An aerial photograph of a coastal area. On the left, there is a road and some buildings. The right side is dominated by a large body of water with a coral reef, showing various shades of blue and green. The text is overlaid on the image.

# 2006 Training Course

for Capacity Building  
on Coastal Geological Survey

**28 August - 10 September 2006**

**Organized by: KIGAM**  
**Supported by: MOST, UNDP**

# Training Course

## for Capacity Building on Coastal Geological Survey

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Organized by:

**Korea Institute of Geoscience and Mineral Resources (KIGAM)**

Supported by:

**Ministry of Science and Technology (MOST)**

**United Nations Development Programme (UNDP)**





## 1. Preparation of Polished Thin Sections

(<http://lucas.lakeheadu.ca/images/geoprep.pdf#search=%22Thin%20Section%20Preparation%22>)

*Lakehead University, Geology Department, 955 Oliver Road, Thunder Bay, ON, P7b 5el*



Rock samples-typically cores or individual grab samples, require processing before they can be used for mineral analysis by either polarizing light microscope or electron microscope (Microprobe or Scanning Electron Microscope/X-ray Microanalysis). The sample has to be thin enough for light to pass through in a light microscope and have a polished surface for electron microscope studies.

### STEP 1: CUTTING A SLAB



A suitable size slab for mounting on a slide is cut from a piece of rock or drill core with a diamond saw

## STEP 2: Initial Lapping of the Slab



The slab is labelled on one side and the other side is lapped flat and smooth first on a cast iron lap with 400grit carborundum, then finished on a glass plate with 600 grit carborundum.

## STEP 3: Glass Slide is Added

After drying on a hot plate, a glass slide is glued to the lapped face of the slab with epoxy.



## STEP 4: Slab is sectioned



Using a thin section saw, the slab is cut-off close to the slide. The thickness is further reduced on a thin section grinder. Powders are mixed with epoxy, then spread on a slide and allowed to cure. The surface is ground flat on the thin section grinder, then finished similarly to a thin sections.

### STEP 5: Final Lapping



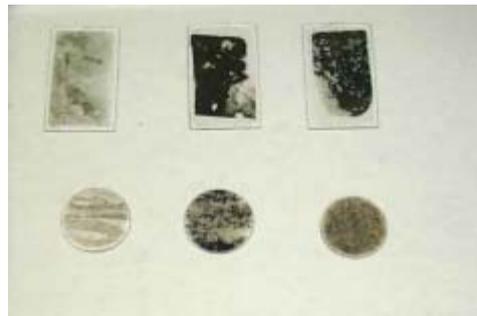
A finished thickness of 30 microns is achieved by lapping the section by hand on a glass plate with 600 grit carborundum. A fine grinding with 1000 grit prior to polishing is optional.

### STEP 6: Polishing

The section is placed in a holder and spun on a polishing machine using nylon cloth and diamond paste until a suitable polish is achieved for microscope or SEM studies.



### STEP 7: Final inspection



Examples of finished thin sections.

## **Keys to Better Thin Section Preparation**

(<http://wardsci.com/article.asp?ai=71>)

Specimen flatness, and an even epoxy layer must be worked out before the final steps on the equipment. We will assume the quality of the glass slide and its index of refraction are adequate, the mounting medium being used is a good workable product with a refractive index equal to that of the glass slide, and the specimen to be sectioned has been impregnated, if necessary, to afford a smooth, flat, mounting surface.

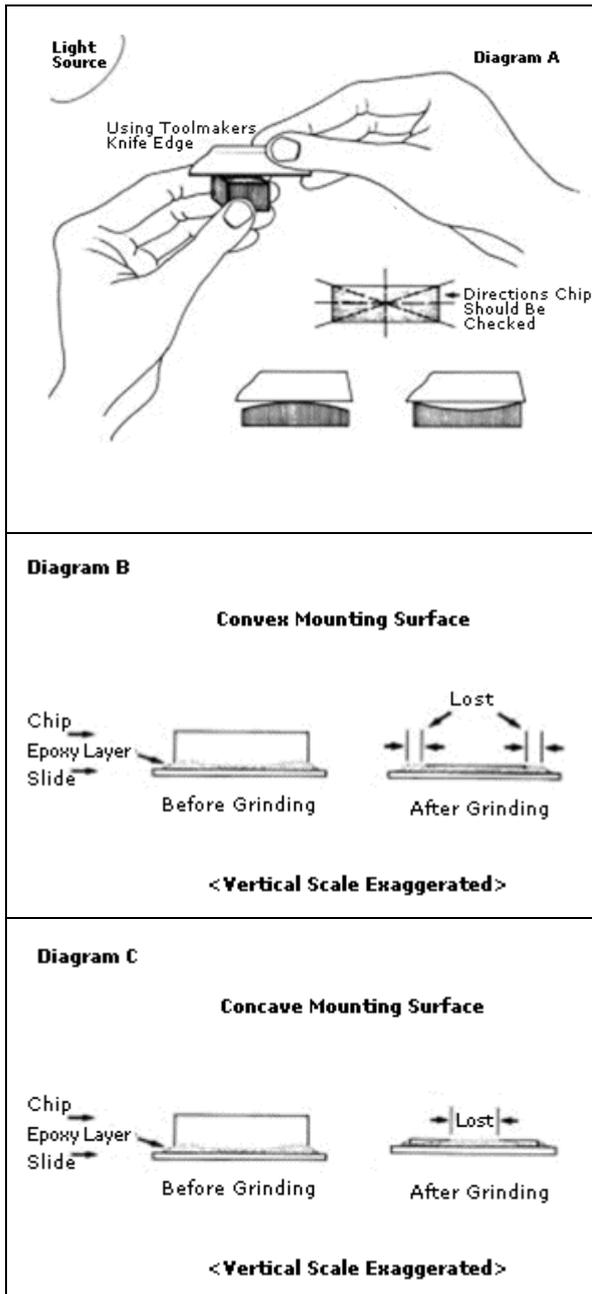
Of first concern is specimen flatness. In preparing a specimen for mounting, you can use sophisticated lapping machines with diamond, or a simple glass plate with silicon carbide. Whatever you use may be dictated by cost and desired output, but the characteristics of the finished chip or specimen should be the same. After lapping with a progression of silicon carbide grits or diamond wheels, the surface of the chip to be mounted must be perfectly flat and smooth. Check for surface flatness periodically with a proven technique. A "tool-maker's" knife edge can be used for this purpose. (See Diagram A.) Rest the knife edge on the ground surface of the chip while holding it up to a light source. Looking straight across the flat plane of the chip, check for light passing between the knife edge and the ground surface of the chip. Rotate the knife edge slowly to check both diagonals and the entire surface from length to width for any irregularities or low points. If light does appear between this contact zone in any one of the positions, the chip is not uniformly flat and should not be mounted. Lapping should continue until no variations are noted.

Lapping is one of the most critical areas of the thin section preparation. When not performed correctly, it will result in the loss of the thin section later on during final grinding. (See Diagrams B & C.) A few minutes spent developing a good lapping procedure can save many hours of doing slides over again. Once this procedure has been worked out, it will become less necessary to check for flatness as you become more accustomed to your own technique and lapping ability.

The second area to consider is the glass slide. Many slides will vary in thickness, from one end to the other, by more than .001 inches. This variation will make it almost impossible to get a flat, uniform thin section from any equipment used. Since the machine chuck and cutting surface are parallel and have been ground in to exacting tolerances, both slide surfaces must be perfectly parallel to achieve a flat finished section.

All slides should be checked with a micrometer before being used. This applies to both plain and frosted slides. Most frosted slides are either chemically frosted or sandblasted, and any irregularities in parallelism of top and bottom surfaces would still

remain. Using the micrometer, measure the thickness at both ends and the middle of the slide. Subtract the lowest reading from the highest reading. Any difference greater than .0005 inches will cause serious problems when grinding your section to a tolerance of .001 inches (the standard thickness of most petrographic thin sections). The usual result is a loss of specimen area during final grinding or the need to do excessive hand finishing.

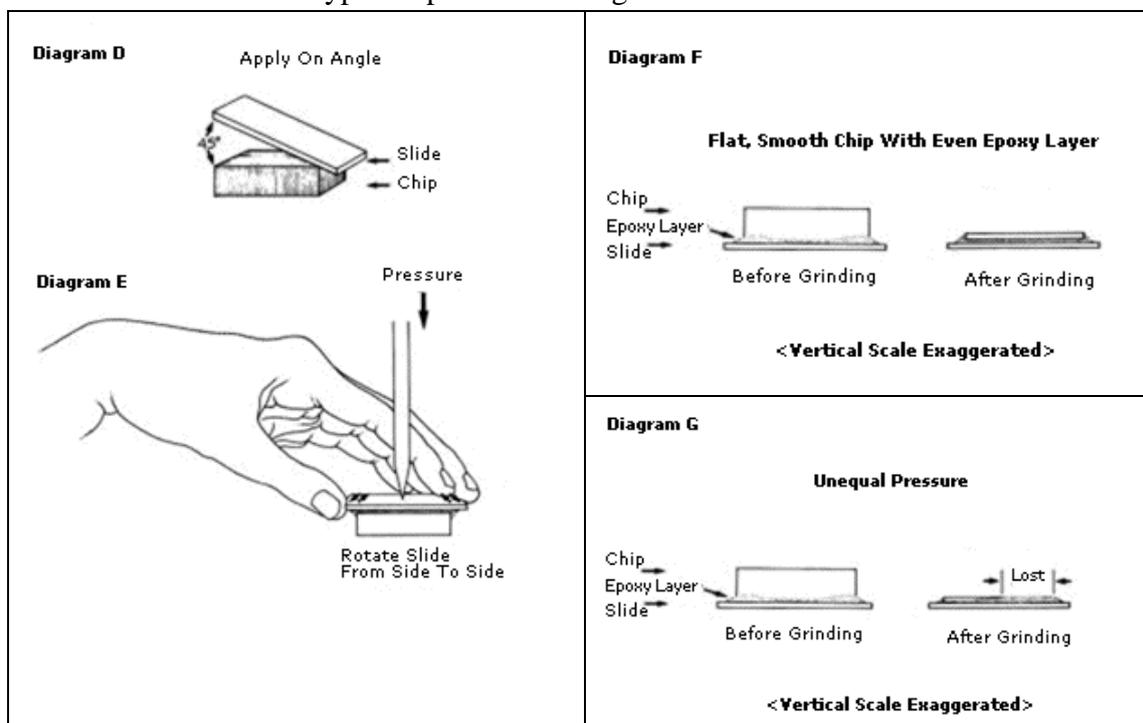


To solve this problem, you could place the blank slide on the Ingram-Ward grinder and grind it down to uniform thickness before mounting the chip, or you can simply continue checking your stock of slides with the micrometer until slides of uniform thickness are found. Generally, sections with slight slide variations of .0006 inches or less can be salvaged fairly easily by removing the slide from the grinder before fracturing or loss of specimen area occurs. The section can then be hand finished to completion by selectively grinding on a glass plate using silicon carbide 600 grit. Taking these relatively simple steps of checking slide uniformity before mounting can save hours of wasted effort and many ruined sections.

The last major area of concern is achieving an even medium (epoxy) layer between specimen and glass slide when mounting. It always seems enough of a problem mounting a chip without air bubbles. It is important to remove these bubbles, but it is only secondary to producing an even, uniform medium layer. The type of medium you select for

this operation can save you time and effort. Epoxy, Canada Balsam, and Lakeside 70C are among those media in general use. Epoxies tend to form a stronger bond, are easier

to use, and have a better index of refraction. Your choice of medium may be dictated by desired results and the type of specimens being mounted.



Whether hot or cold mounting the specimen, and regardless of the medium you choose, the following procedure has proven effective once the medium has been applied to the chip. To avoid as many air bubbles as possible, position the glass slide at a 45 degree angle along the edge of the chip. (See Diagram D.) Carefully lower the glass slide until complete contact has been made between the chip surface and the slide. Work out any trapped air bubbles by applying light pressure to the back surface of the slide with the eraser end of a pencil or a pointed wooden dowel.

After all air is removed, press firmly in the center of the slide with the dowel (or pencil eraser) and rotate the slide in a circular motion with your other hand. (See Diagram E.) This pressure and slide movement will disperse the medium evenly and result in a uniform layer between chip and slide. (See Diagram F.) Failure to achieve this uniform layer may result in loss of specimen area during machine grinding. (See Diagram G.) Do not apply too much pressure, or the medium might be completely forced from the center, creating a dry spot. If these problem areas we've discussed are eliminated, we guarantee the Ingram-Ward equipment will grind a perfectly flat thin section to the desired thickness. The closer you come to reducing these variables, the flatter the section will be and the less hand finishing you will need to do. No matter how you slice it, improved technique prior to machine operation is the key to greater success in your thin section preparations.

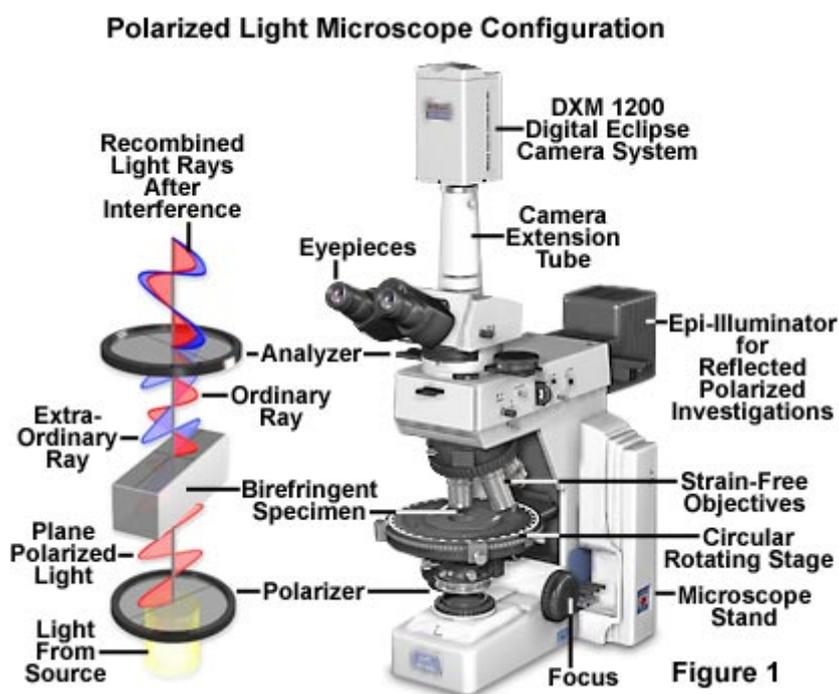
## 2. Introduction to Polarized Light Microscopy

(<http://www.microscopyu.com/articles/polarized/polarizedintro.html>)

**Philip C. Robinson** - Department of Ceramic Technology, Staffordshire Polytechnic, College Road, Stoke-on-Trent, ST4 2DE United Kingdom.

**Michael W. Davidson (digital images and graphics)** - National High Magnetic Field Laboratory, 1800 East Paul Dirac Dr., The Florida State University, Tallahassee, Florida, 32310.

Although much neglected and undervalued as an investigative tool, polarized light microscopy (Figure 1) 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.

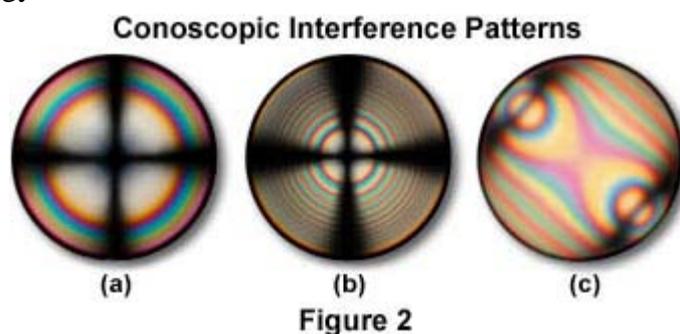


As well as providing information on absorption color and boundaries between minerals of differing refractive indices obtainable in brightfield microscopy, polarized light microscopy can distinguish between isotropic and anisotropic materials. The technique exploits optical properties of anisotropy to reveal detailed information about the structure and composition of materials, which are invaluable for identification and diagnostic purposes.

Isotropic materials, which include gases, liquids, unstressed glasses and cubic crystals, demonstrate the same optical properties in all directions. They have only one refractive

index and no restriction on the vibration direction of light passing through them. Anisotropic materials, in contrast, 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 parts (as illustrated in Figure 1). 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 these materials.

Polarized light microscopy is perhaps best known for its geological applications--primarily for the study of minerals in rock thin sections, but it can also be used to study many other materials. These include both natural and industrial minerals whether refined, extracted or manufactured, composites such as cements, ceramics, mineral fibers and polymers, and crystalline or highly ordered biological molecules such as DNA, starch, wood and urea. The technique can be used both qualitatively and quantitatively and is an outstanding tool for materials science, geology, chemistry, biology, metallurgy and even medicine.



While an understanding of the analytical techniques of polarized microscopy may be perhaps more demanding than other forms of microscopy, it is well worth pursuing, simply for the enhanced information that can be obtained over brightfield imaging. An awareness of the principles of polarizing microscopy is also essential for the effective interpretation of differential interference contrast (DIC) microscopy.

### **Polarized Light**

The wave model of light describes light waves vibrating at right angles to the direction of travel of light with all vibration directions being equally probable. This is "common" light. In plane-polarized light there is only one vibration direction (Figure 1). The human eye-brain system has no sensitivity to the vibration directions of light, and

plane-polarized light can only be detected by an intensity or color effect, for example, by reduced glare when wearing polarized sun glasses.

The most widely used material is Polaroid™ film. Invented by Land in 1932, Polaroid film consists of long chain polymers, treated with light absorbing dyes, and stretched so that the chains are aligned. Light vibrating parallel with the chains is absorbed while light perpendicular to the chains is transmitted.

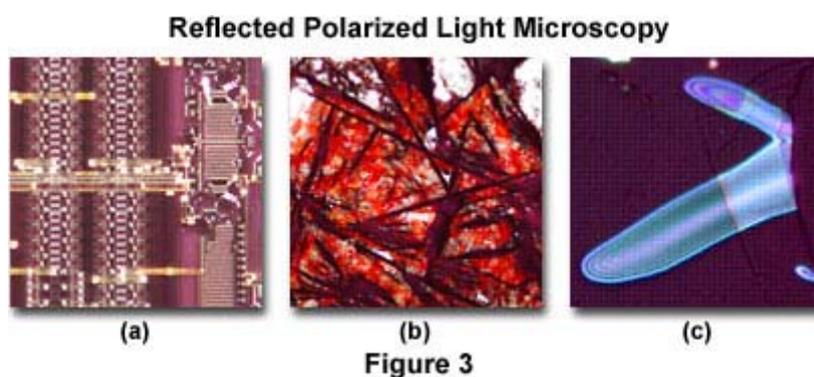
There are two polarizing filters in a polarizing microscope - the polarizer and analyzer (see Figure 1). The polarizer is situated below the specimen stage usually with its permitted vibration direction fixed in the left-to-right, East-West direction, although this is usually rotatable through 360 degrees. The analyzer, usually aligned North-South but again rotatable on some microscopes, is sited above the objectives and can be moved in and out of the light path as required. When both the analyzer and polarizer are in the optical path, their permitted vibration directions 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 field of view present in the eyepieces.

The polarizer and analyzer are the essential components of the polarizing microscope - but other desirable features include:

- A 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.
- Strain free objectives – stress in assembly can produce optical effects under polarized light, a factor that could complicate observations.
- An eyepiece fitted with a cross wire graticule to mark the center of the field of view. Often, the cross wire graticule is substituted for a photomicrography graticule 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.
- A Bertrand lens – to enable easy examination of the objective rear focal plane, to allow accurate adjustment of the illuminating aperture diaphragm and to view interference figures, as presented in Figure 2.
- A slot to allow the insertion of compensators/retardation plates between the 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.

Polarizing microscopy can be used both with reflected and transmitted light. Reflected light is useful for the study of opaque materials such as mineral oxides and sulphides, metals and silicon wafers (Figure 3). Reflected light techniques require a dedicated set of objectives that have not been corrected for viewing through the coverslip, and those for polarizing work should, again, be stress free.



Illustrated in Figure 3 is a series of reflected polarized light photomicrographs of typical specimens imaged utilizing this technique. On the left (Figure 3(a)) is a digital image revealing surface features of a microprocessor integrated circuit. Birefringent elements employed in the fabrication of the circuit are clearly visible in the image, which displays a portion of the chip's arithmetic logic unit. The polished surface of a ceramic superconducting tape (Yttrium-1,2,3) is presented in Figure 3(b), which shows birefringent crystalline areas with interference colors interspersed in a matrix of isotropic binder. Metallic thin films are also visible with reflected polarized light. Figure 3(c) illustrates blisters that form imperfections in an otherwise confluent thin film of copper (about 0.1 micron thick) sandwiched over a nickel/sodium chloride substrate to form a metallic superlattice assembly.

Careful specimen preparation is essential for good results. The method chosen will depend on the type of material studied. In geological applications the standard thickness for rock thin sections is 25-30 micrometers. Specimens can be ground down with diamond impregnated wheels and then hand finished to the correct thickness using abrasive powders of successively decreasing grit size. Softer materials can be prepared in a manner similar to biological samples using a microtome. Slices between one and 40 micrometers thick are used for transmitted light observations. These should be strain-free and free from any knife marks. Specimens are mounted between the slide and the

coverslip using a mounting medium whose composition will depend on the chemical and physical nature of the specimen. This is particularly significant in the study of synthetic polymers where some media can chemically react with and cause structural changes to the material being studied.

### Making Use of Anisotropy

Different levels of information can be obtained in plane-polarized light (analyzer out of the optical path) or with crossed polarizers (analyzer inserted into the optical path). Observations in plane-polarized light reveal details of the **optical relief** of the specimen, which is manifested in the "visibility" of boundaries, and increases with the increase of refractive index across them. Differences in the refractive indices of the mounting adhesive and the specimen determine the extent to which light is scattered as it emerges from the uneven specimen surface. Materials with high relief, which appear to stand out from the image, have refractive indices, which are appreciably different from that of the mountant. Immersion refractometry is used to measure substances having unknown refractive indices by comparison with oils of known refractive index.

Examinations of transparent or translucent materials in plane-polarized light will be similar to those seen in natural light until the specimen is rotated about the optical axis of the microscope. Then observers may see changes in the brightness and/or the color of the material being examined. This pleochroism, that is, variation of absorption color with vibration direction of the light, depends on the orientation of the material in the light path and is a characteristic of anisotropic materials only. An example of a material showing pleochroism is crocidolite, more commonly known as blue asbestos. This effect helps in its identification.

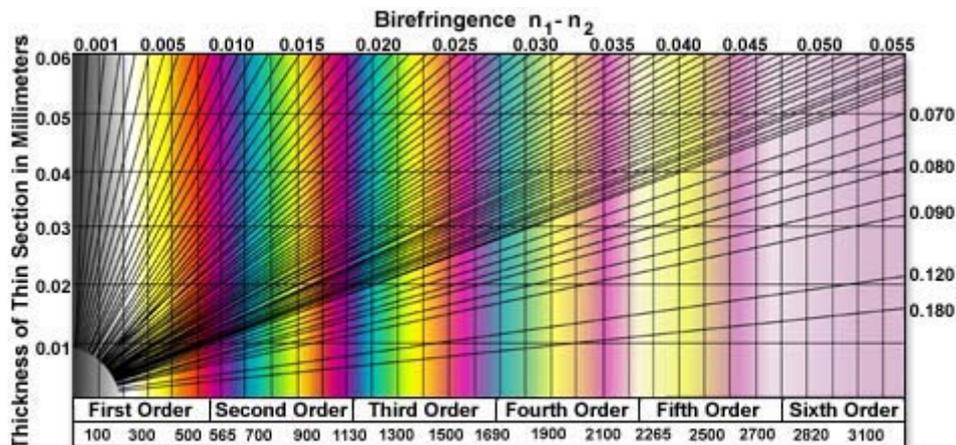


Figure 4

Polarization colors result from the interference of the two components of light split by the anisotropic specimen and may be regarded as white light minus those colors that are interfering destructively. Figure 2 illustrates conoscopic images of uniaxial and biaxial crystals observed at the objective rear focal plane. Interference patterns are formed by light rays traveling along different axes of the crystal being observed. Uniaxial crystals (Figures 2(a) and 2(b)) display an interference pattern consisting of two intersecting black bars (termed **isogyres**) that form a Maltese cross-like pattern. When illuminated with white (polarized) light, birefringent specimens produce circular distributions of interference colors (Figure 2), with the inner circles, called **isochromes**, consisting of increasingly lower order colors (see the Michel-Levy interference color chart, Figure 4). A common center for both the black cross and the isochromes is termed the **melatope**, which denotes the origin of the light rays traveling along the optical axis of the crystal. Biaxial crystals display two melatopes (Figure 2(c)) and a far more complex pattern of interference rings.

The two components of light travel at different speeds through the specimen and have different refractive indices, or **refringences**. **Birefringence** is the numerical difference between these refringences. The faster beam emerges first from the specimen with an optical path difference (**OPD**), which may be regarded as a "winning margin" over the slower one. The analyzer recombines only components of the two beams traveling in the same direction and vibrating in the same plane. The polarizer ensures that the two beams have the same amplitude at the time of recombination for maximum contrast.

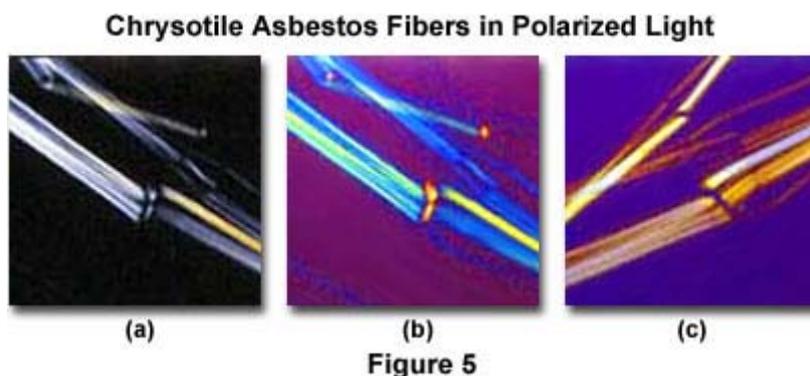
There is constructive and destructive interference of light in the analyzer, depending on the OPD on the specimen and the wavelength of the light, which can be determined from the order of polarization color(s). This relies on the properties of the specimen, including the thickness difference between the refractive index and the birefringence of the two beams, which has a maximum value dependent on the specimen and on the direction of travel of light through the specimen. Optical path differences can be used to extract valuable "tilt" information from the specimen.

Superimposed on the polarization color information is an intensity component. As the specimen is rotated relative to the polarizers, the intensity of the polarization colors varies cyclically, from zero (**extinction**) up to a maximum after 45 degrees and back down to zero after a 90-degree rotation. That is why a rotating stage and centration are provided, which are critical on a polarizing microscope. Centration of the objective and stage ensures that the center of the stage rotation coincides with the center of the field of view, a great convenience, as anyone who has tried to manage without it will know.

Whenever the specimen is in extinction, the permitted vibration directions of light

passing through are parallel with those of either the polarizer or analyzer. This can be related to geometrical features of the specimen, such as fiber length, film extrusion direction, and crystal faces. In crossed polarizers, isotropic materials can be easily distinguished from anisotropic materials as they remain permanently in extinction (remain dark) when the stage is rotated through 360 degrees.

To help in the identification of fast and slow beams, or to improve contrast when polarization colors are of low order, such as dark grey, accessory plates can be inserted in the optical path. These will cause color changes in the specimen, which can be interpreted with the help of a polarization color chart (**Michel-Levy** chart; see Figure 4). These charts show the polarization colors provided by optical path differences from 0 to 1800-3100 nanometers together with birefringence and thickness values. The wave plate produces its own optical path difference. When the light passes first through the specimen and then the accessory plate, the OPDs of the wave plate and the specimen are either added together or subtracted from one another in the way that "winning margins" of two races run in succession are calculated. They are added when the slow vibration directions of the specimen and accessory plate are parallel, and subtracted when the fast vibration direction of the specimen coincides with the slow vibration direction of the accessory plate. If the slow and fast directions are known for the accessory plate (they are usually marked on the mount of commercially available plates), then those of the specimen can be deduced. Since these directions are characteristic for different media, they are well worth finding out and are essential for orientation and stress studies.



The strengths of polarizing microscopy can best be illustrated by examining particular case studies and their associated images. All images illustrated in this section were recorded with a Nikon Eclipse E600 microscope equipped with polarizing accessories, a research grade microscope designed for analytical investigations.

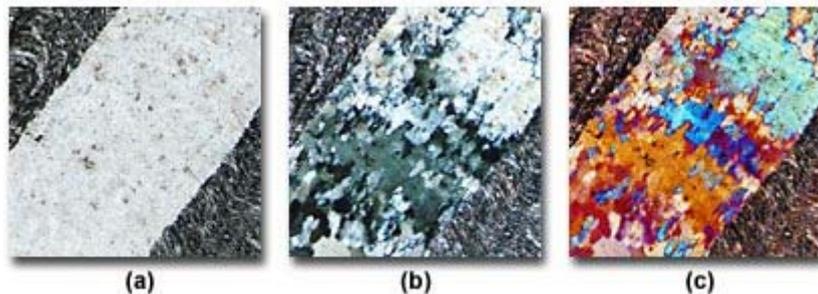
### **Identification of Asbestos Fibers**

Asbestos is a generic name for a group of naturally occurring mineral fibers, which have been widely used, for example, in insulating materials, brake pads and to reinforce concrete. They can be harmful to health when inhaled and it is important that their presence in the environment be easily identified. Samples 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 – chrysotile, crocidolite and amosite. From a health care point of view, it is believed that the amphibole asbestos varieties (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 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, amosite 1.692 and crocidolite, 1.695.

With the use of crossed polars it is possible to deduce the permitted vibration direction of the light as it passes through the specimen, and with the whole wave plate, a determination of the slow and fast vibration directions (Figure 5). Under crossed polars, chrysotile shows pale interference colors - low order whites (Figure 5(a)). When a full wave plate is added (530-560 nanometers), the colors are transformed. Aligned Northeast-Southwest, the wave plate is additive and gives blue and yellow in the fiber (Figure 5(b)). When aligned Northwest-Southeast (Figure 5(c)) the plate is subtracting to give a paler yellow fiber with no blue. From this it is possible to deduce that the slow vibration direction is parallel with the long axis of the fiber. Amosite is similar in this respect.

**Phyllite Thin Section in Polarized Light**



**Figure 6**

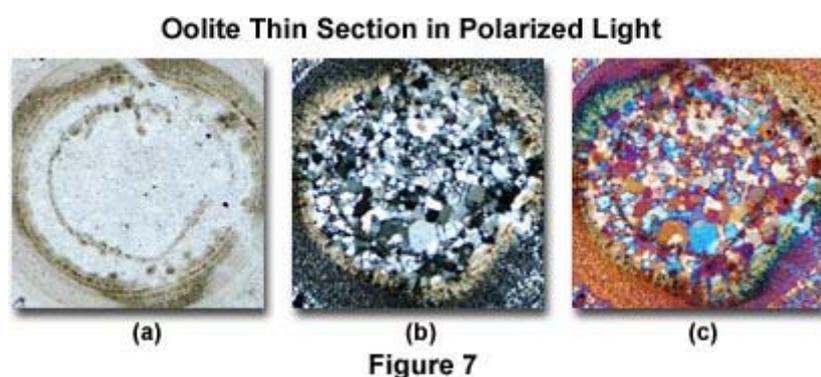
Crocidolite displays blue colors, pleochroism and murky brown polarization colors

and has its fast vibration direction parallel with its length. 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** - An examination of geological thin sections using polarizing microscopy, as well as providing information on component minerals, 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 (Figure 6(a)) and more clearly defined under crossed polars (Figure 6(b)) with and without the wave plate. The crossed polars image reveals that there are several minerals present--quartz in grey and whites and micas in higher order colors. The alignment of the micas is clearly apparent. Addition of the wave plate (Figure 6(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 - caviar! It 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 (Figure 7(a-c)) on which the build up of carbonate mineral occurred.

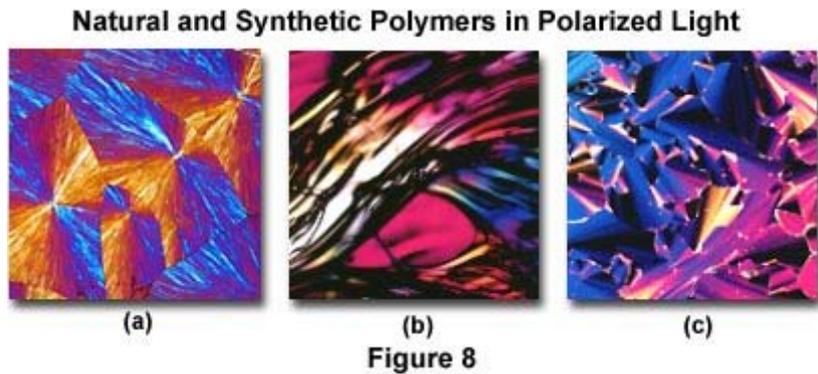


In plane-polarized light (Figure 7(a)), the quartz is virtually invisible having the same refractive index as the cement, while the carbonate mineral with a different refractive index shows high contrast. The crossed polarizer image (Figure 7(b)) shows quartz grains in grays and whites and the calcium 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 full-wave retardation plate is inserted into the optical path (Figure 7(c)), optical path differences become apparent in the specimen, and contrast is enhanced.

### Natural and Synthetic Polymers

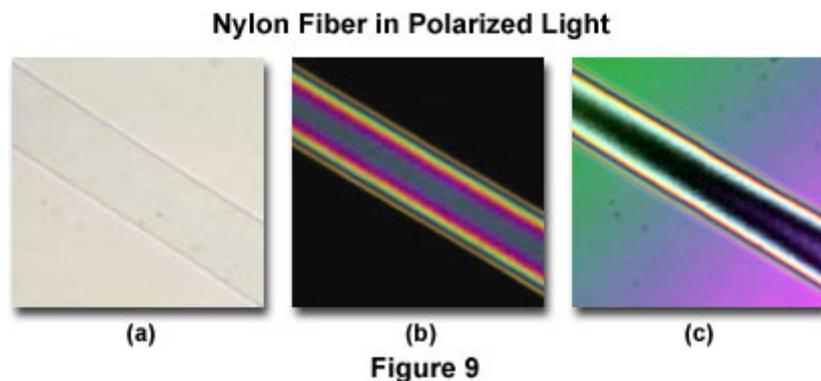
During the solidification of polymer melts there may be some organization of the polymer chains, a process that is often dependent upon the annealing conditions. When nucleation occurs, the synthetic polymer chains often arrange themselves tangentially and the solidified regions grow radially. These can be seen in crossed polarized illumination as white regions with the black extinction crosses. When these spherulites impinge, their boundaries become polygonal. This can be clearly seen in crossed polars but not under plane-polarized light.



The addition of the whole wave plate (Figure 8(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 Figure 8(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. Figure 8(c) illustrates a birefringent columnar-hexatic liquid crystalline phase exhibited by DNA at very high concentrations (exceeding 300 milligrams/milliliter).

**Nylon Fibers** - Observations under plane-polarized light (Figure 9(a)) reveal refractive index differences between the fiber and the mountant and the presence of opacifying titanium dioxide particles. The image under crossed polars (Figure 9(b)) shows third order polarization colors and their distribution across the fibers indicates that this is a cylindrical and not a lobate fiber useful in predicting mechanical strength. The use of the quartz wedge (Figure 9(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.

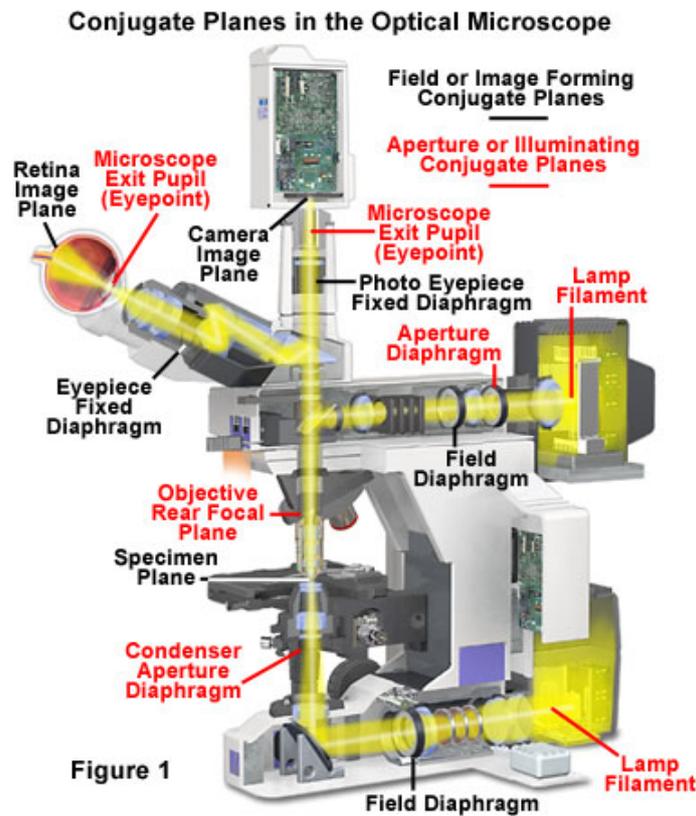
## **Basic Concepts and Formulas in Microscopy**

In order to realize the full potential of the optical microscope, one must have a firm grasp of the fundamental physical principles surrounding its operation. Important topics for understanding the microscope, such as resolution, numerical aperture, depth of field, image brightness, objective working distance, field of view, conjugate planes, and the useful magnification range, are discussed in the review articles linked below.

### **1. Conjugate Planes in Optical Microscopy**

In a properly focused and aligned optical microscope, a review of the geometrical

properties of the optical train demonstrates that there are two sets of principal conjugate focal planes that occur along the optical pathway through the microscope. One set consists of four **field** planes and is referred to as the field or **image-forming** conjugate set, while the other consists of four **aperture** planes and is referred to as the **illumination** conjugate set. Each plane within a set is said to be conjugate with the others in that set because they are simultaneously in focus and can be viewed superimposed upon one another when observing specimens through the microscope.

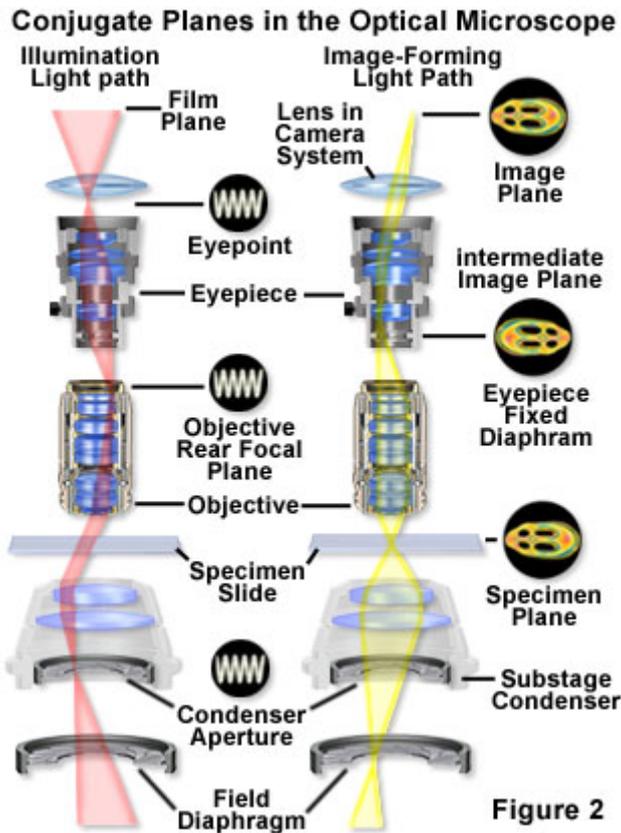


Presented in Figure 1 is a cutaway diagram of a modern microscope (a Nikon Eclipse E600), which illustrates the strategic location of optical components comprising the two sets of conjugate planes in the optical pathways for both transmitted and incident (reflected or epi) illumination modes. Components that reside in the field set of conjugate planes are described in black text, while those comprising the aperture set are described in red text. Note that conjugate planes are illustrated for both observation and digital imaging (or photomicrography) modes. Table 1 lists the elements that make up each set of conjugate planes, including alternate nomenclature (listed in parentheses) that has often been employed and may be encountered in the literature. A minor difference exists in the relative location of the field and condenser apertures between the incident and transmitted modes of illumination, which will be explained later.

In normal observation mode (using the eyepieces), the conjugate set of object or field planes can all be simultaneously viewed when the specimen is in focus. This observation mode is referred to as the **orthoscopic** mode, and the image is known as the orthoscopic image. Observing the other conjugate set of aperture or diffraction planes requires the ability to focus on the rear aperture of the objective, which may be accomplished by using an eyepiece telescope in place of an ocular, or a built-in Bertrand lens on microscopes that are so equipped. This observation mode is termed the **conoscopic**, aperture, or diffraction mode and the image observed at the objective rear aperture is known as the conoscopic image. Although the terms orthoscopic and conoscopic are scattered widely throughout the literature, many microscopists favor using normal mode and aperture mode because the latter nomenclature more clearly relates to the operation of the microscope. Planes belonging to the pair of conjugate sets alternate in succession through the optical train from the light source filament to the final microscope image produced on the retina or the image plane of an electronic sensor. A thorough understanding of the relationships between these conjugate plane sets, and their location within the microscope, is essential in understanding image formation and carrying out correct adjustment of illumination. In addition, the location of principal conjugate planes is often a key factor in the proper placement of optical components such as phase plates, differential interference contrast (DIC) Wollaston prisms, polarizers, modulators, filters, or gratiules.

