

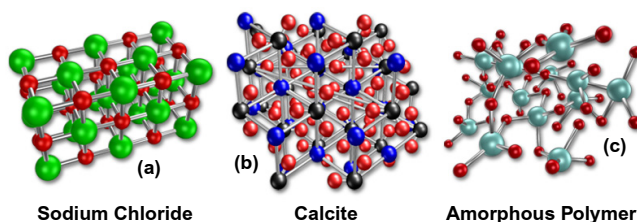


Principles of Birefringence

Introduction to Optical Birefringence

Birefringence is formally defined as the double refraction of light in a transparent, molecularly ordered material, which is manifested by 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 (sodium chloride, illustrated in **Figure 1(a)**), many polymers, and a wide variety of both organic and inorganic compounds.

Figure 1 - Crystalline Structure of Isotropic and Anisotropic Materials



The simplest crystalline lattice structure is cubic, as illustrated by the molecular model of sodium chloride in **Figure 1(a)**, an arrangement where all of the sodium and chloride ions are ordered with uniform spacing along three mutually perpendicular axes. Each chloride ion is surrounded by (and electrostatically bonded to) six individual sodium ions and vice versa for the sodium ions. The lattice structure illustrated in **Figure 1(b)** represents the mineral calcite (calcium carbonate), which consists of a rather complex, but highly ordered three-dimensional array of calcium and carbonate ions. Calcite has an anisotropic crystalline lattice structure that interacts with light in a totally different manner than isotropic crystals. The polymer illustrated in **Figure 1(c)** is amorphous and devoid of any recognizable periodic crystalline structure. Polymers often possess some degree of crystalline order and may or may not be optically transparent.

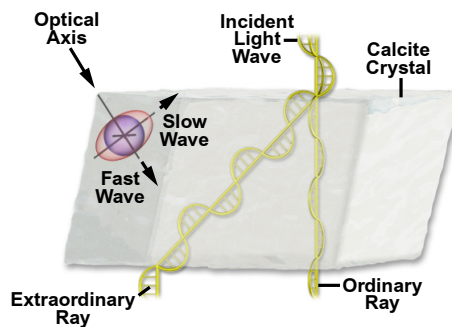
Crystals are classified as being either isotropic or anisotropic depending upon their optical behavior and whether or not their crystallographic axes are equivalent. All isotropic crystals have equivalent axes that interact with light in a similar manner, regardless of the crystal orientation with respect 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 results 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, as mentioned above, isotropic properties remain symmetrical, regardless of the direction of measurement, with each type of probe reporting identical results.

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 (mutually perpendicular) 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.

Electromagnetic radiation propagates through space with oscillating electric and magnetic field vectors alternating in sinusoidal patterns that are perpendicular to one another and to the direction of wave propagation. Because visible light is composed of both electrical and magnetic components, the velocity of light through a substance is partially dependent upon the electrical conductivity of the material. Light waves passing through a transparent crystal must interact with localized electrical fields during their journey. The relative speed at which electrical signals travel through a material varies with the type of signal and its interaction with the electronic structure, and is determined by a property referred to as the **dielectric constant** of the material. The vectorial relationship defining the interaction between a light wave and a crystal through which it passes is governed by the inherent orientation of lattice electrical vectors and the direction of the wave's electric vector component. Therefore, a careful consideration of the electrical properties of an anisotropic material is fundamental to the understanding of how a light wave interacts with the material as it propagates through.

Figure 2 - Light Path Through a Calcite Crystal



The phenomenon of double refraction is based on the laws of electromagnetism, first proposed by British mathematician James Clerk Maxwell in the 1860s. His elaborate series of equations demonstrate that the velocity of light through a material equals the speed of light in a vacuum (c) divided by the product of the square root of the material's dielectric constant (ϵ) multiplied by the magnetic permeability (m) of the medium. In general, biological and related materials have a magnetic permeability very near 1.0, as do many conducting and non-conducting specimens of interest to the microscopist. The dielectric constant of a material is therefore related to the refractive index through a simple equation:

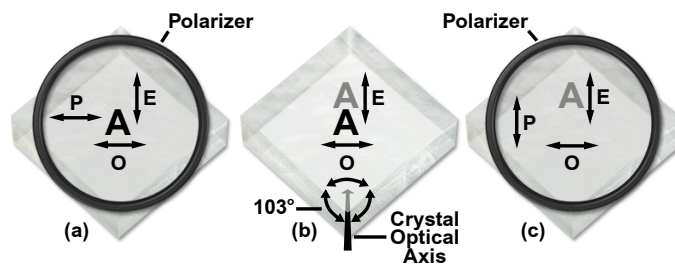
$$\epsilon = n^2$$

where ϵ is a variable representing the dielectric constant, and n is the material's measured refractive index. This equation was derived for specific frequencies of light and ignores dispersion of polychromatic light as it passes through the material. Anisotropic crystals are composed of complex molecular and atomic lattice orientations that have varying electrical properties depending upon the direction from which they are being probed. As a result, the refractive index also varies with direction when light passes through an anisotropic crystal, giving rise to direction-specific trajectories and velocities.

Perhaps one of the most dramatic demonstrations of double refraction occurs with calcium carbonate (calcite) crystals, as illustrated in **Figure 2**. The rhombohedral cleavage block of calcite produces two images when it is placed over an object, and then viewed with reflected light passing through the crystal. One of the images appears as would normally be expected when observing an object through clear glass or an isotropic crystal, while the other image appears slightly displaced, due to the nature of doubly-refracted light. When anisotropic crystals refract light, they split the incoming rays into two components that take different paths during their journey through the crystal and emerge as separate light rays. This unusual behavior, as discussed above, is attributed to the arrangement of atoms in the crystalline lattice. Because the precise geometrical ordering of the atoms is not symmetrical with respect to the crystalline axes, light rays passing through the crystal can experience different refractive indices, depending upon the direction of propagation.

One of the rays passing through an anisotropic crystal obeys the laws of normal refraction, and travels with the same velocity in every direction through the crystal. This light ray is termed the **ordinary** ray. The other ray travels with a velocity that is dependent upon the propagation direction within the crystal, and is termed the **extraordinary** ray. Therefore, each light ray entering the crystal is split into an ordinary and an extraordinary ray that emerge from the distant end of the crystal as linearly polarized rays having their electric field vectors vibrating in planes that are mutually perpendicular.

Figure 3 - Birefringent Calcite Crystal Electric Vector Orientations



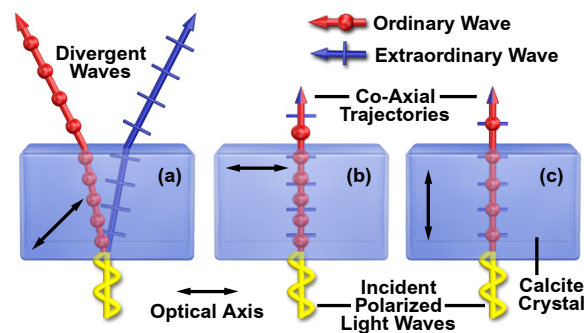
These phenomena are illustrated in **Figures 2 through 4**. The calcite crystal presented in **Figure 3(b)** is positioned over the capital letter **A** on a white sheet of paper demonstrating a double image observed through the crystal. If the crystal were to be slowly rotated around the letter, one of the images of the letter will remain stationary, while the other precesses in a 360-degree circular orbit around the first. The orientation of the electric vector vibration planes for both the ordinary (**O**) and extraordinary (**E**) rays are indicated by lines with doubled arrows in **Figure 3(b)**. Note that these axes are perpendicular to each other. The crystal optical axis, which makes an equal angle (103 degrees) with all three crystal faces joined at the corner, is also indicated at the lower portion of the crystal. The degree of birefringence in calcite is so pronounced that the images of the letter **A** formed by the ordinary and extraordinary rays are completely separated. This high level of birefringence is not observed in all anisotropic crystals.

