Stereo Microscope

A typical stereomicroscope is as shown above. It has three key parts:

- Viewing Head/Body that houses the optical components in the upper part of the microscope.
- Focus Block that attaches the microscope head to the stand and focuses the microscope.
- Stand that supports the microscope and houses any integrated illumination.

Optical Components

As in a compound microscope, there are two optical systems in a compound microscope: Eyepiece Lenses and Objective Lenses.

- **Eyepieces or Oculars:** These are what you look through at the top of the microscope. Typically, standard eyepieces have a magnifying power of 10x. Optional eyepieces of varying powers are available, typically from 5x-30x.
- **Eyepiece tube:** This holds the eyepieces in place above the objective lens.
- **Diopter** adjustment ring that allows for the possible inconsistencies of our eyesight in one or both eyes. Binocular microscopes also swivel (Inter-pupillary Adjustment) to allow for different distances between the eyes of different individuals.
- **Objective Lenses:** These are the primary optical lenses on a microscope. In a low power microscope, they provide fixed magnification or zoom magnification. Zoom magnification is typically offered in a Greenough design or with a common main objective.
- **Stereo head:** There are two eyepieces mounted to a stereo microscope. The stereo head holds the eyepieces and contains prisms which turn the image right side up.

- **Focus Control:** Most stereo microscopes have only coarse focus controls. The focus knob raises and lowers both the objectives as well as the eyepieces. This is a difference to the compound microscopes, where the stage is raised and lowered.
- **Working Stage:** Stage plate is where the specimen to be viewed is placed. Pole and track stands have simple stages since lower magnification powers require less subtle movements than high power microscopes.
- **Stage Clips:** The clips can be used to hold down microscopy slides. It also holds down the glass plate which covers the lamp.
- **Transmitted Illumination:** Since most specimens examined on a stereo microscope are opaque, a top light (Transmitted Illumination) is used to shed light on the specimen. Some stereo microscopes also include a bottom light (Incident Illumination).

Differences between Stereomicroscopes and Compound Microscopes

Stereoscopic microscope, or simply stereo microscope, is an optical tool different from other types of microscopes in instrumentation and working principles. The regular microscope has one eyepiece and one objective lens. In contrary to this, the working of a stereo microscope involves two set of optic systems, which in turn result in formation of two different light paths. The objective of this lens configuration is to create a clearer three-dimensional image. Thus, in comparison to other microscopes that give two-dimensional images, the stereo microscope is superior in terms on creating a better, three-dimensional image.

A stereoscopic microscope is a binocular magnifying tool, used for viewing a three-dimensional (3D) image of the specimen. In a compound microscope, the magnified image of the sample under observation is formed by transmitted illumination. In simple terms, light passes through the specimen and then reaches the eyes. On the other hand, a stereoscopic dissecting microscope works by means of reflected illumination. Over here, light doesn't transmit through the object, but it is reflected back to form a 3D image of the sample.

The size of this microscope is larger than a compound microscope, with the former measuring about 1-2 feet height. Coming to its parts, it has two ocular lenses or eyepiece lenses, and one objective lens. They are connected by a body tube, which can be lowered or elevated to give clear images. The rotating objective is located below the movable eyepiece, and above the stage plate. Based on the model, the lenses are made up of plastic or glass. While some of the models are configured with a lighting source, others require external supply of light. There are also adjustment knobs for regulating light and focus.

Use of Stereoscopic Microscope

The main stereoscopic microscope uses are attributed to examining whole objects with depth perception, but not a part of the object. The magnified image created by this optical tool is through reflected illumination. Considering this, the major application of a stereo microscope is viewing opaque or thick objects, in which transmission of light is not possible. Say for instance, rock samples, coins, flowers, insects and circuit boards are very difficult to observe under a compound microscope. The reason being inability of light to pass through the slide specimens. In such cases, a stereo microscope is used to get realistic images of the objects.

While using a stereo microscope, you need to use both eyes simultaneously. Very often, this particular type of laboratory equipment is known as **stereoscopic dissecting microscope**. The reason is the frequent use of this laboratory tool for laboratory studies that call for close examination of a specimen, like dissection, surgical

procedure and other applications. However, stereoscopic microscope magnification is low, with a maximum magnifying power of 10X to 40X. Thus, very small or minute specimens that require magnification to hundred times or more are viewed under compound microscope.

