Chapter 4.3: Optical Microscopy

Optical Microscopy

The most common, and certainly the oldest, form of microscopy uses visible light to illuminate the sample so that our eyes can directly “see” the magnified image. Generally, this form of microscopy is called optical microscopy. There are, however, a number variations on this theme that form the core of forensic microscopy. To place optical microscopy in context, Figure 4.3.1 shows the relative size of objects that the various types of microscopy can resolve. All of these microscopic systems employ the basic principles that have been described already but have unique advantages and disadvantages that make them useful for specific forensic purposes. In this section, the most important or do you mean often used or generally useful of these methods will be presented.

Bright Field Optical Microscopy

When you simply think of microscopy, you’re probably thinking of bright field microscopy. It is the most basic form of light microscopy where the sample is illuminated by white light, usually from below, which then passes directly through the sample on its way to the magnifying lenses and our eyes (Figure 4.3.2). The ability to distinguish the various parts of the sample (contrast) arises from the different absorances of the various components in the sample. In other words, the light is absorbed or scattered differently by the various features and chemical compounds in the sample – some places absorb a lot of light and appear darker while other areas absorb less light and appear relatively lighter to our eyes. Since light that either does not pass through the sample or is absorbed the least appears brightest to our eyes, the term bright field microscopy indicates that the background appears the brightest in the view and the sample appears relatively darker in comparison. This technique typically is useful in “seeing” objects in the range of 1 µm (1000 mm) to 5 mm (a strand of human hair is typically about 20 mm in thickness). Several examples of bright field micrographs are shown in Figure 4.3.3.

The great advantage of this approach is its simplicity of use and easy sample preparation. It has, however, a number of limitations that arise from problems relating to image resolution and
contrast. Bright field microscopy typically has relatively low contrast for biological samples since most biological materials do not absorb light very well. When a sample is colored, however, it provides much better contrast. Some ways of improving contrast have been developed, foremost among these is staining the sample (*vide infra*). Sometimes, instead of white light, colored light can be used to more successfully visualize the details of the sample. Contrast can also be improved by adjusting the amount of light entering the sample by using a condenser that focuses the light from the source onto the sample. Nonetheless, bright field microscopy provides the basic tool for visualizing small features of both biological and non-living samples.

A variety of modifications of this technique have been developed to enhance contrast by changing the setup of the microscope or by modifying the type of light used, as described in the following sections.

**Dark Field Optical Microscopy**

Sometimes in bright field microscopy, staining or other contrast enhancing techniques designed to allow us to see the details in a sample are either insufficient or not possible. One alternative technique for enhancing contrast that is often useful in these circumstances is referred to as **dark field microscopy**.

In this technique, a physical light block is used to stop all the directly transmitted light that would travel in a straight line from the light source into the lenses. The *only* light that enters the lens, therefore, is light that has been scattered into the lens by the sample. This is shown schematically in Figure 4.3.4. In dark field microscopy, light is aimed only from the edges of the condenser lens onto the sample in such a way that it would miss the lens entirely unless the path of the light is changed by the sample (scattered). If you would look into a dark field microscope without a sample under the lens, you would see a black field – no light would enter the lens since it is blocked by the annular stop (Fig. 4.3.4.). When a sample is present, however, it deflects some of the light into the lens so that it appears illuminated against a black
background, hence the name dark field microscopy. Figure 4.3.5 shows a comparison of a sample as seen under bright field and dark field conditions. Color is sometimes seen in dark field microscopy that does not appear in the sample under normal illumination, aiding contrast further.

Advantages of the dark field method include an enhanced ability to see details in biological samples that do not have enough contrast to be seen well in bright field microscopy and the fact that the technique is experimentally quite simple and inexpensive. An important disadvantage, however, is that the sample often appears to have a high degree of glare, leading to the image showing up in silhouette rather than as a bright object clearly defined.

Polarized Light Microscopy

Light, as electromagnetic radiation, is made up of both electrical and magnetic waves that oscillate as they move through space. These two kinds of waves are perpendicular to one another, as shown in Figure 4.3.6, and allow energy to travel through a vacuum. Visible light, heat, radio waves, X-rays, microwaves, and WIFI are all just forms of electromagnetic radiation that differ in their wavelengths.