Transparent dichroic polarizers can be utilized to determine the electric vector directions for the extraordinary and ordinary rays in a calcite crystal, as presented in **Figures 3(a) and Figure 3(c)**. When the polarizer is oriented so that all light waves having electric vectors oriented in the horizontal direction are transmitted (**Figure 3(a)**), waves having similar vectors in the vertical direction are absorbed, and vice versa (**Figure 3(c)**). In the calcite crystal presented in **Figure 3**, the extraordinary ray has a vertical electric vector vibration angle, which is absorbed when the polarizer is oriented in a horizontal direction (**Figure 3(a)**). In this case, only light from the ordinary ray is passed through the polarizer and its corresponding image of the letter **A** is the only one observed. In contrast, when the polarizer is turned so that the vibration transmission direction is oriented vertically (**Figure 3(c)**), the ordinary ray is blocked and the image of the letter **A** produced by the extraordinary ray is the only one visible.

In **Figure 3**, the incident light rays giving rise to the ordinary and extraordinary rays enter the crystal in a direction that is oblique with respect to the optical axis, and are responsible for the observed birefringent character. The behavior of an anisotropic crystal is different, however, if the incident light enters the crystal in a direction that is either parallel or perpendicular to the optical axis, as presented in **Figure 4**. When an incident ray enters the crystal perpendicular to the optical axis, it is separated into ordinary and extraordinary rays, as described above, but instead of taking different pathways, the trajectories of these rays are coincident. Even though the ordinary and extraordinary rays emerge from the crystal at the same location, they exhibit different optical path lengths and are subsequently shifted in phase relative to one another (**Figure 4(b)**). The two cases just described are illustrated in **Figure 4(a)**, for the oblique case (see **Figures 2 and 3**), and **Figure 4(b)** for the situation where incident light is perpendicular to the optical axis of a birefringent crystal.

In the case where incident light rays impact the crystal in a direction that is parallel to the optical axis (**Figure 4(c)**), they behave as ordinary light rays and are not separated into individual components by an anisotropic birefringent crystal. Calcite and other anisotropic crystals act as if they were isotropic materials (such as glass) under these circumstances. The optical path lengths of the light rays emerging from the crystal are identical, and there is no relative phase shift.

Figure 4 - Separation of Light Waves by a Birefringent Crystal



Although it is common to interchangeably use the terms double refraction and birefringence to indicate the ability of an anisotropic crystal to separate incident light into ordinary and extraordinary rays, these phenomena actually refer to different manifestations of the same process. The actual division of a light ray into two visible species, each refracting at a different angle, is the process of double refraction. In contrast, birefringence refers to the physical origin of the separation, which is the existence of a variation in refractive index that is sensitive to direction in a geometrically ordered material. The difference in refractive index, or

birefringence, between the extraordinary and ordinary rays traveling through an anisotropic crystal is a measurable quantity, and can be expressed as an absolute value by the equation:

$$\text{Birefringence (B)} = |n_e - n_o|$$

where $n(e)$ and $n(o)$ are the refractive indices experienced by the extraordinary and ordinary rays, respectively. This expression holds true for any part or fragment of an anisotropic crystal with the exception of light waves propagated along the optical axis of the crystal. Because the refractive index values for each component can vary, the absolute value of this difference can determine the total amount of birefringence, but the **sign of birefringence** will be either a negative or positive value. A determination of the birefringence sign by analytical methods is utilized to segregate anisotropic specimens into categories, which are termed either positively or negatively birefringent. The birefringence of a specimen is not a fixed value, but will vary with the orientation of the crystal relative to the incident angle of the illumination.

The **optical path difference** is a classical optical concept related to birefringence, and both are defined by the relative phase shift between the ordinary and extraordinary rays as they emerge from an anisotropic material. In general, the optical path difference is computed by multiplying the specimen thickness by the refractive index, but only when the medium is homogeneous and does not contain significant refractive index deviations or gradients. This quantity, as well as the value of birefringence, is usually expressed in nanometers and grows larger with increasing specimen thickness. For a system with two refractive index values ($n(1)$ and $n(2)$), the optical path difference (D) is determined from the equation:

$$\text{Optical Path Difference } D = (n_1 - n_2) \cdot t \text{ (Thickness)}$$

In order to consider the phase relationship and velocity difference between the ordinary and extraordinary rays after they pass through a birefringent crystal, a quantity referred to as the **relative retardation** is often determined. As mentioned above, the two light rays are oriented so that they are vibrating at right angles to each other. Each ray will encounter a slightly different electrical environment (refractive index) as it enters the crystal and this will affect the velocity at which the ray passes through the crystal. Because of the difference in refractive indices, one ray will pass through the crystal at a slower rate than the other ray. In other words, the velocity of the slower ray will be **retarded** with respect to the faster ray. This retardation value (the relative retardation) can be quantitatively determined using the following equation:

$$\text{Retardation } (I) = \text{Thickness } (t) \times \text{Birefringence } (B)$$

