)/02%3A_How_We_See_the_Invisible…)

Components of a typical brightfield microscope.

The item being viewed is called a specimen. The specimen is placed on a glass slide, which is then clipped into place on the stage (a platform) of the microscope. Once the slide is secured, the specimen on the slide is positioned over the light using the x-y mechanical stage knobs. These knobs move the slide on the surface of the stage, but do not raise or lower the stage. Once the specimen is centered over the light, the stage position can be raised or lowered to focus the image. The coarse focusing knob is used for large-scale movements with 4x and 10x objective lenses; the fine focusing knob is used for small-scale movements, especially with 40x or 100x objective lenses.

When images are magnified, they become dimmer because there is less light per unit area of image. Highly magnified images produced by microscopes, therefore, require intense lighting. In a brightfield microscope, this light is provided by...
an illuminator, which is typically a high-intensity bulb below the stage. Light from the illuminator passes up through condenser lens (located below the stage), which focuses all of the light rays on the specimen to maximize illumination. The position of the condenser can be optimized using the attached condenser focus knob; once the optimal distance is established, the condenser should not be moved to adjust the brightness. If less-than-maximal light levels are needed, the amount of light striking the specimen can be easily adjusted by opening or closing a diaphragm between the condenser and the specimen. In some cases, brightness can also be adjusted using the rheostat, a dimmer switch that controls the intensity of the illuminator.

A brightfield microscope creates an image by directing light from the illuminator at the specimen; this light is differentially transmitted, absorbed, reflected, or refracted by different structures. Different colors can behave differently as they interact with chromophores (pigments that absorb and reflect particular wavelengths of light) in parts of the specimen. Often, chromophores are artificially added to the specimen using stains, which serve to increase contrast and resolution. In general, structures in the specimen will appear darker, to various extents, than the bright background, creating maximally sharp images at magnifications up to about 1000x. Further magnification would create a larger image, but without increased resolution. This allows us to see objects as small as bacteria, which are visible at about 400x or so, but not smaller objects such as viruses.

At very high magnifications, resolution may be compromised when light passes through the small amount of air between the specimen and the lens. This is due to the large difference between the refractive indices of air and glass; the air scatters the light rays before they can be focused by the lens. To solve this problem, a drop of oil can be used to fill the space between the specimen and an oil immersion lens, a special lens designed to be used with immersion oils. Since the oil has a refractive index very similar to that of glass, it increases the maximum angle at which light leaving the specimen can strike the lens. This increases the light collected and, thus, the resolution of the image (Figure \(\PageIndex{2}\)). A variety of oils can be used for different types of light.

![Image of microscope and immersion oil](https://bio.libretexts.org/Bookshelves/Microbiology/Book%3A_Microbiology_(OpenStax)/02%3A_How_We_See_the_Invisible...)\(\PageIndex{2}\)

Figure \(\PageIndex{2}\): (a) Oil immersion lenses like this one are used to improve resolution. (b) Because immersion oil and glass have very similar refractive indices, there is a minimal amount of refraction before the light reaches the lens. Without immersion oil, light scatters as it passes through the air above the slide, degrading the resolution of the image.

**MICROSCOPE MAINTENANCE: BEST PRACTICES**

Even a very powerful microscope cannot deliver high-resolution images if it is not properly cleaned and maintained. Since lenses are carefully designed and manufactured to refract light with a high degree of precision, even a slightly dirty or scratched lens will refract light in unintended ways, degrading the image of the specimen. In addition, microscopes are rather delicate instruments, and great care must be taken to avoid damaging parts and surfaces.
Among other things, proper care of a microscope includes the following:

- cleaning the lenses with lens paper
- not allowing lenses to contact the slide (e.g., by rapidly changing the focus)
- protecting the bulb (if there is one) from breakage
- not pushing an objective into a slide
- not using the coarse focusing knob when using the 40\(^\circ\) or greater objective lenses
- only using immersion oil with a specialized oil objective, usually the 100\(^\circ\) objective
- cleaning oil from immersion lenses after using the microscope
- cleaning any oil accidentally transferred from other lenses
- covering the microscope or placing it in a cabinet when not in use