With a stereo microscope, there is less issue for eyestrain as you do not need to squint your eyes for viewing the images. Now the stereoscopic microscope is used in association with a computer that is programmed with three-dimensional image viewing feature. The important images observed under this dissecting microscope are captured by a camera, which are then stored in the computer for later use. Thus, instead of viewing them individually through the eyepieces, the images can be studied by many people at a time.

Types of Stereo Microscopes

Stereo microscopes are designed based on two basic groups

- Greenough
- Common Main Objective

And they can have two basic configurations

- fixed magnification
- continuously variable zoom option

Greenough Design

The **Greenough** stereo microscope is the older of the two and is a wonderful tool used in production processes for soldering on a miniature scale and dissecting specimens in biology. It is easy to maintain and simple to use.
Horatio S. Greenough is credited with designing the use of two angled objectives, thus producing a slightly different view and creating the three dimensional effect. These twin body tubes each have their own objective and ocular lens.
The advantage of the Greenough design is the similarity to a compound microscope since it allows selection of high apertures.
The disadvantage is what's known as the **keystone effect**. This is a slight tilt in the focal plane due to the two lenses viewing the same image at slight angles.
As the lenses are not exactly parallel, this results in the outside of the image in the field of view to be slightly over focused or under focused. Therefore, only the central regions of the image are correctly focused at identical magnifications.

Common Main Objective Design

The **common main objective design** or **CMO** is the usual stereo microscope used in research and development because of its high resolution and the optical and illumination accessories available.
The CMO uses a large, single objective lens, which is shared by a pair of ocular channels and lens assembly.
This design virtually eliminates any image tilt in the focal plane. However, it creates an optical anomaly that makes the viewed object appear to elevate in the center, also referred to as "**perspective distortion**".
This is more of a problem in the less expensive CMO stereo microscopes so those used in top research, being more expensive and made by a major manufacturer, should not have this distortion.

Both the Greenough design and CMO are available in the fixed and zoom variety, with zoom being a more versatile option in stereo microscopy. Of course your choice is dependent on the task to be achieved.

Stereo Fixed Microscope

The fixed magnification uses two objective lenses, which refer to the optical element gathering and focusing the light rays on the image. The magnification has a fixed degree and is limited to the capability of the lens. Changing to a stronger eyepiece increases the magnification. This can be useful as you cannot change to a higher or lower magnification while viewing a specimen, therefore it keeps a stable focus.

These types of microscopes come in a variety of mountings, one of which is a turret style. Objective turret is another term for this type of mounting and indicates that an additional objective lens can be rotated into viewing position. This easily allows the viewer to change magnifications by simply rotating the turret mounting. Stereo turret microscopes are less flexible than the zoom type but are a more economical choice.

Stereo Zoom Microscope

The stereo zoom microscope behaves much as its name implies and is very popular. This microscope can zoom in or out to increase or decrease the desired magnification. The available range can also be altered by changing to a stronger eyepiece.

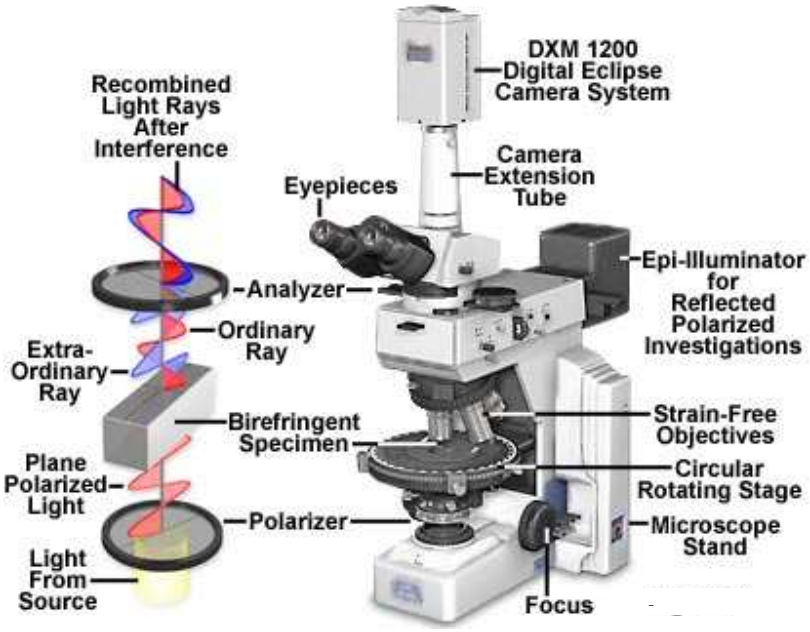
The stereo zoom microscope comes with a choice of stands:

- A simple stand allows room for observation and repair of items such as watches, coins, entomology.
- A **boom stand** is for larger applications and can be mounted to the floor and have an added boom to act as a counterweight. These stands are used for commercial inspection applications such as soldering, circuit board inspection and repair and engraving. Boom stands are more expensive than simple stands.

Two types of optical heads can be chosen and stereo zoom microscopes can be categorized as,

- **Binocular Stereo Zoom Microscope:** The “binocular” indicates that there are two eyepieces mounted to the zoom microscope. Changing the focus requires turning a knob, which slides the microscope up and down.
- **Trinocular Stereo Zoom Microscope:** A “trinocular” head means that a third eyepiece, or “phototube”, can be added without affecting the microscope operation. This third eyepiece allows the attachment of a camera that can be used to take still or video pictures. The trinocular option added to the stereo zoom capability is a popular choice due to this feature.

Polarizing Microscope



A typical polarizing microscope is as shown above.

A petrographic or polarizing microscope is the ideal choice for birefringent materials, which have measurable refracting differences determined by observation direction. A petrographic microscope is usually a modified compound microscope, although stereo microscopes can also be altered to achieve polarization. This microscope differs from others because it contains the following components:

- A polarizer and analyzer
- A circular rotating stage
- Special plates or filters placed between the object and light path.
- Bertrand lens

A polarizer only allows certain light waves or vibrations to pass through it. An analyzer, often a second polarizer located above the sample, determines the amount and direction of light that illuminates a sample. At its most basic, the polarizer focuses the different wavelengths and vibrations of light onto a single plane.

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, which are termed the ordinary and the extraordinary wavefronts 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.

Isotropic materials, which include a variety of gases, liquids, unstressed glasses and cubic crystals, demonstrate the same optical properties when probed in all directions. These materials have only one refractive index and no restriction on the vibration direction of light passing through them. In contrast, anisotropic materials, which

include 90 percent of all solid substances, have optical properties that vary with the orientation of incident light with the crystallographic axes. They demonstrate a range of refractive indices depending both on the propagation direction of light through the substance and on the vibrational plane coordinates. More importantly, anisotropic materials act as beam splitters and divide light rays into two orthogonal components. The technique of polarizing microscopy exploits the interference of the split light rays, as they are re-united along the same optical path to extract information about anisotropic materials.

There are two polarizing filters in a polarizing microscope - termed the polarizer and analyzer. The polarizer is positioned beneath the specimen stage usually with its vibration azimuth fixed in the left-to-right, or East-West direction, although most of these elements can be rotated through 360 degrees. The analyzer, usually aligned with a vibration direction oriented North-South, but again rotatable on some microscopes, is placed above the objectives and can be moved in and out of the light path as required. When both the analyzer and polarizer are inserted into the optical path, their vibration azimuths are positioned at right angles to each other. In this configuration, the polarizer and analyzer are said to be crossed, with no light passing through the system and a dark view field present in the eyepieces.