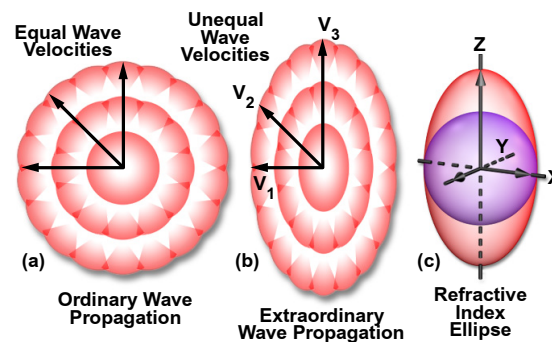
or

$$\Gamma = t \cdot |n_e - n_o|$$

Where Γ is the quantitative retardation of the material, t is the thickness of the birefringent crystal (or material) and B is the measured birefringence, as defined above. Factors contributing to the value of retardation are the magnitude of the difference in refractive indices for the environments seen by the ordinary and extraordinary rays, and also the specimen thickness. Obviously, the greater the thickness or difference in refractive indices, the greater the degree of retardation between waves. Early observations made on the mineral calcite indicated that thicker calcite crystals caused greater differences in splitting of the images seen through the crystals, such as those illustrated in [Figure 3](#). This observation agrees with the equation above, which indicates retardation will increase with crystal (or sample) thickness.

The behavior of an ordinary light ray in a birefringent crystal can be described in terms of a spherical wavefront based on the Huygens' principle of wavelets emanating from a point source of light in a homogeneous medium (as illustrated in [Figure 5](#)). The propagation of these waves through an isotropic crystal occurs at constant velocity because the refractive index experienced by the waves is uniform in all directions ([Figure 5\(a\)](#)). In contrast, the expanding wavefront of extraordinary waves, which encounter refractive index variations as a function of direction (see [Figure 5\(b\)](#)), can be described by the surface of an ellipsoid of revolution.

Figure 5 - Wavefront Propagation in Anisotropic Crystals



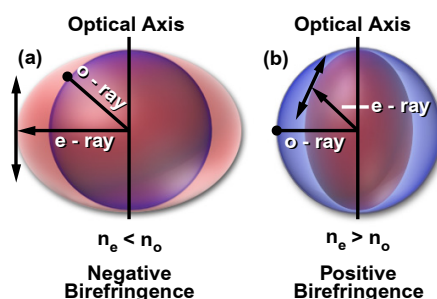
The upper and lower limits of extraordinary wave velocities are defined by the long and short axes of the ellipsoid ([Figure 5\(c\)](#)). The wavefront reaches its highest velocity when propagating in the direction parallel to the long axis of the ellipsoid, which is referred to as the **fast** axis. On the other hand, the slowest wavefronts occur when the wave travels along the short axis of the ellipsoid. This axis is termed the **slow** axis. Between these two extremes, wavefronts traveling in other directions experience a gradient of refractive index, which is dependent upon orientation, and propagate with velocities of intermediate values.

Transparent crystalline materials are generally classified into two categories defined by the number of optical axes present in the molecular lattices. **Uniaxial** crystals have a single optical axis and comprise the largest family of common birefringent specimens, including calcite, quartz, and ordered synthetic or biological structures. The other major class is **biaxial** crystals, which are birefringent materials that feature two independent optical axes. The ordinary and extraordinary wavefronts in uniaxial crystals coincide at either the slow or the fast axis of the ellipsoid, depending upon the distribution of refractive indices within the

crystal (illustrated in **Figure 6**). The optical path difference or relative retardation between these rays is determined by the lag of one wave behind the other in surface wavefronts along the propagation direction.