### Darkfield Microscopy

A darkfield microscope is a brightfield microscope that has a small but significant modification to the condenser. A small, opaque disk (about 1 cm in diameter) is placed between the illuminator and the condenser lens. This opaque light stop, as the disk is called, blocks most of the light from the illuminator as it passes through the condenser on its way to the objective lens, producing a hollow cone of light that is focused on the specimen. The only light that reaches the objective is light that has been refracted or reflected by structures in the specimen. The resulting image typically shows bright objects on a dark background (Figure \(\PageIndex{3}\))

![Diagram of darkfield microscopy](https://bio.libretexts.org/Bookshelves/Microbiology/Book%3A_Microbiology_(OpenStax)/02%3A_How_We_See_the_Invisible…)

Figure \(\PageIndex{3}\): An opaque light stop inserted into a brightfield microscope is used to produce a darkfield image. The light stop blocks light traveling directly from the illuminator to the objective lens, allowing only light reflected or refracted off the specimen to reach the eye.

An opaque light stop inserted into a brightfield microscope is used to produce a darkfield image. The light stop blocks light traveling directly from the illuminator to the objective lens, allowing only light reflected or refracted off the specimen.
to reach the eye.

Darkfield microscopy can often create high-contrast, high-resolution images of specimens without the use of stains, which is particularly useful for viewing live specimens that might be killed or otherwise compromised by the stains. For example, thin spirochetes like *Treponema pallidum*, the causative agent of syphilis, can be best viewed using a darkfield microscope (Figure \(\PageIndex{4}\)).

![Image of spirochetes](https://bio.libretexts.org/Bookshelves/Microbiology/Book%3A_Microbiology_(OpenStax)/02%3A_How_We_See_the_Invisible/Part2/Section2.1/figure4.png)

Figure \(\PageIndex{4}\): Use of a darkfield microscope allows us to view living, unstained samples of the spirochete *Treponema pallidum*. Similar to a photographic negative, the spirochetes appear bright against a dark background. (credit: Centers for Disease Control and Prevention/C.W. Hubbard)

Use of a darkfield microscope allows us to view living, unstained samples of the spirochete *Treponema pallidum*. Similar to a photographic negative, the spirochetes appear bright against a dark background. (credit: Centers for Disease Control and Prevention/C.W. Hubbard)

Exercise \(\PageIndex{1}\)

Identify the key differences between brightfield and darkfield microscopy.

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**PART 2**

Wound infections like Cindy’s can be caused by many different types of bacteria, some of which can spread rapidly with serious complications. Identifying the specific cause is very important to select a medication that can kill or stop the growth of the bacteria.

After calling a local doctor about Cindy’s case, the camp nurse sends the sample from the wound to the closest medical laboratory. Unfortunately, since the camp is in a remote area, the nearest lab is small and poorly equipped. A more modern lab would likely use other methods to culture, grow, and identify the bacteria, but in this case, the technician decides to make a wet mount from the specimen and view it under a brightfield microscope. In a wet mount, a small drop of water is added to the slide, and a cover slip is placed over the specimen to keep it in place before it is positioned under the objective lens.

Under the brightfield microscope, the technician can barely see the bacteria cells because they are nearly transparent...
against the bright background. To increase contrast, the technician inserts an opaque light stop above the illuminator. The resulting darkfield image clearly shows that the bacteria cells are spherical and grouped in clusters, like grapes.

- Why is it important to identify the shape and growth patterns of cells in a specimen?
- What other types of microscopy could be used effectively to view this specimen?

