Light and Color

Light is a complex phenomenon that is classically explained with a simple model based on rays and wavefronts. The Molecular Expressions Microscopy Primer explores many of the aspects of visible light starting with an introduction to electromagnetic radiation and continuing through to human vision and the perception of color. Each section outlined below is an independent treatise on a limited aspect of light and color. We hope you enjoy your visit and find the answers to your questions.

Electromagnetic Radiation - Visible light is a complex phenomenon that is classically explained with a simple model based on propagating rays and wavefronts, a concept first proposed in the late 1600s by Dutch physicist Christiaan Huygens. Electromagnetic radiation, the larger family of wave-like phenomena to which visible light belongs (also known as **radiant energy**), is the primary vehicle transporting energy through the vast reaches of the universe. The mechanisms by which visible light is emitted or absorbed by substances, and how it predictably reacts under varying conditions as it travels through space and the atmosphere, form the basis of the existence of color in our universe.

Light: Particle or a Wave? - Many distinguished scientists have attempted to explain how electromagnetic radiation can display what has now been termed **duality**, or both particle-like and wave-like behavior. At times light behaves as if composed of particles, and at other times as a continuous wave. This complementary, or dual, role for the properties of light can be employed to describe all of the known characteristics that have been observed experimentally, ranging from refraction, reflection, interference, and diffraction, to the results with polarized light and the photoelectric effect.

Sources of Visible Light - A wide variety of sources are responsible for emission of electromagnetic radiation, and are generally categorized according to the specific spectrum of wavelengths generated by the source. Relatively long radio waves are produced by electrical current flowing through huge broadcast antennas, while much shorter visible light waves are produced by the energy state fluctuations of negatively charged electrons within atoms. The shortest form of electromagnetic radiation, gamma waves, results from decay of nuclear components at the center of the atom. The visible light that humans are able to see is usually a mixture of wavelengths whose varying composition is a function of the light source.

Fluorescence - The phenomenon of fluorescence was known by the middle of the nineteenth century. British scientist Sir George G. Stokes first made the observation that the mineral **fluorspar** exhibits fluorescence when illuminated with ultraviolet light, and he coined the word "fluorescence". Stokes observed that the fluorescing light has longer wavelengths than the excitation light, a phenomenon that has become to be known as the **Stokes shift**. Fluorescence microscopy is an excellent method of studying material that can be made to fluoresce, either in its natural form (termed **primary** or **auto** fluorescence) or when treated with chemicals capable of fluorescing (known as **secondary** fluorescence). The fluorescence microscope was devised in the early part of the twentieth century by August Köhler, Carl Reichert, and Heinrich Lehmann, among others. However, the potential of this instrument was not realized for several decades, and fluorescence microscopy is now an important (and perhaps indispensable) tool in cellular biology.

Speed of Light - Starting with Ole Roemer's 1676 breakthrough endeavors, the speed of light has been measured at least 163 times by more than 100 investigators utilizing a wide variety of different techniques. Finally in 1983, more than 300 years after the first serious measurement attempt, the speed of light was defined as being 299,792.458 kilometers per second by the

Seventeenth General Congress on Weights and Measures. Thus, the meter is defined as the distance light travels through a vacuum during a time interval of 1/299,792,458 seconds. In general, however, (even in many scientific calculations) the speed of light is rounded to 300,000 kilometers (or 186,000 miles) per second.

Reflection of Light - Reflection of light (and other forms of electromagnetic radiation) occurs when the waves encounter a surface or other boundary that does not absorb the energy of the radiation and bounces the waves away from the surface. The incoming light wave is referred to as an ***incident*** wave and the wave that is bounced away from the surface is called the ***reflected*** wave. The simplest example of visible light reflection is the glass-like surface of a smooth pool of water, where the light is reflected in an orderly manner to produce a clear image of the scenery surrounding the pool. Throw a rock into the pool, and the water is perturbed to form waves, which disrupt the image of the scene by scattering the reflected light in all directions.

Refraction of Light - As light passes from one substance into another, it will travel straight through with no change of direction when crossing the boundary between the two substances head-on (perpendicular, or a 90-degree angle of incidence). However, if the light impacts the boundary at any other angle it will be bent or refracted, with the degree of refraction increasing as the beam is progressively inclined at a greater angle with respect to the boundary. As an example, a beam of light striking water vertically will not be refracted, but if the beam enters the water at a slight angle it will be refracted to a very small degree. If the angle of the beam is increased even further, the light will refract with increasing proportion to the entry angle. Early scientists realized that the ratio between the angle at which the light crosses the media interface and the angle produced after refraction is a very precise characteristic of the material producing the refraction effect.

Diffraction of Light - Depending on the circumstances that give rise to the phenomenon, diffraction can be perceived in a variety of different ways. Scientists have cleverly utilized diffraction of neutrons and X-rays to elucidate the arrangement of atoms in small ionic crystals, molecules, and even such large macromolecular assemblies as proteins and nucleic acids. Electron diffraction is often employed to examine periodic features of viruses, membranes, and other biological organisms, as well as synthetic and naturally occurring materials. No lens exists that will focus neutrons and X-rays into an image, so investigators must reconstruct images of molecules and proteins from the diffraction patterns using sophisticated mathematical analysis. Fortunately, magnetic lenses can focus diffracted electrons in the electron microscope, and glass lenses are very useful for focusing diffracted light to form an optical image that can easily be viewed.

Polarization of Light - The human eye lacks the ability to distinguish between randomly oriented and polarized light, and plane-polarized light can only be detected through an intensity or color effect, for example, by reduced glare when wearing polarized sun glasses. In effect, humans cannot differentiate between the high contrast real images observed in a polarized light microscope and identical images of the same specimens captured digitally (or on film), and then projected onto a screen with light that is not polarized. The first clues to the existence of polarized light surfaced around 1669 when Erasmus Bartholin discovered that crystals of the mineral Iceland spar (more commonly referred to as **calcite**) produce a double image when objects are viewed through the crystals in transmitted light. During his experiments, Bartholin also observed a quite unusual phenomenon. When the calcite crystals are rotated about their axis, one of the images moves in a circle around the other, providing strong evidence that the crystals are somehow splitting the light into two different beams.

Fundamentals of Interference - The seemingly close relationship between diffraction and interference occurs because they are actually manifestations of the same physical process and produce ostensibly reciprocal effects. Most of us observe some type of optical interference almost every day, but usually do not realize the events in play behind the often-kaleidoscopic display of color produced when light waves interfere with each other. One of the best examples of interference is demonstrated by the light reflected from a film of oil floating on water. Another example is the thin film of a soap bubble, which reflects a spectrum of beautiful colors when illuminated by natural or artificial light sources.

Optical Birefringence - Anisotropic crystals, such as quartz, calcite, and tourmaline, have crystallographically distinct axes and interact with light by a mechanism that is dependent upon the orientation of the crystalline lattice with respect to the incident light angle. When light enters the **optical axis** of anisotropic crystals, it behaves in a manner similar to the interaction with isotropic crystals, and passes through at a single velocity. However, when light enters a non-equivalent axis, it is refracted into two rays each polarized with the vibration directions oriented at right angles to one another, and traveling at different velocities. This phenomenon is termed **double refraction** or **birefringence** and is exhibited to a greater or lesser degree in all anisotropic crystals.

Color Temperature - The concept of color temperature is of critical importance in photography and digital imaging, regardless of whether the image capture device is a camera, microscope, or telescope. A lack of proper color temperature balance between the microscope light source and the film emulsion or image sensor is the most common reason for unexpected color shifts in photomicrography and digital imaging. If the color temperature of the light source is too low for the film, photomicrographs will have an overall yellowish or reddish cast and will appear **warm**. On the other hand, when the color temperature of the light source is too high for the film, photomicrographs will have a blue cast and will appear **cool**. The degree of mismatch will determine the extent of these color shifts, with large discrepancies leading to extremes in color variations. Perhaps the best example is daylight film used in a microscope equipped with a tungsten-halogen illumination source without the benefit of color balancing filters. In this case, the photomicrographs will have a quite large color shift towards warmer reddish and yellowish hues. As problematic as these color shifts may seem, they are always easily corrected by the proper use of conversion and light balancing filters.

Primary Colors - The human eye is sensitive to a narrow band of electromagnetic radiation that lies in the wavelength range between 400 and 700 nanometers, commonly known as the visible light spectrum, which is the only source of color. When combined, all of the wavelengths present in visible light, about a third of the total spectral distribution that successfully passes through the Earth's atmosphere, form colorless white light that can be refracted and dispersed into its component colors by means of a prism. The colors red, green, and blue are classically considered the **primary** colors because they are fundamental to human vision. Light is perceived as white by humans when all three cone cell types are simultaneously stimulated by equal amounts of red, green, and blue light.

Light Filters - A majority of the common natural and artificial light sources emit a broad range of wavelengths that cover the entire visible light spectrum, with some extending into the ultraviolet and infrared regions as well. For simple lighting applications, such as interior room lights, flashlights, spot and automobile headlights, and a host of other consumer, business, and technical applications, the wide wavelength spectrum is acceptable and quite useful. However, in many cases it is desirable to narrow the wavelength range of light for specific applications that require a selected region of color or frequency. This task can be easily accomplished through the use of specialized filters that transmit some wavelengths and selectively absorb, reflect, refract, or diffract unwanted wavelengths.

Human Vision and Color Perception - Human stereo color vision is a very complex process that is not completely understood, despite hundreds of years of intense study and modeling. Vision involves the nearly simultaneous interaction of the two eyes and the brain through a network of neurons, receptors, and other specialized cells. The first steps in this sensory process are the stimulation of light receptors in the eyes, conversion of the light stimuli or images into signals, and transmission of electrical signals containing the vision information from each eye to the brain through the **optic nerves**. This information is processed in several stages, ultimately reaching the **visual cortices** of the cerebrum.

Light and Energy - Mankind has always been dependent upon energy from the sun's light both directly - for warmth, to dry clothing, to cook, and indirectly to provide food, water, and air. Our awareness of the value of the sun's rays revolves around the manner in which we benefit from the energy, but there are far more fundamental implications from the relationship between light and energy. Whether or not mankind devises ingenius mechanisms to harness the sun's energy, our planet and the changing environment contained within is naturally driven by the energy of sunlight.

Introduction to Lenses and Geometrical Optics - The action of a simple lens, similar to many of those used in the microscope, is governed by the principles of refraction and reflection and can be understood with the aid of a few simple rules about the geometry involved in tracing light rays through the lens. The basic concepts explored in this discussion, which are derived from the science of **Geometrical Optics**, will lead to an understanding of the magnification process, the properties of real and virtual images, and lens **aberrations** or defects.

Basic Properties of Mirrors - Reflection of light is an inherent and important fundamental property of mirrors, and is quantitatively gauged by the ratio between the amount of light reflected from the surface and that incident upon the surface, a term known as **reflectivity**. Mirrors of different design and construction vary widely in their reflectivity, from nearly 100 percent for highly-polished mirrors coated with metals that reflect visible and infrared wavelengths, to nearly zero for strongly absorbing materials.

Prisms and Beamsplitters - Prisms and beamsplitters are essential components that bend, split, reflect, and fold light through the pathways of both simple and sophisticated optical systems. Cut and ground to specific tolerances and exact angles, prisms are polished blocks of glass or other transparent materials that can be employed to deflect or deviate a light beam, rotate or invert an image, separate polarization states, or disperse light into its component wavelengths. Many prism designs can perform more than one function, which often includes changing the line of sight and simultaneously shortening the optical path, thus reducing the size of optical instruments.

Laser Fundamentals - Ordinary natural and artificial light is released by energy changes on the atomic and molecular level that occur without any outside intervention. A second type of light exists, however, and occurs when an atom or molecule retains its excess energy until **stimulated** to emit the energy in the form of light. Lasers are designed to produce and amplify this stimulated form of light into intense and focused beams. The word laser was coined as an acronym for **L**ight **A**mplification by the **S**timulated **E**mission of **R**adiation. The special nature of laser light has made laser technology a vital tool in nearly every aspect of everyday life including communications, entertainment, manufacturing, and medicine.

Light and Color Java Tutorials - Difficult concepts in the physics of light and the science of optics are much easier to understand with the aid of interactive tutorials that demonstrate various aspects of the principles involved. Check out these cool Java tutorial-applets that explore a wide range of concepts in light, color, and optics.

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Electromagnetic Radiation

Visible light is a complex phenomenon that is classically explained with a simple model based on propagating rays and wavefronts, a concept first proposed in the late 1600s by Dutch physicist Christiaan Huygens. Electromagnetic radiation, the larger family of wave-like phenomena to which visible light belongs (also known as **radiant energy**), is the primary vehicle transporting energy through the vast reaches of the universe. The mechanisms by which visible light is emitted or absorbed by substances, and how it predictably reacts under varying conditions as it travels through space and the atmosphere, form the basis of the existence of color in our universe.

The Nature of Electromagnetic Radiation - Coined by Sir James Clerk Maxwell, the term **electromagnetic radiation** is derived from the characteristic electric and magnetic properties common to all forms of this wave-like energy, as manifested by the generation of both electrical and magnetic oscillating fields as the waves propagate through space. Visible light represents only a small portion of the entire spectrum of electromagnetic radiation, which extends from high-frequency cosmic and gamma rays through X-rays, ultraviolet light, infrared radiation, and microwaves, down to very low frequency long-wavelength radio waves.

James Clerk Maxwell (1831-1879) - James Clerk Maxwell was one of the greatest scientists of the nineteenth century. He is best known for the formulation of the theory of electromagnetism and in making the connection between light and electromagnetic waves. He also made significant contributions in the areas of physics, mathematics, astronomy and engineering. He considered by many as the father of modern physics.

William Herschel (1738-1822) - Friedrich William Herschel was an eighteenth century German astronomer who is credited with the discovery of the planet Uranus. In addition, Herschel measured the heights of about one hundred mountains on the moon, carefully recorded the data, and prepared papers that were presented to the Royal Society of London. In the late 1700s, he began to build and sell telescopes. The high quality of Herschel's optics was soon widely known outside of England, and he utilized them to publish three catalogues containing data on 2500 heavenly objects, including the sixth and seventh moons of Saturn, Enceladus and Mimas. Herschel continued making observations and cataloging his discoveries until his death in 1822 at age 84.

Christiaan Huygens (1629-1695) - Christiaan Huygens was a brilliant Dutch mathematician, physicist, and astronomer who lived during the seventeenth century, a period sometimes referred to as the Scientific Revolution. Huygens, a particularly gifted scientist, is best known for his work on the theories of centrifugal force, the wave theory of light, and the pendulum clock. His theories neatly explained the laws of refraction, diffraction, interference, and reflection, and Huygens went on to make major advances in the theories concerning the phenomena of double refraction (birefringence) and polarization of light.

Interactive Java Tutorials

Electromagnetic Radiation - This interactive tutorial explores the classical representation of an electromagnetic wave as a sine function, and enables the visitor to vary amplitude and wavelength to demonstrate how this function appears in three dimensions. Whether taking the form of a signal transmitted to a radio from the broadcast station, heat radiating from a fireplace, the dentist's X-rays producing images of teeth, or the visible and ultraviolet light emanating from the sun, the various categories of electromagnetic radiation all share identical and fundamental wave-like properties.

Basic Electromagnetic Wave Properties - Electromagnetic radiation is characterized by a broad range of wavelengths and frequencies, each associated with a specific intensity (or amplitude) and quantity of energy. This interactive tutorial explores the relationship between frequency, wavelength, and energy, and enables the visitor to adjust the intensity of the radiation and to set the wave into motion.

Electromagnetic Wave Propagation - Electromagnetic waves can be generated by a variety of methods, such as a discharging spark or by an oscillating molecular dipole. Visible light is a commonly studied form of electromagnetic radiation, and exhibits oscillating electric and magnetic fields whose amplitudes and directions are represented by vectors that undulate in phase as sinusoidal waves in two mutually perpendicular (orthogonal) planes. This tutorial explores propagation of a virtual electromagnetic wave and considers the orientation of the magnetic and electric field vectors.

Electron Excitation and Emission - Electrons can absorb energy from external sources, such as lasers, arc-discharge lamps, and tungsten-halogen bulbs, and be promoted to higher energy levels. This tutorial explores how photon energy is absorbed by an electron to elevate it into a higher energy level and how the energy can subsequently be released, in the form of a lower energy photon, when the electron falls back to the original ground state.

Jablonski Diagram - Fluorescence activity can be schematically illustrated with the classical Jablonski diagram, first proposed by Professor Alexander Jablonski in 1935 to describe absorption and emission of light. This tutorial explores how electrons in fluorophores are excited from the ground state into higher electronic energy states and the events that occur as these excited molecules emit photons and fall back into lower energy states.

Tuning a Radio Wave Receiver - Variable capacitors are used in conjunction with inductor coils in tuning circuits of radios, television sets, and a number of other devices that must isolate electromagnetic radiation of selected frequencies in the radio wave region. This interactive tutorial explores how a variable capacitor is coupled to a simple antenna transformer circuit to tune a radiofrequency spectrum.

Selected Literature References

Reference Listing - The reference materials listed in this section are an excellent source of additional information on the diverse topic of electromagnetic radiation. Included are references to books, book chapters, and review articles, which discuss the theory and applications of electromagnetic radiation and how they relate to the physics of light and color.

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Light: Particle or a Wave?