**Table 1. Conjugate Focal Planes**

Aperture or Illuminating Conjugate Plane Set	Field or Image-Forming Conjugate Plane Set
Microscope Exit Pupil: Eye Iris Diaphragm, Ramsden Disc, and Eyepoint	Retina of the Eye Camera Image Plane
Objective Rear Focal Plane (Objective Rear Aperture)	Intermediate Image Plane (Eyepiece Fixed Diaphragm)
Condenser Aperture Diaphragm (Condenser Front Focal Plane)	Specimen Plane (Object Plane)
Lamp Filament	Field Diaphragm (Field Stop) (Köhler Diaphragm)



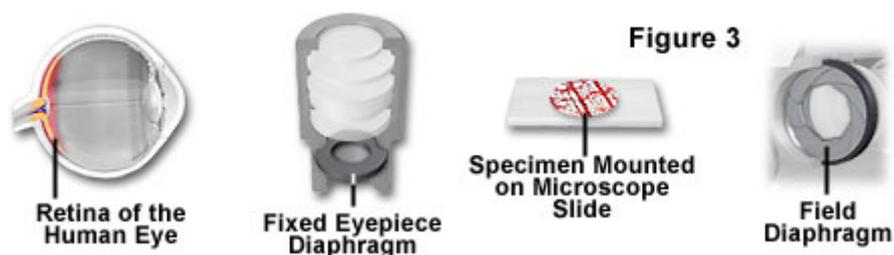
The imaging and illumination ray paths through a microscope adjusted for Köhler illumination are presented in Figure 2, with the focal conjugates of each plane set indicated by crossover points of the ray traces. Illustrated diagrammatically in the figure is the reciprocal nature of the two sets of conjugate planes that occur in the microscope. The optical relationship between the conjugate plane sets is based upon the fact that, in the illuminating ray path (shown in red), the spherical wave fronts converge and are brought into focus onto the aperture planes, while in the imaging ray path (shown in yellow), the spherical waves converge into focused rays in the field planes. Light rays that are focused in one set of conjugate planes are nearly parallel when passing through the other set of conjugate planes. The reciprocal relationship between the two sets of conjugate planes determines how the two ray paths fundamentally interact in forming an image in the microscope, and it also has practical consequences for operation of the microscope.

Illumination is perhaps the most critical factor in determining the overall performance of the optical microscope. The full aperture and field of the instrument is usually best achieved by adjusting the illumination system following the principles first introduced by August Köhler in the late nineteenth century. It is under the conditions of Köhler

illumination that the requirements are met for having two separate sets of conjugate focal planes, field planes and aperture planes, in precise physical locations in the microscope. The details of adjusting a given microscope to satisfy the Köhler illumination conditions depend to some extent upon how the individual manufacturer meets the requirements, and are not discussed here.

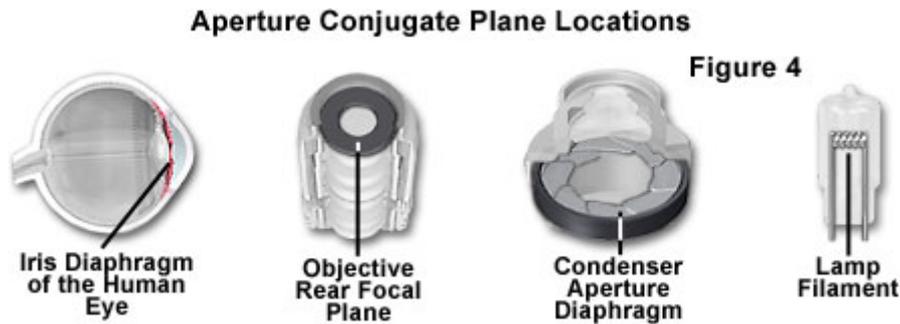
The basic requirements of Köhler illumination are very simple. A collector lens on the lamp housing is required to focus light emitted from the various points on the lamp filament at the front aperture of the condenser while completely filling the aperture. Simultaneously, the condenser must be focused to bring the two sets of conjugate focal planes (when the specimen is also focused) into specific locations along the optical axis of the microscope. Meeting these conditions will result in a bright, evenly illuminated specimen plane, even with an inherently uneven light source such as a tungsten-halogen lamp filament (the filament will not be in focus in the specimen plane). With the specimen and condenser in focus, the focal conjugates will be in the correct position so that resolution and contrast can be optimized by adjusting the field and condenser aperture diaphragms.

### Image-Forming Conjugate Plane Locations



The concept that specific planes in the optical path of the microscope are conjugate indicates that they are equal. That is, whatever appears in focus in one plane of a conjugate set will appear in focus in all the other planes belonging to the same set. On the other hand, the reciprocal nature of the two sets of microscope conjugate planes requires that an object appearing in focus in one set of planes, will not be focused in the other set. The existence of two interrelated optical paths and two sets of image planes characterize Köhler illumination and is the foundation that allows the various adjustable diaphragms and aperture stops in the microscope to be used to control both the cone angle of illumination, and the size, brightness and uniformity of illumination of the field of view. The planes belonging to the field set are sometimes referred to as the **field-limiting** planes because a diaphragm placed in any one of these planes will limit the diameter of the image field. Figure 3 illustrates each of these planes, which will be focused and coincident with the specimen image. The aperture planes may be

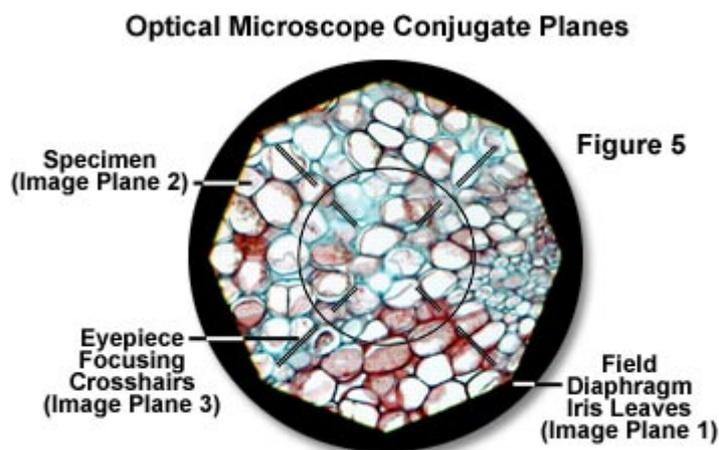
considered **aperture-limiting** because the numerical aperture of the optical system can be controlled by fixed or adjustable (iris) diaphragms inserted at any of these positions. Components containing the set of aperture planes, which are not in focus with the specimen image, are presented (removed from their locations in the microscope) in Figure 4.



A microscopist may not be aware that in the normal observation mode, the specimen image is actually a combined view of four in-focus conjugate image planes (including the specimen plane) whose optical characteristics are determined or modulated by another set of four out-of-focus aperture planes. In a properly adjusted microscope, this fact can be easily ignored. However, the mechanism of the image formation becomes much more apparent, and of immediate practical interest, if there is something seriously wrong with the image. Knowledge of the location and image-forming function of each of the conjugate image and aperture planes is critical in troubleshooting problems that arise in the microscope image. It is also necessary for proper placement and use of filters, stops, phase rings, DIC prisms, and other optical components so that they do not introduce problems. Modern microscope design takes into consideration the location of the conjugate planes and the need for the microscopist to have access to them.

One of the more common ways in which the conjugate nature of the various field planes (including the specimen) and aperture planes (including the illumination source) is exploited, is in the placement of filters and other illumination-modifying optical components. These optical elements are likely to be contaminated with dust or fingerprints, due to frequent handling, and are often not optically designed to be included with the objective, oculars, and other elements of the optical path that are intended to form the focused final image. Therefore, auxiliary components should never be placed in any plane conjugate with the specimen, because any defects, dust, or debris may appear in the final image of the specimen formed at the retina or at a camera imaging plane.

In contrast, measurement graticules, scales, pointers, and other devices that are intended to be in focus and registered on the specimen must be placed at one of the field conjugate planes in order to be imaged with the specimen. For practical reasons, most of the commercially available measuring graticules are designed to be placed at the eyepiece field stop, coincident with the real intermediate image plane. Figure 5 illustrates a microscope viewfield containing three simultaneously focused conjugate planes that will appear, in focus, on the retina of the eye or the imaging plane of a camera. Phase plates, and their corresponding annuli, are intended to control the light path (not to appear in the specimen image), and are placed at conjugate planes in the aperture series. The phase plate is usually placed at the rear aperture (at or very near the rear focal plane) of the objective, so placing the correct annulus at the previous conjugate plane of the aperture series (the front aperture of the substage condenser) will ensure that the images of the two components are superimposed in the illumination optical path. Likewise, Wollaston prisms, which shear and recombine light beams in differential interference contrast microscopy, are placed in the condenser front focal plane and objective rear focal plane for the same reason. Polarizers and analyzers, utilized in polarized light and DIC microscopy, are generally placed far away from either conjugate plane set to avoid introducing contaminating dust or fingerprints into the specimen image or degrading the illumination conditions.



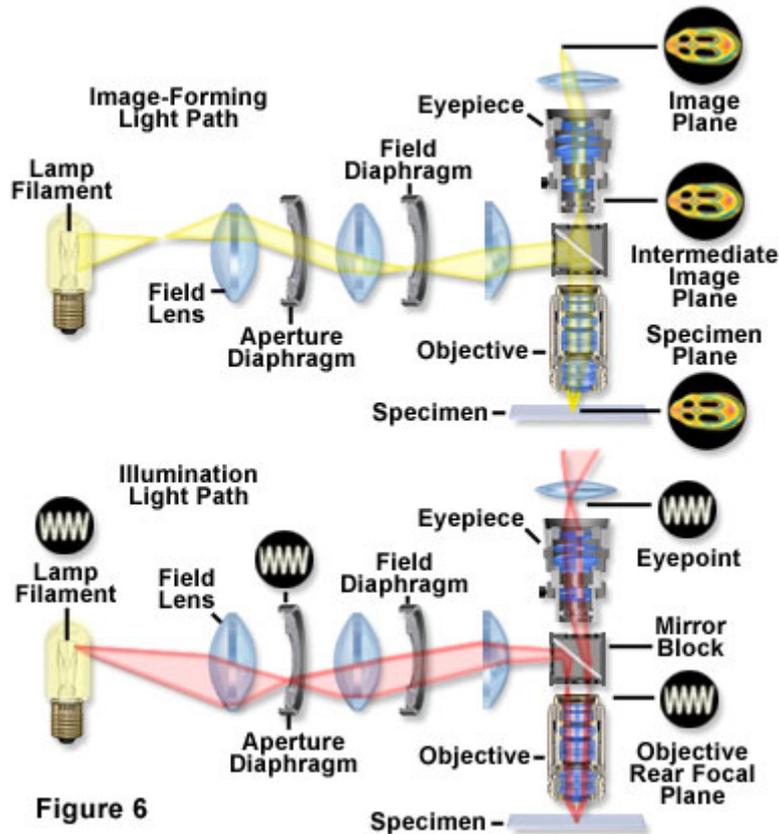
If problems arise that affect the image quality obtained in the microscope, two avenues should be explored when attempting to correct them. In the normal microscope view through the eyepieces, the conjugate field planes are observed in focus. If dust or other imperfections appear distinct and in sharp focus with the image, then the source of the problem is probably one of the glass components located in or very near the field planes of the microscope optical path. The most likely components to contain dust and

debris (that can be observed with the specimen) are the specimen slide itself, the eye lenses of the eyepieces, the objective front lens, the condenser top lens, graticules, and any lenses near the field diaphragm. Often, it is a simple matter to rotate the eyepieces, objective, and condenser and determine if the offending debris rotates in the viewfield, thereby confirming its location.

If this technique does not identify the problem, then the best way to continue troubleshooting is by observing the rear focal plane of the objective to evaluate possible obstructions or misalignments in the illumination path. This aperture, or conoscopic, view is obtained by replacing a normal eyepiece with a specialized eyepiece telescope or using a Bertrand lens (built into the microscope observation head). Although, it is possible to simply remove an eyepiece and peer down the observation tube at the rear aperture of the objective, the image is too small to easily evaluate without a telescope. Observing the aperture planes in this manner will reveal any obstructions such as poorly-centered lenses, dirt or contaminants in the lenses, illumination irregularities such as a poorly centered lamp filament, air bubbles that might be present in immersion oil, and improper adjustment of the condenser diaphragm. The condenser aperture diaphragm determines the effective numerical aperture of the objective-condenser combination, and should normally be adjusted to fill about 70 to 80 percent of the objective rear aperture with light, as an optimum compromise between maximum resolution, depth of field, and adequate contrast.

As stated previously, use of the incident (reflected light) illumination mode requires a minor change in the arrangement of the field and aperture diaphragms in the microscope. This adjustment is necessary because, with incident illumination, the objective plays a dual role and also functions as the condenser, which requires an aperture diaphragm that does not lie in the imaging ray path between the objective lens and the eye or camera. To meet this requirement, lenses in the illumination system are employed to create a conjugate plane between the lamp and the field diaphragm for placement of an aperture diaphragm that is conjugate with the lamp filament. The aperture diaphragm is then projected by relay lenses and mirrors into the rear focal plane (exit pupil) of the objective where these two conjugate planes become coincident. Figure 6 illustrates the illuminating ray path (shown in red) and the imaging ray path (shown in yellow) in an incident light system.

### Conjugate Planes in the Incident Light Optical System



The best image quality can be achieved in the microscope only with a thorough understanding of the central role played by the two sets of reciprocal conjugate focal planes. For optimum results, the microscopist must be familiar with their interrelationships and where they are located in the microscope when the conditions required for Köhler illumination are met. With this understanding, the primary optical components of the microscope, as well as any accessories that might be added, can be utilized in a manner that exploits the reciprocal nature of the conjugate plane sets to maximum benefit.

## 2. Microscope Alignment for Köhler Illumination

Perhaps one of the most misunderstood and often neglected concepts in optical microscopy is proper configuration of the microscope with regards to illumination, which is a critical parameter that must be fulfilled in order to achieve optimum performance. The intensity and wavelength spectrum of light emitted by the illumination source is of significant importance, but even more essential is that light emitted from various locations on the lamp filament be collected and focused at the

plane of the condenser aperture diaphragm. This interactive tutorial reviews both the filament and condenser alignment procedures necessary to achieve Köhler illumination.

The tutorial initializes with a randomly selected specimen image appearing in the virtual microscope viewport and a variable amount of illumination passing through the optical train, which has an intensity level dependent upon the (randomized) initialization state of the lamp filament. Two windows are utilized by the tutorial, and they can be accessed (toggled) using the **Filament Alignment** and **Condenser Alignment** radio buttons. The virtual microscope is assumed to be using a 10x objective to image the specimen selected either randomly at initialization or by using the **Choose A Specimen** pull-down menu. The **Reset** button can be used to re-initialize the tutorial (choose a new specimen and lamp filament position) without reloading the browser.

In order to operate the tutorial, first select the **Filament Alignment** radio button to display the lamp filament in the microscope viewport. The **Filament Control** set of (three) sliders can be employed to adjust the lamp **Intensity** (ranging from zero to 12 volts), **Focus** (position of the lamp along the optical axis), and the **Rotation** axis of the lamp with regard to the lamphouse. In addition, the **Filament Position** sliders translate the filament laterally along the **x** and **y** axes of the virtual microscope.

Once the lamp filament has been centered, focused, and brought to an operating potential of approximately 9.0 volts, click on the **Condenser Alignment** radio button to view the specimen and condenser adjustment control sliders. If the lamp filament has been properly adjusted, the specimen should be evenly illuminated regardless of the fine focus state, condenser height, or field diaphragm opening size. To align the condenser, first focus the specimen using the **Specimen Fine Focus** slider, and then use the **Condenser Height** slider to bring the field diaphragm iris leaves into focus. Next, use the **Condenser Lateral Adjustment** sliders to translate the field diaphragm iris opening to the center of the viewport. Finally, use the **Diaphragm Opening Size** slider to open the field diaphragm to its maximum size. If the diaphragm opens off-center, use the **x-translation** and **y-translation** sliders to bring the opening into the center of the field. Alternatively, the mouse cursor can be placed in the small window (containing a set of crosshairs) and used to drag the image of the field diaphragm (appearing as a white circle) into the center. After the filament has been properly aligned and the virtual microscope adjusted for Köhler illumination, the **Condenser Aperture** slider can be utilized to simulate how varying the numerical aperture affects specimen contrast and resolution.

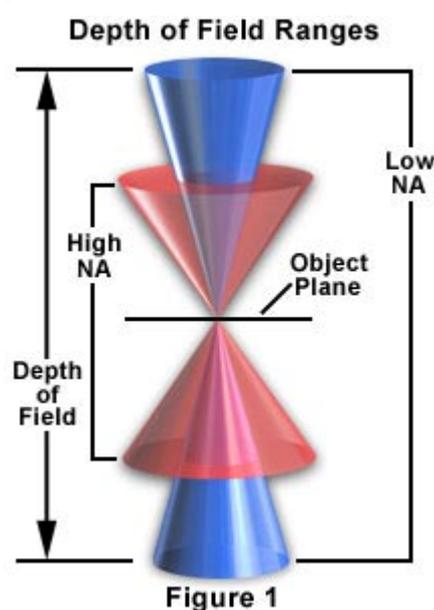
The currently accepted method of microscope illumination was first described by Dr. August Köhler in the late 1800s, and is still widely (almost exclusively) employed for

modern microscopes over 100 years later. Köhler's technique requires a **collector lens** in or near the lamphouse that can be adjusted to focus an image of the lamp filament at the front focal plane of the condenser where the aperture diaphragm resides. If the lamp filament image is properly centered and completely fills the aperture, then illumination of the specimen plane is bright and even. In order to ensure that the filament image appears in the condenser focal plane, the height of the condenser itself must often be adjusted (a technique reviewed in the tutorial). This critical adjustment brings two sets of conjugate focal planes (referred to as the **field** set and the **aperture** set) into precise physical locations within the microscope optical train, and maximizes the performance of the instrument.

### 3. Depth of Field and Depth of Focus

The depth of field is the thickness of the specimen that is acceptably sharp at a given focus level. In contrast, depth of focus refers to the range over which the image plane can be moved while an acceptable amount of sharpness is maintained. The two concepts are often incorrectly used interchangeably when referring to the depth of field of a microscope objective.

When considering resolution in optical microscopy, a majority of the emphasis is placed on point-to-point **lateral** resolution in the plane perpendicular to the optical axis (Figure 1). Another important aspect to resolution is the axial (or **longitudinal**) resolving power of an objective, which is measured parallel to the optical axis and is most often referred to as depth of field.



Axial resolution, like horizontal resolution, is determined only by the numerical

aperture of the objective (Figure 2), with the eyepiece merely magnifying the details resolved and projected into the intermediate image plane. Just as in classical photography, depth of field is determined by the distance from the nearest object plane in focus to that of the farthest plane also simultaneously in focus. In microscopy depth of field is very short and usually measured in units of microns. The term depth of focus, which refers to image space, is often used interchangeably with depth of field, which refers to object space.

This interchange of nomenclature can lead to confusion, especially when the terms are both used specifically to denote depth of field in microscope objectives. The geometric image plane might be expected to represent an infinitely thin section of the specimen, but even in the absence of aberrations, each image point is spread into a diffraction figure that extends above and below this plane. The Airy disk, a basic unit of the diffraction pattern produced by the microscope objective, represents a section through the center of the intermediate image plane. This increases the effective in-focus depth of the Z-axis Airy disk intensity profile that passes through slightly different specimen planes.

Table 1. Depth of Field and Image Depth

<b>Magnification</b>	<b>Numerical Aperture</b>	<b>Depth of Field (mm)</b>	<b>Image Depth (mm)</b>
<b>4x</b>	<b>0.10</b>	<b>55.5</b>	<b>0.13</b>
<b>10x</b>	<b>0.25</b>	<b>8.5</b>	<b>0.80</b>
<b>20x</b>	<b>0.40</b>	<b>5.8</b>	<b>3.8</b>
<b>40x</b>	<b>0.65</b>	<b>1.0</b>	<b>12.8</b>
<b>60x</b>	<b>0.85</b>	<b>0.40</b>	<b>29.8</b>
<b>100x</b>	<b>0.95</b>	<b>0.19</b>	<b>80.0</b>

Depth of focus varies with numerical aperture and magnification of the objective, and under some conditions, high numerical aperture systems (usually with higher magnification power) have deeper focus depths than do those systems of low numerical aperture, even though the depth of field is less (see Table 1). This is particularly important in photomicrography because the film emulsion or digital camera sensor must be exposed or illuminated in a plane that falls within the focus region. Small errors made to focus at high magnification are not as critical as those made with very low magnification objectives. Table 1 presents calculated variations in the depth of field and

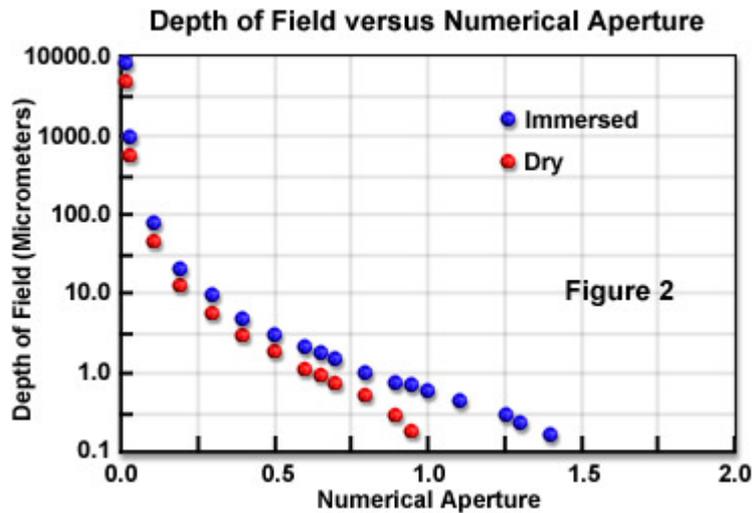
image depth in the intermediate image plane in a series of objectives with increasing numerical aperture and magnification.

At high numerical apertures of the microscope, depth of field is determined primarily by wave optics, while at lower numerical apertures, the geometrical optical **circle of confusion** dominates the phenomenon. Using a variety of different criteria for determining when the image becomes unacceptably sharp, several authors have proposed different formulas to describe the depth of field in a microscope. The total depth of field is given by the sum of the wave and geometrical optical depths of fields as:

$$d_{\text{tot}} = \frac{\lambda_0 n}{\text{NA}^2} + \frac{n}{\mathbf{M} \cdot \text{NA}} e$$

Where **d(tot)** represents the depth of field, **l** is the wavelength of illuminating light, **n** is the refractive index of the medium (usually air (1.000) or immersion oil (1.515)) between the coverslip and the objective front lens element, and **NA** equals the objective numerical aperture. The variable **e** is the smallest distance that can be resolved by a detector that is placed in the image plane of the microscope objective, whose lateral magnification is **M**. Using this equation, depth of field (**d(tot)**) and wavelength (**l**) must be expressed in similar units. For example, if **d(tot)** is to be calculated in micrometers, **l** must also be formulated in micrometers (700 nanometer red light is entered into the equation as 0.7 micrometers). Notice that the diffraction-limited depth of field (the first term in the equation) shrinks inversely with the square of the numerical aperture, while the lateral limit of resolution is reduced in a manner that is inversely proportional to the first power of the numerical aperture. Thus, the axial resolution and thickness of optical sections that can be attained are affected by the system numerical aperture much more so than is the lateral resolution of the microscope.

The human eye can normally accommodate from infinity to about 25 centimeters, so that the depth of field can considerably greater than that given by the equation above when one observes the microscope image through the eyepieces. On the other hand, a video sensor or photographic emulsion lies in a thin fixed plane so that the depth of field and axial resolution using those sensors are given by the parameters in the equation. In these cases, the axial resolution is defined by convention as one-quarter of the distance between the first minima, above and below focus, along the axis of the three-dimensional diffraction image produced by the objective.

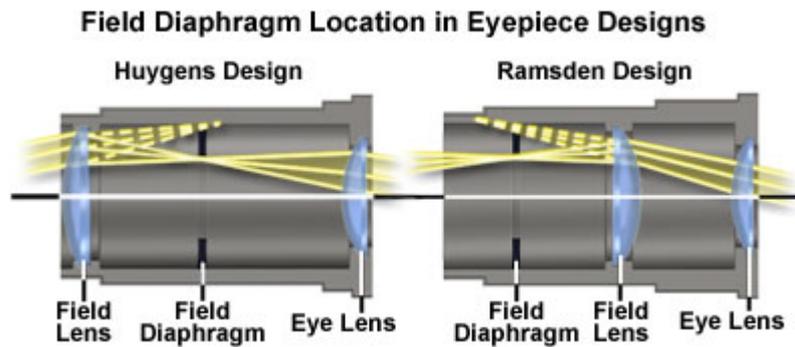


These values for the depth of field, and the distribution of intensities in the three-dimensional diffraction pattern, are calculated for incoherently illuminated (or emitting) point sources where the numerical aperture of the condenser is greater than or equal to that of the objective. In general, the depth of field increases, up to a factor of 2, as the coherence of illumination increases (as the condenser numerical aperture approaches zero). However, the three-dimensional point spread function (**PSF**) with partially coherent illumination can depart in complex ways from that so far discussed when the aperture function is not uniform. In a number of phase-based, contrast-generating modes of microscopy, the depth of field may turn out to be unexpectedly shallower than that predicted from the equation above and may yield extremely thin optical sections.

In digital and video microscopy, the shallow focal plane in the target of the camera tube or CCD, the high contrast achievable at high objective and condenser numerical apertures, and the high magnification of the image displayed on the monitor all contribute to reducing the depth of field. Thus, with video, we can obtain very sharp and thin **optical sections**, and can define the focal level of a thin specimen with very high precision.

#### 4. Field of View

The diameter of the field in an optical microscope is expressed by the **field-of-view number**, or simply the **field number**, which is the diameter of the view field in millimeters measured in the intermediate image plane. In most cases, the eyepiece field diaphragm opening diameter determines the view field size.



**Figure 1**

In most cases, the eyepiece field diaphragm opening diameter determines the view field size. The field size in the specimen plane is then defined as the field number divided by the magnification of the objective:

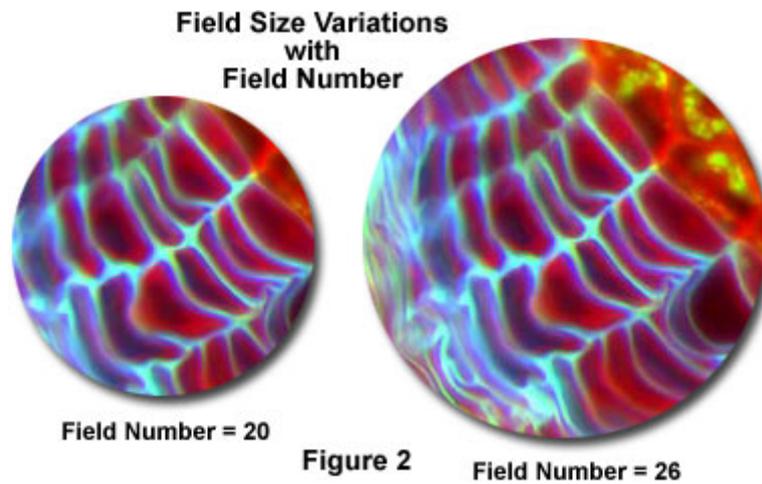
$$\text{Field Size} = \text{Field Number (fn)} \div \text{Objective Magnification (M}_o\text{)}$$

If an auxiliary lens is inserted between the objective and eyepiece, the magnification factor of this lens should also be employed in the equation by multiplication with the objective magnification (prior to the division operation). Although the field number is usually limited by the magnification and field diaphragm (stop) size of the eyepiece, there is clearly a limit that is also imposed by the design of the objective lens system. In early microscope objectives, the maximum usable field diameter tended to be about 18 millimeters or considerably less, but with modern plan apochromats and other specialized flat-field objectives, the maximum usable field can sometimes exceed 28 millimeters.

In modern microscope eyepieces, the field diaphragm either precedes the optical system or is located between the lens element groups, as illustrated in Figure 1. This figure presents cutaway diagrams of a Ramsden and Huygens eyepiece showing ray traces through the field lens, eye lens, and field diaphragm. The Ramsden eyepiece (illustrated on the right in Figure 1) has an optical system consisting of two plano-convex lenses having a fixed separation distance according to their focal lengths. The first lens is termed the **field lens** because it is closer to the plane where the intermediate image is formed in the microscope. The field diaphragm is located between the tube opening and the field lens in the Ramsden eyepiece design.

In all modern eyepiece designs, the field lens is placed a sufficient distance away from the intermediate image plane to ensure that dust or other surface debris and defects on the lens surface are not visualized along with the specimen. The top lens in both eyepiece designs is referred to as the **eye lens** because it is closest to the eye of the

observer.



The Huygens eyepiece, illustrated on the left in Figure 1, is designed with the field lens positioned before the intermediate image plane, which coincides with the field diaphragm. Unlike the Ramsden eyepiece, the Huygens design has a focal point (at the intermediate image plane) residing between the eye and field lenses. In this case, the eye lens acts as a loupe to magnify the intermediate image.

The field number of typical eyepieces varies between 6 and 28 millimeters and (in general) decreases with the magnification of the eyepiece. For example, an eyepiece having a magnification of 10x typically has a field number ranging between 16 and 18 millimeters, while a lower magnification eyepiece (5x) has a field number of about 20 millimeters. Presented in Figure 2 is a comparison between the view fields available with similar eyepieces, one having a field number of 20 and the other a field number of 26. Note the greater range of specimen features visible through the eyepiece having the larger field number.

The size of the eyepiece field diaphragm opening is also dependent upon the correction for off-axial aberrations (coma, astigmatism, and lateral chromatic) of the objective. Recent eyepieces feature highly corrected glass that enables **wide-field** designs having field numbers of 26 millimeters and greater.

## **5. Refractive Index (Index of Refraction)**

Refractive index is a value calculated from the ratio of the speed of light in a vacuum to that in a second medium of greater density. The refractive index variable is most commonly symbolized by the letter **n** or **n'** in descriptive text and mathematical equations.

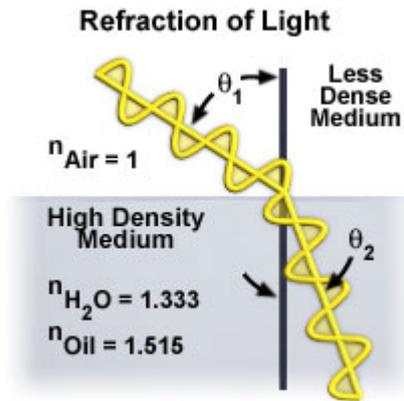


Figure 1

As presented in the figure above, a wavefront incident upon a plane surface separating two media is refracted upon entering the second medium if the incident wave is oblique to the surface. The incident angle ( $q(1)$ ) is related to the refraction angle ( $q(2)$ ) by the simple relationship known as **Snell's law**:

$$n_1 \cdot \sin(q_1) = n_2 \cdot \sin(q_2)$$

Where  $n$  represents the refractive indices of material 1 and material 2 and  $q$  are the angles of light traveling through these materials with respect to the normal. There are several important points that can be drawn from this equation. When  $n(1)$  is greater than  $n(2)$ , the angle of refraction is always smaller than the angle of incidence. Alternatively when  $n(2)$  is greater than  $n(1)$  the angle of refraction is always greater than the angle of incidence. When the two refractive indices are equal ( $n(1) = n(2)$ ), then the light is passed through without refraction.

In optical microscopy, refractive index is an important variable in calculating numerical aperture, which is a measure of the light-gathering and resolving power of an objective. In most instances, the imaging medium for microscopy is air, but high-magnification objectives often employ oil or a similar liquid between the objective front lens and the specimen to improve resolution. The numerical aperture equation is given by:

$$NA \text{ (numerical aperture)} = n \cdot \sin(q)$$

where  $n$  is the refractive index of the imaging medium and  $q$  is the angular aperture of the objective. It is obvious from the equation that increasing the refractive index by replacing the imaging medium from air (refractive index = 1.333) with a low-dispersion oil (refractive index = 1.515) dramatically increases the numerical aperture.

Snell's law was originally defined by the relationship between the incident angles and the ratio of the velocities of light in the two media. The **refractive index** or **index of refraction** is the ratio between the velocity of light ( $c$ ) in free space (for all practical

purposes, either air or a vacuum) and its velocity  $n$  in a particular medium:

$$n = c/v$$

As the refractive index of a material increases, the greater the extent to which a light beam is deflected (or refracted) upon entering or leaving the material. The refractive index of a medium is dependent (to some extent) upon the frequency of light passing through, with the highest frequencies having the highest values of  $n$ . For example, in ordinary glass the refractive index for violet light is about one percent greater than that for red light. A consequence of this phenomenon is that each wavelength experiences a slightly different degree of refraction when a heterogeneous light beam containing more than one frequency enters or leaves the medium. This effect is termed **dispersion** and is responsible for chromatic aberration in microscope objectives.

## 6. Numerical Aperture

The numerical aperture of a microscope objective is a measure of its ability to gather light and resolve fine specimen detail at a fixed object distance. All modern microscope objectives have the numerical aperture value inscribed on the lens barrel, which allows determination of the smallest specimen detail resolvable by the objective and an approximate indication of the depth of field.

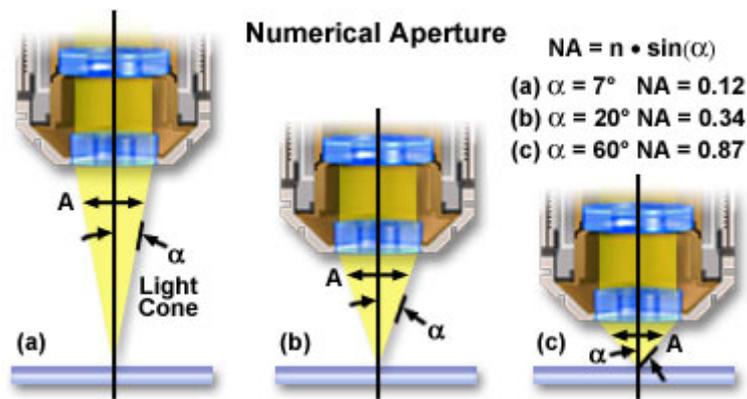
**Numerical Aperture** (also termed **Object-Side Aperture**) is a value (often symbolized by the abbreviation **NA**) originally defined by Abbe for microscope objectives and condensers. It is given by the simple expression:

$$\text{Numerical Aperture (NA)} = n \cdot \sin(m) \text{ or } n \cdot \sin(a)$$

Note: Many authors use the variable  $m$  to designate the one-half angular aperture while others employ the more common term  $a$ , and in some instances,  $q$ .

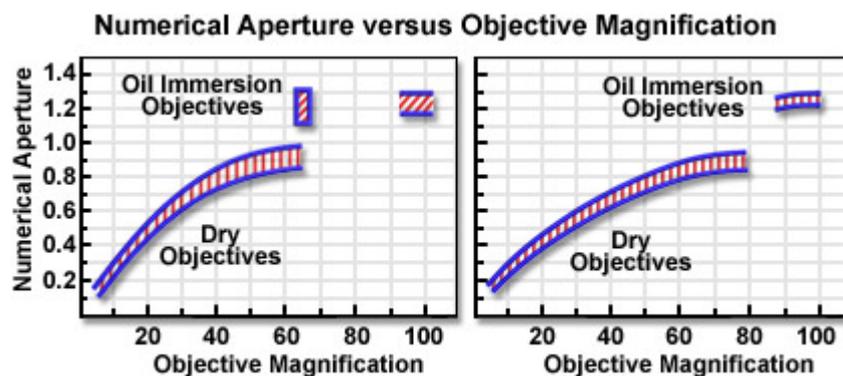
In the numerical aperture equation,  $n$  represents the refractive index of the medium between the objective front lens and the specimen, and  $m$  or  $a$  is the one-half angular aperture of the objective.

The numerical aperture of a microscope objective is a measure of its ability to gather light and resolve fine specimen detail at a fixed object distance. Image-forming light waves pass through the specimen and enter the objective in an inverted cone as illustrated in Figure 1 (above). A longitudinal slice of this cone of light reveals the angular aperture, a value that is determined by the focal length of the objective.



**Figure 1**

In practice, it is difficult to achieve numerical aperture values above 0.95 with dry objectives. Figure 1 illustrates a series of light cones derived from objectives of varying focal length and numerical aperture. As the light cones grow larger, the angular aperture (a) increases from 7° to 60°, with a resulting increase in the numerical aperture from 0.12 to 0.87, nearing the limit when air is utilized as the imaging medium. Higher numerical apertures can be obtained by increasing the imaging medium refractive index (n) between the specimen and the objective front lens. Microscope objectives are now available that allow imaging in alternative media such as water (refractive index = 1.33), glycerin (refractive index = 1.47), and immersion oil (refractive index = 1.51). The numerical aperture of an objective is also dependent, to a certain degree, upon the amount of correction for optical aberration.



**Figure 2**

Highly corrected objectives tend to have much larger numerical apertures for the respective magnification as illustrated in Figure 2 and Table 1 below. If we take a series of typical 10x objectives as an example, we see that for flat-field corrected plan objectives, numerical aperture increases correspond to enhanced correction for chromatic and spherical aberration: plan achromat, N.A. = 0.25; plan fluorite, N.A. = 0.30; and plan apochromat, N.A. = 0.45.

This feature of increasing numerical aperture across an increasing optical correction factor in a series of objectives of similar magnification holds true throughout the range of magnifications as shown in Table 1. Most manufacturers strive to ensure that their objectives have the highest correction and numerical aperture that is possible for each class of objective.

Table 1. Numerical Aperture versus Optical Correction

Magnification	Plan Achromat (NA)	Plan Fluorite (NA)	Plan Apochromat (NA)
<b>0.5x</b>	<b>0.025</b>	<b>n/a</b>	<b>n/a</b>
<b>1x</b>	<b>0.04</b>	<b>n/a</b>	<b>n/a</b>
<b>2x</b>	<b>0.06</b>	<b>n/a</b>	<b>0.10</b>
<b>4x</b>	<b>0.10</b>	<b>0.13</b>	<b>0.20</b>
<b>10x</b>	<b>0.25</b>	<b>0.30</b>	<b>0.45</b>
<b>20x</b>	<b>0.40</b>	<b>0.50</b>	<b>0.75</b>
<b>40x</b>	<b>0.65</b>	<b>0.75</b>	<b>0.95</b>
<b>40x (oil)</b>	<b>n/a</b>	<b>1.30</b>	<b>1.00</b>
<b>60x</b>	<b>0.75</b>	<b>0.85</b>	<b>0.95</b>
<b>60x (oil)</b>	<b>n/a</b>	<b>n/a</b>	<b>1.40</b>
<b>100x (oil)</b>	<b>1.25</b>	<b>1.30</b>	<b>1.40</b>
<b>150x</b>	<b>n/a</b>	<b>n/a</b>	<b>0.90</b>

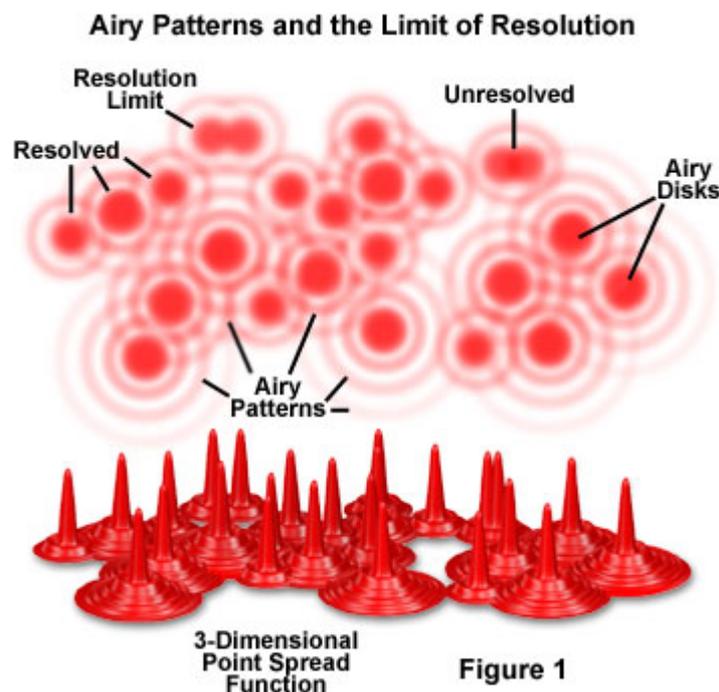
A majority of objectives in the magnification range between 60x and 100x (and higher) are designed for use with immersion oil. By examining the numerical aperture equation presented above, we find that the highest theoretical numerical aperture obtainable with common immersion oils is 1.51 (when  $\sin(\alpha) = 1$ ). In practice, however, most oil immersion objectives have a maximum numerical aperture of 1.4, with the most common numerical apertures ranging from 1.0 to 1.35.

Note: Unless specified by image-side, the term **numerical aperture** refers to the object side.

## 7. Resolution

The resolving power of a microscope is the most important feature of the optical system and influences the ability to distinguish between fine details of a particular specimen. As discussed in this section, the primary factor in determining resolution is the objective numerical aperture, but resolution is also dependent upon the type of specimen, coherence of illumination, degree of aberration correction, and other factors such as contrast enhancing methodology either in the optical system of the microscope or in the specimen itself.

The resolution of an optical microscope is defined as the shortest distance between two points on a specimen that can still be distinguished by the observer or camera system as separate entities. An example of this important concept is presented in the figure below (Figure 1), where point sources of light from a specimen appear as Airy diffraction patterns at the microscope intermediate image plane.



The limit of resolution of a microscope objective refers to its ability to distinguish between two closely spaced Airy disks in the diffraction pattern (noted in the figure). Three-dimensional representations of the diffraction pattern near the intermediate image plane are known as the **point spread function**, and are illustrated in the lower portion of Figure 1. The specimen image is represented by a series of closely spaced point light sources that form Airy patterns and is illustrated in both two and three dimensions.

Resolution is a somewhat subjective value in optical microscopy because at high magnification, an image may appear unsharp but still be resolved to the maximum

ability of the objective. Numerical aperture determines the resolving power of an objective, but the total resolution of the entire microscope optical train is also dependent upon the numerical aperture of the substage condenser. The higher the numerical aperture of the total system, the better the resolution.