**Phase-Contrast Microscopes**

Phase-contrast microscopes use refraction and interference caused by structures in a specimen to create high-contrast, high-resolution images without staining. It is the oldest and simplest type of microscope that creates an image by altering the wavelengths of light rays passing through the specimen. To create altered wavelength paths, an annular stop is used in the condenser. The annular stop produces a hollow cone of light that is focused on the specimen before reaching the objective lens. The objective contains a phase plate containing a phase ring. As a result, light traveling directly from the illuminator passes through the phase ring while light refracted or reflected by the specimen passes through the plate. This causes waves traveling through the ring to be about one-half of a wavelength out of phase with those passing through the plate. Because waves have peaks and troughs, they can add together (if in phase together) or cancel each other out (if out of phase). When the wavelengths are out of phase, wave troughs will cancel out wave peaks, which is called destructive interference. Structures that refract light then appear dark against a bright background of only unrefracted light. More generally, structures that differ in features such as refractive index will differ in levels of darkness (Figure 5).

![Figure 5](https://bio.libretexts.org/Bookshelves/Microbiology/Book%3A_Microbiology_(OpenStax)/02%3A_How_We_See_the_Invisible\...)
Because it increases contrast without requiring stains, phase-contrast microscopy is often used to observe live specimens. Certain structures, such as organelles in eukaryotic cells and endospores in prokaryotic cells, are especially well visualized with phase-contrast microscopy (Figure \(\PageIndex{6}\)).

![Figure \(\PageIndex{6}\): This figure compares a brightfield image (left) with a phase-contrast image (right) of the same unstained simple squamous epithelial cells. The cells are in the center and bottom right of each photograph (the irregular item above the cells is acellular debris). Notice that the unstained cells in the brightfield image are almost invisible against the background, whereas the cells in the phase-contrast image appear to glow against the background, revealing far more detail. (credit: “Clearly kefir”/Wikimedia Commons)](https://bio.libretexts.org/Bookshelves/Microbiology/Book%3A_Microbiology_(OpenStax)/02%3A_How_We_See_the_Invisible…)

**Differential Interference Contrast Microscopes**

Differential interference contrast (DIC) microscopes (also known as Nomarski optics) are similar to phase-contrast microscopes in that they use interference patterns to enhance contrast between different features of a specimen. In a DIC microscope, two beams of light are created in which the direction of wave movement (polarization) differs. Once the beams pass through either the specimen or specimen-free space, they are recombined and effects of the specimens cause differences in the interference patterns generated by the combining of the beams. This results in high-contrast images of living organisms with a three-dimensional appearance. These microscopes are especially useful in distinguishing structures within live, unstained specimens. (Figure \(\PageIndex{7}\)).
A DIC image of *Fonsecaea pedrosoi* grown on modified Leonian’s agar. This fungus causes chromoblastomycosis, a chronic skin infection common in tropical and subtropical climates.

Exercise

What are some advantages of phase-contrast and DIC microscopy?

Fluorescence Microscopes

A fluorescence microscope uses fluorescent chromophores called fluorochromes, which are capable of absorbing energy from a light source and then emitting this energy as visible light. Fluorochromes include naturally fluorescent substances (such as chlorophylls) as well as fluorescent stains that are added to the specimen to create contrast. Dyes such as Texas red and FITC are examples of fluorochromes. Other examples include the nucleic acid dyes 4’,6’-diamidino-2-phenylindole (DAPI) and acridine orange.

The microscope transmits an excitation light, generally a form of EMR with a short wavelength, such as ultraviolet or blue light, toward the specimen; the chromophores absorb the excitation light and emit visible light with longer wavelengths. The excitation light is then filtered out (in part because ultraviolet light is harmful to the eyes) so that only visible light passes through the ocular lens. This produces an image of the specimen in bright colors against a dark background.

Fluorescence microscopes are especially useful in clinical microbiology. They can be used to identify pathogens, to find particular species within an environment, or to find the locations of particular molecules and structures within a cell. Approaches have also been developed to distinguish living from dead cells using fluorescence microscopy based upon whether they take up particular fluorochromes. Sometimes, multiple fluorochromes are used on the same specimen to show different structures or features.