Generally, a light source emits tiny packets of light, called photons, in all directions. Therefore, a beam of light contains photons with their
waves vibrating in all possible orientations (unpolarized light). It is possible, however, to remove all of the photons except those vibrating in just one direction by passing the light through very tiny, thin rectangular slits as shown in Figure 4.3.7. The only photons that can pass through the slit are those in which its plane of oscillation (wave action) lines up with the long direction of the slit. This geometry produces something called **plane polarized light** – light oscillating in only one direction. If you put a second set of slits after the light is polarized (Polarizer 2 in the Figure), two possibilities exist. If the second set of slits lines up with the first set, then the light can pass through and we see light on the other side. If, however, the second polarizer is lined up in another fashion, the polarized light is cut out – it can’t make it through the slit.

A simple analogy of this comes from an old-fashioned comedy movie gag where a person carrying a long board tries to go thru a door. If the board lines up with the door, they can pass through, if not, they are blocked. This is similar to how the plane polarized light works.

Polarized light can play an important role in forensic microscopy. In this application, the light that passes through the sample is first polarized by a polarizing lens. Some chemical compounds have the ability to “twist” the plane of orientation of polarized light when they interact with it. When the polarized light encounters these molecules, the plane of the light is twisted, therefore, into a new orientation (Figure 4.3.8). The light passing through the sample is then passed through a second polarizing lens that can be turned to determine the orientation of the

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**Polarized Applications**

A common form of polarizing lenses comes in the form of polarized sunglasses. These lenses are actually made up of tiny parallel slits that allow only light of one orientation through – plane polarized light. These lenses are very good at blocking reflected light that tends to be horizontally polarized since it does not align well with the direction of the slits in the lenses of the glasses. This greatly reduces glare from reflections on water, roads or other shiny surfaces.

Polarized light is also used to produce a 3D effect in movies. When you put on the “magic 3D glasses”, images seem to jump off the screen. This is because the slits in the right and left lenses of the glasses are oriented perpendicular to one another. The movie then projects two images simultaneously on the screen that are polarized 90° to each other. Each lens of the glasses admits only one of the two polarized images while blocking the other. In this way, each eye sees a slightly different image giving the illusion of 3D.
newly “twisted” light – all other light that is not “twisted” is excluded since it is misaligned with the second polarizing lens and cannot pass through.

Polarized light microscopy works particularly well when molecules that “twist” the light are present in the sample. This is very common for minerals but living samples also can contain such molecules. The contrast when these compounds are present can be very striking, as shown in Figure 4.3.9. The technique is often employed for forensic samples to identify the compositions of fibers, minerals, and soils.

**Phase Contrast Microscopy**

One major problem in bright field microscopy is that most biological samples are colorless and do not absorb enough light to provide suitable contrast to clearly show the features in the sample. **Phase contrast microscopy** is a technique that can be used to enhance the contrast in these types of sample.

When light passes through a substance, it is slightly slowed relative to its speed in air (or in a vacuum). Our eyes cannot detect this very small amount of slowing but we can use this phenomenon to enhance contrast. The light that has been slowed by the sample is *out of phase* with light that bypasses the sample - the waves don’t match up properly, as shown at the bottom of Figure 4.3.10. Out of phase means that the like points on two waves, for example the high points, do not align when they are superimposed. This would be similar to two runners on a track who are initially running at the same speed - shoulder-to-shoulder. Then, one runner is just momentarily slowed by a bad piece of track. After the slowing, the two runners are once again moving at the same speed but they are no longer shoulder-to-shoulder – they are out of phase when they were initially in phase. When two out of phase waves are added together, the resulting new wave shows a lower peak height (amplitude). When two waves are completely out of phase (when the highest point of one wave lines up exactly with the lowest point of a second wave) they cancel each other out entirely and no light is observed at all.

In phase contrast microscopy, light that has been slowed by the sample is recombined in a special way with light that is not slowed by the sample. When this happens, the waves partially destructively cancel each other, causing a darkened image for the sample with enhanced contrast.
This enhancement is illustrated in Figure 4.3.11 where a bright field image is compared with a phase contrast image.

**Fluorescence Microscopy**

In the search for ways to enhance the contrast in biological samples, the technique of fluorescence microscopy has become one of the most intensely explored techniques in forensic optical microscopy. In order to understand how it works, it is first necessary to understand how fluorescence itself operates.

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**Figure 4.3.12.** Fluorescence emission through the absorption of shorter wavelength light to emit longer wavelength light (http://scienceblogs.com/purepedantry/2007/11/two_photon.php).

