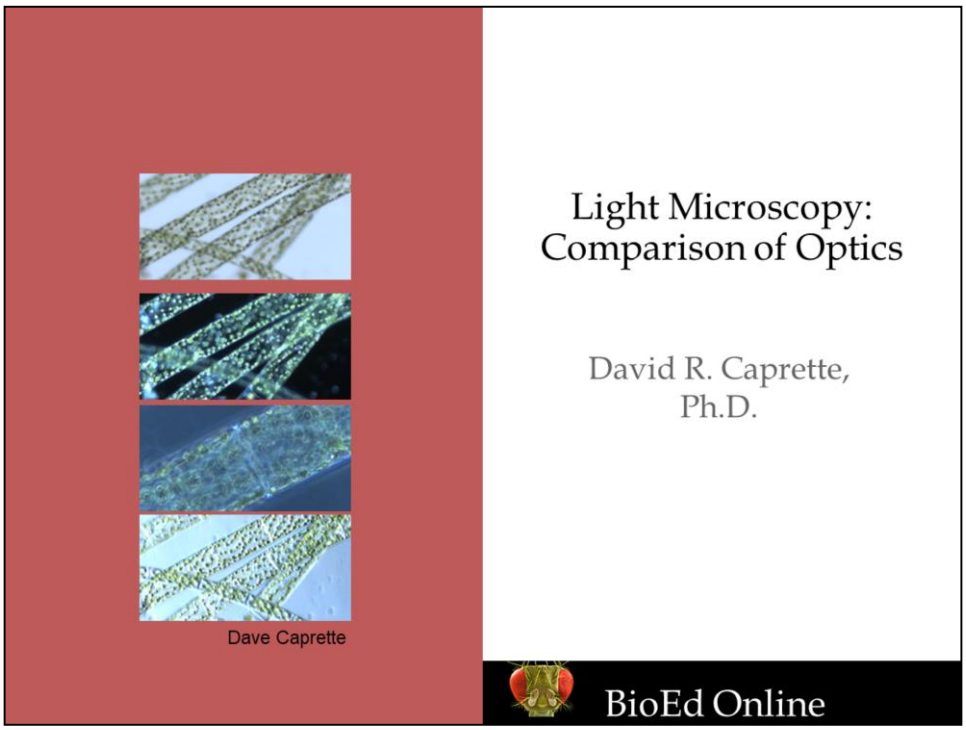


Light Microscopy: Comparison of Optics

References:

Alberts, B., et al. (2002). *Molecular biology of the cell* (4th ed.). New York: Garland Science.

Caprette, D. (2005). Light microscopy. Retrieved 09-12-2005 from http://www.ruf.rice.edu/~bioslabs/methods/microscopy/microscopy.html

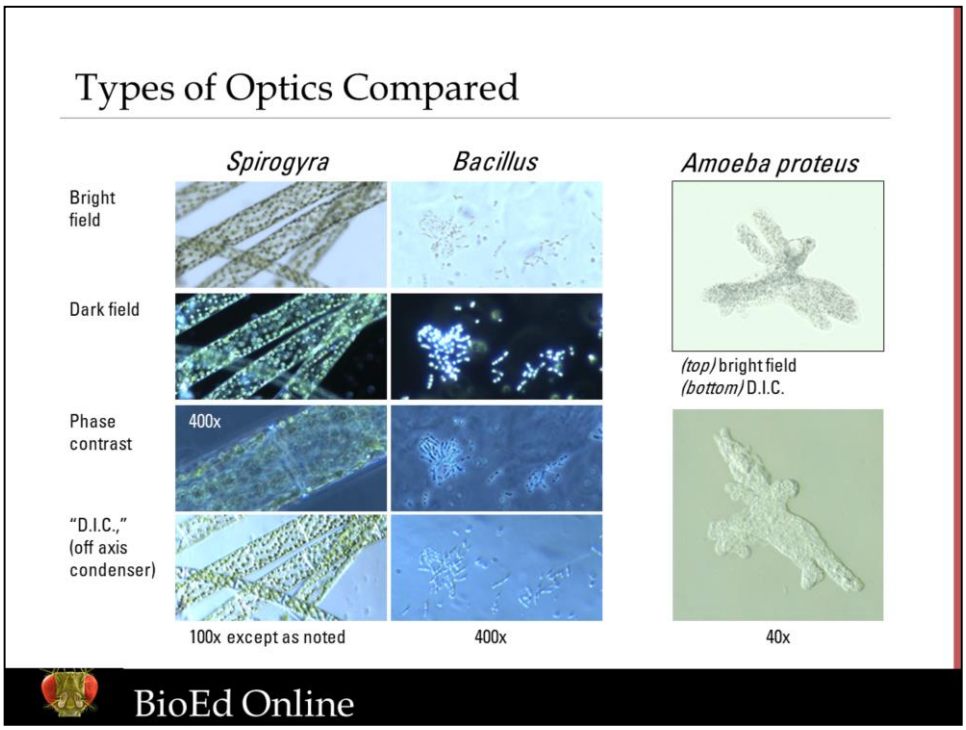
Lodish, H.,et al. (2000). *Molecular cell biology* (4th ed.). New York: W.H. Freeman and Co.