One of the most important applications of fluorescence microscopy is a technique called immunofluorescence, which is
used to identify certain disease-causing microbes by observing whether antibodies bind to them. (Antibodies are protein molecules produced by the immune system that attach to specific pathogens to kill or inhibit them.) There are two approaches to this technique: direct immunofluorescence assay (DFA) and indirect immunofluorescence assay (IFA). In DFA, specific antibodies (e.g., those that target the rabies virus) are stained with a fluorochrome. If the specimen contains the targeted pathogen, one can observe the antibodies binding to the pathogen under the fluorescent microscope. This is called a primary antibody stain because the stained antibodies attach directly to the pathogen.

In IFA, secondary antibodies are stained with a fluorochrome rather than primary antibodies. Secondary antibodies do not attach directly to the pathogen, but they do bind to primary antibodies. When the unstained primary antibodies bind to the pathogen, the fluorescent secondary antibodies can be observed binding to the primary antibodies. Thus, the secondary antibodies are attached indirectly to the pathogen. Since multiple secondary antibodies can often attach to a primary antibody, IFA increases the number of fluorescent antibodies attached to the specimen, making it easier to visualize features in the specimen (Figure \(\PageIndex{8}\)).

![Image of immunofluorescence techniques](https://bio.libretexts.org/Bookshelves/Microbiology/Book%3A_Microbiology_(OpenStax)/02%3A_How_We_See_the_Invisible\(\PageIndex{8}\).)

Exercise \(\PageIndex{3}\)
Why must fluorochromes be used to examine a specimen under a fluorescence microscope?

Confocal Microscopes

Whereas other forms of light microscopy create an image that is maximally focused at a single distance from the observer (the depth, or z-plane), a confocal microscope uses a laser to scan multiple z-planes successively. This produces numerous two-dimensional, high-resolution images at various depths, which can be constructed into a three-dimensional image by a computer. As with fluorescence microscopes, fluorescent stains are generally used to increase contrast and resolution. Image clarity is further enhanced by a narrow aperture that eliminates any light that is not from the z-plane. Confocal microscopes are thus very useful for examining thick specimens such as biofilms, which can be examined alive and unfixed (Figure \(\PageIndex{9}\)).

![Confocal microscopy can be used to visualize structures such as this roof-dwelling cyanobacterium biofilm. (credit: modification of work by American Society for Microbiology)](https://bio.libretexts.org/Bookshelves/Microbiology/Book%3A_Microbiology_(OpenStax)/02%3A_How_We_See_the_Invisible/10)

Two-Photon Microscopes

While the original fluorescent and confocal microscopes allowed better visualization of unique features in specimens, there were still problems that prevented optimum visualization. The effective sensitivity of fluorescence microscopy when viewing thick specimens was generally limited by out-of-focus flare, which resulted in poor resolution. This limitation was greatly reduced in the confocal microscope through the use of a confocal pinhole to reject out-of-focus background fluorescence with thin (<1 μm), unblurred optical sections. However, even the confocal microscopes lacked the resolution needed for viewing thick tissue samples. These problems were resolved with the development of the two-photon microscope, which uses a scanning technique, fluorochromes, and long-wavelength light (such as infrared) to visualize specimens. The low energy associated with the long-wavelength light means that two photons must strike a location at the same time to excite the fluorochrome. The low energy of the excitation light is less damaging to cells, and the long wavelength of the excitation light more easily penetrates deep into thick specimens. This makes the two-photon microscope useful for examining living cells within intact tissues—brain slices, embryos, whole organs, and even entire animals.

Currently, use of two-photon microscopes is limited to advanced clinical and research laboratories because of the high costs of the instruments. A single two-photon microscope typically costs between $300,000 and $500,000, and the
lasers used to excite the dyes used on specimens are also very expensive. However, as technology improves, two-photon microscopes may become more readily available in clinical settings.