When light shines on a molecule, it can excite some of the electrons in the molecule to a higher energy condition (state). In some special molecules, when they absorb light to become excited they can later re-emit the light but at a slightly longer wavelength that the original excitation light, as shown in Figure 4.3.12. In this example, blue light, first absorbed by the molecule, excites it to a higher energy state. Then, after the molecule loses some energy through one of several ways such as vibration, the molecule emits the remaining excitation energy as lower energy green light. This three-step process is termed **fluorescence** [(1)](http://scienceblogs.com/purepedantry/2007/11/two_photon.php).

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**Figure 4.3.13.** Fluorescent strip in a US 20-dollar bill (http://science.howstuffworks.com/innovation/black-light2.htm).

**Figure 4.3.14.** Bright field (top) and fluorescence micrograph (bottom) of sample containing human sperm cells. The bottom image shows the fluorescent sperm heads clearly after a sperm-specific fluorescent dye has been added to the sample (www.paxit.com/sperm_analysis.asp).
absorption of a photon, (2) loss of some energy, and (3) re-emission of a lower wavelength photon). It is possible to filter out the wavelength of the original exciting light so that you only “see” the fluorescence emission. This process is particularly noticeable when the compound is excited by “invisible” ultraviolet light but emits visible light. You probably have seen this “glow-in-the-dark” effect when “black lights” are used to make items such as clothing, posters, or minerals glow. The US treasury, for example, embeds a fluorescent strip in 20-dollar bills that can only be seen using ultraviolet lamps (black lights) as a method to detect counterfeit bills, Figure 4.3.13.

Some naturally occurring chemicals fluoresce under ultraviolet radiation, including some body fluids such as semen and the residues from fingerprint. Specially designed fluorescent compounds may also be added to a forensic sample that bond specifically with certain chemicals to help with visualizing biological structures.

In fluorescence microscopy, a fluorescent dye, which binds specifically with certain biological materials, such as the nuclei in sperm cells, is often added. Light is then shone on the sample to excite the dye molecules and then the wavelength of the original light is filtered out before it reaches the viewer. The only light that is seen, therefore, comes from the fluorescent glow from the dye in the sample.

In forensic investigations, fluorescence microscopy has found a number of very important uses. One use involves analyzing evidence from rape investigations. Sperm cells are often difficult to differentiate from other cells through other microscopic techniques. Using an appropriate sperm-specific fluorescent stain, the sperm cells readily stand out from all other cells, as shown in Figure 4.3.14. Many fluorescent dyes have now been developed that bind to specific biological structures (and not others in the same sample) and are used to readily see these structure.

In art fraud cases, the determination of the authenticity of older works of art can be supported through fluorescence studies. Older paints and pigments often do not contain fluorescent materials but modern paints and pigments very often contain chemicals that fluoresce under ultraviolet light. This is especially true of the brighter colors where fluorescent dyes are added to make them stand out better because the ultraviolet portion of the sunlight makes them emit visible light – the paints are actually glowing a little bit in the sunlight and that makes them seem more brilliant. The same thing happens with the “whiteners” added to laundry detergent making the “whites brighter” by helping them emit visible light in the sunshine.

Limitations on fluorescence microscopy relate to reduced resolution and the requirement that the sample be rather thin. This later requirement is being circumvented in more recent forensic work through the use of two-photon fluorescence microscopy (using two photons of light to excite the molecule instead of just one).

**Infrared Microscopy**

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**Figure 4.3.15.** Infrared spectrum for 1-propanol (CH₃(CH₂)₂OH) indicating the characteristic IR “fingerprint” region used to identify the compound (www.chemguide.co.uk/analysis/ir/fingerprint.html).
Light that has a wavelength between about 700 nm and 1400 nm, referred to as infrared radiation (IR), is not visible to the unaided eye. Many compounds of forensic interest, however, readily absorb light in this region. For example, many drugs, fibers, pigments, and dyes have specific “signatures” in the IR spectrum, most often in a part of the IR spectrum called the IR “fingerprint” region – the “fingerprint” of a molecule between 500 and 1500 cm⁻¹ (Figure 4.3.15). Comparing the pattern from this region of an unknown with a standard may allow the unknown compound to be identified unambiguously.

In infrared microscopy (actually called infrared microspectrophotometry if we use it to identify specific compounds), IR light is shone on the sample and an IR-sensitive detector, such as film or an electronic detector, is used to allow us “see” the sample since our eyes cannot detect IR light. The way certain very small regions of the field of view absorb infrared light (called absorbance) can be chemically analyzed to help identify the material (see Chapter 12 on spectroscopy). This technique allows for the non-destructive analysis of an image by “mapping” the chemical composition of the entire sample. In this fashion, specific inks, drugs, gunshot residues, explosives, and even fingerprints (Figure 4.3.16) have been determined from very tiny, microscopic samples. Figure 4.3.17 shows an example of how IR microspectrophotometry was be used to prove that a painting was a forgery by determining that the acrylic chemical composition of a brush fiber found embedded within the paint was not known to exist at the time the painting was claimed to have been done.