Nave, C.R. (2005). Hyperphysics (light and vision). Retrieved 09-12-2005 from http://hyperphysics.phy-astr.gsu.edu/hbase/hframe.html

Wolfe, S.L. (1993). *Molecular and cellular biology*. Belmont, CA: Wadsworth Publishing Company.

Image Reference:

Caprette, D. (2005). Comparison of optics.



Types of Optics Compared

The best choice of optics depends on the specimen to be viewed. Specialized optics can give wonderfully detailed views of objects that are disappointing in bright field, but bright field often is a better choice. Bright field optics are usually—but not always—the best choice for viewing stained tissue.

A naturally pigmented specimen, such as Spirogyra may appear more dramatic with dark field or D.I.C. optics, but the cell divisions and chloroplasts are distinct in bright field, and the colors are true to nature. Phase contrast does not contribute additional information, and the halo that typically surrounds a specimen actually detracts from ideal contrast. On the other hand, phase contrast optics give the best view of spore-forming bacteria such as Bacillus thuringiensis. Dark field optics show the cell walls and spores with excellent resolution. To see such features in bright field, one must stop down the condenser aperture, causing distortion of the details.

Differential interference contrast does not do much for our views of a specimen such as Spirogyra or Bacillus. On the other hand, D.I.C. optics increase the depth of focus, making features of an object such as an amoeba very distinct even if they do overlap each other.

References:

Alberts, B., et al. (2002). *Molecular biology of the cell* (4th ed.). New York: Garland Science.

Caprette, D. (2005). Light microscopy. Retrieved 09-12-2005 from http://www.ruf.rice.edu/~bioslabs/methods/microscopy/microscopy.html

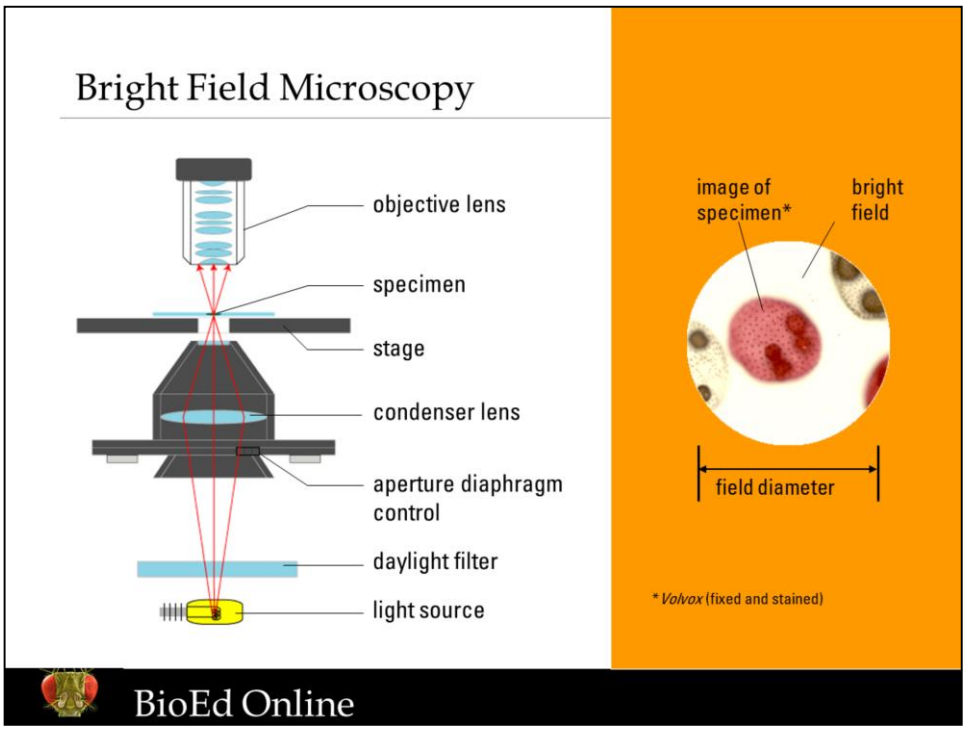
Lodish, H.,et al. (2000). *Molecular cell biology* (4th ed.). New York: W.H. Freeman and Co.