Exercise \(\PageIndex{4}\)

What types of specimens are best examined using confocal or two-photon microscopy?

**Electron Microscopy**

The maximum theoretical resolution of images created by light microscopes is ultimately limited by the wavelengths of visible light. Most light microscopes can only magnify 1000?, and a few can magnify up to 1500?, but this does not begin to approach the magnifying power of an electron microscope (EM), which uses short-wavelength electron beams rather than light to increase magnification and resolution.

Electrons, like electromagnetic radiation, can behave as waves, but with wavelengths of 0.005 nm, they can produce much better resolution than visible light. An EM can produce a sharp image that is magnified up to 100,000?. Thus, EMs can resolve subcellular structures as well as some molecular structures (e.g., single strands of DNA); however, electron microscopy cannot be used on living material because of the methods needed to prepare the specimens.

There are two basic types of EM: the transmission electron microscope (TEM) and the scanning electron microscope (SEM)(Figure \(\PageIndex{10}\)). The TEM is somewhat analogous to the brightfield light microscope in terms of the way it functions. However, it uses an electron beam from above the specimen that is focused using a magnetic lens (rather than a glass lens) and projected through the specimen onto a detector. Electrons pass through the specimen, and then the detector captures the image (Figure \(\PageIndex{11}\)).

Figure \(\PageIndex{10}\): (a) A transmission electron microscope (TEM). (b) A scanning electron microscope (SEM).

(credit a: modification of work by “Deshi”/Wikimedia Commons; credit b: modification of work by “ZEISS Microscopy”/Flickr)
Electron microscopes use magnets to focus electron beams similarly to the way that light microscopes use lenses to focus light.

Figure \(\PageIndex{11}\): Electron microscopes use magnets to focus electron beams similarly to the way that light microscopes use lenses to focus light.

For electrons to pass through the specimen in a TEM, the specimen must be extremely thin (20–100 nm thick). The image is produced because of varying opacity in various parts of the specimen. This opacity can be enhanced by staining the specimen with materials such as heavy metals, which are electron dense. TEM requires that the beam and specimen be in a vacuum and that the specimen be very thin and dehydrated. The specific steps needed to prepare a specimen for observation under an EM are discussed in detail in the next section.

SEM's form images of surfaces of specimens, usually from electrons that are knocked off of specimens by a beam of electrons. This can create highly detailed images with a three-dimensional appearance that are displayed on a monitor (Figure \(\PageIndex{12}\)). Typically, specimens are dried and prepared with fixatives that reduce artifacts, such as shriveling, that can be produced by drying, before being sputter-coated with a thin layer of metal such as gold. Whereas transmission electron microscopy requires very thin sections and allows one to see internal structures such as organelles and the interior of membranes, scanning electron microscopy can be used to view the surfaces of larger objects (such as a pollen grain) as well as the surfaces of very small samples (Figure \(\PageIndex{13}\)). Some EMs can magnify an image up to 2,000,000.

Figure \(\PageIndex{12}\): These schematic illustrations compare the components of transmission electron microscopes and scanning electron microscopes.
Using Microscopy to study Biofilms

A biofilm is a complex community of one or more microorganism species, typically forming as a slimy coating attached to a surface because of the production of an extrapolymeric substance (EPS) that attaches to a surface or at the interface between surfaces (e.g., between air and water). In nature, biofilms are abundant and frequently occupy complex niches within ecosystems (Figure \(\PageIndex{14}\)). In medicine, biofilms can coat medical devices and exist within the body. Because they possess unique characteristics, such as increased resistance against the immune system and to antimicrobial drugs, biofilms are of particular interest to microbiologists and clinicians alike.