**Stereo Microscopy**

In the types of microscopy presented thus far, the light travels from the source, directly through the sample, and finally to the viewer or detector (transmitted light through the sample). It is also possible, however, to use scattered and reflected light that bounces off the sample to look specifically at its surface. A stereo microscope, sometimes called a dissecting microscope, does just that using separate lenses for each eye to provide a low magnification 3D image of the sample’s surface. This is essentially similar to using two magnifying lenses, one for each eye, to obtain a magnified 3D look at a sample. This technique allows us to look at samples that are far too thick or
opaque to see using transmitted light microscopy and allows us to focus on surface structures and features.

![Stereo Microscope Diagram](en.wikipedia.org/wiki/File:Stereomic.png)

**Figure 4.3.18.** Optical paths for the two “views” provided by a stereo microscope used to obtain a 3D image of the surface of a sample (en.wikipedia.org/wiki/File:Stereomic.png).

Stereo microscopes are relatively simple in design, Figure 4.3.18 and 4.3.19. It consists of two optical pathways, one for each eye, which collects light bouncing off the surface of the sample. The view from each pathway is slightly different, giving a 3D perspective of the surface. This allows us to see fine details, such as shown for a coin surface in Figure 4.3.20.

**Comparison Microscopy**

In forensic investigations, it is often useful to compare the fine details of two samples, often an unknown collected at a crime scene and a reference sample of known origin. This could be done, of course, by looking at the samples one at a time through the microscope. It is far more convenient and accurate, however, to use an apparatus that allows us to look at both samples simultaneously and compare them side-by-side at once. This is accomplished very effectively through the use of a comparison microscope.

![Comparison Microscope Design](www.ibotz.com/stereo-microscope-st-30c-6led.html)

**Figure 4.3.19.** Design of one type of stereo microscope (www.ibotz.com/stereo-microscope-st-30c-6led.html).

![Stereo Micrographic Image](commons.wikimedia.org/wiki/File:Deutsche_Mark_Anaglyph_2.jpg)

**Figure 4.3.20.** Stereo micrographic image of a Deutsche Mark coin, showing wear and die striation marks (commons.wikimedia.org/wiki/File:Deutsche_Mark_Anaglyph_2.jpg).
In essence, a comparison microscope consists of two separate and complete microscopes that are joined together at the eyepieces, as shown in Figure 4.3.21. In this fashion, the image from one microscope is viewed in one eyepiece while the image from the other microscope is seen through the other eyepiece. This allows us to simultaneously view two different samples and to move them around independently to get the best comparative orientation for both.

The comparison microscope has become essential to many types of forensic investigations including firearm, questioned document, counterfeiting, arson, hair and fiber, soil, and theft cases, among others. For example, when a bullet is fired from a gun, the scratches and imperfections always present in the barrel of the gun are “imprinted” onto the bullet as striations (scratches and grooves). These striations are unique to an individual gun, arising both from its manufacturing process and through wear by use. When a bullet is recovered from a crime scene, investigators are often very concerned about which particular gun fired the bullet found. If a gun is recovered, then a test bullet of the same brand as the recovered bullet can be fired from the suspect gun. The recovered bullet and the test-fired bullet can then be examined in a comparison microscope to look for microscopic similarities and differences. In this process, the bullets are placed on the two different microscope stages and turned around to try to see if the striations (scratches) on the two bullets match up, indicating a high probability that they were fired from the same weapon. Similarly, a recovered casing (the container that originally held the bullet and gunpowder) can be microscopically compared with one obtained by test firing. An example of this is shown in Figure 4.3.22 for two bullet casings. You should notice that the fine striations between the two casings match up very well, indicating the likelihood that both were fired from the same gun.

Comparison microscopes are also commonly employed in forgery and questioned document cases. For example, two bills are shown from a comparison microscope view in Figure 4.3.23. To
the unaided eye, these two are virtually indistinguishable but when placed side-by-side, it is clear that they don’t match up – when the top of the image is lined up the bottom portions are misaligned.