Correct alignment of the microscope optical system is also of paramount importance to ensure maximum resolution. The substage condenser must be matched to the objective with respect to numerical aperture and adjustment of the aperture iris diaphragm for accurate light cone formation and specimen illumination. The wavelength spectrum of light used to image a specimen is also a determining factor in the degree of resolution afforded by the microscope. Shorter wavelengths are capable of resolving details to a greater degree than are the longer wavelengths. There are several equations that have been derived to express the relationship between numerical aperture, wavelength, and resolution:

$$\text{Resolution (r)} = \lambda/(2\text{NA}) \quad (1)$$

$$\text{Resolution (r)} = 0.61\lambda/\text{NA} \quad (2)$$

$$\text{Resolution (r)} = 1.22\lambda/(\text{NA}(\text{obj}) + \text{NA}(\text{cond})) \quad (3)$$

Where **r** is resolution (the smallest resolvable distance between two objects), **NA** is a general term for the microscope numerical aperture,  $\lambda$  is the imaging wavelength, **NA(obj)** equals the objective numerical aperture, and **NA(cond)** is the condenser numerical aperture. Notice that equation (1) and (2) differ by the multiplication factor, which is 0.5 for equation (1) and 0.61 for equation (2). These equations are based upon a number of factors (including a variety of theoretical calculations made by optical physicists) to account for the behavior of objectives and condensers, and should not be considered an absolute value of any one general physical law. In some instances, such as confocal and fluorescence microscopy, the resolution may actually exceed the limits placed by any one of these three equations. Other factors, such as low specimen contrast and improper illumination may serve to lower resolution and, more often than not, the real-world maximum value of **r** (about 0.25 **mm** using a mid-spectrum wavelength of 550 nanometers) and a numerical aperture of 1.35 to 1.40 are not realized in practice. The following table (Table 1) provides a list resolution (**r**) and numerical aperture (**NA**) values by objective magnification and correction.

When the microscope is in perfect alignment and has the objectives appropriately matched with the substage condenser, then we can substitute the numerical aperture of the objective into equations (1) and (2), with the added result that equation (3) reduces to equation (2). An important fact to note is that magnification does not appear as a factor in any of these equations, because only numerical aperture and wavelength of the

illuminating light determine specimen resolution.

Table 1. Resolution and Numerical Aperture by Objective Correction

	Objective Type					
	Plan Achromat		Plan Fluorite		Plan Apochromat	
Magnification	N.A.	Resolution (μm)	N.A.	Resolution (μm)	N.A.	Resolution (μm)
<b>4x</b>	<b>0.10</b>	<b>2.75</b>	<b>0.13</b>	<b>2.12</b>	<b>0.20</b>	<b>1.375</b>
<b>10x</b>	<b>0.25</b>	<b>1.10</b>	<b>0.30</b>	<b>0.92</b>	<b>0.45</b>	<b>0.61</b>
<b>20x</b>	<b>0.40</b>	<b>0.69</b>	<b>0.50</b>	<b>0.55</b>	<b>0.75</b>	<b>0.37</b>
<b>40x</b>	<b>0.65</b>	<b>0.42</b>	<b>0.75</b>	<b>0.37</b>	<b>0.95</b>	<b>0.29</b>
<b>60x</b>	<b>0.75</b>	<b>0.37</b>	<b>0.85</b>	<b>0.32</b>	<b>0.95</b>	<b>0.29</b>
<b>100x</b>	<b>1.25</b>	<b>0.22</b>	<b>1.30</b>	<b>0.21</b>	<b>1.40</b>	<b>0.20</b>
<b>N.A. = Numerical Aperture</b>						

As we have mentioned (and can be seen in the equations) the wavelength of light is an important factor in the resolution of a microscope. Shorter wavelengths yield higher resolution (lower values for  $r$ ) and visa versa. The greatest resolving power in optical microscopy is realized with near-ultraviolet light, the shortest effective imaging wavelength. Near-ultraviolet light is followed by blue, then green, and finally red light in the ability to resolve specimen detail. Under most circumstances, microscopists use white light generated by a tungsten-halogen bulb to illuminate the specimen. The visible light spectrum is centered at about 550 nanometers, the dominant wavelength for green light (our eyes are most sensitive to green light). It is this wavelength that was used to calculate resolution values in the Table 1. The numerical aperture value is also important in these equations and higher numerical apertures will also produce higher resolution. The effect of the wavelength of light on resolution, at a fixed numerical aperture (0.95), is listed in Table 2, with longer wavelengths producing lowered degrees of resolution.

Table 2. Resolution versus Wavelength

Wavelength (Nanometers)	Resolution (Micrometers)
<b>360</b>	<b>.19</b>

<b>400</b>	<b>.21</b>
<b>450</b>	<b>.24</b>
<b>500</b>	<b>.26</b>
<b>550</b>	<b>.29</b>
<b>600</b>	<b>.32</b>
<b>650</b>	<b>.34</b>
<b>700</b>	<b>.37</b>

The resolving power of a microscope is the most important feature of the optical system and influences the ability to distinguish between fine details of a particular specimen. As discussed above, the primary factor in determining resolution is the objective numerical aperture, but resolution is also dependent upon the type of specimen, coherence of illumination, degree of aberration correction, and other factors such as contrast enhancing methodology either in the optical system of the microscope or in the specimen itself. In the final analysis, resolution is directly related to the useful magnification of the microscope and the perception limit of specimen detail.

### **8. Useful Magnification Range**

In order to observe fine specimen detail in the optical microscope, the minute features present must be of sufficient contrast and project an intermediate image at an angle that is somewhat larger than the angular resolving power of the human eye. At a selected numerical aperture, when the microscope provides a magnified image that has a magnitude equal to the resolution limit of the human eye, additional magnification beyond this point does not result in the resolution of even smaller specimen detail.

The **range of useful magnification** for an objective/eyepiece combination is defined by the numerical aperture of the microscope optical system. There is a minimum magnification necessary for the detail present in an image to be resolved, and this value is usually rather arbitrarily set as 500 times the numerical aperture (500 x NA) and defined by the equation:

**Useful Magnification (total) = 500 to 1000 • NA (Objective)**

At the other end of the spectrum, the maximum useful magnification of an image is usually set at 1000 times the numerical aperture (1000 x NA) as given by the equation above. Magnifications higher than this value will yield no further useful information or finer resolution of image detail, and will usually lead to image degradation. Table 1

catalogs the common objective/eyepiece combinations that lie in the range of useful magnification.

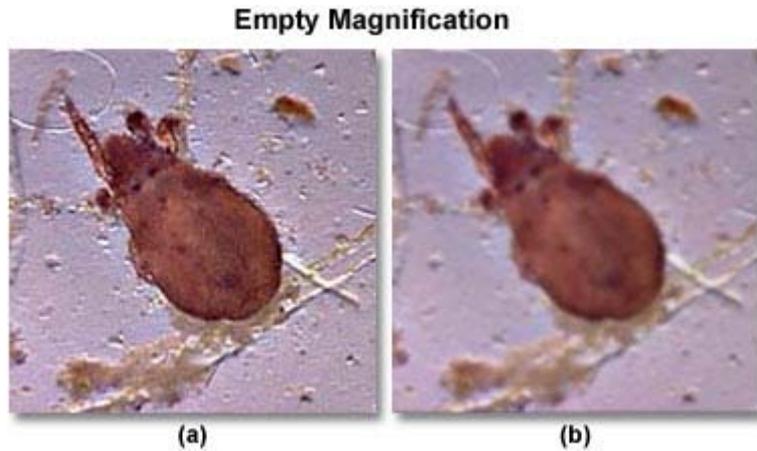


Table 1. Range of Useful Magnification (500-1000 x NA of Objective)

Objective (NA)	Eyepieces				
	10x	12.5x	15x	20x	25x
<b>2.5x</b> <b>(0.08)</b>	---	---	---	<b>x</b>	<b>x</b>
<b>4x</b> <b>(0.12)</b>	---	---	<b>x</b>	<b>x</b>	<b>x</b>
<b>10x</b> <b>(0.35)</b>	---	<b>x</b>	<b>x</b>	<b>x</b>	<b>x</b>
<b>20x</b> <b>(0.55)</b>	<b>x</b>	<b>x</b>	<b>x</b>	<b>x</b>	<b>x</b>
<b>40x</b> <b>(0.70)</b>	<b>x</b>	<b>x</b>	<b>x</b>	<b>x</b>	---
<b>60x</b> <b>(0.95)</b>	<b>x</b>	<b>x</b>	<b>x</b>	---	---
<b>100x</b> <b>(1.42)</b>	<b>x</b>	<b>x</b>	---	---	---

Exceeding the limit of useful magnification causes the image to suffer from the phenomenon of **empty magnification** (illustrated in Figure 1(b)), where increasing magnification through the eyepiece or intermediate tube lens only causes the image to become more magnified with no corresponding increase in detail resolution. In contrast, the image shown in Figure 1(a) was captured using the correct objective and eyepiece combination to effectively utilize the numerical aperture to achieve optimum resolution.

In fact, excessive magnification introduces artifacts, diffraction boundaries, and halos into the image that obscure specimen features and complicate the interpretation of visual observations. Microscope observations are also affected by the sensitivity of the human eye to the intensity and color temperature of the illumination, the age of the observer, the presence of **floaters** in the eye, and whether the eye is rested or fatigued.

For visual observation, the image of the specimen fine structure must be viewed at an angle slightly larger than the resolving power of the human eye. With a microscope having good illumination, the distance between two resolved points in the specimen viewed at the reference visual distance of 250 millimeters is about 0.15 millimeters, corresponding to a **visual acuity** angle of about 2 minutes of arc. This limiting angle is restricted by the separation distance of visual elements in the retina, which are spaced about five microns apart.

To relate the limit of resolution of the eye and the resolving power of the objective, two closely spaced points in the specimen can be considered. If the two points reside at the limit of the objective's resolving power, then:

$$\mathbf{r \text{ (separation distance)} = l/2NA}$$

where **r** is the distance separating the two points, **l** is the wavelength of illumination, and **NA** is the objective numerical aperture. In order to magnify the distance until the specimen points appear to the eye at a separation distance of 0.15 millimeters (representing 2 minutes of arc), we examine the relationship:

$$\mathbf{0.15 \text{ mm} = M \cdot l/2NA}$$

which can be rearranged to:

$$\mathbf{M = (2NA \cdot 0.15)/l}$$

where **M** is the optimum microscope magnification. When the illuminating wavelength is assumed to lie in the green region of the visible light spectrum (550 nanometers or 0.00055 millimeters), we can substitute into the equation:

$$\mathbf{M = (NA \cdot 0.30)/0.00055) = (\text{approximately}) 500 \cdot NA}$$

The result is the minimum magnification for visual observation of the finely spaced specimen detail, which is about 500 times the objective numerical aperture. This discussion applies to specimens having medium contrast, but with specimens of higher contrast the two points can be resolved by higher magnifications even if they are closer to each other. In practice, magnifications deviating considerably from the useful magnification range are often employed. For example, very low magnifications (1x through 4x) are often used to topographically map a specimen (such as a histologically stained thin section) where a wide field of view is desirable in order to quickly note all available specimen features. In many cases, a 2.5x objective may be combined with a

wide field eyepiece at 10x magnification to reveal an area having a diameter of 8 millimeters or greater.

At high magnifications, the limit of useful magnification is sometimes exceeded in order to view the image more comfortably. This is often the case when small particles or organisms are observed and counted at very high numerical apertures and magnifications. Sharpness in the specimen details is then sacrificed, which usually does not interfere with quantitative analysis of the image.

Care should be taken in choosing eyepiece/objective combinations to ensure the optimal magnification of specimen detail without adding unnecessary artifacts. For instance, to achieve a magnification of 250x, the microscopist could choose a 25x eyepiece coupled to a 10x objective. An alternative choice for the same magnification would be a 10x eyepiece with a 25x objective. Because the 25x objective has a higher numerical aperture (approximately 0.65) than does the 10x objective (approximately 0.25), and considering that numerical aperture values define an objective's resolution, it is clear that the latter choice would be the best. If photomicrographs of the same viewfield were made with each objective/eyepiece combination described above, it would be obvious that the 10x eyepiece/25x objective duo would produce photomicrographs that excelled in specimen detail and clarity when compared to the alternative combination.

## **9. Working Distance and Parfocal Length**

Microscope objectives are generally designed with a short free working distance, which is defined as the distance from the front lens element of the objective to the closest surface of the coverslip when the specimen is in sharp focus. In the case of objectives designed to be used without coverslips, the working distance is determined by the linear measurement of the objective front lens to the specimen surface. The parfocal length represents the distance between the specimen plane and the shoulder of the flange by which the objective is supported on the revolving nosepiece.

### Objective Working and Parfocal Distance



Presented in Figure 1 is a schematic illustration of an objective showing the parfocal and working distance length specifications, as well as other descriptions engraved or printed on the objective barrel. In general, the objective working distance decreases as the magnification and numerical aperture both increase, as presented in Table 1 for a highly corrected series of Nikon plan fluorite and plan apochromatic objectives. The current trend is to produce dry objectives having working distances as long as possible, but the demand is somewhat limited by the need for high numerical apertures with their higher resolving power. This often leads manufacturers to a compromise between these two parameters.

Table 1. Common Objective Working Distances

Manufacturer	Correction	Magnification	Numerical Aperture	Working Distance
Nikon	PlanApo	10x	0.45	4.0 mm
Nikon	PlanFluor	20x	0.75	0.35 mm
Nikon	PlanFluor (oil)	40x	1.30	0.20 mm
Nikon	PlanApo (oil)	60x	1.40	0.21 mm
Nikon	PlanApo (oil)	100x	1.40	0.13 mm

Immersion objectives, which operate with a liquid medium of defined refractive index between the front lens element and the coverslip, are more restricted in working distance lengths. If the working distance is too large, maintaining a confluent network of immersion liquid between the objective front lens and specimen can be a problem, leading to introduction of aberrations with subsequent deterioration of the image.

Objectives that have extremely close working distances are spring loaded so the entire front lens assembly will retract when brought into contact with the coverslip. These mounts are often termed **retraction stoppers** and guarantee adequate protection against accidental damage to either the specimen or the objective front lens.

For many applications a long free working distance is highly desirable (and often necessary), and specialized objectives are designed for such use despite the difficulty involved in achieving large numerical apertures and the necessary degree of correction for optical aberrations. Long working distance objectives are particularly useful when examining specimens *in vitro* through thick glass walls and for chemical and metallurgical microscopy, where the objective front lens must be protected against environmental hazards such as heat, caustic vapors, and volatile chemicals by a thick coverslip. The working distance of these objectives often exceeds two to three times that of comparable objectives having the same or a slightly greater numerical aperture. Table 2 lists infinity-corrected Nikon objectives having extra long working distances (**ELWD**) and super long working distances (**SLWD**). Note that working distance decreases with magnification and numerical aperture, but not as dramatically as the objectives listed in Table 1. Also note that the SLWD objectives exhibit significantly longer working distances, but correspondingly lower numerical apertures, than the ELWD series of objectives.

Table 2. Long Working Distance Objectives

Designation	Magnification	Numerical Aperture	Working Distance
<b>ELWD</b>	<b>20x</b>	<b>0.40</b>	<b>11.0 mm</b>
<b>ELWD</b>	<b>50x</b>	<b>0.55</b>	<b>8.7 mm</b>
<b>ELWD</b>	<b>100x</b>	<b>0.80</b>	<b>2.0 mm</b>
<b>SLWD</b>	<b>10x</b>	<b>0.21</b>	<b>20.3 mm</b>
<b>SLWD</b>	<b>20x</b>	<b>0.35</b>	<b>20.5 mm</b>
<b>SLWD</b>	<b>50x</b>	<b>0.45</b>	<b>13.8 mm</b>
<b>SLWD</b>	<b>100x</b>	<b>0.73</b>	<b>4.7 mm</b>

It has become practical with modern manufacturing techniques to considerably improve the mechanical precision of microscope objectives, including their centration and parfocal distance, the distance between the specimen plane and the shoulder of the flange by which the objective lens is supported on the revolving nosepiece (see Figure

1). Thus, on modern research-grade microscopes, the specimen can be kept quite closely in focus (within a micron or so), as well as centered in the field of view, when one turns the revolving nosepiece and switches from one objective to another.

For many years, objectives designed for biological applications from most manufacturers all conformed to an internationally recognized convention, a parfocal distance of 45.0 millimeters, the Royal Microscopical Society (RMS) standard defining the dimension of the thread that supports the objective lens in the nosepiece, and a mechanical tube length of 160 millimeters. Thus, objectives from different manufacturers appeared to be interchangeable. However, with the introduction of infinity-corrected objectives, the convenient interchangeability between objective lenses from different manufacturers has once again disappeared. This is due primarily to different design criteria used to correct for aberrations in the objective and tube lenses and an increased demand for greater flexibility to accommodate the need for ever-greater working distances coupled with higher numerical apertures and increased field size.

Recently, the introduction of the Nikon **CFI-60** optical system, featuring "Chrome Free" objectives, tube lenses, and oculars, has enabled the company to separately correct each component without one being used to achieve correction for another. This system has also introduced a 60-millimeter parfocal length and a 25-millimeter thread diameter for the objectives to replace the RMS thread size.

## **10. Image Brightness**

Regardless of the imaging mode utilized in optical microscopy, image brightness is governed by the light-gathering power of the objective, which is a function of numerical aperture. Just as the brightness of illumination is determined by the square of the condenser working numerical aperture, the image brightness is proportional to the square of the objective numerical aperture.

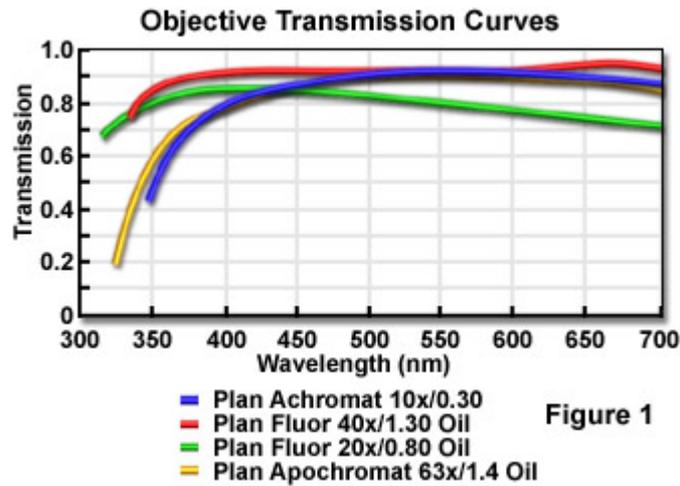


Figure 1

Unlike the situation for the microscope illuminating system, however, image magnification also plays an important role in determining image brightness. In fact, the image brightness is inversely proportional to the square of the lateral magnification:

$$\text{Image Brightness} \propto (\text{NA}/M)^2$$

where **NA** is the objective numerical aperture and **M** is the magnification. The ratio given in the equation above expresses the light-gathering power of the objective in transillumination (note: the case with epi-illumination is somewhat different, as discussed below). Examples of the light-gathering power of selected Nikon objectives having varying degrees of optical correction are listed in Table 1. In general, objectives with high numerical apertures are also better corrected for aberrations. Thus, for the same magnification, higher numerical aperture objectives collect more light, produce a brighter and better-corrected image (see Table 1), and the overall image is better resolved.

It is evident from examining the data in Table 1 that when an objective is used in transillumination, image brightness decreases rapidly as the magnification increases. Utilization of a specific objective for epi-illumination produces increasingly brighter images as the magnification increases, whereas the reverse is often true for the same objective with transmitted light. The terms **F(trans)** and **F(epi)** refer to the light-gathering power of an objective and were calculated according to the following equations:

$$F_{(\text{trans})} = 10^4 \cdot \text{NA}^2/M^2$$

$$F_{(\text{epi})} = 10^4 \cdot (\text{NA}^2/M)^2$$

In theory, the intensity of illumination depends on the square of the condenser numerical aperture and the square of the demagnification of the light source image (in effect, the field diaphragm image becomes brighter as it is made smaller, according to

the square law). The result is that brightness of the specimen image is directly proportional to the square of the objective numerical aperture as it reaches the eyepiece (or camera system), and also inversely proportional to the objective magnification. Therefore, when examining specimens in transmitted light, changing the objective without altering the condenser affects image brightness in response to changes in numerical aperture and magnification.

Table 1. Light-Gathering Power of Selected Objectives

Correction	Magnification	Numerical Aperture	F(trans)	F(epi)
<b>Plan Achromat</b>	<b>10x</b>	<b>0.25</b>	<b>6.25</b>	<b>0.39</b>
<b>Plan Apo</b>	<b>10x</b>	<b>0.45</b>	<b>20.2</b>	<b>4.10</b>
<b>Plan Fluorite</b>	<b>20x</b>	<b>0.50</b>	<b>6.25</b>	<b>1.56</b>
<b>Plan Apo</b>	<b>20x</b>	<b>0.75</b>	<b>14.0</b>	<b>7.90</b>
<b>Plan Achromat</b>	<b>40x</b>	<b>0.65</b>	<b>2.64</b>	<b>1.11</b>
<b>Plan Apo</b>	<b>40x (oil)</b>	<b>1.30</b>	<b>11.0</b>	<b>18.0</b>
<b>Plan Fluorite</b>	<b>60x</b>	<b>0.85</b>	<b>2.01</b>	<b>1.45</b>
<b>Plan Apo</b>	<b>60x (oil)</b>	<b>1.40</b>	<b>5.4</b>	<b>10.6</b>
<b>Plan Apo</b>	<b>100x (oil)</b>	<b>1.40</b>	<b>1.96</b>	<b>3.84</b>
<b>Plan Apo</b>	<b>100x (oil)</b>	<b>1.45</b>	<b>2.10</b>	<b>4.42</b>

In the case of epi-illumination, the same considerations apply, except that the objective also acts as the condenser, and this must be taken into account when considering image brightness. As the objective magnification increases, the light source image is reduced (demagnified) by an equivalent amount, resulting in a brightness level that is less dependent on objective magnification and more dependent on numerical aperture (brightness is governed by the fourth power of numerical aperture in epi-illumination). In practice, the image brightness numbers vary (see Table 1) due to objective rear aperture size differences.

When the light level is limiting, the highest numerical aperture objective should be employed, yet the magnifications of the both the objective and eyepiece should be kept at the lowest level compatible with the desired resolution. In many cases, manufacturers are now providing oil immersion objectives with higher numerical apertures, and correspondingly higher image brightness values, than high-dry counterparts of similar

magnification. For example, the 40x plan apochromatic objective in Table 1 has twice the numerical aperture of the plan achromat 40x dry objective, and produces four times the image brightness in transmitted light. These objectives yield a 16-fold difference in image brightness under epi-fluorescence illumination, with the high numerical aperture oil immersion version producing the brightest images.

The amount of light transmitted through the optical components of the microscope, as a function of incident intensity, is especially critical in fluorescence microscopy. In situations where high resolution fluorescence imaging requires high magnifications with a minimal loss of image brightness, the highest numerical aperture objectives having the greatest degree of light transmission should be employed. As discussed above, overall image brightness decreases rapidly as magnification increases, so the components of a fluorescence microscope should be carefully chosen to maximize the amount of light passing through the optical train.

Fluorescence microscopes that utilize epi-illumination are equipped with objectives that serve the dual purpose of both condenser and objective. Light passing through the excitation filter and reflected from the dichromatic mirror surface in the filter cube is first passed through the objective to form a cone of illumination necessary to excite the specimen. Secondary fluorescence emitted by fluorophores attached to the specimen is then gathered by the same objective lens system and passed back through the dichromatic mirror and barrier filter before being projected into the eyepieces or the imaging system. A high numerical aperture objective acting as a condenser will increase the signal (light) intensity in a manner that is proportional to the square of the numerical aperture. Because the light gathering power of the objective is also proportional to the numerical aperture squared, image brightness will vary as the fourth power of the objective numerical aperture according to the equation:

$$\text{Image Brightness (Fluorescence)} \propto \text{NA}^4/M^2$$

Note that in fluorescence microscopy, brightness is also inversely proportional to the objective magnification squared. Thus, for objectives of identical magnification, image brightness of both the illumination field and the fluorescence image increases dramatically with objective numerical aperture, which is the primary reason manufacturers produce objectives designed with very high numerical apertures for fluorescence microscopy.

The eyepieces employed to observe the specimen further magnify the diffraction-limited image projected into the microscope intermediate image plane, and also serve to decrease the overall observed intensity of the specimen. In fact, image brightness is inversely proportional to the square of the eyepiece magnification, requiring the use of

eyepieces having the lowest possible magnification necessary to conveniently observe specimen fluorescence. It is therefore possible to maximize image brightness in fluorescence microscopy by using the highest available numerical aperture objective coupled to the lowest power eyepieces (although 10x eyepieces are most commonly employed). These remarks apply primarily to large specimen areas that have an even degree of illumination. In the case of sharply focused point sources of light (for example, minute fluorescent spheres), the particle images should be diffraction-limited and have dimensions that are independent of magnification.

In fluorescence microscopy, image brightness is determined by the intensity of illumination, the quantum yield of the fluorophore, and the light-gathering power of the microscope. The greater the intensity of illumination and the higher the quantum yield, the greater the fluorescent signal and the brighter the image becomes until all of the fluorophores are saturated. Likewise in polarized light, image brightness is governed by the illumination intensity and birefringence retardation of the specimen. At values up to a quarter-wavelength of retardation, larger retardations produce a greater birefringence, and thus a stronger signal.

In both of these cases, image brightness is governed by the signal from the specimen, which is a product of the intensity of illumination and the change in light introduced by the specimen. In the case of luminescence, in which the specimens themselves emit light, the brightness of the image is clearly governed by the magnitude of light, or the signal, emitted by the specimen.

In other modes of microscopy, such as phase contrast, differential interference contrast (DIC), darkfield, Hoffman modulation contrast, etc., the intensity of illumination and image contrast can be varied independently. Nevertheless, the signal from the image, namely, the increment of image brightness per unit of variation in an optical parameter (for instance, a difference in path length), would still be determined by the product of the illumination intensity and the contrast produced per unit variation in the optical parameter. To detect small variations in any optical parameter, the microscopist should maximize the signal arising from variations in that particular parameter.

For a given condenser and objective numerical aperture, magnification, and illuminator brightness, the image brightness produced in the microscope can still vary depending on the light transmission through the optical components. The transmission depends on several factors, including absorption by lens elements and cements, reflection losses at the optical interfaces, and transmittances of the lamp jacket, diffusing screen, filters, polarizers, and other auxiliary optical components. Typical

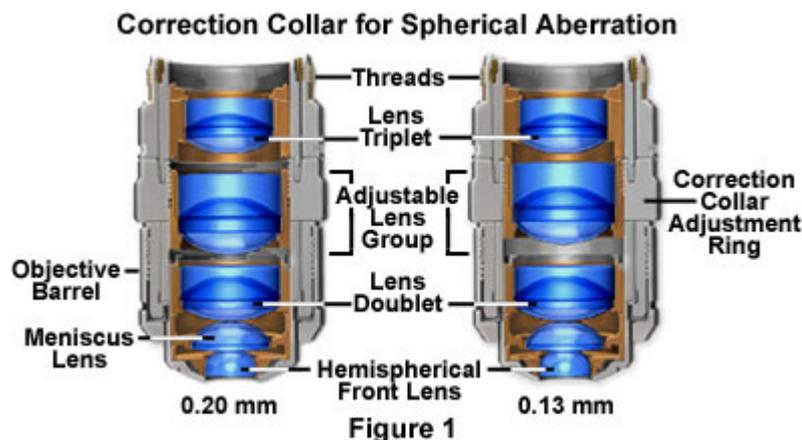
transmission curves for a selected set of high numerical aperture objectives are illustrated in Figure 1. These values are more or less representative of any class of objectives from a particular manufacturer, but even among the specific types for which these curves were measured, the exact transmission values can vary somewhat depending on, for example, the antireflection coating and the batch of glass used in individual lenses.

The transmittance (intensity transmitted as a percentage of incident intensity) of some optical components can be wavelength-dependent even within the visible range, as shown in Figure 1. Also, at wavelengths outside the visible range, the transmittances of lenses, prisms, slides, and the mounting medium for the specimen can drop appreciably. This fact is exemplified by the 20x plan fluor objective transmission curve illustrated in Figure 1, which shows a steady decrease in transmittance as wavelength is increased between 400 and 700 nanometers. Other objectives in the series do not exhibit such a marked degree of wavelength dependence.

### 11. Coverslip Correction

Non-immersion **high-dry** microscope objectives having a numerical aperture exceeding 0.75 are prone to introduction of aberration when imaging through coverslips that deviate from standard thickness and refractive index. To prevent artifacts, many objectives are equipped with correction collars that help compensate for coverslip thickness variations.

For microscope objectives having high numerical apertures, the optical properties and thickness of the medium lying between the front lens element and the specimen critically affect the calculations necessary to satisfy the aplanatic and sine conditions and otherwise to correct for image aberrations.



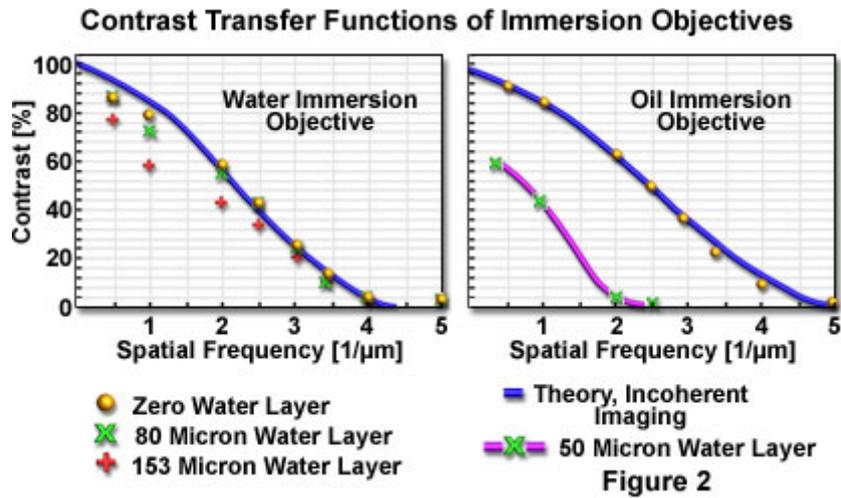
If homogeneous immersion objectives are designed to be used with the refractive indices and dispersion of the immersion oil, coverslip, and medium imbibing the specimen matching that of the objective front lens element, then the calculation is straightforward because all of the media can be considered an extension of the front lens element.

However, with non-immersion objectives, the coverslip can become a source of chromatic aberration, which increases with coverslip thickness and dispersion. Another optical artifact, spherical aberration, is also proportional to the thickness of the coverslip. In designing objectives that are not to be used with homogeneous immersion, one assumes the presence of a standard coverslip and other specific optical media between the front lens element and the specimen. As conditions depart from these designated specifications, spherical aberration and coma increase with the numerical aperture of the objective. This occurs because the difference between the tangent and the sine of the incident angle (which is responsible for departure from the sine condition needed to correct for these aberrations) becomes greater with longer numerical apertures.

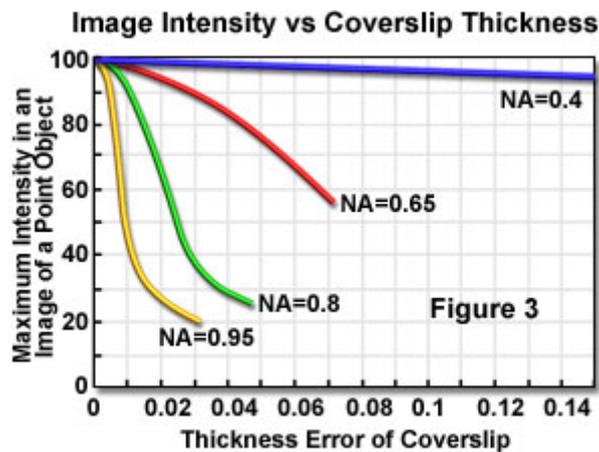
When using oil immersion objectives, it may appear that coverslip thickness is of only limited concern, because its refractive index approximately matches that of the immersion oil. That is true when the specimen is mounted in Canada balsam or other mounting media with refractive indexes similar to that of the coverslip. However, it is no longer true when the specimen is mounted, for example, in physiological saline or other aqueous media whose refractive index is significantly different from that of the coverslip. In these conditions, even focusing through a thin layer of water only 10 microns thick can lead to significant aberrations and a point spread function (**PSF**) that is no longer symmetrical above and below the focal plane. This is because oil immersion objectives are designed assuming that the specimen slide is homogeneously immersed. Unless the specimen region is adjacent to the coverslip (even in an aqueous environment), optical assumptions employed to calculate the aberration corrections are no longer valid.

Once this characteristic behavior of oil immersion objectives was recognized, microscope manufacturers began to produce well-corrected water immersion objectives (around 1994). Plan Apo water immersion objectives equipped with correction collars have a numerical aperture around 1.2, and (even though they have a smaller numerical aperture than their oil immersion counterparts) are able to overcome the inherent limitations of imaging through aqueous solutions (see Figure 2). The latest water immersion objective designs can now focus through approximately 200 microns of aqueous media and still retain excellent correction. Unfortunately, the price of these

objectives is very high, and the image can still degrade when focusing deep within refractile tissue or cell elements.



Contrast transfer values of a 60x/1.2 numerical aperture water immersion and a 60x/1.4 numerical aperture oil immersion objective (both Plan Apochromats), measured with and without layer of water between the coverslip and test gratings are presented in Figure 2. The graphs demonstrate that the water immersion objective performs at the theoretical limit of contrast transfer even with water layers as thick as 153 microns, whereas the contrast transfer function of the oil immersion objective is dramatically reduced and the resolution limit is cut by half when the test grating is imaged through a 50-micron thick water layer. The continuous lines are theoretically calculated contrast transfer functions of aberration-free objective lenses of corresponding numerical aperture at a wavelength of 546 nanometers.



For objectives designed to be used with a standard coverslip thickness and refractive index/dispersion (0.17 millimeters and  $n(D) = 1.515$ ), departure from the standard thickness is not overly critical when the objective has a numerical aperture of 0.4 or less (as presented in Table 1). However, for high numerical aperture non-homogeneous immersion objectives, the problem becomes especially critical so that a departure of coverslip thickness of even a micrometer or two degrades the image with **high-dry** objectives of numerical aperture 0.65 or greater. This concept is illustrated in Figure 3, which presents the calculated maximum intensity in the image of a point object versus the deviation of the coverslip thickness, from the ideal value of 0.17 millimeters, as a function of objective numerical aperture. To compensate for such error, well-corrected, high-dry objectives are equipped with correction collars that can be utilized to adjust the spacing of their intermediate lens elements according to the coverslip thickness. In the same manner, objectives designed for viewing through layers of silicone or plastic, or of different immersion media (water, glycerol, oil), are equipped with correction collars.

Table 1. Performance Reduction with Coverslip Thickness Variation

Numerical Aperture	0.01 mm Deviation	0.02 mm Deviation
<b>0.30</b>	<b>none</b>	<b>none</b>
<b>0.45</b>	<b>none</b>	<b>none</b>
<b>0.70</b>	<b>2 percent</b>	<b>8 percent</b>
<b>0.85</b>	<b>19 percent</b>	<b>57 percent</b>
<b>0.95</b>	<b>55 percent</b>	<b>71 percent</b>

The standard thickness for cover glasses is 0.17 millimeters, which is designated as a number **1½** cover glass. Unfortunately, not all 1½ cover glasses are manufactured to this close tolerance and many specimens have media between them and the cover glass. Compensation for cover glass thickness can be accomplished by adjusting the mechanical tube length of the microscope, or (as previously discussed) by the utilization of specialized **correction collars** that change the spacing between critical lens elements inside the objective barrel. The correction collar is adjusted to compensate for these subtle differences and ensure optimum objective performance. Proper application of objective lenses with correction collars demands that the microscopist is experienced and alert enough to reset the collar using appropriate image criteria. In most cases, focus may shift and the image may wander during adjustment of the correction collar. Use the

steps listed below to make small incremental adjustments to an objective's correction collar while observing changes in the specimen image.

Position the correction collar so that the indicator mark on the objective barrel coincides with the 0.17-millimeter scale mark engraved on the collar housing.

Place a specimen on the stage and focus the microscope on a small specimen feature.

Rotate the correction collar very slightly and re-focus the objective to determine if the image has improved or degraded. Due to the fact that most specimen preparations suffer from cover glass/media sandwiches that are too thick, start the rotation experiment by trying larger compensation values (0.18-0.23) first.

Repeat the previous step to determine if the image is improving or degrading as the correction collar is turned in a single direction.

If the image has degraded, follow the same steps and rotate the correction collar in the opposite direction (toward lower values) to find the position offering optimum resolution and contrast.

Figure 1 illustrates an example of a 60x objective having a numerical aperture of 0.85 equipped with a correction collar for focusing at the surface or through a coverslip up to 1.5 millimeters in thickness without altering the focal setting of the lens system. The adjustable lens group in the center of the optical train is a cemented triplet that can be re-positioned to accommodate variations in coverslip thickness.

## 12. Adjustment of Objective Correction Collars

Most microscope objectives are designed to be used with a cover glass that has a standard thickness of 0.17 millimeters and a refractive index of 1.515, which is satisfactory when the objective numerical aperture is 0.4 or less. However, when using high numerical aperture dry objectives (numerical aperture of 0.8 or greater), cover glass thickness variations of only a few micrometers result in dramatic image degradation due to aberration, which grows worse with increasing cover glass thickness. To compensate for this error, the more highly corrected objectives are equipped with a correction collar to allow adjustment of the central lens group position to coincide with fluctuations in cover glass thickness. This interactive tutorial explores how a correction collar is adjusted to achieve maximum image quality.

The tutorial initializes with a randomly selected specimen appearing in the **Microscope Image** window and a cut-away view of a correction collar objective positioned to the right of the image window. Also, upon initialization, the tutorial automatically selects a random cover glass thickness for the specimen being viewed in the **Microscope Image** window. In order to operate the tutorial, use the **Correction**

**Collar** slider (note that the **Movable Lens Group** in the objective is also translated by this slider) to adjust this value to approximately 0.17, or the standard cover glass thickness. Next, use the **Microscope Focus** slider to obtain the sharpest image specimen possible. Depending upon the pre-selected cover glass thickness (determined by the tutorial), the specimen image may degrade or become sharper as the sliders are moved to the right or left. Continue to use both the **Correction Collar** and **Microscope Focus** sliders to improve the image of the specimen. Cover glass correction in this tutorial covers a variation in thickness ranging from 0.10 to 0.25 millimeters.

The standard thickness for cover glasses is 0.17 millimeters (170 micrometers), which is designated as a number **1½** cover glass. Unfortunately, not all 1½ cover glasses are manufactured to this close tolerance and many specimens have media of widely varying refractive index between them and the cover glass. Compensation for cover glass thickness can be accomplished by adjusting the mechanical tube length of the microscope or by the utilization of specialized **correction collars** that change the spacing between critical lens elements inside the objective barrel. The correction collar is adjusted to compensate for these subtle differences and ensure optimum objective performance. Proper application of objective lenses with correction collars demands that the microscopist is experienced and alert enough to reset the collar using appropriate image criteria. In most cases, focus may shift and the image may wander during adjustment of the correction collar. Use the steps listed below to make small incremental adjustments to an objective's correction collar while observing changes in the specimen image.

- Position the correction collar so that the indicator mark on the objective barrel coincides with the 0.17-millimeter scale mark engraved on the collar housing.
- Place a specimen on the stage and focus the microscope on a small specimen feature.
- Rotate the correction collar very slightly and re-focus the objective to determine if the image has improved or degraded. Due to the fact that most specimen preparations suffer from cover glass/media sandwiches that are too thick, start the rotation experiment by trying larger compensation values (0.18-0.23) first.
- Repeat the previous step to determine if the image is improving or degrading as the correction collar is turned in a single direction.
- If the image has degraded, follow the same steps and rotate the correction collar in the opposite direction (toward lower values) to find the position offering optimum resolution and contrast.

When engineers design a high numerical aperture dry objective, they include the optical properties and thickness of the media positioned between the front lens and the cover glass in the calculations to satisfy the aplanatic and sine conditions for optical correction. Objectives intended for use with oil as an imaging medium are treated as if the medium were an extension of the front lens, and are corrected accordingly. With non-immersion objectives used with air between the front lens and cover glass, minute variations in cover glass thickness and dispersion properties can become a source of chromatic, spherical, and coma aberrations. This problem also occurs with objectives designed for imaging specimens immersed in glycerin or water. Using an objective having a correction collar requires a considerable amount of practice and careful attention. When the collar is adjusted, focus tends to shift and the image often wanders, which can lead to focus errors and an increase in aberration artifacts.

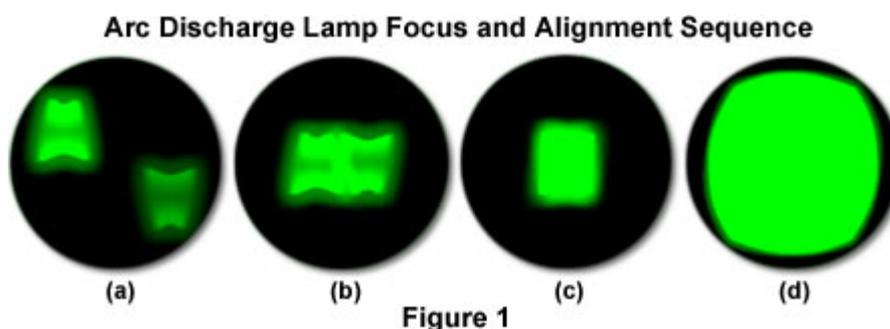
### **13. Focusing and Alignment of Arc Lamps**

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 interactive tutorial, which simulates how the lamp is adjusted in a fluorescence microscope.

Each time the tutorial initializes, the arc lamp adjustment sliders are reset to a random position with the arc image projected onto the stage plate in some condition that deviates from optimum adjustment. To operate the tutorial, first select a lamp type (either **Mercury** or **Xenon**) with the radio buttons in the lower portion of the tutorial window. Next, adjust the **Collector Lens Focus** slider until one or two bow-tie shaped images (simulating a focused image of the arc and its mirror image) appear in the window. Use the **Lamp House Mirror Position** slider to make the intensity of the two arc images approximately equal. The **Arc Lamp Horizontal** and **Vertical** sliders are utilized to overlay the two arc images, so they should be adjusted to merge the images into a single image. When the sliders have been adjusted to produce a small arc image in the center of the window, use the **Collector Lens Focus** slider to enlarge the arc image until it fills the entire window with a uniform field of illumination in a symmetrical manner to ensure the lamp arc is correctly aligned. In order to reset the tutorial to another random set of adjustment positions, use the mouse cursor to click on the blue **Reset** button.