Because biofilms are thick, they cannot be observed very well using light microscopy; slicing a biofilm to create a thinner specimen might kill or disturb the microbial community. Confocal microscopy provides clearer images of biofilms because it can focus on one z-plane at a time and produce a three-dimensional image of a thick specimen. Fluorescent dyes can be helpful in identifying cells within the matrix. Additionally, techniques such as immunofluorescence and fluorescence in situ hybridization (FISH), in which fluorescent probes are used to bind to DNA, can be used.

Electron microscopy can be used to observe biofilms, but only after dehydrating the specimen, which produces undesirable artifacts and distorts the specimen. In addition to these approaches, it is possible to follow water currents through the shapes (such as cones and mushrooms) of biofilms, using video of the movement of fluorescently coated beads (Figure \(\PageIndex{15}\)).
Scanning Probe Microscopy

A scanning probe microscope does not use light or electrons, but rather very sharp probes that are passed over the surface of the specimen and interact with it directly. This produces information that can be assembled into images with magnifications up to 100,000,000?. Such large magnifications can be used to observe individual atoms on surfaces. To date, these techniques have been used primarily for research rather than for diagnostics.

There are two types of scanning probe microscope: the scanning tunneling microscope (STM) and the atomic force microscope (AFM). An STM uses a probe that is passed just above the specimen as a constant voltage bias creates the potential for an electric current between the probe and the specimen. This current occurs via quantum tunneling of electrons between the probe and the specimen, and the intensity of the current is dependent upon the distance between the probe and the specimen. The probe is moved horizontally above the surface and the intensity of the current is measured. Scanning tunneling microscopy can effectively map the structure of surfaces at a resolution at which individual atoms can be detected.

Similar to an STM, AFMs have a thin probe that is passed just above the specimen. However, rather than measuring variations in the current at a constant height above the specimen, an AFM establishes a constant current and measures variations in the height of the probe tip as it passes over the specimen. As the probe tip is passed over the specimen,
forces between the atoms (van der Waals forces, capillary forces, chemical bonding, electrostatic forces, and others) cause it to move up and down. Deflection of the probe tip is determined and measured using Hooke’s law of elasticity, and this information is used to construct images of the surface of the specimen with resolution at the atomic level (Figure \(\PageIndex{16}\)).

![STM image](https://bio.libretexts.org/Bookshelves/Microbiology/Book%3A_Microbiology_(OpenStax)/02%3A_How_We_See_the_Invisible\_Microscopes/02.9_Figure_16.jpg)

![AFM image](https://bio.libretexts.org/Bookshelves/Microbiology/Book%3A_Microbiology_(OpenStax)/02%3A_How_We_See_the_Invisible\_Microscopes/02.9_Figure_17.jpg)

Figure \(\PageIndex{16}\): STMs and AFMs allow us to view images at the atomic level. (a) This STM image of a pure gold surface shows individual atoms of gold arranged in columns. (b) This AFM image shows long, strand-like molecules of nanocellulose, a laboratory-created substance derived from plant fibers. (credit a: modification of work by “Erwinrossen”/Wikimedia Commons).

Exercise \(\PageIndex{6}\)

1. Which has higher magnification, a light microscope or a scanning probe microscope?
2. Name one advantage and one limitation of scanning probe microscopy.