The exact nature of visible light is a mystery that has puzzled man for centuries. Greek scientists from the ancient Pythagorean discipline postulated that every visible object emits a steady stream of particles, while Aristotle concluded that light travels in a manner similar to waves in the ocean. Even though these ideas have undergone numerous modifications and a significant degree of evolution over the past 20 centuries, the essence of the dispute established by the Greek philosophers remains to this day.

One point of view envisions light as wave-like in nature, producing energy that traverses through space in a manner similar to the ripples spreading across the surface of a still pond after being disturbed by a dropped rock. The opposing view holds that light is composed of a steady stream of particles, much like tiny droplets of water sprayed from a garden hose nozzle. During the past few centuries, the consensus of opinion has wavered with one view prevailing for a period of time, only to be overturned by evidence for the other. Only during the first decades of the twentieth century was enough compelling evidence collected to provide a comprehensive answer, and to everyone's surprise, both theories turned out to be correct, at least in part.

The Duality of Light - In the early eighteenth century, the argument about the nature of light had turned the scientific community into divided camps that fought vigorously over the validity of their favorite theories. One group of scientists, who subscribed to the wave theory, centered their arguments on the discoveries of Dutchman Christiaan Huygens. The opposing camp cited Sir Isaac Newton's prism experiments as proof that light traveled as a shower of particles, each proceeding in a straight line until it was refracted, absorbed, reflected, diffracted or disturbed in some other manner. About 200 years later, quantum mechanics was born from the research of Einstein, Planck, de Broglie, Neils Bohr, Erwin Schrödinger, and others who attempted to explain how electromagnetic radiation can display what has now been termed **duality**, or both particle-like and wave-like behavior. At times light behaves as a particle, and at other times as a wave. This complementary, or dual, role for the behavior of light can be employed to describe all of the known characteristics that have been observed experimentally, ranging from refraction, reflection, interference, and diffraction, to the results with polarized light and the photoelectric effect. Combined, the properties of light work together and allow us to observe the beauty of the universe.

Neils Bohr (1885-1962) - Building on Ernest Rutherford's work on the nucleus, Bohr developed a new theory of the atom, which he completed in 1913. The work proposed that electrons travel only in certain orbits and that any atom could exist only in a discrete set of stable states. Bohr further held that the outer orbits, which could hold more electrons than the inner ones, determine the atom's chemical properties and conjectured that atoms emit light radiation when an electron jumps from an outer orbit to an inner one. Although Bohr's theory was initially viewed with skepticism, it earned him the Nobel Prize in physics in 1922 and was eventually expanded by other physicists into quantum mechanics.

Albert Einstein (1879-1955) - Albert Einstein was one of the greatest and most famous scientific minds of the 20th century. The eminent physicist is best remembered for his theories of relativity, as well as his revolutionary notion concerning the nature of light. However, his innovative ideas were often misunderstood and he was frequently ridiculed for his vocal involvement in politics and social issues. The birth of the Manhattan Project yielded an inexorable connection between Einstein's name and the atomic age. However, Einstein did not take part in any of the atomic research, instead preferring to concentrate on ways that the use of bombs might be avoided in the future, such as the formation of a world government.

Christiaan Huygens (1629-1695) - Christiaan Huygens was a brilliant Dutch mathematician, physicist, and astronomer who lived during the seventeenth century, a period sometimes referred to as the Scientific Revolution. Huygens, a particularly gifted scientist, is best known for his work on the theories of centrifugal force, the wave theory of light, and the pendulum clock. His theories neatly explained the laws of refraction, diffraction, interference, and reflection, and Huygens went on to make major advances in the theories concerning the phenomena of double refraction (birefringence) and polarization of light.

Sir Isaac Newton (1642-1727) - Sir Isaac Newton, who was ironically born the same year that Galileo died, is popularly known as one of history's greatest scientists. Many of his discoveries and theories in the areas of light, color, and optics form the basis for current scientific thought in these disciplines. In addition to his extensive work in optics, Newton is perhaps best known for his theory of universal gravitation. He also is considered one of the inventors of calculus along with German mathematician Gottfried Leibniz. Newton's three laws of motion are considered basic to any physics student's education.

Max Planck (1858-1947) - Max Planck, a German physicist, is best known as the originator of the quantum theory of energy for which he was awarded the Nobel Prize in 1918. His work contributed significantly to the understanding of atomic and subatomic processes. Planck made significant contributions to science throughout his life. He is recognized for his successful work in a variety of fields including, thermodynamics, optics, statistical mechanics, and physical chemistry.

Thomas Young (1773-1829) - Thomas Young was an English physician and a physicist who was responsible for many important theories and discoveries in optics and in human anatomy. His best known work is the wave theory of interference. Young was also responsible for postulating how the receptors in the eye perceive colors. He is credited, along with Hermann Ludwig Ferdinand von Helmholtz, for developing the Young-Helmholtz trichromatic theory.

Interactive Java Tutorials

Particle and Wave Reflection - One point of view envisions light as wave-like in nature, producing energy that traverses through space in a manner similar to the ripples spreading across the surface of a still pond after being disturbed by a dropped rock. The opposing view holds that light is composed of a steady stream of particles, much like tiny droplets of water sprayed from a garden hose nozzle. This interactive tutorial explores how particles and waves behave when reflected from a smooth surface.

Particle and Wave Refraction - When a beam of light travels between two media having different refractive indices, the beam undergoes **refraction**, and changes direction when it passes from the first medium into the second. According to the wave theory, a small portion of each angled wavefront should impact the second medium before the rest of the front reaches the interface. This portion will start to move through the second medium while the rest of the wave is still traveling in the first medium, but will move more slowly due to the higher refractive index of the second medium. Because the wavefront is now traveling at two different speeds, it will bend into the second medium, thus changing the angle of propagation. In contrast, particle theory has a rather difficult time explaining why particles of light should change direction when they pass from one medium into another.

Particle and Wave Diffraction - Particles and waves should behave differently when they encounter the edge of an object and form a shadow. Newton was quick to point out in his 1704 book ***Opticks***, that "Light is never known to follow crooked passages nor to bend into the shadow". This concept is consistent with the particle theory, which proposes that light particles must always travel in straight lines. If the particles encounter the edge of a barrier, then they will cast a shadow because the particles not blocked by the barrier continue on in a straight line and cannot spread out behind the edge. On a macroscopic scale, this observation is almost correct, but it does not agree with the results obtained from light diffraction experiments on a much smaller scale.

Thomas Young's Double Slit Experiment - In 1801, an English physicist named Thomas Young performed an experiment that strongly inferred the wave-like nature of light. Because he believed that light was composed of waves, Young reasoned that some type of interaction would occur when two light waves met. This interactive tutorial explores how coherent light waves interact when passed through two closely spaced slits.

Selected Literature References

Reference Listing - Whether light can be better described as a continuous wave or a collection of particles has challenged many great physicists over the ages, resulting in a variety of theories to support both models. The dual nature of light, which is manifested in properties that are at times consistent with either or both corpuscular (particle) and wave theories, is discussed in the review articles and books listed in this literature reference section.

Sources of Visible Light

Visible light comprises only a tiny fraction of the entire electromagnetic radiation spectrum, yet it contains the only region of frequencies to which the rods and cones of the human eye will respond. The wavelengths that humans are typically able to visualize lie in a very narrow range between approximately 400 and 700 nanometers. Humans can observe and respond to stimuli created by visible light because the eyes contain specialized nerve endings that are sensitive to this range of frequencies. The remainder of the electromagnetic spectrum is invisible to humans.

Introduction to Visible Light Sources - A wide variety of sources are responsible for emission of electromagnetic radiation, and are categorized according to the spectrum of wavelengths generated by the source. Relatively long radio waves are produced by electrical current flowing through huge broadcast antennas, while much shorter visible light waves are produced by the energy state fluctuations of negatively charged electrons within atoms. The shortest form of electromagnetic radiation, gamma waves, results from decay of nuclear components at the center of the atom. The visible light that humans are able to see (the spectrum is illustrated in Figure 1) is usually a mixture of wavelengths whose varying composition is a function of the light source.

Introduction to Lasers - Ordinary natural and artificial light is released by energy changes on the atomic and molecular level that occur without any outside intervention. A second type of light exists, however, and occurs when an atom or molecule retains its excess energy until **stimulated** to emit the energy in the form of light. Lasers are designed to produce and amplify this stimulated form of light into intense and focused beams. The word laser was coined as an acronym for **L**ight **A**mplification by the **S**timulated **E**mission of **R**adiation. The special nature of laser light has made laser technology a vital tool in nearly every aspect of everyday life including communications, entertainment, manufacturing, and medicine.

Light Emitting Diode Fundamentals - The past few decades have brought a continuing and rapidly evolving sequence of technological revolutions, particularly in the digital arena, which has dramatically changed many aspects of our daily lives. The developing race among manufacturers of light emitting diodes (**LEDs**) promises to produce, literally, the most visible and far-reaching transition to date. Recent advances in the design and manufacture of these miniature semiconductor devices may result in the obsolescence of the common light bulb, perhaps the most ubiquitous device utilized by modern society.

Light Sources for Optical Microscopy - Modern microscopes usually have an integral light source that can be controlled to a relatively high degree. The most common source for today's microscopes is an incandescent tungsten-halogen bulb positioned in a reflective housing that projects light through the collector lens and into the substage condenser. Other sources include arc-discharge lamps, light emitting diodes (**LEDs**), and lasers.

Laser Systems for Optical Microscopy - The lasers commonly employed in optical microscopy are high-intensity monochromatic light sources, which are useful as tools for a variety of techniques including optical trapping, lifetime imaging studies, photobleaching recovery, and total internal reflection fluorescence. In addition, lasers are also the most common light source for scanning confocal fluorescence microscopy, and have been utilized, although less frequently, in conventional widefield fluorescence investigations.

Fluorescence Microscopy Light Sources - In order to generate enough excitation light intensity to furnish secondary fluorescence emission capable of detection, powerful light sources are needed. These are usually either mercury or xenon arc (burner) lamps, which produce high-intensity illumination powerful enough to image faintly visible fluorescence specimens.

Focusing and Alignment of Arc Lamps - Mercury and xenon arc lamps are now widely utilized as illumination sources for a large number of investigations in widefield fluorescence microscopy. Visitors can gain practice aligning and focusing the arc lamp in a **Mercury** or **Xenon Burner** with this Nikon MicroscopyU interactive tutorial, which simulates how the lamp is adjusted in a fluorescence microscope.

Interactive Java Tutorials

Lightning: A Natural Capacitor - Lightning is one of the naturally occurring mechanisms that provided early mankind with the ability to understand and harness fire. This meteorological phenomenon occurs when water-filled clouds and the ground act in unison to mimic a huge natural capacitor. View the build-up of static electrical charges between storm clouds and the wet ground during a thunderstorm with this tutorial, which simulates capacitor-like lightning discharges.

Color Temperature - Investigate the apparent "color" of a virtual radiator (in this case, a black metal pot) as it is slowly heated through a wide temperature range by external energy. The concept of color temperature is based on the relationship between the temperature and radiation emitted by a theoretical standardized material, termed a **black body radiator**, cooled down to a state in which all molecular motion has ceased. Hypothetically, at cessation of all molecular motion, the temperature is described as being at **absolute zero** or 0 Kelvin, which is equal to -273 degrees Celsius.

Incandescent Lamp Filaments - Nearly every source of light depends, at the fundamental level, on the release of energy from atoms that have been excited in some manner. Standard incandescent lamps, derived directly from the early models of the 1800s, now commonly utilize a tungsten filament in an inert gas atmosphere, and produce light through the resistive effect that occurs when the filament temperature increases as electrical current is passed through. This interactive tutorial demonstrates the sub-atomic activity within a conducting incandescent lamp filament that results in resistance to current flow, and ultimately leads to the emission of infrared and visible light photons (with a corresponding rise in color temperature).

Compact Disk Lasers - A pre-recorded compact disk is read by tracking a finely focused laser across the spiral pattern of lands and pits stamped into the disk by a master diskette. This tutorial explores how the laser beam is focused onto the surface of a spinning compact disk, and how variations between the height of pits and lands determine whether the light is scattered by the disk surface or reflected back into a detector.

Light Emitting Diodes - Light emitting diodes (**LEDs**) are a general source of continuous light with a high luminescence efficiency, and are based on the general properties of a simple twin-element semiconductor diode encased in a clear epoxy dome that acts as a lens. This interactive tutorial explores how two dissimilar doped semiconductors can produce light when a voltage is applied to the junction region between the materials.

Argon-Ion Lasers - As a distinguished member of the common and well-explored family of **ion lasers**, the argon-ion laser operates in the visible and ultraviolet spectral regions by utilizing an ionized species of the noble gas argon. Argon-ion lasers function in continuous wave mode when plasma electrons within the gaseous discharge collide with the excited laser species to produce light.

Diode Lasers - Semiconductor diode lasers having sufficient power output to be useful in optical microscopy are now available from a host of manufacturers. In general these devices operate in the infrared region, but newer diode lasers operating at specific visible wavelengths are now available. Diode lasers coupled to internal optical systems that improve beam shape have sufficient power and stability to rival helium-neon lasers in many applications. This interactive tutorial explores the properties of typical diode lasers and how specialized anamorphic prisms can be utilized for beam expansion.

Ti:Sapphire Mode-Locked Lasers - The self mode-locked Ti:sapphire pulsed laser is currently one of the preferred laser excitation sources in a majority of multiphoton fluorescence microscopy investigations. This tutorial explores the operation of Ti:sapphire lasers over a broad range of near-infrared wavelengths with variable pulse widths and an adjustable applet speed control.

Nd:YLF Mode-Locked Pulsed Lasers - An increasing number of applications, including new illumination techniques in fluorescence optical microscopy, require a reliable high average-power laser source that enables efficient frequency conversion to ultra violet and visible wavelengths. Several variants of the diode-pumped solid state laser have been developed, and of these, the Nd:YLF (neodymium: yttrium lithium fluoride) laser produces the highest pulse energy and average power in the repetition rate ranging from a single pulse up to approximately 6 kHz. This tutorial explores the operation of a Nd:YLF multi-pass slab laser side-pumped by two collimated diode-laser bars.

Selected Literature References

Reference Listing - The reference materials listed in this section, gathered from our vast optical microscopy library, are an excellent source of additional information on microscope illumination. Included are references to review articles and original research reports that discuss how microscopes are configured to take advantage of various illumination scenarios and the critical illumination required for high-quality photomicrography. In addition, basic articles describing the properties of a variety of natural and artificial light sources are included.

Fluorescence

The phenomenon of fluorescence was known by the middle of the nineteenth century. British scientist Sir George G. Stokes first made the observation that the mineral **fluorspar** exhibits fluorescence when illuminated with ultraviolet light, and he coined the word "fluorescence". Stokes observed that the fluorescing light has longer wavelengths than the excitation light, a phenomenon that has become to be known as the **Stokes shift**. Fluorescence microscopy is an excellent method of studying material that can be made to fluoresce, either in its natural form (termed **primary** or **autofluorescence**) or when treated with chemicals capable of fluorescing (known as **secondary fluorescence**). The fluorescence microscope was devised in the early part of the twentieth century by August Köhler, Carl Reichert, and Heinrich Lehmann, among others. However, the potential of this instrument was not realized for several decades, and fluorescence microscopy is now an important (and perhaps indispensable) tool in cellular biology.

Introduction to Fluorescence - Fluorescence microscopy is a rapidly expanding and invaluable tool of investigation. Its advantages are based upon attributes not as readily available in other optical microscopy techniques. The use of fluorochromes has made it possible to identify cells and sub-microscopic cellular components and other entities with a high degree of specificity amidst non-fluorescing material. What is more, the fluorescence microscope can reveal the presence of fluorescing material with exquisite sensitivity. An extremely small number of fluorescent molecules (as few as 50 molecules per cubic micrometer) can be detected. In a given sample, through the use of multiple staining, different probes will reveal the presence of individual target molecules. Although the fluorescence microscope cannot provide spatial resolution below the diffraction limit of the respective specimens, the presence of fluorescing molecules below such limits is made remarkably visible.

Overview of Excitation and Emission Fundamentals - When electrons go from the excited state to the ground state, there is a loss of vibrational energy. As a result, the emission spectrum is shifted to longer wavelengths than the excitation spectrum (wavelength varies inversely to radiation energy). This phenomenon is known as **Stokes Law** or Stokes shift. The greater the Stokes shift, the easier it is to separate excitation light from emission light. The emission intensity peak is usually lower than the excitation peak; and the emission curve is often a mirror image of the excitation curve, but shifted to longer wavelengths. To achieve maximum fluorescence intensity, the fluorochrome is usually excited at the wavelength at the peak of the excitation curve, and the emission is selected at the peak wavelength (or other wavelengths chosen by the observer) of the emission curve. The selections of excitation wavelengths and emission wavelengths are controlled by appropriate filters. In determining the spectral response of an optical system, technical corrections are required to take into account such factors as glass transmission and detector sensitivity variables for different wavelengths.