The recommended sequence for focusing and aligning an arc lamp is presented in

Figure 1. Initially, a lamp that is freshly installed and unaligned may have a variety of orientations when the arc is focused by adjusting the collector lens. An example is illustrated in Figure 1(a), where the arc image is positioned in the upper left and the mirror image is displaced to the lower right. After aligning the arc images and adjusting the mirror position and focus, an image similar to that shown in Figure 1(b) should appear. Merging the arc image into its mirror image will result in an overlap such as the one illustrated in Figure 1(c). Finally, when the collector lens is defocused to illuminate the entire viewfield (Figure 1(d)), an evenly distributed and symmetrical beam should result. If this is not the case, the arc should be re-focused and the alignment procedure started fresh. Visitors are encouraged to practice using the tutorial until they can easily achieve these results.



The mercury short-arc lamps commonly utilized in fluorescence microscopy are gas-discharge lamps that contain a mixture of liquid mercury and an inert gas (such as argon or xenon), housed within a glass envelope together with a pair of closely spaced electrodes. In contrast, xenon arc lamps contain pure xenon gas. When current is applied to the electrodes, a discharge electrical arc occurs in the gap between them, which produces enough heat to vaporize the mercury and create a high-pressure internal atmosphere. Because the arc is limited to only a few millimeters in size, arc discharge lamps approximate an ideal point source of illumination that is useful for microscopy. These lamps emit a very intense light having a color temperature around 5500K. In a mercury arc lamp, plasma balls are located adjacent to both the cathode and anode, and each has approximately the same intensity, which is twice that in the center of the arc. In contrast, the single plasma ball in a xenon arc lamp is located closer to the anode and is nearly five times as bright as the light intensity surrounding the cathode.

After installing a new bulb in a mercury or xenon arc lamphouse, the arc must be carefully aligned and focused to achieve a homogeneous field of illumination for observing and imaging specimens. The arc itself is very small (about 1 or 2 millimeters

in length), and the image of the arc must be positioned along the optical axis of the microscope, at the center of the condenser aperture in the vertical illuminator, to ensure even illumination. The average lifetime of a mercury arc discharge lamp varies between 200 and 300 hours, depending upon the burn switch cycle and the design specifications. Xenon arc lamps typically have a lifetime between 400 and 600 hours. Always follow the manufacturer's instructions for operation and maintenance of arc lamps to ensure maximum lamp lifetime and operator safety.

### **Aligning and Focusing Arc Lamps**

Turn off the power supply and allow the old lamp to cool before installing a new lamp according to the manufacturer's instructions. Pay careful attention to the bulb orientation during installation. Most bulbs (an example is illustrated in Figure 2) are designed to be operated in a vertical position with the anode (+ electrode) underneath, and have a larger end cap on the anode side of the bulb. Bulb mounting sockets in the microscope lamphouse have different diameters to aid in bulb orientation. Because arc lamp glass envelopes are filled with mercury or xenon gas at moderately high pressure, never handle these lamps when they are hot to avoid applying a mechanical force that might cause the lamp to explode. Avoid touching the new lamp with ungloved fingers, because oils from the hands are acidic and may etch the quartz envelope enough to weaken it. In addition, residue from fingerprints can become fused to the exterior of the bulb when it becomes hot. If a bulb does explode, refer to local safety procedures and practices for handling a mercury cleanup and decontamination.



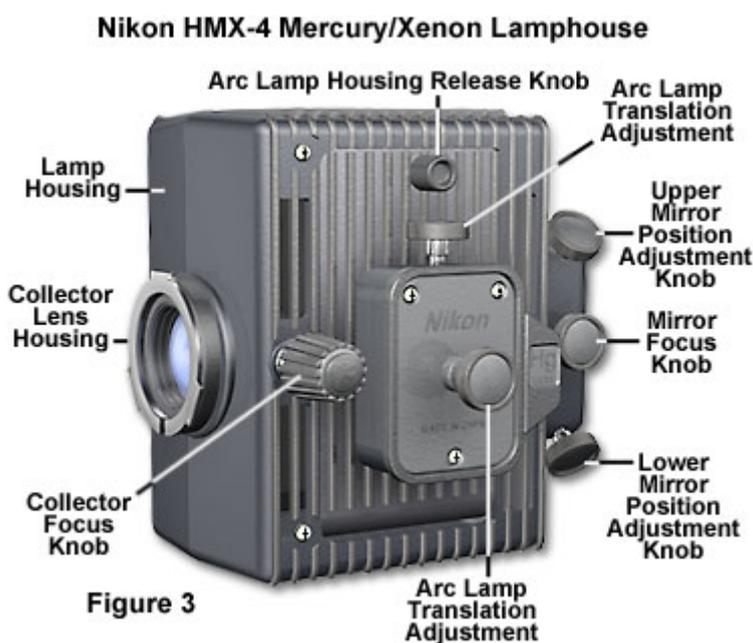
After installing the new lamp, turn on the power supply and allow the lamp to stabilize for 10 to 15 minutes (**Important Note:** always allow a new lamp to **burn in** for at least an hour when it is first turned on). A burn in period is necessary in order for a small pit to be etched into the anode and create a path of least resistance, which enables the arc to remain steady and not wander (flicker) during the useful lifetime of the bulb.

Place neutral density filters in the light path that are sufficiently dense to block approximately 90 to 95 percent of the incident light. A majority of the fluorescence

vertical illuminators on modern microscopes are equipped with neutral density filters built into slider frames that can be pushed into the light path to reduce illumination intensity. If the microscope is not equipped with a filter holder of this type, investigate a suitable location for placement of an aftermarket neutral density filter.

Select a suitable fluorescence filter cube for viewing the lamp arc and place it into the light path. Most manufacturers recommend a cube having an excitation filter that passes light in the green region of the spectrum for this purpose.

Place a white piece of paper or note card on the microscope stage directly under the nosepiece. Remove an objective from the nosepiece and rotate the empty hole into the light path, directly over the white paper. Next, open the shutter slider or knob on the vertical illuminator to enable light to pass through to the nosepiece. At this point an illuminated circle of light should be visible on the white paper with a **hot** spot that may be off center. If the light is too bright, add more neutral density filters. It is also a good idea to wear glasses (either polymer or glass) or to install a tinted breath shield on the microscope to block reflected ultraviolet light from entering the eyes.

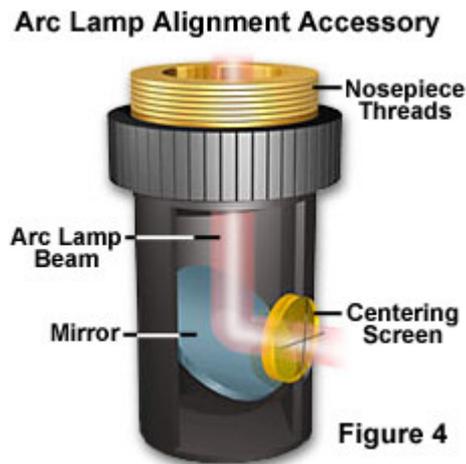


To begin alignment of the arc lamp, focus the collector lens (see Figure 3) to produce a sharply defined image of the arc on the white paper. Centering knobs built onto the lamphouse exterior can then be used to translate the focused arc image directly into the center of the illumination circle produced on the white paper. Some lamphouses have an internal mirror system that directs a more intense illumination into the aperture.

Microscopes equipped with this type of lamphouse will produce two arc images (the actual arc and its mirror image). Use the mirror centering and lamp translation knobs (Figure 3) to position the real arc and its mirror image (which is usually less intense) side-by-side, and then use the mirror focus knob to adjust the intensities until they are approximately equal (lamphouses lacking the mirror system will also lack the adjustment knobs, so carefully check the manufacturer's instructions on lamp adjustment). Finally, use the lamp adjustment knobs to overlay the arc and mirror images as closely as possible.

After the focused arc image (and its mirror image, in lamphouses so equipped) is perfectly aligned in the center of the optical path (and the illumination spot on the white paper), slowly defocus the lamp collector lens with the appropriate adjustment knob. As the lens is defocused, observe the beam as it expands to ensure that it fills the area in a uniform manner and is not displaced to one side. If the arc image does not expand symmetrically, refocus the arc and repeat the alignment procedure. Finally, bring the arc image back into focus and reinsert the objective. To ensure completely uniform illumination, it may be necessary to make additional minor adjustments to the collector lens while viewing a uniform specimen through the eyepieces with the objective in place.

A majority of the microscope manufacturers offer an optional alignment accessory to facilitate the centering of the image of the lamp arc to the rear aperture of the objective. At its upper end, this accessory either has the standard Royal Microscopical Society (RMS) thread or 25-millimeter threads to accommodate newer nosepieces, and can be screwed into the nosepiece in place of an objective. In order to use the accessory, it is first placed in the nosepiece and then rotated into the light path. The lower face of the accessory has a frosted, orange-colored glass window with an inscribed crosshair (as illustrated in Figure 4). Light passing through the microscope dichromatic mirror strikes the built-in reflector of the centering screen and is reflected onto the crosshairs. As the lamp condenser knob and the centering screws on the lamp socket are manipulated, the image can be observed and translated so that it is centered to the crosshairs. The size of the image of the arc can be made bigger or smaller by manipulating the collector lens focus position. After the arc is aligned, the centering accessory can be replaced by a regular objective.



Regardless of whether alignment of the arc is accomplished with the aid of an accessory or with a white piece of paper placed on the microscope stage, both methods project the arc image that is present at the entrance pupil of the objective, in effect, the rear objective aperture. When the objective is in place, the image of the arc at the rear aperture is out of focus and the illumination at the image plane (that of the field diaphragm) is uniform. This is the essence of Köhler illumination.

As digital imaging workstations become more popular and microscopes equipped with high-end camera systems grow more complex, it is important to remember how dangerous the arc lamp power supply can be to electronic equipment. Always turn the arc lamp on **before** powering on auxiliary computer or camera equipment that is in close proximity to the power supply, and always turn this equipment off before turning off the arc lamp. The cable supplying current to the lamp from the power supply is generally quite well shielded, but a momentary 20,000 to 50,000 volt surge is possible when the lamp is fired. This large voltage can generate a magnetic field strong enough to damage sensitive integrated circuits that are nearby.

#### **14. Linear Measurements (Micrometry)**

Performing measurements at high magnifications in compound optical microscopy is generally conducted by the application of eyepiece reticles in combination with stage micrometers. A majority of measurements made with compound microscopes fall into the size range of 0.2 micrometers to 25 millimeters (the average field diameter of widefield eyepieces). Horizontal distances below 0.2 micrometers are beneath the resolving power of the microscope, and lengths larger than the field of view of a widefield eyepiece are usually (and far more conveniently) measured with a stereomicroscope.

The first reported measurements performed with an optical microscope were undertaken in the late 1600s by the Dutch scientist Antonie van Leeuwenhoek, who used fine grains of sand as a gauge to determine the size of human erythrocytes. Since then, countless approaches have been employed for measuring linear, area, and volume specimen dimensions with the microscope (a practice known as **micrometry** or **morphometrics**), and a wide variety of useful techniques have emerged over the past few hundred years.

**Introduction to Micrometry** - Performing measurements at high magnifications in compound optical microscopy is generally conducted by the application of eyepiece reticles in combination with stage micrometers. A majority of measurements made with compound microscopes fall into the size range of 0.2 micrometers to 25 millimeters (the average field diameter of widefield eyepieces). Horizontal distances below 0.2 micrometers are beneath the resolving power of the microscope, and lengths larger than the field of view of a widefield eyepiece are usually (and far more conveniently) measured with a stereomicroscope.

**Eyepiece Reticle Calibration** - Calibration of an eyepiece reticle (determination of the micrometer graduation relationship) for a particular objective is typically conducted by following standard recommended procedures. Note that calibration of an eyepiece reticle holds only for the specific objective/eyepiece combination being tested, and for the mechanical tube length of the microscope. This interactive tutorial explores calibration of various eyepiece reticles with a stage micrometer, and demonstrates how the reticle can then be employed to determine linear specimen dimensions.

**Reference Listing** - In addition to basic linear measurements, the optical microscope is often employed to measure the area or volume of a specimen and for counting particles and fibers. Several books and review articles focused on micrometry and stereology been published by leading researchers in the field and were utilized as references to prepare the measurement discussions included in the MicroscopyU website. This section contains periodical location information about these articles, as well as providing a listing of selected review articles and books describing quantitative microscopy.

### 3. Important Features Visible in Thin Section

(<http://www.uwgb.edu/dutchs/petrology/thinsect.htm>)

*Steven Dutch, Natural and Applied Sciences, University of Wisconsin - Green Bay*

#### 3-1. Minerals to learn

##### A. Silicates

1. Nesosilicates: independent tetrahedra ( $\text{SiO}_4$ )
  - Olivine  $(\text{Mg,Fe})_2\text{SiO}_4$  : Mg - Forsterite, Fe - Fayalite
  - Garnet: Almandine  $\text{Fe}_3\text{Al}_2\text{Si}_3\text{O}_{12}$   
Grossularite  $\text{Ca}_3\text{Al}_2\text{Si}_3\text{O}_{12}$
  - Sillimanite, Andalusite, Kyanite  $\text{Al}_2\text{SiO}_5$
  - Titanite (Sphene)  $\text{CaTiSiO}_5$
  - Staurolite Fe,Al Silicate with (OH)
2. Sorosilicate: double-tetrahedral groups ( $\text{Si}_2\text{O}_7$ )
  - Epidote  $\text{Ca,Fe}^{+3}$ , Al silicate with (OH)
3. Cyclosilicates: rings of linked tetrahedra ( $\text{Si}_6\text{O}_{18}$ )
  - Beryl  $\text{Be}_3\text{Al}_2\text{Si}_6\text{O}_{18}$
  - Tourmaline Na,Mg,Fe,Al etc borosilicate with ( $\text{BO}_3$ ), (OH)
4. Inosilicates: chains of linked tetrahedra
  - (a) Pyroxenes - single chains ( $\text{SiO}_3$ )
    - Diopside  $\text{CaMgSi}_2\text{O}_6$
    - Augite  $(\text{Ca,Mg,Fe})_2\text{Si}_2\text{O}_6$
    - Orthopyroxene (enstatite, hypersthene)  $(\text{Mg,Fe}) \text{SiO}_3$
  - (b) Amphiboles - double chains ( $\text{Si}_4\text{O}_{11}$ ) with (OH)
    - Tremolite, actinolite  $\text{Ca}_2(\text{Mg,Fe})_5(\text{Si}_8\text{O}_{22}) (\text{OH})_2$
    - Hornblende  $\text{NaCa}_2(\text{Mg,Fe})_5(\text{AlSi}_7\text{O}_{22}) (\text{OH})_2$
5. Phyllosilicates: sheets of linked tetrahedra ( $\text{Si}_2\text{O}_5$ ) with (OH)
  - Kaolinite  $\text{Al}_4(\text{Si}_4\text{O}_{10}) (\text{OH})_8$
  - Serpentine  $\text{Mg}_6(\text{Si}_4\text{O}_{10}) (\text{OH})_8$
  - Talc  $\text{Mg}_3(\text{Si}_4\text{O}_{10}) (\text{OH})_2$
  - Chlorite  $\text{Mg}_5\text{Al}(\text{AlSi}_3\text{O}_{10}) (\text{OH})_8$
  - Biotite  $\text{K}(\text{Fe,Mg})_3(\text{AlSi}_3\text{O}_{10}) (\text{OH})_2$
  - Muscovite  $\text{KAl}_2(\text{AlSi}_3\text{O}_{10}) (\text{OH})_2$
6. Tectosilicates: 3-D frameworks of linked tetrahedra ( $\text{SiO}_2$  etc.)
  - Quartz (high, low T)  $\text{SiO}_2$
  - Orthoclase (alkali feldspar)  $\text{KAlSi}_3\text{O}_8$  - (K, Na)  $\text{AlSi}_3\text{O}_8$
  - Plagioclase  $(\text{Na,Ca})\text{Al}(\text{Si,Al})\text{Si}_2\text{O}_8$ : albite -  $\text{NaAlSi}_3\text{O}_8$ ,  
anorthite -  $\text{CaAl}_2\text{Si}_2\text{O}_8$
  - Zeolites complex Ca,Na, Al silicates with  $\text{H}_2\text{O}$

B. Non - silicates

1. Native elements

Graphite C

Sulfur S

Copper Cu

2. Halides

Halite NaCl

Fluorite CaF<sub>2</sub>

3. Sulfides

Pyrite, Marcasite FeS<sub>2</sub>

Pyrrhotite Fe<sub>1-x</sub>S

Sphalerite ZnS

Galena PbS

Chalcopyrite CuFeS<sub>2</sub>

Arsenopyrite FeAsS

4. Oxides

Rutile TiO<sub>2</sub>

Pyrolusite MnO<sub>2</sub>

Hematite Fe<sub>2</sub>O<sub>3</sub>

Ilmenite FeTiO<sub>3</sub>

Corundum Al<sub>2</sub>O<sub>3</sub>

Magnetite Fe<sub>3</sub>O<sub>4</sub>

Chromite FeCr<sub>2</sub>O<sub>4</sub>

5. Hydroxides etc.

Goethite, limonite FeO(OH)

Bauxite, gibbsite, Al(OH)<sub>3</sub> and diaspore AlO(OH)

6. Carbonates

Calcite, Aragonite CaCO<sub>3</sub>

Dolomite CaMg (CO<sub>3</sub>)<sub>2</sub>

Siderite FeCO<sub>3</sub>

Azurite Cu, (CO<sub>3</sub>), (OH)

Malachite Cu, (CO<sub>3</sub>), (OH)

7. Sulfates

Anhydrite CaSO<sub>4</sub>

Barite BaSO<sub>4</sub>

Gypsum CaSO<sub>4</sub> · 2H<sub>2</sub>O

8. Phosphates

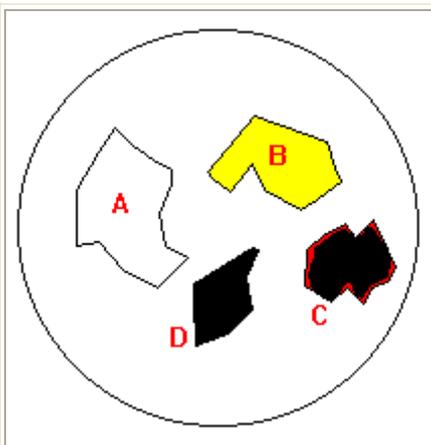
Apatite Ca<sub>5</sub>(PO<sub>4</sub>)<sub>3</sub> [(OH), F, Cl]

## 3-2. Features in Thin Section – Plane Polarized Light

### The Cardinal Rule of Thin Sections

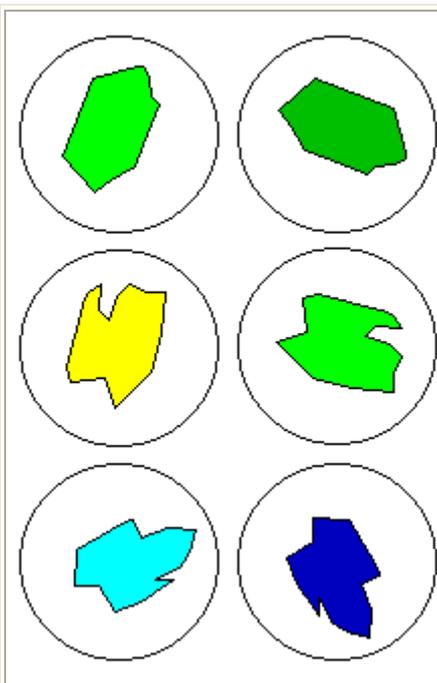
What you see in thin section is a random cross-section through a grain whose properties vary *in three dimensions*. Therefore, always view as many grains of a mineral as possible to get an idea of the total range of properties you observe. Also, bear in mind that you may not get a grain that displays some property to optimum effect.

### Color



Color in thin section tends to be more consistent than in hand specimen. Most major rock forming minerals are colorless (A). Some have distinctive colors (B). Some minerals like hematite (C) which appear opaque in hand specimen are transparent on thin edges in thin section. The most common truly opaque minerals (D) are metallic oxides (magnetite, ilmenite) and sulfides (pyrite).

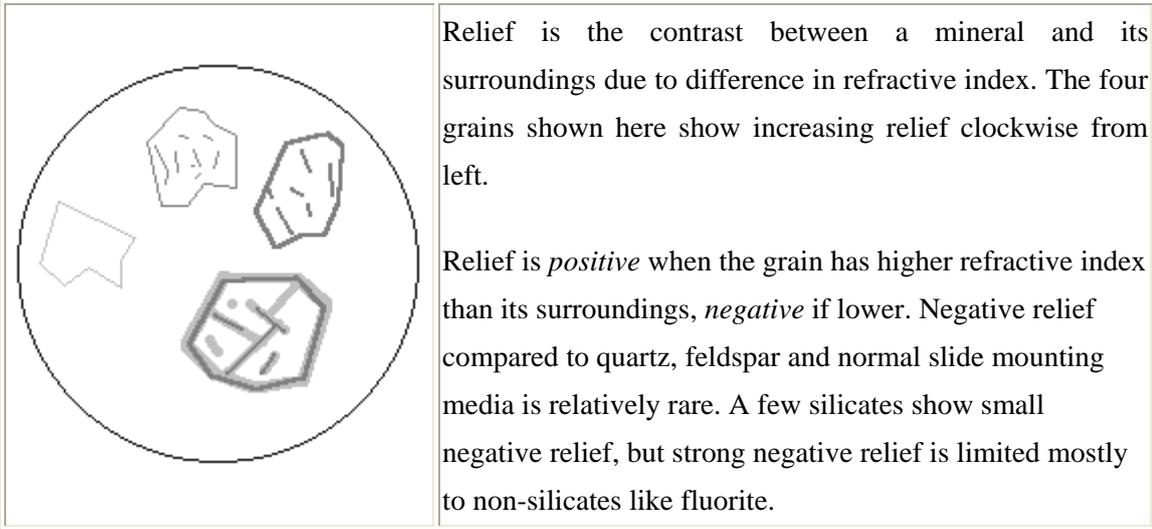
### Pleochroism



Colored minerals often show different colors in thin section depending on how the grain is oriented relative to the polarizer directions.

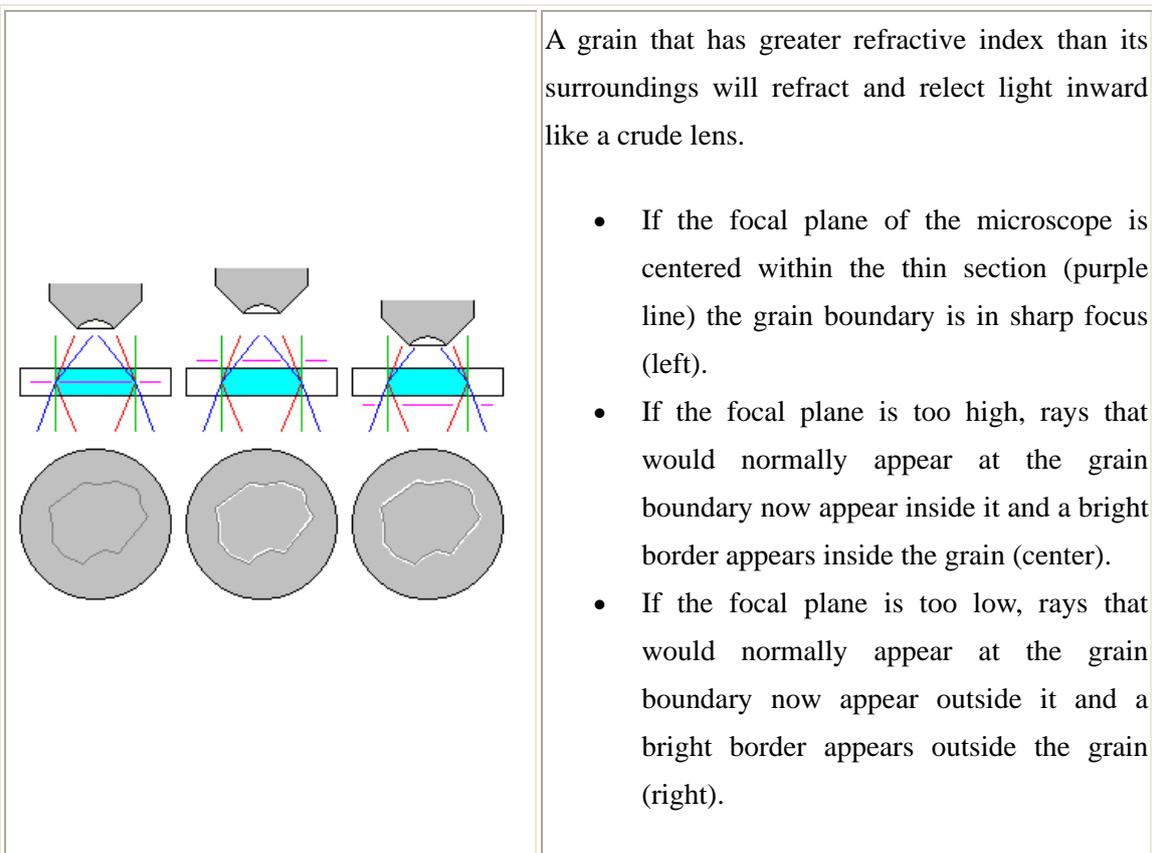
- Top: Most minerals change from lighter to darker as the stage is rotated.
- Middle: Some minerals change color entirely as the grain is rotated.
- Bottom: In a few cases the color change is so extreme that the mineral is, in effect, a natural polarizer. Thin slices of tourmaline were often used as polarizing filters before good synthetic filters became available.

## Relief



## Becke Line

If a grain is not perfectly in focus, it will often appear to be bordered by a bright line called the *Becke Line*. The Becke Line is useful for determining which of two neighboring grains has the highest refractive index.

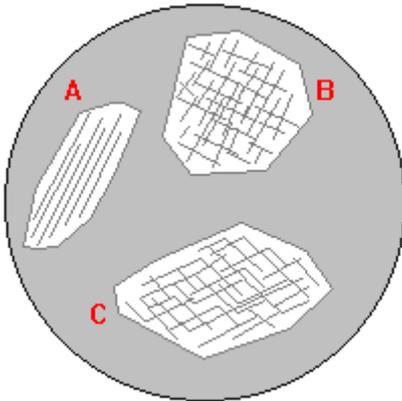


	<p>A grain that has lower refractive index than its surroundings will refract and reflect light outward like a crude diverging lens.</p> <ul style="list-style-type: none"> <li>• If the focal plane of the microscope is centered within the thin section (purple line) the grain boundary is in sharp focus (left).</li> <li>• If the focal plane is too high, rays that would normally appear at the grain boundary now appear outside it and a bright border appears outside the grain (center).</li> <li>• If the focal plane is too low, rays that would normally appear at the grain boundary now appear inside it and a bright border appears inside the grain (right).</li> </ul>
--	--

**To sum up:** As you move *away* from the thin section (raising the objective or lowering the stage), the Becke Line appears to move *into the material with greater refractive index*. Although it's not a wholly accurate analogy, picture the grain focusing rays like a lens and picture what happens as you move along the cone of converging rays.

	<p>Minerals may be surrounded by grains of both higher and lower refractive index, so the Becke Line may move both in and out around the margins of a grain.</p> <p>Carbonates have a maximum refractive index much higher than most rock forming minerals, and a minimum index much lower. It shows high relief all around, but some is positive and some is negative. A lone carbonate grain in a uniform mounting medium will still show a Becke Line that moves in along some parts of the grain boundary and out along others.</p>
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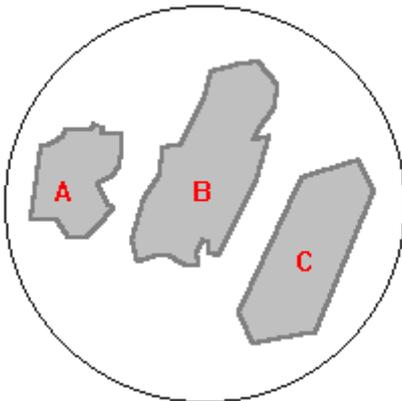
## Cleavage



Cleavage is much easier to see in thin section than in hand specimen. Cleavage along the length of the grain is exhibited by many minerals (A). Pyroxenes viewed end on (B) usually show the characteristic 87-degree cleavage, while cross-sections of amphibole show the characteristic 56-degree cleavage (C).

What you see will depend on the orientation of the grain. A true cross-section of an amphibole will show 56-degree cleavages but an oblique section will show other angles and a longitudinal section will show longitudinal cleavage as in (A).

## Crystal Form



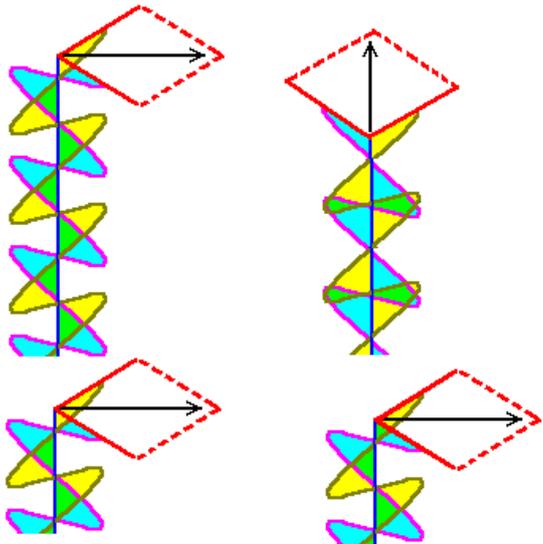
- Grains that show no recognizable crystal form are said to be *anhedral* (A).
- Grains that show imperfect but recognizable crystal form are said to be *subhedral* (B).
- Grains that show sharp and clear crystal form are said to be *euhedral* (C).

## 3-3. Features in Thin Section – Crossed Polarisers

- Extinction
- [Interference Colors](#)
- Isotropic Materials
- [Anomalous Interference Colors](#)
- Zoning
- Undulose Extinction
- Twinning
- Exsolution

## Interference Colors

### Interference Criteria

	<p>As the two beams of polarized light pass through a crystal, they travel at different speeds and get out of phase. The slow ray is said to be retarded and the phase difference is called <i>retardation</i></p> <p>If the retardation is a whole number of wavelengths (left), the beams recombine with the same orientation as when they entered the crystal. These wavelengths will be blocked by the upper polarizer.</p> <p>If the retardation is a whole number of wavelengths <i>plus one-half</i>(right), the beams recombine with an orientation perpendicular to the original direction of polarization. These wavelengths will be fully transmitted by the upper polarizer.</p>
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### Retardation and Interference Color

What determines the retardation of a crystal? Consider a crystal of thickness  $h$ . The index of refraction of its slow ray is  $n_s$  and that of its fast ray is  $n_f$ . The value of  $n_s$  is greater than  $n_f$ .

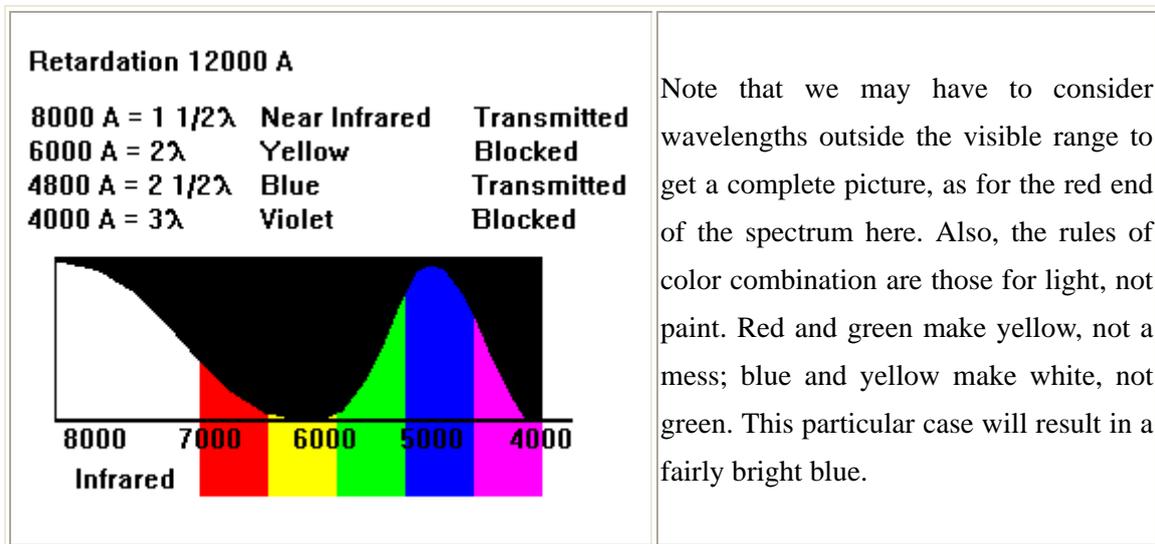
We can calculate:

- Velocities of the Beams
  - Velocity of fast ray:  $v_f = c/n_f$
  - Velocity of slow ray:  $v_s = c/n_s$
- Travel time through the crystal:
  - For the fast ray:  $t_f = h/v_f = hn_f/c$
  - For the slow ray:  $t_s = h/v_s = hn_s/c$
- Lag time of the slow ray:  $t_s - t_f = h(n_s - n_f)/c$
- Amount fast ray leads slow ray once both exit

- Lead = lag time x c = h(ns - nf) = Retardation

Thus **Retardation = h(ns - nf)**. Retardation depends only on the thickness of the material and the difference in refractive index for the two beams, called the *birefringence*. Thickness is controlled by grinding thin sections to a standard thickness, usually .035 mm. This figure is not arbitrary; it is designed to give quartz a consistent appearance in thin sections.

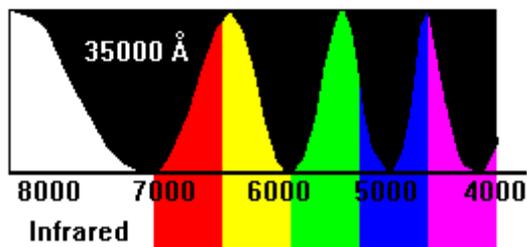
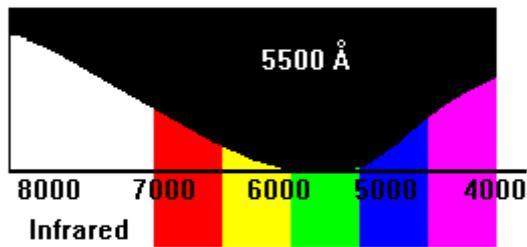
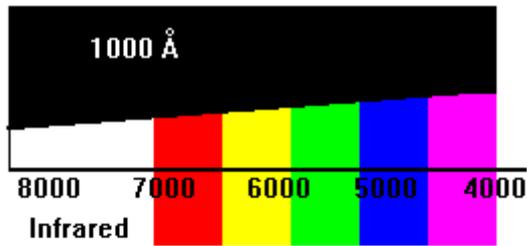
For a given retardation, some wavelengths will satisfy the transmission condition (retardation = integer plus one-half wavelength), while others will be blocked (retardation = whole number of wavelengths). Consider the case below.



Note that we may have to consider wavelengths outside the visible range to get a complete picture, as for the red end of the spectrum here. Also, the rules of color combination are those for light, not paint. Red and green make yellow, not a mess; blue and yellow make white, not green. This particular case will result in a fairly bright blue.

**A given retardation always results in the same combination of wavelengths and always results in the same color.** The sequence of colors that results from increasing retardation is one of the basic facts of optical mineralogy.

Note that zero retardation satisfies the blockage criteria for *all* wavelengths. If there is zero retardation, the light recombines in the same orientation as it had originally and is blocked by the upper polarizer. Some materials have the same refractive index in all directions and always produce zero retardation. Such materials are called *isotropic*. Noncrystalline materials like glass are isotropic, so are isometric minerals like garnet or fluorite. All other materials are termed *anisotropic*.



For low retardations, no visible wavelengths are blocked or fully transmitted. We see part of the whole spectrum and a neutral gray or white. As retardation reaches 4000 Å, the first blockage reaches the blue end of the spectrum and the transmitted color shifts to yellow, then red.

At 5500 Å the blockage is in the green, and we see magenta, the result of mixing red and violet. Each multiple of 5500 Å will result in a magenta hue. The sequence of colors from one magenta to the next is termed an *order*.

For very large retardations, several wavelengths are blocked while others are transmitted. As more and more windows in the spectrum appear, the colors become progressively more pale. Finally they approach white, but a warmer off-white rather than the cold neutral white of low retardation.

Retardation Å	Order	Colors	Notes
0	Zero	Black	
0-5500	First	Gray, White, Yellow, Red	Neutral colors are cold, yellows dull.
5500-11000	Second	Violet Through Spectrum to Red	Purest colors, though not totally pure
11000-16500	Third	Violet Through Spectrum to Red	Have a "fluorescent" appearance
16500 and up	Fourth and higher	Mostly greens and pinks	Colors become more washed out with increasing retardation

### Anomalous interference colors

Some minerals show colors that are not part of the standard interference color sequence.

Such colors are termed *anomalous*. Anomalous colors can result when:

- The mineral is strongly colored and its natural color combines with the interference color.
- The refractive index of the material changes markedly with wavelength, so that different wavelengths satisfy different interference criteria. In some cases the mineral may be isotropic for some wavelengths but not others.
- The privileged directions of the material vary with wavelength, so the mineral never goes completely to extinction at any one time.

Anomalous interference colors tend to be dark. Blue, violet, green and brown are all possible. These are actually useful diagnostic features. Chlorite tends to show anomalous greens and browns. Epidote commonly shows a dark "denim" blue color near extinction. When combined with its normal bright yellow and red interference colors, the effect is quite colorful.

### **Intrinsic Color**

Minerals with relatively weak color like chlorite, actinolite, glaucophane or hypersthene will show interference colors not very different from colorless minerals. The weak intrinsic color is not strong enough to affect the interference color very much. Strongly colored minerals like biotite or hornblende will be dominated by the intrinsic color. Colors very different from the intrinsic color are absorbed so strongly that they contribute little to the interference color. A deep green mineral will look green in crossed polarizers because everything but green is absorbed regardless of the retardation.

### **Variation of Optical Properties With Wavelength**

#### **Birefringence and Refractive Index**

Some minerals are isotropic at certain wavelengths. Obviously that wavelength cannot contribute to an interference color. For example, a mineral that is isotropic in the green but not in the red or violet cannot show a green interference color. Its interference colors will only be red-violet. Since variations in optical properties with wavelength tend to be small, a mineral that is isotropic in one part of the spectrum will have low birefringence everywhere else, so it will not show brilliant interference colors. Anomalous interference colors tend to be dark. Instead of first order gray, a mineral that is isotropic in the green would appear dark purple.

### Optical Orientation

Sometimes the privileged directions in a mineral vary with wavelength. This obviously cannot happen with uniaxial minerals, where the privileged directions are locked to a symmetry axis, so minerals whose optical orientations vary with wavelength tend to be biaxial. If the privileged directions vary with wavelength, all wavelengths will not go extinct at the same time and the mineral will appear colored instead of black. Since the mineral is near extinction even for those wavelengths, the color will be dark.

### 3-4. Identification Table for Common Minerals in Thin Section

#### Definition of Descriptive Terms

Index of Refraction		Birefringence		
Descriptive Term	Range of Values	Descriptive Term	Range of Values	Interference Color
Very low	Below 1.51	Very low	Less than .006	1 Black to Gray
Low	1.51 - 1.571	Low	.006 - .014	1 White-Yellow
Moderate	1.571 - 1.620	Moderate	.014 - .020	1 Red - 2 Blue
Moderately high	1.621 - 1.650	Moderately high	.021 - .025	2 Green - Yellow
High	1.653 - 1.73	High	.025 - .045	2 Orange - 3 Green
Very high	1.731 - 1.90	Very high	.045 - .060	3 Green - 4 Green
Extremely high	Above 1.90	Extremely high	.06 or higher	4 Green and up

#### Summary of Colorless Minerals

R.I. \ Int. Color	1 Blk-Gray	1 Wh-Y	1 R - 2 Bl	2 Gr - Y	2 Or - 3 Gr	3 Gr - 4 Gr	4 Gr +
Very low	Leucite, Analcite, Fluorite, Sodalite						
Low	Nepheline	Quartz, Cordierite, Feldspars		Cancrinite, Scapolite			
Moderate			Tremolite		Muscovite,		

					Talc		
Moderately high	Apatite	Topaz, Wollasonite	Anthophyllite				
High		Enstatite, Kyanite, Clinzoisite, Zoisite		Sillimanite	Olivine (Forsterite), Diopside, Augite, Pigeonite		
Very high	Garnet						
Extremely high						Zircon	Calcite, Aragonite

### Summary of Colored Minerals

R.I. \ Int. Color	1 Blk-Gray	1 Wh-Y	1 R - 2 Bl	2 Gr - Y	2 Or - 3 Gr	3 Gr - 4 Gr	4 Gr +
Very low	Noselite, Hauynite						
Low							
Moderate	Chlorite						
Moderately high			Actinolite	Tourmaline			
High	Spinel	Chloritoid, Andalusite	Hypersthene	Epidote, Hornblende, Glaucofane	Hedenbergite, Humite	Acmite	
Very high		Staurolite		Piedmontite		Fayalite	
Extremely high							Sphene (Titanite), Rutile

### Can't Find It?

- Refractive index and birefringence vary somewhat depending on mineral composition. Try minerals in neighboring boxes.
- Bear in mind that interference color in thin-section can be much lower than the

theoretical maximum, depending on grain orientation. Judge birefringence by the *highest* color you see for that mineral, and check minerals with somewhat higher birefringence if you can't find an exact match.

- Weakly-colored minerals can sometimes be so pale they appear colorless, and some normally colorless minerals can at times be colored. Check both colored and colorless minerals.
- The odds are at least 99 per cent that what you're looking for is on this chart. But you may have one of the one per cent.