Nave, C.R. (2005). Hyperphysics (light and vision). Retrieved 09-12-2005 from http://hyperphysics.phy-astr.gsu.edu/hbase/hframe.html

Wolfe, S.L. (1993). *Molecular and cellular biology*. Belmont, CA: Wadsworth Publishing Company.

Image Reference:

Dave Caprette. (2005).



Bright Field Microscopy

Any light microscope requires a fairly intense light source, preferably built into the microscope and preferably with an intensity control. A typical light source is a tungsten lamp, which produces a yellowish light compared to daylight. On a good quality microscope, light passes through a blue or polarized filter to remove some of the light of yellow wavelength selectively, so that the colors we see are true colors.

Light from the source is collected by a component called a condenser, which serves to focus the light toward the specimen. By concentrating the light, the condenser increases the intensity of illumination. Lenses and/or devices in a condenser permit the use of specialized optics if they are available. On a bright field microscope, a device in the condenser, called an aperture diaphragm, allows a user to optimize contrast and resolution. With some microscopes, the position of the condenser is adjustable so that it can be centered in the light path or moved up and down.

The light path is through the condenser lens, directly through the specimen, and into an objective lens. The user can select from several objective lenses, adjust the condenser, and vary the intensity of illumination at the source.

The expression “bright field” refers to the fact that absorbance or scattering of light causes the object to show up against a bright background, called the field of view, or simply the field. The specimen absorbs light due to natural

pigmentation, because we deliberately stain it, and/or because it is dense enough to scatter a significant amount of light. Many specimens, especially live specimens, are invisible or nearly so in a bright field microscope. Viewing them requires staining or the use of specialized optics. The first order of business, then, is to consider the amount of contrast you can expect from a given specimen, including whether or not it is stained or naturally pigmented.

References:

Alberts, B., et al. (2002). *Molecular biology of the cell* (4th ed.). New York: Garland Science.

Caprette, D. (2005). Light microscopy. Retrieved 09-12-2005 from http://www.ruf.rice.edu/~bioslabs/methods/microscopy/microscopy.html

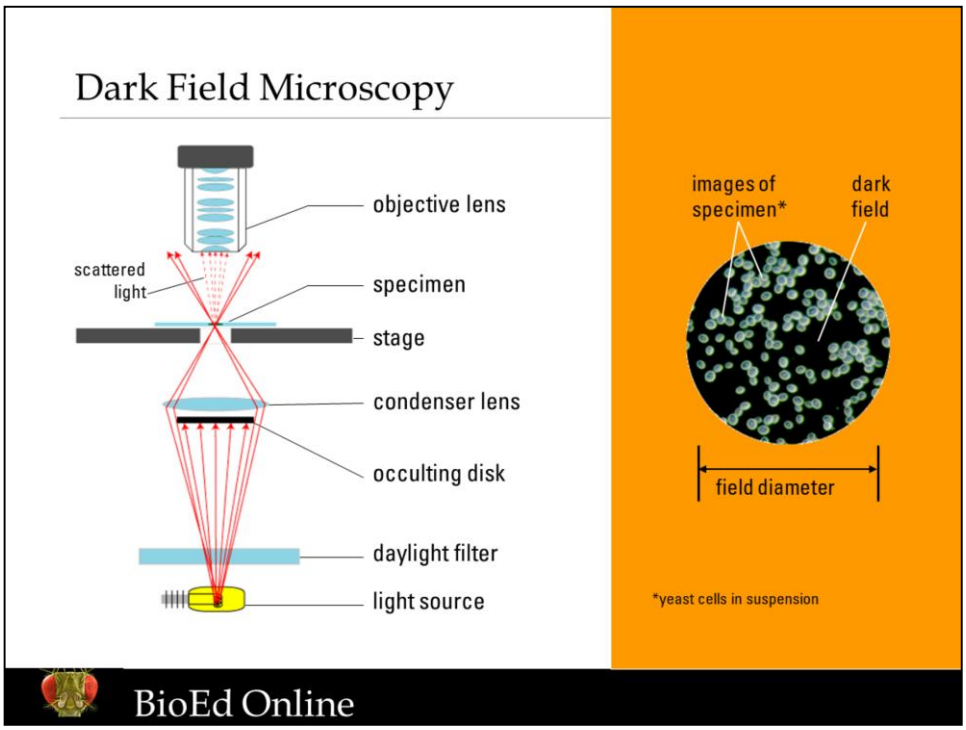
Lodish, H.,et al. (2000). *Molecular cell biology* (4th ed.). New York: W.H. Freeman and Co.