John Frederick William Herschel (1792-1871) - John Herschel was the only child of renowned scientist and astronomer William Herschel. In 1820, the younger Herschel was one of the founding members of the Royal Astronomical Society, and when his father died in 1822 he carried on with the elder Herschel's work, making a detailed study of double stars. In collaboration with James South Herschel compiled a catalog of observations that was published in 1824. The work garnered the pair the Gold Medal from the Royal Astronomical Society and the Lalande Prize from the Paris Academy of Sciences. In 1839, Herschel developed a technique for creating photographs on sensitized paper, independently of William Fox Talbot, but did not attempt to commercialize the process. However, he published several papers on photographic processes and was the first to utilize the terms **positive** and **negative** in reference to photography. Particularly important to the future of science, in 1845 Herschel reported the first observation of the fluorescence of a quinine solution in sunlight.

Alexander Jablonski (1898-1980) - Born in the Ukraine in 1898, Alexander Jablonski is best known as the father of fluorescence spectroscopy. Jablonski's primary scientific interest was the polarization of photoluminescence in solutions, and in order to explain experimental evidence gained in the field, he differentiated the transition moments between absorption and emission. His work resulted in his introduction of what is now known as a **Jablonski Energy Diagram**, a tool that can be used to explain the kinetics and spectra of fluorescence, phosphorescence, and delayed fluorescence.

George Gabriel Stokes (1819-1903) - Throughout his career, George Stokes emphasized the importance of experimentation and problem solving, rather than focusing solely on pure mathematics. His practical approach served him well and he made important advances in several fields, most notably hydrodynamics and optics. Stokes coined the term **fluorescence**, discovered that fluorescence can be induced in certain substances by stimulation with ultraviolet light, and formulated **Stokes Law** in 1852. Sometimes referred to as **Stokes shift**, the law holds that the wavelength of fluorescent light is always greater than the wavelength of the exciting light. An advocate of the wave theory of light, Stokes was one of the prominent nineteenth century scientists that believed in the concept of an ether permeating space, which he supposed was necessary for light waves to travel.

Interactive Java Tutorials

Electron Excitation and Emission - Electrons can absorb energy from external sources, such as lasers, arc-discharge lamps, and tungsten-halogen bulbs, and be promoted to higher energy levels. This tutorial explores how photon energy is absorbed by an electron to elevate it into a higher energy level and how the energy can subsequently be released, in the form of a lower energy photon, when the electron falls back to the original ground state.

Fluorescence Filter Spectral Transmission Profiles - Fluorescence microscopes are equipped with a combination of three essential filters (often termed a **filter set**) that are positioned in the optical pathway between the light source in the vertical illuminator and the objective. The filters are strategically oriented within a specialized **cube** or block that enables the illumination to enter from one side and pass to and from the specimen in defined directions along the microscope optical axis. This tutorial explores the spectral overlap regions of fluorescence filter combinations, and how changes to the individual filter properties help determine the bandwidth of wavelengths passed through the various filter sets.

Jablonski Energy Diagram - Fluorescence activity can be schematically illustrated with the classical Jablonski diagram, first proposed by Professor Alexander Jablonski in 1935 to describe absorption and emission of light. Prior to excitation, the electronic configuration of the molecule is described as being in the ground state. Upon absorbing a photon of excitation light, usually of short wavelengths, electrons may be raised to a higher energy and vibrational excited state, a process that may only take a quadrillionth of a second (a time period commonly referred to as a femtosecond, 10E-15 seconds). This tutorial explores how electrons in fluorophores are excited from the ground state into higher electronic energy states and the events that occur as these excited molecules emit photons and fall back into lower energy states.

Selected Literature References

Reference Listing - The field of fluorescence spectroscopy and microscopy is experiencing a renaissance with the introduction of new techniques such as confocal, multiphoton, deconvolution, time-resolved investigations, and total internal reflection, coupled to the current advances in chromophore and fluorophore technology. Green Fluorescence Protein is rapidly becoming a labeling method of choice for molecular and cellular biologists who can now explore biochemical events in living cells with natural fluorophores. Taken together, these and other important advances have propelled the visualization of living cells tagged with specific fluorescent probes into the mainstream of research in a wide spectrum of disciplines. The reference materials listed below were utilized in the construction of the introductory fluorescence section in the Molecular Expressions Microscopy Primer.

Speed of Light

Starting with Roemer's 1676 breakthrough endeavors, the speed of light has been measured at least 163 times utilizing a wide variety of different techniques by more than 100 investigators (see Table 1 for a compilation of methods, investigators, and dates). As scientific methods and devices were refined, the error limits of the estimates narrowed, although the speed of light has not significantly changed since Roemer's seventeenth century calculations. Finally in 1983, more than 300 years after the first serious measurement attempt, the speed of light was defined as being 299,792.458 kilometers per second by the Seventeenth General Congress on Weights and Measures. Thus, the meter is defined as the distance light travels through a vacuum during a time interval of 1/299,792,458 seconds. In general, however, (even in many scientific calculations) the speed of light is rounded to 300,000 kilometers (or 186,000 miles) per second. Arriving at a standard value for the speed of light was important for establishing an international system of units that would enable scientists from around the world to compare their data and calculations.

Measuring the Speed of Light - Light traveling in a uniform substance, or medium, propagates in a straight line at a relatively constant speed, unless it is refracted, reflected, diffracted, or perturbed in some other manner. This well-established scientific fact is not a product of the Atomic Age or even the Renaissance, but was originally promoted by the ancient Greek scholar, Euclid, somewhere around 350 BC in his landmark treatise ***Optica***. By the late 1960s, lasers were becoming stable research tools with highly defined frequencies and wavelengths. It quickly became obvious that a simultaneous measurement of frequency and wavelength would yield a very accurate value for the speed of light, similar to an experimental approach carried out by Keith Davy Froome using microwaves in 1958. Several research groups in the United States and in other countries measured the frequency of the 633-nanometer line from an iodine-stabilized helium-neon laser and obtained highly accurate results. In 1972, the National Institute of Standards and Technology employed the laser technology to measure the speed at 299,792,458 meters per second (186,282 miles per second), which ultimately resulted in the redefinition of the meter through a highly accurate estimate for the speed of light.

Alhazen (965-1040) - Born in Iraq as Abu Ali Hasan Ibn al-Haitham, the great Arab physicist is more often known by the Latinized version of his first name, Alhazen. The efforts of Alhazen resulted in over one hundred works, the most famous of which was "***Kitab-al-Manadhirn***", rendered into Latin in the Middle Ages. The translation of the book on optics exerted a great influence upon the science of the western world, most notably on the work of Roger Bacon and Johannes Kepler. A significant observation in the work contradicted the beliefs of many great scientists, such as Ptolemy and Euclid. Alhazen correctly proposed that the eyes passively receive light reflected from objects, rather than emanating light rays themselves.

Dominique-François-Jean Arago (1786-1853) - In 1811, Arago, in collaboration with Augustin-Jean Fresnel, discovered that two beams of light polarized in perpendicular directions do not interfere, eventually resulting in the development of a transverse theory of light waves. Arago was also instrumental in the success and funding of Louis-Jacques-Mandé Daguerre's photographic process, known as the **daguerreotype**, and directed studies that directly led to the discovery of the location of Neptune by Urbain-Jean-Joseph Le Verrier.

Roger Bacon (1214-1294) - Roger Bacon was an English scholastic philosopher who was also considered a scientist because he insisted on observing things for himself instead of depending on what other people had written. Bacon's writings included treatises on optics (then called perspective), mathematics, chemistry, arithmetic, astronomy, the tides, and the reformation of the calendar. His skill in the use of optical and mechanical instruments caused him to be regarded by many as a sorcerer. Bacon was acquainted with the properties of mirrors, knew the powers of steam and gunpowder, had a working knowledge in microscopy, and possessed an instrument very much like a modern telescope.

Albert Einstein (1879-1955) - Albert Einstein was one of the greatest and most famous scientific minds of the 20th century. The eminent physicist is best remembered for his theories of relativity, as well as his revolutionary notion concerning the nature of light. However, his innovative ideas were often misunderstood and he was frequently ridiculed for his vocal involvement in politics and social issues. The birth of the Manhattan Project yielded an inexorable connection between Einstein's name and the atomic age. However, Einstein did not take part in any of the atomic research, instead preferring to concentrate on ways that the use of bombs might be avoided in the future, such as the formation of a world government.

Armand Fizeau (1819-1896) - Armand Fizeau is best known for being the first to develop a reliable experimental method of determining the speed of light on the Earth. Previously, the speed of light was measured based upon astronomical phenomena. Fizeau also conducted experiments that demonstrated that the velocity of light is a constant, regardless of the motion of the medium it is passing through. It was previously established that light traveled at different rates through different mediums, but prior to Fizeau's discovery, it was believed that if the medium was in motion, the velocity of the speed of light would be increased by the movement of the medium.

Jean-Bernard-Leon Foucault (1819-1868) - Jean-Bernard-Leon Foucault was a French physicist who is considered one of the most versatile experimentalists of the nineteenth century. Together with the French physicist Armand Fizeau, Foucault developed a way to measure the speed of light with extreme accuracy. He also proved independently that the speed of light in air is greater than it is in water. Foucault's other contributions to the field of optics included a method of measuring the curvature of telescope mirrors, an improved technique to silver astronomical mirrors, a method of testing telescope mirrors for surface defects, and the invention of a polarizing prism to analyze polarized light.

Johannes Kepler (1571-1630) - Johannes Kepler was a sixteenth century German astronomer and student of optics who first delineated many theories of modern optics. In 1609, he published "***Astronomia Nova***" delineating his discoveries, which are now called Kepler's first two laws of planetary motion. This work established Kepler as the "father of modern science", documenting how, for the first time, a scientist dealt with a multitude of imperfect data to arrive at a fundamental law of nature.

Albert Michelson (1852-1931) - Albert Abraham Michelson, a Polish-American physicist, was awarded the Nobel Prize in Physics in 1907. He is best known for his experiments in which he proved that the hypothetical medium of light, the "***ether***", did not exist, and his many attempts at accurately measuring the speed of light. Michelson is also well known for developing a means to more accurately measure the speed of light and the size of stars.

Ole Christensen Roemer (1644-1710) - Roemer' s greatest achievement was the first relatively accurate measurement of the speed of light, a feat he accomplished in 1676. At the Royal Observatory in England, Roemer's studies of Jupiter's moon Io and its frequent eclipses enabled him to predict the periodicity of an eclipse period for the moon. By applying the relatively inaccurate calculations for the distances between Earth and Jupiter available during the seventeenth century, Roemer was able to approximate the speed of light to be 137,000 miles (or 220,000 kilometers) per second.

Interactive Java Tutorials

Speed of Light in Transparent Materials - When light traveling in a vacuum enters a new transparent medium, such as air, water, or glass, the speed is reduced in proportion to the refractive index of the new material. This interactive tutorial explores the reduction in the speed of light as a function of refractive index in common substances.

Selected Literature References

Reference Listing - Understanding and measuring the speed of light has challenged the minds of the great physicists from around the world over the ages. Even in today's technologically rich society, new discoveries still produce scientific debate and advances. Included in this section are references to books and review articles that discuss theoretical and applied aspects relating to the measurement of the speed of light, and how this constant effects our understanding of the physics of light and color.

Reflection of Light

Reflection of light (and other forms of electromagnetic radiation) occurs when the waves encounter a surface or other boundary that does not absorb the energy of the radiation and bounces the waves away from the surface. The simplest example of visible light reflection is the surface of a smooth pool of water, where the light is reflected in an orderly manner to produce a clear image of the scenery surrounding the pool. Throw a rock into the pool, and the water is perturbed to form waves, which disrupt the reflection by scattering the incident and reflected light.

The reflection of visible light is a property of the behavior of light that is fundamental in the function of all modern microscopes. Light is often reflected by one or more plane (or flat) mirrors within the microscope to direct the light path through lenses that form the virtual images we see in the oculars (eyepieces). Microscopes also make use of beamsplitters to allow some light to be reflected while simultaneously transmitting other light to different parts of the optical system. Other optical components in the microscope, such as specially designed prisms, filters, and lens coatings, also carry out their functions in forming the image with a crucial reliance on the phenomenon of light reflection.

Introduction to the Reflection of Light - Because light behaves in some ways as a wave and in other ways as if it were composed of particles, several independent theories of light reflection have emerged. According to wave-based theories, the light waves spread out from the source in all directions, and upon striking a mirror, are reflected at an angle determined by the angle at which the light arrives. The reflection process inverts each wave back-to-front, which is why a reverse image is observed. The shape of light waves depends upon the size of the light source and how far the waves have traveled to meet the mirror. Wavefronts that originate from a source near the mirror will be highly curved, while those emitted by distant light sources will be almost linear, a factor that will affect the angle of reflection.

Augustin-Jean Fresnel (1788-1827) - Augustin-Jean Fresnel, was a nineteenth century French physicist, who is best known for the invention of unique compound lenses designed to produce parallel beams of light, which are still used widely in lighthouses. In the field of optics, Fresnel derived formulas to explain reflection, diffraction, interference, refraction, double refraction, and the polarization of light reflected from a transparent substance.

Christiaan Huygens (1629-1695) - Christiaan Huygens was a brilliant Dutch mathematician, physicist, and astronomer who lived during the seventeenth century, a period sometimes referred to as the Scientific Revolution. Huygens, a particularly gifted scientist, is best known for his work on the theories of centrifugal force, the wave theory of light, and the pendulum clock. His theories neatly explained the laws of refraction, diffraction, interference, and reflection, and Huygens went on to make major advances in the theories concerning the phenomena of double refraction (birefringence) and polarization of light.

Interactive Java Tutorials

Reflection of Light - Reflection of light (and other forms of electromagnetic radiation) occurs when the waves encounter a surface or other boundary that does not absorb the energy of the radiation and bounces the waves away from the surface. This tutorial explores the incident and reflected angles of a single light wave impacting on a smooth surface.

Specular and Diffuse Reflection - The amount of light reflected by an object, and how it is reflected, is very dependent upon the smoothness or texture of the surface. When surface imperfections are smaller than the wavelength of the incident light (as in the case of a mirror), virtually all of the light is reflected equally. However, in the real world most objects have convoluted surfaces that exhibit a diffuse reflection, with the incident light being reflected in all directions. This interactive tutorial explores how light waves are reflected by smooth and rough surfaces.

Concave Spherical Mirrors - Concave mirrors have a curved surface with a center of curvature equidistant from every point on the mirror's surface. An object beyond the center of curvature forms a real and inverted image between the focal point and the center of curvature. This interactive tutorial explores how moving the object farther away from the center of curvature affects the size of the real image formed by the mirror. Also examined in the tutorial are the effects of moving the object closer to the mirror, first between the center of curvature and the focal point, and then between the focal point and the mirror surface (to form a virtual image).

Concave Spherical Mirrors (3-Dimensional Version) - Concave mirrors have a curved surface with a center of curvature equidistant from every point on the mirror's surface. An object beyond the center of curvature forms a real and inverted image between the focal point and the center of curvature. This interactive tutorial explores how moving the object farther away from the center of curvature affects the size of the real image formed by the mirror.

Convex Spherical Mirrors - Regardless of the position of the object reflected by a convex mirror, the image formed is always virtual, upright, and reduced in size. This interactive tutorial explores how moving the object farther away from the mirror's surface affects the size of the virtual image formed behind the mirror.

Convex Spherical Mirrors (3-Dimensional Version) - Regardless of the position of the object reflected by a convex mirror, the image formed is always virtual, upright, and reduced in size. This interactive tutorial explores how moving the object farther away from the mirror's surface affects the size of the virtual image formed behind the mirror. This tutorial utilizes three-dimensional graphics.

Reflection and Refraction with Huygens Wavelets - Near the beginning of the eighteenth century, Dutch physicist Christiaan Huygens proposed that each point in a wave of light can be thought of as an individual source of illumination that produces its own spherical **wavelets**, which all add together to form an advancing wavefront. This interactive Java tutorial is designed to illustrate the reflection and refraction of light according to the multiple wavelet concept, now known as the **Huygens' principle**.

Antireflection Surface Coatings - The concept behind **antireflection** technology is to control the light used in an optical device in such a way that the light rays reflect from surfaces where it is intended and beneficial, and do not reflect from surfaces where this would have a deleterious effect on the image being observed. One of the most significant advances made in modern lens design, whether for microscopes, cameras, or other optical devices, is the significant improvement in antireflection coating technology. This tutorial explores various coatings and their reflectivities as a function of incident angle.

The Critical Angle of Reflection - An important concept in optical microscopy is the **critical angle of reflection**, which is a necessary factor to consider when choosing whether to use dry or oil immersion objectives to view a specimen at high magnification. Upon passing through a medium of higher refractive index into a medium of lower refractive index, the path taken by light waves is determined by the incident angle with respect to the boundary between the two media. This interactive tutorial explores the transition from refraction to total internal reflection as the angle of the incident wave is increased at constant refractive index.

Common Reflecting Prisms - The angular parameters displayed by various prism designs cover a wide gamut of geometries that dramatically extend the usefulness of prisms as strategic optical components. Reflecting prisms are often designed to be located in specific orientations where the entrance and exit faces are both parallel and perpendicular to the optical axis. This interactive tutorial explores image deviation, rotation, and displacement exhibited by common reflecting prisms.

Right-Angle Prisms - The right-angle prism possesses the simple geometry of a 45-degree right triangle, and is one of the most commonly used prisms for redirecting light and rotating images. This interactive tutorial explores light reflection and image rotation, inversion, and reversion by a right-angle prism as a function of the prism orientation with respect to incident light.