For incident light polarized microscopy, the polarizer is positioned in the vertical illuminator and the analyzer is placed above the half mirror. Most rotatable polarizers are graduated to indicate the rotation angle of the transmission azimuth, while analyzers are usually fixed into position (although advanced models can be rotated either 90 or 360 degrees). The polarizer and analyzer are the essential components of the polarizing microscope, but other desirable features include:

- **Specialized Stage** - A 360-degree circular rotating specimen stage to facilitate orientation studies with centration of the objectives and stage with the microscope optical axis to make the center of rotation coincide with the center of the field of view. Many stages designed for polarized light microscopy also contain a vernier scale so that rotation angle can be measured to an accuracy of 0.1 degree. For advanced studies of conoscopic images, a universal stage having multiple axes of rotation can also be employed to enable observation of the specimen from any direction.
- **Strain Free Objectives** - Stress introduced into the glass of an objective during assembly can produce spurious optical effects under polarized light, a factor that could compromise performance. Objectives designed for polarized light observation are distinguished from ordinary objectives with the inscription **P**, **PO**, or **Pol** on the barrel. The performance of an objective is limited by several factors, including the anti-reflection coatings used on lens surfaces, and the refractive properties due to angle of incident light on the front lens. In addition, lens strain can be introduced at the cement junction between elements in a lens group or from a single or group of lenses that has been mounted too tightly in the frame.
- **Centerable Revolving Nosepiece** - Because the objective optical axis position varies from one assembly to another, many polarized light microscopes are equipped with a specialized nosepiece that contains a centering mechanism for individual objectives. This enables each objective to be centered with respect to the stage and microscope optical axis so that specimen features remain in the center of the viewfield when the stage is rotated through 360 degrees.
- **Strain Free Condenser** - Condensers designed for polarized light microscopy have several features in common, including the use of strain free lenses. Some condensers are equipped with a receptacle for the polarizer or have the polarizing element mounted directly into the condenser, beneath the aperture diaphragm. Many polarized light condensers have a top lens that can be removed (a **swing-lens** condenser) from the light path to generate nearly parallel illumination wavefronts for low magnification and birefringence observations.
- **Eyepieces** - Polarized light microscope eyepieces are fitted with a cross wire reticle (or graticule) to mark the center of the field of view. Often, the cross wire reticle is substituted for a photomicrography reticle that assists in focusing the specimen and composing images with a set of frames bounding the

area of the viewfield to be captured either digitally or onto film. Orientation of the eyepiece with respect to the polarizer and analyzer is guaranteed by a point pin that slides into the observation tube sleeve.

- **Bertrand Lens** - A specialized lens mounted in an intermediate tube or within the observation tubes, a Bertrand lens projects an interference pattern formed at the objective rear focal plane into focus at the microscope image plane. The lens is designed to enable easy examination of the objective rear focal plane, to allow accurate adjustment of the illuminating aperture diaphragm and to view interference figures.
- **Compensator and Retardation Plates** - Many polarized light microscopes contain a slot to allow the insertion of compensators and/or retardation plates between the crossed polarizers, which are used to enhance optical path differences in the specimen. In most modern microscope designs, this slot is placed either in the microscope nosepiece or an intermediate tube positioned between the body and eyepiece tubes. Compensation plates inserted into the slot are then situated between the specimen and the analyzer.

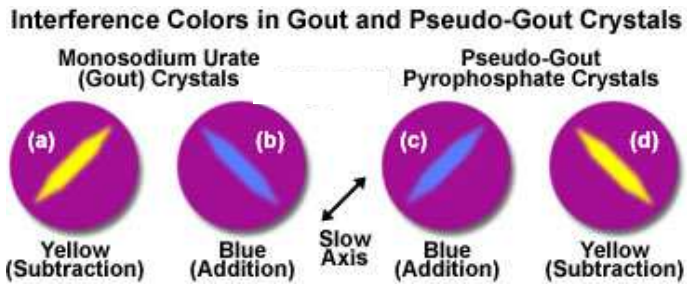
Polarized light microscopy can be used both with reflected (incident or **epi**) and transmitted light. Reflected light is useful for the study of opaque materials such as ceramics, mineral oxides and sulfides, metals, alloys, composites, and silicon wafers. Reflected light techniques require a dedicated set of objectives that have not been corrected for viewing through the cover glass, and those for polarizing work should also be strain free.

Applications of Polarized Light Microscopy

The strengths of polarizing microscopy can best be illustrated by examining particular case studies and their associated images. As described above, polarized light microscopy is utilized in a broad range of disciplines, including medicine, biology, geology, materials science, and the food industry. The specimens that are readily examined between crossed polarizers originate from a variety of natural and synthetic sources and include gout crystals, amyloid, muscle tissue, teeth, minerals, solid crystals, liquid crystals, fibers, fats, glasses, ceramics, metals, alloys, among others.