<table>
<thead>
<tr>
<th>LIGHT MICROSCOPES</th>
<th>Magnification: up to about 1000x</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscope Type</td>
<td>Key Uses</td>
</tr>
<tr>
<td>Brightfield</td>
<td>Commonly used in a wide variety of laboratory applications as the standard microscope; produces an image on a bright background. Example: Bacillus sp. showing endospores.</td>
</tr>
<tr>
<td>Darkfield</td>
<td>Increases contrast without staining by producing a bright image on a dark background; especially useful for viewing live specimens. Example: Bordetella burgdorferi</td>
</tr>
<tr>
<td>Phase contrast</td>
<td>Uses refraction and interference caused by structures in the specimen to create high contrast, high-resolution images without staining; making it useful for viewing live specimens, and structures such as endospores and organelles. Example: Pseudomonas sp.</td>
</tr>
<tr>
<td>Differential interference contrast (DIC)</td>
<td>Uses interference patterns to enhance contrast between different features of a specimen to produce high-contrast images of living organisms with a three-dimensional appearance, making it especially useful in distinguishing structures within live, unstained specimens; images viewed reveal detailed structures within cells. Example: Escherichia coli O157:H7</td>
</tr>
<tr>
<td>Fluorescence</td>
<td>Uses fluorescent dyes to produce an image; can be used to identify pathogens, to find particular species, to distinguish living from dead cells, or to find locations of particular molecules within a cell, also used for immunofluorescence. Example: P. putida stained with fluorescent dyes to visualize the capsule.</td>
</tr>
<tr>
<td>Confocal</td>
<td>Uses a laser to scan multiple z-planes successively, producing numerous two-dimensional, high-resolution images at various depths that can be constructed into a three-dimensional image by a computer, making this useful for examining thick specimens such as biofilms. Example: Escherichia coli stained with acridine orange dye to show the nucleoid regions of the cells.</td>
</tr>
<tr>
<td>Two-photon</td>
<td>Uses a scanning technique, fluorochromes, and long-wavelength light (such as infrared) to penetrate deep into thick specimens such as biofilms. Example: Mouse intestine cells stained with fluorescent dye.</td>
</tr>
</tbody>
</table>
"Darkfield": modification of work by American Society for Microbiology; credit "Phase contrast": modification of work by American Society for Microbiology; credit "DIC": modification of work by American Society for Microbiology; credit "Fluorescence": modification of work by American Society for Microbiology; credit "Confocal": modification of work by American Society for Microbiology; credit "Two-photon": modification of work by Alberto Diaspro, Paolo Bianchini, Giuseppe Vicidomini, Mario Faretta, Paola Ramoino, Cesare Usai.

<table>
<thead>
<tr>
<th>ELECTRON MICROSCOPES</th>
<th>Magnification: 20–100,000x or more</th>
<th>Key Uses</th>
<th>Sample Images</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transmission (TEM)</td>
<td>Uses electron beams that pass through a specimen to visualize small images; useful to observe small, thin specimens such as tissue sections and subcellular structures.</td>
<td>Example: Ebola virus</td>
<td></td>
</tr>
<tr>
<td>Scanning (SEM)</td>
<td>Uses electron beams to visualize surfaces; useful to observe the three-dimensional surface details of specimens.</td>
<td>Example: Campylobacter jejuni</td>
<td></td>
</tr>
</tbody>
</table>

Figure \(\PageIndex{18}\): (credit "TEM": modification of work by American Society for Microbiology; credit "SEM": modification of work by American Society for Microbiology)

<table>
<thead>
<tr>
<th>SCANNING PROBE MICROSCOPES</th>
<th>Magnification: 100–100,000,000x or more</th>
<th>Key Uses</th>
<th>Sample Images</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scanning tunneling (STM)</td>
<td>Uses a probe passed horizontally at a constant distance just above the specimen while the intensity of the current is measured; can map the structure of surfaces at the atomic level; works best on conducting materials but can also be used to examine organic materials such as DNA, if fixed on a surface.</td>
<td>Example: Image of surface reconstruction on a clean gold [Au(100)] surface, as visualized using scanning tunneling microscopy.</td>
<td></td>
</tr>
<tr>
<td>Atomic force (AFM)</td>
<td>Can be used in several ways, including using a laser focused on a cantilever to measure the bending of the tip of a probe passed above the specimen while the height needed to maintain a constant current is measured; useful to observe specimens at the atomic level and can be more easily used with nonconducting samples.</td>
<td>Example: AFM height image of carboxymethylated noncellulose adsorbed on a silica surface.</td>
<td></td>
</tr>
</tbody>
</table>

Figure \(\PageIndex{19}\): Microscopy techniques for scanning probe microscopes.