Transmission and Reflection by Beamsplitters - A beamsplitter is a common optical component that partially transmits and partially reflects an incident light beam, usually in unequal proportions. In addition to the task of dividing light, beamsplitters can be employed to recombine two separate light beams or images into a single path. This interactive tutorial explores transmission and reflection of a light beam by three common beamsplitter designs.

Selected Literature References

Reference Listing - The reference materials listed in this section are an excellent source of additional information on the diverse topic of reflection from both specular and diffuse surfaces. Included are references to books, book chapters, and review articles, which discuss the theory and applications of the reflection of electromagnetic radiation and how they relate to the physics of light and color.

Refraction of Light

When electromagnetic radiation, in the form of visible light, travels from one substance or medium into another, the light waves may undergo a phenomenon known as **refraction**, which is manifested by a bending or change in direction of the light. Refraction occurs as light passes from one medium to another only when there is a difference in the **index of refraction** between the two materials. The effects of refraction are responsible for a variety of familiar phenomena, such as the apparent bending of an object that is partially submerged in water and the mirages observed on a dry, sandy desert. The refraction of visible light is also an important characteristic of lenses that enables them to focus a beam of light onto a single point.

Introduction to the Refraction of Light - As light passes from one substance into another, it will travel straight through with no change of direction when crossing the boundary between the two substances head-on (perpendicular, or a 90-degree angle of incidence). However, if the light impacts the boundary at any other angle it will be bent or refracted, with the degree of refraction increasing as the beam is progressively inclined at a greater angle with respect to the boundary. As an example, a beam of light striking water vertically will not be refracted, but if the beam enters the water at a slight angle it will be refracted to a very small degree. If the angle of the beam is increased even further, the light will refract with increasing proportion to the entry angle. Early scientists realized that the ratio between the angle at which the light crosses the media interface and the angle produced after refraction is a very precise characteristic of the material producing the refraction effect.

Friedrich Johann Karl Becke (1855-1931) - Friedrich Johann Karl Becke was an Austrian geologist, mineralogist and petrologist from the University of Prague, who developed a method for determining the relationship between light refraction and refractive index differences observed in microscopic specimens. The phenomenon, which is now referred to as the formation of **Becke lines**, has been named for him.

Augustin-Jean Fresnel (1788-1827) - Augustin-Jean Fresnel, was a nineteenth century French physicist, who is best known for the invention of unique compound lenses designed to produce parallel beams of light, which are still used widely in lighthouses. In the field of optics, Fresnel derived formulas to explain reflection, diffraction, interference, refraction, double refraction, and the polarization of light reflected from a transparent substance.

Willebrord Snell (1580-1626) - Willebrord Snell was an early seventeenth century Dutch mathematician who is best known for determining that transparent materials have different indices of refraction depending upon the composition. Snell discovered that a beam of light would bend as it enters a block of glass, and that the angle of bending was dependent upon the incident angle of the light beam. Light traveling in a straight line into the glass will not bend but, at an angle, the light is bent to a degree proportional to the angle of inclination. In 1621, Snell found a characteristic ratio between the angle of incidence and the angle of refraction. Snell's law demonstrates that every substance has a specific bending ratio-the "***refractive index***. The greater the angle of refraction, the higher the refractive index for a substance.

Interactive Java Tutorials

Refraction of Light - Refraction occurs as light passes from one medium to another only when there is a difference in the **index of refraction** between the two materials. The effects of refraction are responsible for a variety of familiar phenomena, such as the apparent bending of an object that is partially submerged in water and the mirages observed on a dry, sandy desert. The refraction of visible light is also an important characteristic of lenses that enables them to focus a beam of light onto a single point. This interactive tutorial explores how changes to the incident angle and refractive index differential between two dissimilar media affect the refraction angle of light at the interface.

Observing Objects in Water - An object seen in the water will usually appear to be at a different depth than it actually is, due to the refraction of light rays as they travel from the water into the air. This tutorial explores how fish, observed from the bank of a pond or lake, appear to be closer to the surface than they really are.

Refraction by an Equilateral Prism - Visible white light passing through an equilateral prism undergoes a phenomenon known as **dispersion**, which is manifested by wavelength-dependent refraction of the light waves. This interactive tutorial explores how the incident angle of white light entering the prism affects the degree of dispersion and the angles of light exiting the prism.

The Critical Angle of Reflection - An important concept in optical microscopy is the **critical angle of reflection**, which is a necessary factor to consider when choosing whether to use dry or oil immersion objectives to view a specimen at high magnification. Upon passing through a medium of higher refractive index into a medium of lower refractive index, the path taken by light waves is determined by the incident angle with respect to the boundary between the two media. This interactive tutorial explores the transition from refraction to total internal reflection as the angle of the incident wave is increased at constant refractive index.

Beam Steering by Wedge Prisms - Circular prisms having plane surfaces positioned at slight angles with respect to each other are termed **optical wedges**, and deflect light by refraction rather than reflection. Although wedges are prismatic in nature, they can be manipulated to act as beamsplitters or **beam steerers**. This interactive tutorial explores how two wedge prisms operate together to deflect an incident light beam.

Refraction of Monochromatic Light - Refraction occurs as light passes from one medium to another only when there is a difference in the **index of refraction** between the two materials. The effects of refraction are responsible for a variety of familiar phenomena, such as the apparent bending of an object that is partially submerged in water and the mirages observed on a dry, sandy desert. The refraction of visible light is also an important characteristic of lenses that enables them to focus a beam of light onto a single point. This interactive tutorial explores how changes to the incident angle and refractive index differential between two dissimilar media affect the refraction angle of monochromatic light at the interface.

Selected Literature References

Reference Listing - The reference materials listed in this section are an excellent source of additional information on the diverse topic of light refraction and dispersion by isotropic and anisotropic media. Included are references to books, book chapters, and review articles, which discuss the theory and applications of the refraction and refractive index, and how they relate to the physics of light and color.

Diffraction of Light

In his 1704 treatise on the theory of optical phenomena (***Opticks***), Sir Isaac Newton wrote that "light is never known to follow crooked passages nor to bend into the shadow". He explained this observation by describing how particles of light always travel in straight lines, and how objects positioned within the path of light particles would cast a shadow because the particles could not spread out behind the object. Depending on the circumstances that give rise to the phenomenon, diffraction can be perceived in a variety of different ways. Scientists have cleverly utilized diffraction of neutrons and X-rays to elucidate the arrangement of atoms in small ionic crystals, molecules, and even such large macromolecular assemblies as proteins and nucleic acids. Electron diffraction is often employed to examine periodic features of viruses, membranes, and other biological organisms, as well as synthetic and naturally occurring materials. No lens exists that will focus neutrons and X-rays into an image, so investigators must reconstruct images of molecules and proteins from the diffraction patterns using sophisticated mathematical analysis. Fortunately, magnetic lenses can focus diffracted electrons in the electron microscope, and glass lenses are very useful for focusing diffracted light to form an optical image that can easily be viewed.

Introduction to Diffraction - When we view a specimen, whether directly or with a microscope, telescope, or other optical instrument, the image we see is composed of a myriad of overlapping points of light emanating from the plane of the specimen. Therefore, the appearance and integrity of the image from a single point of light holds a significant amount of importance with regards to formation of the overall image. Because the image-forming light rays are diffracted, a single point of light is never really seen as a point in the microscope, but rather as a diffraction pattern containing a central disk or spot of light having a finite diameter and encircled by a fading series of rings. As a result, the image of a specimen is never an exact representation of the specimen, and a lower limit is imposed on the smallest detail in the specimen that can be resolved. The resolving power is the ability of an optical instrument to produce clearly separated images of two adjacent points. Up to the point at which diffraction causes the resolution to be limited, the quality of the lenses and mirrors in the instrument, as well as the properties of the surrounding medium (usually air), determine the final resolution.

Sir George Biddell Airy (1801-1892) - Sir George Airy was a distinguished nineteenth century English Astronomer Royal who carried out optical research and first drew attention to the visual defect of astigmatism. Airy manufactured the first correcting eyeglasses (1825) using a cylindrical lens design that is still in use. The diffraction disks that bear his name (Airy Disks) were discovered in the spherical center of a wavefront traveling through a circular aperture. These diffraction patterns form the smallest unit that comprises an image, thus determining the limits of optical resolution.

Jacques Babinet (1794-1872) - Jacques Babinet was a French physicist, mathematician, and astronomer born in Lusignan, who is most famous for his contributions to optics. Among Babinet's accomplishments are the 1827 standardization of the Ångström unit for measuring light using the red cadmium line's wavelength, and the principle (bearing his name) that similar diffraction patterns are produced by two complementary screens.

William Henry Bragg (1862-1942) - Sir William Henry Bragg was a noted British physicist and President of the Royal Society who had numerous research interests, but the work that earned him a rank as one the great leaders in science was his historic advancements in X-ray crystallography. Working with his son William Lawrence Bragg, he developed a method of bombarding single crystals with high-energy X-rays emitted by specially constructed vacuum tubes. By examining the pattern of X-rays diffracted by various crystals, Bragg and his son were able to establish some fundamental mathematical relationships between an atomic crystal structure and its diffraction pattern. For this achievement, William Henry Bragg and William Lawrence Bragg were awarded the Nobel Prize in Physics in 1915.

Augustin-Jean Fresnel (1788-1827) - Augustin-Jean Fresnel, was a nineteenth century French physicist, who is best known for the invention of unique compound lenses designed to produce parallel beams of light, which are still used widely in lighthouses. In the field of optics, Fresnel derived formulas to explain reflection, diffraction, interference, refraction, double refraction, and the polarization of light reflected from a transparent substance.

Lord Rayleigh (John William Strutt) - (1842-1919) - Lord Rayleigh was a British physicist and mathematician who worked in many disciplines including electromagnetics, physical optics, and sound wave theory. The criteria he defined still act as the limits of resolution of a diffraction-limited optical instrument. Rayleigh wrote over 446 scientific papers, but is perhaps best known for his discovery of the inert gas argon, which earned him a Nobel Prize.

Interactive Java Tutorials

Diffraction of Light - Several of the classical and most fundamental experiments that help explain diffraction of light were first conducted between the late seventeenth and early nineteenth centuries by Italian scientist Francesco Grimaldi, French scientist Augustin Fresnel, English physicist Thomas Young, and several other investigators. These experiments involve propagation of light waves though a very small slit (aperture), and demonstrate that when light passes through the slit, the physical size of the slit determines how the slit interacts with the light. This interactive tutorial explores the diffraction of a monochromatic light beam through a slit of variable aperture.

Particle Size and Diffraction Angles - The phenomenon of diffraction is observed when a specimen consisting of fine particles is illuminated with a beam of semi-coherent, collimated light. Good examples of this effect are a microscope slide containing particles of various sizes, and the spreading of automobile headlights on a foggy night. In both cases, diffraction is manifested through the scattering of light by small particles having linear physical dimensions similar to the wavelength of the illumination. This interactive tutorial demonstrates the effects of diffraction at an aperture and explores the spreading of light by a specimen composed of individual particles.

Line Spacing Calculations from Diffraction Gratings - By definition, a diffraction grating is composed of a planar substrate containing a parallel series of linear grooves or rulings, which can be transparent, semi-transparent, or opaque. When the spacing between lines on a diffraction grating is similar in size to the wavelength of light, an incident collimated and coherent light beam will be strongly diffracted upon encountering the grating. This interactive tutorial examines the effects of wavelength on the diffraction patterns produced by a virtual periodic line grating of fixed line spacing.

Light Diffraction Through a Periodic Grating - A model for the diffraction of visible light through a periodic grating is an excellent tool with which to address both the theoretical and practical aspects of image formation in optical microscopy. Light passing through the grating is diffracted according to the wavelength of the incident light beam and the periodicity of the line grating. This interactive tutorial explores the mechanics of periodic diffraction gratings when used to interpret the Abbe theory of image formation in the optical microscope.

Airy Pattern Formation - When an image is formed in the focused image plane of an optical microscope, every point in the specimen is represented by an Airy diffraction pattern having a finite spread. This occurs because light waves emitted from a point source are not focused into an infinitely small point by the objective, but converge together and interfere near the intermediate image plane to produce a three-dimensional Fraunhofer diffraction pattern. This interactive tutorial explores the origin of Airy diffraction patterns formed by the rear aperture of the microscope objective and observed at the intermediate image plane.

Airy Pattern Basics - The three-dimensional diffraction pattern formed by a circular aperture near the focal point in a well-corrected microscope is symmetrically periodic along the axis of the microscope as well as radially around the axis. When this diffraction pattern is sectioned in the focal plane, it is observed as the classical two-dimensional diffraction spectrum known as the Airy pattern. This tutorial explores how Airy pattern size changes with objective numerical aperture and the wavelength of illumination; it also simulates the close approach of two Airy patterns.

Numerical Aperture and Image Resolution - The Airy pattern formed at the microscope intermediate image plane is a three-dimensional diffraction image, which is symmetrically periodic both along the optical axis of the microscope, and radially across the image plane. This diffraction pattern can be sectioned in the focal plane to produce a two-dimensional diffraction pattern having a bright circular disk surrounded by an alternating series of bright and dark higher-order diffraction rings whose intensity decreases as they become further removed from the central disk. Usually only two or three of the circular luminous rings are visible in the microscope (this number is dependent upon the objective numerical aperture), because the higher orders are absorbed by stray light and are not visible.

Conoscopic Images of Periodic Gratings - The purpose of this tutorial is to explore the reciprocal relationship between line spacings in a periodic grid (simulating a specimen) and the separation of the conoscopic image at the objective aperture plane. When the line grating has broad periodic spacings, several images of the condenser iris aperture appear in the objective rear focal plane. If white light is used to illuminate the line grating, higher order diffracted images of the aperture appear with a blue fringe closer to the zeroth order (central) image and with a green-yellow-red spectrum appearing further out towards the objective aperture periphery.

Spatial Frequency and Image Resolution - When a line grating is imaged in the microscope, a series of conoscopic images representing the condenser iris opening can be seen at the objective rear focal plane. This tutorial explores the relationship between the distance separating these iris opening images and the periodic spacing (spatial frequency) of lines in the grating.

Airy Patterns and the Rayleigh Criterion - Airy diffraction pattern sizes and their corresponding radial intensity distribution functions are sensitive to both objective numerical aperture and the wavelength of illuminating light. For a well-corrected objective with a uniform circular aperture, two adjacent points are just resolved when the centers of their Airy patterns are separated by a distance **r**. This tutorial examines how Airy disk sizes, at the limit of optical resolution, vary with changes in objective numerical aperture and illumination wavelength and how these changes affect the resolution of the objective.

Periodic Diffraction Images - When a microscope objective forms a diffraction-limited image of an object, it produces a three-dimensional diffraction pattern that is periodic both along the optical axis and laterally within the intermediate image plane. This tutorial explores diffraction images produced by a periodic object at several focal depths.

Selected Literature References

Reference Listing - The reference materials listed in this section are an excellent source of additional information on the diverse topic of light diffraction and scattering by gratings and through apertures. Included are references to books, book chapters, and review articles, which discuss the theory and applications of these diverse topics, and how they relate to the physics of light and color.

Polarization of Light

Sunlight and almost every other form of natural and artificial illumination produces light waves whose electric field vectors vibrate in all planes that are perpendicular with respect to the direction of propagation. If the electric field vectors are restricted to a single plane by filtration of the beam with specialized materials, then the light is referred to as **plane** or **linearly polarized** with respect to the direction of propagation, and all waves vibrating in a single plane are termed **plane parallel** or **plane-polarized**.

Introduction to Polarized Light - The human eye lacks the ability to distinguish between randomly oriented and polarized light, and plane-polarized light can only be detected through an intensity or color effect, for example, by reduced glare when wearing polarized sun glasses. In effect, humans cannot differentiate between the high contrast real images observed in a polarized light microscope and identical images of the same specimens captured digitally (or on film), and then projected onto a screen with light that is not polarized. The first clues to the existence of polarized light surfaced around 1669 when Erasmus Bartholin discovered that crystals of the mineral Iceland spar (more commonly referred to as **calcite**) produce a double image when objects are viewed through the crystals in transmitted light. During his experiments, Bartholin also observed a quite unusual phenomenon. When the calcite crystals are rotated about their axis, one of the images moves in a circle around the other, providing strong evidence that the crystals are somehow splitting the light into two different beams.

Polarized Light Microscopy -The polarized light microscope is designed to observe and photograph specimens that are visible primarily due to their optically anisotropic character. In order to accomplish this task, the microscope must be equipped with both a **polarizer**, positioned in the light path somewhere before the specimen, and an **analyzer** (a second polarizer), placed in the optical pathway between the objective rear aperture and the observation tubes or camera port. Image contrast arises from the interaction of plane-polarized light with a **birefringent** (or doubly-refracting) specimen to produce two individual wave components that are each polarized in mutually perpendicular planes. The velocities of these components are different and vary with the propagation direction through the specimen. After exiting the specimen, the light components become out of phase, but are recombined with constructive and destructive interference when they pass through the analyzer. Polarized light is a contrast-enhancing technique that improves the quality of the image obtained with birefringent materials when compared to other techniques such as darkfield and brightfield illumination, differential interference contrast, phase contrast, Hoffman modulation contrast, and fluorescence.