In cases where the ordinary and extraordinary wavefronts coincide at the long or major axis of the ellipsoid, then the refractive index experienced by the extraordinary wave is greater than that of the ordinary wave (**Figure 6(b)**). This situation is referred to as positive birefringence. However, if the ordinary and extraordinary wavefronts overlap at the minor axis of the ellipsoid (**Figure 6(a)**), then the opposite is true. In effect, the refractive index through which the ordinary wave passes exceeds that of the extraordinary wave, and the material is termed negatively birefringent. A diagrammatic ellipsoid relating the orientation and relative magnitude of refractive index in a crystal is termed the **refractive index ellipsoid**, and is illustrated in Figures 5 and 6.

Figure 6 - Refractive Index Ellipsoids



Returning to the calcite crystal presented in **Figure 2**, the crystal is illustrated having the optical axis positioned at the top left-hand corner. Upon entering the crystal, the ordinary light wave is refracted without deviation from the normal incidence angle as if it were traveling through an isotropic medium. Alternatively, the extraordinary wave deviates to the left and travels with the electric vector perpendicular to that of the ordinary wave. Because calcite is a negatively birefringent crystal, the ordinary wave is the slow wave and the extraordinary wave is the fast wave.

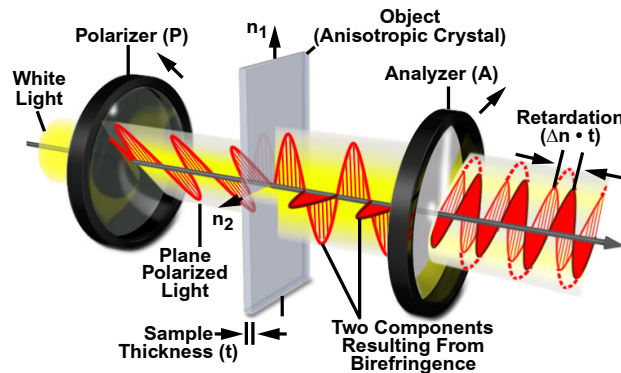
Birefringent Crystals in a Polarizing Optical Microscope

As mentioned above, light that is doubly refracted through anisotropic crystals is polarized with the electric vector vibration directions of the ordinary and extraordinary light waves being oriented perpendicular to each other. The behavior of anisotropic crystals under crossed polarized illumination in an optical microscope can now be examined. **Figure 7** illustrates a birefringent (anisotropic) crystal placed between two polarizers whose vibration directions are oriented perpendicular to each other (and lying in directions indicated by the arrows next to the polarizer and analyzer labels).

Non-polarized white light from the illuminator enters the polarizer on the left and is linearly polarized with an orientation in the direction indicated by the arrow (adjacent to the polarizer label), and is arbitrarily represented by a red sinusoidal light wave. Next, the polarized light enters the anisotropic crystal (mounted on the microscope stage) where it is refracted and divided into two separate components vibrating parallel to the crystallographic axes and perpendicular to each other (the red open and filled light waves). The polarized light waves then travel through the analyzer (whose polarization position is indicated by the arrow next to the analyzer label), which allows only those components of the light waves that are parallel to the analyzer transmission azimuth to pass. The relative retardation of one ray with respect to another is indicated by an equation (thickness multiplied by refractive index difference) that

relates the variation in speed between the ordinary and extraordinary rays refracted by the anisotropic crystal.