**Key Concepts and Summary**

- Numerous types of microscopes use various technologies to generate micrographs. Most are useful for a particular type of specimen or application.
- Light microscopy uses lenses to focus light on a specimen to produce an image. Commonly used light microscopes include brightfield, darkfield, phase-contrast, differential interference contrast, fluorescence, confocal, and two-photon microscopes.
- Electron microscopy focuses electrons on the specimen using magnets, producing much greater magnification than light microscopy. The transmission electron microscope (TEM) and scanning electron microscope (SEM) are two common forms.
- Scanning probe microscopy produces images of even greater magnification by measuring feedback from sharp probes that interact with the specimen. Probe microscopes include the scanning tunneling microscope (STM) and the atomic force microscope (AFM).

**Footnotes**

Glossary

**atomic force microscope**
-a scanning probe microscope that uses a thin probe that is passed just above the specimen to measure forces between the atoms and the probe

**binocular**
-having two eyepieces

**brightfield microscope**
a compound light microscope with two lenses; it produces a dark image on a bright background

**coarse focusing knob**
a knob on a microscope that produces relatively large movements to adjust focus

**chromophores**
pigments that absorb and reflect particular wavelengths of light (giving them a color)

**condenser lens**
a lens on a microscope that focuses light from the light source onto the specimen

**confocal microscope**
a scanning laser microscope that uses fluorescent dyes and excitation lasers to create three-dimensional images

**darkfield microscope**
a compound light microscope that produces a bright image on a dark background; typically a modified brightfield microscope

**diaphragm**
a component of a microscope; typically consists of a disk under the stage with holes of various sizes; can be adjusted to allow more or less light from the light source to reach the specimen

**differential interference-contrast microscope**
a microscope that uses polarized light to increase contrast

**electron microscope**
a type of microscope that uses short-wavelength electron beams rather than light to increase magnification and resolution

**fine focusing knob**
a knob on a microscope that produces relatively small movements to adjust focus

**fluorescence microscope**
a microscope that uses natural fluorochromes or fluorescent stains to increase contrast

**fluorochromes**
chromophores that fluoresce (absorb and then emit light)

**illuminator**
the light source on a microscope

**immunofluorescence**
a technique that uses a fluorescence microscope and antibody-specific fluorochromes to determine the presence of
specific pathogens in a specimen

**monocular**
having a single eyepiece

**objective lenses**
on a light microscope, the lenses closest to the specimen, typically located at the ends of turrets

**ocular lens**
on a microscope, the lens closest to the eye (also called an eyepiece)

**oil immersion lens**
a special objective lens on a microscope designed to be used with immersion oil to improve resolution

**phase-contrast microscope**
a light microscope that uses an annular stop and annular plate to increase contrast

**rheostat**
a dimmer switch that controls the intensity of the illuminator on a light microscope

**scanning electron microscope (SEM)**
a type of electron microscope that bounces electrons off of the specimen, forming an image of the surface

**scanning probe microscope**
a microscope that uses a probe that travels across the surface of a specimen at a constant distance while the current, which is sensitive to the size of the gap, is measured

**scanning tunneling microscope**
a microscope that uses a probe that is passed just above the specimen as a constant voltage bias creates the potential for an electric current between the probe and the specimen

**stage**
the platform of a microscope on which slides are placed

**total magnification**
in a light microscope is a value calculated by multiplying the magnification of the ocular by the magnification of the objective lenses

**transmission electron microscope (TEM)**
a type of electron microscope that uses an electron beam, focused with magnets, that passes through a thin specimen

**two-photon microscope**
a microscope that uses long-wavelength or infrared light to fluoresce fluorochromes in the specimen

**x-y mechanical stage knobs**
knobs on a microscope that are used to adjust the position of the specimen on the stage surface, generally to center it directly above the light