Max Berek (1886-1949) - Max Berek was a German physicist and mathematician, associated with the firm of E. Leitz, who designed a wide spectrum of optical instruments, in particular for polarized light microscopy and several innovative camera lenses. Professor Berek is credited as the inventor of the Leica camera lens system at their Wetzlar factory.

Sir David Brewster (1781-1868) - Sir David Brewster was a Scottish physicist who invented the kaleidoscope, made major improvements to the stereoscope, and discovered the polarization phenomenon of light reflected at specific angles. In his studies on polarized light, Brewster discovered that when light strikes a reflective surface at a certain angle (now known as Brewster's Angle), the light reflected from that surface is plane-polarized. He elucidated a simple relationship between the incident angle of the light beam and the refractive index of the reflecting material.

Shinya Inoué (1921-Present) - Shinya Inoué is a microscopist, cell biologist, and educator who has been described as the grandfather of modern light microscopy. The pioneering microscopist heavily influenced the study of cell dynamics during the 1980s through his developments in video-enhanced contrast microscopy (**VEC**), which is a modification of the traditional form of differential interference contrast (**DIC**) microscopy. Inoué also made significant contributions to the investigation of biological systems with polarized light microscopy. His seminal work, "***Video Microscopy***," was published in 1986, and a second revised and updated edition, co-authored with Kenneth Spring, followed in 1997. The book is a cornerstone of microscopical knowledge and is highly regarded throughout the scientific community.

Edwin Herbert Land (1909-1991) - The founder of the Polaroid Corporation, Edwin Herbert Land was an American inventor and researcher who dedicated his entire adult life to the study of polarized light, photography and color vision. Perhaps Land's most famous contribution to science, however, was his development of instant photography. The invention was inspired by his three-year old daughter when she asked him why she could not instantly see a picture he had just taken of her on vacation. The one-step dry photographic process took Land three years to perfect, but his success was phenomenal.

Henri Hureau de Sénarmont (1808-1862) - Sénarmont was a professor of mineralogy and director of studies at the École des Mines in Paris, especially distinguished for his research on polarization and his studies on the artificial formation of minerals. He also contributed to the Geological Survey of France by preparing geological maps and essays. Perhaps the most significant contribution made by de Sénarmont to optics was the polarized light retardation compensator bearing his name, which is still widely utilized today.

Interactive Java Tutorials

Brewster's Angle - Light that is reflected from the flat surface of a dielectric (or insulating) material is often partially polarized, with the electric vectors of the reflected light vibrating in a plane that is parallel to the surface of the material. Common examples of surfaces that reflect polarized light are undisturbed water, glass, sheet plastics, and highways. In these instances, light waves that have the electric field vectors parallel to the surface are reflected to a greater degree than those with different orientations. This tutorial demonstrates the polarization effect on light reflected at a specific angle (the **Brewster** angle) from a transparent medium.

Double Refraction (Birefringence) in Iceland Spar - The first clues to the existence of polarized light surfaced around 1669 when Erasmus Bartholin discovered that crystals of the mineral Iceland spar (more commonly referred to as **calcite**) produce a double image when objects are viewed through the crystals in transmitted light. This interactive tutorial simulates viewing of a ball-point pen and a line of text through a crystal of Iceland spar, producing a double image.

Polarized Light Waveforms - The ordinary and extraordinary light waves generated when a beam of light traverses a birefringent crystal have plane-polarized electric vectors that are mutually perpendicular to each other. In addition, due to differences in electronic interaction that each component experiences during its journey through the crystal, there is usually a phase shift that occurs between the two waves. This interactive tutorial explores the generation of linear, elliptical, and circularly polarized light by a pair of orthogonal light waves (as a function of the relative phase shift between the waves) when the electric field vectors are added together.

Polarization of Light - When light travels through a linear polarizing material, a selected vibration plane is passed by the polarizer, while electric field vectors vibrating in all other orientations are blocked. Linearly polarized light transmitted through a polarizer can be either passed or absorbed by a second polarizer, depending upon the electric vector transmission azimuth orientation of the second polarizing material. This tutorial explores the effect of rotating two polarizers on an incident beam of white light.

Polarization of Light (3-D Version) - When non-polarized white light encounters a linear polarizer that is oriented with the transmission azimuth positioned vertically to the incident beam, only those waves having vertical electric field vectors will pass through. Polarized light exiting the first polarizer can be subsequently blocked by a second polarizer if the transmission axis is oriented horizontally with respect to the electric field vector of the polarized light waves. The concept of using two polarizers oriented at right angles with respect to each other is commonly termed **crossed polarization** and is fundamental to the concept of polarized light microscopy. This tutorial explores the effects of two polarizers having adjustable transmission axes on an incident beam of white light, and enables the visitor to translate the optical train in three dimensions.

Nicol Prisms - Several versions of prism-based polarizing devices were once widely available, and these were usually named after their designers. The most common polarizing prism (illustrated in the tutorial window) was named after William Nicol, who first cleaved and cemented together two crystals of Iceland spar with Canada balsam in 1829. Nicol prisms were first used to measure the polarization angle of birefringent compounds, leading to new developments in the understanding of interaction between polarized light and crystalline substances. This interactive tutorial explores the generation of orthogonal or mutually perpendicular (**ordinary** and **extraordinary**) waves as the result of light transmission through a Nicol prism.

Electromagnetic Wave Propagation - Electromagnetic waves can be generated by a variety of methods, such as a discharging spark or by an oscillating molecular dipole. Visible light is a commonly studied form of electromagnetic radiation, and exhibits oscillating electric and magnetic fields whose amplitudes and directions are represented by vectors that undulate in phase as sinusoidal waves in two mutually perpendicular (orthogonal) planes. This tutorial explores propagation of a virtual electromagnetic wave and considers the orientation of the magnetic and electric field vectors.

Polarized Light Virtual Microscopy Java Tutorials

Polarized Light Virtual Microscopes - When a birefringent material is placed between crossed polarizers in an optical microscope, light incident upon the material is split into two component beams whose amplitude and intensity vary depending upon the orientation angle between the polarizer and permitted vibration directions of the material. Use this link to explore our tutorials on polarized light microscopy.

Selected Literature and Web Resources

Polarized Light Literature References - A number of high-quality books and review articles on polarized light microscopy have been published by leading researchers in the field. This section contains periodical location information about these articles, as well as providing a listing of selected original research reports and books describing the classical techniques of optical crystallography and polarized light microscopy.

Polarized Light Microscopy Web Resources - Although much neglected and undervalued as an investigative tool, polarized light microscopy provides all the benefits of brightfield microscopy and yet offers a wealth of information, which is simply not available with any other optical microscopy technique. This section is a compendium of web resources focused on all aspects of polarized light microscopy, optical crystallography, and related techniques.

Interference of Light Waves

The formation of an image in the microscope relies on a complex interplay between two critical optical phenomena: diffraction and interference. Light passing through the specimen is scattered and diffracted into divergent waves by tiny details and features present in the specimen. Some of the divergent light scattered by the specimen is captured by the objective and focused onto the intermediate image plane, where the superimposed light waves are recombined or summed through the process of **interference** to produce a magnified image of the specimen.

Fundamentals of Interference - The seemingly close relationship between diffraction and interference occurs because they are actually manifestations of the same physical process and produce ostensibly reciprocal effects. Most of us observe some type of optical interference almost every day, but usually do not realize the events in play behind the often-kaleidoscopic display of color produced when light waves interfere with each other. One of the best examples of interference is demonstrated by the light reflected from a film of oil floating on water. Another example is the thin film of a soap bubble, which reflects a spectrum of beautiful colors when illuminated by natural or artificial light sources.

Augustin-Jean Fresnel (1788-1827) - Augustin-Jean Fresnel, was a nineteenth century French physicist, who is best known for the invention of unique compound lenses designed to produce parallel beams of light, which are still used widely in lighthouses. In the field of optics, Fresnel derived formulas to explain reflection, diffraction, interference, refraction, double refraction, and the polarization of light reflected from a transparent substance.

Christiaan Huygens (1629-1695) - Christiaan Huygens was a brilliant Dutch mathematician, physicist, and astronomer who lived during the seventeenth century, a period sometimes referred to as the Scientific Revolution. Huygens, a particularly gifted scientist, is best known for his work on the theories of centrifugal force, the wave theory of light, and the pendulum clock. His theories neatly explained the laws of refraction, diffraction, interference, and reflection, and Huygens went on to make major advances in the theories concerning the phenomena of double refraction (birefringence) and polarization of light.

Samuel Tolansky (1907-1973) - Born in Newcastle upon Tyne, England as Samuel Turlausky, Tolansky performed a significant amount of his research and developed the interference contrast microscopy technique that bears his name. Other research interests of Tolansky included the analysis of spectra to investigate nuclear spin and the study of optical illusions. Although he was primarily concerned with the spectrum of mercury, during World War II Tolansky was asked to ascertain the spin of uranium-235, the isotope capable of fission in a nuclear chain reaction.

Thomas Young (1773-1829) - Thomas Young was an English physician and a physicist who was responsible for many important theories and discoveries in optics and in human anatomy. His best known work is the wave theory of interference. Young was also responsible for postulating how the receptors in the eye perceive colors. He is credited, along with Hermann Ludwig Ferdinand von Helmholtz, for developing the Young-Helmholtz trichromatic theory.

Interactive Java Tutorials

Wave Interactions in Optical Interference - The classical method of describing interference includes presentations that depict the graphical recombination of two or more sinusoidal light waves in a plot of amplitude, wavelength, and relative phase displacement. In effect, when two waves are added together, the resulting wave has an amplitude value that is either increased through constructive interference, or diminished through destructive interference. This interactive tutorial illustrates the effect by considering a pair of light waves from the same source that are traveling together in parallel, but can be adjusted with respect to coherency (phase relationship), amplitude, and wavelength.

Interference Phenomena in Soap Bubbles - Most of us observe some type of optical interference almost every day, but usually do not realize the events in play behind the often-kaleidoscopic display of color produced when light waves interfere with each other. One of the best examples of interference is demonstrated by the light reflected from a film of oil floating on water. Another example is the thin film of a soap bubble, which reflects a spectrum of beautiful colors when illuminated by natural or artificial light sources. This interactive tutorial explores how the interference phenomenon of light reflected by a soap bubble changes as a function of film thickness.

Thomas Young's Double Slit Experiment - In 1801, an English physicist named Thomas Young performed an experiment that strongly inferred the wave-like nature of light. Because he believed that light was composed of waves, Young reasoned that some type of interaction would occur when two light waves met. This interactive tutorial explores how coherent light waves interact when passed through two closely spaced slits.

Interference Filters - Recent technological achievements in bandpass filter design have led to the relatively inexpensive construction of thin-film interference filters featuring major improvements in wavelength selection and transmission performance. These filters operate by transmitting a selected wavelength region with high efficiency while rejecting, through reflection and destructive interference, all other wavelengths. Explore how interference filters operate by selectively transmitting constructively reinforced wavelengths while simultaneously eliminating unwanted light with this interactive tutorial.

Interference Between Parallel Light Waves - If the vibrations produced by the electric field vectors (which are perpendicular to the propagation direction) from waves that are parallel to each other (in effect, the vectors vibrate in the same plane), then the light waves may combine and undergo interference. If the vectors do not lie in the same plane, and are vibrating at some angle between 90 and 180 degrees with respect to each other, then the waves cannot interfere with one another. Analogous to the wave tutorial linked above, this interactive tutorial illustrates the effect by considering a pair of light waves from the same source that are coherent (having an identical phase relationship) and traveling together in parallel.

Complex Waveforms and Beat Frequencies in Superposed Waves - In general, the process of describing interference through the superposition of sine waves generates simple waveforms that can be adequately represented by a resultant sine curve in a plot of amplitude, wavelength, and relative phase displacement. If the recombined waves have appreciably different frequencies, the resulting waveform is often complex, yielding a contour that is no longer a sine function with a simple, single harmonic. This interactive tutorial explores the complex waveforms and beat frequencies generated by the superposition of two light waves propagating in the same direction with different relative frequencies, amplitudes, and phases.

Selected Literature References

Reference Listing - Leading investigators in the fields of optics and photonics have published a number of high-quality review articles on a variety of interference phenomena. This section contains periodical and book location information about these articles, as well as providing a listing of the chapter titles for appropriate sections dealing with the interference between wavefronts and related effects.

Optical Birefringence

Birefringence is formally defined as the double refraction of light in a transparent, molecularly ordered material, which is a manifestation of the existence of orientation-dependent differences in refractive index. Many transparent solids are optically isotropic, meaning that the index of refraction is equal in all directions throughout the crystalline lattice. Examples of isotropic solids are glass, table salt, many polymers, and a wide variety of both organic and inorganic compounds.

Crystals are classified as being either isotropic or anisotropic depending upon their optical behavior and whether or not their crystallographic axes are equivalent. All isotropic crystals have equivalent axes that interact with light in a similar manner, regardless of the crystal orientation with respect to incident light waves. Light entering an isotropic crystal is refracted at a constant angle and passes through the crystal at a single velocity without being polarized by interaction with the electronic components of the crystalline lattice.

The term **anisotropy** refers to a non-uniform spatial distribution of properties, which result in different values being obtained when specimens are probed from several directions within the same material. Observed properties are often dependent on the particular probe being employed and often vary depending upon the whether the observed phenomena are based on optical, acoustical, thermal, magnetic, or electrical events. On the other hand, isotropic properties remain symmetrical, regardless of the direction of measurement with each type of probe reporting identical results.

Introduction to Birefringence - Anisotropic crystals, such as quartz, calcite, and tourmaline, have crystallographically distinct axes and interact with light by a mechanism that is dependent upon the orientation of the crystalline lattice with respect to the incident light angle. When light enters the **optical axis** of anisotropic crystals, it behaves in a manner similar to the interaction with isotropic crystals, and passes through at a single velocity. However, when light enters a non-equivalent axis, it is refracted into two rays each polarized with the vibration directions oriented at right angles to one another, and traveling at different velocities. This phenomenon is termed **double refraction** or **birefringence** and is exhibited to a greater or lesser degree in all anisotropic crystals.

Interactive Java Tutorials

Acoustical Model of Anisotropy - The anisotropic character of materials relates to those properties that have different values when measurements are made in different directions within the same material. This interactive tutorial explores how sound waves exhibit anisotropic character as a function of grain structure when traveling through a wooden block, which serves as an excellent model for the behavior of light passing through anisotropic crystals.

Double Refraction (Birefringence) - Calcite is a form of calcium carbonate, commonly referred to as **Iceland spar**, which has a rhombohedral crystalline shape. Light passing through a crystal of calcite is refracted into two rays, which are separated by a wide margin due to the strong birefringence of the crystal. This interactive tutorial simulates viewing of a ball-point pen and a line of text through a crystal of Iceland spar, producing a double image from the refracted light rays.

Birefringence in Calcite Crystals - As light travels through an anisotropic material, the electromagnetic waves become split into two principal vibrations, which are oriented mutually perpendicular to each other and perpendicular to the direction that the waves propagate. The wave whose electric vector vibrates along the major axis of the index ellipse is termed the **slow wave**, because the refractive index for this wave is greater than the refractive index for the other wave. The wave vibrating perpendicular to the slow wave is termed the **fast wave**. This tutorial explores double refraction or birefringence in calcite (calcium carbonate), a colorless, transparent, rhombohedral crystalline salt that is the most common such material found naturally.

The Fresnel or Refractive Index Ellipsoid - The Fresnel, or refractive index, ellipsoid describes the dielectric properties measured in all directions through a material. Measurements through the radius yields the refractive index (**n**) or the square root of the dielectric constant for waves whose electric displacement vectors lie in the direction of the ellipsoid radius. This tutorial explores variations in the shape and dimensions of the ellipsoid as a function of refractive index.

Birefringence Variations with Crystal Orientation - When a beam of light is incident on a birefringent crystal, the waves are split upon entry into orthogonal polarized components (termed **ordinary** and **extraordinary**) that travel through the molecular lattice along different pathways, depending on their orientation with respect to the crystalline optical axis. If the incident beam is oblique to the optical axis, the waves diverge during their journey through the crystal. In contrast, the orthogonal wave components follow a co-linear pathway when the incident light beam enters the crystal either parallel or perpendicular to the optical axis. This interactive tutorial explores variations in birefringence that result from orientational variations between the crystal optical axis and the incident light beam.

Birefringent Crystals in Polarized Light - In order to examine how birefringent anisotropic crystals interact with polarized light in an optical microscope, the properties of an individual, isolated crystal can be considered. The specimen material in this tutorial is a hypothetical tetragonal birefringent crystal having an optical axis oriented in a direction that is parallel to the long axis of the crystal. Light entering the crystal from the polarizer will be traveling perpendicular to the optical axis of the crystal, regardless of the crystal orientation with respect to the polarizer and analyzer transmission axes. The virtual microscope viewport presents the crystal as it would appear in the eyepieces of a microscope under crossed-polarized illumination as it is rotated around the microscope optical axis.

Interactive Michel-Levy Birefringence Chart - Quantitative analysis of the interference colors observed in birefringent samples is usually accomplished by consulting a Michel-Levy chart similar to the one illustrated in the tutorial window below. As is evident from this graph, the polarization colors visualized in the microscope and recorded onto film or captured digitally can be correlated with the actual retardation value, thickness, and birefringence of the specimen. The chart is relatively easy to use with birefringent samples if two of the three required variables are known. This interactive tutorial enables visitors to determine the interference color associated with all three values by clicking on selected regions of the interactive chart. A **large version** of the tutorial is also available.