Nave, C.R. (2005). Hyperphysics (light and vision). Retrieved 09-12-2005 from http://hyperphysics.phy-astr.gsu.edu/hbase/hframe.html

Wolfe, S.L. (1993). *Molecular and cellular biology.* Belmont, CA: Wadsworth Publishing Company.

Image Reference:

Dave Caprette. (2005).



Dark Field Microscopy

Dark field optics are very useful for finding a target, especially when the target is small, unstained, and/or moving. Organelles such as cilia, flagella, contractile vacuoles, and cell nuclei may be obscure or invisible in a bright field microscope. They show up readily in dark field, and with much better resolution than can be obtained using bright field optics. Dark field optics can resolve objects with diameters as little as 25 nanometers (1/40 of a micrometer), which is far superior to the resolution attainable using bright field optics.

Magnification is usually not a problem when looking for the specimen. The smallest known living things are the bacteria, and the smallest of bacteria can be seen at 40x total magnification, which is typically the lowest magnification on a compound microscope. The issue is contrast. Living bacteria are essentially invisible in bright field because they are too small to absorb or scatter sufficient light to be detected. Living bacteria are readily detected in dark field.

The term “dark field” refers to the fact that specimens appear as bright images against a nearly black background. To obtain dark field imaging, an opaque (occulting) disk is placed in the light path on the opposite side from the specimen. The disk blocks light from traveling directly through the condenser to the objective lens. The only light that can reach the condenser lens is light that passes around the edges of the occulting disk. For a given objective, the disk is sized so that light that does pass around the edges and is transmitted

through the specimen cannot reach the objective lens.

Light that passes through a clear area of a specimen will travel in a fairly straight line and shoot right past the objective lens. If there is any object in the way, no matter how small, it will scatter some of the light. Some of the scattered light will be diverted into the objective lens, making the object visible as a bright object against a dark background. Because dark field optics rely on scattered light, one generally needs the maximum illumination intensity possible. Dark field should not be used for color determinations such as Gram stain interpretation, because scattered light will be of a different wavelength than the incident light. A true pink may appear purple, for example, and a purple stained object may appear pink or even gold colored.

References:

Alberts, B., et al. (2002). *Molecular biology of the cell* (4th ed.). New York: Garland Science.

Caprette, D. (2005). Light microscopy. Retrieved 09-12-2005 from http://www.ruf.rice.edu/~bioslabs/methods/microscopy/microscopy.html

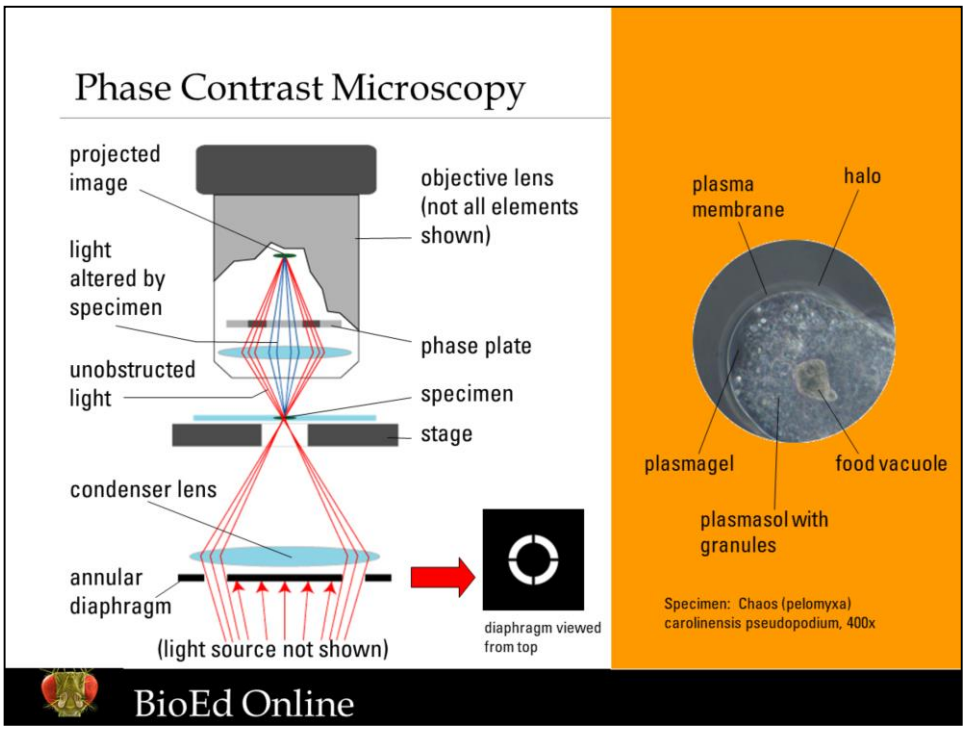
Lodish, H.,et al. (2000). *Molecular cell biology* (4th ed.). New York: W.H. Freeman and Co.