Biological samples can also be compared using this technique. In Figure 4.3.24, two human hairs from the Wayne Williams case were compared (Chapter 7). One hair was found in William’s car while the other was taken from the???. The two were found to be very similar what features?, supporting the idea that both came from the victim.

**Staining Techniques in Microscopy**

Most biological samples do not provide sufficient contrast between the key features of the sample to be easily seen. In the preceding sections, we discussed a variety of microscopic techniques that have been developed to enhance the contrast in a sample using optical tools. It is also possible, however, to enhance the contrast of a sample by modifying the sample itself through staining techniques.

<table>
<thead>
<tr>
<th>Stain</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carmine</td>
<td>Stains glycogen red</td>
</tr>
<tr>
<td>Crystal violet</td>
<td>Stains cell walls purple</td>
</tr>
<tr>
<td>Fuchsin</td>
<td>Stains bacteria, collagen, smooth muscle and mitochondria magenta</td>
</tr>
<tr>
<td>Malachite green</td>
<td>Stains bacteria and spores blue-green</td>
</tr>
<tr>
<td>Neutral red</td>
<td>Stains neurons red</td>
</tr>
<tr>
<td>Osmium tetroxide</td>
<td>Stains lipids black</td>
</tr>
<tr>
<td>Rhodamine</td>
<td>A fluorescent stain for proteins</td>
</tr>
<tr>
<td>Safranin</td>
<td>Stains nuclei red</td>
</tr>
</tbody>
</table>

**Figure 4.3.23.** Comparison micrographs of two banknotes: the original (left) clearly does not align with the suspected forgery (right) (www.upei.ca/~morph/webct/Modules/Light_Microscopy/Stains/H&E.html).

**Figure 4.3.24.** Comparison micrograph showing two human hairs (unknown and reference sample) from the Wayne Williams case (http://geradts.com/anal/ij/vol_006_no_002/reviews/pb/page001ex.html).

**Table 4.3.1.** Examples of Stains used in Microscopy

**Figure 4.3.25.** Example of a tissue with contrast enhanced by two different stains and their mixture (www.aperi.ca/~morph/webct/Modules/Light_Microscopy/Stains/H&E.html).
Staining simply refers to adding a colored or light absorbing chemicals that bind selectively to specific parts of a sample so as to allow us to more readily see the various features of the sample. Many, many dyes have been developed that are useful for imparting color to a biological sample. Several of these are listed in Table 4.3.1. Ideally, the stain used will specifically localize only in the structure that you wish to see. For example, iodine will bind to starch and color it dark blue while methylene blue is used to stain animal cell nuclei blue. Stains can also be combined to give greater enhanced contrast, as illustrated in Figure 4.3.25. Fluorescent stains, as already mentioned, are good examples of the specific use of stains in visualizing biological features.

**Other Forms of Optical Microscopy**

There are many other types of optical microscopy, including numerous variants on those described here, but these have largely not yet made their way into widespread use in forensic investigations. This may seem like a bewildering array of techniques, but each microscopic technique provides an important tool in the arsenal to explore the very small features of forensic samples. Table 4.3.2 is an attempt to summarize the main features, advantages, and limitations of the different microscopic techniques presented in this chapter to aid in understanding their place in scientific examinations.

In this section, we have focused upon microscopy that uses “light” (actually electromagnetic radiation of a variety of wavelengths) as the means for illuminating the sample. In the next section, however, we explore the important use of another type of “illumination” for a sample: electrons.
Microscopy and the DC Snipers

For three weeks in October 2002, the metro-Washington DC area was on edge. During this time, an unknown sniper shot and killed 10 innocent people and wounded a number of others in a seemingly random, lunatic rampage. It was the top story in every newspaper, and in TV and radio broadcasts for hours on end. The crime spree finally ended with the arrest of two men, John Allen Muhammad and Lee Boyd Malvo, who were apprehended while they sleeping in their car at an interstate rest stop in the DC area.

Forensic microscopy played a significant role in the identification, tracking and later conviction of the two men in this killing spree. Using comparison microscopy, the bullets from the various shootings were examined to link many of the crimes together and, later, to the gun found in Muhammad’s car and other shootings around the country. But, microscopy also helped to provide critical early leads in the case.