Color Temperature

The color temperature model is based on the relationship between the temperature of a theoretical standardized material, known as a **black body radiator**, and the energy distribution of its emitted light as the radiator is brought from absolute zero to increasingly higher temperatures. As the name implies, black body radiators completely absorb all radiation, without any transmission or reflection, and then re-emit all incident energy in the form of a continuous spectrum of light representing all frequencies in the electromagnetic spectrum. Although the black body radiator does not actually exist, many metals behave in a manner very similar to a theoretical radiator.

Basic Principles of Color Temperature - The concept of color temperature is of critical importance in photography and digital imaging, regardless of whether the image capture device is a camera, microscope, or telescope. A lack of proper color temperature balance between the microscope light source and the film emulsion or image sensor is the most common reason for unexpected color shifts in photomicrography and digital imaging. If the color temperature of the light source is too low for the film, photomicrographs will have an overall yellowish or reddish cast and will appear **warm**. On the other hand, when the color temperature of the light source is too high for the film, photomicrographs will have a blue cast and will appear **cool**. The degree of mismatch will determine the extent of these color shifts, with large discrepancies leading to extremes in color variations. Perhaps the best example is daylight film used in a microscope equipped with a tungsten-halogen illumination source without the benefit of color balancing filters. In this case, the photomicrographs will have a quite large color shift towards warmer reddish and yellowish hues. As problematic as these color shifts may seem, they are always easily corrected by the proper use of conversion and light balancing filters.

Interactive Java Tutorials

Color Temperature in a Virtual Radiator - Investigate the apparent "color" of a virtual radiator (in this case, a black metal pot) as it is slowly heated through a wide temperature range by external energy. The concept of color temperature is based on the relationship between the temperature and radiation emitted by a theoretical standardized material, termed a **black body radiator**, cooled down to a state in which all molecular motion has ceased. Hypothetically, at cessation of all molecular motion, the temperature is described as being at **absolute zero** or 0 Kelvin, which is equal to -273 degrees Celsius.

Color Temperature Nomograph - The color temperature nomograph is a useful tool with which to determine the necessary color balancing and/or correction filter(s) that are necessary to convert a light source from one color temperature to another. To use this type of graph, a straight edge ruler is placed at the color temperature of the original source and is pivoted to connect to the desired color temperature. The region where the ruler intersects the central axis identifies the necessary filter to achieve the color conversion. This interactive Java nomograph tutorial can be employed to quickly determine the appropriate filter under a variety of illumination scenarios.

White and Black Balance - The overall color of a digital image captured with an optical microscope is dependent not only upon the spectrum of visible light wavelengths transmitted through or reflected by the specimen, but also on the spectral content of the illuminator. In color digital camera systems that employ either charge-coupled device (**CCD**) or complementary metal oxide semiconductor (**CMOS**) image sensors, **white** and/or **black balance** (baseline) adjustment is often necessary in order to produce acceptable color quality in digital images.

Incandescent Lamp Filaments - Nearly every source of light depends, at the fundamental level, on the release of energy from atoms that have been excited in some manner. Standard incandescent lamps, derived directly from the early models of the 1800s, now commonly utilize a tungsten filament in an inert gas atmosphere, and produce light through the resistive effect that occurs when the filament temperature increases as electrical current is passed through. This interactive tutorial demonstrates the sub-atomic activity within a conducting incandescent lamp filament that results in resistance to current flow, and ultimately leads to the emission of infrared and visible light photons (with a corresponding rise in color temperature).

Contributing Authors

Primary Colors

The human eye is sensitive to a narrow band of electromagnetic radiation that lies in the wavelength range between 400 and 700 nanometers, commonly known as the visible light spectrum, which is the only source of color. When combined, all of the wavelengths present in visible light, about a third of the total spectral distribution that successfully passes through the Earth's atmosphere, form colorless white light that can be refracted and dispersed into its component colors by means of a prism. The colors red, green, and blue are classically considered the **primary** colors because they are fundamental to human vision. Light is perceived as white by humans when all three cone cell types are simultaneously stimulated by equal amounts of red, green, and blue light.

The complementary colors (cyan, yellow, and magenta) are also commonly referred to as the **primary subtractive** colors because each can be formed by subtracting one of the primary additives (red, green, and blue) from white light. For example, yellow light is observed when all blue light is removed from white light, magenta forms when green is removed, and cyan is produced when red is removed. The color observed by subtracting a primary color from white light results because the brain adds together the colors that are left to produce the respective complementary or subtractive color.

Introduction - Pigments and dyes are responsible for most of the color humans see in the real world. Eyes, skin, and hair contain natural protein pigments that reflect the colors visualized in the people around us (in addition to any assistance by colors used in facial makeup and hair dyes). Books, magazines, signs, and billboards are printed with colored inks that create colors through the process of color subtraction. In a similar manner, automobiles, airplanes, houses, and other buildings are coated with paints containing a variety of pigments. The concept of color subtraction is responsible for most of the color produced by the objects just described. For many years, artists and printers have searched for substances containing dyes and pigments that are particularly good at subtracting specific colors.

Interactive Java Tutorials

Primary Additive Colors - Light is perceived as white by humans when all three cone cell types are simultaneously stimulated by equal amounts of red, green, and blue light. Because the addition of these three colors yields white light, the colors red, green, and blue are termed the **primary additive** colors. This tutorial explores how the three primary additive colors interact with each other, both in pairs or all together.

Primary Subtractive Colors - The complementary colors (cyan, yellow, and magenta) are also commonly referred to as the **primary subtractive** colors because each can be formed by subtracting one of the primary additives (red, green, and blue) from white light. This tutorial explores how the three primary subtractive colors interact with each other, both in pairs or all together.

Color Filters - Examine how color filters operate to change the color of objects visualized under filtered illumination. The tutorial enables visitors to drag and drop red, green, and blue virtual color filters over objects illuminated both with white light and also previously filtered with one of the primary additive colors.

Color Separation - Pigments and dyes are responsible for most of the color that humans see in the real world. Books, magazines, signs, and billboards are printed with colored inks that create colors through the process of color subtraction. This interactive tutorial explores how individual subtractive primary colors can be separated from a full-color photograph, and then how they can be reassembled to create the original scene.

Selected Literature References

Reference Listing - Presented in this section are selected literature references on various topics concerning both the additive and subtractive primary colors from our extensive library. The perception of color in the human visual system, whether in paint, printed materials or video displays depends on the interactions between the primary colors. Included are references to books and review articles that discuss numerous aspects of color theory and how they may be applied.

Light and Color

Light is a complex phenomenon that is classically explained with a simple model based on rays and wavefronts. The Molecular Expressions Microscopy Primer explores many of the aspects of visible light starting with an introduction to electromagnetic radiation and continuing through to human vision and the perception of color. Each section outlined below is an independent treatise on a limited aspect of light and color. We hope you enjoy your visit and find the answers to your questions.

Electromagnetic Radiation - Visible light is a complex phenomenon that is classically explained with a simple model based on propagating rays and wavefronts, a concept first proposed in the late 1600s by Dutch physicist Christiaan Huygens. Electromagnetic radiation, the larger family of wave-like phenomena to which visible light belongs (also known as **radiant energy**), is the primary vehicle transporting energy through the vast reaches of the universe. The mechanisms by which visible light is emitted or absorbed by substances, and how it predictably reacts under varying conditions as it travels through space and the atmosphere, form the basis of the existence of color in our universe.

Light: Particle or a Wave? - Many distinguished scientists have attempted to explain how electromagnetic radiation can display what has now been termed **duality**, or both particle-like and wave-like behavior. At times light behaves as if composed of particles, and at other times as a continuous wave. This complementary, or dual, role for the properties of light can be employed to describe all of the known characteristics that have been observed experimentally, ranging from refraction, reflection, interference, and diffraction, to the results with polarized light and the photoelectric effect.

Sources of Visible Light - A wide variety of sources are responsible for emission of electromagnetic radiation, and are generally categorized according to the specific spectrum of wavelengths generated by the source. Relatively long radio waves are produced by electrical current flowing through huge broadcast antennas, while much shorter visible light waves are produced by the energy state fluctuations of negatively charged electrons within atoms. The shortest form of electromagnetic radiation, gamma waves, results from decay of nuclear components at the center of the atom. The visible light that humans are able to see is usually a mixture of wavelengths whose varying composition is a function of the light source.

Fluorescence - The phenomenon of fluorescence was known by the middle of the nineteenth century. British scientist Sir George G. Stokes first made the observation that the mineral **fluorspar** exhibits fluorescence when illuminated with ultraviolet light, and he coined the word "fluorescence". Stokes observed that the fluorescing light has longer wavelengths than the excitation light, a phenomenon that has become to be known as the **Stokes shift**. Fluorescence microscopy is an excellent method of studying material that can be made to fluoresce, either in its natural form (termed **primary** or **auto** fluorescence) or when treated with chemicals capable of fluorescing (known as **secondary** fluorescence). The fluorescence microscope was devised in the early part of the twentieth century by August Köhler, Carl Reichert, and Heinrich Lehmann, among others. However, the potential of this instrument was not realized for several decades, and fluorescence microscopy is now an important (and perhaps indispensable) tool in cellular biology.

Speed of Light - Starting with Ole Roemer's 1676 breakthrough endeavors, the speed of light has been measured at least 163 times by more than 100 investigators utilizing a wide variety of different techniques. Finally in 1983, more than 300 years after the first serious measurement attempt, the speed of light was defined as being 299,792.458 kilometers per second by the Seventeenth General Congress on Weights and Measures. Thus, the meter is defined as the distance light travels through a vacuum during a time interval of 1/299,792,458 seconds. In general, however, (even in many scientific calculations) the speed of light is rounded to 300,000 kilometers (or 186,000 miles) per second.

Reflection of Light - Reflection of light (and other forms of electromagnetic radiation) occurs when the waves encounter a surface or other boundary that does not absorb the energy of the radiation and bounces the waves away from the surface. The incoming light wave is referred to as an ***incident*** wave and the wave that is bounced away from the surface is called the ***reflected*** wave. The simplest example of visible light reflection is the glass-like surface of a smooth pool of water, where the light is reflected in an orderly manner to produce a clear image of the scenery surrounding the pool. Throw a rock into the pool, and the water is perturbed to form waves, which disrupt the image of the scene by scattering the reflected light in all directions.

Refraction of Light - As light passes from one substance into another, it will travel straight through with no change of direction when crossing the boundary between the two substances head-on (perpendicular, or a 90-degree angle of incidence). However, if the light impacts the boundary at any other angle it will be bent or refracted, with the degree of refraction increasing as the beam is progressively inclined at a greater angle with respect to the boundary. As an example, a beam of light striking water vertically will not be refracted, but if the beam enters the water at a slight angle it will be refracted to a very small degree. If the angle of the beam is increased even further, the light will refract with increasing proportion to the entry angle. Early scientists realized that the ratio between the angle at which the light crosses the media interface and the angle produced after refraction is a very precise characteristic of the material producing the refraction effect.

Diffraction of Light - Depending on the circumstances that give rise to the phenomenon, diffraction can be perceived in a variety of different ways. Scientists have cleverly utilized diffraction of neutrons and X-rays to elucidate the arrangement of atoms in small ionic crystals, molecules, and even such large macromolecular assemblies as proteins and nucleic acids. Electron diffraction is often employed to examine periodic features of viruses, membranes, and other biological organisms, as well as synthetic and naturally occurring materials. No lens exists that will focus neutrons and X-rays into an image, so investigators must reconstruct images of molecules and proteins from the diffraction patterns using sophisticated mathematical analysis. Fortunately, magnetic lenses can focus diffracted electrons in the electron microscope, and glass lenses are very useful for focusing diffracted light to form an optical image that can easily be viewed.

Polarization of Light - The human eye lacks the ability to distinguish between randomly oriented and polarized light, and plane-polarized light can only be detected through an intensity or color effect, for example, by reduced glare when wearing polarized sun glasses. In effect, humans cannot differentiate between the high contrast real images observed in a polarized light microscope and identical images of the same specimens captured digitally (or on film), and then projected onto a screen with light that is not polarized. The first clues to the existence of polarized light surfaced around 1669 when Erasmus Bartholin discovered that crystals of the mineral Iceland spar (more commonly referred to as **calcite**) produce a double image when objects are viewed through the crystals in transmitted light. During his experiments, Bartholin also observed a quite unusual phenomenon. When the calcite crystals are rotated about their axis, one of the images moves in a circle around the other, providing strong evidence that the crystals are somehow splitting the light into two different beams.

Fundamentals of Interference - The seemingly close relationship between diffraction and interference occurs because they are actually manifestations of the same physical process and produce ostensibly reciprocal effects. Most of us observe some type of optical interference almost every day, but usually do not realize the events in play behind the often-kaleidoscopic display of color produced when light waves interfere with each other. One of the best examples of interference is demonstrated by the light reflected from a film of oil floating on water. Another example is the thin film of a soap bubble, which reflects a spectrum of beautiful colors when illuminated by natural or artificial light sources.

Optical Birefringence - Anisotropic crystals, such as quartz, calcite, and tourmaline, have crystallographically distinct axes and interact with light by a mechanism that is dependent upon the orientation of the crystalline lattice with respect to the incident light angle. When light enters the **optical axis** of anisotropic crystals, it behaves in a manner similar to the interaction with isotropic crystals, and passes through at a single velocity. However, when light enters a non-equivalent axis, it is refracted into two rays each polarized with the vibration directions oriented at right angles to one another, and traveling at different velocities. This phenomenon is termed **double refraction** or **birefringence** and is exhibited to a greater or lesser degree in all anisotropic crystals.

Color Temperature - The concept of color temperature is of critical importance in photography and digital imaging, regardless of whether the image capture device is a camera, microscope, or telescope. A lack of proper color temperature balance between the microscope light source and the film emulsion or image sensor is the most common reason for unexpected color shifts in photomicrography and digital imaging. If the color temperature of the light source is too low for the film, photomicrographs will have an overall yellowish or reddish cast and will appear **warm**. On the other hand, when the color temperature of the light source is too high for the film, photomicrographs will have a blue cast and will appear **cool**. The degree of mismatch will determine the extent of these color shifts, with large discrepancies leading to extremes in color variations. Perhaps the best example is daylight film used in a microscope equipped with a tungsten-halogen illumination source without the benefit of color balancing filters. In this case, the photomicrographs will have a quite large color shift towards warmer reddish and yellowish hues. As problematic as these color shifts may seem, they are always easily corrected by the proper use of conversion and light balancing filters.

Primary Colors - The human eye is sensitive to a narrow band of electromagnetic radiation that lies in the wavelength range between 400 and 700 nanometers, commonly known as the visible light spectrum, which is the only source of color. When combined, all of the wavelengths present in visible light, about a third of the total spectral distribution that successfully passes through the Earth's atmosphere, form colorless white light that can be refracted and dispersed into its component colors by means of a prism. The colors red, green, and blue are classically considered the **primary** colors because they are fundamental to human vision. Light is perceived as white by humans when all three cone cell types are simultaneously stimulated by equal amounts of red, green, and blue light.

Light Filters - A majority of the common natural and artificial light sources emit a broad range of wavelengths that cover the entire visible light spectrum, with some extending into the ultraviolet and infrared regions as well. For simple lighting applications, such as interior room lights, flashlights, spot and automobile headlights, and a host of other consumer, business, and technical applications, the wide wavelength spectrum is acceptable and quite useful. However, in many cases it is desirable to narrow the wavelength range of light for specific applications that require a selected region of color or frequency. This task can be easily accomplished through the use of specialized filters that transmit some wavelengths and selectively absorb, reflect, refract, or diffract unwanted wavelengths.

Human Vision and Color Perception - Human stereo color vision is a very complex process that is not completely understood, despite hundreds of years of intense study and modeling. Vision involves the nearly simultaneous interaction of the two eyes and the brain through a network of neurons, receptors, and other specialized cells. The first steps in this sensory process are the stimulation of light receptors in the eyes, conversion of the light stimuli or images into signals, and transmission of electrical signals containing the vision information from each eye to the brain through the **optic nerves**. This information is processed in several stages, ultimately reaching the **visual cortices** of the cerebrum.

Light and Energy - Mankind has always been dependent upon energy from the sun's light both directly - for warmth, to dry clothing, to cook, and indirectly to provide food, water, and air. Our awareness of the value of the sun's rays revolves around the manner in which we benefit from the energy, but there are far more fundamental implications from the relationship between light and energy. Whether or not mankind devises ingenius mechanisms to harness the sun's energy, our planet and the changing environment contained within is naturally driven by the energy of sunlight.

Introduction to Lenses and Geometrical Optics - The action of a simple lens, similar to many of those used in the microscope, is governed by the principles of refraction and reflection and can be understood with the aid of a few simple rules about the geometry involved in tracing light rays through the lens. The basic concepts explored in this discussion, which are derived from the science of **Geometrical Optics**, will lead to an understanding of the magnification process, the properties of real and virtual images, and lens **aberrations** or defects.

Basic Properties of Mirrors - Reflection of light is an inherent and important fundamental property of mirrors, and is quantitatively gauged by the ratio between the amount of light reflected from the surface and that incident upon the surface, a term known as **reflectivity**. Mirrors of different design and construction vary widely in their reflectivity, from nearly 100 percent for highly-polished mirrors coated with metals that reflect visible and infrared wavelengths, to nearly zero for strongly absorbing materials.