Figure 7 - Birefringent Crystals Between Crossed Polarizers

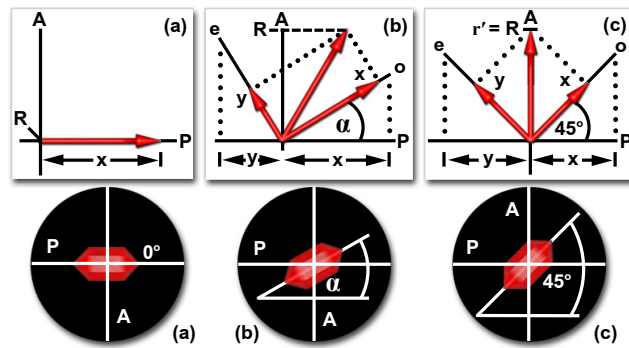


In order to examine more closely how birefringent, anisotropic crystals interact with polarized light in an optical microscope, the properties of an individual crystal will be considered. The specimen material is a hypothetical tetragonal, birefringent crystal having an optical axis oriented in a direction that is parallel to the long axis of the crystal. Light entering the crystal from the polarizer will be traveling perpendicular to the optical (long) axis of the crystal. The illustrations in **Figure 8** present the crystal as it will appear in the eyepieces of a microscope under crossed-polarized illumination as it is rotated around the microscope optical axis. In each frame of **Figure 8**, the axis of the microscope polarizer is indicated by the capital letter **P** and is oriented in an East-West (horizontal) direction. The axis of the microscope analyzer is indicated by the letter **A** and is oriented in a North-South (vertical) direction. These axes are perpendicular to each other and result in a totally dark field when observed through the eyepieces with no specimen on the microscope stage.

Figure 8(a) illustrates the anisotropic tetragonal, birefringent crystal in an orientation where the long (optical) axis of the crystal lies parallel to the transmission azimuth of the polarizer. In this case, light passing through the polarizer, and subsequently through the crystal, is vibrating in a plane that is parallel to the direction of the polarizer. Because none of the light incident on the crystal is refracted into divergent ordinary and extraordinary waves, the isotropic light waves passing through the crystal fail to produce electric vector vibrations in the correct orientation to traverse through the analyzer and yield interference effects (see the horizontal arrow in **Figure 8(a)**, and the discussion below). As a result the crystal is very dark, being almost invisible against the black background. For the purposes of illustration, the crystal depicted in **Figure 8(a)** is not totally extinct (as it would be between crossed polarizers) but passes a small portion of red light, to enable the reader to note the position of the crystal.

Microscopists classically refer to this orientation as being a **position of extinction** for the crystal, which is important as a reference point for determining the refractive indices of anisotropic materials with a **polarizing microscope**. By removing the analyzer in a crossed polarizing microscope, the single permitted direction of light vibration passing through the polarizer interacts with only one electrical component in the birefringent crystal. The technique allows segregation of a single refractive index for measurement. Subsequently, the remaining refractive index of a birefringent material can then be measured by rotation of the polarizer by 90 degrees.

Figure 8 - Birefringent Crystal Orientation in Polarized Light

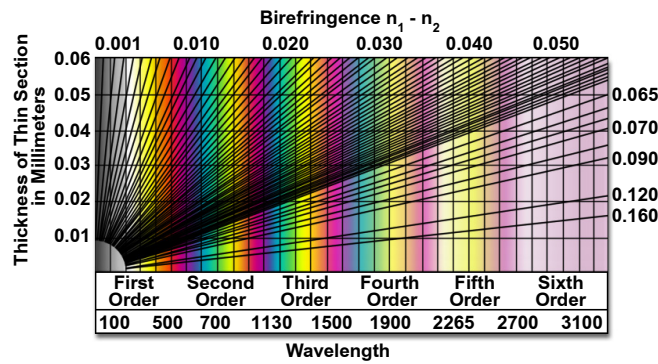


The situation is very different in **Figure 8(b)**, where the long (optical) axis of the crystal is now positioned at an oblique angle (α) with respect to the polarizer transmission azimuth, a situation brought about through rotation of the microscope stage. In this case, a portion of the light incident upon the crystal from the polarizer is passed on to the analyzer. To obtain a quantitative estimate of the amount of light passing through the analyzer, simple vector analysis can be applied to solve the problem. The first step is to determine the contributions from the polarizer to **o** and **e** (see **Figure 8(b)**); the letters refer to the ordinary (**o**) ray and extraordinary (**e**) ray, which are discussed above). Projections of the vectors are dropped onto the axis of the polarizer, and assume an arbitrary value of 1 for both **o** and **e**, which are proportional to the actual intensities of the ordinary and extraordinary ray. The contributions from the polarizer for **o** and **e** are illustrated with black arrows designated by **x** and **y** on the polarizer axis (**P**) in **Figure 8(b)**. These lengths are then measured on the vectors **o** and **e** (illustrated as red arrows designating the vectors), which are then added together to produce the resultant vector, **r'**. A projection from the resultant onto the analyzer axis (**A**) produces the absolute value, **R**. The value of **R** on the analyzer axis is proportional to the amount of light passing through the analyzer. The results indicate that a portion of light from the polarizer passes through the analyzer and the birefringent crystal displays some degree of brightness.