Early on in the shooting spree, the sniper had taunted police by saying that he had been involved a month earlier in the murder of two women in Montgomery, Alabama. This was the break investigators were hoping for. They were able to quickly identify the crime that the sniper described and found the ballistic evidence from the Alabama case that matched the microscopic evidence from the DC area. Fingerprints were also found on the Alabama bullet magazine and, by searching the national fingerprint database, a match was found – teenager Lee Boyd Malvo. Malvo had been previously arrested but, more importantly, another name also surfaced with Malvo’s previous record of arrest in Tacoma, Washington - that of John Allen Muhammad. FBI agents quickly located a target “practice” tree stump associated with Muhammad in a yard in Tacoma, Washington and microscopy was again used to link this Tacoma evidence to the Alabama and DC shootings. Now the police had two key suspects – and the license plate number for Muhammad’s blue Chevy Caprice with New Jersey plates NDA-21Z. ATF records also showed that Muhammad also had a Bushmaster gun in his possession, the same type of gun used in the DC shootings. It only took one day after a media blitz of this information for the car to be spotted and the two suspects to be arrested.

Comparison microscopy of ballistic evidence played a key role both in identifying and convicting the two men responsible for the shootings. Muhammad and Mulvo were convicted and Muhammad was executed on Nov. 10, 2009 in Virginia.
<table>
<thead>
<tr>
<th>Type of Microscopy</th>
<th>Operation and Uses</th>
<th>Source</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Mag.</th>
<th>Resol.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bright Field Optical Microscopy</td>
<td>Uses visible light absorbed by parts of a sample to provide contrast</td>
<td>Visible Light</td>
<td>Easy, rapid and most readily available instrument</td>
<td>Difficult to see many types of samples due to poor contrast, especially biological samples; light must go thru the sample</td>
<td>100X to 1,000X</td>
<td>~ 500 nm</td>
</tr>
<tr>
<td>Dark Field Optical Microscopy</td>
<td>Displays only light refracted or scattered by the sample</td>
<td>Visible Light</td>
<td>Allows improved contrast of some biological samples</td>
<td>Limited improvement in contrast; light must go thru the sample</td>
<td>100X to 1,000X</td>
<td>~ 500 nm</td>
</tr>
<tr>
<td>Polarized Light Microscopy</td>
<td>Uses plane polarized light to enhance contrast</td>
<td>Visible Light</td>
<td>Allows improved contrast of some biological samples</td>
<td>Limited improvement in contrast; light must go thru the sample</td>
<td>100X to 1,000X</td>
<td>~ 500 nm</td>
</tr>
<tr>
<td>Phase Contrast Microscopy</td>
<td>Uses phase differences between light passing thru the sample and light bypassing the sample to enhance contrast</td>
<td>Visible Light</td>
<td>Allows improved contrast of some biological samples</td>
<td>Limited improvement in contrast; light must go thru the sample</td>
<td>100X to 1,000X</td>
<td>~ 500 nm</td>
</tr>
<tr>
<td>Fluorescence Microscopy</td>
<td>Uses dyes added to sample or natural fluorescence in sample to enhance contrast</td>
<td>Visible Light</td>
<td>Allows improved contrast of some biological samples</td>
<td>Limited improvement in contrast; light must go thru the sample</td>
<td>100X to 1,000X</td>
<td>~ 500 nm</td>
</tr>
<tr>
<td>Stereo Microscopy</td>
<td>Uses scattered and reflected light to provide a 3D image of the sample</td>
<td>Visible Light</td>
<td>View surfaces of samples in 3D</td>
<td>Limited magnification and images only the surface</td>
<td>10x to 100x</td>
<td>~ 0.01 mm</td>
</tr>
<tr>
<td>Comparison Microscopy</td>
<td>Two tandem stereo microscopes</td>
<td>Visible Light</td>
<td>Able to compare surfaces of two samples simultaneously</td>
<td>Limited magnification and images only the surface</td>
<td>10X to 100X</td>
<td>~ 0.01 mm</td>
</tr>
<tr>
<td>Scanning Electron Microscopy (SEM)</td>
<td>Contrast created by electron scattering from sample</td>
<td>Electron beam</td>
<td>View surfaces of very small samples with high resolution</td>
<td>Non-living samples; view only scattered electrons from the surface</td>
<td>~100,000 to 1,000,000 X</td>
<td>0.2 – 2 nm</td>
</tr>
<tr>
<td>Transmission Electron Microscopy (TEM)</td>
<td>Contrast by electron transmission through sample</td>
<td>Electron Beam</td>
<td>View internal structure of very small samples with high resolution</td>
<td>Non-living samples; view only internal structure</td>
<td>Up to ~5,000,000X</td>
<td>0.5 – 10 nm</td>
</tr>
</tbody>
</table>