Prisms and Beamsplitters - Prisms and beamsplitters are essential components that bend, split, reflect, and fold light through the pathways of both simple and sophisticated optical systems. Cut and ground to specific tolerances and exact angles, prisms are polished blocks of glass or other transparent materials that can be employed to deflect or deviate a light beam, rotate or invert an image, separate polarization states, or disperse light into its component wavelengths. Many prism designs can perform more than one function, which often includes changing the line of sight and simultaneously shortening the optical path, thus reducing the size of optical instruments.

Laser Fundamentals - Ordinary natural and artificial light is released by energy changes on the atomic and molecular level that occur without any outside intervention. A second type of light exists, however, and occurs when an atom or molecule retains its excess energy until **stimulated** to emit the energy in the form of light. Lasers are designed to produce and amplify this stimulated form of light into intense and focused beams. The word laser was coined as an acronym for **L**ight **A**mplification by the **S**timulated **E**mission of **R**adiation. The special nature of laser light has made laser technology a vital tool in nearly every aspect of everyday life including communications, entertainment, manufacturing, and medicine.

Light and Color Java Tutorials - Difficult concepts in the physics of light and the science of optics are much easier to understand with the aid of interactive tutorials that demonstrate various aspects of the principles involved. Check out these cool Java tutorial-applets that explore a wide range of concepts in light, color, and optics.

Contributing Authors

Human Vision and Color Perception

Human stereo color vision is a very complex process that is not completely understood, despite hundreds of years of intense study and modeling. Vision involves the nearly simultaneous interaction of the two eyes and the brain through a network of neurons, receptors, and other specialized cells. The first steps in this sensory process are the stimulation of light receptors in the eyes, conversion of the light stimuli or images into signals, and transmission of electrical signals containing the vision information from each eye to the brain through the **optic nerves**. This information is processed in several stages, ultimately reaching the **visual cortices** of the cerebrum.

Introduction to Human Vision - The human eye is equipped with a variety of optical components including the cornea, iris, pupil, aqueous and vitreous humors, a variable-focus lens, and the retina. Together, these elements work to form images of the objects that fall into the field of view for each eye. When an object is observed, it is first focused through the convex **cornea** and lens elements, forming an inverted image on the surface of the **retina**, a multi-layered membrane that contains millions of light-sensitive cells. In order to reach the retina, light rays focused by the cornea must successively traverse the **aqueous humor** (in the anterior chamber), the crystalline lens, the gelatinous vitreous body, and the vascular and neuronal layers of the retina before they reach the photosensitive outer segments of the cone and rod cells. These photosensory cells detect the image and translate it into a series of electrical signals for transmission to the brain.

Sir George Biddell Airy (1801-1892) - Sir George Airy was a distinguished nineteenth century English Astronomer Royal who carried out optical research and first drew attention to the visual defect of astigmatism. Airy manufactured the first correcting eyeglasses (1825) using a cylindrical lens design that is still in use. The diffraction disks that bear his name (Airy Disks) were discovered in the spherical center of a wavefront traveling through a circular aperture. These diffraction patterns form the smallest unit that comprises an image, thus determining the limits of optical resolution.

Alhazen (965-1040) - Born in Iraq as Abu Ali Hasan Ibn al-Haitham, the great Arab physicist is more often known by the Latinized version of his first name, Alhazen. The efforts of Alhazen resulted in over one hundred works, the most famous of which was "***Kitab-al-Manadhirn***", rendered into Latin in the Middle Ages. The translation of the book on optics exerted a great influence upon the science of the western world, most notably on the work of Roger Bacon and Johannes Kepler. A significant observation in the work contradicted the beliefs of many great scientists, such as Ptolemy and Euclid. Alhazen correctly proposed that the eyes passively receive light reflected from objects, rather than emanating light rays themselves.

Euclid (325-265 BC) - Though often overshadowed by his mathematical reputation, Euclid is a central figure in the history of optics. He wrote an in-depth study of the phenomenon of visible light in ***Optica***, the earliest surviving treatise concerning optics and light in the western world. Within the work, Euclid maintains the Platonic tradition that vision is caused by rays that emanate from the eye, but also offers an analysis of the eye's perception of distant objects and defines the laws of reflection of light from smooth surfaces. ***Optica*** was considered to be of particular importance to astronomy and was often included as part of a compendium of early Greek works in the field. Translated into Latin by a number of writers during the medieval period, the work gained renewed relevance in the fifteenth century when it underpinned the principles of linear perspective.

Thomas Young (1773-1829) - Thomas Young was an English physician and a physicist who was responsible for many important theories and discoveries in optics and in human anatomy. His best known work is the wave theory of interference. Young was also responsible for postulating how the receptors in the eye perceive colors. He is credited, along with Hermann Ludwig Ferdinand von Helmholtz, for developing the Young-Helmholtz trichromatic theory.

Interactive Java Tutorials

Human Eye Accommodation - Accommodation of the eye refers to the act of physiologically adjusting crystalline lens elements to alter the refractive power and bring objects that are closer to the eye into sharp focus. This tutorial explores changes in the lens structure as objects are relocated with respect to the eye.

Ishihara Color Blindness Test - Color blindness, a disruption in the normal functioning of human photopic vision, can be caused by host of conditions, including those derived from genetics, biochemistry, physical damage, and diseases. Partial color blindness, a condition where the individual has difficulty discriminating between specific colors, is far more common than total color blindness where only shades of gray are recognized. This interactive tutorial explores and simulates how full-color images appear to colorblind individuals, and compares these images to the Ishihara diagnostic colorblind test.

Selected Literature References

Reference Listing - References are presented in this section that explore human stereovision and the perception of colors. Included are pertinent review articles and books that discuss theoretical and applied aspects of color perception and the complexities of the human visual system. Several of the reference materials also address the topics of color blindness, astigmatism, and other physiological defects of the human visual system.

Light and Energy

The amount of energy falling on the Earth's surface from the sun is approximately 5.6 billion billion (quintillion) megajoules per year. Averaged over the entire Earth's surface, this translates into about 5 kilowatt-hours per square meter every day. The energy input from the sun in a single day could supply the needs for all of the Earth's inhabitants for a period of about 3 decades. Obviously, there is no means conceivable (nor is it necessary) to harness all of the energy that is available; equally obvious is that capturing even a small fraction of the available energy in a useable form would be of enormous value.

Introduction - Only in the last few decades has mankind begun to search in earnest for mechanisms to harness the tremendous potential of solar energy. This intense concern has resulted from a continuing increase in energy consumption, growing environmental problems from the fuels that are now consumed, and an ever-present awareness about the inevitable depletion of fossil fuel resources upon which we have become so heavily dependent. Among the topics discussed in this section are photosynthesis, the photoelectric effect, solar cells, charge-coupled devices, fuel cells, and nuclear fusion.

Electronic Imaging Detectors - The range of light detection methods and the wide variety of imaging devices currently available to the microscopist make the selection process difficult and often confusing. This discussion is intended to aid in understanding the basics of light detection and to provide a guide for selecting a suitable electronic detector (CCD or video camera system) for specific applications in optical microscopy.

The MOS Capacitor - At the heart of all charge-coupled devices (CCDs) is a light-sensitive metal oxide semiconductor (MOS) capacitor, which has three components consisting of a metal electrode (or gate), an insulating film of silicon dioxide, and a silicon substrate.

Introduction to CMOS Image Sensors - CMOS image sensors are designed with the ability to integrate a number of processing and control functions, which lie beyond the primary task of photon collection, directly onto the sensor integrated circuit. These features generally include timing logic, exposure control, analog-to-digital conversion, shuttering, white balance, gain adjustment, and initial image processing algorithms. Inexpensive CMOS image sensors are entering the field of optical microscopy in educational instruments that combine acceptable optical quality with user-friendly control and imaging software packages.

Photomultiplier Tubes - A photomultiplier tube, useful for light detection of very weak signals, is a photoemissive device in which the absorption of a photon results in the emission of an electron. These detectors work by amplifying the electrons generated by a photocathode exposed to a photon flux.

Interactive Java Tutorials

Photosynthesis - Green plants absorb water and carbon dioxide from the environment, and utilizing energy from the sun, turn these simple substances into glucose and oxygen. With glucose as a basic building block, plants synthesize a number of complex carbon-based biochemicals used to grow and sustain life. This process is termed **photosynthesis**, and is the cornerstone of life on Earth. The tutorial demonstrates the basic molecular steps in the photosynthetic process.

Solar Cell Operation - Solar cells convert light energy into electrical energy either indirectly by first converting it into heat, or through a direct process known as the **photovoltaic effect**. The most common types of solar cells are based on the photovoltaic effect, which occurs when light falling on a two-layer semiconductor material produces a potential difference, or voltage, between the two layers. The voltage produced in the cell is capable of driving a current through an external electrical circuit that can be utilized to power electrical devices. This tutorial explores the basic concepts behind solar cell operation.

Incandescent Lamp Filaments - Nearly every source of light depends, at the fundamental level, on the release of energy from atoms that have been excited in some manner. Standard incandescent lamps, derived directly from the early models of the 1800s, now commonly utilize a tungsten filament in an inert gas atmosphere, and produce light through the resistive effect that occurs when the filament temperature increases as electrical current is passed through. This interactive tutorial demonstrates the sub-atomic activity within a conducting incandescent lamp filament that results in resistance to current flow, and ultimately leads to the emission of infrared and visible light photons (with a corresponding rise in color temperature).

Hydrogen Fuel Cell Basics - Fuel cells are designed to utilize a catalyst, such as platinum, to convert a mixture of hydrogen and oxygen into water. An important byproduct of this chemical reaction is the electricity generated when hydrogen molecules interact (through oxidation) with the anode to produce protons and electrons. This interactive tutorial explores the major steps in fuel cell operation.

Interaction of Photons with Silicon - In a charge-coupled device (CCD) incident light must first pass through a silicon nitride passivation coating as well as several thin films of silicon dioxide and polysilicon gate structures before being absorbed into the silicon substrate. This interactive tutorial explores the interaction of photons with silicon as a function of wavelength.

Building A Charge-Coupled Device - Explore the steps utilized in the construction of a charge-coupled device (CCD) as a portion of an individual pixel gate is fabricated on a silicon wafer simultaneously with thousands or even millions of neighboring elements. The interactive tutorial examines and illustrates each individual stage in the fabrication of the CCD photodiode sensor element.

Full-Frame CCD Operation - Full-frame charge-coupled devices (CCDs) feature high-density pixel arrays capable of producing digital images with the highest resolution currently available. This popular CCD architecture has been widely adopted due to the simple design, reliability, and ease of fabrication.

Photomultiplier Tube Operation - In the end-on photomultiplier tube design, photons impact an internal photocathode and transfer their energy to electrons, which then proceed through a chain of electron multipliers termed **dynodes** ending in the **anode**. This tutorial explores how electrons are generated by the photocathode and amplified by passing through a dynode chain.

Selected Literature References

Reference Listing - Gathered from our vast library of literature on optical microscopy, the listed reference materials are an excellent source of additional information on the topic of light and energy and their interrelationships. Included in this section are references to books, select book chapters, and review articles that discuss various aspects of the theory and applications regarding how light is converted into energy and vice versa.

Lenses and Geometrical Optics

The action of a simple lens, similar to many of those used in the microscope, is governed by the principles of refraction and reflection and can be understood with the aid of a few simple rules about the geometry involved in tracing light rays through the lens. The basic concepts explored in this discussion, which are derived from the science of **Geometrical Optics**, will lead to an understanding of the magnification process, the properties of real and virtual images, and lens **aberrations** or defects.

Introduction to Lenses and Geometrical Optics - The term **lens** is the common name given to a component of glass or transparent plastic material, usually circular in diameter, which has two primary surfaces that are ground and polished in a specific manner designed to produce either a **convergence** or **divergence** of light passing through the material. The optical microscope forms an image of a specimen placed on the stage by passing light from the illuminator through a series of glass lenses and focusing this light either into the eyepieces, on the film plane in a traditional camera system, or onto the surface of a digital image sensor.

Common Aberrations in Lens Systems - Microscopes and other optical instruments are commonly plagued by lens errors that distort the image by a variety of mechanisms associated with defects (commonly referred to as aberrations) resulting from the spherical geometry of lens surfaces. There are three primary sources of non-ideal lens action (errors) that are observed in the microscope. Of the three major classes of lens errors, two are associated with the orientation of wavefronts and focal planes with respect to the microscope optical axis. These include **on-axis** lens errors such as **chromatic** and **spherical** aberration, and the major **off-axis** errors manifested as **coma**, **astigmatism**, and **field curvature**. A third class of aberrations, commonly seen in stereomicroscopes that have zoom lens systems, is geometrical distortion, which includes both **barrel** distortion and **pincushion** distortion.

Lens Interactive Java Tutorials

Simple Bi-Convex Thin Lenses - A simple thin lens has two **focal** planes that are defined by the geometry of the lens and the relationship between the lens and the focused image. Light rays passing through the lens will intersect and are physically combined at the focal plane, while extensions of the rays passing through the lens will intersect with the rays emerging from the lens at the principal plane. The focal length of a lens is defined as the distance between the principal plane and the focal plane, and every lens has a set of these planes on each side (front and rear). This interactive tutorial explores how changes to focal length and object size affect the size and position of the image formed by a simple thin lens.

Simple Magnification - A typical magnifying glass consists of a single thin bi-convex lens that produces a modest magnification in the range of 1.5x to 30x, with the most common being about 2-4x for reading or studying rocks, stamps, coins, insects, and leaves. Magnifying glasses produce a virtual image that is magnified and upright. This interactive tutorial demonstrates how a simple, thin bi-convex magnifying lens works to produce a magnified virtual image on the retina.

Magnification with a Bi-Convex Lens - Single lenses capable of forming images (like the bi-convex lens) are useful in tools designed for simple magnification applications, such as magnifying glasses, eyeglasses, single-lens cameras, loupes, viewfinders, and contact lenses. This interactive tutorial explores how a simple bi-convex lens can be used to magnify an image.

Image Formation with Converging Lenses - Positive, or converging, thin lenses unite incident light rays that are parallel to the optical axis and focus them at the focal plane to form a real image. This interactive tutorial utilizes ray traces to explore how images are formed by the three primary types of converging lenses, and the relationship between the object and the image formed by the lens as a function of distance between the object and the focal points.

Image Formation with Diverging Lenses - Negative lenses diverge parallel incident light rays and form a virtual image by extending traces of the light rays passing through the lens to a focal point behind the lens. In general, these lenses have at least one concave surface and are thinner in the center than at the edges. This interactive tutorial utilizes ray traces to explore how images are formed by the three primary types of diverging lenses, and the relationship between the object and the image formed by the lens as a function of distance between the object and the focal points.

Geometrical Construction of Ray Diagrams - A popular method of representing a train of propagating light waves involves the application of geometrical optics to determine the size and location of images formed by a lens or multi-lens system. This tutorial explores how two representative light rays can establish the parameters of an imaging scenario.

Perfect Lens Characteristics - The simplest imaging element in an optical microscope is a perfect lens, which is an ideally corrected glass element that is free of aberration and focuses light onto a single point. This tutorial explores how light waves propagate through and are focused by a perfect lens.

Perfect Two-Lens System Characteristics - During investigations of a point source of light that does not lie in the focal plane of a lens, it is often convenient to represent a perfect lens as a system composed of two individual lens elements. This tutorial explores off-axis oblique light rays passing through such a system.

Radius and Refractive Index Effects on Lens Action - The action of a simple bi-convex thin lens is governed by the principles of refraction (which is a function of lens curvature radius and refractive index), and can be understood with the aid of a few simple rules about the geometry involved in tracing light rays through the lens. This interactive tutorial explores how variations in the refractive index and radius of a bi-convex lens affect the relationship between the object and the image produced by the lens.

Optical Aberration Interactive Java Tutorials

Astigmatism - Astigmatism aberrations are similar to comatic aberrations, however these artifacts are not as sensitive to aperture size and depend more strongly on the oblique angle of the light beam. The aberration is manifested by the off-axis image of a specimen point appearing as a line or ellipse instead of a point. Depending on the angle of the off-axis rays entering the lens, the line image may be oriented in either of two different directions, tangentially (meridionally) or sagittally (equatorially). The intensity ratio of the unit image will diminish, with definition, detail, and contrast being lost as the distance from the center is increased.

Chromatic Aberration - Chromatic aberrations are wavelength-dependent artifacts that occur because the refractive index of every optical glass formulation varies with wavelength. When white light passes through a simple or complex lens system, the component wavelengths are refracted according to their frequency. In most glasses, the refractive index is greater for shorter (blue) wavelengths and changes at a more rapid rate as the wavelength is decreased.

Comatic Aberration - Comatic aberrations are similar to spherical aberrations, but they are mainly encountered with off-axis light fluxes and are most severe when the microscope is out of alignment. When these aberrations occur, the image of a point is focused at sequentially differing heights producing a series of asymmetrical spot shapes of increasing size that result in a comet-like (hence, the term coma) shape to the Airy pattern.

Curvature of Field - Modern microscopes deal with field curvature by correcting this aberration using specially designed objectives. These specially-corrected objectives have been named **plan** or **plano** (for flat-field) and are the most common type of objective in use today, providing ocular fields ranging between 18 and 26 millimeters, which exhibit sharp detail from center to edge.