Identification of Gout Crystals

One of the most common medical applications for polarized light microscopy is the identification of gout crystals (monosodium urate) with a first order retardation plate. This practice is so common that many microscope manufacturers offer a **gout kit** attachment for their laboratory brightfield microscopes that can be purchased by physicians. Gout is an acute, recurrent disease caused by precipitation of urate crystals and characterized by painful inflammation of the joints, primarily in the feet and hands. In practice, several drops of fresh synovial fluid are sandwiched between a microscope slide and cover glass and sealed with nail polish to prevent drying. After the specimen has been prepared, it is examined between crossed polarizers with a first order retardation plate inserted into the optical path.



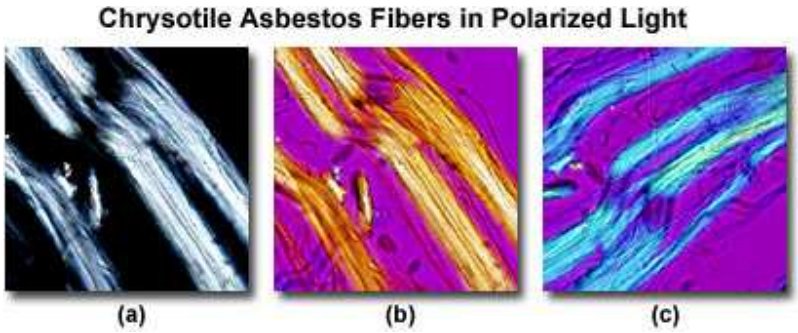
Monosodium urate crystals grow in elongated prisms that have a negative optical sign of birefringence, which generates a yellow (subtraction) interference color when the long axis of the crystal is oriented parallel to the slow axis of the first order retardation plate (Fig (a)). Rotating the crystals through 90 degrees changes the

interference color to blue (addition color; Fig (b)). In contrast, pseudo-gout pyrophosphate crystals, which have similar elongated growth characteristics, exhibit a blue interference color (Fig (c)) when oriented parallel to the slow axis of the retardation plate and a yellow color (Fig (d)) when perpendicular. The sign of birefringence can be employed to differentiate between gout crystals and those consisting of pyrophosphate. Gout can also be identified with polarized light microscopy in thin sections of human tissue prepared from the extremities. Polarized light is also useful in the medical field to identify amyloid, a protein created by metabolic deficiencies and subsequently deposited in several organs (spleen, liver, kidneys, brain), but not observed in normal tissues.

Identification of Asbestos Fibers

Asbestos is a generic name for a group of naturally occurring mineral fibers, which have been widely used as insulating materials, brake pads, and to reinforce concrete. These materials can be harmful to the health when inhaled and it is important that their presence in the environment be easily identified. Specimens are commonly screened using scanning electron microscopy and x-ray microanalysis, but polarizing microscopy provides a quicker and easier alternative that can be utilized to distinguish between asbestos and other fibers and between the major types asbestos, including chrysotile, crocidolite, and amosite. From a health care point of view, it is believed that the amphibole asbestos derivatives (crocidolite and amosite) are more harmful than the serpentine, chrysotile.

Plane-polarized light provides information about gross fiber morphology, color, pleochroism, and refractive index. Glass fibers and others that are isotropic will be unaffected by rotation under plane-polarized light while asbestos fibers will display some pleochroism. Chrysotile asbestos fibrils may appear crinkled, like permed or damaged hair, under plane-polarized light, whereas crocidolite and amosite asbestos are straight or slightly curved. Chrysotile has a refractive index of about 1.550, while that of amosite is 1.692, and crocidolite has the highest, with a value of 1.695. Note that the refractive index value of the amphibole asbestos products is much higher than chrysotile.