### **Very Low R. I. (Less than Mounting Medium)**

#### **Colorless;**

##### **Leucite**

Isotropic, often weakly birefringent. Complex twins, trapezohedral habit, radial rows of inclusions,

##### **Analcite**

Lack of complex twins, absence of oriented inclusions, R.I. less than that of leucite, Mainly as alteration product. Isotropic.

##### **Fluorite**

Isotropic, octahedral. Octahedral cleavage and twinning, crystal form and extremely low R.I. characteristic.

##### **Sodalite**

Isotropic, alteration rims characteristic.

#### **Colored;**

##### **Noselite and Hauynite**

Similar to sodalite, but generally colored blue or green,

### **Low R. I.**

#### **Colorless**

##### **Nepheline**

Very low birefringence, Moderate R. I. Uniaxial negative, alteration common

##### **Quartz**

Low birefringence Uniaxial positive, absence of cleavage and alteration

**Feldspars**

Low birefringence, 2 sets of cleavage, alteration common, twinning and zoning common. Biaxial with high 2V

**Cordierite**

Low birefringence, Biaxial negative with high 2V, pleochroic halo around tiny-inclusions, complex twinning, alteration to pinite.

**Cancrinite**

Moderately high to high birefringence, all R.I.'s less than mounting medium., uniaxial negative

**Scapolite**

Moderately high to high birefringence, all R.I.'s greater than mounting medium., uniaxial negative

**Moderate R. I.****Colorless;****Muscovite**

Very fine cleavage in one set, high birefringence, straight extinction, mottling in extinction position, Biaxial negative with moderate 2V.

**Talc**

Similar in all respects to muscovite but lower 2V and associated with Mg-rich rocks (altered ultrabasic or metamorphosed impure Ca-Mg-rich sediments).

**Colored;****Chlorite**

Pleochroic in shades of light green, one set of fine micaceous cleavage, straight extinction. Low to very low birefringence, often ultrablue interference color, Lamellar twinning common

**Biotite**

Strong pleochroism in shades of yellow, brown and red- brown, high birefringence, mottling in extinction position, straight extinction, Biaxial negative with low 2V.(R. I. high in some instances).

**Moderately High R. I.**

**Colorless;**

**Apatite**

Water clear, extremely low birefringence, uniaxial negative (sometimes with high R. I.)

**Topaz**

Water clear, low birefringence, biaxial positive with moderately high 2V

**Anthophyllite**

Occasionally greenish, 2 sets of cleavage at 54 degrees, extinction straight, 2V high, optically positive, birefringence moderate to moderately high.

**Wollastonite**

3 sets of cleavage, low birefringence, optically negative with moderate 2V, inclined extinction.

**Colored;**

**Tremolite-Actinolite**

Feebly pleochroic in shades of green (sometimes colorless), 2 sets of cleavage at 57 degrees, birefringence moderate to moderately high, extinction inclined (15-20 degrees), biaxial negative with very high 2V

**Tourmaline**

Strongly pleochroic in shades of yellow, brown and blue, extinction straight, birefringence moderately high, uniaxial negative, elongation negative, cracks normal to elongation - (sometimes with high R. I.).

**High R. I.**

**Colorless;**

**Olivine (Forsterite)**

Water clear, high birefringence, straight extinction, six-sided grains, reticulate cracks with alteration, 2V almost 90 degrees.

**Sillimanite**

Water clear, moderately high birefringence, low 2V, biaxial positive, elongation positive, prismatic grains with transverse cracks, straight extinction. Often aggregates of very fine needle-like fibers.

**Enstatite**

Water clear, low birefringence, 2 sets of cleavage at 87 degrees, extinction straight, 2V high, optically positive.

**Kyanite**

Sometimes ash gray, birefringence low to moderate, 3 sets of very good cleavage, extinction inclined, biaxial negative with very high 2V, lamellar twinning often present.

#### **Clinozoisite**

Low birefringence with ultrablue interference color, 2V very high, optically negative, extinction inclined.

#### **Zoisite**

Similar to clinozoisite but straight extinction and optically positive with moderate 2V.

#### **Diopside**

High birefringence, 2 sets of cleavage at 87 degrees, 38 degree extinction angle, biaxial positive with moderate to moderately high 2V.

#### **Augite**

Similar to diopside but with extinction angle greater than 80 degrees (pleochroic in shades of violet or pink in case of titanaugite).

#### **Pigeonite**

Similar to augite but with 2V less than 30 degrees.

#### **Colored;**

#### **Epidote (Pistacite)**

Pleochroic in shades of light pistachio green, moderately high birefringence, optically negative with 2V nearly 90°, inclined extinction -

#### **Chloritoid**

Strongly pleochroic in shades of slate blue, green and pale yellow, low birefringence, one set of micaceous cleavage, inclined extinction, generally positive with moderate 2V, lamellar twinning common -

#### **Hedenbergite**

Pleochroic in shades of yellowish green to green, high birefringence, 2 sets of cleavage at 57° with each other, extinction angle around 47°, biaxial positive with moderately high 2V -

#### **Acmite (Aegerine)**

Strongly pleochroic in shades of green, brown and brownish yellow, very high birefringence, 2 sets of cleavage at 87° with each other, biaxial positive with high 2V, very small extinction angle (about 5°) -

#### **Common hornblende**

Pleochroic in shades of green and bluish green, moderately high birefringence, 2 sets of cleavage at 57°, extinction inclined (about 200), biaxial negative with very high 2V -

#### **Glaucophane**

Strongly pleochroic in shades of blue, refractive index slightly lower than hornblende, typical amphibole cleavage at 570, biaxial negative with moderately high 2V, extinction angle 4"-60 -

#### Hypersthene

Pleochroic in shades of light green and pink, 2 sets of cleavage at 87a, extinction straight, birefringence low to moderate, biaxial negative with 2V high to very high - -

#### **Andalusite**

Pleochroic in patchy pink shade, low birefringence, straight extinction, elongation negative, optically negative with 2V nearly 90", square cross section and oriented inclusions present -

#### **Humite group of minerals**

Pleochroic from almost colorless to yellow, biaxial positive with moderately high 2V, in all other properties similar to forsterite.

#### **Spinel**

Often green, nonpleochroic, isotropic, octahedral habit (R. I. is very high in some varieties.)

#### **Very high R. I.**

#### **Colorless;**

##### Garnet

Nonpleochroic - Generally pink, isotropic, dodecahedral habit

#### **Colored;**

##### Staurolite

Strongly pleochroic in shades of deep yellow and brownish yellow, birefringence low, extinction straight, biaxial positive with 2V nearly 90", sieve structure because of inclusions common.

#### **Piedmontite**

Strongly pleochroic in shades of orange, violet and bright red, birefringence high to very high, extinction inclined, biaxial positive with high 2V.

#### **Fayalite**

Pleochroic in shades of yellow, birefringence very high, biaxial negative with moderately high 2V, extinction straight.

## **Extremely High R. I**

### **Zircon**

Nonpleochroic in thin section with very high birefringence, straight extinction, uniaxial positive sign, and characteristic square prisms

### **Sphene**

Pleochroic in shades of pink, extremely high birefringence, inclined extinction, biaxial positive with low 2V

### **Rutile**

strongly pleochroic in shades of red-brown, extremely high birefringence, but interference color often masked by strong absorption, uniaxial positive, often occurs as needles and slender prisms

### **Calcite Group of Carbonates**

Variegated R. I. ranging from very low to very high, twinkling due to sharp change in refringence. Uniaxial negative.

### **Aragonite group of Carbonates**

Biaxial negative. Low to high and very high relief, extremely high birefringence

## **3-5. Distinguishing the Feldspars**

### **The Principal Feldspars**

Potassium Feldspars:  $\text{KAlSi}_3\text{O}_8$

- Microcline
- Orthoclase
- Sanidine

### **Plagioclase Series**

- Albite:  $\text{NaAlSi}_3\text{O}_8$
- Anorthite  $\text{CaAl}_2\text{Si}_2\text{O}_8$
- Other plagioclases form a continuous solid-solution series with any mixture of albite and anorthite possible. The composition of plagioclase is usually described in terms of the anorthite component. The plagioclase series consists of:
  - Albite: 0-10% anorthite
  - Oligoclase: 10-30% anorthite

- Andesine: 30-50% anorthite
- Labradorite: 50-70% anorthite
- Bytownite: 70-90% anorthite
- Anorthite: 90-100% anorthite.

### Mixtures of Feldspars

- Anorthoclase: a solid solution of about equal proportions of K-feldspar and albite.
- Perthite: an exsolution texture with K-feldspar enclosing plagioclase. If the texture is very fine it can be termed microperthite or cyptoperthite.
- Antiperthite: an exsolution texture with plagioclase enclosing K-feldspar.
- Peristerite: a microscopic- or smaller-scale exsolution texture in albite-rich plagioclase.

### Other Feldspars

- Celsian:  $\text{BaAl}_2\text{Si}_2\text{O}_8$
- Hyalophane:  $(\text{K,Na,Ba})(\text{Al,Si})_4\text{O}_8$
- The **feldspar structure** consists of zigzag chains of silica (and aluminum) tetrahedra enclosing rather large spaces where the alkali cations occur. The enclosing spaces are large, thus only large cations can be retained and there are no iron or magnesium feldspars. The closest mineral in composition to an iron-magnesium feldspar is probably the iron-manganese aluminosilicate **cordierite**, which has a completely different and unrelated structure.

### Summary of Feldspar Occurrence

#### Potassium Feldspars: $\text{KAlSi}_3\text{O}_8$

- Microcline: The principal potassium feldspar in granitic and metamorphic rocks. The low-temperature potassium feldspar.
- Orthoclase: A high-temperature polymorph found in volcanic rocks and high-temperature metamorphic rocks.
- Sanidine: The highest-temperature polymorph of potassium feldspar, found in volcanic rocks and very high-temperature metamorphic rocks.

### Plagioclase Series

- Albite (0-10% anorthite) is found mostly in very Na-rich environments. It forms from more anorthite-rich plagioclase by ion-exchange with sea water, and as an authigenic

mineral in marine sedimentary rocks. Magmas are rarely sufficiently Na-rich to form albite.

- Oligoclase (10-30% anorthite) is the principal plagioclase in granitic rocks.
- Andesine (30-50% anorthite) is the principal plagioclase in intermediate rocks.
- Labradorite (50-70% anorthite) is the principal plagioclase in gabbroic rocks and anorthosites.
- Bytownite (70-90% anorthite) is the least common plagioclase. It is too calcic to form from most magmas and too sodic to form in most metamorphic rocks. It is the first feldspar to form in mafic magmas and may be present in the cores of zoned feldspars. It also forms from sodic metasomatism of metamorphic rocks.
- Anorthite (90-100% anorthite) is too calcic to form magmatically and forms mostly from metamorphism of calc-silicate rocks.

### Mixtures of Feldspars

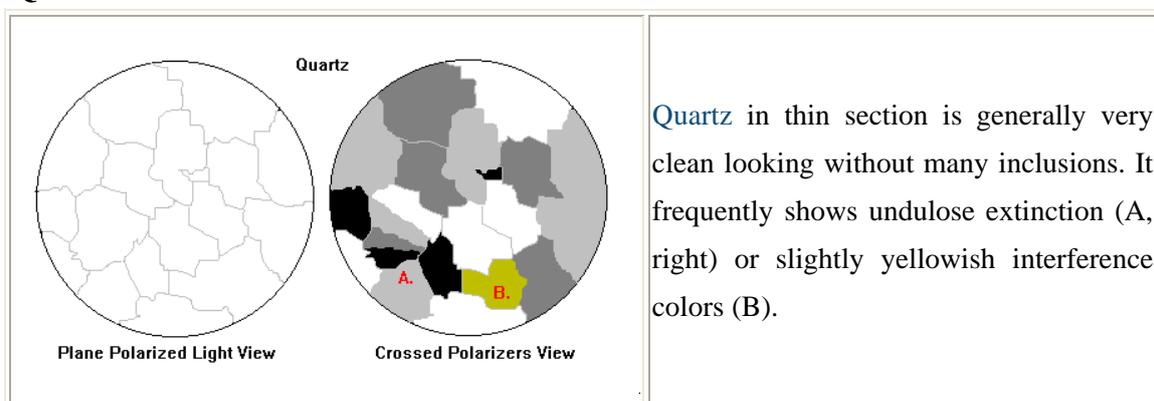
- Anorthoclase: a solid solution of about equal proportions of K-feldspar and albite. Generally a high-temperature mineral. The sodium-rich equivalent of orthoclase and sanidine.
- Perthite is widely found in microcline in granitic rocks.

### Other Feldspars

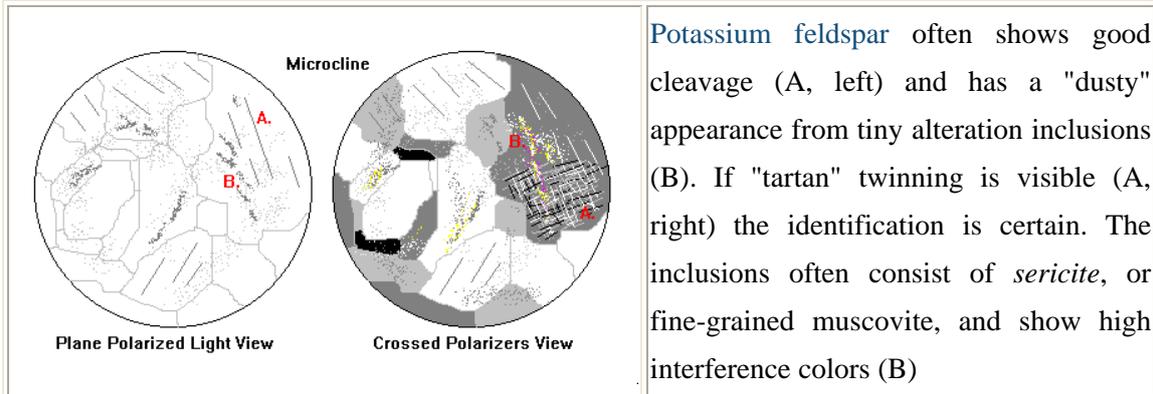
- Celsian:  $BaAl_2Si_2O_8$  and Hyalophane:  $(K,Na,Ba)(Al,Si)_4O_8$  are rare and found, for some reason, mostly in manganese or deposits.

## 3-6. Distinguishing Quartz and Its Look-Alikes

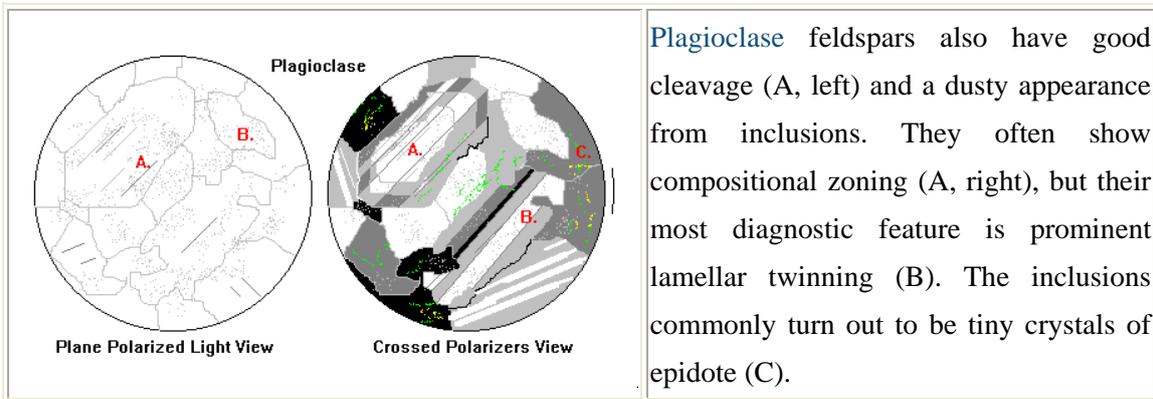
### Quartz



## Potassium Feldspar



## Plagioclase Feldspar

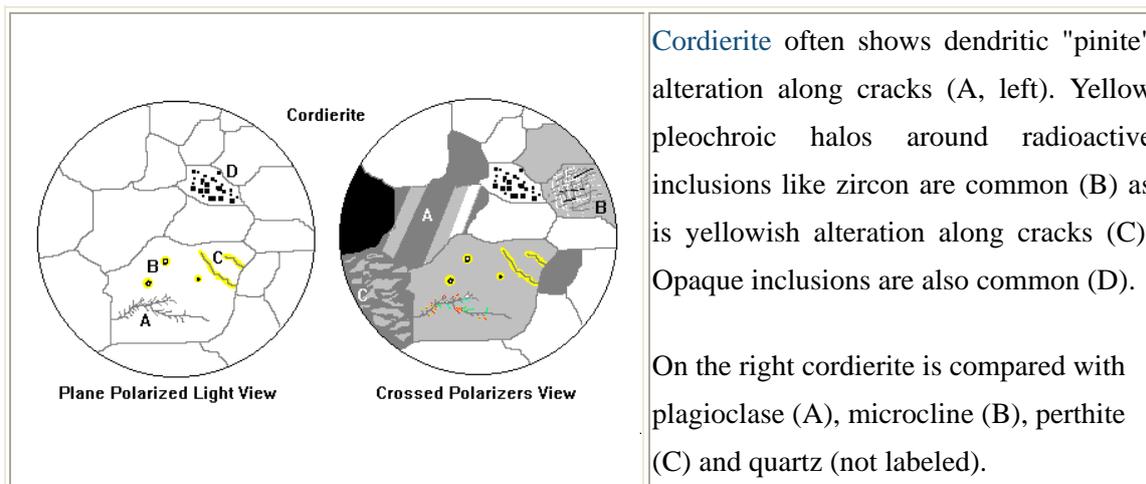


If your feldspar lacks twinning, it can be hard to tell them apart. Usually the dusty appearance, cleavage and alteration separate them from quartz. Sometimes the easiest approach is to use one of several staining methods.

## Nepheline

Nepheline *never* occurs with quartz, unlike the other minerals shown here. It is most often found in silica poor igneous rocks that are rich in alkali feldspar. Nepheline is uniaxial, but in contrast to quartz is optically negative. It lacks cleavage, distinguishing it from the feldspars. Nepheline commonly alters to *cancrinite*, a Na- and Ca- bearing aluminosilicate. Cancrinite typically shows first order interference colors but the crystals are typically much larger than the very fine sericite grains that form in K-feldspar, and are easily distinguishable.

## Cordierite



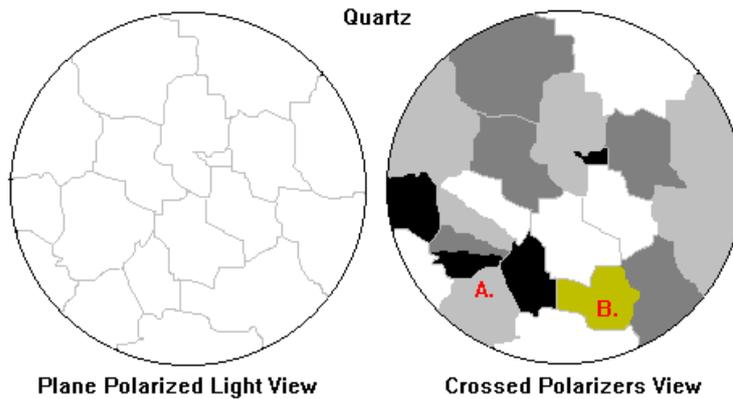
### 3-7. Distinguishing Andalusite, Kyanite and Sillimanite in Thin Section

	Andalusite	Kyanite	Sillimanite
<b>Color</b>	Clear to pleochroic in patchy shades of pink	Colorless to grayish	Colorless, clear
<b>Crystal Habit</b>	Square prisms. Chiastolite cross-markings in cross-section are distinctive but not invariably present	Bladed grains	Needles or slender prisms. Masses of fine needles common.
<b>Twinning</b>	Lamellar twinning common		
<b>Cleavage</b>	Three sets nearly perpendicular	Prismatic cleavage, two sets	Pinacoidal cleavage, transverse cracks characteristic.
<b>Refractive Index</b>	High	Highest of the three	High
<b>Birefringence</b>	Low (.011)	Moderate (.014)	Moderately high (.023)
<b>Extinction</b>	Parallel	Inclined	Parallel
<b>Elongation</b>	Negative	Generally Positive	Positive
<b>Optic Sign</b>	Negative, $2V=86$ degrees	Negative, $2V=83$ degrees	Positive, low $2V$ (24 degrees)

## 4. Rock-forming Minerals

### 4-1. Essential Minerals

#### Quartz



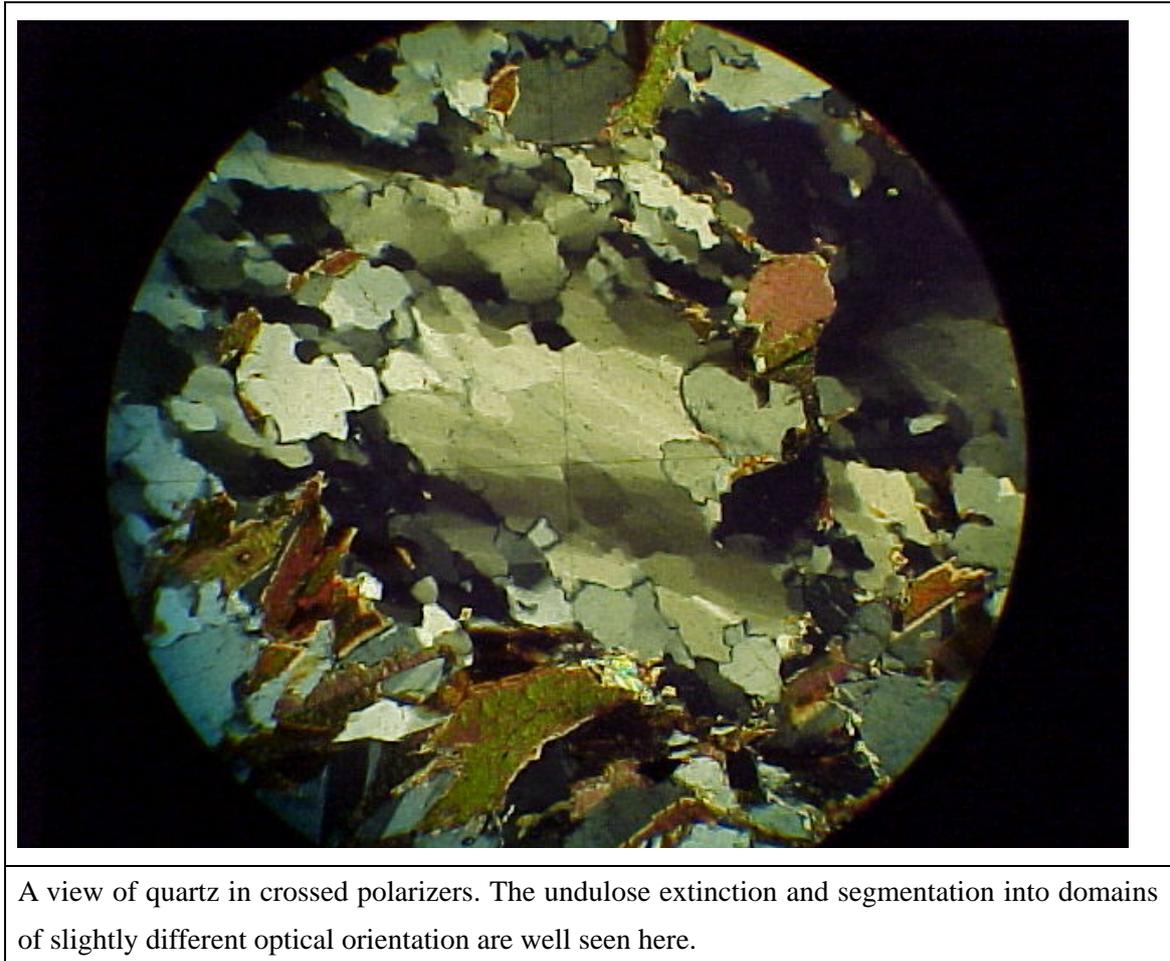
#### Plane-Polarized Light

- Low relief
- Clear
- No cleavage
- Usually free of inclusions

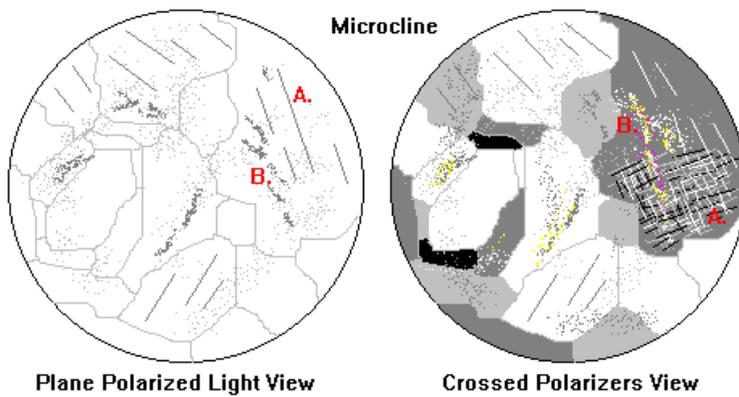
#### Crossed Polarizers

- Gray to white interference colors, sometimes to light yellowish white
- Undulose extinction common, often with a fan-like pattern (A)
- Grains with highest interference color sometimes look yellowish-brown near extinction (B)

Quartz, the feldspars, cordierite and nepheline all have low relief and gray to white interference colors. Beginners find them hard to tell apart. [View Quartz and its Look-Alikes.](#)



### Microcline

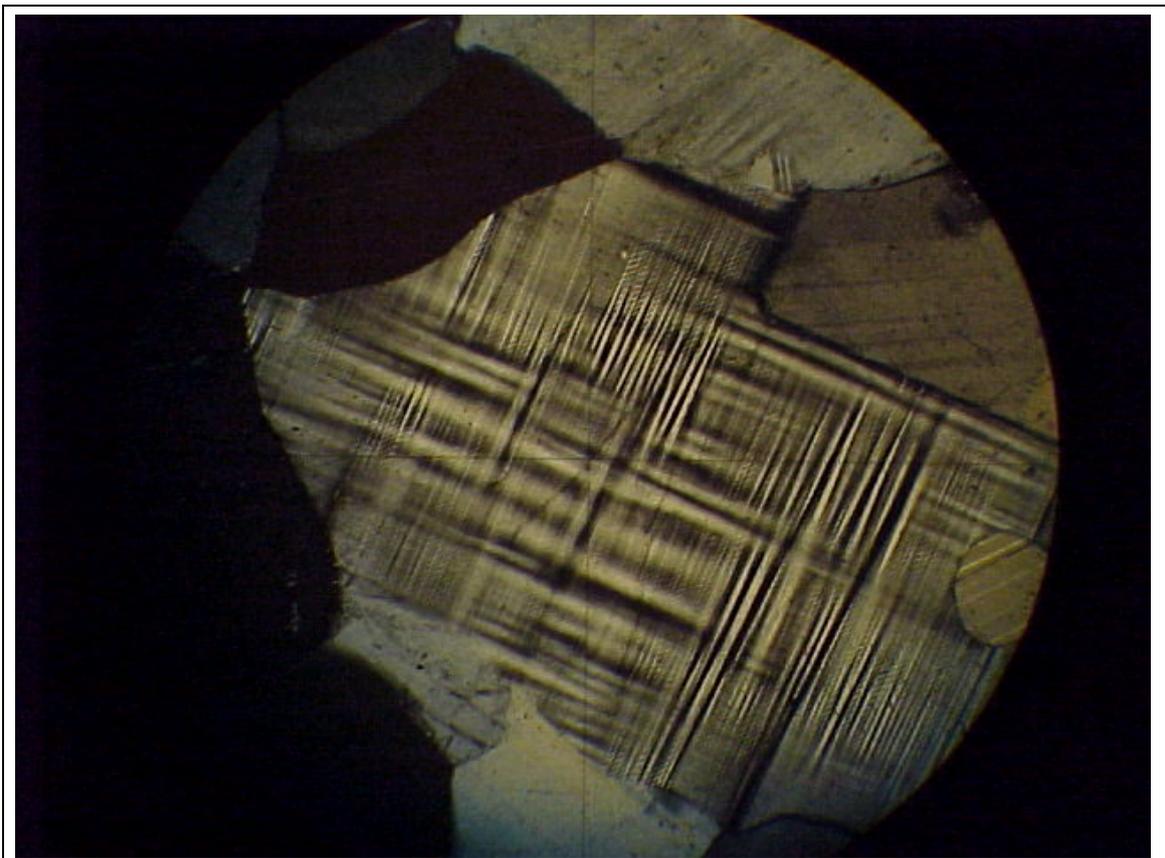


Plane-Polarized Light

- Low relief
- Clear
- Cleavage often visible (A)
- Usually numerous inclusions, giving it a much dustier look than quartz. The inclusions are due to microscopic alteration (B)

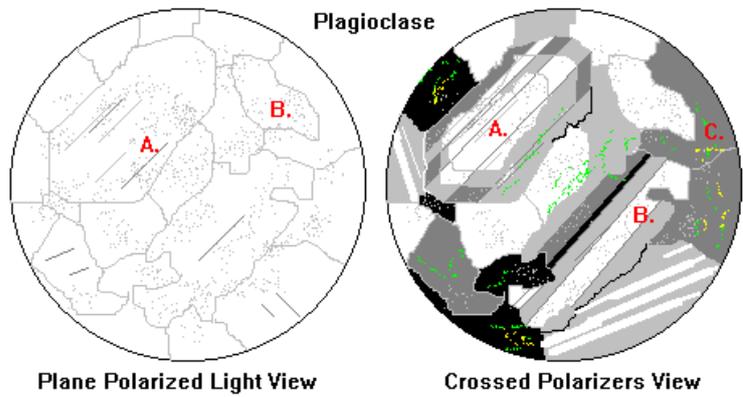
#### Crossed Polarizers

- Gray to white interference colors, slightly lower than quartz
- Alteration products often have high interference colors (A)
- Often has a distinctive crosshatch or "tartan" twinning pattern.(B)
- Perthitic texture, due to exsolution of plagioclase, is very common (C). It is easily visible in hand specimen as irregular milky streaks.



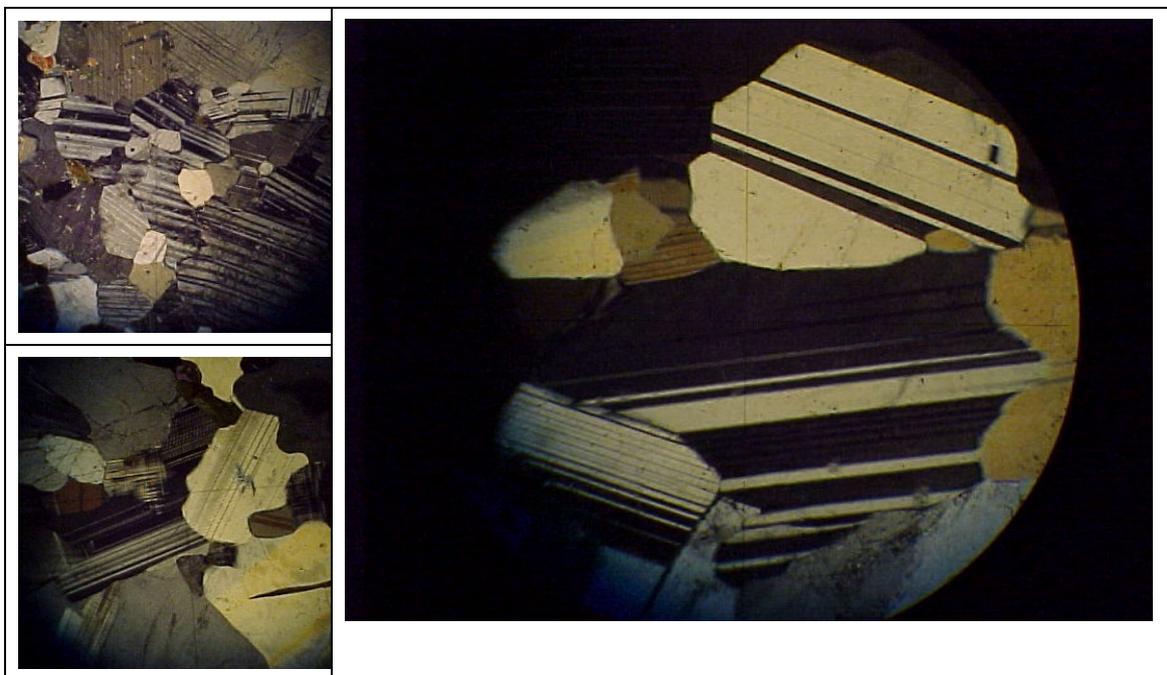
The photo shows tartan twinning clearly.

## Plagioclase



### Plane-Polarized Light

- Low relief
- Clear
- Cleavage often visible (A)
- Usually numerous inclusions, giving it a much dustier look than quartz. The inclusions are due to microscopic alteration (B)



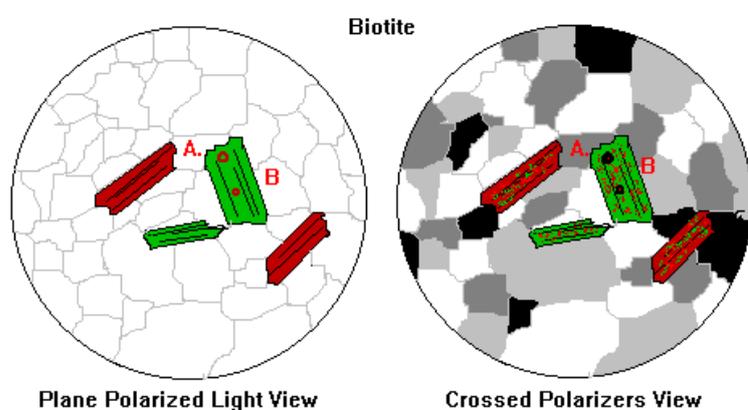
### Crossed Polarizers

- Gray to white interference colors, slightly lower than quartz
- The tiger-stripe twinning is far and away the most distinctive feature, but beware! Not all plagioclase shows twinning.
- Euhedral crystals are common and frequently zoned (A). Zoning is a valuable tracer of magmatic history.
- Corroded crystals (B) are also common due to alteration or reaction with magma or other minerals
- Alteration products often have high interference colors (C). Tiny euhedral epidote crystals are common as alteration products.

## Quartz and Its Looks-Alike

*Refer to chapter 3.6.*

## Biotite

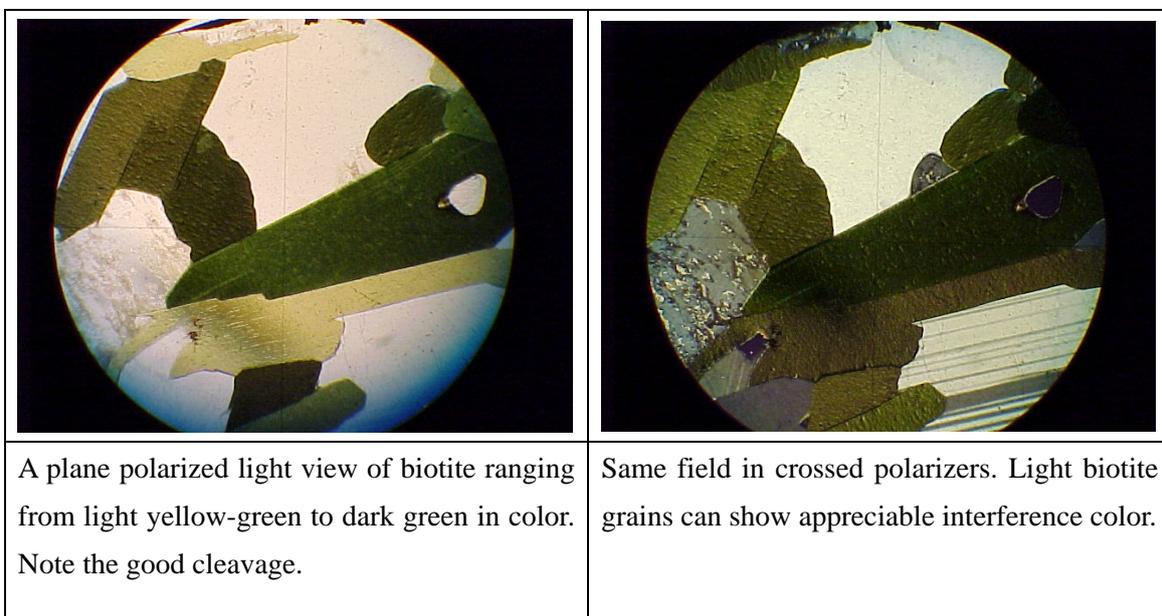
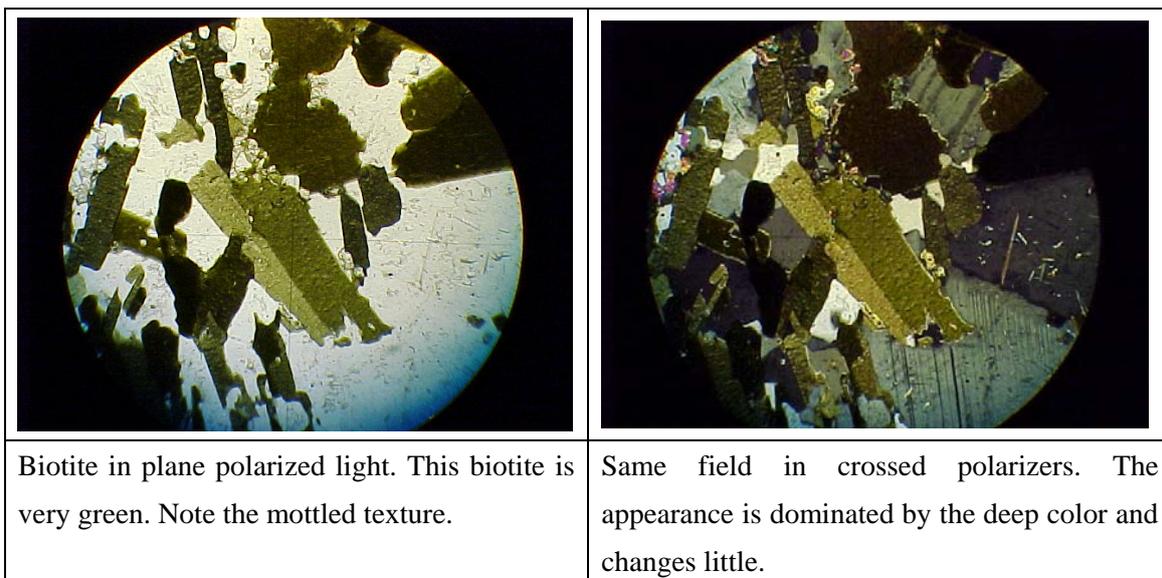


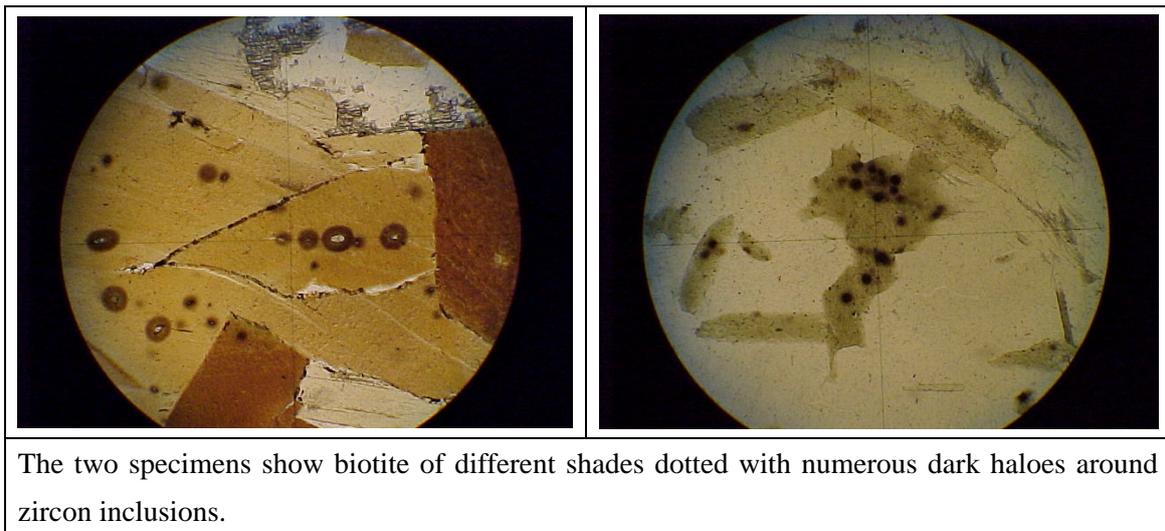
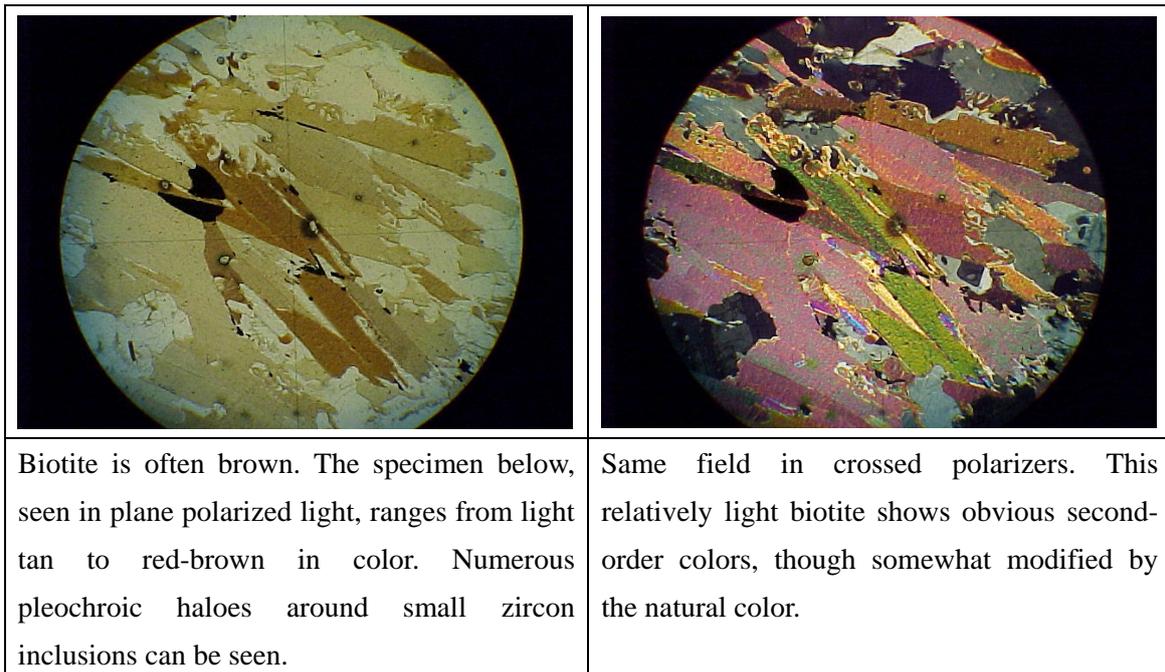
### Plane-Polarized Light

- Moderate relief
- Orange, brown or dark green (A)
- Perfect micaceous cleavage
- May be dark pleochroic halos around inclusions of zircon or other mildly radioactive minerals (B)

### Crossed Polarizers

- Second-and third-order interference colors usually not strikingly evident because of the strong natural coloration.
- Mottling common, giving the mineral a gnarly or "birds-eye maple" texture (A)
- May be dark pleochroic halos around inclusions of zircon or other mildly radioactive minerals (B)



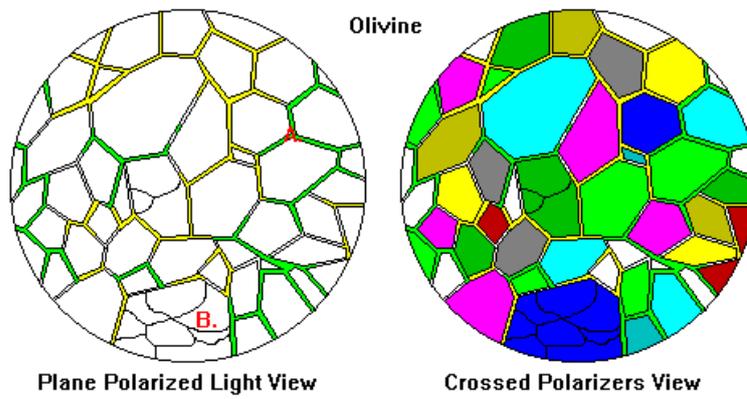


## Olivine

### Plane-Polarized Light

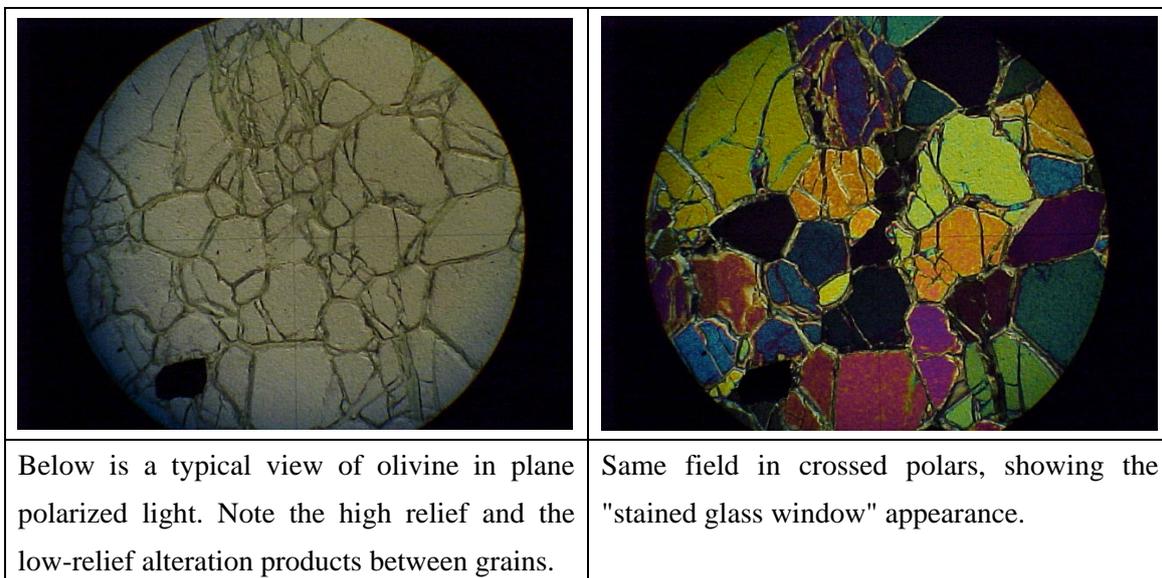
- Moderately high relief
- Clear, occasionally very light yellowish or greenish
- No cleavage
- Commonly rimmed with greenish alteration products (A)
- Internal fracturing of grains common (B)

- Never occurs with quartz

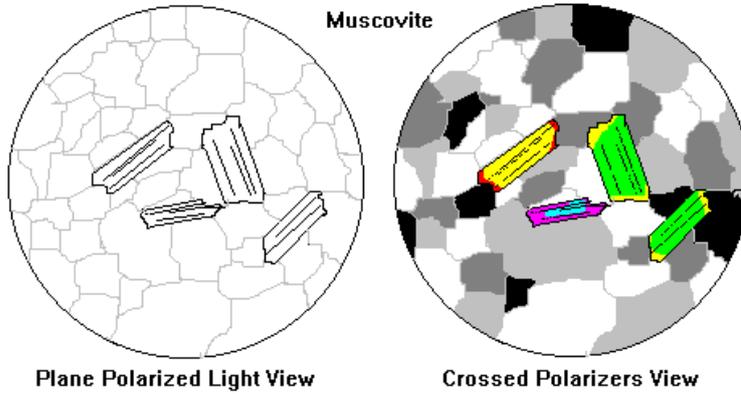


### Crossed Polarizers

- Bright second- and third-order interference colors.
- Alteration products tend to have low interference colors.