Nave, C.R. (2005). Hyperphysics (light and vision). Retrieved 09-12-2005 from http://hyperphysics.phy-astr.gsu.edu/hbase/hframe.html

Wolfe, S.L. (1993). *Molecular and cellular biology.* Belmont, CA: Wadsworth Publishing Company.

Image Reference:

Dave Caprette. (2005



Phase Contrast Microscopy

There is too little difference in transparency or color among most intracellular structures to enable them to be seen in a bright field light microscope. However, structures such as cell nuclei, cytoplasm, contractile vacuoles, cilia, and flagella differ markedly in refractive index. A phase contrast microscope causes images of such structures to differ in brightness, producing high contrast with similar resolution to that of a bright field instrument.

Light passing through an object with high refractive index is scattered so that the light path is longer than for objects that do not refract light as much. Scattered light also loses some velocity when traveling through the object. The maximum difference in path length between biological structures of low versus high refractive index typically is 1/4 of a wavelength. A phase contrast lens contains an element, called a “phase plate,” that is matched to an annular diaphragm in the condenser. A hollow cone of light passes through the diaphragm, the condenser lens, and then through the specimen. Light that is not significantly scattered by the specimen passes through the central part of the phase plate, which does not alter the properties of the light. Highly scattered light passes through a ring in the phase plate with high refractive index, causing the light to lose another 1/4 wavelength.

The image comes to a focus at the back of the objective lens, where the altered light is recombined with the unobstructed light. Light that has been retarded a full 1/2 wavelength is completely out of phase with the unobstructed light, causing interference that shows up as a dark spot. Unscattered light

recombines in phase and thus shows up as a bright spot. By centering the condenser so that the annular diaphragm is concentric with the phase plate, one can optimize the dynamic range (i.e., obtain maximum contrast in brightness among parts of a specimen with varying indexes of refraction). Organelles that are nearly or completely invisible in bright field often are strikingly apparent in phase contrast.

References:

Alberts, B., et al. (2002). *Molecular biology of the cell* (4th ed.). New York: Garland Science.

Caprette, D. (2005). Light microscopy. Retrieved 09-12-2005 from http://www.ruf.rice.edu/~bioslabs/methods/microscopy/microscopy.html