The maximum brightness for the birefringent material is observed when the long (optical) axis of the crystal is oriented at a 45 degree angle with respect to both the polarizer and analyzer, as illustrated in **Figure 8(c)**. Dropping the projections of the vectors **o** and **e** onto the polarizer axis (**P**) determines the contributions from the polarizer to these vectors. When these projections are then measured on the vectors, the resultant can be determined by completing a rectangle to the analyzer axis (**A**). The technique just described will work for the orientation of any crystal with respect to the polarizer and analyzer axis because **o** and **e** are always at right angles to each other, with the only difference being the orientation of **o** and **e** with respect to the crystal axes.

When the ordinary and extraordinary rays emerge from the birefringent crystal, they are still vibrating at right angles with respect to one another. However, the components of these waves that pass through the analyzer are vibrating in the same plane (as illustrated in **Figure 8**). Because one wave is retarded with respect to the other, interference (either constructive or destructive) occurs between the waves as they pass through the analyzer. The net result is that some birefringent samples acquire a spectrum of color when observed in white light through crossed polarizers.

Figure 9 - 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 [Figure 9](#). 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, 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. When the specimen is placed between crossed polarizers in the microscope and rotated to a position of maximum brightness with any one of a variety of retardation plates, the color visualized in the eyepieces can be traced on the retardation axis to find the wavelength difference between the ordinary and extraordinary waves passing through the specimen. Alternatively, by measuring the refractive indices of an anisotropic specimen and calculating their difference (the birefringence), the interference color(s) can be determined from the birefringence values along the top of the chart. By extrapolating the angled lines back to the ordinate, the thickness of the specimen can also be estimated.

The lower section of the Michel-Levy chart (x-axis) marks the orders of retardation in multiples of approximately 550 nanometers. The area between zero and 550 nanometers is known as the **first order** of polarization colors, and the magenta color that occurs in the 550 nanometer region is often termed **first-order red**. Colors between 550 and 1100 nanometers are termed **second-order** colors, and so on up the chart. The black color at the beginning of the chart is known as **zero-order black**. Many of the Michel-Levy charts printed in textbooks plot higher-order colors up to the fifth or sixth order.

The most sensitive area of the chart is first-order red (550 nanometers), because even a slight change in retardation causes the color to shift dramatically either up in wavelength to cyan or down to yellow. Many microscope manufacturers take advantage of this sensitivity by providing a full-wave **retardation plate** or **first-order red compensator** with their polarizing microscopes to assist scientists in determining the properties of birefringent materials.

Categories of Birefringence

Although birefringence is an inherent property of many anisotropic crystals, such as calcite and quartz, it can also arise from other factors, such as structural ordering, physical stress, deformation, flow through a restricted conduit, and strain. **Intrinsic** birefringence is the term utilized to describe naturally occurring materials that have asymmetry in refractive index that is direction-dependent. These materials include many anisotropic natural and synthetic crystals, minerals, and chemicals.

Structural birefringence is a term that applies to a wide spectrum of anisotropic formations, including biological macromolecular assemblies such as chromosomes, muscle fibers, microtubules, liquid crystalline DNA, and fibrous protein structures such as hair. Unlike many other forms of birefringence, structural birefringence is often sensitive to refractive index fluctuations or gradients in the surrounding medium. In addition, many synthetic materials

also exhibit structural birefringence, including fibers, long-chain polymers, resins, and composites.

Stress and **strain** birefringence occur due to external forces and/or deformation acting on materials that are not naturally birefringent. Examples are stretched films and fibers, deformed glass and plastic lenses, and stressed polymer castings. Finally, **flow** birefringence can occur due to induced alignment of materials such as asymmetric polymers that become ordered in the presence of fluid flow. Rod-shaped and plate-like molecules and macromolecular assemblies, such as high molecular weight DNA and detergents, are often utilized as candidates in flow birefringence studies.

In conclusion, birefringence is a phenomenon manifested by an asymmetry of properties that may be optical, electrical, mechanical, acoustical, or magnetic in nature. A wide spectrum of materials display varying degrees of birefringence, but the ones of specific interest to the optical microscopist are those specimens that are transparent and readily observed in polarized light.

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