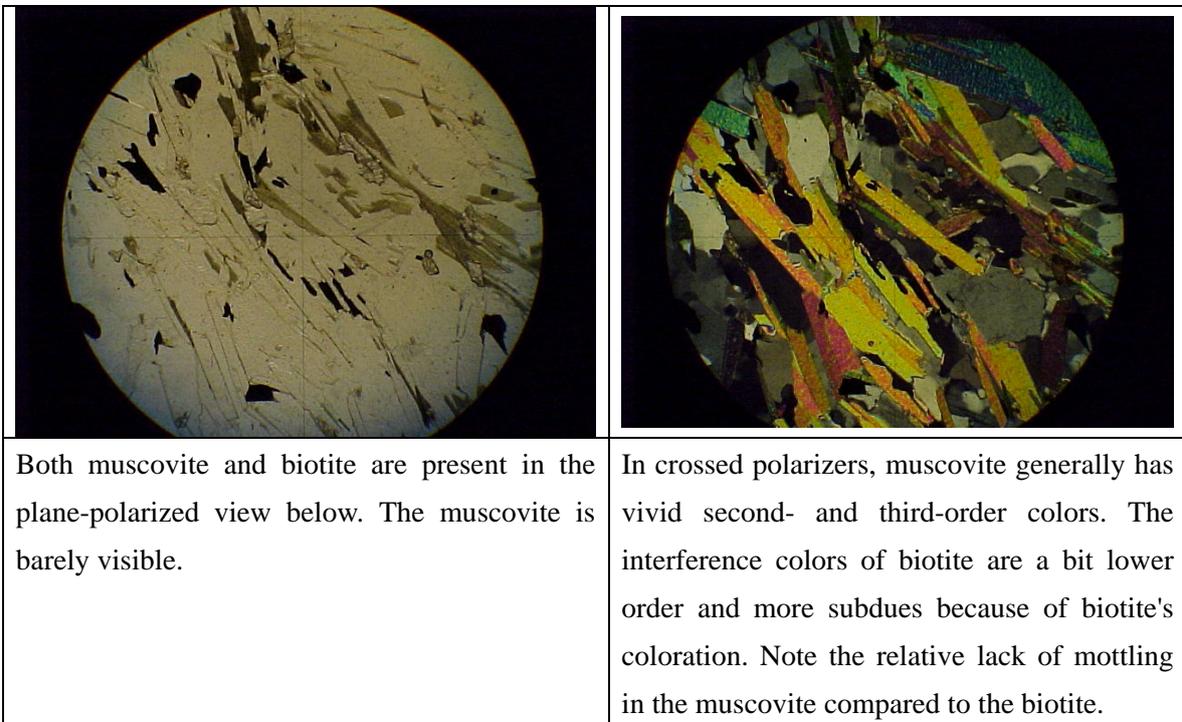


## Muscovite



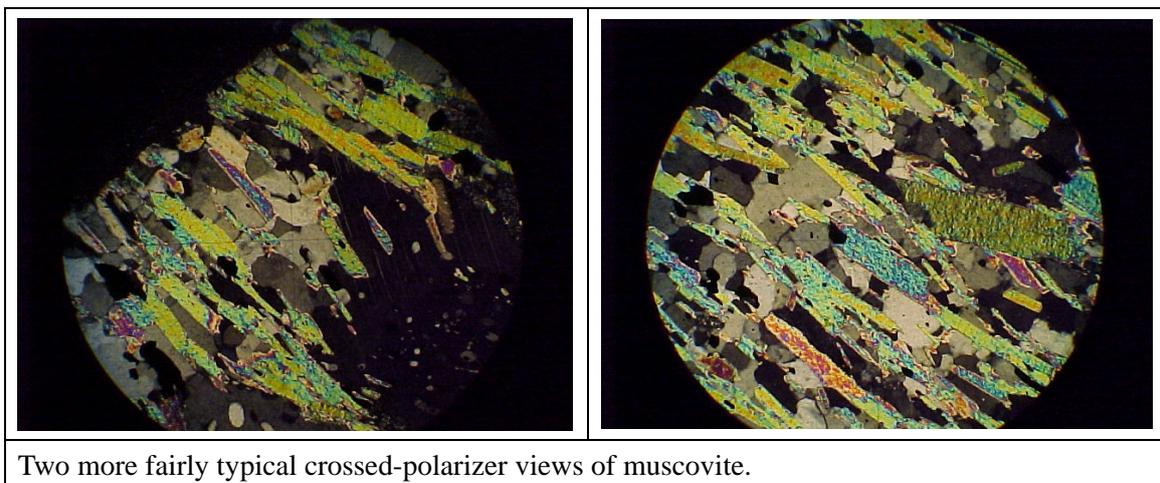
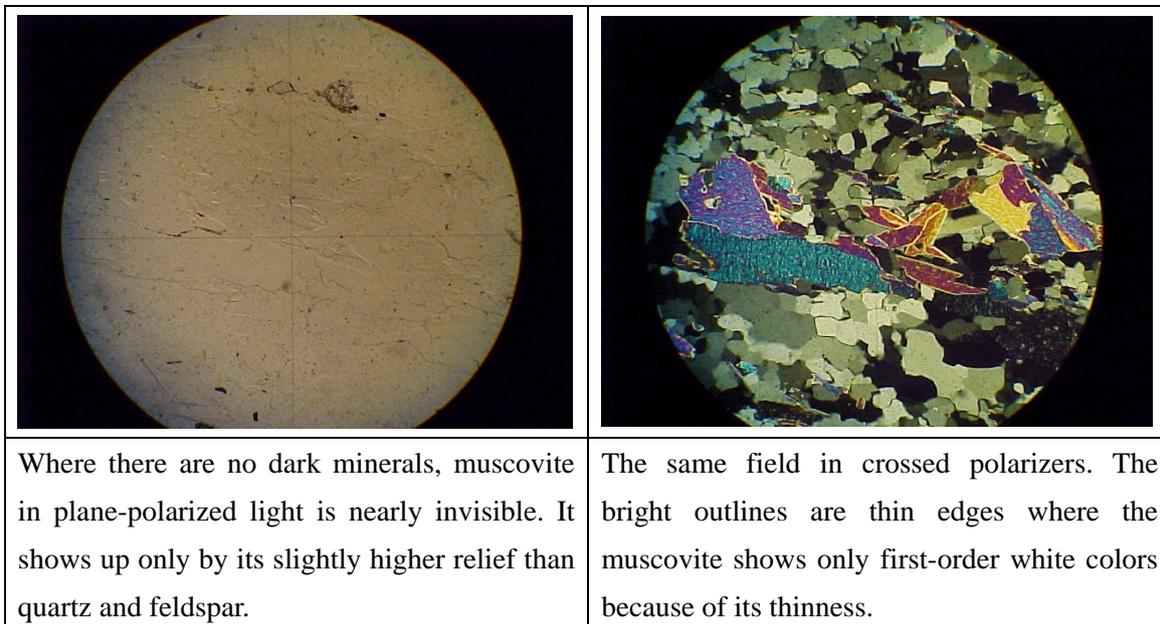
### Plane-Polarized Light

- Low relief
- Clear
- Perfect micaceous cleavage

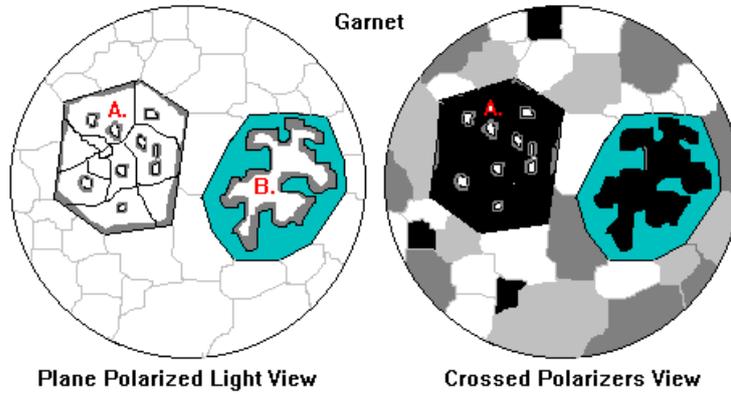


### Crossed Polarizers

- Bright second- and third-order interference colors
- Generally not as much mottling as biotite.



## Garnet



### Plane-Polarized Light

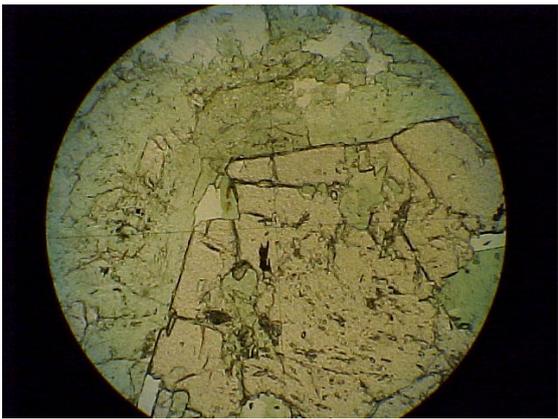
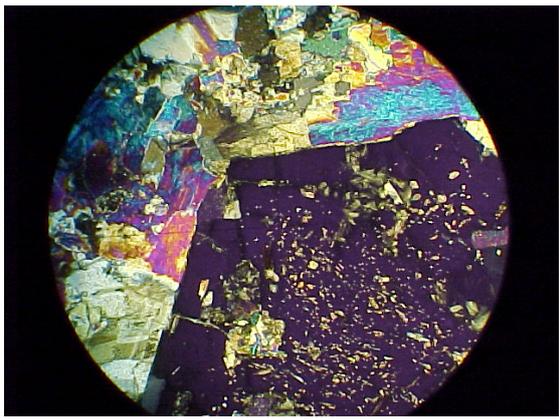
- High relief
- Clear, occasionally very light pinkish
- No cleavage
- Euhedral crystals common (A)
- Retrograde metamorphism common (B). Garnet will revert to greenish amphiboles or chlorite, still retaining the original outline of the crystal. Remnants of garnet are often preserved in the interior
- Internal fracturing of grains common

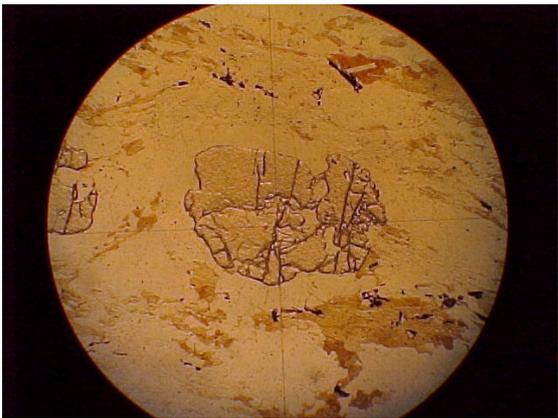
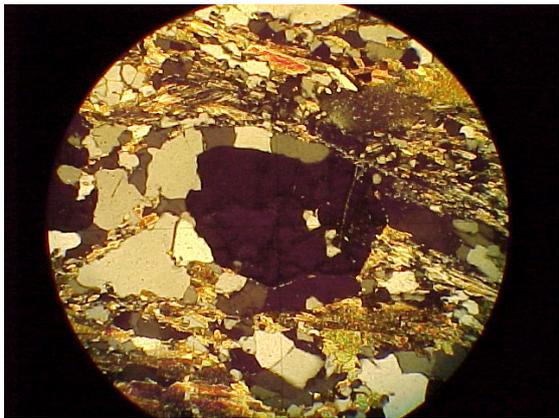
<p>In plane-polarized light, garnet is clear and has very high relief. The light brown material is biotite. Most of the clear material is quartz.</p>	<p>In crossed polarizers, garnet is isotropic. The black areas are larger than the visible garnet above because of plucking - part of the garnet broke off the slide during the grinding process.</p>

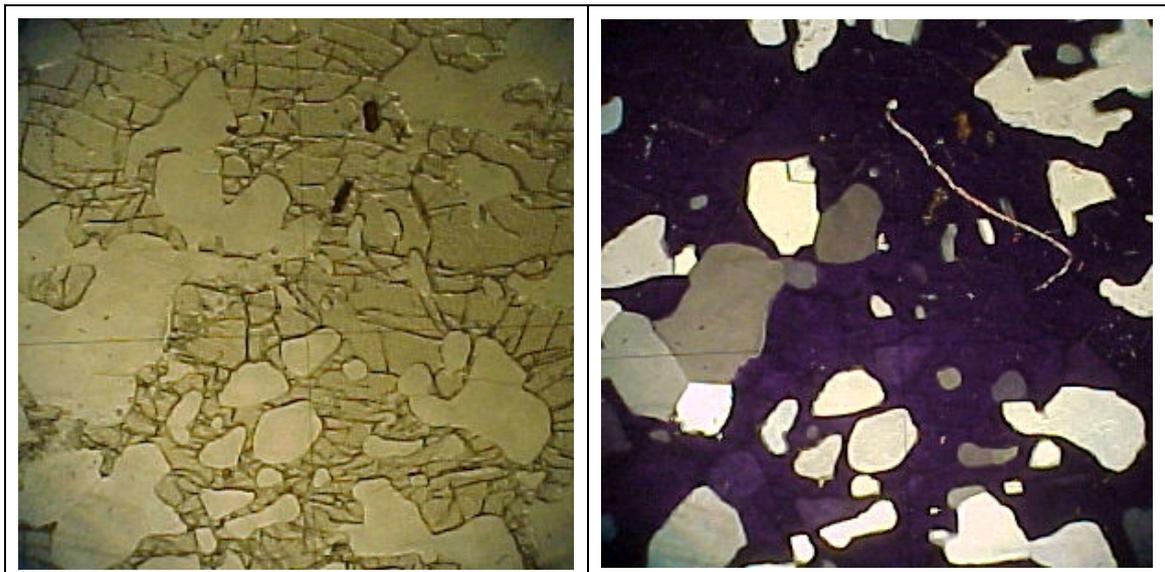
- Crystals commonly contain inclusions, incorporated as the garnet grew.

### Crossed Polarizers

- The most common isotropic mineral

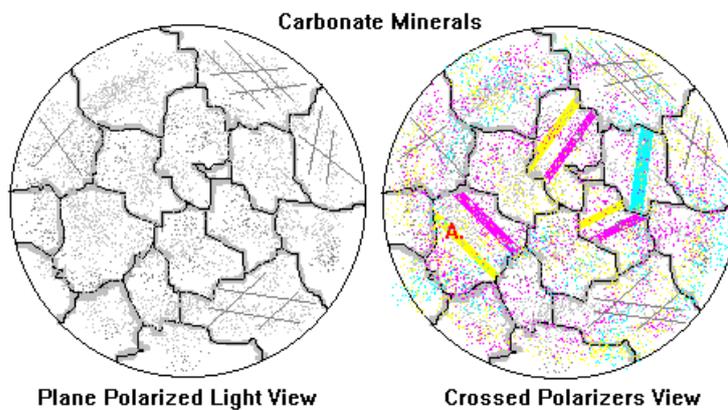
	
<p>Garnet often is euhedral, as in the plane-polarized view below. This garnet is light pinkish. The light green material around it is an amphibole called omphacite. The rock is an eclogite, a metamorphic rock from the upper mantle.</p>	<p>In crossed polarizers the euhedral form of the garnet stands out sharply. Note the numerous inclusions.</p>

	
<p>A fairly typical small garnet seen in plane-polarized light.</p>	<p>The same field is seen in crossed polarizers.</p>



At left is a very large garnet filling the entire field and enclosing numerous quartz inclusions. At right is the same field under crossed polarizers.

## Carbonate Minerals

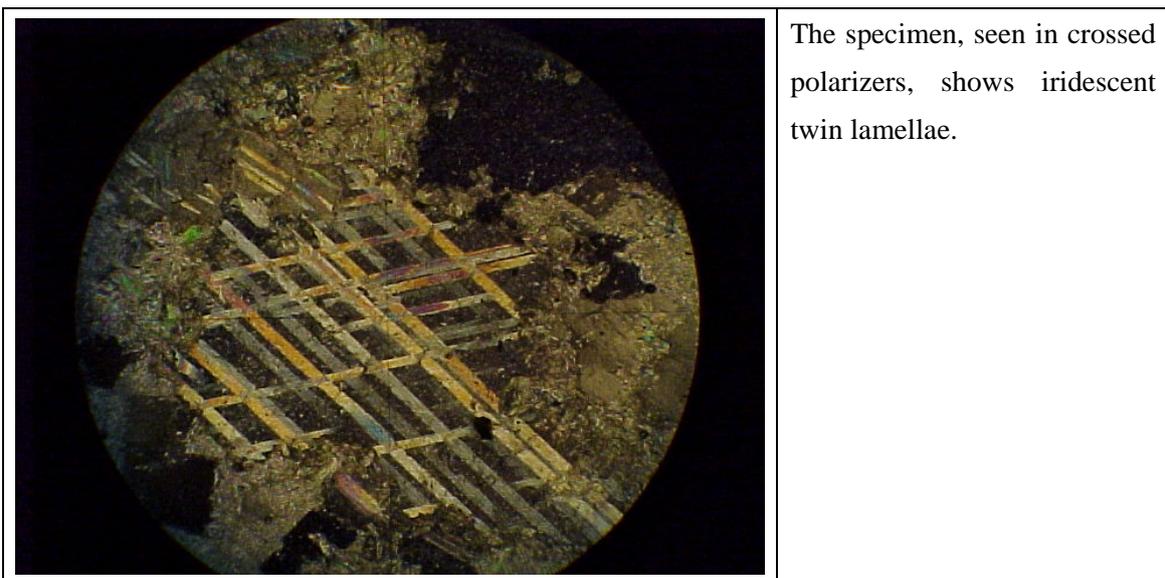
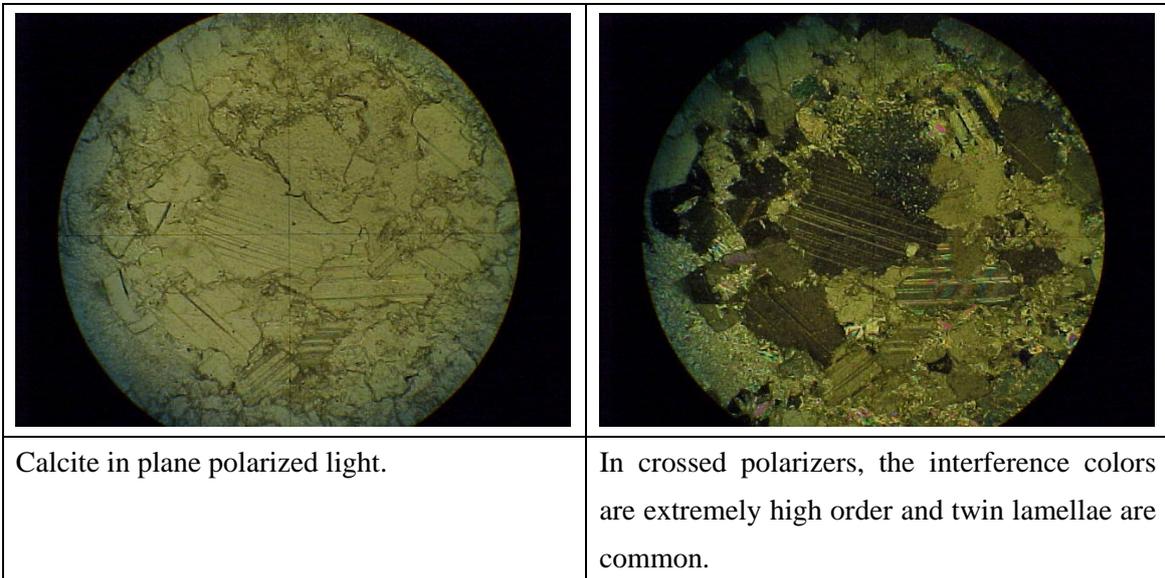


### Plane-Polarized Light

- Calcite, Dolomite, Siderite and other common carbonates are almost indistinguishable in thin section.
- Extremely high relief
- Colorless, but tends to be grainy in appearance. The extremely high relief means even the tiniest irregularities stand out.
- Perfect rhombohedral cleavage

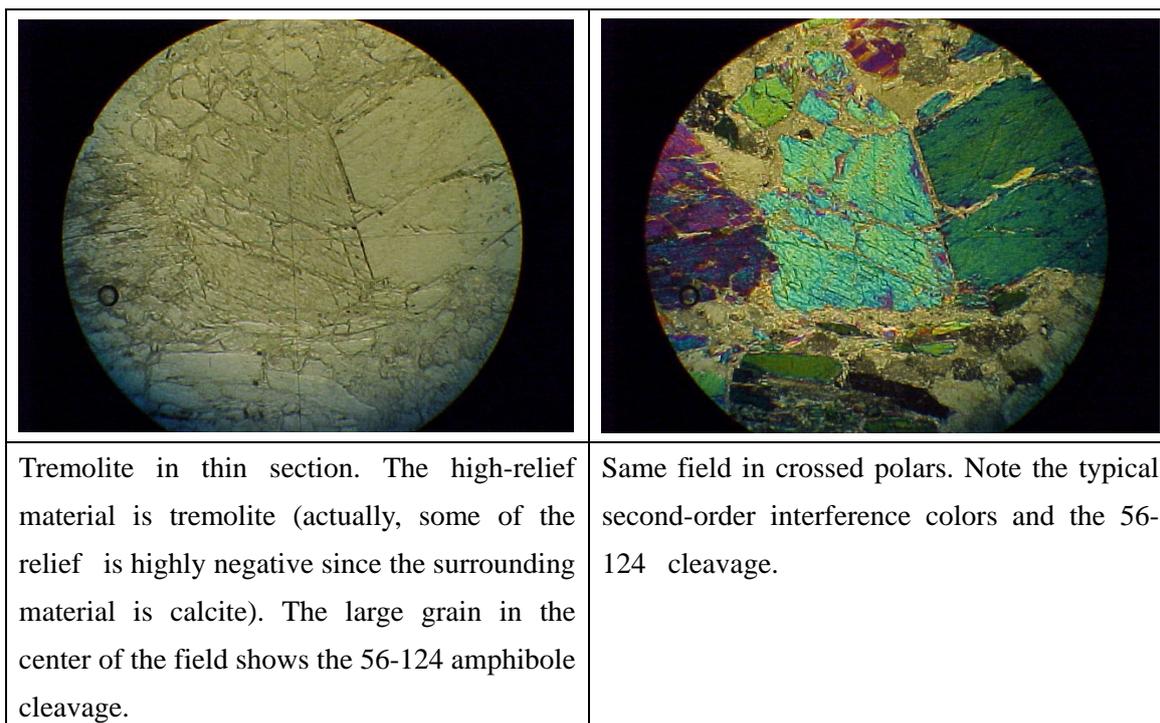
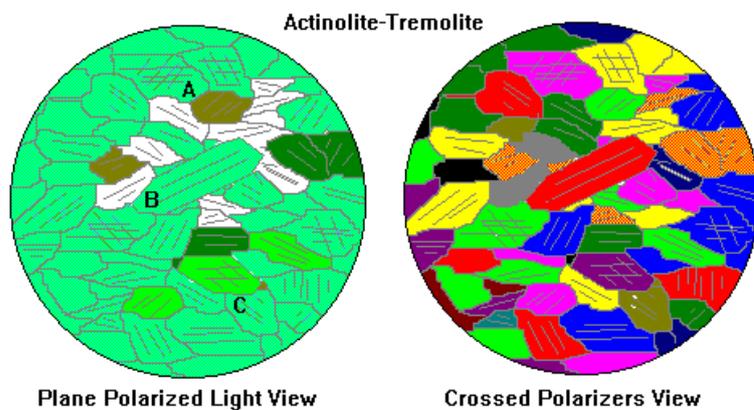
## Crossed Polarizers

- Extremely high order interference colors. Has a pearly appearance because of tiny patches of color. Even the slightest variation in thickness due to grinding results in noticeable color variation.
- Twin bands are common (A). These often have moderate but highly mottled color because the crystallographic orientation in the twin band partially cancels out the interference color of the rest of the crystal. Calcite is extremely ductile and these are often due to deformation.



## 4-2. Amphiboles and Pyroxenes

### Actinolite-Tremolite



#### Plane-Polarized Light

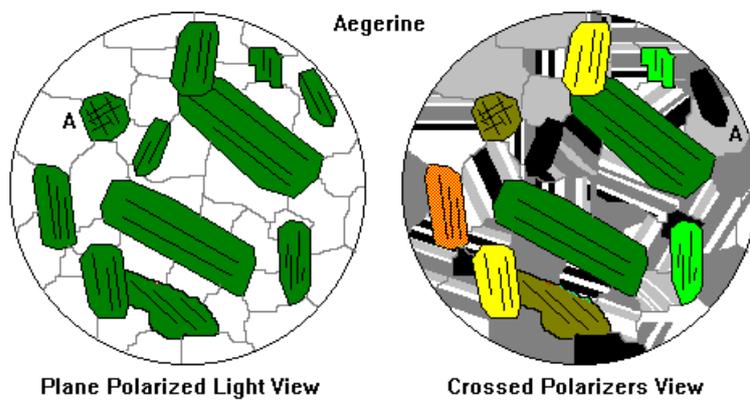
- A series of amphiboles ranging from pure magnesium to iron-rich compositions. The more iron, the deeper the color (A).
- Actinolite is a very common amphibole in medium-grade metamorphic rocks. It is pleochroic in shades of light green, with moderate relief. Often shows good 56-124 degree amphibole cleavage (C).

- Tremolite occurs in magnesium-rich settings like metamorphosed, impure dolomites. It is like actinolite in properties but colorless (B).

### Crossed Polarizers

- Mostly first-order interference colors, sometimes second order, not too much affected by the generally light color.

### Aegerine



<p>Acmite in plane-polarized light. Note the near right-angle pyroxene cleavage in the central grain. Pyroxene cleavage this good is actually fairly uncommon, and much more often seen in thin section than hand specimen.</p>	<p>Same field in crossed polarizers. The deep green color still pretty much dominates, though some grains with red interference colors shift to orange or yellow, a blend of interference color and natural color. Note also the lath-like plagioclase feldspar crystals.</p>

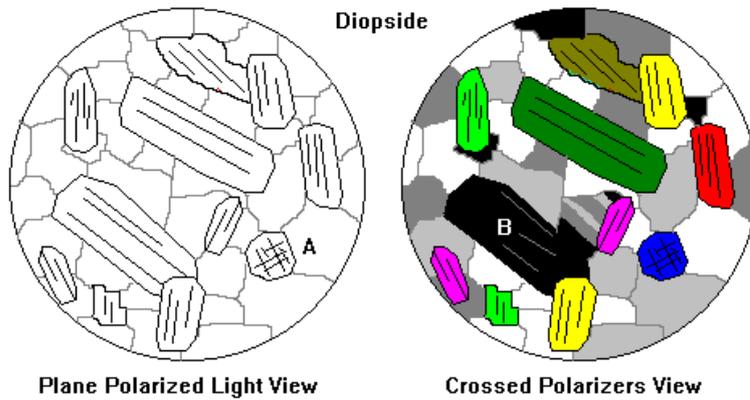
### Plane-Polarized Light

- A sodium-bearing pyroxene of highly-alkaline igneous rocks. Mostly identified by its deep green color. May show characteristic 87-93 degree pyroxene cleavage.

Crossed Polarizers

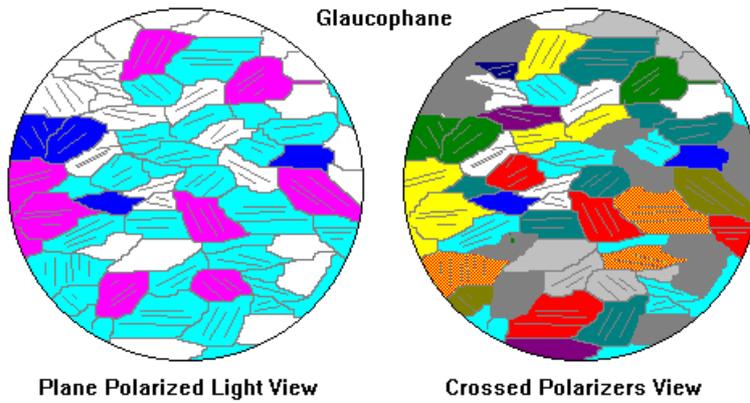
- The deep color pretty much dominates the interference colors.

**Diopside**



<p>The rounded grains with good cleavage in the plane-polarized view below are diopside. The surrounding material is mostly calcite. The diopside has high relief because its refractive index is strongly different from the calcite.</p>	<p>Same field in crossed polarizers. The bright interference colors are typical, as is the occurrence. Diopside is a common mineral in calc-silicate marbles.</p>

## Glaucophane

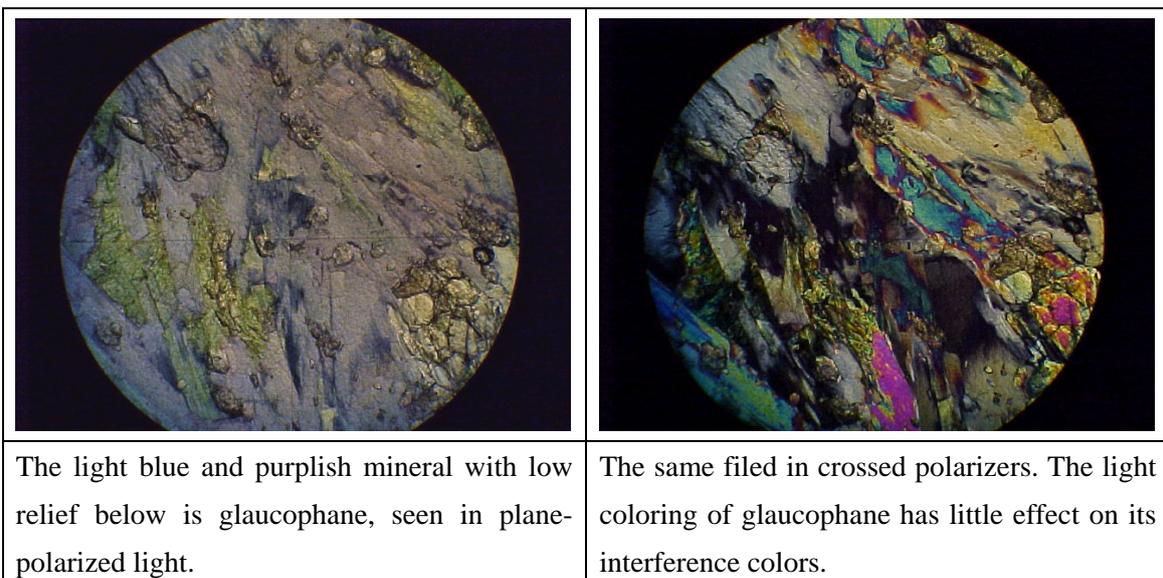


### Plane-Polarized Light

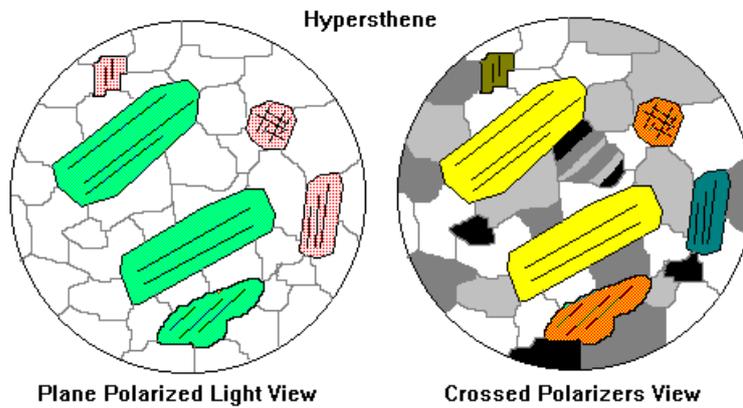
- A somewhat uncommon mineral but very important geologically because it is pretty much confined to high-pressure, low temperature subduction zone settings. Thus it is of great tectonic significance. It's a sodium-bearing amphibole.
- Moderate relief, pleochroic in shades from near colorless through light blue to lavender. The color is the most distinctive feature.

### Crossed Polarizers

- Moderate birefringence, resulting in first- and second-order colors that are not too badly modified by its natural color.

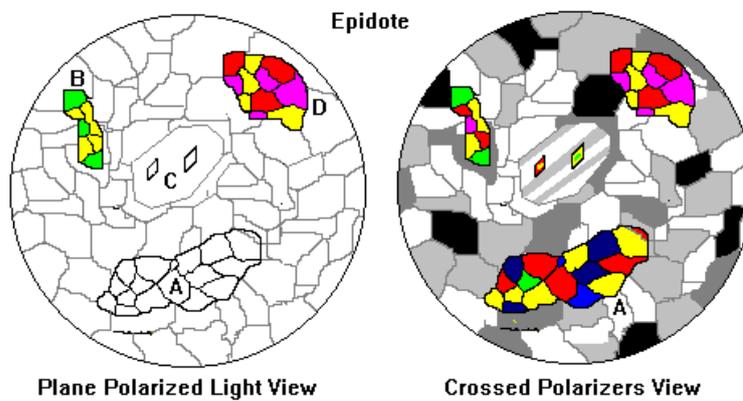


## Hypersthene



## 4-3. Important Igneous Minerals

### Epidote

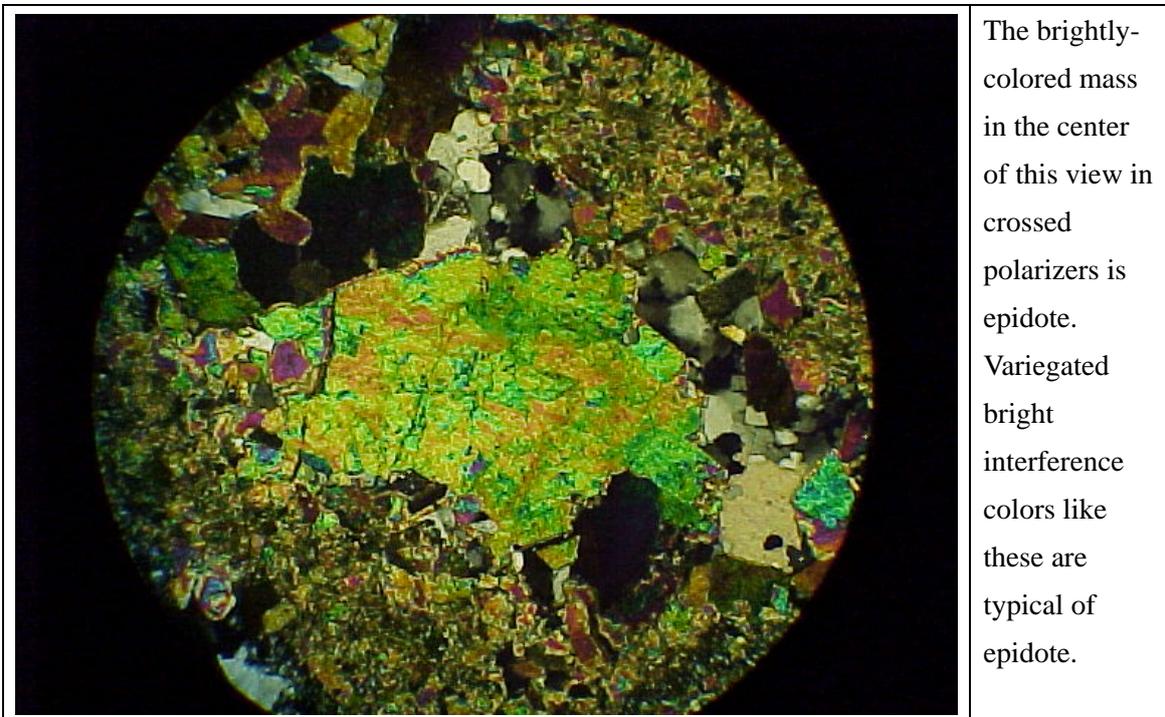
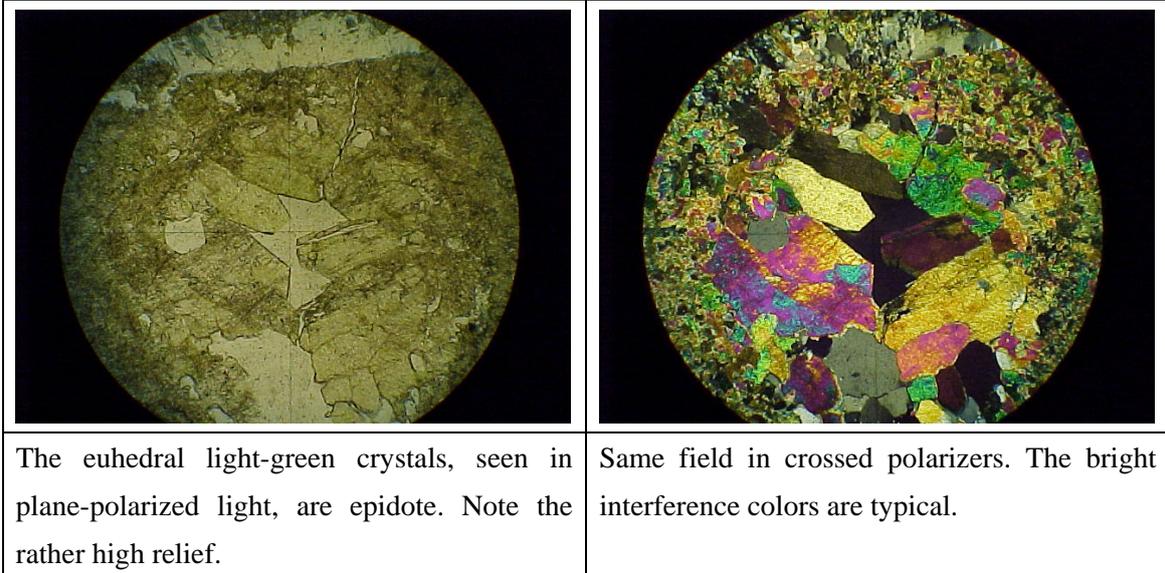


#### Plane-Polarized Light

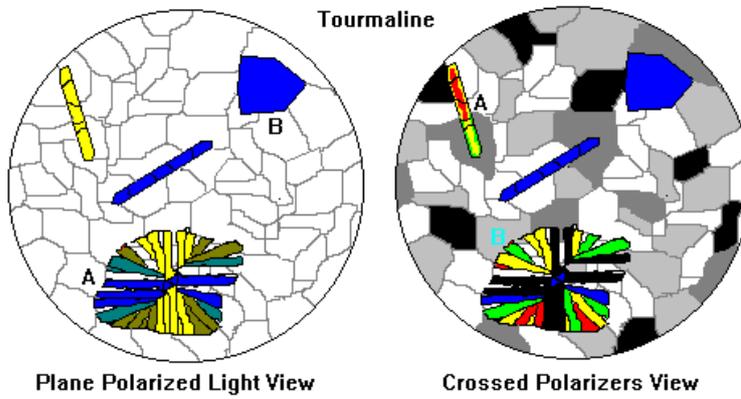
- High relief. Can occur as colorless irregular grains (A) or be tinted yellowish or greenish (B). Common alteration or metamorphic mineral derived from plagioclase, and small euhedral crystals in plagioclase are common (C).
- *Piemontite*, an uncommon manganese-bearing epidote, is one of the showpieces of geology. It has a dazzling pleochroism in yellow, orange, red and magenta (D). It cannot be mistaken for anything else.