Geometrical Distortion - Distortion is an aberration commonly seen in stereoscopic microscopy, which is manifested by changes in the shape of an image rather than the sharpness or color spectrum. The two most prevalent types of distortion, positive and negative (often termed pincushion and barrel, respectively), can often be present in very sharp images that are otherwise corrected for spherical, chromatic, comatic, and astigmatic aberrations. In this case, the true geometry of an object is no longer maintained in the image.

Spherical Aberration - The most serious of the **monochromatic defects** that occurs with microscope objectives, spherical aberration, causes the specimen image to appear hazy or blurred and slightly out of focus. The effect of spherical aberration manifests itself in two ways: the center remains more in focus than the edges of the image and the intensity of the edges falls relative to that of the center. This defect appears in both on-axis and off-axis image points.

Basic Properties of Mirrors

Reflection of light is an inherent and important fundamental property of mirrors, and is quantitatively gauged by the ratio between the amount of light reflected from the surface and that incident upon the surface, a term known as **reflectivity**. Mirrors of different design and construction vary widely in their reflectivity, from nearly 100 percent for highly-polished mirrors coated with metals that reflect visible and infrared wavelengths, to nearly zero for strongly absorbing materials.

The images formed by a mirror are either **real** or **virtual**, depending upon the proximity of the object to the mirror, and can be accurately predicted with respect to size and location from calculations based on the geometry of any particular mirror. Real images are formed when the incident and reflected rays intersect in front of the mirror, whereas virtual images occur at points where extensions from incident and reflected rays converge behind the mirror. Planar (flat) mirrors produce virtual images because the focal point, at which extensions from all incident light rays intersect, is positioned behind the reflective surface.

Introduction to Mirrors - In order to reflect light waves with high efficiency, the surface of a mirror must be perfectly smooth over a long range, with imperfections that are much smaller than the wavelength of light being reflected. This requirement applies regardless of the shape of the mirror, which can be irregular or curved, in addition to the planar mirror surfaces commonly seen in households. Curved mirrors are roughly divided into two categories, **concave** and **convex**, terms that are also used to describe the geometry of simple thin lenses. With mirrors, the curved surface is referred to as either concave or convex depending upon whether the center of curvature occurs on the side of the reflecting surface or the opposite side.

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Concave Spherical Mirrors - Concave mirrors have a curved surface with a center of curvature equidistant from every point on the mirror's surface. An object beyond the center of curvature forms a real and inverted image between the focal point and the center of curvature. This interactive tutorial explores how moving the object farther away from the center of curvature affects the size of the real image formed by the mirror. Also examined in the tutorial are the effects of moving the object closer to the mirror, first between the center of curvature and the focal point, and then between the focal point and the mirror surface (to form a virtual image).

Concave Spherical Mirrors (3-Dimensional Version) - Concave mirrors have a curved surface with a center of curvature equidistant from every point on the mirror's surface. An object beyond the center of curvature forms a real and inverted image between the focal point and the center of curvature. This interactive tutorial explores how moving the object farther away from the center of curvature affects the size of the real image formed by the mirror.

Convex Spherical Mirrors - Regardless of the position of the object reflected by a convex mirror, the image formed is always virtual, upright, and reduced in size. This interactive tutorial explores how moving the object farther away from the mirror's surface affects the size of the virtual image formed behind the mirror.

Convex Spherical Mirrors (3-Dimensional Version) - Regardless of the position of the object reflected by a convex mirror, the image formed is always virtual, upright, and reduced in size. This interactive tutorial explores how moving the object farther away from the mirror's surface affects the size of the virtual image formed behind the mirror. This tutorial utilizes three-dimensional graphics.

Selected Literature References

Reference Listing - This selection of review articles on the basic properties of mirrors reflects their importance in understanding the physics of light and color. The reference section contains book location information for these articles, as well as providing a listing of the appropriate chapter titles dealing with mirror systems.

Prisms and Beamsplitters

Beamsplitters and prisms are not only found in a wide variety of common optical instruments, such as cameras, binoculars, microscopes, telescopes, periscopes, range finders, and surveying equipment, but also in many sophisticated scientific instruments including interferometers, spectrophotometers, and fluorimeters. Both of these important optical tools are critical for laser applications that require tight control of beam direction to precise tolerances with a minimum of light loss due to scatter or unwanted reflections. Illustrated in Figure 1 is a diagram of a typical binocular microscope observation tube configuration. In order to divert light collected by the objective into both eyepieces, it is first divided by a beamsplitter and then channeled through reflecting prisms into parallel cylindrical optical light pipes. Thus, the binocular observation tube utilizes both prism and beamsplitter technology to direct beams of light having equal intensity into the eyepieces.

Introduction - Prisms and beamsplitters are essential components that bend, split, reflect, and fold light through the pathways of both simple and sophisticated optical systems. Cut and ground to specific tolerances and exact angles, prisms are polished blocks of glass or other transparent materials that can be employed to deflect or deviate a light beam, rotate or invert an image, separate polarization states, or disperse light into its component wavelengths. Many prism designs can perform more than one function, which often includes changing the line of sight and simultaneously shortening the optical path, thus reducing the size of optical instruments.

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Common Reflecting Prisms - The angular parameters displayed by various prism designs cover a wide gamut of geometries that dramatically extend the usefulness of prisms as strategic optical components. Reflecting prisms are often designed to be located in specific orientations where the entrance and exit faces are both parallel and perpendicular to the optical axis. This interactive tutorial explores image deviation, rotation, and displacement exhibited by common reflecting prisms.

Right-Angle Prisms - The right-angle prism possesses the simple geometry of a 45-degree right triangle, and is one of the most commonly used prisms for redirecting light and rotating images. This interactive tutorial explores light reflection and image rotation, inversion, and reversion by a right-angle prism as a function of the prism orientation with respect to incident light.

Refraction by an Equilateral Prism - Visible white light passing through an equilateral prism undergoes a phenomenon known as **dispersion**, which is manifested by wavelength-dependent refraction of the light waves. This interactive tutorial explores how the incident angle of white light entering the prism affects the degree of dispersion and the angles of light exiting the prism.

Transmission and Reflection by Beamsplitters - A beamsplitter is a common optical component that partially transmits and partially reflects an incident light beam, usually in unequal proportions. In addition to the task of dividing light, beamsplitters can be employed to recombine two separate light beams or images into a single path. This interactive tutorial explores transmission and reflection of a light beam by three common beamsplitter designs.

Dielectric Plate Beamsplitters - The simplest configuration for a beamsplitter is an uncoated flat glass plate (such as a microscope slide), which has an average surface reflectance of about 4 percent. When placed at a 45-degree angle, the plate will transmit most of the light, but reflect a small amount at a 90-degree angle to the incident beam. **Plate** beamsplitters are, as the name implies, optical crown glass plates having a partially silvered coating designed to produce a desired transmission-to-reflection ratio. These ratios usually vary between 50:50 and 20:80, depending upon the application.

Beam Steering by Wedge Prisms - Circular prisms having plane surfaces positioned at slight angles with respect to each other are termed **optical wedges**, and deflect light by refraction rather than reflection. Although wedges are prismatic in nature, they can be manipulated to act as beamsplitters or **beam steerers**. This interactive tutorial explores how two wedge prisms operate together to deflect an incident light beam.

Birefringent Polarizing Prisms - Polarizing prisms are utilized in a wide spectrum of applications ranging from optical microscopy and spectroscopy to complex laser systems. This interactive tutorial explores how various common birefringent polarizing prisms operate to split light waves into ordinary and extraordinary components.

Selected Literature References

Selected References - A number of high-quality review articles on prisms and beamsplitters have been published by leading investigators in the fields of optics and photonics. This section contains periodical and book location information about these articles, as well as providing a listing of the chapter titles for appropriate sections dealing with beamsplitters and prism systems.

Laser Fundamentals

In a few decades since the 1960s, the laser has gone from being a science fiction fantasy, to a laboratory research curiosity, to an expensive but valuable tool in esoteric scientific applications, to its current role as an integral part of everyday tasks as mundane as reading grocery prices or measuring a room for wallpaper. Any substantial list of the major technological achievements of the twentieth century would include the laser near the top. The pervasiveness of the laser in all areas of current life can be best appreciated by the range of applications that utilize laser technology. At the spectacular end of this range are military applications, which include using lasers as weapons to possibly defend against missile attack, and at the other end are daily activities such as playing music on compact disks and printing or copying paper documents. Somewhere in between are numerous scientific and industrial applications, including microscopy, astronomy, spectroscopy, surgery, integrated circuit fabrication, surveying, and communications.

Introduction to Lasers - Ordinary natural and artificial light is released by energy changes on the atomic and molecular level that occur without any outside intervention. A second type of light exists, however, and occurs when an atom or molecule retains its excess energy until **stimulated** to emit the energy in the form of light. Lasers are designed to produce and amplify this stimulated form of light into intense and focused beams. The word laser was coined as an acronym for **L**ight **A**mplification by the **S**timulated **E**mission of **R**adiation. The special nature of laser light has made laser technology a vital tool in nearly every aspect of everyday life including communications, entertainment, manufacturing, and medicine.

Laser Systems for Optical Microscopy - The lasers commonly employed in optical microscopy are high-intensity monochromatic light sources, which are useful as tools for a variety of techniques including optical trapping, lifetime imaging studies, photobleaching recovery, and total internal reflection fluorescence. In addition, lasers are also the most common light source for scanning confocal fluorescence microscopy, and have been utilized, although less frequently, in conventional widefield fluorescence investigations.

Laser Safety - The two major concerns in safe laser operation are exposure to the beam and the electrical hazards associated with high voltages within the laser and its power supply. While there are no known cases of a laser beam contributing to a person's death, there have been several instances of deaths attributable to contact with high voltage laser-related components. Beams of sufficiently high power can burn the skin, or in some cases create a hazard by burning or damaging other materials, but the primary concern with regard to the laser beam is potential damage to the eyes, which are the part of the body most sensitive to light.

John Kerr (1824-1907) - John Kerr was a Scottish physicist who discovered the electro-optic effect that bears his name and invented the **Kerr cell**. Pulses of light can be controlled so quickly with a modern Kerr cell that the devices are often used as high-speed shutter systems for photography and are sometimes alternately known as **Kerr electro-optical shutters**. In addition, Kerr cells have been used to measure the speed of light, are incorporated in some lasers, and are becoming increasingly common in telecommunications devices.

Theodore Harold Maiman (1927-Present) - Theodore Maiman is best remembered for constructing the world's first laser, a device that has transcended the field of optics to find a diversity of applications in the modern world. In May of 1960, Maiman built his prototype laser using a synthetic ruby rod silvered at both ends to reflect light. Small enough to be held in the palm of the hand, when the atoms in the rod were excited by an intense beam of light from a xenon lamp, a release of energy was initiated and an internal chain reaction occurred that caused the energy to bounce back and forth within the rod. When the energy built up to a certain level, it escaped from one end of the ruby rod to form an intense beam of monochromatic light centered at 694.3 nanometers.

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Laser Cavity Resonance Modes and Gain Bandwidth - In a typical laser, the number of cavity resonances that can fit within the gain bandwidth is often plotted as a function of laser output power versus wavelength. This interactive tutorial explores how varying the appropriate frequencies can alter curves describing the number of cavity modes and gain bandwidth of a laser.

Laser Energy Levels - A population inversion can be produced through two basic mechanisms, either by creating an excess of atoms or molecules in a higher energy state, or by reducing the population of a lower energy state. This tutorial explores metastable states for both three-level and four-level laser systems.

Spontaneous and Stimulated Processes - One of the most important concepts necessary in understanding laser operation is the fact that quantization of energy in the atom results in discrete energy levels. In addition, transitions from one energy level to another must be possible in order for light emission to occur, and these transitions include both spontaneous and stimulated emission. This tutorial explores the concepts of spontaneous absorption and emission, as well as stimulated emission.

Stimulated Emission in a Laser Cavity - The amplification of light by stimulated emission is a fundamental concept in the basic understanding of laser action. This interactive tutorial explores how laser amplification occurs starting from spontaneous emission of the first photon to saturation of the laser cavity and the establishment of a formal equilibrium state.

Argon-Ion Lasers - As a distinguished member of the common and well-explored family of **ion lasers**, the argon-ion laser operates in the visible and ultraviolet spectral regions by utilizing an ionized species of the noble gas argon. Argon-ion lasers function in continuous wave mode when plasma electrons within the gaseous discharge collide with the excited laser species to produce light.

Diode Lasers - Semiconductor diode lasers having sufficient power output to be useful in optical microscopy are now available from a host of manufacturers. In general these devices operate in the infrared region, but newer diode lasers operating at specific visible wavelengths are now available. Diode lasers coupled to internal optical systems that improve beam shape have sufficient power and stability to rival helium-neon lasers in many applications. This interactive tutorial explores the properties of typical diode lasers and how specialized anamorphic prisms can be utilized for beam expansion.

Helium-Neon Lasers - Helium-neon lasers are among the most widely utilized laser systems for a broad range of biomedical and industrial applications, and display the most superior Gaussian beam quality of any laser. These lasers are readily available at relatively low cost, have compact size dimensions, and exhibit a long operating life (often reaching 40,000 to 50,000 hours). The low power requirements, superior beam quality (virtually a pure Gaussian profile), and simple cooling requirements (convection) make helium-neon lasers the choice system for many confocal microscopes.

Helium-Cadmium Lasers - Helium-cadmium (**He**/**Cd**) lasers are finding an increasing number of important applications in confocal microscopy due to their three primary emission spectral lines (322, 354, and 442 nanometers) in the ultraviolet and blue-violet regions. The shortest wavelength (322 nanometers) requires specialized ultraviolet transparent optics and is seldom used in microscopy, but membrane probes (such as indo-1 and fura-2) can be efficiently excited with the 354-nanometer line. The blue-violet spectral line is useful for a host of common fluorophores and fluorescent proteins in single, double, or triple labeling experiments. This interactive tutorial explores a simplified model of the helium-cadmium laser cavity operation.

Krypton-Argon Lasers - Air-cooled lasers using krypton-argon mixtures have become popular in confocal microscopy when several illumination wavelengths are required for dual or multiple-fluorophore studies. Such **mixed-gas** lasers are only capable of producing stable output on major lines that are well separated in the wavelength spectrum. Of the three laser lines typically utilized for confocal microscopy, the 488-nanometer and 568-nanometer lines have approximately equal power (10 to 15 milliwatts), while the 647-nanometer line has about 50 percent more (15 to 25 milliwatts). This interactive tutorial simulates the three major spectral lines produced by an krypton-argon mixed-gas laser.

Ti:Sapphire Mode-Locked Lasers - The self mode-locked Ti:sapphire pulsed laser is currently one of the preferred laser excitation sources in a majority of multiphoton fluorescence microscopy investigations. This tutorial explores the operation of Ti:sapphire lasers over a broad range of near-infrared wavelengths with variable pulse widths and an adjustable applet speed control.

Nd:YLF Mode-Locked Pulsed Lasers - An increasing number of applications, including new illumination techniques in fluorescence optical microscopy, require a reliable high average-power laser source that enables efficient frequency conversion to ultra violet and visible wavelengths. Several variants of the diode-pumped solid state laser have been developed, and of these, the Nd:YLF (neodymium: yttrium lithium fluoride) laser produces the highest pulse energy and average power in the repetition rate ranging from a single pulse up to approximately 6 kHz. This tutorial explores the operation of a Nd:YLF multi-pass slab laser side-pumped by two collimated diode-laser bars.

Pockels Cell Laser Modulators - All lasers are susceptible to noise introduced by their power supplies. **Switching** power supplies, which have become common because of their efficiency and small size, are particularly likely to introduce laser system ripple at frequencies ranging into the tens of kilohertz. Such interference, when it affects the light beam in confocal microscopy systems, can be especially troublesome to diagnose and remove. The beam intensity of continuous wave lasers can be stabilized by either electronic control of the tube current or through utilization of external components that modulate the light intensity. This interactive tutorial examines how the Pockels cell modulator operates to stabilize laser beam intensity.

Compact Disk Lasers - A pre-recorded compact disk is read by tracking a finely focused laser across the spiral pattern of lands and pits stamped into the disk by a master diskette. This tutorial explores how the laser beam is focused onto the surface of a spinning compact disk, and how variations between the height of pits and lands determine whether the light is scattered by the disk surface or reflected back into a detector.

Acousto-Optic Tunable Filters - Wavelength selection is of fundamental importance in many arenas of the optical sciences, including fluorescence spectroscopy and confocal microscopy. Electro-optic devices, such as the acousto-optic tunable filter (**AOTF**), are increasingly being employed to modulate the wavelength and amplitude of illuminating laser light in the latest generation of confocal microscopes. These filters do not suffer from the mechanical constraints, speed limitations, image shift, and vibration associated with rotating filter wheels, and can easily accommodate several laser systems tuned to different output wavelengths. In addition, acousto-optic filters do not deteriorate when exposed to heat and intense light as do fluorescence interference filters.

Selected Literature References

Reference Listing - Lasers have emerged from advanced research laboratories and military arsenals into our everyday lives as the technology advances and the fabrication costs decline. Leading researchers in the field have published a number of high-quality books and review articles on laser fundamentals and applications. This section contains a listing of selected books and book chapters from this cutting-edge field of research.