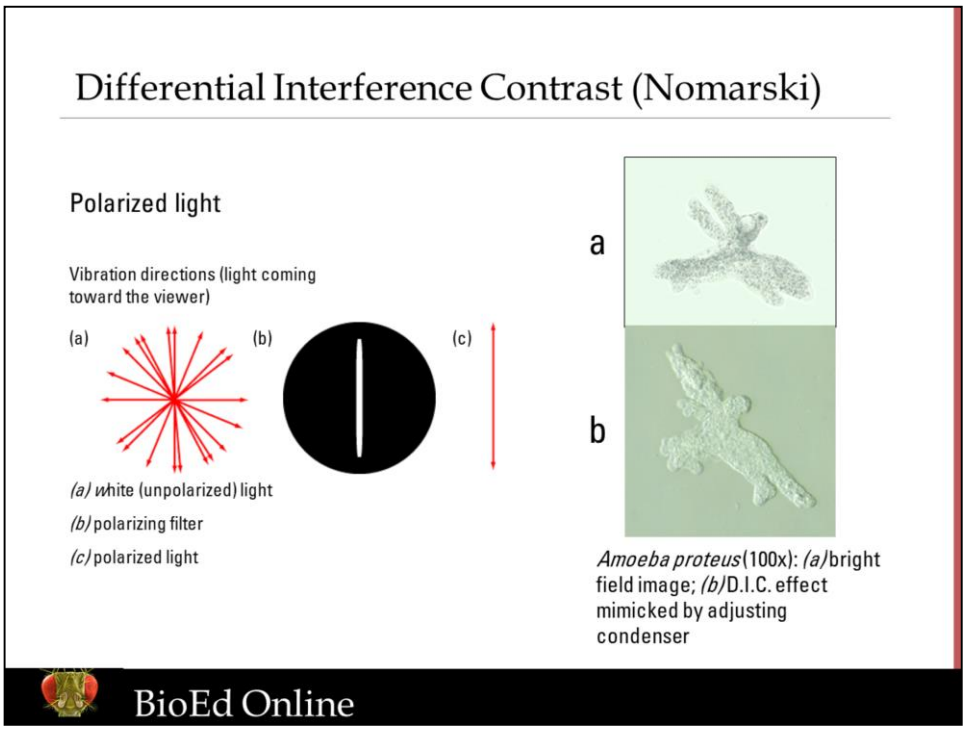
Lodish, H.,et al. (2000). *Molecular cell biology* (4th ed.). New York: W.H. Freeman and Co.

Nave, C.R. (2005). Hyperphysics (light and vision). Retrieved 09-12-2005 from http://hyperphysics.phy-astr.gsu.edu/hbase/hframe.html

Wolfe, S.L. (1993). *Molecular and cellular biology.* Belmont, CA: Wadsworth Publishing Company.

Image Reference:

Dave Caprette. (2005).



Differential Interference Contrast

Differential interference contrast (D.I.C.) optics also are known as Nomarski optics, after their inventor, Jerzy Nomarski. As with phase contrast microscopy, D.I.C. enhances contrast by taking advantage of differences in refractive index among parts of a specimen. In D.I.C., however, the optics make use of polarized light.

Light consists of electromagnetic waves that have the properties of particles as well as of waves. Suppose you view a beam of white light coming directly toward you from the source. If you could see the individual waves somehow, you would see them vibrating in random directions. If, on the way to you, the beam passed through a polarizing filter, the waves that pass through would be seen to vibrate only in one plane, that is, in two opposite directions such as up and down, left and right, or at some other angle, depending on the position of the filter. Without going into too much detail, let's just acknowledge that by using polarizing filters, one can direct more than one beam of light at an object and select one or the other beam using appropriate filters.

D.I.C. works by using a prism to direct two beams of polarized light at a specimen, with one beam slightly offset from the other. A second prism reassembles the beams to produce a shadowing effect. The result is an apparent three-dimensional image, not unlike a scanning electron micrograph or the three-dimensional effect one sees when viewing the terminator on the Moon. The terminator is the boundary between the lit and unlit face of the Moon, where the shadowing effect highlights craters and other features. In D.I.C., the contrast we see is due to differences in refractive index within a specimen, rather than to topography. In fact, the three-dimensional image does not necessarily correspond to the actual shape of the specimen.

A D.I.C. microscope typically is very expensive and not likely to be used in a teaching laboratory. However, one can mimic a D.I.C. effect using a bright field microscope with an adjustable condenser. It is necessary to move the condenser slightly off axis to produce a shadowing effect. A very dramatic effect can be achieved through trial and error.

References:

Alberts, B., et al. (2002). *Molecular biology of the cell* (4th ed.). New York: Garland Science.

Caprette, D. (2005). Light microscopy. Retrieved 09-12-2005 from http://www.ruf.rice.edu/~bioslabs/methods/microscopy/microscopy.html

Lodish, H.,et al. (2000). *Molecular cell biology* (4th ed.). New York: W.H. Freeman and Co.

Nomarski, G. (2003). Molecular Expressions. Retrieved 10-18-2005 from http://micro.magnet.fsu.edu/optics/timeline/people/nomarski.html

Nave, C.R. (2005). Hyperphysics (light and vision). Retrieved 09-12-2005 from http://hyperphysics.phy-astr.gsu.edu/hbase/hframe.html

Wolfe, S.L. (1993). *Molecular and cellular biology*. Belmont, CA: Wadsworth Publishing Company.

Image Reference:
Dave Caprette. (2005).