### Crossed Polarizers

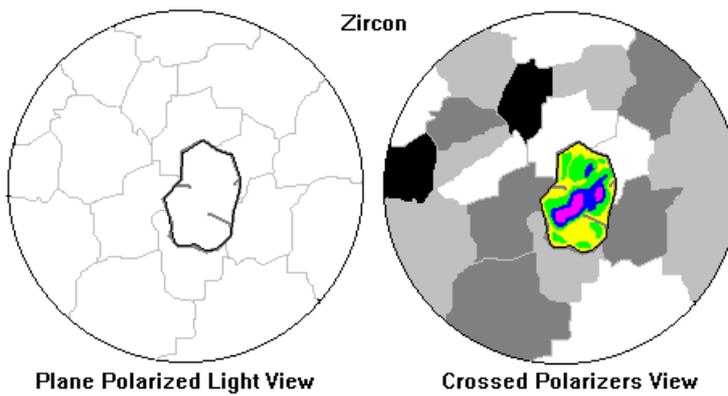
- Moderately high birefringence resulting in vivid second- and third- order colors. Anomalous interference colors are common in the variety *zoisite* (A). Piemontite's bright colors totally dominate its interference colors.



## Tourmaline



## Zircon

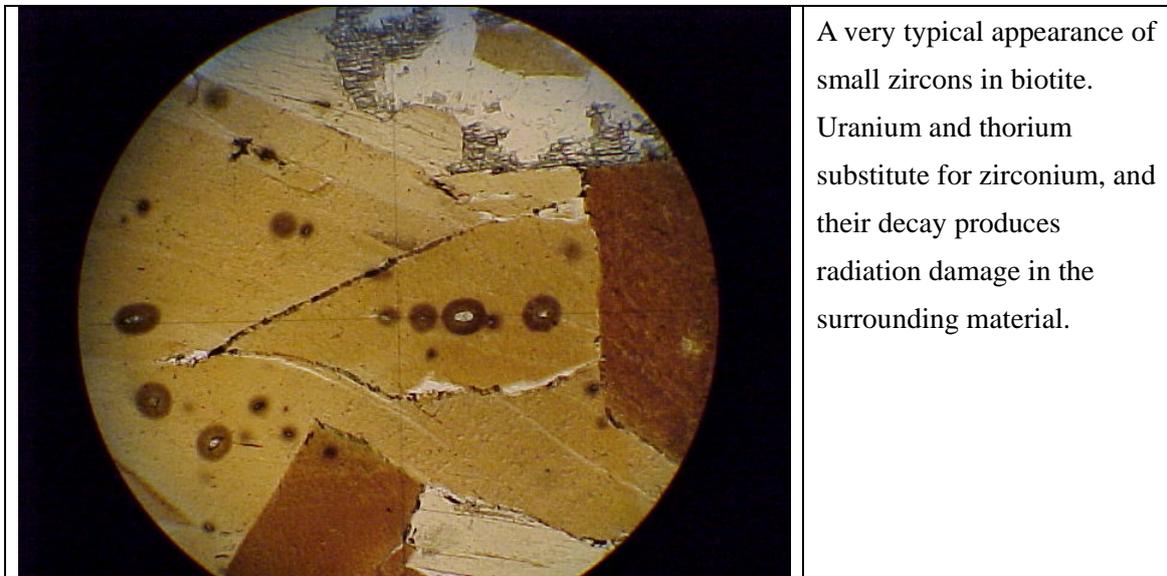
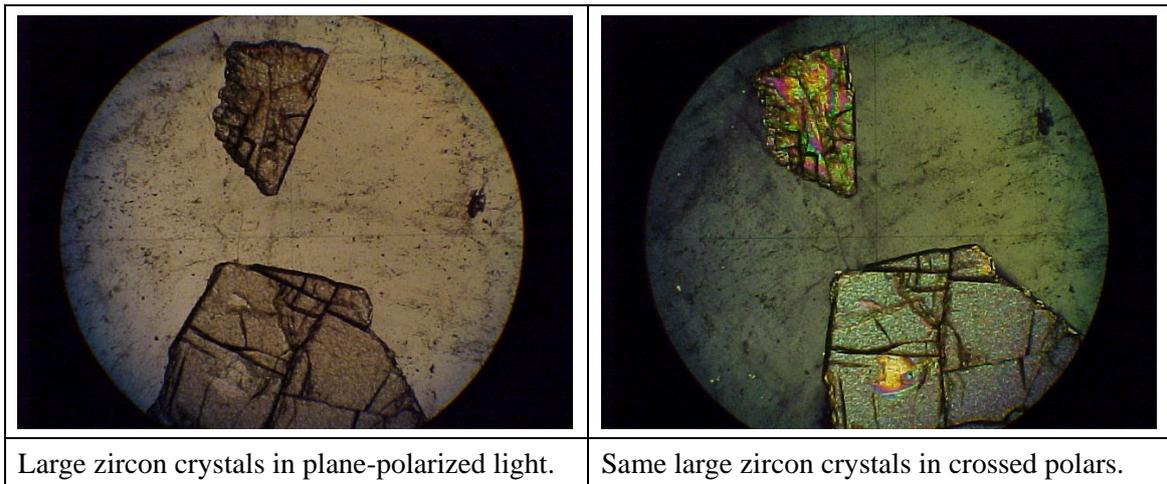


### Plane-Polarized Light

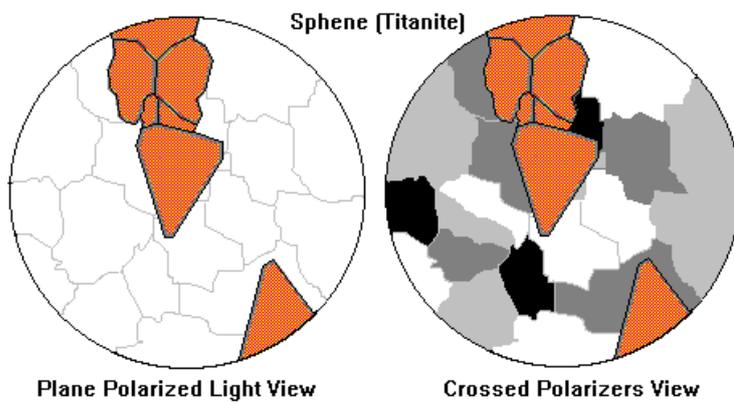
- Very high relief
- Usually clear but may be colored
- When surrounded by biotite or cordierite, often create pleochroic haloes due to radiation damage.

### Crossed Polarizers

- Very high (third- or fourth-order) interference colors



**Sphene (Titanite)**

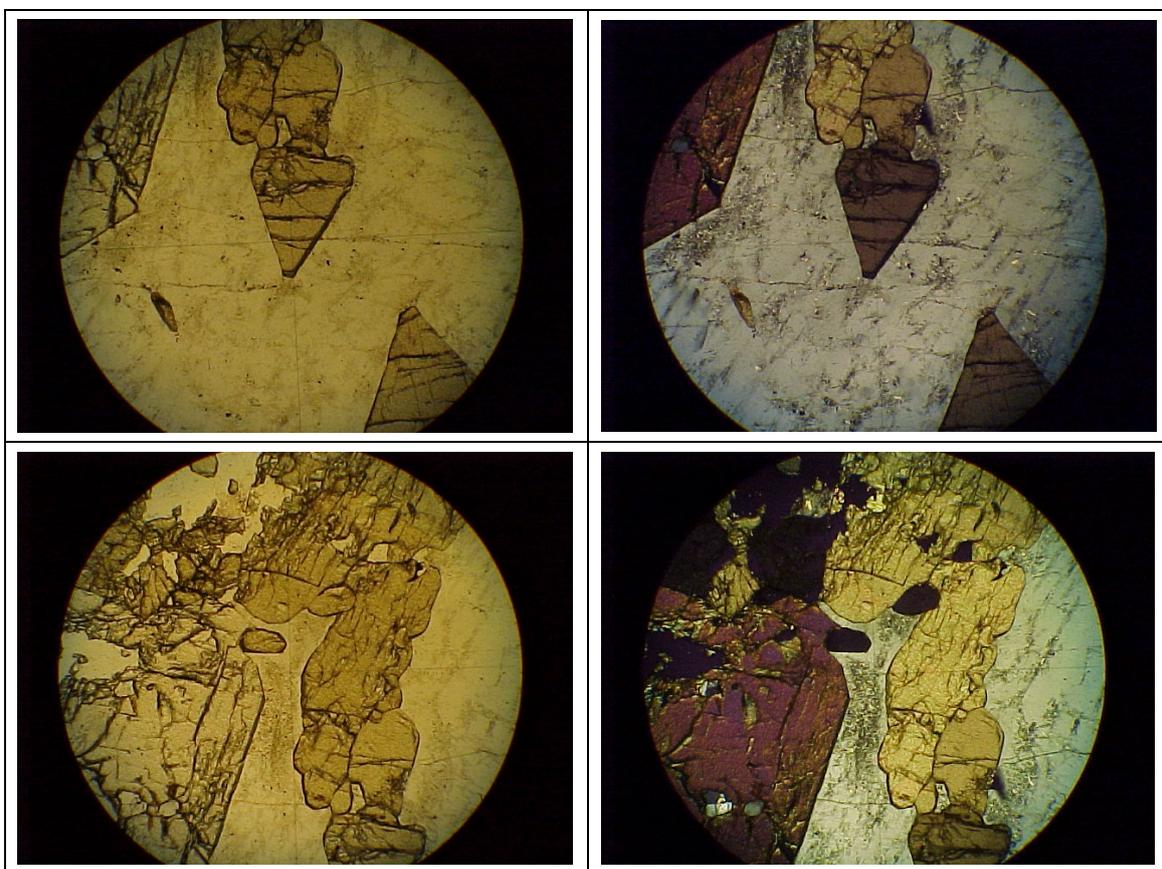


## Plane-Polarized Light

- High relief
- Often brownish or pinkish
- Wedge- or rhomb-shaped crystals common

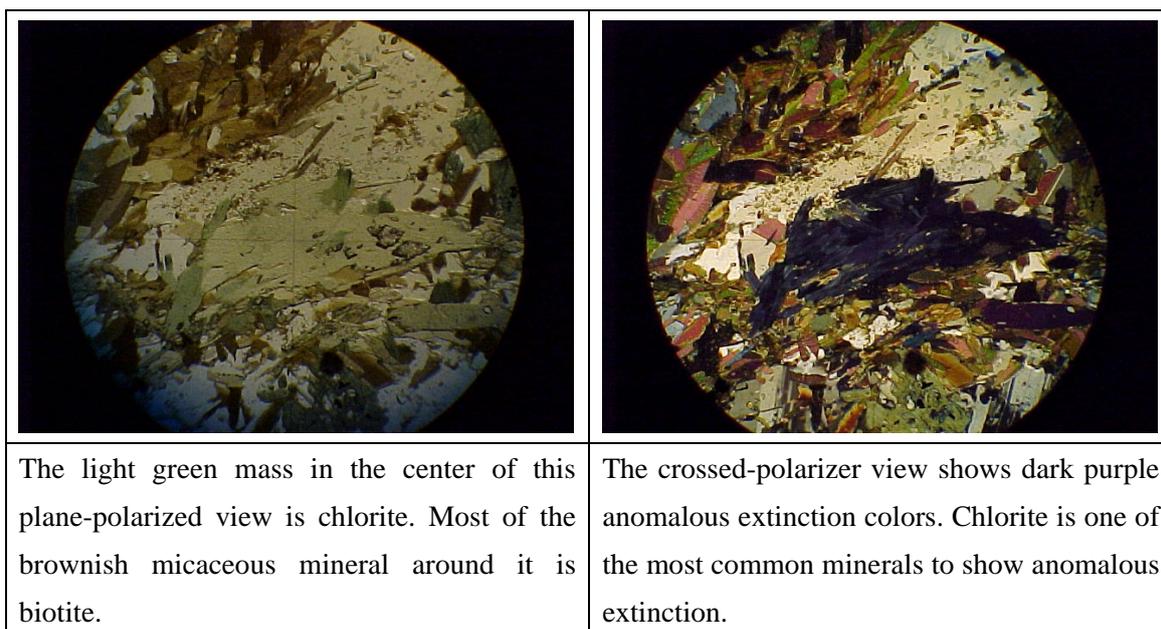
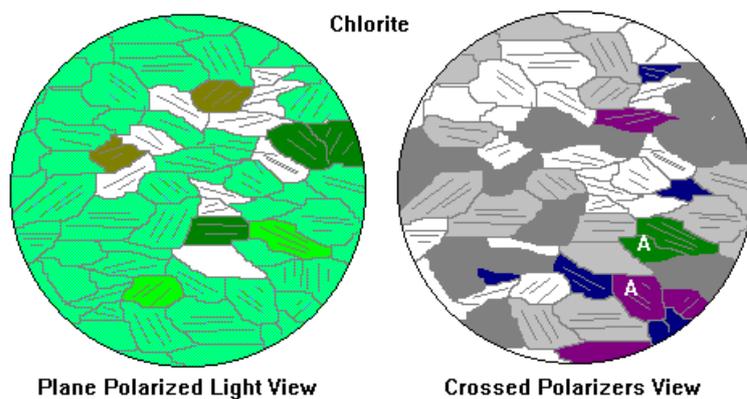
## Crossed Polarizers

- Extremely high birefringence - ultra-high-order whites result in little change in appearance.



## 4-4. Important Metamorphic Minerals

### Chlorite



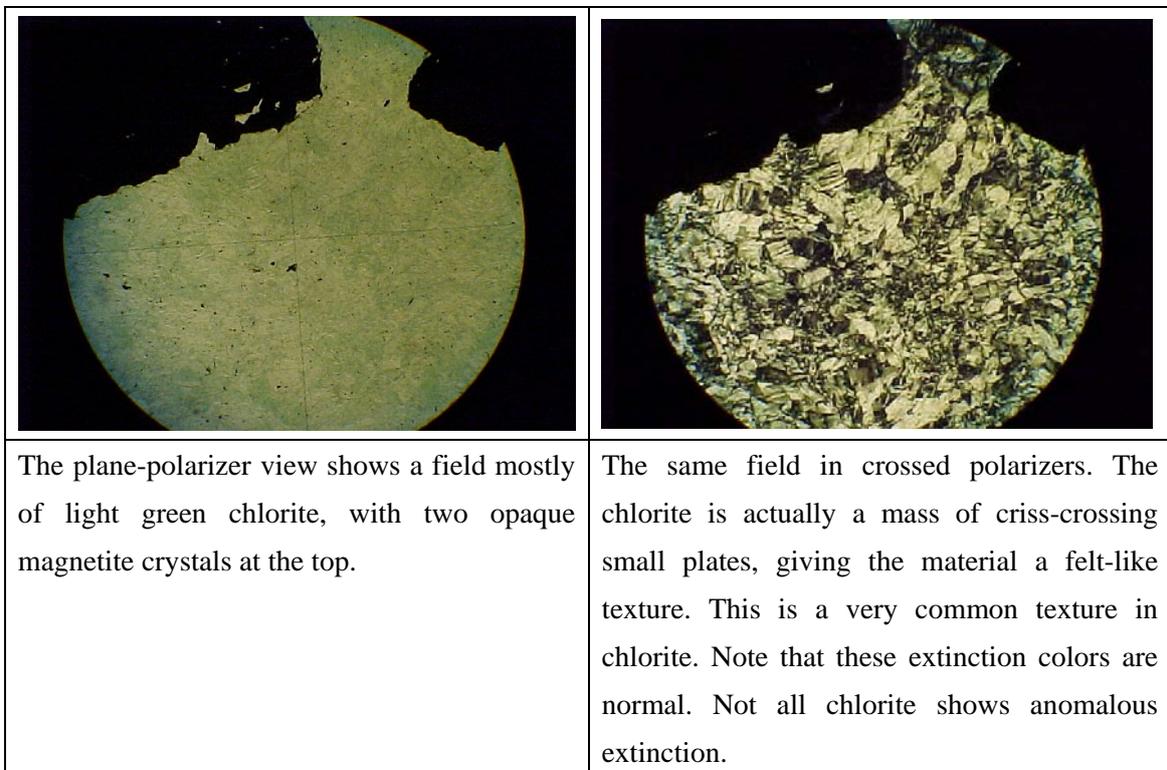
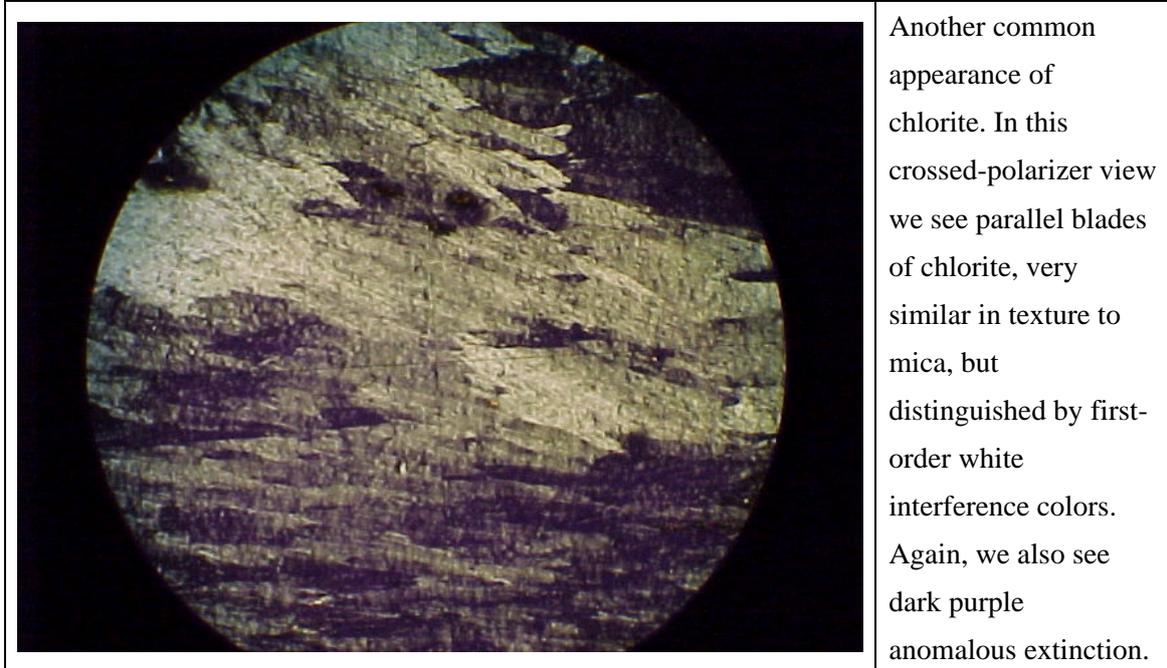
#### Plane-Polarized Light

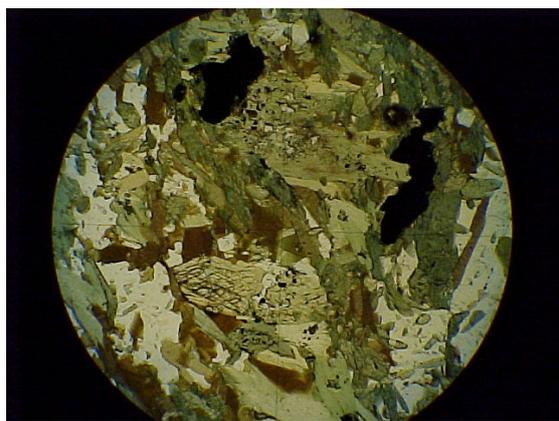
- Very common mineral in low-grade metamorphic rocks and as an alteration product of ferromagnesian minerals. Low relief, colorless to green. Light green is most common shade. Micaceous cleavage, often matted or feltlike appearance.

#### Crossed Polarizers

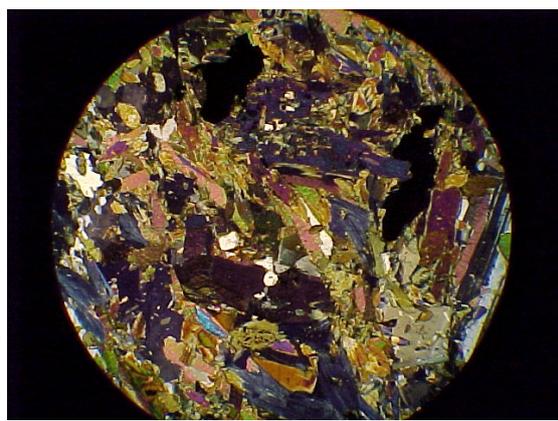
- Low birefringence results in first-order whites. Anomalous interference colors very

common (A). Dark blue, brown, purple and green are possible. Fine-grained, matted-looking varieties often show undulose extinction.

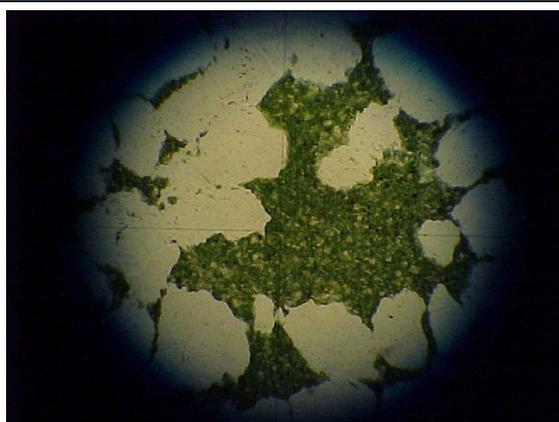




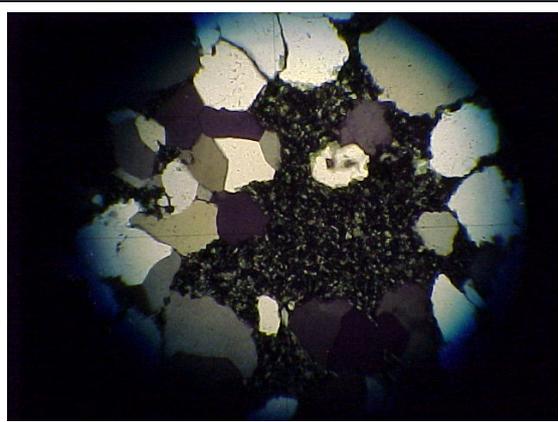
The plane-polarizer view below is pretty typical of greenschist facies metamorphic rocks. The brown is biotite, the light green is mostly chlorite, and the darker green includes hornblende (note one grain with 56-124 cleavage just below left center).



In the crossed-polarizer view, the chlorite stands out because of its anomalous extinction colors.

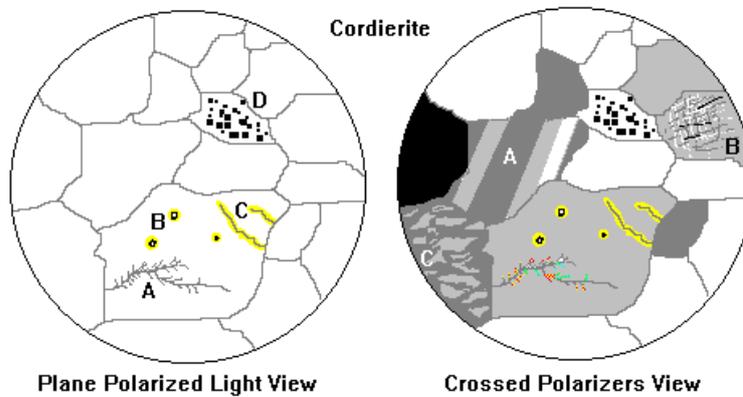


In the impure quartzite, the clay filling between the quartz grains has been metamorphosed to bright green chlorite.



The same field in crossed polarizers. The chlorite is a fine-grained felt-like mass showing first-ordred whites and normal extinction colors.

## Cordierite



### Plane-Polarized Light

A ferromagnesian metamorphic mineral that can easily be confused with quartz and feldspar. Occurs in low-pressure metamorphic rocks and contact metamorphic rocks. Distinguishing characteristics:

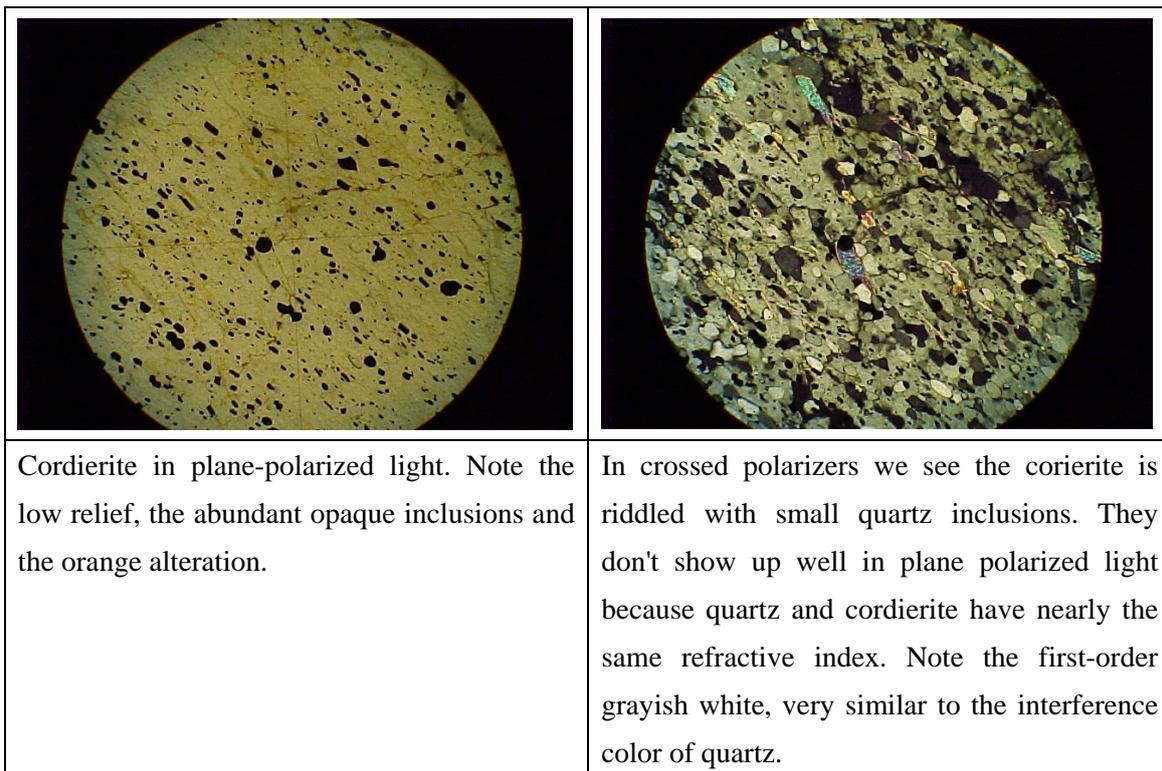
- Commonly shows feathery alteration veinlets (pinite - A).
- Pleochroic yellow halos around inclusions of zircon are common (B).
- Iron-staining alteration along cracks (C).
- Often has numerous spinel inclusions (D).

### Crossed Polarizers

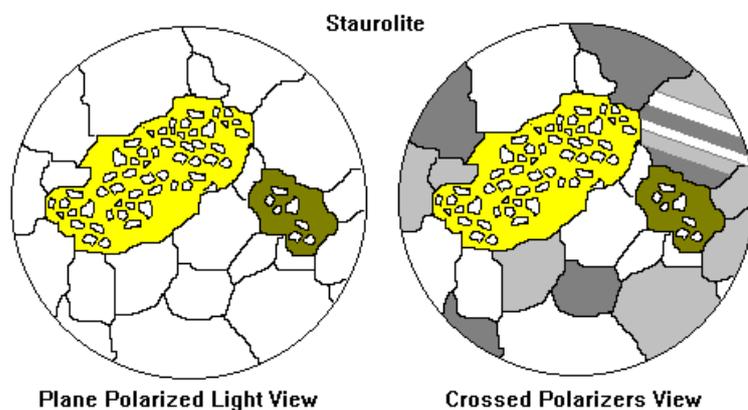
In relief and interference color, looks much like quartz and feldspar. However:

- Quartz lacks alteration
- Plagioclase can be distinguished by its lamellar twinning (A).
- Microcline can be distinguished by its tartan twinning (B).
- Feldspars often have perthitic texture (C).

Pinite is mostly muscovite and shows high interference colors.



## Staurolite

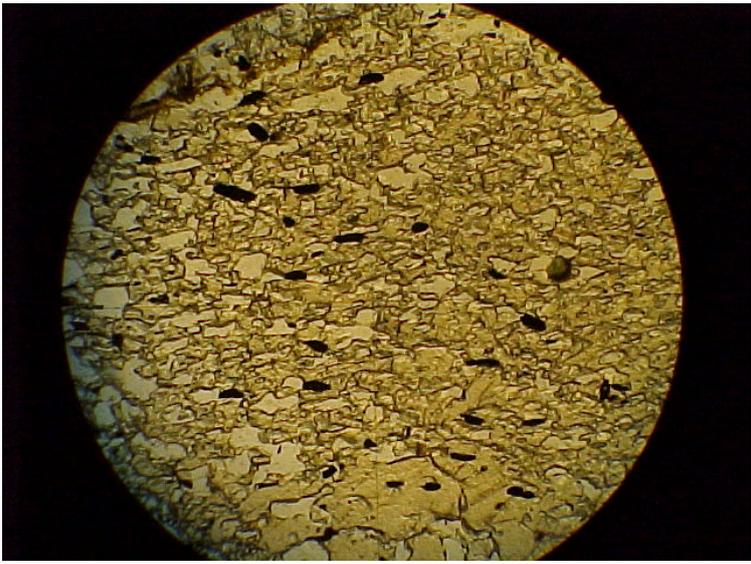
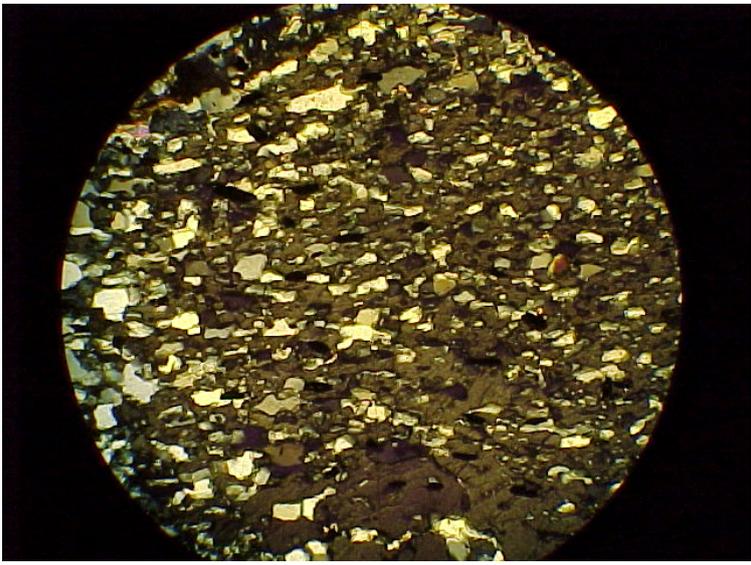
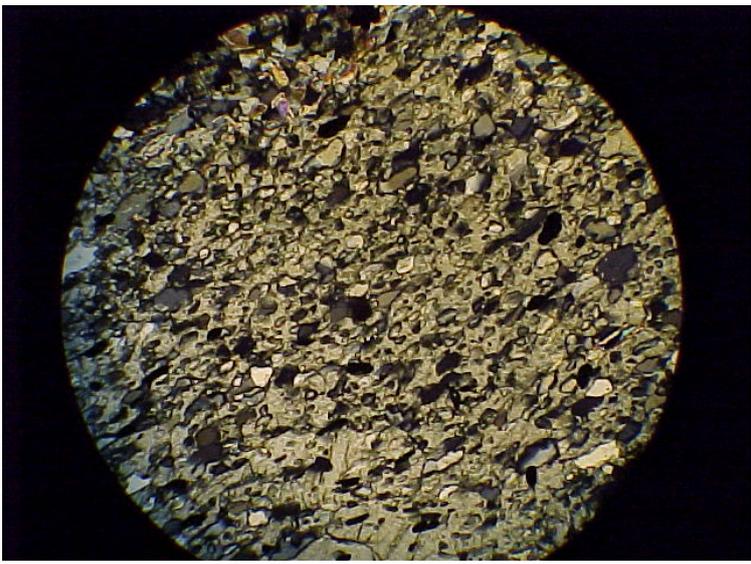


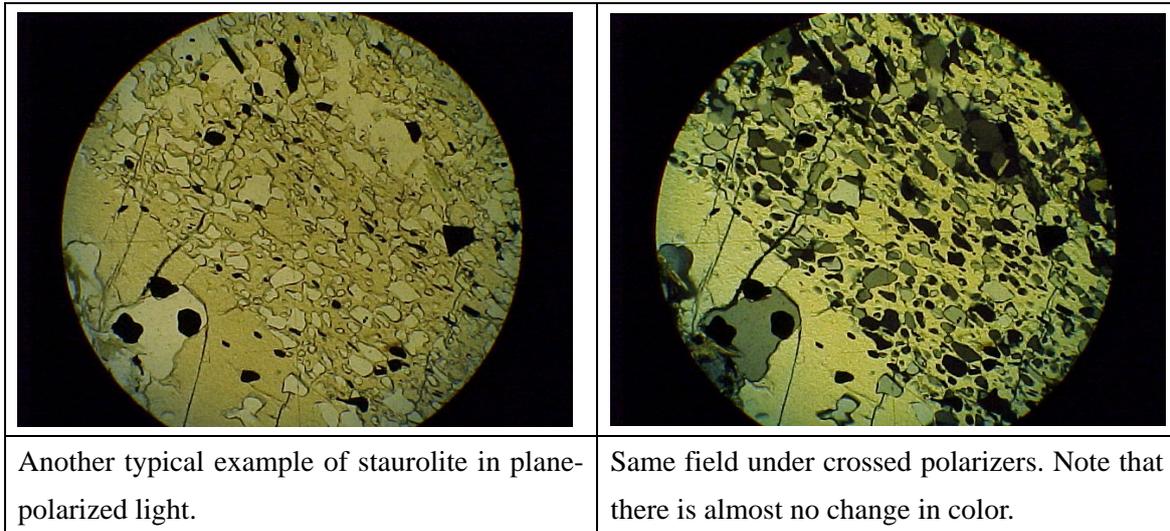
### Plane-Polarized Light

- A mineral of high-grade and high-pressure metamorphic rocks. Usually fairly good crystal form. Moderately high relief. Pleochroic in shades of yellow, and often riddled with inclusions. This texture is called *poikiloblastic*.

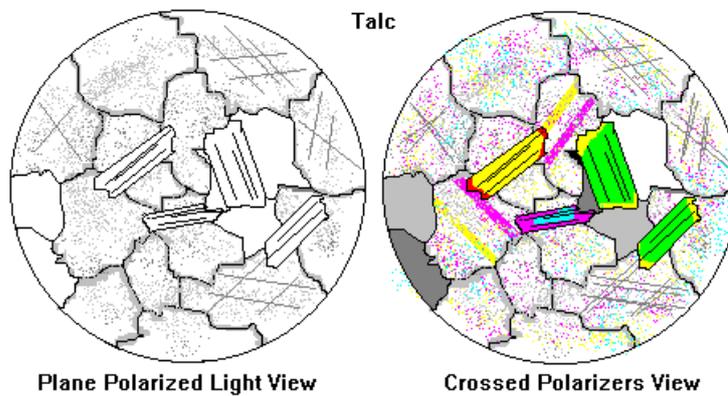
### Crossed Polarizers

- Has low birefringence and looks pretty much the same in both plane and crossed polarized light.

	<p>A very typical example of staurolite in plane-polarized light, showing high relief, yellow color and poikiloblastic texture.</p>
	<p>Same field under crossed polarizers. The dark color is due to being near extinction.</p>
	<p>Same field rotated to maximum color.</p>



## Talc



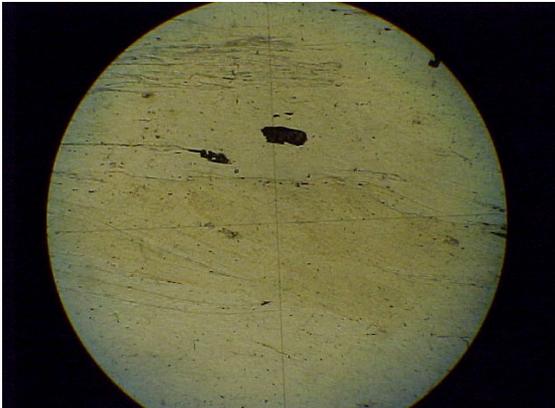
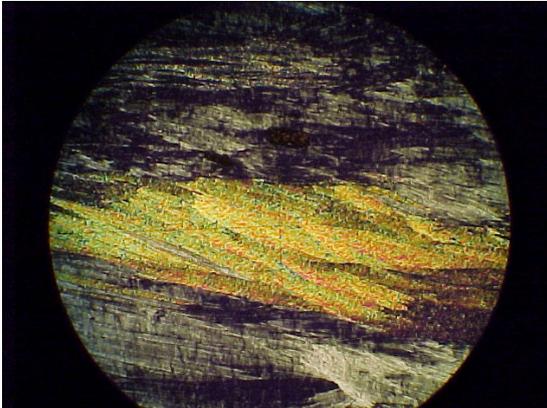
### Plane-Polarized Light

- Low relief
- Clear
- Perfect micaceous cleavage

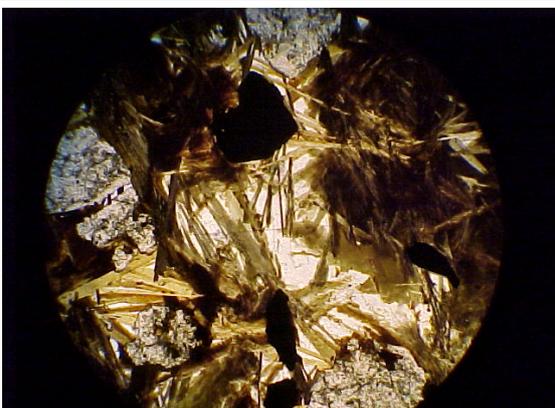
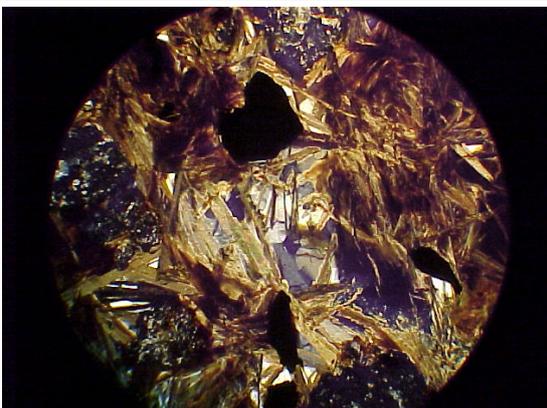
### Crossed Polarizers

- Bright second- and third-order interference colors
- Essentially indistinguishable from muscovite

- Best clue to identity is occurrence: talc is most likely to occur in calc-silicate and other magnesium-rich rocks. The sketch above shows a groundmass of calcite and quartz.