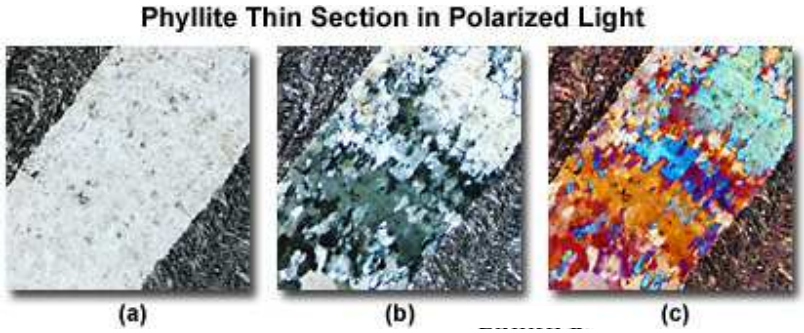


With the use of crossed polarizers it is possible to deduce the permitted vibration direction of the light as it passes through the specimen, and with the first order retardation plate, a determination of the slow and fast vibration directions can be ascertained. Under crossed polarizers, chrysotile displays pale interference colors, which are basically restricted to low order whites (Fig (a)). When a first order retardation plate is added (retardation value of one wavelength, or 530-560 nanometers), the colors of the fiber are transformed. If the fiber is aligned Northwest-Southeast, the retardation plate is additive (white arrow in Fig (b)) and produces primarily yellow subtractive interference colors in the fiber. When the fiber is aligned Northeast-Southwest (Fig (c)), the plate is additive to produce a higher order blue tint to the fiber with no yellow hues. From this evidence it is possible to deduce that the slow vibration direction of the retardation plate (denoted by the white arrows in Fig (b) and (c)) is parallel with the long axis of the fiber. Amosite is similar in this respect.

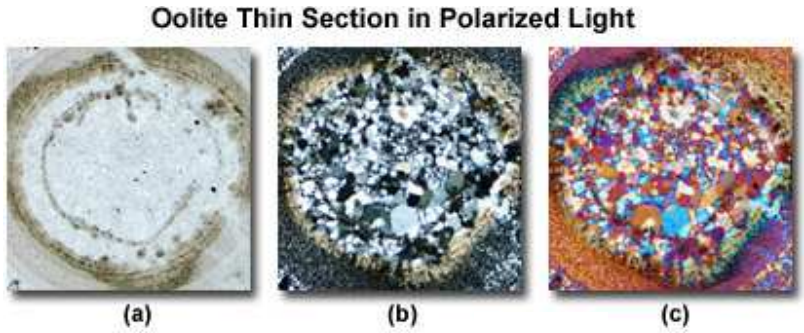
Crocidolite displays blue colors, pleochroism, and murky brown polarization colors. The fast vibration for this fiber is parallel with the long axis. In summary, identification of the three asbestos fiber types depends on shape, refractive indices, pleochroism, birefringence, and fast and slow vibration directions.

Uncovering the History of Rock Formation

Phyllite - As well as providing information on component minerals, an examination of geological thin sections using polarizing microscopy can reveal a great deal about how the rock was formed. Phyllite, a metamorphic rock, clearly shows the alignment of crystals under the effects of heat and stress. Small-scale folds are visible in the plane-polarized image (Fig (a)) and more clearly defined under crossed polarizers (Fig (b)) with and without the first order retardation plate. The crossed polarizers image reveals that there are several minerals present, including quartz in gray and whites and micas in higher order colors. The alignment of the micas is clearly apparent. Addition of the first order retardation plate (Fig (c)) improves contrast for clear definition in the image.



Oolite - Oolite, a light gray rock composed of siliceous oolites cemented in compact silica, is formed in the sea. The mineral's name is derived from its structural similarity to fish roe, better known as caviar. Oolite forms in the sea when sand grains are rolled by gentle currents over beds of calcium carbonate or other minerals. These minerals build up around the sand grains and subsequent cementation transforms the grains into coherent rock. The thin sections show the original quartz nuclei (Fig (a-c)) on which the buildup of carbonate mineral occurred.