	
<p>A mass of chlorite and talc. The talc is visible in plane polarized light by a slight color difference, perhaps due to a tiny amount of iron.</p>	<p>In crossed polars the chlorite has very low interference colors while the talc has bright second order colors.</p>

### Stilpnomelane

	
<p>A plane-polarized view of stilpnomelane. Although similar in many ways to biotite, it lacks mottling and is never greenish in color.</p>	<p>The same field in crossed polarizers. The dark colors are not anomalous interference colors but are due to the very dark brown color of much of the stilpnomelane.</p>

### 4-5. Identification Chart (Akira Ishiwatari, 2002)

([http://earth.s.kanazawa-u.ac.jp/ishiwata/min\\_id\\_e.htm](http://earth.s.kanazawa-u.ac.jp/ishiwata/min_id_e.htm))

Identification Chart for the Rock-Forming Minerals by Polarizing Microscope

		A	B	C	D	E	F	G	H		
Optic Axial Angle 2Vz Optical Character		Optically Isotropic	0	20	60 90	-60	-20	0	Most Important Minerals		
Plane-Polarized Light			Uniaxial	Biaxial	Optically Anisotropic			Biaxial			Uniaxial
		Positive	Positive	Conoscopic Observation			Negative	Negative	Crossed Polarized Light		
1 Colorless	Refractive Index Low Cleavage Obscure (*Cleavage Distinct) n<=1.54-1.58	Fluorite*	Chabazite	Cryolite	Mordenite*	Wairakite	Stilbite\$*	Chabazite	Elongation Sign +/-		
		Opal	Heulandite_*	Clinoptilolite*	Tridymite	Epistilbite^*	Scolecite_*	Sepiolite+*	Straight Extinction +/-		
		Cristobalite	Chalcedony +/-	Thomsonite\$	Natrolite +	Fenaksite	Laumontite^*	Cristobalite	Low-angle Extinction ^/		
		Analcite	Leucite	GypsumX*	Mesolite\$*	Canasite*	Orthoclase* (Perthite)	Cancrinite*	High-angle Extinction X		
		Sodalite	F,OH Apophyllite Na		Phillipsite^*	Microcline#*		Kaliophilite	Elongation both signs \$		
		Nosean(gray)	Quartz	Catapleite*	Low Ab, An50-80 Plagioclase -*			Sanidine*	Nepheline	Twins	
		Cement Resin	Xonotlite+*	Anhydrite+*	Petalite*	Cordierite -	Anortho-	Scapolite-*	Polysynthetic =		
2 Colorless	Refractive Index High Cleavage Obscure	Zunyte	Eudialyte	Topaz +	Pectolite	Andalusite -	Bohmite	Beryl -	Polysynth. in 2 directions #		
		Helvite	Phenakite	Mullite +	Chondrodite	Danburite%	Serpentine^	Corundum-	Birefringence(Interference)		
		Garnet MgAl pink, CaAl yel., FeAl red, CaFe brown, MnAl red, CaCr green	Mellite + (Akermanite)	Sillimanite +	(Clino)Humite	Datolite	Kaolinite^	Mellite-(Gehlenite)	Small (Low)		
				Pumpellyite	Chrysoberyl	Axinite	Smectite		Medium (Med)		
				Stishovite	Larnite	Fo Olivine Fo<90			Pyrophyllite +	Apatite-	Large (High)
		Zircon +	Titanite(Sphene)%	Diaspore	Monticellite	Lepidolite+	Vesuvianite	Abnormal Interference Color			
		3 Colorless	Refractive Index High Cleavage Distinct	Alunite	Coesite 65°	Prehnite-	Tremolite^	Margarite+	Talc +15°	Conoscopic Dispersion Strong %	
Brucite	Barite Celestite			Lawsonite-	Cumming-	Paragonite+ Phengite+	Sericite Illite	PPL Color Zoning Distinct @			
(Clino)Zoisite\$				Anthophyllite+	Gedrite Grunerite	Muscovite +	Phengite+				
Periclase	Kotoite 21°			AugiteX	En Orthopyroxene+ En<85		Wollastonite\$	Calcite Dolomite	Opaque Minerals(Reflection)		
Diamond	PigeoniteX			DiopsideX	JadeiteX	Kyanite^	Aragonite	Magnesite Siderite	Ilmenite (black, platy, ani)		
4 Blue-Purple		Fluorite	Schedite	Clinoenstatite	Rhodonite(red)	Lazulite	Arfvedsonite-@		Magnetite (bk, bulky, iso)		
		Hauyne	Osumilite	Chloritoid _= (Blue Green, Green)		Sapphirine%	Glaucophane^@ Crossite\$@		Hematite (red transl. ani)		
		Lazurite	Yoderite Turquoise			Azurite Riebeckite_ \$	Winchite	Tourmaline+(highly pleochroic)	Pyrite (yellow, bulky, iso)		
5 Brown		Perovskite Melanite@	Cassiterite@Thorite Monazite Aenigmatite	Perovskite Melanite Allanite \$@		Barkevikite			Chalcopyrite (yel. bul. ani)		
		Cr-Spinel Chromite	Brookite @%	Titanite(Sphene)%	Pargasite^	Common	Richterite	Biotite+ Phlogopite	Pyrrhotite (brown, platy, ani)		
6 Green-Blue Green		Al-Spinel Hercynite		Antlerite	OmphaciteX%	Horndblende^	Barroisite^	Stilpnomelane	Graphite (black, platy, ani)		
		Manganosite	Chlorite _	HedenbergiteX	Aegirine Augite @	Actinolite^	Malachite	Chlorite^	isotropic/ anisotropic		
7 Yellow-Yellow Green		Pyrochlore	Xenotime-Y	Pumpellyite\$	Staurolite^	Aegirine^@	Serpentine^	Glaucosite^			
		Limonite Helvite	Rutile	Lamprophyllite	Astrophyllite, Epidote \$, Pistacite \$@			Anatase			
8 Red-Red Purple-Pink		Garnet, Cuprite	Eudialyte	TitanaugiteX@	Piemontite\$@	Hypersthene+	Bustamite	Biotite+, Hematite			

Compiled by A.Ishiwatari (2002),  
Kanazawa University, Japan

## 5. Sediment Core Analysis

(<http://www1.union.edu/~rodbell/CoreLab.html>)

*Geology Department, Union College, NY, USA*

### 5-1. Procedure for Splitting, Describing, and Sampling Cores, and for Determination of Water Content and Bulk Density

#### Core Splitting

1. After whole core magnetic susceptibility is complete, unwrap core and roll onto a layer of saran wrap on a flat table
2. Slide two split PVC tubes under saran wrap and buttress or tape in place



**Picture showing a core cutting machine**

3. Carefully cut core with a sharp knife so that the two sides are completely free of one another
4. Pull the split PVC tubes away from split cores allowing the core halves to roll onto their backs sides
5. Wrap the best preserved half up in two layers of saran wrap; use the plywood to enable you to flip the core into the split PVC tubes to complete your wrapping
6. Label the archive half as: *Name*; *Core A* (or B, C, etc.), *Trip Number* (1,2,3, etc.) and *Depth of trip* in cm (e.g., 100-200 cm); include an *up-arrow*, and label the top as *TOP*; write clearly on the core Archive **\* Use masking tape for all labels**



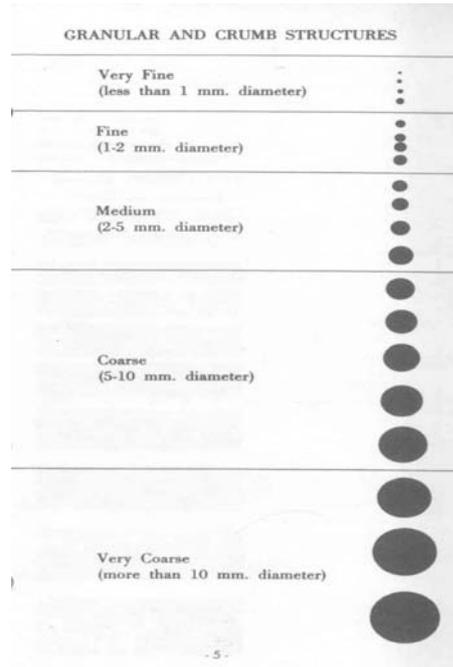
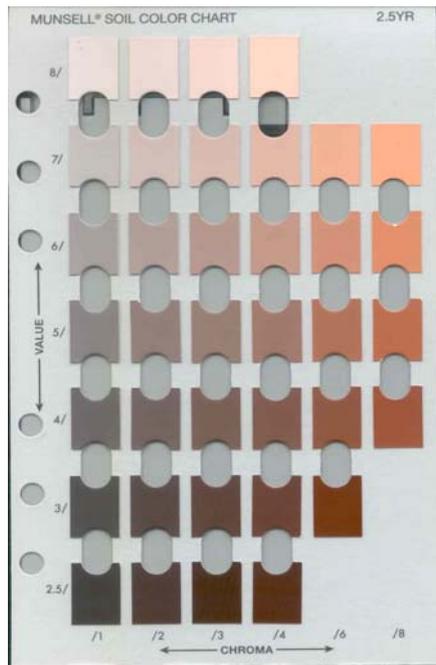
**Picture showing half-cut cores archived in a low temperature (4°C) room**

7. Tape the core into the trough with strips of masking tape so that it cannot fall out if the trough were to roll over
8. Repeat for the working half and label it accordingly

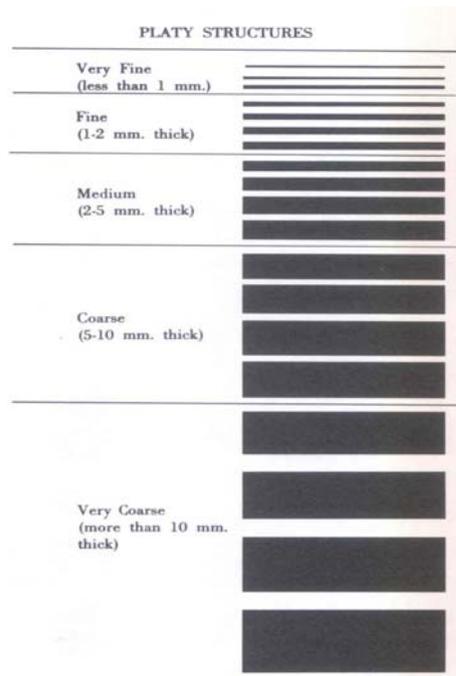
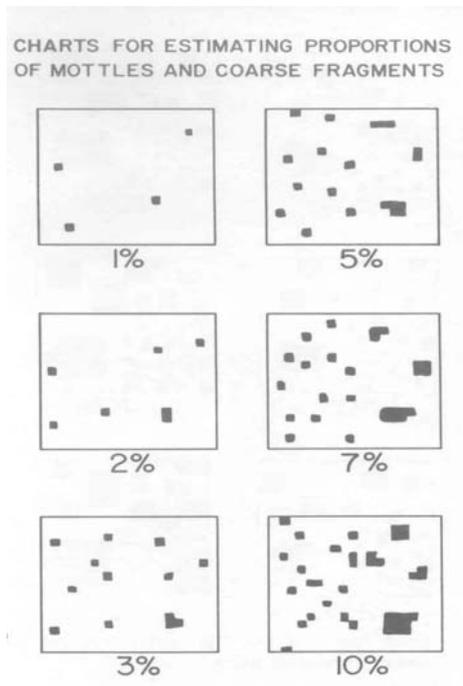
### **Core Description**

1. Carefully scrape the surface of the core parallel to bedding planes so that you have a clean surface
2. Note any obvious sedimentologic breaks and mark them on the core description sheets
3. In the column named UNIT shade the relative changes in darkness/lightness of the sediment
4. In the column named Description, include the following:
  - Munsell Color- use color book and start on 10YR page
  - Approximate dominant grain size (gravel, sand, silt, clay)
  - Note whether massive or laminated, and if laminated, note the thickness of the laminae
  - If you can see sand grains, use a handheld lens and note their roundness
  - Using the Folk Sorting chart decide on the degree of sorting of the layers
  - Look carefully at the sediment and decide whether or not the unit (or





Examples of Munsel soil color (left) and granular and crumb structure (right) charts



Examples of mottles/coarse fragments (left) and platy structures (right) charts

laminae therein) fines upward, coarsens upward, or neither

- Note the nature of the lower contact- is it *planar*, *angular* or *wavy*?
- Note the abruptness of the lower contact- is it abrupt or gradational?  
If gradational, over what interval (cm) does the gradation occur.

### Description of Sand Fraction

1. For each layer scrape off a small amount of material and place it on a 75  $\mu\text{m}$  sieve
2. With a squirt bottle (or carefully under a faucet), hose the sediment through the beaker until all that remains are particles that are larger than 75  $\mu\text{m}$ .
3. Wash all of these particles into a glass vial, that you have labelled as (sample number, >75  $\mu\text{m}$ )
4. With a pipette draw off about 5 ml of sample and place it on a large, labelled glass slide
5. Place slide on microscope and observe the sand grains that are present
6. Describe their shape according to the roundness chart above.
7. Describe the presence of other grains such as charcoal (shiny, angular, and black), leaves, twigs, etc.) as **n** (not-present), **m** (minor), or **a** (abundant); WRITE THESE VALUES IN THE COLUMN LABELLED SAMPLES TAKEN FOR ANALYSES IN DESCRIPTION SHEET AS:
  - **c (charcoal)=n,m, or a**
  - **pf (plant fragments)=n,m, or a**
  - **s (sand)= n, m, or a; rounded/subrounded, etc.**
  - **o (other)= describe**

### Core Sampling

1. Decide upon a sampling interval and indicate where samples are to be obtained on the far right-hand side of the sample description sheet (in the margin on the right side)
2. Label enough small snap-cap vials
3. Weigh these vials to the nearest 0.001 g on a top loading balance *WITHOUT TOPS ON*
4. With a clean volumetric sampler, take a 1 cc sample at each of your sampling localities and extrude this into a prelabeled and preweighed vial
5. Weigh vial and sample *WITHOUT TOPS ON* to the nearest 0.001 g on a top loading

balance.

6. Place vial in a vial holder (block of wood with holes)
7. For pollen samples, cap vials
8. For all other analyses, leave caps off and place in freeze-dryer for 24 hours
9. Return and weigh sample and calculate
  - % original water content (((wet weight-dry weight)/wet weight))\*100)
  - dry bulk density (g/cc of sediment)

## 5-2. Procedures for the Measurement of Magnetic Susceptibility (MS) in Cores and Sediment Samples



### Measurement of the MS of Sediment Cores Using the Surface Scanning Sensor

1. Select Start>Programs>*Multisus2*
2. Select File>New Data File>Surface Scanning Sensor (MS2E1)
3. Enter Core Reference (Core and Drive #)
4. Select the following
5. Drift warning level=5

6. Measurement interval = 20 mm (this is the highest resolution)
7. Alignment= strata
8. Units= SI
9. Range=0.1 for maximum precision
10. Select OK, and you will be prompted to zero the meter
11. Hold surface scanning sensor away from any solid object and Zero Unit
12. Place sensor on core at first interval so that centerline of detector aligns with start depth
13. Measure (F9), and repeat when measurement is complete
14. Apply correction every 25 cm of core depth *and at the end of your run*; to do this follow the prompt and be sure that the sensor is well away from any solid object.
15. Save File in a folder with your lake name within the MagData Folder, which is on the desk top

### **Measurement of the MS of Sediment Cores Using the 75-mm- and 100-mm-core loops**

1. Select Start>Programs>*Multisus2*
2. Select File>New Data File>Core Logging Sensor (MS2C)
3. Enter Core Reference (Core and Drive #)
4. Drift warning level=5
5. Measurement interval = 20 mm (this is probably the maximum resolution possible, and should be chosen for most cores)
6. Core Diameter= 50 mm for standard square rod core; measure for percussion cores)
7. Sensor Diameter= 75 mm for small loop; 100 mm for large loop
8. Units= SI
9. Range=0.1 for maximum precision
10. select OK, and you will be prompted to zero the meter
11. Slide core to your starting depth so that centerline of loop aligns with start depth
12. Measure (F9), and repeat when measurement is complete
13. Apply correction every 25 cm of core depth and at the end of your run; to do this follow the prompt and be sure that the sensor is well away from any solid object
14. Save File in a folder with your lake name within the MagData folder, which is

on the desk top

### Measurement of the MS of Sediment Samples Using the Dual Frequency Sensor

1. Select Start>Programs>*Multisus2*
2. Select File>New Data File>Dual Frequency Sensor (MS2B)
3. Enter Batch Reference (outcrop, soil pit, or core ID number)
4. Drift warning level=5
5. Measurement type = mass specific (if you have a known mass of oven dried [50;C max] sediment in your cubes)
6. Units= SI
7. Range=0.1 for maximum precision
8. Container Susceptibility Correction= none
9. Select container type
10. Select OK and you will be prompted to zero the meter. Remove any /all solid objects from the vicinity of the meter and select OK. From here on, do not move the meter from its present position
11. Enter sample name
12. Enter units range=0.1
13. Enter frequency to match the setting on the MS2B. Note, start with low frequency and make all measurements at the low frequency, then repeat at high frequency
14. Select OK to begin measuring
15. First take an air reading (no sample in cell), then take a sample reading (sample in cell), and finally take a second air reading.
16. Repeat step #15 at least 3 times per sample
17. Select save average
18. Repeat for all samples
19. If you are interested in determining the % frequency dependence of your samples, enter sample name *exactly* as you did for the low frequency
20. Select the high frequency option frequency option, change to high frequency on the MS2B
21. Follow prompt to zero the meter
22. Start measuring and repeat as in step #14

## 5-3. Procedure For Extraction of Biogenic Silica

### Materials and Laboratory Equipment

- mortar and pestle
- centrifuge
- 35 ml centrifuge tubes
- hotplate
- thermometer
- balance (analytical)
- 100 ml beaker
- pipette (25 ml)
- pipette (1 ml)
- ion chromatograph vials

### Reagents

- Sodium hydroxide (NaOH), 0.1 M solution
- Distilled water
- Hydrochloric acid (HCl)

### Extraction

Biogenic silica is extracted from sediment by dissolving samples in a strong basic solution. The extraction of biogenic silica from sediment dissolves all forms silica in the sample. The silica components in sediment can be separated between diatoms, sponge spicules, and silicate minerals (Conley et al., 1993). Distinguishing the source of biogenic silica is possible because of the different dissolution rates of the silica components. Diatoms have been shown to completely dissolve within the first two hours of digestion (DeMaster, 1981; Krausse et al., 1983). Sponge spicules are larger in size and have longer dissolution rates, and minerals have the longest dissolution rates.

Extractions run for 5-hours have been shown to yield the concentration of biogenic silica from diatoms (DeMaster, 1981; Conley et al., 1993)

### Procedure

1. Dry samples either in 50°C oven or by freeze drying samples
2. Crush in agate mortar and pestle to eliminate clumps of sediment
3. Weigh between 10 and 15 mg of sediment to the nearest 0.0001 g and transfer via weighing paper to 35 ml plastic centrifuge tubes

4. Heat about 500 ml of 0.1 M NaOH to 85<sup>0</sup> C
5. Using a pipette, add 25 ml of heated NaOH to each sample tube.
6. Write down precise time of step #5
7. Shake each tube and place in an 85<sup>0</sup> C bath of deionized water.
8. Every 30 minutes for the next 5 hours, remove each centrifuge tube from the water bath and shake it vigorously.
9. At precisely 2 hours after step #5, shake each sample, place in centrifuge and spin for about 1 minute
10. Draw off 1 ml of solution and place into a ion chromatograph vial
11. Repeat steps 9 and 10, at 3 hours, 4 hours, and 5 hours since step #5
12. Finally, add 1 ml of DI water to each sample.

### **Analysis**

Analysis of aliquots is by ion chromatography. In order to obtain the concentrations of diatoms and sponge spicules contributing to the total biogenic silica of a sample, two slopes must be fit to the 24-hour digestion (Conley et al., 1993). The first slope is mainly a function of the dissolution of sponge spicules, and minerals; the second slope is the dissolution of only minerals (Conley et al., 1993). Using the slopes of these two lines, the amount of diatom biogenic silica and sponge spicule biogenic silica can be extrapolated. By fitting a line to the 2, 3, 4, and 5 hour extractions the slope of the sponge spicule and mineral components is determined. Extrapolating these best-fit-lines to the y-axis will give the values of the diatom silica, and the spicule silica. The amount of diatom silica can be interpreted by only using the y-intercept from 2, 3, 4, and 5 hours.

## 5-4. Procedure for Preparing Samples for AMS Radiocarbon Dating

### Items Needed

- hot plate
- 1N HCl
- 1M NaOH
- Ultra pure H<sub>2</sub>O

### Procedure

1. Heat 250 ml beaker that are 1/2 filled with water to 90°C on hotplate
2. Place sample in labeled glass vial, and place vial in hot bath
3. Add enough 1N HCl to cover all of the sediment
4. Leave sample on hot plate in acid for 30 minutes
5. Remove from hot plate and carefully pipette off acid
6. Add NaOH and place back on hot plate
7. After 15 minutes, remove from heat and carefully pipette off NaOH
8. Repeat #6.
9. Repeat # 6 until supernatant is clear and this may take 6 or more NaOH leaches
10. Repeat step #3
11. Rinse with ultra pure H<sub>2</sub>O
12. Add a few ml of HCl to sample and dilute with ultra pure water until sample is covered in solution.

## 5-5. Procedure for Freeze Drying Sediment Samples



### Procedure

1. Place samples in open vials on the shelves of the freeze drier
2. VERY CAREFULLY lift bell cover and set in place being sure not to disturb any of your carefully placed samples
3. Be sure that all valves are closed (in up, that is 12:00, position)
4. Press the condenser button and wait for about 15 minutes
5. Press and hold the vacuum button until you hear the vacuum motor kick on
6. Leave samples for at least 12 hours
7. Turn vacuum off by pressing and holding the vacuum button until you hear the vacuum motor kick off
8. Turn off condenser by pressing that button
9. Very slowly release the vacuum by opening one of the valves in the bell cover until you hear the sound of air entering the chamber
10. Wait for a few minutes until the full vacuum has been released
11. Carefully lift off bell cover and retrieve samples
12. Samples should still be placed in the drying oven at 50°C to ensure complete air-dryness.

## 5. Geochemical Sediment Analysis Procedures

(<http://pubs.usgs.gov/of/2002/of02-371/>)

*U.S. Geological Survey Open-File Report 02-371*

*by S.A. Jablonski, E.L. Mecray, J.M. Munson, and D.S. Blackwood*

### 6-1. Carbon

Coulometer and CHN Analyzers are generally used to measure organic, inorganic, and total carbon content. Total and organic carbon percentages are determined using the Perkin Elmer 2400 Series II CHN Analyzer (Fig. 1). From these analyses, one can determine the percent inorganic carbon of a sediment sample by subtracting the percent organic carbon from the percent total carbon:

$\% \text{total carbon} = \% \text{inorganic carbon} + \% \text{organic carbon}$ . Another instrument available for the determination of inorganic carbon content in sediment, is the UIC Coulometrics, Inc. Carbon Dioxide Coulometer with an acidification unit (Fig. 2).

The organic carbon fraction in sediments is important because it serves as a binding site for contaminant metals. The abundance of organic carbon controls many diagenic processes. Normalizing contaminant data by the % organic carbon allows for the distinction of specific anthropogenic sources.

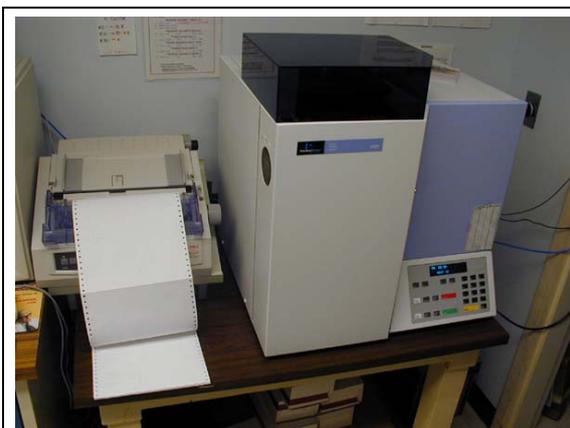


Fig. 1. Perkin Elmer Series II CHN Analyzer.

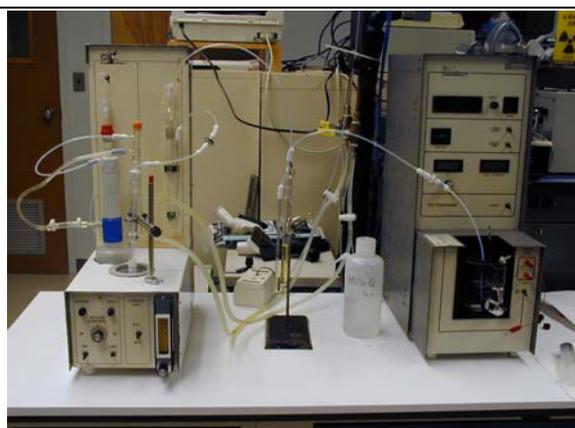


Fig. 2. Coulometer with Acidification Unit

The CHN Analyzer uses a combustion method to convert the sample elements to simple gases ( $\text{CO}_2$ ,  $\text{H}_2\text{O}$ , and  $\text{N}_2$ ). The dried and ground sediment sample is first oxidized using classical reagents like Silver Vanadate, Silver Tungstate, and EA-1000, which is a mixture of chrome and nickel oxides. Products produced in the combustion

zone include CO<sub>2</sub>, H<sub>2</sub>O, and N<sub>2</sub>. Elements such as halogens and sulfur are removed by scrubbing agents in the combustion zone. The resulting gases are homogenized and controlled to exact conditions of pressure, temperature, and volume. The homogenized gases are allowed to de-pressurize through a column where they are separated in a stepwise steady-state manner and quantified as a function of their thermal conductivities (Perkin Elmer Instruction Manual).

In order to measure %organic carbon in sediment samples, one must first acidify the samples to remove all inorganic matter. The inorganic material is removed by adding sulfurous acid to the sediment so the inorganics will turn to gas and leave the sample. Using this method, only the organic material is analyzed by the CHN Analyzer (Verardo, David J. *et. al*).

The Coulometer provides an accurate and absolute determination of the concentration of carbon dioxide (CO<sub>2</sub>) evolved from an acidification process. The coulometer cell is filled with a cathode and an anode solution (proprietary through UIC) with a colorimetric indicator. A platinum cathode and a silver anode are positioned in the cell and the assembly is positioned between a light source and a photodetector in the coulometer. When a gas stream passes through the solution, CO<sub>2</sub> is quantitatively absorbed, reacting with the elements in the cathode/anode solutions to form a titratable acid. This acid causes the color indicator to fade. Photodetection monitors the change in the solution's color as percent transmittance (%T). As the %T increases, the titration current is automatically activated to stoichiometrically generate a base at a rate proportional to the %T. When the solution returns to its original color (original %T), the current stops (UIC Coulometrics Instruction Manual).

Equipment used for these procedures includes the instruments described above (Perkin Elmer 2400 Series II CHN Analyzer and a UIC Coulometrics Coulometer with an Acidification Unit), as well as a ball-mill grinder used to grind the dried sediment sample to a fine powder, ovens used to insure the sample is dry, desiccators used to cool and store samples in a moisture-free environment, and the chemicals involved in the analysis process.

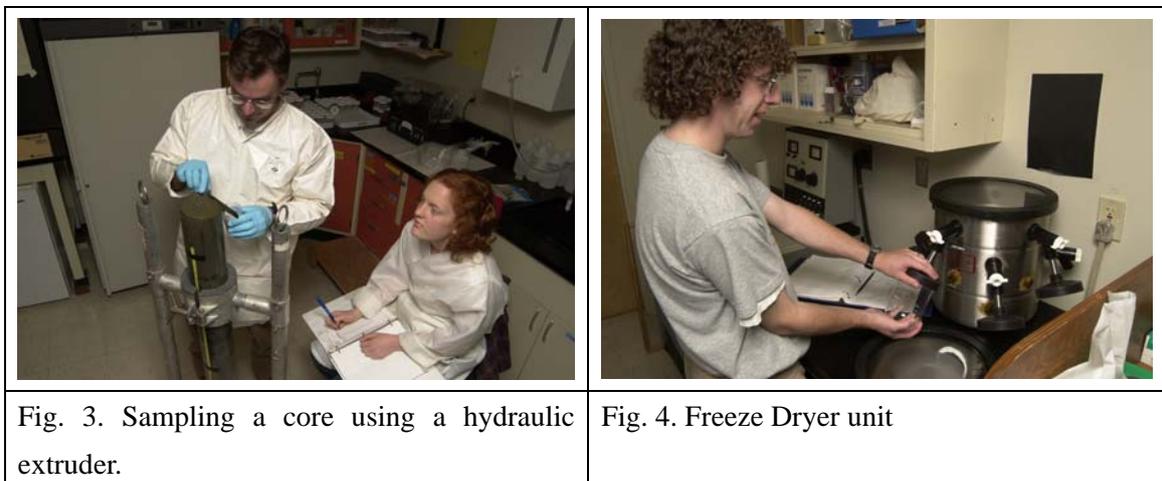
## **6-2. Geochemistry**

This laboratory is equipped to handle wet sediment samples (cores and grabs) as they are returned from field collection. Samples are collected from marine and coastal areas and returned to the laboratory for analysis. Cores are sectioned and sub-sampled using titanium tools to minimize contamination (Fig. 3). Samples from cores and grabs are

freeze-dried (Fig. 4) and sent to contract laboratories for various analyses including toxicity tests, mercury concentrations, foraminifera identification, pollen counting, nitrogen isotope analysis, and organic and inorganic contaminant concentrations.

Freeze drying is a process whereby water is removed from frozen materials by converting the frozen water directly into its vapor without the intermediate formation of liquid water. The basis for this sublimation process involves: the use of a vacuum pump to enhance the removal of water vapor from the surface of the sample; the transfer and deposition of water vapor onto the condenser; the removal of heat, due to ice formation, from the condenser by means of a refrigeration system. In essence, the freeze dry process is a balance between the heat absorbed by the sample to vaporize the water and the heat removed from the condenser to convert the water vapor into ice (LABCONCO Instruction Manual).

The vacuum environment is important as it speeds the sublimation process by removing atmospheric pressure which would act to contain the molecules in liquid form. Equipment used for the procedures in this laboratory includes the instrumentation mentioned above (hydraulic core extruder and freeze dryer), as well as acid-washing baths to clean the titanium spatulas, and a sample splitter to get a non-biased sub-sample.



### 6-3. Radiochemistry

This laboratory is equipped to determine gamma decay emissions from dried sediment samples. This method allows for the determination of the sample's age and the sedimentation rates in sediment cores. Two types of gamma detectors are used: well-type (Fig. 5) and planar-type (Fig. 6). These high purity germanium (HPGe) detectors

are in constant use and are located in a shared laboratory on the Woods Hole Oceanographic campus.

Radioisotopes, such as Pb-210 and Cs-137, can be measured by gamma emission and used in conjunction with a numerical model to estimate sedimentation rates. These measurements are important in modeling chronological changes such as heavy metal pollution. The WHFC conducts studies in the New York Bight (NYB) and in Long Island Sound (LIS) because they are in close proximity to high-density population centers and sewage dumping. Anthropogenic sources have added metals, carbon, bacteria, and organic contaminants to the sea floor. Although these pollutants have been dispersed over time, they are still present in the sedimentary record and can be measured. Cs-137 is a fallout isotope produced from bomb tests in the 1950's and 1960's and since it is not a naturally occurring isotope, it is known that the onset date for this isotope is 1954 and this information can be used to date the sediment cores collected from those areas (Santschi *et al*, 1999).



Fig. 5. Well-type Gamma Detector.



Fig. 6. Planar-type Gamma Detector.

#### 6-4. Biogenic Silica

The WHFC has the capability to digest sediment samples to be further analyzed by Inductively Coupled Plasma - Emission Spectrometry (ICP-ES) for biogenic silica. Biogenic silica fluxes indicate ecosystem productivity over time. The WHFC does not, at this time, have the instrumentation to analyze the biogenic silica extracts, so contract laboratories are used.

Equipment for this procedure includes a heated shaker bath (Fig. 7) to resuspend the sediment and a centrifuge (Fig. 8) to help remove the supernatant, as well as ovens to dry sediment samples, a mortar and pestle to grind the sediment samples, a balance to

weigh samples, a vortexer to swirl the samples, a sonicator to break up sample particles, and the chemicals used in the preparation of the sediment samples.



Fig. 7. Heated Shaker Bath.



Fig. 8. Centrifuge.

## CORE PREPARATION

### CORE PREPARATION FOR SECTIONING

#### **Materials Required:**

- Piston
- Utility Knife
- Tape
- Gloves
- Siphoning System (not shown)
- Spatula



Remove tape from the top cap of the core.

Use the spatula to vent the core tube while removing the cap.



Siphon off overlying water.



Be careful not to siphon out any sediment.



Retape the top cap onto the core tube.



Remove the tape from the bottom cap.



Using the utility knife, make two vertical cuts on opposite sides of the bottom end cap.



Using the utility knife, make a horizontal cut along the bottom edge of the end cap, connecting the two vertical cuts.



Detach 1/2 of the cap (the section without the bottom attached).



Place a patch of tape over the hole on the face of the piston.

Slide the core and end cap onto the piston.

Use an o-ring on the piston for muddy cores.

\*Do not use an o-ring for sandy cores\*.



Slide out the remaining portion of the end cap.



Press downward on the core barrel until the piston is all the way inside the core barrel.



Mount the core barrel in the clamp of the extruder stand.



Close the clamp, but do not tighten.



Press downward on the core barrel until the sediment is almost at the top edge.

Tighten the clamp.



## CORE SECTIONING

### CORE EXTRUDING AND SECTIONING

#### **Materials Required:**

- Core
- Extrusion Apparatus
- Syringe
- Ruler
- Tape Measure
- Color Chart
- Log Book
- Spatula
- Balance
- Sample Containers



Remove the remaining overlying water with a syringe.



Extrude the sediment until the lowest point on the surface is at the top of the core barrel.

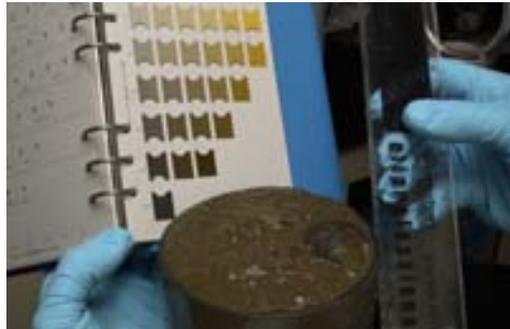
Measure the amount of surface relief from the lowest to the highest point of the exposed core.



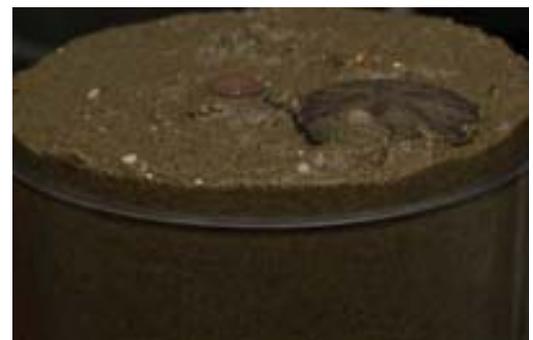
Secure a tape measure to the side of the core barrel and record the lowest point on the piston in centimeters.



Describe and record the surface sediment using the color chart.



Use the hydraulic jack to extrude the desired amount of sediment up from the core tube that you wish to section.



Monitor the piston movement relative to the tape measure and record the lowest point on the piston in centimeters.



Tare an empty container on the balance.



Using a titanium spatula, trim the edge of the sediment into the container.

Record the weight of the trimmings from the balance.



Describe, record, and remove biota from exposed core.



Use the titanium spatula to sample the remaining sediment into labeled sample containers.

\*You may need to follow the surface slope for the first few intervals, depending on the amount of surface relief.



Homogenize the sample in the sample container using the same titanium spatula from which you sampled that level of the core.



Make the required aliquots and record the weights from the balance.



Using vice grips with taped jaws, crimp the hinges of the aliquot vials to ensure proper closure.



Place bulk samples in the freezer or the refrigerator.



Place texture and *c. perf* aliquots in the refrigerator.



Record data in the log book.

For each core level sampling, use a clean titanium spatula and repeat steps above.



## DRYING SAMPLES - Oven or Freeze Dry (preparation)

### OVEN DRYING SAMPLES

#### **Materials Required:**

- Oven
- Sample to be dried



Several ovens are available for use:

- THELCO in XRay Lab for "clean" samples
- THELCO in Filter Room for desiccant and chemically treated samples
- Imperial III with N2 in Geochem Lab for samples and some small equipment (might change to air pump)

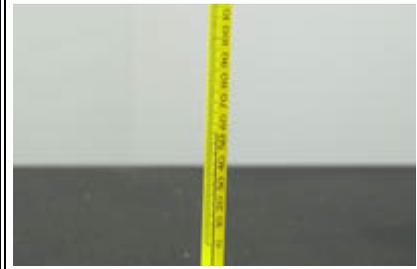
Check contents of oven with other users before placing new items in it to avoid cross-contamination to open vessels.

Remove top from sample container.



Place samples in oven at 60 degrees F for at least 24 hours or until cracks appear in sediment.

Check oven door to make sure it latches.



Place samples in desiccator when removed from oven to cool and to avoid getting moisture from the air in your samples.

Weigh samples on a balance to get the dry weight.

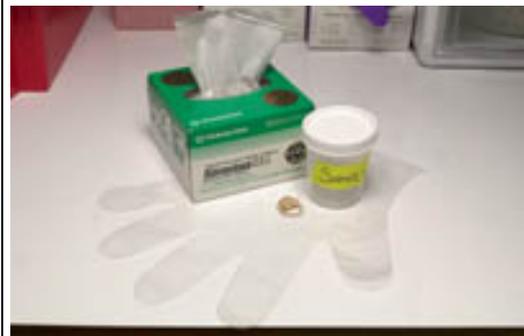
Record dry weights on a log sheet, in a data log book, or a personal log book.



## FREEZE DRYING PROCEDURES

### **Materials Required:**

- Small Kimwipe
- Rubberbands
- Plastic Glove
- Sample



After core cutting, samples are frozen and put into storage.

When ready to analyze, locate samples in storage and wipe sides of sample container with a Kimwipe so there is no frost.



Place container lid in a plastic glove so there is no contamination of sediment on the lid.

Rip a Kimwipe in half along fold so 1 layer fits on mouth of sample container.

Place Kimwipe on sample and secure with a rubber band.

\*Make sure that both lid and sample container are labeled *before* removing lid!

\*Prior to freeze drying, check to see that wet weights have been recorded on the sample.



## FREEZE DRYING SAMPLES

### **Materials Required:**

- Samples
- Freeze Dryer
- Instrument Notebook
- Sharpie
- Basin
- Paper Towels
- Vacuum Pump Oil 19 (VWR Scientific Inc.)



Weigh the samples without lids to determine which one is the heaviest. Record the wet weights in the freeze dryer notebook. Mark the heaviest sample with a Sharpie on the Kimwipe. This sample is used as a standard to compare the drying progress of the other samples.

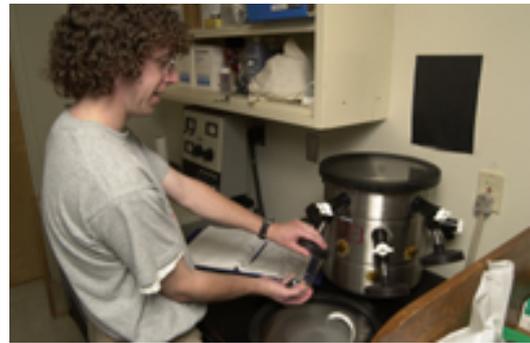
Place a stack of containers in the freeze dryer using the racks provided.

If the heaviest sample is completely freeze dried, then the others will be.



Remove exterior attachable jars, or, if necessary, place additional samples in the jars.

If using the exterior jars, make sure the vacuum seal is good.



Turn the white knob to 'vent' if there is no jar attached.

Turn the white knob to 'vac' if there is a jar attached.



Check the vacuum pump oil level and oil quality.

Oil level should be at the midline or just above - check to ensure the oil is clear and free of sediment, bubbles, and water. Check the instrument notebook for a record of the last oil change. Oil should be changed once a month during periods of

frequent use.

Start the machine by pushing the Refrigeration button ON to Man. Vac. (Manual Vacuum). Once the temperature reaches -40 degrees C (this takes about 20 min), turn the vacuum on by pushing the Vacuum button up to the ON position.

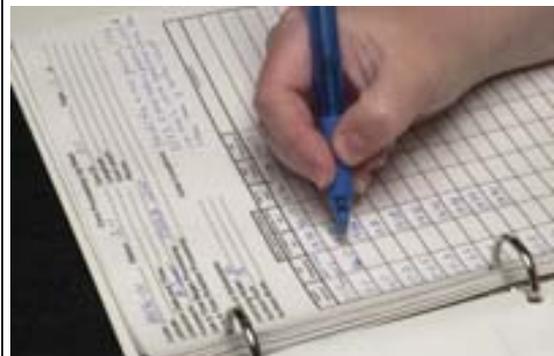
You will see the word "HI" for high vacuum displayed in the window. Once numbers start appearing, you should check it periodically to make sure the numbers are decreasing. The system is functioning properly when the number drops to 200 millibars or below.

Record the pressure and temperature in the notebook and watch periodically for 3 days until all samples are dried.

To determine if samples are dry, release the vacuum slowly by venting from the black ports. Turn off the vacuum pump, open the chamber, weigh the sample, place it back in the freeze dryer, and restart the system.

Return after 2 hours - if the weight has changed, continue the drying process.

Remove samples from machine and remove the kimwipe and rubberband - remove cap from glove and replace cap over sample so you can weigh the sample immediately.



To shut down after use:

- shut down the refrigerator and vacuum
- open the lid to the condensing chamber
- turn on the DEFROST to speed the process
- position the basin beneath the drain hose
- remove the drain hose plug and place in a secure location
- leave machine to defrost
- once defrosted, dry off chamber with paper towels, replace plug in the drain hose, and close the lid.