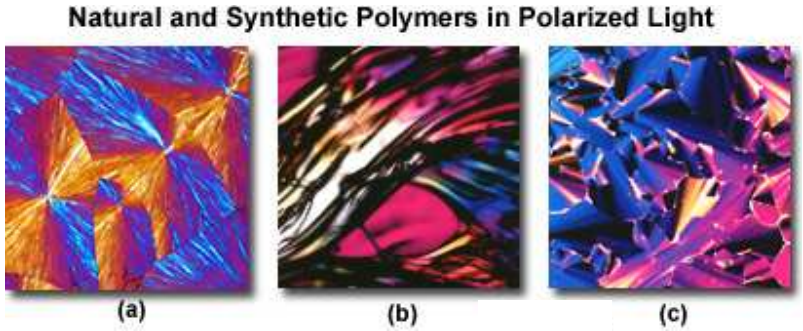


In plane-polarized light (Fig (a)), the quartz is virtually invisible having the same refractive index as the cement, while the carbonate mineral, with a different refractive index, shows high contrast. The crossed polarizer image (Fig (b)) reveals quartz grains in grays and whites and the calcium carbonate in the characteristic biscuit colored, high order whites. The groups of quartz grains in some of the cores reveal that these are polycrystalline and are metamorphic quartzite particles. When a first order retardation plate is inserted into the optical path (Fig (c)), optical path differences become apparent in the specimen, and contrast is enhanced.

Natural and Synthetic Polymers

During the solidification of polymer melts there may be some organization of the polymer chains, a process that is often dependent upon the annealing conditions. When nucleation occurs, the synthetic polymer chains often arrange themselves tangentially and the solidified regions grow radially. These can be seen in crossed polarized

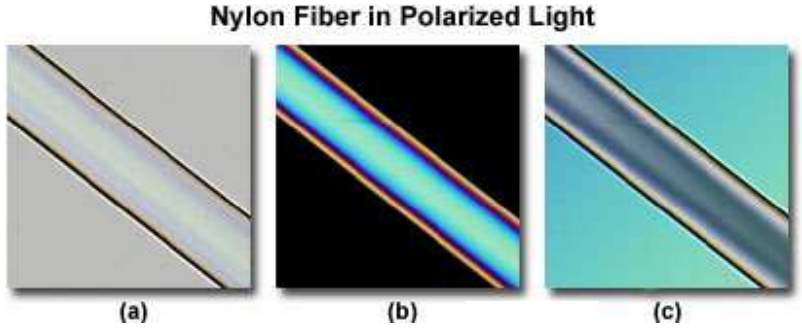
illumination as white regions, termed spherulites, with the distinct black extinction crosses. When these spherulites impinge, their boundaries become polygonal. This can be clearly seen in crossed polarizers but not under plane-polarized light.



The addition of the first order retardation plate (Fig (a)) confirms the tangential arrangement of the polymer chains. The banding occurring in these spherulites indicates slow cooling of the melt allowing the polymer chains to grow out in spirals. This information on thermal history is almost impossible to collect by any other technique. Nucleation in polymer melts can take place as the result of accidental contamination or contact with a nucleating surface and can lead to substantial weakening of the product. Identification of nucleation can be a valuable aid for quality control.

Other polymers may not be birefringent (evidenced by the polycarbonate specimen illustrated in Fig (b)), and do not display substantial secondary or tertiary structure. In other cases, both biological and synthetic polymers can undergo a series of lyotropic or thermotropic liquid crystalline phase transitions, which can often be observed and recorded in a polarized light microscope. Fig (c) illustrates a birefringent columnar-hexatic liquid crystalline phase exhibited by rod-like DNA molecules at very high aqueous solution concentrations (exceeding 300 milligrams/milliliter).

Nylon Fibers - Observations under plane-polarized light (Fig (a)) reveal refractive index differences between a nylon fiber and the mounting medium, and the presence of opacifying titanium dioxide particles. The image under crossed polarizers (Fig (b)) reveals second and third order polarization colors and their distribution across the fibers indicate that this is a cylindrical and not a lobate fiber useful in predicting mechanical strength. The use of the quartz wedge (Fig (c)) enables the determination of optical path differences for birefringence measurements.



In summary, polarizing microscopy provides a vast amount of information about the composition and three-dimensional structure of a variety of samples. Virtually unlimited in its scope, the technique can reveal information about thermal history and the stresses and strains to which a specimen was subjected during formation. Useful in manufacturing and research, polarizing microscopy is a relatively inexpensive and accessible investigative and quality control tool, which can provide information unavailable with any other technique.